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THE ISOLATION OF 6-*O*-ACETYL-2,3,4-TRI-*O*-[(+)-3-METHYLVALERYL]-β-D-GLUCOPYRANOSE FROM TOBACCO*

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ABSTRACT

A crystalline mixed ester, 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucopyranose, was obtained from Turkish tobacco by column chromatography and liquid–liquid partitions of a hexane extract. Saponification of the ester gave glucose, identified by paper chromatography, and acetic and 3-methylpentanoic (3-methylvaleric) acids, identified by gas chromatography and by the formation of their *p*-phenylphenacyl esters. Exhaustive methylation of the mixed ester, followed by saponification of the methylated ester gave methyl β-D-glucopyranoside. Confirmation of the structure of the ester was provided by its synthesis from D-glucose, (+)-3-methylvaleric acid, and acetic acid in 43% yield.

DISCUSSION

Organic acids of low molecular weight are obtained from the volatile oils of tobacco[1–9], small amounts occurring seemingly in the free state[1–3,9]. However, these acids generally are found as esters of aliphatic alcohols, terpene alcohols, and polyhydric alcohols, such as glycerol and D-glucitol[4–8].

The isolation of 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucose marks the first time that D-glucose, esterified with low-molecular-weight fatty acids, has been isolated in a pure crystalline form from tobacco. The occurrence of this ester in Nature is unique, because it is a mixed ester which still exhibits reducing properties, and a naturally occurring sugar ester, esterified to the degree found in this compound, has never been reported.

The glucose ester was isolated from a hexane extract of Turkish tobacco, grown in the Souyalassian region of Greece, as follows: the extract was partitioned between 9:1 methanol–water and hexane on a Podbielniak centrifugal extractor[10], and the material from the 9:1 methanol–water layer was chromatographed on a Magnesol–Celite column. The effluent was thoroughly fractionated by partitions and chromatography to give the mixed tetraester of D-glucose.

*Dedicated to the memory of Professor M. L. Wolfrom.

The i.r.-absorption spectrum (Fig. 1) of this compound showed that it was a sugar ester containing at least one free hydroxyl group. On saponification, the sugar moiety was identified as a glucose by paper chromatography with the procedure of Partridge[11] and that of Hough, Jones, and Wadman[12]. Gas–liquid chromatography[13] showed the acids to be mainly (a) six-carbon acid(s) with a small amount of acetic or formic acid. The acids were converted into their *p*-phenylphenacyl esters by the method of Shriner and Fuson[14], and separated chromatographically according to Kirchner, Prater, and Haagen-Smit[15]. *p*-Phenylphenacyl acetate and *p*-phenylphenacyl (+)-3-methylvalerate were obtained.

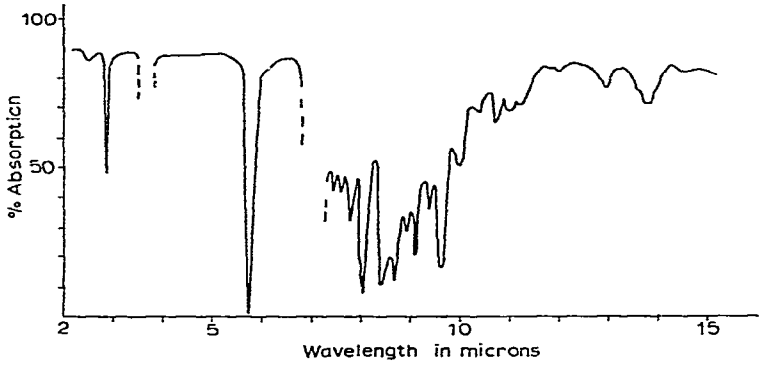

Fig. 1. I.r. spectrum of 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucose determined on a Nujol mull.

Methylation of the isolated D-glucose ester with methyl iodide and silver oxide[16] followed by saponification yielded methyl β-D-glucopyranoside. This showed that only one free hydroxyl group was present and that it was attached to the C-1 of the D-glucose molecule. This likewise indicated the presence of four ester groups. Furthermore, the elementary analysis and the saponification equivalent (118) indicated the presence of three 3-methylvaleryl groups and one acetyl group. The position of the acetyl group was determined by synthesis, and the β-D-configuration was established by mutarotation of the natural and synthetic products.

The glucose ester, 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucopyranose, was synthesized by a modification of the method used by Reynolds and Evans[17] for the synthesis of α- and β-D-glucose 1,2,3,4-tetraacetate and of the method of Wolfrom and Christman[18] involving further esterification after detritylation. D-Glucose was converted into the 6-*O*-trityl derivative, which was further esterified with (+)-3-methylvaleryl chloride to give 1,2,3,4-tetra-*O*-[(+)-3-methylvaleryl]-6-*O*-trityl-D-glucose. The primary hydroxyl group at C-6 was regenerated by detritylation with hydrogen bromide, and then esterified with acetyl chloride. The (+)-3-methylvaleryl group was removed from the 1-position with hydrogen bromide (more drastic conditions than in the detritylation). The resulting 1-bromo compound was treated with silver carbonate to give 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-α- and -β-D-glucose. The β anomer crystallized, whereas the α anomer remained as a syrup. With time, the

α anomer mutarotated to the α,β-equilibrium mixture from which an additional quantity of the β anomer crystallized. The yield of the β anomer was 43% based on D-glucose.

The synthetic product was shown to be identical with the ester isolated from tobacco. Thus, the structure of the isolated material has been unequivocally identified as 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucopyranose.

A small amount of a crystalline by-product was obtained from this synthesis. The i.r. spectrum and the elementary analysis suggested that it was 2,3,4,6-tetra-*O*-[(+)-3-methylvaleryl]-D-glucose, which was confirmed by synthesis.

EXPERIMENTAL

General. — All melting points were determined with a Fisher–Johns melting-point apparatus. Melting points and boiling points were uncorrected. The elementary analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Colorado.

Isolation and identification of 6-O-acetyl-2,3,4-tri-O-[(+)-3-methylvaleryl]-β-D-glucopyranose from tobacco. — The tobacco (55 kg) was shredded on a small cutting machine and extracted three times in a stainless-steel column with hexane at 30°. The combined hexane extracts were concentrated to 40 gallons on a Turba-Film evaporator. The hexane extract was passed through a Podbielniak extractor[10] where it contacted a stream of 9:1 methanol–water. The methanol–water extract was concentrated to a small volume on the Turba-Film evaporator, and water was removed at 45–50° under 28 mm pressure to give an oil (yield 438 g).

This material was divided into three parts (125, 153, and 160 g, respectively). Each of these was dissolved in 1:1 hexane–ether (500 ml) and the solution was passed through a column of Magnesol–Celite (5:1) prewashed with 1:1 hexane–ether (1000 ml). Development was effected with the same solvent mixture (6–8 liters). The column of adsorbent was extruded after collecting 6 l of effluent, and cut into 3 sections as indicated by the colored zones. The sections were eluted with methanol, and the solvent was removed under reduced pressure. The combined yields from the three columns were: 70 g from effluent (Fraction A), 120 g from bottom zone (Fraction B), 172 g from middle zone (Fraction C), and 29 g from top zone (Fraction D). Total yield was 391 g (89%).

Fraction A was partitioned between three 800-ml layers of hexane and four 800-ml layers of 9:1 methanol–water. Evaporation under diminished pressure of the combined lower layers yielded a syrup (47.4 g). It was dissolved in 1:1 hexane–ether (800 ml) and divided into 8 fractions (each 100 ml). Each fraction was passed through a silicic acid column (14 × 8 cm) which had been activated by washing with 0.2, 1.0, 0.8, and 1.0 vol. (solvent needed to wet the entire column) of ether, 1:1 ether–acetone, ether, and hexane, respectively. The column was developed with hexane–ether solutions; the amount of ether was increased from 10% to 100%, as needed to elute the material from the column. After evaporation of the solvent, similar syrupy fractions of the 8 separate chromatograms were combined according to their i.r.

spectra: 1.4 g (Fraction 1), 8.9 g (Fraction 2), 22.5 g (Fraction 3), and 10.5 g (Fraction 4).

The material from Fraction 3 was partitioned between 25 100-ml hexane–ether (3:1) layers and 25 100-ml methanol–water (3:1) layers, with an H. O. Post countercurrent-distribution apparatus[19]. After evaporation at 50° under diminished pressure, the material from upper layers 3–12, 13–16, and 17–22 was combined to give Fractions A, B, and C (3.13 g, 1.95 g, and 2.38 g, respectively). Fractions A and B partially crystallized, and were filtered off after the addition of pentane. More crystalline material was obtained from the filtrate and Fraction C. Recrystallization from hexane of the total solid obtained gave needles (1.2 g), m.p. 104–106°, $[\alpha]_D^{25}$ +30.21° (*c* 4.7, chloroform), $[\alpha]_D^{25}$ +15.45 → +54.50° (*c* 4.0, 4:1 methanol–water), constant after 25 h. This substance also gave a positive Somogyi[20] test for reducing sugars.

Anal. Calc. for $C_{26}H_{44}O_{10}$: C, 60.44; H, 8.58; Mol. wt. 516.6. Found: C, 60.16, 60.41; H, 8.56, 8.61; Mol. wt., 415 (with dec.; Rast method).

To a solution of the ester (100 mg) in methanol, cooled to −20°, was added a solution of 0.2M sodium methoxide in methanol (0.3 ml). After being kept at −20° for 62 h, acetone (6 ml) was added. A white solid precipitated. It was filtered off, dissolved in methanol, and the solution was concentrated under diminished pressure to give a syrup (29.5 mg). The filtrate was concentrated to give the sodium salts of the acids.

Identification of the sugar moiety. — The syrup was chromatographed according to the method of Partridge[11] along with D-glucose, D-ribose, D-xylose, D-arabinose, and D-rhamnose, on Whatman No. 1 paper with 4:1.1:1.9 butyl alcohol–ethanol–water (v/v) for 40 h. The spots were revealed[12] by spraying with a saturated solution of *p*-anisidine hydrochloride in butyl alcohol followed by drying at 100°. The syrup gave only one spot which coincided exactly with D-glucose. It was compared in a second chromatogram with maltose, sucrose, D-fructose, D-mannose, and D-glucose, and gave one spot coinciding with D-glucose.

Identification of acids. — The sodium salts of the acids were converted into the methyl esters by heating at reflux with methanol (10 ml) containing 10 drops of concentrated sulfuric acid. The esters were chromatographed on a Perkin–Elmer Vapor Fractometer, Model 154-B with a column (1 m) packed with purified Celite containing 20% of the adipate polyester of di-(2-hydroxyethyl) ether[13]. The chromatogram showed the presence of methyl acetate (or methyl formate) and (a) methyl ester(s) of a C_6 acid.

To a solution of the ester (100 g) in acetone (15 ml) cooled to 0° was added 0.1M sodium hydroxide (25 ml), and the mixture was kept for 2 h at 0°. It was neutralized with 0.1M hydrochloric acid (phenolphthalein). A control experiment was performed with glucose (33 mg) instead of the glucose ester to correct for any acid formation due to the action of alkali on the glucose portion. The saponification equivalent was found to be 118.2.

The sodium salts of the acids were recovered, dissolved in water (2 ml), and neutralized with two drops of dilute hydrochloric acid. Ethanol (5 ml) and *p*-phenylphenacyl bromide (200 mg) were added and the solution was heated at reflux for 1 h.

The reaction mixture was evaporated to dryness. The residue was dissolved in benzene and the solution was chromatographed on a silicic acid column (14 × 2 cm) according to the procedure of Kirchner, Prater, and Haagen-Smit[15]. The column was developed with 1:1 hexane–benzene, and fractions of 20 ml were collected. Fractions 4–6 yielded *p*-phenylphenacyl (+)-3-methylvalerate (51 mg), m.p. 46° (lit.[21]: m.p. 47°). This m.p. was not depressed when the product was mixed with an authentic sample prepared from (+)-3-methylvaleric acid. Fractions 8 and 9 gave 11 mg of *p*-phenylphenacyl acetate, m.p. 110–111°. A mixed melting point with an authentic sample gave no depression. The i.r.-absorption spectra of both esters were identical with those of the corresponding authentic samples.

Methylation of the ester[16]. — A mixture of the ester (300 mg), methyl iodide (2 ml), and silver oxide (200 mg) was warmed (oil bath) with vigorous stirring. Additional methyl iodide (2-ml portions) was added periodically over two days. Ether was added, the suspension was filtered, and evaporation of the filtrate gave a syrup (300 mg). The i.r. spectrum showed no hydroxyl peak. The methylated product was saponified in acetone (25 ml) at 0° with 0.1M sodium hydroxide (49 ml). After neutralization with 0.1M hydrochloric acid and evaporation to dryness under diminished pressure, the residue was dissolved in water (20 ml) and the solution was deionized by successive passage through (6 × 1.2 cm) columns of Amberlite IR-120 and Duolite A-4. The final effluent was concentrated to dryness under reduced pressure to give a syrup (100 mg). This was dissolved in abs. ethyl alcohol and the solution was chromatographed on a (6 × 1.2 cm diameter) column of Florex XXX–Celite (5:1 by wt.) prewashed with abs. ethyl alcohol (50 ml). The column was developed with abs. ethyl alcohol and 25-ml fractions were collected. On the basis of the i.r. spectra, fractions 3–6 were combined, dissolved in ethyl alcohol, and kept to crystallize. Recrystallization from ethyl alcohol gave methyl β-D-glucopyranoside (60 mg), m.p. 112–113°, $[\alpha]_D^{25}$ −32.5° (*c* 3.0, water). A mixed m.p. with an authentic sample[22] gave no depression. The i.r. absorption spectra of both products were identical.

(+)-*3-Methylvaleryl chloride.* — Attempts to resolve racemic 3-methylvaleric acid according to the methods of Levene and Marker[23] were unsatisfactory for our purpose. The dextrorotatory 3-methylvaleric acid (120 g), a known constituent of tobacco, was obtained from tobacco (70 kg) according to the procedure of Sabetay and Panouse[4], b.p. 196°, $[\alpha]_D^{25}$ +7.40° (lit.[25]: b.p. 196–198°, $[\alpha]_D^{25}$ +7.94).

(+)-3-Methylvaleric acid (20 g) was mixed with thionyl chloride (13 ml) and kept for 15 h. The product was distilled on a short glass helices-packed column to give (+)-3-methylvaleryl chloride, b.p. 140–142°.

1,2,3,4-Tetra-O-[(+)-*3-methylvaleryl*]-*6*-O-*trityl*-D-*glucose.* — D-Glucose (5 g), powdered and dried over phosphorus pentoxide, and chlorotriphenylmethane (8 g recrystallized from acetyl chloride by the procedure of Reynolds and Evans[17]), were dissolved in anhydrous pyridine (45 ml) by continuous shaking for 5 h. The solution was kept for 15 h and freshly prepared (+)-3-methylvaleryl chloride (16 g) was added carefully. After being kept for 7 h at room temperature, the reaction mixture was poured into ice–water (200 ml), and the aqueous mixture was extracted

twice with equal portions of ether. The ether extracts were combined, concentrated, decolorized with Darco G-60, and dried with sodium sulfate. Removal of the ether under reduced pressure gave a crude syrup product (25 g, incompletely dry).

1,2,3,4-Tetra-O-[(+)-*3-methylvaleryl*]-D-*glucose.* — The trityl ester was dissolved in acetic acid (60 ml) and the solution was cooled in an ice bath. Acetic acid (12 ml) saturated with hydrogen bromide gas at 0° was added. Bromotriphenylmethane precipitated and was immediately removed by filtration. The filtrate was quickly poured into ice–water (300 ml) and thoroughly stirred. The oily layer was dissolved in ether, decolorized with Darco G-60, and dried with sodium sulfate. Evaporation gave a noncrystalline product (17 g), contaminated with a small percentage of triphenylcarbinol.

6-O-Acetyl-1,2,3,4-tetra-O-[(+)-*3-methylvaleryl*]-D-*glucose.* — The crude 1,2,3,4-tetraester was immediately dissolved in anhydrous pyridine (25 ml) and treated with acetyl chloride (4 ml). After being kept for 4 h at room temperature, the reaction mixture was poured into ice–water (200 ml). The solid product was dissolved in ether, the solution was decolorized with Darco G-60, and dried with sodium sulfate. After filtration, evaporation of the filtrate under reduced pressure gave a crude syrup (16.4 g).

6-O-Acetyl-2,3,4-tri-O-(+)-*3-methylvaleryl*]-D-*glucopyranosyl bromide.* — The mixed ester just described was dissolved in 1,1,2-trichloroethane (25 ml). Acetic acid (15 ml) saturated at 0° with hydrogen bromide was added, and the mixture was kept for 24 h. The solvents were removed under diminished pressure, and benzene was added to form an azeotrope with acetic acid to yield 16.5 g of crude product. The free (+)-3-methylvaleric acid was not removed from the product at this time.

6-O-Acetyl-2,3,4-tri-O-[(+)-3-*methylvaleryl*]-β-D-*glucose.* — The glycosyl bromide was dissolved in acetone (45 ml), water (1 ml) and freshly prepared silver carbonate (9 g) were added, and the mixture was shaken continuously on a water bath for 7 h at 50°. The mixture was filtered through a short column (7 cm × 1.5 cm) containing a mixture of Darco G-60, Celite, and sodium sulfate (1:1:1). The filtrate was concentrated to give an almost colorless syrup (14.5 g) still containing one-mole equivalent of (+)-3-methylvaleric acid.

This crude product was dissolved in pentane and kept for 15 h at −15°, whereby 1.1 g of material crystallized. Recrystallization from hexane gave fluffy white needles, m.p. 105–106°; $[\alpha]_D^{25}$ +30.90° (*c* 2.32, chloroform), $[\alpha]_D^{25}$ +16.01 → +55.00° (*c* 1.82, 4:1 methanol–water, constant after 25 h). A mixed melting point with the glucose ester isolated from tobacco showed no depression. The crude mother liquor of the product described above had $[\alpha]_D^{25}$ +50.90 → +39.52° (*c* 5.0, 9:1 methanol–water). It was partitioned between 40 10-ml layers of pentane and 40 10-ml layers of 3:1 methanol–water. The solvent was removed from the upper and lower layers under reduced pressure, and the material in these layers was combined according to their i.r.-absorption spectra. The material in lower layers 15–39 and upper layers 25–39 were combined (6.1 g) which gave additional crystalline product (5.1 g), for a total yield of 6.2 g (43% based on D-glucose).

Lower layers 0–14 yielded a material (3.6 g) which contained mostly (+)-3-methylvaleric acid. Upper layers 0–4 gave 1.8 g of syrup which showed a weak i.r. absorption for a hydroxyl group. This indicated that the reactions involving the formation of a free hydroxyl group on C-1 were incomplete.

Upper layers 5–24 gave a material (80 mg) which crystallized. Recrystallization from hexane gave 52 mg of crystals, m.p. 52–53°. The i.r. spectrum of this material suggested that it was 2,3,4,6-tetra-*O*-[(+)-3-methylvaleryl]-D-glucose, formed by the incomplete tritylation, or by detritylation in the initial esterification step. This substance was then synthesized as described below. A mixed melting point of 2,3,4,6-tetra-*O*-[(+)-3-methylvaleryl]-D-glucose and of the crystalline material from upper layers 5–24 gave no melting-point depression.

2,3,4,6-*Tetra*-O-[(+)-3-*methylvaleryl*]-D-*glucose*. — D-Glucose (2 g) was mixed with anhydrous pyridine (24 ml) and heated on the steam bath for 30 min. On cooling, (+)-3-methylvaleryl chloride (8 ml) was added. The mixture was heated for 1 h at 60°, and then poured into cold water (200 ml). The ester was extracted with ether; the ether solution was washed with 5% sulfuric acid (10 ml) and water (20 ml), and dried with sodium sulfate. Subsequent removal of the ether gave 5.65 g of the glucose ester. The i.r. spectrum showed no hydroxyl group. The penta(methylvaleryl)-glucose was dissolved in 1,1,2-trichloroethane (6 ml) and treated for 2 h with acetic acid (6 ml) saturated with hydrogen bromide at 0°. Toluene (15 ml) was added, and the mixture was concentrated under diminished pressure to give the glycosyl bromide (4.5 g). This compound was dissolved in dry acetone (10 ml) and the solution was cooled to 0°. Freshly prepared silver carbonate (2 g) and water (0.25 ml) were added. The reaction mixture was continuously stirred for 45 min at 0°, heated for several min at 55° and then filtered. The filtrate was concentrated to a syrup, which partially crystallized at −15°. The crystalline product was filtered and recrystallized from hexane–petroleum ether, m.p. 52–53°, $[\alpha]_D^{25}$ +22.67 → +62.00° (*c* 3.0, 4:1 methanol–water, constant after 27 h).

Anal. Calc. for $C_{30}H_{52}O_{10}$: C, 62.91; H, 9.51. Found: C, 63.14; H, 9.09.

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BENZOYL DERIVATIVES OF D-GLUCONOLACTONES AND THEIR REACTION WITH ALCOHOLS*

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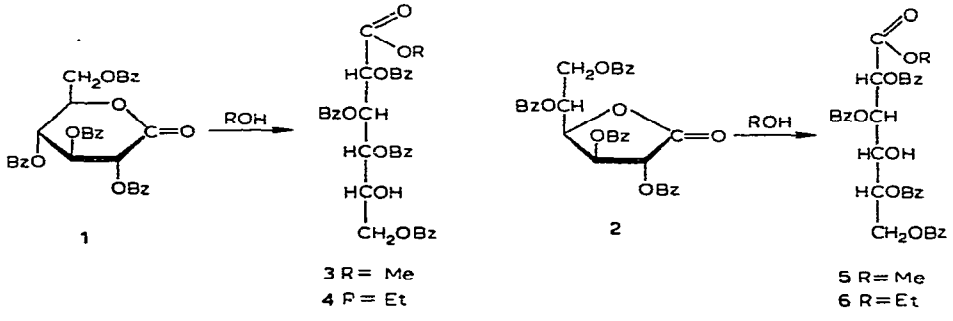
ABSTRACT

Benzoylation of D-glucono-1,4-lactone and D-glucono-1,5-lactone afforded the corresponding tetra-*O*-benzoyl derivatives. They showed the optical rotation and i.r. spectral absorption characteristic for 1,4 or 1,5-lactones, respectively. Opening of the lactone ring was effected by treatment with alcohols to give tetra-*O*-benzoyl-D-gluconates having an unesterified hydroxyl group at C-4 or C-5, respectively.

RESULTS AND DISCUSSION

Benzoylation of D-glucono-1,4-lactone and D-glucono-1,5-lactone afforded the corresponding tetra-*O*-benzoyl derivatives, which showed the optical rotation and i.r. spectral absorption[1] characteristic for 1,4 or 1,5-lactones, respectively.

The fact that the 2,3,4,6-tetra-*O*-benzoyl-D-glucono-1,5-lactone (**1**) could be obtained by direct benzoylation of the 1,5-lactone is of interest, since almost all known derivatives of 1,5-lactones have been obtained by oxidation of the corresponding aldose derivatives[2,3]. Upson and Bartz[4] reported that the acetylation of D-glucono-1,5-lactone in the presence of zinc chloride gave a tetra-*O*-acetyl-D-gluconic acid monohydrate.



On benzoylation of D-glucono-1,4-lactone, the 2,3,5,6-tetra-*O*-benzoyl-D-glucono-1,4-lactone (**2**) was obtained. When the crude product was dissolved in

*Dedicated to the memory of Professor M. L. Wolfrom.

**Research Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas.

methanol, opening of the lactone ring took place, and after a few days crystalline methyl 2,3,5,6-tetra-*O*-benzoyl-D-gluconate (**5**) was obtained. Treatment of the benzoylated lactone with ethanol afforded the crystalline ethyl ester (**6**). The opening of the lactone ring was probably catalyzed by traces of acid that still remained in the crude product. Ethyl 2,3,5,6-tetra-*O*-benzoyl-D-gluconate (**6**) was also obtained by heating at boiling pure **2** in ethanol containing a catalytic amount of hydrogen chloride until dissolution. However, the reaction was slow and was completed only after ten days. As reported for the unsubstituted lactones[5], opening of the 1,5-lactone ring was faster than that of the 1,4-lactone. Alcoholysis of **1** was completed in a few hours, and it could be easily followed by t.l.c. on silica gel, as the ester having one hydroxyl group free has a much lower R_F value than the benzoylated lactone.

The n.m.r. spectra of substances **3**–**6** were measured in chloroform-*d*. They all showed the presence of four *O*-benzoyl groups (τ 2.1, 2.6). The methyl ester protons appeared at τ 6.38 for compound **3** and at τ 6.4 for **5**. The ethoxy carbonyl group of both ethyl esters (**4** and **6**) was observed as a triplet of intensity 3 at τ 8.88, and as a quartet of intensity 2 at τ 5.9.

As Horton and Wander[6] reported for other acyclic derivatives of sugars, H-3 is the lowest-field signal for all four compounds. This signal appeared as a triplet at τ 3.7, J 3.2 Hz for substances **3** and **4**, whereas it was superposed with the H-5 signal, at a higher field, for compounds **5** and **6**, because of the stronger deshielding effect of the adjacent benzoate group at C-4 of **3** and **4** compared with the hydroxyl group of substances **5** and **6**. The H-2 signal showed as a sharp doublet at τ 4.38, $J_{2,3}$ 3.2 Hz. In the spectra of **3** and **4** the H-2 doublet overlapped the H-4 signal. At higher field, all derivatives showed a 3-proton multiplet due to the superposed signals of H-6, H-6', and the proton at the carbon atom attached to the unesterified hydroxyl group (H-5 for **3** and **4**, and H-4 for **5** and **6**). All four compounds showed a broad signal centered at τ 7.1, due to the hydroxyl proton, which disappeared on deuteration.

The partially benzoylated esters having a free hydroxyl group at C-4 or C-5 could be used as intermediates for the preparation of sugar derivatives having a particular substituent at C-4 or C-5.

EXPERIMENTAL

General. — Melting points were determined with a Thomas-Hoover apparatus (Arthur Thomas Co., Philadelphia, Pennsylvania) and are uncorrected. Specific rotations were measured with a 1-dm tube. Infrared spectra were recorded with a Perkin–Elmer Infracord spectrophotometer. N.m.r. spectra were determined in deuteriochloroform with tetramethylsilane as the external reference, by using a Varian A-60 spectrometer. T.l.c. was performed on Silica Gel G (E. Merck, Darmstadt, Germany) using 1:9 ethyl acetate–benzene as the mobile phase; the materials were detected with iodine vapor or by spraying with 5% ethanolic sulfuric acid, followed by heating for 10 min at 110–120°.

2,3,4,6-Tetra-O-benzoyl-D-glucono-1,5-lactone (**1**). — To a suspension of D-glucono-1,5-lactone (1.0 g) in anhydrous pyridine (5 ml), cooled to 0°, was added

with stirring benzoyl chloride (4.5 ml). After being shaken for 90 min at room temperature, the mixture was poured with stirring into ice–water. The gum that separated was washed several times with water by decantation until it solidified; yield 4.2 g (84%). The dry product was washed with cold ether to remove benzoic acid, and was recrystallized from benzene to give needles, m.p. 178–179°, $[\alpha]_D^{19}$ +112.9 (6 min)→+90.0 (4 h)→+19.3° (after 51 h, *c* 0.8, 90% acetone). This mutarotation is comparable to that of 2,3,4,6-tetra-*O*-methyl-D-glucono-1,5-lactone for which Haworth and coworkers[7] reported $[\alpha]_D^{18}$ +101→+29.6° (after 8 h,water). I.r. data: ν_{max}^{Nujol} 1755 (1,5-lactone) and 1720 cm^{-1} (benzoate carbonyl), no hydroxyl peak.

Anal. Calc. for $C_{34}H_{26}O_{10}$: C, 68.68; H, 4.41. Found: C, 69.00; H, 4.70.

2,3,5,6-Tetra-O-benzoyl-D-glucono-1,4-lactone (**2**). — D-Glucono-1,4-lactone was prepared by heating at reflux D-glucono-1,5-lactone in acetic acid according to Isbell and Frush[8]. The lactone was recrystallized from *p*-dioxane; m.p. 134–135°, $[\alpha]_D^{20}$ +65.2 (12 min)→+62.3° (after 24 h, *c* 1, water), in agreement with the reported values. This compound (1 g) was added with stirring to a mixture of benzoyl chloride (5 ml) and pyridine (5 ml) cooled to 0°. After being shaken for 2 h at room temperature, the reaction mixture was poured into ice–water. The oil formed was washed several times with ice–water, and then dried in a vacuum desiccator; yield 4.16 g (83.7%). The product was soluble in ether and benzene. The chromatographically pure compound, R_F 0.69, had $[\alpha]_D^{20}$ +44.5° (*c* 0.6, 90% acetone); the rotation did not change after three days. I.r. data: ν_{max}^{Nujol} 1790 (1,4-lactone) and 1720 cm^{-1} (benzoate carbonyl), no hydroxyl peak.

Anal. Calc. for $C_{34}H_{26}O_{10}$: C, 68.68; H, 4.41. Found: C, 68.87; H, 4.40.

Methyl 2,3,4,6-tetra-O-benzoyl-D-gluconate (**3**). — Compound **1** (630 mg) was dissolved by shaking with 50 ml of methanol containing two drops of ether saturated with hydrogen chloride. After 3 h, only one spot, R_F 0.44, was detected. The solution was poured into cold dilute sodium hydrogen carbonate, and an amorphous solid precipitated, yield 620 mg (93%). The product could not be crystallized, and it was purified by precipitation with water from a methanolic solution. The chromatographically pure compound, R_F 0.44, had $[\alpha]_D^{23}$ +20.2° (*c* 1, chloroform); i.r. data: ν_{max}^{KBr} 3450 (OH), 1720 (benzoate carbonyl), and 1750 cm^{-1} (Et ester carbonyl); n.m.r. data: τ 2.1, 2.6 (20-proton multiplets, 4 Bz), 3.7 (1-proton triplet, *J* 3.2 Hz, H-3), 3.85–4.45 (2 proton multiplet, H-2,4), 5.18–5.82 (3-proton multiplet, H-5,6,6'), 6.38 (3-proton singlet, OMe), and 7.08 (1-proton broad signal, disappears on deuteration, OH).

Anal. Calc. for $C_{35}H_{30}O_{11}$: C, 67.08; H, 4.79. Found: C, 66.80; H, 4.62.

Ethyl 2,3,4,6-tetra-O-benzoyl-D-gluconate (**4**). — After a solution of compound **1** (200 mg) in abs. ethanol (20 ml) had been heated for 2 h at reflux, t.l.c. showed one main spot (R_F 0.33) and a faint spot corresponding to the starting material that disappeared after 24 h at room temperature. The syrup, obtained on evaporation of the ethanol under reduced pressure, was soluble in cold ethanol, ether, and benzene, and could not be induced to crystallize. Chromatographically pure **4** was obtained

as an amorphous solid by precipitation with water from the ethanolic solution; it showed $[\alpha]_D^{20}$ +14.8° (*c* 0.8, chloroform); i.r. data: ν_{max}^{Film} 3400 (OH), 1720 (benzoate carbonyl), and 1750 cm^{-1} (Et ester carbonyl); n.m.r. data: τ 2.1, 2.6 (20-proton multiplets, 4 Bz), 3.7 (1-proton triplet, *J* 3.2 Hz, H-3), 3.85–4.5 (2-proton multiplet, H-2,4), 5.2–5.8 (3-proton multiplet H-5,6,6'), 5.9 (2-proton quartet, *J* 7 Hz, CH_2 of EtO carbonyl), 7.2 (1-proton broad signal that disappeared on deuteration, OH), and 8.88 (3-proton triplet, Me).

Anal. Calc. for $C_{36}H_{32}O_{11}$: C, 67.49; H, 5.03. Found: C, 67.10; H, 4.94.

*Methyl 2,3,5,6-tetra-*O*-benzoyl-*D*-gluconate* (**5**). — Crude compound **2**, obtained from 1 g of D-glucono-1,4-lactone, was dried in a desiccator, and then dissolved in boiling methanol. Crystals appeared in the solution after approximately one week at room temperature. They were filtered off, and on being kept, more product was obtained from the mother liquors. Total yield 2.33 g (53%) in five crops. Pure **5** was obtained by recrystallization from benzene, m.p. 182–183°, $[\alpha]_D^{20}$ +35.5° (*c* 1, chloroform); i.r. data: ν_{max}^{Nujol} 3450 (OH), 1720 (benzoate carbonyl), and 1750 cm^{-1} (Me ester carbonyl); n.m.r. data: τ 2.1, 2.6 (20-proton multiplets, 4 Bz), 4.05 (2-proton multiplet, H-3,5), 4.38 (1-proton doublet, $J_{2,3}$ 3.2 Hz, H-2), 4.85–5.7 (3-proton multiplets, H-4,6,6'), 6.4 (3-proton singlet, OMe), and 7.1 (1-proton broad signal that disappeared on deuteration, OH).

Anal. Calc. for $C_{35}H_{30}O_{11}$: C, 67.08; H, 4.79. Found: C, 67.14; H, 4.95.

*Ethyl 2,3,5,6-tetra-*O*-benzoyl-*D*-gluconate* (**6**). — Compound **2** (100 mg) was dissolved by boiling in ethanol (10 ml) containing one drop of ether saturated with hydrogen chloride at 0°. The reaction was followed by t.l.c.; after 10 days at room temperature, the spot of R_F 0.69, corresponding to the lactone, had completely disappeared, and a spot of R_F 0.37 was detected. After pouring the solution into an equal volume of dilute sodium hydrogen carbonate, a crystalline product was obtained; yield 60 mg (58%), m.p. 170–172°. Recrystallization from 2:3 ethanol–acetone gave pure **6**, m.p. 175°, $[\alpha]_D^{23}$ +30.7° (*c* 0.8, chloroform); i.r. data: ν_{max}^{KBr} 3450 (OH), 1720 (benzoate carbonyl), and 1750 cm^{-1} (Et ester carbonyl); n.m.r. data: τ 2.1, 2.6 (20-proton multiplets 4 Bz), 4.05 (2-proton multiplet, H-3,5), 4.4 (1-proton doublet, $J_{2,3}$ 3.2 Hz, H-2), 4.85–5.65 (3-proton multiplet, H-4,6,6'), 5.92 (2-proton quartet, *J* 7.1 Hz, CH_2 of EtO carbonyl), 8.88 (3-proton triplet, Me), and 7.1 (1-proton broad signal that disappeared on deuteration, OH).

Anal. Calc. for $C_{36}H_{32}O_{11}$: C, 67.49; H, 5.03. Found: C, 67.92; H, 5.03.

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5-THIO-D-GLUCOPYRANOSE 1-PHOSPHATE AND 6-PHOSPHATE*

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ABSTRACT

5-Thio-D-glucopyranosyl phosphate was prepared by reaction of the acetylated glycopyranosyl bromide with silver diphenyl phosphate followed by hydrogenolysis of the phenyl groups and deacetylation. 5-Thio-D-glucopyranose 6-phosphate was prepared from methyl 5-thio-2,3,4,6-tetra-*O*-(trimethylsilyl)-α-D-glucopyranoside by preferential removal of the trimethylsilyl group at O-6 by methanolysis, phosphorylation with diphenyl phosphorochloridate, and removal of the trimethylsilyl and phenyl groups. Both phosphates were obtained crystalline as their dicyclohexylammonium salts.

INTRODUCTION

In recent years this laboratory has been interested in the biochemistry of 5-thio-D-glucose. During the course of this work it became evident that 5-thio-α-D-glucopyranosyl phosphate (**4**) and 5-thio-D-glucose 6-phosphate (**10**) would be useful products to examine as enzyme substrates. Consequently the synthesis of these compounds was undertaken.

RESULTS AND DISCUSSION

Because of the thermal sensitivity[1] of 5-thio-D-glucopyranose pentaacetate[1], its fusion with phosphoric acid† could not be used to produce 5-thio-α-D-glucopyranosyl phosphate. Consequently, the older reaction[2] of an acetylated glycopyranosyl bromide with silver diphenyl phosphate was used. The resulting 2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl diphenyl phosphate (**3**) was obtained crystalline. Integration of the n.m.r. spectrum gave the expected number of protons. The H-1 resonance appeared as a quartet centered at τ 3.94. It showed a $J_{H\text{-}1,P}$ coupling of 8.5 Hz and $J_{H\text{-}1,H\text{-}2}$ coupling of 2.5 Hz. The n.m.r. spectrum of α-D-glucopyranosyl phosphate in deuterium oxide exhibited the same type of quartet for the H-1 resonance,

*Dedicated to the memory of Professor M. L. Wolfrom.
†Called 110% phosphoric acid, Matheson, Colemann, and Bell, East Rutherford, N. J., U. S. A.

with its center at τ 4.63 and with coupling constants $J_{H\text{-}1,P}$ of 7.5 Hz and $J_{H\text{-}1,H\text{-}2}$ of 3 Hz. D-Aldopyranoses in the *C1* conformationshow spin–spin coupling constants for H-1, H-2 in equatorial–axial relation of about 3 Hz. Thus, the weak coupling for the sulfur-containing sugar is indicative of the α-D anomeric configuration. Its high positive specific optical rotation (+136°) was evidence also for an α-D structure.

The phenyl ester groups were removed by hydrogenolysis over platinum and the acetyl groups by saponification with lithium hydroxide solution. The product was separated as the barium salt and converted into the crystalline dicyclohexylammonium salt.

For the preparation of 5-thio-D-glucopyranose 6-phosphate, methyl 5-thio-α-D-glucopyranoside[3] was used as the starting material. This was first converted into the tetra(trimethylsilyl)ether[4]. The trimethylsilyl group at C-6 was preferentially removed by methanolysis[5] and the product was phosphorylated by diphenyl phosphorochloridate. Removal of the trimethylsilyl groups in refluxing methanol and removal of the phenyl groups by hydrogenolysis over platinum provided the monophosphoric ester, which was isolated as the crystalline dicyclohexylammonium salt.

EXPERIMENTAL

General. — Thin-layer chromatography was used for monitoring reactions and estimating the purity of products. The ascending technique was employed with 5 × 13 cm and 2.5 × 7.5 cm glass plates coated with Silica Gel* G according to the method described by Whistler, Lamchen, and Rowell[6]. Components were located on thin-layer slides by spraying with 5% sulfuric acid in ethanol and then charring by heating on a hot plate. Quantitative column chromatography was made on silica gel†. Irrigants used were: *A*, benzene–ethyl acetate; *B*, hexane–ethyl acetate; *C*, chloroform–methanol; *D*, ethanol–ammonium hydroxide–water. Ratios represent parts by volume. All chemicals were reagent grade.

Melting points were determined on a Fisher–Johns melting-block apparatus. N.m.r. spectra were obtained with a Varian Associates A-60 instrument at 40°. Signal positions were taken from tetramethylsilane as an internal standard. Optical rotations were determined on a Perkin–Elmer 141 recording polarimeter. Solutions were concentrated on a rotary evaporator at less than 40° under diminished pressure.

*2,3,4,6-Tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl diphenyl phosphate* (3). — Syrupy 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranosyl bromide[1] (**2**, 5.4 g) was dissolved in 20 ml of dry benzene and 5 g of powdered silver diphenyl phosphate[7] was added. The mixture was refluxed for 1 h on a steam bath with exclusion of light and moisture. Silver salts were removed by centrifugation and washed with dry benzene. The benzene solution was concentrated to a syrup, which was dissolved in chloroform and treated with activated charcoal.

*Brinkman Instruments, Inc., Westbury, N. Y. 11590, U. S. A.
†J. T. Baker Chemical Co., Philippsburg, N. J., U. S. A.

After filtration from charcoal and removal of the chloroform, the product was dissolved in dry ethanol and crystallized at −5°; yield 4.1 g (56% from compound **1**), m.p. 94–95°. Recrystallization from ether–hexane gave crystals having m.p. 96–97°, $[\alpha]_D^{25}$ +136.2° (*c* 2.05, chloroform).

Anal. Calc. for $C_{26}H_{29}O_{12}PS$: C, 52.35; H, 4.90; P, 5.19; S, 5.38. Found: C, 52.36; H, 4.86; P, 5.00; S, 5.60.

The n.m.r. spectrum showed identified signals at τ 2.47 (singlet, two Ph groups), 3.94 (quartet, $J_{H\text{-}1,H\text{-}2}$ 2.5 Hz, $J_{H\text{-}1,P}$ 8.5 Hz, H-1), 7.91 (singlet, three $COCH_3$), and 8.19 (singlet, $COCH_3$).

5-Thio-α-D-glucopyranosyl phosphate (**4**). — Into a 50-ml round-bottomed flask equipped with a magnetic stirring bar was placed 0.65 g of 2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl diphenyl phosphate (**3**), 0.65 g of platinum oxide (Adams' catalyst), and 12 ml of anhydrous methanol. The gas inside the apparatus was partially removed by evacuation and was replaced with hydrogen. After repeating this process, the reaction mixture was kept under a slight pressure of hydrogen gas and stirred vigorously for 1 h. The catalyst was removed by filtration and washed with methanol. The filtrate was neutralized with dilute sodium hydrogen carbonate solution and concentrated. Lithium hydroxide solution (2M, 3 ml) was added and the mixture was kept for 16 h at 25°. Cations were removed by slowly passing the solution through a water-jacketed column of IR-120 (H^+) ion-exchange resin (5 ml) at 15°. The effluent was collected in a flask containing 3 ml of freshly distilled cyclohexylamine and the column was washed with water until the effluent was neutral. Excess cyclohexylamine was removed by the addition of water and subsequent evaporation under diminished pressure until the odor of the amine could not be detected. The volume was adjusted to about 5 ml and crystallization was subsequently induced by the careful addition of acetone until incipient turbidity developed. Fluffy white crystals of the cyclohexylammonium salt formed upon standing. They were isolated and dried in a vacuum desiccator over phosphorus pentaoxide; yield 0.468 g (89%), $[\alpha]_D^{25}$ +136.6° (*c* 3.02, water).

Anal. Calc. for $C_{18}H_{39}N_2O_8PS{\cdot}H_2O$: C, 43.89; H, 8.39; N, 5.69. Found: C, 43.64; H, 8.30; N, 5.52.

The titration curve obtained by reaction of the product **4** with barium hydroxide showed two inflections, similar to those given by α-D-glucopyranosyl phosphate.

Methyl 2,3,4,6-tetra-O-acetyl-5-thio-α-β-D-glucopyranoside. — Oxygen was removed from a sodium methoxide solution (60 mg of sodium in 50 ml of anhydrous methanol) by bubbling dry nitrogen gas through it. 5-Thio-D-glucose pentaacetate (**1**, 3.5 g) was added to the solution and the mixture was stirred with a magnetic bar at 25° under nitrogen. Deacetylation, which was monitored by t.l.c. (irrigant *C*, 3:1) was complete within 10 min, affording 5-thio-D-glucose, which was not isolated. Excess methanol-washed IR-120 (H^+) ion-exchange resin (15 ml) was added, which removed the sodium ions and supplied the acid catalyst for glycosidation. Stirring was continued for 3 days at 25° under nitrogen. The resin was removed by filtration

and washed several times with methanol until no product could be detected by t.l.c. (irrigant *C*, 3:1) in the wash. The methanol solutions and washings were concentrated to a syrup, which was dried in a vacuum desiccator over calcium chloride for 30 h.

The above product, a mixture of the α-D and β-D anomers of **5**, was acetylated in 30 ml of anhydrous pyridine with 20 ml of acetic anhydride at 25°. The reaction was complete after 3 h as shown by t.l.c. (irrigant *C*, 3:1). Excess acetic anhydride was destroyed by cooling the reaction mixture and adding 15 ml of methanol. After 30 min the reaction mixture was poured into 800 ml of ice and water and the products were extracted with three 200-ml portions of toluene. The toluene extracts were washed with two 300-ml portions of ice-cold 5% (w/v) sodium hydrogen carbonate solution and then with 10% (w/v) aqueous sodium chloride solution (4×200 ml). The separated toluene solution was dried over anhydrous sodium sulfate and concentrated to a syrup.

The α-D and β-D anomers were separated into three fractions on a silica gel column with irrigant *B*, 7:3; yields: α-D anomer (2.06 g), β-D anomer (0.37 g), mixture of anomers (0.45 g); total isolated yield, 2.9 g (91%). The pure anomers were crystallized from ether–hexane.

Methyl 2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranoside (**6**) had m.p. 98–99°, $[\alpha]_D^{25}$ +224.8° (*c* 1.8, chloroform).

Anal. Calc. for $C_{15}H_{22}O_9S$: C, 47.61; H, 5.86; S, 8.47. Found: C, 47.65; H, 5.92; S, 8.60.

Methyl 2,3,4,6-tetra-*O*-acetyl-5-thio-β-D-glucopyranoside had m.p. 92–93°, $[\alpha]_D^{25}$ −26.9° (*c* 1.85, chloroform).

Anal. Calc. for $C_{15}H_{22}O_9S$: C, 47.61; H, 5.86; S, 8.47. Found: C, 47.73; H, 5.84; S, 8.55.

Methyl 5-thio-α-D-glucopyranoside (**5**). — Methyl 2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranoside (3.33 g) was dissolved in 30 ml of anhydrous methanol and a catalytic amount of sodium methoxide (20 mg of sodium in 10 ml of methanol) was added with stirring. After 30 min the solution was neutralized with 1 ml of IR-120 (H^+) ion-exchange resin. The resin was removed by filtration and washed repeatedly with methanol. Concentration of the filtrate and washings afforded the pure product in quantitative yield as a syrup which crystallized on standing; m.p. 124–125°. Recrystallization from methanol raised the melting point to 126°; $[\alpha]_D^{25}$ +326.3° (*c* 1.61, methanol).

Anal. Calc. for $C_7H_{14}O_5S$: C, 39.99; H, 6.71; S, 15.25. Found: C, 40.05; H, 6.64; S, 15.23.

The β-D anomer was deacetylated as described above. The product, obtained in quantitative yield, did not crystallize from absolute ethanol, methanol, ethyl acetate, or methanol–chloroform, but formed a gel in each trial; $[\alpha]_D^{25}$ −51.4° (*c* 2.7, methanol).

Methyl 2,3,4,6-tetra-O-(trimethylsilyl)-5-thio-α-D-glucopyranoside (**6**). — To a solution of 2.3 g of methyl 5-thio-α-D-glucopyranose (**5**) in 90 ml of dry pyridine were added 4 ml of hexamethyldisilazane and 10 ml of chlorotrimethylsilane. The

resulting milky mixture was shaken, allowed to stand for 10 min, and then concentrated to a thick, white syrup. Dry hexane was added, the salts were removed by filtration and were washed with small portions of hexane. The clear filtrate was concentrated to a syrup that was methanolyzed directly to remove the primary trimethylsilyl group.

Methyl 5-thio-2,3,4-tri-O-(*trimethylsilyl*)-α-D-*glucopyranoside* (7). — The above syrup was dissolved in 230 ml of dry methanol and cooled to 0°. A solution of 127 mg of potassium carbonate in 40 ml of dry methanol was added with stirring. The temperature was maintained at 0° and the reaction was monitored by t.l.c. (irrigant *B*, 6:1). After 70 min the catalyst was neutralized by addition of 0.11 ml of glacial acetic acid. Since the product did not crystallize from methanol–water, methanol, or ethanol, the solution was concentrated to a syrup. Water and alcohol were removed by evaporation with anhydrous pyridine (40 ml). Insoluble salts were removed by filtration and were washed with anhydrous pyridine. Pyridine (40 ml) was added to the filtrates and washings, and the solution was concentrated to a thick syrup to remove alcohol and moisture. This syrup was phosphorylated without further purification.

Methyl 5-thio-α-D-glucopyranoside 6-diphenyl phosphate (**8**). — To the above syrup in 10 ml of dry pyridine at 0° was added 2.7 ml of diphenyl phosphorochloridate, dropwise, with stirring. The reaction was monitored by t.l.c. with irrigant *B*, 6:1. Since the product, methyl 5-thio-2,3,4-tri-*O*-trimethylsilyl-α-D-glucopyranoside 6-diphenyl phosphate, moved at about the same rate as the starting material, a mixture of the starting material and reaction mixture was used for comparison. After 1 h at 0°, 50 ml of toluene was added and the reaction mixture was washed with 75 ml of ice-cold 5% (w/v) sodium hydrogen carbonate solution. The separated toluene layer was washed with 5% (w/v) aqueous sodium chloride solution until neutral, dried over anhydrous sodium sulfate, and concentrated to a syrup. Attempted high-vacuum distillation at 3.5×10^{-3} mtorr resulted in degradation of the product. Attempts at crystallization were unsuccessful. Therefore, the product was de(trimethylsilyl)ated by dissolving in 50 ml of methanol, water was added until turbidity appeared, and the mixture was refluxed for 10 min. The solution was concentrated to a syrup, which was purified by silica gel column chromatography with irrigant *B*, 6:1. The resulting product (**8**) did not crystallize; yield: 3.1 g (80%) from **5**, $[\alpha]_D^{25}$ +130.7° (*c* 2.0, chloroform).

Methyl 5-thio-α-D-glucopyranoside 6-phosphate (**9**). — Methyl 5-thio-α-D-glucopyranoside 6-diphenyl phosphate (**8**, 0.50 g) was dissolved in 10 ml of methanol and 1 g of platinum oxide (Adams' catalyst) was added. The flask was partially evacuated and hydrogen gas admitted. After repeating this process twice the reaction mixture was shaken vigorously for 6 h at 25° under a slight overpressure of hydrogen. The catalyst was removed by filtration and the filtrate was concentrated to a syrup. Water (10 ml) was added and the solution was treated with activated charcoal. After removal of the charcoal the barium salt of **9** was formed by addition of 400 mg of barium acetate. After removal of insoluble material by centrifugation, the barium

salt was precipitated by the gradual addition of three volumes of ethanol at 5°. After the mixture had been kept overnight in the refrigerator, the precipitate was collected by centrifugation, dissolved in water, and insoluble material removed by centrifugation. The product was converted into the cyclohexylammonium salt by passing it through a cooled, 3-ml column of IR-120 (H^+) ion-exchange resin. The effluent was collected in a flask containing 3 ml of freshly distilled cyclohexylamine. Excess cyclohexylamine was removed by evaporation under diminished pressure until the odor of the amine could not be detected. The syrup was dissolved in a minimum quantity of methanol, and acetone was carefully added to incipient turbidity. After being kept at 25° crystals formed, which were isolated by filtration and dried over phosphorus pentaoxide in a vacuum desiccator; yield 0.34 g (62%), $[\alpha]_D^{25}$ +123° (*c* 1.29, water).

Anal. Calc. for $C_{19}H_{41}N_2O_8PS \cdot H_2O$: C, 45.05; H, 8.56; N, 5.53; P, 6.11; S, 6.33. Found: C, 44.98; H, 8.40; N, 5.72; P, 6.28; S, 6.52.

Titration of the product **9** with barium hydroxide gave a curve showing two inflections, similar to that given by D-glucose 6-phosphate.

Periodate oxidation[8] of **9** was considered complete after 32 h when the periodate consumption slowed considerably and the production of formic acid leveled off. It was found that 2.8 moles of periodate per mole of sample were consumed and 0.8 to 1.1 moles of formic acid per mole of sample were produced. Periodate consumption in excess of that required for glycol cleavage was attributed to oxidation of the sulfur atom[9].

5-Thio-D-glucopyranose 6-phosphate (**10**). — To 0.20 g of methyl 5-thio-α-D-glucopyranoside 6-phosphate (**9**) in 5 ml of water was added 1.5 ml of IR-120 (H^+) ion-exchange resin and the mixture was refluxed under a nitrogen atmosphere, as were all operations and manipulations of this compound. The progress of the reaction was followed by t.l.c. (irrigant *D*, 5:3:1). After 6 h traces of starting material persisted, but another impurity, presumed to be a disulfide, continued to increase. Therefore, the reaction mixture was cooled and the resin was removed and washed by filtration. Barium acetate (150 mg) was added to the filtrate, which was concentrated to a small volume. After removal of insoluble material by centrifugation, the barium salt of **10** was precipitated by the gradual addition of three volumes of ethanol at 5°. After 2 h at 5° the precipitate was collected by centrifugation, dissolved in water, and any soluble material was removed by centrifugation. The cyclohexylammonium salt was formed by passing the barium salt through a cooled column of IR-120 (H^+) ion-exchange resin (2 ml) and collecting the effluent in a flask containing 1 ml of freshly distilled cyclohexylamine. Excess cyclohexylamine was removed by addition of water and subsequent evaporation under diminished pressure until the smell of amine could not be detected. The product, in a small volume of water, was crystallized by carefully adding acetone to incipient turbidity. The crystals, which formed at 25°, were isolated by filtration and dried over phosphorus pentaoxide under vacuum; yield 71 mg (38%). Examination by t.l.c. (irrigant *E*, 5:3:1) revealed that the crystals

contained traces of impurities, one of which could not be removed by recrystallization from water–acetone.

Anal. Calc. for $C_{18}H_{39}N_2O_8PS$: C, 45.56; H, 8.28; N, 5.90. Found: C, 45.00; H, 8.25; N, 5.68.

The product **10** was titrated with barium hydroxide yielding a curve with two inflections, similar to that obtained with D-glucose 6-phosphate.

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1,3,4,6-TETRA-*O*-BENZYL-D-FRUCTOFURANOSE AND SOME OF ITS DERIVATIVES*

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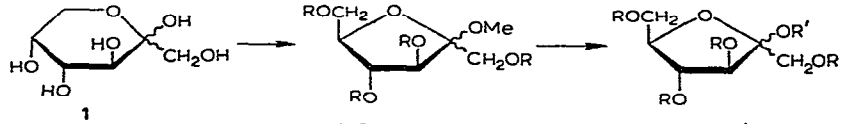
ABSTRACT

Crystalline 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**) has been obtained in 60% overall yield from D-fructose through the following steps: D-fructose→methyl D-fructofuranoside→methyl tetra-*O*-benzyl-D-fructofuranoside→**4**. The new D-fructofuranose derivative has been further characterized through the preparation of a *p*-nitrobenzoate, a *p*-phenylazobenzoate and a benzoate — all crystalline derivatives. To confirm the structure of **4**, it was also made through the hydrolysis of octa-*O*-benzylsucrose and by the partial oxidation of 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**); the preparation of **11** and of two crystalline derivatives therefrom is described.

DISCUSSION

During a general study of the chemistry of the ketoses, 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**) was required. Initial attempts to prepare **4** from D-fructose (**1**) were patterned after the route used earlier for the conversion of D-arabinose into 2,3,5-tri-*O*-benzyl-D-arabinose[1-3]. A crude mixture of syrupy methyl D-fructofuranosides (**2**), obtained directly from D-fructose (**1**), was benzylated and the product (**3**) was hydrolyzed under relatively mild conditions (aqueous acetic acid at *ca.* 92°) to yield a crude, amorphous tetra-*O*-benzylhexulose from which crystalline *p*-nitro-



1

2, R = H
3, R = CH_2Ph

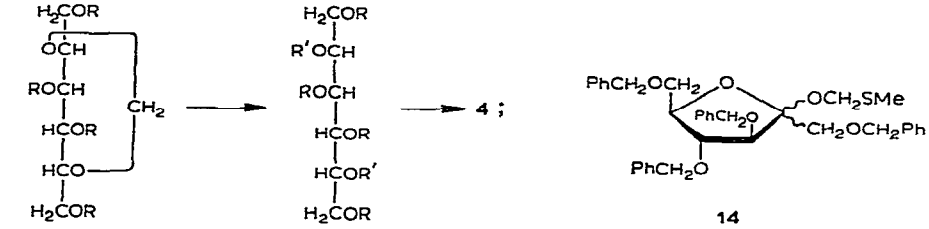
4, R = CH_2Ph ; R' = H
5, R = CH_2Ph ; R' = $C(=O)-C_6H_4-NO_2$ (*p*)
6, R = CH_2Ph ; R' = $C(=O)-C_6H_4N_2Ph$ (*p*)
7, R = CH_2Ph ; R' = Bz
8, R = CH_2Ph ; R' = Ac

benzoyl (**5**) and *p*-phenylazobenzoyl (**6**) esters were prepared. To provide unequivocal proof that the syrupy product was indeed 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**),

*Dedicated to the memory of Professor M. L. Wolfrom.

two further and wholly independent syntheses of this substance were undertaken. The readily accessible cyclic acetal 2,5-*O*-methylene-D-mannitol[4] (**9**) was converted into its crystalline tetrabenzyl ether (**10**) and the acetal bridge was cleaved by acidic hydrolysis in the presence of phloroglucinol. The 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**) thus prepared proved to be a readily crystallizable substance; it was further characterized through its dibenzoate (**12**) and *p*-phenylazobenzoate (**13**).

The end-to-end symmetry of mannitol makes the hydroxyl groups in 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**) equivalent. It was expected that oxidation of one of these hydroxyl groups would be followed very rapidly by hemiacetal formation, effectively protecting the second hydroxyl group from further oxidation and yielding 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose. Actually, the oxidation of **11** with methyl sulfoxide–acetic anhydride[5] proved to be a somewhat complex reaction, although its progress could readily be monitored by polarimetry and by t.l.c. Preparative chromatography of the crude product allowed isolation (in 14% yield) of a product which proved to be identical with that obtained earlier from D-fructose. In addition, two other amorphous products were separated. One of these had the properties expected of a 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**8**); the other had the elemental composition of a (methylthio)methyl 1,3,4,6-tetra-*O*-benzyl-D-fructofuranoside (**14**).



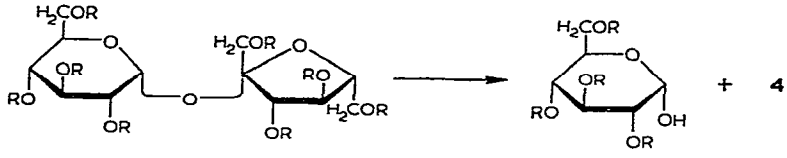
14

9, R = H
10, R = CH_2Ph

11, R = CH_2Ph ; R' = H
12, R = CH_2Ph ; R' = Bz
13, R = CH_2Ph ; R' = $\overset{O}{\overset{\|}{C}}-C_6H_4N_2Ph$ (*p*)

Another approach to the synthesis of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**) was to use the D-fructofuranoside, sucrose (**15**). Because this disaccharide is markedly labile toward alkali, it cannot be benzylated with benzyl chloride and potassium hydroxide. However, the Kuhn-type of alkylation, with benzyl bromide, barium oxide, and silver oxide in *N*,*N*-dimethylformamide, was successfully applied to sucrose (**15**) by Tate and Bishop[6] and this procedure was repeated in the present work. The octa-*O*-benzylsucrose (**16**) was obtained, after chromatography, as a syrup having the appropriate elemental composition. Hydrolysis of **16** was effected in a mixture of water, acetic acid, and sulfuric acid at 65°, the highly insoluble 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose[7] (**17**) crystallizing directly from the reaction mixture in quantitative yield. Chromatography of a sample of the material remaining in the mother liquor led to the crystallization, for the first time, of 1,3,4,6-tetra-*O*-

benzyl-D-fructofuranose (**4**). With seed crystals available, the syrupy products obtained from D-fructose (**1**) and from 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**) readily crystallized, and it was then found possible to obtain crystalline **4** from D-fructose in 60% overall yield. A crystalline benzoate (**7**) and an amorphous acetate (**8**) were



15, R = H
16, R = CH_2Ph

17, R = CH_2Ph

prepared from crystalline **4**; the optical rotation of the latter derivative ($[\alpha]_D^{20}$ +13.0°, chloroform) was reasonably close to that of the amorphous acetate ($[\alpha]_D^{20}$ +9.7°, chloroform) obtained as a byproduct in the methyl sulfoxide–acetic anhydride oxidation of 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**).

The preparation of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**) from 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**) and from sucrose (**15**) constitutes unequivocal proof of its structure. Crystalline **4** shows a very slight dextromutarotation when dissolved in chloroform or in aqueous *p*-dioxane, but convincing evidence for the anomeric configuration of this substance as well as of the various esters of **4** reported here (**5**–**8**) is not presently available. Some chemical properties of **4** will be reported in a future communication.

EXPERIMENTAL

General methods. — Melting points are equivalent to corrected values. Qualitative t.l.c. was carried out on Silica Gel GF (250 μm, Analtech, Inc., Wilmington, Del.), with the solvent systems specified. Components were detected with a Gelman–Camag universal u.v. lamp Model 51402 and also by spraying with 10% sulfuric acid and heating. Preparative t.l.c. was performed on Silica Gel GF-254 (2 mm, E. Merck AG, Darmstadt). Column chromatography was carried out with Silica Gel No. 7734 (0.05–0.20 mm) of E. Merck.

1,3,4,6-Tetra-O-benzyl-D-fructofuranose (**4**) *from* D-*fructose* (**1**). — D-Fructose (45.0 g) was added to anhydrous methanol (900 ml) and the mixture was stirred at room temperature until the sugar was largely dissolved. Concentrated sulfuric acid (3.4 ml) was added cautiously and stirring was continued for 25 min, by which time the solution gave but a very faintly positive Fehling test. Amberlite IR-45 (CO_3^{2-}) (60 g) was added and the suspension was stirred for 5 min before being poured onto a column (4 × 44 cm) of Amberlite IR-45 (CO_3^{2-}). When the solution had passed through the column, the resin was washed with methanol (1 liter), the washings being added to the main solution. The solution was then evaporated *in vacuo* at 35° (bath) to a syrup to which was added benzyl chloride (125 ml) and powdered potassium hydroxide (50 g). The mixture was stirred and heated to 115–120°; while stirring

and heating were continued, more potassium hydroxide (175 g) was added over the course of 20 min. Inasmuch as the reaction is exothermic, the source of heat was removed from time to time in order to maintain the temperature at 115–116°. When addition of the potassium hydroxide was complete, the heating and stirring were continued for 5.5 h; the mixture was then cooled slightly and subjected to steam distillation for *ca.* 3 h in order to remove benzyl chloride and benzyl alcohol. The mixture (*ca.* 1 liter) was then cooled and the two phases were separated. The aqueous layer was extracted with four 100-ml portions of dichloromethane and the combined extracts were added to the organic layer which was then washed with water (1 liter) containing *ca.* 35 ml of acetic acid. (Omission of the acetic acid leads to the formation of intractable emulsions). The aqueous extract was washed with four 100-ml portions of dichloromethane, these extracts being added to the main organic solution. Moisture was removed from this solution with magnesium sulfate and the solution was filtered through a layer of decolorizing carbon. Evaporation of the solution *in vacuo*, eventually at *ca.* 1 mmHg and 140° (bath), gave a syrup (124 g) which was dissolved in acetic acid (500 ml). Water (120 ml) was added and the cloudy suspension was heated on a steam bath, becoming a clear solution as it warmed. After 2 h at 92° (steam bath), the solution was examined by t.l.c. (1:4 ether–benzene) and hydrolysis of the benzylated glycoside (**3**) was found to be complete. Cooled to room temperature, the mixture was diluted with water (1 liter) and the crude product was extracted with four 150-ml portions of dichloromethane. The combined extracts were shaken gently with water (1 liter) containing sodium hydrogen carbonate (80 g) until the evolution of carbon dioxide had ceased*. The organic layer was separated and washed with water (1 liter). Moisture was removed with magnesium sulfate and the solution was evaporated *in vacuo* (35° bath) to a thick syrup (125 g) which was dissolved, without heating, in isopropyl ether (437 ml, 3.5 ml/g). The solution was cooled to −5° and seeded with crystals of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**), obtained as described later in this paper. The solution was kept at −5° and stirred from time to time over the course of several days as crystallization progressed. When crystallization appeared to have ceased, a chilled mixture of pentane (25 ml) and isopropyl ether (50 ml) was stirred into the mass and the solid was removed by filtration, the crystals being washed at −5° with a chilled mixture of pentane (50 ml) and isopropyl ether (100 ml) and then dried thoroughly *in vacuo* at room temperature: yield, 81.1 g (60%), m.p. 39–41°. For recrystallization, the 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose was dissolved in isopropyl ether (324 ml) without heating. The solution was filtered through a layer of decolorizing carbon (Darco X, 3 g) and the carbon was washed with 81 ml of isopropyl ether (to make a total of 5 ml/g); the solution was then cooled to −5° and seeded. The mixture was stirred from time to time over the course of 2 days as crystallization progressed. A chilled mixture of pentane (50 ml) and isopropyl ether (100 ml) was stirred into the crystalline mass and the pure product

*Owing to the serious foaming which can take place at this stage, it is advisable to conduct the operation in a capacious vessel; the authors used a 6-l flask.

was removed by filtration and dried *in vacuo* at room temperature; yield 70 g, m.p. 42–43°, $[\alpha]_D^{20}$ +6.5→+8.7° (25 h, *c* 1.43, chloroform); $[\alpha]_D^{20}$ −3.3→+5.7° (equilibrium, 28 h, *c* 1.07, 1:9 water–*p*-dioxane.)

Anal. Calc. for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.63; H, 6.90.

1,3,4,6-Tetra-O-*benzyl-2*-O-p-*nitrobenzoyl*-D-*fructofuranose* (**5**). — *p*-Nitrobenzoyl chloride (0.34 g) was added to pyridine (2.5 ml) and the mixture, cooled to −5°, was stirred while 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (0.5 g) was added. Stirring of the reaction mixture was continued at −5° overnight; t.l.c. (1:4 ether–benzene) then showed the acylation to be incomplete. The mixture was brought to room temperature and stirred for a day; t.l.c. then showed the reaction to be complete, two products having been formed in approximately equal quantities. The reaction mixture was worked up in conventional fashion to give a syrup (0.55 g) which was dissolved in a mixture of acetone (2.5 ml) and methanol (7 ml). At +5° crystallization progressed rapidly to give 0.28 g (45%) of **5**. Recrystallized from acetone–methanol, the product had m.p. 66–67° and $[\alpha]_D^{20}$ +35.7° (*c* 1.77, chloroform).

Anal. Calc. for $C_{41}H_{39}NO_9$: C, 71.40; H, 5.70; N, 2.03. Found: C, 71.24; H, 5.69; N, 1.98.

Prior to the crystallization of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**), the *p*-nitrobenzoate (**5**) was obtained through *p*-nitrobenzoylation of crude, syrupy **4**, the yield of **5** being 29%, based on D-fructose.

1,3,4,6-Tetra-O-*benzyl-2*-O-p-*phenylazobenzoyl*-D-*fructofuranose* (**6**). — A mixture of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**, 2.0 g), *p*-phenylazobenzoyl chloride (2.0 g), and pyridine (10 ml) was stirred for 2 days at room temperature. T.l.c. (1:4 ether–benzene) then showed the presence of two major colored products as well as of a trace of a third colored product; starting material was absent. A few drops of water were added and the reaction mixture was left at room temperature for 15 min to decompose the excess of *p*-phenylazobenzoyl chloride. Dichloromethane (20 ml) was added and the solution was washed with 1.5M sulfuric acid, the *p*-phenylazobenzoic acid thus precipitated then being removed by filtration. After being washed successively with aqueous sodium hydrogen carbonate solution and with water, the solution was dried with magnesium sulfate and evaporated *in vacuo* to a syrup (2.6 g) which was dissolved in acetone. On standing at −5° overnight, the solution deposited 1.5 g (54%) of fine needles, m.p. 123–124°. Recrystallization from acetone failed to change this m.p.: the pure substance (**6**) had $[\alpha]_D^{20}$ +34.6° (*c* 1.0, chloroform).

Anal. Calc. for $C_{47}H_{44}N_2O_7$: C, 75.38; H, 5.92; N, 3.74. Found: C, 75.29; H, 5.95; N, 4.04.

Compound **6** may be prepared directly from crude, syrupy **4**, the yield of **6** from D-fructose then being 26%.

2-O-*Benzoyl-1,3,4,6-tetra*-O-*benzyl*-D-*fructofuranose* (**7**). — Crystalline **4** (5.0 g) was added to a chilled mixture of benzoyl chloride (1.7 ml) and pyridine (6 ml) and the resulting mixture was kept for 26 h at room temperature; t.l.c. (11:1 benzene–ether) then showed that **4** was no longer present. The mixture was worked up in conventional fashion to yield, from absolute alcohol solution, 1.75 g (29%) of

crystalline product. After recrystallization from isopropyl alcohol, the material had m.p. 52–54° and $[\alpha]_D^{20}$ +37.0° (*c* 2.5, chloroform).

Anal. Calc. for $C_{41}H_{40}O_7$: C, 76.37; H, 6.25. Found: C, 76.23; H, 6.09.

2-O-Acetyl-1,3,4,6-tetra-O-benzyl-D-fructofuranose (**8**). — A solution of **4** (2.00 g) in a mixture of acetic anhydride (2.5 ml) and dry pyridine (10 ml) was heated for 3 h at 100°; examination by t.l.c. (5:1 benzene–ether) then showed the presence of only a small amount of **4**. The reaction mixture was worked up in conventional fashion to yield a crude product which was subjected to preparative t.l.c. (5:1 benzene–ether) to yield a major component (0.83 g, 38%) which was rechromatographed and finally obtained as a clear syrup which was dried for 8 h at 0.1 mm and 100°: $[\alpha]_D^{20}$ +13° (*c* 2.4, chloroform).

Anal. Calc. for $C_{36}H_{38}O_7$: C, 74.20; H, 6.57. Found: C, 73.95; H, 6.59.

1,3,4,6-Tetra-O-benzyl-2,5-O-methylene-D-mannitol (**10**). — A well-stirred mixture of 2,5-*O*-methylene-D-mannitol[4] (**9**, 15 g), benzyl chloride (150 ml) and powdered potassium hydroxide (75 g) was slowly heated at 115–125° and kept for 4.5 h in this temperature range (stirring being continued). The mixture was cooled, water (500 ml) was added, and the whole was subjected to steam distillation for 3.5 h. Again the mixture was cooled and now the organic (lower) layer was removed in a separatory funnel. The aqueous layer was washed with five 100-ml portions of ether, the extracts being added to the organic layer. The latter was extracted with three 100-ml portions of water, a few ml of acetic acid being added to break the emulsion which formed. Moisture was removed with magnesium sulfate and the solution was evaporated *in vacuo* to a syrup (39.2 g) that was dissolved in methanol (70 ml). Crystallization was allowed to progress, first at room temperature and then at −5°, to give 31 g (72%) of product, m.p. 54–56°, which appeared homogeneous by t.l.c. (1:25 ether–dichloromethane). The material was dissolved in isopropyl ether (2 ml/g) and the solution was treated with decolorizing carbon; crystallization was allowed to proceed at −5° and the crystals were washed with chilled 1:2 pentane–isopropyl ether to give 30 g of pure 1,3,4,6-tetra-*O*-benzyl-2,5-*O*-methylene-D-mannitol (**10**), m.p. 55–56°. Further recrystallization failed to change this m.p. and yielded material having $[\alpha]_D^{20}$ −6.6° (*c* 1.02, chloroform).

Anal. Calc. for $C_{35}H_{38}O_6$: C, 75.78; H, 6.91. Found: C, 75.52; H, 6.98.

1,3,4,6-Tetra-O-benzyl-D-mannitol (**11**). — The hydrolytic cleavage of the methylene acetal (**10**) was patterned after a procedure described by Wolfrom, Lew, and Goepp[8]. Tetra-*O*-benzyl-2,5-*O*-methylene-D-mannitol (**10**, 20 g) and phloroglucinol (38 g) were dissolved in *p*-dioxane (1100 ml) and the solution was diluted with 0.6M hydrochloric acid (770 ml). The reaction mixture was boiled gently under reflux, the progress of the hydrolysis being monitored by t.l.c. with 1:4 ether–dichloromethane. After 21 h, concentrated hydrochloric acid (81 ml) was added and the heating was continued for a further 10 h. The cooled mixture was then evaporated *in vacuo* at 35° (bath) to a dry, crystalline mass which was shaken with dichloromethane (200 ml) at room temperature. Unchanged phloroglucinol was removed by filtration and washed with more (100 ml) dichloromethane; the combined filtrate

and washings were washed with aqueous sodium hydrogen carbonate solution and then with water. Moisture was removed with magnesium sulfate and the solution was filtered through a small amount of decolorizing carbon and evaporated *in vacuo* to a syrup (17.3 g), which was dissolved in a mixture of ether (100 ml) and pentane (130 ml). Crystallization progressed at −5° to give 15.1 g (77%) of product in two crops; recrystallized from ether–pentane and then from isopropyl ether, the pure 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**) had m.p. 55–56° and $[\alpha]_D^{20}$ +31.2° (*c* 0.87, chloroform).

Anal. Calc. for $C_{34}H_{38}O_6$: C, 75.25; H, 7.06. Found: C, 75.20; H, 6.96.

2,5-Di-O-benzoyl-1,3,4,6-tetra-O-benzyl-D-mannitol (**12**). — A mixture of 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**, 100 mg), benzoyl chloride (0.1 ml), and pyridine (0.5 ml) was heated for 2 h at 45° and the cooled reaction mixture was then diluted with water. Rubbed with fresh water, the precipitated syrup crystallized: yield, 78 mg (57%). Recrystallized twice from alcohol, the product had m.p. 68–69° and $[\alpha]_D^{20}$ +15.4° (*c* 1.0, chloroform).

Anal. Calc. for $C_{48}H_{46}O_8$: C, 76.78; H, 6.18. Found: C, 76.78; H, 5.93.

1,3,4,6-Tetra-O-benzyl-2,5-di-O-p-phenylazobenzoyl-D-mannitol (**13**). — A mixture of 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**, 200 mg), *p*-phenylazobenzoyl chloride (360 mg) and pyridine (1 ml) was heated for 1.3 h at 45° and then cooled. Water was added and the product was extracted with dichloromethane; after washing the extract with cold 1.5M sulfuric acid it was filtered (to remove *p*-phenylazobenzoic acid) and washed with sodium hydrogen carbonate solution. Moisture was removed with magnesium sulfate and the solution was evaporated *in vacuo* to a syrup to which ethanol (15 ml) was added. On standing at room temperature, the mixture afforded fine needles: yield, 240 mg (68%). The crude product thus obtained was stirred with isopropyl ether (6 ml) and the insoluble solid (30 mg) was removed by filtration. The filtrate was concentrated *in vacuo* to dryness and the residue was dissolved in dichloromethane (1 ml); absolute ethanol (10 ml) was added, and crystallization was allowed to progress; yield 190 mg, m.p. 62–63°, $[\alpha]_D^{20}$ −15.0° (*c* 1.15, chloroform).

Anal. Calc. for $C_{60}H_{54}N_2O_8$: C, 75.14; H, 5.68; N, 5.84. Found: C, 75.25; H, 5.90; N, 5.95.

The oxidation of 1,3,4,6-tetra-O-benzyl-D-mannitol (**11**) *with methyl sulfoxide–acetic anhydride.* — In a preliminary experiment, a solution of **11** (2.05 g) in methyl sulfoxide (90 ml) was diluted with acetic anhydride (60 ml) and the optical rotation of the resulting reaction mixture was observed in a 1-dm tube at 20°; the rotation is plotted in Fig. 1 as a function of time. At 125 min (A), 198 min (B), 231 min (C), 389 min (D), and 50 h (E) a small portion of the reaction mixture was removed and treated with 40 times its volume of water. The aqueous layer was separated from the syrup and extracted with dichloromethane; both the syrup and the dichloromethane extract together were examined by t.l.c. (1:1 ether–carbon tetrachloride). At time A, 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**) preponderated, the 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**) representing less than *ca.* 5% of the mixture; at time B, the same two components were detected, the **4** then representing *ca.* 20%

of the mixture. At time C, **11** and **4** were present in approximately equal quantities and were accompanied by traces of an acetate of **4** (see below). By time D, no **11**

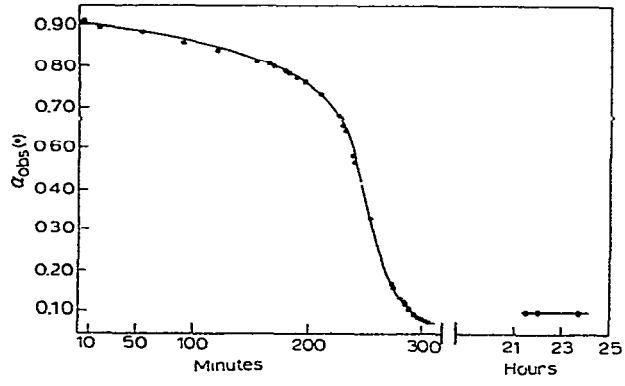


Fig. 1. Optical rotation of 1,3,4,6-tetra-*O*-benzyl-D-mannitol (**11**) in methyl sulfoxide–acetic anhydride.

could be detected; the mixture was approximately 50% **4**, 30% of an acetate of **4**, and 20% of a compound which was assumed to be a (methylthio)methyl 1,3,4,6-tetra-*O*-benzyl-D-fructofuranoside (**14**, see below). By time E, the **4** had disappeared, leaving **14** and the acetate of **4** in a ratio of approximately 3:7.

Acetic anhydride (57 ml) was added to a solution of **11** (1.92 g) in methyl sulfoxide (85 ml). When the optical rotation of the resulting mixture was close to the minimum (4.3 h), the solution was poured into ice–water (800 ml). The mixture was extracted with dichloromethane and the extract was washed thoroughly with aqueous sodium hydrogen carbonate solution. Moisture was removed with magnesium sulfate and the solution was evaporated *in vacuo* to a syrup which was dissolved in methanol. Methanolic barium methoxide was added to cleave the acetate of **4** (t.l.c.); the methanolic solution was evaporated *in vacuo* to give a semi-crystalline mass which was subjected to preparative t.l.c. (1:6 ether–benzene). The band having the migration rate of **4** was eluted with ether and the eluate was evaporated to give crystalline material which was recrystallized from isopropyl ether to yield 268 mg (14%) of **4**, m.p. 41–42°; a mixture m.p. with **4**, derived from D-fructose as described earlier, was undepressed.

A reaction, similar to that described above, was carried out with 199.1 mg of **11** and halted after 4.75 h. The syrupy product was subjected to preparative t.l.c. (1:1 ether–carbon tetrachloride) and separated into three bands. The slowest-moving component (56.6 mg) was **4**; the middle band afforded a component (48.3 mg) which had $[\alpha]_D^{20}$ +9.7° (*c* 1.3, chloroform) and strong absorption at 1700 cm^{-1} (neat). After treatment of this material with barium methoxide in methanol solution, it was examined by t.l.c. (1:1 ether–carbon tetrachloride) and then found to be chromatographically identical with **4**. The fastest-moving band yielded a syrupy material (51.4 mg) which had $[\alpha]_D^{20}$ +6° (*c* 2.4, chloroform) and an i.r. spectrum devoid of carbonyl and hydroxyl absorption. Its elemental composition is consistent with the

conclusion that it is a (methylthio)methyl 1,3,4,6-tetra-*O*-benzyl-D-fructofuranoside (**14**).

Anal. Calc. for $C_{36}H_{40}O_6S$: C, 71.97; H, 6.71; S, 5.34. Found: C, 72.12; H, 7.01; S, 5.33.

Octa-O-benzylsucrose (**16**). — Sucrose (**15**) was benzylated in *N,N*-dimethylformamide solution with benzyl bromide, barium oxide, and silver oxide as described by Tate and Bishop[6], the temperature of the stirred mixture being kept for 3 h at 50–55°. After removal of the excess of the reactants, the crude product was chromatographed on a column of silica gel, benzyl ether and benzyl alcohol being eluted with 1:5 isopropyl ether–cyclohexane. The octa-*O*-benzylsucrose (**16**) was eluted with 1:1 isopropyl ether–cyclohexane and obtained as a syrup which showed $[\alpha]_D^{20}$ +38.6° (*c* 1.31, chloroform); lit.[6] $[\alpha]_D^{20}$ +31.6° (*c* 1.65, chloroform), $[\alpha]_D^{20}$ +38.6° (*c* 1.62, chloroform)[9].

Anal. Calc. for $C_{68}H_{71}O_{11}$: C, 76.81; H, 6.64. Found: C, 76.74; H, 6.17.

Hydrogenolysis of a small sample of **16** over a palladium catalyst readily led to the isolation of crystalline sucrose.

The hydrolysis of octa-O-benzylsucrose (**16**). — Octa-*O*-benzylsucrose (**16**, 5.4 g) was dissolved in glacial acetic acid (50 ml) and the solution was heated at 65° (water bath) and stirred while 2M sulfuric acid was added in 4-ml portions, care being taken to avoid the precipitation of a syrup. After 10 min, 12 ml of the dilute sulfuric acid had been added and the precipitation of crystalline 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**17**) began. Crystallization was allowed to proceed in the stirred mixture over the course of 6 h at 65°, 2M sulfuric acid being added cautiously from time to time (to a total of 26 ml) without precipitating syrup. Finally, the reaction mixture was stirred overnight at room temperature and the 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**17**) was removed by filtration: 2.8 g (102%). Recrystallized from hot propyl alcohol, the **17** (2.1 g, 76%) had m.p. and mixture m.p. 153–154° and $[\alpha]_D^{20}$ +19.1° (*c* 1.57, chloroform); lit.[7] $[\alpha]_D^{20}$ +21.2 ±0.6° (*c* 3.5, chloroform).

The filtrate remaining after the removal of the 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose was diluted with water and extracted with dichloromethane. The extract was washed successively with aqueous sodium hydrogen carbonate solution and with water. Moisture was removed with magnesium sulfate and the solution was evaporated *in vacuo* (35° bath) to a syrup (1.8 g) which was dissolved in isopropyl ether and seeded* to give 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (**4**); yield, 0.48 g (17%). After recrystallization from isopropyl ether at −5°, the product (0.25 g) had m.p. 42–43°; a mixture m.p. with a sample of **4**, prepared from D-fructose as described earlier, was undepressed.

*A sample of the material from a similar preparation was chromatographed on a column of silica gel with 1:1 ether–carbon tetrachloride. Saturated solutions of the chromatographically homogeneous product in pentane and in hexane gave the first crystals of **4** when they were kept for several days at +5°.

ACKNOWLEDGMENTS

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DIPHENYL DITHIOACETALS OF D-RIBOSE, D-XYLOSE, AND D- AND L-ARABINOSE. CONFORMATIONAL STUDIES AND FORMATION OF A KETENE DIPHENYL DITHIOACETAL*†

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ABSTRACT

D-Ribose, D-xylose, D-arabinose, and L-arabinose have been converted in good yield into their respective diphenyl dithioacetals, **1**, **5**, **8**, and **3**. The tetraacetate **4** of **3** adopts a planar, zigzag conformation in chloroform solution, but the D-*xylo* analog **6** and D-*ribo* analog **2** adopt conformations that have no parallel 1,3-interactions of acetoxyl groups. A crystalline diisopropylidene acetal **7** was obtained from **1**. On treatment with a strong base, the corresponding acetal **9** from the D-*arabino* derivative **8** underwent elimination of acetone to give the ketene diphenyl dithioacetal **10**, characterized as its crystalline 3-*p*-nitrobenzoate **11** and its remarkably stable 3-methyl ether **12**.

INTRODUCTION

The uncatalyzed addition of benzenethiol to chloral was reported[3] in 1870, although the product was neither characterized nor identified. In 1885, Baumann demonstrated the formation of diphenyl dithioacetals from pyruvic acid, acetone, benzaldehyde, and chloral[4], and the use of this reaction in natural-product chemistry was demonstrated in 1887 with the preparation of the 3-(diphenyl dithioacetal) of 3,7,11-trioxocholanic acid[5]. Seven years later, Emil Fischer[6] reported the preparation of dialkyl dithioacetals of sugars, and stated that benzenethiol does not react with aldoses. This assertion was repeated[7] in 1909.

In 1958, El-Hewehi[8] challenged Fischer's dictum, and reported the isolation of the crystalline diphenyl dithioacetal of D-galactose by mercaptolysis of lactose. Treatment of D-ribose with benzenethiol in the presence of zinc chloride and hydrochloric acid, followed by acetylation and distillation of the product, gave an unspecified yield of an oil that was identified as tetra-*O*-acetyl-D-ribose diphenyl dithioacetal on the basis of carbon and hydrogen analyses. In 1965, Horton and Wander[2] briefly noted the preparation of crystalline diphenyl dithioacetals of D-ribose and L-arabinose,

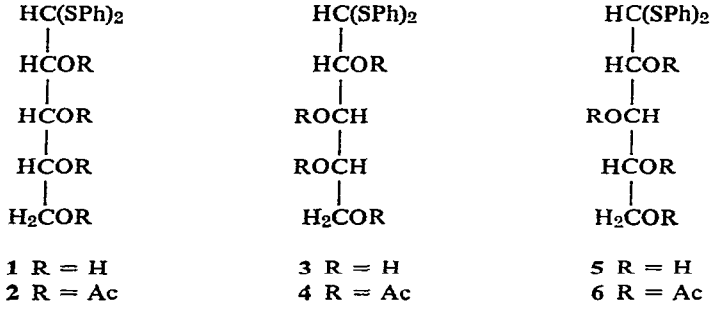
*Dedicated to the memory of Professor M. L. Wolfrom.
†This paper is part 4 of a series entitled "Conformation of Acyclic Derivatives of Sugars". For part 3, see ref. 1. For a preliminary report of part of this work, see ref. 2.

and, in the following year, Zissis, Clingman, and Richtmyer[9] described the diphenyl dithioacetals of D-glucose and D-mannose.

This report describes in detail the preparation of diphenyl dithioacetals of D-ribose, D-xylose, and D- and L-arabinose, together with three *O*-acetyl and two *O*-isopropylidene derivatives, and also describes a 1,2-elimination reaction to give a ketene dithioacetal derivative that was noted briefly in the preliminary report[2].

DISCUSSION

Preparation of diphenyl dithioacetals. — Conversion of D- and L-arabinose into their crystalline diphenyl dithioacetals **8** and **3**, respectively, proceeded satisfactorily with benzenethiol and concentrated hydrochloric acid within ~1 h at room temperature, to give the products in 70% yield. The D-xylose analog **5** was obtained similarly; a crystalline sample was first obtained by saponification of the tetraacetate **6** of **5**. Richtmyer *et al.*[9] reported that an extended time of reaction was required for preparation of the diphenyl dithioacetals of D-glucose, D-mannose, and D-galactose. The crystalline diphenyl dithioacetal of D-ribose (**1**) was obtained by the general procedure used for **3**, **5**, and **8**, but careful recrystallization was necessary for removal of a second component (a mixture of thioglycosides) from the reaction product.



1 R = H
2 R = Ac

3 R = H
4 R = Ac

5 R = H
6 R = Ac

Acetylation of **1**, **3**, and **5** with acetic anhydride in pyridine gave the corresponding tetraacetates **2**, **4**, and **6** in excellent yield; the L-*arabino* and D-*xylo* products **4** and **6** were crystalline, but the D-*ribo* analog **2** was obtained after distillation as an extremely viscous, colorless oil.

Conformational analysis of acetylated diphenyl dithioacetals. — The conformations of the three acetates in solution were examined by n.m.r. spectroscopy. In previous reports from this laboratory[1,10,11], it has been shown that acyclic sugar chains in solution tend to adopt a planar, zigzag arrangement[10] of carbon atoms, unless such a conformation would lead to a 1,3-eclipsed interaction of polar substituents[1,11]. The latter interaction, which resembles the *syn*-diaxial arrangement in a disubstituted, 6-membered ring-system, has been shown to be a powerful destabilizing factor, forcing the molecule to adopt a "sickle" conformation, resulting from rotation about a carbon–carbon bond, in order to relieve the 1,3-interaction.

Such a situation has also been observed in the solid state for the ribitol chain in riboflavin[12], and recently, in the crystallographic work of Jeffrey and coworkers, for ribitol[13] and xylitol[14].

The acetates **2**, **4**, and **6** in chloroform-*d* gave n.m.r. spectra that were readily analyzed by the methods already reported[1], and the observed chemical-shift and spin-coupling data for protons on the backbone chain are recorded in Table I, together

TABLE I

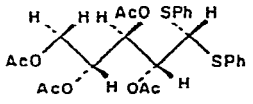
CHEMICAL SHIFTS AND COUPLING CONSTANTS FROM 100-MHz P.M.R. SPECTRA, IN CHLOROFORM-*d*, OF TETRA-*O*-ACETYL-D-RIBOSE DIPHENYL DITHIOACETAL (**2**), TETRA-*O*-ACETYL-L-ARABINOSE DIPHENYL DITHIOACETAL (**4**), TETRA-*O*-ACETYL-D-XYLOSE DIPHENYL DITHIOACETAL (**6**), AND THEIR DIETHYL ANALOGS

	Tetra-O-acetylpentose dithioacetal					
	D-ribo		D-xylo		L-arabino	
	Diethyl[a]	*Diphenyl*	*Diethyl*[a]	*Diphenyl*	*Diethyl*[a]	*Diphenyl*
Couplings						
$J_{1,2}$	6.2	3.6	5.2	3.0	8.3	5.4
$J_{2,3}$	5.7	7.2	5.9	7.0	2.8	3.1
$J_{3,4}$	3.6	3.7	4.2	3.1	7.9	8.5
$J_{4,5}$	3.1	3.8	4.3	5.1	2.9	3.8
$J_{4,5'}$	7.7	7.3	6.6	7.2	6.0	5.2
$J_{5,5'}$	12.0	12.2	11.8	11.9	12.3	12.8
Chemical shifts						
H-1	6.01	5.51	6.02	5.59	6.09	5.46
H-2	4.67	4.53	4.65	4.58	4.70	4.61
H-3	4.37	4.26	4.26	4.22	4.28	4.19
H-4	4.62	4.67	4.62	4.62	4.87	4.91
H-5	5.55	5.70	5.67	5.77	5.70	5.81[b]
H-5'	5.89	5.93	5.99	6.13	5.98	5.92[b]
Acetate[c]	7.89	8.06	7.96 (6)	7.95	7.92	7.93
	7.95	8.09	7.98	7.99	7.93	7.99
	7.97	8.12 (6)	8.02	8.02	7.99	8.06
	8.00			8.09	8.00	8.38

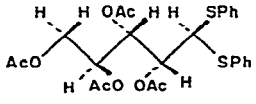
[a]Data from ref. 1. [b]By ABX analysis. [c]Three-proton singlets, unless otherwise noted in parentheses.

with corresponding data[1] for the diethyl dithioacetal analogs. By comparison of the spin couplings in the three acetates **2**, **4**, and **6**, and of those in the diethyl analogs, it may be seen that there is very little difference in the magnitudes of corresponding couplings, except for the value of $J_{1,2}$. By the arguments already presented[1] for the diethyl analogs, the favored conformation of tetra-*O*-acetyl-L-arabinose diphenyl dithioacetal (**4**) may be formulated as the planar, zigzag arrangement **4a**, corresponding to maximum relief of steric interactions between small–medium–large sets of groups along each carbon–carbon bond without the generation of parallel 1,3-interactions of substituents.

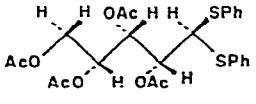
The spin-coupling data for tetra-*O*-acetyl-D-ribose diphenyl dithioacetal (**2**) clearly do not accord with the planar, zigzag arrangement **2a**; such a conformation would require large values for $J_{2,3}$ and $J_{3,4}$, as H-2, H-3, and H-4 would be mutually



2a
not favored



4a
favored



6a
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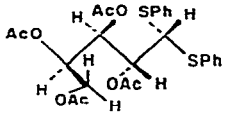

2b
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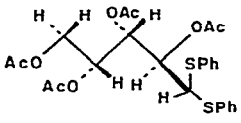

6b
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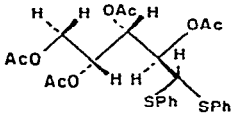


6c

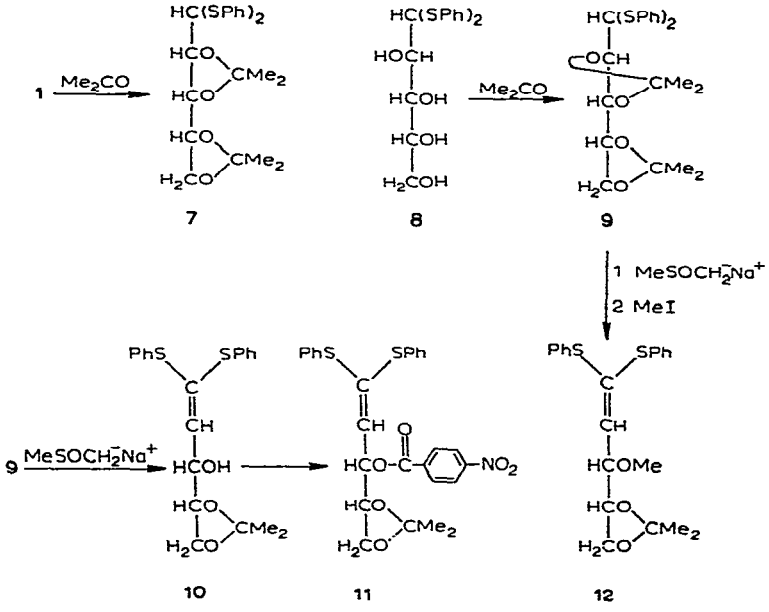
antiparallel. The value of 7.2 Hz observed for $J_{2,3}$ accords with an antiparallel disposition of H-2 and H-3, but the low value (3.7 Hz) of $J_{3,4}$ requires that H-3 and H-4 be gauche-disposed. The observed data are accommodated by the "sickle" conformation (**2b**) derived from the planar, zigzag arrangement **2a** by rotation along C-3–C-4 in order to remove the parallel interaction with the 2-acetoxyl group. The representation **2b** depicts the C-1–C-2 rotamer state that has H-1 and H-2 gauche-disposed (to accommodate the observed $J_{1,2}$ value of 3.6 Hz) and that has no parallel interaction between the 3-acetoxyl group and one phenylthio group. Such a parallel interaction would destabilize the other two C-1–C-2 rotamers, even though the one having H-1 and H-2 antiparallel would have the maximum staggering of small–medium–large sets of groups along C-1–C-2.

In the case of tetra-*O*-acetyl-D-xylose diphenyl dithioacetal (**6**), the observed spin-couplings again do not support the planar, zigzag formulation **6a**, because such a conformation would require small values for $J_{2,3}$ and $J_{3,4}$. Although $J_{3,4}$ is small (3.1 Hz), the value of $J_{2,3}$ (7.0 Hz) indicates that H-2 and H-3 are antiparallel in the favored rotamer state. The coupling data indicate that the molecule exists principally in the sickle conformation **6b**, derived from **6a** by rotation along C-2–C-3 in order to remove the 1,3-parallel interaction between the acetoxyl groups at C-2 and C-4, and to bring H-2 and H-3 into an antiparallel disposition. The C-1–C-2 rotamer state depicted in **6b** has H-1 and H-2 gauche-disposed, in order to accord with the observed small value (3.0 Hz) of $J_{1,2}$. That C-1–C-2 rotamer having maximum staggering of bulky groups, namely, **6c**, would have H-1 and H-2 antiparallel, giving a large value of $J_{1,2}$. It is probable that steric interference between the 4-acetoxyl group and the (bulky) phenylthio group causes **6c** to be less stable than **6b**, because the latter conformer is free from such interaction.

For the diphenyl derivatives **2**, **4**, and **6**, the H-1 signal appears ~0.5 p.p.m.

to lower field than its position for the diethyl analogs (see Table I), indicating a substantial difference in the electronic environment of H-1 in the two series of derivatives. In the light of the demonstrated[15] dependence of coupling constants on substituent character, it is possible that this factor may also make some contribution to the differences in $J_{1,2}$ values observed between the diphenyl derivatives and their diethyl analogs.

Formation of ketene dithioacetals. — Acetonation of D-arabinose diphenyl dithioacetal **8** in the presence of copper(II) sulfate and a trace of sulfuric acid gave, in good yield, a distillable, liquid diisopropylidene acetal that was identified as the 2,3:4,5 isomer **9** by analogy with the corresponding diethyl dithioacetal[16], and on the basis of mass-spectral data (see Experimental section and Table III) and subsequent reactions. Similar acetonation of the D-ribose analog **1** gave, in good yield, a crystalline diisopropylidene acetal presumed also to be the 2,3:4,5 isomer, namely, **7**, by analogy with the corresponding diethyl dithioacetal[17] and by analysis of its mass-spectral fragmentation pattern (see Experimental section and Table III).



The fully protected diphenyl dithioacetals **7** and **9** had been prepared with a view to generating a carbanion at C-1 by abstracting H-1, as a proton, with a strong base. The formation of alkoxide anions was to be prevented by the use of the *O*-isopropylidene group, normally alkali-stable, and it was supposed that the arylthio groups could stabilize the C-1 carbanion by resonance. Alkylation of such a carbanion might afford a route to higher ketoses. The D-*arabino* derivative **9** was therefore treated at room temperature with sodium methylsulfinylcarbanion in methyl sulfoxide, and an exothermic reaction ensued. Treatment of the product with methyl iodide caused a second exothermic reaction. Removal of the solvents and the excess of

reagents gave a syrupy product, $C_{21}H_{24}O_3S_2$, that was remarkably stable to alkali and to oxidizing agents; it could be recovered unchanged after treatment with concentrated permanganate (alkaline). The same product could be obtained by use of potassium *tert*-butoxide or butyllithium as the base, or of methyl sulfate as the methylating reagent.

TABLE II

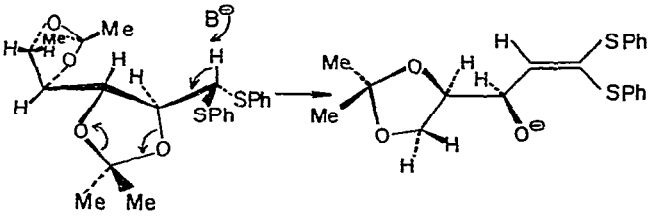
N.M.R. SPECTRAL DATA (100 MHz) FOR COMPOUNDS **7, 9, 10, 11, 12,** AND THE DIETHYL ANALOG OF **12,** IN CHLOROFORM-*d*

Spectral parameter	**7**	**9**	**10**[a]	**11**	**12**	*Diethyl analog of* **12**[a,b]
First-order couplings, Hz						
$J_{1,2}$	1.2	0.9				
$J_{2,3}$	[c]	4.3	9	8.6	8.8	8
$J_{3,4}$	[c]	[c]	5	4.0	5.6	[c]
$J_{4,5}$	[c]	[c]	[c]	7.0	[c]	[c]
$J_{4,5'}$	[c]	[c]	[c]	6.7	[c]	[c]
$J_{5,5'}$	[c]	[c]	[c]	8.5	[c]	[c]
Chemical shifts, τ						
H-1	5.30	5.17				
H-2	5.76[d]	5.64	3.78	4.03	4.08	4.00
H-3	6.02	5.75[d]	5.04	3.75	5.70	5.35[d]
H-4	6.16[d]	|	5.64[d]	5.60	5.97[d]	|
H-5	|	|	|	5.90	|	|
H-5′	6.31	6.20	6.14	6.14	6.32	6.14
Ph_2	2.45–2.80	2.50–2.86	2.50–2.78	2.74, 1.76[e]	2.84	7.08[f] 8.60[f]
CMe_2	8.56, 8.60 (2) 8.67	8.51, 8.63 8.73, 8.77	8.60 (2), 8.63 (2)	8.66 (2)	8.74 8.81	8.60 (2)
OMe					6.82	6.61
OH			6.60[g]			

[a]Measured at 60 MHz. [b]1,2-Dideoxy-1,1-bis(ethylthio)-3-*O*-methyl-D-*erythro*-pent-1-enitol. [c]Not determined. [d]Unresolved multiplet. [e]Four protons of the *p*-nitrobenzoyl group. [f]Protons of two EtS groups. [g]Exchanged by deuterium oxide.

The n.m.r. spectrum of the product $C_{21}H_{24}O_3S_2$ (see Table II) showed that two phenyl groups were still present, but that only one *O*-isopropylidene group remained. A 3-proton signal was observed in the region characteristic of methoxyl-group resonances. A 3-proton group of signals appeared in the spectral region where the starting acetal **9** showed resonances for H-4, H-5, and H-5′. A one-proton doublet showing a spacing of 8.8 Hz was observed at τ 4.08, and a one-proton quartet that also showed this spacing, together with a spacing of 5.6 Hz, was observed at τ 5.70. These data indicate that the product is formed by 1,2-elimination from **9**, loss of a molecule of acetone, and methylation of an alkoxide anion. The structure 1,2-dideoxy-4,5-*O*-isopropylidene-3-*O*-methyl-1,1-bis(phenylthio)-D-*erythro*-pent-1-enitol (**12**) is,

therefore, proposed for this compound; the doublet signal at τ 4.08 was assigned to the vinylic proton (H-2), and the quartet at τ 5.70 was assigned to H-3. Evidently, the base abstracts H-1 from **9**, but a synchronous or subsequent process leads to displacement of O-2 as an anion, and loss of acetone; and the resultant oxyanion is methylated in the subsequent step, as shown in the following scheme.



Treatment of **9** with base, without subsequent *O*-alkylation but with the addition of water, leads to the 3-hydroxy analog **10** of **12**, as a syrup. The n.m.r. spectrum of **10** (see Table II) closely resembles that of **12**, except that the methoxyl-group signal is absent and an additional signal is observed, namely, that of a proton that was labile to exchange with deuterium oxide. A crystalline *p*-nitrobenzoate **11** was prepared from **10**, and its n.m.r. spectrum (see Fig. 1 and Table II) could be completely analyzed on a first-order basis. The spectrum of **11** resembled that of the

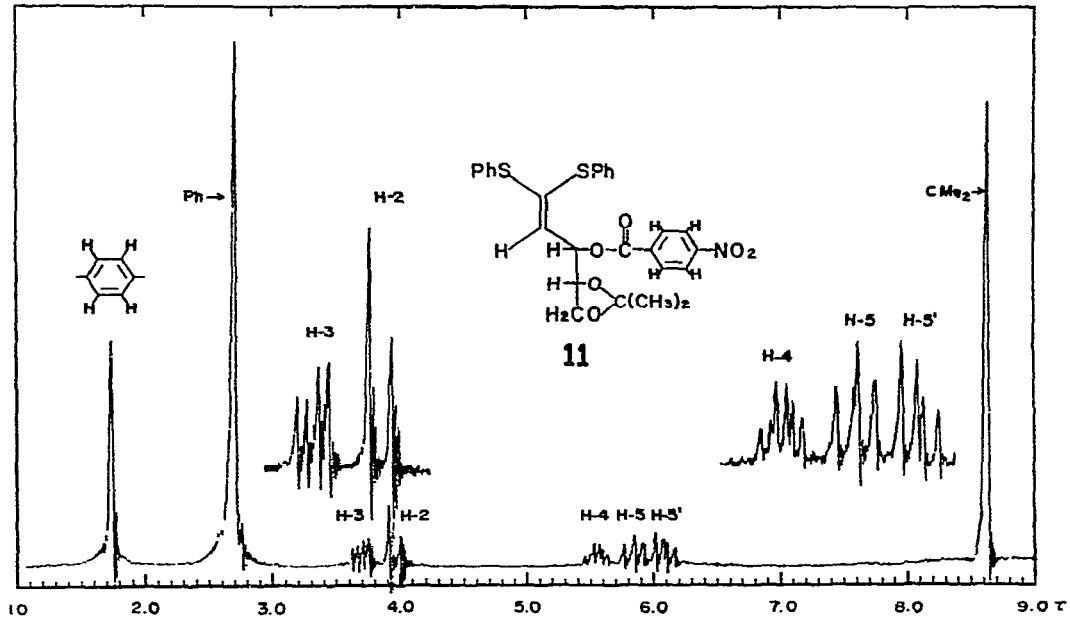


Fig. 1. The 100-MHz n.m.r. spectrum of 1,2-dideoxy-4,5-*O*-isopropylidene-3-*O*-(*p*-nitrobenzoyl)-1,1-bis(phenylthio)-D-*erythro*-pent-1-enitol (**11**) in chloroform-*d*. The main spectrum to which the scale refers was measured at a sweep-width of 1000 Hz; insets measured at a sweep-width of 500 Hz show fine structure of the signals of H-2, 3, 4, 5, and 5'.

3-methyl ether **12**, except that H-3 in **11** resonated at considerably lower field than H-3 in **12**, the result of the greater deshielding effect of the *p*-nitrobenzoyloxy group.

The mass spectrum of the unsaturated ether **12** showed a major ion having *m/e* 123 that was present to a considerably smaller extent in the spectrum of the precursor **9** (see Table III). The ion having *m/e* 123 is, presumably, that of $C_7H_7S^+$, and it can be expected to have arisen from **12** by the process shown in the following scheme.

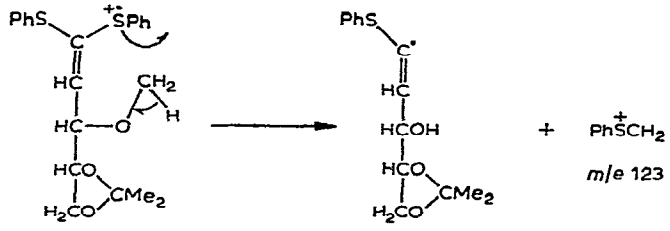


TABLE III

PRINCIPAL IONS FROM MASS-SPECTRAL DECOMPOSITION AT 70 eV (BASE PEAK = 100.0)

m/e	*Compound*			*Assignment*
	7	**9**	**12**	
43	78.0	100.0	28.5	CH_3CO^+
77	14.4	8.0	28.0	$C_6H_5^+$, phenyl
78	9.3	4.1	55.2	$C_6H_6^+$
91	14.9	14.4	48.9	$C_7H_7^+$, tropylium
101	10.0	33.3	0.2	[a]$C_5H_9O_2^+$
109	33.7	25.0	65.8	$C_6H_5S^+$, probably thiatropylium
110	43.5	11.1	10.3	$C_6H_6S^+$
123	69.0	32.2	100.0	$C_7H_7S^+$ ($PhSCH_2^+$)
135	50.0	41.8	0.2	unidentified
207	65.2	33.3	>0.1	unidentified
264	100.0	58.3	0.1	unidentified
388	—	—	>0.1	M^+
417	3.5	2.1	—	M^+–CH_3
432	5.0	9.5	—	M^+

[a]See Experimental.

In the case of **9**, a pathway involving scission of a $-CH_2-$ group from the 2,3-*O*-isopropylidene group would be unfavored on steric and electronic grounds.

Treatment of the diethyl analog[16] of **9** with strong base, followed by methylation, gave a product identified by its n.m.r. spectrum as the diethyl analog of **12**. It is evident, therefore, that aryl groups attached to sulfur are not essential to occurrence of the elimination reaction.

The base-induced elimination **9**→**10** resembles the 1,2-eliminations observed with certain α-substituted acetals of aldehydes[18] and with α-substituted thioethers[19];

α-haloaldehyde dialkyl dithioacetals eliminate hydrogen halide spontaneously upon formation[20]. For cyclic acetal groups, alkaline displacements bearing a formal resemblance to the behavior observed with **9** include the alkaline removal of the 3,5-*O*-isopropylidene group of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-nitro-α-D-galactofuranose[21], and the debenzylidenation of methyl 4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucopyranoside[22].

Although some dialkyl dithioacetals of simple ketenes have been reported[18–20,23], the products **10**, **11**, and **12**, and their dialkyl analogs, represent a new class of unsaturated sugar. Studies on the reactivity of these ketene dithioacetals of sugars will be reported at a later date.

EXPERIMENTAL

General methods. — Melting points were measured with a Thomas–Hoover "Unimelt" oil-bath apparatus. Optical rotations were measured with a Perkin–Elmer Model 141 automatic polarimeter and a 1-dm tube. T.l.c. was performed with Silica Gel G, and column chromatography with Silica Gel 7734 (E. Merck, Darmstadt, Germany); plates were activated at 110°. Indication was effected with sulfuric acid. U.v. spectra were recorded with a Cary Model 14 spectrophotometer, and i.r. spectra were recorded with a Perkin–Elmer Model 137 i.r. spectrophotometer. N.m.r. spectra were recorded at 100 MHz with a Varian HA-100 spectrometer in the frequency-sweep mode, with 5% of tetramethylsilane as a lock signal and internal standard, according to the general procedures reported earlier[1]. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities. Elemental analyses were performed by W. N. Rond, by Galbraith Laboratories, and by Huffman Laboratories. Mass spectra were recorded by C. R. Weisenberger under the supervision of Dr. R. C. Dougherty; an AEI MS-902 instrument was used with a direct-insertion probe, at an inlet temperature of 250°, an ionizing potential of 70 eV, and an accelerating potential of 8 kV.

D-*Ribose diphenyl dithioacetal* (**1**). — A mixture of D-ribose (3.0 g), benzenethiol (5.6 ml), and 9.0 ml of concentrated hydrochloric acid pre-saturated at 0° with hydrogen chloride gas was shaken for 2 h at 0° and then for 20 min at room temperature. The resultant, homogeneous solution was poured into cold water (100 ml), and the syrupy product that formed was separated by decantation of the water layer. The syrup was dissolved in ethyl acetate (50 ml), and the solution was dried (sodium carbonate) and evaporated. A solution of the product in benzene (100 ml) was kept for 2 days at 0° to give the crude dithioacetal **1**; yield 3.0 g (42%) in two crops, m.p. 96–98°. Recrystallization from ethanol–ether and then from ethanol–water gave pure **1** as fluffy, white clusters of very fine needles, m.p. 101.5–102.0°, $[\alpha]_D^{27}$ +42.3 ±0.6° (*c* 1, pyridine); λ_{max}^{KBr} 6.35, 6.75, 6.95 (aryl C=C), 13.60, 14.20, and 14.55 μm (aryl); λ_{max}^{EtOH} 256 (ε 12,000) and 217 (sh) nm (15,000); X-ray powder

diffraction data: 13.69 m (3), 10.84 m, 9.16 m, 5.31 vs (1), 4.74 s (2,2), 4.54 m, 4.04 s (2,2), 3.77 m, 3.07 m, 2.93 m, 2.45 w, 2.11 w, and 1.96 w.

Anal. Calc. for $C_{17}H_{20}O_4S_2$: C, 57.95; H, 5.68; S, 18.18. Found: C, 58.25; H, 5.98; S, 17.99.

A second component from the reaction product, less soluble than **1** in ethanol, was isolated; yield ~0.1 g, m.p. 178–180°, $[\alpha]_D^{27}$ −48 ±1° (*c* 1.1, pyridine).

Anal. Calc. for $C_{11}H_{14}O_4S$: C, 54.54; H, 5.78; S, 13.22. Found: C, 54.30; H, 6.04; S, 13.09.

From the data, the side product appears to be a phenyl 1-thio-D-riboside; its ring size and anomeric configuration have not yet been established. After acetylation with acetic anhydride in pyridine, a syrup was obtained (R_F 0.6 in dichloromethane) whose n.m.r. spectrum in chloroform-*d* showed signals for one phenyl group and a total of three acetyl groups; the n.m.r. spectrum indicated that the product was a mixture of isomeric forms.

D-*Arabinose diphenyl dithioacetal* (**8**). — A solution of D-arabinose (50 g) in concentrated hydrochloric acid (125 ml) was shaken with benzenethiol (85 ml) at room temperature until the mixture had become solid (30–45 min). The off-white solid was dispersed in 1 liter of water in a blender, and the slurry was filtered. The resultant solid was dissolved in boiling, abs. ethanol (3 liters), and the solution was allowed to cool slowly overnight. After an additional 24 h at 0°, the mixture was filtered to give **6** as matted, white needles; yield 80 g (70%) in two crops, m.p. 186.5–187.0°, $[\alpha]_D^{29}$ +24.0 ±0.6° (*c* 1, pyridine); λ_{max}^{KBr} 6.35, 6.80, 6.95, 7.20 (aryl C=C), 13.50, 14.20, and 14.55 μm (aryl); λ_{max}^{EtOH} 256 (ε 9,000) and 216 (sh) nm (17,000); X-ray powder diffraction data: 12.99 s (2), 11.70 m, 9.35 s (3,3), 8.30 m, 5.38 m, 5.16 vw, 4.70 s, 4.33 s (3,3), 4.12 vs (1), 4.00 vw, 3.89 w, 3.61 vw, 3.47 vw, and 2.67 w.

Anal. Calc. for $C_{17}H_{20}O_4S_2$: C, 57.95; H, 5.68; S, 18.18. Found: C, 57.94; H, 5.95; S, 18.24.

L-*Arabinose diphenyl dithioacetal* (**3**). — The previous experiment was repeated, but with L-arabinose as the starting material. The product (**3**) had m.p. 186–186.8°, $[\alpha]_D^{29}$ −25.9 ±0.6° (*c* 1.2, pyridine); the i.r. and u.v. spectra and X-ray powder diffraction pattern were superposable on those of **8**.

Anal. Calc. for $C_{17}H_{20}O_4S_2$: C, 57.95; H, 5.68; S, 18.18. Found: C, 57.81; H, 5.90; S, 18.24.

D-*Xylose diphenyl dithioacetal* (**5**). — A solution of D-xylose (10 g) in 15 ml of concentrated hydrochloric acid was shaken with 17 ml of benzenethiol for 1 h at room temperature. The homogeneous mixture was poured into ice–water (400 ml), and the mixture was extracted with two 100-ml portions of ethyl acetate. The extracts were combined, dried (magnesium sulfate), and evaporated, to give crude **5** as a clear, pale-yellow syrup, yield 11.5 g (50%).

Crystalline **5** was obtained by saponification of the tetraacetate **6**. To a solution of **6** (1.0 g) in methanol (50 ml) was added sodium (~10 mg); after 6 h, Dry Ice (~2 g) was added, and the mixture was filtered. The filtrate was evaporated, and

the residue was dried at 150°/0.04 torr; trituration of the residue with ethanol caused it to crystallize. Recrystallization from ethanol gave **5** as white granules, m.p. 98–100°, yield 475 mg (77%); after being dried at 150° and further recrystallized, it had m.p. 100–101.5°, $[\alpha]_D^{26}$ −8 ±1° (*c* 0.5, ethanol); λ_{max}^{KBr} 2.95 (OH), 6.20, 8.85, 9.35, 11.60, 13.45, and 14.60 μm (aryl); λ_{max}^{EtOH} 256 (ε 9,000) and 217 (sh) nm (16,000); X-ray powder diffraction data: 8.20 s (2), 5.82 m, 4.77 vs (1), 4.44 m, 4.21 s (3), and 2.21 m.

Anal. Calc. for $C_{17}H_{20}O_4S_2$: C, 57.95; H, 5.68; S, 18.18. Found: C, 57.95; H, 5.91; S, 18.05.

Tetra-O-acetyl-D-xylose diphenyl dithioacetal (**6**). — A solution of crude, syrupy **5** (10.5 g) in pyridine (60 ml) and acetic anhydride (20 ml) was kept overnight at room temperature, and then poured into ice–water (750 ml). After 15 min, the mixture was extracted with two 250-ml portions of dichloromethane, and the extracts were combined, dried (magnesium sulfate), and evaporated. A solution of the product in dichloromethane was passed through a short column (2.5 × 1 cm) of silica gel. Evaporation of the effluent, and crystallization of the product from ether (40 ml) and petroleum ether (b.p. 30–60°, ~10 ml) at 0° gave **6** as a white solid; yield 10.6 g (67%), m.p. 81–90°. Recrystallization from hot ethanol gave 9.1 g of **6** as broad needles, m.p. 90–91°, $[\alpha]_D^{25}$ +54.5° (*c* 1, ethanol); λ_{max}^{KBr} 5.65 (OAc), 6.35, 7.25, 11.20, 11.95, 13.50, and 14.55 μm (aryl); λ_{max}^{EtOH} 256 (ε 9,000) and 216 (sh) nm (18,700); for n.m.r. data, see Table I; X-ray powder diffraction data: 9.02 s (2,2), 8.34 vs (1), 6.47 m, 6.03 s (2,2), 5.58 s (2,2), 4.39 m, 4.01 s (2,2), 3.85 s (2,2), 3.65 s (2,2), and 2.82 w.

Anal. Calc. for $C_{25}H_{28}O_8S_2$: C, 57.69; H, 5.38; S, 12.30. Found: C, 57.40; H, 5.41; S, 12.13.

Tetra-O-acetyl-D-ribose diphenyl dithioacetal (**2**). — A solution of **1** (5 g) in dry pyridine (25 ml) and acetic anhydride (10 ml) was kept for 18 h at room temperature, and then poured into ice–water (500 ml). The mixture was extracted with dichloromethane, and the extract was dried (magnesium sulfate) and evaporated, to give **2** as a pale-yellow syrup, yield 6.3 g (84%). The analytical sample was obtained by distillation at 205° (bath)/5 torr; $[\alpha]_D^{26}$ +79.8 ±0.5° (*c* 1.18, chloroform); R_F 0.4 (dichloromethane); λ_{max}^{Film} 5.60, (OAc), 6.35, 6.80, 6.85 (aryl C=C), 7.20, 13.45, and 14.55 μm (aryl); λ_{max}^{EtOH} 252 (ε 7,200) and 217 (sh) nm (14,500); for n.m.r. data see Table I.

Anal. Calc. for $C_{25}H_{28}O_8S_2$: C, 57.69; H, 5.38; S, 12.30. Found: C, 57.71, H, 5.64; S, 12.10.

El-Hewehi[8] described **2** as a yellowish oil boiling at 140–150° under "high vacuum".

Tetra-O-acetyl-L-arabinose diphenyl dithioacetal (**4**). — A solution of **3** (5.0 g) in pyridine (25 ml) and acetic anhydride (25 ml) was kept for 18 h at room temperature, and then poured into ice–water (500 ml). The resultant solid was filtered off, and recrystallized from ether–petroleum ether (b.p. 30–60°) to give **4** as dense prisms; yield 6.4 g (80%), m.p. 80–81°. A small sample was freed of minor impurities by

passing a solution in dichloromethane through a short column (2.5 × 1 cm) of silica gel; recrystallization, as before, gave pure **4**, m.p. 82–83°; $[\alpha]_D^{27}$ −60.3 ±0.6° (*c* 1, pyridine); λ_{max}^{KBr} 5.65 (OAc), 6.30, 6.70, 6.95 (aryl C=C), 12.95, 13.10, 13.35, 14.40, and 14.55 μm (aryl); λ_{max}^{EtOH} 257; (ε 10,000) and 217 (sh) nm (20,000); for n.m.r. data, see Table I; X-ray powder diffraction data: 9.35 s (2,2), 8.28 vs (1), 7.35 m, 6.56 m, 5.60 m, 4.64 s (2,2), 4.14 m, 3.95 w, and 3.69 w.

Anal. Calc. for $C_{25}H_{28}O_8S_2$: C, 57.69; H, 5.38; S, 12.30. Found: C, 57.53; H, 5.54; S, 12.06.

2,3:4,5-Di-O-isopropylidene-D-ribose diphenyl dithioacetal (**7**). — D-Ribose diphenyl dithioacetal (**1**, 1.0 g) was shaken for 24 h at room temperature with acetone (50 ml), anhydrous copper(II) sulfate (5 g), and 1 drop of concentrated sulfuric acid. The acid was neutralized with solid sodium carbonate (~1 g), and the mixture was filtered. Evaporation of the filtrate gave a syrup that began to crystallize after 12 h. Trituration with chloroform (0.3 ml) and ethanol (2 ml) gave white platelets of **5** that were filtered off and washed twice with 1.5-ml portions of ethanol; yield 1.0 g (80%), m.p. 124.5–126°, $[\alpha]_D^{26}$ +17.7 ±0.9° (*c* 0.78, chloroform): λ_{max}^{KBr} 6.95 (aryl C=C), 7.25 (CMe_2), 9.35, 11.10, 13.40, 14.00, and 14.55 μm (aryl); λ_{max}^{EtOH} 260 (ε 14,000) and 218 (sh) nm (21,000); for n.m.r. data, see Table II; X-ray powder diffraction data: 11.51 m, 7.74 m, 6.90 s (2,2), 4.94 s (2,2), 4.47 vs (1), 3.84 s (3), 3.47 w, 3.25 w, and 2.27 m; for mass-spectral data, see Table III.

Anal. Calc. for $C_{23}H_{28}O_4S_2$: C, 63.88; H, 6.46; S, 14.81. Found: C, 63.97; H, 6.71; S, 14.59.

The presence in the mass spectrum of **7** (see Table III) of a fragment having *m/e* 101 is diagnostic for a 4-monosubstituted 2,2-dimethyl-1,3-dioxolane residue, such as is seen for the exocyclic 5,6-acetal grouping of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose[24], and indicates that the substitution pattern in **7** is 2,3:4,5.

2,3:4,5-Di-O-isopropylidene-D-arabinose diphenyl dithioacetal (**9**). — A mixture of **8** (40 g) and anhydrous copper(II) sulfate (40 g) in acetone (500 ml) containing concentrated sulfuric acid (4 drops) was shaken for 72 h at room temperature. The acid was neutralized by stirring with anhydrous sodium carbonate (2 g), the mixture was filtered, and the filtrate was evaporated, to give **7** sufficiently pure for further conversions; yield 32.2 g (55%). An analytical sample was prepared by passing a solution of the product in dichloromethane through a short column of silica gel, evaporating off the solvent, and distilling the syrup at 210° (bath)/5 torr. The product **9** had $[\alpha]_D^{27}$ −35.7 ±0.6° (*c* 1, chloroform); R_F 0.5 (dichloromethane); λ_{max}^{Film} 6.35, 6.70, 6.95 (aryl C=C), 7.20 (CMe_2), 13.50, and 14.50 μm (aryl); λ_{max}^{EtOH} 257 (ε 13,000) and 225 (sh) nm (25,000); for n.m.r. data, see Table II; for mass-spectral data, see Table III.

Anal. Calc. for $C_{23}H_{28}O_4S_2$: C, 63.88; H, 6.46; S, 14.81. Found: C, 63.63; H, 6.51; S, 15.03.

As with **7**, the ion at *m/e* 101 in the mass spectrum of **9** results[24] from cleavage of the C-3–C-4 bond with retention of the charge at C-4, and indicates that the order of attachment of the isopropylidene groups is 2,3 and 4,5.

The product **9** decomposed on being stored, even when it had been carefully purified, to give acetone and the starting dithioacetal **8**. This decomposition was greatly accelerated by traces of acid. The unchanged acetal **9** could be extracted from the partially decomposed product by use of ether.

*1,2-Dideoxy-4,5-*O*-isopropylidene-3-*O*-methyl-1,1-bis(phenylthio)*-D-erythro-*pent-1-enitol* (**12**). — To a solution of sodium methylsulfinylcarbanion prepared by dissolving sodium (5 g) in freshly distilled methyl sulfoxide (80 ml) was added 2,3:4,5-di-*O*-isopropylidene-D-arabinose diphenyl dithioacetal (**9**, 23.4 g, 50 mmoles), with cooling during the ensuing exothermic reaction to keep the temperature below 40°. When the reaction had subsided, methyl iodide was added dropwise to the dark-red solution, with cooling (30°) to control the exothermic reaction. When addition of methyl iodide no longer elicited liberation of heat (~10–15 ml added), a final 2 ml was added. After a few minutes, the resultant sludge was transferred to a 1-liter separatory funnel with ice and water (500 ml) and ether (250 ml), and the mixture was shaken. The layers were separated, and the aqueous layer was extracted with 300 ml of ether. The extracts were combined, washed with cold water (500 ml), dried (magnesium sulfate), and evaporated, to give **10** as a reddish orange syrup; yield 14.2 g (68%), R_F 0.5 (dichloromethane). The product was chromatographically homogeneous, and its n.m.r. spectrum indicated that substantial proportions of contaminants were absent. Some of the colored contaminants could be removed by passing a solution of the product in dichloromethane through a column (5×2 cm) of silica gel, but attempts to distil the product at 250° (bath)/3 torr led to extensive charring and decomposition, and the yield of distillate was insignificant. Treatment of the crude product (2 g) with water (5 ml), potassium hydroxide (3 g), and potassium permanganate (3 g) at room temperature led to an initially exothermic reaction. After 24 h, an excess of 40% formaldehyde solution was added to the dark-green solution, inorganic solids were filtered off, and the filtrate was extracted with chloroform. The extract was passed through a short column of silica gel and then evaporated, to give pure **10** as a red-orange syrup, with little net loss; $[\alpha]_D^{27}$ +35.7 ±0.5° (*c* 1.2, chloroform); λ_{max}^{Film} 6.30, 6.70, 6.95 (aryl C=C), 7.20 (CMe_2), 13.45, and 14.55 μm (aryl); λ_{max}^{EtOH} 246 (ε 17,500) and 212 (sh) nm (45,000); for n.m.r. data, see Table II; for mass-spectral data, see Table III.

Anal. Calc. for $C_{21}H_{24}O_3S_2$: C, 64.96; H, 6.18; S, 16.49; OCH_3, 7.99. Found: C, 64.72; H, 6.27; S, 16.20; OCH_3, 8.11.

Essentially the same results were obtained when potassium *tert*-butoxide in *tert*-butyl alcohol, or butyllithium in ether or methyl sulfoxide, was used as the base, or when methyl sulfate was used instead of methyl iodide.

When 2,3:4,5-di-*O*-isopropylidene-D-arabinose diethyl dithioacetal[16] was used instead of the diphenyl analog, a syrupy product was obtained whose n.m.r. spectrum (see Table II) indicated that it was the diethyl analog of **12**.

*1,2-Dideoxy-4,5-*O*-isopropylidene-1,1-bis(phenylthio)*-D-erythro-*pent-1-enitol* (**10**). — To a solution prepared by dissolving sodium (0.6 g) in methyl sulfoxide (30 ml) was added **9** (3.0 g, 6.9 mmoles). After the ensuing, exothermic reaction had subsided,

the mixture was shaken in a separatory funnel containing ice and water (400 ml) and ether (250 ml). The layers were separated, and the aqueous phase was extracted with 100 ml of ether. The extracts were combined, washed successively with water and saturated aqueous sodium chloride, dried (magnesium sulfate), and evaporated, to give **10** as a yellow-orange syrup, yield 1.2 g (44%); for n.m.r. data, see Table II. The crude product was used directly in the next experiment.

1,2-Dideoxy-4,5-O-isopropylidene-3-O-(p-nitrobenzoyl)-1,1-bis(phenylthio)-D-erythro-pent-1-enitol (**11**). — The syrupy **10** from the preceding experiment was dissolved in 20 ml of pyridine, *p*-nitrobenzoyl chloride (6.0 g) was added, and the mixture was kept overnight at room temperature. The mixture was poured into 600 ml of ice–water, and the resultant solid was filtered off, washed with 500 ml of water, and dried. The solid was leached with ether (25 ml), to dissolve the product and leave most of the contaminating *p*-nitrobenzoic acid undissolved. The extract was evaporated, the resultant, crude product was dissolved in dichloromethane (400 ml), and the solution was passed through a column (20 × 1 cm) of silica gel to remove the remaining *p*-nitrobenzoic acid. Evaporation of the eluate and crystallization of the residue from ether–petroleum ether (b.p. 30–60°) gave **12** as a yellowish solid; yield 1.15 g (74%), m.p. 100–102°. Recrystallization from the same solvents gave **12** as a microcrystalline, off-white powder, m.p. 102–103°, $[\alpha]_D^{28}$ +37.9 ±0.5° (*c* 1.2, chloroform); λ_{max}^{KBr} 5.80 (C=O), 6.30, 6.55 (aryl), 7.45 (doublet, CMe_2), 13.50, 13.90, and 14.55 μm (aryl); λ_{max}^{EtOH} 257 (ε 22,000) and 206 nm (26,500); for n.m.r. data, see Table II; X-ray powder diffraction data: 10.67 s (2,2), 8.64 m, 7.37 m, 5.37 m, 5.10 vs (1), 4.88 w, 4.34 m, 4.15 m, 3.80 s (2,2), 3.46 s (2,2), 3.35 w, and 3.19 s (3).

Anal. Calc. for $C_{27}H_{25}NO_6S_2$: C, 61.95; H, 4.78; N, 2.68; S, 12.24. Found: C, 61.79; H, 4.83; N, 2.70; S, 12.24.

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REACTION OF GLYCOSYL CHLORIDES WITH SILVER TETRAFLUOROBORATE*,[1]

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ABSTRACT

A new method for the synthesis of glycosyl fluorides under very mild conditions in a homogeneous system is described. When glycosyl chlorides (**1–4**) are mixed with silver tetrafluoroborate in anhydrous ether, reaction occurs immediately and anomeric mixtures of glycosyl fluorides are obtained in good yield. Changing the solvent to toluene results in anomerization and the preponderant formation of α-D-glucosyl fluorides. An unexpected result was obtained in the reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride with silver tetrafluoroborate in ether. After a short period of reaction 2,3,4,6-tetra-*O*-acetyl-α- (48%) and -β-D-glucopyranosyl fluorides (33%) were obtained, but 1,2-*O*-acetoxonium-3,4,6-tri-*O*-acetyl-α-D-glucopyranose tetrafluoroborate could not be obtained. After a longer period, only 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride was obtained, in 81.5% yield. Similar results were obtained when 1,2-*O*-acetoxonium-3,4,6-tri-*O*-acetyl-α-D-glucopyranose tetrafluoroborate was treated with anhydrous ether.

INTRODUCTION

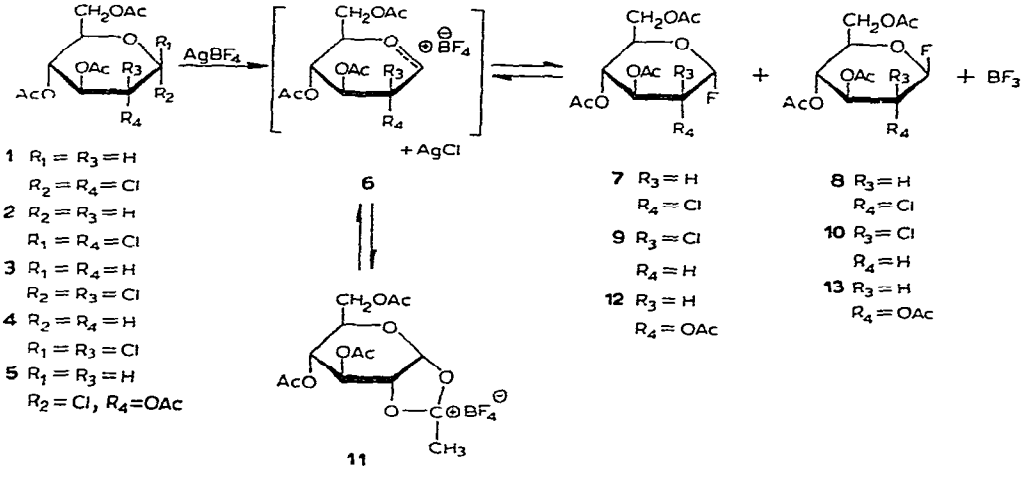
Recently, there has been a marked increase in interest in the synthesis of fluorinated carbohydrates[2–8] Brauns[9] first reported the preparation of acetylated α-D-glycosyl fluorides, by the action of anhydrous, liquid hydrogen fluoride (prepared from potassium hydrogen fluoride) on fully acetylated sugars. Micheel and co-workers[10] used the commercially available, anhydrous hydrogen fluoride. The less stable anomers of the acetylated glycosyl fluorides were prepared by treating 1,6-anhydro-β-D-glucopyranose triacetate with hydrogen fluoride[10] or[11] by the action of silver fluoride on the acetylated glycosyl bromides or chlorides having the more stable configuration at C-1. Kent and co-workers[5] have reported the addition of halogen fluorides (XF, prepared *in situ* from the *N*-halosuccinimide and hydrogen fluoride) to glycals, giving 2-deoxy-2-haloglycopyranosyl fluorides. Hall and Manville[7] have also reported the addition of XF (prepared from halogens and silver fluoride) to D-glucal triacetate. In the course of other studies we have found a new

*Dedicated to the memory of Professor M. L. Wolfrom.

method for the synthesis of glycosyl fluorides under very mild, homogeneous conditions.

RESULTS AND DISCUSSION

First, we chose the anomers of 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy-D-gluco- and -D-mannopyranosyl chlorides as starting materials having a weak participating group at C-2, since the four isomers have been isolated in crystalline form[12] and three of the four glycosyl fluorides anticipated are already known[7]. When the glycosyl chlorides (**1**–**4**) were mixed with a solution of silver tetrafluoroborate in anhydrous ether at 0° with stirring, silver chloride was immediately precipitated. The reaction was complete within 10 min and anomeric mixtures of the glycosyl fluorides were obtained in good yield. The reaction was studied under various conditions and the results of analyses by gas–liquid chromatography (g.l.c.) are summarized in Table I.



Under the conditions used, no anomerization of either the starting glycosyl chlorides or the products was observable, and the α- and β-D-glycopyranosyl fluorides obtained correspond to the products of kinetic control. Furthermore, chlorine at C-2 did not react with silver tetrafluoroborate at all. As shown in Table I, the fact that the proportion of the mannosyl fluorides obtained is almost constant regardless of the configuration of the starting chlorides indicates that the reaction proceeds *via* a common intermediate such as **6**. The same situation substantially applies to the glucose series.

When anhydrous toluene was used as the solvent instead of ether, equilibrated mixtures of the anomers were obtained in good yields (see Experimental).

These results appear reasonable. It is well known that boron trifluoride forms a very stable complex with ether but not with toluene; it also has a tendency to

TABLE I

G.L.C. ANALYSIS[a] OF GLYCOSYL FLUORIDES (7–10) OBTAINED IN THE REACTION OF GLYCOSYL CHLORIDES (1–4) WITH SILVER TETRAFLUOROBORATE

Compound	*Method of*[b] *mixing*	*Proportion of glycosyl fluoride*		*Total yield* (%)
		7 α-D-Glucosyl	**8** β-D-Glucosyl	
1	*A*	67.9	32.1	99.4
	B	67.7	32.3	98.8
	C	60.8	39.2	94.2
	D	60.3	39.7	92.8
	E	55.6	44.4	94.8
2	*A*	75.3	24.7	99.2
	B	74.2	25.8	100
	C	69.7	30.3	95.3
	D	68.4	31.6	96.5
	E	68.7	31.3	98.8
		9 α-D-Mannosyl	**10** β-D-Mannosyl	
3	*A*	82.1	17.9	100
	B	84.4	15.6	97.6
	C	86.9	13.1	92.7
	D	89.0	11.0	94.9
	E	88.0	12.0	100
4	*A*	75.0	25.0	100
	B	84.6	15.4	95.9
	C	86.3	13.7	98.4
	D	90.5	9.5	94.7
	E	87.2	12.8	100

[a]For the details, see Experimental. [b]All reactions were carried out at 0° in thermostatted bath with stirring. *Method A:* 100 mg of glycosyl chloride was added all at once to a solution of 68 mg of silver tetrafluoroborate in 2.3 ml of anhydrous ether. *Method B:* to a solution of 100 mg of the glycosyl chloride in 3.17 ml of anhydrous ether cooled to 0° was added all at once a solution of 68 mg of silver tetrafluoroborate in 0.33 ml of anhydrous ether cooled to 0°. *Method C:* same as *method B* except that the concentration of the reagents is decreased to one-tenth. *Method D:* a solution of 50 mg of the glycosyl chloride in 9.7 ml of anhydrous ether was added dropwise during 10 min to a solution of 34 mg of silver tetrafluoroborate in 7.8 ml of anhydrous ether. *Method E:* a solution of 34 mg of silver tetrafluoroborate in 7.8 ml of anhydrous ether was added dropwise during 10 min to a solution of 50 mg of glycosyl chloride in 9.7 ml of anhydrous ether.

coordinate with active fluorine of an organic compound to yield the tetrafluoroborate ion (BF_4^-) in which each fluorine atom is equivalent. In ether, the reverse reaction from glycopyranosyl fluorides and boron trifluoride etherate, to give the glycopryanosyl tetrafluoroborate ion-pair, is prevented owing to the stability of the boron trifluoride etherate. However, in toluene the reverse reaction can easily take place.

An unexpected result was obtained in the reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride with silver tetrafluoroborate. In ether, the reaction was complete in 5 min at 0° and 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranosyl fluorides were obtained in 48 and 33% yields, but 1,2-*O*-acetoxonium-3,4,6-tri-*O*-

acetyl-α-D-glucopyranose tetrafluoroborate[13] could not be obtained. When the reaction mixture was kept for 15 h at room temperature, it was shown by g.l.c. that the product was an equilibrated mixture of the anomers, and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride was isolated in 81.5% yield. The preponderant formation of the α-D-glucosyl fluoride can be explained by considering an equilibrium between the glycosyl fluorides and boron trifluoride, the 1,2-*O*-acetoxonium tetrafluoroborate (**11**), and the glycosyl tetrafluoroborate (**6**), which would favor the more stable α-D-glucosyl fluoride since boron trifluoride is stabilized as the etherate. In other words, it is shown that, even in the presence of a large excess of ether, boron trifluoride etherate can be equilibrated with the tetrafluoroborate ion. The boron trifluoride abstracts fluorine from an organic compound having an active fluorine substituent, provided that the compound has an acetoxy group (a good neighboring group for participation) *trans*-disposed to a vicinal fluorine atom. It has also been reported that methyl tetra-*O*-acetyl-β-D-glucopyranoside is easily anomerized[14] with titanium tetrachloride, whereas anomerization of methyl 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy-β-D-glucopyranoside is very difficult[15].

The D-glucosyl fluorides were also obtained in similar yields when 1,2-*O*-acetoxonium-3,4,6-tri-*O*-acetyl-α-D-glucopyranose tetrafluoroborate was treated with anhydrous ether at 0°.

A surprising result was obtained when a mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride and silver tetrafluoroborate in 1,2-dichloroethane was stirred for one h at 0°. In this reaction an anomeric mixture of D-glucopyranose pentaacetates (9.4%) was obtained, together with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride (29.5%) and a partially hydrolyzed compound (about 48%). The yield of the pentaacetate was increased to 17% when the reaction was performed at room temperature. The formation of the pentaacetate cannot yet be interpreted fully. Acetylation of the partially hydrolyzed compound gave mainly α-D-glucopyranose pentaacetate. Isolation by preparative t.l.c. gave 2,3,4,6-tetra-*O*-acetyl-D-glucose, which was assumed to form by hydrolysis of the 1,2-*O*-acetoxonium tetrafluoroborate. Although Paulsen[13] has isolated 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose by this hydrolysis, the difference may be attributed to the difference of the reagent for the hydrolysis.

When the reaction was performed for 24 h at −15° the tetra-*O*-acetyl-D-glucose portion was the major product together with small proportions of tetra-*O*-acetyl-α-D-glucopyranosyl fluoride and a penta-*O*-acetyl-D-glucose. This result can reasonably be interpreted by considering that in 1,2-dichloroethane the equilibrium mentioned above is inclined to the 1,2-*O*-acetoxonium tetrafluoroborate ion, since boron trifluoride does not form a complex with 1,2-dichloroethane.

EXPERIMENTAL

General. — Melting points were measured with a Monoscope (H. Boch, Frankfurt am Main, Germany) and are uncorrected. N.m.r. spectra were measured in chloroform-*d* with a Varian A-60 spectrometer. Tetramethylsilane was used as an

internal reference. Specific rotations were measured in chloroform with a Perkin–Elmer Model 141 polarimeter. Solvents were evaporated under diminished pressure below 35° with a rotary evaporator. Preparative t.l.c. were performed by using Silica Gel G (E. Merck AG, Darmstadt, Germany) with 1:1 benzene–ether as the developer. Zones were detected, after spraying with 0.01% morin solution in methanol, in u.v. light as bright yellow bands. The zones were collected, and extracted with dichloromethane containing a small proportion of methanol. Silver tetrafluoroborate was prepared by Olah's method[16] and purified by Meerwein's method[17].

3,4,6-Tri-O-acetyl-2-chloro-2-deoxy-α- (**7**) *and -β-D-glucopyranosyl fluorides* (**8**). — *A. In ether.* To a solution of 136 mg of silver tetrafluoroborate in 4.6 ml of anhydrous ether cooled to 0° was added at once 200 mg of **1** with stirring. After stirring for 15 min the mixture was poured into ice-cold, 10% sodium hydrogen carbonate solution. The organic layer was separated and the water layer was extracted with dichloromethane. The combined ether and dichloromethane extracts were washed with water, dried with sodium sulfate, and evaporated. The product was fractionated by preparative t.l.c. From the faster-moving zone (R_F 0.68) 115 mg (60.5%) of **7**, m.p. 98.5–99.5°, $[\alpha]_D^{21}+152.3\pm1.8°$ (*c* 1.064) [lit.[7] m.p. 95–97°, $[\alpha]_D^{25}+203°$ (*c* 1.13, chloroform)], was obtained as colorless leaflets after recrystallization from ether and petroleum ether (b.p. 30–50°). From the slower moving zone (R_F 0.63) 33.7 mg (17.7%) of **8**, m.p. 79.5–80.5°, $[\alpha]_D^{21}+92.3\pm1.3°$ (*c* 0.997) [lit.[7] m.p. 77–78°, $[\alpha]_D^{25}+124°$ (*c* 1.13, chloroform)], was similarly obtained as colorless leaflets. Both compounds showed only one peak on g.l.c. and were proved to be identical with authentic samples prepared by Hall's method[7]. When 200 mg of 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy-β-D-glucopyranosyl chloride (**2**) was treated as above, **7** and **8** were obtained in 62 and 15% yields, respectively.

B. In toluene. To a solution of 136 mg of silver tetrafluoroborate in 7.4 ml of anhydrous toluene was added 200 mg of **1** at 0° with stirring*. After one h, the mixture was treated as above. Compounds **7** and **8** were obtained in 76.3 and 1% yields, respectively. In a similar manner, **7** and **8** were obtained in 77.2 and 1% yields from **2**. Quantitative analysis by g.l.c. (see below) showed that in both cases **7** and **8** were obtained in a ratio of 95.5:4.5.

3,4,6-Tri-O-acetyl-2-chloro-2-deoxy-α- (**9**) *and -β-D-mannopyranosyl fluorides* (**10**). — *A. In ether.* When 3,4,6-tri-O-acetyl-2-chloro-2-deoxy-α- (**3**) or -β-D-mannopyranosyl chloride (**4**) was treated as in the case of **1**, **9** (R_F 0.67), m.p. 122.5–123.5°, $[\alpha]_D^{21}-17.3\pm0.6°$ (*c* 1.021), [lit.[7] m.p. 120–121°, $[\alpha]_D^{25}-23.4°$ (*c* 0.96)]; and **10** (R_F 0.49), m.p. 88.5–89.5°, $[\alpha]_D^{21}-44.8\pm0.8°$ (*c* 1.038), were obtained in 70 and 18% yield from **3**, and 72.8 and 19.8% yields from **4**, respectively.

Anal. Calc. for $C_{12}H_{16}ClFO_7$ (**10**): C, 44.11; H, 5.28; Cl, 10.85; F. 5.82. Found: C, 44.40; H, 5.11; Cl, 11.10; F, 5.69.

Compound **9** proved to be identical with an authentic sample[7].

*Although in a preliminary report it was reported that 2 moles of silver tetrafluoroborate was needed, it is found that 1.2 moles is sufficient.

B. In toluene. When **3** or **4** was treated as in the case of **2**, compounds **9** and **10** were obtained in 91 and 1% yields in both cases. Quantitative analysis by g.l.c. showed that **9** and **10** were obtained in a ratio of 98.9:1.1.

Quantitative analysis by g.l.c. of **7**, **8**, **9**, *and* **10**. — G.l.c. analysis was performed with a Yanagimoto Gas Chromatograph GCG-550F (Osaka, Japan) equipped with a flame-ionization detector by using a 75 cm × 3 mm inside diameter stainless-steel column packed with 1.5% XE-60 on Gaschrom Q (80/100 mesh) under the following conditions: column temperature, 133°; injection temperature, 155°; nitrogen as a carrier gas, 0.9 kg.cm^{-2}, 89 ml.min^{-1}; hydrogen, 40 ml.min^{-1}. 3,4,6-Tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hexitol (dihydro-D-glucal triacetate) was used as the internal standard. Retention times in min were: **7**, 5.83; **8**, 7.5; **9**, 4.5; **10**, 13.3; the standard, 3.25. Areas were determined by the product of the peak height and width at half-height. To establish identity, comparisons were made both by retention times and by simultaneous injection of a pure compound with a compound to be tested to observe peak enhancement.

2,3,4,6-Tetra-O-acetyl-α- (**12**) *and -β-D-glucopyranosyl fluorides* (**13**). — To a solution of 140 mg of silver tetrafluoroborate in 7 ml of anhydrous ether was added at once 220 mg of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride (**5**) at 0° with stirring. After 15 min the mixture was treated as above in the preparation of **7** and **8**. The product (209 mg) was fractionated by preparative t.l.c. From the portion having R_F 0.52 101 mg (48%) of **12**, m.p. 108–110°, was obtained. Recrystallization from ether gave pure **12** as prisms, m.p. 111–112°, $[\alpha]_D^{26}$ +90.9±1.2° (*c* 1.106) (lit.[7] m.p. 108°, $[\alpha]_D^{26}$ +90.1°). From the portion having R_F 0.45, 71 mg (33%) of **13**, m.p. 81–84°, was obtained by crystallization from ether and petroleum ether. Recrystallization from ether gave pure **13** as prisms, m.p. 88–89°, $[\alpha]_D^{26}$ +19.8±0.5° (*c* 1.103) (lit.[10,11] m.p. 87–89°, $[\alpha]_D^{20}$ +20° (ref.10); m.p. 85-87°, $[\alpha]_D^{18}$ +19.48° (ref. 11).

When a suspension of 1,2-*O*-acetoxonium-3,4,6-tri-*O*-acetyl-α-D-glucopyranose tetrafluoroborate[13] in anhydrous ether was stirred at 0°, the compound dissolved gradually and after one h the mixture became clear. Treatment as above gave **12** and **13** in 49 and 24% yields, respectively.

When the reaction mixture from **5** and silver tetrafluoroborate was kept for 15 h at room temperature, compound **12** was obtained in 81.5% yield.

Reaction of **5** *with silver tetrafluoroborate in 1,2-dichloroethane.* — To a solution of 140 mg of silver tetrafluoroborate in anhydrous 1,2-dichloroethane was added 220 mg of **5** at 0° with stirring. After 1.5 h, the mixture was treated as before. From the portion having R_F 0.52, 62 mg (29.5%) of **12** (m.p. 111–112°) was obtained. From the portion having R_F 0.39, 22 mg (9.4%) of syrup was obtained. The syrup was proved to be a mixture of α- and β-D-glucose pentaacetates by comparison of i.r. and n.m.r. spectra, and by t.l.c. and g.l.c. From the portion having R_F 0.21 (100 mg), 10 mg of a crystalline compound, m.p. 116–119°, or sometimes m.p. 120–124°, $[\alpha]_D^{27}$ +8.4±0.5° (*c* 0.960) was obtained. This product was identical with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose prepared by Lemieux's method[18]. Lemieux and Morgan did not crystallize it but we did; m.p. 116–119° or m.p. 120–124°, $[\alpha]_D^{27}$ +6.8±0.4°

(*c* 1.080). Acetylation of the mother liquor gave mainly α- and β-D-glucopyranose pentaacetate (g.l.c.).

When the reaction was performed for 25 min at room temperature, penta-*O*-acetyl-α,β-D-glucopyranose was formed (yield 17%) from which penta-*O*-acetyl-α-D-glucopyranose, m.p. 114–116°, $[\alpha]_D^{26} +102.4 \pm 1.4°$ (*c* 1.002) was obtained. This product was identical with an authentic sample by comparative i.r. and n.m.r. spectra, t.l.c. and g.l.c. In this reaction **12** and tetra-*O*-acetyl-D-glucopyranose were obtained in 25.8 and 27% yield, respectively.

When the reaction mixture was kept for 24 h at −20°, t.l.c. of the product showed that main product was tetra-*O*-acetyl-D-glucopyranose together with a small proportion of **12** and penta-*O*-acetyl-D-glucose. Crystallization of the tetra-*O*-acetyl-D-glucose portion from ether and petroleum ether, and then from ether, gave 2,3,4,6-tetra-*O*-acetyl-D-glucose.

G.l.c. of **12**, **13**, *and penta-*O*-acetyl-α- and -β-D-glucopyranose.* — G.l.c. analyses of these compounds were performed with a 75 cm × 3 mm (inside diameter) stainless-steel column packed with 1.5% DEGS on Gaschrom Q (80/100 mesh) under the following conditions: column temperature, 180°; injection temperature, 210°; nitrogen as a carrier gas, 1.07 kg.cm^{-2}, 43.5 ml.min^{-1}; hydrogen, 30 ml.min^{-1}. Retention times in min were: **12**, 2.0; **13**, 2.9; α-D-glucose pentaacetate, 5.9; β-D-glucose pentaacetate, 7.5.

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STUDIES ON DEHYDRO-L-ASCORBIC ACID ARYLOSAZONES

PART IV. OLEFINIC MONOHYDRAZONES AND ANHYDRO BIS(HYDRAZONES)*

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ABSTRACT

Acetylation and benzoylation of dehydro-L-ascorbic acid phenylhydrazone result in the formation of olefinic derivatives; treatment of the bis(phenylhydrazone) mono-*p*-toluenesulfonate with sodium iodide affords an anhydro derivative having a diazine ring.

DISCUSSION

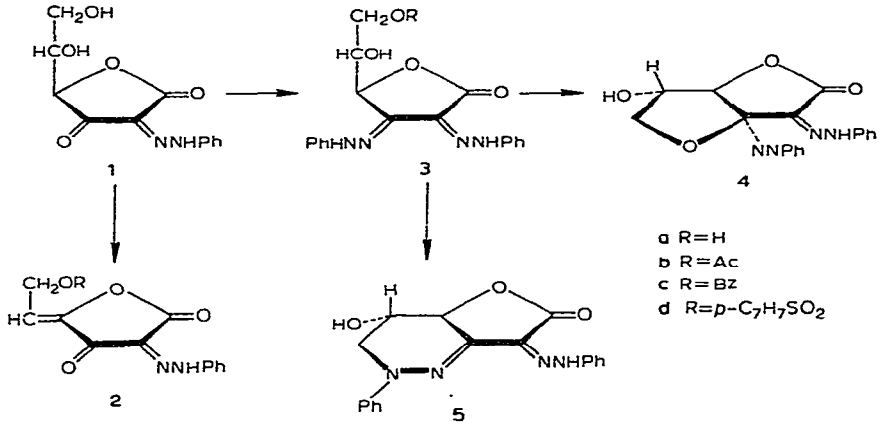
In continuation of our work[1-3], we have synthesized dehydro-L-ascorbic acid monophenylhydrazone[4] (**1**) from dehydro-L-ascorbic acid and 1-acetyl-2-phenylhydrazine. The n.m.r. spectrum of **1** in pyridine-d_5 showed the C-4 proton as a doublet at δ 5.5 p.p.m., split by the C-5 proton (J 2 Hz); the latter appeared as a triplet at δ 4.8 split by the C-6 protons (J 6 Hz); a minor coupling-constant of 2 Hz, caused by the C-4 proton, was observed in each branch of the well-defined triplet. The C-6 methylene protons appeared as a doublet at δ 4.2 (J 6 Hz), and the imino proton as a singlet at δ 9.2.

Acetylation or benzoylation of **1** caused simultaneous dehydration and formation of optically inactive olefinic compounds (**2b** and **2c**); a similar dehydration to azoethylenes is known to occur on acetylation of sugar hydrazones[5,6]. The n.m.r. spectrum of **2b** showed one acetyl group at δ 2.2, a doublet at δ 4.9 (J 6 Hz) attributed to the C-6 methylene group, and a triplet of one-proton intensity at δ 6.0 (J 6 Hz) assigned to the adjacent C-5 proton. The spectrum of benzoate **2c** was very similar; it showed a doublet at δ 5.2 (J 6 Hz) and a triplet at δ 6.2 (J 6 Hz). The imino protons appeared at δ 12.5 for **2b** and at δ 10.0 for **2c**. Benzoylhydrazine converted **2b** into a crystalline hydrazone, but phenylhydrazine gave an amorphous product from which **3a** could not be obtained by deacetylation.

The n.m.r. data given indicate clearly that dehydro-L-ascorbic acid monophenylhydrazone (**1**) and its olefinic acetate (**2b**) and benzoate (**2c**) exist in the 1,4-lactone form. As compound **1** can be converted quantitatively into the bis(phenylhydrazone) without evidence of ring opening (such as the formation of azo-

*Dedicated to the memory of Professor M. L. Wolfrom.

pyrazolones[2]), it must be concluded that dehydro-L-ascorbic acid bis(phenylhydrazone) and its dehydro derivative[3] exist also in the 1,4-lactone form (structures **3** and **4**), not in the 1,5-lactone form suggested earlier.



p-Toluenesulfonylation of **3a** yielded the mono- (**3d**) and di-*p*-toluenesulfonates, depending on the reaction time. The primary sulfonyloxy group of **3d** reacted with sodium iodide in acetone yielding, by nitrogen participation, the anhydro derivative **5a**. Compound **5a** showed one imino proton at δ 11.0 instead of two for the parent osazone[1] (**3a**). The methylene protons resonated at δ 3.6 and δ 4.2, the C-5 proton at δ 4.4 (multiplet), and the C-4 proton at δ 5.0 (doublet, J 5 Hz). Acetylation, benzoylation, and *p*-toluenesulfonylation of **5a** afforded **5b**, **5c**, and **5d**, respectvely, and treatment with alkali converted **5a** into a hydrate which, on acetylation, gave **5b**.

Treatment of dehydro-L-ascorbic acid bis(phenylhydrazone) di-*p*-toluenesulfonate with sodium iodide afforded the 6-iodo-5-*O*-*p*-tolylsulfonyl derivative; no anhydro or olefinic derivative was detected.

EXPERIMENTAL

General. — I.r. and u.v. spectra were recorded with Unicam SP-200 and SP-800 spectrophotometers. The n.m.r. spectra were measured in pyridine-d_5 on Varian A-60 and T-60 spectrometers.

Dehydro-L-ascorbic acid mono(phenylhydrazone) (**1**). — A solution of dehydro-L-ascorbic acid, obtained by oxidizing a solution of L-ascorbic acid (10 g) with an excess of iodine in ethanol, was heated for 30 min on a boiling-water bath with 1-acetyl-2-phenylhydrazine and a few drops of acetic acid. After one week at room temperature, the monohydrazone was filtered off, and recrystallized from ethanol; yellow needles, m.p. 167–170°; λ_{max}^{EtOH} 234, 246 (sh), 254, and 376 nm (log ε 4.05, 3.98, 3.94, and 4.34), λ_{min}^{EtOH} 252 and 292 nm (log ε 3.93 and 3.25); ν_{max}^{KBr} 1675 (CO), 1755 (COO), and 3400 (OH) cm^{-1}.

Anal. Calc. for $C_{12}H_{12}N_2O_5$: C, 54.5; H, 4.6; N, 10.6. Found: C, 54.4; H,4.6; N, 10.5.

Heating of **1** with an equivalent of phenylhydrazine in ethanol for 5 min gave the bis(phenylhydrazone) **3a** in quantitative yield; m.p. and mixed m.p. 223°.

Olefinic mono(phenylhydrazone) acetate (**2b**). — A solution of dehydro-L-ascorbic acid mono(phenylhydrazone) (**1**; 0.5 g) in pyridine (10 ml) was treated with acetic anhydride (7 ml), and the solution was kept overnight at room temperature. The mixture was poured onto crushed ice, and the product was filtered off, washed with water, and recrystallized from chloroform–ethanol; yellow needles, m.p. 158–161°; λ_{max}^{EtOH} 223 (sh), 239, 356 (sh), 275 (sh), and 374 nm (log ε 4.12, 4.14, 3.92, 3.84, and 4.30), λ_{min}^{EtOH} 312 nm (log ε 3.83); ν_{max}^{KBr} 1670 (CO), 1735 (OAc), and 1780 (COO) cm^{-1}.

Anal. Calc. for $C_{14}H_{12}N_2O_5$: C, 58.3; H, 4.2; N, 9.7. Found: C, 58.3; H, 4.1; N, 10.0.

Benzoylhydrazone of **2b**. — Heating an ethanol solution of **2b** with an equivalent of benzoylhydrazine and a few drops of acetic acid afforded the benzoylhydrazone, m.p. 203–204°.

Anal. Calc. for $C_{21}H_{18}N_4O_5 \cdot H_2O$: C, 59.5; H, 4.7; N, 13.2. Found: C, 59.8; H, 4.9; N, 13.2.

Olefinic mono(phenylhydrazone) benzoate (**2c**). — A solution of **1** (0.5 g) in pyridine (10 ml) was treated with benzoyl chloride (2 ml) for 24 h at room temperature, and then poured onto crushed ice. Compound **2c** crystallized from chloroform–ethanol in yellow needles, m.p. 156–159°; λ_{max}^{EtOH} 233, 275 (sh), 282 (sh), and 374 nm (log ε 4.42, 3.96, 3.93, and 4.31), λ_{min}^{EtOH} 300 nm (log ε 3.89); ν_{max}^{KBr} 1640 (CO), 1708 (OBz), and 1780 (COO) cm^{-1}.

Anal. Calc. for $C_{19}H_{14}N_2O_5$: C, 65.1; H, 4.0; N, 8.0. Found: C, 65.3; H, 3.9; N, 8.3.

Dehydro-L-ascorbic acid bis(phenylhydrazone) mono-p-toluenesulfonate (**3d**). — Dehydro-L-ascorbic acid bis(phenylhydrazone) (1 g) in pyridine (20 ml) was treated with *p*-toluenesulfonyl chloride (1 g), and the mixture was kept overnight at room temperature. The mixture was poured onto crushed ice, and the product (0.9 g) was filtered off, washed, and recrystallized from chloroform–ethanol; orange-red needles, m.p. 190–192°; λ_{max}^{EtOH} 206, 228, 262, 282 (sh), 353, and 445 nm (log ε 4.18, 4.23, 4.2, 4.13, 3.88, and 4.19), λ_{min}^{EtOH} 214, 242, 321, and 387 nm (log ε 4.09, 3.99, 3.61, and 3.71); ν_{max}^{KBr} 1600 (CN), 1740 (COO), and 3500 (OH) cm^{-1}.

Anal. Calc. for $C_{25}H_{24}N_4O_6S$: C, 59.0; H, 4.8; N, 11.0. Found: C, 59.4; H, 4.9; N, 10.5.

Dehydro-L-ascorbic acid bis(phenylhydrazone) di-p-toluenesulfonate. — Dehydro-L-ascorbic acid bis(phenylhydrazone), or **3d** (0.5 g) in pyridine (20 ml) was treated with *p*-toluenesulfonyl chloride (1 g). The mixture was kept for two days at room temperature, and poured onto crushed ice. The product was recrystallized from chloroform–ethanol; red needles, m.p. 175–177°; λ_{max}^{EtOH} 207, 228, 262, 285 (sh), 356, and 453 nm (log ε 4.00, 4.15, 3.91, 3.75, 3.58, and 3.86), λ_{min}^{EtOH} 213, 243, 324, and 390 nm (log ε 3.96, 3.77, 3.33, and 3.43); ν_{max}^{KBr} 1600 (CN) and 1725 (COO) cm^{-1}.

Anal. Calc. for $C_{32}H_{30}N_4O_8S_2$: C, 58.0; H, 4.6; N, 8.5. Found: C, 57.7; H, 4.4; N, 9.0.

Anhydride of dehydro-L-ascorbic acid bis(phenylhydrazone) (**5a**). — A solution of ester **3d** (0.5 g) and sodium iodide (0.6 g) in dry acetone was heated for 2 h in a sealed tube at 100°. After the precipitated sodium *p*-toluenesulfonate had been filtered off, the acetone solution was concentrated, and then diluted with water. The anhydro derivative crystallized from chloroform–ethanol in orange needles; m.p. 257–259°; λ_{max}^{EtOH} 206, 262, 285, 353, and 450 nm (log ε 4.17, 4.23, 4.18, 3.96, and 4.34), λ_{min}^{EtOH} 221, 273, 320, and 390 nm (log ε 3.87, 4.14, 3.56, and 3.76); ν_{max}^{KBr} 1600 (CN), 1740, 1755 (COO), and 3400 (OH) cm^{-1}.

Anal. Calc. for $C_{18}H_{16}N_4O_3$: C, 64.3; H, 4.8; N, 16.7. Found: C, 64.0; H, 4.6; N, 17.0.

A suspension of the anhydro derivative **5a** (0.1 g) in 2M sodium hydroxide (10 ml) was heated for a few minutes at 70–80° until complete dissolution occurred. On acidification with acetic acid, the hydrate separated; it was recrystallized from ethanol–water in orange needles, m.p. 170–173°.

Anal. Calc. for $C_{18}H_{16}N_4O_3 \cdot H_2O$: C, 61.0; H, 5.1; N, 15.8. Found: C, 61.1; H, 5.0; N, 16.2.

Acetate **5b**. — *Method A*. The anhydro bis(phenylhydrazone) **5a** or its hydrate was acetylated with acetic anhydride in pyridine. The acetate had m.p. 289–291°; λ_{max}^{EtOH} 205, 260, 285, 356, and 450 nm (log ε 4.02, 4.18, 4.11, 3.88, and 4.28), λ_{min}^{EtOH} 221, 272, 318, and 386 nm (log ε 3.85, 4.08, 3.51, and 3.77); ν_{max}^{KBr} 1590 (CN), 1740 (OAc), and 1770 (COO) cm^{-1}.

Anal. Calc. for $C_{20}H_{18}N_4O_4$: C, 63.5; H, 4.8; N, 14.8. Found: C, 63.2; H, 5.1; N, 15.2.

Method B. The same product was obtained by refluxing a solution of ester **3d** (0.2 g) and sodium iodide (0.5 g) in acetic anhydride (10 ml) for 30 min, and pouring it onto crushed ice.

Benzoate **5c**. — Benzoylation of anhydro derivative **5a** with benzoyl chloride in pyridine, in the usual way, afforded yellow needles from chloroform–ethanol; m.p. 254–255°; λ_{max}^{EtOH} 206, 234, 259, 276 (sh), 283, and 446 nm (log ε 3.92, 4.09, 4.01, 3.93, 3.94, 3.69, and 4.11), λ_{min}^{EtOH} 214, 247, 272, 319, and 384 nm (log ε 3.78, 3.96, 3.71, 3.26, and 3.56); ν_{max}^{KBr} 1600 (CN), 1720 (OBz), and 1765 (COO) cm^{-1}.

Anal. Calc. for $C_{25}H_{20}N_4O_4$: C, 68.2; H, 4.6; N, 12.7. Found: C, 68.2; H, 5.0; N, 12.4.

p-*Toluenesulfonate* **5d**. — The anhydro derivative **5a** (0.1 g) in pyridine (5 ml) was treated with *p*-toluenesulfonyl chloride (0.2 g). The mixture was kept for five days at room temperature, and then poured onto crushed ice. The product was recrystallized from chloroform–ethanol; m.p. 225–228°; λ_{max}^{EtOH} 207, 228, 259, 275 (sh), 283, 354, and 446 nm (log ε 4.12, 4.25, 4.19, 4.10, 4.11, 3.89, and 4.27), λ_{min}^{EtOH} 212, 242, 271, 318, and 384 nm (log ε 4.08, 4.06, 4.09, 3.49, and 3.75); ν_{max}^{KBr} 1590 (CN) and 1775 (COO) cm^{-1}.

Anal. Calc. for $C_{25}H_{22}N_4O_5S$; C, 61.2; H, 4.5; N, 11.4. Found: C, 61.2; H, 4.3; N, 11.8.

*6-Deoxy-6-iodo-5-*O-p-*tolylsulfonyl derivative of dehydro-L-ascorbic acid bis(phenylhydrazone)*. — A solution of dehydro-L-ascorbic acid bis(phenylhydrazone)

di-*p*-toluenesulfonate (1 g) and sodium iodide (1 g) in dry acetone (20 ml) was heated in a sealed tube for 2 h at 100°. The product crystallized from chloroform–ethanol in orange needles; m.p. 193–195°; λ_{max}^{EtOH} 221, 290, and 370 nm (log ε 4.46, 4.51, and 3.95), λ_{min}^{EtOH} 255 and 347 nm (log ε 4.20 and 3.93); ν_{max}^{KBr} 1620 (CN) and 1725 (COO) cm^{-1}.

Anal. Calc. for $C_{25}H_{23}IN_4O_5S$: C, 48.6; H, 3.8; N, 9.1. Found: C, 48.4; H, 3.4; N, 9.4.

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MONOSACCHARIDES AS *O*-GLYCOSYL LEAVING GROUPS FROM 3-HYDROXY AMINO ACIDS DURING BASE-CATALYZED ELIMINATION*,[1]

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ABSTRACT

The synthesis and characterization of 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-L-serine and -threonine methyl esters are described. The kinetics of base-catalyzed elimination were determined to be pseudo-first-order under various conditions of reaction for these glycosides as well as for the analogous 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-L-serine and -threonine methyl esters. The marked base sensitivity of 3-*O*-glycosides of substituted amino acids is emphasized. These data are useful in structural studies on such glycosidic linkages in naturally occurring glycopeptides.

INTRODUCTION

Standard methodology for determining the presence of *O*-glycosidic linkages to 3-hydroxy amino acids[2,3] in glycopeptides involves use of base-catalyzed elimination[4] of the glycosyl residue (mono- or oligo-saccharide) followed by reduction of the double bond concomitantly introduced into the amino acid portion[5–8]. The susceptibility of glycosidic linkages at the 3-position of serine and threonine to elimination reactions was first pointed out by Anderson and Meyer[9] and also by Tanaka and Pigman[10]. The base lability of this particular glycosidic linkage has been tested on appropriate model compounds by several investigators[2–3,11–17]. The elimination of various leaving groups from substituted 3-hydroxy acids was studied in great detail by Linstead[18] and has been reviewed by Cram[19], Banthorpe[20], and McLennon[4].

The evidence presented by Koshland[21], Bunnett[22], or Photaki[23,24] for ease of elimination reactions with substituted 3-hydroxy-2-amino acids does not delineate clearly either the E1cB mechanism[4] or the concerted E2 reaction[20]. However, the ease of racemization of other substituted 2-amino acids does reflect the acidity of the 2-proton[25–27]. This is particularly true when a third, strong, negative inductive-effect is introduced at the 2-proton by substituents on the amino acid chain in addition to

*Dedicated to the memory of Professor M. L. WOLFROM.

the contributions from the substituted 2-amino and carboxyl groups. Photaki[23,24] and Riley[28] found that the nature of the group eliminated from substituted 3-hydroxy amino acids was less a rate-determining factor than solvent or base type.

The present work does not differentiate specifically the above two mechanisms of elimination from *O*-glycosyl derivatives of hydroxy amino acids. Kinetic data are presented for the elimination of variously substituted monosaccharides in glycosidic linkage to L-serine and threonine. These rate data might be useful in future structural work on glycoproteins. The benzyl and acetyl substituents were chosen for these monosaccharide leaving groups as representative of widely differing group polarities which should mirror various solvation effects and, consequently, susceptibility to base-catalyzed reaction. In addition, the perbenzylated D-glucopyranosyl glycosides synthesized for this study are α-D-linked to L-serine (**1**) and L-threonine (**2**), respectively, whereas the peracetylated 2-amino-2-deoxy-D glucopyranosyl glycosides to L-serine[2] (**3**) and L-threonine[3] (**4**) possess the β-D configuration. The *N*-dinitrophenyl blocking group on the 3-hydroxy amino acid esters was found useful as a chromophore in the spectrophotometric monitoring of the reaction. No previous reports of actual kinetic rate-constants for this elimination reaction are known to the authors. The sensitivity to u.v. absorption exhibited by the 2,4-dinitrophenyl group permitted determination of very small changes in substrate concentration.

DISCUSSION

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-L-serine methyl ester (**1**) and its L-threonine analog (**2**) were synthesized by established methods[29–31]. Although an anomeric mixture is expected from the glycosidation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride, the preponderant products in the syntheses of **1** and **2** were α-D-linked, as shown by n.m.r. spectroscopy and optical rotatory power. Comparison of these results is shown in Table I for glycosides **1**–**5** and their respective aglycons. Application of Hudson's rules of isorotation[32–33] as well as the Karplus spin-coupling relationship[33] suggests the configurations assigned. Since the present syntheses were completed, an improved route to 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride has been published[34].

The lesser products of the glycosidation (see Experimental section) are thought to be either the product of racemization in the amino acid moiety of a single anomer, or the other possible anomer. In any case, although the α-D-linked glycoside preponderated in each reaction, a substantial proportion of a second characterizable glycoside was present in both mixtures. The anomeric distribution during glycosidation of tetra-*O*-benzyl-D-glucosyl chloride with suitably blocked hydroxy amino acids is under detailed study[35]. A syrupy product (in 6% yield) from the glycosidation of *N*-(2,4-dinitrophenyl)-L-threonine methyl ester was identified as 3-*O*-benzyl-*N*-(2,4-dinitrophenyl)-L-threonine methyl ester. By way of explanation, the cleavage of *O*-benzyl groups during the formation of 2,3,4,6-tetra-*O*-benzyl-D-glucosyl chloride[30] could have led to benzyl chloride in the presence of thionyl chloride, which in turn

TABLE I

N.M.R. AND OPTICAL ROTATORY DATA FOR α-D AND β-D LINKED 3-*O*-GLYCOSIDES OF *N*-(2,4-DINITROPHENYL)SERINE AND THREONINE

Compound	*Name*	$[\alpha]_D^{25}$	*Chemical shift H-1'* (τ)	$J_{1',2'}$ (*Hz*)
1	3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-gluco-pyranosyl)-*N*-(2,4-dinitrophenyl)-L-serine methyl ester	+27°	5.13	3
2	3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-L-threonine methyl ester	+24°	4.95	3.5
3	3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-L-serine methyl ester[2]	−37°	5.11	9
4	3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-DL-threonine methyl ester[3]	+4°	5.06	8.5
5	4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-hydroxyproline methyl ester[37]	−400°	5.20	9
	N-(2,4-dinitrophenyl)-L-serine methyl ester[2]	−26°		
	N-(2,4-dinitrophenyl)-L-threonine methyl ester	−54°		
	N-(2,4-dinitrophenyl)-DL-threonine methyl ester[3]	0°		

might have benzylated some of the L-threonine derivative present. The use of the purer glycosyl chloride mentioned above[35] could preclude such complications.

Stability to base and base-catalyzed elimination data are summarized in Tables II–V. The isolation of 2-(2,4-dinitroanilino)acrylic acid methyl ester and the crotonic acid analog from these elimination reactions followed procedures previously developed[36]. The considerably lower rate of formation of 2-methoxycarbonyl-6-nitrobenzimidazole-1-oxide from the 2-(2,4-dinitroanilino)acrylate and crotonate[36] caused no interference with pseudo-first-order plots for the elimination reactions. Other than this latter side-reaction, no rearrangement or hydrolysis of the unsaturated amino acids was detectable, nor was the carbohydrate portion degraded under the conditions employed.

In general, the rate constants given in Table II are illustrative of behavior documented for other such elimination reactions[18–20]. As shown by Photaki[23,24], for *beta*-eliminations from cystine, the nature of the leaving group is of less importance than the nature of the solvent and base, as well as the type of substitution on the nitrogen atom and carboxyl group of the amino acid. Rough calculations from Derevitskaya's work[14] on the elimination of monosaccharide from serine indicate that the rate constants would be comparable with those in Table II, assuming a linear disappearance of the glycoside. The kinetic data presented here are reproducible within a few percent error, and computerized least-squares treatment of the data gave reproducible, linear, first-order plots.

TABLE II

KINETIC RATE CONSTANTS FOR FOUR 3-*O*-GLYCOSYL AMINO ACIDS UNDER VARIOUS CONDITIONS OF β-ELIMINATION[a]

Compound[b]	*Temperature*	*Solvent*	*Base*[a] *(mmoles/l)*	k (h^{-1})	$t_{1/2}$
1	25°	Methanol	1.2×10^{-2}	3.3	0.2
2	25°	Methanol	1.2×10^{-2}	0.4	1.7
3	25°	Methanol	1.2×10^{-2}	0.3	2.0
4	25°	Methanol	1.2×10^{-2}	4.6	0.15
3	25°	Methanol	5×10^{-9}	0.009	75
3	50°	Methanol	5×10^{-9}	0.024	28.4
3	25°	Methanol	None	0.004	142
3	25°	Tetrahydro-furan	1.0×10^{-1}	0.35	1.97

[a]Concentration of substrate 2.4×10^{-1} mmoles/l. Pseudo-first-order rate plots for reactions as described in Experimental with sodium-carbonate catalysis. [b]See Table I for compounds corresponding to numbers.

There are no immediate explanations for the differences in rates shown in Table II observed for compounds **1–4** under identical reaction conditions. Solvation of the intermediate carbanion could possibly be one stabilization factor, if the ElcB mechanism of elimination[4] is assumed. A second possible factor in the change of rate from one monosaccharide type to another is the contribution attributable to solvation of the leaving group (benzylated *vs.* acetylated monosaccharide). However, no clear-cut distinction exists. Thus, data for the elimination reaction occurring in methanol alone, without base (Table II), apply only to the acetylated monosaccharides **3** and **4**. The reaction of **3** in methanol alone is undoubtedly an elimination reaction, because the resulting 2-(2,4-dinitroanilino)-acrylic and -crotonic acid methyl esters from the reaction were isolated and identified. Solvation could play a significant role in this differentiation of **3** and **4** from **1** and **2** in alcohol (Table II). Contrasted to this behavior of **3** is the inertness of **1** in methanol alone without catalyst, as illustrated in Table III-1. The order of magnitude of difference of rates between these leaving groups is not considered to be highly significant. Neither the extra methyl group on threonine nor the anomeric configuration of **1–4** appear critical in affecting the rate. In fact, from our own data[36] as well as those of others[18,23,24] there seems to be little difference in the rate of elimination of alkoxide or acylate anions from the amino acids as compared with these monosaccharide derivatives.

Tables III, IV, and V list properties of individual glycosides: polarity of solvent, base type, concentration of substrate, and temperature for the elimination reaction. Data of this type are important for assessing stability of the compounds under various conditions of elimination. Pseudo-first-order plots were not made for each of the samples listed, although the data are entirely reproducible. Data listed for sodium carbonate parallel the reactivity in sodium methoxide and sodium hydroxide. Among the bases used, for a given solvent, the order of reactivity was: anionic

TABLE III

DATA ON RELATIVE REACTIVITY FOR THE β-ELIMINATION OF 3-*O*-(2,3,4,6-TETRA-*O*-BENZYL-α-D-GLUCOPYRANOSYL)-*N*-(2,4-DINITROPHENYL)-L-SERINE METHYL ESTER (1) IN VARIOUS SOLVENTS AND WITH DIFFERENT BASES

Solvent	*Base*	*Concentration of base (mmoles/l)*	*Time*	*% Glycoside reacted*[a]
1 Methanol			3 days	0
2 Methanol	Na_2CO_3	0.1	5 sec	100
3 Methanol	Na_2CO_3	6×10^{-2}	20 min	75
4 Benzene			3 days	0
5 *N,N*-Dimethylformamide			1 day	0
6 *N,N*-Dimethylformamide	Et_3N	1.7×10^{3}	1 day	100
7 *N,N*-Dimethylformamide	Na_2CO_3	0.1	3 sec	100
8 Tetrahydrofuran			3 days	0
9 Tetrahydrofuran	Na_2CO_3	0.1	1 day	100
10 Tetrahydrofuran	Et_3N	1.7×10^{3}	1 day	48

[a]Concentration of substrate 2.4×10^{-1} mmoles/l with reaction at 25°.

base > amine > other Lewis bases (*i.e.*, polar solvents). Interestingly, among these solvent–base selections the base is totally unable to produce dissociation of the proton under certain conditions. Of peculiarly poor proton-abstracting power was the benzene–triethylamine pair (Table IV-3,6) and tetrahydrofuran–triethylamine (Table V-8). These data could reflect lack of formation of a carbanionic intermediate because of poor availability of base under the solvation conditions.

TABLE IV

DATA ON RELATIVE REACTIVITY FOR β-ELIMINATION OF 3-*O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-*N*-(2,4-DINITROPHENYL)-L-SERINE METHYL ESTER[2] (3) IN VARIOUS SOLVENTS WITH DIFFERENT BASES

Solvent	*Temperature*	*Base*	*Base*[a] *(mmoles/l)*	*Time*	*% Glycoside reacted*[a]
1 Benzene	25°			4 days	0
2 Benzene	50°			3 days	0
3 Benzene	25°	Et_3N	2.4×10^{-7}	2 days	0
4 Benzene	25°	Et_3N	2.4×10^{-4}	2 days	0
5 Benzene	25°	Et_3N	2.4×10^{-1}	2 days	0
6 Benzene	50°	Et_3N	2.4×10^{-1}	3 days	0
7 Tetrahydrofuran	25°			2 days	0
8 Tetrahydrofuran	50°			1 day	11

[a]Concentration of substrate 2.4×10^{-1} mmoles/l.

In Table II, compound 3 is used to illustrate the effect of temperature on the rate of elimination. By raising the reaction temperature from 25° to 50° the kinetic

7 (32 mg, 90%). Recrystallization of the product from ethanol gave pure **7**; m.p. 171–173° $[\alpha]_D^{25}$ −13.6° (*c* 0.65, chloroform); ν_{max}^{Nujol} 3500, 3450 (OH), and 1775 cm^{-1} (γ-lactone); c.d. $\Delta\varepsilon$ −0.759 (λ_{max} 225 nm, *c* 0.0045, methanol), $[\theta]_{225}^{30}$ −2507; n.m.r. [$CDCl_3$ and $(CD_3)_2CO$]: τ 3.95 (d, 1, $J_{1,2}$ 3.8 Hz, H-1), 5.03 (d, 1, $J_{2,3}$ 3.5 Hz, H-3), 5.53 (q, 1, H-2), 8.39, and 8.62 (2 s, 6, CH_3).

Anal. Calc. for $C_{10}H_{14}O_7$: C, 48.78; H, 5.73. Found: C, 48.45; H, 5.62.

3-O-Acetyl-4-C-carboxyl-1,2-O-isopropylidene-5-O-tosyl-α-D-gulofuranose 4',6-lactone (**6**). — Compound **5** (50 mg) was dissolved in anhydrous pyridine (1 ml) at 0°, *p*-toluenesulfonyl chloride (66 mg) was added, and the resulting solution was heated for two days at 50°. The cooled mixture was diluted with ice–water (5 ml) and extracted with dichloromethane (2 × 10 ml). The combined organic extracts were washed with saturated, aqueous sodium hydrogencarbonate (10 ml), dried, and evaporated to afford a syrup (80 mg). T.l.c. (developed with 4:1 benzene–ethyl acetate) of the product indicated the presence of some starting material. Chromatography of the mixture on t.l.c.-grade silica (23 × 1 cm) developed with the same solvent afforded the pure sulfonate **6** (37 mg) in 61% yield (based on starting material consumed). Recrystallization of the product from benzene gave an analytical sample; m.p. 160.5–161°, $[\alpha]_D^{26}$ −48.9° (*c* 0.62, chloroform); n.m.r. ($CDCl_3$): τ 2.05 (m, 2, H-2', H-6'), 2.6 (m, 2, H-3', H-5'), 3.93 (d, 1, $J_{1,2}$ 3.7 Hz, H-1), 4.38 (q, 1, $J_{5,6}$ 2 Hz, H-5), 4.82 (d, 1, $J_{2,3}$ 4.5 Hz, H-3), 4.99 (q, 1, H-2), 5.46 (m, 2, H-6, H-6'), 7.58 (s, 3, CH_3), 7.88 (s, 3, OAc), 8.4, and 8.67 (2 s, 6, CH_3).

Anal. Calc. for $C_{19}H_{22}O_{10}S$: C, 51.58; H, 5.01. Found: C, 51.43; H, 4.82.

4-C-Carboxyl-α-(and β)-D-gulofuranose 4',6-lactone (**8**). — Compound **7** (20 mg) was dissolved in 80% aqueous trifluoroacetic acid (0.5 ml) and the solution was stirred for 15 min at room temperature after which time toluene (2 × 2 ml) was evaporated from the solution to afford a mixture of α and β-anomers of **8** (12 mg, 75%); ν_{max}^{film} 1770 cm^{-1} (γ-lactone); n.m.r. [$(CD)_3CO$ and D_2O]: 4.70 (1, d, $J_{1\alpha,2}$ 4 Hz, H-1α) and 4.85 (1, d, $J_{1\beta,2}$ 1.5 Hz, H-1β).

Anal. Calc. for $C_7H_{10}O_7$: C, 40.78; H, 4.89. Found: C, 40.61; H, 4.93.

3-C-Carbamoyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**19**). — 3-*O*-Acetyl-3-*C*-carbamoyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**3**, 0.10 g) was deacetylated as described for the preparation of **4**, to afford compound **19** (80 mg, 90%); m.p. 156–157°, $[\alpha]_D^{26}$ −1.75° (*c* 0.6, chloroform); $\nu_{max}^{CHCl_3}$ 3575 (OH), 3450, 3300, and 1700 cm^{-1} ($CONH_2$); n.m.r. ($CDCl_3$): τ 3.05, 3.6 (2 broad s, NH_2), 4.0 (d, 1, $J_{1,2}$ 3.9 Hz, H-1), 5.34 (d, 1, H-2), 6.5 (s, 1, OH, exchanges in D_2O), 8.44, 8.60, 8.64, and 8.7 (4 s, 12, CH_3).

Anal. Calc. for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.50; H, 6.79; N, 4.60.

3-O-Acetyl-3-C-carbamoyl-1,2-O-isopropylidene-α-D-allofuranose (**20**). — Compound **3** (0.60 g) in methanol (50 ml) and 5% aqueous hydrochloric acid (1 ml) was kept for 10 h at room temperature. The solution was then neutralized with Amberlite IR-45 (OH^-) resin and evaporated to yield **20** (0.49 g, 93%) as a syrup; $[\alpha]_D^{26}$ +114.4° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 3500–3300 (OH), 3200, 1695 ($CONH_2$), and 1750 cm^{-1}

Chromagram Sheets (type K301R2, silica gel). Separations were made with 2:1 ethyl acetate–ethyl ether (Solvent *A*), 2:1 petroleum ether (b.p. 30–60°)–ethyl acetate (Solvent *B*), 3:1:1 butyl alcohol–acetic acid–water (Solvent *C*), 6:1 ethyl ether–ethyl acetate (Solvent *D*), 4:1 petroleum ether (b.p. 30–60°)–ethyl acetate (Solvent *E*), chloroform (Solvent *F*), 9:1 benzene–ethyl acetate (Solvent *G*), 1:1 ethyl ether–ethyl acetate (Solvent *H*), 3:1 petroleum ether (b.p. 30–60°)–ethyl acetate (Solvent *I*). Zones were located with sulfuric acid; with ninhydrin (0.2% ethanol); with silver nitrate–sodium hydroxide spray reagent[39]; with iodine vapors; with u.v. light; or visually for the colored derivatives. Optical rotations were measured in 1-dm and 2-dm tubes. Melting points were determined on a National Instruments Co. "Melt Meter". I.r. spectra were measured with a Perkin–Elmer Model 137 "Infracord" i.r. spectrophotometer. N.m.r. spectra were measured with a Varian A-60A spectrometer, with saturated solutions in chloroform-*d* and tetramethylsilane as internal reference. Samples were not degassed before use. U.v. spectra were recorded with a Bausch and Lomb Spectronic 600 spectrophotometer. Microanalyses were determined by Dr. G. Weiler and Dr. F. B. Strauss, Oxford, England. Microcrystalline cellulose is Avicel, a product of the American Viscose Division of the Food Machinery Corp., Marcus Hook, Pa.[40]. Least-squares analysis of kinetic data was performed with an Olivetti–Underwood Programma 101 computer.

3-O-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)-N-(2,4-dinitrophenyl)-L-serine methyl ester (**1**). — A modification of the procedure of Austin[30] was used for the glycosidation reaction. *N*-(2,4-dinitrophenyl)-L-serine methyl ester[2] (2.0 g, 7 mmoles), silver carbonate (2.5 g, 14.8 mmoles)[41], and Drierite (7.5 g, standard grade) were added to pure, dry benzene (50 ml) and the mixture was stirred overnight in the dark. Silver perchlorate (0.1 g, 4.8 mmoles) was added and a solution of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride[30,42] (2 g, 3.5 mmoles) in benzene (25 ml) was introduced during 4 h with stirring in the dark. The mixture was stirred until t.l.c. (Solvent *E*) revealed no further formation of product (36 h). The solution was filtered through a Celite pad, extracted three times with 100 ml of water, dried with sodium sulfate, and evaporated to dryness under diminished pressure. The combined syrups from five such glycosidations were taken up in 200 ml of ethyl ether and kept cold for 2 days to precipitate any further silver salts. The solution was again filtered through a Celite pad and evaporated. A portion of the resultant syrup (649 mg) was dissolved in benzene (100 ml). Preparative t.l.c. on Silica Gel G (0.6 mm adsorbent thickness, 200 × 200 mm glass plates, activated for 2 h at 100°, 20 mg syrup per plate, Solvent *F*) revealed seven yellow zones in the reaction mixture. To extend the time of elution, Whatman No. 1 filter-paper wicks were hung over the back of the plates and pressed close to the adsorbent on the front by means of a 50 × 200 mm glass plate which was held tightly with rubber bands. Yellow zones having the following R_F values were obtained: 0.1 [*N*-(2,4-dinitrophenyl)serine methyl ester], 0.18, 0.26, 0.35, 0.51, 0.78, and 0.92 [2-(2,4-dinitroanilino)acrylic acid methyl ester]. Silver salts appeared at the base line. Each zone was scraped from the plate and eluted with acetone. The solution was filtered through a Celite pad and evaporated at room

temperature. The principal yellow zones (R_F 0.26 and 0.35) were decolorized in benzene with charcoal and filtered through a Celite pad. Each zone was re-chromatographed as above on Silica Gel G (Solvent *G*), and yellow zones (R_F 0.43 and 0.48, respectively) were obtained for each of the previously obtained zones (R_F 0.26 and 0.35). The sulfuric acid spray reagent indicated zones at the base line and solvent front as well as each of the yellow zones. The yellow zones were isolated as before and each syrup was rechromatographed (Silica Gel G, Solvent *H*) to give yellow zones having R_F 0.23 and 0.28, respectively. After drying at 0.01 torr the zone having R_F 0.23 (**1**) weighed 60.2 mg (10.8% of syrup chromatographed), $[\alpha]_D^{25}$ +27.1° (*c* 0.5, chloroform); λ_{max}^{Film} 2.62 (OH, N–H), 3.20 (C–H), 5.68 ($-CO_2CH_3$), 6.12, 6.23 (Ar–), 6.55, 6.70, 7.49 ($-NO_2$), 9.2, 9.3 (–C–O–C), 6.78, 8.2, 9.58, 10.75, 11.4, 11.75, 13.15 μm; n.m.r. data: τ 0.93 (1 proton, broad singlet, N–H), 1.07 (1-proton doublet, H-3′, $J_{3',5'}$ 3 Hz), 1.80 (1-proton quartet, H-5′, $J_{5',6'}$ 10 Hz), 2.73 (20 protons, broad singlet, benzyl aromatic protons), 3.01 (1-proton doublet, H-6′), 5.13 (1-proton doublet, H-1″, $J_{1'',2''}$ 3 Hz), 5.78–6.48 (17-proton multiplet, amino acid chain, carbohydrate ring protons and benzylic $-CH_2-$), 6.20 (3-proton singlet, OCH_3).

Anal. Calc. for $C_{44}H_{45}N_3O_{12}$: C, 65.40; H, 5.32; N, 5.20. Found: C, 65.76; H, 5.11; N, 5.44.

The zone having R_F 0.28 was a second glycoside; yield 53.5 mg (8.2%), $[\alpha]_D^{25}$, +21.4° (*c*, 0.6, chloroform); λ_{max}^{Film} 2.55, 2.6 (OH, N–H), 3.19 (C–H), 5.62 ($-CO_2CH_3$), 6.10, 14.35 (Ar–), 6.51, 6.92, 7.45 ($-NO_2$), 9.3 (–C–O–C–), 2.83, 3.30, 4.05, 8.2, 8.62, 9.0, 10.75, 11.4, 11.75, 13.15 μm; n.m.r. data: τ 0.82 (1-proton, broad singlet, N–H); 0.93 (1-proton doublet, H-3′, $J_{3',5'}$ 3 Hz), 1.78 (1-proton quartet, H-5′, $J_{5',6'}$ 8 Hz), 2.73 (20-proton broad singlet, benzyl aromatic protons), 3.19 (1-proton doublet, H-6′), 5.13 (1-proton doublet, H-1″, $J_{1'',2''}$ 3 Hz), 5.22–6.52 (17-proton multiplet, amino acid chain, carbohydrate ring protons and benzylic $-CH_2-$), 6.17 (3 proton singlet, $-OCH_3$). Although no satisfactory elementary analysis could be obtained for this syrupy, chromatographically homogeneous substance, instrumental data clearly identify it as a second glycoside.

The u.v. absorption data for both glycosides were identical in methanol (maxima at 260 nm and 340 nm, minima at 290 nm). Glycosides **1** and the component having R_F 0.28 (5 mg) in methanol (2 ml) containing 2 drops of 12M hydrochloric acid were hydrolyzed at reflux. Products that corresponded in chromatographic mobility to *N*-(2,4-dinitrophenyl)-L-serine and 2,3,4,6-tetra-*O*-benzyl-α-D-glucose were detected by t.l.c. (Solvent *I*, silver nitrate–sodium hydroxide spray reagent).

Treatment of the two glycosides (10 mg) in methanol (2 ml) with 2mM sodium carbonate (0.5 ml) gave 2-(2,4-dinitroanilino)acrylic acid methyl ester and 2-methoxycarbonyl-6-nitrobenzimidazole-1-oxide[36], separated by t.l.c. (Solvent *I*, R_F 0.00 and 0.84, respectively), and identified by u.v. spectroscopy: 2-(2,4-dinitroanilino)acrylic acid methyl ester (in methanol), maxima at 250 nm and 343 nm, minimum at 295 nm; 2-methoxycarbonyl-6-nitrobenzimidazole-1-oxide (in methanol), maxima at 250 nm and 300 nm, minimum at 280 nm. These constants accord with literature values[36].

N-(2,4-Dinitrophenyl)-L-threonine. — Following the procedure of Sanger[43],

L-threonine (5.00 g, 42 mmoles) was dissolved in water (100 ml), to which solid sodium hydrogen carbonate (7.50 g, 89.3 mmoles) was added. 1-Fluoro-2,4-dinitrobenzene (11.2 g, 62 mmoles) in 95% ethanol (100 ml) was slowly added with shaking. The mixture was stirred and the reaction monitored by t.l.c. (Solvent *A*, ninhydrin). The starting material (R_F 0.32) disappeared after 3 h. The mixture was concentrated to remove ethanol, and neutralized with concentrated hydrochloric acid. A syrup precipitated, which solidified to an amorphous yellow solid; yield, 11.7 g (98%). After recrystallization from methanol–water the product had m.p. 128–132°; R_F 0.77; λ_{max}^{KBr} 2.95 (OH, N–H), 3.35, 5.80 (CO_2H), 6.18, 6.30 (Ar–), 6.57 ($-NO_2$), 7.45 ($-NO_2$), 7.05, 8.10, 8.60, 9.05, 9.25, 9.80, 10.85, 11.4, 12.0, 12.25, 13.45 μm.

N-(*2,4-dinitrophenyl*)-L-*threonine methyl ester*. — Crude *N*-(2,4-dinitrophenyl)-L-threonine (6.50 g, 0.0228 mole) in tetrahydrofuran (80 ml) was treated with ethereal diazomethane[44] in small increments, and the reaction was monitored by t.l.c. on Silica Gel G (Solvent *B*, visual detection). An excess of diazomethane was avoided because of formation of side products. After all of the acid (R_F 0.12) had reacted the solution was evaporated to a syrup that was dissolved in acetone. The solution, filtered through a Celite pad, was evaporated to a syrup, which was dissolved in a minimum volume of benzene and refrigerated for 1 day to give yellow crystals; yield 4.85 g (65%); m.p. 113–114°, $[\alpha]_D^{25}$ −54° (*c* 3.33, acetone); R_F 0.86; λ_{max}^{KBr} 2.87 (OH), 2.95 (N–H), 3.35 (C–H), 5.75 ($-CO_2CH_3$), 6.15, 6.30 (Ar–), 6.57, 7.00, 7.45 ($-NO_2$), 7.95, 8.05, 9.0, 10.8, 12.10, 13.41, 13.80 μm; n.m.r. data: τ 0.83 (1-proton, broad multiplet, –NH), 0.99 (1-proton doublet, $J_{3',5'}$ 3 Hz, H-3'), 1.74 (1-proton quartet, H-5'), 2.84 (1-proton doublet, $J_{5',6'}$ 10 Hz, H-6'), 5.22–5.45 (2-proton multiplet, H-2 and H-3), 6.26 (3-proton singlet, OCH_3), 7.21 (1-proton singlet, OH), 8.68 (3-proton doublet, $J_{4,3}$ 6 Hz, H-4).

These data are in essential accord with those of Derevitskaya and coworkers[15]. The procedures are repeated here because the present workers found difficulty in reproducing this synthesis to give crystalline products.

3-O-(*2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl*)-N-(*2,4-dinitrophenyl*)-L-*threonine methyl ester* (**2**). — The Koenigs–Knorr synthesis and isolation of this glycoside followed the method described for **1**, but *N*-(2,4-dinitrophenyl)-L-threonine methyl ester was used. The resulting syrup, in acetone (20 ml), was resolved chromatographically on 25 plates as described for **1**, and each plate was eluted for 3 h. The R_F values for the yellow zones (Silica Gel G, Solvent *G*, sulfuric acid) were: 0.76, 0.42 mixed with 0.44, 0.32, 0.21, 0.11, and 0.00.

The bands of adsorbent containing the zones having R_F 0.32 and 0.42 were separated, transferred to small columns, and eluted with acetone. The eluates were concentrated to syrups, taken up in acetone (10 ml), and each was preparatively rechromatographed on plates of Silica Gel G (solvent *F*). The zone having R_F 0.32 in solvent *B* was separated into two zones (R_F 0.15 and 0.28). After scraping the desired zones from the plates, the adsorbent was again packed into small columns and eluted with acetone. The eluates were concentrated to syrups. All three zones failed to crystallize from a variety of solvents although they were found to be chro-

matographically homogeneous in several different solvent systems. The syrups were dried in vacuum (1 mm) to constant weight.

The zone having R_F 0.15 (Solvent *C*) yielded 0.125 g (2.47%); $[\alpha]_D^{25}$ 0.00° (*c* 3.3, chloroform); λ_{max}^{Film} 2.92 (OH, N–H), 3.37 (C–H), 5.70 (–CO_2CH_3), 6.15, 6.27, 13.48, 14.38 (Ar–), 6.55, 6.98, 7.50 (–NO_2), 9.25, 9.35 (–COC–), 3.24, 3.44, 6.89, 7.80, 8.35, 8.62, 8.78, 9.74, 10.85, 12.00, 12.25 μm; n.m.r. data: τ 0.85 (1-proton, broad multiplet, N–H), 0.96 (1 proton quartet, $J_{3',5'}$ 2.5 Hz, H-3'), 1.83 (1-proton quartet, $J_{5',6'}$ 10 Hz, H-6'), 2.77 (20-proton, broad singlet, benzyl aromatic protons), 3.25 (1-proton multiplet, H-6'), τ 5.14–6.39 (16-proton multiplet, H-2, H-3, carbohydrate ring protons and benzylic –CH_2–), 6.27 (3-proton singlet, OCH_3), 8.66 (3-proton doublet, $J_{3,4}$ 7 Hz, H-4).

Anal. Calc. for $C_{45}H_{47}N_3O_{12}$: C, 65.75; H, 5.75; N, 5.11. Found: C, 65.48; H, 6.09; N, 4.87.

The zone having R_F 0.28 (Solvent *F*) yielded glycoside **2**, 0.286 g (5.82%); $[\alpha]_D^{25}$ +23.7° (*c* 3.3, chloroform); the i.r. spectrum was almost identical with that of zone R_F 0.15; n.m.r. data: τ 0.85 (1-proton, broad multiplet, N–H), 1.00 (1-proton doublet, $J_{3',5'}$ 3 Hz, H-3'), 1.77 (1-proton quartet, $J_{5',6'}$ 10 Hz, H-5'), 2.77 (20-proton, broad singlet, benzyl aromatic protons), 3.23 (1-proton doublet, H-6'), 4.95 (1-proton doublet, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.10–6.38 (15-proton multiplet, H-2, H-3, carbohydrate ring protons and benzylic –CH_2–), 6.62 (3 protons, singlet, OCH_3), 6.42 (1 proton, H-2''), 8.58 (3 protons, doublet, $J_{3,4}$, 6 Hz, H-4), λ_{max}^{MeOH} 260 nm and 342 nm (ε 1.8×10^4).

Anal. Calc. for $C_{45}H_{47}N_3O_{12}$: C, 65.75; H, 5.76, N, 5.11. Found: C, 65.59; H, 6.05; N, 5.09.

The zone having R_F 0.42 (Solvent *B*) yielded 0.156 g (6.08%); $[\alpha]_D^{25}$ −3° (*c* 1.5, chloroform); λ_{max}^{film} 2.93 (N–H), 3.39 (C–H), 5.72 (–CO_2CH_3), 6.19, 6.30, 13.48, 14.38 (–Ar), 6.60, 7.04, 7.60 (–NO_2), 9.27 (–COC–), 3.47, 7.85, 8.30, 8.64, 9.84, 10.90, 12.01, 12.25, 13.85 μm; n.m.r. data: τ 0.84 (2-proton, broad multiplet, $J_{3',5'}$ 3 Hz, N–H and H-3'), 1.78 (1-proton quartet, $J_{5',6'}$ 10 Hz, H-5'), 2.75 (5-proton, broad singlet, benzyl aromatic protons), 3.30 (1-proton doublet, H-6'), 5.37–5.80 (4-proton multiplet, H-2, H-3, benzylic –CH_2–), 6.27 (3 protons, singlet, OCH_3), 8.64 (3 protons, doublet, $J_{3,4}$ 6 Hz, H-4). This compound is the *O*-benzyl ether of *N*-(2,4-dinitrophenyl)-L-threonine methyl ester (See Discussion).

To solutions of the glycoside **2** and the product having R_F 0.42 (5 mg) in methanol (2 ml) were added 2 drops of 12M hydrochloric acid. The solutions were refluxed for 2 days, at the end of which time, t.l.c. on Silica Gel G (Solvent *B*) indicated in each experiment the formation of the second yellow zone having R_F 0.11 [mobility identical with that of *N*-(2,4-dinitrophenyl)-L-threonine methyl ester] and 2,3,4,6-tetra-*O*-benzyl-D-glucose.

Treatment of glycoside **2** with methanolic sodium carbonate as for the serine analog yielded 2,3,4,6-tetra-*O*-benzyl-α-D-glucose and 2-(2,4-dinitroanilino)crotonic acid methyl ester. This latter[36] gave u.v. absorption data similar to those of the

derivative from serine. 2-Methoxycarbonyl-6-nitrobenzimidazole-1-oxide[36] was also detected.

Rate measurements for β-elimination with O-serine and threonine glycosides. — A glycoside solution of known concentration was prepared in the desired solvent. The base solutions were prepared so that 0.01 ml of the solution would contain the desired number of equivalents of base per ml of the glycoside solution. The acid solutions used for neutralization were made on the same principle. At the start of the reaction the base was added to the reaction mixture and at each of the desired time intervals a 1-ml portion was withdrawn and neutralized with 0.01 ml of acid. The volume of the solution was assumed to remain constant at 1 ml. The solution was immediately evaporated to ~0.1 ml under diminished pressure at room temperature. The entire solution resulting was spotted onto commercial chromatography sheets. The tube was washed with a few drops of the reaction solvent, and the washings were also spotted onto the sheet. The reactants and products were separated (Solvent *I*) by t.l.c. The zone representing the unreacted glycoside was scraped from the plate, eluted from the silica gel with 3 ml of methanolic hydrogen chloride containing 0.77 μmoles/l acid, centrifuged for 20 sec, and the absorbance of the supernatant was read at 340 nm. The concentration (μg/ml) of the remaining glycoside was obtained from a Beer–Lambert plot established previously. Pseudo-first-order kinetics were assumed for the reaction because the base is regenerated after the elimination. The rate and half-life of the reaction were then determined by plotting log $a/(a-x)$ *vs.* time (h). The initial concentration of glycoside was obtained by taking 0.01 ml of the acid solution and 0.01 ml of the base solution and adding to that mixture 1 ml of the pure glycoside solution. This mixture was treated as described above, and the concentration from the Beer–Lambert plot was designated as the initial concentration of glycoside. Each kinetic run was made at least in duplicate. An average of the two concentrations of unreacted glycoside from each aliquot was used to obtain the rate constant and the half-life. The mean standard deviation was ±2%. The data were subjected to least-squares analysis by means of a computer program and fitted to first-order kinetics plots.

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ADDITION OF NITRYL IODIDE TO UNSATURATED CARBOHYDRATE DERIVATIVES*†

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ABSTRACT

The addition of nitryl iodide (prepared *in situ* by the reaction of silver nitrite and iodine in ether solution) to unsaturated sugars yields β-iodonitro adducts, which can be dehydrohalogenated by sodium hydrogen carbonate in boiling benzene to give the highly reactive α-nitroolefins. The double bonds in the latter compounds can be selectively reduced with sodium borohydride in ethanol.

INTRODUCTION

In 1932 Birchenbach *et al.*[2] reported the reaction of silver nitrite and iodine in the presence of cyclohexene to give dinitro and iodonitro adducts. Only recently, however, have the synthetic utility and mechanism of the reaction been described[3]. The reaction in the presence of olefins has the characteristics of a nitryl iodide (NO_2I) addition and appears to proceed by a free-radical attack of an NO_2 species on the double bond. The method provides a selective synthesis of β-iodonitro-, vinylnitro-, and nitro-alkanes under mild reaction conditions. In connection with our studies on use of pseudohalogens as a method of stereospecific introduction of nitrogen functions into carbohydrate molecules[4], we decided to try the addition of nitryl iodide to unsaturated carbohydrate derivatives. In this paper we describe complete details of the reaction with some unsaturated sugars.

RESULTS AND DISCUSSION

Treatment of 3-*O*-acetyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose[5] (**1**) (prepared by acetylation of 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose[6]) with an excess of silver nitrite and iodine in ether gave predominantly an iodo-*C*-nitro adduct (**2**). The adduct was unstable and liberated iodine on standing, but could be dehydrohalogenated by sodium hydrogen carbonate

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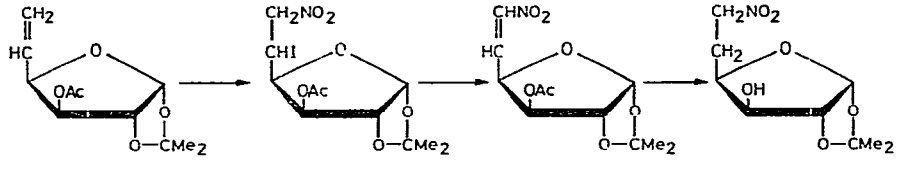
in boiling benzene to the known 3-*O*-acetyl-5,6-dideoxy-1,2-*O*-isopropylidene-6-nitro-α-D-*xylo*-hex-5-enofuranose[7] (**3**), thereby establishing the position of the nitro group. A higher yield of nitroolefin **3** could be obtained by treatment of the adduct **2** with pyridine containing acetic anhydride; a rapid elimination reaction occurred at room temperature, and **3** was isolated in 80% yield after purification by column chromatography. The double bond in compound **3** was selectively reduced with sodium borohydride in ethanol[8a] to yield 5,6-dideoxy-1,2-*O*-isopropylidene-6-nitro-α-D-*xylo*-hexose (**4**). Such reductions, in the carbohydrate field, have been achieved[8b] by hydrogenation over palladium black. The observed regiochemistry* of the reaction of silver nitrite and iodine in the presence of the unsaturated sugar **1** is consistent with the free-radical pathway described by Hassner *et al.*[3] for the addition of nitryl iodide to terminal olefins.



1 2 3 4

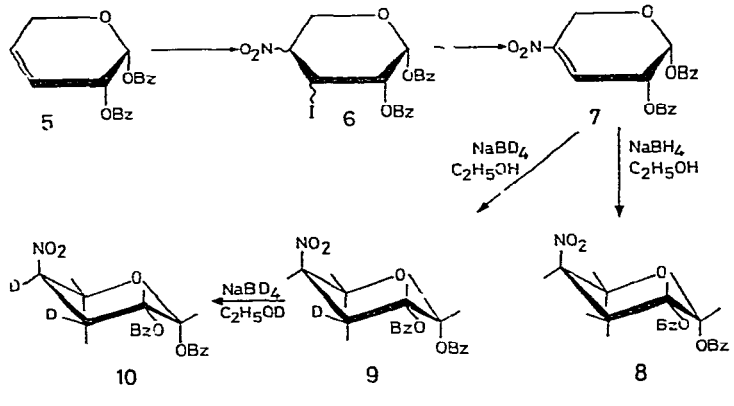
The configuration of the double bond in **3** was determined to be *trans* by n.m.r spectroscopy. In nitroethylene the vicinal proton coupling constants J_{trans} and J_{cis} have been estimated[9] to be 13.4 Hz and 4.9 Hz, respectively. The vinyl protons, H-5 and H-6, in the spectrum of **3** resonate in the region τ 2.55–3.07 as the AB part of an ABX type of pattern; the coupling constant for the protons is approximately 14 Hz, indicating that the nitroolefin has the *trans* stereochemistry.

Nitryl iodide has been added also to the cyclic, unsaturated sugar benzyl 2-*O*-benzyl-3,4-dideoxy-α-D-*glycero*-pent-3-enoside[10] (**5**). A crystalline adduct **6** was obtained which decomposed on standing. Dehydrohalogenation with sodium hydrogen carbonate in boiling benzene yielded the cyclic α-nitroolefin, benzyl 2-*O*-benzyl-3,4-dideoxy-4-nitro-α-D-*glycero*-pent-3-enoside (**7**). The position of the nitro function in **7** was proven as follows.

Treatment of compound **7** with sodium borohydride in ethanol afforded crystalline benzyl 2-*O*-benzyl-3,4-dideoxy-4-nitro-β-L-*threo*-pentoside (**8**). In the n.m.r. spectrum of **8** a 1-proton doublet at τ 5.18 having a splitting of 3 Hz was assigned to H-1. A complex, 2-proton multiplet in the region τ 7.19–8.19 was assigned to the two H-3 protons, and a 1-proton multiplet in the region τ 5.58–5.83 was attributed to H-4. If sodium borodeuteride in ethanol is used for the selective reduction of the double bond in compound **8**, only one deuterium atom is incorporated into the molecule, and the product is benzyl 2-*O*-benzyl-3,4-dideoxy-3-deuterio-4-nitro-β-L-*arabino*-pentoside (**9**), as shown by n.m.r. spectroscopy. Thus, the signal for the H-3

*"Regio" is used to describe directional effects in bond making or breaking: compare A. Hassner, *J. Org. Chem.*, 33 (1968) 2684.

proton appeared as a quartet with splittings of 11.5 Hz ($J_{2,3}$) and 4.5 Hz ($J_{3,4}$), and the signal for the H-2 proton appeared as a quartet with splittings of 3 Hz ($J_{1,2}$) and 11.5 Hz ($J_{2,3}$). These n.m.r. data are consistent with compound **9** being in the *C1* (L) conformation and the deuterio and nitro groups possessing the equatorial and axial orientations at C-3 and C-4, respectively. The configuration at C-4 does not give any information about the steric course of the reduction, since it has been found that the proton at C-4 is exchanged under the reaction conditions. Thus, treatment of compound **9** with sodium borodeuteride in deuterioethanol (C_2H_5OD) gave benzyl 2-*O*-benzyl-3,4-dideoxy-3-deuterio-4-nitro-β-L-*arabino*-pentoside-4-*d* (**10**). In the n.m.r. spectrum of **10** the signal for H-3 now appeared as a doublet at τ 7.88 with a splitting of 11.5 Hz ($J_{2,3}$); no signal was observed in the region τ 5.58–5.82 attributable to an H-4 signal. Since a nitronate anion would be expected to be an intermediate in the exchange reaction, the configuration at C-4 is determined by a proton transfer to an sp^2 carbon atom. It is remarkable that, in the absence of substituents on C-3 and C-5, the isolated product has the nitro group at C-4 in an axial orientation. In contrast, for example, in the oxidation of 4-*t*-butylcyclohexanone oxime with peroxytrifluoroacetic acid, a reaction involving a nitronate anion or nitronic acid as an intermediate, there is a slight preference (3:1) for axial protonation to give the more stable *trans*-4-*t*-butylnitrocyclohexane[11]. The stereochemistry of protonation of nitronic acids and nitronate anions in tetrahydropyran systems is being investigated, and the results will be reported separately. To our knowledge, compound **8** is the first example of an aldopyranose derivative with a nitro group at C-4.



(Bz = CH_2Ph)

A further noteworthy feature of the reduction of nitroolefin **7** with sodium borodeuteride in ethanol is that the addition of deuteride ion at C-3 occurs stereospecifically. None of the compound epimeric with **9** at C-3 could be detected by n.m.r. spectroscopy.

In the present work, methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enoside[12] has been found to be resistant to the addition of nitryl iodide in ether. This alkene has been reported to be unreactive also toward the pseudohalogen iodine

azide in either acetonitrile or *N,N*-dimethylformamide[13], and unreactive or of low reactivity toward reagents of the carbenoid and nitrene type[14]. Horton *et al.*[14] have reported also that the alkene reacted with nitrosyl chloride below room temperature, but that the product decomposed to regenerate the starting alkene on attempted isolation.

The addition of nitryl iodide to unsaturated carbohydrate derivatives and dehydrohalogenation of the resultant adducts provides a convenient procedure for the preparation of unsaturated nitro sugars. These highly reactive compounds are susceptible to many nucleophilic addition reactions, and several examples have been reported by Baer *et al.*[15].

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 automatic polarimeter at 21 ±2°. I.r. spectra were recorded with a Beckman-IR5A spectrophotometer. N.m.r. spectra were recorded at 60 MHz in chloroform-*d* with tetramethylsilane as the internal standard. Thin-layer chromatography (t.l.c.) was performed with Silica Gel G as the adsorbent. The developed plates were air-dried, sprayed with 5% ethanolic sulfuric acid, and heated at about 150°. The term "petroleum ether" refers to a fraction of b.p. 60–80°.

3-O-Acetyl-5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hex-5-enofuranose (**1**). — 5,6-Dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose[6] (0.25 g) was dissolved in dry pyridine (3 ml), and treated with acetic anhydride (1 ml). After 8 h at room temperature, the solution was poured into ice–water, and the product was isolated in the usual manner in almost quantitative yield. Distillation at 70°/0.01 torr gave an oil having the same physical constants as those previously reported[5] for compound **1** prepared by a Wittig reaction.

Reaction of compound **1** *with silver nitrite and iodine in ether.* — Iodine (0.40 g) and freshly prepared silver nitrite (0.22 g) were stirred in dry ether (26 ml), in a flask covered with aluminum foil, for 1 min at room temperature. A solution of the olefin **1** (0.30 g) in a small volume of dry ether was added, and stirring was continued. The progress of the reaction was followed by t.l.c. in 2:3 ethyl acetate–petroleum ether, and additional amounts of silver nitrite and iodine were added at intervals until the starting material (R_F 0.75) had all reacted. The reaction mixture was filtered, and the filtrate was concentrated to an iodine-colored syrup, which was chromatographed on silica gel, with the same solvent as used for t.l.c. as eluent. The iodo-*C*-nitro adduct **2** was obtained as a syrup (0.27 g), R_F 0.45. This material was unstable on standing. The infrared spectrum (film) of the crude material showed absorptions at 5.69–5.78 μm (OAc), 6.41 μm (C–NO_2), and 6.55 μm (olefinic NO_2).

3-O-Acetyl-5,6-dideoxy-1,2-O-isopropylidene-6-nitro-α-D-xylo-hex-5-enofuranose (**3**). — *A.* A portion (0.15 g) of the crude adduct obtained above was stirred for 6 h in boiling benzene (6 ml) with anhydrous sodium hydrogen carbonate (0.30 g). The

mixture was then cooled and filtered, and the filtrate was shaken with a few drops of concentrated aqueous sodium thiosulfate solution until the iodine color had disappeared. The solution was dried (sodium sulfate) and concentrated to a syrup which crystallized at $-10°$ from petroleum ether containing a little benzene; yield 36 mg (35%), m.p. 98–102°. Recrystallization gave pure compound **3**, m.p. 108–109°, $[\alpha]_D$ $+5.3 \pm 1.5°$ (*c* 1.1, chloroform); n.m.r. data: τ 2.55–3.07 (2 protons, $J_{5,6} \sim 14$ Hz, $J_{4,5} \sim 2.5$ Hz, $J_{4,6} \sim 0.5$ Hz, H-5 and H-6), 3.98 (1-proton doublet, $J_{1,2}$ 3.5 Hz, H-1), 4.64 (1-proton doublet, $J_{2,3} < 0.5$ Hz, $J_{3,4}$ 3 Hz, H-3), 4.84–5.00 (1-proton multiplet, H-4), 5.36 (1-proton doublet, $J_{1,2}$ 3.5 Hz, H-2), 7.94 (3-proton singlet, OAc), 8.45, 8.63 (3-proton singlets, CMe_2); lit.[7] for compound **3**, m.p. 109–110°, $[\alpha]_D$ +6.5° (*c* 1, chloroform).

B. A solution of the crude adduct (0.27 g) in pyridine (2 ml) containing acetic anhydride (1 ml) was kept at room temperature for 30 min. The solution was poured into ice–water, and the product was isolated in the usual manner. After purification by chromatography on silica gel, with 2:3 ethyl acetate–petroleum ether as eluent, and crystallization [yield 0.13 g (80%)] the product had the same physical constants as compound **3** obtained in *A*.

*5,6-Dideoxy-1,2-*O*-isopropylidene-6-nitro-α-*D*-xylo-hexose* (**4**). — A mixture of compound **3** (50 mg), ethanol (3 ml), and sodium borohydride (20 mg) was stirred for 1 h at room temperature. An excess of M sulfuric acid was then added, followed by sufficient water to dissolve the solid. The solution was extracted with two 5-ml portions of chloroform, and the combined extracts were washed with water and evaporated to a syrup. Crystallization from ether–petroleum ether gave compound **4**, yield 21 mg (49%), m.p. 103.5–104°, $[\alpha]_D -15 \pm 2°$ (*c* 0.6, chloroform); λ_{max}^{KBr} 2.95 (OH), 6.47 (C–NO_2), 7.20, 7.25 μm (CMe_2).

Anal. Calc. for $C_9H_{15}NO_6$: C, 46.3; H, 6.5; N, 6.0. Found: C, 46.5; H, 6.7; N, 5.9.

*Reaction of benzyl 2-*O*-benzyl-3,4-dideoxy-α-*D*-glycero-pent-3-enoside*[10] (**5**) *with silver nitrite and iodine.* — Iodine (3.84 g) and freshly prepared silver nitrite (1.62 g) were stirred in dry ether (200 ml), in the dark, for 10 min at 5°. Compound **5** (3.96 g) was added, and stirring was continued for 1 h at 5° and then for 23 h at room temperature. The mixture was filtered, and the filtrate was washed with a solution of sodium thiosulfate, dried (magnesium sulfate), and concentrated to a syrup (4.89 g). A portion (2.00 g) of this material was dissolved in ethyl acetate (2 ml), and petroleum ether (6 ml) was added; the oil that separated crystallized from ethanol (20 ml). Recrystallization from ethanol gave a sample (53 mg) of the adduct **6** as an unstable solid, m.p. 139–141°; λ_{max}^{KBr} 6.42 μm (C–NO_2). A satisfactory elemental analysis could not be obtained.

*Benzyl 2-*O*-benzyl-3,4-dideoxy-4-nitro-α-*D*-glycero-pent-3-enoside* (**7**). — The crude syrupy adduct (2.89 g) obtained above was stirred in boiling benzene (100 ml) with sodium hydrogen carbonate (9 g) for 16 h. The mixture was then cooled and filtered, and the filtrate was decolorized with a concentrated solution of sodium thiosulfate. The solution was washed with water, dried (magnesium sulfate), and concentrated to

a syrup that crystallized from ethanol, yield 0.12 g, m.p. 115–119°. The mother liquors were concentrated, and the residue was again treated with sodium hydrogen carbonate (10 g) in boiling benzene (200 ml) for 3 days; a further crop of crystals was obtained, yield 0.50 g, m.p. 121–124°. Recrystallization of the combined products from ethanol gave the pure nitroolefin **7**, m.p. 127.5–128.5°, $[\alpha]_D$ +83.5° (*c* 1.3, chloroform); λ_{max}^{KBr} 6.56, 7.44 μm (olefinic NO_2); n.m.r. data: τ 2.58–2.75 (10-proton multiplet, aromatic protons), 2.89–2.95 (1-proton multiplet, H-3), 5.01 (1-proton doublet, $J_{1,2}$ 4 Hz, H-1), 5.29 (2-proton doublet, *J* 4 Hz, benzyl-methylene), 5.43 (2-proton singlet, benzyl-methylene), 5.48–5.59 (2-proton multiplet, 2 H-5), 5.62–5.88 (1-proton multiplet, H-2).

Anal. Calc. for $C_{19}H_{19}NO_5$: C, 66.9; H, 5.6; N, 4.1. Found: C, 67.1; H, 5.6; N, 4.0.

*Benzyl 2-O-benzyl-3,4-dideoxy-4-nitro-β-*L-threo-*pentoside* (**8**). — A mixture of nitroolefin **7** (100 mg), ethanol (5 ml), and sodium borohydride (50 mg) was stirred for 1 h. The mixture was neutralized with carbon dioxide, and then slowly diluted with water until crystallization was complete. The solid product was washed with water and recrystallized from ethanol–water to give pure **8**, yield 48 mg (48%), m.p. 112–113°, $[\alpha]_D$ +155° (*c* 0.9, chloroform); n.m.r. data: τ 2.52–2.88 (10-proton multiplet, aromatic protons), 5.18 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), 5.41 (2-proton doublet, *J* 5 Hz, benzyl-methylene), 5.55 (2-proton singlet, benzyl-methylene), 5.58–5.83 (1-proton multiplet, H-4), 5.85–6.06 (2-protons, 2 H-5), 6.06–6.45 (1-proton, H-2), 7.19–8.19 (2-proton multiplet, 2 H-3).

Anal. Calc. for $C_{19}H_{21}NO_5$: C, 66.5; H, 6.1; N, 4.1. Found: C, 66.2; H, 6.4; N, 4.2.

*Benzyl 2-O-benzyl-3,4-dideoxy-3-deuterio-4-nitro-β-*L-arabino-*pentoside* (**9**). — Nitroolefin **7** was treated with sodium borodeuteride in ethanol and the product isolated as described above, m.p. 112–114°. In the n.m.r. spectrum of **9** the signal for H-2 now appeared as a 1-proton quartet at τ 6.23 with $J_{1,2}$ 3 Hz and $J_{2,3}$ 11.5 Hz, and the signal for H-3 appeared as a 1-proton quartet at 7.88 with $J_{2,3}$ 11.5 Hz and $J_{3,4}$ 4.5 Hz.

*Benzyl 2-O-benzyl-3,4-dideoxy-3-deuterio-4-nitro-α-*L-arabino-*pentoside-4-*d (**10**). — A mixture of **9** (20 mg), deuterioethanol (C_2H_5OD) (1 ml), and sodium borodeuteride (20 mg) was stirred for 30 min. The product (**10**) was isolated as described for compound **8**, yield 15 mg, m.p. 111–112°. In the n.m.r. spectrum of **10** the signal for H-3 now appeared as a 1-proton doublet at τ 7.88 with $J_{2,3}$ 11.5 Hz; no signal was observed in the region τ 5.58–5.82 attributable to an H-4 signal.

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REVERSE OSMOSIS STUDIES ON DESALINATION MEMBRANES FORMED FROM CHEMICALLY MODIFIED CELLULOSE ACETATE*

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ABSTRACT

Secondary cellulose acetate was chemically modified by introducing *O*-(carboxymethyl), *O*-nitro, and *O*-(cyanoethyl) groups, and then tested for semipermeability. Introducing *O*-(cyanoethyl) groups (0.1–0.68 cyanoethyl group per C_6 unit) gave an elastic film that was tougher and of better desalination characteristics than the standard cellulose acetate membrane.

INTRODUCTION

Reverse osmosis is one of the most potentially competitive methods for desalination that is ready for commercial development. Table I summarizes typical values for the energy requirements, calculated theoretically, that are necessary to remove the dissolved salts from 1000 gallons of sea water[1]. The difficulties with

TABLE I

ENERGY REQUIRED TO REMOVE SALTS FROM 1000 GALLONS OF SEA WATER

Procedure	*Theoretical requirements*
Distillation	300 KWh
Freezing	60 KWh
Reverse osmosis	50 KWh

reverse osmosis are the low permeability to water of salt-retaining membranes, the rapid flux-decay due to compaction of the membrane under the high influent pressure, and the limited lifetime of the membrane because of poor chemical resistance of the cellulose acetate membranes most commonly used.

The presently demonstrated success of cellulose acetate membranes, compared with other plastic membranes[2], indicates that the former material may be selected for

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use in future desalination plants[3]. Loeb and Sourirajan[4] considerably improved the rate of permeation with membranes of cellulose acetate by modifying the film-casting technique and by developing what are known as modified and asymmetric membranes. Another approach is by chemical modification of the cellulose acetate, with the aim of improving its performance in desalination. In the present work, the main skelton of secondary cellulose acetate has been maintained, and chemical modification has been effected by introducing small proportions of specific functional groups, such as cyanoethyl, nitro, and carboxymethyl groups, along the cellulose acetate macromolecule. The semipermeability of these new membranes was tested.

EXPERIMENTAL

Raw material. — A low-grade, Egyptian, fly-cotton waste was purified[5] to give a satisfactory bleached cotton. *Anal.*: α-cellulose, 98.4; ether extractives, 0.16; ash, 0.17; d.p., 2077.

Derivatives of cellulose acetate. — A. Primary and secondary acetates of cellulose. The viscosity of purified cotton was adjusted by steeping it with 17.5% sodium hydroxide, centrifuging the slurry, and keeping the product in glass bottles for 40 h. The material was then washed and dried. "Primary" cellulose acetate and acetone-soluble cellulose acetate (d.s. 2.44) were prepared essentially by the method of Malm *et al.*[6], except that the ratio of acetic anhydride added, with respect to cotton, was doubled. The time of hydrolysis of "primary" cellulose acetate to "secondary" acetate was 60 h and 90% aqueous acetic acid was used.

B. O-Acetyl-O-(cyanoethyl)cellulose. Purified cotton (10 g) was shaken with 84.3 ml, of 17% sodium hydroxide (w/w) for 3 h and the slurry was filtered. The filter was washed with 100 ml of 2% sodium hydroxide, the filtered cake was shaken for 1 h with 100 ml of 2% sodium hydroxide, and the slurry was filtered and washed with 100 ml of 2% sodium hydroxide. The filtered cake (34 g) was then mixed with 1.2 ml of acrylonitrile, 6 ml of 2% sodium hydroxide, and the mixture was shaken for 20 h at room temperature. The slurry was then warmed for 3 h at 35° with occasional shaking. After acidification to pH 5–6 with 20% acetic acid, the product was filtered off, washed with water, and dried. The *O*-(cyanoethyl)cellulose thus formed (10 g) was acetylated as described above, and then hydrolyzed for 60 h with 90% acetic acid, to give *O*-acetyl-*O*-(cyanoethyl)cellulose. (*Anal.*: acetyl, 2.41; OH, 0.46; cyanoethyl, 0.13 per C_6 unit.) More-extensive cyanoethylation by using 3.1 ml of acrylonitrile (first preparation) and 6.2 ml of acrylonitrile (second preparation), followed by acetylation as above, gave *O*-acetyl-*O*-(cyanoethyl)cellulose containing *O*-cyanoethyl, 0.39 per C_6 unit, and 0.68 per C_6 unit, respectively.

C. O-Acetyl-O-(carboxymethyl)cellulose. *O*-(Carboxymethyl)cellulose (d.s. 0.074) was prepared as described by Harpham *et al.*[7]. Acetylation of this material was effected as before, except that the hydrolysis was conducted without the addition of magnesium acetate, and the time of hydrolysis with 90% acetic acid was extended to 60 h. (*Anal.*: acetyl, 2.35; OH, 0.57; carboxymethyl, 0.074 per C_6 unit).

D. O-Acetyl-O-nitrocellulose. Nitrocellulose of low d.s. was prepared from purified cotton as previously described[8] by using a nitrating mixture (31.76% nitric acid, 33.66% water, and 34.57% sulfuric acid). The washed and dried nitrocellulose (20 g, d.s. 0.13) was acetylated as before, and hydrolyzed for 60 h with 90% acetic acid, to give *O*-acetyl-*O*-nitrocellulose. (*Anal.*: acetyl, 2.4; OH, 0.47; nitro, 0.13 per C_6 unit.)

All of the cellulose derivatives prepared were soluble in acetone.

Casting of membranes. — Membranes were cast essentially as described by Loeb *et al.*[4], by incorporating an aqueous solution of magnesium perchlorate in the cellulose derivative–acetone solution. The solution was cast at -5 to $-11°$ to control the rate of evaporation of acetone, and after an evaporation period of 1.5 min the film was immersed in a water bath for 1 h at 0 to $-2°$ to leach out the soluble components. This procedure was followed by a heat treatment for a fixed time at 71.5°.

The casting solution contained the following (wt. %): acetone, 69.46; cellulose derivative, 23.11; magnesium perchlorate, 1.65; hydrochloric acid, 0.33; water, 5.45.

Testing of membranes. — A laboratory osmotic cell was constructed as described by Loeb[4], with a slight modification concerning the exit of the fresh-water product. The salt feed-solution (0.5% aq. solution of sodium chloride) was pressurized to 600 lb.in^{-2}, and fed at a rate of 350 ml per h onto the top of the active surface of the membrane, which was supported by a Millipore filter-paper (0.45 μm pore size) that rested on a porous plate of stainless steel. A portion of the water permeated through the membrane and was collected as fresh water at the bottom of the unit, while the concentrated brine was discharged from the top of the unit. The results of the permeability tests are shown in Table II. The percent rejection is given as:

$$\% \text{ rejection} = \left(100 - \frac{\text{wt.\% of solute in product}}{\text{wt.\% of solute in feed}} \times 100\right)$$

DISCUSSION

The present investigation was an attempt to modify cellulose acetate with the aim of improving its performance for desalination. The main skelton of "secondary" cellulose acetate was maintained, while a low d.s. of specific functional groups was introduced. Acetone-soluble derivatives of cellulose containing $\sim$2.4 acetyl groups per C_6 unit were successfully prepared from purified "chemical" cotton. These derivatives were: "secondary" cellulose acetate, *O*-acetyl-*O*-(carboxymethyl)cellulose (0.047 carboxymethyl group per C_6 unit), *O*-acetyl-*O*-nitrocellulose (0.13 nitro group per C_6 unit), and *O*-acetyl-*O*-(cyanoethyl)cellulose (0.13, 0.39, and 0.68 moles of cyanoethyl group per C_6 unit, respectively). The cellulose derivatives prepared were cast successfully according to the general procedure of Loeb[4] to give membranes suitable for testing in a special osmotic cell. The films were tested with an input solution containing 5000 p.p.m. of sodium chloride at an operating pressure of 600 lb.in^{-2}.

The effect of functional groups in the cellulose derivatives on the permeability properties of the membranes is given in Table II. Three sets of experiments (Expts.

TABLE II

PERMEABILITY OF CELLULOSE DERIVATIVES

Experiment No.	*Cellulose derivative*	*Degree of substitution of groups introduced*	*Film-casting conditions*		*Desalination properties*		*Comparative performance*[b]	
			Acetone/ acetate derivative	*Time of heating, (min)*[a]	*Flux, ($ml.h^{-1}.in^{-2}$)*	*Salt rejection (%)*	*Flux*	*Salt rejection (%)*
1	Acetate (Eastman 398-3)		3:1	4	13.5	91.9	100	100
2	Acetate (Egyptian)		3:1	4	13	87.3	96	95
3	*O*-Acetyl-*O*-(carboxymethyl)	0.074 carboxymethyl	3:1	4	7.5	40.1	56	44
4	*O*-Acetyl-*O*-nitro	0.13 (nitro)	3:1	4	14.4	83.47	107	91
5	*O*-Acetyl-*O*-(cyanoethyl)	0.13 cyanoethyl	3:1	4	16.3	91.4	121	99.4
6	Acetate (Eastman)		3:1	2.5	42	82.6	311	90
7	*O*-Acetyl-*O*-(cyanoethyl)	0.1 cyanoethyl	3:1	2.5	39	77.5	289	84
8	*O*-Acetyl-*O*-(cyanoethyl)	0.39 cyanoethyl	3:1	2.5	42	85.4	311	93
9	*O*-Acetyl-*O*-(cyanoethyl)	0.68 cyanoethyl	3:1	2.5	32	79.2	237	86
10	Acetate (Eastman)		4:1	2.5	57	63.2	422	69
11	Acetate (Egyptian)		4:1	2.5	36.6	65	272	71
12	*O*-Acetyl-*O*-(carboxymethyl)	0.074 carboxymethyl	4:1	2.5	24	15.3	177	17
13	*O*-Acetyl-*O*-nitro	0.13 (nitro)	4:1	2.5	29.2	63.5	216	69
14	*O*-Acetyl-*O*-(cyanoethyl)	0.1 cyanoethyl	4:1	2.5	33	62	244	67
15	*O*-Acetyl-*O*-(cyanoethyl)	0.39 cyanoethyl	4:1	2.5	47.5	83.3	352	91
16	*O*-Acetyl-*O*-(cyanoethyl)	0.68 cyanoethyl	4:1	2.5	66	67.4	490	73

[a]At 71.5°. [b]Taking the Eastman acetate (Expt. 1) as standard of 100 flux and salt rejection.

1–5, 6–9, and 10–16, Table II) were performed by using three different sets of conditions for casting the films. The results indicate that heating the membrane for a longer period (4 min), with a 3:1 acetone–cellulose derivative (Expts. 1–5, Table II) produced a "tighter" film of lower flux and better salt-rejection. In accordance with previous work[9], by using a higher ratio of acetone to the cellulose derivative, or a shorter time of heating the film (2.5 min, Expts. 6–16, Table II) produced membranes that were less dense and had higher flux-rates, but they showed lower salt-rejection. To compare the performance of the different films, the Eastman cellulose acetate (Expt. 1) was chosen as a standard, since its capacity for salt rejection is higher, when compared with the films cast in Expts. 6 and 10, in which the same cellulose acetate was used, but under less suitable conditions of casting.

The results obtained show that Egyptian cellulose acetate (Expt. 2, Table II) is similar to the standard Eastman film (Expt. 1), being only 4% lower in flux and salt rejection.

It has been reported that osmotic membranes prepared from sodium alginate[10] and certain seaweeds[11] found off the coast of India are semipermeable to sea water. However, the results of Table II show that introducing a small proportion of carboxymethyl groups (0.074 carboxymethyl groups per C_6 unit) into the molecule of cellulose acetate is detrimental to performance of the membrane, since it resulted in a 44% lower flux and 56% less salt rejection in comparison with the standard Eastman film.

Earlier reports[12] that cellulose nitrate (collodion) is more effective in salt-rejection and is less permeable to water than cellulose acetate film, were investigated. By introducing *O*-nitro groups (d.s. 0.13) along the molecule of "secondary" cellulose acetate (Expt. 4, Table II) there resulted a 6% improvement in flux and 9% less salt-rejection, in comparison with the standard Eastman cellulose acetate. One disadvantage of introducing the nitrate ester group is that it causes the film to be delicate and brittle.

The results also show that introducing *O*-cyanoethyl groups to a d.s. of 0.13 (Expt. 5, Table II) produced 21% more flux for the same salt rejection (~91%) as the standard Eastman film. Another encouraging feature is that *O*-acetyl-*O*-(cyanoethyl)-cellulose (0.39 cyanoethyl groups per C_6 unit) (Expt. 15, Table II) performed fairly well at higher acetone–cellulose ratios and produced a 352% higher flux at a slightly lower level of salt rejection (83.3%), in comparison with the standard film. By using three different casting conditions, the cyanoethylated product (Expts. 5, 8, and 15) showed an improvement over control experiments (1, 6, and 10, respectively). The cyanoethylated products are tougher and more elastic than the standard acetate film. They are also interesting since the cyanoethyl group is known to confer considerable resistance to attack on the membrane mildew and bacteria, a factor that is detrimental to the performance of the membrane[13].

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THE EFFECT OF STEREOCHEMISTRY ON THE OXIDATION OF SUBSTITUTED HEXITOLS*

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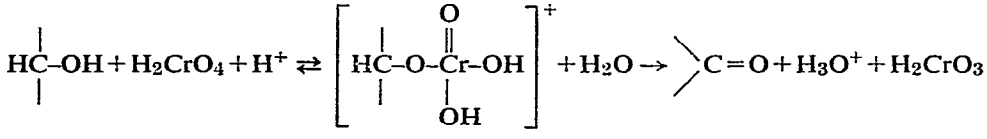
ABSTRACT

1-*O*-Benzoyl-2,3:5,6-di-*O*-isopropylidene-D-gulitol (**3**) and -D-allitol (**4**) were found to be inert toward oxidation by chromium trioxide in pyridine, confirming previous findings of apparent effects of 1,4-interaction. 1-*O*-Benzoyl-2,3:5,6-di-*O*-isopropylidene-D-mannitol (**2**), though previously established to be inert toward chromium trioxide in pyridine, was smoothly oxidized to 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D-*arabino*-3-hexulose (**5**) by methyl sulfoxide in acetic anhydride. However, the latter converted 1,5-di-*O*-benzoyl-2,4-*O*-benzylidene-xylitol (**6**) and -ribitol (**7**) into 3-(methylthio)methyl ethers.

INTRODUCTION

The reaction mechanism proposed by Westheimer[1] for the oxidation of secondary alcohols to ketones by chromic acid has had general acceptance. A rapid, reversible formation of chromate ester is followed by the rate-determining abstraction of a proton from the carbinol carbon atom. However, the initial step may become rate



controlling when an alcohol that is highly sterically hindered is applied[2].

Oxidation of substituted alditols with chromic acid as a route to ketoses has given varied results. 1-*O*-Benzoyl-2,3:5,6-di-*O*-isopropylidene-DL-galactitol (**1**) was smoothly oxidized to the expected ketose by chromium trioxide in pyridine[3]. On the other hand, a stereoisomer, 1-*O*-benzoyl-2,3:5,6-di-*O*-isopropylidene-D-mannitol (**2**), was inert[4,5]. The extent of interaction of the hydroxyl group on C-4 with the benzoyloxy group on C-1 was presumed[4] to be the important variable. In order to understand better this marked difference in reactivity, we have prepared two additional stereoisomers of **1** and **2** and have established their reactivity toward chromic acid.

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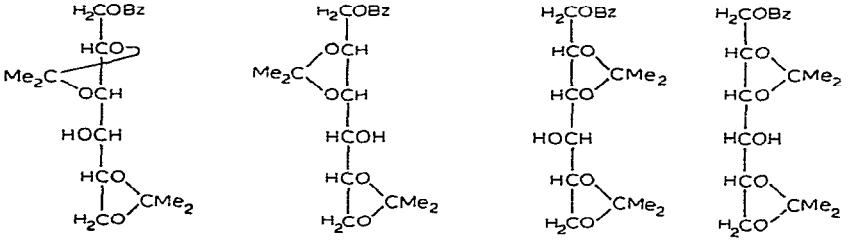
Methyl sulfoxide in acetic anhydride and with phosphorus pentaoxide have been applied as additional oxidizing agents for comparative purposes.

RESULTS AND DISCUSSION

Crystalline 1-*O*-benzoyl-2,3:5,6-di-*O*-isopropylidene-D-gulitol (**3**) was obtained by reduction of 2,3:5,6-di-*O*-isopropylidene-D-gulono-1,4-lactone[6], followed by selective benzoylation of the primary hydroxyl group. The i.r. absorption of the starting lactone near 1780 cm^{-1} (1,4-lactone) supports the structural assignment made[6]. Acetonation of D-allono-1,4-lactone[7] provided the 2,3:5,6-diisopropylidene acetal, which exhibited i.r. absorption near 1780 cm^{-1}. Reduction of the latter acetal, followed by selective benzoylation, gave crystalline 1-*O*-benzoyl-2,3:5,6-di-*O*-isopropylidene-D-allitol (**4**).



Neither **3** nor **4** gave a ketose under reaction conditions (chromium trioxide–pyridine)[3] that gave 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-DL-*xylo*-3-hexulose from **1**. In compounds **2**, **3**, and **4**, the hydroxyl groups on C-2 and C-3 are disposed *cis* in the Fischer projection formula. Thus acetonation to give the 2:3-isopropylidene acetal provides a dioxolane ring on which C-1 and C-4 are *cis* (Fig. 1). In the galactitol derivative (**1**), these substituents are placed in a *trans*-relationship (Fig. 2). Formation of a chromate ester would probably be hindered seriously when the substituents at

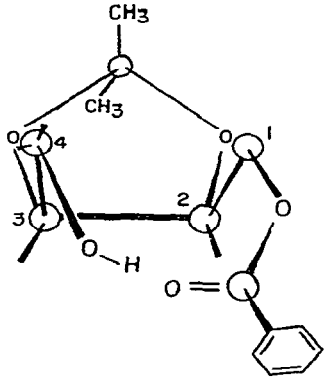


Fig. 1. *cis*-Disposition of substituents on 2,3-acetal ring.

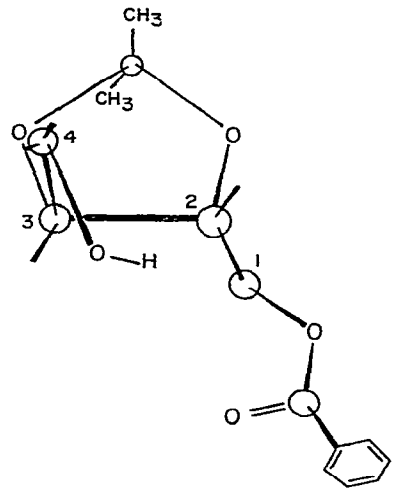
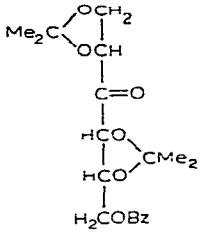


Fig. 2. *trans*-Disposition of substituents on 2,3-acetal ring.

C-1 and C-4 are disposed *cis*, as compared with the opposite configuration. We propose that hindrance in this esterification step causes the marked difference in reactivity, since it is not evident how removal of a proton from the carbinyl carbon atom, should esterification occur, be appreciably different among the four isomers.

Some comparative studies have been made by using methyl sulfoxide (Me_2SO) and acetic anhydride[8] as the oxidizing agent. The mannitol derivative (**2**) was converted into a crystalline product. The n.m.r. spectrum showed signals assignable without ambiguity to aromatic, methine and methylene, and methyl protons in the ratio, 5:7:12, consistent with the structure of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D-*arabino*-3-hexulose (**5**). Hydrolysis of **5** in acetone with Amberlite IR-120 (H^+) resin gave crystalline 6-*O*-benzoyl-D-*arabino*-3-hexulose, whose n.m.r. spectrum showed no signals attributable to methyl protons. Application of the same procedure to the gulitol (**3**) and the allitol (**4**) derivatives provided syrups that have not been crystallized.



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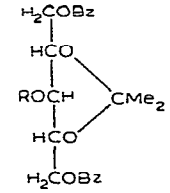
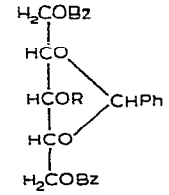


6 R = H
8 R = CH_2SCH_3
10 R = $COCH_3$



7 R = H
9 R = CH_2SMe
11 R = Ac

The behavior of some additional substrates toward methyl sulfoxide and acetic anhydride has been determined. 1,5-Di-*O*-benzoyl-2,4-*O*-methylenexylitol[9] and

1,5-di-*O*-benzoyl-2,4-*O*-methyleneribitol[4] have previously been oxidized by chromium trioxide in acetic acid, but not in pyridine, to the same product, 1,5-di-*O*-benzoyl-2,4-*O*-methylene-*erythro*-3-pentulose. Under the same reaction conditions, 1,5-di-*O*-benzoyl-2,4-*O*-benzylidene-xylitol (**6**) and -ribitol (**7**) were not oxidized[4]. Both **6** and **7**, when treated with methyl sulfoxide–acetic anhydride, yielded crystalline products, presumed to be the 3-(methylthio)methyl ethers (**8** and **9**, respectively), based upon the presence of signals at τ 7.80 in the n.m.r. spectra, assignable to methyl protons in the SCH_3 groups[10]. Melting points were depressed when **8** and **9** were mixed with 3-*O*-acetyl-1,5-di-*O*-benzoyl-2,4-*O*-benzylidenexylitol (**10**) and -ribitol (**11**), respectively. A mechanism describing the formation of these ethers has been previously described[8].

No products of oxidation were obtained when **2**, **3**, and **4** were treated with methyl sulfoxide and phosphorus pentaoxide[11] in phosphoric acid.

EXPERIMENTAL

General methods. — Analyses were made by the Australian Microanalytical Service. N.m.r. spectra were recorded on a Varian A-60A n.m.r. spectrometer for solutions in deuteriochloroform. I.r. spectra of Nujol mulls or potassium bromide pellets were recorded on a Beckman IR-10 spectrophotometer. Melting points were determined on a Fisher–Johns apparatus and are not corrected. Optical rotations were taken on a Shimadzu spectropolarimeter in 1-dm tubes.

2,3:5,6-Di-O-isopropylidene-D-gulitol. — A solution of 2,3:5,6-di-*O*-isopropylidene-D-gulono-1,4-lactone[6] (2 g) in 250 ml of dry ether was added dropwise during 60 min at room temperature into 100 ml of an ethereal solution of 0.80 g powdered lithium aluminum hydride. The mixture was refluxed for 2 h and the excess hydride destroyed by gradual addition of wet ether, with external cooling and stirring. The mixture was filtered through a layer of packed Celite (Johns–Manville). Concentration of the filtrate at room temperature with seeding provided a crystalline product, yield 1.4 g (70%), m.p. 72–74°, $[\alpha]_D^{22}$ +9.35° (*c* 0.11, ethanol). The first crystalline product was obtained by vacuum distillation of the syrup at <150°; the i.r. spectrum showed no band at 1,790 cm^{-1}. An analytical sample, m.p. 75.5–76°, was prepared by three recrystallizations from ligroin.

Anal. Calc. for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 54.65; H, 8.66.

1-O-Benzoyl-2,3:5,6-di-O-isopropylidene-D-gulitol (**3**). — To an ice-cold, vigorously stirred solution of 2.62 g (10 mmoles) of 2,3:5,6-di-*O*-isopropylidene-D-gulitol in 13 ml of dry pyridine was added dropwise a mixture of 1.4 ml (1.54 g, 11 mmoles) of benzoyl chloride in anhydrous pyridine (13 ml). After 12 h at 0° the resulting suspension was poured with stirring into 200 ml of crushed ice. The mixture was kept overnight in the cold to give a layer of syrup, which was crystallized from ethanol giving 2.4 g (70%) of **3**, m.p. 98–100°. An analytical sample, m.p. 99–100°, $[\alpha]_D^{22}$ −12.5° (*c* 1.1, ethanol), was prepared by three additional recrystallizations from ligroin.

Anal. Calc. for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.26; H, 7.10.

2,3:5,6-Di-O-isopropylidene-D-allono-1,4-lactone. — A mixture of D-allono-1,4-lactone[7] (2 g), anhydrous acetone (100 ml), and 18M sulfuric acid (0.70 ml) was shaken for 40 h at room temperature. The mixture was neutralized with 2.8 ml of 15M ammonium hydroxide. The precipitated ammonium sulfate was removed by filtration through Celite and the filtrate was evaporated to a viscous syrup by a stream of air. The diisopropylidene acetal was extracted into benzene. Removal of solvent gave crystalline 2,3:5,6-di-*O*-isopropylidene-D-allono-1,4-lactone; yield 2.3 g (63%), m.p. 57–60°. An analytical sample, m.p. 57–60°, $[\alpha]_D^{22}$ −73.5° (*c* 0.66, ethanol), was prepared by several recrystallizations from ligroin; i.r. data: ν_{max} 1,782 cm^{-1} (1,4-lactone); n.m.r. data: τ 5.2–6.3 (multiplet, 6 protons, methylene and methine), 8.5 (singlet, 6 protons, methyl), 8.6, 8.67 (two singlets, 6 protons, methyl).

Anal. Calc. for $C_{12}H_{18}O_6$: C, 55.80; H, 7.02. Found: C, 55.89; H, 6.92.

2,3:5,6-Di-O-isopropylidene-D-allitol. — A suspension of lithium aluminum hydride (6.0 g) in 1000 ml of anhydrous ether was cooled externally with a methanol–ice bath. A solution of 2,3:5,6-di-*O*-isopropylidene-D-allono-1,4-lactone (18 g) in anhydrous ether (200 ml) was added dropwise during 2 h with vigorous stirring. After continued stirring for 12 h, ether saturated with water was added slowly to destroy excess hydride. The resulting suspension was filtered through Celite, and the ether layer was evaporated to a syrup. Absolute ethanol was repeatedly evaporated at room temperature from the syrup to remove water, and 13.5 g (75%) of a liquid 2,3:5,6-di-*O*-isopropylidene-D-allitol was obtained, which was characterized as mono- and di-*O*-benzoyl derivatives.

1-O-Benzoyl-2,3:5,6-di-O-isopropylidene-D-allitol (**4**). — To a solution of 2,3:5,6-di-*O*-isopropylidene-D-allitol (13.5 g) in 80 ml of anhydrous pyridine cooled at 0° was slowly added with stirring a mixture of benzoyl chloride (7.0 ml) and dry pyridine (30 ml) during 2 h. After being kept overnight in a refrigerator the mixture was poured onto 200 g of crushed ice. The syrup deposited was dissolved in chloroform and washed successively with 5% hydrochloric acid, 5% sodium hydrogen carbonate, and water. The chloroform solution was dried (magnesium sulfate) and evaporated to give 14 g of syrupy **4**. This syrup gave crystals upon mixing with ligroin. An analytical sample, m.p. 62.3°, $[\alpha]_D^{22}$ −36.7° (*c* 0.67, ethanol), was prepared by three additional recrystallizations from ethanol.

Anal. Calc. for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.20; H, 6.93.

1,4-Di-O-benzoyl-2,3:5,6-di-O-isopropylidene-D-allitol. — When an excess of benzoyl chloride was used in the above preparation, a crystalline 1,4-di-*O*-benzoyl-2,3:5,6-di-*O*-isopropylidene-D-allitol was obtained from ethanol; m.p. 107–108°, $[\alpha]_D^{22}$ +7.4° (*c* 2.1, ethanol).

Anal. Calc. for $C_{26}H_{30}O_8$: C, 66.37; H, 6.43. Found: C, 66.10; H, 6.25.

6-O-Benzoyl-1,2:4,5-di-O-isopropylidene-D-arabino-3-hexulose (**5**). — A mixture of 1-*O*-benzoyl-2,3:5,6-di-*O*-isopropylidene-D-mannitol[4] (2.75 g), 23 ml of methyl sulfoxide (distilled over calcium hydride) and acetic anhydride (15.3 ml) was kept for 76 h at room temperature. The mixture was poured, with vigorous stirring, onto crushed ice–water containing potassium carbonate. The suspension was kept in the

cold and a syrup resulted. Several washings of the syrup with ice-cold water and keeping in the cold afforded a solid, which crystallized from absolute ethanol to give **5**; yield 2,4 g (51%), m.p. 76°. An analytical sample, m.p. 74–75°, $[\alpha]_D^{22}$ +60.5° (*c* 0.8, ethanol), was prepared after four recrystallizations from ligroin; n.m.r. data: τ 1.8–2.1 (multiplet, 2 protons, *ortho*-aromatic), 2.4–2.7 (multiplet, 3 protons, *meta*- and *para*-aromatic), 4.9–6.1 (multiplet, 7 protons, methylene and methine), 8.4, 8.56, 8.6, 8.64 (4 three-proton singlets, methyl).

Anal. Calc. for $C_{19}H_{24}O_7$: C, 62,62; H, 6.64. Found: C, 62.64; H, 6.63.

*6-O-Benzoyl-*D-arabino-*3-hexulose.* — A mixture of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-D-*arabino*-3-hexulose (1 g), acetone (50 ml), water (15 ml), and 5 g of Amberlite IR-120 (H^+) resin was refluxed for 5 h. The mixture was filtered and the filtrate was evaporated to provide crystalline 6-*O*-benzoyl-D-*arabino*-3-hexulose (89%), m.p. 147–148°, $[\alpha]_D^{22}$ −82.0 (*c* 0.91, ethanol); n.m.r. data (in chloroform-*d* after exchange with deuterium oxide): τ 1.9–2.7 (multiplet, aromatic), 5.3–6.3 (multiplet, methylene and methine), no signal between τ 8–9.

Anal. Calc. for $C_{13}H_{16}O_7$: C, 54.93; H, 5.68. Found: C,54.84; H, 5.67.

1,5-Di-O-benzoyl-2,4-O-benzylidene-3-O-(methylthio)methylxylitol (**8**). — A solution of 3.45 g (10 mmoles) of 1,5-di-*O*-benzoyl-2,4-*O*-benzylidene-xylitol[12] (**6**), 30 ml of methyl sulfoxide (distilled over calcium hydride), and 20 ml of acetic anhydride was kept for 20 h at room temperature. The mixture was poured, with stirring, onto ice–water containing potassium carbonate. The powder deposited was collected on filter paper, washed several times with cold water, and crystallized from ethanol to give 1.2 g (41%) of **8**, m.p. 156–157°. An analytical sample, m.p. 160–161°, was prepared by three recrystallizations from ligroin; n.m.r. data: τ 7.8 (3-proton singlet, SCH_3), 1.8–2.8 (multiplet, 15 aromatic protons, 4.3–6.0 (multiplet, 10 protons, methylene and methine).

Anal. Calc. for $C_{28}H_{28}O_7S$: C, 66.12; H, 5.55. Found: C, 66.00; H, 5.58.

*3-O-Acetyl-1,5-*di-O-*benzoyl-2,4-O-benzylidenexylitol* (**10**). — To 1,5-di-*O*-benzoyl-2,4-*O*-benzylidenexylitol[12] (1 g) in pyridine (10 ml) was added 1 ml of acetic anhydride. The mixture, after being kept overnight at room temperature, was poured, with stirring, into an ice-cold solution of potassium carbonate. The precipitate was filtered on paper, washed several times with cold water, and crystallized from absolute ethanol to give 1.3 g (91%) of **10**, m.p. 124–127°. An analytical sample, m.p. 128–129°, was prepared by three recrystallizations from ethanol; n.m.r. data: τ 7.81 (singlet, 3 protons, acetyl methyl) 1.82–2.7 (multiplet, 15 protons, aromatic), 4.2–5.8 (multiplet, 8 protons, methylene and methine).

Anal. Calc. for $C_{28}H_{26}O_8$: C, 68.56; H, 5.34. Found: C, 68.89; H, 5.55.

1,5-Di-O-benzoyl-2,4-O-benzylidene-3-O-(methylthio)methylribitol (**9**). — A solution of 3.45 g (10 mmoles) of 1,5-di-*O*-benzoyl-2,4-*O*-benzylideneribitol[4] (**7**), 30 ml of methyl sulfoxide, and 20 ml of acetic anhydride was kept for 42 h at room temperature. The solution was poured into aqueous potassium carbonate with cooling and stirring. The mixture was kept in the cold for several days and the residue was filtered off, washed several times with cold water, and crystallized from ethanol to

give 1.75 g of **9**, m.p. 98°. After several recrystallizations from ethanol, the compound had m.p. 98–99°. After heating the sample in a high vacuum at 78°, the m.p. increased to 116–118°; n.m.r. data: τ 7.8 (3-proton singlet, SCH_3), 1.7–2.7 (multiplet, 15 aromatic protons, 4.2–6.0 (multiplet, 10 protons, methylene and methine).

Anal. Calc. for $C_{28}H_{28}O_7S$: C, 66.12; H, 5.55. Found: C, 66.69; H, 5.96.

3-O-Acetyl-1,5-di-O-benzoyl-2,4-O-benzylideneribitol (**11**). — To 1 g of 1,5-di-*O*-benzoyl-2,4-*O*-benzylideneribitol[4] in pyridine (10 ml) was added 1 ml of acetic anhydride. The mixture, after being kept overnight, was poured, with stirring, into an ice-cold solution of potassium carbonate. The precipitate deposited was filtered off and washed with cold water. An analytical sample, m.p. 108–109°, was prepared by three recrystallizations from ethanol; n.m.r. data: τ 7.94 (singlet, 3 protons, acetyl methyl), 1.8–2.8 (multiplet, 15 protons, aromatic), 4.1–6.0 (multiplet, 8 protons, methylene and methine).

Anal. Calc. for $C_{28}H_{26}O_8$: C, 68.56; H, 5.34. Found: C, 68.25; H, 5.50.

Attempted oxidation of 1-O-benzoyl-2,3:5,6-di-O-isopropylidene-D-gulitol and -D-allitol with chromium trioxide in pyridine. — A mixture of 1 g of the hexitol derivative, 0.4 g of chromium trioxide, and 25 ml of anhydrous pyridine was heated for 120 min at 60°. The mixture was poured with vigorous stirring into ice–water containing sodium hydrogen carbonate. The suspension was extracted several times with chloroform. The chloroform was dried (magnesium sulfate) and the starting gulitol and allitol derivatives were recovered in yields of 70% and 65%, respectively.

Attempted oxidation of 1-O-benzoyl-2,3:5,6-di-O-isopropylidene-D-mannitol (**2**), *-D-gulitol* (**3**), *and -D-allitol* (**4**) *with methyl sulfoxide, phosphorus pentaoxide, and phosphoric acid.* — A solution of phosphorus pentaoxide in 85% phosphoric acid (1:1, by wt) was prepared. To 20 ml of this mixture, methyl sulfoxide (10 ml) and 1 g of the hexitol derivative were added with stirring. The reaction mixture was kept overnight at room temperature and then poured into an aqueous solution of potassium carbonate, cooled externally. The precipitate obtained was crystallized from ligroin. Compounds **2**, **3**, and **4** were recovered in yields of 70%, 65%, and 65%, respectively.

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STUDIES ON THE SYNTHESIS OF UNSATURATED NUCLEOSIDE ANALOGS*

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ABSTRACT

An unsaturated 6-deoxy-L-hexopyranosyltheophylline diacetate was synthesized, starting from L-rhamnopyranose tetraacetate (**1**), by the fusion procedure with the use of *p*-toluenesulfonic acid, and also through dehydration with phosphorus pentaoxide by way of 2,3,4-tri-*O*-acetyl-L-rhamnopyranose (**2**) in *N*,*N*-dimethylformamide. It was found that 2,3,4-tri-*O*-acetyl-1,5-anhydro-1,6-dideoxy-L-*arabino*-hex-1-enitol (2,3,4-tri-*O*-acetyl-2-hydroxy-L-rhamnal) (**3**) was involved as an intermediate. The following glycal derivatives were condensed with theophylline by the fusion procedure: 2-hydroxy-D-glucal tetraacetate, D-galactal triacetate, 2-hydroxy-D-glucal tetraacetate, D-galactal triacetate, 2-hydroxy-D-galactal tetraacetate (**6**), 2-hydroxy-D-xylal triacetate, L-rhamnal diacetate, and 1,2,4,6-treta-*O*-acetyl-3-deoxy-D-*threo*-hex-2-enopyranose (**7**). The unsaturated nucleoside analogs thus synthesized are described.

INTRODUCTION

The synthesis of an unsaturated nucleoside has been reported by Bowles *et al.*[1] who utilized various glycal derivatives in an acid-catalyzed, fusion reaction. In a similar fashion Leutzinger *et al.*[2] synthesized 9-(2,3-dideoxy-D-*erythro*-hex-2-enopyranosyl)guanine. The introduction of 2′,3′-unsaturation has also been described in the synthesis of pyrimidine[3,4] and purine[5] nucleosides, based largely on the intermediate formation of a 2′,3′-anhydronucleoside or an oxetane ring in a base-catalyzed, elimination reaction. McCarthy *et al.*[6,7] synthesized angustmycin A (decoyinine) from 1′,3′,4′-*O*-orthoformylpsicofuranine.

In the course of our studies on the synthesis of nucleosides[8], it was found that a double bond was formed in the sugar moiety of the products obtained when theophylline was condensed with the tetraacetate **1** by the fusion procedure or when the triacetate **2** reacted with theophylline by the dehydrating procedure. The present paper describes a new reaction for the synthesis of 7-(2,4-di-*O*-acetyl-3,6-dideoxy-α-L-*erythro*-hex-2-enopyranosyl)theophylline (**5**) and the synthesis of several theophylline nucleoside derivatives having 2′,3′-unsaturation.

*Dedicated to the memory of Professor M. L. Wolfrom.

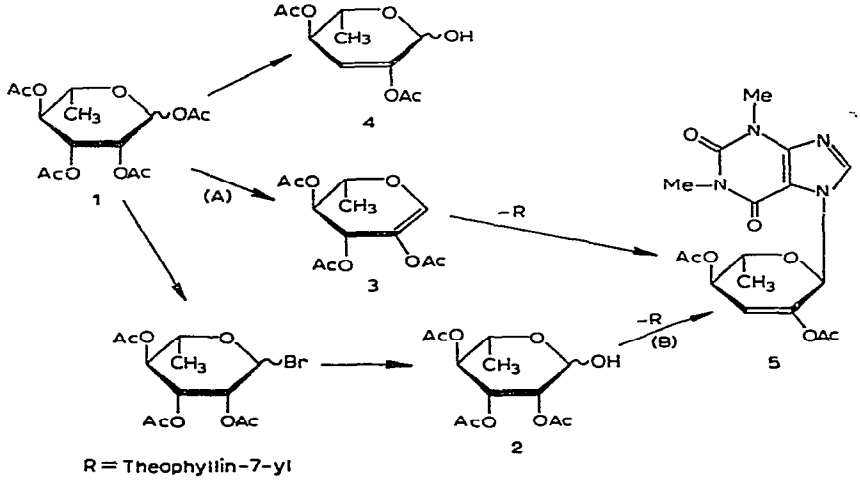
RESULTS AND DISCUSSION

Condensation of **1** with theophylline was effected by fusion in the presence of *p*-toluenesulfonic acid. White, fine needles were obtained in 25% yield. The n.m.r. spectrum of the product showed signals for two acetoxy groups, two *N*-methyl groups, and four protons attributable to the sugar moiety, indicating absence of a proton at C-2′. The anomeric configuration was tentatively assigned on the basis of the observed negative optical rotation and the negative, plain o.r.d. curve.

The position of substitution on the theophylline residue was determined to be *N*-7 by u.v. spectroscopy. The *J* values ($J_{3',4'}$ 2.7 Hz, $J_{4',5'}$ 7.5 Hz) obtained by n.m.r. spectroscopy on **5**, as reported in the previous paper[9], suggest that the double bond is probably between C-2′ and C-3′, and that the acetoxy group is attached to C-2′ (compare the n.m.r. data obtained with 1,2,4-tri-*O*-acetyl-3,6-dideoxy-α-D-*erythro*-hex-2-enopyranose[10]). The analytical results also supported this suggestion. Consequently, the product was identified as 7-(2,4-di-*O*-acetyl-3,6-dideoxy-α-L-*erythro*-hex-2-enopyranosyl)theophylline (**5**). The best yield of **5** in the fusion procedure was obtained by heating the mixture for 30 min at 160–170° under diminished pressure.

Deacetylation of the product with methanolic ammonia caused fission of the glycosidic bond and theophylline was recovered. Compound **5** did not undergo hydrogenation over platinum oxide in methanol, possibly because of steric reasons.

Other addends such as *p*-nitrophenol and 6-benzamidopurine were used in place of theophylline, but no unsaturated condensation products were detected in the reaction mixture. The use of acetylated hexopyranoses in place of **1** gave the usual condensation products and no unsaturated products were detected.



R = Theophyllin-7-yl

(A) By the fusion procedure, (B) By the dehydration procedure

Condensation of **2** with theophylline occurred on heating in *N*,*N*-dimethylformamide for 140 h at 60–70°, with phosphorus pentaoxide as a dehydrating agent.

The reaction product was an unsaturated L-hexopyranosyltheophylline, obtained in 4.5% yield; the product was proved to be identical with **5** produced by the fusion procedure described above.

In order to verify the structure of **5** and to deduce the mechanism of these reactions, some related reactions were investigated. The reaction of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-L-mannopyranose with theophylline by the fusion procedure did not give an unsaturated product, but instead produced a saturated compound 7-(2,3,4-tri-*O*-acetyl-6-deoxy-L-mannopyranosyl)theophylline in 61% yield. The structure of this product was determined from the signals of three acetoxy groups and five protons attributed to the sugar moiety in the n.m.r. spectrum, and from elementary analytical data. It was considered that the substituent at C-5′ took little or no part in the formation of a double bond in the reaction product.

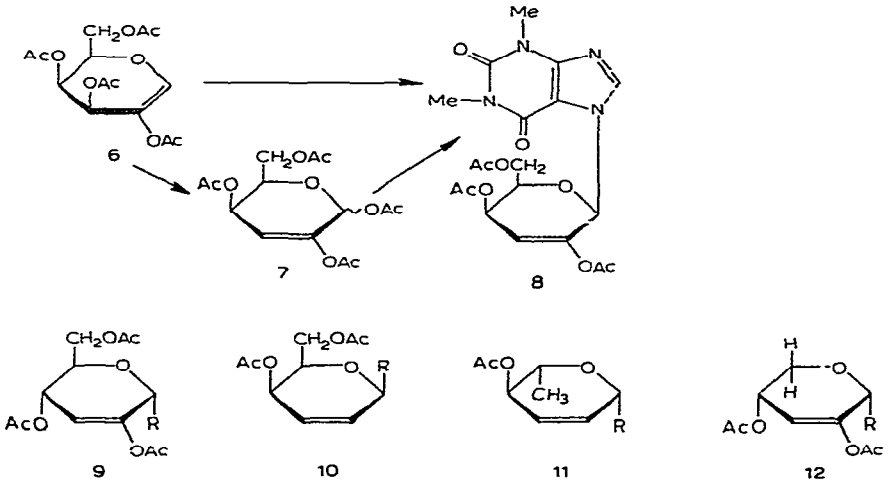
7-(2,3,4-Tri-*O*-acetyl-6-deoxy-α-L-galactopyranosyl)theophylline was prepared by condensation of 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-galactopyranosyl bromide[11] with chloromercuritheophylline in boiling xylene, with subsequent removal of the protecting groups followed by acetylation in pyridine[12]. Although this compound was treated under the same reaction conditions as those used for synthesis of **5**, the compound was recovered unchanged. Therefore, it was considered that the unsaturation was formed in the sugar moiety before *N*-glycosylation. Subsequently, **1** was heated for 15 min at 130–140° in presence of *p*-toluenesulfonic acid under diminished pressure. Two spots [R_F 0.44 (*A*) and R_F 0.69 (*B*)] were observed by t.l.c. on Kieselgel-G with 1:9 (v/v) ethyl acetate–benzene as the developer. The two fractions were isolated as syrups by column chromatography with use of the same solid phase and eluting solvent. The n.m.r. spectrum of the product from fraction *A* showed the signals of three acetoxy groups and four other protons, and the existence of a double bond was indicated by the appearance of u.v. absorption at λ_{max}^{MeOH} 228 nm. It was also found that no specific absorption appeared at 3200–3300 cm^{-1} that could be assigned to a hydroxyl group in the i.r. spectrum. The product showed the same mobility as that of synthetic 2-hydroxy-L-rhamnal triacetate (2,3,4-tri-*O*-acetyl-1,5-anhydro-1,6-dideoxy-L-*arabino*-hex-1-enitol, **3**).

The syrup obtained from fraction *B* was considered to be 2,4-di-*O*-acetyl-3,6-dideoxy-L-*erythro*-hex-2-enopyranose (**4**), as shown by n.m.r., i.r., and u.v. spectral measurements. The n.m.r. spectrum showed that two acetoxy groups and four other protons were present. A hydroxyl group was indicated by the i.r. spectral absorption at 3200–3300 cm^{-1}. A broad absorption at λ_{max}^{MeOH} 228 nm indicated the presence of a double bond in the product.

These results suggest that the double bond in **5** is formed in the sugar moiety before *N*-glycosylation.

Theophylline was condensed with **3** under the same conditions. Apparently degradation of **3** took place to a large extent, and the uncrystallized reaction product was subjected to t.l.c. on Kieselgel-G with 80% butyl alcohol (*i*) and 3:7 (v/v) ethyl acetate–benzene (*ii*) as the developer. The R_F value of the product was 0.66 with solvent (*i*) and 0.54 with solvent (*ii*), respectively. These values are the same as those

of **5** synthesized by the fusion procedure, suggesting, therefore, that *N*-glycosylation takes place between the unsaturated sugar molecule and theophylline.



R = Theophyllin-7-yl

In order to investigate further the structure of the product from **3**, and to attempt to explain the mechanism of formation of a double bond in the sugar molecule, some unsaturated nucleoside analogs were synthesized, by using glycal derivatives. Theophylline was condensed in presence of a catalytic amount of *p*-toluenesulfonic acid with the fully acetylated derivatives of following glycals: 2-hydroxy-D-galactal (**6**), 2-hydroxy-D-glucal, D-galactal, L-rhamnal, 2-hydroxy-D-xylal, and 1,2,4,6-tetra-*O*-acetyl-3-deoxy-D-*threo*-hex-2-enopyranose (**7**); to give 7-(2,4,6-tri-*O*-acetyl-3-deoxy-β-D-*threo*-hex-2-enopyranosyl)theophylline (**8**), 7-(2,4,6-tri-*O*-acetyl-3-deoxy-α-D-*erythro*-hex-2-enopyranosyl)theophylline (**9**), 7-(4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*threo*-hex-2-enopyranosyl)theophylline (**10**), 7-(4-*O*-acetyl-2,3,6-trideoxy-β-L-*erythro*-hex-2-enopyranosyl)theophylline (**11**), 7-(2,4-di-*O*-acetyl-3-deoxy-α-D-*glycero*-pent-2-enopyranosyl)theophylline (**12**), and **8**, respectively.

Further investigations on the mechanism for the formation of unsaturation in the acetylated L-rhamnopyranose molecule are desirable.

EXPERIMENTAL

General methods. — Melting points were measured on a hot stage. Specific rotations were determined with Yanagimoto direct-reading polarimeter. N.m.r. spectra were observed with a Varian A-60 spectrometer at *ca.* 30°. Tetramethylsilane in chloroform-*d* was used as internal standard. Chemical shifts are expressed on the τ scale in p.p.m. and the coupling constants in Hz were obtained by direct measurement of spacings of the spectral lines. Multiplet spacings were measured at a sweep-width of 250 Hz. The concentration of the sample solutions was about 10%. I.r.

spectra were measured with Shimadzu Ar-7 spectrometer. O.r.d. curves were obtained with a Yanagimoto ORD-185 instrument. U.v. spectra were measured with Hitachi multipurpose spectrometer. T.l.c. was performed on Kieselgel-G with 86:14 butyl alcohol–water, or 1:9 and 3:7 ethyl acetate–benzene as developing solvents, and detection was performed with 50% sulfuric acid.

*2,3,4-Tri-*O*-acetyl-6-deoxy-α-*L*-mannopyranose* (**2**). — The syrupy tetraacetate **1** (10 g), prepared by acetylation of L-rhamnopyranose monohydrate with acetic anhydride–pyridine, was dissolved in a mixture of acetic acid (4 ml) and acetic anhydride (4 ml) at 0°. To the solution was added 30 ml of acetic acid saturated at 0° with hydrogen bromide, and the reaction mixture was kept for 3 h at 16°. The reddish solution was poured dropwise into ice–water with vigorous stirring during 1 h. The reaction product was extracted twice with 50 ml of chloroform. The chloroform layer was washed with an aqueous solution of sodium hydrogen carbonate and water, dried over calcium chloride and evaporated *in vacuo* to a syrup. Ether (80 ml) was added to the syrup and the resultant solution was decolorized with activated carbon. Refrigeration of the solution and recrystallization from ether gave fine needles; yield 5.6 g (64%); m.p. 90–91°, $[\alpha]_D^{16}$ −130° (*c* 0.5, chloroform); n.m.r. data (chloroform-*d*): τ 4.83 (3 protons, H-1, H-2, H-3), 5.85 (quartet, H-4), 6.5 (quartet, H-5), 7.85, 7.93, 8.0 (9 protons, OAc), 8.76 (3 protons, doublet, H-6), λ_{max}^{KBr} 3430 (OH), 1730 (C=O), 1225 cm^{-1} (OAc).

Anal. Calc. for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25. Found: C, 49.30; H. 6.61.

*Fusion reaction of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-*L*-mannopyranose* (**1**) *to give* (**3**) *and* (**4**). — Compound **1** (3 g) was heated for 15 min at 130–140° with a catalytic amount of *p*-toluenesulfonic acid under diminished pressure. The reaction mixture was dissolved in a small volume of 1:9 (v/v) ethyl acetate–benzene and subjected to column chromatography (monitored by t.l.c.) to give two fractions.

Evaporation of the first fraction gave **3** as a syrup; n.m.r. data (chloroform-*d*): τ 5.29 (doublet, H-1), 4.8 (doublet, H-3), 4.68 (quartet, H-4), 6.32 (octet, H-5), 7.9, 7.99, 8.05 (9 protons, OAc), 8.72 (3 protons, H-6); ν_{max}^{liq} 1780 (C=O), 1225 cm^{-1} (OAc); λ_{max}^{MeOH} 228 nm. The second fraction gave **4**, also as a syrup; n.m.r. data (chloroform-*d*): τ 4.9 (singlet, H-1), 4.3 (doublet, H-3), 4.8 (quartet, H-4), 6.2 (octet, H-5), 7.83, 7.95 (6 protons, OAc), 8.70 (3 protons, H-6); ν_{max}^{liq} 3450 (OH), 1740 (C=O), 1225 (OAc), 835 cm^{-1} (C=C); λ_{max}^{MeOH} 228 nm.

*7-(2,4-Di-*O*-acetyl-3,6-dideoxy-α-*L*-*erythro*-hex-2-enopyranosyl)theophylline* (**5**). — Syrupy **1** (5 g) and 2.7 g of theophylline were heated at 160–170° in an oil bath. To the molten mixture 0.1 g of *p*-toluenesulfonic acid was added with vigorous stirring. After 30 min at 160–170° under diminished pressure, the mixture was dissolved in a small volume of ethanol, decolorized with active carbon and kept overnight in a refrigerator. The crystalline product was recrystallized twice from ethanol to afford fine needles; yield 1.5 g (25%); m.p. 192–193°, $[\alpha]_D^{21}$ −121° (*c* 0.55, chloroform); λ_{max}^{EtOH} 274 nm, λ_{min}^{EtOH} 244 nm; n.m.r. data (chloroform-*d*): τ 2.12 (1 proton, H-8), 3.25 (broadened singlet, H-1′), 3.90 (quartet, H-3′), 4.69 (octet, H-4′), 6.21 (octet, H-5′), 6.40, 6.59 (MeN-1 and -3), 7.86, 7.91 (6 protons, OAc), 8.76 (3 protons,

doublet, H-6'), ν_{max}^{Nujol} 1770 (C=O), 1695, 1717 (C=O), 1560 (C=C), 1225 (OAc), 840, 835 cm^{-1} (C=C); o.r.d. data (*c* 0.1, chloroform, 20°): negative plain curve.

Anal. Calc. for $C_{17}H_{20}N_4O_7$: C, 52.54; H, 5.11; N, 14.28. Found: C, 52.04; H, 5.12; N, 14.29.

Compound **2** (5 g) and theophylline (4.5 g) were dissolved in 100 ml of *N,N*-dimethylformamide containing 2.5 g of phosphorus pentaoxide. The mixture was heated for 140 h at 60–70° with exclusion of moisture, and the reaction mixture was poured into separatory funnel containing equal volumes of chloroform and ice–water. The chloroform layer was washed with ice–water three times, dried over anhydrous sodium sulfate and evaporated under diminished pressure. The residual syrup was dissolved in a small volume of hot ethanol. After decolorization, the ethanolic solution was kept overnight in a refrigerator. The crystalline product obtained was recrystallized from ethanol; yield 350 mg (4.5%), m.p. 192–193°, $[\alpha]_D^{21}$ −121.3° (*c* 0.55, chloroform); λ_{min}^{EtOH} 244 nm.

Anal. Calc. for $C_{17}H_{20}N_4O_7$: C, 52.04; H, 5.11; N, 14.28. Found: C, 51.95; H, 5.05; N, 14.26.

The n.m.r., i.r., u.v., and o.r.d. data for this compound were identical with those of **5** produced by the fusion procedure.

7-(2,4,6-Tri-O-acetyl-3-deoxy-β-D-threo-hex-2-enopyranosyl)theophylline (**8**). — Compound **6** (7.8 g) and 4.0 g of theophylline were condensed by heating for 20 min at 180–200° in the presence of *p*-toluenesulfonic acid. After treatment of the reaction mixture as described above, white crystals were obtained in 23% yield; m.p. 203–205°, $[\alpha]_D^{22}$ −10.7° (*c* 0.28, chloroform); λ_{max}^{EtOH} 275 nm, λ_{min}^{EtOH} 246 nm; o.r.d. data (*c* 0.1, chloroform, 20°): a negative plain curve; n.m.r. data: τ 2.27 (1 proton, H-8), 3.12 (doublet, H-1'), 3.67 (quartet, H-3'), 4.57 (quartet, H-4'), 5.97 (octet, H-5'), 5.78 (quartet, H-6'), 6.40, 6.58 (MeN-1 and -3), 7.89, 7.93, 8.05 (9 protons, OAc); ν_{max}^{KBr} 1775, 1723 (C=O), 1560 (C=C), 1225 (OAc), 835 (C=C) cm^{-1}.

Compound **7** (5 g) and theophylline (2.8 g) were condensed as described above. The product was obtained in 33% yield and its physical properties were identical with those of compound **8** prepared by the condensation of **6** with theophylline.

Anal. Calc. for $C_{19}H_{22}N_4O_9$: C, 50.66; H, 4.92; N, 12.44. Found: C, 50.53; H, 5.03; N, 12.53.

7-(2,4,6-Tri-O-acetyl-3-deoxy-α-D-erythro-hex-2-enopyranosyl)theophylline (**9**). — 2-Hydroxy-D-glucal tetraacetate (3 g) and theophylline (2 g) were melted at 130° in an oil bath. After the addition of 0.1 g of *p*-toluenesulfonic acid, condensation was performed for 20 min at 130–140° under diminished pressure. To the reaction mixture was added 10 ml of ethanol and the mixture was then decolorized with active carbon. A crystalline product was obtained, yield 1.7 g (42%); m.p. 86–88°, $[\alpha]_D^{26}$ +80° (*c* 1.0, chloroform); λ_{max}^{EtOH} 276 nm, λ_{min}^{EtOH} 245 nm; n.m.r. data (chloroform-*d*): τ 2.08 (1 proton, H-8), 3.17 (singlet, H-1'), 3.84 (quartet, H-3'), 4.39 (octet, H-4'), 5.80 (2 protons, H-6'), 6.13 (octet, H-5'), 6.40, 6.60 (MeN-1 and -3), 7.83, 7.85, 7.96 (9 protons, OAc); ν_{max}^{KBr} 1750, 1710 (C=O), 1550 (C=C), 1220 (OAc), 840, 835 cm^{-1} (C=C); o.r.d. data (*c* 0.1, chloroform, 20°): a positive plain curve.

Anal. Calc. for $C_{19}H_{22}N_4O_9 \cdot 0.5\ H_2O$): C, 49.64; H, 5.05; N, 12.20. Found: C, 49.83; H, 5.13; N, 12.39.

*7-(4,6-Di-O-acetyl-2,3-dideoxy-β-*D-threo-*hex-2-enopyranosyl)theophylline* (**10**). — Condensation of D-galactal triacetate (4.9 g) with theophylline (3.9 g) was effected for 30 min at 140–150° in the presence of *p*-toluenesulfonic acid and under diminished pressure. The reaction mixture was dissolved in 22 ml of ethanol and the crystalline product that formed was recrystallized from ethanol; yield, 12%; m.p. 208–210°, $[\alpha]_D^{22}$ 0° (*c* 0.25, chloroform); λ_{max}^{EtOH} 274 nm, λ_{min}^{EtOH} 245 nm; o.r.d. data (*c* 0.1, chloroform, 20°): a negative plain curve, n.m.r. data (chloroform-*d*): τ 2.22 (1 proton, H-8), 3.09 (H-1′), 4.98 (H-2′), 4.73 (H-3′), 4.55 (1 proton, H-4′), 5.80 (2 protons, H-6′), 6.02 (1 proton, H-5′), 6.42, 6.60 (MeN-1 and -3), 7.82, 7.94 (6 protons, OAc); ν_{max}^{KBr} 1750, 1710 (C=O), 1550 (C=C), 1220 (OAc), 835 cm^{-1} (C=C).

Anal. Calc. for $C_{17}H_{20}N_4O_7$: C, 52.04; H, 5.14; N, 14.28. Found: C, 51.80; H, 4.93; N, 14.13.

*7-(4-O-Acetyl-2,3,6-trideoxy-β-*L-erythro-*hex-2-enopyranosyl)theophylline* (**11**). — The condensation reaction was performed for 15 min at 130–140° in the presence of *p*-toluenesulfonic acid with 1 g of L-rhamnal diacetate and 0.9 g of theophylline; yield, 23.4%; m.p. 85–86°, $[\alpha]_D^{16}$ +71.7° (*c* 0.55, chloroform); λ_{max}^{EtOH} 274 nm, λ_{min}^{EtOH} 247 nm; o.r.d. data (*c* 0.1, chloroform, 20°): a positive plain curve; n.m.r. data (chloroform-*d*): τ 2.22 (1 proton, H-8), 3.30 (H-1′), 5.12 (H-2′), 4.22 (H-3′), 4.67 (H-4′), 5.86 (H-5′), 6.40, 6.60 (MeN-1 and -3), 8.10 (3 protons, OAc), 8.66 (H-6′); ν_{max}^{KBr} 1720, 1690 (C=C), 1220 cm^{-1} (OAc).

Anal. Calc. for $C_{15}H_{18}N_4O_5$: C, 53.88; H, 5.43; N, 16.76. Found: C, 53.88; H, 5.67; N, 16.65.

*7-(2,4-Di-O-acetyl-3-deoxy-α-*D-glycero-*pent-2-enopyranosyl)theophylline* (**12**). — Condensation of 2-hydroxy-D-xylal triacetate (1.9 g) with theophylline (1.35 g) was performed for 20 min at 140° under diminished pressure. The product was obtained amorphous; $[\alpha]_D^{20}$ +15° (*c* 0.1, chloroform); λ_{max}^{EtOH} 276 nm, λ_{min}^{EtOH} 247 nm; o.r.d. data (*c* 0.1, chloroform, 20°): a positive plain curve; n.m.r. data (chloroform-*d*): τ 2.23 (1 proton, H-8), 3.18 (H-1′), 3.80 (H-3′), 4.61 (H-4′), 6.18 (2 protons, H-5_a, 5′), 6.40, 6.60 (MeN-1 and -3), 7.87, 7.89 (6 protons, OAc); ν_{max}^{KBr} 1770, 1710 (C=O), 1550 (C=C), 1230 cm^{-1} (OAc).

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NUCLEOSIDES VI: THE SYNTHESIS AND OPTICAL PROPERTIES OF THE 5'-(ADENIN-9-YL)-5'-DEOXY DERIVATIVES OF THYMIDINE AND 2'-DEOXYADENOSINE*

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ABSTRACT

Two "double-headed" nucleosides, 5'-(adenin-9-yl)thymidine (**3**) and 5'-(adenin-9-yl)-2,5'-dideoxyadenosine (**4**) have been synthesized from a 5'-deoxy-5'-tosyl precursor through displacement of the tosyl group by adenine (sodium salt) in *N,N*-dimethylformamide. The site of attachment of the incoming adenine moiety was established by degradation of **3** to 9-(2-hydroxyethyl)adenine. Some intramolecular base–base interactions in **3** and **4** were indicated by their u.v. and c.d. spectra.

INTRODUCTION

During our previous study of nucleoside derivatives as potential antiviral agents, a group of 5'-deoxy-5'-substituted derivatives of ribosyl, D-*erythro*-pentofuranosyl, and arabinosyl nucleosides were investigated as novel nucleotide analogs that are permeable and nonincorporable[1]. These derivatives possess a polar substituent at C-5', which was selected to mimic the ionic structure and the speculated stereochemistry of the 5'-mono and triphosphates. It was noted that the nature of 5'-substituent exerted a profound influence on the o.r.d. and c.d. spectra. Anomalous circular dichroism (c.d.) bands near 260 nm were displayed by several 5'-adenylic acid derivatives[2], such as adenosine 5'-[(imidazol-1-yl)phosphonate] and adenosine 5'-[(pyrazol-1-yl)phosphonate] and several 5'-thiosubstituted 5'-deoxyadenosines[3], presumably attributable to some interaction between the functional group at C-5' with the adenosine chromophore.

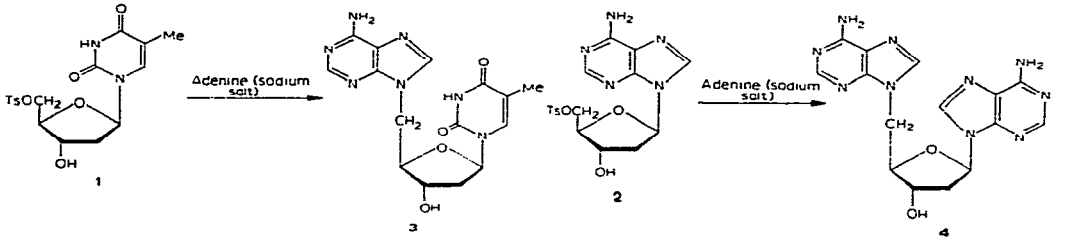
In order to extend this observation we have synthesized two "double-headed" nucleosides, the 5'-(adenin-9-yl)-5'-deoxy derivatives of thymidine (**3**) and 2'-deoxyadenosine (**4**), respectively. The selection of adenine as the 5'-substituent was based on the well-known stacking interaction of two purine or pyrimidine bases *ca.* 3.4 Å apart in oligo or polynucleotides. Models of **3** and **4** show that the two heterocyclic ring-systems at both ends of the 2-deoxy-D-*erythro*-pentofuranose molecule can rotate freely to assume several conformations that would allow maximum π-inter-

*Dedicated to the memory of Professor M. L. Wolfrom.
†To whom all enquiries should be addressed.

actions. In the study of charge-transfer complexes related to nicotinamide adenine dinucleotide, a pronounced interaction was demonstrated when the donor-acceptor pair was linked covalently by two methylene groups[4]. Very recently Brown, Eisinger and Leonard[5] have described a thorough spectrophotometric study of dinucleotide analogs having two bases linked by a flexible oligomethylene chain. The 2′-deoxy-D-*erythro*-pentose moiety in compounds **3** and **4** not only provides a more rigid backbone but also permits comparison of their c.d. and o.r.d. spectra with those of the corresponding dinucleotides.

DISCUSSION

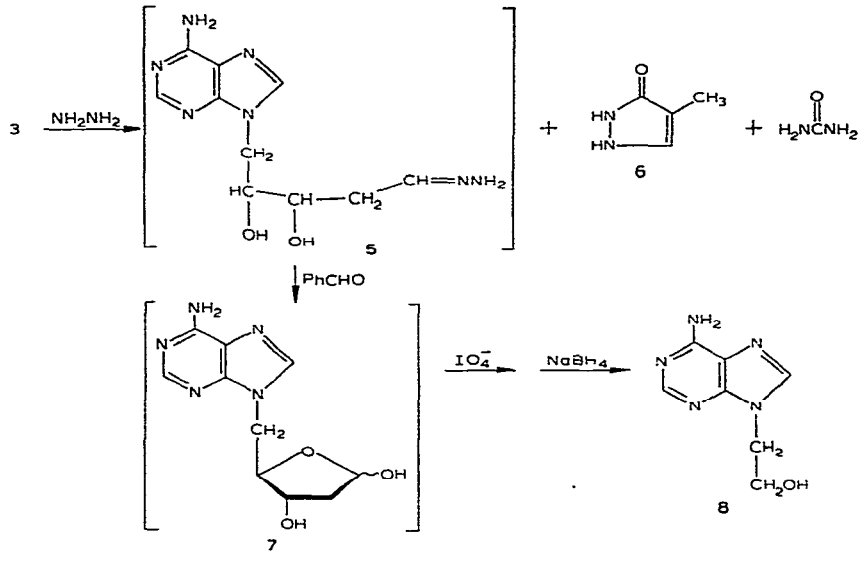
Of the possible synthetic routes available to form "double-headed" nucleosides, direct alkylation of the sodium salt of adenine by preformed 5′-deoxy-5′-*O*-tosyl-nucleosides in *N*,*N*-dimethylformamide proved to be the most convenient.



From the alkylation mixture of **1**, compound **3** was readily isolated as a crystalline monohydrate, m.p. 239–240° in *ca.* 30% yield. In the case of **2**, a substance, seemingly homogeneous, was obtained first. It gave a single spot in three t.l.c. and two paper-strip chromatography systems, and had the empirical formula expected ($C_{15}H_{16}N_{10}O_2$) for **4**. However, the n.m.r. spectrum of this solid indicated extraneous peaks in the region for the signals of H-2–H-8 in adenosine. After careful chromatography a major component (*ca.* 75%) was isolated and identified (see below) as the desired 5′-(adenin-9-yl)-2′,5′-dideoxyadenosine (**4**), m.p. 305–306°. The minor component (*ca.* 25%) is presumably an isomer of **4**, but further investigation will be needed to establish its structure.

It has been demonstrated[5] previously that alkylation of the sodium salt of adenine by alkyl halides in *N*,*N*-dimethylformamide produces mainly substituted adenine at N-9. The n.m.r. spectra of **3** and **4** are indicative of a 2′-deoxy-D-*erythro*-pentofuranose having base substitutions at C-1′ and C-5′. The u.v. spectra of **3** and **4** are relatively insensitive to pH changes, a characteristic of 9-alkyladenines[6], and the u.v. spectrum of **4** is almost identical with that of 5′-(adenin-9-yl)-5′-deoxyadenosine[7] synthesized by an unambiguous route.

To avoid any ambiguity that might arise from the intramolecular interaction of the two u.v.-absorbing chromophores at C-1′ and C-5′, compound **3** was degraded chemically by the following sequence of reactions to a known reference compound, 9-(2-hydroxyethyl)adenine (**8**).



First, the thymine moiety in **3** was eliminated by hydrazinolysis[8]. The resulting hydrazone (**5**) was cleaved with benzaldehyde to liberate the 2′-deoxy-D-*erythro*-pentose derivative (**7**). Treatment of **7** with periodate followed by reduction with borohydride yielded 9-(2-hydroxyethyl)adenine (**8**), identified by direct comparison with an authentic specimen[9]. Both samples gave identical u.v. and i.r. spectra, and identical t.l.c. and paper-strip chromatographic data. In addition, in their mass spectra both samples showed a molecular ion having *m/e* 179 and identical fragmentation patterns. Consequently, it was concluded that the adenine moiety in compound **3** is attached to C-5′ through N-9.

The u.v. spectra of **3** and **4** in comparison with those of their components (thymidine, 2′-deoxyadenosine, and 9-alkyladenine) show a blue shift and hypochromicity[10]. Brown *et al*[5]. have compared for hypochromism the u.v. spectra of the model compounds* $Ad(CH_2)_3Ty$ and $Ad(CH_2)_3Ad$ and their components $Ad(CH_2)_3H$ and $Ty(CH_2)_3H$. Although we have not calculated the hypochromism for **3** and **4**, the extinction coefficients have approximately the same magnitude as the extinction coefficients for $Ad(CH_2)_3Ty$ and $Ad(CH_2)_3Ad$. Presumably, similar base–base interaction could exist in both cases.

The c.d. spectra of **3** and **4** reflect significant changes from the spectra of adenosine (disregarding the 2′-deoxy group), and thymidine. In a similar manner as does ApA, the absorption band near 257 nm in both **3** and **4** gives rise to two pronounced c.d. bands of opposite sign. However, the latter compounds exhibit *negative* c.d. bands at the longer wavelength, as opposed to ApA where the long-wavelength c.d. band is *positive*. Further investigation of the significance of these optical properties is in progress.

*The following abbreviations are used: Ad, adenin-9-yl; Ty, thymin-1-yl; C_3, *n*-propyl; ApA, adenylyl-(3′→5′)-adenosine.

TABLE I

ULTRAVIOLET SPECTRAL DATA

Compound (pH 7)	λ_{max} (*nm*)	$\varepsilon \times 10^3$	λ_{min} (*nm*)	$\varepsilon \times 10^3$	*Ref.*
9-Propyladenine	261.0	14.3	227	2.47	*a*
2′-Deoxyadenosine	259	15.0	225		*b*
4	256	24.9	228	5.2	
$Ad(CH_2)_3Ad$	256	24.4	228.5	2.34	*a*
Thymidine	267	9.65	235		*b*
3	257	19.84	225	6.80	
$Ad(CH_2)_3Ty$	261.5	19.85	232	5.03	*a*

[a]Ref. 5. [b]Handbook of Biochemistry.

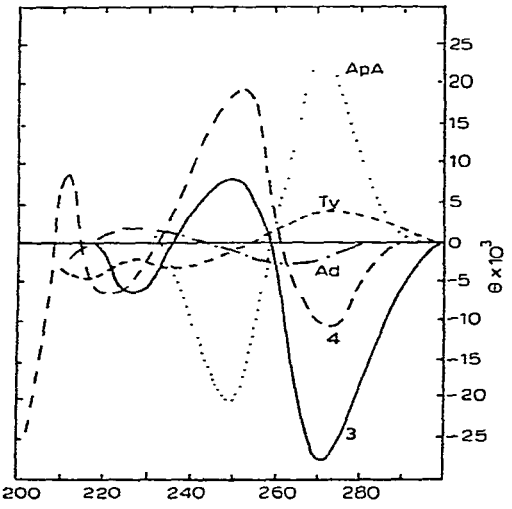


Fig. 1

EXPERIMENTAL

5′-(Adenin-9-yl)-5′-deoxythymidine (**3**). — To a suspension of adenine (2.0 g., 14.8 mmoles) in *N,N*-dimethylformamide (60 ml) under nitrogen at room temperature was added 408 mg (8.35 mmoles) of sodium hydride (52% dispersed in mineral oil) and the mixture was stirred for 2.5 h. 5′-*O*-Tosylthymidine[12] (**1**) (3.2 g, 8.07 mmoles) was added, and the reaction flask was heated in an oil bath for 15–30 min at 75–90°. The reaction was monitored by t.l.c. (silica gel) with 10:1 (v/v) chloroform–methanol; the product was detected as a new u.v.-absorbing spot that also gave a positive Buchanan test[13].

The solvent was evaporated off *in vacuo* and the residue was triturated with 50 ml of ice–water containing a small amount of acetic acid. The precipitate was collected and recrystallized from 275 ml of boiling water to give 0.94 g (31%) of a crude product, m.p. 227–234°. An analytically pure monohydrate (m.p. 239–240°)

was obtained by a second recrystallization from water; u.v. data: $\lambda_{max}^{H_2O}$ 257 (ε 19,840), $\lambda_{min}^{H_2O}$ 235 (ε 6,800), $\lambda_{max}^{.01MNaOH}$ 260 (ε 18,200), $\lambda_{min}^{.01MNaOH}$ 238 (ε 9,100), $\lambda_{max}^{.01MHCl}$ 260 (ε 18,700), $\lambda_{min}^{.01MHCl}$ 233 nm (ε 5,600); n.m.r. data: (Me_2SO-d_6): τ 8.1 (singlet, 3 protons, CH_3), 7.85 (multiplet, 2–3 protons, H-2′), 5.60 (multiplet, 4–5 protons, H-3′, 4′, 5′), 3.81 (triplet, 1 proton, H-1′), 2.80 (singlet, 2 protons, NH_2), 2.63 (singlet, 1 proton, thymine H-6), 1.89 (singlet, 1 proton, adenine H-2 or H-8), 1.80 (singlet, 1 proton, adenine H-2 or H-8), −1.29 (singlet, 1 proton, thymine NH); c.d. data (20% MeOH–H_2O, pH 7): θ_{300} (0), θ_{273} negative max (−27,500), θ_{258} (0), θ_{250} positive max (8,600), θ_{237} (0), θ_{225} negative max (−6,500), θ_{220} (0).

Anal. Calc. for $C_{15}H_{17}N_7O_4 \cdot H_2O$: C, 47.74; H, 5.08; N, 25.98; Found: C, 47.46; H, 4.95; N, 25.64.

5′-(Adenin-9-yl)-2′,5′-dideoxyadenosine (**4**). — To a suspension of the sodium salt of adenine (1 mmole) in *N*,*N*′-dimethylformamide (5 ml) was added 2′-deoxy-5′-*O*-tosyladenosine[14] (**2**) (405 mg, 1 mmole). The thick suspension was heated on a steam bath until solution had occurred and further heated for 0.5 h. The darkened solution was concentrated *in vacuo* at 50° and from the residue was evaporated repeatedly portions of water (3 × 15 ml) and ethanol (2 × 15 ml). The residue was triturated successively with dichloromethane (3 × 15 ml), ether, and methanol (3 × 15 ml) to give 300 mg of a tan solid. Additional solid could be obtained by concentrating the methanolic mother liquors to half volume and keeping them overnight. The crude solid was dissolved in hot water and cooled to a gel, which yielded 150 mg of solid after trituration.

A 60 mg sample of the above solid was chromatographed on three 2-mm Brinkmann silica-gel plates (F-254) with 3:3:2 ethyl acetate–isopropyl alcohol–water as the eluent. The major front-running band (R_F 0.4–0.5) was scraped off and extracted with ethanol overnight to yield a solid, which was recrystallized from hot water to give 23 mg of **4** (14%) as a multiple hydrate (12.9% H_2O). The crystalline hydrate lost water upon heating to 100° and melted at 305–306°; u.v. data: $\lambda_{max}^{H_2O}$ 256 (ε 25,900), $\lambda_{min}^{H_2O}$ 228 (ε 5,200), $\lambda_{max}^{.01MNaOH}$ 255.5 (ε 24,800), $\lambda_{min}^{.01MNaOH}$ 229 (ε 5,500), $\lambda_{max}^{.01MHCl}$ 256 (ε 25,500), $\lambda_{min}^{.01MHCl}$ 229 nm (ε 5,900); n.m.r. data (Me_2SO-d_6): τ 3.60 (triplet, 1 proton, H-1′), 2.70 (singlet, 2 protons, NH_2), 2.82 (singlet, 2 protons, NH_2), 2.09 (singlet, 1 proton, adenine H-2 or H-8), 1.82 (singlet, 2 protons, adenine H-2 or H-8), 1.75 (singlet, 1 proton, adenine H-2 or H-8); c.d. data pH 7, θ_{290} (0), θ_{274} negative max (−10,500), θ_{262} (0), θ_{253} positive max (20,000), θ_{233} (0), θ_{222} negative max (−6,900), θ_{215} (0), θ_{212} positive max (9,000), θ_{209} (0).

Anal. Calc. for $C_{15}H_{16}N_{10}O_2$: C, 48.91; H, 4.38; N, 38.03. Found: C, 49.03; H, 4.42; N, 37.99.

Degradation of **3** *to* **7**. — A solution of 0.5 g of (**3**) in 50 ml of 97% hydrazine was heated for 6 h at 60–65° and evaporated to dryness *in vacuo* at 50°. The residual hydrazine was removed by repeatedly suspending the crude reaction mixture in 25 ml water and evaporating to dryness *in vacuo*. The final residue was triturated with 10 ml of water in an ice-bath, and the insoluble 4-methyl-5-pyrazolone (**6**), (66 mg, $\lambda_{max}^{H_2O}$ 244, λ_{max}^{HCl} 257 nm) was filtered off. The filtrate was shown by t.l.c. to contain a

mixture of unchanged **3**, **5**, and a small amount of **6**. After the addition of 25 ml water and 2 ml of benzaldehyde, enough ethanol was added to give a clear solution. A solid began to precipitate immediately. The suspension was stirred for 12 h and the precipitate (benzalazine, λ_{max}^{EtOH} 302 nm) was filtered off and washed with a small amount of water. The filtrate was combined with the washing and extracted 3 times with 50 ml of ether. The aqueous phase was evaporated to dryness *in vacuo* and triturated with 10 ml of methanol. The supernatant was evaporated to dryness and triturated with methanol again. The process was repeated for three times to yield 125 mg of slightly contaminated 5′-(adenin-9-yl)-2′-deoxy-D-*erythro*-pentose (**7**), which was used in the following experiment without further purification.

9-(2-Hydroxyethyl)adenine (**8**) *from* **7**. — A solution of 213 mg of sodium metaperiodate in 5 ml of water was added to 100 mg of **7** in 4 ml of water. The mixture was stirred for 20 min and then passed through a 10-ml column of Dowex-1 (OAc^-). The column was washed with 100 ml of 20 mM acetic acid and to the combined effluent was added 200 mg of sodium borohydride. After 20 h, 10 ml of M acetic acid was added and the solution was passed through a 10-ml column of Dowex-50-4H (H^+). The column was washed with 100 ml of water and the product was eluted with 100 ml of 5 M ammonium hydroxide[15]. The ammonia solution was evaporated to dryness *in vacuo*, redissolved in water, and evaporated to dryness again. The residue was dissolved in 10 ml of methanol and cooled. The precipitate was collected and tentatively identified as 5′-(adenin-9-yl)-2′-deoxy-D-*erythro*-pentitol (**9**) on the basis of its i.r. spectrum, which was almost identical with that of **8** and also by elementary analysis. The mother liquor was evaporated to dryness and the residue was freed from the remaining 2′-deoxy-D-*erythro*-pentitol derivative by t.l.c. chromatography (2-mm Brinkmann silica-gel plates (F-254) eluted with 1:4 methanol–dichloromethane. The recovered 9-(2-hydroxyethyl)adenine (**8**) was recrystallized* from methanol, m.p. 234–235°.

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*Less than quantitative yields were obtained in all of the degradation steps. To make sure that no minor isomers were overlooked during isolation, each crude reaction mixture was examined by t.l.c. and paper-strip chromatography. Only three u.v.-absorbing chromophores, corresponding to compound **6**, benzalazine, and 9-substituted adenines (that is, **5**, **7**, **8** and **9**), were found.

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SYNTHESIS OF BRANCHED-CHAIN NITRO AND AMINO SUGARS BY THE NITROMETHANE ROUTE*

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ABSTRACT

Addition of 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranos-3-ulose (**1**) to excess nitromethane and 1 molar equivalent of sodium methoxide in methanol gave 5-*O*-benzyl-1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-D-ribofuranose (**2**) in 60% yield. Under essentially the same conditions, 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (**4**) yielded 1,2:5,6-di-*O*-isopropylidene-3-*C*-nitromethyl-α-D-glucofuranose (**5**) in 71% yield. Treatment of the ketose **4** with nitromethane and sodium hydride gave **5** and the *allo*-epimer **5a** in a total yield of 91%. The proof of structure of the branched-chain nitro sugars is described. Selective hydrolysis of **5** gave the partially blocked nitro sugar **6**, which was converted by catalytic hydrogenation into the branched-chain amino sugar **9** (characterized as its *N*-acetyl derivative). Oxidation of **6** with sodium metaperiodate, followed by reduction of the aldehydo-derivative with sodium borohydride, afforded 1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-D-xylofuranose (**7**). Reduction of **7** yielded the partially blocked amino sugar **13**. Compounds **7** and **13** were unblocked to afford the unsubstituted, branched-chain, nitro and amino sugars. The 3-acetate of **5** was converted by a Schmidt–Rutz reaction into a nitroolefin (not isolated), which was hydrogenated with sodium borohydride in ethanol to yield mainly 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-nitromethyl-α-D-glucofuranose (**17**, assigned on basis of its n.m.r. spectrum).

INTRODUCTION

During recent years the nitro sugar derivatives have been much investigated[1,2]. In early work the nitromethane synthesis served as a supplementary synthetic method for preparing higher-carbon aldoses by use of the Nef reaction[3]. From the acetylated nitro alditols, by way of the Schmidt–Rutz[4] reaction, the unsaturated nitro carbohydrates became available, and these in turn, by way of the Nef reaction, provided 2-deoxyaldoses. About a decade later the "sugar dialdehydes" were elegantly cyclized with nitromethane to afford deoxynitro sugar intermediates[5]. When nitroethane was substituted for nitromethane, branched-chain deoxynitro sugars were produced[6].

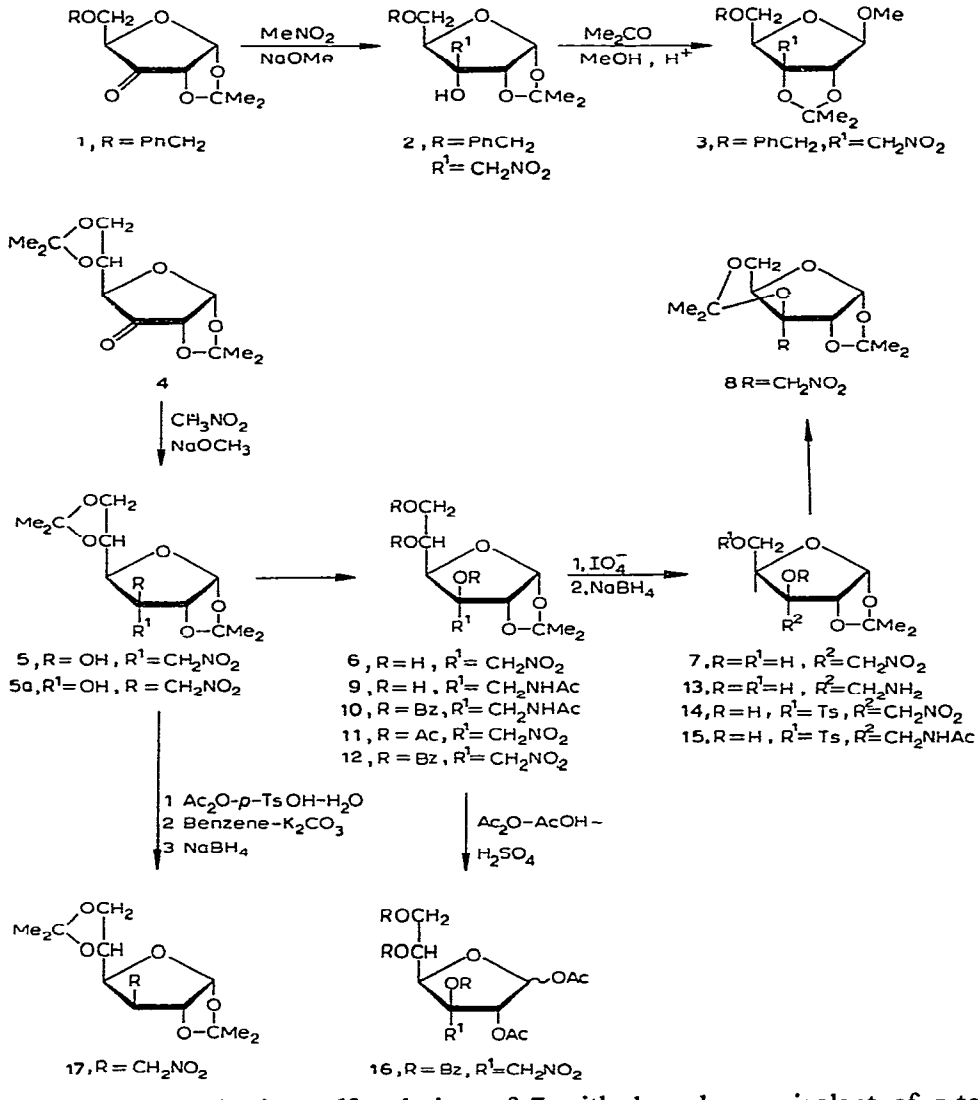
*Dedicated to the memory of Professor M.L. Wolfrom.

Michael addition of nitroalkanes to suitable unsaturated nitro sugar derivatives has yielded branched-chain dinitro sugar derivatives[7], and amination of the unsaturated nitro sugars afforded an entry into the diamino sugars[8]. The utility of nitro sugar derivatives as intermediates in the synthesis of aminodeoxy sugars has been well investigated and reviewed[9]. The wide occurrence of amino sugars as constituents of antibiotics[10], coupled with the recent finding[11] that a nitro sugar (evernitrose) occurs in Nature, led us to begin an investigation[12] of the reaction of nitromethane with suitably blocked 3-oxo sugars in order to prepare branched-chain nitro sugars. A further objective of the research described herein was to prepare branched-chain amino sugars and to use these as intermediates in the synthesis of nucleosides containing branched-chain amino sugars.

RESULTS AND DISCUSSION

Addition of 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranos-3-ulose[13] (**1**) to an excess of nitromethane and 1 molar equivalent of sodium methoxide in methanol for 16 h at room temperature gave the crystalline branched-chain nitro sugar **2** in 60% yield, and together with a minor component (not isolated), which was readily removed by fractional crystallization or by column chromatography on silicic acid. The configuration at C-3 in **2** was deduced from the fact that **2** readily condensed with acetone to yield a methyl 2,3-*O*-isopropylidene glycoside, thus indicating the presence of a cis glycol at C-2 and C-3. The assignment of the β-configuration to **3** was made by n.m.r. spectroscopy on the basis of the low value (~0 Hz) of the $J_{1,2}$ coupling constant. Therefore, compound **3** is undoubtedly methyl 5-*O*-benzyl-2,3-*O*-isopropylidene-3-*C*-nitromethyl-α-D-ribofuranose, and **2** must be 5-*O*-benzyl-1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-D-ribofuranose. Interestingly, the i.r. spectrum of **2** (5mM in carbon tetrachloride) showed hydroxyl peaks at 3560 and 3430 cm^{-1}, thus indicating intramolecular hydrogen bonding[14] of the free 3-hydroxyl group with an oxygen atom of the 1,2-*O*-isopropylidene group. The configuration at C-3 of **2** is consistent with the known steric control exerted by the 1,2-*O*-isopropylidene group toward a nucleophilic reagent[15].

Quite unexpectedly, the addition of nitromethane to 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose[16](**4**) yielded 1,2:5,6-di-*O*-isopropylidene-3-*C*-nitromethyl-α-D-glucofuranose (**5**), in 71% yield. In the preliminary communication[12], **5** was erroneously assigned, on the basis of mechanistic considerations, the *allo* configuration. Although the assigment of the *gluco*-configuration to **5** is not consistent with the reported steric control exerted by 1,2-*O*-isopropylidene groups[15], nor with the conversion of **1** into **2**, it is supported strongly by the following evidence. Selective hydrolysis of **5** afforded **6** having a free terminal glycol group. Compound **6** was degraded with sodium metaperiodate, with subsequent reduction of the *aldehydo* intermediate with sodium borohydride, to yield 1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-D-xylofuranose (**7**). This nitro sugar exhibited a positive circular dichroism (c.d.) spectrum, whereas **3**, in contrast, showed a negative c.d. maximum, thus indicating that **7** and **3** are epimeric[17] at C-3. Compound **5**, therefore, has the *gluco*-



configuration. Selective sulfonylation of **7** with 1 molar equivalent of *p*-toluenesulfonyl chloride gave a monosulfonate (**14**). The n.m.r. spectrum of **14** in anhydrous dimethyl sulfoxide-d_6, showed a hydroxyl peak as a singlet at τ 3.8 (lost on addition of D_2O), thus confirming the presence of a free, tertiary hydroxyl-group on C-3, and thus **14** must be the 5-*p*-toluenesulfonate. Comparison of the n.m.r spectrum of **14** with the spectrum reported[12b] for 1,2-*O*-isopropylidene-3-*C*-nitromethyl-5-*O*-tosyl-α-D-ribofuranose showed the two to be different, thus indicating that compound **14** has the *xylo*-configuration, and therefore **5** must have the *gluco*-configuration*.

*Lourens[12b] reported the application of nitromethane to 1,2-*O*-isopropylidene-5-*O*-triphenylmethyl-α-D-*erythro*pentofuranos-3-ulose.

Reduction of **14** over palladium on charcoal, followed by acetylation, gave the *N*-acetyl derivative **15**, which failed to cyclize to form a sugar having nitrogen in the ring[18]. In contrast, the corresponding *ribo* compound of Lourens[12b] readily yielded such a product. Additional positive evidence corroborating the hypothesis that **7** had the *xylo* configuration was provided by the fact that **7** readily condensed with acetone to form 1,2:3,5-di-*O*-isopropylidene-3-*C*-nitromethyl-α-D-xylofuranose (**8**), and therefore, **5** must be 1,2:5,6-di-*O*-isopropylidene-3-*C*-nitromethyl-α-D-glucofuranose.

When the *ribo* ketose **4** was allowed to react with nitromethane in 1,2-dimethoxyethane in the presence of about 0.1 molar equivalents of sodium hydride, a mixture of two C-3 epimeric [(gluco (**5**) and allo (**5a**)] branched-chain nitro sugars (90% yield) was formed, in the ratio of about 4:1. Preparative t.l.c. of the mixture on silica gel, with benzene–ethyl acetate as developer, gave pure **5** and impure **5a** as evidenced by the n.m.r. spectrum of the latter.

Hydrogenation of the partially blocked nitro sugars **6** and **7** over palladium on charcoal in the presence of hydrochloric acid readily yielded the branched-chain amino sugars **9** and **13**. Accompanying contaminants were removed by passage of the amino sugars through Dowex-50 W-X resin, followed by elution of the resin with dilute ammonium hydroxide. The amino sugar from **6** was characterized as the *N*-acetyl derivative **9** and as the tribenzoate **10**. Direct reduction of the blocked nitro sugar **5** with lithium aluminum hydride in tetrahydrofuran also gave the amino sugar (as evidenced by a positive ninhydrin test). Three side products were formed, which could not be separated.

The unsubstituted, branched-chain nitro sugar was obtained by hydrolysis of **7** with aqueous trifluoroacetic acid. On the basis of n.m.r. spectroscopy it was deduced that the free sugar probably existed as an anomeric mixture of furanose and pyranose forms[21]. Reduction of the free nitro sugar in dilute aqueous hydrochloric acid afforded the hydrochloride of the branched-chain amino sugar. On the basis of its n.m.r. spectrum ($J_{1,2}$ = 7 Hz) it was inferred that the sugar existed in solution mainly as 3-*C*-aminomethyl-β-D-xylopyranose hydrochloride.

Because the acetamido derivative **10** was required as an intermediate in the synthesis of nucleosides of branched-chain amino sugars, an attempt was made to remove the 1,2-*O*-isopropylidene group by acetolysis. Although acetolysis removed the 1,2-*O*-isopropylidene group, the 1-acetoxy group appeared to be ejected (as evidenced by the n.m.r. spectrum of the acetolysis product) by the participating *N*-acetyl group at C-3. Such participation, which is well documented in the chemical literature[18], led us to abandon the use of the branched-chain amino sugar as an intermediate in the attempted synthesis of a nucleoside containing a branched-chain amino sugar.

An alternative approach to the synthesis of a nucleoside of a branched-chain amino sugar appeared to be *via* use of the 1,2-diacetate of the branched-chain nitro sugar, followed by reduction of the nitro group in the nucleoside. As a consequence, the blocked nitro sugar **12** was converted, *via* acetolysis, into the 1,2-diacetate **16**. Repeated attempts to condense **16** with 6-benzamido-9-chloromercuripurine to yield

a nucleoside, by the titanium tetrachloride method[19] or by a fusion method, were unsuccessful.

Application of the Schmidt–Rutz reaction to the 3-acetate of **5**, with potassium carbonate[12b] as base in refluxing benzene, gave a nitroolefin. The latter was immediately reduced with sodium borohydride in ethanol to yield a mixture of two 3-deoxy nitro sugars, which were separated by column chromatography on silica gel, with benzene–ethyl acetate as developer. The major component, isolated in about 20% yield, was presumed to be 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-nitromethyl-α-D-glucofuranose (**17**) on the basis of its n.m.r. and c.d. spectra. The n.m.r spectrum of **17** showed two doublets of equal coupling at τ 4.02 and 5.25. Irradiation of the H-1 doublet (at τ 4.02) collapsed the doublet at τ 5.25 to a singlet, thus establishing the latter as the H-2 signal. The H-3 signal occurred as a multiplet at τ 7.65. It has been previously established[19] that H-2 of 3-deoxyfuranose derivatives having the *ribo* or *allo* configurations exhibits a triplet because of the coupling of H-2 with the cis protons at H-3 and H-1. On the other hand, H-2 of the corresponding glucofuranose derivatives exhibits a doublet[20] (the trans $J_{2,3}$ coupling is either not measurable or is less than 0.7 Hz). This evidence, coupled with the facts that the n.m.r. spectrum of **17** was different from that of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-nitro-methyl-α-D-allofuranose[21] and the c.d. spectrum of **17** gave a positive maximum, indicated that **17** had the *gluco*-configuration[17]. Attempts to prepare the 3-deoxy branched-chain nitro sugar by application of the Schmidt–Rutz reaction to the 3,5,6-triacetate **11** were unsuccessful.

EXPERIMENTAL

General. — N.m.r. spectra were obtained in chloroform-*d* solution (unless otherwise stated), with tetramethylsilane as the internal standard (set at τ 10) by using a Jeolco 60 or Varian HA-100 spectrometer (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). Mass spectra were recorded with an A.E.I.MS-9 spectrometer. I.r. spectra were obtained with a Perkin–Elmer Model 457 spectrometer. The c.d.[17] spectra were measured with a Jasco Model ORD/UV-5 spectropolarimeter. All melting points (micro hot-stage) are corrected. Solutions were concentrated *in vacuo*. Silica Gel G was used for t.l.c. and column chromatography. Elemental analyses were performed by the microanalytical laboratory of the University of British Columbia.

*Reaction of 5-*O*-benzyl-1,2-*O*-isopropylidene-α-*D*-erythro-pentofuranos-3-ulose* (**1**) *with nitromethane to yield 5-*O*-benzyl-1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-*D*-ribo-furanose* (**2**). — A M solution of sodium methoxide in methanol (1.45 ml, 1.45 mmoles) was added dropwise with stirring to a solution of compound **1** (0.400 g, 1.45 mmoles) in 6 ml of anhydrous nitromethane. The reaction mixture was stirred for 16 h at room temperature and then was deionized with Rexyn 101 (H^+) resin (prewashed with cold methanol) and the filtrate was evaporated to a syrup. Crystallization of the syrup from ethyl acetate–petroleum ether (b.p. 30–60°) gave 0.093 g of a crystalline product contaminated with a minor component, as evidenced by t.l.c. with 3:1 benzene–ethyl

acetate as developer. One further recrystallization gave pure compound **2**. Column chromatography of the residue from the mother liquor, with 9:1 benzene–ethyl acetate as developer, gave an additional 0.150 g of pure **2** (60% yield), m.p. 103–104°, $[\alpha]_D^{22}$ +41° (*c* 2, chloroform); *v* (0.005 molar CCl_4) 3560 (s), 3430 (w, OH), 1550 (NO_2); τ^{CDCl_3} 4.12 (d, H-1, $J_{1,2}$ 3.8 Hz), 5.1–5.5 (m), 5.42 (s, CH_2 Ph), 5.6–6.0 (m), 6.3 (d, *J* 4 Hz), 4.75 (OH), 8.40 and 8.60 (CH_3).

Anal. Calc. for $C_{16}H_{21}NO_7$: C, 56.63; H, 6.24; N, 4.13. Found: C, 56.80; H, 6.14; N, 4.25.

Methyl 5-O-benzyl-2,3-O-isopropylidene-3-C-nitromethyl-β-D-ribofuranoside (**3**). — A solution of 50 mg of **2** in anhydrous acetone (1 ml) was treated with 1 ml of 3% (w/w) hydrogen chloride in methanol. The solution was kept for 3 days at room temperature after which time none of **2** remained, a trace of free sugar was present, and a fast-moving, major component **3** was formed (monitored by t.l.c. with 4:1 benzene–ethyl acetate as developer). The reaction mixture was neutralized with solid sodium hydrogen carbonate, filtered, and the filtrate was evaporated. The residue was dissolved in water (5 ml) and the aqueous solution was extracted with chloroform (2 × 5 ml). The chloroform extract was dried (magnesium sulfate) and evaporated to a syrup (0.056 g). This syrup was chromatographed on 5 g of silica gel (activity II), with 98:2 benzene–ethyl acetate as developer, to give 0.023 g of the acetal **3**, $[\alpha]_D^{22}$ −49° (*c* 1, chloroform); c.d. (*c* 0.0004, methanol): $[\theta]_{279}$ −1440; τ^{CDCl_3} 2.8 (C_6H_5), 5.07 (an AB system, $J_{a,b}$ 12.5 Hz, methylene protons a and b on C-1′), 5.2 (s, H-1), 5.38 (s, H-2), 5.52 (s, CH_2Ph), 5.4–5.6 (overlapping signals of one H), 6.5 (H-5), 6.70 (OCH_3), 8.5, 8.7 (CMe_2).

Anal. Calc. for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.53; N, 3.96. Found: C, 58.23; H, 6.80; N, 3.88.

Addition of 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranose-3-ulose (**4**) *to nitromethane to yield 1,2:5,6-di-O-isopropylidene-3-C-nitromethyl-α-D-glucofuranose* (**5**). — A solution of M sodium methoxide in methanol (1.95 ml, 1.95 mmoles) was added dropwise with stirring to a solution of **4** (0.5 g, 1.95 mmoles) in 5 ml of nitromethane. The reaction mixture was stirred for 16 h at room temperature and then deionized, and the filtrate then evaporated to a syrup. Crystallization from petroleum ether (b.p. 60–110°) gave 0.430 g (71%) of pure, crystalline nitro derivative **5**, m.p. 138–140°, $[\alpha]_D^{22}$ +31° (*c* 2, chloroform); *v* (CCl_4) (5 mM), 3560 (s) (OH), 1560 (NO_2); τ^{CDCl_3} 4.05 (d, H-1, $J_{1,2}$ 3.5 Hz), 5.13 (an AB system, $J_{a,b}$ 12.5 Hz, methylene protons a and b on C-1′), 5.38 (d, H-2, $J_{1,2}$ 3.5 Hz), 5.5–6.3 (m), 6.50 (OH), 8.40, 8.55 (two CH_3), 8.62 and 8.66 (two CH_3).

Anal. Calc. for $C_{13}H_{21}NO_8$: C, 48.89; H, 6.63; N, 4.39. Found: C, 48.73; H, 6.49; N, 4.54.

1,2:5,6-Di-O-isopropylidene-3-C-nitromethyl-α-D-allofuranose (**5a**) +**5**. — Compound **4** (0.513 g) was added to a magnetically stirred solution of anhydrous nitromethane (0.5 ml) and anhydrous 1,2-dimethoxyethane ("glyme", 1 ml) containing sodium hydride (~0.025 g). The reaction was performed at room temperature in a dry box under nitrogen. After 24 h the reaction mixture was neutralized with IR-120

(H^+) resin, filtered (resin washed with glyme), and the filtrate then evaporated to a syrup. Crystallization from ether–petroleum ether (b.p. 30–60°) gave 0.55 g (91%) of a crystalline mixture of **5** and **5a**, in the ratio of 4:1. The mixture was partially separated by preparative t.l.c., with 4:1 chloroform–ether as developer, to give pure **5** and impure **5a** (contaminated with **5**). Attempts to prepare pure **5a** were unsuccessful. N.m.r. data for **5a**: τ^{CDCl_3} 4.10 (d, H-1, $J_{1,2}$ 4 Hz), 4.97 (AB system, $J_{a,b}$ 12 Hz, CH_2NO_2), 5.37 (d, H-2, $J_{1,2}$ 4 Hz), 5.4–6.1 (m), 5.9 (broad OH, overlapping multiplet), 6.45 and 6.63 (two s), 8.37, 8.55, 8.6 (CMe_2).

Partial hydrolysis of **5** *to yield 1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-*D*-glucofuranose* (**6**). — To a solution of 1 g of **5** in 15 ml of methanol was added 3 ml of 10% sulfuric acid. The reaction mixture was kept for 2 h room temperature, and then neutralized with barium carbonate, and filtered through Celite. The filtrate was concentrated to a syrup. The residue was dissolved in hot ethyl acetate and filtered through Celite to remove traces of inorganic material. After evaporation of the solvent the residue (0.834 g) was crystallized twice from dichloroethane to give 0.654 g of **6**, m.p. 96–97°, $[\alpha]_D^{22}$ +72° (*c* 3, water); ν(5mM CCl_4) 3650 (w), 3560(s), 3430 (w) (OH); τ^{D_2O} 4.0 (d, H-1, $J_{1,2}$ 4 Hz), 4.95 (AB system with $J_{a,b}$ 11 Hz, CH_2NO_2).

Anal. Calc. for $C_{10}H_{17}NO_8$: C, 42.99; H, 6.14; N, 4.90. Found: C, 42.91; H, 6.22; N, 4.99.

Degradation of **6** *with sodium metaperiodate and reduction to yield 1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-*D*-xylofuranose* (**7**). — To a well-stirred solution of **6** (0.500 g, 1.8 mmoles) in 10 ml of water was added sodium metaperiodate (0.385 g, 1.8 mmoles). The reaction was monitored by t.l.c. (4:1 benzene–ethanol) and by tests with potassium iodide paper. After the oxidation was complete the solution was concentrated, and ethanol was added to precipitate the inorganic material. The filtrate was evaporated to dryness and the residue was twice treated with ethanol and the solvent was evaporated. The residue was then extracted with chloroform (3 × 20 ml). The extract was dried (sodium sulfate) and treated with a small portion of charcoal, and filtered. Evaporation of the filtrate gave a syrup, which was dried by distilling benzene from it, to afford 0.385 g (87% yield) of a syrup, ν (Nujol) 1710 cm^{-1} (CHO). The aldehydo sugar was dissolved in ethanol (5 ml) and reduced with sodium borohydride (0.200 g added during 10 min). The reaction mixture was stirred for 1 h at room temperature and then deionized with an ethanolic mixture of IR-120 (H^+) resin. The filtrate was evaporated to dryness and the residue was thrice treated with methanol, with subsequent evaporation of the solvent, to yield 0.375 g of a syrup. Crystallization (twice) from ether–petroleum ether (b.p. 30–60°) gave **7**, m.p. 101–103°, $[\alpha]_D^{27}$ +32° (*c* 2, chloroform); c.d. (*c* 0.0004, methanol: $[\theta]_{280}$ +2480; ν(5 mM CCl_4), 3640 (w), 3600 (m), 3440 (s) OH); τ^{D_2O} 4.0 (d, H-1, $J_{1,2}$ 4 Hz), 5.16 (an AB system $J_{a,b}$ 12.5 Hz, of methylene protons a and b of CH_2NO_2), 5.28 (H-2, $J_{1,2}$ 4 Hz), 5.4 (DOH), 5.4–6.3 (3-proton m), 8.6 (2-CH_3).

Anal. Calc. for $C_9H_{15}NO_7$: C, 43.37; H, 6.06; N, 5.62 Found: C, 43.46; H, 5.89; N, 5.48.

*1,2:3,5-Di-*O*-isopropylidene-3-*C*-nitromethyl-α-*D*-xylofuranose* (**8**). — A solution

of 0.067 g of **7** in 10 ml of acetone and 2 ml of 2,2-dimethoxypropane was treated with 0.260 g of di-*p*-nitrophenyl hydrogen phosphate and the mixture was stirred for 2 days at room temperature. The course of the reaction was followed by t.l.c. in 10:70:2 benzene–ethyl acetate–methanol; the R_F of **8** was 0.4. The reaction solution was added to 3 ml of 0.1M sodium hydrogen carbonate and the mixture was then extracted with three 10-ml portions of chloroform. After evaporation of the chloroform the residue was chromatographed [2:3 ether–light petroleum ether (b.p. 30–60°)]. Fractions containing the product were pooled and concentrated to a syrup (0.042 g), which crystallized from petroleum ether (b.p. 60–110°), m.p. 83–85°, $[\alpha]_D^{22}$ +50° (*c* 2, chloroform); τ^{CDCl_3} 3.98 (d, H-1, $J_{1,2}$ 4 Hz), 5.14 (AB system, $J_{a,b}$ 12 Hz, CH_2NO_2), 5.35 (d, H-2, $J_{1,2}$ 4 Hz), 5.95 (H-4 and H-5), 8.45–8.62 (CMe_2).

Anal. Calc. for $C_{12}H_{19}NO_7$: C, 49.80; H, 6.58; N, 4.84; Found: C, 49.81; H, 6.60; N, 4.69.

Attempted reduction of **5** *with lithium aluminum hydride.* — When compound **5** (1 g), dissolved in anhydrous tetrahydrofuran (100 ml), was reduced with lithium aluminum hydride (2 g) in 100 ml of tetrahydrofuran for 1 h under reflux and the product worked up in the usual way, a mixture of four compounds (basic and ninhydrin-positive) was obtained. An attempt to separate the components by chromatography was unsuccessful. When the reduction was allowed to proceed for 4 h under reflux the products were neutral and ninhydrin-negative.

Catalytic reduction of **6** *followed by acetylation to yield 3-C-acetamidomethyl-1,2-O-isopropylidene-α-*D*-glucofuranose* (**9**). — The nitro sugar **6** (0.500 g), dissolved in a mixture of water (5 ml) and 1.8 ml of M hydrochloric acid, was hydrogenated in the presence of prehydrogenated platinum oxide (0.25 g) for 16 h. The uptake of hydrogen was 128 ml (calc. value 120 ml). After filtration the solution was passed through a column of Dowex-50W X-8 (H^+) (10 ml). The resin was washed with water until the eluent was neutral and chloride-free. Elution of the column with 0.6 M ammonium hydroxide (100 ml), followed by evaporation of the basic eluent, gave 0.371 g of a syrup consisting of a major component, $R_{\text{2-amino-2-deoxyglucose}} = 2.1$ and one minor component $R_{Gl} = 1.0$ [pyridine–water–ethyl acetate–acetic acid in trough (5:3:5:1, v/v) and pyridine–ethyl acetate–water (11:20:6) in the tank][22]. The major component was ninhydrin positive, τ^{D_2O} 4.1 (d, H-1, $J_{1,2}$ 4.0 Hz), 7.06 (d, CH_2NH_2). The amino sugar (0.51 g) was immediately acetylated with acetic anhydride (0.5 ml) in the presence of methanol for 3 h at room temperature (until it was ninhydrin-negative). After evaporation of the volatile components, the residue was treated successively with ethanol, toluene, and ethanol, followed by subsequent evaporations, to yield 0.43 g (72%) of a syrup. An amount of 0.120 g of this syrup was chromatographed on 10 g of silica gel, with 9:1 benzene–methanol as developer, to yield 0.062 g of a major component as a glass having $[\alpha]_D^{22}$ +90° (*c* 3, chloroform); τ^{CDCl_3} 4.16 (d, H-1, $J_{1,2}$ 3 Hz), 3.24 (broad, NH), 7.97 (s, CH_3 of acetamido group), 8.5, 8.7 (CMe_2).

Anal. Calc. for $C_{12}H_{21}NO_7 \cdot H_2O$: C, 46.61; H, 7.49; N, 4.53. Found: C, 47.04; H, 7.50; N, 4.66.

3-C-Acetamidomethyl-3,5,6-tri-O-benzoyl-1,2-O-isopropylidene-α-D-glucofuranose (**10**). — To a solution of 0.43 g of anhydrous **9** in 8 ml of anhydrous pyridine was added with mixing 0.9 ml of freshly distilled benzoyl chloride. The mixture was kept overnight at room temperature and a few drops of water were added to dissolve the pyridine hydrochloride. The mixture, diluted with 10 ml of chloroform, was washed consecutively with saturated sodium hydrogen carbonate (5 ml), water (5 ml), and it was then dried (sodium sulfate). Evaporation of the filtrate gave 1.02 g of syrup, which was chromatographed on 70 g of silica gel, with 96:4 benzene–ethyl acetate as developer. The major component was an unstable glass (0.49 g), $[\alpha]_D^{22}$ +29° (*c* 4, chloroform); τ^{CDCl_3} 3.8 (broad, NH), 4.01 (d, H-1, $J_{1,2}$ 3.5 Hz), 4.41 (d, H-2), 8.01 (acetamido methyl), 8.5 and 8.75 (CH_3).

Anal. Calc. for $C_{33}H_{33}NO_{10}$: C, 65.67; H, 5.51. Found: C, 67.24; H, 5.40.

Acetolysis of compound **10**. — To a well-stirred solution (kept at 0°) of **10** (0.39 g) in glacial acetic acid (5 ml) and acetic anhydride (0.7 ml) was added dropwise 0.25 ml of concentrated sulfuric acid. The product was worked-up in the usual way[19] to yield 0.32 g of a syrup, which was chromatographed on a column of 20 g of silica gel, with 9:1 benzene–ethyl acetate as developer. The major zone (0.205 g) was fractionally crystallized from benzene–petroleum ether (b.p. 30–60°) to give crystals (0.061 g) that were homogeneous by t.l.c., m.p. 186–187°, $[\alpha]_D^{22}$ −115° (*c* 1, chloroform); *ν*(Nujol) 1730, 1760, 1667 (possibly C=N), τ^{CDCl_3} 2–3 (m, 15 H), 3.4 (d, H-1, $J_{1,2}$ 2 Hz), 4.18 (d, H-2), 5.15 (s, two H), 7.96 (s, three H).

Anal. Calc. for $C_{32}H_{29}NO_{10}$: C, 65.4; H, 4.93; N, 2.39; mol. wt. 588. Found: C, 67.30; H, 5.10; N, 2.60. Mass spectrum: *m/e* 589, 588, 543, 527, 526, 427, 426, 407, 406, 405, 381, 380, 347, 346, 345, 321, 320, 319, 308, 305, 304.

3,5,6-Tri-O-benzoyl-1,2-O-isopropylidene-3-C-nitromethyl-α-D-glucofuranose (**12**). — Compound **6** (0.30 g) was benzoylated in the usual way to give a syrup (0.574 g, 90% yield). The crude benzoate, contaminated with a trace of impurity, was chromatographed with 97:3 benzene–ethyl acetate as developer; $[\alpha]_D^{22}$ +54° (*c* 1, chloroform); τ^{CDCl_3} 7.87–2.95 (3 Ph groups), 3.98 (d, H-1, $J_{1,2}$ 3.5 Hz), 4–4.85 (m), 5.1–5.23 (m), 5.4 (d, *J* 3.5 Hz), 8.48, 8.68 (CH_3).

Anal. Calc. for $C_{31}H_{29}NO_{11}$: C, 62.92; H, 4.94; N, 2.37. Found: C, 62.70; H, 4.99; N, 2.45.

1,2-Di-O-acetyl-3,5,6-tri-O-benzoyl-3-C-nitromethyl-α,β-glucofuranose (**16**). — Acetolysis of **12** (0.35 g) by the procedure already described gave 0.260 g (81%) of mainly the β-anomer as a syrup, which was chromatographed on 15 g of silica gel with 19:1 benzene–ethyl acetate as developer, to afford a glass consisting of a mixture of the α and β anomers in the ratio of about 1:5; $[\alpha]_D^{22}$ 0° (*c* 1, $CHCl_3$), τ^{CDCl_3} 3.8 (s, H-1 of β anomer), 3.5 (d, H-1 of α anomer, $J_{1,2}$ 4 Hz), 7.89, 7.94, 7.97 (equal to six acetyl protons).

Anal. Calc. for $C_{32}H_{29}NO_{13}$: C, 60.47; H, 4.59; N, 2.20. Found: C, 60.27; H, 4.58; N, 2.30.

3-C-Aminomethyl-1,2-O-isopropylidene-α-D-xylofuranose (**13**). — The degraded nitro sugar 7 (0.200 g) was reduced in 80% yield by the same procedure as described

for the reduction of **6**. The crystalline seed crystals of the amino sugar were obtained by first passing the crude product through Dowex-50W X8 (H^+) resin, followed by crystallization from ethanol–petroleum ether (b.p. 30–60°). In subsequent reductions the crude syrup was treated with Rexyn 201 (OH^-) resin only, followed by crystallization from the above solvents with seeding; m.p. 126–128°, $[\alpha]_D^{22}$ +56° (*c* 1, water); τ^{D_2O} 3.96 (d, H-1, $J_{1,2}$ 3.5 Hz), 5.4 (d, H-2, $J_{1,2}$ 3.5 Hz), 7.04 (AB system, $J_{a,b}$ 14 Hz, CH_2NH_2), 8.4 and 8.6 (CH_3).

Anal. Calc. for $C_9H_{17}NO_5$: C, 49.31; H, 7.81; N, 6.38. Found: C, 48.96; H, 7.72; N. 6.33.

3-C-Nitromethyl-D-xylose. — The partially blocked nitro sugar **7** (0.1 g) was treated with 2 ml of 75% trifluroroacetic acid and kept overnight at room temperature. The progress of the reaction was monitored by t.l.c., with 4:1 chloroform–ether as developer. After total disappearance of **7**, the reaction mixture was evaporated with several additions of water until free from acid; final evaporation gave a syrup (70 mg), $[\alpha]_D^{22}$ +26° (*c* 3, water); τ^{D_2O} 4.18 (d, H-1, $J_{1,2}$ 3.8 Hz), 4.85 (s, CH_2NO_2), 5.0 (d, H-2, $J_{1,2}$ 3.8 Hz), 4.6 (s), overlapping peaks in region 4.6–5.1, 5.2 (HOH), 5.5–6.3 (m, equal to 3 H).

Anal. Calc. for $C_6H_{11}NO_7$: C, 34.45; H, 5.30; N, 6.69. Found: C, 34.55; H, 5.81; N, 6.12.

3-C-Aminomethyl-D-xylose hydrochloride. — An amount of 0.075 g of 3-*C*-nitromethyl-D-xylose was reduced in 4 ml of 0.1M hydrochloric acid in the presence of 0.040 g of prehydrogenated platinum dioxide. When the hydrogen uptake had ceased, the solution was filtered and the filtrate evaporated to a syrup (0.070 g) with three evaporations with water; τ^{D_2O} 5.42 (d, H-1, $J_{1,2}$ 7 Hz), 6.15 (s, CH_2NH_2), 6.38 (d, H-2, $J_{1,2}$ 7 Hz), 6.4–6.8 (m, equal to 3 H). The amino sugar hydrochloride was deionized in the usual way through a column of Dowex-50W X8 (H^+) resin to yield a syrup; τ^{D_2O} 4.6–5.1 (broad peak overlapping with HOH peak at τ 5.2), 5.6 (two equal overlapping doublets with *J* 7 Hz), 4.2 (s, CH_2NH_2), 4.2 (overlapping signal equal to 1 H), 6.2–7.2 (m, equal to 3 H).

1,2-O-Isopropylidene-3-C-nitromethyl-5-O-p-tolylsulfonyl-α-D-xylofuranose (**14**). — To a solution of 0.100 g of nitro sugar **7** in 2 ml of anhydrous pyridine was added 0.102 g (1.1 molar equivalent) of *p*-toluenesulfonyl chloride. The reaction mixture, protected from moisture, was stirred for 4 h and monitored by t.l.c. with 4:1 chloroform–ether as developer. Two separate portions of toluene were added to the reaction mixture and were evaporated off to remove pyridine. The residue was extracted with chloroform (10 ml), and the chloroform extract washed with aqueous sodium hydrogen carbonate, water and then dried (sodium sulfate). Evaporation of the filtrate gave a solid residue, which was crystallized from ethanol–petroleum ether (b.p. 30–60°) to give 0.140 g of the sulfonate **14**, m.p. 133–134°, $[\alpha]_D^{22}$ +7° (*c* 1, chloroform); τ^{CDCl_3} 4.1 (d, H-1, $J_{1,2}$ 3.5 Hz), 5.35 (d, H-2, $J_{1,2}$ 3.5 Hz), and AB system centered at 5.28, $J_{a,b}$ 12.5 Hz (methylene protons a and b on C-1'), 5.8 (d, *J* 2.4 Hz), 5.8 (overlapping multiplet), 6.7 (OH), 7.55 (p-$CH_3C_6H_5$) (Irradiation at τ 4.1 collapsed

the doublet at τ 5.35 to a singlet); $\tau^{(CD_3)_2SO}$, 3.8 [1 H, singlet lost on addition of D_2O (OH)], 4.13 (d, H-1, $J_{1,2}$ = 3.5 Hz), 5.22 (s, CH_2NO_2), 5.42 (d, H-2, $J_{1,2}$ 3.5 Hz).

Anal. Calc. for $C_{16}H_{21}NO_4S$: C, 47.63; H, 5.24; N, 3.47. Found: C, 47.49; H, 5.10; N, 3.45.

3-C-Acetamido-1,2-O-isopropylidene-5-O-p-tolylsulfonyl-α-D-xylofuranose (**15**). — The monosulfonate **14** was hydrogenated overnight with hydrogen over palladium-on-charcoal in acidified ethanol. Subsequent acetylation of the product with acetic anhydride and pyridine for 2 days at room temperature gave a mixture of two products, which was chromatographed on a column, with 95:5 chloroform–ether as developer. The faster-moving, major component **15** was isolated as an oil, τ^{CDCl_3} 2–2.7 (aromatic protons), 4.1 (d, H-1, $J_{1,2}$ 3.8 Hz), 4.4–5.3 (broad peak), 5.52 (d, H-2, $J_{1,2}$ 3.8 Hz), 5.6–6.5 (complex multiplet), 7.5 (s, $CH_3C_6H_4$), 7.84 (s, equal to 3 H, *Ac*N).

The second, minor component (very low mobility on t.l.c.) could not be eluted from the column.

Schmidt-Rutz reaction of 3-O-acetyl-1,2:5,6-di-O-isopropylidene-3-C-nitromethyl-α-D-glucofuranose to yield 1,2:5,6-di-O-isopropylidene-3-deoxy-3-C-nitromethyl-α-D-glucofuranose (**17**). — The nitro sugar **5** (0.45 g) was acetylated with acetic anhydride (2.5 ml) and *p*-toluenesulfonic acid (0.10 g) for 15 h at room temperature. The product was worked up in the usual way to yield 0.550 g of an unstable syrup, which was immediately allowed to react with anhydrous potassium carbonate (0.50 g) in benzene (20 ml) for 2 h under reflux. After removal of the potassium carbonate and benzene the nitroolefin was reduced with sodium borohydride (0.100 g) in ethanol for 2 h. The solvent was evaporated and the product was extracted into chloroform. The extract was washed with water and evaporated to a syrup, which was chromatographed on a column, with 10:1 benzene–ethyl acetate as developer, to yield two fractions. The slower-moving, major fraction (0.095 g) crystallized from ethanol–water, m.p. 108–110°, $[\alpha]_D^{24}$ +60° (*c* 2, chloroform); c.d. (*c* 0.00076, methanol): $[\theta]_{280}$ +1530; τ^{CDCl_3} 4.02 (d, H-1, $J_{1,2}$ 4 Hz), 5.1 (AB system, $J_{a,b}$ 14 Hz, CH_2NO_2), 5.25 (d, H-2, $J_{1,2}$ 4 Hz), 5.8–6.4 (two sets of multiplets equal to 4 hydrogens, spikes at τ 5.82 and possibly poorly resolved doublet at τ 6.2), 7.65 (m, equal to 1 H, H-3, presumed to be H-3), 8.4–8.62 (CMe_2); irradiation at τ 4 collapsed the doublet at τ 5.25 to a singlet. The n.m.r. spectrum of **17** was different from that of 1,2:5,6-di-*O*-isopropylidene-3-deoxy-3-*C*-nitromethyl-α-D-allofuranose as reported by Jones and co-workers[21].

Anal. Calc. for $C_{13}H_{21}NO_7$: C, 51.5; H, 6.9; N, 4.6. Found: C, 48.6; H, 6.5; N, 4.3. A trace of inorganic ash was present.

The minor, fast-moving component (0.030 g) was a syrup, τ^{CDCl_3} 4.02 (d, H-1, $J_{1,2}$ 3.8 Hz), 5.15 (AB system, $J_{a,b}$ 14 Hz, CH_2NO_2), 5.28 (d, H-2, $J_{1,2}$ 3.8 Hz), 5.7 (s, overlapping m), 5.95 (m) (from 5.7–6.0 = 4 protons), 7.9 (m, equal to 1 H), 8.43–8.6 (CMe_2).

5,6-Di-O-acetyl-1,2-O-isopropylidene-3-C-nitromethyl-α-D-glucofuranose. — The partially blocked nitro sugar **6** (0.50 g) was treated with acetic anhydride (10 ml) and

boron trifluoride etherate (5 drops) for 1 h at room temperature. The acetic anhydride was removed by evaporating toluene from the product. The product was chromatographed, with 9:1 benzene–methanol as eluant, to yield 0.140 g of material that crystallized from ethanol–light petroleum ether (b.p. 30–60°), m.p., 159–160° $[\alpha]_D^{22}$ +53° (*c* 2, methanol); τ^{CDCl_3} 4.0 (d, H-1, $J_{1,2}$ 4.0 Hz), 5.35 (d, H-2, $J_{1,2}$ 4.0 Hz), 6.2 (OH, lost on addition of D_2O), 7.90 and 7.93 (two acetyl peaks). In methyl sulfoxide-d_6 the hydroxyl peak appeared as a sharp singlet.

Anal. Calc. for $C_{14}H_{21}O_{10}N$: C, 46.3; H, 5.78; N, 3.85. Found: C, 46.7; H, 5.71; N, 3.72.

Attempted conversion of 3,5,6-tri-O-acetyl-1,2-O-isopropylidene-3-C-nitromethyl-α-D-glucofuranose (**11**) *into 5,6-di-O-acetyl-3-deoxy-1,2-O-isopropylidene-3-C-nitromethyl-α-D-glucofuranose.* — The partially blocked nitro sugar **6** (0.50 g) was treated with acetic anhydride (10 ml) and *p*-toluenesulfonic acid (0.33 g) for 15 h at room temperature. The product was added to ice–water and than extracted in the usual way with chloroform; yield, 0.540 g (syrup); τ^{CDCl_3} 3.98 (d, H-1, $J_{1,2}$ 4 Hz), 7.90 and 7.93 (three acetyl groups), 8.46 and 8.62 (CMe_2). The product was not stable and was not analyzed.

The triacetate **11** (0.50 g) was treated with anhydrous potassium carbonate (0.30 g) in anhydrous benzene (25 ml) for 3 h under reflux. After removal of the potassium carbonate the benzene was evaporated and the resulting syrup was then reduced with sodium borohydride (0.070 g) in acetonitrile (15 ml) and water (3 ml). The reaction mixture was kept neutral by addition of 20% acetic acid. T.l.c. of the product showed a complex mixture of products, which could not be separated.

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MICROHETEROGENEITY AND PAUCIDISPERSITY OF GLYCOPROTEINS

PART I. THE CARBOHYDRATE OF CHICKEN OVALBUMIN*†

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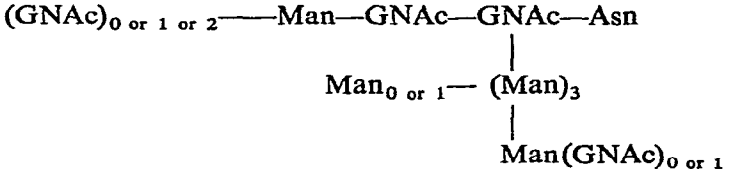
ABSTRACT

Five components of L-β-aspartamido–carbohydrate, prepared from crystalline chicken ovalbumin by digestion with pronase, have been isolated and purified by a combination of ion-exchange chromatography and zone electrophoresis. Their sequences may be represented:



homology being demonstrated by enzymic hydrolysis with α-D-mannosidase (E.C.3.2.1.24) or *N*-acetyl-β-D-glucosaminidase (E.C.3.2.1.30). The molecular dispersity of ovalbumin is discussed in the light of these results.

INTRODUCTION

In a discussion of the molecules present in a preparation of a biopolymer it is necessary to consider, among other things, if (*i*) there are different biopolymer molecules present that are quite unrelated to each other, (*ii*) all the molecules have the same number of monomer units linked together in an identical fashion, (*iii*) the number of monomer units varies in a polymer otherwise identically constructed, (*iv*) some of these monomer residues in the biopolymer are derivatized by the addition of a small group, such as an alkyl, acyl, or glycosyl residue, (*v*) the biopolymer molecules differ in their intramolecular cross-linking so as to give rise to different molecular species, and (*vi*) the biopolymers have more than one stable conformation. These questions relate to the description of the population of molecules in a preparation from some living system, and the answers are necessary in order to know how definitive any elucidation of the primary, covalent structure can be. Statistical

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descriptions of the population may be derived without knowledge of the covalent structure from the hydrodynamic properties of the system. The covalent structure must be determined to give meaning at a molecular level to the various dispersities possible in a biopolymer preparation.

This paper describes an investigation of the carbohydrate structures in ovalbumin by the separation of five L-β-aspartamido–carbohydrate* components, and discusses the molecular dispersity of the glycoprotein in defined terms.

EXPERIMENTAL

Ovalbumin. — Ovalbumin was prepared and crystallized four times, as described previously[1,2], from the fresh eggs of Hy-Line White Leghorns. Five dozen eggs yielded 50–60 g of ovalbumin.

Assay of glycosidases. — Crystalline α-D-mannosidase[3] and *N*-acetyl-β-D-glucosaminidase[4], both kindly supplied by Dr. Y. T. Li, were assayed against 2 mM solutions of the *p*-nitrophenyl glycopyranosides of 2-amino-2-deoxy-β-D-glucose at pH 5.0 and α-D-mannose at pH 4.5 at 25° for 2 min.

Analytical methods. — Hexoses were determined in the carbohydrate-containing fractions without previous acid hydrolysis by the phenol–sulfuric acid method, as described previously[5,6]. Reducing sugars[7], 2-acetamido-2-deoxy-D-glucose[8], and amino acids[9] or amino sugars[9], were determined by the procedures indicated. The specific contents of amino acids and 2-amino-2-deoxy-D-glucose in the glycopeptides were analyzed with a Spinco Model 120C amino acid analyzer, the samples being hydrolyzed with hydrochloric acid at 105° in sealed tubes under nitrogen, 4 to 6 h with 4N acid for the amino sugar and 20 h with 6N acid for the amino acids. It had been determined that these were the optimum conditions of hydrolysis for the materials under study.

Column effluents were monitored continuously[10].

Column preparation. — Cation-exchange resin AG-50W x2 (Bio-Rad Laboratories, 200–400 mesh) was converted into the hydroxyl form by stirring with 2M sodium hydroxide. The resin was then washed successively with distilled water to remove most of the excess alkali and sodium acetate buffer, pH 2.6 and mM in Na^+. A column (0.9 × 150 cm) of this resin was prepared and equilibrated with the sodium acetate buffer for at least two days at room temperature (20–25°) and a constant flow-rate of 27.5 ml/h of buffer. Although the temperature seemed to play little part in the fractionation between 20 and 50°, the temperature was kept reasonably constant (±1°) during the chromatographic separations.

Analyses of the glycopeptides of ovalbumin containing up to 5 mg of D-mannose were carried out on the above column. Samples containing up to 275 mg of D-mannose were separated on a column (2 × 150 cm), prepared similarly but run at a buffer flow-rate of 60 ml/h.

*The mixture of L-β-aspartamido–carbohydrate components from ovalbumin will be abbreviated AC, with each of the five components being represented AC-A, AC-B, AC-C, AC-D, and AC-E.

Preparation of L-*β-aspartamido–carbohydrate components of ovalbumin.* — A solution of ovalbumin, containing 113 g of protein in 2 liters of water (after dialysis until freed from sodium sulfate) was adjusted to pH 7.8 with dilute sodium hydroxide and heated for 15 min at 80–82°. The solution of denatured protein was cooled to room temperature and to it was added 660 ml of 0.2M Tris buffer, which was also 0.04M in calcium chloride. The pH was adjusted to 7.4, the mixture layered with toluene and brought to 40°. Soon after adding the buffer and calcium chloride, the highly concentrated solution of denatured ovalbumin set to a gel, which gradually dissolved after adding pronase (600 mg). Further additions of pronase (600 mg) were made after 6 h and 12 h and the hydrolyzate was centrifuged after 24 h. The insoluble residue was suspended in 500 ml of 0.05M Tris buffer, pH 7.4, containing 0.01M calcium chloride, and incubated at 40° with pronase (200 mg). After 24 h any insoluble residue was again isolated, re-treated with pronase (100 mg) and after 24 h the supernatent liquid was combined with the previous solutions of hydrolyzate and freeze-dried. The dry residue was dissolved in 0.1M acetic acid (350 ml), centrifuged, and the clear liquid was applied to a column (4 × 64 cm) of Sephadex G-25 in 50-ml portions, as described previously[11]. The fractions containing hexose were combined and freeze dried. The recovery of hexose was 81%.

The glycopeptides so produced were treated three more times with pronase as follows. The freeze-dried glycopeptides were dissolved in Tris buffer (200 ml) as before and treated with pronase (400 mg) for 24 h at 40°. The resulting solution was centrifuged, gel-filtered in 50-ml portions, and the fractions containing hexose were combined and freeze-dried. The final product (9.7 g) contained the equivalent of 1.5 g of D-mannose, which represented an overall recovery of hexose of 65%.

The glycopeptide mixture was fractionated on a column (2 × 150 cm) of AG-50W x2 resin, which had been prepared and equilibrated with starting sodium acetate buffer, pH 2.6, mM in Na^+. Fractions (15 ml) of effluent were collected until all the AC had been eluted. The glycopeptides with additional amino acid residues, principally L-leucine, were then eluted with 0.05M sodium acetate, pH 6.0. Fig. 1 shows analyses of two ovalbumins, one prepared in our laboratory (identified Pirkl) and one kindly provided by Dr. E. D. Kaverzneva. Peaks F were eluted with the front and the fractions of AC are identified A through E. Peak G contains those glycopeptides having several amino acid residues. The total recovery of D-mannose from the fractionation procedure was 95%, of which about 30% was AC. At this stage, the amounts of the fractions of AC, expressed as their contents of D-mannose, were AC-A, 23 mg; AC-B, 48 mg; AC-C, 166 mg; AC-D, 125 mg; and AC-E, 76 mg. Additional amounts of AC can be obtained by further treatment of the other glycopeptide fractions with pronase[12].

The separated fractions of AC were each rechromatographed on AG-50W x2 resin as described before until they were completely free from each other, as determined by analysis on the analytical column. An amino acid analysis of the purified fractions showed that AC-D and AC-E were completely free from all but aspartic acid. AC-A, AC-B, and AC-C contained contaminating peptides, which were

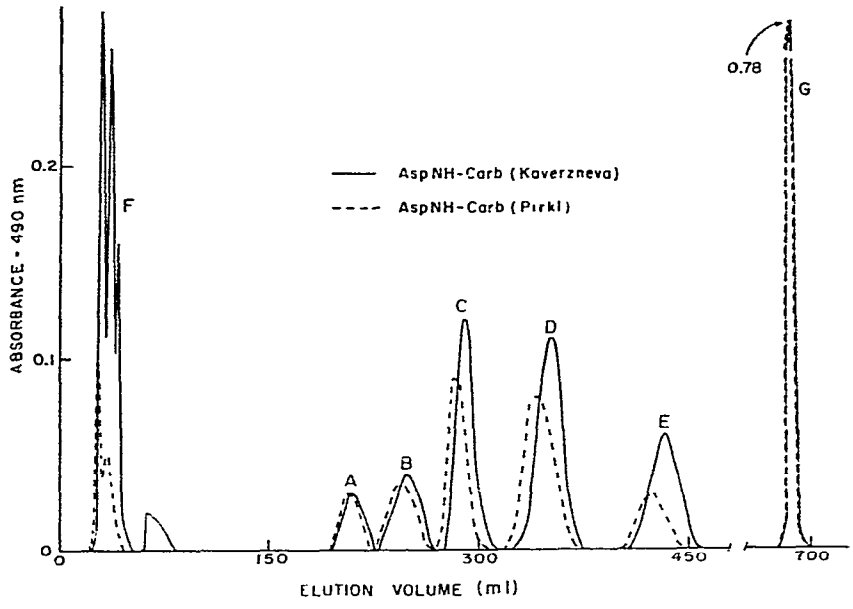


Fig. 1. Fractionation of the L-β-aspartamido–carbohydrates A to E from two sources of chicken ovalbumin on a column of AG-50W x2 resin, 0.9 × 150 cm. Elution was accomplished with sodium acetate buffer, pH 2.6, mM in Na^+ up to 600 ml and with 0.05M sodium acetate, pH 6.0 up to 750 ml. For details of F and G, see text.

removed by zone electrophoresis on a column of ethanolyzed and sodium borohydride-reduced cellulose in 0.1M pyridine acetate, pH 6.4, exactly as described previously[12]. The fractions of AC-A, AC-B, and AC-C so purified were freeze-dried, dissolved in 0.1M acetic acid, and gel-filtered on a column (1.6 × 100 cm) of superfine Sephadex G-25 to remove any trace of pyridine. The composition of the AC-components is given in Table I.

Action of N-*acetyl-β-*D-*glucosaminidase on the components of AC.* — *N*-Acetyl-β-D-glucosaminidase (10 units)[13], kindly supplied by Dr. Y. T. Li, was added to each AC-fraction (containing about 2.6-mg equivalents of D-mannose) dissolved in 0.05M citrate buffer (5 ml) pH 5.0. The reaction, under an atmosphere of toluene, was followed at 25°, by periodically withdrawing aliquots (25 μl). The latter were heated for 3 min on a boiling water bath, evaporated to dryness under diminished pressure, and the free 2-acetamido-2-deoxy-D-glucose was determined in the residue. When the rate of hydrolysis slowed down, more enzyme (5 units) was added until the release of hexosamine stopped. A control experiment was followed concomitantly and the results are summarized in Fig. 2.

At the end of the reaction, the nature of the reducing sugars was checked chromatographically and only 2-acetamido-2-deoxy-D-glucose was detected.

Action of α-D-*mannosidase on the components of AC.* — To each AC-fraction (containing about 2.6-mg equivalents of D-mannose) in 5 ml of 0.05M citrate buffer, pH 4.5, at 25°, was added α-D-mannosidase (25 units). The reactions were continued

TABLE I

COMPOSITION AND ENZYMIC HYDROLYSIS OF THE L-β-ASPARTAMIDO–CARBOHYDRATES FROM CHICKEN OVALBUMIN

AC-Component	*Mole % of AC*	*Composition*[a]		*Molecular weight*		*Sugar residues hydrolyzed by*	
		Man	*GNAc*	*Amino acid analysis*[b]	*Formula*[c]	*α-D-Mannosidase*	*N-Acetyl-β-D-glucosaminidase*
A	5.0	6.01	5.00	2142	2121	1.0	2.9
B	12.5	5.10	5.08	2022	1959	0.0	3.0
C	35.8	5.92	4.05	1970	1918	1.1	2.0
D	27.0	5.94	2.18	1634	1650	4.7	0.4
E	19.7	5.01	1.96	1387	1349	3.8	0.0

[a]Moles per mole of L-aspartic acid. [b]Weight of component that hydrolyzes to give one mole of L-aspartic acid. [c]Empirical formula weight.

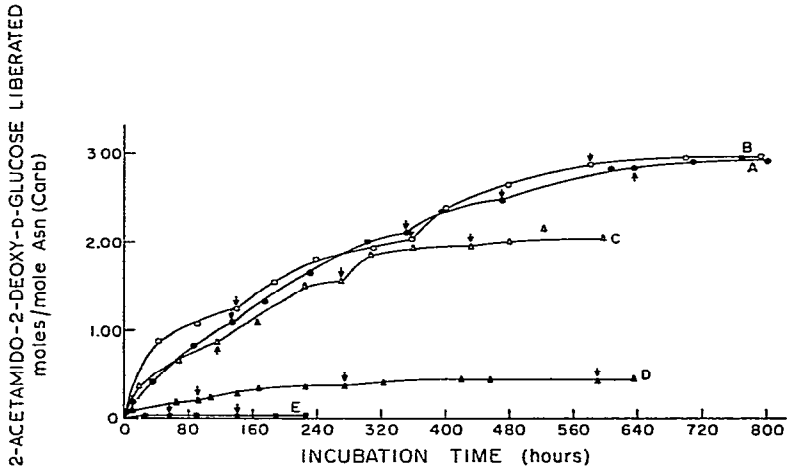


Fig. 2. Rate of hydrolysis of the L-β-aspartamido–carbohydrates by *N*-acetyl-β-D-glucosaminidase. Vertical arrows indicate additions of fresh enzymes.

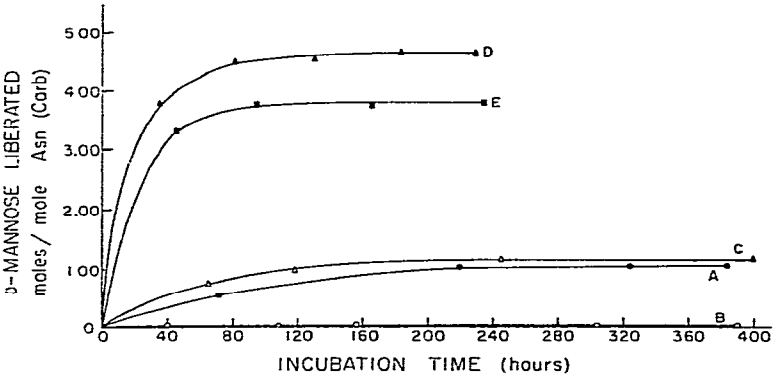


Fig. 3. Rate of hydrolysis of the L-β-aspartamido–carbohydrates by α-D-mannosidase.

in an atmosphere saturated with toluene to prevent bacterial contamination. Periodically, aliquots (5 μl) of the reaction solutions were removed, heated for 3 min in a boiling water bath and evaporated to dryness under diminished pressure. The D-mannose in the residues was determined. The results are summarized in Fig. 3. As determined by paper chromatography, no reducing sugars other than D-mannose were produced.

RESULTS AND DISCUSSION

Ovalbumins from the egg-whites of various birds have been shown to be different immunologically[14] and differ in their amino acid compositions[15,16]. The crystalline ovalbumin from several varieties of chicken has been most extensively studied and can be separated by electrophoresis[17] or ion-exchange chromatography[18] into three fractions that appear to differ only in their content[19] of phosphate ester, the carbohydrate compositions being the same[1]. This microheterogeneity can be removed by enzymic hydrolysis of the phosphate groups[19].

It is well known that the carbohydrate of chicken ovalbumin is present as a single oligosaccharide attached to a unique asparaginyl residue in the polypeptide chain[20]. The carbohydrate, isolated after proteolytic digestion of ovalbumin as the L-β-aspartamido–carbohydrate, was studied by the classical methods of carbohydrate chemistry. Three kinds of structure were proposed[21–23] before it was shown definitely by Cunningham[24,25] that the carbohydrate was a mixture, as suggested earlier[26,27], separable into five fractions by an ion-exchange procedure. The method has been refined further in the present study, the most critical factors in the separation being the prior equilibration of the ion-exchange column and the pH and ionic strength of the starting buffer (sodium acetate). The buffer system was changed from pyridine acetate to reduce the problems of subsequent analyses[10]. The pH of the buffer, as measured potentiometrically with a glass electrode and a reference calomel electrode, should be 2.5 to 2.6, with the cation concentration not exceeding mM. Extensive equilibration of the ion-exchange resin improved the separation of the peaks, the actual volumes of each peak varying somewhat with the length of the equilibration time. Column temperatures up to 50° did not change the peak separations significantly.

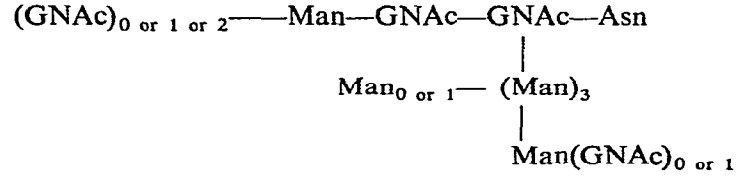
In order to obtain a clear separation of the AC-peaks from the proteolysis mixture it was necessary to carry out four treatments with pronase, after which the L-β-aspartamido–carbohydrate was well separated from the other glycopeptides and fractionated into the five components, identified as AC-A, AC-B, AC-C, AC-D, and AC-E. These fractions were rechromatographed until free from each other but it was necessary to subject AC-A, AC-B, and AC-C to zone electrophoresis[12] to remove the last traces of contaminating peptides. Each of the final products contained no amino acid other than L-asparagine and the carbohydrate was constituted of various proportions of D-mannose and 2-acetamido-2-deoxy-D-glucose, as summarized in Table I. These values differed from those reported by Cunningham[25] who chose not

to pursue the fractionation to completion. Each fraction behaved in a homogeneous fashion by ion-exchange chromatography, zone electrophoresis, ultracentrifugation, and countercurrent distribution of the dinitrophenyl derivatives[28]. Also, the molecular weights, as determined by either the spectrophotometric analysis of the 2,4,6-trinitrobenzene-1-sulfonic acid derivatives[29] or by ultracentrifugation, were within 3% of the formula weight (Table I).

It may be noted that the order of elution of the AC-components from the ion-exchange column was in the order of their molecular size. This molecular-sieve effect is similar to that reported for similar columns in a Li^+ form[30]. The larger glycopeptides, which included Asn(Carbohydrate)-Leu[12], were eluted later at a higher pH and ionic strength. Preliminary experiments have not separated the component carbohydrate–peptides into fractions that would correspond to those in AC.

Although Cunningham had given evidence to suggest that the different oligosaccharide chains in AC were not artefacts of isolation[25] the question of their origin is still properly raised[31], particularly since α-D-mannosidase and *N*-acetyl-β-D-glucosaminidase were reported in the eggwhite of chicken[32]. In this regard it has been shown[28]: (*a*) the relative amounts of the AC-components were not dependent upon the age of the egg, (*b*) every genetic variant of chicken showed the five components in AC, (*c*) the ovalbumins of several birds, including turkey that has no *N*-acetyl-β-D-glucosaminidase in the eggwhite, showed fractions in the AC, (*d*) a retreatment of pure AC-C and AC-D with pronase, under the conditions used for their preparation from ovalbumin, did not produce any degradation or other fraction of AC. These observations suggest strongly that the series of oligosaccharides in AC was the product of biosynthesis and not the degradation of one or two oligosaccharides.

The interrelationships of the carbohydrate groups in the components of AC were studied by the enzymic hydrolysis of each compound with the specific exo-hydrolases α-D-mannosidase and *N*-acetyl-β-D-glucosaminidase. Control experiments produced no D-mannose or 2-acetamido-2-deoxy-D-glucose. The rates of hydrolysis and the times at which fresh enzyme was added to ensure a complete reaction are shown in Figs. 2 and 3. The results, summarized in Table I, are consistent with the sequence of sugar residues in the oligosaccharide chains shown in **1**.



1

The structures represented in **1** are compatible with many of the observations reported earlier on the AC mixture[21–23], with the exception of the preliminary communication[33] in which less highly purified enzymes are used.

When the mole ratios of the components of AC (Table I) are taken into account, there is an average of 1.3 terminal hexosamine residues per mole of AC, approximately that noted earlier[21,34,37] for unfractionated glycopeptide, and an average of 2.4 non-reducing terminal mannose residues, which is close to that proposed before[22,23] from degradations by the Smith procedure and methylation[22]. Further support for the present picture is found in the preliminary results of Levvy *et al.*[31], who showed that fractions of ovalbumin glycopeptides having a high hexosamine content produced more hexosamine per D-mannose residue upon treatment with *N*-acetyl-β-D-glucosaminidase than did the other fractions.

Although the present results indicate that the sequences of sugar residues can be represented by a single general formula (**1**), the possibility that the AC-fractions have the same general constitution is open to question and is under study at present.

Dispersity of ovalbumin. — Several labels have been used to describe populations of biopolymers but they have not been given a precise interpretation at the molecular level. The following definitions are proposed, being related to dispersity and purity of the primary covalent structures by an inspection of the population of molecules with "molecular tweezers". It is recognized that the differentiation of the possible conformers for each molecule has not been made.

Monodisperse. — A biopolymer preparation in which all of the molecules have identical primary, covalent structures. By the very nature of the biosynthetic processes as they are now understood, deoxyribonucleic acid, ribonucleic acid, or a pure monomeric protein cannot be polydisperse or even paucidisperse. The biosynthesis of each of these polymers is controlled by its particular template and the natural error in the copying process is estimated to be extremely small. It is important, however, in the preparation under study, to recognize not only the problems of isolation artefacts, but also the biological variations present in biopolymers from different species and different individuals. The microbiologist works with a pure culture and thus excludes individual variation, but the variations present in a population of higher animals will many times exclude the possibility of genetic homogeneity. It is becoming increasingly clear that the studies of materials from single animals, wherever possible, gives the most definitive results.

Paucidisperse. — A biopolymer preparation in which a few kinds of molecules exist, each having a covalent structure that is related to the others. The operating word that differentiates paucidispersity from heterogeneity in the present discussion is "related". The present concept of paucidispersity is closer to a low level of dispersity than it is to a mixture of a few separable molecules. The relationship is perhaps most clearly recognized by the paths of biosynthesis, the different molecules being the result of the action of the same synthetases, which give rise to molecules that are in a sense "homologous" in the same way as the lacto-*N*-glycoses of human milk or the kestoses are a related series of oligomers.

Polydisperse. — A biopolymer preparation in which the molecules have the same polymeric covalent structure but differ in molecular size. The biosynthesis of the glycosidic bonds in oligosaccharides and polysaccharides involves the stepwise addition

of glycosyl residues. Transglycosylation proceeds from a donor to an acceptor molecule until the growing chain is less and less preferred as an acceptor, or until the product becomes a suitable substrate for another enzyme. This change in the growing biopolymer is not abrupt, as a consequence of which the molecular weights of the individual polymer chains range between two extremes. The number of molecules in this range follow some distribution function of molecular weight, the peak representing the most favored molecular size.

Microheterogeneity. — A preparation is microheterogeneous when all the molecules have the same backbone primary structure, but with limited modifications of some of its monomer units. Microheterogeneity, like paucidispersity, implies a comparison of some property without describing a reference criterion. Since we are concerned in the present framework with the molecular structures of biopolymers, microheterogeneity has been applied to mixtures of molecules with the same primary structure and differing from each other in substitutions of small monomer residues or chemical groups on the primary chain. This description embraces nearly all of the uses of the term microheterogeneity, with the exception of those protein preparations that contain slight variations in the sequence of amino acids. Such examples have now been shown to be due largely to genetic variations in the population of organisms from which the material was derived, and they would now be considered heterogeneous. In the area of the protein portion of a biopolymer some causes of microheterogeneity are (*i*) partial phosphorylation, (*ii*) partial hydrolysis of the glutamine or asparagine as a result of isolation procedures (Asn and Gln are genetically coded into the primary structure), (*iii*) partial acylation of free amino groups either at the amino terminus or the ε-amino groups of lysine[38], (*iv*) disulfide interchange, giving rise to the possibilities for many different species of a protein, as seen in albumin of bovine plasma[39], and (*v*) variability in protein–metal ion complexes.

It is inevitable that the dispersion of a biopolymer may be categorized in more than one way, depending upon the parameter that is used for reference[40]. Thus, pure heparin preparations are polydisperse with reference to the molecular weight but microheterogeneous in terms of sulfate groups[41–44]. The partial acid hydrolysis of such a biopolymer increases both the polydispersity and the microheterogeneity. It is clear that ovalbumin from genetically homogeneous chickens may show a molecular microheterogeneity due to the extent of phosphorylation and a paucidispersity as a result of a "homology" of oligosaccharide groups. The microheterogeneity can be removed by the action of phosphatases, and whether the remaining paucidispersity can account for all the residual complexity of the ovalbumin described by Lush[45] is still to be demonstrated. Some preliminary experiments would suggest that this is the case[28]. This type of paucidispersity should have been anticipated by the results of Roseman and coworkers[46]. Transglycosylation of the first 2-acetamido-2-deoxy-D-glucosyl residue to a specific L-asparagine residue in the ovalbumin polypeptide chain is followed by a step-by-step addition of further hexosamine of D-mannosyl residues to produce the branched-chain oligosaccharides (**1**). The length of each chain reflects a different degree of completion, the ultimate of which being an undecasaccharide in

the mixture studied. Each component represents at least 5% of the total and the paucidispersity is probably not due to inexactness of the transglycosylases[39], although in some instances such processes may add to the dispersity of a glycoprotein.

It will be a matter for future study to demonstrate the generality of molecular paucidispersity in each prosthetic group of other glycoproteins. Indications are reported in several cases[39], including tropocollagen[40], α_2-macroglobulin[41], and porcine submaxillary mucin[42], without however isolating the individual glycopeptide groups. It is probable that no pure glycoprotein will be monodisperse.

ACKNOWLEDGMENTS

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APPLICATIONS OF EMPIRICAL RULES FOR OPTICAL ROTATION TO PROBLEMS OF CONFORMATIONAL ANALYSIS*†

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ABSTRACT

Whiffen's rules for estimating the molar rotation of saturated pyranoid carbohydrates from their geometrical structure are reduced to four rotational parameters, based on *gauche* relationships between carbon atoms, oxygen atoms, and carbon and oxygen atoms, and whether or not these atoms are bridged by C–C or O–C. The numerical values assigned are based on the rotations of simple model compounds as structural units of the more complex molecules. Consideration is given to the favored orientations for the methyl group of methyl glycopyranosides and for the hydroxymethyl group of hexopyranoses and their derivatives. Conclusions based on rotation are substantiated by the coupling of ^{13}C to the anomeric hydrogen atom for the methyl glycopyranosides. Solvation effects on rotation are interpreted in terms of the empirical rules and the effects of hydrogen bonding.

INTRODUCTION AND DISCUSSION

Recent investigations in this laboratory[1,2] have shown that changes in specific rotation at the D-line of sodium can be related to changes in conformational equilibria. These interpretations are based on the empirical rules that were first suggested by Whiffen[3] and later elaborated by Brewster[4]. This communication provides further experimental evidence for the utility of such rules. However, to do so, we need to propose new values for the contributions to rotation made by certain asymmetric conformational units found in *saturated* oxygenated compounds wherein neighboring groups are encountered that have staggered arrangements, or at least approximately so. Whiffen proposed it to be "unwise to obtain numerical values from the minimum requisite number of compounds and better to use such values as a guide and adjust them to give the best overall fit with the experimental values." Our basic assumption in developing the numerical values for contributions to rotation by asymmetric conformational units contradicts this proposal. Instead, we believe it more *useful* to

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‡Postdoctorate fellow with Professor M. L. Wolfrom, 1946–47.
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arrive at values by using the rotations of closely related structures, including whenever possible simple model compounds that contain structural units present in the more complex carbohydrate structures. This procedure is considered more useful since it allows predictions of the rotation of more-complex, related structures of fixed conformation. The resultant values are generally in better accord with observation than are those obtained from the more general approaches employed either by Whiffen[3] or by Brewster[4]. Thus, the procedure should be more reliable in attaching significance to differences between calculated and observed rotations. This expectation appears, as will be seen, well justified by the results achieved. Also, Brewster found it necessary to invoke the concept of permolecular dissymmetry in his treatment of the rotations of carbohydrates. We have found, following Whiffen's procedure, that the rotation thus assigned can be attributed to the occurrence of asymmetric conformational units formed by carbon and oxygen atoms in *gauche* relationship, as long as different values are assigned depending on whether the bridging atoms are both carbon (C/C, O/C or O/O) or one is oxygen (C/C_O or O/C_O).

This procedure is considered to be only a rough first approximation toward a proper understanding of the optical rotations of the sugars and related structures. Kauzmann, Clough, and Tobias[5] have recently speculated on the limitations of this simple "pairwise interaction principle." It was stated: "we need to know more about the effects of solvents on pairwise interaction, the extent to which solvent molecules participate in pairwise interactions, the influence of steric effects on pairwise interactions, and the relative magnitudes of pairwise and higher order interactions" and the opinion was expressed, "we cannot afford to be satisfied with the more limited assumptions of Whiffen and Brewster." Further on, these authors[5] stated, "it is evident that the principle of pairwise interactions cannot lead to very accurate predictions of the rotations of the sugars possibly because of restricted rotation of the hydroxyl groups and solvation effects that vary irregularly with configuration." Furthermore, it was suggested, "if an empirical approach is to be fruitful, it ought to be set up in a manner consistent with what is known about the general theoretical basis of the phenomenon." The facts are, however, that developments occur through attempts to correlate observed data on the basis of simple rules, with subsequent expansion of the theory to higher orders as the experimental data dictate refinements. For example, as will be seen later on, we have dispelled by experiment the fears based on theory that solvation effects (arising from simple solvents) on the rotation may vary irregularly with configuration to an extent that such effects may drastically obscure rotational changes caused by changes in conformation. Empirical rules that are theoretically inadequate have frequently played critically important roles in the development of chemistry. Accordingly, we present here the results of efforts to rationalize the rotations of structurally related carbohydrates, on a strictly pairwise principle as applied to the solute, and involving only the carbon and oxygen atoms. The presentation highlights the deficiencies in the procedure, and as such is of heuristic value to the elaboration of better theoretical approximations. The method clearly provides a useful, though still primitive, tool for the study of conformational

equilibria. There can be no doubt that the pairwise principle, as applied to asymmetric conformational units, will be the most important single parameter in efforts to account for the optical rotations of the complex, saturated, carbohydrate structures.

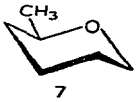
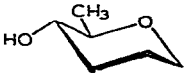
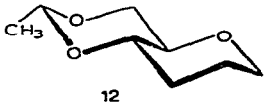
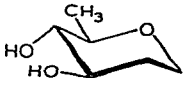
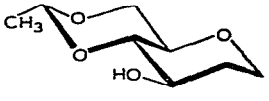
Throughout this discussion, the term rotation will mean molecular rotation* defined as

$$[M]_D = \frac{[\alpha]_D \times \text{Mol. wt.}}{100}$$

The rotations used to establish the empirical rules are those measured in water, since in this solvent the compounds are expected to possess the conformation shown in the Tables. Virtually the same rotations are expected in other solvents if these conforma-

TABLE I

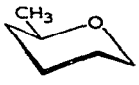
EFFECT ON CHANGE OF SOLVENT ON MOLECULAR ROTATION[a] (°)

Compound	*Solvent*			
	Water	*Methanol*	*Chloroform*	*Carbon tetrachloride*
 7	4	5	3	2
 9	14	—	11	12
 12	2	—	1.8	2.1
 10	−32	—	−23	−26
 13	−44	−38	−38	−39

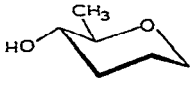
[a] $[\alpha]_D^{25}$ for 0.5–1% solutions, as reported in this paper and ref. 2.

*This definition is that commonly employed in organic chemistry and was used both by Whiffen[3] and Brewster[1]. Traditionally, carbohydrate chemists have used the product of the specific rotation and the molecular weight, especially with reference to Hudson's rules of isorotation. Recently, biochemists have adopted the convention of dividing this product by 10 so that the molecular rotation corresponds to that expected for a molar solution (Style Manual for Biological Journals, American Institute of Biological Sciences, 2000 P Street, N.W., Washington 6, D.C., 1960, p. 29).

tions are maintained[1,2]. This is further displayed, for example, by the data presented in Table I. To determine the rotation of a compound in water it was at times necessary to extrapolate measurements made with various proportions of methanol and water[2].

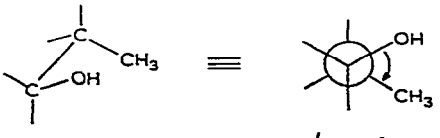


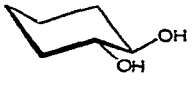
7



9

Analysis of the asymmetric conformation units present in *R*-2-methyltetrahydropyran in conformation **7** which has the methyl group in equatorial orientation shows that these cancel to zero. Both Whiffen[3] and Brewster[4] have pointed out that in the absence of differences in the contributions to rotation arising from such asymmetric conformational units (pair-wise contributions[5]) only small rotations are to be expected; that is, the presence of the asymmetric carbon in **7** on its own is not expected to render the compound appreciably optically active and, indeed, as seen in Table I the molecular rotation was less than 5° in all the solvents reported. In the case of compound **9**, analysis of the asymmetric conformational units present in the chair form indicated shows the following differences: O/C−(O/H+C/H−H/H). The rotation of **9** was found to be +14° in water. As mentioned above, it is *arbitrarily* assumed for the purposes of this presentation that the contributions O/H, C/H, and H/H are negligibly small as compared to the O/C contribution. Since it seems reasonable to expect the term O/H+CH−H/H to be positive, the O/C contribution is estimated to be somewhat lower than the rotation of **9**. Therefore, the difference between the rotation of **9** and **7**, namely 14−4 = 10°, is considered a better approximate value for the O/C contribution than is the rotation of **9**. It is emphasized again that this assumption is made both for reasons of convenience and better overall agreement as are other basically false assumptions implicit to this approach; namely, that the molecules are in perfect chair forms (indeed appreciable contributions to rotation may even arise from neighboring substituents wrongly assumed to be in a perfect antiparallel arrangement), that interactions further extended than for neighboring atoms make no contributions (no permolecular contributions), and that solvent effects are not present. In this light, we assign a contribution to rotation of +10° when in the structures under consideration there exists an oxygen and a carbon in *gauche* relationship and bridged by two carbon atoms and describing a right-handed screw pattern of asymmetry; *i.e.*, as shown in the following asymmetric unit.



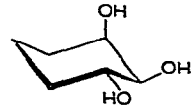
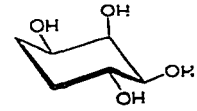
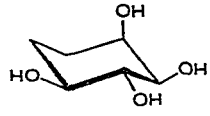
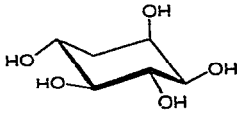
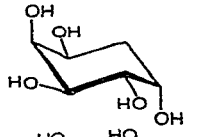
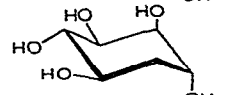
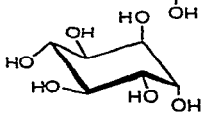


11

D-*threo*-1,2-Cyclohexanediol (**11**) differs in asymmetric conformational units from cyclohexanol by 2(H/H–O/H)–(O/O–H/H), and this difference is considered to be the origin of the molar rotation (−48°) of the compound. Consideration of the content of Table II shows that the choice of O/O = 45°, as initially proposed by

TABLE II

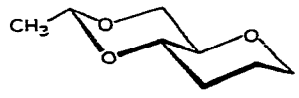
MOLECULAR ROTATIONS (°) OF SELECTED CYCLITOLS

	Analysis	*Molecular rotation*	
		Calc.	*Found*[a]
	−O/O	−45	−48
	−2O/O	−90	−92
	−O/O	−45	−49
	−O/O	−45	−54
	−O/O	−45	−43
	−2O/O	−90	−91
	+2O/O	+90	+100
	O/O	+45	+49
	3O/O	+135	+117

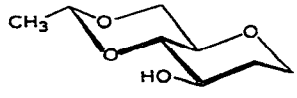
[a]See ref. 3.

Whiffen[3], provides an excellent basis for estimating the molar rotations of compounds with known conformations wherein these asymmetric conformational units occur. In

the case of 1,5-anhydro-2,3-dideoxy-4,6-*O*-ethylidene-D-*erythro*-hexitol ("1,2,3-trideoxy-4,6-*O*-ethylidene-D-*erythro*-hexopyranose", **12**), our analysis would require a rotation of zero. Indeed, a rotation of only 2° was observed (see Table I). The related structure **13** can be analyzed as $-O/O$, giving a predicted molar rotation of $-45°$, in good agreement with the value of $-44°$ found (Table I).



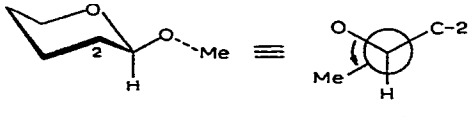
12



13

Brewster assigned a rotatory contribution of 50° for the asymmetric unit defined as *k*(O–H) (C–H) and which we represent simply as O/C. We conclude that, for our procedure, the most useful value for O/C is 10°, with the sign depending on the screw pattern, in view of the analyses presented in Table III for selected pentoses and 6-deoxyhexoses and certain of their deoxy derivatives. Brewster's rules provided a permolecular contribution to rotation of $\pm 60°$ for an axial hydroxyl group at positions 2 or 4 of the compounds listed in Table III. In the case of 6-deoxy-β-D-mannopyranose, for example, a permolecular contribution of $-60°$ was assigned to the axial 2-hydroxytetrahydropyran grouping, and it is this contribution that allowed the use of $+50°$ for the contribution *k*(O–H) (C–H). Examination of Table III clearly shows that our method provides a much better correspondence between the rotations calculated and found than does Brewster's procedure. However, it must be noted that our values are specially chosen for the kinds of structures under consideration and do not, for example, account as well for the rotations observed for the simple 2-methylcyclohexanols as do Brewster's rules, which are based on the rotations of these compounds. The choice of the value for the O/C contribution is of central importance to this communication, since a main purpose is to present arguments for assigning conformational preferences to the hydroxymethyl group of hexopyranose structures. Before discussing this matter further, the rotations of methyl glycopyranosides should be considered.

In his empirical rules to correlate rotation with the structure of methyl glycopyranosides, Brewster[4] assigned a contribution of $\pm 105°$ (depending on the configuration) to the conformational unit defined by the acetal linkage as shown in **14**. That this carbon–oxygen bridged, asymmetric conformational-unit, which we specify as O/C_0,

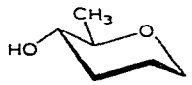
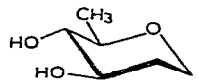
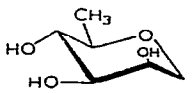
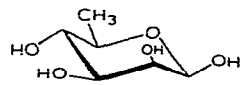
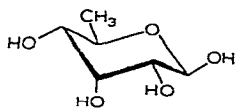
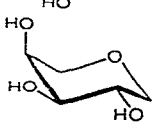
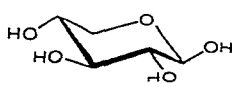
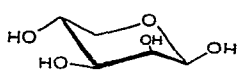


14

should make a strong contribution to rotation is evident from the rotations of the aldopyranoses listed in Table IV having the 1-substituent axial. It is seen that a value of $+115°$ for the O/C_0 unit described by the axial oxygen atom and C-5 provides a

TABLE III

MOLECULAR ROTATIONS (°) OF SELECTED DEOXY DERIVATIVES OF PENTOSES AND HEXOSES

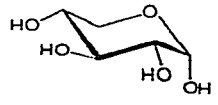
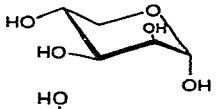
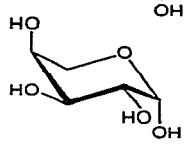
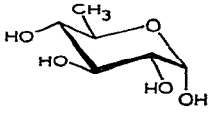
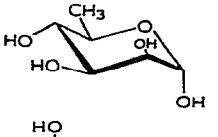
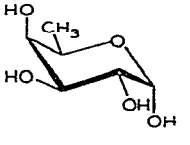
	Analysis	*Molecular rotations*		*Brewster[4] method*	*References*
		Calc.	*Found*		
	O/C	+10	+14	+50	—
	O/C–O/O	−35	−32	+5	—
	2O/C–3O/O	−115	−124	−100	30
	2O/C–2O/O	−70	63	−55	27*c*
	O/C–O/O	−35	−20	+5	27*c*
	3O/O–O'C	+125	+132[a]	+150	31
	−O/O	−45	−25[b]	−45	26, 32
	O/C–2O/O	−80	−109	−105	26

[a]From the rotation of the enantiomer. [b]Estimated from the rotation of the equilibrium mixture.

good correspondence between predicted and observed rotations. The only serious discrepancies are with the configurationally related sugars, α-D-lyxose and 6-deoxy-α-D-mannose. It is now well established by X-ray crystallography that the pyranose ring of the sugars is appreciably distorted from the ideal-chair form and that neighboring substituents in *gauche* relationship can define dihedral angles over an appreciable range. Since it is possible that the magnitude of the contribution is substantially dependent on the dihedral angle, it is not considered useful for the present purposes to try to achieve better agreement through the introduction of further parameters such

TABLE IV

MOLECULAR ROTATIONS OF 1-AXIAL SUGARS

	Analysis	*Molecular rotations*		*References*
		Calc.	*Found*	
	O/O–O/C+O/C$_0$	+150	+140	26, 27*a*
	O/C$_0$–3O/O	−20	8.4	26, 27*a*
	4O/O–2O/C+O/C$_0$	275	303[a]	28
	O/O+O/C$_0$	160	163[a]	29
	O/C–3O/O+O/C$_0$	−10	+16[a]	26
	4O/O–3O/C+O/C$_0$	265	251[a]	26

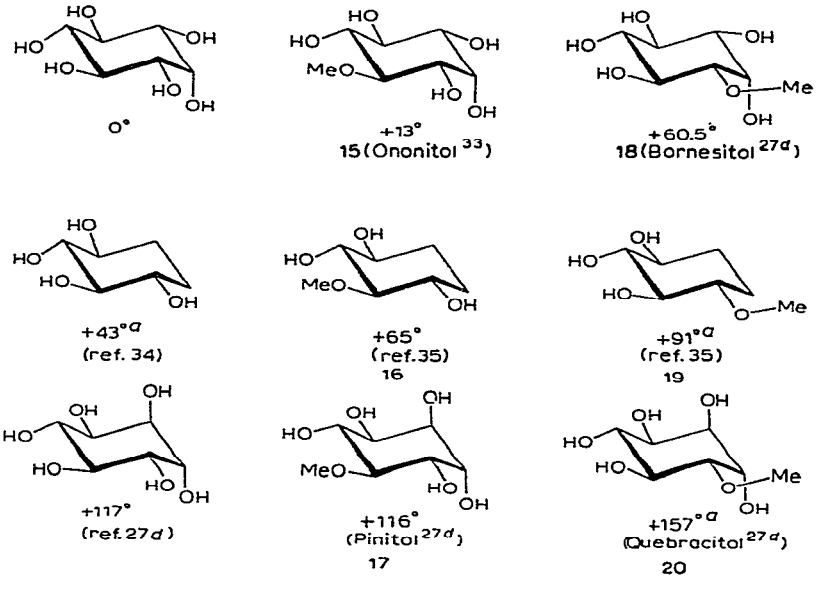
[a]From the rotation of the enantiomer.

as these, or by use of three- and higher-way interactions[5]. Indeed, the main immediate promise of these empirical rules to conformational analysis is for the interpretation of changes in rotation in terms of changes in conformational equilibria as may be brought about through changes in solvation. For these purposes, a basis for precise predictions of rotations, although desirable, is not normally essential. The value of 115° for O/C$_0$ corresponds to the value of 113° assigned by Whiffen[3] to his rotational parameter *J*.

Table V shows that the introduction of an *O*-methyl group in a cyclitol structure can cause a change in rotation as great as 60°. These data should be contrasted with the statement by Whiffen[3] that "there are reasons for believing that the contribution is small and that the formation of methyl ethers has little effect on the molecular rotation." As would be expected, introduction of a methyl group on an equatorial hydroxyl group flanked by two equatorial hydroxyl groups should lead to only a

TABLE V

EFFECTS OF METHYLATION ON ROTATION



[a]From the enantiomer.

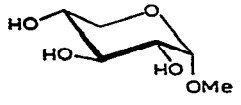
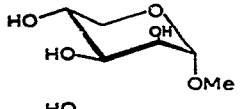
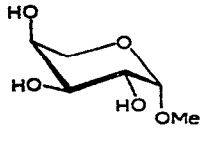
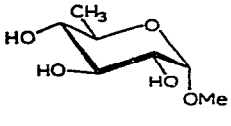
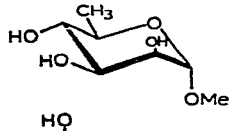
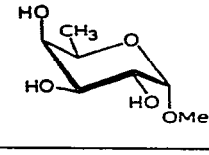
small change in rotation, as is seen for compounds **15**, **16**, and **17** of Table V. However, for compounds **18**, **19**, and **20**, the orientation of the methyl group that brings it into opposition with an equatorial hydrogen atom, as depicted in the formulas of Table V, should be preferred. Thus, the introduction of the methyl group in these cases should lead to conformers where the group is part of a dextrorotatory C/C_0 contribution. Indeed, it can be seen in each case that the rotation of the methyl ether is substantially more positive than the parent alcohol. In view of the rotation of compound **18**, the value of 60° is provisionally assigned to the parameter C/C_0.

In the case of methyl glycopyranosides, there can be no doubt about the favored orientation for the methyl group. Both the exo-anomeric effect[2,6] and the steric factors favor the orientation shown in **14**, where the methyl group is *gauche* to both the ring-oxygen atom and the anomeric hydrogen atom for both of the anomers. A right-handed screw pattern provides a positive contribution, and this situation was seen to apply also for methyl ethers.

Table VI shows that good agreement is achieved in the calculation of the molar rotations for 1-axial methyl α-D-glycopyranosides if it is assumed that the orientation of the methyl group is entirely that of the enantiomer of **14** (that is, $+O/C_0$). However, the agreement is not nearly as acceptable for the 1-equatorial methyl glycopyranosides (Table VII), since in all of the examples given the difference between the calculated and found values is negative. This is not the case for the 1-axial anomers listed in Table VI. The results suggest, therefore, that conformers involving rotation about the

TABLE VI

MOLECULAR ROTATIONS (°) FOR 1-AXIAL METHYL GLYCOPYRANOSIDES

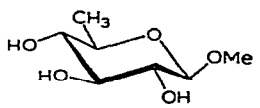
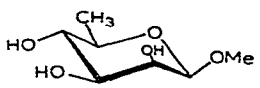
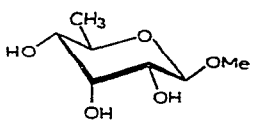
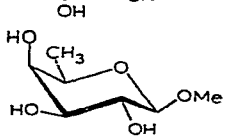
	Analysis	*Molecular rotations*		*References*
		Calc.	*Found*	
	O/O–O/C+2O/C_0	265	252	26
	2O/C_0–3O/O	95	97	26
	4O/O–2O/C+2O/C_0	390	403	26
	O/O+2O/C_0	275	271	3
	O/C–3O/O+2O/C_0	105	111[a]	26
	4O/O–3O/C+2O/C_0	380	351[a]	26

[a]From the rotation of the enantiomer.

C-1–OCH_3 bond, which make positive contributions to rotation relative to **14**, are more abundant for the 1-equatorial methyl glycosides than the corresponding rotamers for their anomers (which make negative contributions). The three staggered orientations are depicted in projections **14a**, **14b**, and **14c** together with the analysis of their asymmetric conformational units. It can be seen that **14b** must be expected to make a positive contribution of at least +60°. Conformer **14c** will also make a numerically positive contribution if indeed $O/C_0 > C/C_0$, as was considered above. Obviously, therefore, the presence of substantial proportions of one or both conformers **14b** and **14c**, relative to the situation for their anomers, is indicated for the 1-equatorial glycosides. In the case of the 1-axial glycosides, evidence was presented which seemed to require an increase in the form corresponding to **14b** when the solvent was changed to water[2]. Attempts are presently being made in this laboratory to establish more precisely these conformational equilibria through studies of ^{13}C

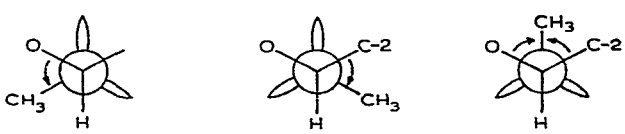
TABLE VII

MOLECULAR ROTATIONS (°) OF 1-EQUATORIAL METHYL GLYCOPYRANOSIDES

	Analysis	*Molecular rotation*		*References*
		Calc.	*Found*	
	−O/O−O/C$_0$	−160	−108	26
	−O/O+O/C−O/C$_0$	−150	−98	26
	−2O/O+O/C−O/C$_0$	−195	−170[a]	26
	−O/O+O/C−O/C$_0$	−150	−109	3
	2O/O−2O/C−O/C$_0$	−45	−29[a]	26

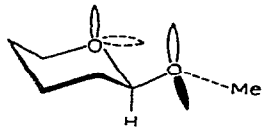
[a]From the rotation of the enantiomer.

to proton coupling over the C-1–O bond[7,8], based on the assumption that vicinal ^{13}C to proton coupling is stronger for a torsional angle of 180° (as in **14c**) than for one of 60° (as in **14a** or **14b**). Indeed, the preliminary results[9] are in accord with the

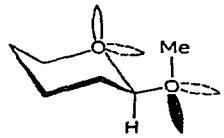


above expectation based on optical rotation. It is found that both methyl α-D-glucopyranoside and its 2-deoxy derivative, labelled in the methyl group with ^{13}C, displayed weak coupling (3.7 and 2.7 Hz, respectively) between the ^{13}C and the anomeric proton. For 1-axial methyl glycopyranosides, the orientation corresponding to **14c** is expected to be inhibited strongly, mainly for steric reasons but also for reasons of the *exo*-anomeric effect. Thus, the methyl group is restricted to the *gauche* relationships with the anomeric hydrogen atom, and the weak coupling observed is

in accord with these expectations. On the other hand, much stronger coupling is observed between the anomeric hydrogen atom and the ^{13}C-labelled methyl group for both methyl β-D-glucopyranoside and its 2-deoxy derivative (4.6 and 4.4 Hz, respectively). This is not surprising, since for 1-equatorial methyl glycopyranosides the conformation **14c** must be expected to be much more stable relative to the favored conformation (**14a**) than in the case of 1-axial glycopyranosides. If the anomeric effect is viewed as arising from the repulsion between unshared pairs of electrons for geminal oxygen atoms, then the *exo*-anomeric effect for 1-equatorial glycopyranosides is equal for rotamers **14a** and **14c**. Although **14a** must be less strained than **14c**, these



≡14a



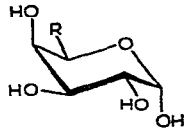
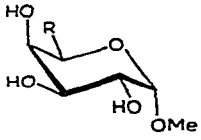
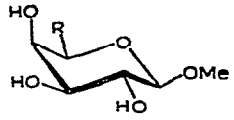
≡14c

rotamers probably do not differ in energy by more than about one kcal/mole. This conclusion is of obvious interest for consideration of the conformational properties of β-glycopyranosides, especially cellulose.

As seen in Table VIII, the effect of introducing an hydroxyl group at C-6 of a 6-deoxy-D-hexopyranose structure is to increase the rotation by about 25° when there

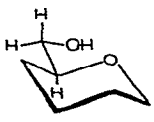
TABLE VIII

EFFECT OF THE 5-HYDROXYMETHYL GROUP ON ROTATION (°)

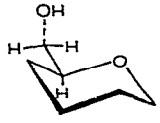
	Molecular rotations[a]		*Difference*
	$R = CH_2OH$	$R = CH_3$	
	+22	+4	+18
	+271	+251	+20
	+380	+351[b]	+29
	−0	−29[b]	+29

[a]Ref. 26. [b]From the rotation of the enantiomer.

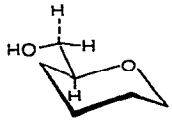
is either no hydroxyl group at C-4 or the hydroxyl group at C-4 is axial. On the basis of the rotational parameters developed earlier, the three staggered orientations for the hydroxymethyl group should make the contributions to the rotation of the compound shown for **4a**, **4b**, and **4c**. Of these conformations, **4b** should be least



4a
O/O = 45°



4b
O/C − O/O = −35°



4c
−O/C = −10°

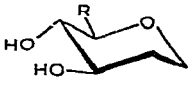
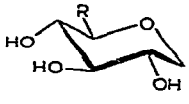
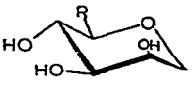
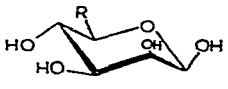
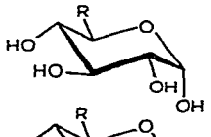
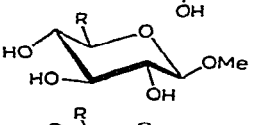
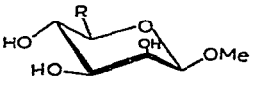
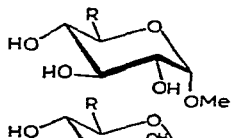
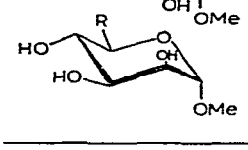
stable and especially so when an axial hydroxyl group is present at C-4. If it is assumed that conformer **4b** occurs to a negligible extent for the *galacto* derivatives given in Table VIII, then the relative amounts of **4a** to **4c** are expected to be about 2:1. This seems reasonable, since the non-bonded interaction HO/O in **4a** should be somewhat less than the HO/CH interaction in **4c**.

The non-bonded interactions in **4c** are expected to be increased greatly through the introduction of an equatorial hydroxyl group at C-4, since this brings these hydroxyl groups into a relationship (see **8c**) similar to the syn-axial orientation of two axial hydroxyl groups. Clearly this situation is strongly disfavored when water is the solvent[10,11]. For these reasons, the conclusions reached by Yamana[12,13], that the hydroxymethyl group of hexoses favors that rotamer corresponding to **8c** for reasons of intramolecular hydrogen bonding, are untenable. Indeed, as seen in Table IX, the effect of the hydroxyl group at C-6 on the rotation of the D-*erythro* compounds shown is much greater than it is for the D-*threo* compounds of Table VIII. If it is assumed that conformation **4c** is entirely prohibited, one would expect the ratio of **4a** to **4b** to be about 3:1, since for the D-*erythro* compounds of Table IX the difference in the non-bonded interactions between conformations **4a** and **4b** is one-half of that between the two chair forms of cyclohexanol in water[14]. On this basis, the differences in rotation for the pairs given in Table IX should be about $0.75 \times 45° - 0.25 \times 35° = 25°$. These considerations leave no doubt that the rotamer **4a** is the favored one for hexoses, and to a greater extent for D-*erythro*-hexopyranoses than for D-*threo*-hexopyranoses, as would be anticipated by conformational analysis.

Hall and Manville[15] have recently discussed the conformational populations arising from rotation about the C-5–C-6 bond of acetylated hexopyranoses, as indicated by n.m.r. spectra, and concluded that the favored rotamer for D-*gluco* compounds was **4b**, and **4c** for the D-*galacto* compounds (as the acetates). These conclusions are not those expected from the above considerations based on rotation. As pointed out[15] they are at variance with conclusions reached by Lemieux and Stevens[16] in favor of acetylated **4a**. We now present data based both on n.m.r. spectroscopy and optical rotation to support the contention that **4a** represents the favored conformation for both the acetylated and unacetylated forms of D-*erythro*-hexopyranoses.

TABLE IX

EFFECT OF THE 5-HYDROXYMETHYL GROUP ON ROTATION (°)

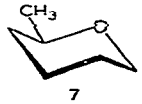
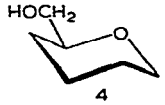
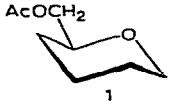
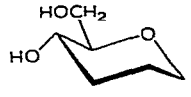
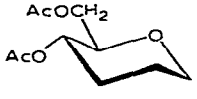
	Molecular rotation		*Difference*	*References*
	$R = CH_2OH$	$R = CH_3$		
	+53	+13	+40	—
	+21	−32	+53	—
	+70	+48 (CH_3OH)	+22	36, 37
	−81	−124	+43	30
	−31	−63[a]	+32	26
	+202	+163[a]	+39	26, 29
	−66	−98	+32	26
	−135	−170[a]	+35	26
	+309	+271	+38	26
	+154	+111[a]	+43	26

[a]From the rotation of the enantiomer.

As seen in Table X, the introduction of either an hydroxyl or an acetoxy group into the methyl group of compound **7** causes approximately the same increase of rotation. Also, the rotations of both 1,5-anhydro-2,3-dideoxy-D-*erythro*-hexitol ("1,2,3-trideoxy-D-*erythro*-hexopyranose", **8**) and its diacetate (**21**) are about the

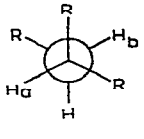
TABLE X

RELATIVE EFFECTS OF HYDROXYLATION AND ACETOXYLATION ON ROTATION

	Molecular rotation (°)
 7	+4
 4	+22
 1	+21
 8	+53
 21	+50

same. Thus, for these simple compounds, there is no reason based on optical rotation to anticipate conformational properties that are markedly different. Instead, the results provide circumstantial evidence for similar distributions of the various rotamers.

In spite of the restraints on conformation provided by chair forms to maintain neighboring atoms in *gauche* relationship, with dihedral angles of near 60°, a dihedral angle of 75±1° exists between the acetoxy groups (ether-oxygen atoms) at C-2, -3, and -4 of tetra-*O*-acetyl-α-D-glucopyranosyl chloride[17]. Surely, therefore, dihedral angles of 60° cannot be assumed for open-chain compounds. For example, a 1,1,2-

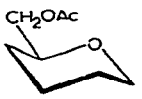
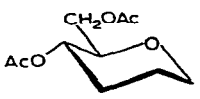
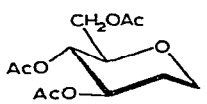
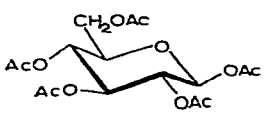
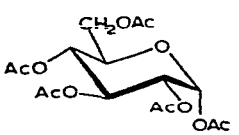


trisubstituted ethane (as shown), may have a dihedral angle defined by H_a and H_b of substantially less than 180° if separation of the *gauche* R groups by rotation about the central C–C bond is accommodated by the greater eclipsing of the R groups by hydrogen, with a net decrease in energy. It is evident, therefore, that an interpretation

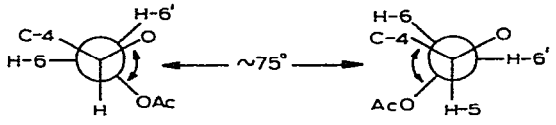
of n.m.r. data for such compounds cannot be made on the basis of an assumption that time-averaged coupling constants reflect the averaging of the coupling constants expected for protons defining dihedral angles of 60° and 180°. This contention is supported by the torsional angles found between the hydroxyl group of the hydroxymethyl group side-chain of numerous glycosidic structures. The orientation found in the crystalline state obviously has no necessary bearing on the favored rotamer in aqueous solution, and neither does the magnitude of the torsional angle. Nevertheless, the possibility exists, in the absence of information to the contrary, that substantial deviation from idealized dihedral angles of 60° and 180° occurs in solution. For example, in 2′-deoxyadenosine[18], the 5′-hydroxyl group is oriented relative to the furanose ring as depicted in **4a**, and in 2′-deoxy-5-fluorouridine[19], where the orientation corresponds to **4c**, the positive torsional angles with the ring oxygen-atom are 68° and 172°, respectively. Indeed, such distortions from the ideal 60° and 180° are found most commonly[18]. Unfortunately, the X-ray crystallographers have not until recently normally presented the structural features in terms of torsional angles between neighboring groups. These angles are now being examined in this laboratory in an attempt to achieve a better calibration of n.m.r. parameters.

TABLE XI

COUPLING CONSTANTS FOR THE HYDROGENS AT THE 6-POSITION OF DERIVATIVES OF D-*erythro*-HEXOPYRANOSE

	$J_{5,6'}$	$J_{5,6}$	$J_{6,6'}$
 1	4.2	6.2	−11.2
 21	5.0	2.7	−12.0
 24	4.9	2.5	−12.0
	4.7	2.5	−12.5
	4.75	2.5	−12.4

Introduction of an acetoxy group at C-4 of compound **1** led to a substantial decrease in one of the two time-averaged coupling constants $J_{5,6'}$ and $J_{5,6}$ (Table XI), and these coupling constants are found in the other 4-substituted compounds listed. Most probably the larger coupling constants of 4.7–5.0 Hz (assigned to $J_{5,6'}$) arise from a large dihedral angle[17] of about 165° for these hydrogen atoms in rotamer **22**

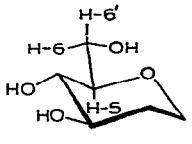


22 23

and a value of near zero for $J_{5,6'}$ in the less abundant rotamer **23**. The coupling constants $J_{5,6} = 6.2$ and $J_{5,6'} = 4.2$ Hz found for compound **1** are very similar to those observed for *galacto* compounds[15], and are in accord with expectation that rotamer **23** is relatively much more important when C-4 is not substituted by an equatorial acetoxy group.

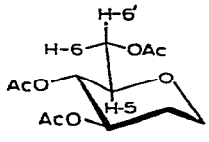
Finally, it must be kept in mind that the coupling constant for a methylenic hydrogen atom of an hydroxymethyl group with a neighboring methine hydrogen atom with which it defines a torsional angle of 180° can be expected to be substantially greater than for two similarly disposed methine hydrogen atoms[20]. As a result, the proportions of rotamers that represent weak coupling-interactions may be seriously underestimated. These considerations place severe limitations on the use of n.m.r. spectroscopy to establish rotamer populations and emphasize the importance of refining alternative methods, such as that based on optical rotatory power.

A third and most compelling reason to assign the greatest stability to the rotamer state shown for **24** resides in the evidence based on optical rotation for assigning the rotamer depicted for **11** and the similarity in the coupling constants for H-6, H-6', and H-5 in compounds **24** and **11**.



11

$J_{5,6} = 2.4$ Hz
$J_{5,6'} = 5.7$ Hz
$J_{6,6'} = -12$ Hz



24

2.5 Hz
4.9 Hz
−12 Hz

We do not consider this matter completely settled. Nevertheless, the study of conformational equilibria by way of changes in optical rotation may well show up limitations in applications of n.m.r. spectroscopy to ethylenic structures, since the numerical changes in contribution to optical rotation with changes in dihedral angle are greater, and therefore more readily interpreted, than are changes in coupling constants with changes in the populations of rotamers.

The effects of change in solvent on the optical rotations of compounds **4** and **8**

are of particular interest. The rotation of **4** is +22° in water, +8° in 1,2-dichloroethane and −65° in methyl sulfoxide. Lemieux, Barlow, and Mills[21] have presented evidence that the *gauche* relationship between two oxygen atoms is favored in water more so than in aprotic solvents. Thus, one would expect **4** to favor **4a** more than **4c** to a greater extent in water than in 1,2-dichloroethane and, therefore, that the compound be more dextrorotatory in water than in 1,2-dichloroethane, as was found. A solution of **4** in methyl sulfoxide must be expected to show strong destabilization of conformation **4a** for the same kinds of reasons; for example those described by Lemieux and Pavia[11] to explain the effect of methyl sulfoxide on the chair–chair equilibrium for methyl 3-deoxy-4-*O*-methyl-β-L-*erythro*-pentopyranoside. Thus, **4** should have more of conformer **4c** in methyl sulfoxide than in either 1,2-dichloroethane or water and should, therefore, have the smallest rotation in this solvent. Indeed, the observed rotation was −6°, close to that expected (−10°) for conformer **4c**. The change of rotation with change in the concentration of methyl sulfoxide in 1,2-dichloroethane is shown in Fig. 1.

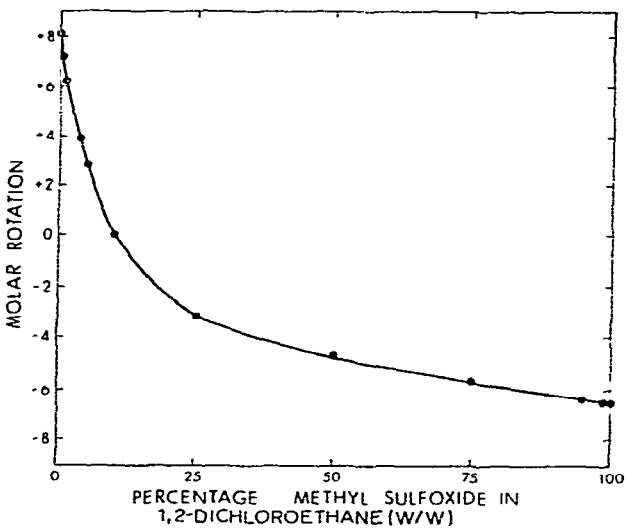
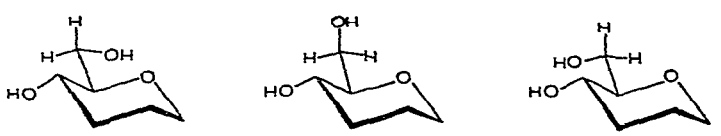


Fig. 1. The effect of methyl sulfoxide on the optical rotation (D-line) of (S)-(+)-2-(hydroxymethyl) tetrahydropyran (**4**) in 1,2-dichloroethane at 25° (*c*, 1.18).



8a o/c + o/o = 55°

8b 2 o/c − o/o = −25°

8c o/c − o/c = 0°

In contrast to **4**, compound **8** has nearly the same rotation in water (+53°) as in methyl sulfoxide (+42°). In both cases, the strong dextrorotation infers a high proportion of rotamer **8a**. For obvious reasons, this rotamer should be the favored

conformation in these solvents. However, in 1,2-dichloroethane, the two hydroxyl groups can become hydrogen-bonded in conformation **8c**. Thus, in this solvent, the weakly optically active rotamer **8c** is expected to be abundant. Indeed, the rotation was only $+20°$ in 1,2-dichloroethane. It was to be expected[2] that the addition of a small proportion of methyl sulfoxide to the solution of **8** in 1,2-dichloroethane would increase the concentration of the hydrogen-bridged form **8c**, and thereby lead to a decrease in rotation. This, indeed, was the case, as is evident from Fig. 2.

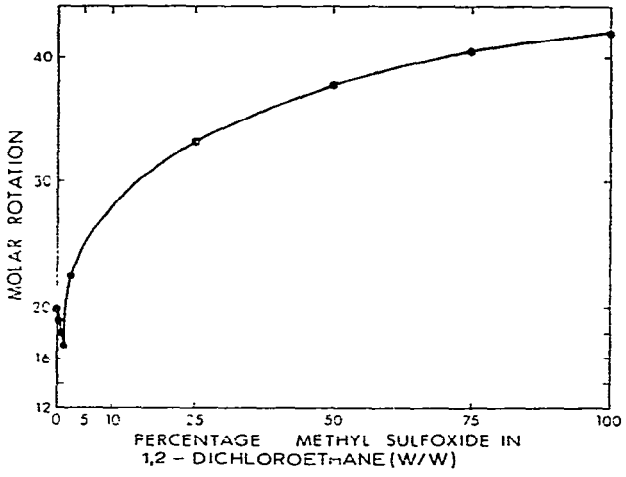


Fig. 2. The effect of methyl sulfoxide on the optical rotation (D-line) of 1,5-anhydro-2,3-dideoxy-D-*erythro*-hexitol (**8**) in 1,2-dichloroethane at 25° (*c*, 1.0).

Compound **1** was readily obtained in about 50% yield by hydrogenation in ethyl acetate of tri-*O*-acetyl-D-galactal (3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol), with either 5% palladium-on-charcoal or platinum as catalyst. This result is in sharp contrast to the lack of this product on the hydrogenation of tri-*O*-acetyl-D-glucal (3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol[22]). Indeed, although the latter compound under these conditions gave about a 20% yield of the product of hydrogenolysis of the 3-acetoxy group and saturation of the double bond, the corresponding product was formed from tri-*O*-acetyl-D-galactal in only 7% yield. Evidently, hydrogenolysis of an acetylated glycal may proceed with reduction of the anomeric center to provide the 2,3-unsaturated intermediate, which may undergo extensive hydrogenolysis at C-4. The axial orientation of the 4-acetoxy group of tri-*O*-acetyl-D-galactal may provide a particularly favorable situation for this second stage.

Judging from the data in Table VIII, the contribution to rotation resulting from the presence of the 6-hydroxyl group in both 1,5-anhydro-2,3-dideoxy-D-*threo*-hexitol ("1,2,3-trideoxy-D-*threo*-hexopyranose", **5**) and 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol ("1,2-dideoxy-D-*lyxo*-hexopyranose", **6**) is expected to be about $+25°$. On this basis, a molar rotation of $25° - O/C = +15°$ is expected for **5**, in good agreement

with the experimental value of +13°. In the case of **6**, a rotation of O/O–O/C+25° = 60° is expected and +68° was found. These results further justify the values given to the O/O and O/C rotation parameters.

Although Hudson's rules of isorotation have played an important role for classifying anomeric structures, it may be noted that these rules are based on the premise that the A-contribution to rotation is derived from the anomeric center only. However, it is now apparent[5] that the presence or absence of an asymmetric conformational unit defined by the aglycon and a substituent at position 2 enters into the calculation of the A-value, and explains the so-called anomaly for the value of the A-contribution in the *manno*-series of sugars and glycosides. Thus, the 2A value for an anomeric pair of D-mannopyranosides is that of the 2-deoxy derivative plus the contribution of an asymmetric unit defined by the 1- and 2-substituents in *gauche* relationship. In the case of D-*gluco* derivatives the 2A-value is two such contributions greater.

EXPERIMENTAL

The procedures for the preparation of 1,5-anhydro-2-deoxy-D-*arabino*-hexitol ("1,2-dideoxy-D-*arabino*-hexopyranose", **11**), 1,5-anhydro-2,3-dideoxy-D-*erythro*-hexitol ("1,2,3-trideoxy-D-*erythro*-hexopyranose", **8**), and their *O*-acetyl and their 4,6-*O*-ethylidene derivatives were reported in a separate communication[2].

(S)-(+)-*2-Acetoxymethyltetrahydropyran* (**1**), *4,6-di-O-acetyl-1,5-anhydro-2,3-dideoxy-D-threo-hexitol* ("*di-O-acetyl-1,2,3-trideoxy-D-threo-hexopyranose*", **2**) *and 3,4,6-tri-O-acetyl-1,5-anhydro-2-deoxy-D-lyxo-hexitol* ("*tri-O-acetyl-1,2-dideoxy-D-lyxo-hexopyranose*", **3**). — Tri-*O*-acetyl-D-galactal (10 g) was hydrogenated at 50 lb.in^{-2} for 7 h in 50 ml of ethyl acetate. The catalyst was either 5% palladium-on-charcoal (1 g) or prereduced platinum oxide (2 g). Diethylamine (0.600 g) was sometimes used as modifier. After the usual work-up, the ratio of the three title compounds was determined by gas chromatography over silicone rubber on Chromosorb at 175°.

	Mole % of products		
Conditions	**1**	**2**	**3**
Pt, Et_2NH	55	5	40
Pd, Et_2NH	54	7	42
Pd	~100	0	0

Distillation under diminished pressure (2 mm) provided (*S*)-(+)-2-acetoxymethyltetrahydropyran (**1**), b.p. 60–62°, $[\alpha]_D^{25}$ +13.3° (*c* 4.5, chloroform); 4,6-di-*O*-acetyl-1,5-anhydro-2,3-dideoxy-D-*threo*-hexitol (**2**), b.p. 92–96°, $[\alpha]_D^{25}$ +9.2° (*c* 1.5, chloroform); and 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hexitol (**3**), b.p. 141–144°, $[\alpha]_D^{25}$ +43.7° (*c* 0.75, chloroform).

Gas-chromatographic analysis indicated high state of purity for **1** and **3**, and

the n.m.r. spectra were in accord with the structures assigned. Compound **2** was further purified by column chromatography on silica gel, with 9:1 Skellysolve B–acetone as eluant.

For compound **1**, named as 6-*O*-acetyl-1,5-anhydro-2,3,4-trideoxy-D-*glycero*-hexitol, the n.m.r. spectrum in benzene-d_6 at 100 MHz showed a ratio of five protons in the range τ 5.8–7.0 to nine protons in the range τ 8.1–9.0, as expected for the assigned structure. A sharp singlet at τ 8.28 corresponded in intensity to the acetoxy-group signal. A sharp, 2-proton multiplet centered at about τ 6.0 was assigned to the methylenic hydrogen atoms of the acetoxymethyl group, a one-proton multiplet at τ 6.18 was assigned to H-5 with a band width suggestive of strong coupling (>9 Hz) presumably with the axial proton at the 4-position. The methylenic protons at the 1-position gave a broad multiplet in the range τ 6.5–7.0. With aid of spin-decoupling, the following approximate coupling constants (Hz) could be estimated: $J_{1,1'}$ −11.0; $J_{1,2}$ 10; $J_{1,2'}$ 3.7; $J_{1',2}$ 4.3; $J_{1',2'}$ 2.0; $J_{5,6}$ 6.2; $J_{5,6'}$ 4.2; $J_{6,6'}$ −11.2.

The n.m.r. spectrum for compound **2** at 60 MHz in benzene-d_6 also gave integrals in accord with the assigned structure. A rough singlet centered at τ 5.09, with a half-band of width 7 Hz, was assigned to H-4, an apparent sharp doublet centered at τ 5.75 was assigned to the C-6 methylene group. H-1 and H-5 produced a broad multiplet in the range τ 5.9–7.2 and the signals for both acetyl groups were at τ 8.26. The methylenic protons at C-2, -3, and -4 gave a broad band in the region τ 7.9–9.2.

The spectrum for compound **3** was very well resolved at 100 MHz in benzene-d_6 and the following assignments could readily be made with the aid of spin decoupling. The chemical shifts were τ 6.88 (H-1), 6.25 (H-1'), 8.65 (H-2), 8.00 (H-2'), 5.13 (H-3), 4.65 (H-4), 6.55 (H-5), and both C-6 protons at 5.88. The acetoxy-group signals were at τ 7.95, 8.07, and 8.12. The following coupling constants (Hz) were estimated, $J_{1,1'}$ −12; $J_{1,2}$ 12; $J_{1,2'}$ 2.2; $J_{1',2}$ 5.5; $J_{1',2'}$ 1.8; $J_{3,4}$ 4.8; $J_{4,5}$ 1.5; $(J_{5,6}+J_{5,6'})/2$ 6.8.

(S)-(+)-*2-Hydroxymethyltetrahydropyran* (**4**). — Deacetylation of (*S*)-(+)-2-acetoxymethyltetrahydropyran (**1**) provided a liquid, which was purified by distillation at 20 mm pressure; b.p. 90–91° (compare ref. 23), $[\alpha]_D^{25}$ 19.2° (*c* 1, water). The compound appeared pure when examined by gas chromatography and by n.m.r. spectroscopy. The molar rotations in various solvents were 22° (water), 15° (carbon tetrachloride), 11° (chloroform), 8.3° (methanol), 8° (1,2-dichloroethane), −5° (acetone), −6.5° (methyl sulfoxide).

1,5-Anhydro-2,3-dideoxy-D-threo-*hexitol* ("*1,2,3-Trideoxy*-D-threo-*hexopyranose*," **5**). — Compound **2** (350 mg) was deacetylated as described above to provide syrupy **5**, $[\alpha]_D$ +10° (*c* 0.5, water). The n.m.r. spectrum suggested a high state of purity but the low yield precluded further attempts at purification.

1,5-Anhydro-2-deoxy-D-lyxo-*hexitol* ("*1,2-dideoxy*-D-lyxo-*hexopyranose*", **6**). — Compound **3** (5.5 g; 0.02 mole) was treated in 40 ml of anhydrous methanol with 10 ml of 2M sodium methoxide in methanol. After 2 h at room temperature, the mixture was neutralized with Amberlite IR-120 (H^+) resin. Evaporation of the

solvent gave crystalline **6**, which, after recrystallization from ethyl acetate, had m.p. 129°, $[\alpha]_D^{25}$ +43.1° (*c* 1.0, water) [lit.[24], m.p. 128–129°, $[\alpha]_D^{20}$ +41.8 (*c* 0.5, water)].

(S)-(+)-*2-p-(Tolylsulfonyloxymethyl)tetrahydropyran.* — (*S*)-(+)-2-(Hydroxymethyl)tetrahydropyran (**4**) (3.48 g, 0.03 mole) was treated with recrystallized *p*-toluenesulfonyl chloride (5.7 g, 0.035 mole) in 20 ml of dry pyridine for 20 h at −5°. The crystalline product, m.p. 76°, was purified by recrystallization from petroleum ether–benzene. The n.m.r. spectrum was consistent with the assigned structure.

(R)-(+)-*2-Methyltetrahydropyran* (**7**). — The foregoing *p*-toluenesulfonate (2 g, 0.075 mole) was refluxed in acetone (30 ml) with sodium iodide (3 g, 0.12 mole) for 12 h. The usual work-up and removal of solvent provided (*S*)-(+)-2-(iodomethyl)-tetrahydropyran as a yellow liquid. The crude material (1.6 g) was dissolved in 30 ml of methanol containing 0.6 g of triethylamine and the mixture was shaken in an hydrogen atmosphere with 0.5 g of 5% palladium-on-charcoal for 5 h. The catalyst was then removed by filtration and the solvent removed under normal pressure. The resultant liquid was fractionally distilled at atmospheric pressure to provide 350 mg of (*R*)-(+)-2-methyltetrahydropyran (**7**), b.p. 97–98° (compare ref. 25), $[\alpha]_D^{25}$ +2.5° (*c* 0.5, chloroform), whose n.m.r. spectrum was consistent with the structure assigned. Gas chromatography over silicone rubber on Chromosorb at 75° indicated a high state of purity. The specific rotations of **7** in various solvents are reported in Table I.

*1,5-Anhydro-2,3,6-trideoxy-*D-erythro-*hexitol* ("*1,2,3,6-tetradeoxy-*D-erythro-*hexopyranose*", **9**). — Compound **8** (1.32 g, 0.01 mole) was treated with recrystallized *p*-toluenesulfonyl chloride (1.9 g, 0.01 mole) in 20 ml of dry pyridine for 20 h at −5°. The mixture was worked up as usual to provide a colorless syrup, which was treated overnight in acetone (40 ml) with sodium iodide (0.6 g, 0.04 mole) at reflux temperature. The crude product, in 20 ml of methanol containing 500 mg of 5% palladium-on-charcoal and 600 mg of triethylamine, was reduced with hydrogen for 5 h. The product isolated was purified by preparative gas chromatography over diethylene glycol succinate on Chromosorb at 150°. The n.m.r. spectrum was in good agreement (chemical shifts and integration of signals) with the proposed structure. The specific rotations of **9** in various solvents are given in Table III.

*1,5-Anhydro-2,6-dideoxy-*D-arabino-*hexitol* ("*1,2,6-trideoxy-*D-arabino-*hexopyranose*," **10**). — Compound **6** (1.48 g, 0.01 mole) was treated with recrystallized *p*-toluenesulfonyl chloride (1.9 g, 0.01 mole) in 10 ml of dry pyridine for 28 h. After work-up, the crude *p*-toluenesulfonate was refluxed with sodium iodide (0.6 g, 0.04 mole) in 25 ml of acetone for 6 h. The pale yellow product was hydrogenolyzed in 50 ml of methanol containing 500 mg of 5% palladium-on-charcoal and 600 mg of triethylamine. The colorless syrupy product (**10**) was purified by distillation, b.p. 94–96° (0.5 mm). The n.m.r. spectrum was consistent with the assigned structure. The specific rotations of **10** in various solvents are given in Table I.

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Note

Photochemical reactions of carbohydrates Part III*. The irradiation of D-galactose azine**

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As a result of a continuing interest in the application of photochemical reactions to carbohydrate chemistry, we have studied the photochemistry of D-galactose azine (**1**). Our reason for selecting this azine system for study was the possibility that it would be photochemically converted into D-galactononitrile (**2**). Such a transformation seemed reasonable since the photolysis of aromatic aldazines in the presence of oxygen produces nitriles in high yield[1,2].

$$PhCH{=}NN{=}CHPh \xrightarrow[O_2]{h\nu} PhC{\equiv}N$$

Since sugar azines are readily obtained from sugars[3], a photochemical conversion of an azine into a nitrile would provide a new and useful route for the synthesis of sugar nitriles. This paper presents the results of a photochemical investigation of **1**.

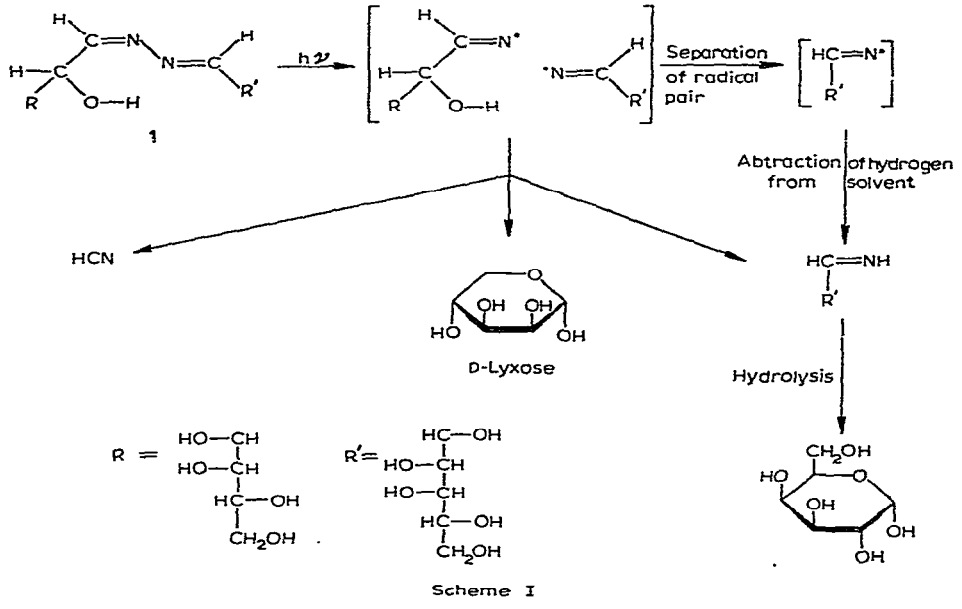
Direct irradiation of 0.50 mmole of **1** in methanol under oxygen with a 450-watt Hanovia mercury-vapor lamp led to essentially complete disappearance of **1** after 2.0 h. Surprisingly, however, the i.r. spectrum of the crude reaction mixture showed no absorption in the nitrile region (4.4–4.6 μm). In addition, analysis of the reaction mixture by t.l.c. in comparison with D-galactononitrile (**2**) showed that, although several photoproducts were formed, none of the expected nitrile **2** was present. A second irradiation, identical with the first but under nitrogen, also showed essentially complete decomposition of the starting material after 2.0 h, but no nitrile **2** was formed (i.r. spectrum, t.l.c.). Of the products formed by irradiation of **1** under nitrogen, only two appeared (by t.l.c.) to be the same as those formed during the similar irradiation under oxygen.

Thick-paper chromatographic analysis of the products from the irradiation made under oxygen led to the isolation of two sugars, identified as D-galactose (37%) and D-lyxose (23%). These two compounds were also isolated in 32% (D-galactose)

*Part II : W. W. Binkley and R. W. Binkley, *Carbohyd. Res.*, 11 (1969) 1.
**Dedicated to the memory of Professor M. L. Wolfrom.

and 14% (D-lyxose) yield from the irradiation under nitrogen. Control experiments showed that D-galactose azine (**1**) was stable in the absence of light under the conditions of reaction and isolation.

A possible mechanism for the formation of D-lyxose and D-galactose from the photolysis of D-galactose azine (**1**) is shown in Scheme I.



Scheme I

EXPERIMENTAL

General methods. — Solutions were evaporated below 50° under diminished pressure unless stated otherwise. Melting points were determined with a Fisher–Johns melting-point apparatus (Fisher Scientific Co., Pittsburgh, Pa.) and are uncorrected. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak.

Irradiation. — In each reaction the stirred solution of D-galactose azine (**1**) was irradiated at 20° with a 450-watt Hanovia high-pressure quartz mercury vapor lamp that had been lowered into a water-cooled, quartz immersion-well. Prepurified nitrogen was passed through the solution for 1 h prior to irradiation and a slow stream of nitrogen was continued during photolysis. No filter was used. Solutions of the irradiation mixtures were evaporated below 30° under diminished pressure before chromatography. Specific information for each photolysis experiment is given in footnotes *a–d* of Table I.

Isolation. — In a typical thick-paper chromatogram, 70–150 mg lots of the irradiated reaction mixture in 2–3 ml of methanol were deposited on a sheet (23 × 53 cm) of Whatman No. 17 filter paper, which was developed at 20° with 150–180 ml of 85:15 (v/v) acetone–water. A strip (3 cm wide), cut from the lengthwise

edge of the chromatogram, was sprayed with *p*-anisidine hydrochloride and was used as a guide in the location of the zones. The yields of materials recovered by water elution are given in Table I.

TABLE I

ZONE LOCATIONS AND YIELDS OF MATERIALS RECOVERED FROM THE THICK-PAPER CHROMATOGRAPHY OF IRRADIATED D-GALACTOSE AZINE

Zone	*Zone location*	*Yields of recovered materials (mg)*				*Compound isolated*
	(cm)[e]	*Expt. A*[a]	*Expt. B*[b]	*Expt. C*[c]	*Expt. D*[d]	
1	0.0–0.9	18.9	25.9	8.8	8.3	
2	0.4–1.3			11.7		
3	1.3–5.8	13.3	14.8	7.9	15.5	
4	5.2–7.0		7.7	8.1		D-Galactose azine
5	7.3–10.9	19.9	25.1	39.8	25.5	D-Galactose
6	13.8–16.9	4.4	3.5	4.6		
7	17.0–21.3	6.6	8.9	8.7	13.2	D-Lyxose
8	24.5–36.1	6.3	5.8	5.5	20.0	
9	36.7–43.5	10.5		4.9	13.0	
10	45.6–53.6	20.1	8.3		4.5	

[a]Irradiated for 1.0 h, 80 mg of material in 400 ml of methanol, under nitrogen. [b]Irradiated for 2.0 h, 154 mg of material in 400 ml of methanol, under nitrogen. [c]Irradiated for 2.0 h, 308 mg of material in 400 ml of water, under nitrogen. [d]Irradiated for 2.0 h, 138 mg of material in 400 ml of methanol, under oxygen. [e]Distance from point of addition of adsorbate.

α-D-*Lyxopyranose tetraacetate* (**3**). — A mixture of D-lyxose (Nutritional Biochemicals Corp., Cleveland, Ohio) (1.00 g), powdered, fused sodium acetate (500 mg), and acetic anhydride (10 ml) was heated at 80° until all of the reactants had dissolved (15 min), and heating was continued for a total of 2 h at 80°. Partial removal of the solvents at 80° was achieved under diminished pressure, chloroform (20 ml) was added to the residue, and the mixture was shaken with saturated aqueous sodium hydrogen carbonate (10 ml) at 10° until all acetic anhydride had been removed. The dried (sodium sulfate) chloroform solution was evaporated, and resulting syrup crystallized spontaneously; yield 2.17 g. These crystals were recrystallized twice from 95% ethanol; m.p. 93–94°, $[\alpha]_D^{20}$ +25.1° (*c* 3.3, chloroform) [lit.[4] m.p. 93–94°, $[\alpha]_D^{25}$ +25° (chloroform), method of preparation not given]; they were designated **3** *lm* (low melting); t.l.c. R_F 0.82 on silica gel developed with 100:1 (v/v) benzene–*tert*-butyl alcohol; X-ray powder diffraction data: 9.87 vs, 7.54 vvs, 5.99 vs, 4.81 s, 4.62 m, 4.373 vs, 4.072 vs, 3.754 s, 3.559 s, 3.450 vs, 3.377 w, 3.207 m, 3.093 s, 2.996 mw, 2.895 m, 2.688 ms, 2.617 m, 2.528 mw, 2.467 mw, 2.412 m, 2.346 w, 2.300 mw, 2.191 m, 2.135 mw, 2.080 mw, 1.936 mw, 1.804 m, 1.637 mw.

In another acetylation of D-lyxose, performed in the same manner, the crystals formed in 95% ethanol had m.p. 113–114°, $[\alpha]_D^{20}$ +25.0° (*c* 2.0, chloroform), and were designated **3** *hm* (high melting). The i.r. spectrum and t.l.c. mobility on silica gel of these crystals were identical with those of **3** *lm*: X-ray powder diffraction data of

3 *hm*: 9.87 vs, 7.57 vvs, 5.99 vs, 4.84 s, 4.61 m, 4.382 vs, 4.072 vs, 3.754 s, 3.570 s, 3.449 vs, 3.370 w, 3.220 m, 3.080 s, 2.988 mw, 2.898 m, 2.698 ms, 2.610 m, 2.535 mw, 2.464 mw, 2.406 m, 2.341 w, 2.292 mw, 2.186 m, 2.131 mw, 2.078 mw, 1.925 mw, 1.802 m, 1.637 mw.

Anal. Calc. for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70. Found: C, 49.07; H, 5.71.

Recovery of unreacted D-*galactose azine* (**1**) *from zone 4 of Experiment B.* — Elongated prisms formed slowly in the residual syrup isolated from this zone (see Table I). These crystals were found to be identical with **1** (i.r., t.l.c., paper chromatography).

Isolation of D-*galactose from zone 5.* — The syrups recovered from this zone yielded elongated prisms having an i.r. spectrum, and R_F values on paper and powdered cellulose by t.l.c. identical with those for D-galactose. A methanolic solution of the residual syrups yielded golden-colored crystals. An aqueous-methanolic solution of these crystals afforded straw-colored crystals, m.p. 165–166° (mixed m.p. with D-galactose unaltered).

Isolation of D-*lyxose tetraacetate* (**3**) *from zone 7.* — The combined, residual syrups from zone 7 possessed R_F values on paper and powdered cellulose by t.l.c. identical with those for D-lyxose. A lot (40 mg) of these syrups was allowed to react with acetic anhydride (5 ml) and sodium acetate (50 mg) for 2 h at 80–90°. The work-up of the reaction mixture was the same as that used in the preparation of **3**; yield 74 mg. An ethanolic solution of the resulting acetate, on nucleation with 3 *hm*, yielded 19 mg of prisms; the i.r. spectrum and t.l.c. R_F value on silica gel were identical with those of **3**. Recrystallization from the same solvent, nucleating with 3 *hm*, afforded prisms, m.p. 112–113°; identical with α-D-lyxopyranose tetraacetate (3 *hm*) by mixed m.p. and X-ray powder diffraction.

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The authors thank Dr. R. Schaffer for the authentic specimen of α-D-lyxopyranose tetraacetate, W. T. Winter for obtaining the X-ray powder diffraction data, and W. F. Altenburg for assistance with the thick-paper chromatography.

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Note

Isolation of L-fucose 4-sulfate from fucoidan*†

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Fucoidan has been isolated from various species of brown seaweed and described as a sulfated polysaccharide composed mainly of L-fucose units. In 1950, Conchie and Percival[1] suggested, on the basis of the results of methylation and alkali treatment, that most of the sulfate groups of fucoidan from *Fucus vesiculosus* are located at position 4 of the fucose units. In spite of this evidence, L-fucose 4-sulfate has not yet been isolated from the seaweed polysaccharide.

In the present paper, a fucoidan[2] containing L-fucose and a small amount of D-galactose, isolated from brown seaweed *Pelvetia wrightii*, was partially hydrolyzed with dilute sulfuric acid, and L-fucose 4-sulfate was isolated from its hydrolyzate.

The fact that L-fucose 4-sulfate was isolated from fucoidan can be considered to be a direct proof for the presence of a sulfate group situated at C-4 of the L-fucose units of the polysaccharide, since migration of an ester sulfate is unlikely to occur in dilute acid solution[3,4]. This appears to be the first instance of the isolation of fucose sulfate from a natural source, and the synthesis of L-fucose 4-sulfate has not hitherto been reported.

EXPERIMENTAL

A solution of fucoidan (3.0 g) in 0.05M sulfuric acid (300 ml) was heated for 1 h at 100°. The hydrolyzate was neutralized with aqueous barium hydroxide, and after centrifugation 3 volumes of ethanol were added to precipitate the undegraded polymer. The supernatant was concentrated and passed through a column (2 × 25 cm) of Dowex-1 (X-2, AcO^-). The column was washed with water (1 l) to separate the neutral components, and then eluted with 0.25M sulfuric acid (2 l) to obtain the acidic fractions. These (660 mg, determined as fucose) were neutralized with barium hydroxide, and then were converted into the potassium salt by passage through a column of Dowex-50 (H^+), followed by neutralization of the effluent with a solution of potassium hydroxide. A portion (150 mg, determined as fucose) of this potassium salt solution was applied to a column (2.2 × 95 cm) of Sephadex G-25 (fine), and

*Dedicated to the memory of Professor M. L. Wolfrom.
†A preliminary communication was presented at the 6th International Seaweed Symposium at Santiago, Spain, September, 1968.

eluted with water. The eluates were analyzed for fucose by the phenol–sulfuric acid test[5]. The main peak contained 2 components, which were detected with alkaline silver nitrate[6], after descending paper chromatography in 50:12:25 butyl alcohol–acetic acid–water, and paper electrophoresis in 0.1M acetic acid–pyridine buffer (pH 6.5, 3000 V, 30 min) on Toyo No. 51 paper. The faster moving component having R_G 0.81 on the paper chromatogram was eluted with water from large sheets of thick paper (Toyo No. 514). The eluates were collected, concentrated to a small volume, and freeze-dried. The yield of this component, calculated as fucose from colorimetric measurement[5], was 25% of the acidic fraction.

The component was homogeneous on either paper chromatography or paper electrophoresis. In the latter test[7], it migrated 6.8 cm from the starting line (L-fucose, 0.8 cm) under the conditions described above. The mobility was about the same as that of galactose 6-sulfate and that of L-fucose monosulfate, prepared by direct sulfation with chlorosulfonic acid[8]. On further hydrolysis (1.0M hydrochloric acid for 3 h at 100°), only L-fucose was detected on a paper chromatogram. The molar ratio of L-fucose[9] to sulfate[10] was 1.00:1.07; $[\alpha]_D^{16}$ −66° (*c* 0.6, water). The degree of polymerization determined by the method of Timell[11] was 1.1. Reduction with sodium borohydride, followed by hydrolysis and g.l.c. of the per(trimethylsilyl) derivatives[12], on a Shimazu model GC-1C chromatograph, gave only fucitol. The i.r. spectrum obtained with a KBr disc had a strong absorption band in the 1240-cm^{-1} region and a sharp band at 845 cm^{-1}, suggesting that the sulfate group is substituted at the axial C-4 position of the L-fucopyranose unit in the stable *C1* conformation[13,14]. With the *p*-anisidine hydrochloride reagent, the compound gave a yellow spot, and showed strong fluorescence under u.v. light on paper as described for D-galactose 4-sulfate[15]. On periodate oxidation in unbuffered solution, it consumed[16] the following moles of oxidant per mole (time in h): 0.70 (0.75); 0.98 (2); 1.34 (6); 1.59(27); 2.22(54); and 2.32(124), and it liberated 1.8 mole of formic acid and no acetaldehyde after 124 h. These results indicate that the sugar sulfate is L-fucose 4-sulfate.

The substance showing a slower moving spot (R_G 0.54) was isolated from the thick-paper chromatogram, and it was tentatively characterized by its properties and periodate oxidation as an α-L-(1→2)-linked fucobiose having an ester sulfate linked to the C-4 of the non-reducing moiety.

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Note

The isolation of *myo*-inositol from roasted *Coffea arabica**

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(Received October 3rd, 1969; in revised form, November 17th, 1969)

Coffee, an important commodity, has been the subject of numerous chemical investigations. A number of workers [1–13] have investigated the mono-, oligo-, and poly-saccharides of green and roasted beans. Herein we report the isolation and identification of another carbohydrate component of roasted coffee, *myo*-inositol.

An aqueous solution of a commercial coffee extract was treated with Somogyi reagent[14], which precipitates the proteinaceous and melanoidin components. The solids in the supernatant were fractionated by column and paper chromatography. One fraction was identified as *myo*-inositol by i.r. spectroscopy. This identification was confirmed by the preparation of hexa-*O*-acetyl-*myo*-inositol. An extract of roasted coffee beans with 80% ethanol also yielded *myo*-inositol, identified as its crystalline hexaacetate.

EXPERIMENTAL

General. — Solutions were evaporated under diminished pressure. Melting points were determined with a Thomas–Hoover "Unimelt" apparatus. I.r. spectra were determined with a Perkin–Elmer Model 221 spectrophotometer. Descending chromatography was performed with Whatman No. 1 paper. Extrusion-column chromatography was performed on Avicel microcrystalline cellulose (Technical Grade, Avicel Sales Division, FMC Corp., Marcus Hook, Pa.) by a technique described elsewhere[15]. Three chromatographic solvent-systems were employed: (*a*) 10:3:3 butyl alcohol–ethanol–water; (*b*) 9:2:2 ethyl acetate–acetic acid–water; (*c*) 6:1:3 propyl alcohol–ethyl acetate–water. The silver nitrate–sodium hydroxide reagent[16] was used for spraying the paper and column chromatograms.

Removal of melanoidins from soluble coffee powder. — The soluble coffee sample was obtained in factory-sealed jars. The powder (100 g, 3% moisture) was dissolved in water (2000 ml) at 50°. After cooling to 20°, 0.15M barium hydroxide (2000 ml) and 5.0% zinc sulfate (2000 ml) were added[14]. After the resulting precipitate had been removed by centrifugation, the supernatant was passed through a column (43 × 410 mm) of Amberlite MB-3 (H^+, OH^-) ion-exchange resin. The eluate was then freeze-dried to yield a light brown solid (*A*); wt. 30 g.

*Dedicated to the memory of Professor M. L. Wolfrom.

Chromatographic fractionation of A. — A column (50 × 260 mm) was prepared[15] with 200 g of Avicel and 800 ml of solvent (*a*) and washed with 300 ml of the solvent. The sample (*A*, 500 mg) was placed on the top of the column as a solution in 10 ml of the solvent. By using a vacuum of 10–20 cm Hg, 800 ml of solvent (*a*) was passed through the column. After draining, the column was extruded and sprayed with silver nitrate–sodium hydroxide reagent[16]. Four zones were revealed. The zone extending from 80 to 120 mm from the column top was excised and eluted with three 250-ml portions of water. The combined eluates were centrifuged and the soluble solids recovered by freeze drying. The solids from 5 such columns were combined and re-chromatographed in the same manner to give one zone. The weight of recovered material (*B*) was 70 mg. Paper chromatography of *B* with solvent (*b*) showed two components. A preparative paper was then run with 7 mg of *B*. Guide strips were cut from the sides of the paper and sprayed with silver nitrate–sodium hydroxide reagent. The horizontal strip of paper corresponding to the slower-moving major (based on visual estimation) component was excised and eluted. The eluate was evaporated to 4 ml.

Identification of myo-*inositol.* — An aliquot of the eluate was taken to dryness in a stream of warm air and the residue was mixed with potassium bromide. The i.r. spectrum of this mixture was identical with that published[17] for *myo*-inositol, and with the i.r. spectrum of an authentic sample (Pfanstiehl Chemical Co., Waukegan, Ill.).

Isolation of myo-*inositol from soluble coffee powder as its hexaacetate.* — Coffee powder (2.5 g) was refluxed for 2 h with 0.6M hydrochloric acid[18] (250 ml). After cooling, the solution was filtered, diluted to 1500 ml, and passed through an MB-3 (H^+, OH^-) ion-exchange column (43 × 410 mm). The eluate was then evaporated to a syrup, which was chromatographed on an Avicel column (50 × 260 mm), prepared as described previously, except that solvent (*c*) was used. After 400 ml of solvent (*c*) had passed through the column the packing was extruded. Spraying with the silver nitrate–sodium hydroxide reagent showed two zones. The slower-moving zone was excised and then eluted with 300 ml of the developing solvent. The eluate was evaporated to a syrup, which was acetylated[19] by refluxing for 2 h with acetic anhydride (10 ml) containing a drop of concentrated sulfuric acid. The acetylation mixture was poured into boiling water (10 ml). The solid that formed was filtered off and taken up in chloroform (25 ml). The filtrate was extracted with an equal volume of chloroform to a total of 3 times. All of the chloroform solutions were combined and washed successively with equal volumes of water, saturated sodium hydrogen carbonate, and water. The chloroform solution was dried over sodium sulfate and evaporated to give a dark-brown syrup. This was dissolved in absolute ethanol (10 ml) and the solution boiled with decoloziring carbon for 2 min. The carbon was filtered off and the ethanol evaporated to a small volume and cooled. A small amount of crystals (10 mg) formed. These melted at 214–215°, unchanged when mixed with authentic hexa-*O*-acetyl-*myo*-inositol.

Isolation of myo-*inositol from roasted beans of* Coffea arabica *as its hexaacetate.*

— Roasted beans (83 g, equivalent to 100 g of green beans) were ground and extracted[9] with petroleum ether (350 ml) and then with 80% aqueous ethanol (500 ml). The alcohol solution was evaporated to a syrup, which was mixed with water (100 ml) and the insoluble solids removed by centrifugation. These solids were washed once with water (100 ml) and centrifuged. The supernatant and washings were combined and evaporated to a syrup. The syrup was hydrolyzed and treated as described above for soluble coffee, to give hexa-*O*-acetyl-*myo*-inositol.

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Note

Attempted formation of an anhydronucleoside from 1-(2,3,5-trideoxy-5-iodo-β-D-*glycero*-pent-2-enofuranosyl)thymine*

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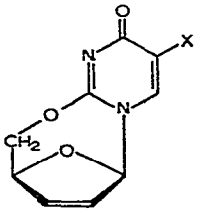
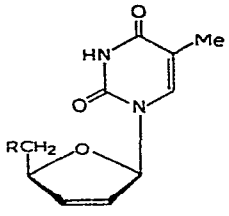
Measurements of circular dichroism on pyrimidine nucleosides[1-3] prompted the attempted synthesis of a nucleoside related to **1**, where X is arbitrary.



1, X = Me

The most suitable route to a molecule of this form appeared to be from 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine[4] (**2**).



2 R = OH
3 R = I

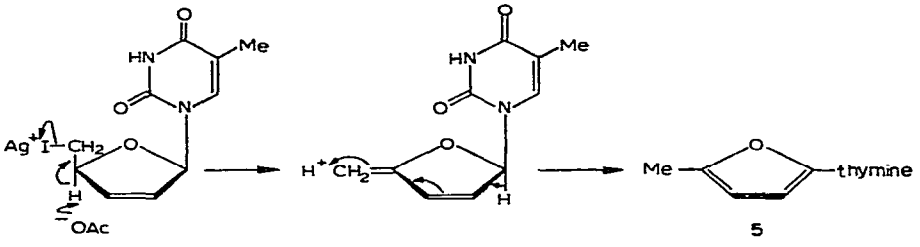
Conversion of the 5′-hydroxymethyl group of **2** into a 5′-iodomethyl group, followed by formation[5,6] of an anhydronucleoside under silver acetate catalysis, would then possibly give **1**.

Treatment of **2** with methyltriphenoxyphosphonium iodide[7] gave a good yield of 1-(2,3,5-trideoxy-5-iodo-β-D-*glycero*-pent-2-enofuranosyl)thymine (**3**) without any evidence of reaction with the isolated 2′,3′-double bond. The structure of **3** was established on the basis of its elemental analysis, p.m.r. spectrum and its conversion into 1-(2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)thymine (**4**). The p.m.r. spectrum of **3**, measured in methyl sulfoxide-d_6, showed a striking resemblance to the published

*Dedicated to the memory of Professor M. L. Wolfrom.

spectrum of 9-(2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl)adenine[8]. The properties of **4** differed from those reported by Todd *et al.*[6] for impure **4**.

Attempts to convert **3** into **1** by treatment with silver acetate in refluxing methanol[5] failed. The rapid formation of a precipitate of yellow silver iodide, such as that which occurs in the preparation of an anhydronucleoside from 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine[5], was not evident. T.l.c. demonstrated the presence of starting material, even after a reaction time of several h. The procedure of Michelson and Todd[6], which was successful in converting 5′-deoxy-5′-iodothymidine into an anhydronucleoside, was investigated. When treated with silver acetate in refluxing acetonitrile, **3** reacted sluggishly and there was no evidence of anhydronucleoside formation after the prescribed time. T.l.c. indicated the presence of a small proportion of a new, slightly faster-moving product. Increase in the quantity of silver acetate and extension of the time of reaction resulted in further conversion into this product. This product proved to be 1-[2-(5-methylfuryl)]thymine (**5**), which would be expected from the sequence of reactions shown[9].



5

The addition of amines to purified acetonitrile in this silver acetate-catalyzed procedure, as recommended by Michelson and Todd[6], gave similar results and still did not produce any anhydronucleoside.

Fox *et al.*[10] recently demonstrated that the conformation of the D-ribose moiety is an important factor in the formation of anhydronucleosides involving the 5′-methylene group. It is probable that the constraint to planarity imposed by the 2′,3′-double bond of **3** prevents this molecule from adopting a conformation favorable for formation of an anhydronucleoside. This limitation is not found, of course, in the flexible sugar moiety of 5′-deoxy-5′-iodo-thymidine and so in this case the anhydronucleoside is formed.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. P.m.r. spectra were measured with appropriate internal standards of tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate with a Varian A-60 n.m.r. spectrometer. First-order *J* values are given. U.v. spectra were determined with a Beckman DK-2 spectrophotometer. I.r. spectra were determined with a Beckman IR-5 spectrophotometer. Detection of

components on SilicAR 7GF (Mallinckrodt) was by u.v. light. Silica gel was chromatographic grade (J. T. Baker Chemical Co.). Solvent proportions were by volume. Evaporations were performed under diminished pressure at 35°.

*1-(2,3,5-Trideoxy-5-iodo-β-D-*glycero*-pent-2-enofuranosyl)thymine* (**3**). — A mixture of 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine[4] (1.63 g) and methyltriphenoxyphosphonium iodide[7] (3.3 g) in dry *N,N*-dimethylformamide (10 ml) was sealed and kept for 3.5 h at room temperature. The solution was evaporated at 30° to give a partially crystalline residue. This residue was dissolved in chloroform and the solution was washed twice with sodium thiosulfate solution and water. The dried ($MgSO_4$) solution was evaporated to a small volume whereupon crystallization occurred; yield, 1.97 g (81%), m.p. 112–113° (unchanged after recrystallization from chloroform); λ_{max}^{KBr} 1670, 1700 cm^{-1} (C=O of thymine moiety); λ_{max}^{pHl} 264 (ε 10,350), λ_{max}^{pHll} 264 nm (ε 8,000); p.m.r. (methyl sulfoxide-d_6) δ 1.81 (3-proton singlet, 5-CH_3), 3.51 (2-proton doublet, *J* 5.0 Hz, 5′-CH_2I), 4.71–5.10 (1-proton multiplet, H-4′), 5.98–6.21 (1-proton multiplet, H-3′), 6.35–6.55 (1-proton multiplet, H-2′), 6.71–6.88 (1-proton multiplet, H-1′), 7.40 (1-proton singlet, H-6), 11.66 (1-proton singlet, NH).

Anal. Calc. for $C_{10}H_{11}IN_2O_3$: C, 35.95; H, 3.32: N, 8.39. Found: C, 35.88; H, 3.17; N, 8.01.

Attempted formation of an anhydronucleoside from **3**. — *A*. A mixture of **3** (0.50 g) and silver acetate (0.80 g) in dry "nanograde" acetonitrile (Mallinckrodt) (45 ml) was heated for 1.25 h with stirring under reflux. A small aliquot of supernatant was treated with hydrogen sulfide and examined by t.l.c. on SilicAR gel 7GF with 1:1 ethyl acetate–chloroform as developer. Several developments revealed the presence of a small proportion of a faster-moving component in addition to starting material. Portions (0.80 g each) of silver acetate were added after 2, 4, and 6 h. After 4 h an additional 45 ml of acetonitrile was also added. The reaction was allowed to continue for 17 h. The mixture was cooled, filtered through Celite and the filtrate was treated with hydrogen sulfide. The resulting precipitate was removed and the filtrate was evaporated to a syrup. This syrup was dissolved in chloroform and applied to the short edge of a SilicAR 7GF plate (2 × 200 × 400 mm) and the plate was developed with 4:1 chloroform–ethyl acetate. The major band was excised and the silica gel was extracted with methanol. Solvent removal gave a crystalline residue. This material was extracted with ethyl acetate and the solvent removed to give 0.07 g (m.p. 158–162°). Recrystallization from ethyl acetate–hexane gave pure **5**; m.p. 164–166°; p.m.r. (methyl sulfoxide-d_6) δ 1.88 (3-proton singlet, 5-CH_3), 2.34 (3-proton singlet, 5′-CH_3), 6.23 (1-proton doublet of doublets, $J_{3',5'}$ 1.0 Hz, H-3′), 6.37 (1-proton doublet, $J_{2',3'}$ 3.5 Hz, H-2′), 7.52 (1-proton doublet, $J_{6,5\text{-}CH_3}$ 1.0 Hz, H-6), 11.91 (1-proton singlet, NH).

Anal. Calc. for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.58. Found: C, 57.97; H, 4.86; N, 13.31.

Horwitz *et al.*[4] reported m.p. 165–166.5°, p.m.r. (methyl sulfoxide-d_6) δ 1.8 and 2.25.

B. To **3** (0.20 g), silver acetate (0.32 g) in dry "nanograde" acetonitrile (20 ml)

was added triethylamine (0.3 ml). The mixture was heated for 1.25 h under reflux with stirring. Examination by t.l.c. revealed two spots, **3** and **5**, with **3** having slightly greater intensity.

*1-(2,3,5-Trideoxy-β-*D*-glycero*-*pentofuranosyl)thymine* (**4**). — A mixture of 1-(2,3,5-trideoxy-5-iodo-β-D-*glycero*-pent-2-enofuranosyl)thymine (0.50 g), sodium acetate (0.50), and 5% palladium on charcoal (0.20 g) in ethanol (75 ml) was hydrogenated at 5 lb.in^{-2} for 0.75 h at room temperature. The mixture was filtered through Celite. Silica gel was added to the filtrate, and the mixture was evaporated to give a dry, free-running powder. This material was added to a dry column (43 × 2.6 cm) of silica gel to give a column having dimensions 55 × 2.6 cm. The column was eluted with chloroform and the first 300 ml of eluate were collected and evaporated to a syrup, which crystallized spontaneously. Trituration with warm heptane followed by cooling gave 0.24 g of white crystals, m.p. 144–145°. This material was dissolved in chloroform and the solution was treated with charcoal. The syrup obtained on removal of solvent was crystallized from chloroform–heptane to give pure **4**; m.p. 148–149°; λ_{max}^{pH1} 267 (ε 9,870), λ_{max}^{pH11} 267 nm (7,550); p.m.r. (methyl sulfoxide-d_6) 1.28 (3-proton doublet, $J_{4',5'}$ 6.0 Hz, 5'-CH_3), 1.85 (3-proton singlet, 5-CH_3), 1.50–2.40 (4-proton multiplet, H-2' and -3'), 3.75–4.27 (1-proton multiplet, H-4'), 5.80–6.10 (1-proton quartet, H-1'), 7.41 (1-proton doublet, $J_{6,5\text{-}CH_3}$ 1.0 Hz, H-6), 11.41 (1-proton singlet, NH).

Anal. Calc. for $C_{10}H_{14}N_2O_3$: C, 57.13; H, 6.71; N, 13.33. Found: C, 57.06; H, 6.70; N, 13.27.

Todd *et al.*[6] reported data on impure **4**.

ACKNOWLEDGMENTS

The author thanks Professor Roland K. Robins for his helpful suggestions, and the National Cancer Institute of the National Institutes of Health, Public Health Service (Grant No. CA-08109-4) for financial support.

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Note

Synthesis of D-arabinose from D-*glycero*-D-*gulo*-heptono-1,4-lactone*

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In the course of our studies involving the synthesis of some rare sugars, use was made of the selective cleavage of certain bonds by periodic acid in unsubstituted carbohydrates. It was reported in the synthesis of 4-*C*-(hydroxymethyl)-L-xylurono-5,2-lactone from 2-*C*-(hydroxymethyl)-D-glucono-1,4-lactone that the side chain of an aldonolactone could be selectively cleaved by periodic acid, while maintaining the lactone ring intact[1]. Another example of this reaction was the production of L-lyxurono-5,2-lactone from D-galactono-1,4-lactone[2].

Oxidation of D-*glycero*-D-*gulo*-heptono-1,4-lactone with one mole of periodic acid, however, yielded not the expected shorter-chained lactone, but D-arabinose in 70% yield, evidently through selective cleavage of the C-2–C-3 bond.

Examination of the structures of the three starting materials indicates that the course of the reaction is dependent upon the configuration of the hydroxyl groups in the lactone ring. In the first two examples, the only vicinal pair of hydroxyl groups in the ring is oriented trans, and is thus unfavorably situated for cleavage by periodic acid. Furthermore, in the case of 2-*C*-(hydroxymethyl)-D-glucono-1,4-lactone, one of the vicinal hydroxyl groups of the ring is tertiary. Apparently, cleavage on the side chain occurs faster than ring cleavage or ring opening (hydrolysis), and the product having an intact lactone ring is obtained.

In the case of D-*glycero*-D-*gulo*-heptono-1,4-lactone, however, there is a vicinal *cis*-diol in the ring, at C-2 and C-3. In this situation, cleavage in the ring is more favorable than side-chain cleavage, and D-arabinose is produced in good yield. The arabinose was identified by physical constants and by preparation of the tetraacetate and arabinitol as derivatives.

EXPERIMENTAL

General. — All evaporations and concentrations were done in a rotary evaporator under diminished pressure at 40° (bath). Melting points are uncorrected.

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D-*Arabinose.* — A solution of D-*glycero*-D-*gulo*-heptono-1,4-lactone (50 g) in water (400 ml), in a low-actinic Erlenmeyer flask, was cooled to 0° in an ice–salt bath and a solution of periodic acid (50 g) in water (200 ml) was added dropwise with stirring over 30 min. The reaction mixture was kept overnight at 4°, and was passed over a column of Amberlite IR-4B(OH^-) (200 ml of wet resin) at 4°. Concentration to a syrup and crystallization from 95% ethanol gave D-arabinose (25 g, 70%) having m.p. and mixture m.p. 163–165°, $[\alpha]_D^{28} - 104°$ (24 h) (*c* 2.06, water) (lit[3] m.p. 160° and $[\alpha]_D^{20} - 104.5°$).

Acetylation of the product gave 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose having m.p. 97–99° (lit.[4] m.p. 97°), and borohydride reduction gave D-arabinitol having m.p. 103–104° (lit.[5] m.p. 102°).

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Note

Chemistry of rutin. Mass-spectral characterization of rutinose heptaacetate*

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That rutinose and quercetin are hydrolysis products of rutin, either through enzymic[1] or weakly acidic[2] catalysis, has long been known. The synthesis of rutinose heptaacetate (**1**) from 2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl bromide and 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose, and subsequent deacetylation of **1**, led to an assignment[3] of the structure 6-*O*-(β-L-rhamnopyranosyl)-D-glucopyranose to rutinose. On the basis of periodate-oxidation studies[4], the structure of rutinose was revised to 6-*O*-(α-L-rhamnopyranosyl)-D-glucopyranose. Quite recently, a novel procedure for preparation of rutinose heptaacetate from rutin decaacetate has been described[5].

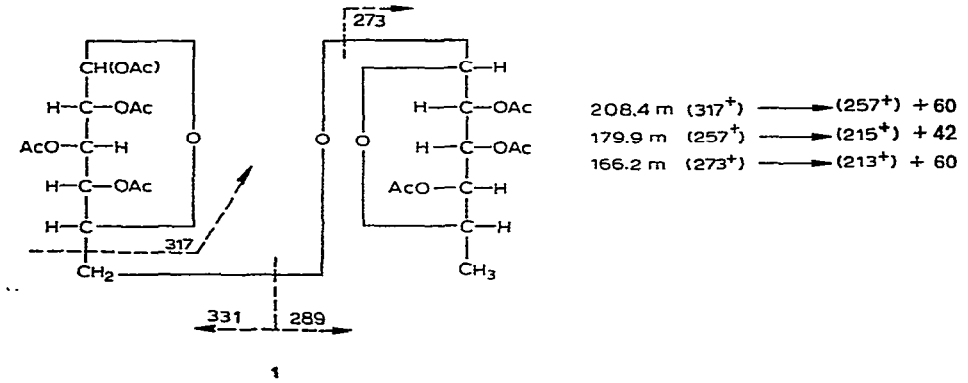
In the first report[2] of hydrolysis of rutin in dilute acetic acid, rutinose heptaacetate (**1**) was obtained in rather low yield by acetylation of the crude sugar fraction. By extending the period of hydrolysis and making other minor procedural modifications, **1** can be obtained in nearly twice the yield previously cited. In addition to customary analytical characterization, we have measured the mass spectrum of **1** (*m/e* values in Experimental).

The spectrum does not show a molecular-ion peak at 620. However, peaks are observed at *m/e* 561 and 560, as would be expected by loss of CH_3CO_2 and CH_3CO_2H fragments, respectively, from an ion of mass 620. The peaks listed (Experimental) are generally of low relative intensity, but can be plausibly accounted for in terms of stepwise losses of CH_3CO_2, CH_3CO_2H, and CH_2CO fragments. Such decomposition is known to be characteristic of acetic esters[6] and, in particular, of sugar polyacetates[7].

By far the strongest peak in the spectrum is that at *m/e* 43, attributed[7] to CH_3CO^+. Other prominent peaks can be accounted for by characteristic ether cleavages[8] as shown by the broken lines in Fig. 1. Moreover, the two strongest peaks in the high-mass region, those at *m/e* 317 and 273, are the two strongest peaks in the entire spectrum when measurement is made at a lower ionizing potential. Smaller peaks occur at *m/e* 331, 330 (from hydrogen migration, also characteristic of ethers)[8c],

*Dedicated to the memory of Professor M. L. Wolfrom.

and 289. Relatively strong peaks at *m/e* 257, 215, and 213 can be shown by metastable peaks to arise by further breakdown of major primary products.



1

Fig. 1. Principal pathways for fragmentation of rutinose heptaacetate (1) in the mass spectrometer. Transitions corresponding to metastable peaks (m) are shown.

In addition to peaks associated with rutinose heptaacetate, a series of peaks occur at *m/e* 534, 474, 445, 432, 415, 414, and 372. These may originate from an acetylated proanthocyanidin hydrate, a specific example of which would be a diarylglycerol derivative [$C_{15}H_{11}O_8(COCH_3)_5$]. The proanthocyanidin area has been reviewed by Freudenberg[9]. A proanthocyanidin hydrate could be a contaminant of rutin, despite purification of the rutin by recrystallization from water. In terms of the presently accepted structure[3], a proanthocyanidin hydrate can not arise by hydrolysis of rutin under non-reductive conditions.

Presence of the rutinose moiety in rutin is clearly demonstrated by hydrolytic experiments[1,2], and more recently by syntheses of rutin in two different laboratories[10,11]. Methylation of rutin has been investigated in the present work in an effort to achieve permethylation of this flavonol glycoside. The procedure employed involves partial methylation by interaction of rutin with methyl sulfate and aqueous alkali, and then repeated further methylation steps with methyl sulfate and dry sodium hydroxide in tetrahydrofuran. The initial methylation process is carried out in such a way that it is violently exothermic, and partial oxidative degradation may well occur. Furthermore, completely consistent results have not been realized. In several instances, especially when an additional *p*-phenylazobenzoylation (azoylation) step was introduced to remove partial methylation products, the procedure has given a colorless solid for which combustion and methoxyl analyses are in agreement with theory for a decamethyl ether of rutin. In other experiments, however, an unstable, crystalline, yellow product or yellow oils were obtained.

Acidic hydrolysis of the colorless methylation product of rutin yields the known quercetin 3′,4′,5,7-tetramethyl ether and a methylated sugar fraction. Azoylation and subsequent chromatographic purification of the azoylated sugars by the general procedure of Reich[12] and Coleman[13] gave a crystalline azoyl derivative

whose analysis and m.p. indicate that it is 1,6-di-*O*-azoyl-2,3,4-tri-*O*-methyl-D-glucopyranose. Thus, methylation and hydrolysis indicate that the D-glucose portion of the rutinose moiety in rutin, as well as in the isolated sugar rutinose, has the pyranoid ring structure. All attempts to detect 2,3,4-tri-*O*-methyl-L-rhamnopyranose were unsuccessful.

EXPERIMENTAL

General. — Melting points were measured in capillary tubes and are uncorrected. Mass spectra were measured with 70-volt and low-voltage (9.5 volts, nominal) electrons on a Consolidated Model 21-103 mass spectrometer, with the source and inlet at 250°. Microanalyses were determined by Alfred Bernhardt, Geller Laboratories, and Micro-Tech Laboratories.

Rutinose heptaacetate (**1**). — Rutin trihydrate (N.F. X, 10 g, recrystallized from water) was heated with 300 ml of 10% aqueous acetic acid for 12 h and the sugar fraction was isolated by the general procedure of Zemplén[2]. The crude sugar was acetylated with 20 ml of acetic anhydride and 4 g of anhydrous sodium acetate for 9 h at steam-bath temperature. The clear reaction mixture was poured into a large excess of water and kept overnight. Evaporation of the resulting mixture to dryness *in vacuo* gave a residue which was washed several times with hot water. The residue then was extracted with chloroform; the chloroform extract was washed with water, dried, and evaporated to dryness. The resulting residue was treated with ether and nucleated with **1**, whereupon a colorless, crystalline product, m.p. 168–169°, was obtained. The oily residue from the ether treatment was dissolved in methanol and refrigerated to give **1** as a colorless, crystalline solid; yield, 1.5 g, m.p. 168–169°. Analytically pure **1** was obtained by repeated recrystallization from ether and drying *in vacuo* for 24 h; m.p. 169–170°, lit.[3] m.p. 168.5–169°.

Anal. Calc. for $C_{12}H_{15}O_{10}(COCH_3)_7$: C, 50.36; H, 5.85; O, 43.87; CH_3CO, 48.55; C (CH_3), 19.38. Found: C, 50.86; H, 5.83; O, 43.28; CH_3CO, 47.04; C (CH_3), 18.23.

The mass spectrum showed peaks at *m/e* 561, 560, 518, 501, 500, 459, 458, 441, 399, 398, 381, 339, 338, 331, 330, 317, 297, 296, 289, 273, 257, 215, 213, and 43, attributed to rutinose heptaacetate, and at *m/e* 534, 474, 445, 432, 415, 414, and 372, seemingly unrelated to the main series and attributed to an impurity. Peaks of *m/e* less than 213, except for the intense peak at 43, are not listed here.

Methylation of rutin. — Rutin (3 g, purified by the Couch procedure[14]) was suspended in 20 ml of methyl sulfate. Aqueous alkali (9 g of sodium hydroxide 97% in 18 ml of water) was added all at once. A violent reaction occurred, which subsided after *ca.* one h. The reaction mixture then was treated with 70 ml of tetrahydrofuran. The organic phase was separated and treated twice with 7 ml of methyl sulfate and excess powdered sodium hydroxide. The mixture was heated for 2 h on a steam bath, filtered, and to the filtrate was added 7 ml of methyl sulfate and powdered sodium hydroxide. The resulting mixture was kept overnight at room temperature. Iterative

treatment (2 to 3 times), with 7 ml of methyl sulfate and excess powdered sodium hydroxide, with the final mixture being kept for 3 days at room temperature and then being refluxed for one h, gave a tetrahydrofuran solution (dried over anhydrous magnesium sulfate) which was chromatographed on acidic alumina (Woelm). The column was developed with tetrahydrofuran or benzene and the total effluent was collected. Removal of the solvent gave a red oil (positive sulfur and Mg–HCl tests, negative $FeCl_3$), which was treated with 30% aqueous sodium hydroxide and heated for 30 min on a steam bath. The aqueous mixture was extracted with tetrahydrofuran, and the dried extract was chromatographed on acidic alumina. Removal of the solvent from the total effluent gave a solid residue (negative $FeCl_3$ and sulfur tests, positive Mg–HCl), m.p. *ca.* 55–66°.

To remove partially methylated material, the solid product in 30 ml of dry pyridine was treated with excess *p*-phenylazobenzoyl (azoyl) chloride at room temperature overnight, with protection from atmospheric moisture. Water (10 ml) and excess sodium hydrogen carbonate were added, followed by acetone. The inorganic material thus precipitated was filtered off, the solvent was removed *in vacuo* from the filtrate, and the resulting residue was dissolved in chloroform. Excess azoyl chloride, azoic acid, and azoic anhydride were removed by chromatography of the solution on acidic alumina. The red chloroform effluent was evaporated and the residue was dissolved in ethanol. Addition of water caused precipitation of reddish, colloidal material. After the addition of charcoal, the mixture was filtered through a funnel at 60° (to avoid oxidation). The filtrate was extracted three times with chloroform and the combined extracts were dried and evaporated. A solution of the residue in tetrahydrofuran was chromatographed on acidic alumina with retention of the total effluent. Removal of the solvent *in vacuo* gave the decamethyl ether of rutin as a solid, colorless, water-soluble residue (negative $FeCl_3$, positive Mg–HCl tests), melting unsharply near 103°, with marked softening at 73°.

Anal. Calc. for $C_{27}H_{20}O_6(OCH_3)_{10}$: C, 59.19; H, 6.71; OCH_3, 41.33; C (CH_3), 2.00. Calc. for $C_{27}H_{19}O_7(OCH_3)_9$: C, 58.69; H, 6.57; OCH_3, 37.9; C (CH_3), 2.04. Found: C, 58.91; H, 6.73; OCH_3, 40.74; C (CH_3), 1.82.

Hydrolysis of methylated rutin. — Methylated rutin (2 g, prepared as in the foregoing experiment in 30 ml of 95% ethanol and 50 ml of 6% hydrochloric acid was refluxed for 8 h. Water (50 ml) was added, and the resulting mixture was refrigerated overnight. The precipitated quercetin 3′,4′,5,7-tetramethyl ether, m.p. 194°, lit.[15] m.p. 193-196°, was filtered off. The filtrate was neutralized with barium carbonate, excess barium carbonate was removed, and the solvent was removed *in vacuo* from the filtrate. The solid residue was extracted with tetrahydrofuran, and the extract was evaporated to give an oil. The latter was dried in a vacuum desiccator and azoylated by the procedure of Coleman[13]. The azoyl derivatives were subjected to two chromatographic steps. First, a chloroform solution was chromatographed on acidic alumina to remove azoyl chloride, azoic acid, and azoic anhydride. The effluent was then concentrated to 10 ml, and chromatographed on silicic acid ($H_2SiO_3 \cdot H_2O$). Development with 3:1 (v/v) petroleum ether–benzene gave a single, brightly colored

zone, which was removed mechanically. Elution with 3:1 (v/v) chloroform–methanol gave the 1,6-bis(azoate) of 2,3,4-tri-*O*-methyl-D-glucopyranose, m.p., after crystallization from 95% ethanol, 138.5–139° (lit.[16] m.p. 133°).

Anal. Calc. for $C_{25}H_{34}N_4O_8$: C, 65.82; H, 5.37; N, 8.77. Found: C, 65.81; H, 5.28; N, 8.86.

ACKNOWLEDGMENT

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Note

Solid-state transition of 1,6-anhydro-β-D-glucopyranose*

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(Received August 29th, 1969; in revised form, December 15th, 1969)

A program has been developed in this laboratory for investigation of the pyrolytic reactions of cellulosic materials and related model compounds. In the course of these investigations it has been found that the methods of thermal analysis, especially differential thermal analysis and scanning calorimetry, provide useful methods for investigation of the pyrolytic reactions of carbohydrate compounds[1,2] as well as some of the physical changes that precede the pyrolytic reactions.

Crystalline carbohydrates, when examined by differential thermal analysis, generally exhibit an endothermic peak corresponding to their melting point. 1,6-Anhydro-β-D-glucopyranose (levoglucosan) behaves quite differently by showing an endothermic peak at 113°, before it melts at 180°. As seen in Fig. 1, if the sample is

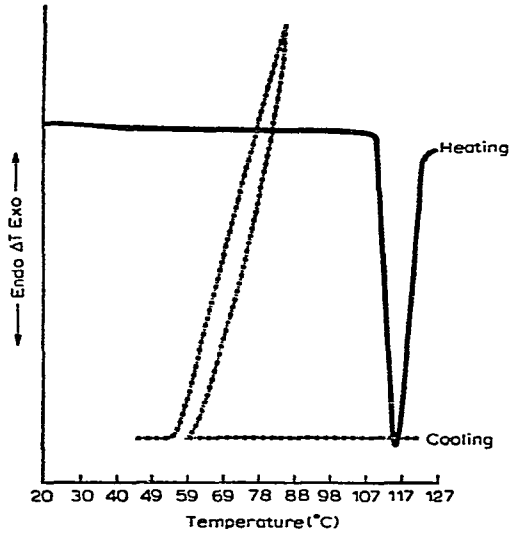

Fig. 1. Thermogram of 1,6-anhydro-β-D-glucopyranose indicating the solid-state transition on heating and its reversal on cooling.

*Dedicated to the memory of Professor M. L. Wolfrom.

†Established through a grant from the Hoerner–Waldorf Corporation of Montana.

cooled after this transition and before melting, the process is reversed, with supercooling (at about 50°). The reverse transition is very fast and gives a sharp exotherm. The enthalpy of the transition has been recorded (Fig. 2) by a differential scanning calorimeter and found to be 6 kcal.mole^{-1} for the initial transition and 4.9 kcal.mole^{-1} for the reverse transition. These values are much larger than the heat of fusion for

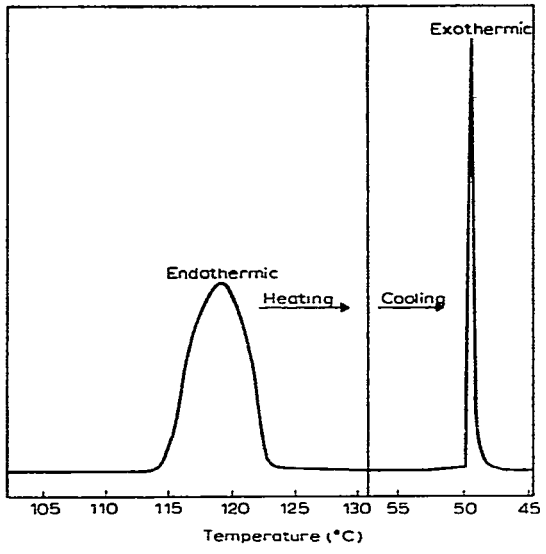

Fig. 2. Measurement of the endothermic and exothermic transitions of 1,6-anhydro-β-D-glucopyranose by differential scanning calorimetry.

levoglucosan, which was found to be 0.8 kcal.mole^{-1}, although the latter value could not be obtained very accurately because of a slight decomposition at this temperature.

The difference between the enthalpies of transition at 113° and the reversal at 50° is an interesting and significant phenomenon. It means that within the temperature range of the supercooling (50–113°), the two forms of levoglucosan have different heat capacities. In other words, the pre-transition form (levoglucosan II) gains less energy on heating than the post-transition form (levoglucosan I) loses on cooling within the same temperature range, as shown in the following scheme:

Levoglucosan II at 50°	$\xrightarrow{X \text{ kcal. mole}^{-1}}$	Levoglucosan II at 113°
↑ -4.9 kcal.mole^{-1}		↓ 6 kcal.mole^{-1}
Levoglucosan I at 50°	$\xleftarrow{-(X+1.1) \text{ kcal. mole}^{-1}}$	Levoglucosan I at 113°

Actual measurement of the heat capacity of the two forms, and the relation of heat capacity to molecular structure and motion will be discussed in another communication.

It should be noted here that recently some changes in the i.r. spectra of sugars with temperature has been reported by different groups[3–5]. These changes, which take place at lower temperatures (in the solid state), show different characteristics from the sharp and highly endothermic transition of levoglucosan. The difference between the two types of transformations has been established by a combination of i.r. spectroscopy and thermal analytic methods and will be reported in the following communication.

EXPERIMENTAL

Dynamic thermal analysis. — The differential thermal analysis was obtained with a DuPont 900 thermal analyzer, programmed at the rate of 5 degrees.min^{-1}. The experiments were conducted in a nitrogen atmosphere with silica beads as the reference and 20 mg of powdered 1,6-anhydro-β-D-glucopyranose as the sample.

The differential-scanning calorimetric data were obtained with a Perkin–Elmer DSC-1B calorimeter, programmed at the rate of 5 degrees.min^{-1}, in a nitrogen atmosphere. The sample consisted of 7 mg of powdered 1,6-anhydro-β-D-glucopyranose. The system was calibrated against the heat of fusion of indium at 156.4°, $\Delta H_m = 6.75$ cal.g^{-1}.

ACKNOWLEDGMENTS

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Note

Formation of 6-deoxy-L-gulonolactone from D-glucofuranurono-6,3-lactone*

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The published synthesis[1] of 6-deoxy-L-gulonolactone (**1**) is long and the product is not well characterized. We here report preparation of **1** in three steps starting with D-glucofuranurono-6,3-lactone (**2**); the key step involves reduction of a *p*-tolylsulfonylhydrazone to a methyl group, by the reaction described by Caglioti and Grasselli[2] and used in the sugar field by deBelder and Weigel[3].

D-Glucuronamide[4] (**3**) was refluxed with *p*-tolylsulfonylhydrazine to yield crystalline D-glucuronamide *p*-tolylsulfonylhydrazone (**4**). Borohydride reduction of **4** yielded 6-deoxy-L-gulonolactone (**1**) instead of the expected amide. Presumably during the workup the amide was hydrolyzed[5] to the ammonium salt and subsequent deionization and concentration of the effluents *in vacuo* yielded the crystalline lactone.

The *p*-tolylsulfonylhydrazone of **2** was prepared as a possible precursor of **4**, but suitable conditions for the conversion into **4** were not established.

This appears to be the first application of the tosylhydrazone–borohydride method for reduction to methyl in the uronic acid series. We believe it can be of general synthetic utility in producing 6-deoxy sugars from hexuronic acids.

EXPERIMENTAL

General. — Microanalyses were performed by Weiler and Strauss Microanalytical Labs, Oxford, England. Melting points are uncorrected. All evaporations and concentrations were done in a rotary evaporator under diminished pressure at 40° (bath).

D-*Glucuronolactone* p-*tolylsulfonylhydrazone.* — A solution of **2** (1.0 g) and *p*-tolylsulfonylhydrazine (1.07 g) in 76% ethanol (25 ml) was brought to boiling on a steam bath. Upon cooling and concentration *in vacuo* to 10 ml, crystallization took place depositing 1.9 g (97%) of D-glucuronolactone *p*-tolylsulfonylhydrazone; m.p. 117–123° after three recrystallizations from abs. ethanol, $[\alpha]_D^{24}$ +3.57 (5 min)→+1.39°

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(24 h) (*c* 1.58, pyridine); λ_{max}^{Nujol} 3470–3290 (OH, NH), 1775 (lactone), 1605 (*p*-substituted aryl).

Anal. Calc. for $C_{13}H_{16}N_2O_7S$: C, 45.35; H, 4.65; N, 8.14. Found: C, 45.34; H, 4.68; N, 8.19.

D-*Glucuronamide* p-*tolylsulfonylhydrazone* (**4**). — A solution of *p*-tolylsulfonylhydrazine (10.0 g) and **3** (10.0 g) in 76% ethanol (100 ml) was refluxed for 45 min. After 2 h at 3°, a crystalline mass (15.38 g, 78%) of **4** appeared; m.p. 134–135° after 3 recrystallizations from 95% ethanol; $[\alpha]_D^{26}$ −24.3° (*c* 3.84, pyridine); λ_{max}^{Nujol} 3410–3200 (OH, NH), 1690–1632 (amide), 1600 (*p*-substituted aryl).

Anal. Calc. for $C_{13}H_{18}N_3O_7S$: C, 43.34; H, 5.00; N, 11.65. Found: C, 43.10; H, 5.28; N, 11.45.

*6-Deoxy-*L*-gulonolactone* (**1**). — A solution of **4** (850 mg) and potassium borohydride (1.5 g) in anhydrous methanol (30 ml) was refluxed for 16 h. Ethyl acetate was added and the solution was refluxed for 30 min more. The cooled reaction mixture was then passed over Dowex-50W X8 (H^+) (100 ml of wet resin prepared with methanol) and then the column was washed with methanol (200 ml). The effluents were concentrated *in vacuo*, and a water solution of the residue was decolorized with carbon and then continually extracted with ether for 48 h. The aqueous layer was concentrated *in vacuo*, depositing crystals (31.2 mg, 8.5%) of **1**; m.p. 184–185.5° after 3 recrystallizations from abs. ethanol; $[\alpha]_D^{24}$ +29.9° (*c* 1.12, water); λ_{max}^{Nujol} 3360 (OH), 1750 (lactone); n.m.r. (60 MHz, D_2O) δ 1.18 (3-proton doublet, $J_{5,6}$ 6 Hz. methyl), 4.3 (4-proton unsymmetrical multiplet, H-2, 3, 4, 5).

Anal. Calc. for $C_6H_{10}O_5$: C, 44.46; H, 6.17. Found: C, 44.61; H, 6.04.

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Preliminary communication

An improved synthesis of 4-deoxy-4-fluoro-D-glucose

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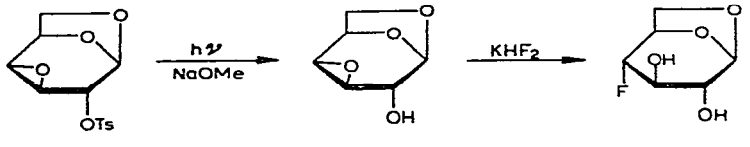
(Received December 15th, 1969)

4-Deoxy-4-fluoro-D-glucose has been synthesised by reaction sequences the key stages of which involve the action of (1) potassium hydrogen fluoride–ethane-1,2-diol on 1,6-anhydro-4-*O*-toluene-*p*-sulphonyl-β-D-glucopyranose[1] and (2) tetrabutylammonium fluoride–acetonitrile on methyl 4-*O*-mesyl-2,3-di-*O*-methyl-6-*O*-trityl-α-D-galactopyranoside[2]. For various reasons neither synthesis is convenient for the preparation of 4-deoxy-4-fluoro-D-glucose on a scale adequate to permit a thorough biological evaluation. We now describe a route that meets this requirement.

1,6:3,4-Dianhydro-2-*O*-toluene-*p*-sulphonyl-D-galactose[3] [1, m.p. 145–147.5°, $[\alpha]_D$ −42° (chloroform)] is readily obtained (48% overall yield) from 1,6-anhydro-β-D-glucopyranose by conversion into the 2,4-ditoluene-*p*-sulphonate followed by treatment with sodium methoxide in methanol–chloroform. Irradiation[4] (quartz vessel, 450 watt medium-pressure Hg lamp, 3 h, ~35°) of a 0.3% methanolic solution of **1** containing an equimolar amount of sodium methoxide, followed by concentration and elution of the residue from Kieselgel (Merck, 7734) with light petroleum (b.p. 40–60°)–ether (1:4) gave 80% of 1,6:3,4-dianhydro-D-galactose[5] (**2**), b.p. 130° (bath)/0.35 mm, m.p. 69–71° (from ether–light petroleum), $[\alpha]_D$ −76° (water). Treatment of **2** with pyridine–toluene-*p*-sulphonyl chloride regenerated **1** (68%).

A solution of **2** (0.5 g) in ethylene glycol (5 ml) containing potassium hydrogen fluoride (1.25 g) was boiled for 1 h and then neutralised with aqueous sodium hydrogen carbonate. Concentration *in vacuo*, and elution of the residue from Kieselgel (Merck, 7734) with chloroform–methanol (25:1) gave 1,6-anhydro-4-deoxy-4-fluoro-D-glucose[1] (**3**, 65%), m.p. and mixed m.p. 120–122°, $[\alpha]_D$ −53° (water). The epoxide **2** has been postulated



1 2 3

★Fellow of the Rockefeller Foundation, 1953–54, working with Professor M. L. Wolfrom at the Ohio State University.

as an intermediate in the conversion[1] of 1,6-anhydro-4-*O*-toluene-*p*-sulphonyl-D-glucose into 3 on treatment with potassium hydrogen fluoride—boiling ethylene glycol. A satisfactory chemical method for effecting the conversion 1 → 2 has not been found.

The acid-catalysed conversion of 3 into 4-deoxy-4-fluoro-D-glucose, which proceeds in high yield, has been reported elsewhere[1].

The above synthesis of 4-deoxy-4-fluoro-D-glucose from 1,6-anhydro-β-D-glucopyranose involves 5 stages and an overall yield of 14%, which undoubtedly can be improved upon.

The ready availability of the dianhydride 2 by the above route offers a convenient approach to 4-substituted derivatives and 2-ethers of D-glucose.

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PREPARATION OF 1,3,6,2′,3′,4′,6′-HEPTA-*O*-ACETYL-β-MALTOSE

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(Received August 18th, 1969)

ABSTRACT

A synthesis of the title compound (**2**, 15.5%) from octa-*O*-acetyl-β-maltose *via* hexa-*O*-acetyl-2-*O*-trichloroacetyl-β-maltosyl chloride (**1**) is described. No intermediate compounds were isolated. Compound **2** could be readily distinguished from the anomerically unsubstituted β-heptaacetate which is the main product formed from hepta-*O*-acetyl-α-maltosyl bromide under conditions which result in acetyl migration from C-2 to C-1 on hydrolysis of the acetylated glycosyl bromides of D-glucose and cellobiose.

INTRODUCTION

Of the various hepta-*O*-acetylmaltoses, the β-anomeric forms of those in which the unsubstituted hydroxyl group is at C-1 (ref. 1, 2), C-3 (ref. 3), C-6 (ref. 4, 5), and C-6′ (ref. 5, 6) are known. Neither the α- nor the β-heptaacetate having the free hydroxyl group at C-2 appears to have been described, although unsuccessful attempts at synthesis have been noted[7] (*cf.* ref. 8). The formation of hexa-*O*-acetyl-2-*O*-trichloroacetyl-β-maltosyl chloride[9] (**1**) from the reaction of phosphorus pentachloride and octa-*O*-acetyl-β-maltose suggested that 1,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (**2**) might be accessible *via* a reaction sequence analogous to that employed for the preparation of 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[10,11].

DISCUSSION

The efficiency of the method described[10,11] for the preparation of 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose from D-glucose depends largely on the yield of the intermediate 3,4,6-tri-*O*-acetyl-2-*O*-trichloroacetyl-β-D-glucopyranosyl chloride, and this is variable [11–13] (30–65%). T.l.c. indicated the formation of only one major product in the reaction of β-maltose octaacetate with phosphorus pentachloride, which previous experience[9] suggested might be an unresolved anomeric mixture. Since both anomers would be of equal value in the proposed synthesis, the next stage was carried out without attempting to isolate any intermediate compound.

Selective ammonolysis removed the trichloroacetyl group to yield an anomeric

mixture of the 3,6,2′,3′,4′,6′-hexa-*O*-acetylmaltosyl chlorides, dissolution of which in anhydrous acetone resulted in rapid conversion of the β-chloride into the more stable α-anomer (*cf.* the isomerization of 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride[10]). On prolonged (more than 3 days at room temperature) solution in acetone, the maltosyl chloride underwent significant conversion into 3,6,2′,3′,4′,6′-hexa-*O*-acetylmaltose (**3**), despite stringent precautions to ensure anhydrous conditions. Examination of the acetone-soluble material by t.l.c. indicated that it consisted largely of a single component, although numerous other constituents were also present in low concentration. Since attempts to crystallize the crude hexa-*O*-acetyl-α-maltosyl chloride failed and attempts to purify it by t.l.c. resulted in almost complete hydrolysis to **3**, it was treated directly with mercuric acetate in acetic acid to yield the title compound (**2**) which was obtained crystalline after purification by t.l.c.

Initial yields of **2** were very low, and 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (**4**), formed by hydrolysis of residual acetylated glycosyl chloride[12,14], was a contaminant. Compounds **2** and **4** were inseparable by t.l.c., and it was therefore necessary to prevent the occurrence of **4** in the final reaction mixture. Extension (to *ca.* 2 h) of the reaction time[9] for the preparation of **1** resulted in a more complete chlorination of the 2-*O*-acetyl group, and conditions favouring hydrolysis during subsequent stages were eliminated as far as possible.

In the procedure described, the over-all yield of the heptaacetate **2** from β-maltose octaacetate was 15.5%. The purified product **2** could be readily distinguished from the anomerically unsubstituted β-heptaacetate (**4**), despite the chromatographic similarity of the two compounds, and mixed melting points were considerably depressed.

Acetylation of **2** in pyridine–acetic anhydride yielded only octa-*O*-acetyl-β-maltose, whereas the α-anomer could also be detected by t.l.c. when **4** was acetylated under the same conditions.

Reaction with methanesulphonyl chloride in pyridine has been used extensively[7,8,15] to distinguish between sugar derivatives in which only alcoholic hydroxyl groups are free, and those in which the anomeric hydroxyl group is unsubstituted. Thus **2** gave 65% of hepta-*O*-acetyl-2-*O*-methanesulphonyl-β-maltose (**5**), whereas **4** underwent partial conversion into hepta-*O*-acetyl-α-maltosyl chloride. From the hexaacetate **3**, hexa-*O*-acetyl-2-*O*-methanesulphonyl-α-maltosyl chloride (**6**) was obtained in moderate yield, and converted into **5** by treatment with mercuric acetate in acetic acid.

In the n.m.r. spectrum of **2** in chloroform-*d*, the anomeric proton resonated as a low-field doublet (τ 4.32, $J_{1,2}$ 7.5 Hz). This is in excellent agreement with the values reported[3] for the corresponding protons in 1,2,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose and in octa-*O*-acetyl-β-maltose (τ 4.36 and 4.25, respectively, $J_{1,2}$ 8 Hz). By contrast, *H-1 in* **4** *appeared further upfield among the other skeletal protons, and could not be clearly differentiated.* Neither compound exhibited signals at the resonance of τ 7.82 reported for axial *O*-acetyl groups[16], and in each case, seven acetyl groups were accounted for by the signals between τ 7.88 and 8.01.

Compound **2** failed to mutarotate in chloroform solution, even after addition of diethylamine. Under the same conditions, 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose also failed to mutarotate, but the α-anomer exhibited significant downward mutarotation when diethylamine was added. This phenomenon, which has also been observed in aqueous pyridine and in acetic acid solution, has been ascribed to acetyl migration from C-1 to C-2 *via* an intermediate orthoester form, and anomerization of the resultant 2,3,4,6-tetra-*O*-acetyl isomer[8,16,17]. Although formation of the intermediate orthoester would appear possible with almost equal ease from both anomeric forms of such D-glucopyranose 1-acetate derivatives, assuming them to possess the *C1*(D) conformation[18], rearrangement may occur more readily in the case of the α-D anomer in which the C-1 acetoxyl group is in the less stable, axial orientation. It appears that this is the case[7,8,16,17], although, under rather different conditions, acetyl migration has also been encountered on methylation of 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[18].

The use of compound **2** for the synthesis of centose[19] and related trisaccharides will be described elsewhere.

Experiments in this laboratory have confirmed that conditions which lead to acetyl migration from C-2 to C-1 on hydrolysis of the acetylated glycosyl bromides of D-glucose[8] and cellobiose[7] do not yield the same result in the case of hepta-*O*-acetyl-α-maltosyl bromide, and that the product was originally identified correctly[1] as the anomerically unsubstituted β-heptaacetate[2] (**4**).

Methanol–benzene mixtures have been widely employed for the separation of carbohydrate acetates by t.l.c. on Silica Gel G, but suffer from the disadvantage that daily replacement of the solvent is necessary because of gradual depletion of the methanol content by adsorption onto the silicic acid[20]. In the present work, acetone–benzene mixtures have given consistently more reliable results. Not only have the polarities of the mixtures remained practically unchanged over long periods of use, but the separation of many anomeric mixtures has proved possible without recourse to multiple-development techniques.

EXPERIMENTAL

General. — Melting points were determined by the Kofler method, and are uncorrected. Mixed m.p.'s were made on the crystalline residue remaining after dissolving equal weights of both compounds in acetone, and removing the solvent under vacuum. Thin-layer chromatography (t.l.c.) was performed on Merck Kieselgel G, using acetone–benzene mixtures, and sulphuric acid as spray reagent; for preparative work, zones were visualized with water[20], and components were eluted with acetone. I.r. spectra were recorded on a Beckman IR-9 spectrophotometer by the potassium bromide disc method; band intensities are indicated: m, moderate; s, strong; w, weak. N.m.r. spectra were measured on a Varian HA-60IL spectrometer with chloroform-*d* as solvent, and tetramethylsilane as internal standard. The anomeric 1,3,4,6-tetra-*O*-acetyl-D-glucopyranoses[11] and 2,3,6,2',3',4',6'-hepta-*O*-

acetyl-β-maltose[1] (**4**) were prepared by literature methods. Octa-*O*-acetyl-β-maltose[2,1] was recrystallized directly from ethanol, and had m.p. 158–159°.

1,3,6,2',3',4',6'-Hepta-O-acetyl-β-maltose (**2**). — Octa-*O*-acetyl-β-maltose (8.5 g) and phosphorus pentachloride (14 g) were heated under reflux at 105°. Vigorous evolution of hydrogen chloride ceased after *ca.* 45 min, and, after 2 h, t.l.c. [8% (v/v) acetone in benzene] revealed only one major reaction product which was very slightly more mobile than, but inseparable from, penta-*O*-acetyl-β-D-glucopyranose. Only a trace of hepta-*O*-acetyl-α-maltosyl chloride, and no residual octa-*O*-acetyl-β-maltose could be detected. The reaction mixture was distilled *in vacuo* at 40°, and the residue was dissolved in chloroform (80 ml) and washed with iced water (2 × 30 ml), saturated, aqueous sodium hydrogen carbonate (30 ml), and water (30 ml). The neutral solution was filtered through anhydrous sodium sulphate, and evaporated to dryness at 30°. The residue was dissolved in sodium-dried ether (15 ml), and added to an ammonia-saturated solution of anhydrous ether (300 ml) at 0°. After 30 min at room temperature, the solution was filtered, and evaporated to dryness at 20°, and the residue (9.6 g) was dissolved in anhydrous acetone. Examination of the solution by t.l.c. [30% (v/v) acetone in benzene] revealed the main constituent to be slightly more mobile than, but only partially separable from, the anomerically unsubstituted hepta-*O*-acetyl-β-maltose[1,2] (**4**). After 2 h, the solvent was evaporated, and the residue (9.6 g) was treated with a solution of mercuric acetate (10 g) in glacial acetic acid (110 ml) for 4 h at room temperature. The solution was diluted with chloroform (175 ml), washed with water (4 × 50 ml), and evaporated to dryness. The heptaacetate **2** was isolated from the residue by t.l.c. [60 plates, 30% (v/v) acetone in benzene], and crystallized from ethanol to give material (1.24 g, 15.5% yield) which after 2 recrystallizations, had m.p. 166.5–168.5° (depressed to below 140° on admixture with **4**), $[\alpha]_D^{27}$ +86.7° (*c* 2.6, chloroform), unchanged 24 h after addition of diethylamine (1 drop) to the chloroform solution (1 ml). {On similar treatment, 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, $[\alpha]_D^{27}$ +25.6° (*c* 2.5, chloroform), was unaffected, whereas the α-anomer, $[\alpha]_D^{27}$ +140.8° (*c* 2.5, chloroform), underwent rapid, downward mutarotation to +88.8° (constant value, 30 min)}; ν_{max}^{KBr} 3465m, 1752s, 1645w, 1435m, 1373s, 1240s, 1130m, 1080s, 1045s, 985w, 945w, 910w, 895w, 878w, 780w, 605m cm^{-1}; n.m.r. data: τ 4.32 (doublet, $J_{1,2}$ 7.5 Hz, H-1); 4.52–6.53 (multiplets, 13 protons); 6.81 (broad singlet, HO-2); 7.88, 7.92, 8.00 (singlets, 21 protons, seven acetyl groups).

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.1; H, 5.7; acetyl, 47.3. Found: C, 48.9; H, 5.7; acetyl, 46.2.

Derivatives of **2**. — With acetic anhydride–pyridine, in the usual manner, **2** gave octa-*O*-acetyl-β-maltose, m.p. (and mixed m.p.) 158–159° (from ethanol). The β-octaacetate was the only product detected in the mother liquor by t.l.c. [12% (v/v) acetone in benzene], whereas when **4** was similarly acetylated, the presence of a slightly less mobile component, corresponding to the α-anomer, was also observed.

With methanesulphonyl chloride–pyridine, in the usual manner, **2** gave the 2-methanesulphonate **5** (0.11 g, 65.3%), m.p. 165–166° (from ethanol), $[\alpha]_D^{27}$ +66.9° (*c* 2.18, chloroform).

Anal. Calc. for $C_{27}H_{38}O_{20}S$: C, 45.4; H, 5.3; S, 4.5. Found: C, 45.0; H, 5.5; S, 4.4.

Attempted methanesulphonylation of **4**. — Compound **4** (1.0 g) was added to a mixture of pyridine (5 ml) and methanesulphonyl chloride (0.8 ml) at −20°. After stirring for 30 min, the mixture was kept for 60 h at 5°, diluted with chloroform (100 ml), and then processed, in the usual manner, to give a residue consisting mainly of **4** and a product which was isolated by t.l.c. [10 plates, 25% (v/v) acetone in benzene] and crystallized from ethanol. The product (86 mg, 8.4%) had m.p. 123–124° alone and in admixture with authentic hepta-*O*-acetyl-α-maltosyl chloride[22]. The identity of the two compounds was further supported by $[\alpha]_D$, i.r., n.m.r., and t.l.c. data.

*3,6,2',3',4',6'-Hexa-*O*-acetylmaltose* (**3**). — In a second experiment, on half the scale described for the preparation of **2**, an attempt was made to purify the intermediate 3,6,2',3',4',6'-hexa-*O*-acetyl-α-maltosyl chloride by t.l.c. [31 plates, 30% (v/v) acetone in benzene]. However, examination (t.l.c.) of the eluted material revealed it to have undergone almost complete hydrolysis. Crystallization from ethanol yielded **3** (0.74 g, 19.9%), which, after several recrystallizations, had constant m.p. 155–157°, $[\alpha]_D^{28}$ +116° (7 min, unchanged after 24 h; *c* 2.36, chloroform). However, t.l.c. [45% (v/v) acetone in benzene] resolved it into two closely moving components, the less mobile of which was the main constituent (α-anomer?). It appears either that the crystalline **3** is a mixture of both anomers in a constant proportion, or that anomerization occurs so rapidly in solution that no mutarotation could be detected.

Anal. Calc. for $C_{24}H_{34}O_{17}$: C, 48.5; H, 5.7; acetyl, 43.4. Found: C, 48.0; H, 5.6; acetyl, 42.6.

*3,6,2',3',4',6'-Hexa-*O*-acetyl-2-*O*-methanesulphonyl-α-maltosyl chloride* (**6**). — Compound **3** (0.4 g) was treated with pyridine (2 ml) and methanesulphonyl chloride (0.3 ml), and the product was isolated by t.l.c. (4 plates) as described for the attempted methanesulphonylation of **4**. Crystallization from ethanol afforded **6** (0.153 g, 31%), m.p. 160–162°, $[\alpha]_D^{27}$ +148.3° (*c* 1.77, chloroform); n.m.r. data: τ 3.85 (doublet, $J_{1,2}$ 4 Hz, H-1); 4.28–6.16 (multiplets, 13 protons); 6.95 (singlet, 3 protons, $MeSO_2$); 7.87, 7.91, 7.95, 8.00, 8.01 (singlets, 18 protons, six acetyl groups).

Anal. Calc. for $C_{27}H_{37}ClO_{19}S$: C, 44.2; Cl, 4.8; H, 5.0; S, 4.4. Found: C, 44.0; Cl, 4.5; H, 5.1; S, 4.7.

Conversion of **6** *into* **5**. — Compound **6** (0.1 g) was dissolved in a solution of mercuric acetate (0.1 g) in glacial acetic acid (2 ml). After 2 h at room temperature, the solution was diluted with chloroform (25 ml), extracted with water (3 × 10 ml), and evaporated to dryness. Crystallization of the residue from ethanol afforded needles (76 mg, 78.0%), identical with **5** by m.p., mixed m.p., $[\alpha]_D$, i.r., n.m.r., and t.l.c. data.

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NITROGEN-CONTAINING CARBOHYDRATE DERIVATIVES

PART XXII*. PERIODATE OXIDATION OF METHYL AMINO-4,6-*O*-BENZYLIDENE-DEOXY-α-D-ALTROPYRANOSIDES

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ABSTRACT

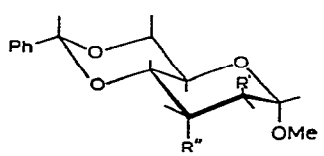
It has been shown that methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-altroside and its 3-amino-3-deoxy isomer are slowly oxidised by periodate in aqueous solution. The reaction, which followed second-order kinetics only up to the consumption of 0.6 mole of oxidant per mole, was accompanied by over-oxidation.

INTRODUCTION

In Part XXI, we described[1] the oxidation of methyl amino-4,6-*O*-benzylidene-deoxy-α-D-glycosides having the *allo*, *gluco*, and *manno* configurations, with the amino group in the 2- and in the 3-positions. It was shown that all six compounds followed the expected, second-order, kinetic pattern, and one molecular proportion of periodate was consumed.

In this paper, we discuss the oxidation of the remaining two compounds that complete the above series, namely, methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-altroside (**1**) and its 3-amino-3-deoxy isomer (**2**). Both compounds, which have the reactive groups in axial positions, were prepared by established methods[2]. The methods used to study the oxidation were precisely those used previously[1], and details of the experimental conditions and procedures will not be repeated here.

Honeyman and Shaw[3] showed that the corresponding diol **3** was unaffected by periodate, whereas compound **2** was slowly oxidised.



1 R' = NH_2 , R" = OH
2 R' = OH , R" = NH_2
3 R' = R" = OH

DISCUSSION

Because the oxidations of the two altrosides were very slow, oxidations were carried out at 25° at pH 6.87 in a phosphate buffer; this pH was used as the oxidation

*Part XXI: Ref. 1.
†To whom enquiries should be addressed.

was too slow at lower pH values. The second-order rate equation was obeyed only over the region of oxidant consumption from 0–0.6 mole/mole; an increase in rate was observed after this period; the rate constants were calculated for this limited range, and are given in Table I. Both the aminoaltrosides were extensively over-oxidised at appreciable rates (see, for example, Fig. 1). The products of the oxidations were the dialdehyde dihydrate[4] (4) and ammonia. Ammonia was shown not to react with periodate under these conditions. Honeyman and Shaw[3] made no comment about over-oxidation, nor on the degree that the second-order rate equation was obeyed, stating only that compound 2 "consumed one mole of periodate in about 20 h". They isolated compound 4 after oxidation.

TABLE I

EXPERIMENTAL $(K_2')^a$ AND TRUE $(K_2)^a$ SECOND-ORDER RATE CONSTANTS (L.MOLE^{-1}.SEC^{-1}) FOR PERIODATE OXIDATION AT 25° AT pH 6.87 IN PHOSPHATE BUFFER OVER THE RANGE 0 TO 0.6 MOLE/MOLE UPTAKE

Methyl 4,6-O-benzylidene-α-D-altroside	10^3 K_2'	10^3 K_2
2-amino-2-deoxy	2.06	0.367
3-amino-3-deoxy	2.15[b]	3.920

[a]See ref. 1. [b]Honeyman and Shaw[3] reported a value of 2.11 at pH 6.93 and 25°.

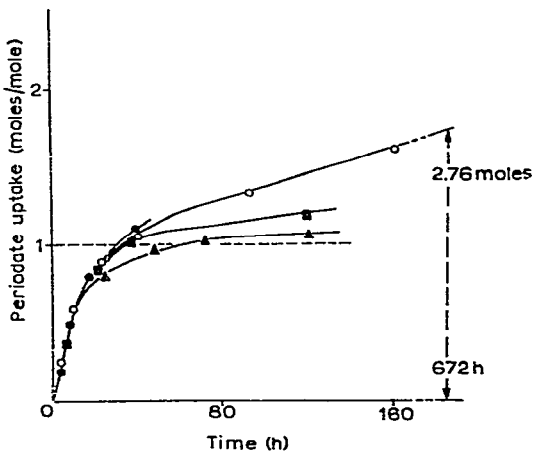


Fig. 1. Over-oxidation studies on methyl 4,6-*O*-benzylidene-α-D-glucoside (▲) in phosphate buffer (pH 6.91) at 25°, and methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-altroside at 25° in (●) arsenate buffer, pH 7.13; (○) phosphate buffer, pH 6.91; and (■) β,β′-dimethylglutaric acid–sodium hydroxide buffer, pH 7.35.

In order to determine whether this anomalous behaviour was attributable to the buffer system, or to the amino sugars, a more-detailed study was carried out. This showed that deviations from second-order kinetics also occurred for the oxidation of the 3-amino-altroside in phosphate, arsenate, and β,β′-dimethylglutaric acid buffers. In contrast, the oxidation of methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-alloside

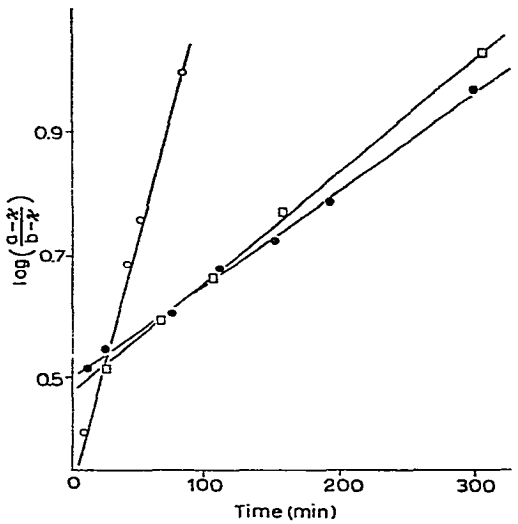


Fig. 2. Oxidation of methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-alloside in (○) phosphate buffer, pH 6.03 at 8.5°; (□) arsenate buffer, pH 5.65 at 0°; (●) β,β′-dimethylglutaric acid–sodium hydroxide buffer, pH 5.35 at 8.5°.

(Fig. 2) in these buffer systems (although of different* pH), and the oxidation of methyl 4,6-*O*-benzylidene-α-D-glucoside in phosphate buffer, obeyed second-order kinetics. Further, it was shown that the 3-amino-altroside in phosphate and arsenate buffers over-oxidised appreciably, but to a far lesser extent in β,β′-dimethylglutaric acid buffer (Fig. 1). The 3-amino-glucoside (Fig. 1) and the corresponding alloside showed no strong tendency to over-oxidise.

From the kinetic and over-oxidation evidence, it seems likely that it was the aminoaltroside itself that was causing the anomalous uptake of periodate, rather than the buffer components, mainly because methyl 4,6-*O*-benzylidene-α-D-glucoside in phosphate buffer, at pH 6.93, showed a second-order kinetic relationship, and, less convincingly, because of the normal behaviour of the 3-amino-alloside in both phosphate and arsenate buffers. In the latter case, the pH of the buffers was different and this may have been critical, because any "periodate–buffer" species[5] capable of reacting with the amino sugars at a fairly low rate would have been masked by the faster, normal, oxidation-reaction species in the *allo* case; in the case of the amino-altroside, the two rates may have been similar. It is unfortunate that it was not possible to work at the same pH with both *allo* and *altro* compounds and obtain a direct comparison of their reactions with periodate.

In conjunction with the fact that methyl 4,6-*O*-benzylidene-α-D-altroside is not oxidised by periodate (explained[3] by the diaxial arrangement of the hydroxyl groups which are therefore unable to form a complex), it is now suggested that an unknown,

*The alloside is oxidised at a much faster rate than the altroside, and accurate rate data could not be obtained at pH values greater than those shown in Fig. 2.

alternative mechanism to that of the periodate oxidation of the six "normal" amino sugars is operative for the aminoaltrosides. Methyl 4,6-*O*-benzylidene-3-deoxy-3-methylamino-α-D-altroside has recently been shown to behave in a similar manner on treatment with periodate[6], with over-oxidation and deviation from the second-order rate equation.

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SYNTHESIS OF 3-AMINO-2,3-DIDEOXYTETROSE DERIVATIVES

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ABSTRACT

Ethyl 2-amino-4,4-diethoxybutyrate (**2**) was prepared from ethyl malonate. In an alternative route, methyl 2-amino-4,4-dimethoxybutyrate (**5**) was prepared from DL-aspartic acid. Reduction of **5** with lithium aluminium hydride yielded 3-amino-4-hydroxybutyraldehyde dimethyl acetal (**6**) from which the *N*-acetyl derivative **7** and the diethyl dithioacetal **9** could be prepared. Acid hydrolysis of the (*S*)-isomer **7'** of compound **7**, prepared from L-aspartic acid, yielded the crystalline 3-acetamido-2,3-dideoxy-D-tetrose (**10'**).

INTRODUCTION

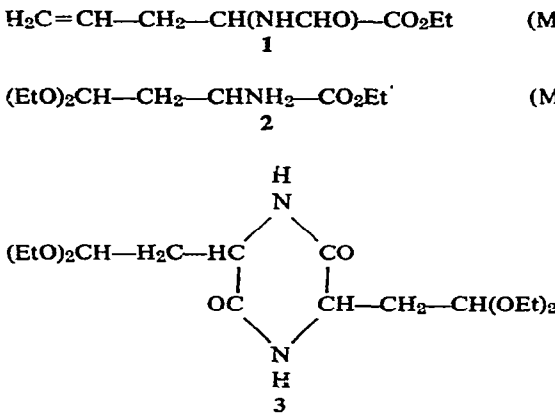
The synthesis of 2-amino-2-deoxy-DL-glyceraldehyde and several of its derivatives by the acid hydrolysis of the corresponding acetals has been described previously[1]. This procedure has now been extended to the synthesis of derivatives of 3-amino-4-hydroxybutyraldehyde, a 3-amino-2,3-dideoxytetrose. The only free amino-deoxytetroses previously described are 2-amino-2-deoxy-D-threose[2], 2-amino-2-deoxy-D-erythrose[2], and 2-amino-3,3-dimethyl-4-hydroxybutyraldehyde[3], the furanoid form of which has been established.

RESULTS AND DISCUSSION

An ester acetal of L-aspart-4-aldehydic acid was required for the synthesis of 3-amino-4-hydroxybutyraldehyde. It was obtained either as a DL mixture from ethyl malonate or, more conveniently, in higher overall yield from L-aspartic acid.

Ethyl 2-allyl-2-formamidomalonate[4] was treated with one equivalent of cold alkali to give the acid ester which was decarboxylated at 120–130° to ethyl 2-formamido-4-pentenoate (**1**) in 69% overall yield. Oxidation of **1** with osmium tetroxide and sodium metaperiodate[5] yielded the ethyl ester of (±)-*N*-formylaspart-4-aldehydic acid as a syrup which failed to give a crystalline derivative. Treatment of the syrupy aldehyde with ethanolic hydrogen chloride removed the formyl protecting group and afforded syrupy ethyl 2-amino-4,4-diethoxybutyrate (**2**), in 22% yield from **1**, which was characterized as its picrate. A crystalline dioxopiperazine **3** was isolated from the

residue after distillation. 2-Amino-4,4-diethoxybutyric acid (**4**), a new amino acid, was obtained by the alkaline hydrolysis of **2**.



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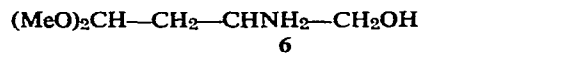

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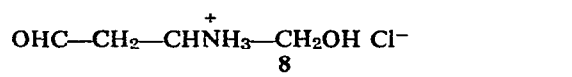

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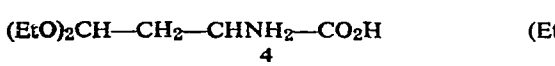

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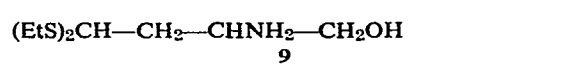

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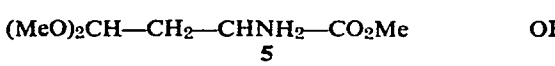

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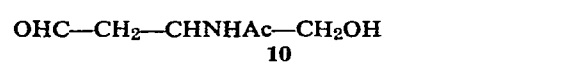

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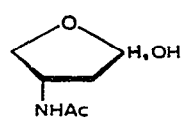

10'

An alternative route to the required ester acetal was suggested by Weygand and Fritz in the synthesis of 3-ethoxycarbonyl-3-trifluoroacetamidopropionaldehyde[6]. Treatment of this compound with ethanolic hydrogen chloride failed to remove the trifluoroacetyl group which can usually be cleaved by methanolic hydrogen chloride[7,8].

3-Methoxycarbonyl-3-trifluoroacetamidopropionaldehyde was chosen as a starting compound in order to avoid a possible transesterification reaction, and was obtained from 3-methoxycarbonyl-3-trifluoroacetamidopropionyl chloride[9] by a Rosenmund reduction. The syrupy product was characterized as its 2,4-dinitrophenylhydrazone. Treatment with methanolic hydrogen chloride at room temperature for 48 h yielded methyl 2-amino-4,4-dimethoxybutyrate (**5**) as a colourless, distillable liquid (overall yield, 40%), which was characterized as the picrate. Reduction of **5** with lithium aluminium hydride yielded 3-amino-4-hydroxybutyraldehyde dimethyl acetal (**6**), as a viscous, distillable syrup, characterized as its neutral oxalate.

The acetamido acetal **7** was obtained in 86% yield by treatment of a methanolic solution of the amino acetal **6** with acetic anhydride.

An amorphous, reducing compound, probably 3-amino-4-hydroxybutyr-

aldehyde hydrochloride, was obtained by the acid hydrolysis of the acetal **6**. This material, which was soluble in water and methanol and was stable at room temperature and in acid media, was characterized as its crystalline 2,4-dinitrophenylhydrazone.

The dithioacetal **9** was obtained in 90% yield from **6** by treatment with ethanethiol and concentrated hydrochloric acid, and was characterized as its neutral oxalate.

Hydrolysis of **7** with 0.02N sulphuric acid for 24 h at room temperature gave the free DL acetamido sugar **10**, characterized as its crystalline semicarbazone.

The synthesis involving L-aspartic acid yielded the (*S*)-isomers, **5′**, **6′**, **7′**, **9′**, and **10′**, of **5**, **6**, **7**, **9**, and **10**. The crystalline compound **10′** exhibited no aldehyde carbonyl absorption in the infrared, but its aqueous solution had reducing properties which enabled its detection with the silver nitrate–sodium hydroxide spray reagent. The compound did not mutarotate in aqueous solution. The n.m.r. spectrum in deuterioacetone showed the absence of an aldehydic proton, and the methyl protons of the *N*-acetyl group resonated as a doublet (τ 8.1), suggesting the presence of tautomers. These properties parallel those of 3-acetamido-2,3-dideoxy-D-ribose, which is known as a highly stable hemiacetal both in the solid state and in solution[10].

EXPERIMENTAL

Solvents were removed under diminished pressure at a bath-temperature of 45°. Melting points were determined on a Kofler hot-stage apparatus and are corrected. N.m.r. spectra were determined on a Varian A-60 spectrometer at 35°. Infrared spectra were recorded with a Perkin–Elmer spectrophotometer (Model 237), either as thin films or Nujol mulls. Microanalyses were performed by the C.N.R.S. Laboratories, Thiais, France.

Ethyl 2-formamido-4-pentenoate (**1**). — A solution of ethyl 2-allyl-2-formamidomalonate[4] (68 g, 0.28 mole) in absolute alcohol (250 ml) was treated with 6N sodium hydroxide (51 ml) for 2 h at room temperature. After the addition of 6N sulphuric acid (51 ml) and ethanol (50 ml), the solution was kept for 1 h at 0°, filtered, and evaporated. The residue which crystallized spontaneously (m.p. 110–115°) was decarboxylated by heating for 20 min at 120–130°. After cooling, the resulting syrup was extracted with ether. An insoluble, crystalline product (6 g, m.p. 210°) was discarded. The ethereal solution was evaporated, and the resulting yellow oil was distilled to yield a colourless liquid (33 g, 69%); b.p. 91–92°/0.1 mm.

Anal. Calc. for $C_8H_{13}NO_3$: C, 56.12; H, 7.65; N, 8.18. Found: C, 56.28; H, 7.82; N, 8.09.

Ethyl 2-amino-4,4-diethoxybutyrate (**2**). — A solution of **1** (2.7 g, 16 mmoles) in *p*-dioxane (40 ml) and water (15 ml) was stirred for 5 min at room temperature with osmium tetroxide (0.041 g, 0.16 mmole). Sodium metaperiodate (7.5 g, 35 mmoles) was added portionwise during 30 min to the brown reaction mixture. After stirring for a further 30 min, the solids were removed by filtration and washed

with ethyl acetate. The pale-yellow filtrate and washing were concentrated to a syrup which contained colloidal osmium. The syrup was dissolved in chloroform and chromatographed on alumina (60 g). 1:4 Chloroform–ethanol eluted a pale-yellow oil (2.25 g, 81%) which could not be crystallized.

The product was treated for 18 h at room temperature with absolute alcohol (25 ml) presaturated at 0° with hydrogen chloride. The solution was concentrated to a thick syrup which was dissolved in chloroform (20 ml). Sodium hydrogen carbonate (4 g) was added, and the mixture was stirred for 4 h at room temperature. Insoluble salts were removed by filtration, and the filtrate was washed with 5% aqueous sodium hydrogen carbonate (5 ml) and saturated, aqueous sodium chloride (5 ml), dried (Na_2SO_4), and evaporated to dryness. The syrupy, brown residue was distilled at 100°/0.1 mm to give a colourless liquid (0.74 g, 22%); ν_{max} 3380 (NH_2), 2980, 2930, 1740 (CO), 1445, 1375, 1265, 1180, 1120, 1060 cm^{-1}.

Anal. Calc. for $C_{10}H_{21}NO_4$: C, 54.77; H, 9.65; N, 6.39. Found: C, 54.69; H, 9.50; N, 6.43.

The picrate was prepared by using water as solvent and was crystallized as the monohydrate, m.p. 55–57°, which melted to a gum on attempted drying *in vacuo* at room temperature. There was a broad i.r. band at 3400 cm^{-1} (bound OH). The analytical sample was air-dried.

Anal. Calc. for $C_{16}H_{24}N_4O_{11}\cdot H_2O$: C, 41.20; H, 5.62; N, 12.01. Found: C, 41.12; H, 5.59; N, 12.12.

Distillation of amino ester **2** left a crystalline residue which was extracted with hot water. The dioxopiperazine **3** crystallized from the cooled solution; m.p. 144–145°; ν_{max}^{Nujol}: 3050 (NH), 1665 (CO), 1340 (ring), 1130, 1055, 1010, 935, 875, 845, 750 cm^{-1}; n.m.r. data (chloroform-*d*): δ 1.22 (triplet, *J* 6 Hz, four CH_3); 2.2 (multiplet, two C–CH_2–C); 3.55 (quadruplet, *J* 6 Hz, four O–CH_2); 4.09, 4.25 (ring CH); 4.65 (triplet, *J* 4.5 Hz, two CHO_2); 6.80, 6.88 (NH). This is indicative of a mixture of *meso* and *racemic* isomers.

Anal. Calc. for $C_{16}H_{30}N_2O_6$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.32; H, 8.48; N, 8.33.

2-Amino-4,4-diethoxybutyric acid (**4**). — A solution of **2** (1.1 g, 5 mmoles) in ethanol (12 ml) was treated with 4N sodium hydroxide (3 ml) for 48 h at 0° and then neutralized with Amberlite IR-50 (H^+) resin. The filtrate was evaporated to dryness, and the residue was dissolved in 95% alcohol. The amino acid **4** crystallized slowly at 0°; m.p. 159–162° (dec.).

Anal. Calc. for $C_8H_{17}NO_4$: C, 50.25; H, 8.96; N, 7.33. Found: C, 49.68; H, 8.62; N, 7.23.

Methyl 2-amino-4,4-dimethoxybutyrate (**5**). — 3-Methoxycarbonyl-3-trifluoroacetamidopropionyl chloride[9] (102 g, 0.39 mole), obtained from DL-aspartic acid, was dissolved in hot, dry xylene (1000 ml) and hydrogenated at 100–105° and atmospheric pressure in the presence of 5% palladium on barium sulphate (30 g) and quinoline–sulphur poison (1 ml). The reaction was terminated when the evolution of hydrogen chloride ceased abruptly. The catalyst was removed by filtration and

washed with hot xylene. Evaporation of the filtrate and washings afforded the aldehyde as a pale-green syrup (91 g) which afforded a 2,4-dinitrophenylhydrazone, m.p. 201–202° (from ethanol).

Anal. Calc. for $C_{13}H_{12}F_3N_5O_7$: C, 38.34; H, 2.97; N, 7.19. Found: C, 38.57; H, 3.19; N, 17.48.

The syrupy aldehyde (91 g) was treated for 48 h at room temperature with dry methanol (1000 ml) presaturated at 0° with hydrogen chloride. The product was isolated as for compound **2**, to give **5** as a colourless liquid (27.5 g, 40%); b.p. 70°/0.3 mm.

Anal. Calc. for $C_7H_{15}NO_4$: C, 47.44; H, 8.53; N, 7.91. Found: C, 47.34; H, 8.42; N, 7.98.

The picrate of **5** was crystallized from water and alcohol, and had m.p. 120° (dec.).

Anal. Calc. for $C_{13}H_{18}N_4O_{11}$: C, 38.42; H, 4.47; N, 13.79. Found: C, 38.42; H, 4.36; N, 13.92.

(*S*)-Methyl 2-amino-4,4-dimethoxybutyrate (**5'**) was prepared in the same way, starting from L-aspartic acid. It had $[\alpha]_D^{21}$ +11° (*c* 2.0, methanol).

3-Amino-4-hydroxybutyraldehyde dimethyl acetal (**6**). — A solution of **5** (27.5 g, 0.16 mole) in dry ether (100 ml) was added dropwise during 3 h to a suspension of lithium aluminium hydride (13 g, 0.34 mole) in dry ether (400 ml). The reaction mixture was kept for 24 h at room temperature. Water (43 ml) was added to the cooled (−15°) mixture, which was then filtered. The inorganic residue was washed with boiling ethanol (5 × 200 ml), and the combined organic filtrates were evaporated. Distillation of the residue gave a colourless, viscous liquid (8.8 g, 38%); b.p. 90–91°/0.2 mm; ν_{max}: 3350, 3290 (OH, NH_2); 2930, 2830, 1595 (NH_2), 1465, 1445, 1370, 1120, 1055, 960 cm^{-1}.

The neutral oxalate of **6** was crystallized from absolute ethanol and had m.p. 147–148°.

Anal. Calc. for $C_{14}H_{32}N_2O_{10}$: C, 43.29; H, 8.30; N, 7.21. Found: C, 43.16; H, 8.21; N, 7.42.

(*S*)-3-Amino-4-hydroxybutyraldehyde dimethyl acetal (**6'**) was prepared from **5'** and gave a neutral oxalate with m.p. 155–156° and $[\alpha]_D^{20}$ +5.5° (*c* 2.0, methanol).

3-Acetamido-4-hydroxybutyraldehyde dimethyl acetal (**7**). — Acetic anhydride (1.5 ml) was added dropwise to a stirred solution of **6** (1 g) in dry methanol (10 ml). After 24 h at room temperature, the solution was evaporated, and last traces of acetic acid were removed by coevaporation with toluene. The residue was distilled to give the acetal **7** (1.1 g, 86%); b.p. 120°/0.06 mm; ν_{max}: 3300, 3090 (NH and OH); 2940, 2840, 1660, 1650 (amide I); 1550 (amide II); 1435, 1375, 1300, 1190, 1125, 1055, 970 cm^{-1}.

Anal. Calc. for $C_8H_{17}NO_4$: C, 50.25; H, 8.96; N, 7.33. Found: C, 49.96; H, 9.12; N, 6.79.

(*S*)-3-Acetamido-4-hydroxybutyraldehyde dimethyl acetal (**7'**) was prepared in the same way, starting from compound **6'**.

3-Amino-4-hydroxybutyraldehyde hydrochloride (**8**). — A solution of **6** (1 g, 6.7 mmoles) in 1.18N hydrochloric acid (15 ml) was stored for 24 h at room temperature. Paper chromatography in 4:1 propyl alcohol–water indicated the presence of only one reducing product (R_F 0.40), as detected by silver nitrate–sodium hydroxide. The solution was evaporated to dryness, and the residue was dried *in vacuo* over KOH and P_2O_5 to yield **8** as a yellow, amorphous solid (0.93 g) which was soluble in water and methanol.

The 2,4-dinitrophenylhydrazone of **8** was recrystallized from methanol and had m.p. 165–178° (dec.).

Anal. Calc. for $C_{10}H_{14}ClN_5O_5$: C, 37.57; H, 4.41; N, 21.91. Found: C, 37.28; H, 4.47; N, 21.18.

(S)-*3-Amino-4-hydroxybutyraldehyde diethyl dithioacetal* (**9′**). — A solution of **6′** (1.49 g, 10 mmoles) in water (1 ml) was treated at 0° with ethane thiol (2.5 ml) and conc. hydrochloric acid (8 ml) with vigorous stirring. After 24 h at room temperature, the reaction mixture was poured into a mixture of 40% aqueous sodium hydroxide (20 ml) and crushed ice (50 g). The product was extracted with chloroform (4 × 10 ml), and the extract was washed with water, dried (Na_2SO_4), and evaporated. The residue was distilled to give a colourless syrup which solidified at room temperature (1.8 g, 90%); b.p. 115–116°/0.1 mm; $[\alpha]_D^{19}$ +2.2° (*c* 2.5, methanol).

Anal. Calc. for $C_8H_{19}NOS_2$: C, 45.89; H, 9.15; N, 6.69; S, 30.63. Found: C, 45.59; H, 9.06; N, 6.56; S, 30.67.

The neutral oxalate of **9′** was crystallized from 95% alcohol and had m.p. 186–187°.

Anal. Calc. for $C_{18}H_{40}N_2O_6S_4$: C, 42.49; H, 7.92; N, 5.51; S, 25.21. Found: C, 42.72; H, 7.73; N, 5.51; S, 25.49.

(S)-*3-Acetamido-4-hydroxybutyraldehyde* (**10′**). — A solution of **7′** (2.4 g, 12 mmoles) in 0.02N sulphuric acid (250 ml) was stored for 24 h at room temperature, neutralized with barium carbonate, and filtered through Celite. The residue obtained by evaporation of the filtrate was dissolved in chloroform and the solution was filtered. Crystallization occurred following the evaporation of solvent and addition of acetone (0.9 g, 50%). The product was recrystallized from ethyl acetate; m.p. 90–91°; $[\alpha]_D^{21}$ −38° (*c* 0.9, water); t.l.c. in silica gel with 24:65:12 isopropyl alcohol–ethyl acetate–water indicated the presence of only one reducing compound (R_F 0.45) as detected with silver nitrate–sodium hydroxide; ν_{max}^{Nujol} 3320, 3170 (OH and NH); 1640 (amide I); 1550 (amide II); 1290, 1132, 1090, 1030, 995, 910 cm^{-1}; n.m.r. data (acetone-d_6): τ 8.1 (doublet, CH_3CO); 8.0 (multiplet, CH_2); 6.2 (multiplet, CH_2–O); 5.5 (broad band, OH); 4.7 and 4.5 (2 multiplets, 2 protons); and 2.7 (broad band, NH).

Anal. Calc. for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.49; H, 7.39; N, 9.67.

A semicarbazone derivative (m.p. 185–186°), which was crystallized from water, was obtained from the syrupy compound given by acid hydrolysis of the racemic acetal **6**.

Anal. Calc. for $C_7H_{14}N_4O_3$: C, 41.58; H, 6.98; N, 27.71. Found: C, 41.53; H, 7.10; N, 27.69.

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THE CHEMICAL STRUCTURE OF THREE TRISACCHARIDES FORMED IN THE LYSOZYME-CATALYZED TRANSGLYCOSYLATION REACTION*

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ABSTRACT

Treatment of the bacterial cell-wall tetrasaccharide, *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-*O*-β-D-GNAc-(1→4)-D-MurNAc (**1**), with hen's egg-white lysozyme in the presence of 2-acetamido-2-deoxy-D-glucose as acceptor, gave a trisaccharide, *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-GNAc (**2**), which was isolated by paper electrophoresis and paper chromatography. This compound was found to be identical with a trisaccharide obtained by degradation of **1** with lysostaphin. Two trisaccharides were isolated by a combination of anion-exchange chromatography, paper electrophoresis, and paper chromatography from reaction mixtures containing the cell-wall tetrasaccharide **1**, lysozyme, and D-galactose or D-xylose as acceptors. They were assigned the following structures: *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→2)-D-Gal (**3**) and *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-Xyl (**4**). The corresponding ^{14}C-labeled trisaccharides were also prepared using radioactive acceptors. For all trisaccharides, structural identification was based on the results of chemical and enzymic experiments which included color reactions, analysis of chemical composition, end-group determination by sodium borohydride reduction, periodate oxidation, and methylation. The trisaccharides were cleaved by lysozyme to give the free-acceptor sugars.

INTRODUCTION

We have previously reported that the bacterial cell-wall tetrasaccharide, *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-*O*-β-D-GNAc-(1→4)-D-MurNAc† (**1**), can serve as a donor of *O*-β-D-GNAc-(1→4)-D-MurNAc residues, and a variety of mono- and oligo-saccharides can serve as acceptors in transglycosylation reactions catalyzed by hen's egg-white lysozyme[2,3]. Similar findings with β-(1→4)-linked oligosaccharides of 2-acetamido-2-deoxy-D-glucose (chitin oligosaccharides) as donors

*A preliminary account has been presented (see ref. 1).
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†Abbreviation used: D-MurNAc for 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (*N*-acetylmuramic acid).

have also been described by other workers[4,5] and by ourselves[6]. However, no detailed evidence on the structure of the transfer products formed was presented. Such evidence is of importance for the understanding of the mechanism of lysozyme action[7].

We now describe the characterization by chemical and enzymic techniques of three trisaccharides which were isolated on a preparative scale from incubation mixtures containing compound **1**, hen's egg-white lysozyme, and one of the following unlabeled or radioactively labeled acceptors: 2-acetamido-2-deoxy-D-glucose, D-galactose, and D-xylose. The trisaccharides were found to be homogeneous in all of the paper-chromatographic and paper-electrophoretic systems tested. The acceptors proved to be located at the reducing end of the trisaccharides so that the general structure *O*-*β*-D-GNAc-(1 → 4)-D-MurNAc-X, where X is the acceptor, represents the compounds obtained. The isolated compounds all exhibited low optical rotations and could be cleaved by lysozyme to yield free-acceptor saccharides. On the basis of the low optical rotations observed for the trisaccharides, the glycosidic bond joining the 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucosyl residue to the acceptor has been assigned the *β*-D configuration. This result shows that the transfer reaction proceeds with retention of configuration. Periodate-oxidation studies of the sodium borohydride-reduced trisaccharides, analysis of the products of methylation, and other reactions led to the assignment of the following structures to the three compounds: *O*-*β*-D-GNAc-(1 → 4)-*O*-*β*-D-MurNAc-(1 → 4)-D-GNAc (**2**), *O*-*β*-D-GNAc-(1 → 4)-*O*-*β*-D-MurNAc-(1 → 2)-D-Gal (**3**), and *O*-*β*-D-GNAc-(1 → 4)-*O*-*β*-D-MurNAc-(1 → 4)-D-Xyl (**4**). Trisaccharide **2** was found to be identical by all criteria tested with a trisaccharide[8] which was isolated after degradation of **1** by lysostaphin. From the structure of the trisaccharides, it is concluded that lysozyme can form and cleave not only *β*-D-(1 → 4) linkages, but *β*-D-(1 → 2) linkages as well.

MATERIALS AND METHODS

General. — Hen's egg-white lysozyme (twice recrystallized, salt-free) was a product of Worthington. Boar-epididyme *exo*-*β*-*N*-acetylglucosaminidase[9] was given by Dr. James Conchie and lysostaphin[10] by Dr. P. A. Tavormina. The cell-wall tetrasaccharide, *O*-*β*-D-GNAc-(1→4)-*O*-*β*-D-MurNAc-(1→4)-*O*-*β*-D-GNAc-(1→4)-D-MurNAc (**1**), and the corresponding disaccharide, *O*-*β*-D-GNAc-(1 → 4)-D-MurNAc (**5**), were prepared from cell walls of *Micrococcus lysodeikticus*[11,12]. Authentic trisaccharide *O*-*β*-D-GNAc-(1 → 4)-*O*-*β*-D-MurNAc-(1 → 4)-D-GNAc was obtained by digestion of **1** with lysostaphin[8], and it was purified by preparative paper-electrophoresis at pH 6.5. 2-Acetamido-2-deoxy-D-glucose was a gift of Pfizer and Co. It was recrystallized from ethanol–acetone to give a melting point of 202–203°. 2-Amino-2-deoxy-D-glucitol and 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucitol were obtained as markers for paper chromatography and paper electrophoresis, as previously described[13], by reduction of 2-acetamido-2-deoxy-D-glucose and of compound **5**, respectively, with sodium borohydride, followed by acid hydrolysis. Radioactive monosaccharides, ^{14}C-labeled either uniformly or only at C-1, were

obtained from the Radiochemical Center, from the New England Nuclear Corporation, and from Calbiochem. Methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside containing a small amount of the corresponding β anomer was purchased from Koch–Light, and methylated standards of D-galactose and D-xylose for g.l.c. were kindly given by Dr. B. Gestetner and Dr. G. O. Aspinall.

Optical rotations were determined on water solutions with a Bendix ETL-NPL automatic polarimeter (Type 143A) at 25 ±1°.

Preparation of trisaccharides. O-β-D-*GNAc*-(*1*→*4*)-O-β-D-*MurNAc*-(*1*→*4*)-D-*GNAc* (**2**). — A typical incubation mixture consisted of tetrasaccharide **1** (40 μmoles), 2-acetamido-2-deoxy-D-glucose (160 μmoles), and lysozyme (4 mg) in 0.1M ammonium acetate buffer, pH 5.25 (4 ml). After incubation for 72 h at 37°, the reaction mixture was applied as a narrow band to two sheets of paper and subjected to electrophoresis at pH 6.5 for 90 min, in the presence of **1**, **5**, and the trisaccharide obtained by degradation of **1** with lysostaphin, as standards. The markers were located with the sodium hydroxide reagent. The material which migrated as the authentic trisaccharide (M_T 0.68) was eluted from the paper, and the eluate was lyophilized. The crude product was freed of compound **5** by paper chromatography in solvent (*a*), and final purification to remove a small amount of **1** was performed by chromatography in solvent (*b*) to give 4 mg (15% yield based on **1**) of compound **2**. In a similar manner, *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-GNAc-1-^{14}C (**2a**) was prepared by using 2-acetamido-2-deoxy-D-glucose-1-^{14}C as acceptor. In this case, guide strips were cut from the electrophoretogram, and the radioactive products were located by scanning.

O-β-D-*GNAc*-(*1*→*4*)-O-β-D-*MurNAc*-(*1*→*2*)-D-*Gal* (**3**). — The tetrasaccharide **1** (100 μmoles), D-galactose (5 mmoles), and lysozyme (3 mg) in acetate buffer (pH 5.25, 10 ml) were incubated at 37° for 18 h. The reaction mixture was lyophilized, and the residue was dissolved in a minimal volume of water and applied to a column (35 × 2 cm) of Dowex-1 (AG1–X-8, AcO^-, 200–400 mesh, Bio-Rad). Unreacted D-galactose was removed from the column with water (200 ml). Fraction II was obtained by elution with 0.45M acetic acid (150 ml), and undigested tetrasaccharide was eluted from the column with 0.75M acetic acid (150 ml). Upon electrophoresis at pH 6.5, fraction II was resolved into three bands having electrophoretic mobilities of M_T 0.84 (compound **5**), M_T 0.88, and M_T 0.68. The material having an M_T 0.68 was eluted from the paper. After lyophilization, further purification was achieved by preparative paper chromatography in solvent (*a*), and the major component (R_{GNAc} 0.55) was isolated to give 9 mg (13% yield based on **1**) of **3**. The corresponding, labeled trisaccharide *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→2)-D-Gal-1-^{14}C (**3a**) was also prepared according to this method, except that the cell-wall tetrasaccharide and D-galactose were used at equimolar concentrations (10mM).

O-β-D-*GNAc*-(*1*→*4*)-O-β-D-*MurNAc*-(*1*→*4*)-D-*Xyl* (**4**). — The conditions used were the same as those used for the preparation of **3**, except that D-xylose replaced D-galactose. The trisaccharide mixture was isolated by fractionation on Dowex-1, followed by preparative paper-electrophoresis at pH 6.5 (M_T 0.70). Preparative

paper-chromatography in solvent (*a*) gave two products having R_{GNAc} 0.59 and R_{GNAc} 0.69. The slower-moving product (5.5 mg, 9%) was homogeneous in all paper-chromatographic and -electrophoretic systems tested and was identified as the title compound **4**. The faster moving product (4.5 mg, 7%) was found to be a mixture of two trisaccharides, analogous to **4** but with (1→3)- and (1→2)-linked D-xylose[1], which could not be separated on a preparative scale. *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-Xyl-1-^{14}C (**4a**) was similarly prepared.

Paper chromatography and paper electrophoresis. — Whatman No. 1 paper was used for analytical experiments and No. 3 for preparative work. The following solvent systems (v/v) were used for descending paper-chromatography: (*a*) 25:6:25 butyl alcohol–acetic acid–water (upper phase), (*b*) 25:6:25 butyl alcohol–acetic acid–water (upper phase), in a chamber saturated with pyridine vapors[14]; (*c*) 2:1:2 ethyl acetate–pyridine–water (upper phase); and (*d*) 6:4:2:1 butyl alcohol–pyridine–water–6% (w/v) aqueous boric acid. The rates of migration in solvents (*a*), (*b*), and (*c*) are given relative to those of 2-acetamido-2-deoxy-D-glucose (R_{GNAc}), and in solvent (*d*) relative to 2-amino-2-deoxy-D-glucose (R_{GN}). Paper electrophoresis was performed at a constant voltage of 50 volts per cm with the following buffer systems: 0.86M acetic acid adjusted with pyridine to pH 3.5; 1.2M pyridine adjusted with acetic acid to pH 6.5; and 0.07M germanium dioxide adjusted with 50% sodium hydroxide to pH 10.7[15]. Electrophoretic mobilities are expressed relative to 2-amino-2-deoxy-D-glucose (M_{GN}) at pH 3.5, relative to the tetrasaccharide **1**, (M_T) at pH 6.5, and relative to D-glucose (M_G) at pH 10.7. The oligosaccharides were detected on paper chromatograms or paper electrophoretograms by one or more of the following reagents: sodium hydroxide[16], silver nitrate[17], and ninhydrin.

Radioactive chromatograms or electrophoretograms were routinely analyzed with a Vanguard gas-flow strip scanner. For quantitative measurements, the strips were divided into sections of 3 to 20 mm, depending on the proximity of the radioactive peaks. The paper sections were then placed into counting vials, and water (0.6 ml) was added to each vial. After the strips had been kept overnight, Bray's dioxane scintillation solution[18] was added, and the samples were counted in a Packard Tri-Carb scintillation counter for 5 min each.

Analytical methods. — 2-Acetamido-2-deoxy-D-glucose and 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose were measured as 2-amino-2-deoxy-D-glucose and 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose, respectively, with a Beckman Spinco amino acid analyzer, after acid hydrolysis (2M hydrochloric acid, 2.5 h, 100°) of the saccharides[13] using as standard compound **5** which was hydrolyzed under the same conditions. Neutral sugars were determined in the original compounds by the phenol–sulfuric acid test[19] with the appropriate saccharide standards. The triphenyltetrazolium test was performed according to Schiffman *et al.*[20].

Gas-liquid chromatography. — The trisaccharides (about 6 mg of each) were methylated according to the method of Kuhn *et al.*[21] by treatment with freshly prepared silver oxide, redistilled *N*,*N*-dimethylformamide, and methyl iodide. The methylated compounds were heated for 12 h at reflux in 5% methanolic hydrogen

chloride. The solutions were neutralized with silver carbonate and evaporated. After repeated additions of chloroform followed by evaporation, the resulting mixtures of methyl glycosides of *O*-methyl sugars were analyzed by g.l.c. with a Packard Model 1800 apparatus (flame ionization detector) using nitrogen flow rates of about 60 ml per min on (i) a coiled glass column (8 ft × 1/8 in) of 15% (w/w) polyethylene glycol adipate on Chromosorb-W (60–80 mesh, acid-washed) at 175°, and (ii) a column (6 ft × 1/8 in) of 5% (w/w) neopentyl glycol adipate on Chromosorb-W (60–80 mesh, acid-washed) at 150°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as standard. Only the methyl ethers of the acceptor saccharide portion of the trisaccharides were examined, since their identification was sufficient for assigning the positions of the new linkage formed. Furthermore, the acceptor portions of the molecule appeared, in all cases, to be completely methylated, since no peaks having slower retention times corresponding to those of incompletely methylated derivatives were observed. As no methylated standards were available for the 2-acetamido-2-deoxy-D-glucose and the 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose moieties of the compounds analyzed, it was not possible to conclude whether these residues were completely methylated. However, an analysis of methylated and methanolyzed *O*-β-D-GNAc-(1→4)-D-MurNAc did not reveal any peaks having retention times corresponding to those of the methylated acceptor saccharides after similar treatment.

Identification of terminal reducing sugar. — In a typical experiment, approximately 1 μmole of trisaccharide was dissolved in 0.1M borate buffer (0.5 ml, 0.025M sodium tetraborate and 0.1M boric acid, pH 8.3). A large excess of sodium borohydride (100 μmoles) was added, and the mixture was kept in the dark for 12 h at 4°; it was then brought to room temperature, and kept for 90 min at this temperature. Hydrochloric acid (4M, 0.5 ml) was added, and the hydrolysis was performed in a sealed tube for 3 h at 105°. An aliquot (0.2 ml) was dried *in vacuo* in the presence of potassium hydroxide, and the residue was dissolved in a minimal volume of water and analyzed by paper electrophoresis (pH 3.5, 45 min) with the standards, 2-amino-2-deoxy-D-glucose and 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (M_{GN} 0.29). The remaining portion (0.8 ml) of the hydrolyzate was applied to a column (5 × 1 cm) of Amberlite MB-3. The neutral compounds were eluted from the column with water (20 ml), and the eluate was evaporated to dryness under diminished pressure. Methanol (5 ml) was added, and the solution was evaporated *in vacuo* to remove the boric acid as volatile methyl borate. The addition of methanol and evaporation was repeated three times. The dried residue was dissolved in water (50 μl) and the solution was deposited on paper for chromatography in solvent (*d*) in the presence of the standards, D-galactose (R_{GN} 1.29), galactitol (R_{GN} 1.02), D-xylose (R_{GN} 1.80), and xylitol (R_{GN} 1.46). The spots were revealed with the silver nitrate reagent.

In the case of compound **2**, the sodium borohydride reduction followed by acid hydrolysis was performed as described in the preceding paragraph. The hydrolyzate was dried *in vacuo*, methanol was added and repeatedly evaporated, and the residue was dissolved in water (100 μl). The solution was divided into four equal

aliquots which were deposited on four different sections of the same paper for electrophoresis at pH 3.5, for 45 min, with standards 2-amino-2-deoxy-D-glucose, 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose, 2-amino-2-deoxy-D-glucitol (M_{GN} 1.00), and 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucitol (M_{GN} 0.29). One section of the electrophoretogram was treated with ninhydrin, another with silver nitrate. From the remaining two sections, the areas corresponding to M_{GN} 1.00 were cut out and sewn onto another sheet of paper for chromatography with solvent (*d*). The areas corresponding to M_{GN} 0.29 were treated similarly, except that chromatography was performed with solvent (*a*). The saccharides were revealed with the ninhydrin and silver nitrate reagents. The trisaccharide obtained by degradation of **1** with lysostaphin was analyzed in the same manner.

Periodate oxidation. — Periodate consumption by untreated and sodium borohydride-reduced trisaccharides was measured with the method of Leyh-Bouille *et al.*[8] except that the amounts of material were scaled up approximately tenfold. In a typical experiment, the untreated saccharide (0.5 μmole) was dissolved in water (400 μl) and 0.05M sodium metaperiodate (100 μl) was added. The reaction mixture was kept in the dark at room temperature and, over a period of 4 to 5 days, aliquots (10 μl) were added to a cuvette containing water (1 ml), and the absorbance was read at 224 nm. After 50 h, duplicate aliquots (100 μl, 0.1 μmole of trisaccharide) were removed. To these aliquots 2M sodium arsenite (100 μl) was added and after 15 min at room temperature the solutions were diluted to 1 ml. Formaldehyde was determined by the 2,4-pentanedione–ammonia method[22] and compared to an erythritol standard similarly oxidized.

For studies of the reduced trisaccharides, the saccharide (0.5–1.5 μmole) in water (200 μl) was mixed with a solution of 0.2M sodium borohydride (100 μl). The solution was kept for 4 h at room temperature, and the pH was then adjusted to about 5 with 0.2M acetic acid (100 μl). Sodium metaperiodate (0.05M, 100 μl) was added, and the solution kept at room temperature in the dark. Aliquots (10 μl) were withdrawn over a period of 80 h, added to a cuvette containing water (1 ml), and the absorbance was read at 224 nm. After 50 h, aliquots (50 to 100 μl) were taken for formaldehyde determination[22].

Sodium borohydride–periodate degradation of trisaccharides ^{14}C-labeled at C-1. — Sodium borohydride (0.2M, 100 μl) was added to the radioactive trisaccharide (0.5 μmole) in water (200 μl). The solution was kept for 4 h at room temperature, the pH was adjusted to about 5 with acetic acid (0.2M, 100 μl), and sodium metaperiodate (0.05M, 100 μl) was added. The oxidation was performed for a period of 2 h in the dark at room temperature. To destroy unreacted periodate, erythritol (0.15M, 100 μl) was added, and the solution was kept for another hour. Sodium borohydride (0.2M, 100 μl) was added, and after 4 h at room temperature the sample was hydrolyzed with 4M hydrochloric acid (700 μl) for 2.5 h at 100°. The hydrolyzate was applied to an Amberlite MB-3 column (5 × 1 cm). The column was washed with water (20 ml), the eluate was evaporated under reduced pressure; methanol was added to the residue and evaporated. The dried residue was dissolved in a known volume of water, and

radioactivity was determined on aliquots by liquid scintillation counting in Bray's solution. Aliquots were analyzed by paper chromatography in the presence of a glycerol marker with solvents (*a*) (R_{GNAc} 1.35) and (*c*) (R_{GNAc} 1.19) and by electrophoresis at pH 10.7 (M_G 0.35). Chromatograms or electrophoretograms of ^{14}C-labeled sugars were analyzed as outlined above.

Treatment with exo-β-N-*acetylglucosaminidase.* — The trisaccharide (1 μmole, labeled or unlabeled), was incubated at 37° with boar-epididyme *exo-β-N*-acetylglucosaminidase solution[9] (0.08 ml, 3×10^6 units per ml per h, in 0.05M citrate buffer, pH 4.2, and 0.1M sodium chloride), bovine serum albumin (0.02 ml, 2 mg per ml), and 0.05M citrate buffer, (0.10 ml, pH 4.2). Duplicate aliquots (45 μ leach) were removed, at zero time and after 24 h of incubation, for analysis by paper electrophoresis, at pH 6.5, using 2-acetamido-2-deoxy-D-glucose and the disaccharide **5** as standards. Part of the electrophoretogram (one set of the 0- and 24-h aliquots) was treated with the sodium hydroxide reagent, while, from the other part, the areas of the reaction mixture corresponding in mobility to the standards were cut out and sewn onto a second paper for chromatography with solvent (*a*). When radioactively labeled trisaccharides were used, the positions of the products of digestion on the chromatograms and electrophoretograms were revealed by strip scanning.

Degradation of trisaccharides with lysozyme. — For testing the susceptibility of the isolated trisaccharides to lysozyme degradation, they were incubated for periods of 72 to 98 h at 37° in 0.1M ammonium acetate–acetic acid buffer, pH 5.25 (prepared by adding 0.1M acetic acid to 0.1M ammonium acetate to the required pH), using concentrations of 2.4 to 7.9×10^{-2}M of trisaccharide and of 1 mg per ml of enzyme. The radioactive products formed during the course of incubation were analyzed by paper electrophoresis at pH 6.5.

RESULTS AND DISCUSSION

Trisaccharide **2**, isolated from an incubation mixture containing the cell-wall tetrasaccharide **1**, 2-acetamido-2-deoxy-D-glucose, and lysozyme, migrated as a single spot or radioactive peak (when **2a** was tested) in all the paper-chromatographic and electrophoretic systems tested. Its rates of migration (Table I) were, in all cases, identical with those of the trisaccharide obtained by degradation of **1** with lysostaphin. After acid hydrolysis, compound **2** contained 95% of the theoretical amount of 2-amino-2-deoxy-D-glucose and the ratio of the latter to 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose was 2.02 to 1.00 (as compared to 1.98 to 1.00 for the authentic trisaccharide). Its optical rotation, $[\alpha]_D^{25} -20.4°$ (*c* 0.52, water), was very close to that of the authentic trisaccharide, $[\alpha]_D^{25} -22°$ (*c* 0.65, water), and considerably lower than that of the disaccharide **5** ($[\alpha]_D^{25} +13°$, ref. 12).

The terminal reducing sugar of compound **2** was shown to be 2-acetamido-2-deoxy-D-glucose and not 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose. Reduction of the trisaccharide with sodium borohydride, followed by acid hydrolysis, gave 2-amino-2-deoxy-D-glucose, 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose, and a third compound which migrated on paper chromatograms and electrophoreto-

TABLE I

RATES OF MIGRATION OF OLIGOSACCHARIDES ON PAPER CHROMATOGRAPHY AND PAPER ELECTROPHORESIS

Compounds[a]	R_{GNAc} *in solvents*			*Electrophoresis*	
	(*a*)	(*b*)	(*c*)	M_T, *pH* 6.5	M_G, *pH* 10.7
Standard compounds					
2-Acetamido-2-deoxy-D-glucose	1.00	1.00	1.00		
D-Galactose	0.70		0.76		
D-Glucose	0.74		0.81		1.00
D-Xylose	0.93		0.94		
O-β-D-GNAc-(1→4)-D-MurNAc (**5**)	1.05	0.73	0.46	0.84	
O-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-*O*-β-D-GNAc-(1→4)-D-MurNAc (**1**)	0.66	0.33	0.21	1.00	
Trisaccharides					
O-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-GNAc (**2**)[b]	0.66	0.50	0.36	0.68	1.03
O-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→2)-D-Gal (**3**)	0.55		0.30	0.68	0.36
O-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-Xyl (**4**)	0.59	0.38	0.33	0.70	0.76

[a]Compounds were revealed with the sodium hydroxide and silver nitrate reagents[16,17]. In the case of trisaccharides, rates of migration were also estimated by the use of the radioactive compounds **2a**, **3a**, and **4a**. [b]Identical in migration rates to the trisaccharide prepared by lysostaphin degradation of the cell-wall tetrasaccharide **1**.

grams at the same rate as that of 2-amino-2-deoxy-D-glucitol and not as that of 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucitol. The same result was observed with the authentic trisaccharide. Hydrolyzates were first separated by paper electrophoresis, and then chromatographed in solvent (*a*) to detect 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucitol (R_{GNAc} 0.85) [as compared to 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (R_{GNAc} 1.05)], and in solvent (*d*) for 2-amino-2-deoxy-D-glucitol (R_{GN} 0.60). Both these sugar alcohols gave weakly positive ninhydrin tests. However, only 2-amino-2-deoxy-D-glucitol gave a reaction with the silver nitrate reagent (brown colored spot), although the intensity of the spot was weak, and therefore difficult to detect in solvent (*d*) which contains boric acid as one of its constituents.

The location of the 2-acetamido-2-deoxy-D-glucose at the reducing end of the trisaccharide **2** was also established by degradation with *exo*-β-*N*-acetylglucosaminidase. Treatment of **2** with the enzyme yielded two products corresponding in electrophoretic and chromatographic mobility to 2-acetamido-2-deoxy-D-glucose and to the disaccharide *O*-β-D-GNAc-(1→4)-D-MurNAc (**5**). However, the spot which migrated as the disaccharide had a different fluorescent color when revealed with the sodium hydroxide reagent. Identical results were obtained with the authentic trisaccharide which was shown by Leyh-Bouille *et al.*[8] to give the disaccharide *O*-β-D-MurNAc-(1→4)-D-GNAc upon treatment with *exo*-β-*N*-acetylglucosaminidase. consumed very rapidly (within minutes), presumably by the vicinal hydroxyl groups

Furthermore, it was found that treatment of the 1-^{14}C-labeled trisaccharide **2a** with *exo*-β-*N*-acetylglucosaminidase gave a sole radioactive compound. This compound migrated on paper chromatography in solvent (*b*), and on paper electrophoresis at pH 6.5, not as 2-acetamido-2-deoxy-D-glucose, but as compound **5**, thus showing that the digestion product is an acidic disaccharide, most probably *O*-β-D-MurNAc-(1→4)-D-GNAc-1-^{14}*C*.

Periodate oxidation studies of sodium borohydride-reduced **2** and of the authentic trisaccharide gave the same results (Fig. 1). One mole of periodate was

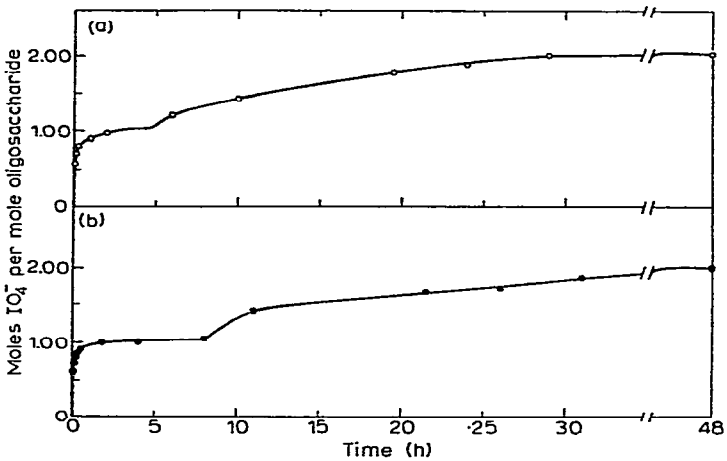

Fig. 1. Comparison of the periodate consumption of *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-GNAc (a), prepared by lysostaphin degradation of the cell-wall tetrasaccharide **1**, and trisaccharide **2** (b), prepared by lysozyme-catalyzed transglycosylation. Both trisaccharides were reduced with sodium borohydride prior to periodate oxidation, and periodate uptake was followed spectrophotometrically at 224 nm by removing aliquots from the reaction mixtures over a period of 48 h.

attached at C-5 and C-6 of the reducing end of the trisaccharides, while a second mole was used by the non-reducing end at a slow rate, over a period of 48 h, in agreement with the results of Leyh-Bouille *et al.*[8]. Furthermore, the total periodate uptake and formaldehyde release were found to be characteristic of a (1→4)-linked 2-acetamido-2-deoxy-D-glucose moiety located at the reducing end of the molecule (Table II).

Compound **3**, $[\alpha]_D^{25} - 13.9°$ (*c* 0.51, water) was homogeneous on paper chromatography and paper electrophoresis (for rates of migration, see Table I). The ratio of 2-amino-2-deoxy-D-glucose to 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose to neutral sugar was 1.00 to 1.00 to 1.07. The purity of compound **3**, based on the content of 2-amino-2-deoxy-D-glucose, was estimated to be 93%. Reduction of **3** with sodium borohydride, followed by acid hydrolysis showed that its D-galactose moiety was converted into galactitol and that the amino sugar moieties were not modified.

When sodium borohydride-reduced **3** was treated with sodium metaperiodate, it was found to consume rapidly 3 moles of periodate (2.8 moles at 1.5 h) per mole of trisaccharide, a fourth mole being reduced after about 50 h. The first 3 moles are presumably required for oxidation of the D-galactose moiety, and the fourth for a

TABLE II

QUANTITATIVE ANALYSIS OF PERIODATE CONSUMPTION AND FORMALDEHYDE RELEASE IN UNTREATED AND SODIUM BOROHYDRIDE-REDUCED TRISACCHARIDES

Trisaccharide O-β-D-*GNAc*-(*1→4*)-O-β-D-*MurNAc-X*		*Moles of periodate (IO_4) consumed and formaldehyde (H_2CO) released per mole of trisaccharide*				
		Calc. for MurNAc-X linkage[a]				*Found*[b]
		(*1→2*)	(*1→3*)	(*1→4*)	(*1→6*)	
X = D-GNAc (2), $NaBH_4$-reduced	IO_4	[c]	3	2	3	2.0
	H_2CO	[c]	1	1	0	0.98
X = D-Gal (3), untreated[d]	IO_4	7*	7*	7*	4	6.36
	H_2CO	1	1	1	0	0.96
X = D-Gal (3), $NaBH_4$-reduced	IO_4	4	7*	7*	5	4.09
	H_2CO	1	2	2	1	0.94
X = D-Xyl (4), untreated[d]	IO_4	6*	6*	3	[e]	3.0
	H_2CO	1	1	0	[e]	0
X = D-Xyl (4), $NaBH_4$-reduced	IO_4	3	6*	3	[e]	3.0
	H_2CO	1	2	1	[e]	0.98

[a]The calculated values are the total number of moles of periodate consumed and formaldehyde released, assuming complete oxidation. For any particular linkage, one mole of the total number of moles of periodate consumed is contributed by oxidation at the non-reducing end of the molecule. Where an asterisk marks the value, overoxidation is expected to occur under the experimental conditions used[24–26]. [b]In the case of sodium borohydride-reduced trisaccharides, the values of periodate uptake were found for 80-h experiments, whereas for unmodified trisaccharides, the consumption was measured after 130 h. Change of optical density at 224 nm (a measure of the periodate consumption) was also determined over these time periods, and at the end of 50 h formaldehyde release was estimated. [c]Excluded because of the 2-acetamido group. [d]Untreated signifies no prior reduction with sodium borohydride. [e]Not possible with D-xylose.

slow oxidation of the 2-acetamido-2-deoxy-D-glucose moiety present at the non-reducing end of the trisaccharide[8]. As indicated in Table II, these results are consistent with a (1→2) linkage to D-galactose at the reducing end of this trisaccharide, since a (1→6)-linked D-galactose would rapidly consume 4 moles (and not 3) of periodate (in addition to 1 mole of periodate degrading the non-reducing end of the trisaccharide) to give a stable, substituted acetaldehyde derivative[23]. Furthermore, both the (1→3)- and (1→4)-linked D-galactose would be over-oxidized and would consume more periodate at room temperature after 80 h. Over-oxidation of this type is known to occur through the formation of an unstable malondialdehyde derivative[24,26].

Additional evidence for the nature of the linkage of D-galactose in **3** comes from periodate-oxidation studies of the unmodified trisaccharide. After 5 h, 3.09 moles of periodate per mole of **3** were reduced. This consumption can be ascribed only to the D-galactose portion of the trisaccharide, since the non-reducing end is apparently not oxidized at all after 5 h, as suggested by the results of the periodate oxidation

of the sodium borohydride-reduced **2** (Fig. 1 and ref. 8). A (1→3)- or (1→4)-linked D-galactose unit at the reducing end of similar trisaccharides should be oxidized rapidly in its pyranose form, resulting in the consumption of 1 or 2 moles of periodate, respectively. Oxidation of the D-galactose moiety in these cases must proceed through a rate-limiting step known to involve the very slow hydrolysis of a formyl ester[25,26]. A trisaccharide having a (1→6)-linked D-galactose unit would, on the other hand, consume 3 moles of periodate at the early stage of the oxidation, in agreement with the observed values; a (1→6) linkage however, can be ruled out for the following reasons: one mole of formaldehyde was released, and the total number of moles of periodate reduced per mole of trisaccharide, after 130 h, was closer to 7 rather than 4 (see Table II). The only alternative remaining is a trisaccharide having a (1→2) bond between 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose and D-galactose, and such a bond is in fact in agreement with the values found (Table II). A (1→2)-linked D-galactose, in the pyranose form, would be oxidized initially by one mole of periodate at the vicinal C-3 and C-4 hydroxyl groups, and in the process a hemiacetal would be formed. This product, in contrast to the formyl ester arising from the oxidation of (1→3)- and (1→4)-linked D-galactose, is unstable and will be cleaved at its hemiacetal bond, giving glyceraldehyde and an unstable malondialdehyde derivative. The latter compound would be gradually overoxidized, while glyceraldehyde would be rapidly cleaved by 2 moles of periodate (per mole of glyceraldehyde) with a release of 1 mole of formaldehyde.

After sodium borohydride–periodate degradation of **3a** (30,000 c.p.m.), the bulk of radioactivity (23,000 c.p.m.) was recovered in the non-volatile fraction. The sole radioactive compound found in this fraction was glycerol-^{14}C, as shown by paper chromatography and paper electrophoresis. This result indicates that the linkage formed, by lysozyme transglycosylation, between the 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose unit of the transferred disaccharide and D-galactose in trisaccharide **3** is (1→2). The incomplete recovery of radioactivity from **3a** may be attributed to losses encountered during the experimental procedure.

Compound **3** (0.8 μmole per 0.1 ml) failed to give a color when treated with the triphenyltetrazolium chloride (TTC) reagent. Sophorose also remained colorless at the same concentration, whereas laminaribiose, cellobiose, and gentiobiose (all at 0.15 μmole per 0.1 ml) gave dark-red formazan precipitates. A negative reaction with the TTC reagent in a test for (1→2) linkages[20].

The results of the methylation procedure applied to compound **2** are given in Table III. Only a 2,3,4-tri-*O*-methyl-D-galactose standard was available, and the retenti ontime *T* for this derivative was found to be just slightly higher, on column (ii), than the value obtained by Dr. G. O. Aspinall. In general, the values observed on this column were almost the same as those of Dr. G. O. Aspinall (personal communication), although column (i) gave *T* values which were higher* by approximately 20%.

*T values are not absolute and vary from column to column. However, the same degree of resolution is found for any liquid phase; and for any particular liquid phase, only a simple conversion factor is necessary (Dr. G. O. Aspinall, personal communication).

Assuming that the *T* values for the 2,3,4-tri-*O*-methyl- and 2,4,6-tri-*O*-methyl-D-galactose derivatives given by Aspinall would in fact correspond to observed values, if such standards were available, it can be seen from Table III that compound **3** exhibits retention times which cannot be attributed to (1→3), (1→4), or (1→6) linkages. Presumably, therefore, the D-galactose unit is (1→2)-linked, as suggested by other results. On the basis of our findings, the structure of compound **3** is *O*-*β*-D-GNAc-(1→4)-*O*-*β*-D-MurNAc-(1→2)-D-Gal. The assignment of a *β*-configuration to the bond between the 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose and the D-galactose units is based on the low rotation-value found for compound **3**, and its similarity to the rotation of compound **2**.

TABLE III

GAS-LIQUID CHROMATOGRAPHIC DATA FOR METHYLATED AND METHANOLYZED OLIGOSACCHARIDES[a]

Methylated and methanolyzed oligosaccharides	*Relative retention times* (T) *of methyl glycosides*	
	Column (i)[b]	*Column (ii)*[c]
2,3,4-Tri-*O*-methyl-D-galactose		6.67, (6.51)[d]
2,3,6-Tri-*O*-methyl-D-galactose		(2.85, 3.76, 4.20)
2,4,6-Tri-*O*-methyl-D-galactose		(3.66, 4.25)
Compound **3**		3.56, 5.97
3,4-Di-*O*-methyl-D-xylose	1.59, 1.96	1.01, 1.21
2,3-Di-*O*-methyl-D-xylose	1.77, 2.17	1.14, 1.39
2,4-Di-*O*-methyl-D-xylose	1.86, 2.49	1.15, 1.53
Compound **4**	1.77, 2.17	1.14, 1.39

[a]Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-*β*-D-glucopyranoside. [b]Column (i); polyethylene glycol adipate. [c]Column (ii), neopentyl glycol adipate. [d]Values in parentheses are those communicated by Dr. G. O. Aspinall.

The D-xylose-containing trisaccharide **4**, $[\alpha]_D^{25} -38.5°$ (*c* 0.54, water), was shown to be homogeneous and 93% pure by the same criteria as those used for compounds **2** and **3**. Sodium borohydride reduction followed by acid hydrolysis revealed the presence of xylitol, but not of D-xylose. The ratio of 2-amino-2-deoxy-D-glucose to 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose to neutral sugar was 1.00 to 1.00 to 1.03. Upon oxidation of compound **4**, 3 moles of periodate were consumed, but no formaldehyde was released (Table II). This is in agreement with a (1→4) linkage to D-xylose, as both (1→2) and (1→3) linked D-xylose tend to be overoxidized under these conditions[24]. In the case of sodium borohydride-reduced **4**, the values found are consistent with either a (1→2) or (1→4) linkage (Table III). A (1→3) linked D-xylose, on the other hand, would be overoxidized *via* the malondialdehyde derivative[24,26], resulting in a periodate uptake higher than that expected for either (1→2)- or (1→4)-linked D-xylose.

Upon sodium borohydride–periodate degradation of **4a** (96,000 c.p.m.), only an insignificant amount of radioactivity (400 c.p.m.) was recovered in the non-volatile fraction, showing that no ^{14}C-labeled glycerol was formed. Trisaccharide **4** gave a red formazan precipitate with TTC indicating the presence of a linkage other than (1→2).

The results of the methylation procedure (Table III) are in agreement with the periodate oxidation studies and other experiments, and show that the D-xylose moiety in compound **4** is (1→4)-linked. The structure of this trisaccharide is, therefore, *O*-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-Xyl. The assignment of the β-configuration to the glycosidic bond linking the D-xylose in compound **4** is based on the low optical rotation of the trisaccharide.

Digestion by lysozyme. — The trisaccharides described were cleaved by lysozyme at a slow rate, to release the terminal, reducing moieties of their molecules (Table IV).

TABLE IV

CLEAVAGE BY LYSOZYME OF TRISACCHARIDES RESULTING FROM TRANSGLYCOSYLATION[a]

Trisaccharide	*Molar concentration*[b]	*Time of incubation (h)*	*Percent of cleavage*[c]
O-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-GNAc-1-^{14}C (2a)	2.4×10^{-2}	98	1
O-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→2)-D-Gal-1-^{14}C (3a)	3.7×10^{-2}	72	12
O-β-D-GNAc-(1→4)-*O*-β-D-MurNAc-(1→4)-D-Xyl-1-^{14}C (4a)	7.9×10^{-2}	98	4

[a]For experimental conditions, see Material and Methods. [b]An appropriate amount of unlabeled trisaccharide was added to give the required concentration. [c]The percent of cleavage by lysozyme was determined from the amount of radioactive monosaccharide released after paper electrophoresis of the reaction mixture at pH 6.5.

The structure of the trisaccharides and the mechanism of action of lysozyme. — The foregoing experiments, which establish the structure of three trisaccharides formed in the lysozyme-catalyzed transglycosylation reaction, show that the enzyme can both form and cleave linkages other than β-D-(1→4), such as β-D-(1→2), and that the transfer reaction proceeds with retention of configuration. Formation of linkages other than β-D-(1→4) was in fact suggested from a close examination of the three-dimensional model of the enzyme, especially of subsite $E^{1,27}$. Retention of configuration in the transfer reaction shows that the leaving and incoming groups occupy the same site on the enzyme, and strongly implicates the existence of a long-lived intermediate, most likely a carbonium ion[7].

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SPECIFIC AND IRREVERSIBLE LYSOZYME INHIBITORS: 2′,3′-EPOXYPROPYL β-D-GLYCOSIDES OF 2-ACETAMIDO-2-DEOXY-D-GLUCOSE AND ITS OLIGOMERS

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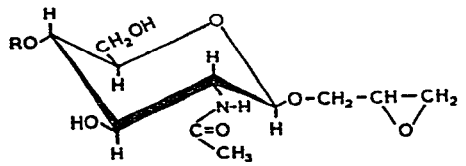
ABSTRACT

The acetylated allyl β-glycosides of 2-acetamido-2-deoxy-D-glucose, di-*N*-acetylchitobiose, and tri-*N*-acetylchitotriose are described. Epoxidation with peroxyphthalic acid was followed by catalytic deacetylation to give the corresponding 2′,3′-epoxypropyl β-D-glycosides. These compounds are specific and irreversible inhibitors of hen's egg-white lysozyme.

INTRODUCTION

The title compounds (**1–3**) were synthesized as possible specific reagents for the active site of hen's egg-white lysozyme, using the principle[1] of "affinity labelling". 2-Acetamido-2-deoxy-D-glucose and its oligomers, di-*N*-acetylchitobiose and tri-*N*-acetylchitotriose (**4**), are known to bind to lysozyme, and the association constants increase with chain length[2]. It seemed possible to use these saccharides as carriers of alkylating groups strategically placed for reaction with an amino acid residue in the active site. Synthetic considerations dictated that the alkylating group be incorporated into the aglycone portion of a β-D-glycoside; an epoxide function was chosen, for reasons stated below.



1 R = H
2 R = GlcNAc
3 R = GlcNAc-β-(1→4)-GlcNAc

RESULTS AND DISCUSSION

Condensation of the appropriate acetylated glycosyl chloride with allyl alcohol under the usual Koenigs–Knorr conditions gave the corresponding allyl β-D-glyco-

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sides. In each case, epoxidation with peroxyphthalic acid gave the pure epoxide. Catalytic *O*-deacetylation the ngave compounds **1–3**, formulated as the 2′,3′-epoxypropyl β-D-glycosides. Each glycoside reacted readily with dilute sodium thiosulphate solution, liberating hydroxide ions, as expected of epoxides[3].

As reported elsewhere[4], compounds **2** and **3** proved to be potent, irreverisble inhibitors of lysozyme, **3** being the more effective on a molar basis, as expected in light of its higher association constant. By using ^{14}C-labelled **2**, 1 mole of inhibitor was found to be covalently bound to 1 mole of enzyme at 100% inactivation. This demonstrates the high specificity achieved.

The design of these inhibitors followed examination of a model of the lysozyme–**4** complex[5]. Compounds **1–3** would be expected to bind in a "non-productive" fashion, occupying sub-sites *C*, *B–C*, and *A–B–C*, respectively. The β-epoxypropyl function would not significantly interfere with this mode of binding. Further, the epoxide group would then be positioned in sub-site *D*, which contains residues 35(Glu) and 52(Asp) — the side chains postulated to take part in the bond-breaking step[5]. The reaction of epoxides with carboxyl groups, in particular carboxylate ions, is well documented[3]. It is hoped that these inhibitors will be useful both in studying aspects of the mechanism of action of hen's egg-white lysozyme, and also in probing the active-site geometry of lysozymes from other organisms.

EXPERIMENTAL

Rotations were determined in semi-micro tubes with a Perkin–Elmer 141 polarimeter. All melting points are uncorrected.

Di-*N*-acetyl-hexa-*O*-acetylchitobiose and tri-*N*-acetyl-octa-*O*-acetylchitotriose were prepared by acetolysis of chitin[6]. They were converted into the corresponding glycosyl chlorides by treatment with glacial acetic acid pre-saturated at 0° with dry hydrogen chloride. After 20 h, at room temperature, the mixtures were worked up as previously described[7]. Peroxyphthalic acid was prepared as described (*Org. Syn.*, Coll. Vol. III, p. 619) and stored at −5° in methylene chloride solution.

All reactions were followed by t.l.c. on silica gel with ethyl acetate–acetone (acetates) or methanol–acetone (free sugars).

*Allyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside.* — Dry allyl alcohol (150 ml), silver carbonate (4.25 g), and powdered anhydrous calcium sulphate (15 g) were stirred with exclusion of light and moisture for 2 h. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucosyl chloride (5.6 g) and silver perchlorate (200 mg) were added, and stirring was continued for 6 h. The mixture was then filtered through Celite, the filtrate evaporated to dryness, and the residue dissolved in methylene chloride. After washing with N HCl, 10% aqueous $KHCO_3$, and water, the organic layer was dried ($MgSO_4$) and evaporated to a colourless syrup which crystallized on triturating with propan-2-ol. Recrystallisation from methanol–ether gave material (3.5 g, 52%) having m.p. 160°, $[\alpha]_D^{23}$ −15.1° (*c* 2.06, chloroform).

Anal. Calc. for $C_{17}H_{25}NO_9$: C, 52.7; H, 6.5; N, 3.6. Found: C, 52.5; H, 6.7; N, 3.85.

The chitobiose analogue, allyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside, obtained, as above, in 50% yield from the acetylated glycosyl chloride of chitobiose, had m.p. 242–245°, $[\alpha]_D^{23}$ −38° (*c* 2.09, chloroform).

Anal. Calc. for $C_{29}H_{42}N_2O_{16}$: C, 51.5; H, 6.2; N, 4.15. Found: C, 51.45; H, 6.1; N, 4.4.

The chitotriose analogue, allyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside, obtained in 50% yield from the acetylated glycosyl chloride of chitotriose, had m.p. 280° (dec.), $[\alpha]_D^{23}$ −23.0° (*c* 0.945, chloroform–methanol, 1:1).

Anal. Calc. for $C_{41}H_{59}N_3O_{23}$: C, 51.2; H, 6.2; N, 4.4. Found: C, 50.9; H, 6.0; N, 4.4.

Epoxidation. — The appropriate allyl glycoside (1 mmole) was dissolved in methylene chloride (10 ml) containing peroxyphthalic acid (*ca.* 2 mmoles), and the solution was refluxed gently for 3 h. After cooling to 0°, phthalic acid was filtered off, and the filtrate was extracted twice with cold 0.5M $KHCO_3$ and twice with water. After drying ($MgSO_4$) and evaporation, the corresponding epoxide (essentially one component as shown by t.l.c.) was crystallized from methanol–ether. Yields usually exceeded 80%. The following compounds were obtained:

2′,3′-Epoxypropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside, m.p. 162–163°, $[\alpha]_D^{23}$ −34.4° (*c* 0.36, chloroform).

Anal. Calc. for $C_{17}H_{25}N_1O_{10}$: C, 50.6; N, 6.25; N, 3.5. Found: C, 50.7; H, 6.4; N, 3.6.

2′,3′-Epoxypropyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside, m.p. 260°, $[\alpha]_D^{23}$ −53.2° (*c* 2.15, chloroform).

Anal. Calc. for $C_{29}H_{42}N_2O_{17}$: C, 50.4; H, 6.1; N, 4.1. Found: C, 50.5; H, 6.0; N, 4.2.

2′,3′-Epoxypropyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside, m.p. 288–290° (dec.), $[\alpha]_D^{23}$ −29.7° (*c* 0.59, chloroform–methanol, 1:1).

Anal. Calc. for $C_{14}H_{59}N_3O_{24}$: C, 50.35; H, 6.1; H, 4.3. Found: C, 49.9; H, 5.8; N, 4.55.

Deacetylation. — The appropriate epoxypropyl glycoside was suspended in dry methanol (10% w/v) with rapid magnetic stirring, and treated with methanolic barium methoxide to give a final concentration of 0.02M. After dissolution, precipitation of the *O*-deacetylated products **2** and **3** began within 5 min. After 12 h at 3°, the solids were collected, and crystallized from aqeous acetone. Compound **1** was isolated by first neutralizing the deacetylation mixture with CO_2, evaporating to dryness, and then proceeding as above. Yields ranged from 50–70%. Compounds **1** and **2** were homogeneous by t.l.c., having R_F values of 0.3 and 0.2, respectively, in acetone–methanol

(9:1). Glycoside **3** gave one spot on paper chromatograms (isopropyl alcohol–water, 4:1 v/v) when detected by a chlorination procedure[8]. The following compounds were obtained:

2′,3′-Epoxypropyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**), m.p. 175–178°, $[\alpha]_D^{23}$ −37.4° (*c* 2.02, water).

Anal. Calc. for $C_{11}H_{19}NO_7$: C, 47.65; H, 6.9; N, 5.05; for $C_{11}H_{19}NO_7 \cdot H_2O$: C, 44.7; H, 7.1; N, 4.7. Found: C, 44.6; H, 7.0; N, 5.3.

2′,3′-Epoxypropyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**2**), m.p. 280–285° (dec.), $[\alpha]_D^{23}$ −33.2° (*c* 2.45, water).

Anal. Calc. for $C_{19}H_{32}N_2O_{12}$: C, 47.5; H, 6.7; N, 5.8; for $C_{19}H_{32}N_2O_{12} \cdot H_2O$: C, 45.7; H, 6.4; N, 5.6. Found: C, 45.9; H, 6.9; N, 5.6.

2′,3′ Epoxypropyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**3**), m.p. >300°, $[\alpha]_D^{23}$ −26.9° (*c* 1.2, water).

Anal. Calc. for $C_{27}H_{45}N_3O_{17}$: N, 6.2; for $C_{27}H_{45}N_3O_{17} \cdot H_2O$: N, 6.0. Found: N, 5.8, 6.1.

Solutions of glycosides **1–3** in 0.1N sodium thiosulphate solution (containing 1 drop of phenolphthalein indicator) became alkaline within 5 min at room temperature[3].

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SYNTHESIS OF 2,4-DI-*O*-METHYL-D-FUCOSE*

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ABSTRACT

2,4-Di-*O*-methyl-D-fucose has been synthesized from methyl 6-*O*-benzyl-α-D-galactopyranoside by acetonation, methylation at O-2, deacetonation, benzylidenation, benzylation at O-3, debenzylidenation, tosylation at O-6, methylation at O-4, and removal of protective groups.

INTRODUCTION

Methylation studies in this laboratory on a fucose-containing lipopolysaccharide isolated from a strain of *E. coli* required the di-*O*-methyl ethers of D-fucose as authentic standards for identification purposes. Of the six possible di-*O*-methyl ethers of D- and L-fucopyranose, only the 2,3-D[1,2] and -L[2], and 3,4-L[3] dimethyl ethers have been synthesized[1–3]. The 2,4-dimethyl ether of D-fucose is known, having been isolated from the antibiotic labilomycin[4]; 2,4-di-*O*-methyl-L-fucose is also known, having been isolated as a product from partial methylation of methyl L-fucosides[5], but no unambiguous synthesis has been reported. The present communication reports a synthesis of 2,4-di-*O*-methyl-D-fucose and a description of its properties.

RESULTS AND DISCUSSION

Methyl 6-*O*-benzyl-α-D-galactopyranoside (**3**), obtained[6] from 1,2:3,4-isopropylidene-α-D-galactopyranoside (**1**) through the derivative **2**, gave, on acetonation and subsequent methylation, methyl 6-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-methyl-α-D-galactopyranoside (**5**). Compound **5** was partially hydrolyzed to give methyl 6-*O*-benzyl-2-*O*-methyl-α-D-galactopyranoside (**6**) which on debenzylation afforded methyl 2-*O*-methyl-α-D-galactopyranoside (**7**). Benzylidenation of **7** and subsequent benzylation yielded methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methyl-α-D-galactopyranoside (**9**). Debenzylidenation of **9** followed by *p*-tolylsulfonylation gave methyl

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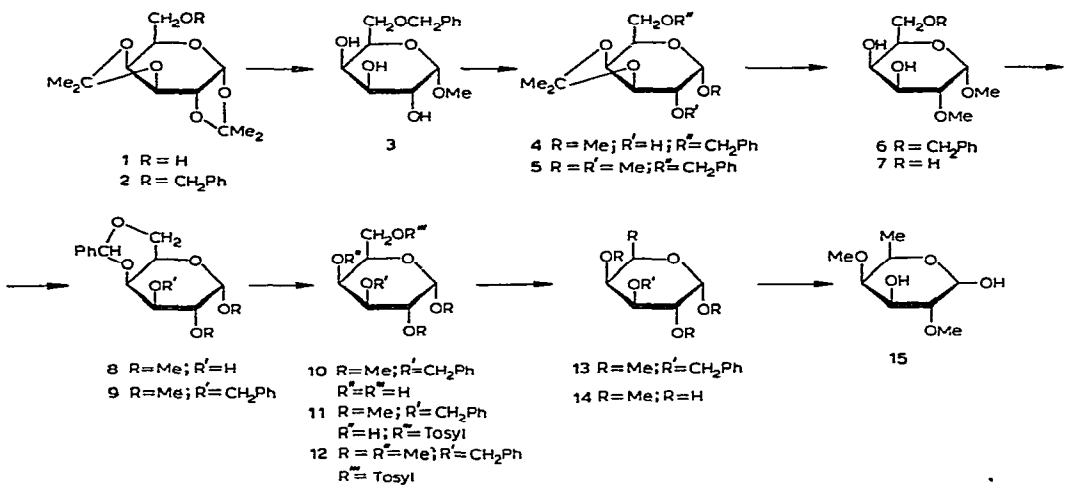
3-*O*-benzyl-2-*O*-methyl-6-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (**11**). Methylation of **11** yielded methyl 3-*O*-benzyl-2,4-di-*O*-methyl-6-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (**12**), which when treated with lithium aluminium hydride gave methyl



3-*O*-benzyl-6-deoxy-2,4-di-*O*-methyl-α-D-galactopyranoside (**13**). Debenzylation of **13** followed by hydrolysis gave pure 6-deoxy-2,4-di-*O*-methyl-D-galactose (2,4-di-*O*-methyl-D-fucose).

Chromatographic examination of the synthetic 2,4-di-*O*-methyl-D-fucose showed a single spot by paper chromatography and two peaks by g.l.c. (attributed to the α and β anomers). The crystalline sugar had a m.p. similar to that of the L enantiomorph, but the specific rotation, opposite in sign as would be expected, was significantly greater than that reported for the L form. It gave a crystalline anilide.

2,4-Di-*O*-methyl-D-fucose is readily distinguished from the 2,3- and 3,4-di-*O*-methyl derivatives of D- or L-fucose by comparison of their melting points, specific optical rotations, and reaction with periodate[1–3].

EXPERIMENTAL

General. — Evaporations were carried out under reduced pressure. Melting points were determined on a Fisher-Johns hot stage and are corrected. Optical rotations, determined on a Perkin-Elmer 141 polarimeter, are equilibrium values unless otherwise stated. Methoxyl determinations were made according to Steyermark[7]. Paper chromatography was performed on Whatman No. 1 paper in butanone saturated with water (solvent *A*) and in 4:1:5 butyl alcohol–ethanol–water (solvent *B*). R_F and R_G values are with reference to solvent front and 2,3,4,6-tetra-*O*-methyl-D-glucose, respectively. G.l.c. was performed on glass columns (120 × 0.5 cm) packed with 10% (w/w) butane-1,4-diol succinate polyester on Chromosorb W (100–120 mesh)

(column A) at 150°, and with 10% polyphenyl ether on Chromosorb W (100–120 mesh) (column B) at 193°. The gas (argon) pressure was 30 lb. in^{-2}. T_G values are retention times with reference to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside. N.m.r. spectra were recorded in chloroform-*d* (tetramethylsilane as internal standard) with a Varian A-60A n.m.r. spectrometer. I.r. spectra were recorded with a Perkin-Elmer Infracord spectrometer. Hydrolysis was carried out with 0.5M sulfuric acid for 4 h on a boiling water bath; the hydrolyzate was neutralized by passing through a column of Duolite A-4 (OH^-), and the eluate was concentrated.

Methyl 6-O-benzyl-α-D-galactopyranoside[6] (3). — D-Galactose (C.P. grade, Pfanstiehl Laboratories) (100 g) was converted into the 1,2:3,4-di-*O*-isopropylidene derivative **1** by treatment with acetone, anhydrous zinc chloride, and sulfuric acid[8]. Benzylation of **1** in *N*,*N*-dimethylformamide with benzyl bromide and silver oxide afforded 6-*O*-benzyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**). Compound **2** was heated at reflux with 3.0% methanolic hydrogen chloride for 3 h. The solution was cooled and neutralized with silver oxide, and the filtrate was evaporated to give crystalline **3**. Recrystallization from ethanol afforded pure **3** (41.0 g, 26%), m.p. 143–145°; $[\alpha]_D^{25}$ +113.2° (*c* 1, water). Lit.[6]: m.p. 144–145°; $[\alpha]_D$ +113° (water).

Methyl 6-O-benzyl-3,4-O-isopropylidene-α-D-galactopyranoside (4). — Compound **3** (40.0 g) was stirred overnight with acetone (2 l) and *p*-toluenesulfonic acid (1.0 g). The solution was neutralized with M sodium hydroxide and concentrated. The oily residue was extracted with ether, and the ether extract, after washing (water) and drying (sodium sulfate), was evaporated to a thick syrup (35 g, 76%); $[\alpha]_D^{25}$ +101.0° (*c* 1, methanol); n.m.r. data: τ 5.2 (doublet, *J* 3.7 Hz, anomeric proton), 5.41 (singlet, benzylic methylene group), 6.59 (singlet, glycosidic Me group), and 8.58 (doublet, *J* 11 Hz, $C{=}Me_2$ group).

Methyl 6-O-benzyl-3,4-O-isopropylidene-2-O-methyl-α-D-galactopyranoside (5). — Compound **4** (34.0 g) was twice methylated with Purdie's reagents (silver oxide and methyl iodide) to yield **5** (33.0 g, 93%) as a thick syrup. A small portion of the syrup was distilled (b.p. 150°/0.2 mm), to give a product having $[\alpha]_D^{25}$ +84.0° (*c* 2.0, chloroform); n.m.r. data: additional singlet at τ 6.48 (Me at O-2). No hydroxyl-group absorption was detected in the i.r. spectrum.

Anal. Calc. for $C_{18}H_{26}O_6$: C, 63.75; H, 7.71; OMe, 18.3. Found: C, 63.88; H, 7.74; OMe, 18.0.

Methyl 6-O-benzyl-2-O-methyl-α-D-galactopyranoside (6). — A solution of compound **5** (30.0 g) in methanol (200 ml) and 0.5M hydrochloric acid (70 ml) was heated for 1 h at reflux and neutralized with 2M sodium hydroxide solution, and the solvents were evaporated. The residue was extracted with ether, and the ether extract was dried (sodium sulfate) and evaporated to give a syrup (24 g, 90%); $[\alpha]_D^{25}$ +105.4° (*c* 0.8, chloroform); the n.m.r. spectrum did not show any doublet at τ 8.58, thus establishing the removal of the isopropylidene group.

Anal. Calc. for $C_{15}H_{22}O_6$: OMe, 20.8. Found: 18.2.

Methyl 2-O-methyl-α-D-galactopyranoside (7). — Compound **6** (23 g) was debenzylated[9] with sodium metal (30 g) and absolute ethanol (100 ml) for 4 h at 70°.

The suspension was then diluted with water and passed through Rexyn 101 (H^+). The effluent was evaporated to give **7** as a thick syrup (12.2 g, 64%); $[\alpha]_D^{25}$ +180° (*c* 1, methanol).

Anal. Calc. for $C_8H_{16}O_6$: OMe, 29.81. Found: 29.60.

Methyl 4,6-O-benzylidene-2-O-methyl-α-D-galactoside (**8**). — Compound **7** (12 g) was shaken overnight with freshly distilled benzaldehyde (30 ml) and anhydrous zinc chloride (10 g). A saturated solution of sodium carbonate was added to the mixture, and the precipitate that formed was filtered off and washed repeatedly with methanol. The filtrate and washings were concentrated to a dry residue which was extracted twice with petroleum ether (60–80°), and the solvent extracts were discarded. The residue was extracted with chloroform, and the chloroform extract, after drying (sodium sulfate), was evaporated to a solid mass which crystallized from ethanol. Recrystallization gave pure **8** (8.5 g, 50%); m.p. 152°; $[\alpha]_D^{25}$ +165.2° (*c* 1.6, methanol). The n.m.r. spectrum showed the benzylidene tertiary proton as a singlet at τ 4.48.

Anal. Calc. for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 61.04; H, 6.96.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-methyl-α-D-galactopyranoside (**9**). — Compound **8** (8 g) was benzylated as described earlier to give **9** which was crystallized from ethanol. Recrystallization gave pure **9** (8.1 g, 77%), m.p. 131.5°; $[\alpha]_D^{25}$ +164.1° (*c* 2.5, chloroform). No hydroxyl-group absorption was detected in the i.r. spectrum. The n.m.r. spectrum showed the benzylic methylene group as a singlet at τ 5.28.

Anal. Calc. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.10; H, 6.69.

Methyl 3-O-benzyl-2-O-methyl-α-D-galactopyranoside (**10**). — A solution of **9** (8 g) in methanol (100 ml) and M hydrochloric acid (8.5 ml) was heated for 5 h at reflux. The solution was neutralized with sodium hydrogen carbonate and was concentrated to remove methanol. Water (50 ml) was added, and the mixture was concentrated again; this procedure was repeated three times. The aqueous suspension was extracted with chloroform, and the extract was washed with water, dried (sodium sulfate) and evaporated to give a syrup (6.2 g, 99%), $[\alpha]_D^{25}$ +127.8° (*c* 2, methanol). The n.m.r. spectrum showed the absence of the one-proton signal at τ 4.48, thus indicating the removal of the benzylidene group.

Anal. Calc. for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 59.74; H, 7.26.

Methyl 3-O-benzyl-2-O-methyl-6-O-p-tolylsulfonyl-α-D-galactopyranoside (**11**). — To a solution of compound **10** (6 g) in dry pyridine (10 ml), cooled to 0°, was added a cold solution of *p*-toluenesulfonyl chloride (2.8 g) in dry pyridine (5 ml). The mixture was kept for 3 days at room temperature, and on pouring into ice–water compound **11** crystallized out. Recrystallization from ethanol gave pure **11** (7.5 g, 86%), m.p. 108–109°; $[\alpha]_D^{25}$ +103° (*c* 1, methanol). The n.m.r. spectrum showed a singlet at τ 7.58 attributed to the toluenic methyl group.

Anal. Calc. for $C_{22}H_{28}O_8S$: C, 58.40; H, 6.24; S, 7.07. Found: C, 58.04; H, 6.41; S, 6.97.

Methyl 3-O-benzyl-2,4-di-O-methyl-6-O-p-tolylsulfonyl-α-D-galactopyranoside (**12**). — Compound **11** (7 g) was methylated as described before to give **12** as a syrup (7.1 g, 98%), $[\alpha]_D^{25}$ +95° (*c* 1, methanol). No hydroxyl-group absorption was detected

in the i.r. spectrum. The n.m.r. spectrum showed an additional methyl group as a singlet at τ 6.41.

Anal. Calc. for $C_{23}H_{30}O_8S$: OMe, 19.95. Found: 19.3.

Methyl 3-O-benzyl-6-deoxy-2,4-di-O-methyl-α-D-galactopyranoside (**13**). — Compound **12** (7 g) was dissolved in 2:1 dry ether–benzene (300 ml), and the solution was heated with lithium aluminium hydride for 15 h at reflux. The excess of lithium aluminium hydride was decomposed with ethyl acetate and ice. The salts were filtered off and washed with ether. The filtrate, after being washed with water, was dried (sodium sulfate) and was evaporated to a syrupy residue (3.6 g, 71%); $[\alpha]_D^{25}$ +137.1° (*c* 1, methanol). The n.m.r. spectrum showed the disappearance of the singlet at τ 7.58 (toluenic methyl group) and the appearance of a doublet at τ 8.77 (*J* 6.1 Hz) due to the secondary methyl group.

Anal. Calc. for $C_{16}H_{24}O_5$: OMe, 31.4. Found: 30.8.

Methyl 6-deoxy-2,4-di-O-methyl-α-D-galactopyranoside (**14**). — Compound **13** (3.4 g) was debenzylated with sodium metal (4 g) and absolute ethanol (10 ml) as described earlier. The solution was diluted with water, and passed through Rexyn 101 (H^+). The eluate was then evaporated to dryness, and the residue extracted 3 times with ether. The ether extract was dried with sodium sulfate, and was evaporated to a thin syrup which crystallized. Recrystallization from ether–petroleum ether (30–60°) gave **14** (1.4 g, 58%); m.p. 86°; $[\alpha]_D^{25}$ +164.6° (*c* 1, methanol); lit.[4]: m.p. 86°; $[\alpha]_D^{30}$ +176° (chloroform).

Anal. Calc. for $C_9H_{18}O_5$: C, 52.41, H, 8.80; OMe, 45.15. Found: C, 52.3; H, 8.67; OMe, 44.9.

6-Deoxy-2,4-di-O-methyl-D-galactose (*2,4-di-O-methyl-D-fucose*) (**15**). — Compound **14** on hydrolysis gave **15** (1.2 g, 100%), which showed, on paper chromatograms, only one spot having R_F and R_G values respectively of 0.41 and 0.52 (solvent *A*) and 0.68 and 0.83 (solvent *B*). Heating **15** (10 mg) with 3% methanolic hydrogen chloride for 6 h at reflux yielded the glycosides which on g.l.c. analysis showed two peaks having R_T values of 0.96 (medium) and 1.16 (strong; column A) and 0.66 (medium) and 0.75 (strong; column B). The syrup crystallized after some time, and on recrystallization from ether gave pure 2,4-di-*O*-methyl-D-fucose, m.p. 131.5–132°; $[\alpha]_D^{25}$ +146→+100° (*c* 1, water); reported values[4] for 2,4-di-*O*-methyl-D-fucose, m.p. 129°; $[\alpha]_D^{27}$ +82° (water); and for 2,4-di-*O*-methyl-L-fucose[5], m.p. 131–132°; $[\alpha]_D^{18}$ −85° (water); R_F 0.73; R_G 0.75 (solvent *B*).

Anal. Calc. for $C_8H_{16}O_5$: OMe, 32.29. Found: 32.14.

Compound **15** (50 mg) was heated for 6 h at reflux with aniline (31 mg) in abs. ethanol (10 ml). The solvent was removed by evaporation, and the aniline derivative was crystallized from ethyl acetate–petroleum ether (30–60°). Recrystallization gave 2,4-di-*O*-methyl-*N*-phenyl-D-fucosylamine, m.p. 154°, $[\alpha]_D^{25}$ −86→+61° (3 days, *c* 1, ethanol).

Anal. Calc. for $C_{14}H_{22}NO_4$: N, 5.22. Found: N, 4.92.

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LOCATION OF ACYL GROUPS ON TWO PARTLY ACYLATED GLYCOLIPIDS FROM STRAINS OF *Ustilago* (SMUT FUNGI)*

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ABSTRACT

Partly acylated ustilagic acids **8** [from *Ustilago maydis* (DC) Corda (= *U. zeae* Unger) PRL-119], consisting of partially esterified β-cellobiosyl residues glucosidically linked to long-chain, hydroxylated fatty acids, and a mixture of partially acylated derivatives of 4-*O*-β-D-mannopyranosyl-D-erythritol from *Ustilago sp.* [probably *U. nuda* (Jens.) Rostr. = *U. tritici* (Pers.) Rostr.] PRL-627 were acetalated with methyl vinyl ether, deacylated, and methylated. Vigorous acid hydrolysis of a methylated **8** gave 2- and 6-*O*-methyl-D-glucose (11:9 molar ratio) corresponding to 2′- and 6-*O*-acyl substitution of the cellobiose residue. Graded acid hydrolysis of methylated **8** gave an *O*-methyl derivative (**9**) which yielded, on Smith degradation, glycerol and 4-*O*-methyl-D-erythritol. Comparison of the p.m.r. spectra of **8** and the product of its oxidation by lead tetraacetate suggested C-6 and C-2′ as the locations of the acetate and hydroxy acid ester-groups, respectively. Vigorous acid hydrolysis of the methylated PRL-627 glycolipid gave D-mannose, its 2- and 4-methyl, and 2,6- and 4,6-dimethyl ethers, and erythritol and its D-1- and 2-methyl-, and 1,3-dimethyl ethers. The positions of the methyl groups corresponded to those of the ester groups in the glycolipid.

INTRODUCTION AND DISCUSSION

Water-insoluble glycolipids have been isolated from *Ustilago maydis* (corn smut), PRL-119, and *Ustilago nuda* (loose smut of wheat) PRL-627. PRL-119 produces compounds termed ustilagic acids (**8**), which consist of partly acylated cellobiosyl residues glycosidically linked in the β-form to the hydroxyl groups of long-chain fatty acids[1–7] **8**. The metabolite from PRL-627 consists of partly acylated 4-*O*-β-D-mannopyranosyl-D-erythritols[8–10]. In order to further elucidate the structures of these compounds, the locations of their acyl groups were determined.

Several methods are now available for location of acyl groups in carbohydrates. P.m.r. spectroscopy may be used with those glycolipids that give well-resolved spectra[11,12]. A more general method is one in which acyl groups are replaced by

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O-methyl groups in accordance with the following reaction sequence: (*1*) protection of free hydroxyl groups, (*2*) deacylation, (*3*) *O*-methylation, (*4*) removal of protecting groups. The relative merits of several protecting groups in determining *O*-acetyl substitution in polysaccharides have been evaluated by de Belder and Norrman[13]. The phenylcarbonyl protecting group (step *1*) formed by reaction of polyols with phenylisocyanate[14–16] was considered to have disadvantages compared with the acetals obtained by using 2,3-dihydro-4*H*-pyran, methyl vinyl ether or ethyl vinyl ether in the presence of *p*-toluenesulfonic acid[17]. De Belder and Norrman showed that complete substitution of an *O*-acetyldextran could be achieved by using the methyl vinyl ether reagent, provided that the product was transferred directly to a Sephadex column and then chromatographically fractionated. Another comparable method for detection of acyl groups, involving nitration at step *1*, has been described by Fink and Hay[18].

Application of the methyl vinyl ether method to model compounds. — In the present investigation the applicability of the methyl vinyl ether method to glycolipids was tested by using a number of model compounds (see Fig. 1). Methyl 2-*O*-acetyl-

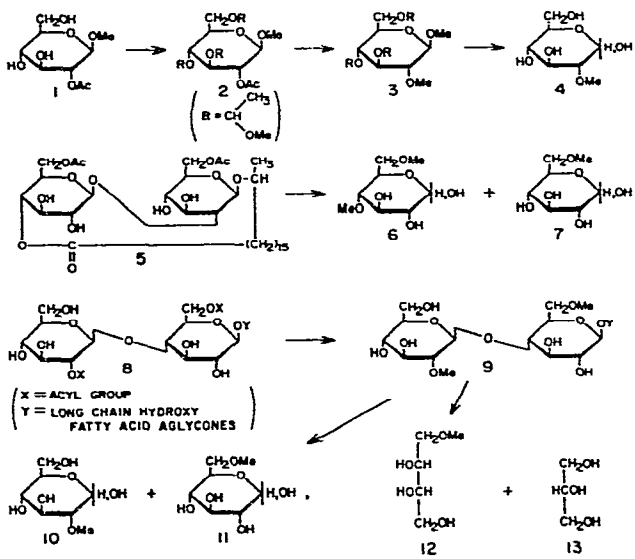

Fig. 1. Model compounds **1–13** for testing the applicability of the methyl vinyl ether method to glycolipids.

β-D-glucopyranoside (**1**) was substituted with *O*-(1′-methoxyethyl) groups by treatment with methyl vinyl ether in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid. The product **2** did not absorb in the hydroxyl region (3600 cm^{-1}) of the infrared. Subsequent consecutive deacetylation and methylation with Purdie's reagent to give **3**, followed by acid hydrolysis, gave 2-*O*-methyl-D-glucose (**4**). In a similar manner, methyl 6-*O*-acetyl-β-D-glucopyranoside was converted into 6-*O*-methyl-D-glucose. Also, a sophoroside lactone **5** was converted into a mixture of 6-*O*-methyl-D-glucose (**7**) and 4,6-di-*O*-methyl-D-glucose (**6**), in accord with the

structure based on p.m.r. spectroscopy and glycol-cleavage reactions[11]. Methyl 2,3-di-*O*-acetyl-β-D-glucopyranoside, on treatment with methyl vinyl ether–acid, gave a mixture of 4,6-di-*O*-(1′-methoxyethyl)- and 4,6-*O*-ethylidene derivatives, which both gave rise to 2,3-di-*O*-methyl-D-glucose after following the usual reaction sequence. In all of these cases crystalline products were obtained and byproducts were not detected.

Location of acyl groups in acylated ustilagic acids. — *Ustilago maydis* PRL-119, when grown in an aqueous medium, produces a mixture of insoluble solid glycolipids called ustilagic acids[1–3], which have antibiotic activity[1]. The glycolipids are partly acylated. Deacylation provides two compounds: one has a cellobiosyl residue[7] β-linked to a hydroxyl group of 15,16-dihydroxyhexadecanoic acid, and the other has a similar carbohydrate portion with 2,15,16-trihydroxyhexadecanoic acid as the aglycon[6]. The mixed, partly acylated glycolipids have a neutralization equivalent of 801, a saponification equivalent of 396, and 1.96 D-glucose units. Each molecule contains, on the average, 2.0 acyl substituents, which are acetate (1.0 mole/mole), L-3-hydroxyhexanoate (0.7 mole/mole), L-3-hydroxyoctanoate (0.3 mole/mole), and hexanoate (0.05 mole/mole)[4,5].

The crude *Ustilago maydis* PRL-119 glycolipids were acetalated with methyl vinyl ether in the presence of *p*-toluenesulfonic acid. The product, which did not show hydroxyl absorption in the infrared, was successively deacylated and methylated. Hydrolysis of the methylated product with aqueous acid gave, on a paper chromatogram, a spot in the region for a mono-*O*-methyl-D-glucose. Since the acylated glycolipids contain 2.0 moles of acyl groups per mole, the D-glucose residues rather than hydroxy fatty acid units are partially acylated.

The mono-*O*-methyl-D-glucoses were isolated by preparative paper chromatography and shown to be free of 3-*O*-methyl-D-glucose, since the derived methyl D-glucosides were completely oxidized with sodium periodate. Gas–liquid chromatography (g.l.c.) of the trimethylsilyl (t.m.s.) ethers of the mono-*O*-methyl-D-glucoses gave two peaks having retention times of 49 and 65 min (11:29 ratio) eliminating 4-*O*-methyl-D-glucose (retention time of t.m.s. ether; 40 min) as a possible component. Since the t.m.s. ethers of 2-*O*-methyl-D-glucose give two peaks having retention times of 49 and 67 min (1:1 ratio) and the corresponding 6-derivative gives a single peak having a retention time of 65 min, the unknown must contain 2-*O*- and 6-*O*-methyl-D-glucose (**10** and **11**). The peak areas of the t.m.s. derivatives therefore indicate a respective ratio of 11:9. This in turn shows that the 2′- and 6-positions of the two D-glucopyranosyl units of the acylated ustilagic acids are each substituted with acyl groups to an almost equal extent.

These results are ambiguous, however, since it is not known which of the D-glucopyranosyl units is acylated in the 2- or 6-positions. In order to determine the exact location of substitution, the acylated ustilagic acids **8** were successively treated with methyl vinyl ether–acid, deacylated, methylated, and partially hydrolyzed to remove the (1′-methoxyethyl) acetal groups. Periodate oxidation of the product **9**, followed by sodium borohydride reduction and acid hydrolysis, gave glycerol (**13**)

and 4-*O*-methyl-D-erythritol (**12**) as the only significant products. These were detected by g.l.c. of their acetates and by paper chromatography. They arise, respectively, from a 2′-*O*-acylated non-reducing D-glucopyranosyl end-unit and an adjacent D-glucopyranosyl residue acylated in the 6-position. These results indicate that the PRL-119 glycolipid consists largely of structure **8**.

The locations of the acetyl, hydroxyhexanoyl and hydroxyoctanoyl groups on the acylated ustilagic acids were determined following oxidation by lead tetraacetate. By using pyridine[19] (dried over phosphorus pentaoxide) as solvent almost 2 moles/mole of oxidant were consumed at a measurable rate until the 1,2-glycols were almost cleaved (Table I). Under these conditions the related methyl 2-*O*-acetyl-

TABLE I

UPTAKES OF LEAD TETRAACETATE IN PYRIDINE AT 0° (MOLE/MOLE)

Substrate	*Time*					
	0.2 *h*	0.5 *h*	1 *h*	2 *h*	3 *h*	5 *h*
Acylated ustilagic acids	0.22	0.59	0.91	1.42	1.50	1.72
Methyl 2-*O*-acetyl-β-D-glucopyranoside	0.13	0.31	0.51	0.60	0.71	0.77

β-D-glucopyranoside consumed almost 1 mole/mole of oxidant, no deacetylation taking place (Table I). The oxidation product obtained from the acylated ustilagic acids was isolated and its p.m.r. spectrum was compared with that of the starting material. The spectrum of the latter in pyridine (Fig. 2) showed four signals connected

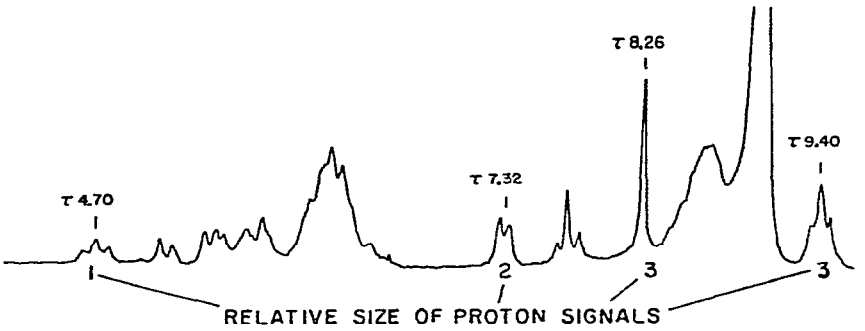

Fig. 2 P.m.r. spectrum of the starting material.

with acyl substitution. These are: (*1*) the triplet at τ 4.70; J = 8 Hz, from H-2′ on the sugar end-unit; (*2*) the doublet at τ 7.32, J = 7 Hz from the two α-protons of the hydroxyhexanoyl and hydroxyoctanoyl esters; (*3*) a 3-proton signal at τ 8.26, from the acetate group; and (*4*) a 3-proton triplet at τ 9.40; J = 7 Hz, from the C-CH_3 group of the hydroxy esters. In the less well-defined spectrum of the oxidation product it was noticeable that the acetate signals remained at τ 8.26 and the α-protons of the hydroxyhexanoate and hydroxyoctanoate esters could not be detected at

τ 7.32. These observations are consistent with the majority of acetate groups being located on C-6 of the middle D-glucose unit of the acylated ustilagic acids and hydroxyesters situated at C-2′ of the D-glucose end-unit. The hydroxyester methylene signal was affected, in the oxidation product, either by the presence of a newly created aldehyde group or, more likely, the hydroxy ester was hydrolyzed during the isolation procedure. Esters α-related to an aldehyde group are hydrolyzed readily in the presence of water[20].

Location of acyl groups in acylated 4-O-β-D-mannopyranosyl-D-erythritol. — *Ustilago nuda* PRL-627, in aqueous medium, forms a mixture of a soluble metabolite[8,9] and an "oil", heavier than water[8]. The soluble material is 4-*O*-β-D-mannopyranosyl-D-erythritol[9,10] and the "oil" consists of the disaccharide partially esterified with acetate and C_6 to C_{18} (and possibly C_{22}) fatty acids. Myristate, palmitate, and stearate esters predominate[9]. In the present study, the oil from *Ustilago nuda* PRL-627 was fractionated by column chromatography on silicic acid. Elution with chloroform gave 26% of free fatty acids, and washing with methanol gave 74% of glycolipids. The acids had 12 to 18 carbon atoms, with palmitic and oleic acids as the main components (Table II), as shown by g.l.c. of their methyl esters. Methanolysis of the glycolipids gave fatty acid methyl esters of the same chain-length, but in somewhat different proportions (Table II).

TABLE II

FATTY ACID COMPONENTS IN FREE ACIDS AND GLYCOLIPID FROM *Ustilago nuda* PRL-627

Fatty acids from U. nuda	% *Composition, as methyl esters*	
	From free acid mixture	*From acyl residues bound to glycolipid*
Lauric acid	3.9	6.2
Myristic acid	7.8	13.5
Myristoleic acid	2.7	4.8
Palmitic acid	52.4	41.0
Palmitoleic acid	6.5	11.2
Stearic acid	4.4	2.1
Oleic acid	22.1	21.1
C_{20} acids	trace	trace
C_{22} acids	trace	—

The mixture of fatty acid and glycolipid was acetalated with methyl vinyl ether–*p*-toluenesulfonic acid, and the product was successively deacylated, methylated, and hydrolyzed with hot aqueous acid. Paper chromatography of the resulting mixture showed that it was complex, consisting of erythritol, mono-*O*-methyl-D-erythritol(s), di-*O*-methyl-D-erythritol(s), mannose, mono-*O*-methyl-D-mannose(s), and di-*O*-methyl-D-mannose(s).

The erythritol derivatives were characterized as erythritol, 1-*O*-, and 2-*O*-methyl-D-erythritol and 1,3-di-*O*-methyl-D-erythritol, by g.l.c. of acetylated mixture.

The acetate of the latter was chromatographically distinguishable from the acetates of 1,2- and 2,3-di-*O*-methylerythritol. The peak area ratios of the four components are presented in Table III.

TABLE III

G.L.C. IDENTIFICATION AND RELATIVE PEAK AREAS OF DERIVATIVES OF *O*-METHYL FRAGMENTS OBTAINED FROM PRL-627 GLYCOLIPID

Peracetate of[a]	*Relative peak area*	*Retention time (min)*
Erythritol	2.0	12
1-*O*-Methyl-D-erythritol	2.8	6.6
2-*O*-Methyl-D-erythritol	2.1	7.5
1,3-Di-*O*-Methyl-D-erythritol	2.1	4
T.m.s. derivative(s) of methyl glycosides of[b]		
D-Mannose	2.2	47
2-*O*-Methyl-D-mannose	1.8	34
4-*O*-Methyl-D-mannose	1.8	26
2,6-Di-*O*-Methyl-D-mannose	3.0	38
4,6-Di-*O*-Methyl-D-mannose	3.5	22
Unknown	1.8	29

[a]The peracetates of 1,3-, 2,3-, and 1,2-di-*O*-methyl-erythritol have retention times of 4.0, 4.4 and, 3.6 min, respectively on the 8 ft × 1/4 in. nitrile silicone gum XE-60 column maintained at 170°. [b]The t.m.s. derivatives of the methyl 6-*O*-methyl-α,β-D-mannopyranosides have a retention time of 40 min on the 12 ft × 1/4 in. neopentyl glycol succinate column maintained at 140°.

The di-*O*-methyl-D-mannose fraction, contaminated with mono-*O*-methyl-D-erythritol, was isolated from paper chromatograms and was shown to consist of a mixture of 2,6- and 4,6-di-*O*-methyl-D-mannose by g.l.c. of the methyl glycosides and the t.m.s. ethers of the methyl glycosides[21]. The mono-*O*-methyl-D-mannose fraction, isolated from paper, was contaminated with erythritol. It was shown to be free of 3-*O*-methyl-D-mannose since the methyl glycosides obtained by refluxing with methanolic hydrogen chloride were completely oxidized with sodium periodate. G.l.c. of the t.m.s. ethers of the mono-*O*-methyl-D-mannose mixture showed that 2-*O*- and 4-*O*-methyl-D-mannose were present and 6-*O*-methyl-D-mannose was absent (for retention times see Table III). P.m.r. spectroscopy of the free sugars gave two signals of almost equal intensity in the H-1 region arising from the two possible isomers, 2-*O*- and 4-*O*-methyl-D-mannose. A peak-area ratio of mannose, mono-*O*-, and di-*O*-methyl-D-mannoses was obtained on g.l.c. of the t.m.s. derivatives of the original mixture of mannose and erythritol derivatives. The ratio of peak areas given in Table III show the compounds present in significant amounts. It can be seen that PRL-627 metabolite is a complex mixture, consisting of 4-*O*-β-D-mannopyranosyl-D-erythritol with acyl substitution on several of the hydroxyl groups. The chemical nature of the substituent(s) at each hydroxyl group was not determined.

EXPERIMENTAL

Evaporations were carried out under diminished pressure. I.r. spectra were obtained from films by using a Perkin–Elmer Model 21 spectrophotometer. G.l.c. analyses of sugar derivatives were performed with an apparatus of conventional design with thermal-conductivity detectors and helium (40 lb.in^{-2} injection pressure) as the mobile phase. A copper column (12 ft × 1/4 in. outside diameter), packed with 2% neopentyl glycol succinate on Chromosorb W, was used at 140° for t.m.s. derivatives. Methyl di-*O*-methyl-D-hexopyranosides were fractionated at 190°. An 8-ft column of 2% nitrile silicone gum XE-60 at 170° was used to fractionate acetates of erythritol derivatives. For analyses of fatty acids g.l.c. was carried out on a column (8 ft × 1/8 in.) of 5% 1,3-propanediol succinate on Chromosorb W (acid-washed; 60–80 mesh) at 185°, with a flame-ionization detector. Flow rates of helium and hydrogen were 40 ml/min and 30 ml/min respectively. P.m.r. spectra were obtained with a Varian HA-100 spectrometer. Tetramethylsilane was used as standard and chemical shifts are expressed as τ values. T.l.c. was carried out on Silica Gel G, and 50% sulfuric acid was used as a charring reagent. Specific rotations were determined at 25°. Gaseous methyl vinyl ether, obtained from K and K Laboratoires, Inc., Plainview, N. Y., was condensed by passage through an acetone–Dry Ice bath.

All starting materials were dried, and reactions were performed under anhydrous conditions. Paper chromatography was carried out by using 40:11:19 (v/v/v) butyl alcohol–ethanol–water as solvent and ammoniacal silver nitrate and *p*-anisidine hydrochloride as spray reagents were used when appropriate.

General procedure for the replacement of O-*acyl groups with* O-*methyl groups.* — *A. Reactions with methyl vinyl ether.* The partially acylated starting material (0.1–1.0 g) was dissolved in 10–20 ml of a suitable solvent (benzene, *N,N*-dimethylformamide). *p*-Toluenesulfonic acid (10–50 mg), initially dissolved in a small amount of solvent containing Drierite was added to the cooled (10–15°) solution. The mixture was stirred for 2 min and excess methyl vinyl ether (2–5 ml) was added, and stirring was continued for 30–90 min at 10–15° until a yellow color developed. Anhydrous potassium carbonate was then added, the suspension stirred for 1 h and the unreacted methyl vinyl ether was allowed to evaporate. The reaction mixture was diluted with more solvent, filtered, and evaporated to dryness. Complete substitution of hydroxyl groups with *O*-(1′-methoxyethyl) groups was shown by the absence of i.r. absorption in the 3600 cm^{-1} region. In several cases, one or two further treatments with methyl vinyl ether were necessary in order to achieve complete substitution.

B. O-*Deacylation of* O-(*1′-methoxyethyl*) *derivatives.* Deacylation could be effected by treatment with 0.1 equivalent of 0.1M sodium methoxide in methanol or with lithium aluminum hydride in ether–dichloromethane (in the absence of a potential reducing sugar). In most cases, complete deesterification could be shown by the absence of i.r. absorption in the 1720 cm^{-1} region.

C. Methylation. Deesterified material was methylated according to Purdie's method[22] or by Kuhn's procedure[23].

D. Hydrolysis. *O*-(1′-methoxyethyl) groups were removed by treating the methylated product with refluxing 3% methanolic hydrogen chloride for 15 min, by heating with 50% aqueous acetic acid for 10 min at 100°, or with M sulfuric acid for 18 h at 100°.

Conversion of several known O-acetyl derivatives to their corresponding methyl ethers. — A. Methyl 2-O-acetyl-β-D-glucopyranoside into 2-O-methyl-D-glucose. Methyl 2-*O*-acetyl-β-D-glucopyranoside[24] (100 mg) was converted into 2-*O*-methyl-D-glucose (70 mg) by the above reaction sequence with *N,N*-dimethylformamide as acetalation solvent and hydrolysis with M sulfuric acid. The product was homogeneous by paper chromatography. It crystallized from methanol, having m.p. 153°, and was identical with an authentic sample of 2-*O*-methyl-β-D-glucose[25] (by g.l.c. of t.m.s. derivatives and mixed m.p. 153–155°).

B. Methyl 6-O-acetyl-β-D-glucopyranoside into 6-O-methyl-D-glucose. Methyl 6-*O*-acetyl-β-D-glucopyranoside[26] (100 mg) was similarly converted into 6-*O*-methyl-α-D-glucose (75 mg) identical in all respects (g.l.c. and mixed m.p. 140–142°) with an authentic specimen[27]. 6-*O*-Methyl-α-D-glucose (15 mg) also could be obtained from methyl 6-*O*-stearoyl-β-D-glucopyranoside[24] (50 mg) by the application of the same process.

C. Methyl 2,3-di-O-acetyl-β-D-glucopyranoside into 2,3-di-O-methyl-D-glucose. The methylated product obtained from methyl 2,3-di-*O*-acetyl-β-D-glucopyranoside[28] (250 mg) by the described reaction sequences (benzene as acetalation solvent) when de-esterified, gave a mixture (200 mg), a part of which was resolved into two fractions by silicic acid column chromatography [solvent 2:1 (v/v) chloroform–acetone].

Fraction *1* (8 mg) could be identified as methyl 4,6-*O*-ethylidene-2,3-di-*O*-methyl-β-D-glucopyranoside, having m.p. 108–110° and $[\alpha]_D^{25}$ −45° (*c* 0.5, chloroform) and p.m.r. data (CCl_4): τ 5.45 (quartet, J = 5 Hz, ethylidene H), 5.96 (doublet, J = 7 Hz, anomeric H), 6.53, 6.56, 6.70 (singlets, 3 OCH_3), 8.73 (doublet, J = 5 Hz, CH_3 of ethylidene). Lit.[29] m.p. 109–111° and $[\alpha]_D^{25}$ −47.8° (chloroform).

Fraction *2* (50 mg) was hydrolyzed with M sulfuric acid at 100° and was identified as 2,3-di-*O*-methyl-β-D-glucose (identical g.l.c. retention time and mixed m.p. 108–110°[30]).

D. Conversion of sophoroside lactone **5** *into 6-O-methyl- and 4,6-di-O-methyl-D-glucose* (**7** *and* **6** *respectively*). The sophoroside lactone[11] **5** (200 mg) was treated with methyl vinyl ether in *N,N*-dimethylformamide and the resulting product was deesterified with lithium aluminum hydride in 1:1 dichloromethane–diethyl ether for 45 min. The product gave no ester absorption in the i.r. Purdie methylation of the deesterified product and subsequent hydrolysis with M sulfuric acid (10 h, 100°) gave a material which contained mono- and di-*O*-methyl hexoses, as shown by paper chromatography. Preparative paper chromatography of a portion of the above material gave 5 mg of 6-*O*-methyl-D-glucose, which was crystallized from ethanol and had m.p. and mixed m.p. 140–142°[27]. Also 6 mg of 4,6-di-*O*-methyl-α-D-glucose was isolated (crystallized from ethyl acetate), which had m.p. and mixed m.p. 154–156°. Lit.[31]: m.p. 156–158°.

Effect on O-*acetyl substituent during the introduction and removal of* O-(1′-

methoxyethyl)-groups. — Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (100 mg) was treated with methyl vinyl ether for 1 h in benzene according to the general procedure. Crystallization of the colored material from methanol gave 86 mg of the starting material (m.p. and p.m.r. spectrum).

The p.m.r. spectrum of the reaction product of methyl 2-*O*-acetyl-β-D-glucopyranoside with methyl vinyl ether was examined, although it was very complicated. The ratio of the CH_3–C–H to CH_3–C–O– was 3:1. When the above material was heated for 10 min at 100° with 50% aqueous acetic acid and the solvent was subsequently removed under diminished pressure, only methyl 2-*O*-acetyl-β-D-glucopyranoside was formed (identified by p.m.r. spectrum and mixed m.p.).

Investigation of unknown, partially esterified natural products. — *Partially acylated ustilagic acids from PRL-119.* Crude partially acylated ustilagic acids[5] (**8**, 1 g) in *N,N*-dimethylformamide were treated with methyl vinyl ether. After two treatments, a portion of the product (0.8 g) was soluble in chloroform or benzene. The soluble portion did not contain free hydroxyl groups (i.r. spectrum). The insoluble portion (0.3 g) was partially substituted and could be dissolved in chloroform or benzene after further treatment with methyl vinyl ether. The completely substituted material (0.4 g) was treated (6 h) with sodium methoxide in methanol, and then methylated. Hydrolysis of the methylated product with *M* sulfuric acid (16 h, 100°) gave a colored syrup (100 mg) which gave one spot on a paper chromatogram with the *p*-anisidine and silver nitrate sprays. Purification of the above syrup (50 mg) by preparative paper chromatography afforded a colorless material (35 mg) which consumed 1.2 moles/mole of lead tetraacetate, and a part of the starting material remained as unoxidized mono-*O*-methyl-D-glucose as shown by t.l.c. [solvent 1:1 (v/v) chloroform–acetone] and paper chromatography. The purified syrup was refluxed with 3% methanolic hydrogen chloride for 4 h to give methyl D-glucosides, p.m.r. spectrum (in D_2O: doublets (J = 4 Hz) at τ 4.52 and 4.76 for two anomeric protons in a ratio of 1:1). Treatment of the D-glucoside mixture with sodium periodate oxidized all of the starting material (t.l.c.) [solvent 2:1 (v/v) chloroform–acetone] eliminating the possibility of methyl 3-*O*-methyl-D-glucopyranoside. Formation of formic acid indicated the presence of methyl 6-*O*-methyl-α,β-D-glucopyranosides. G.l.c. (t.m.s. derivatives) comparison of the mono-*O*-methyl-D-glucose fraction with standard samples also suggest that is consisted of 2-*O*- and 6-*O*-methyl-D-glucoses (retention times of 49 and 67 min, 1:1 ratio and 65 min, respectively). The t.m.s. ether of 4-*O*-methyl-D-glucose has a retention time of 40 min.

In another experiment, the *O*-methylated, *O*-(1′-methoxyethyl) derivatives obtained from acylated ustilagic acids was heated with 50% aqueous acetic acid (10 min, 100°) to remove *O*-(1′-methoxyethyl) groups. The partially *O*-methylated, hydroxy fatty acid glycoside obtained after removal of acetic acid was oxidized with sodium periodate (excess was destroyed by ethylene glycol), reduced with sodium borohydride and hydrolyzed with M sulfuric acid (4 h, 100°). The hydrolyzate contained glycerol and 4-*O*-methyl-D-erythritol only [paper chromatography, t.l.c. with solvent 1:1 (v/v) chloroform–acetone, and g.l.c. of acetates).

Lead tetraacetate oxidation of acylated ustilagic acids **8** *and methyl 2-O-acetyl-β-D-glucopyranoside* (**1**). — The oxidations were carried out according to the procedure of Goldschmid and Perlin[19], with one modification. The pyridine (250 ml) used was dried by shaking for 30 min with phosphorus pentaoxide (15 g) followed by refluxing for 1 h prior to distillation. Compounds **1** and **8** were oxidized with approx. 2% lead tetraacetate in pyridine (10 ml) at 0°. The uptakes of oxidant over a period of time are recorded in Table I. The foregoing reaction was repeated on **8** (100 mg). After 18 h erythritol (1 g) in pyridine (20 ml) was added and after a further 3 h at 0° the solution was evaporated to a syrup. This was dissolved in a mixture of ethyl acetate and sufficient M sulfuric acid to acidify the solution. The precipitate of $PbSO_4$ was filtered off, the ethyl acetate shaken twice with water, dried ($MgSO_4$), filtered and evaporated to a syrup (82 mg).

Water insoluble glycolipids from Ustilago nuda *PRL-627.* — *Ustilago nuda* PRL-627 glycolipid (1 g) was treated in *N,N*-dimethylformamide with methyl vinyl ether in the usual manner, and the product was deesterified and then methylated. The hydrolyzate (200 mg) obtained by heating with M sulfuric acid (6 h, 100°) was examined by paper chromatography, which revealed the presence of mannose, mono-*O*-methyl-D-mannose(s), di-*O*-methyl-D-mannose(s), erythritol and mono-*O*-methyl-D-erythritol(s). A portion of the above hydrolyzate was resolved by preparative paper chromatography, and the following fractions were isolated. Fraction *1* (5 mg) comprised of D-mannose only. Fraction *2* (10 mg) contained erythritol and mono-*O*-methyl-D-mannose(s), as indicated by paper chromatography. The p.m.r. spectrum of this mixture showed two aldose H-1 doublets (J = 1.5 Hz) with equal intensity at τ 4.42 and τ 4.70 (2-*O*-methyl and 4-*O*-methyl-D-mannose) respectively. There were two other minor signals (J = 2 Hz) in the H-1 region. Treatment of fraction *2* with 3% methanolic hydrogen chloride (4 h, reflux) gave methyl glycosides that were completely oxidized by sodium periodate [t.l.c. with 2:1 (v/v) chloroform–acetone] indicating the absence of 3-*O*-methyl-D-mannose. This fraction, therefore, contained 2-*O*-methyl-D-mannose and 4-*O*-methyl-D-mannose in equal amounts, in addition to erythritol since the possibility of 6-*O*-methyl-D-mannose could be eliminated on the basis of g.l.c. comparison with standard compounds.

The following compounds were used in characterization of Fraction *2*. Samples of 6-*O*-methyl-D-mannose and 4-*O*-methyl-D-mannose could be obtained by treating methyl 6-*O*-methyl-2,3-*O*-methylene-α-D-mannofuranoside[32] and 1,6-anhydro-2,3-*O*-benzylidene-4-*O*-methyl-β-D-mannopyranose[21], respectively, with M sulfuric acid (16 h, 100°). 2-*O*-Methyl-D-mannose was obtained by methylation of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[33]. T.m.s. derivatives of 4-*O*-methyl-, 2-*O*-methyl-, and 6-*O*-methyl-D-mannoses have retention times of 30, 32, and 34 min, respectively. Fraction **3** (10 mg) consisted of mono-*O*-methyl-D-erythritol(s) and di-*O*-methyl-D-mannose(s), as indicated by paper chromatography. G.l.c. examination[21] of this fraction following treatment with methanolic hydrogen chloride showed the presence of the methyl glycosides 2,6- and 4,6-di-*O*-methyl-D-mannose.

In order to characterize and roughly estimate erythritol and its derivatives

the complex mixture of mannose, erythritol and their partly methylated derivatives were converted into their acetates and fractionated by g.l.c. on an 8-ft silicone nitrile gum XE-60 column at 170°. With this column each of the possible mono-*O*- and di-*O*-methyl-erythritol peracetates could be resolved (see Table III). 1,3-Di-*O*- and 1,2-di-*O*-methylerythritol standards were prepared from 1,3-*O*-[34] and 1,2-*O*-(2′-hydroxyethylidene)-erythritols[35], respectively, by treatment with Purdie's reagent followed by acid hydrolysis. 4-*O*-Methyl-D-erythritol and 2-*O*-methyl-D-erythritol were prepared from 6-*O*- and 4-*O*-methyl-D-glucose, respectively, by oxidation with lead tetraacetate[36] followed by reduction with sodium borohydride.

The D-mannose derivatives could also be estimated roughly by conversion of the same mixture to the t.m.s. derivatives which were then subjected to g.l.c. on neopentyl glycol succinate (see Table III).

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THE RELATIONSHIP BETWEEN METHOXYL CONTENT AND GELLING TEMPERATURE OF AGAROSE

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ABSTRACT

The gelling temperature of 1.5% agarose sols was found to increase with increasing methoxyl content of the agarose. Of about 50 preparations tested, only 3 failed to follow this pattern. High methoxyl-type agaroses could be fractionated into portions of higher and lower methoxyl content having correspondingly higher and lower gelling temperatures.

INTRODUCTION

Since Araki's[1] original fractionation of agar into a neutral, gelling fraction, agarose, and an anionic non-gelling fraction, agaropectin, other methods have been developed to effect the fractionation. For small-scale preparation, the quaternary ammonium salt precipitation method of Hjerten[2] proved advantageous by yielding stronger-gelling agarose of better color with greater ease. A modification of this process, wherein carrageenan or a similar polyelectrolyte is added to the agar to coprecipitate with the agaropectin as the quaternary ammonium salt, was patented by Blethen[3] and thus provided a commercially feasible process. At approximately the same time, Russell, Mead, and Polson[4] devised another means of fractionating agar. By adding polyethylene glycol of molecular weight about 6000 to an agar sol, agarose was precipitated. It is claimed in a commercial process patented by Hyland Laboratories[5] that treatment of agar with pectinase or a calcium sequestrant renders the sulfated polysaccharides cold-water soluble so that subsequent washing removes them, leaving behind the neutral, strong-gelling polymer. More recently, the use of diethylaminoethyl cellulose to remove agaropectin was patented by Zabin[6].

In the course of testing agar for agarose preparation, a number of variations were observed in commercial agar samples. In addition to such familiar differences as gel strength and sulfate content, it was noted that agars from certain sources generally led to slight gelation of the 2-propanol when the agarose was precipitated in alcohol as part of the normal process. Since the gelation indicated a moderate degree of solubility in alcohol, it was concluded that this type of agarose must be functionally different from that which did not cause gelation. Araki[7] reported in 1965 that agarose from certain agarophytes (among them, *Gracilaria* spp.) had much higher content of

6-*O*-methyl-D-galactose than the agarose from certain others. Since a methyl ether could confer some measure of alcohol solubility upon a relatively small polysaccharide such as agarose, which already contains one hydrophobic group in the form of 3,6-anhydro-L-galactose, methoxyl analyses were carried out to seek a correlation between methoxyl content and alcohol solubility. Furthermore, it was known that some of the agaroses which caused gelation of the alcohol came from *Gracilaria* agar. Another general characteristic of *Gracilaria* agars is their high gelling temperature[8]. Thus, it appeared that partial alcohol solubility, methoxyl content, and gelling temperature might, in some way, be related.

This paper presents the results of experiments that show a correlation between the methoxyl (6-*O*-methyl-D-galactose) content and gelling temperature of a number of agarose samples.

RESULTS AND DISCUSSION

Table I presents the data on the various preparations investigated in this study. All agaroses were prepared by the method of Blethen[3] in this laboratory, using U.S.

TABLE I

ANALYSES AND PROPERTIES OF AGAROSE PREPARATIONS

Sample No.	*Gel temp.*[a] *(degrees)*	*OMe (%)*	*SO_4 (%)*	*Ash (%)*	*Gel strength (g)*[b]	*Source*
1	35	0.39	0.66	0.75	1193	Unknown
2	35.5	0.37	0.31	0.33	840	Unknown
3	35.5	0.43	0.39	0.46	759	Unknown
4	36.3	0.61	0.44	0.58	750	Unknown
5	36.5	0.72	0.57	0.97	830	Unknown
6	36.5	0.73	0.40	0.92	865	Unknown
7	36.5	0.82	0.69	1.12	733	Unknown
8	38.5	0.64	0.11	0.24	1270	*Gracilaria*[c]
9	39	0.89	0.17	0.49	[d]	*Gracilaria*[c]
10	39.5	1.39	0.48	0.58	535	Unknown
11	40	1.07	0.17	0.43	1133	*Gracilaria*[c]
12	40	1.55	0.45	0.61	718	Unknown
13	40	1.62	0.35	0.84	980	*Gracilaria*[c]
14	40.5	1.61	0.41	0.62	873	Unknown
15	40.5	1.63	0.35	0.31	970	*Gracilaria*[c]
16	40.5	1.65	0.33	0.62	980	*Gracilaria*[c]
17	40.5	2.20	0.68	0.88	673	Unknown
18	41	1.72	0.38	0.61	1000	*Gracilaria*[c]
19	41	1.74	0.33	0.66	736	Kobe
20	41	1.76	0.38	0.62	1150	*Gracilaria*[c]
21	41	2.03	0.36	0.64	1155	*Gracilaria*[c]
22	41.5	1.49	0.77	0.89	1067	Unknown
23	41.5	1.68	0.23	0.53	808	Unknown
24	42	2.00	2.08	1.34	803	Unknown
25	42	2.26	0.32	0.61	1013	Japan
26	42	2.53	0.13	0.34	594	*Gracilaria*[e]
27	42.5	1.87	0.57	0.75	1020	Japan

TABLE I (*continued*)

Sample No.	*Gel temp.*[a] *(degrees)*	*OMe (%)*	*SO_4 (%)*	*Ash (%)*	*Gel strength (g)*[b]	*Source*
28	42.5	2.62	0.33	0.65	883	Unknown
29	43	2.07	0.36	0.56	1068	*Gracilaria*[c]
30	43	2.20	0.21	0.55	963	Unknown
31	43.5	2.23	0.12	0.40	964	Japan
32	43.5	2.32	0.21	0.59	793	Chile
33	43.5	2.48	0.17	0.45	1003	Unknown
34	43.5	2.65	0.30	0.49	803	Unknown
35	44	2.62	0.27	0.56	858	Unknown
36	44	2.80	0.38	0.83	681	Kobe
37	44	2.85	0.15	0.37	920	Japan
38	44	3.29	0.40	0.76	877	*Gracilaria*[c]
39	44	3.47	0.54	0.65	717	*Gracilaria*[c]
40	45	3.00	0.37	0.66	980	Japan
41	45	3.46	0.44	0.59	833	*Gracilaria*[c]
42	45.5	4.58	0.72	0.72	613	*Gracilaria*[c]
43	46	5.04	0.67	0.93	669	*Gracilaria*[c]
44	47.5	7.94	0.48	5.72	[d]	Unknown
45	48	6.59	0.78	0.77	617	*Gracilaria*[c]
46	48	2.09	0.18	0.66	458	Unknown
47	52	2.37	0.60	1.20	639	Argentina *Gracilaria*
48	53	1.91	0.49	0.95	[d]	Argentina *Gracilaria*

[a]At concentration of 1.5%. [b]At concentration of 1%. [c]These agars were manufactured in Chile from native seaweed. The only species commonly known to be used from this region is *G. lemaeniformis*[9] (*sic*; probably should be *lemanaeformis*), although it is highly likely that the seaweed is actually *Gracilariopsis lemanaeformis*[10]. [d]Not determined. [e]Alkali-treated sample No. 41.

Pharmacopoeia grade agar. Where known, seaweed source or country of manufacture of the agar is given. The relationship between methoxyl content and gelling temperature is shown graphically in Fig. 1. Plots of gelling temperature *vs.* percent of sulfate, percent of ash, or gel strength showed completely random distribution for the overall group of samples, although for a group of 11 samples (see below) prepared from one agar, gelling temperature was related linearly to each of these properties (least significantly to ash). This might be anticipated in consideration of the common source of these samples, although the reason for a high sulfate content of the agaroses having a high methoxyl content is not clear from the available data. Clearly, the methoxyl content and gelling temperature follow the same general pattern as in the other samples, and it can be reasoned that the variations in the type of process brought about the wide spread of methoxyl content of the samples due to different solubility relationships. Reduced gel strength of the high methoxyl-high sulfate samples may be due to interference from the sulfate groups. Investigations are under way to determine whether the sulfate group is part of a separate polysaccharide and to what extent those sugars containing the ester sulfate are converted into 3,6-anhydro-

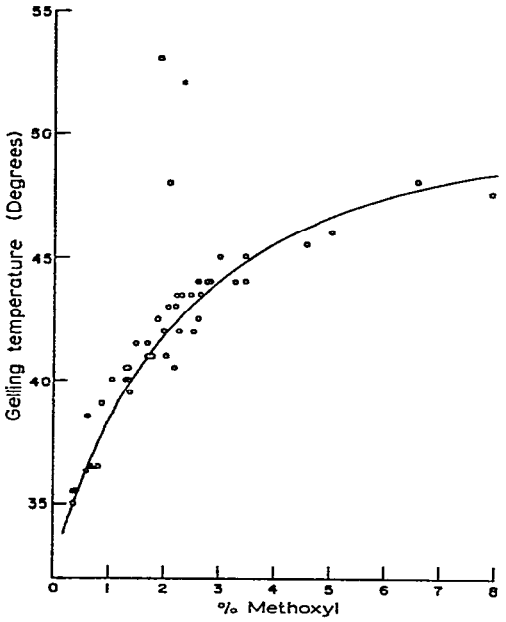

Fig. 1. Relationship between 1.5%-sol gelling temperature and methoxyl content of agarose preparations.

L-galactose by alkaline treatment. The evidence for the presence of 6-*O*-methyl-D-galactose in the polymer came from methoxyl analyses, chromatographic data on selected specimens, and literature references to the presence of 6-*O*-methyl-D-galactose in agaroses, particularly in those obtained from *Gracilaria* spp.[7,11–13]. No attempt was made to isolate and characterize 6-*O*-methyl-D-galactose from hydrolyzates of any of the agarose samples. Hydrolyzates of samples No. 2, 5, 11, and 43 (see Table I) were compared to D-galactose and 6-*O*-methyl-D-galactose by paper chromatography in solvent *A*. The intensity of the 6-*O*-methyl-D-galactose spot was seen to increase from not detectable with samples No. 2 and 5 to nearly as intense as D-galactose with sample 43 (5.04% methoxyl is equivalent to 28.6% 6-*O*-methyl-D-galactose, and therefore represents an approximately equimolar amount relative to D-galactose). An unidentified spot located between D-galactose and 6-*O*-methyl-D-galactose, was clearly given by samples No. 11 and 43, barely by sample No. 2. Another chromatogram in solvent *B* with 4-*O*-methyl-D-galactose included as a standard, indicated that the unidentified spot was not this sugar, although it has been reported as a minor constituent of agar[12].

That high methoxyl agarose is composed of a continuum of low to high methoxyl fractions rather than discrete high and low methoxyl fractions is suggested by the varying results obtained when working with one sample of agar. For example, preparations 8, 9, 11, 13, 15, 26, 38, 41, 42, 43, and 45 were all made from the same agar. Table II shows the variations in process *i.e.*, fractionation, solvent changes, or alkaline treatment, which brought about the diversity in gelling temperatures and

TABLE II

AGAROSE PREPARED FROM ONE AGAR BY VARIATIONS IN PROCEDURE

Sample No.	*Filtrate temp. (degrees)*	*Precipitating solvent*		
		Type	*Relative vol.*	*Temp. (degrees)*
8	73	methanol	1.5	42
9	60	2:1 methanol–2-propanol	1.5	25
11	75	methanol	1.5	40
13	75	propanol-2	1.5	40
15	74	propanol-2	1.5	40
26[a]	61	propanol-2	1.5	38
38	65	acetone	1.5	25
41	70	acetone	2.0	43
42	75[b]	acetone	2.0	42
43	75[b]	acetone	2.0	42
45	74[b]	acetone	2.0	40

[a]Alkali-treated sample obtained from No. 41. [b]After removal of alcohol by evaporation.

methoxyl contents. Thus, samples No. 8 and 42, No. 11 and 43, and No. 15 and 45 represent fractionated pairs, whereas No. 38 and 41 are unfractionated agaroses obtained by acetone precipitation. Samples No. 9 and 13 are agaroses having a relatively low methoxyl content equivalent to that of the first fractions. In these cases, however, the second fractions were not isolated. Finally, sample No. 26 is an alkali-treated, acetone-precipitated agarose which underwent some fractionation upon alcohol precipitation after treatment; again, the second fraction was not isolated. Table III shows a balance of the material with respect to total methoxyl present in 2 of the fractionated pairs and in the unfractionated sample No. 41. Calculations take into account yield and moisture content of the agaroses and are based on 100 g of agar as the starting material. Similar calculations involving yield and gelling temperatures lead to gelling temperatures of 43.3° and 43.6° for mixtures of samples No. 15 and

TABLE III

PROPORTION OF MATERIAL AND OF METHOXYL GROUPS IN FRACTIONATED AND UNFRACTIONATED AGAROSE

Sample no.	*Gel temp. (degrees)*	*Agarose*		*Methoxyl groups (g)*[b]	
		Yield[a] *(per cent)*	*Methoxyl content (per cent)*	*In agarose fraction*	*In starting material*
15	40.5	44.55	1.63	0.726	2.60
45	48	28.44	6.59	1.874	
8	38.5	19.86	0.64	0.127	2.62
42	45.5	54.37	4.58	2.490	
41	45	73.94	3.46	2.558	2.56

[a]Yield of anhydrous agarose based on agar. [b]Based on 100 g of agar.

No. 45 and No. 8 and 42, respectively, when recombined in proportion to their respective percent yield. Gelling temperatures of 44° were actually observed for both combinations, suggesting that gelling temperatures of mixtures can be calculated.

In an effort to further demonstrate that the methoxyl content controls the gelling temperature of agarose, portions of agarose No. 5 were subjected to methylation with methyl sulfate. In order to minimize degradation through peeling reactions in the highly alkaline medium (1.15M sodium hydroxide), end groups were reduced with sodium borohydride, and a product similarly treated with alkali but unmethylated, was used as a control. The results are presented in Table IV. The control

TABLE IV

PROPERTIES OF ALKALI-TREATED AND METHYLATED AGAROSE SAMPLE NO. 5

Sample	*Methyl sulfate used (ml)*	*Gel temp. (degrees)*	*Gel strength (g)*[a]	*Methoxyl (per cent)*	*Sulfate (per cent)*	*Ash (per cent)*
Original	—	36.5	830	0.72	0.57	0.97
Alkali-treated	—	36	807	[b]	0.03	0.20
Methylated	5	31	323	2.87	0.10	0.08
Methylated	10	27	260	5.03	0.10	0.14
Methylated	20	17	25	9.06	0.11	0.10

[a]At concentration of 1%. [b]Not determined.

showed very little change in either gel strength or gelling temperature, indicating that the borohydride treatment was effective in preventing degradation. However, the methylated samples showed a drop in both gel strength and gelling temperature with increasing methoxyl content. It was concluded that random introduction of methyl groups with methyl sulfate interferes with the gelation mechanism, thus producing weaker gels which form with greater difficulty, as evidenced by their lower gelling temperatures. Probably due to poor resolution in solvent system *A*, hydrolyzates of the 3 methylated samples showed only spots which corresponded perfectly to the 3 spots of hydrolyzates of high methoxyl agarose namely D-galactose, 6-*O*-methyl-D-galactose, and an unknown material located between these two.

One further interesting feature of this work was the observation that agaroses of Argentinian origin (from *Gracilaria* spp.) failed to fit into the pattern established. Samples No. 47 and 48 both had higher gelling temperatures than one would predict from the methoxyl content. (Similarly, sample No. 46 is also inconsistent, but the agar was obtained from a jobber, and the source is unknown.) Both samples No. 47 and 48 were unusual in another respect — their viscosities were much higher than is expected for agar or agarose (at 1.5% and 75°, about 25 times as great). Molecular weight may play a large part in both these observed properties, but may not be the entire answer. As suggested by gel strength to gelling temperature relationships for a series of degraded agar samples (the same *Gracilaria* agar used to prepare the fractionated agaroses described in the preceding paragraphs), gel strength and gelling temperature are not

interdependent in the region examined (Table V). Additional work should be done to gain an understanding of these agaroses having an extremely high gelling temperature.

TABLE V

RELATIONSHIP BETWEEN GEL STRENGTH AND GELLING TEMPERATURE FOR A SERIES OF DEGRADED AGAR SAMPLES

Gel strength (g)[a]	*Gelling temp. (degrees)*
558	43.8
397	44
312	44
266	44
237	43.5
190	43.5
99	43.5

[a]At a concentration of 1%.

EXPERIMENTAL

Materials. — U.S. Pharmacopoeia grade agar was used for the preparation of agarose and was obtained from a number of jobbers, although in a few cases, it came directly from manufacturers. In these instances, information was available with respect to the genus of agarophyte from which the agar had been extracted and this, rather than the manufacturer name was included in Table I. In other cases, country of origin is indicated.

Hyamine 1622, a quaternary ammonium salt, was purchased as a 50% solution from Rohm and Haas, Philadelphia, Pa. Carrageenan was from Marine Colloids, Inc., Rockland, Me., the type sold as "Viscarin". Filter aids used were Hyflo Super-cel (Johns-Manville, New York, N.Y.) and Special Speedflow (Dicalite Div., Grefco, Inc., Los Angeles, Calif.).

Analytical procedures. — Gel-strength determinations were made on 1% gels prepared by boiling agarose (2.00 g) in water (200 ml) for 5 min, cooling the solution to 70°, adding water to replace evaporation losses, and pouring into 50×70 mm crystallizing dishes, which were then covered with a glass plate and kept for 2 h at 10°. After this time, the cover was removed, the gel removed from the dish, inverted, and returned to the dish, care being taken to remove air bubbles trapped between the gel and the bottom of the dish. The dish was placed on a dietary scale of about 1300-g capacity which was positioned on the base of a Cherry-Burrell curd-tension meter (Cherry Burrell Corp., Cedar Rapids, Iowa), equipped with a 1.1-cm diameter plunger. The gel was placed so that the plunger contacted the surface about halfway between the center and the edge. After the reading was made, the dish was rotated 120°, and the process repeated. A third determination was similarly made and the results averaged.

Gelling temperatures were determined on 1.5%-agarose sols prepared by boiling agarose (750 mg) in water (50 ml) for 5 min on a hot plate, with stirring. Enough of the sol was poured into an 18 × 150-mm test tube to just reach the immersion line of a thermometer placed in the test tube. The tube was immediately placed into a rack, in a bath containing water at about 60°. Up to 7 such tubes could conveniently be watched simultaneously. When the temperature of all the tubes was the same as that of the bath, cold water was passed through a copper coil in the stirred bath (20-l battery jar). After the temperature reached 50°, the flow rate of the water through the coil was reduced to give a steady drop in temperature of about 0.3–0.5° per min. The bath temperature was never allowed to differ from the temperature in the tubes by more than 0.5°. Periodically, the thermometers were carefully withdrawn from the liquid in the tubes. When gelation had occurred, a distinct depression or neat hole remained; otherwise, the sol flowed back to form a normal meniscus. Gelation generally took place rather abruptly, within the span of 0.5°, and with few exceptions, gelling temperatures are reported to the nearest 0.5°.

Moisture analyses were performed overnight, in duplicate, on 100-mg samples in a vacuum oven at <10 mm and 70°: ash was determined by first charring the samples under i.r. lamps, and then heating them to constant weight in a muffle furnace at 550°. Sulfate determinations were generally performed by the normal gravimetric method when sufficient sample was available. When it was not, a micro method[14] was employed. Because of the low-sulfate content of the material, gravimetric analyses were performed with 2-g samples. The agarose was digested with hot, conc. nitric acid in a 500-ml Kjeldahl flask. Excess acid was destroyed with sulfate-free formaldehyde. The solution was transferred to a 150-ml beaker, taking care to keep the volume to a maximum of about 100 ml. Barium chloride was then added and the usual procedure followed. It was learned through experience that if smaller samples (0.2–0.3 g) were used, or if the volume of the digest were too large, low values were obtained. Methoxyl determinations were made by the Zeisel method[15].

Paper chromatography was performed on Whatman No. 1 paper with either 4:1:2 butyl alcohol–ethanol–water[11] (Solvent *A*), or 4:1:2 butyl alcohol–acetic acid–water[12] (Solvent *B*) with detection by the alkaline silver nitrate method[16]. The agarose samples (1.0 g) were hydrolyzed with 0.5M sulfuric acid (13.3 ml) for 15–16 h on a boiling water bath. The solution was diluted to 50 ml and phenolphthalein added. The hot solution was neutralized with 0.2M barium hydroxide solution, then carbon dioxide was bubbled through until the pink color disappeard, filter-aid (3 g) was mixed in, the hot suspension was filtered (Whatman No. 5 paper precoated with 2–3 g of filter-aid), and the solution evaporated *in vacuo*. The residue, dissolved into 25 ml of water was spotted on the chromatograms.

Preparation[3] *of agarose.* — A mixture of agar (12 g), carrageenan (2.4 g), and filter-aid (35-40 g) was blended and added to water (800 ml) with good agitation. The mixture was boiled, with stirring, for 3 to 5 min to insure complete dissolution of the agar, 200 ml of 5% Hyamine 1622 solution was added, rather rapidly, and the mixture was reheated nearly to boiling. Generally, within a few seconds after the

Hyamine addition was complete, a bulky, curd-like precipitate formed. Filtration was carried out in a pressure bomb using Whatman No. 5 paper. Pressures of 10–20 p.s.i. were usually adequate, although in a few instances up to 70-80 p.s.i. was required. The cake was rinsed with hot water (about 100 ml), about one-half the pressure used for the filtration being needed. The temperature of the combined filtrate and rinse was adjusted to 60–62° in the case of low methoxyl agars and to 72–75° in the case of high methoxyl ones. The solution was then poured, in a small stream, into 1.5 time its volume of 99% 2-propanol. Hand-stirring was employed throughout the process. With low methoxyl agars, the alcohol was initially at room temperature; with high methoxyl agars, the alcohol was warmed to 35–40° prior to precipitation. The short fibers of precipitated agarose settled to the bottom where they joined to form a firm mat upon cooling. The purpose of using higher filtrate and alcohol temperatures with high-methoxyl agaroses was to insure that the temperature of the mixture was about 10° higher than the gelling temperature of the agarose; otherwise, a weak gel formed in the alcohol before the fibers settled and matted together, thus making subsequent work-up virtually impossible. After the mixture had cooled to room temperature, the agarose mat was lifted out and squeezed dry, under a rubber dam, between two sheets of polypropylene filter cloth on a Büchner funnel. It was then soaked in 60% 2-propanol (150–200 ml) for a minimum of 15 min, returned to the funnel, and again squeezed dry. After 3 such washings, the mat was shredded by hand, dried at 60°, and ground through a 20-mesh screen in a Wiley mill.

Separation of agarose into high and low methoxyl fractions. — When a high methoxyl agarose was the product of the above scheme, the alcohol was found to be gelled after cooling. (This did not impair removal of the agarose mat.) The weak gel was melted, and the alcohol was removed by evaporation on a boiling water bath or in a rotary vacuum evaporator. The resulting aqueous solution was added at about 70° with stirring, to 1.5 vol. of acetone at 40°. A second fraction of agarose having a higher methoxyl content settled out as fibers. It was washed as before, except that 60% acetone was used. When fractionation was avoided, acetone was used initially, in place of 99% 2-propanol and all the agarose precipitated. By substituting methanol for 2-propanol in the initial precipitation, a different distribution of the two fractions was obtained.

Alkaline treatment of agarose. — A modification of the method used by Rees[17] for carrageenan was developed for agarose. Agarose (6 g) was boiled in water (225 ml) and the solution cooled to 80°. Sodium borohydride (0.35 g) was added (and generally octanol, as an antifoam), and the solution was kept, covered, for 10–15 min in the 80° bath, which was sufficient for end-group reduction, as shown by the lack of degradation in the products obtained. A solution of sodium hydroxide (15 g) in water (100 ml) was added (70–80°), and the solution was held for 2 h at 80°. It was then cooled to about 50° and neutralized with 3M acetic acid; the product was isolated by precipitation with 1.5 volume of 99% 2-propanol. The coagulum was washed 3 times with 60% 2-propanol, shredded, dried at 60°, and ground through a 20-mesh screen in a Wiley mill. For further purification, it was dissolved by boiling in water to make a

1% solution which, on cooling, formed a gel which was then frozen. It was thawed in about 3 vol. of water, separated on plastic filter cloth on a Büchner funnel, and squeezed under a rubber dam. The thawed gel was given a brief wash with about half the amount of water used to thaw it, and again pressed out on the funnel. To hasten drying and prevent sticking to the container, the gel was then soaked for about half an hour in twice its volume of 99% propanol-2, separated by filtration, and given a second 99% propanol-2 wash. Finally, it was broken up, dried at 60°, and ground through a 20-mesh screen in a Wiley mill.

Methylation of agarose. — The same procedure and amounts used for alkaline treatment were employed. After admixture of the sodium hydroxide, methyl sulfate was added. For the 3 preparations, 5, 10, and 20 ml were used, respectively. Work-up was the same as in the modification.

Acid degradation of agar. — For comparison of gelling temperatures of agars of different gel strengths, *Gracilaria* agar having a 1% gel strength of 558 g was degraded in the following manner: the pH of a 1%-agar sol kept at 60° was adjusted to 3.0, and samples were withdrawn periodically (0.5, 1.0, 1.5, 2, 3, and 4 h). These were neutralized and allowed to gel. The gels were frozen, thawed, washed, and dried as described in the section on alkaline treatment. Gelling temperatures and gel strengths are recorded in Table V.

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STUDIES ON DILUTE SOLUTIONS AND DISPERSIONS OF THE POLYSACCHARIDE FROM *Xanthomonas campestris* NRRL B-1459 *

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ABSTRACT

Some properties of the extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* NRRL B-1459 were measured in partially purified culture fluids and in liquid systems prepared from thoroughly purified and lyophilized material. High intrinsic viscosities resulted from dispersions and solutions of polysaccharide B-1459 in 0.01M ammonium acetate and 4M urea. Heating a dispersion of B-1459 in 4M urea yielded a true macromolecular solution in which the polysaccharide had an apparent molecular weight of approximately 2×10^6. Light-scattering measurements on culture fluids of B-1459 made 4M in urea gave molecular weights of 13×10^6 and 50×10^6. Various solution-properties and factors that affect the viscosity of dispersions prepared from lyophilized polysaccharide are presented.

INTRODUCTION

The extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* NRRL B-1459 has gained widespread use industrially[1]. A useful characteristic of polysaccharide B-1459 is its ability to produce highly viscous aqueous dispersions and solutions that maintain their viscosity in the presence of many salts. Some characteristics of its dispersions and behavior under conditions of varying temperature, pH, and salt addition have been reported[2]. B-1459, isolated as the potassium salt, is composed[3] of D-glucose, D-mannose, D-glucuronic acid, acetic acid (as *O*-acetyl), and pyruvic acid (as an acetal substituent) in the molar proportions: 2.8:3.0:2.0:1.7:0.51–0.63. The repeating unit, composed of 16 hexose residues and their substituents[4], has β-D-(1→2) and β-D-(1→4)-linkages[4,5]. The macromolecular properties of B-1459 were investigated in an aqueous environment because the main industrial uses of this material involve fluid systems containing water. Two aspects of some fluid systems containing B-1459 are reported here: (*i*) solution parameters in 4M urea, and (*ii*) dilute dispersion viscosities in 0.01M ammonium acetate (NH_4OAc) and in 4M urea.

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**This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

EXPERIMENTAL

A. Materials. — A lot of B-1459, designated MP-84, came from a batch-type fermentation process[6]. Recovery of polysaccharide involved clarification by repeated passages through a Sharples* centrifuge, three successive precipitations from solution by ethanol in the presence of potassium chloride to remove protein, dialysis against deionized water, and adjustment of retained solution to pH 6.0–6.5 with dilute potassium hydroxide[2]. The resulting solution was filtered through sintered glass to remove a small amount of extraneous matter, concentrated to about 0.5% solids on a rotary evaporator under diminished pressure, and lyophilized. After the lyophilized polymer had been equilibrated in air at 20° and 50% relative humidity as previously described[2], it had a moisture content of 15.6%.

A second batch of polymer, designated X-FD-1, was obtained as a culture broth sample from a continuous fermentation run in a pilot plant. This liquid was diluted with eight volumes of distilled water and then passed twice through a Sharples centrifuge to remove cells and particulate matter. The diluted and clarified culture fluid was then dialyzed against 0.01M NH_4OAc for 3 days at room temperature. The retained solution was adjusted to pH 7.3 with NH_4OH upon completion of the dialysis and was refrigerated until used. Sufficient mercuric iodide (HgI_2) to leave a visible precipitate was added to all B-1459 systems containing 0.01M NH_4OAc to discourage microbial growth.

A portion of this NH_4OAc-dialyzed culture fluid then was dialyzed against distilled water for several days with no adjustment of pH. A fibrous mass of polysaccharide resulted by lyophilizing this portion of culture fluid. Both the culture liquid and lyophilized material retained a brownish pigment. Polysaccharide concentrations in dialyzed solutions and in culture fluid were determined by the phenol–sulfuric acid colorimetric procedure[7].

Another portion, 900 ml, of X-FD-1 culture fluid was taken from the refrigerated stock bottle after 7 months' storage. (Microorganism growth was inhibited, but not completely stopped during storage.) This portion was dialyzed overnight at 5° against distilled water and then centrifuged at $40 \times 10^3 \times g$ for 1 h. The resulting, clarified liquid (light yellow) was divided into two equal volumes. One volume was lyophilized directly; the other was made 1% in potassium chloride, an equal volume of ethanol was added, and the system was stirred for 1 h. The gel of B-1459 that formed upon addition of alcohol was gathered by decanting the alcohol–water solution. The gel was then redispersed into distilled water to its original concentration of 0.195%. After dialysis against distilled water for 60 h at 5°, the alcohol–potassium chloride-treated B-1459 system was lyophilized.

A third batch of polymer came from two frozen samples of culture fluid taken from batch fermentation runs. The samples[8], designated X-C-9(68) Fertrol No. 2

*Mention of firm names or trade products does not imply an endorsement or recommendation by the Department of Agriculture over other firms or similar products not mentioned.

and X-L-10(68) Fertrol No. 1, were each thawed, then combined, and diluted with distilled water to a concentration of 0.4 to 0.5%. The diluted culture fluid was centrifuged at $40 \times 10^3 \times g$ for 1.5 h to remove particulate matter and some polymer, which appeared as a gel on the pad of debris in the centrifuge tube. The light yellow, clarified liquid was dialyzed in a refrigerator for 1 week against 0.01M NH_4OAc and then part of it was lyophilized. Another portion of this culture fluid was then made 4M in urea and dialyzed against 4M urea at 5° before use.

Dispersions were prepared by weighing lyophilized samples of B-1459 into 25-ml volumetric flasks. About 10 to 15 ml of the desired solvent was added, and the flasks were shaken overnight. More solvent was added the next day, and shaking was continued for a few h. The proper amount of solvent was then added to bring the liquid to volume. If the polymer was not dispersed well, a stirring bar was placed in the flask and the contents were stirred, with occasional shaking, to ensure proper mixing. Sometimes, it was sufficient to shake the stoppered sample vigorously to obtain an even dispersion. Concentrations up to about 0.25% were dispersed evenly by these methods. The 25 ml of dispersed material was used as stock from which dilutions were made. Dispersion in 4M urea sometimes required 2 to 7 days' storage at room temperature before an apparently stable viscosity resulted.

Many samples of B-1459 were vacuum-dried before being dispersed. One technique (No. *1*) used was to place the lyophilized samples in a vacuum oven at room temperature, turn on both vacuum and heat, and allow the samples dry overnight (*ca.* 17 h). The vacuum oven reached the desired drying temperature of 70° to 75° in about 1 h. Another method (No. *2*) was to vacuum-dry the samples overnight at room temperature (*ca.* 23°) and then heat under vacuum the next day for 5 to 6 h. Dispersion viscosity depended upon which of these two methods was used to dry the lyophilized sample from which the dispersion was prepared.

Solutions in 4M urea were prepared from batch MP-84. A lightly stoppered, 25-ml volumetric flask containing lyophilized sample and about 15 ml of 4M urea was placed into a water bath for 3 h at 90°. The flask was shaken occasionally to dissolve the polysaccharide during heating. After the flask had cooled, solvent was added to the volumetric mark and the flask was shaken vigorously to ensure even mixing of contents.

B. Methods. — A model A Zimm viscometer[9] was used to measure viscosities at shear rates that varied from about 0.5 to 2.0 sec^{-1}. Sedimentation velocity and synthetic-boundary measurements were made in a Spinco ultracentrifuge in double sector, 30-mm cells. From the sedimentation velocity measurements, a sedimentation coefficient (s) was calculated by the standard formula[10]. Partial specific volumes of B-1459 in solution were calculated from density measurements made at 25.00 $\pm$0.05° in 35-ml double-column pycnometers. Diffusion was measured at 0.9° in a Tiselius cell in a Spinco Model H electrophoresis instrument. Density, diffusion, molecular weight, and sedimentation measurements were made on solutions prepared from batch MP-84. Estimated errors are indicated in values presented in Tables I and II.

Molecular weight determined by light scattering, $\overline{M}_{LS}$, was measured in a

modified Brice Phoenix instrument, which was calibrated on the basis of a value of 16.3×10^{-6} for the Rayleigh ratio of benzene at 546 nm at 25.0°. Measurements were made at 546 nm and 436 nm. Samples used for light-scattering measurements were dialyzed for at least 2 weeks before measurements were made. These samples were centrifuged in a Spinco preparative centrifuge as follows:

Sample	*Preparative centrifugation treatment*
B-1459 solution in 4M urea	1 h at $105 \times 10^3 \times g$
X-FD-1 culture fluid	3 h at $78 \times 10^3 \times g$
XC-XL culture fluid	1.5 h at $78 \times 10^3 \times g$
XC-XL culture fluid heated for 3.5 h at 85°	20 min at $35 \times 10^3 \times g$

Two problems encountered were those of achieving a true macromolecular solution and deciding when the polymer was in a true state of solution. A solution of B-1459 in 4M urea could be produced by heating the system for 1 to 4 h at 90°. Major proportions of gel component in unheated or incompletely solubilized B-1459–4M urea systems were detected by measurements in the ultracentrifuge. Sometimes, gel or high-molecular-weight aggregates were detected by the rapid appearance of material at the bottom of a cell during a sedimentation run. Measurement of area in a synthetic-boundary pattern served as the criterion of concentration in solution when the presence was suspected of gel that would not be exhibited clearly in a sedimentation run. The high concentration-dependence shown by B-1459 required use of low concentrations to observe solution properties best.

RESULTS

Molecular weight studies. — The molecular weight of B-1459 was studied in 4M urea because initially it was considered to be a better solvent than 0.01M NH_4OAc. Solutions of B-1459 prepared from lyophilized samples of batch MP-84 were used in determining mocular weights, $\bar{M}_{SD}$, from sedimentation and diffusion measurements. A molecular weight was calculated according to the Svedberg equation:

$$\bar{M}_{SD} = \frac{RTs^\circ}{D^\circ(1-\bar{v}\rho)} \tag{1}$$

where R is the gas constant, T is temperature (k°), $\bar{v}$ is the partial specific volume, ρ is the density of the solvent, and s° and D° are the sedimentation and diffusion constants extrapolated to zero concentration. Fig. 1 displays the sedimentation behavior of B-1459 in 4M urea and in 0.01M NH_4OAc. There is a distinct difference in concentration dependence between the week-old and 2-month-old samples in 4M urea as indicated by the different slopes of lines A and B. A portion of the sample in 4M urea was dialyzed to 0.01M NH_4OAc. Some $1/s$ values of this sample are indicated in Fig. 1.

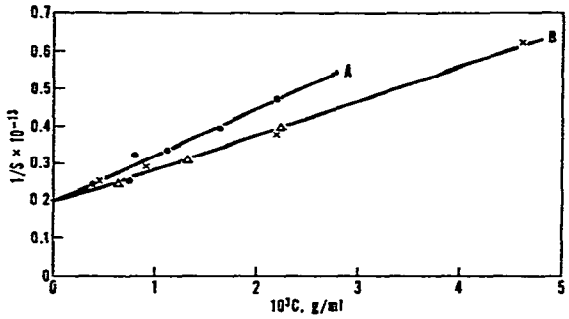

Fig. 1. Sedimentation behavior of polysaccharide B-1459 from *Xanthomonas campestris* (MP-84). ● = Sample in 4M urea for 1 week, line A. × = Sample in 4M urea for 2 months, line B. △ = Sample in 0.01M NH_4OAc for 1 month, line B. All samples stored at 5°. All $1/s$ values measured at or corrected to 25°. Values of $1/s$ for 0.01M NH_4OAc solutions corrected to 4M urea solution. All concentrations corrected for radial dilution.

Results of diffusion measurements are shown in Fig. 2. Solutions were stored at 5°, and it was necessary to dialyze them for 2 to 3 weeks to obtain meaningful

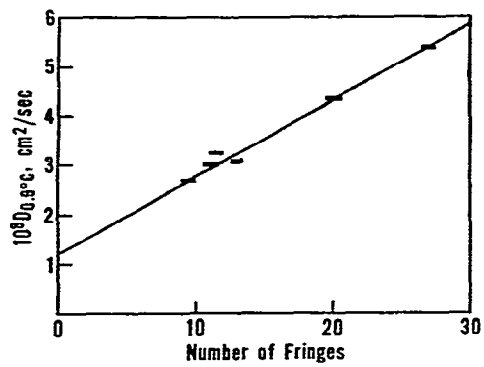

Fig. 2. Diffusion behavior of B-1459 (MP-84) in 4M urea. Concentration expressed in number of Rayleigh fringes (27 fringes = 0.21%).

diffusion patterns. Diffusion coefficients were determined by the height-area method using the equation:

$$A/\lambda_{max} = (4\pi Dt)^{1/2} \tag{2}$$

where A is the area under and λ_{max} is the maximum height of the refractive index gradient curve, and t is the time from the start of the diffusion process. It was extremely difficult to obtain sharp, zero-time boundaries in the diffusion cell with B-1459. Zero-time corrections of the order of 20 to 40 h were encountered. A photograph of a typical asymmetric schlieren pattern of diffusing B-1459 polymer is shown in Fig. 3. The diffusion constant measured at 0.9° was corrected to 25° by the usual formula[11] to yield a value of $D_{25} = (2.36 \pm 0.40) \times 10^{-8}$ cm²/sec. Uncertainties encountered in extrapolation of diffusion and sedimentation constants to zero concentration led to an estimated possible error of about 22% in $\bar{M}_{SD}$.

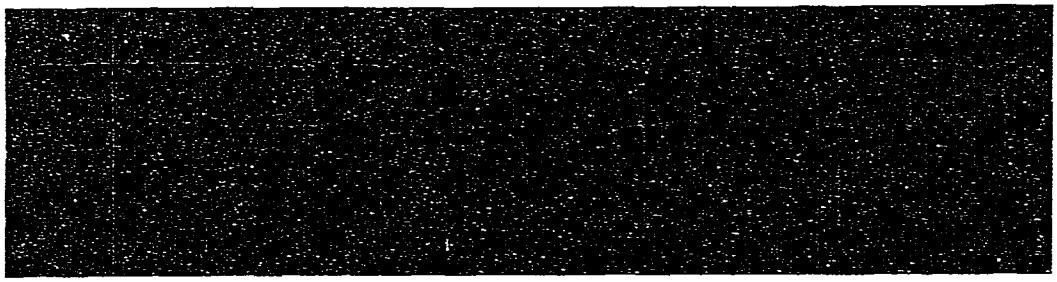

Fig. 3. Diffusion pattern of B-1459 (MP-84) in 4M urea. $c = 0.1\%$, T = 0.9; bar angle = 35°. Time from formation of boundary = 290 h. Solvent region is on the left, solution on the right.

Light-scattering measurements were made in 4M urea on a solution prepared from batch MP-84 and on culture fluids from X-FD-1 and the combined XC-XL samples. Preparative centrifugation, as previously described, was used in an attempt to rid these systems of a possible "micro-gel" component. Systems from MP-84 and X-FD-1 were first centrifuged until their original concentration was reduced by a factor of at least one-third. The XC-XL culture fluid sample was of low concentration, $c \sim 0.060\%$, and it lost a negligible amount of polymer during preparative centrifugation. The Zimm plot of the MP-84 solution (Fig. 4) showed curvature in

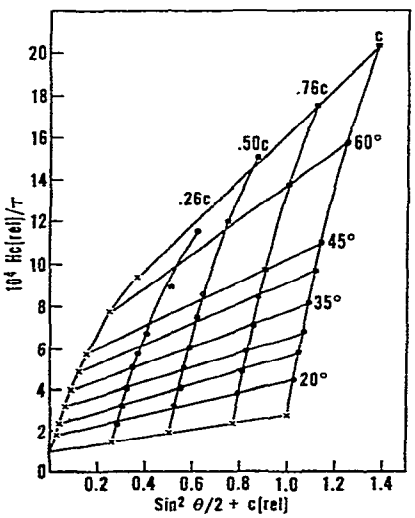

Fig. 4. Zimm plot of B-1459 (MP-84) solution in 4M urea prepared by heating a lyophilized sample for 3 h at 90°. Initial concentration, $c = 3.4 \times 10^{-3}$ g/ml. Angles of 20° to 45° from the incident beam are indicated in 5° increments. The two highest angles are 60° and 75° from the incident beam. ● = Measured values. × = Extrapolated values at zero angle and zero concentration.

the lines of constant concentration, as did the Zimm plot (not shown) of culture fluid X-FD-1. Molecular-weight ranges in MP-84 solution were estimated by extrapolating to zero angle those measurements that were 45° and less from the incident beam. Values of molecular weight determined by light scattering, $\bar{M}_{LS}$, of 13×10^6

and 50×10^6 were obtained from the low angle extrapolations of Zimm plots from culture fluid batches X-FD-1 and XC-XL, respectively. (Since batches X-FD-1 and XC-XL were not identically grown or treated, one should not expect identical $\bar{M}_{LS}$ values.) The solution prepared from batch MP-84 had a range of $\bar{M}_{SD}$ that was lower than its range of $\bar{M}_{LS}$, which was lower than values of $\bar{M}_{LS}$ determined on culture fluid samples from other batches. Some parameters of B-1459 solution in 4M urea are shown in Table I.

TABLE I

B-1459 (MP-84) IN 4M UREA

$(dn/dc)_{546}$	0.135 ml/g
$[\eta]_G = 0$	29×10^2 ml/g
$\bar{V}_{25}$	0.59 ml/g
$D^0_{.09}$	$(1.2 \pm 0.2) \times 10^{-8}$ cm^2 sec^{-1}
s^0_{25}	$(5.0 \pm 0.2) \times 10^{-13}$
$\bar{M}_{SD}$	$(1.4 \pm 0.3) \times 10^6$
$\bar{M}_{LS}$	$(3.6 \pm 0.7) \times 10^6$ (Fig. 4)

Heating either the lyophilized B-1459 or the culture fluid decreases molecular size. This observation is supported by two measurements: (*i*) The intrinsic viscosity of an aged dispersion in 4M urea is about twice as great as the intrinsic viscosity of a solution prepared by heating a portion of the dispersion for 3 h at 90° and (*ii*) the $\bar{M}_{LS}$ value of the XC-XL culture fluid decreased from 50×10^6 to 11×10^6 after the culture fluid was heated for 3.5 h at 85°.

Viscosity studies. — Changes in viscosity values were the basis for judging the approximate stability of dispersions and solutions. Dispersions of B-1459 in redistilled water and in 0.01M NH_4OAc were prepared from samples of batch MP-84 and were stored at room temperature and at 5°. Storage at 5° helped maintain viscosity in both solvent systems. The dispersion in water suffered a 50% decrease in relative viscosity within 3 weeks at room temperature (*ca.* 25°). The dispersion in 0.01M NH_4OAc was stable for 2 months at room temperature and was stable for about 8 months when stored at 5°. Solutions in 4M urea, prepared from batch MP-84, yielded stable viscosity values for at least 4 months when stored at 5°.

Some representative viscosity characteristics of dispersions prepared from lyophilized and vacuum oven dried B-1459 obtained from culture fluid batch X-FD-1 are displayed in Fig. 5. The polyelectrolytic nature of the polymer is clearly indicated by the thixotropic behavior and higher viscosity of the dispersion in distilled water (curve A). The behavior of a dispersion in 4M urea, that was aged 7 days at 5°, is shown by line B_1. After 2 months' storage at 5°, the viscosity of this system, represented by line B_2, had markedly increased. The viscosity of a dispersion aged 7 days at 5° in 0.01M NH_4OAc is shown by line D. Even after 2 months' storage at 5°, the viscosity of this system remained constant.

Viscosity behavior of B-1459 polymer not isolated from the culture fluid was different from that of lyophilized and dried material. Line C (Fig. 5) represents the

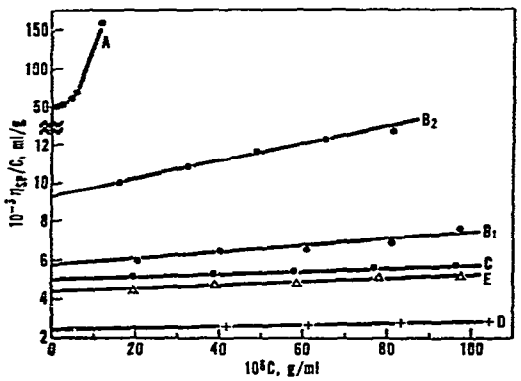

Fig. 5. Viscosity behavior of B-1459 (from X-FD-1). (A) Lyophilized, not dried, polymer dispersed overnight in redistilled water. Note scale change. (B_1) Lyophilized and dried polymer, dispersed 7 days in 4M urea at 5°. (B_2) Sample B_1 aged 2 months at 5°. (C) Clarified culture fluid dialyzed against 0.01M NH_4OAc and stored 4 weeks at 5°. (D) Lyophilized and dried polymer, dispersed 7 days in 0.01M NH_4OAc at 5°. (E) Sample C made up to 4M urea for 4 days at 5°. Lyophilized samples dried by technique *1*, but not equilibrated in constant temperature-humidity room before drying. Ethanol–KCl precipitation not used on these samples.

behavior of clarified culture fluid that had been dialyzed against 0.01M NH_4OAc for several days. The dialyzed system had been stored for 4 weeks at 5°. Curve E represents the viscosity of the dialyzed culture fluid that has been made about 4M in urea. Five milliliters of 0.01M NH_4OAc dialyzed culture fluid was diluted to 100 ml with 4M urea, and the fluid was aged 5 days at 5° before measurements were taken. Dilutions were made with 4M urea.

Some effects upon the intrinsic viscosity, $[\eta]$, of dispersions prepared from a lyophilized batch of B-1459 from X-FD-1 culture fluid are summarized in Table II. In this table are compared the effects of (*i*) dehydrating versus not dehydrating the sample, (*ii*) 0.01M NH_4OAc versus 4M urea as a solvent, (*iii*) dehydrating the samples by methods 1 or 2, and (*iv*) ethanol–potassium chloride precipitation from the culture fluid versus direct lyophilization.

TABLE II

INTRINSIC VISCOSITY OF B-1459 DISPERSIONS

Sample	$[\eta]$ *ml/g* $\times 10^{-3}$	
	0.01M NH_4OAc	*4M Urea*
(A) Culture fluid not treated with ethanol–KCl		
Dehydrated	1.7 ±0.1 (9.0 ±0.2)	8.1 ±0.3 (8.1 ±0.3)
Not dehydrated	5.8 ±0.1	5.9 ±0.1
(B) Culture fluid treated with ethanol–KCl		
Dehydrated	9.2 ±0.2 (9.0 ±0.2)	8.2 ±0.2 (8.1 ±0.2)
Not dehydrated	7.7 ±0.1	7.0 ±0.1

Lyophilized polymer obtained from culture fluid batch X-FD-1 stock solution that was stored for 7 months at 5°. Values for samples dehydrated by drying method *2* are indicated in parentheses.

Precipitation of polysaccharide from the culture fluid with alcohol–potassium chloride removes protein. Lyophilized material (X-FD-1) after alcohol–potassium chloride precipitation contained 0.24 weight percent nitrogen; that prepared directly from the clarified culture fluid contained 1.71 weight percent nitrogen. (Batch MP-84, which is highly purified, contained 0.06% nitrogen.) Estimates of 10% protein in directly lyophilized X-FD-1 samples and 1.4% in ethanol–potassium chloride precipitated X-FD-1 samples are made by using a conversion factor of 6.0 between weight of nitrogen and weight of protein. Values of $[\eta]$ in part (A) of Table II have been calculated by using concentrations 0.91 times those measured on a weight basis. This procedure is used to correct for the greater amount of protein in the directly lyophilized samples. Values of $[\eta]$ in part (B) of Table II are calculated on a direct concentration by weight basis.

DISCUSSION

The highly concentration-dependent behavior of B-1459 (MP-84) solution in 4M urea is shown in several ways. One may use the data in Fig. 1 to calculate a concentration dependence coefficient, K_s, from the equation:

$$1/s = 1/s^{\circ}[1 + K_s c] \tag{3}$$

where c is the concentration in g/ml. Values of K_s in 4M urea equal to 614 ml/g and 435 ml/g are calculated for the week-old solution and the 2-month-old solution, respectively. The asymmetry shown by the diffusion photograph (Fig. 3) is a reflection of concentration dependence or polydispersity, or both of these properties. Sedimentation patterns (not shown) of a fresh B-1459 solution in 4M urea and culture fluids in 0.01M NH_4OAc appear to have infinite boundaries at polymer concentrations above 0.2%. The schlieren patterns of a fresh solution in 4M urea appear as sharp spikes at concentrations of about 0.15 to 0.1%; at a 0.05% concentration the pattern opens to give the appearance of a sawtooth. This non-Gaussian character of the sedimentation pattern is another indication of concentration dependence.

The difference in molecular-weight values calculated for the 4M urea solution of B-1459 (MP-84) is possibly caused by a mode of high-molecular-weight particles, or micro-gel, which would be heavily weighted in the $\bar{M}_{LS}$ average. A few percent of micro-gel could remain in the solutions despite preparative ultracentrifugation and could also remain undetected in the synthetic boundary measurements. Light scattering becomes less concentration dependent at angles closer to the incident beam as is indicated by decreasing slopes of lines at constant angle (Fig. 4). Because of this, and the fact that lines at constant concentration have less curvature at lower scattering angles, we chose measurements between 45° and 20° from the incident beam as being most meaningful. Of course, a weight average molecular weight would be obtained from the $\bar{M}_{LS}$ value in the absence of micro-gel. The $\bar{M}_{SD}$ value does not represent a simple average. This is particularly true for B-1459 solutions in which asymmetric schlieren patterns are obtained in measurements of s and D. Therefore,

our molecular-weight determinations should be considered as qualitative indications of the molecular system in solution.

Light-scattering measurements on both the solution and the culture fluids indicate polydisperse systems of high molecular weight. We cannot say whether the observed $\bar{M}_{LS}$ values are representative of B-1459 polymer molecules or aggregates. Despite the probability that some micro-gel contributes to the $\bar{M}_{LS}$ values measured on the culture fluids, molecular weights in the culture fluids are well above those in the 4M urea solution.

Attempts to detect measurable amounts of gel by ultracentrifuge synthetic-boundary measurements yielded negative results in both dilute 4M urea solutions or dialyzed culture fluid samples in 0.01M NH_4OAc. Therefore, under these conditions of low gravity and concentration, $c < 0.2\%$, we conclude that B-1459 is capable of existing in a solution state. Sedimentation measurements made on 0.1% dispersions in 0.01M NH_4OAc prepared from lyophilized material show gel to be present. Since the dialyzed culture fluid in NH_4OAc and 4M urea behaves as a truly solvated system, we conclude the process of lyophilization alters the solubility of B-1459, as well as its viscosity properties. Perhaps lyophilization in some manner "denatures" the polysaccharide.

Our studies show that a variety of factors may affect dispersion viscosity. We noted an "aging" effect of B-1459 systems in 4M urea. The viscosity of B-1459 initially placed in 4M urea is lower than its viscosity later. This behavior, illustrated in Fig. 5 by lines B_1 and B_2, is most easily observed in lyophilized samples although it sometimes may be seen in culture-fluid samples. The rate of viscosity change increases when the sample is exposed to higher temperatures. A lyophilized sample stored at room temperature may reach higher viscosities within 24 to 48 h. Sedimentation patterns of B-1459 samples that have been in 4M urea at 5° for 6 months or longer show peaks that have a more normal appearance than those seen in nonaged samples. The change in concentration dependence illustrated by lines A and B in Fig. 1 is another example of B-1459 undergoing change in 4M urea. The changes in dispersion viscosity and solution properties of an already solvated entity suggest to us that B-1459 could be reacting slowly with 4M urea. These changes might involve a disaggregation of molecular aggregates or a chemical reaction between urea and polysaccharide. (Formation of carbohydrate carbamates prepared by heating starch with urea has been reported by Paschall[12].)

Our experience with dispersion viscosity measurements has shown that extreme care must be used to ensure that time-dependent behavior does not make comparisons invalid. All of the values in Table II were measured after dispersions had aged at room temperature for at least 5 days. Values of η_{sp}/c were then checked to ensure that no changes occurred during the time span of these measurements. After an initial period of instability, viscosities of dispersions in 0.01M NH_4OAc and 4M urea at room temperature decrease slowly with time.

Some general conclusions about dispersions prepared from lyophilized polysaccharide (X-FD-1) are indicated in Table II. The only extreme viscosity difference

occurred in 0.01M NH_4OAc dispersions of high-protein content because drying method *1* lowered dispersion viscosity of the samples. Provided drying method *2* is used, dehydration of sample increased viscosity in all situations investigated. Samples precipitated with ethanol–potassium chloride, but not dried, yielded higher viscosities than samples neither ethanol–potassium chloride precipitated nor dried. Perhaps the polysaccharide is "denatured" in some manner by the precipitation and drying process. A final comment concerns $[\eta]$ values in Table II, part A, Dehydrated, as compared with $[\eta]$ values illustrated by lines B_2 and D (Fig. 5), which would be even higher had a correction for protein content been made. Measurements listed in Table II were made on material stored for 7 months at 5° as partially clarified culture fluid. During this time the polysaccharide had altered, as indicated by lower $[\eta]$ values, and hence identical materials were not used for measurements in Table II and Fig. 5.

The purpose of this study has been to determine some molecular parameters and viscosity characteristics of polysaccharide B-1459 in dilute solution and dispersion. The dilute solution systems consist of high-molecular-weight entities that interact strongly. High intrinsic viscosity is maintained under the conditions of our experiments. Dispersion viscosity may be enhanced by appropriate treatment of the polymer before dispersion. The most interesting question remains to be answered. What specific subunits and structures of the polysaccharide chain cause the property of high viscosity observed in fluid systems of B-1459?

ACKNOWLEDGMENTS

We thank Dr. Allene Jeanes for providing the purified sample of B-1459, Mr. S. P. Rogovin for preparing culture fluid batch X-FD-1, and Mr. R. W. Silman for the XC-XL sample. Discussions with Drs. Jeanes and J. H. Sloneker were most helpful.

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UNSATURATED CARBOHYDRATES

PART XIII [1]. THE MASS SPECTROMETRY OF HEX-2- AND HEX-3-ENOPYRANOSYL DERIVATIVES

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ABSTRACT

The mass spectra of a series of hex-2-enopyranoside derivatives indicated that fragmentation of members of this class occurs by two general paths: (*i*) by loss of the substituents attached to C-1, C-4, and C-6, giving a series of cyclic ions related to pyran, and (*ii*) by retrodienic cleavage. Recognition of the ions produced by this latter mode can provide means for allocating the positions of double bonds in unsaturated carbohydrates, but since allylic rearrangements can, in some circumstances, precede fragmentations, care has to be taken in interpreting the spectra.

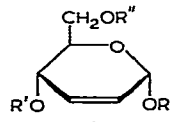
The spectrum of 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-*glycero*-pent-2-enopyranose indicates that isomerisation to 5,5,6-triacetoxy-5,6-dihydro-2*H*-pyran occurs prior to fragmentation. Such an isomerisation has not been observed before with unsaturated sugars.

INTRODUCTION

The scant attention given so far to the mass spectrometry of unsaturated carbohydrate derivatives was stimulated by the belief that such compounds might represent intermediates in the degradations undergone by saturated analogues on electron impact. Finan *et al.*[2] thus concluded that D-glucal was formed from the methyl D-glucopyranosides and noted that all of these compounds gave similar spectra, and other workers have postulated that cyclic enol esters are first produced during the fragmentation of saturated glycose peracetates[3]. In the only investigation of unsaturated derivatives previously reported[4], tri-*O*-acetyl-D-glucal, its 2-acetoxy derivative, methyl 4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranoside, and 1,2,4,6-tetra-*O*-acetyl-3-deoxy-α-D-*erythro*-hex-2-enopyranose were investigated, and it was concluded that such unsaturated compounds are intermediates in the breakdown of hexose peracetates. The work now reported was undertaken to assess the value of mass spectrometry as a means of locating the position of double bonds in unsaturated carbohydrate derivatives; in particular, we wished to apply it do compounds known to be either hex-2- or hex-3-enopyranosides some of which could not be characterised readily by proton magnetic resonance methods.

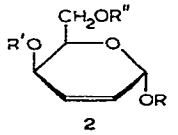
RESULTS AND DISCUSSION

Initially, the spectra of compounds (**1a–f**) and (**2a, b**), which had previously been characterised by chemical methods, were investigated (Fig. 1–8).



1

	R	R'	R''	Ref.
1a	Et	H	H	5
1b	i-Pr	H	H	6
1c	Et	Ac	Ac	5
1d	$pC_6H_4NO_2$	Ac	Ac	7



2

	R	R'	R''	Ref.
1e	X*	Ac	Ac	6
1f	Me	Bz	Bz	6
2a	Me	Ac	Ac	8
2b	Et	Bz	Ms	9

*X=6-deoxy-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranos-6-yl

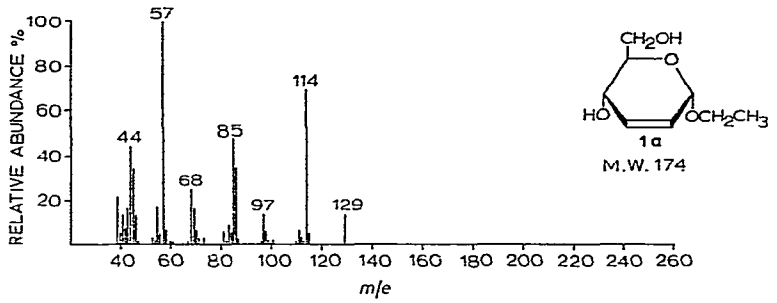


Fig. 1.

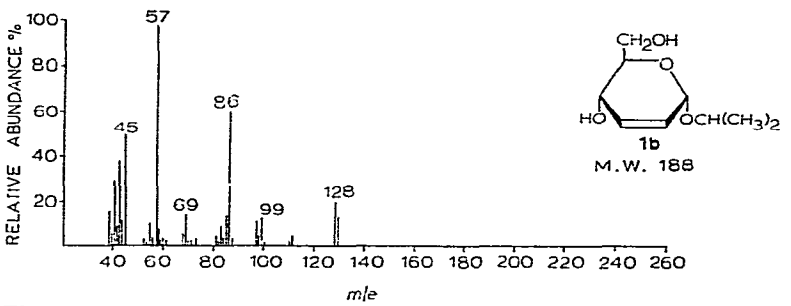


Fig. 2.

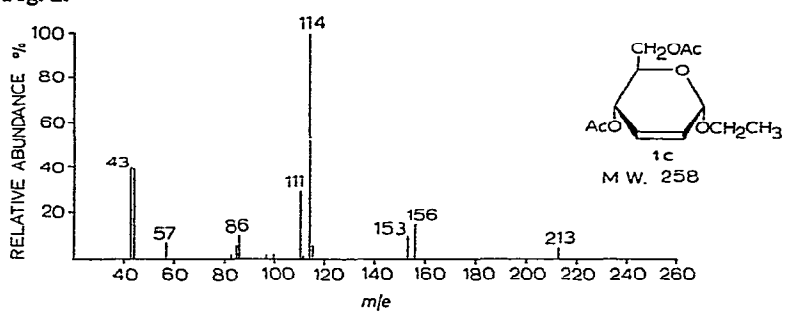


Fig. 3.

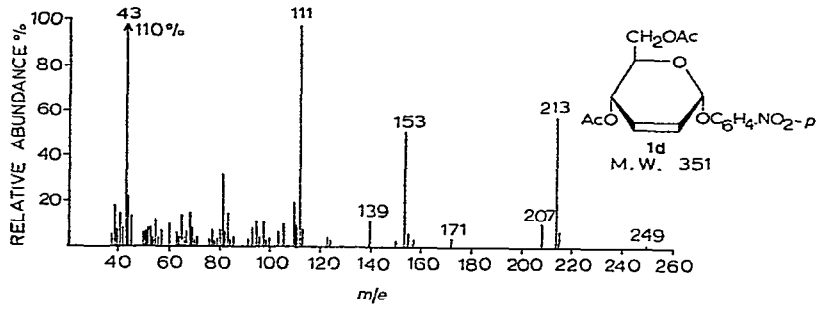


Fig. 4.

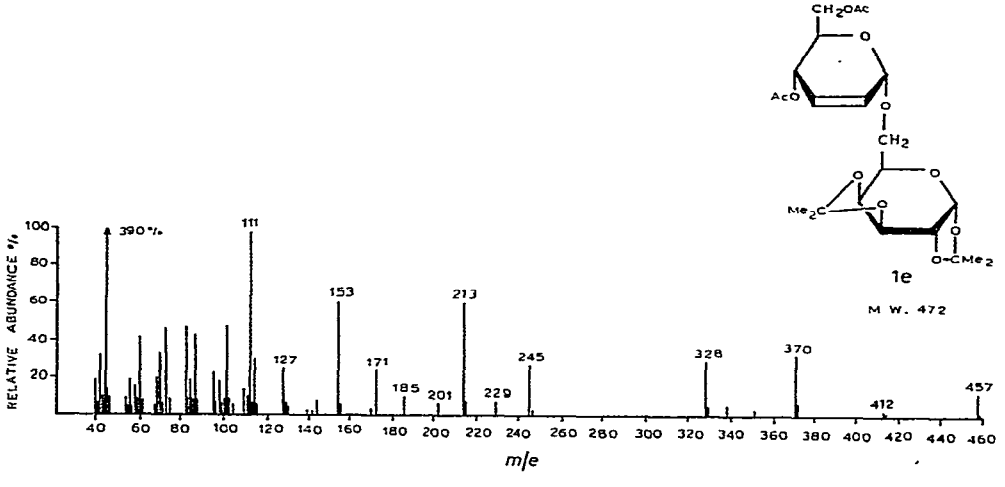


Fig. 5.

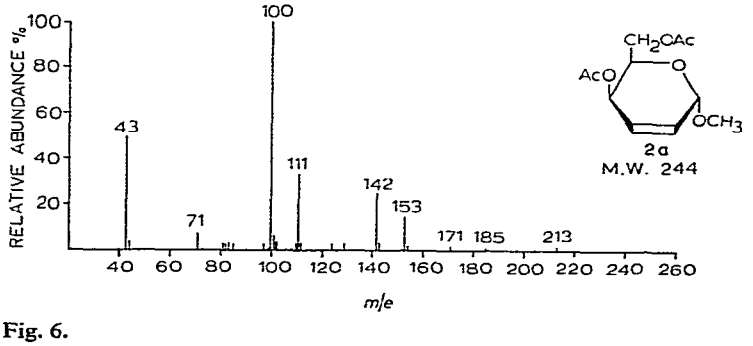


Fig. 6.

For the dihydroxy compounds (**1a** and **1b**, Figs. 1 and 2), postulated fragmentation patterns are shown in Scheme 1; initial breakdown of the molecular ion occurs either by direct loss of individual ring substituents to give ions which retain the pyranoid ring (P fragmentation), or by retrodiene cleavage to give ions (RD series)

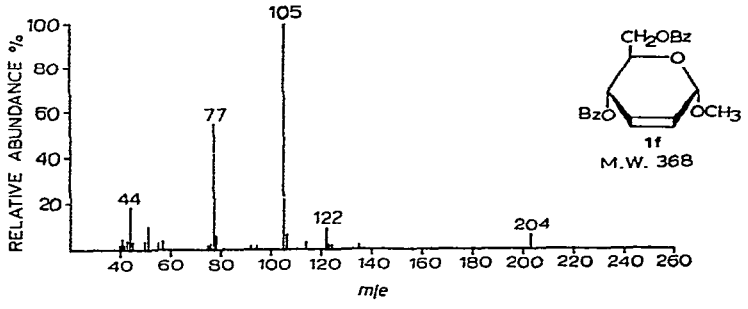

RELATIVE ABUNDANCE %
m/e
105
77
44
122
204
CH2OBz
BzO
OCH3
1f
M.W. 368

Fig. 7.

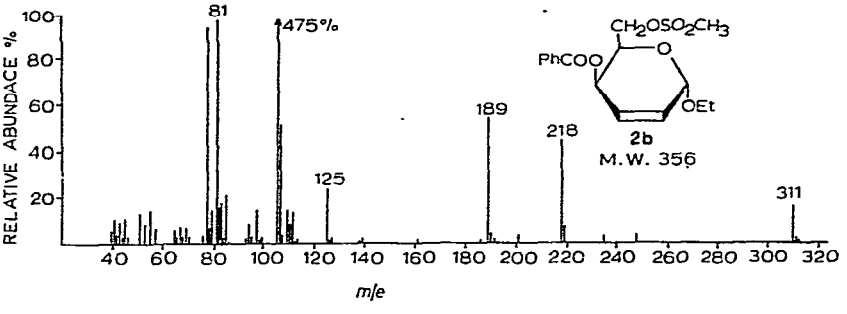
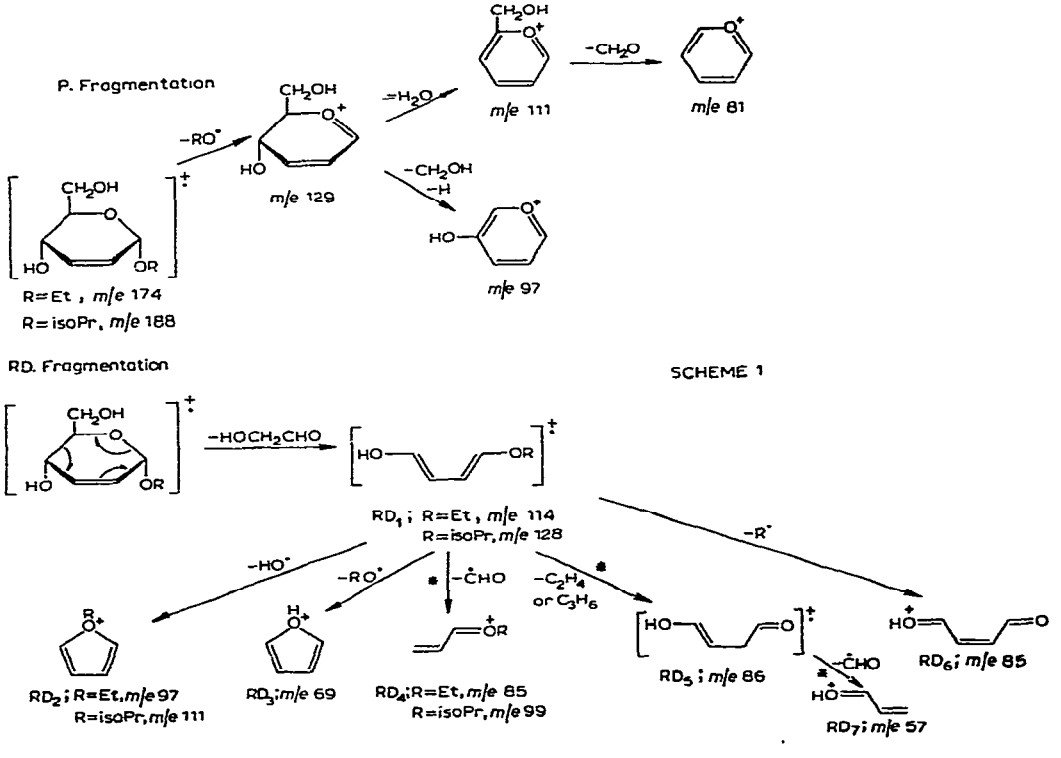
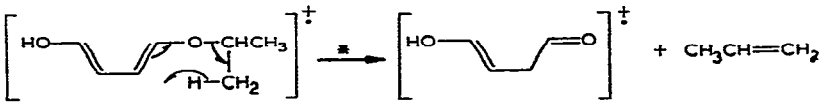

RELATIVE ABUNDANCE %
m/e
81
475%
189
218
125
311
CH2OSO2CH3
PhCOO
OEt
2b
M.W. 356

Fig. 8.


P. Fragmentation
-RO·
-H2O
-CH2O
m/e 111
m/e 81
m/e 129
-CH2OH
-H
m/e 97
R=Et , m/e 174
R=isoPr, m/e 188
RD. Fragmentation
SCHEME 1
-HOCH2CHO
RD1 ; R=Et, m/e 114
R=isoPr, m/e 128
-HO·
-RO·
-·CHO
-C2H4
or C3H6
-R·
RD2 ; R=Et, m/e 97
R=isoPr, m/e 111
RD3 ; m/e 69
RD4 ; R=Et, m/e 85
R=isoPr, m/e 99
RD5 ; m/e 86
RD6 ; m/e 85
RD7 ; m/e 57

containing initially C-1, C-2, C-3, and C-4 and the substituents attached to C-1 and C-4. The most intense ion is RD_7, and it is noticeable that RD_1 gives more RD_4 and/or RD_6 (both *m/e* 85) than RD_5 (*m/e* 86) in the case of the ethyl glycoside (**1a**), whereas this ratio is reversed (by virtue of increase in the *m/e* 86 ion) for the isopropyl compound for which the McLafferty rearrangement[10] (Scheme 2) is more favoured.

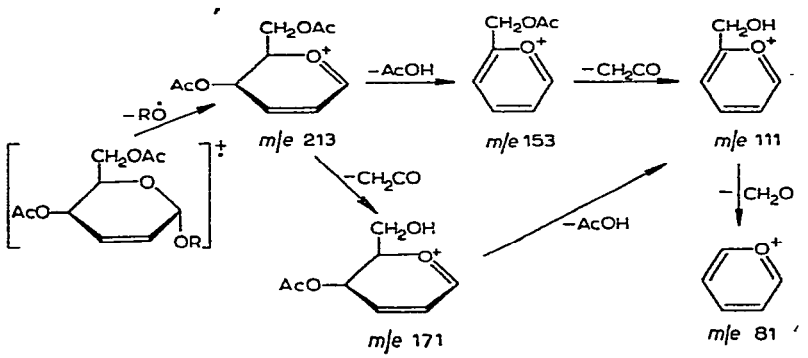


RD_1 ; *m/e* 128 RD_5 : *m/e* 86

SCHEME 2

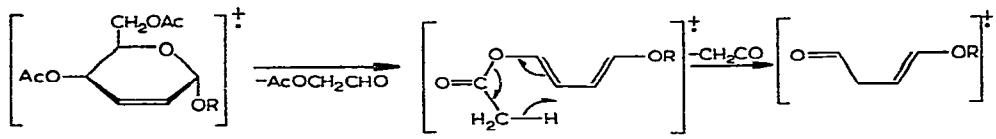
Evidence in support of the proposed RD fragmentation mechanism was obtained by recognition of metastable peaks for the transitions $RD_1 \rightarrow RD_4$, $RD_1 \rightarrow RD_5$, $RD_5 \rightarrow RD_7$, and of ions corresponding to RD_1+1, RD_3+1, RD_5+1 (possibly RD_6+1) and RD_7+1 in the spectra of *O*-deuterated samples of the glycosides.

For each of the acetates (**1c–e** and **2a**, Figs. 3–6), five ions produced by loss of the aglycon (P mechanism) and subsequent degradations were observed at *m/e* 213, 171, 153, 111, and 81 (Scheme 3; 81 and 171 of low intensity for the alkyl glycosides **1c** and **2a**), and this represents the most important P fragmentation pathway occurring in these compounds.



SCHEME 3

Retrodiene ions (Scheme 4, RD_1) are also readily apparent in the spectra of the acetates (**1c–e, 2a**), and these then simply lose ketene as shown in Scheme 4. Other subsequent fragmentations are relatively unimportant, so the degradation of the RD_1 ions is appreciably simpler than that of the corresponding hydroxyl-containing ions (Scheme 1, RD_1). The spectra (Figs. 3–6) reveal that the relative significance of the P and RD degradation pathways depend on structure; those compounds having simple aglycons (**1c** and **2a**) favour the retrodiene mechanism, whereas the more complex glycosides (**1d** and **1e**) give intense P fragments. Evidently, the C-1–O-1 bonds in the latter pair cleave relatively readily as has already been noted for methylated phenyl glycosides[11,12] and disaccharides[13].



SCHEME 4

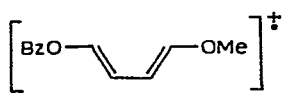
RD$_1$: R = Me, *m/e* 142; R = Et, *m/e* 156; R = *p*-$C_6H_4NO_2$, *m/e* 249; R = X*, *m/e* 370

R = Me, *m/e* 100; R = Et, *m/e* 114; R = *p*-$C_6H_4NO_2$, *m/e* 207; R = X*, *m/e* 328

* X = 6-deoxy-1,2 : 3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl

The two general modes of degradation observed for these acetates are those already proposed by Rosenthal[4] for the fragmentation of methyl 4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranoside, but under the conditions used in the present work much greater specificity within the P series was found. Whereas Rosenthal found appreciable proportions of ions derived by loss of the substituents at C-4 and C-5, in the present study P fragmentation occurred almost exclusively by loss of the aglycon.

Replacement of the acetyl groups in such compounds by benzoyl groups leads to almost complete domination of the fragments derived from the carbohydrate moieties by ions having their origin in the ester groups. Thus, in the spectrum of methyl 4,6-di-*O*-benzoyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (**1f**, Fig. 7), the only structurally significant ion of any intensity was the retrodienic ion **3**, *m/e* 204



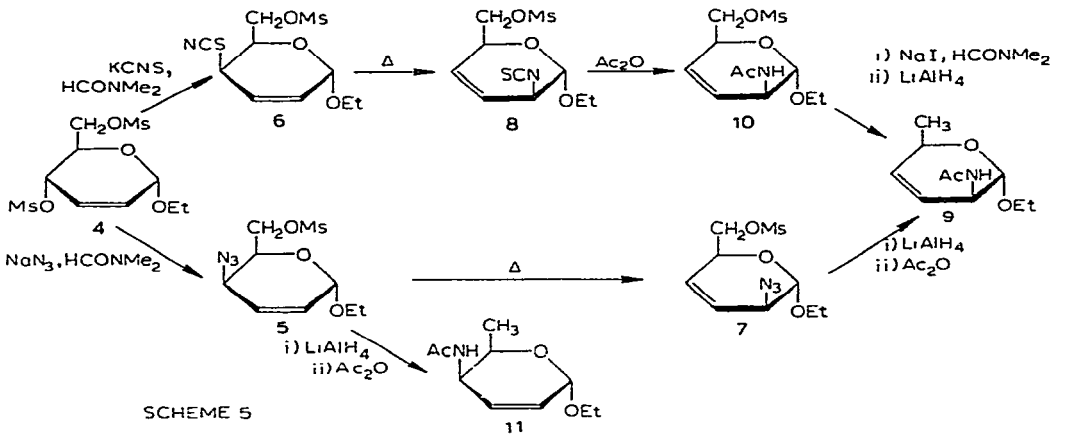
3

which, however, established that the molecule underwent the expected type of fragmentation. The main peaks had *m/e* 122 $(PhCOOH)^{+\cdot}$, 105 $(PhCO)^+$, and 77 $(Ph)^+$. From the spectrum of ethyl 4-*O*-benzoyl-2,3-dideoxy-6-*O*-methanesulphonyl-α-D-*threo*-hex-2-enopyranoside (**2b**, Fig. 8), expanded to amplify the structurally significant ions, fragments of the RD and P series were detected as follows: *m/e* 218 $(M-MsOCH_2CHO)^{+\cdot}$, 311 $(M-EtO\cdot)^+$, 189 $(M-EtO\cdot-PhCOOH)^+$, 125 $(M-EtO\cdot-PhCOOH-SO_2)^+$, 81 (pyrilium ion, favoured, in this case, by the electron-withdrawing properties of the methanesulphonyloxy group).

From the mass spectra of the aforementioned glycosides, it is concluded that for 2,3-dideoxy-hex-2-enopyranoside derivatives with different groups at C-1, C-4, and C-6 the two main directions of fragmentation are direct loss of the ring substituents and retrodiene cleavage. In the latter case, the primary dienic fragment contains C-1–C-4 and the C-1 and C-4 substituents, so recognition of this ion confirms that the species undergoing cleavage contained a 2,3-double bond. This generalisation was then utilised in the structural analysis of compounds obtained by nucleophilic displacements of allylic substituents from C-4 of hex-2-enopyranoside derivatives,

and which could have been derived by either a direct substitution mechanism or by entry of the nucleophiles at C-2 and migration of the double bond to the 3,4-position.

Treatment of ethyl 2,3-dideoxy-4,6-di-*O*-methanesulphonyl-α-D-*erythro*-hex-2-enopyranoside[14] (**4**) with sodium azide and potassium thiocyanate in *N*,*N*-dimethylformamide gave products **5** and **6**, respectively, and these isomerised on heating to give compounds **7** and **8**. The rearranged products were then each converted into ethyl 2-acetamido-2,3,4,6-tetradeoxy-α-D-*threo*-hex-3-enopyranoside (**9**) as illustrated in Scheme 5, and the 2,3-unsaturated isomer **11** of the product **9** was obtained by direct reduction of compound **5** followed by acetylation. All of the compounds in Scheme 5 have given correct chemical and p.m.r. spectra, although the positions of the double bonds were difficult to assign by n.m.r. means; full details of this work will be reported in a separate communication[9].



SCHEME 5

In the spectrum of compound **11** (Fig. 9), the most intense ions were observed at *m/e* 155, 113, and 84, indicating clearly that retrodienic fragmentation of a 2,3-unsaturated hexoside derivative had occurred (Scheme 6), and establishing that the displacement of the mesyloxy group (from compound **4**) took place at C-4 without rearrangement of the allylic system. On the other hand, the spectrum of the 6-deoxy compound **9** obtained from both the azide **5** and the thiocyanate **6** revealed no such ions (Fig. 10). Instead, the most abundant ions had *m/e* 125 and 83, which can be

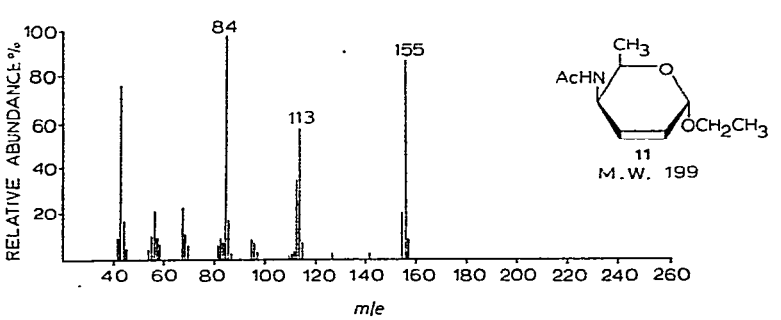


Fig. 9.

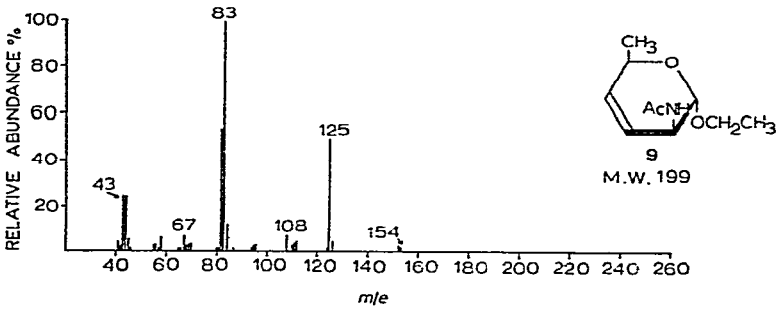


Fig. 10.

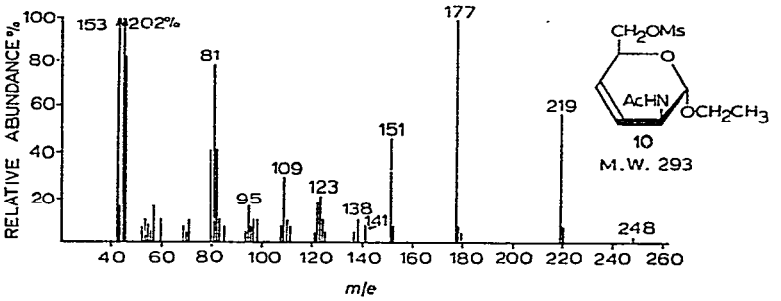
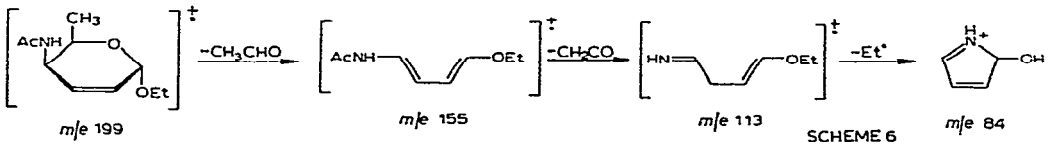


Fig. 11.

accounted for by the fragmentation shown in Scheme 7, *i.e.*, by retrodiene fragmentation of a 3,4-unsaturated glycoside. Compound **9** therefore was formed after sigma-

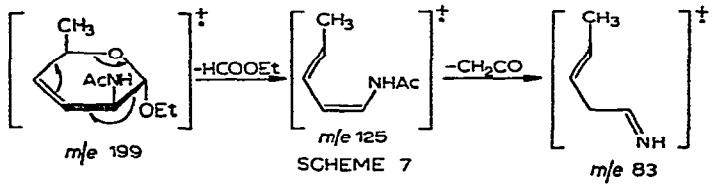


SCHEME 6

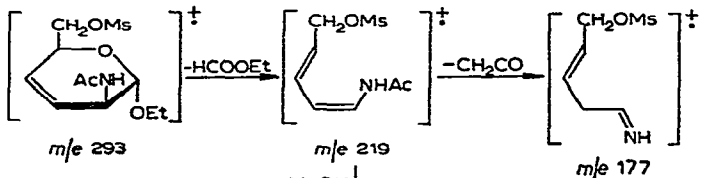
tropic rearrangements of the azide **5** and the thiocyanate **6** as shown in Scheme 5. The spectrum of compound **10** (Fig. 11) confirms this by showing intense retrodienic ions (Scheme 8) derived from a 3,4-unsaturated compound, and no ion with *m/e* 155 $(M-MsOCH_2CHO)^+$ expected for the 2,3-unsaturated isomer.

The spectrum of the azide **5** obtained by selective displacement of a mesyloxy group from the disulphonate **4** showed ions of *m/e* 139, 111, and 82 (Fig. 12), indicative of retrodienic fragmentation of a 2,3-unsaturated glycoside (Scheme 9). No *m/e* 203 ion $(M-HCOOEt)^+$ was observed, confirming the absence of a 3,4-double bond. Other ions of high *m/e* ratios in the spectrum of this compound are attributable to P fragmentations.

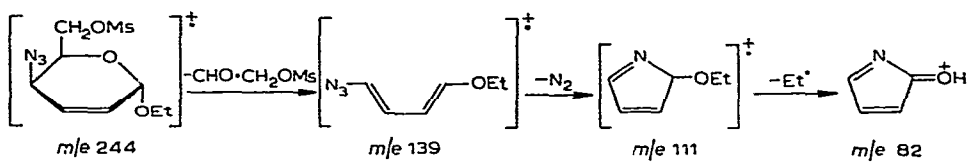
Thiocyanate **6**, expected to be structurally analogous to the azide **5**, surprisingly gave a spectrum (Fig. 13) identical to that of the isothiocyanate **8** into which it was converted on heating. This thermal rearrangement therefore also occurred in the mass spectrometer prior to fragmentation. That the thermodynamically stable isomer contains a 3,4-unsaturated ring is evidenced by the formation from it of an ion *m/e* 219



SCHEME 7

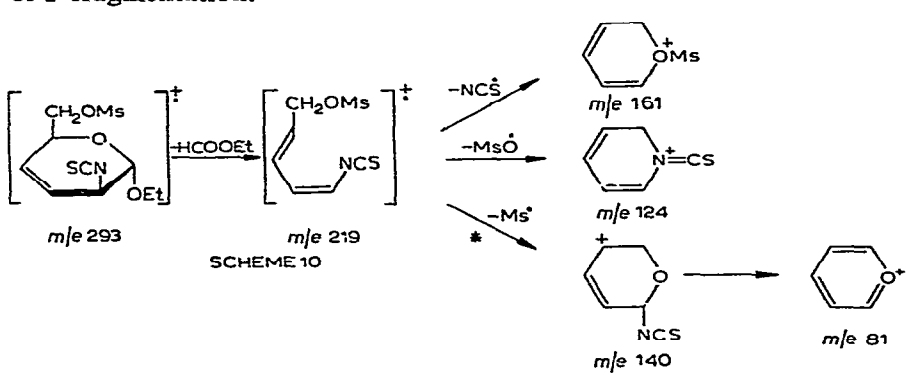


SCHEME 8



SCHEME 9

and of related fragments (Scheme 10), and further by the absence of an ion of *m/e* 155 $(M-MsOCH_2CHO)^+$. Other ions in this spectrum are readily assignable to products of P fragmentation.



SCHEME 10

Mass spectrometry therefore failed as a means of characterising compound **6**. However, when the *erythro*-thiocyanate (**12**, obtained from the *threo*-isomer of the dimethanesulphonate **4**) and the isothiocyanate [9] (**13**) were examined, it was ascertained that the former had a 2,3-unsaturated structure. The spectrum of compound **13** was

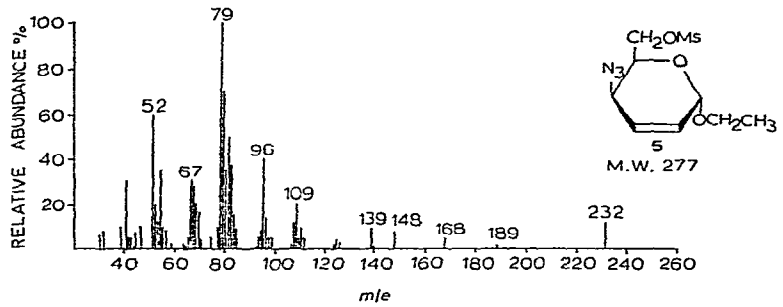


Fig. 12.

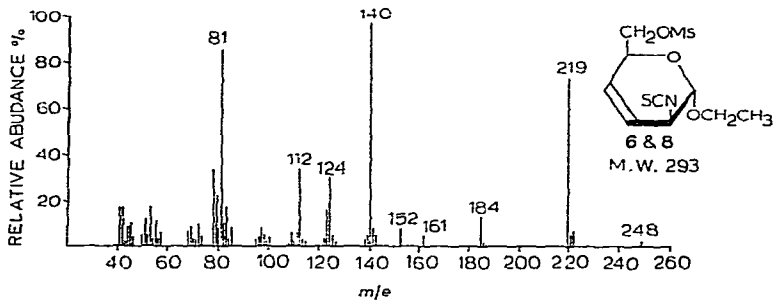
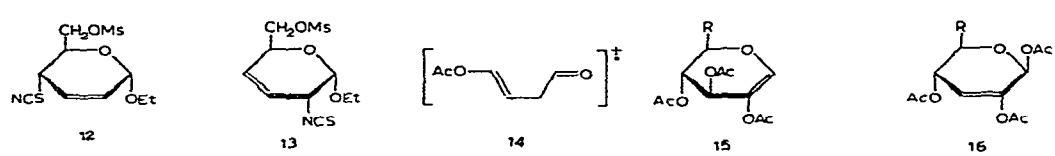


Fig. 13.

very similar to those of the *threo*-isomers (**6** and **8**), but in the spectrum of the thiocyanate **12** the ion *m/e* 219 $(M-HCOOEt)^{+}$ had an intensity of only 20% (65–75% for the other three isomers), whereas the ion *m/e* 155 $(M-MsOCH_2CHO)^{+}$ now also had intensity 20%. This indicates that compound **12** has the illustrated structure, and that the thermal isomerisation occurs less readily with this *erythro* glycoside than with the *threo* isomer **6**; this conclusion is consistent with results obtained on heating the compounds in solution[9], and with expectations, since the thiocyanate group is *quasi*-axial and thus more suitably oriented for migration in the *threo*-compound **6**.

The spectra of such thermally unstable allylic derivatives as the thiocyanate **6** clearly must be interpreted with caution, since retrodienic fragments may not be derived from the initial compounds. Allylic acetates are subject to this difficulty, and allylic rearrangements have been described for ions containing this structural feature and which were derived from saturated aldose peracetates[15]. Since tri-*O*-acetyl-D-glucal rearranges on heating to 1,4,6-tri-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enose[16], it might be expected to give on fragmentation an ion *m/e* 170 $(M-AcOCH_2CHO)^{+}$; this was formed in significant intensity, and the expected daughter radical-ion (**14**, *m/e* 128) was also present[4].



In related fashion, 1-deoxyald-1-enose esters (hydroxyglycal esters) (*e.g.*, **15**, R = H or CH_2OAc) rearrange thermally to 3-deoxyald-2-enoses esters[17] (*e.g.*, **16**, R = H or CH_2OAc), and it is not surprising, therefore, that Rosenthal found an ion *m/e* 228 $(M-AcOH_2CCHO)^+$ in the mass spectrum of tetra-*O*-acetyl-2-hydroxy-D-glucal (**15**, R = CH_2OAc). The intensity of this ion, however, was minute in his spectrum. In our spectrum of this compound, this ion had appreciable intensity (*ca.* 8% of base peak *m/e* 126), but, as revealed by a metastable ion at *m/e* 180.5, was derived (at least in part) by loss of acetic acid from the ion *m/e* 288, and not directly from the molecular ion; allylic isomerisation prior to fragmentation is therefore not established in this case. Surprisingly, when 1,2,4-tri-*O*-acetyl-3-deoxy-β-D-*glycero*-

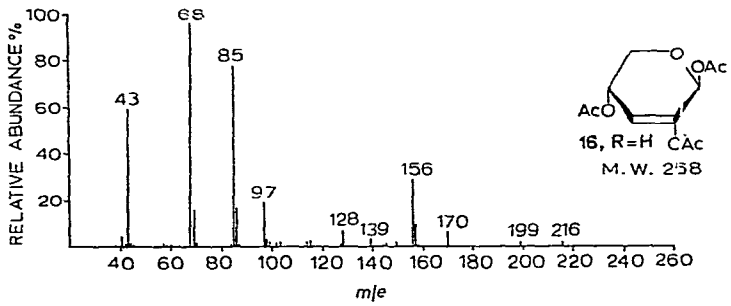
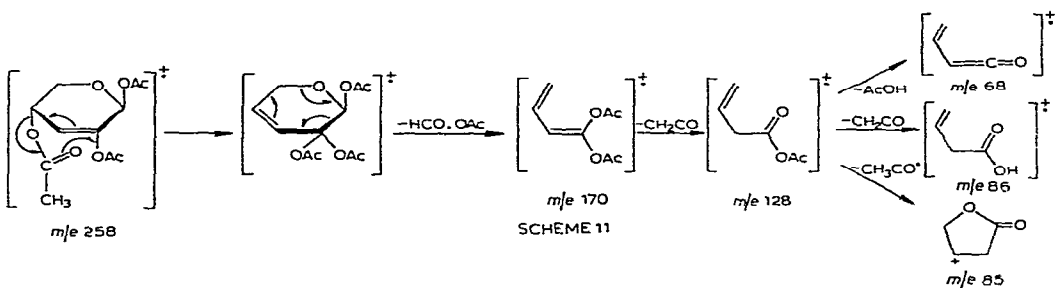


Fig. 14.

pent-2-enopyranose[17] (**16**, R = H) was examined, no ion (*m/e* 228) formed by retrodienic loss of formaldehyde was produced (Fig. 14), but instead a series was present which indicated that 2,3- to 3,4-migration of the double bond had preceded fragmentation (Scheme 11). This is somewhat unexpected, since this derivative (**16**, R = H) can be obtained by thermal rearrangement of the hydroxyglycal ester[17] (**15**, R = H), and since efforts to carry out further isomerisations of several 2,3-



unsaturated compounds failed[18]. However, it is consistent with the fact that the system

OAc OAc

| |

–CH = CH–CH–O– is thermodynamically more stable than –CH–CH = CH–O–,

as evidenced by the rearrangements[17] **15**→**16**, and tri-*O*-acetyl-D-glucal to 1,4,6-tri-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enose[16]. We have observed ions of low intensity at *m/e* 242, 200, 140, and 98 in the spectrum of tetra-*O*-acetyl-2-

hydroxy-D-glucal (**15**, R = CH_2OAc) which suggests that this compound may also isomerise partially to a 3,4-unsaturated isomer before fragmentation.

EXPERIMENTAL

The mass spectra were measured with an MX-1303 instrument at an ionising potential of 70 eV. Samples were introduced into the ionisation chamber either by way of a stainless-steel reservoir heated at 100–120° (compounds **1a**, **1b**, **1c**, **2a**, **9**, **11**), or directly into this ion source at 30–50°.

ACKNOWLEDGMENTS

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ACETAL MIGRATION IN THE METHANOLYSIS OF 1,2:5,6-DI-*O*-ISOPROPYLIDENE-α-D-ALLOFURANOSE; THE FOUR METHYL D-ALLOSIDES

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ABSTRACT

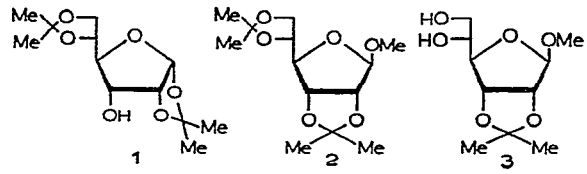
The acid-catalysed methanolysis of 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose gives at least six products in proportions depending on the reaction conditions. The products are the methyl glycosides of α- and β-D-allopyranose and α- and β-D-allofuranose, methyl 2,3-*O*-isopropylidene-β-D-allofuranoside, and methyl 2,3:5,6-di-*O*-isopropylidene-β-D-allofuranoside. The last two compounds are products of acetal migration.

INTRODUCTION

Methyl α- and β-D-allopyranosides were required in connection with other work, and although methods for their synthesis have been reported recently[1,2], the methanolysis of the readily available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose appeared to offer a convenient route to both compounds which could then be conveniently separated by ion-exchange chromatography.

RESULTS AND DISCUSSION

When 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**1**) (prepared by an improved method) was refluxed for 20 h in 2.4% methanolic hydrogen chloride, the products were shown by n.m.r. spectroscopy to contain *O*-isopropylidene compounds. Crystallization of the product mixture gave, in low yield, a methyl di-*O*-isopropylidenehexoside **2**, and, subsequently, methyl β-D-allopyranoside. Chloroform extraction removed the remaining *O*-isopropylidene compounds, and fractionation of the aqueous layer on a strongly basic anion-exchange resin gave, in order of elution, syrupy methyl mono-*O*-isopropylidenealloside **3** (contaminated with small proportions of other compounds), methyl α-D-allopyranoside (14%, characterised as the tetraacetate), methyl β-D-allopyranoside (total yield, 32%), methyl α-D-allofuranoside (2.5%), and methyl β-D-allofuranoside (5.5%). The chloroform extract contained mainly two compounds which were separated by several partitions between chloroform and water to give more di-*O*-isopropylidenehexoside **2** (total yield, 1.6%) and mono-*O*-isopropylidenealloside **3** (total yield, 26%).



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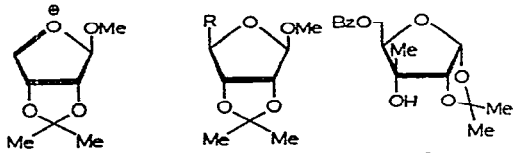

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The structure of **3** was established by n.m.r. and mass spectroscopy. The mass spectrum showed a strong P−15 peak at 219, which is due to loss of Me from the *O*-isopropylidene function[3], and a weak P−31 peak due to loss of OMe from C-1. The ion **4** at 173, resulting from cleavage of the C-4–C-5 bond, established the furanoside structure and also the position of the *O*-isopropylidene function. The β-D configuration followed from the n.m.r. spectrum ($J_{1,2}$ <0.5 Hz) and optical rotation ($[\alpha]_D$ −43° in water). Compound **3** gave a crystalline ditoluene-*p*-sulphonate which did not have a sharp melting point, but which had n.m.r. and mass spectra consistent with its structure as the ditoluene-*p*-sulphonate of **3**; the mass spectrum contained a very weak parent ion at 542 and a strong P−15 ion, which was shown to have the correct formula by accurate mass measurement. Compound **3** consumed one equivalent of periodate with concomitant liberation of formaldehyde, and on acid hydrolysis gave a mixture of allose and 1,6-anhydroallose.

That **2** was methyl 2,3:5,6-di-*O*-isopropylidene-β-D-allofuranoside was suggested by the n.m.r. data; the values of $J_{1,2}$ (~0 Hz), $J_{2,3}$ (6 Hz), and $J_{3,4}$ (~0 Hz) were similar to those[4,5] of **3** and other 2,3-*O*-isopropylidenefuranose derivatives of type **5**. The mass spectrum of **2** showed a strong P−15 peak at 259, and a weak P−31 peak at 243 (loss of OMe). The 173 ion could be formed by cleavage of the C-4–C-5 bond in the furanoside **2**, or by "h-rupture" in the 2,3:4,6-di-*O*-isopropylidenepyranoside*. The presence of a very strong peak at 101, however, suggested[3] the furanoside structure **2**, and this was proved by the formation of **2** from the mono-*O*-isopropylideneallofuranoside **3** under mild, neutral conditions. The β-D configuration followed from the value of $J_{1,2}$ and the optical rotation ($[\alpha]_D$ −54°).

The methyl α- and β-D-allofuranosides, not previously reported, gave a mixture of allose and 1,6-anhydroallose on acid hydrolysis. The anomeric configurations were assigned from the values of $J_{1,2}$ (3 Hz for α-anomer, 2 Hz for β-anomer) and $[\alpha]_D$ (+131[c] for α-anomer, −57.5° for β-anomer).

The unfractionated products of methanolysis could be conveniently analysed by n.m.r. spectroscopy by integration of the signals in the OMe region (assuming all

*The possibility of an "h-type" fragmentation[6] was pointed out by a referee.

products were methyl glycosides). Thus, di-*O*-isopropylideneallose **1** in 2.4% methanolic hydrogen chloride gave, after refluxing for 20 h, a product containing 46% of β-pyranoside, 23% of *O*-isopropylidene derivative **3**, and 23% of α-pyranoside + β-furanoside. An increase in the reaction time to 48 h decreased the amount of compound **3**, and the major products were then the β-pyranoside (51%) and α-pyranoside (30%). Milder reaction conditions [*e.g.*, methanol containing cation-exchange resin (H^+ form) at room temperature] gave, after three days, a product in which the *O*-isopropylidenealloside **3** predominated (58% estimated by n.m.r.). Controlled hydrolysis of **3** in methanol–N hydrochloric acid (9:1) gave methyl β-D-allofuranoside as the major product.

The *O*-isopropylidene compounds **2** and **3** are products of acetal migration; presumably **3** is formed from 1,2-*O*-isopropylidene-α-D-allofuranose, since this compound was shown by t.l.c. to be formed in the early stages of the reaction*. 2,3-*O*-Isopropylidene-D-allose is presumably an intermediate in the rearrangement, since it has been shown[7] that in the methanolysis of 1,2-*O*-isopropylidene-α-D-glucofuranose D-glucose is formed prior to methyl D-glucofuranosides. Similarly, 2,3:5,6-di-*O*-isopropylidene-D-allose would be an intermediate in the formation of **2**.

The observation of these acetal migrations is important because such migrations are not normally considered to occur under mild, acidic conditions[8,9]. Attempts by Baggett *et al.*[10] to detect, by n.m.r. spectroscopy, migration of *O*-isopropylidene groups in alditol acetals led to the conclusion that such migrations did not occur under hydrolytic conditions. The closely related rearrangement of the 1,2-*O*-isopropylidene-D-ribofuranose derivative **6** to the 2,3-*O*-isopropylidene derivative in acidic methanol solution has recently been reported[11]. Migration of an *O*-isopropylidene function on a pyranose ring is now known also; the 2,3-*O*-isopropylidene derivative is formed as an intermediate in the hydrolysis, in aqueous acetic acid of 1,6-anhydro-3,4-*O*-isopropylidene-β-D-talopyranose[12]. The recently reported[13] rearrangement of 1,2-*O*-isopropylidene-α-D-apio-L-furanose differs from those described above in that the mechanism involves opening of the furanose ring.

The α- and β-D-allopyranosides can therefore be obtained in good yield from 1,2:5,6-di-*O*-isopropylidene-α-D-allose (**1**) provided that the conditions ensure complete reaction of the relatively stable (in acidic, anhydrous methanol) 2,3-*O*-isopropylidene-D-alloside **3**.

EXPERIMENTAL

General methods. — Solutions were concentrated under diminished pressure. Melting points are uncorrected. Thin-layer chromatography (t.l.c.) was performed on Silica Gel G (Merck), and compounds were detected by spraying with 5% ethanolic sulphuric acid followed by heating for 10 min at 120°. Gas–liquid chromatography (g.l.c.) was carried out on the trimethylsilyl ethers[14] on 8-foot glass columns of 3.4% silicone rubber UCW 98 (F and M, LP166) on silanized 60–80 Chromosorb W, and

*The detection by paper chromatography of allose also in the early stages of the reaction demonstrates that methanolysis and migration of the acetal group are competing processes.

10% Carbowax 20M on 60–80 Chromosorb W, using an F and M Model 810 gas chromatograph with flame-ionization detector. N.m.r. spectra were recorded on a Varian HA-100 MHz spectrometer. Mass spectra were measured on an A.E.I. MS9 spectrometer by using the direct-insertion probe.

1,2:5,6-Di-O-isopropylidene-α-D-allofuranose (**1**). — The method of Sowa and Thomas[15] for the preparation of **1** from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose was modified by purifying the intermediate hex-3-ulose acetal *via* its bisulphite addition compound[16]. The regenerated hexulose derivative was reduced, without isolation, to **1** in 61% overall yield. Thus, column chromatography was unnecessary.

Methanolysis of 1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**1**). — The acetal **1** (9.4 g) was refluxed in anhdyrous methanol (315 ml) containing 2.4% (w/v) of hydrogen chloride (generated from 15 ml of acetyl chloride) for 20 h. The neutralised [Amberlite CG-45 (HO^-) resin] solution was shown by t.l.c. (ethanol) to contain three major and three minor products. Filtration and evaporation of the methanolic solution gave a syrup which when dissolved in water deposited methyl 2,3:5,6-di-*O*-isopropylidene-β-D-allofuranoside (**2**, 29 mg), m.p. 67–68°. Aliquots were removed from the filtrate for n.m.r and g.l.c. analysis. The n.m.r. spectrum (pyridine) contained two doublets (τ 8.50 and 8.56) due to *O*-isopropylidene groups and four OMe signals (τ 6.46, 6.59, 6.61*, and 6.71; estimated relative intensity 6:1:3:3, respectively). G.l.c. analysis was of limited use, since methyl α- and β-D-allopyranosides had the same retention times on each column.

The syrup obtained on concentrating the aqueous solution gave, when diluted with absolute ethanol (7 ml), a solid (1.62 g, m.p. 155–159°) which, on recrystallisation from absolute ethanol, gave methyl β-D-allopyranoside (1.22 g), m.p. 158–160°; lit.[1] m.p. 154–155°). The mother liquor was concentrated to dryness, and the residue was dissolved in water (70 ml) and extracted with chloroform (7 × 60 ml). The aqueous layer was concentrated to dryness, and another crop (0.38 g) of methyl β-D-allopyranoside obtained on crystallisation from ethanol. The residue from the mother liquor was dissolved in water (4 ml) and fractionated on a column (2.5 × 54 cm) of Deacidite FF (HO^-) resin[17] by eluting with carbon dioxide-free water and collecting *ca.* 10-ml fractions. Fractions 11–16 gave methyl 2,3-*O*-isopropylidene-β-D-allofuranoside (**3**, 118 mg), shown by n.m.r. and t.l.c. to contain small proportions of other compounds. Fraction 17 contained **3** and methyl α-D-allopyranoside. Fractions 18–21 gave the pure α-D-pyranoside (0.99 g, characterised by n.m.r.[1] and as its tetraacetate, m.p. and mixed m.p. 121–122°). Fraction 22 gave a mixture of α- and β-D-pyranosides (24 mg). Fractions 23–32 gave slightly impure β-D-pyranoside (0.9 g), recrystallisation of which gave material (0.63 g) having m.p. 160–161°. Fractions 36–45 gave methyl α-D-allofuranoside (174 mg) which, after recrystallisation from ethanol, had m.p. 108–109°, $[\alpha]_D^{25}$ +131° (*c* 0.265, water).

Anal. Calc. for $C_7H_{14}O_6$: C, 43.3; H, 7.26. Found: C, 43.3; H, 6.81.

*This peak was subsequently resolved into two in expanded spectra (250 Hz sweep width).

Fractions 78–100 gave a syrup (387 mg) which was crystallised from ethyl acetate to give methyl β-D-allofuranoside, m.p. 86–88°, $[\alpha]_D^{25}$ −57.5° (*c* 0.195, water).

Anal. Calc. for $C_7H_{14}O_6$: C, 43.3; H, 7.26. Found: C, 43.2; H, 7.26.

The chloroform extract contained mainly the two compounds having highest R_F values (0.4 and 0.8, t.l.c. in ethyl acetate). These were separated by extraction of the chloroform solution (45 ml) with water (5×45 ml). Compound **2** (R_F 0.8) remained in the chloroform layer, concentration of which gave a syrup (0.21 g) which crystallised on standing. Recrystallisation from aqueous ethanol, followed by sublimation, gave methyl 2,3:5,6-di-*O*-isopropylidene-β-D-allofuranoside (**2**, 130 mg), m.p. 67–68°, $[\alpha]_D^{23}$ −54° (*c* 0.19, chloroform); mass spectrum: *m/e* 259 (50%), 243 (2%), 173 (11%), 141 (20%), 126 (12.5%), 115 (16%), 113 (12.5%), 101 (100%); n.m.r. data ($CDCl_3$): τ 5.11 (1-proton singlet, H-1), 5.19 (1-proton doublet, *J* 6 Hz, H-3), 5.44 (1-proton doublet, *J* 6 Hz, H-2), 5.92–6.14 (4-proton multiplet, H-4, H-5, H-6a, H-6b), 6.75 (3-proton singlet, OMe), 8.52, 8.56, 8.65, 8.67 (four 3-proton singlets, CMe).

Anal. Calc. for $C_{13}H_{22}O_6$: C, 56.9; H, 8.08. Found: C, 56.9; H, 8.40.

Concentration of the aqueous layer gave methyl 2,3-*O*-isopropylidene-β-D-allofuranoside (**3**) as a syrup (2.07 g), $[\alpha]_D^{25}$ −43° (*c* 0.35, water); i.r. data: ν_{max}^{film} 3450 cm^{-1} (strong, broad OH absorption); mass spectrum: *m/e* 219 (11%), 203 (2%), 173 (20%), 159 (6%), 141 (2%), 127 (8%), 115 (11%), 113 (17%), 59 (100%); n.m.r. data (pyridine): τ 3.8 (broad singlet, OH), 4.63 (1-proton broadened doublet, $J_{2,3}$ 6, $J_{3,4} \leqslant 1$ Hz, H-3), 4.85 (1-proton singlet, $J < 0.5$ Hz, H-1), 5.23 (1-proton doublet, $J_{2,3}$ 6 Hz, H-2), 5.35 (1-proton broadened doublet, $J_{3,4} \leqslant 1$, $J_{4,5}$ 9 Hz, H-4), 5.72–6.13 (3-proton multiplet, H-5, H-6a, H-6b), 6.72 (3-proton singlet, OMe), 8.48 and 8.68 (3-proton singlets, CMe); the presence of two exchangeable protons was shown by the $CDCl_3$ and $CDCl_3/D_2O$ spectra. The *O*-isopropylidenealloside **3**, when treated with 0.015M sodium metaperiodate, consumed 1 equivalent of periodate (determined spectrophotometrically)[18] in 3 and 48 h. In a separate experiment, formaldehyde was isolated as the dimedone derivative (32%, m.p. and mixed m.p. 196° after two recrystallisations) after oxidation for 20 h.

When the methanolysis was carried out for a longer period (48 h), n.m.r. analysis of the product (in pyridine) gave the following results.

Compound	*OMe τ value*	%[a]
β-Pyranoside	6.47	51
α-Furanoside	6.60	4
α-Pyranoside	6.62	30
β-Furanoside	6.63	6
Acetal **3**	6.71	9

[a]Calculated from the average of three integrals.

Methyl 2,3-O-isopropylidene-5,6-di-O-toluene-p-sulphonyl-β-D-allofuranoside. — The acetal **3** (106 mg) in anhydrous pyridine (5 ml) was treated with toluene-*p*-

sulphonyl chloride (260 mg). Reaction was shown by t.l.c. to be complete after 18 h. The reaction mixture was poured into ice-cold water, and the emulsion which formed slowly precipitated. Recrystallisation of the filtered solid (m.p. 90–100°) from ethanol gave very fine needles (73 mg), m.p. 100–120°. Recrystallisation from chloroform–light petroleum gave material (67 mg), m.p. 104–120°, which was homogeneous on t.l.c.; the i.r. spectrum showed the absence of hydroxyl and the presence of tosyl groups; mass spectrum: *m/e* 542 (very weak), 527 (14%), 511 (~0.2%), 467 (<0.1%), 424 (~0.2%), 252 (10%), 173 (17%), 155 (100%), 141 (5%), 139 (7%), 127 (7%), 115 (9%), 113 (9%); n.m.r. data ($CDCl_3$): τ 2.20–2.38 (4-proton multiplet, ArH), 2.62–2.78 (4-proton multiplet, ArH), 5.14 (1-proton singlet, H-1), 5.28–5.46 (2-proton multiplet), 5.55 (1-proton doublet, *J* 6 Hz, H-2 or H-3), 5.63–6.02 (3-proton multiplet), 6.78 (3-proton singlet, OMe), 7.57 (3-proton singlet, ArMe), 8.59, 8.76 (two 3-proton singlets, CMe). Microanalysis was unsatisfactory, but the molecular formula was established by accurate mass measurement of the P − 15 ion; calc. for $C_{23}H_{17}O_{10}S_2$: 527.1046; found 527.1023±0.0025.

Hydrolysis of the methyl D-*allofuranosides and monoacetal* **3**. — Samples (*ca.* 30 mg) of methyl α-D-allofuranoside, methyl β-D-allofuranoside, and **3** were hydrolysed separately in water (10 ml) containing Bio Rad AG 50W-x2 (H^+) resin (*ca.* 1 ml) for 2.5 h at 100°. Analysis by paper chromatography revealed (detection with alkaline $AgNO_3$) spots corresponding to allose and 1,6-anhydroallose. The same mixture was obtained on hydrolysis of 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose. The combined, filtered hydrolysates were concentrated to a syrup which crystallised. Recrystallisation from absolute ethanol gave β-D-allose, m.p. 129–132°, lit.[19] m.p. 128°.

Controlled hydrolysis of the monoacetal **3**. — A solution of **3** (92 mg) in methanol (4.5 ml) containing N hydrochloric acid (0.5 ml) was refluxed for 35 min. The neutralised [Amberlite CG-45 (HO^-) resin] and filtered solution was concentrated to a syrup which was shown to contain mainly methyl β-D-allofuranoside by n.m.r. and g.l.c. data. The relative retention times (min) on the Carbowax column (operated at 172°) were methyl α- and β-D-allopyranoside (1.0), methyl β-D-allofuranoside (1.43), methyl α-D-allofuranoside (2.28), and methyl 2,3-*O*-isopropylidene-β-D-allofuranoside (2.39); the β-furanoside was formed in 76% yield, determined by using a disc integrator and assuming that all compounds present gave equivalent detector responses. The yield of β-furanoside was estimated to be 68% from the integral of the n.m.r. spectrum.

*Controlled methanolysis of 1,2:5,6-di-*O*-isopropylidene-α-*D*-allofuranose* (**1**). — The acetal **1** (0.43 g) in anhydrous methanol (10 ml) was magnetically stirred with Bio Rad resin (4 ml, AG 50W-x2, H^+ form, washed with anhydrous methanol) at room temperature, and the reaction was followed by t.l.c. and paper chromatography. After 5 min, the mixture contained mainly starting material and 1,2-*O*-isopropylidene-α-D-allofuranose, together with a trace of allose. After 30 min, **3** was also present and at least one other unidentified compound. After 3 days, **3** was the major component, and was estimated by n.m.r. to constitute 58% of the mixture.

*Formation of methyl 2,3:5,6-di-*O*-isopropylidene-β-*D*-allofuranoside* (**2**) *from the*

mono-O-isopropylideneallofuranoside **3**. — Anhydrous copper sulphate (*ca.* 100 mg) was added to a solution of **3** (63 mg) in dry acetone (5 ml). T.l.c. analysis after 3 h at room temperature indicated that the solution contained the di-*O*-isopropylidene-alloside **2**, together with a small proportion of a compound having the same R_F value as **3**. The latter compound was still present after 23 h. The product was isolated crystalline by evaporation of the filtered solution; sublimation gave the di-*O*-isopropylidenealloside, m.p. 65–66°, mixed m.p. with **2** isolated above, 65–67°.

ACKNOWLEDGMENT

The gift of a sample of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranoside from Professor S. J. Angyal is gratefully acknowledged.

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Note

Hydrazinadditionsverbindungen von 1,2-*O*-Isopropyliden-α-D-*xylo*-hexofuranurono-6,3-lacton-5-ulose*

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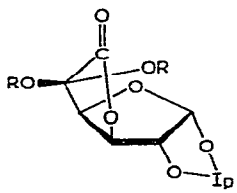
1,2-*O*-Isopropyliden-α-D-*xylo*-hexofuranurono-6,3-lacton-5-ulose ist auf mehreren Wegen[1,2], am besten durch katalytische Oxydation[3] von 1,2-*O*-Isopropyliden-α-D-glucofuranurono-6,3-lacton zugänglich. Die Ulose kristallisiert stets als Hydrat **1**, denn sie läßt sich mit Hexamethyldisilazan und Chlortrimethylsilan in Pyridin zum Bis(trimethylsilyl)äther **2** umsetzen. Das n.m.r.-Spektrum stimmt mit der Struktur **2** überein und das Massenspektrum zeigt zwar kein Molekülion (MZ 376), dafür aber ein Ion der größten Masse MZ 361 = $M^+ - 15$. Dieses Ion kommt durch Abspaltung eines Methylradikals aus der Isopropyliden- oder Trimethylsilylgruppe des Molekülions zustande. Die Additionsverbindung der Ulose mit Wasser **1** besitzt somit eine hohe Stabilität, die durch die benachbarte Lactoncarbonylgruppierung bedingt ist, die die Additionsfreudigkeit nucleophiler Reagenzien entsprechend erhöht.

Es wurde gefunden, daß **1** auch stickstoffhaltige Addukte zu bilden vermag. Mit Phenylhydrazin in Äthanol setzt sich **1** in hoher Ausbeute zum kristallinen Addukt **3** um. Ein Phenylhydrazon entsteht nicht und kann auch nicht durch Wasserabspaltung aus **3** dargestellt werden. Der Lactonring wird durch das schwach basische Phenylhydrazin nicht gespalten, denn die Lactonbande ist im i.r.-Spektrum von **3** (1780 cm^{-1}) unverändert vorhanden. Mit Hexamethyldisilazan und Chlortrimethylsilan in Pyridin ergibt **3** den Mono(trimethylsilyl)äther **4**. Im n.m.r.-Spektrum von **4** ist eine Trimethylsilylgruppe sichtbar. Das Massenspektrum von **4** zeigt ein Molekülion MZ 394. Als Hauptschritte der Primärspaltungen werden die Abspaltung von Trimethylsilanol ($M^+ - 90$) und Phenyldiimin ($M^+ - 106$) beobachtet. Verbindung **3** stellt somit ein Carbinolhydrazin dar. Nur wenige offenkettige Verbindungen von diesem Typ sind bisher bekannt[4-6]. Die Reaktionsprodukte von Dioxobernsteinsäurepropylester und Oxalessigsäureäthylester mit Phenylhydrazin werden von Anschütz und Pauly[6] als Carbinolhydrazine formuliert.

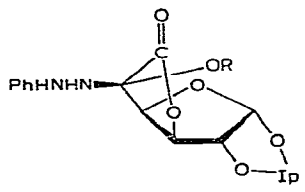
Die Kopplungskonstanten (Versuchsteil) im n.m.r.-Spektrum von **3** entsprechen weitgehend den Werten, wie man sie allgemein in 1,2-Isopropylidenfuranosen findet[7], so daß eine entsprechende Verdrehung des Furanoseringes zu einer „skew"-Konformation anzunehmen ist[7]. Das Molekülmodell von **3** läßt erkennen, daß die

*Hydrazinreaktionen VIII; VII. Mitteil.: H. Paulsen und D. Stoye, *Chem. Ber.*, 102 (1969) 3833.

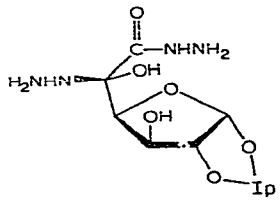
Konfiguration am neuen asymmetrischen Atom C-5 vermutlich so ist, daß der große Phenylhydrazin-Rest in *exo*-Stellung zum ankondensierten Furanosering steht, wie es in Formel **3**, **4**, und **5** gezeigt wird. Eine *endo*-Stellung erscheint aus sterischen Gründen recht unwahrscheinlich.



1 R = H
2 R = Si(Me)$_3$



3 R = H
4 R = Si(Me)$_3$



5

Mit Hydrazin setzt sich **1** ebenfalls zu einem Carbinolhydrazin **5** um. Das stark basische Hydrazin öffnet hierbei den Lactonring zum Hydrazid. Im i.r.-Spektrum von **5** ist die Lactonbande (1780 cm^{-1}) verschwunden, und hierfür erscheint eine Hydrazidbande (1660 cm^{-1}). Das n.m.r.-Spektrum von **5** in Dimethylsulfoxid-d_6 zeigt ein Dublett bei τ 4.13 (H-1) und ein Doppelsignal für die Isopropylmethylgruppen bei τ 8.71. Die restlichen 11 Protonen liegen im Multiplett bei τ 5.6. Von den 11 Protonen sind 8 austauschbar, denn nach dem Austausch mit Deuteriumoxid bleibt ein Multiplett von 3 Protonen des Furanoseringes zurück. Dieses Verhalten steht mit Formel **5** in Übereinstimmung.

Die Carbinolhydrazine vom Typ **3** und **5** besitzen offenbar eine erhöhte Stabilität. Es ist nicht gelungen, Amine, wie Anilin, Benzylamin oder Semicarbazid mit **1** zu entsprechenden Additionsverbindungen umzusetzen. Mit Hydroxylamin wird kein einheitliches Produkt erhalten. Auch die Ergebnisse der Hydrierung von **3** und **5** sprechen dafür, daß aus **1** gebildete Carbinolamine wesentlich instabiler sind. Die Hydrierung von Carbinolaminen verläuft in der Regel so, daß eine C–O-Bindung bevorzugt vor einer C–N-Bindung hydrierend gespalten wird, so daß bei der Hydrierung Amine erhalten werden[8]. Bei analoger Reaktionsfolge müssten aus den Carbinolhydrazinen primär Hydrazino-Verbindungen erhalten werden, die unter N–N-Spaltung in Amine übergehen. Bei der Hydrierung von **3** und **5** wird jedoch primär stets die N–N-Bindung des Hydrazins gespalten. Die so gebildeten Carbinolamine sind instabil und zerfallen unter Aminabspaltung, so daß als Hydrierungsprodukte 1,2-*O*-Isopropyliden-α-D-glucofuranurono-6,3-lacton bzw. dessen Amid zurückerhalten wird.

EXPERIMENTELLER TEIL

Allgemeines. — Alle Reaktionen wurden dünnschichtchromatographisch verfolgt (Kieselgel G nach Stahl), mit Benzol–Äthanol (4:1) als Laufmittel und *N*,*N*-Dimethyl-*p*-phenylendiamin in Schwefelsäure (20%ig) als Sprühmittel. Die n.m.r.-Spektren wurden mit dem Gerät Varian A-60 aufgenommen. Die Massenspektren wurden mit dem Gerät Atlas MAT-SM1 aufgenommen.

1,2-O-Isopropyliden-5-di-O-(trimethylsilyl)-α-D-xylo-hexofuranurono-6,3-lacton-5-ulose-hydrat (**2**). — 1,2-*O*-Isopropyliden-α-D-*xylo*-hexofuranurono-6,3-lacton-5-ulose-hydrat[3] (100 mg) wird in abs. Pyridin (5 ml) gelöst und mit Hexamethyldisilazan (150 mg) und drei Tropfen Chlortrimethylsilan versetzt. Man läßt 4 Stdn. bei Raumtemperatur stehen und engt dann ein. Den Kristallbrei extrahiert man mit Benzol und engt ein. Der erhaltene Sirup wird an der Kapselpumpe getrocknet (163.5 mg, 100%).

Anal. Ber. für $C_{15}H_{28}O_7Si_2$: C, 48.27; H, 7.32. Gef.: C, 48.41; H, 7.28.

5-Dehydro-1,2-O-isopropyliden-5-phenylhydrazino-α-D-gluco-(β-L-ido)-furanurono-6,3-lacton (**3**). — 1,2-*O*-Isopropyliden-α-D-*xylo*hexofuranurono-6,3-lacton-5-ulose-hydrat[3] (100 mg) wird in Äthanol (30 ml) gelöst und unter Rühren mit Phenylhydrazin (50 mg) in Äthanol (10 ml) versetzt. Die Reaktionslösung wird 2 bis 3 Stdn. bei Raumtemperatur gehalten und dann bei −15° stehengelassen. Man filtriert die ausgefallenen Kristalle ab, wäscht gut mit Petroläther (60–70°) und trocknet. Umkristallisation aus Äther ergibt farblose Nadeln (123 mg, 89%); Schmp. 206–207°; n.m.r.: in Dimethylsulfoxid-d_6, innerer Standard Tetramethylsilan, τ 4.03 (1-Proton Dublett, $J_{1,2}$ 3.7 Hz, H-1), τ 5.15 (1-Proton Dublett, H-2), τ 4.98 (1-Proton Dublett, $J_{2,3} < 0.5$ Hz, H-3), τ 5.43 (1-Proton Dublett, $J_{3,4}$ 3.2 Hz, H-4), τ 4.50 (1-Proton Singulett, NH), τ 8.65 (3-Protonen, Singulett), τ 8.71 [3-Protonen, Singulett, Me_2C], und τ 2.7–3.6 (5-Protonen, Multiplett, Phenylringprotonen).

Anal. Ber. für $C_{15}H_{18}N_2O_6$: C, 56.39; H, 5.63; N, 8.69. Gef.: C, 56.29; H, 5.48; N, 8.72.

5-Dehydro-1,2-O-isopropyliden-5-phenylhydrazino-5-O-trimethylsilyl-α-D-gluco-(β-L-ido)-furanurono-6,3-lacton (**4**). — 5-Dehydro-1,2-*O*-isopropyliden-5-phenylhydrazino-α-D-*gluco*-(β-L-*ido*)-furanurono-6,3-lacton (100 mg) wurde in abs. Pyridin (5 ml) gelöst und mit Hexamethyldisilazan (160 mg) und drei Tropfen Chlortrimethylsilan versetzt. Man läßt 4 Stdn. bei Raumtemperatur stehen und engt dann ein. Der Kristallbrei wird mit Benzol extrahiert, die benzolische Lösung eingeengt und der Sirup an der Kapselpumpe getrocknet. Aus Äthanol erhält man Kristalle (169.0 mg, 98%); Schmp. 212°.

Anal. Ber. für $C_{18}H_{26}N_2O_6Si$: C, 54.80; H, 6.64; N, 7.10. Gef.: C, 55.06; H, 6.73; N, 6.82.

5-Dehydro-1,2-O-isopropyliden-5-hydrazino-α-D-gluco-(β-L-ido)-furanuronsäure-hydrazid (**5**). — 1,2-*O*-Isopropyliden-α-D-*xylo*-hexofuranurono-6,3-lacton-5-ulose-hydrat[3] (100 mg) werden in Äthanol (30 ml) gelöst und unter Rühren mit Hydrazin (30 mg) in Äthanol (10 ml) versetzt. Die ausgefallenen Kristallen werden abgesaugt und aus Methanol umkristallisiert (109 mg, 91%); Schmp. 134°.

Anal. Ber. für $C_9H_{18}N_4O_6$: C, 38.85; H, 6.52; N, 20.14. Gef.: C, 38.64; H, 6.49; N, 19.54.

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Note

Mercaptolysis of 3-deoxyoctulosonic acids

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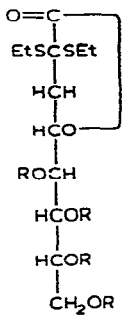
(Received September 12th, 1969)

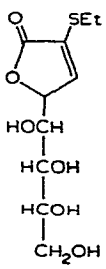
3-Deoxyglyculosonic acids are a biochemically important class of carbohydrates, but relatively little is known of their chemical properties. Thus, only two derivatives of 3-deoxy-D-*manno*-octulosonic acid (pentaacetate[1] and the pentaacetate[2] of the methyl ester) have been described. Further investigation of the chemistry of 3-deoxyoctulosonic acids may be of value for synthetic work and for the analysis of biopolymers containing these compounds. Thus, we now report on the mercaptolysis of 3-deoxyoctulosonic acids.

Treatment of the 3-deoxyoctulosonic acids, obtained by condensation of D-arabinose with oxalacetic acid[2], with ethane thiol–conc. hydrochloric acid at room temperature (conditions used[3] for *N*-acetylneuraminic acid) gave two products in the ratio *ca.* 5:1, which were separated by cellulose-column chromatography.

The major, crystalline product (**1**) had i.r. carbonyl bands at 1770 and 1744 cm^{-1}. The double absorption may be due to hydrogen bonding, since the i.r. spectrum of **1** in pyridine solution showed a single carbonyl absorption at 1760 cm^{-1}. The i.r. spectrum of the per-*O*-trimethylsilylated derivative of **1** also exhibited a single carbonyl band at 1775 cm^{-1}. Thus, **1** is a γ-lactone. Mild, periodate oxidation of **1** resulted in the rapid consumption of 3 mol. of oxidant, with subsequent slow over-oxidation, and the release of 1.65 mol. of formic acid. These data, together with elemental analysis, show that **1** is the diethyl dithioacetal of 3-deoxyoctulosono-1,4-lactone. With mercuric chloride in aqueous acetone, in the presence of mercuric



1 R = H
2 R = Ac



3

oxide, **1** gave 3-deoxy-D-*gluco*-octulosonic acid (identified by gas–liquid chromatography[4]).

Since the synthetic mixture of 3-deoxyoctulosonic acids is reported[2] to contain a considerable proportion of 3-deoxy-D-*manno*-octulosonic acid, the mother liquor, after separation of **1**, was investigated. However, acetylation, followed by chromatographic purification, gave the crystalline acetate (**2**) of **1** as the only identifiable product.

The second, crystalline, minor product (**3**) of mercaptolysis migrated slower on paper chromatography (R_F 0.61, *cf.* 0.81 for **1**) and had a lower retention time in g.l.c. Its u.v. spectrum ($\lambda_{max}^{H_2O}$ 276 nm, ε 5520) and i.r. spectrum (ν_{max} 1754 cm^{-1}; *cf.* 2-buten-4-olide, 1585 cm^{-1}, C=C–SR) indicated the presence of a 2-alkylmercapto-2-buten-4-olide group.

Although chromatographically homogeneous, **3** appeared to be a mixture of C-4 epimers. Oxidative degradation of the acetate of **3** with osmium tetroxide and sodium metaperiodate in aqueous *N*,*N*-dimethylformamide, with subsequent hydrolysis, gave a mixture of glucose, mannose, and arabinose (paper chromatography). The shortening of a carbon chain by three carbon atoms upon oxidative degradation of 2-buten-4-olides bearing functional substituents at C-2 has been described[5]. The formation of glucose and mannose not only shows that **3** is a mixture of C-4 epimers, but also establishes the position of the double bond.

EXPERIMENTAL

Paper chromatography was performed on paper "C" by the ascending technique with butyl alcohol saturated with water (system *A*) and by the descending technique with 9:1:1 butanone–acetic acid–saturated aqueous boric acid (system *B*). Warren's reagent[6], periodate–benzidine reagent, and aniline hydrogen phthalate were used for detection. Column and thin-layer chromatography (t.l.c.) was carried out on silica gel KSK with 7:3 benzene–ether (system *C*) for t.l.c. and conc. sulphuric acid for detection. G.l.c. was carried out on a Pye–Argon Chromatograph equipped with a β-ionisation detector, using glass columns (120 × 0.5 cm) packed with 5% silicon gum rubber SE-30 on DMCS-treated Chromosorb W (60–80 mesh) (column *1*) and with 5% poly(neopentylglycol succinate) on Chromosorb W (80–100 mesh) (column *2*). Operating temperatures were 196° and 180°, respectively. Melting points were determined on a Kofler micro-heating stage. Unicam SP-700 and UR-20 spectrometers were used for recording of u.v. and i.r. spectra, respectively. Solutions were evaporated under diminished pressure at 40°. Periodate oxidation was carried out at 0° by using 0.01562M sodium metaperiodate. The course of oxidation was monitored spectrometrically[7] at 305 nm, and the formic acid liberated was determined by potentiometric titration with 0.045M sodium hydroxide.

Mercaptolysis of 3-deoxyoctulosonic acids. — 3-Deoxyoctulosonic acids[2] (2.71 g) were dissolved at 0° in conc. hydrochloric acid (20 ml), ethane thiol (40 ml) was added, and the mixture vigorously stirred magnetically for 16 h at room tempera-

ture. Water (100 ml) was added, the aqueous layer was separated, washed with hexane (2 × 50 ml), and stirred with basic lead carbonate added portionwise to pH 5–6. The precipitate was filtered off and washed with hot water (1 litre), and the combined filtrate and washings were evaporated to dryness. The residue was treated with a warm mixture of ethyl acetate and alcohol (1:1, 100 ml). The filtered extract was passed through a small column of silica gel which was eluted with 250 ml of 2:3 ethyl acetate–alcohol. The eluate was evaporated to give 2.43 g of pale-yellow syrup that contained two components (R_F 0.81 and 0.61, system *A*) detectable with periodate–benzidine reagent. Analysis of this product after trimethylsilylation[8] revealed the presence of two peaks having retention times of 11.65 and 23.1 min, in the ratio *ca.* 1:5 (column *I*).

The solution of the mercaptolysis product (0.54 g) in butyl alcohol saturated with water (2 ml) was placed on a cellulose column (42 × 4 cm) and eluted with solvent system *A*; 14-ml fractions were collected. The appropriate fractions were combined and evaporated to give a syrupy product (330 mg, R_F 0.81) that crystallised on standing, and a second component (70 mg, R_F 0.61); the retention times of the per-*O*-trimethylsilyl derivatives were 23.1 and 11.65 min, respectively. The faster-migrating component was recrystallised from 1:4 ethyl acetate–ether to give 3-deoxy-D-*gluco*-octulosono-1,4-lactone diethyl dithioacetal (**1**, 120 mg), m.p. 94–95°, $[\alpha]_D^{20}$ +1.6 ±0.6° (*c* 3.74, methanol) (Found: C, 43.70; H, 6.85; S, 19.33. $C_{12}H_{22}O_6S_2$ calc.: C, 44.16; H, 6.80; S, 19.65%). Periodate oxidation of **1** proceeded as follows (time in min and oxidant consumed in moles per mole of substrate are given): 15, 3.14; 30, 3.22; 60, 3.37; 120, 3.56. The release of formic acid was 1.65 moles per mole of **1**.

The product having R_F 0.61 was recrystallised from 3:2 methanol–ether to give 2-ethylmercapto-4-(D-*arabino*-tetrahydroxybutyl)-2-buten-4-olide (**3**) (Found: C, 45.42; H, 6.05; S, 12.03. $C_{10}H_{16}O_6S$ calc.: C, 45.43; H, 6.10; S, 12.14%).

5,6,7,8-Tetra-O-*acetyl-3-deoxy*-D-gluco-*octulosono-1,4-lactone diethyl dithioacetal* (**2**). — (*a*) The mother liquor after separation of **1** was taken to dryness, and the residue was treated with pyridine (2 ml) and acetic anhydride (2 ml) and kept overnight at room temperature. The solution was evaporated, and toluene (3 × 2 ml) was distilled from the residue. A solution of the syrupy product in benzene was added to a column (25 × 1 cm) of silica gel which was eluted with 50 ml of benzene–ether mixtures (95:5, 90:10, and 80:20). The fractions containing a homogeneous product (R_F 0.7, system *C*) were evaporated, and the syrupy residue was crystallised from ether–light petroleum to give **2** (100 mg), m.p. 90–91.5°, $[\alpha]_D^{20.5}$ −2 ±0.3° (*c* 2.52, chloroform) (Found: C, 48.54; H, 6.22; S, 12.99. $C_{20}H_{30}O_{10}S_2$ calc.: C, 48.55; H, 6.12; S, 12.97%).

(*b*) Compound **1** (40 mg) was acetylated with acetic anhydride and pyridine as in (*a*) to yield 15 mg of **2**, m.p. 88–91° (from ether–light petroleum). The i.r. spectra of both of the samples of **2** were superimposable.

Identification of 3-deoxy-D-gluco-*octulosonic acid.* — (*a*) To a solution of **1** (30 mg) in acetone (2 ml) and water (0.2 ml), yellow mercuric oxide (50 mg) and mercuric chloride (50 mg) were added, and the mixture was stirred magnetically for

2 days at room temperature. Examination of the reaction mixture by paper chromatography (system *A*) revealed a product having R_F 0.0–0.1, which could be detected by Warren's reagent[6]. The mixture was diluted with water (2 ml), several drops of pyridine were added, and after being cooled for 5 h the precipitate was filtered off. The filtrate was subjected to preparative, paper chromatography (system *A*), and the zone having R_F 0.0–0.1 was excised and extracted with water. The product from the extract was refluxed with 5 ml of 0.2N methanolic hydrogen chloride for 2 h. The solution was evaporated, and the dry residue was trimethylsilylated[8]. G.l.c. of the product obtained was carried out on column *2* by using derivatives of authentic 3-deoxy-D-*gluco*- and -D-*manno*-octulosonic acids[4] as reference compounds.

(*b*) A solution of **2** (50 mg) in 1.5 ml of dry methanol was treated with 0.15 ml of N sodium methoxide for 3 h at room temperature, neutralised with cation-exchanger resin KU-2(H^+), filtered, and evaporated. The residue (*ca.* 40 mg) was treated with mercuric chloride in aqueous acetone, followed by isolation, methanolysis, and trimethylsilylation as described above, to give 3-deoxy-D-*gluco*-octulosonic acid, identified by g.l.c.

Oxidative degradation of compound **3**. — Compound **3** (15 mg) was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) in the usual manner, and the product (*ca.* 20 mg) was dissolved in 80% aqueous *N*,*N*-dimethylformamide (1 ml). A small crystal of osmium tetroxide was added, and the mixture was stirred for 30 min. The darkened solution was treated with sodium metaperiodate (50 mg). After 30 min, the filtered solution was diluted with chloroform (5 ml), washed with saturated, aqueous thiosulphate (3 × 5 ml) and water, dried ($MgSO_4$), and evaporated. The residue was heated under reflux with 0.5N methanolic hydrogen chloride (2 ml) for 2 h, the solution was evaporated, and the residue was heated with 2N hydrochloric acid (1 ml) for 2 h at 80–85°. The solution was neutralised with silver carbonate, filtered, and passed through a column (5 × 0.7 cm) of KU-2(H^+) resin by elution with water. Paper chromatography of the eluate in system *B* revealed the presence of glucose, mannose, and arabinose.

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Note

A synthesis of 2-deoxy-2-fluoro-D-xylose*

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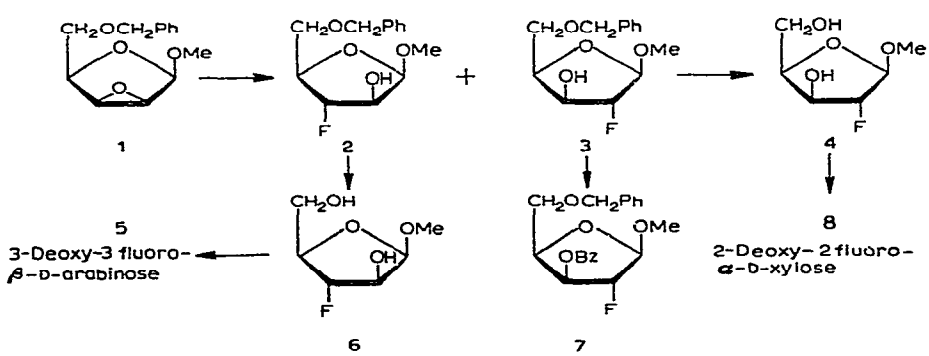
Recent interest[1] in the field of fluorocarbohydrates prompts us to report the synthesis of the previously unknown 2-deoxy-2-fluoro-D-xylose (**8**). As part of a program of synthesis of fluorocarbohydrates and nucleosides thereof[2], we studied the action of potassium hydrogen fluoride–sodium fluoride on methyl 2,3-anhydro-5-*O*-benzyl-β-D-lyxofuranoside (**1**) in ethylene glycol at reflux. Previous results[2,3] led us to expect a mixture of 2- and 3-fluorinated products, and this was indeed found to be the case.

Fractionation of the syrupy reaction mixture on a short column of Silica Gel G (Merck) resulted in the isolation of two major products (**2**) (31%) and **3** (23%) as chromatographically-pure syrups.

Compound **2** was identified as methyl 5-*O*-benzyl-3-deoxy-3-fluoro-β-D-arabinofuranoside by hydrogenolysis to the glycoside **6**, followed by acid hydrolysis to give 3-deoxy-3-fluoro-D-arabinose (**5**), identical in all respects with authentic material[4].



The structure of compound **3** was established by benzoylation of the free hydroxyl group, which caused a downfield shift in the p.m.r. signal assigned to the geminal proton, resulting in a compound, **7**, possessing a clearly-resolved first order

*This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service (Grant No. CA 08748).

p.m.r. spectrum. Signals at δ 5.64 (octet, $J_{3,F}$ 16.5, $J_{3,4}$ 5.5, $J_{3,2}$ 1.6 Hz), 5.07 (doublet, $J_{1,F}$ 13.4 Hz), and 5.00 p.p.m. (quartet, $J_{2,F}$ 49.5 Hz) were assigned to H-3, H-1, and H-2 respectively, and are consistent with a 2-deoxy-2-fluoro structure for **7**, and therefore for **3**. The very small (< 1.0 Hz) H-1–H-2 coupling establishes the 1-2-*trans*-configuration, and on the basis of *trans*-scission of the epoxide **1**, compound **3** is therefore methyl 5-*O*-benzyl-2-deoxy-2-fluoro-β-D-xylofuranoside.

Hydrogenolysis of **3** afforded a quantitative yield of the glycoside **4** as a colorless, mobile syrup, which upon acid hydrolysis led to the free deoxyfluoropentose **8** as colorless needles, m.p. 121–122°.

On the basis of a mutarotation study ($[\alpha]_D^{25}$ +69 → +32°, after 1 h, of the free sugar **8**, it was assigned the α-configuration. Compound **8** exhibited a medium-intensity, type-3 absorption[5] band at 757 cm^{-1} which suggests that it possesses the pyranoid structure.

EXPERIMENTAL

Melting points were determined with a Hoover-Thomas capillary apparatus, and are corrected. Thin-layer chromatography (t.l.c) was performed on microscope slides coated with Silica Gel GF 254 (Merck) using 1:2 ethyl acetate–light petroleum (30–60°) (solvent *A*) and ethyl acetate (solvent *B*). Compounds were detected by spraying with 20% (v/v) sulfuric acid in ethanol, followed by heating to 130°. Reducing sugars were detected with the aniline hydrogen phthalate reagent. Evaporations were carried out *in vacuo*.

P.m.r. and i.r. spectra were recorded on Varian A-60 and Perkin–Elmer 221 instruments, respectively, and were found to be consistent with the proposed structures. Analytical samples of non-crystalline compounds (except **1**) were purified by preparative t.l.c., and elementary analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Methyl 2,3-anhydro-5-O-benzyl-β-D-lyxofuranoside (**1**). — Methyl 2,3-anhydro-β-D-lyxofuranoside[6] (26.5 g), silver oxide (37 g), benzyl bromide (32 ml), and anhydrous *N,N*-dimethylformamide (135 ml) were shaken together for 20 h at room temperature. The mixture was partitioned between chloroform (1 liter) and water (1 liter). The chloroform layer was separated, filtered, treated with pyridine (100 ml), and washed successively with water (6 × 500 ml), 2M hydrochloric acid (2 × 500 ml), saturated sodium hydrogen carbonate (500 ml), and water (500 ml), and then dried with magnesium sulfate and evaporated. The residual oil was vacuum-distilled to give **1** (32.9 g) as a mobile, colorless oil, b.p. (0.03 mmHg) 135–140°, $[\alpha]_D^{23}$ −67° (*c* 2.0, ethanol).

Anal. Calc. for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 66.17; H, 6.79.

Methyl 5-O-benzyl-2-deoxy-2-fluoro-β-D-xyloside (**3**) *and methyl 5-O-benzyl-3-deoxy-3-fluoro-β-D-arabinoside* (**2**). — The epoxide **1** (3.0 g), potassium hydrogen fluoride (3.0 g), and sodium fluoride (3.0 g) were heated for 2 h at reflux in ethylene glycol (60 ml). The cooled mixture was poured into saturated sodium hydrogen

carbonate (500 ml) and extracted with chloroform (3 × 100 ml). Evaporation of the chloroform layer, dried with magnesium carbonate afforded a yellow oil which was chromatographed on a column of Silica Gel G (150 g). It was eluted with solvent *A* and 12-ml fractions were collected. Evaporation of fractions 34–48 gave compound **3** (0.75 g) as a pale-yellow syrup, $[\alpha]_D^{23}$ −42° (*c* 0.9, ethanol).

Anal. Calc. for $C_{13}H_{17}FO_4$: C, 60.92; H, 6.69; F, 7.41. Found: C, 60.68; H, 6.68; F, 7.19.

Evaporation of fractions 59–90 gave compound **2** (1.00 g) as a colorless syrup. It was identified by hydrogenolysis, followed by acidic hydrolysis, as described below, to give 3-deoxy-3-fluoro-D-arabinose (**5**), m.p. 118–120° (lit.[4]: m.p. 120°). P.m.r. and i.r. spectra of **5** were identical with those of authentic material.

Benzoylation of **3** with 2 moles of benzoyl chloride in pyridine for 2 h at 0° gave an 87% yield of the monobenzoyl ester **7** as a stiff syrup, $[\alpha]_D^{23}$ −63° (*c* 1.2, ethanol); p.m.r. signals (chloroform-*d*, TMS internal standard) at δ 7.1–8 2 (11–12 protons, benzoyl and benzyl), 5.64 (1-proton octet, H-3, $J_{3,F}$ 16.5, $J_{3,4}$ 5.5, $J_{3,2}$ 1.6 Hz), 5.07 (1-proton doublet, H-1, $J_{1,F}$ 13.4 Hz), 5.00 (1-proton quartet, H-2, $J_{2,F}$ 49.5 Hz), 4.71 (1-proton pseudo-quartet, H-4, $J_{4,5}$ 5.5 Hz), 4.45 (2-proton singlet, benzyl CH_2), 3.71 (2-proton doublet, H-5) and 3.39 p.p.m. (3-proton singlet, OMe). Coupling constants quoted are first order. P.m.r. and i.r. spectra indicated the presence of a small amount of benzoyl chloride in the sample.

Anal. Calc. for $C_{20}H_{21}FO_5$: C, 63.82; H, 5.62; F, 5.04. Found: C, 66.55; H, 6.03; F, 4.73.

Methyl 2-deoxy-2-fluoro-β-D-xylofuranoside (**4**). — A solution of **3** (500 mg) in ethanol (100 ml) containing 10% palladium-on-charcoal (100 mg) was hydrogenated on a Parr apparatus at ~ 24 p.s.i. until the uptake of hydrogen ceased (30 min). The filtered solution was evaporated to give a colorless oil (328 mg), $[\alpha]_D^{23}$ −102° (*c* 1.4, ethanol).

Anal. Calc. for $C_6H_{11}FO_4$: C, 43.37; H, 6.67; F, 11.44. Found: C, 43.39; H, 6.60; F, 11.72.

2-Deoxy-2-fluoro-D-xylose (**8**). — To a solution of **4** (496 mg) in water (50 ml), Dowex 50-W (X-8, H^+, 5 ml, previously treated with boiling water) was added, and the mixture was heated for 1 h at reflux. After filtration, the solution was evaporated to dryness, and the syrupy residue was crystallized from ethanol–ether (294.5 mg, m.p. 109–111°). Two recrystallizations from propyl alcohol gave a product having m.p. 121–122°. Evaporation of the mother liquors, after treatment with charcoal, gave chromatographically pure **8** (102 mg) as a colorless syrup which slowly crystallized. A mixed m.p. of **8** and **5** gave a depression (101–116°), and on paper chromatography (4:1:5 butyl alcohol–ethanol–water, downward elution) **8** and **5** showed R_F 0.38 and 0.32, respectively. Compound **8** showed $[\alpha]_D^{23}$ +69° (1.5 min) → +32° (1 h) (*c* 0.9, ethanol).

Anal. Calc. for $C_5H_9FO_4$: C, 39.48; H, 5.96; F, 12.49. Found: C, 39.60; H, 6.03; F, 12.48.

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Note

Methyl 2,3-di-*O*-methyl-α-D-glucopyranoside 4,6-carbonate

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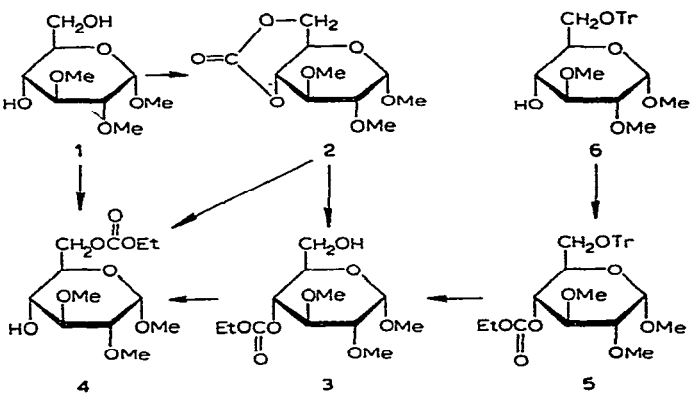
Reactive, five-membered, *trans* cyclic carbonates of sugars have been prepared by treating pyranoid sugars having vicinal diequatorial hydroxyl groups with ethyl chloroformate and triethylamine[1]. These compounds undergo facile ring-opening reactions at the carbonate center with such nucleophiles as alcohols, thiols, and amines to give acyclic carbonates, monothiolcarbonates, and carbamates, respectively[2].

In continuing studies on the preparation and properties of carbohydrate carbonates, it was desirable to prepare a six-membered cyclic carbonate of a pyranose. Such a derivative should be highly reactive since it is known[3] that simple six-membered cyclic esters are less stable and more susceptible to ring-opening reactions than the corresponding five-membered ring compounds.

In 1938 a sugar was reported that contained a six-membered carbonate fused to a furanose ring[4]. D-Xylose was treated with acetone and phosgene to give 1,2-*O*-isopropylidene-α-D-xylofuranose 3,5-carbonate. Ring opening of the carbonate took place readily in the presence of methanol.

We chose methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (**1**) for an attempt to form a cyclic carbonate at C-4 and C-6 since hydroxyl groups here react readily to form six-membered cyclic acetals. When **1** was treated with ethyl chloroformate and triethylamine in the molar proportions of 1:23:8, t.l.c. showed a major component, which was recovered by crystallization from chloroform–hexane. This component



was characterized as methyl 2,3-di-*O*-methyl-α-D-glucopyranoside 4,6-carbonate (**2**) by i.r. and n.m.r. data, microanalyses, and its molecular weight. For comparison of i.r.[5] and n.m.r. spectral data, the 4-*O*- and 6-*O*-(ethoxycarbonyl) derivatives (**3** and **4**) of **1** were prepared. Compound **3** was isolated in quantitative yield on detritylation of methyl 4-*O*-(ethoxycarbonyl)-2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (**5**) with silica gel[6]. When detritylation of **5** was performed with hydrochloric acid in methanol[7,8] followed by neutralization with ammonium hydroxide, a different compound was formed which was shown to be **4**.

Compound **4** was also prepared by the direct reaction of **1** with ethyl chloroformate and pyridine and by reaction of **2** with ethanol containing 10% of triethylamine. Although no **3** was detected in either of these reaction products, it is probable that **3** was initially present and transformed into **4** in the presence of base. When lower concentrations of triethylamine were used in the ring-opening of **2**, nearly equal amounts of **3** and **4** were formed initially. Subsequently, the amount of **4** increased while that of **3** decreased. Pure **3** was converted into a mixture of **3** and **4** under these reaction conditions.

Comparison of the reactivity of **2** with that of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate in ring-opening reactions revealed that the 4,6-carbonate was converted into the acyclic carbonate at a rate more than twice that for the 2,3-carbonate.

EXPERIMENTAL

I.r. spectra for films cast onto plates of silver chloride were recorded with a Perkin–Elmer* Model 137 spectrophotometer. A Perkin–Elmer Model 621 spectrophotometer was used to record the spectrum of **2** in bromoform. Wavelengths were calibrated with polystyrene film. N.m.r. spectra were recorded for solutions in chloroform-*d* by means of a Varian HA-100 spectrometer with tetramethylsilane (τ 10.00) as the internal reference standard. Melting points were determined in sealed capillaries in an oil bath and are uncorrected. Optical rotations were measured with a Rudolph polarimeter. Molecular weights were measured with a Mechrolab Model 301A vapor-pressure osmometer. T.l.c. was performed on Silica Gel G (E. Merck, Germany) with ether or 4:1 (v/v) ether–amyl acetate, and detection was with either 19:1 (v/v) methanol–sulfuric acid for charring or Rhodamine 6-G (1 mg per liter of water) as a fluorescent indicator.

*Methyl 2,3-di-*O*-methyl-α-D-glucopyranoside 4,6-carbonate* (**2**). — A solution of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside[6] (**1**, 500 mg, 2.25 mmoles) in tetrahydrofuran (5 ml) was cooled to 5° and mixed with ethyl chloroformate (5 ml, 52 mmoles). Triethylamine (2.5 ml, 18 mmoles) in tetrahydrofuran (20 ml) was added dropwise during 30 min. The mixture was kept for 18 h at −15° and filtered, and the

*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.

filtrate evaporated to an oil which contained impure **2**. This oil was extracted first with four 5-ml portions of hexane at 25° and then with four 5-ml portions of hexane at 65°. Each extract was cooled to 5° before being decanted. When the remaining semisolid was dissolved in chloroform and hexane was added, 430 mg (77%) of product separated. Three recrystallizations from chloroform–hexane gave transparent, waxy needles, m.p. 52–55°. This product was an unstable solvate of **2** which effloresced during 2 h to give opaque, white needles of pure **2**, m.p. 75–77°, $[\alpha]_D^{23}$ +63.3° (*c* 1.17, chloroform); λ_{max}^{film} 1770 (C = O), 1200 (O–C–O), and 765 cm^{-1} [O(C = O)O]; $\lambda_{max}^{CHBr_3}$ 1772 and 1764 cm^{-1} (C = O).

Anal. Calc. for $C_{10}H_{16}O_7$: C, 48.4; H, 6.50; mol. wt., 248. Found: C, 48.3; H, 6.50; mol. wt., 253.

Compound **2** was soluble in water and most polar organic solvents, but not in hexane. Compound **2** in aqueous solution decomposed to **1** within several hours, as shown by t.l.c.

Methyl 4-O-(ethoxycarbonyl)-2,3-di-O-methyl-α-D-glucopyranoside (**3**). — A solution of methyl 2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside[6,7] (**6**) (1.0 g, 2.16 mmoles) in pyridine (20 ml) was cooled to 5° and ethyl chloroformate (5 ml, 52 mmoles) was added dropwise with continuous stirring. After 18 h at 25° the mixture was treated with ether (200 ml), the resulting suspension filtered through Celite, and the filtrate evaporated to a syrup that crystallized spontaneously. Recrystallization from hexane and ethanol gave 0.85 g (73%) of product. Three recrystallizations from ethanol gave pure methyl 4-*O*-(ethoxycarbonyl)-2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (**5**), m.p. 127–129°, $[\alpha]_D^{24}$ +66.2° (*c* 1.32, chloroform); λ_{max} 1750 (C = O), 1260 (O–C–O), and 787 cm^{-1} [O(C = O)O]; n.m.r. data: τ 5.35 (1-proton modified triplet, H-4), τ 6.85 (2-proton doublet, H-6 and H-6'), τ 6.00 and τ 8.83 (2-proton split quartet or octet and 3-proton triplet, respectively, OEt). The product was fused at 120–140° and vacuum dried prior to analysis.

Anal. Calc. for $C_{31}H_{36}O_8$: C, 69.4; H, 6.76. Found: C, 69.2; H, 6.74.

Compound **5** (510 mg, 0.95 mmole) in benzene (20 ml) was detritylated with silica gel (Davison Grade 12, 50 g)[8] in a column (2.7 × 14 cm). After 1 h the column was eluted successively with benzene (100 ml) and 1:9 ethyl acetate–benzene (300 ml) to remove triphenylmethanol, and then with 1:3 methanol–ethyl acetate (300 ml) to remove the carbohydrate product. Evaporation of the latter eluate gave a syrup, 290 mg (100%), which crystallized from ether–hexane. Three recrystallizations from ether–hexane gave **3**, which was pure by t.l.c., m.p. 81–83°, $[\alpha]_D^{24}$ +107.4° (*c* 2.36, chloroform); λ_{max} 1750 (C = O), 1260 (O–C–O), and 790 cm^{-1} [O(C = O)O]. The n.m.r. spectrum of **3** showed τ 5.35 (1-proton modified triplet, H-4) analogous to **5**.

Anal. Calc. for $C_{12}H_{22}O_8$: C, 49.0; H, 7.54. Found: C, 49.0; H, 7.64.

Methyl 6-O-(ethoxycarbonyl)-2-3-di-O-methyl-α-D-glucopyranoside (**4**). — (*a*) *Ring-opening of* **2**. A solution of **2** (50 mg, 0.20 mmole) in ethanol (1 ml, 17.4 mmoles) and triethylamine (0.1 ml, 0.72 mmole) was kept in a closed container for 18 h at 25°. The solution was diluted with chloroform (50 ml) and shaken with acetate buffer [10 ml, containing 10% (v/v) acetic acid and 10% (w/v) sodium acetate] to neutralize

the amine. The chloroform phase was separated, dried with sodium sulfate, and evaporated to a syrup, 46 mg (78%), which showed a single component by t.l.c. Crystallization from hexane gave m.p. 56–57°, $[\alpha]_D^{23}$ +99.7° (*c* 1.52, chloroform); λ_{max} 1740 (C = O), 1260 (O–C–O), and 793 cm^{-1} [O(C = O)O]; n.m.r. data: τ 5.62 (2-proton multiplet, H-6 and H-6'), τ 5.82 and τ 8.70 (2-proton quartet and 3-proton triplet, respectively, OEt). The appearance of the characteristic resonances for H-6 and H-6' at τ 5.62 represented a downfield displacement of 0.73 p.p.m. from compounds unsubstituted by ethoxycarbonyl at C-6, and confirmed substitution at this position.

Anal. Calc. for $C_{12}H_{22}O_8$: C, 49.0; H, 7.54. Found: C, 49.0; H, 7.67.

(*b*) *Direct ethoxycarbonylation of* **1**. To a solution of **1** (500 mg, 2.25 mmoles) in pyridine (5 ml) at 5° was added ethyl chloroformate (0.4 ml, 4.2 mmoles). After being kept for 18 h at 25°, the mixture was diluted with chloroform (100 ml) and the solution was successively washed with dilute hydrochloric acid and sodium hydrogen carbonate solution, dried (sodium sulfate), and evaporated under diminished pressure at 40° to a syrup. This syrup was resolved into monoethoxycarbonyl and diethoxycarbonyl fractions by preparative t.l.c. The zone of lower R_F (monoethoxycarbonyl fraction) was separated by sectioning and eluted with ethyl acetate, and the eluate was evaporated. The syrupy residue (89 mg, 13%) crystallized when kept for 18 h at 25°. After recrystallization from hexane it had m.p. 56–58°. The mixed m.p. of this product with that from part (*a*) showed no depression; the i.r. spectra of (*a*) and (*b*) were identical.

(*c*) *Detritylation of* **5**. Compound **5** (340 mg, 0.635 mmole) was mixed with 0.03M hydrochloric acid in methanol (20 ml) and stirred for 2.5 h at 50° to remove the trityl group. The mixture was made neutral with ammonium hydroxide, mixed with ice–water (100 ml), and extracted with four 20-ml portions of hexane to remove methyl triphenylmethyl ether. The aqueous-alcoholic solution was then extracted with four 20-ml portions of chloroform and the chloroform solution was dried and evaporated to a syrup that crystallized from hexane to give 90 mg (48%), m.p. 55–57°. The n.m.r. and i.r. spectra were identical with those of authentic **4** and the mixed m.p. with authentic **4** showed no depression.

Comparison between ring-opening reactions of **2** *and of methyl 4,6-O-benzylidene-α-D-glucopyranoside 2,3-carbonate.* — Equimolar portions (0.1 mmole) of the title compounds were separately dissolved in 10 ml of a stock solution of chloroform containing ethanol (1 mmole) and triethylamine (0.1 mmole). The progress of the reaction was monitored by t.l.c. and i.r. spectroscopy. After 18 h, **2** was completely converted into an equimolar mixture of **3** and **4**. In the same interval, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate showed only about 50% conversion into acyclic products. The large proportion of chloroform used retards the intramolecular migration of the ethoxycarbonyl group from C-4 to C-6. Within the interval studied, 0.1 mmole of **3** in 10 ml stock solution showed about 10% conversion into **4**, and 0.1 mmole of **4** under these conditions showed no change.

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Note

Labeling of acid mucopolysaccharides with tritium
Part II. Purification and fractionation of tritium-labeled heparin*

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With the use of various methods for labeling heparin with tritium[1,2], it has become evident that purification of the crude labeled material is the step which requires the greatest care.

The various labeling methods cause considerable degradation of heparin: 80% with the unmodified Wilzbach's method[1], 13%[1] and 50%[2] with catalytic methods, and 28% with the labeling under electric discharge[1]. These losses reflect the amount of the starting material which has been modified so that it does not precipitate on addition of quaternary ammonium compounds[2] or ethyl alcohol containing appropriate cations[1]. However, even the material harvested by precipitation may be heterogeneous and includes compounds differing in chemical structure, physical properties, and biological activity.

The following method, adopted in our laboratories for the purification and fractionation of tritiated heparin, may be useful whenever a relatively uniform preparation of tritium-labeled heparin is required.

EXPERIMENTAL

Sodium heparinate (500 mg, Calbiochem grade, Lot 63525), labeled by exposure to tritium gas under electric discharge[1], was transferred to a plastic beaker containing water (50 ml) and Dowex-1 (20 g; X-2, 200–400 mesh, Cl^-). The resin, with the adsorbed heparin, could be frozen and stored indefinitely, or could be poured into a column and washed with water. The effluent was tested for hexuronic acid to verify whether the capacity of the resin had been exceeded. Thereafter, the water effluent was monitored; when the tritium radioactivity became constant, 0.5M sodium chloride

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(2 litres) was passed through the column. Abundance of hexuronic acid-containing material in this eluate indicated extensive heparin breakdown. The column was then eluted with solutions of sodium chloride (500 ml) of increasing molarity[3], and the effluents separately collected were tested for hexuronic acid. Those found to be positive were diluted with water to a final sodium chloride molarity of 0.75. The heparin, precipitated with a 10% solution of cetylpyridinium chloride in water, was collected by centrifugation, washed with a 10% solution of potassium acetate in 95% ethanol, 95% ethanol, and abs. ethanol, and dried with ether. Each precipitate was dissolved in water, and the solution was passed through a column (5 × 0.9 cm) of Dowex-50 (X-8; 200–400 mesh, H^+) to remove the last traces of cetylpyridinium chloride. The effluent was collected, neutralized, and precipitated with 3 vol. of a 10% solution of potassium acetate in 95% ethanol. The ppt. was washed and dried with ether, as described previously. Aliquots of the fractions obtained were used for analyses, mol. weight determination, measurement of radioactivity and biological activity, and ultrafiltration.

The various analytical procedures used have been described in detail previously[4,5]. Weight-average molecular weights were determined from plots of *ln y versus* x^2 at sedimentation equilibrium[6]. Measurements were made on 0.4–0.5% solutions of the various heparin fractions in 0.15M sodium chloride. All runs were performed at 20,000 r.p.m. and lasted 20–24 h. The partial specific volume for heparin[7] was assumed to be 0.42. Radioactivity was measured in a scintillation counter, as previously described[1].

The biological activity of the original sodium heparinate or of the various fractions obtained after treatment was assessed by measuring the prothrombin time[1]. Ultrafiltration of aqueous solutions of heparin was performed in a 50-ml Diaflo apparatus (Amicon Corp., Lexington, Mass.) provided with a UM-1 membrane, and operated at a pressure of 40 p.s.i. The ultrafiltrate was collected in 5-ml fractions and analyzed for tritium radioactivity.

Eighty-nine per cent of the sodium heparinate initially used was recovered from the Dowex-1 column (Table I), most of the heparin being eluted with 1.6 and

TABLE I

ANALYTICAL DATA OF THE ORIGINAL HEPARIN AND OF THE VARIOUS FRACTIONS OF TRITIATED HEPARIN

Fraction[a]	*Amount* (*mg*)	*Hexuronic acid* (%)	*S* (%)	*Mol. weight*	^3H (*d.p.m./μg*)
1.0	3.8	22.3	5.24		54,500
1.20	9.4	29.9	7.17	3,240	12,520
1.40	28.9	26.2	7.41	5,320	2,650
1.60	203.8	25.2	7.08	7,040	1,000
1.80	190.0	26.1	8.00	8,950	800
2.0	10.2	28.2	8.72	11,000	1,600
Heparin[b]		27.8	8.70	8,240	

[a]Eluted from Dowex-1 column; molarity of sodium chloride eluent. [b]Starting material.

1.8M sodium chloride solutions. With the exception of the material eluted by M sodium chloride, the hexuronic acid content of the various fractions is quite constant, whereas their sulfur content and molecular weight increase with the molarity of the sodium chloride eluent. Fig. 1 shows that a plot of log molarity of sodium chloride used for

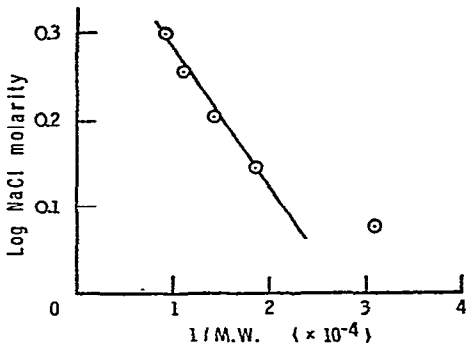

Fig. 1. Relationship between the log molarity of the sodium chloride eluent and the inverse of the molecular weight of heparin fractions eluted from a Dowex-1 column.

elution of the various fractions against the reciprocal of the respective molecular weights gives a straight line, in agreement with the data of Laurent and Scott[8] on chondroitin 4-sulfate and keratan sulfate. As reported in that study[8], our fraction of lowest molecular weight also deviates from the straight-line relationship. The specific activity of the various fractions (expressed as d.p.m./μg heparin) decreased with the increase of the molarity of the sodium chloride eluent.

The biological activity of the various fractions is illustrated in Fig. 2. The material eluted from Dowex-1 with 1.0, 1.2, and 1.4M sodium chloride was completely inactive, whereas that eluted with 1.6, 1.8, and 2.0M sodium chloride was much more active than the original preparation. When aliquots of labeled heparin (1.8 and 1.6M sodium chloride fractions, corresponding to $11.5 \cdot 10^6$ and $15.5 \cdot 10^6$ c.p.m.), respectively were concentrated by passage through a UM-1 membrane, less than 0.1% of the radioactivity was found in the ultrafiltrates.

The heparin fractions possessing high biological activity (1.6, 1.8, and 2.0M sodium chloride) have specific activities ranging between 8 and $16 \cdot 10^5$ d.p.m./mg, values one order of magnitude smaller than those reported[2] for unfractionated heparin labeled catalytically with tritium. However, our fractions have a biological activity far superior to that of the starting material, while the labeled preparation of Barlow and Cardinal[2] had lost 20% of the original biological activity. It is conceivable that the latter preparation represented a mixture of various molecular species, varying in size, specific activity, and biological activity.

The inverse relationship existing between specific activity of heparin fractions and their molecular weight and biological activity confirms the observation that the material more directly exposed to the effect of radiation becomes more labeled but also is degraded to a larger extent[1]. Thus, one should strive to achieve a useful compro-

mise between specific activity and biological activity. Since it is quite difficult to reproduce exactly the technique and the results of the labeling procedures, the fractionation described in the present communication is a convenient method for obtaining a rather uniform material having useful biological activity.

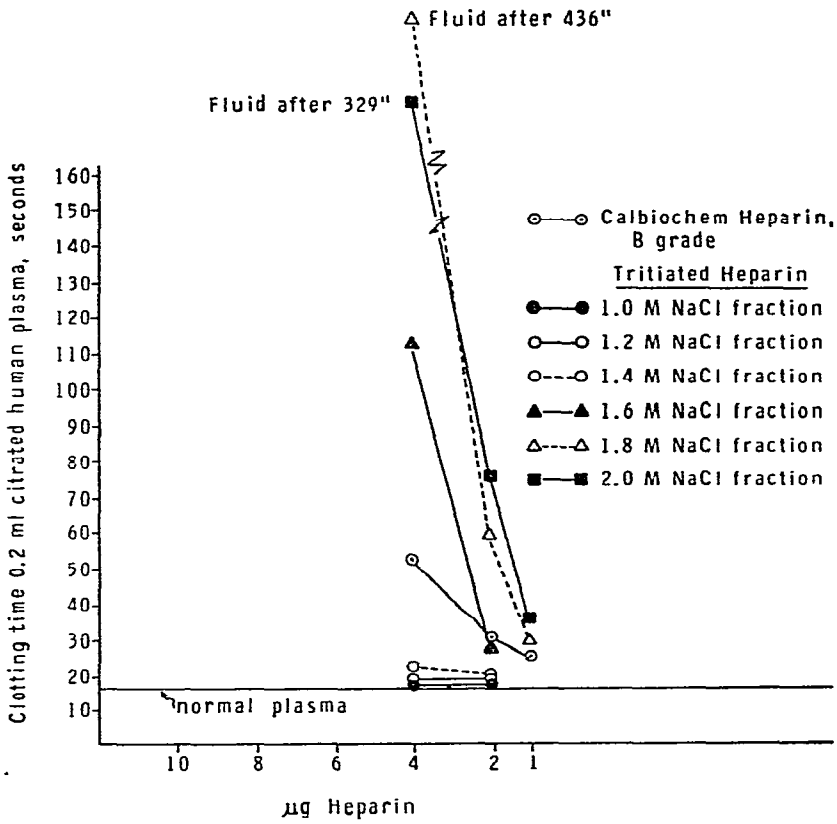

Fig. 2. Biological activity (prothrombin time) of the original, nonradioactive heparin and of the various radioactive fractions obtained by chromatography on a Dowex-1 column.

Fractionation of heparin into fractions of increasing molecular weight and biological activity was performed in 1961 by Laurent[7] using cetylpyridinium chloride precipitation or chromatography on Ecteola cellulose, and by Lasker and Stivala[9] using differential solubility in alcohol–water mixtures or chromatography on Ecteola cellulose. The result presented here demonstrates that Dowex-1 may also separate a given acid mucopolysaccharide into fractions of different molecular weight. Thus, despite the opinion to the contrary of Pearce, Mathieson, and Grimmer[10], Dowex-1 does behave in this respect like Ecteola cellulose and cetylpyridinium chloride[7,8], as one would expect considering the essentially similar mechanism by which they bind acid mucopolysaccharides and the general validity of the concept of critical electrolyte concentration[8].

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Note

Stereoselective hydroxylation of glycals

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(Received August 6th, 1969; in revised form, September 19th, 1969)

The mode of hydroxylation of glycals with perbenzoic acid, which probably involves an intermediate 1,2-epoxide, is dependent on the nature of the substituent at C-3. The hydroxyl group at C-2 is preferentially introduced *cis* to the substituent at C-3 when the latter is a hydroxyl group and *trans* if HO-3 is substituted. Numerous examples are known[1–3].

Treatment of glycals with hydrogen peroxide and osmium tetroxide in *tert*-butyl alcohol gave[4], predominantly, the aldose having *trans*-substituents at positions 2 and 3, regardless of substitution of HO-3.

Hydroxylation of glycals by perbenzoic acid has been used for the preparation of some relatively rare aldoses, for example, D-talose (32%) from D-galactal[2], and L-ribose (40%) from L-arabinal[3]. The disadvantage of this method is the instability of perbenzoic acid and the poor stereoselectivity of the reaction.

We have found that the hydroxylation of D-galactal by hydrogen peroxide in the presence of osmium tetroxide gives D-galactose and D-talose in the ratio 4:1. In the presence of selenium dioxide, vanadium pentoxide, or chromium trioxide, the ratio was *ca.* 2:1. However, the yield was relatively low, due to the formation of by-products. Whereas the hydroxylation of D-galactal in the presence of OsO_4, SeO_2, V_2O_5, or CrO_3 leads preferentially to products having *trans* 2,3-substituents, hydroxylation in the presence of molybdenum trioxide or tungsten trioxide affords almost exclusively *cis* 2,3-substituted products (Table I).

TABLE I

HYDROXYLATION OF D-GALACTAL BY HYDROGEN PEROXIDE IN THE PRESENCE OF VARIOUS OXIDES

Catalyst	*Yield* (%) D-*talose*+D-*galactose*	*Ratio* D-*talose*: D-*galactose*
MoO_3	90	9.5:0.5
WO_3	90	9:1
OsO_4	10	1:4
SeO_2	10	1:2
V_2O_5	5	1:2
CrO_3	5	1:2

The stereoselective, catalytic action of molybdenum trioxide was further demonstrated with D-glucal, D-arabinal, and D-xylal, by the formation of D-mannose, D-ribose, and D-lyxose, respectively (Table II). In addition to the main product, small proportions of the epimeric aldose and the corresponding 2-deoxyaldose are formed, the latter being the result of the addition of water due to the relatively acidic medium (pH 2.8). The stereoselectivity may be due, at least in part, to complex formation between HO-3 and peroxymolybdenic acid.

TABLE II

PRODUCTS ISOLATED FROM REACTION MIXTURES OBTAINED BY HYDROXYLATION OF GLYCALS (5 g) BY HYDROGEN PEROXIDE IN THE PRESENCE OF MOLYBDENUM TRIOXIDE

Glycal	*Reaction products*	(*g*)	*Yield* (%)	*Ratio of aldoses*	R_F *values*
D-Glucal	D-Mannose	4.9	80.3	95.2	0.12
(R_F 0.49)	D-Glucose	0.3		4.8	0.09
	2-Deoxy-D-*arabino*-hexose	0.45			0.25
D-Galactal	D-Talose	4.8	78.7	95.0	0.15
(R_F 0.42)	D-Galactose	0.25		5.0	0.07
	2-Deoxy-D-*lyxo*-hexose	0.4			0.22
D-Arabinal	D-Ribose	5.75	89.1	97.5	0.19
(R_F 0.65)	D-Arabinose	0.15		2.5	0.12
	2-Deoxy-D-*erythro*-pentose	traces			0.34
D-Xylal	D-Lyxose	4.85	75.2	96.0	0.16
(R_F 0.65)	D-Xylose	0.2		4.0	0.14
	2-Deoxy-D-*threo*-pentose	0.3			0.37

EXPERIMENTAL

Melting points were determined on a Kofler microstage apparatus. Specific rotations were measured with a Bendix–Ericsson polarimeter, Type 143A. The fractionation of reaction mixtures was carried out by chromatography on columns (110 × 6 cm) of Whatman CF 12 cellulose with 5:1:4 butyl alcohol–ethanol–water. Sugars were identified by paper chromatography on Whatman No. 1 paper with detection by diphenylamine[5], and the amounts were determined by direct scanning of the detected chromatograms with an ERI-10 densitometer (Zeiss, Jena).

The starting glycals were synthesized by literature procedures: D-glucal[6], m.p. 57–60°, $[\alpha]_D^{24}$ −8° (*c* 2, water); D-galactal[6], m.p. 98–100°, $[\alpha]_D^{24}$ −6.6° (*c* 2, water); D-xylal, $[\alpha]_D^{24}$ −245.4° (*c* 2, water); D-arabinal (prepared from 3,4-di-*O*-acetyl-D-arabinal[7] by Zemplén deacetylation), m.p. 80–82°, $[\alpha]_D^{24}$ −197.4° (*c* 2, water).

Hydroxylation of D-*galactal in the presence of various oxides.* — D-Galactal (0.5 g) was dissolved in 5% aqueous hydrogen peroxide (5 ml), and one of the following oxides (10–15 mg) was added: MoO_3, WO_3, OsO_4, SeO_2, V_2O_5, and CrO_3. When OsO_4, SeO_2, V_2O_5, or CrO_3 was used, the reaction was complete within 4 h. In the presence of MoO_3 or WO_3, all of the D-galactal had reacted within 40 h. The reaction products were analysed by paper chromatography (Table I).

Hydroxylation of glycals in the presence of molybdenum trioxide. — To a solution of D-glycal (5 g) in 5% aqueous hydrogen peroxide (50 ml), molybdenum trioxide (40–50 mg) was added. After 48 h at room temperature, undissolved oxide was removed, and the excess of hydrogen peroxide was decomposed by treatment for 24 h at 22° with 5% palladised charcoal (0.1–0.2 g). The filtered mixture was then evaporated *in vacuo*. A solution of the residue in methanol (100 ml) was treated with charcoal and then chromatographed on cellulose. The results are summarized in Table II.

Preparation of D-*talose.* — A mixture of D-galactal (100 g) and molybdenum trioxide (1 g) in 5% aqueous hydrogen peroxide (1 litre) was kept for 48 h at room temperature. After filtration, 5% palladised charcoal (1–2 g) was added, and the mixture was stored for 48 h at 22°. The filtered mixture was evaporated *in vacuo*, and the syrupy residue was dried by two-fold evaporation with methanol. A solution of the residue in methanol (600 ml) was treated with charcoal and, after storage for 24 h at room temperature and thereafter for 24 h at 4°, gave D-talose (64 g), $[\alpha]_D^{24}$ +19.8° (*c* 1.6, water). Concentration of the mother liquor to half volume gave a further crop (21 g). The final mother liquor was subjected to cellulose chromatography to give D-talose (8.2 g), D-galactose (4.8 g), and 2-deoxy-D-*lyxo*hexose (7 g).

The crude, crystalline D-talose obtained above contained a small proportion of 2-deoxy-D-*lyxo*hexose. Recrystallization from methanol (8 ml per 1 g of crude D-talose) gave material having m.p. 130–133°, $[\alpha]_D^{24}$ +20.6° (*c* 1.5, water); lit.[2] m.p. 133–134°, $[\alpha]_D$ +21° (water).

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Preliminary communication

2-Deoxy-2-fluoro-D-galactose

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(Received February 25th, 1970)

Terminal, nonreducing D-galactopyranosyl residues occur in many antigens of biological interest, and lactose has been employed as a hapten in numerous immunochemical studies[1]. For use in studies of the mechanism of binding of D-galactose-containing oligosaccharides to antibodies, the complete series of deoxyfluoro-D-galactopyranose derivatives was required. The 3- and 6-fluoro derivatives have been described[2,3]. We now report the synthesis of 2-deoxy-2-fluoro-D-galactose.

The reaction of 3,4,6-tri-*O*-acetyl-D-galactal (**1**) with trifluoro-(fluoro-oxy)methane in trichlorofluoromethane[4] at −78° is closely analogous to that of the corresponding D-glucal[5] derivative, in that *cis* addition occurs[6] preponderantly on the lower side of the double bond[7]. The four-product mixture was resolvable by t.l.c. [Kieselgel (Merck) 7731, 1:2 light petroleum (b.p. 40–60°)–ether, detection with concentrated sulfuric acid]. Elution of the mixture from Kieselgel (Merck 7734) with 2:1 light petroleum (b.p. 40–60°)–ether gave, first, trifluoromethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranoside (**2**, 39%), m.p. 67–68° (from light petroleum–ether), $[\alpha]_D$ +151° (*c* 3.2, chloroform) (Found: C, 42.2; H, 4.4; F, 20.5. $C_{13}H_{16}F_4O_8$ calc.: C, 41.5; H, 4.3; F. 20.2%), and then 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl fluoride (**3**, 37%), m.p. 71–72° (from light petroleum–ether), $[\alpha]_D$ +136° (*c* 2.8, chloroform) (Found: C, 46.8; H, 5.3; F, 12.5. $C_{12}H_{16}F_2O_7$ calc.: C, 46.4; H, 5.2; F, 12.3%). Further elution with 1:1 light petroleum–ether gave small amounts (~ 5%) of the (presumed) corresponding talose derivatives.

Hydrolysis of **2** with boiling 2M hydrochloric acid was complete in 2.5 h (t.l.c., 2:1 ethyl acetate–ethanol), to give a single product (R_F 0.65). Dilution with ethanol, neutralization ($PbCO_3$), and elution of the product from Kieselgel, gave 2-deoxy-2-fluoro-β-D-galactopyranose (**6**, 79%), having m.p. 131–135° (from ethyl acetate–methanol) and $[\alpha]_D$ +78.5 (5 min) → +92° (12 h) (*c* 2.3, water) (Found: C, 39.8; H, 6.3; F, 10.7. $C_6H_{11}FO_5$ calc.: C, 39.5; H, 6.0; F, 10.4%). Hydrolysis of **3** with 2M hydrochloric acid was complete in 1 h, and 2-deoxy-2-fluoro-β-D-galactopyranose (**4**, 63%), m.p. 131–135°, was isolated as described for **6**

The n.m.r. spectrum (94.1 MHz) of a solution of the fluoro sugar in D_2O showed ^{19}F resonances at 3822.5 Hz ($J_{F,1} < 0,5$, $J_{F,2}$ 50, $J_{F,3}$ 12.6, and $J_{F,4} \sim 3.5$ Hz) and at

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TABLE I

COUPLING CONSTANTS [a], J(IN Hz), FOR THE PRODUCTS FROM THE REACTION OF 3,4,6-TRI-*O*-ACETYL-D-GALACTAL WITH CF_3OF

Compound	*H-1/H-2*	*H-2/H-3*	*H-3/H-4*	*H-4/H-5*	*F-1/H-2*	*F-2/H-1*	*F-2/H-3*	*F-2/H-4*	*F-1/F-2*
2	3.7	10.5	3.5	1.0		<0.5	11.0	3.5	
3	2.7	9.7	3.2	1.0	23.0	<0.5	11.0	3.5	18.0

[a] Data refer to solutions in $CDCl_3$; they were obtained with a modified, Varian HA-100 spectrometer, operating in the field-sweep mode at 100 MHz for ^1H spectra, and in the frequency-sweep mode at 94.1 MHz for ^{19}F spectra.

3802 Hz ($J_{F,1}$ 3.5, $J_{F,2}$ 50.5, $J_{F,3}$ 15.3, and $J_{F,4} \sim 3.5$ Hz) upfield from C_6F_6, with relative integrated intensities of 2:3, that were assignable[5] to the α- and β-D anomers of 2-deoxy-2-fluoro-D-galactopyranose. The n.m.r. spectrum in Me_2SO-d_6 showed the anomeric hydroxyl proton, τ 3.17 (doublet, J 7 Hz) and one other hydroxyl proton, τ 5.0 (doublet, J 5 Hz; assigned to OH-3) (acetonitrile at τ 8.0). Addition of D_2O removed the hydroxyl protons, leaving one anomeric proton, τ 5.41 (triplet, J 6 Hz).

The structures of 2 and 3 were established by n.m.r. spectroscopy, and the essential data are included in Table I. The α-D- galacto configuration follows from the magnitude of the vicinal, $^1H-^1H$ coupling-constants[8]. The vicinal $^1H-^{19}F$ coupling-constants confirm this assignment, and the ^4J coupling (3.5 Hz) between F-2 and H-4 is consistent with a planar-W relationship[9] inherent in the D-galactopyrano configuration in the *C1* (D) conformation. The fluorine atoms of the OCF_3 group were coupled (J 1.5 Hz) to F-2, as shown by decoupling experiments[10], with simultaneous removal of the residual coupling (J 0.5 Hz) of the ring protons to the OCF_3 group.

The reaction sequence outlined gives 2-deoxy-2-fluoro-D-galactose from D-galactose in an overall yield of 30%.

ACKNOWLEDGMENTS

We thank P. N. Jenkins of Imperial College, London, for recording the n.m.r. spectra, and Professor A. B. Foster for his interest. The work was supported by grants to the Chester Beatty Research Institute, Institute of Cancer Research:Royal Cancer Hospital, from the Medical Research Council and the British Empire Cancer Campaign for Research. One of the authors (DMM) is Career Investigator of the Health Research Council of the City of New York and is also Eleanor Roosevelt Fellow, International Union against Cancer, 1969–1970.

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Preliminary Communication

New route for the synthesis of 3-deoxy-D-*erythro*-hexos-2-ulose★

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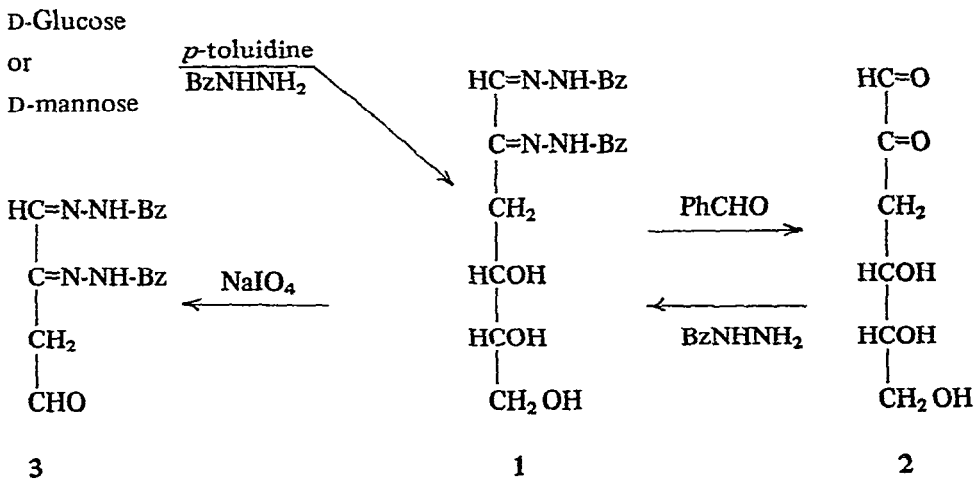
(Received March 6th, 1970)

It is known[1] that aldoses are converted into 3-deoxy-aldos-2-uloses by prolonged heating with amines. We have added benzoylhydrazine to the aldose–amine mixture, and have obtained a 3-deoxy-aldos-2-ulose bis(benzoylhydrazone) which, upon transhydrazonation with benzaldehyde, gives the 3-deoxy-aldos-2-ulose. In view of the high yields and good crystallizing properties of these new bis(benzoylhydrazones), the present route constitutes an improvement over existing methods[1,2] for the preparation of 3-deoxy-aldos-2-uloses.



The reaction is illustrated by the preparation of 3-deoxy-D-*erythro*-hexos-2-ulose (2) as follows: a solution of D-glucose (2.5 g), benzoylhydrazine (3.2 g), and *p*-toluidine (1 g) in ethanol (50 ml), water (10 ml), and acetic acid (0.5 ml) was boiled

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for 7 h under reflux. The product (1.9 g) crystallized from ethanol, m.p. 191°; $[\alpha]_D^{21}$ + 20.0° (*c* 0.2, pyridine); ν_{max}^{KBr} 1660(CONH) and 3350 cm^{-1} (OH); by mixed m.p. and X-ray powder diffraction pattern, it was identical with 3-deoxy-D-*erythro*-hexos-2-ulose bis-(benzoylhydrazone) (1) obtained from an authentic sample of 3-deoxy-D-*erythro*-hexos-2-ulose[2]. Upon periodate oxidation, compound 1 consumed 2 moles of oxidant per mole, and gave aldehyde 3, m.p. 212°, ν_{max}^{KBr} 1660 (CONH) and 1700 cm^{-1} (CHO). Treatment of the bis(benzoylhydrazone) 1 with benzaldehyde in the same way as described for the preparation of D-*arabino*-hexosulose[3] afforded 0.6 g of 3-deoxy-D-*erythro*-hexos-2-ulose[1,2] (2), identified by conversion into the known[2] bis[(2,4-dinitrophenyl)hydrazone], m.p. and mixed m.p. 265°, and a new bis(phenylhydrazone), m.p. 190°; $[\alpha]_D^{31}$ −73.1° (*c* 0.12, pyridine).

Full details of this synthesis and of the synthesis of other 3-deoxyaldos-2-uloses will be published later.

ACKNOWLEDGMENTS

The authors thank Dr. E. F. L. J. Anet, Commonwealth Scientific and Industrial Research Organization, Australia, for providing generous samples of 3-deoxy-D-*erythro*-hexos-2-ulose and its bis[(2,4-dinitrophenyl)hydrazone].

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3 S. Bayne, *Methods Carbohyd. Chem.*, 2 (1963) 421.

CORRIGENDA

Carbohyd. Res., 12 (1970) page 101, line 1 should read:
pic à *m/e* 88 $MeOCH^{+}$ CHOMe qui a été trouvé pour les méthyl 3,5-di-*O*-acétyl-2-*O*-

page 105, line 4:
(10:1) (2,3 mg), p.f. 90–91°. Le Tableau II indique le temps de rétention de ces anomères

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la c.p.g. des produits obtenus après déméthylation sont donnés dans le Tableau I.

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Book review

The Amino Sugars. The Chemistry and Biology of Compounds Containing Amino Sugars; *Volume IA, Chemistry of Amino Sugars:* edited by ROGER W. JEANLOZ, Academic Press, New York and London, August 1969, xxi+480 pp., $27.00 (252 s).

The importance of this unique series of volumes may be inferred from the fact that amino sugar derivatives are remarkably widespread; indeed, Volume IIA of the series even contained a chapter entitled "Glycosaminoglycans in the Connective Tissue of the Electric Organ". Research in the chemistry of amino monosaccharides has been quite incredibly stimulated by the discovery of a large number of unusual amino sugars as components of antibiotic and carcinostatic agents, and, in general, the synthetic efforts of the organic chemist have kept pace with the isolation of these compounds from natural sources.

In Chapter 1 (by D. Horton), which constitutes over 50% of the textual material of the book, is undertaken the heroic task of describing the chemistry of monomeric amino sugars. This is as much an exercise in permutations and combinations as any branch of organic chemistry, since the amino sugars now known include those in which one or more nitrogen atoms (each with a large variety of possible substituents) occupy virtually any position, either attached to the carbon chain of the sugar or engaged in a heterocycle.

Regrettably, the production of this book has been plagued by innumerable delays, and, as a result, Chapter 1 covers only the material published prior to January, 1965, except that a list of the author's subsequent publications in the field is appended. Nevertheless, this chapter is very worthwhile, as much of the material published between 1955 and 1965 has not been adequately reviewed before, and the reader who desires fuller coverage of more recent advances in the field can look forward to the appearance of Volume III, in which (hopefully) Volume IA and the previously published Volumes IIA and IIB will be brought up to date. The quantity of new material available is such that it could not possibly have been included in a single volume, especially as the authors have been encouraged to write inclusively, instead of selectively. Chapter 1 contains a number of previously unpublished infrared spectra of positional isomers of methylated hexosamine derivatives. Unfortunately, these spectra are all quite similar (and unassigned), and it appears that more cogent structural or "fingerprint" information could have resulted from the inclusion of some high-field nuclear magnetic resonance or mass spectra.

Chapter 2 (by G. Blix and R. W. Jeanloz) is devoted to sialic acids (acylated derivatives of 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid) and muramic acid [2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose]. These interesting

natural products are considered from the point of view of their discovery, structural characterization, estimation, physical and chemical properties, synthesis, and derivatives. The unpublished physical constants of a number of derivatives of *N*-acetylmuramic acid are tabulated, together with data from the literature, which is surveyed mainly to the end of 1966, with a limited number of references for 1967 and 1968.

Similar coverage is given in Chapter 3 (by H. H. Baer) to a large variety of amino oligosaccharides, ranging from lactosamine to the tetradecasaccharid, difucotri(lacto-*N*-tetraose). Approximately 80 individual amino oligosaccharides are considered in some detail, and Haworth–Hirst formulas are given for about 75% of these—a useful feature, as, in many cases, their systematic names are rather cumbersome. The elegant work of Kuhn and his associates on the amino oligosaccharides of human milk is particularly well described, as are also the complexities of the Morgan–Elson reaction as applied to these, and to related, compounds.

Chapter 4 (by J. L. Strominger) is a short, but rather fascinating, synopsis of nucleotide derivatives of amino-sugars.

Although all of the contributors to this volume are extremely well qualified, the native tongue of several of them is not English; they have, nevertheless, made a very commendable effort to write in good style. The relatively few errors of this type are not without some humor or innate truth; for example (from Chapter 2): "Definition of the anomery has often led to confusion". In general, this book is biased towards aspects of synthesis, isolation, analysis, and derivatization, and, with the possible exception of Chapter 1, little attention is paid to conformational and mechanistic factors. Determination of structure by periodate oxidation techniques is discussed often, and whilst use of this oxidant for materials of high molecular weight is, of course, still valid, such oxidations of monosaccharide derivatives have now been largely superseded by spectroscopic techniques. A number of very useful Tables of amino sugar derivatives are interspersed throughout the book, and a combined Bibliography and Author Index and a comprehensive Subject Index are provided.

This volume is recommended to all carbohydrate chemists, and to those members of the biochemical and biomedical fraternity who wish to extend further their appreciation of the relationships of amino sugars to other natural products and to life processes.

National Bureau of Standards, Washington, D.C. BRUCE COXON

CORRIGENDUM

Carbohyd. Res., 13 (1970) page 47, Ref. 16, line 2, schould read:

Burg, and G. Rembarz, *Chem. Ber.*, 92 (1959) 1614.

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MODEL PARAMETERS FOR THE ANALYSIS OF SKEW CONFORMATIONS OF CARBOHYDRATES BY P.M.R. SPECTROSCOPY*

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ABSTRACT

The p.m.r. spectrum of 3-*O*-benzoyl-1,2,4-*O*-benzylidyne-α-D-ribopyranose[1] (**1**) at 100 MHz has been analyzed by an iterative, least-squares method. The resulting geminal, vicinal, and long-range coupling-constants have been correlated with the stereochemistry of the locked, skew conformation of **1**, and used to test the applicability of equations that relate coupling constant to dihedral angle. The mean deviation of the approximate proton–proton dihedral angles as calculated from a Karplus equation from those measured from a molecular model was 11°.

INTRODUCTION

Although the relative importance of chair and nonchair conformations is of considerable interest in the conformational analysis of carbohydrate molecules, there is a paucity of n.m.r. data from genuine, or widely accepted, examples of nonchair conformations[2]. This is especially true because several of the earlier assignments of the skew-boat conformation to carbohydrates[3] or to other heterocyclic molecules[4] have been questioned[5–9], or withdrawn[8]. In order to determine the magnitudes of some of the proton-resonance parameters that may be expected to be characteristic of the skew conformations of pyranose compounds, the p.m.r. spectrum (at 100 MHz) of 3-*O*-benzoyl-1,2,4-*O*-benzylidyne-α-D-ribopyranose[1] (**1**) has been analyzed by iterative, computer methods[10]. This rigid system provides a suitable model for the testing of theoretical and empirical equations that relate various types of coupling constant with dihedral angle.

DISCUSSION

Inspection of molecular models of **1** shows that the molecule is rigidly locked in a skew conformation (**2**) by the three-point attachment of the benzylidyne group. In contrast to the skew conformations of many other monocyclic[11] and polycyclic systems[2,3,12], that of **1** is quite inflexible, and it therefore contains a group of protons

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of well-defined geometry which is ideally suitable for a study of the magnitudes of coupling constants. In this case, there is no possibility that the coupling constants observed could be modified by contributions from populations of other conformers in solution.

The assignments of the signals of the ring-protons in the spectrum (see Fig. 1a) of **1** in chloroform-*d* were confirmed by noting the solvent shifts of solutions in pyridine, and by frequency-swept, double-resonance experiments. Some of the double-resonance spectra are shown in Fig. 1 (c and d).

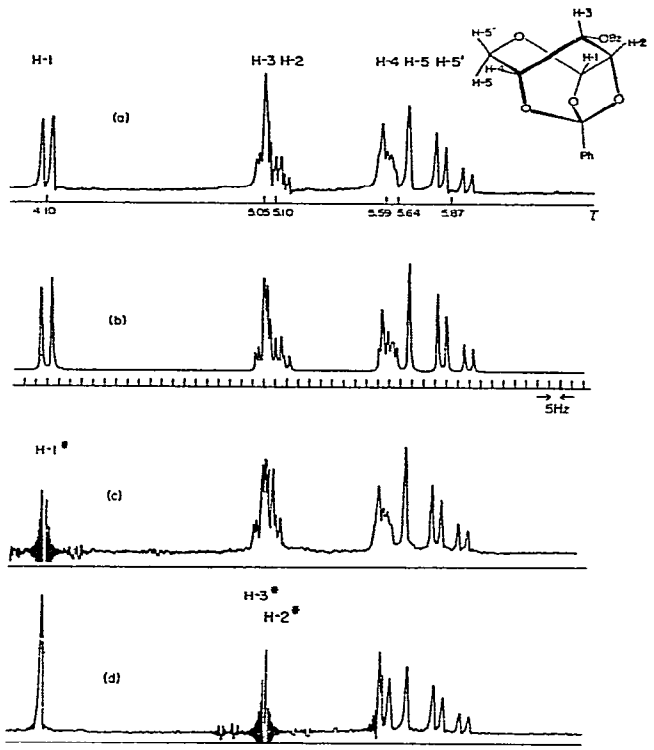


Fig. 1. P.m.r. spectra of 3-*O*-benzoyl-1,2,4-*O*-benzylidyne-α-D-ribopyranose in chloroform-*d* at 100 MHz; the signals of the aromatic protons are not shown. (a) Single resonance spectrum, (b) theoretical spectrum from iterative analysis, (c) irradiation of H-1, and (d) irradiation of H-2 and H-3.

Initial parameters for the iterative analysis of the spectra were derived by ABX analysis[1-3] of the H-3, H-2, and H-4 nuclei with H-1 decoupled (Fig. 1c), and by ABX analysis of the H-5, H-5′, and H-4 signals in the single-resonance spectrum (Fig. 1a) (H-5 resonates at lower field than H-5′). With H-1 decoupled, H-2 still resonated as a quartet (Fig. 1c) whose spacings suggested long-range coupling with H-4. This situation was confirmed by irradiation of H-4.

The initial p.m.r. parameters, and refined parameters after a total of thirteen iterations, are shown in Table I. Good agreement was obtained between the observed spectrum (see Fig. 1a) and the theoretical spectrum (Fig. 1b) calculated from the

refined parameters. Analysis of the observed spectrum was also attempted by using a negative sign for the long-range coupling $J_{2,4}$. In the trial theoretical spectrum calculated for this case, the relative intensities of the H-4 lines, and of the lines in the multiplet which may be assigned mainly to H-3, did not agree with the observed spectrum so well as when $J_{2,4}$ was made positive. Evidently, there is sufficient mixing of the spin states of H-2 and H-3 for reversal of the sign of $J_{2,4}$ to affect the intensities of the (mainly) H-3 lines. Iterative computations in which a negative initial value was used for $J_{2,4}$ failed to converge to a satisfactory fit, and hence $J_{2,4}$ may be assumed to be positive.

TABLE I

P.M.R. SPECTRAL PARAMETERS (Hz) OF 3-*O*-BENZOYL-1,2,4-*O*-BENZYLIDYNE-α-D-RIBOPYRANOSE (**1**) AT 100 MHz

Parameter	*Initial*	*Refined (13 iterations)*	
ν_1	590.32	590.31	±0.02
ν_2	489.99	489.77	±0.04
ν_3	495.36	495.34	±0.04
ν_4	440.72	440.62	±0.03
ν_5	435.68	435.79	±0.03
$\nu_{5'}$	412.43	412.64	±0.03
$J_{1,2}$	4.50	5.22	±0.05
$J_{1,3}$	0	−0.54	±0.05
$J_{1,4}$	0	−0.06	±0.03
$J_{1,5}$	0	0.05	±0.03
$J_{1,5'}$	0	−0.14	±0.04
$J_{2,3}$	3.37	3.58	±0.03
$J_{2,4}$	2.27	2.45	±0.03
$J_{2,5}$	0	0.04	±0.03
$J_{2,5'}$	0	−0.04	±0.04
$J_{3,4}$	1.08	1.33	±0.04
$J_{3,5}$	0	−0.18	±0.05
$J_{3,5'}$	0	−0.02	±0.05
$J_{4,5}$	0.30	0.27	±0.04
$J_{4,5'}$	3.30	4.09	±0.04
$J_{5,5'}$	−11.69	−11.71	±0.03

From Table I, it may be seen that the iterative process caused little change in the chemical shifts, although there was a general tendency for the numerical values of the coupling constants to increase. For the particular values of proton–proton dihedral angles (ϕ) represented in **2**, the corresponding values of the vicinal coupling-constants (Table I) may be used to test the applicability of the Karplus equation[14,15]

$$\phi = \cos^{-1}\left\{\frac{0.5 \pm [0.25 + 36(J + 0.28)]^{1/2}}{18}\right\} \quad (1)$$

to carbohydrate systems. The mathematical form[15] of this equation is convenient, in that the solution of the quadratic equation in cos ϕ provides two roots, one of which

corresponds to $0° \leqslant \phi \leqslant 88°$, and the other, to $88° \leqslant \phi \leqslant 180°$, with the use of the same set of constants for each type of angle*.

TABLE II

APPROXIMATE PROTON–PROTON DIHEDRAL ANGLES (ϕ, DEGREES) IN 3-*O*-BENZOYL-1,2,4-*O*-BENZYLIDYNE-α-D-RIBOPYRANOSE

Angle	*Proton orientations*[a]	*From molecular models*[b]	*Calculated from J*	*For C_2 twist form*[c]
$\phi_{1,2}$	*e, q*	22	36 (139)[d]	33
$\phi_{2,3}$	*q, a*	50	47 (129)	33
$\phi_{3,4}$	*a, e*	72	63 (113)	71
$\phi_{4,5}$	*e, q*	100	103 (74)	87
$\phi_{4,5'}$	*e, q*	20	44 (132)	33

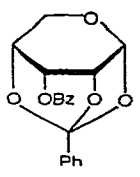
[a]Equatorial (*e*), axial (*a*), quasi (*q*). [b]Dreiding. [c]Pyranoid ring in idealized symmetrical twist form with ring-atom dihedral angles of 33 or 71°. [d]The figures in parentheses are the alternative values of ϕ calculated from the Karplus equation.

In Table II are shown (*a*) the approximate dihedral angles of the vicinal protons obtained by measurement of Dreiding molecular models of **2**, (*b*) the two angles which were calculated from each vicinal coupling-constant by using equation (1), and (*c*) the angles that would be expected for an idealized, symmetrical C_2 skew form[16] of the pyranoid ring (ignoring the symmetry-destroying effect of the ring oxygen-atom). It may be seen that, in a molecular model of **2**, the molecule is somewhat distorted from the idealized, C_2 skew form, as only the corresponding values of $\phi_{3,4}$ are in close agreement. Part of this distortion is due to the unsymmetrical attachment of the five-, six-, and seven-membered rings formed by the benzylidyne substituent. The values of $\phi_{2,3}$, $\phi_{3,4}$, and $\phi_{4,5}$ calculated from equation (1) agree quite well with the values estimated by study of molecular models, although, in order to obtain good agreement for $\phi_{4,5}$, it is necessary to choose the larger value (103°) from the two possible values. The agreement is less good for $\phi_{1,2}$ and $\phi_{4,5'}$. Although $J_{1,2}$ (5.22 Hz) for this molecule (**1**) is quite large, it is not large enough to yield a calculated value of $\phi_{1,2}$ approaching 22°, probably because the magnitude of $J_{1,2}$ is lessened[17] by the electronegative atoms that are bound to C-1 and C-2. The reason for the deviation of the calculated from the observed value of $\phi_{4,5'}$, amounting to 24°, is not yet clear. The expected dependence[14] of vicinal coupling-constants on bond angle and bond length has not been verified for carbohydrate systems, possibly because of the difficulty in obtaining suitable data from solutions, and the inadequacy of most types of molecular model for such studies.

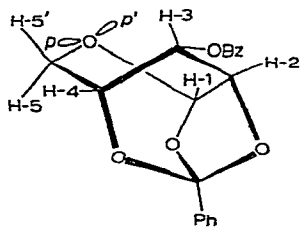
The value of the geminal coupling ($J_{5,5'}$, 11.7 Hz) of **1** is intermediate between those (10.8–10.9 and 13.4–14.0 Hz) reported[18] for α anomers (A) or β anomers (B) of D-ribopyranose tetrabenzoate or of the related 1-halides, for which a dependence[19]

*The effect on vicinal coupling constants of molecular parameters other than dihedral angle has been emphasized previously[14].

on the orientation of the vicinal, electronegative substituent (benzoyloxy) on C-4 has been indicated[18]. Although the value of 11.7 Hz for $J_{5,5'}$ is closer to those of compounds A, the orientation of O-4 with respect to H-5′ and H-5 more nearly resembles the *trans-gauche* arrangement found[18] in compounds B. However, for nonchair conformations of six-membered heterocycles, a further influence on the geminal coupling-constant must be considered. The results of theoretical[19] and experimental[20–22] studies have suggested that proximity of the σ and p orbitals of a C–H bond and an oxygen atom, respectively, can lead to a $p \rightarrow \sigma$ electron transfer which results in a positive increment to the geminal coupling-constant, in addition to that due to σ-electron withdrawal by a suitably disposed, electronegative substituent. In comparisons of the geminal coupling-constants of the two chair conformations of a pentopyranose, it is not necessary to consider the $p \rightarrow \sigma$ effect, as, for each conformation, the methylene H–C-5 bonds have the same type of orientation with respect to the p orbitals of the ring oxygen-atom. However, in passing from these conformations to the skew conformation **2**, the dihedral angles $\phi_{5,p}$ and $\phi_{5',p'}$ of the p orbitals decrease from $\sim 60°$ to $\sim 44°$ (as estimated from Dreiding molecular models), thus leading to more effective $p \rightarrow \sigma$ transfer and an increased, positive contribution to $J_{5,5'}$. The fact that $J_{5,5'}$ is more positive than expected is qualitatively explained by this mechanism.



1



2

The long-range coupling $J_{2,4}$ of **1**, amounting to 2.45 Hz, is probably the largest thus far observed over four saturated bonds of a carbohydrate system, although values as high as 3.0 Hz have been found[23] for *m*-dioxane derivatives. In the skew conformation **2**, the dihedral angles ϕ' (of H-2 and C-4, about the C-2–C-3 bond) and ϕ''' (of H-4 and C-2, about the C-3–C-4 bond) are $\sim 170°$ and $\sim 171°$, respectively, and, therefore, the orientation of H-2, C-2, C-3, C-4, and H-4 is very close to the planar W-arrangement that is considered[24] to be optimal for coupling (^4J) over four saturated bonds. In **2**, H-2 and H-4 have quasi and equatorial orientations, with respect to the pyranose ring.

The positive sign of $J_{2,4}$ is in agreement with the indirect, through-bond mechanism proposed[25] by Barfield, but does not agree with an alternative, indirect, coupling mechanism[26] based on intra-atomic Hund coupling between electron spins. The latter mechanism does not depend on the stereochemistry of the nuclei coupled, and cannot therefore explain the planar W rule. However, Barfield's mechanism[25] does not satisfactorily account for the recently reported[27] variation of the

sign of ^4J with the equatorial-equatorial, or equatorial-axial orientations of ring protons of the *chair* conformations of pyranoid sugars. Barfield's theory[25] has been interpreted[28] in terms of the equation

$$^4J = 0.7(\cos^2\phi' + \cos^2\phi''') - 0.3 \quad (2)$$

Substitution of the values of ϕ' and ϕ''' for **2** in this equation gives a value for ^4J of 1.06 Hz, a value that does not agree with the observed value of $J_{2,4}$, namely, 2.45 Hz. Such disagreement has been noted[28] for ^4J in a series of tetrachlorobicyclo[2,2,1]-heptane derivatives, and it was suggested[28] by Bystrov and Stepanyants, that better agreement with the experiment could be obtained by use of the equation

$$^4J = A\cos^2\phi'\cos^2\phi''' - B \quad (3)$$

where $B = 0.35$, and $A = 0.31$ for $0° \leqslant \phi', \phi''' \leqslant 90°$; 1.07 for $0° \leqslant \phi' \leqslant 90° \leqslant \phi''' \leqslant 180°$; or 3.61 for $90° \leqslant \phi', \phi''' \leqslant 180°$. For **2**, equation (3) yields ^4J 3.07 Hz, which agrees more closely with the observed value of $J_{2,4}$.

The applicability of equations (2) and (3) to long-range couplings of other carbohydrate derivatives was next examined. The couplings $J_{1,3}$ +1.6 and $J_{3,5}$ +1.5 Hz have been reported[27] for 1,6-anhydro-β-D-mannopyranose triacetate (**3**); these values are the largest that were found in a survey[27] of the magnitudes and relative signs of ^4J (over saturated bonds of carbohydrate ring-systems). Considering $J_{1,3}$ first, measurement of Dreiding molecular models of **3** yielded dihedral angles ϕ' (of H-1 and C-3, about the C-1–C-2 bond) and ϕ''' (of H-3 and C-1, about the C-3–C-2 bond) of 180° and 167°, respectively. The application of equations (2) and (3) to these angles gives theoretical values of $J_{1,3}$ of 1.06, or 3.08 Hz, respectively. Similarly, for $J_{3,5}$, the dihedral angles ϕ' 167° (of H-3 and C-5, about the C-3–C-4 bond) and ϕ''' 172° (of H-5 and C-3, about the C-5–C-4 bond) obtained from models of **3** yield theoretical couplings of 1.05 (equation 2), or 3.01 Hz (equation 3). Thus, for compound **3**, and other similar derivatives[27,29], the ^4J couplings are predicted more accurately by equation (2), than by equation (3). These derivatives do not have completely rigid ring-systems, whereas the D-ribopyranose compound **1** and the tetrachlorobicyclo-[2,2,1]heptane derivatives[28] do. For the bicycloheptane derivatives and **1**, it is less likely that molecular vibration (or conformational interconversion) forces the interacting protons into orientations less favorable for coupling. It has often been observed that the ^4J couplings in conformationally locked ring-systems are abnormally large[24,30,31], although, in some cases, this must be due to a greater number of "through-bond" coupling-paths.

Although H-5 and H-5' each have the same quasi orientation with respect to the skew shape (**2**) of the pyranoid ring, the results in Table I show that H-5 resonates at 0.23 p.p.m. to low field of H-5'. Measurements of Dreiding molecular models of **2** indicate that the distances of H-5 from the axially oriented O-1 and O-4 are about 2.4 and 2.5 Å, respectively. As these distances are less than the sum (2.6 Å) of the van der Waals radii, deshielding of H-5 by a van der Waals mechanism[32,33] may be expected to be important.

Several other types of locked-ring system are present in **1**. The 1,3-dioxolane ring formed by C-1, C-2, O-1, O-2, and the benzylidyne carbon atom (C-1,2,4) must adopt a twist shape. A flattened chair-conformation[3,4] of a *m*-dioxane ring is formed by C-2, C-3, C-4, O-2, O-4, and C-1,2,4, and the seven-membered ring comprised by C-1, C-4, C-5, O-1, O-4, O-5, and C-1,2,4 is forced to adopt a twisted chair form. Each ring-system bears at least one pair of vicinal protons whose coupling constants (see Table I) may be useful as examples that are characteristic of locked conformations.

EXPERIMENTAL

General. — P.m.r. spectra of **1** were recorded at 100 MHz, in the frequency-sweep mode, with the spectrometer locked internally on the signal of tetramethylsilane (τ 10.00). Frequency-swept double-resonance experiments were performed with an external, audio oscillator. The concentrations of the solutions of **1** were 83 mg in chloroform-*d* (0.5 ml) (saturated solution), and 183 mg in pyridine (0.8 ml). Spectra for analysis were recorded at a sweep-width of 250 Hz; however, the sweep-offset was calibrated from spectra measured at the 1 kHz sweep-width. Sweep widths were verified by means of an electronic frequency counter.

Spectral assignments and preliminary analysis. — Because of their proximity to electronegative or anisotropic atoms or groups, it was expected that H-1 and H-3 would resonate at low field. Spectra of **1** in chloroform-*d* showed a sharp doublet (with weak, outlying lines) at τ 4.1 which was assigned to H-1, complex multiplets at τ 5.1 and 5.6, and a sharp quartet at τ 5.9. Results of integration of the spectrum at 60 MHz indicated that each complex multiplet represented two protons. However, in spectra of **1** in pyridine, the complex multiplet at higher field was separated into a less complex, one-proton multiplet at τ 5.36, and a sharp, one-proton doublet (spacing 11.4 Hz) at τ 5.56. Moreover, in pyridine, H-1 resonated as a complex multiplet composed of at least six lines, and the bandwidth of the remaining two-proton complex multiplet at τ 4.8 was smaller than that at τ 5.1 for solutions in chloroform-*d*. On irradiating solutions in pyridine at τ 4.8, or in chloroform-*d* at τ 5.1 (see Fig. 1d), the doublet or complex multiplet, respectively, at low field collapsed to a sharp singlet (H-1, decoupled), thereby demonstrating that the multiplet at τ 4.8 or 5.1 contains the H-2 signal, in addition to that of H-3. Also observed in these spin-decoupling experiments was the collapse of the complex multiplet at τ 5.36 (in pyridine) or at τ 5.6 (in chloroform-*d*, see Fig. 1d) to a sharp doublet which contained a spacing of medium size that also appeared in the quartet at highest field. Since both the latter quartet and the doublet at slightly lower field each contained the same large spacing ($\sim$12 Hz) characteristic of nonequivalent, geminal protons, these multiplets were assigned to H-5 and H-5', and the remaining complex multiplet, to H-4.

On irradiating H-1 (in chloroform-*d*), the portion of the complex multiplet (τ 5.1) at higher field collapsed to a quartet, whereas the part of the multiplet at lower field was not changed (see Fig. 1c). On this basis, the H-2 signal could be assigned as being mainly an octet overlapping with a quartet (H-3, mainly) at slightly lower field.

On irradiation of H-4, the H-3 signal was simplified to a doublet, and the H-2 signal to a quartet, thereby confirming the existence of the long-range coupling between H-2 and H-4.

Irradiation of H-1 of **1** (in pyridine) simplified the complex multiplet due to H-2 and H-3 to a sharp doublet, thus suggesting that, in this solvent, H-2 and H-3 have the same chemical shift[35].

In order to obtain initial parameters for iterative analysis of the spectra, the H-2 and H-3 quartets in the spectrum (Fig. 1c) for H-1 decoupled were subjected to an ABX analysis[13], with H-3, H-2, and H-4 regarded as the A, B, and X nuclei, respectively. The recalculated and experimental line-positions agreed to within 0.1 Hz. The results of the ABX analysis suggested that the relative signs of J_{AX} ($J_{3,4}$) and J_{BX} ($J_{2,4}$) are the same. As vicinal, proton–proton coupling-constants (*e.g.*, $J_{3,4}$) are normally positive[27], it was therefore assumed, initially, that $J_{2,4}$ is also positive. Because the chemical shifts of H-2 and H-3 from H-1 were large and similar (~100 Hz), it was assumed that the H-2 and H-3 signals experienced approximately the same Bloch–Seigert shift[36] on irradiation of H-1. The chemical-shift difference $\nu_3 - \nu_2$ (δ_{AB}) calculated from the double-resonance spectrum (see Fig. 1c) was then used for estimating the chemical shifts of H-2 and H-3 in the single-resonance spectrum (see Fig. 1a) by reference to their observed line-positions.

As already mentioned, the H-5 signal is a doublet in both chloroform-*d* and pyridine; therefore, since, despite their small chemical-shift difference, H-4 and H-5 are obviously only weakly coupled, initial parameters for H-5, H-5′, and H-4 were estimated from an ABX analysis[13] of this sub-system of protons. From this analysis, the recalculated and experimental line-positions agreed within 0.09 Hz, and the parameters ν_4 440.72, ν_5 435.68, $\nu_{5'}$ 412.43 Hz, $J_{4,5}$ 0.93, $J_{4,5'}$ 3.30, and $J_{5,5'}$ 11.69 Hz were obtained.

The parameters from the two ABX analyses were combined with first-order parameters from the H-1 signal, and, on the assumption[27] that the geminal coupling $J_{5,5'}$ is negative, a trial theoretical spectrum for six spins (H-1–H-5′) was computed by using the LAOCN3 program[10]. The resulting machine-drawn*, theoretical spectrum agreed well with the spectrum observed, except that the theoretical signal for H-5 was a quartet that contained a small spacing, and the chemical-shift difference of H-2 and H-3 was too great.

Iterative computations. — Suitable adjustments were made to the initial parameters (see Table I) and a new trial theoretical spectrum was computed; H-5 now appeared as a doublet.

Iterative computations were then performed in which 88 of the theoretical transitions were assigned to 26 experimental line-positions, and in which variation of all of the 21 chemical-shift and coupling parameters was permitted. Computation proceeded for five iterations, with root-mean-square (r.m.s.) errors of 0.414, 0.250, 0.233, 0.230, and 0.230. The computed probable errors of the iterated parameters

*By a digital, incremental, x–y plotter with Lorentzian and Gaussian line-widths of 0.6 Hz.

were then $\leqslant 0.11$. However, the frequency errors ($|\Delta\nu|$) of 23 of the assigned theoretical lines were >0.2 Hz. Hence, their experimental frequencies were re-assigned to other theoretical lines.

With these modifications, the iterated parameters were re-inserted in the program, and four more iterations were performed, giving r.m.s. errors of 0.124, 0.098, 0.095, and 0.095, and probable errors of parameters $\leqslant 0.05$. With further minor modifications to line assignments, four further iterations were conducted, affording r.m.s. errors of 0.129, 0.114, 0.109, and 0.108, probable errors of parameters $\leqslant 0.05$, and $\Delta\nu \leqslant 0.29$. In Table I, the refined parameters (and their computed, probable errors) are compared with the initial parameters.

The computations were repeated for the case where the same initial parameters were used, except that $J_{2,4}$ was made negative. In the trial theoretical spectrum resulting for this, the relative intensities of the two downfield-lines of H-3, and of two lines of the H-4 multiplet, were reversed, so that poorer agreement with the observed spectrum (see Fig. 1a) was obtained. Iterative computations (seven cycles, and three cycles, successively) for this gave r.m.s., frequency, and probable errors that were appreciably larger than where $J_{2,4}$ was positive. In addition, the theoretical spectrum calculated from the iterated parameters was in poor agreement with the observed spectrum.

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SYNTHESE EINES DI-α-D-GLUCOFURANOSE-3,6'-ANHYDRIDS

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ABSTRACT

5,6-Anhydro-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose reacts either in the melted state or in boiling dioxane with the 3-sodio derivative of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose to give 1,2:5,6:1',2'-tri-*O*-isopropylidene-3'-*O*-methyl-di-α-D-glucofuranose 3,6'-anhydride. Acid hydrolysis and acetylation yielded the crystalline hepta-acetate. The structure was established by chemical transformation and mass spectrometry.

ZUSAMMENFASSUNG

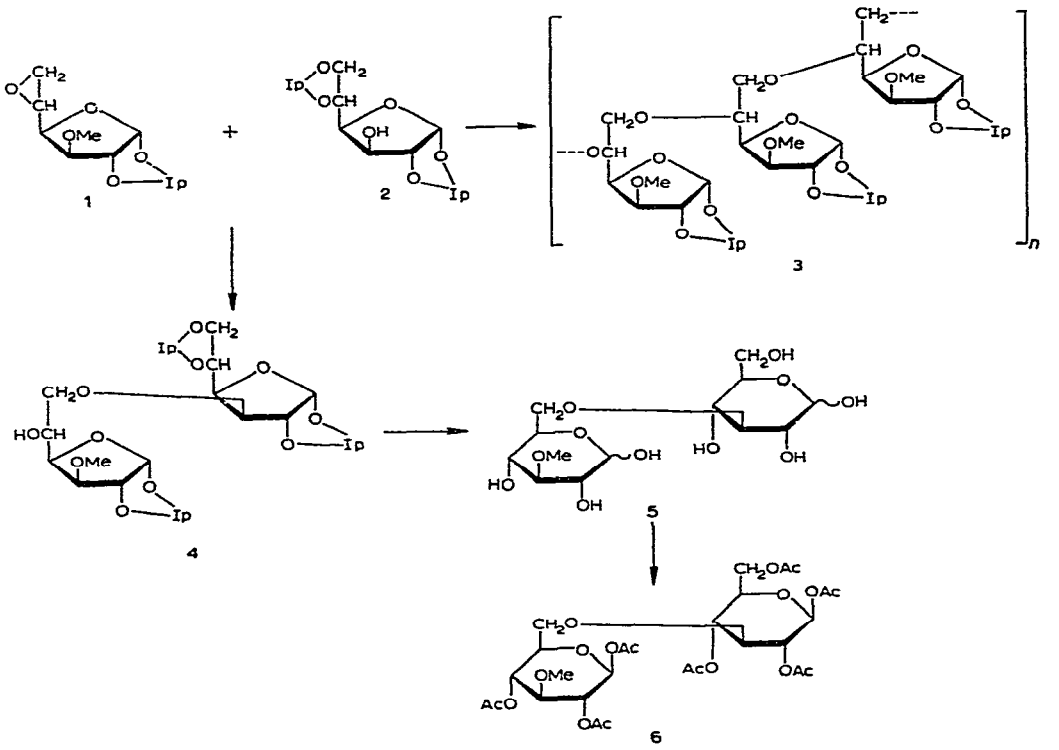
5,6-Anhydro-1,2-*O*-isopropyliden-3-*O*-methyl-α-D-glucofuranose ergibt in der Schmelze oder in siedendem Dioxan mit der 3-Natriumverbindung der 1,2:5,6-Di-*O*-isopropyliden-α-D-glucofuranose das 1,2:5,6:1',2'-Tri-*O*-isopropyliden-3'-*O*-methyl-di-α-D-glucofuranose-3,6'-anhydrid. Saure Hydrolyse und Acetylierung ergeben das kristalline Hepta-acetat. Die Struktur wird durch chemische Untersuchungen und Massenspektrometrie sichergestellt.

EINFÜHRUNG

5,6-Anhydro-1,2-*O*-isopropyliden-α-D-glucofuranose ist verschiedentlich zu Disaccharidsynthesen verwendet worden. So wird der Epoxidring durch 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl-bromid unter Bildung von 6-Brom-6-desoxy-5-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl]-1,2-*O*-isopropyliden-α-D-glucofuranose geöffnet[1]. Auch endständige Hydroxylgruppen geeigneter Zuckerderivate greifen den Epoxidring nucleophil an. Wird z.B. 5,6-Anhydro-1,2-*O*-isopropyliden-α-D-glucofuranose mit 1,2-*O*-Isopropyliden-α-D-glucofuranose geschmolzen, so entsteht als überwiegendes Hauptprodukt das Di-*O*-isopropyliden-Derivat eines Di-α-D-glucofuranose-6,6'-anhydrids[2]. Arbeitet man mit 5,6-Anhydro-1,2-*O*-isopropyliden-3-*O*-methyl-α-D-glucofuranose (**1**) in Gegenwart basischer Katalysatoren, so tritt hauptsächlich Polymerisation des Anhydrozuckers unter 5,6-Verknüpfung ein. Diese Reaktion ist von Schuerch und Mitarbeitern systematisch untersucht worden[3].

ERGEBNISSE UND DISCUSSION

Wir führten die Umsetzung von **1** mit der 3-Natriumverbindung der 1,2:5,6-Di-*O*-isopropyliden-α-D-glucofuranose (Diacetonglucose) (**2**) durch. In der Schmelze oder in siedendem Dioxan erhielten wir hauptsächlich das bisher unbekannte 1,2:5,6:1′,2′-Tri-*O*-isopropyliden-3′-*O*-methyl-di-α-D-glucofuranose-3,6′-anhydrid (**4**) neben einer kleinen Menge des von Schuerch bereits beschriebenen Linearpolymeren **3**. Die Isolierung von **3** und **4** erfolgte durch Säulenchromatographie an Kieselgel oder Sephadex LH-20; **4** und das durch saure Hydrolyse daraus erhaltene 3′-*O*-Methyl-di-α-D-glucopyranose-3,6′-anhydrid (**5**) sind sirupöse, nicht ganz reine Substanzen. Durch Acetylierung überführten wir **5** in das kristalline, analysenreine Hepta-acetat **6**.



Schema 1.

Das ebenfalls kristalline Nebenprodukt **3** besitzt ein mittleres Mol.-Gew. von 2300. Seine analytischen Daten und besonders sein i.r.-Spektrum stimmen mit den von Schuerch und Mitarbeitern[3] gemachten Angaben überein.

Die Struktur des Di-α-D-glucose-3,6′-anhydrids haben wir auf folgendem Wege sichergestellt. Nach der Hydrolyse des Isopropylidenderivates (**4**) mit 5%-iger Salzsäure zeigte das Papierchromatogramm ein einziges, einheitliches Produkt **5** mit einem R_F-Wert, der einem Disaccharid mit einer *O*-Methylgruppe entspricht. Freie D-Glucose oder 3-*O*-Methyl-D-glucose waren nicht nachzuweisen. Das zeigt, daß eine reine Ätherbindung vorliegt. Tritylierungsversuche an **4** ergaben Hinweise auf die

erwartete 3,6′-Verknüpfung. Wie das i.r.-Spektrum zeigt, besitzt **4** eine freie Hydroxylgruppe. In das Anhydrid **4** ließ sich keine Tritylgruppe einführen. Dies zeigt in Analogie zu den umfangreichen Umsetzungen dieser Art[4] eine 6-Verknüpfung an.

Durch massenspektrometrische Untersuchungen an **4** und dem Hepta-acetat **6** ließen sich neben den exakten Molekulargewichten der untersuchten Derivate die Stellen der Verknüpfung exakt festlegen. Das Massenspektrum von **4** (Abb. 1) ließ

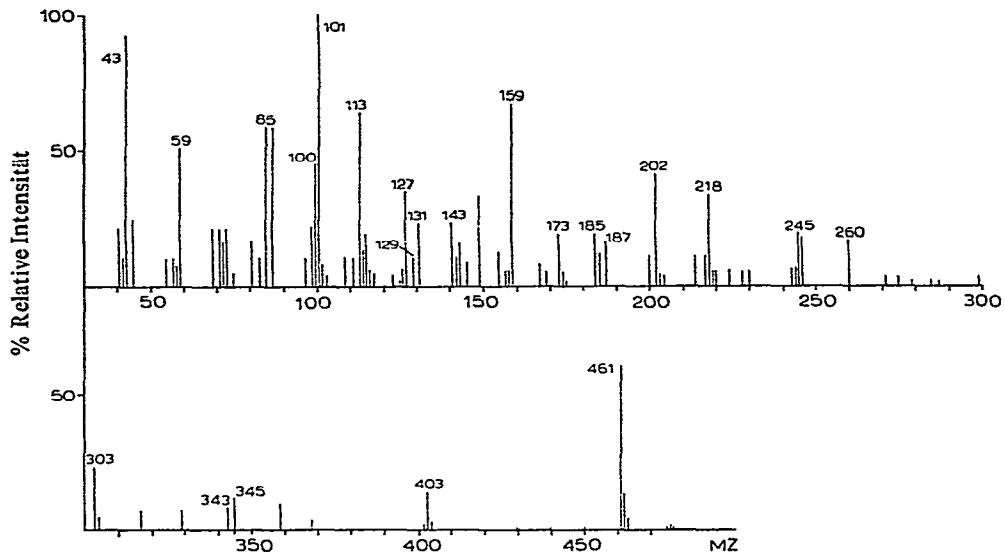
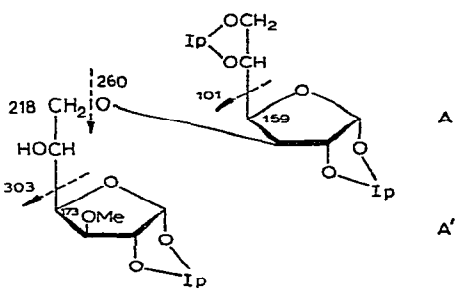


Abb. 1. Massenspektrum von 1,2:5,6:1′,2′-Tri-*O*-isopropyliden-3′-*O*-methyl-di-α-D-glucofuranose-3,6′-anhydrid (**4**).

sich unter Berücksichtigung der Arbeiten von DeJongh und Biemann[5] weitgehend deuten. Ein Molekülpeak wird nicht gefunden. Die Abspaltung einer der geminalen Methylgruppen der Isopropylidenreste gibt jedoch ein stabiles Ion, so daß ein intensiver Peak bei Massenzahl (MZ) 461 (M−15) auftritt. Dieses Ion zerfällt unter Eliminierung von Aceton und Essigsäure. Auf diese Weise kommen die Fragmente MZ 403 (M−15−58) und MZ 343 (M−15−58−60) zustande. Eine ähnliche Serie entsteht durch Zerfall eines Fragmentes MZ 260 (Diacetonglucose), das durch Spaltung der "Disaccharidbindung" in der in Schema 2 angegebenen Weise gebildet



Schema 2.

wird. Es sind dies die Peaks bei MZ 245 (200−15), MZ 187 (260−15−58), MZ 185 (260−15−60) und MZ 127 (260−15−58−60). Besonders leicht wird bei Hexofuranosen die C-4–C-5-Bindung gespalten. Die positive Ladung übernimmt bevorzugt das C-5–C-6-Fragment, so daß das Spektrum von einem intensiven Peak bei MZ 101 beherrscht wird. Das C-1–C-2–C-3–C-4-Fragment MZ 159 wird im A′-Teil des Moleküles durch die *O*-Methylgruppe zu MZ 173 verschoben. Nach diesem Zerfallsmechanismus werden auch die Fragmente MZ 303 (M−173) und MZ 202 (M−173 −101) gebildet, in denen die 3,6′-Bindung intakt blieb.

Durch einen weiteren charakteristischen Zerfall, bei dem die C-3–C-4-Bindung aufgeht, entstehen die Fragmente MZ 345, MZ 131, MZ 129 und MZ 143. Außerdem gibt es eine Reihe weniger charakteristischer Peaks, die in den Spektren aller Isopropylidenverbindungen auftreten. Es sind dies die Ionen MZ 100, MZ 85 und MZ 113. Intensive Peaks finden sich auch bei MZ 43 (CH_3CO^+) und MZ 59 (protoniertes Aceton). Das Massenspektrum von **4** steht somit in sehr guter Übereinstimmung mit der Struktur eines 3-verknüpften Dimeren.

Die Verknüpfung in dem 3-*O*-Methylglucoseteil (5′ oder 6′) ist daraus jedoch nicht zu ersehen. Hierzu zogen wir das Massenspektrum von **6** (Abb. 2) heran. Bei

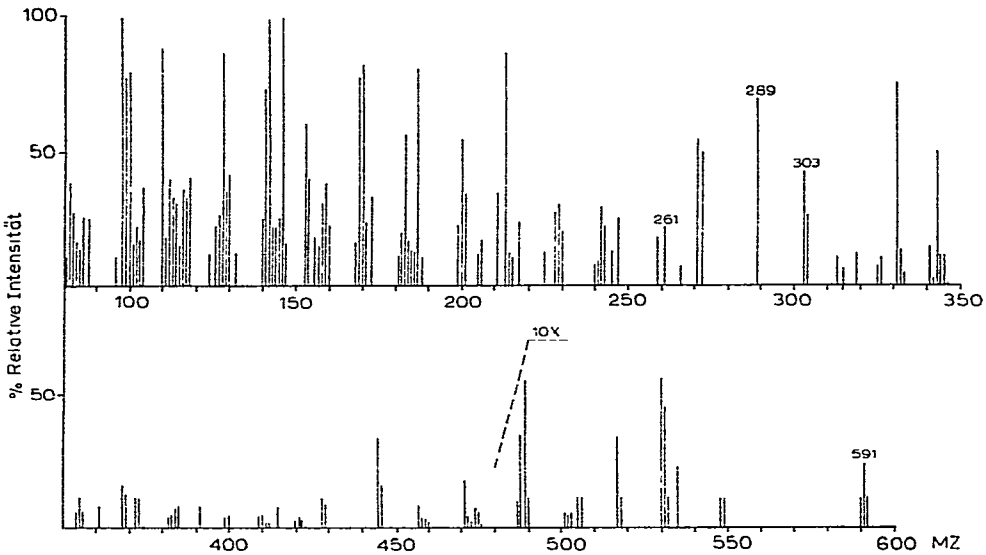
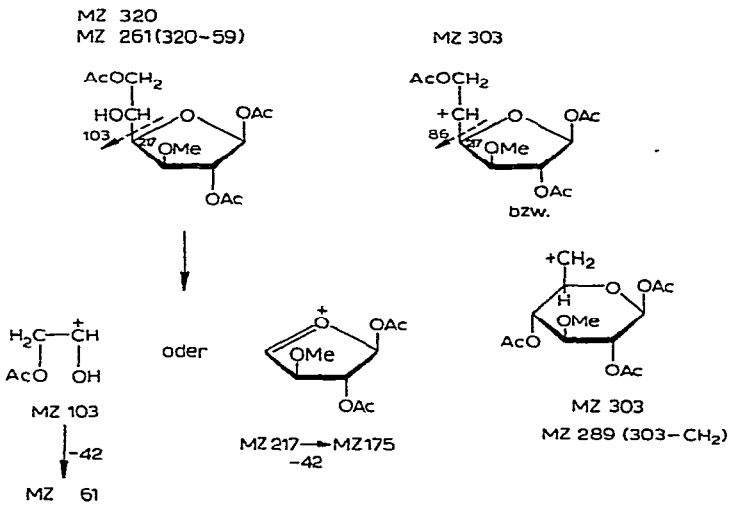


Abb. 2. Massenspektrum von 3′-*O*-Methyl-di-α-D-glucose-3,6′-anhydrid-hepta-acetat (6).

einer 6′-Verknüpfung liegen beide Glucosemoleküle in der pyranoiden Form vor. In diesem Fall dürfen die sehr charakteristischen Peaks für die furanoide Form[6,7] (Siehe S. 5) im Massenspektrum nicht aufteten. Diese Peaks kommen im Spektrum nicht mit der erwarteten Intensität vor[6,7]. Dagegen leitet sich z.B. vom Fragment MZ 303 ein Ion ab, das nur bei Vorliegen eines Sechsrings entstehen kann. Es ist das Ion MZ 289 (303−CH_2) mit einer relativen Intensität von 75%.



Schema 3.

EXPERIMENTELLER TEIL

Allgemeines. — Das Eindampfen der Lösungen geschah im Vakuum bei einer Badtemperatur von 35°. Die i.r.-Spektren wurden mit einem Perkin–Elmer Spektrophotometer Modell 157 aufgenommen. Die Substanzen kamen als KBr-Presslinge in den Strahlengang. Auf mit Kieselgel G (E. Merck AG) bestrichenen Glasplatten wurden die Dünnschichtchromatogramme ausgeführt. Als Laufmittel fand ein Lösungsmittelgemisch, 200:47:15:1 Benzol–Äthanol–Wasser–Ammoniak, Verwendung. Die Entwicklung der Chromatogramme geschah durch Sprühen mit konzentrierter Schwefelsäure und anschließendes Erhitzen bei 120°. Die Papierchromatogramme wurden mit Whatman Nr. 1-Papier aufsteigend mit 8:1:4 Butanol–Äthanol–Wasser als Laufmittel angefertigt. Besprüht wurde mit Anilinphthalat-Lösung. Die Massenspektren wurden mit einem Massenspektrometer Model SM 1-B der Firma Varian-MAT nach einer direkten Einlaßmethode unter folgenden Bedingungen aufgenommen: Arbeitstemperatur 160°, Elektronenenergie 70 eV.

1,2:5,6:1′,2′-Tri-O-isopropyliden-3′-O-methyl-di-α-D-glucofuranose-3,6′-anhydrid (**4**). — (*a*) *Darstellung in einem Lösungsmittel.* Zu einer Lösung von 1,2:5,6-Di-*O*-isopropyliden-α-D-glucofuranose[8] (**2**, 1,00 g, Schmp. 110°) in absolutem Dioxan wurde Natriumdraht (*ca.* 3 g) gepreßt und die Mischung 5 Stdn. unter Rückfluß und Feuchtigkeitsausschluß bei Zimmertemperatur stehengelassen. Sodann wurde der Natriumdraht herausgezogen und eine äquimolare Menge von 5,6-Anhydro-1,2-*O*-isopropyliden-3-*O*-methyl-α-D-glucofuranose[9] (**1**, 0,83 g, bzw. 0,68 ml des Sirups) hinzugegeben. Daran schloß sich ein 15-stündiges Erhitzen unter Rückfluß und Feuchtigkeitsausschluß an. Anschließend wurde zu der Dioxanlösung Wasser gegeben und die stark alkalische Lösung mit verdünnter Schwefelsäure vorsichtig neutralisiert. Nach dem Eindampfen und Aufnehmen des Rückstandes mit

Benzol konnten die anorganischen Salze abfiltriert werden. Die Isolierung durch Chromatographie ist im folgenden Paragraphen beschrieben.

(*b*) *Darstellung in der Schmelze.* Verbindung **2**[8] (1,00 g) wurde mit einer äquimolaren Menge Natrium (0,09 g) 1 Std. bei 110° im Ölbad unter getrocknetem Stickstoff geschmolzen. Dabei sublimierte ein geringer Teil von **2**. Zu der klaren Schmelze wurde eine äquimolare Menge Verbindung **1**[9] (0,83 g) hinzugegeben und anschließend die Mischung 2 Stdn. bei 80–100° gehalten. Die abgekühlte Schmelze wurde in Methanol–Wasser aufgenommen, wobei man, um die Rückstände besser lösen zu können, zweckmäßig etwas Aceton hinzufügt. Die stark alkalische Lösung wurde mit verdünnter Schwefelsäure neutralisiert. Im übrigen wurde wie vorher beschrieben verfahren.

Chromatographische Reinigung und Trennung. — In ein Chromatographierrohr wurde Kieselgel G Pulver unter 0,08 mm mit 9:1 Benzol–Äthanol eingeschlämmt. Nachdem sich die Suspension über Nacht abgesetzt hatte, wurde der Sirup, gelöst im bereits angegebenen Lösungsmittelgemisch, in der üblichen Weise auf die Säule gebracht. Anschließend wurde mit dem gleichen Lösungsmittel nach der Durchlaufmethode mit Hilfe eines Fraktomaten eluiert. Ausgetestet wurden die Fraktionen auf Kieselgel G-Dünnschichtplatten. Die entsprechenden Fraktionen wurden vereinigt und eingedampft. Zu der Fraktion, die **4** neben gewissen Mengen des Linearpolymeren **3** enthielt, wurde wenig trockenes Äthanol gegeben und vorsichtig eingeengt. Dabei kristallisierte die Verunreinigung **3** aus und **4** fiel als Sirup an, der beim scharfen Trocknen im Vakuum zu einem Glas erstarrte; 620 mg (34%), $[\alpha]_D^{20}$ −32,9° (*c* 1,8, Aceton).

Anal. Ber. für $C_{22}H_{36}O_{11}$ (476,5): C, 55,44; H, 7,71; OCH_3, 6,51. Gef.: C, 55,76; H, 7,70; OCH_3, 8,89; Mol.-Gew.: 545 (dampfdruckosmometrisch), 476 (massenspektrometrisch).

Die Ausbeute von Poly(5,6-anhydro-1,2-*O*-isopropyliden-3-*O*-methyl-α-D-glucofuranose) (**3**) war 80 mg (9%), bezogen auf ein mittleres Molekulargewicht von 2300; Schmp. 155–157°; $[\alpha]_D^{20}$ −32,2° (*c* 1,1, Aceton).

Anal. Ber. für $C_{10}H_{16}O_5$ $[(216,2)_n]$: C, 55,50; H, 7,40; OCH_3, 14,35. Gef.: C, 55,39; H, 7,43; OCH_3, 13,96; Mol.-Gew.: *ca.* 2300 (dampfdruckosmometrisch).

3'-O-Methyl-di-α-D-glucose-3,6'-anhydrid-hepta-acetat (**6**). — Verbindung **4** (600 mg) wurde zur Abspaltung der Acetonreste 2 Stdn. mit 50%-iger Essigsäure (10 ml) zum Sieden erhitzt und anschließend zur Trockne eingedampft. Im Vakuum über Phosphorpentoxid getrocknetes **5** (450 mg) wurde mit wasserfreiem Natriumacetat (250 mg) und Acetanhydrid (3,0 ml) unter häufigem Umschütteln im Verlauf von 1 Std. im Ölbad auf 100° erhitzt. Nach einer weiteren Std. wurde die Reaktionslösung für 10 Min auf 120° gebracht. Nach dem Abkühlen wurde die Lösung in Eiswasser (100 ml) eingerührt. Der dabei ausfallende Sirup kristallisierte nach einiger Zeit. Die Lösung wurde mit festem Natriumhydrogenkarbonat neutralisiert und 12 Stdn. stehengelassen. Danach wurden die Kristalle abgesaugt und das Produkt aus absolutem Äthanol zwei- bis drei-mal umkristallisiert; **6** war dünnschichtchromatographisch einheitlich. Weiteres Umkristallisieren änderte den Schmelzpunkt nicht;

530 mg (73 %), Schmp. 189–190°.

Anal. Ber. für $C_{27}H_{38}O_{18}$ (650,5): C, 49,85; H, 5,88. Gef.: C, 49,87; H, 6,07. Mol.-Gew.: 636 (dampfdruckosmometrisch), 650 (massenspektrometrisch).

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STRUCTURES OF THE L-RHAMNO-D-MANNAN FROM *Ceratocystis ulmi* AND THE D-GLUCO-D-MANNAN FROM *Ceratocystis brunnea**

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ABSTRACT

The structures of mannose-containing polysaccharides of cell walls from *Ceratocystis ulmi* and *Ceratocystis brunnea* have been determined. The L-rhamno-D-mannan from *Ceratocystis ulmi* (the causative agent of Dutch elm disease) contains a (1→6)-linked α-D-mannopyranose main-chain substituted in the 3-positions mainly by single-unit α-L-rhamnopyranosyl side-chains (**1**) and probably by a small proportion of O-L-rhamnopyranosyl-(1→4)-L-rhamnopyranosyl side-chains (**2**). The D-gluco-D-mannan from *Ceratocystis brunnea* also has an (1→6)-linked α-D-mannopyranose main-chain, which is substituted in most of the 2-positions by α-D-glucopyranosyl side-chains (**3**) and also by *O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-mannopyranosyl (**4**) and D-mannopyranosyl side-chains (**5**). The mannan main-chain (**10**) of the D-gluco-D-mannan was prepared by conversion of the side-chain units (**6** and **8**) into the 3,6-anhydro-derivatives (**7** and **9**), which were then removed preferentially by partial acid hydrolysis.

INTRODUCTION

Recent studies on identification and taxonomy of yeasts, based on the structures of the mannose-containing polysaccharides of the cell wall and on their proton magnetic resonance (p.m.r.) spectra[1-11], have been extended to fungi of the genus *Ceratocystis*. Some members of this genus resemble yeasts since they can exist in the unicellular form and multiply by budding.

The present investigation concerns the determination of chemical structure of two polysaccharides which appear to be representative of a number of the *Ceratocystis* spp. These are the L-rhamno-D-mannan of *Ceratocystis ulmi* (Dutch elm disease fungus) and the D-gluco-D-mannan of *Ceratocystis brunnea* (from the insect galleries in subalpine fir) in terms of their main-chain and side-chain structures.

RESULTS AND DISCUSSION

Cells of *Ceratocystis* species were extracted with hot aqueous alkali and mannose-containing polysaccharide components of the extracts purified via the

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insoluble copper complexes formed with Fehling solution. The H-1 portions of the p.m.r. spectrum of each polysaccharide were compared. Thirteen distinguishable types of p.m.r. spectra were obtained from 46 species of *Ceratocystis*. Of these types, the two largest groups of H-1 spectra consisted of the spectra of the L-rhamno-D-mannans of *Ceratocystis ulmi* CBS 374.67, *Ceratocystis ips* CBS 137.36, *Ceratocystis populina* CBS 212.67, *Ceratocystis leptographioides* CBS 144.59, *Ceratocystis auracariae* CBS 114.68, and *Ceratocystis tremulo-aurea* CBS 361.68 (Fig. 1, spectrum A), and the spectra of the D-gluco-D-mannans of *Ceratocystis brunnea* CBS 161.61, *Ceratocystis paradoxa* CBS 128.32, *Ceratocystis coerulescens* CBS 142.53, *Ceratocystis adiposa* CBS 127.27, and *Ceratocystis radicicola* CBS 114.47 (Fig. 1, spectrum B). Details on the determination of the structures of *C. ulmi* L-rhamno-D-mannan and *C. brunnea* D-gluco-D-mannan are given below.

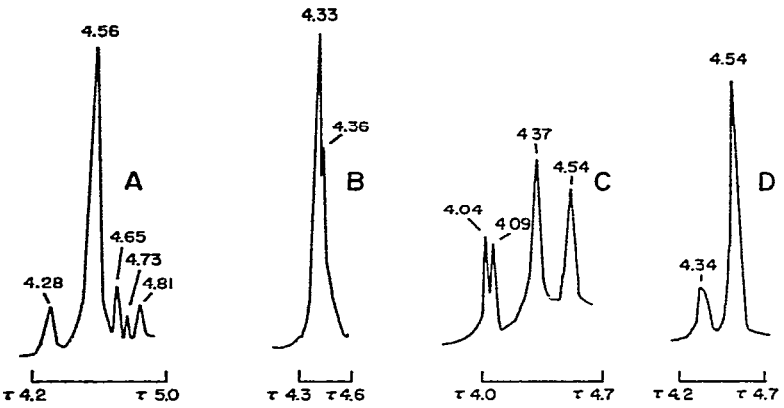

Fig. 1. P.m.r. spectra from species of *Ceratocystis*.

A. Structure of the L-*rhamno*-D-*mannan from* Ceratocystis ulmi. — The L-rhamno-D-mannan was obtained from cells of *C. ulmi* in 3.4% yield and it gave a single peak on ultracentrifugation. Acid hydrolysis gave L-rhamnose and D-mannose. The p.m.r. spectrum of the L-rhamno-D-mannan in deuterium oxide at 70° showed an C–CH_3 doublet (anhydrorhamnose) at τ 8.18 (J = 7 Hz) and a principal H-1 signal at τ 4.56 (Fig. 1, spectrum A). Comparison of the area of the H-1 and C–CH_3 signals showed that the L-rhamno-D-mannan contains rhamnose and hexose residues in the molar ratio 57:43. The mannose residues were found to be in the main chain of the polymer since a Smith degradation[12], incorporating mild hydrolytic conditions, gave a mannan, specific rotation +73°, which contained α-(1→6)-D-mannopyranose residues. The structure of the mannan was recognized by its p.m.r. spectrum and by periodate-oxidation characteristics. The specific rotation (−10°) of the heteropolymer indicates that most of the glycosidic linkages have the α-configuration.

The position of attachment of the rhamnose-containing side chains to the mannan main-chain was determined as follows. A methylation–fragmentation experiment provided 2,4-di-*O*-methyl-D-mannose, 2,3,4-tri-*O*-methyl-L-rhamnose.

and an unidentified di-*O*-methylrhamnose. The unknown fragment gave a methyl glycoside having a gas–liquid chromatographic (g.l.c.) retention time differing from that of the 3,4-di-*O*-methyl isomer, thus eliminating the possibility of 2-*O*-substituted rhamnopyranosyl units. Since the polyalcohol derived from the rhamnomannan by periodate oxidation followed by borohydride reduction gave mannose and 4-deoxy-L-erythritol on hydrolysis, 4-*O*-substituted rhamnopyranosyl residues are indicated. The linkage between the residues units and the main chain were found to be α-L since partial acetolysis[13] of the rhamnomannan followed by deacetylation gave a mixture from which 3-*O*-α-L-rhamnopyranosyl-D-mannose was isolated; its structure was determined as follows. The disaccharide gave rhamnose and mannose on hydrolysis and the alditol obtained by sodium borohydride reduction was hydrolyzed to rhamnose and mannitol. 3-*O*-Substitution of the mannose residues was shown by the uptake of 1 mole per mole of lead tetraacetate[14]. The rhamnose residues have the α-L configuration since the molecular rotation of the derived 3-*O*-α-L-rhamnopyranosyl-D-mannitol is −11,200°, close to −11,100° reported for methyl α-L-rhamnopyranoside[15].

The above data indicates that the L-rhamno-D-mannan contains an α-(1→6)-D-mannopyranosyl main-chain substituted in the 3-positions mainly by α-L-rhamnopyranosyl side-chains (**1**) and also by L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl (**2**) and possibly larger (1→4)-linked side-chains. The rhamnomannan consumed 0.92 mole per mole of sodium periodate per mole of aldose unit, with production of 0.43 mole of acid, values that are consistent with relatively few 4-*O*-substituted L-rhamnopyranosyl units.

B. Structure of the D-*gluco*-D-*mannan from* Ceratocystis brunnea. — The yield of D-gluco-D-mannan from dry cells of *Ceratocystis brunnea* was 2.5% and ultracentrifugation of the polymer gave a single peak. The ratio of 1:1.3 for glucose to mannose residues was determined by g.l.c. analysis of the alditol acetates obtained by hydrolysis of the polymer followed by sodium borohydride reduction and acetylation. The free hexoses were characterized as the D enantiomers. The specific rotation of the polysaccharide, +93°, indicates that α-D linkages preponderate.

The D-gluco-D-mannan was converted, by the methylation–fragmentation technique, into a mixture of *O*-methylated hexoses which were fractionated by cellulose column chromatography. Pure 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,-di-*O*-methyl-D-mannose were isolated, but the tri-*O*-methyl fraction, which was equivalent to 26% of the tetra-*O*-methyl fraction on a molar basis, was a mixture. This mixture was converted into methyl glycosides and trimethylsilyl (t.m.s.) ethers of the methyl glycosides. The g.l.c. retention times of the ethers corresponded to those of derivatives of 3,4,6- and 2,3,4-tri-*O*-methyl-D-mannose[16], in an approximate ratio of 1:0.71. A g.l.c. analysis of the t.m.s. derivatives[16] of the tetra-*O*-methyl-D-hexose fraction showed that it contained 20% of 2,3,4,6-tetra-*O*-methyl-D-mannose. These observations are consistent with the formation of glycerol only by acid hydrolysis of the polyalcohol obtained from the D-gluco-D-mannan by successive treatments with sodium periodate and sodium borohydride.

Partial acetolysis[13] of the D-gluco-D-mannan followed by deacetylation and

cellulose-column chromatography gave di- and tri-saccharide fractions in 25% and 11% yields, respectively. These were characterized as 2-*O*-α-D-glucopyranosyl-D-mannose and *O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose, respectively, as follows. The disaccharide gave glucose and mannose on hydrolysis and the alditol formed on sodium borohydride reduction was hydrolyzed to a glucose and a hexitol. The disaccharide was inert to lead tetraacetate[14] and is therefore 2-*O*-D-glucopyranosyl-D-mannose. The configuration of the glycosidic linkage was found to be α-D since the $[M]_D$ of 2-*O*-D-glucopyranosyl-D-mannose minus the $[M]_D$ of 2-*O*-α-D-mannopyranosyl-D-mannose[13] is +12,500°, close to the difference (+15,200°) of $[M]_D$ values of methyl α-D-glucopyranoside[17] and methyl α-D-mannopyranoside[18]. Furthermore the H-1 p.m.r. signals of the glucopyranosyl residue of the disaccharide has *J* 3.5 Hz, corresponding to an α configuration. The trisaccharide gave, on hydrolysis, a mannose and a smaller amount of a glucose. The hydrolysis product from the sodium borohydride-reduced trisaccharide contained glucose and mannose in approximately equal amounts, as shown by paper chromatography by using the *p*-anisidine hydrochloride spray. The trisaccharide contains a reducing end-unit of 2-*O*-linked mannose, consistent with its stability to lead tetraacetate[14]. Methylation–fragmentation analysis of the trisaccharide after reduction with sodium borohydride gave *O*-methyl derivatives which, by g.l.c. analysis of their methyl glycosides and corresponding t.m.s. derivatives were shown to be 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-mannose. These data, combined with the strong positive specific rotation (+86°) of the trisaccharide fraction, show that it is predominantly *O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose.

The above methylation–fragmentation and partial acetolysis data are consistent with an α-(1→6)-D-mannopyranose main-chain substituted in most of the 2-positions by single-unit α-D-glucopyranosyl side-chains (**3**) with smaller amounts of mannopyranosyl (**5**) and *O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-mannopyranosyl (**4**)* side-chains.

The mannan main chain might also contain (1→2) linkages, but this possibility was eliminated by preparation of a (1→6)-linked α-D-mannan main chain. Attempts to remove the side chains of the D-gluco-D-mannan by partial acid hydrolysis or with α-D-glucosidases were unsuccessful so that another degradative method had to be used. Since Haworth, Jackson, and Smith[19] hydrolyzed the α and β anomers of methyl 3,6-anhydro-D-galactopyranoside to 3,6-anhydro-D-galactose with 0.1M sulfuric acid at room temperature, the side-chain units (**6** and **8**) of the D-gluco-D-mannan might be easily hydrolyzed if they were converted into 3,6-anhydro-derivatives. Accordingly, a 5-step process with the first 4 steps analogous to those described by Ingle and Whistler[20] for conversion of amylose into 3,6-anhydroamylose was used. This involves successive (*1*) tritylation, (*2*) acetylation, (*3*) detritylation by using HBr, (*4*)

*A side chain with this structure, but with an additional (1→2)-linked α-D-mannopyranose unit, may be present.

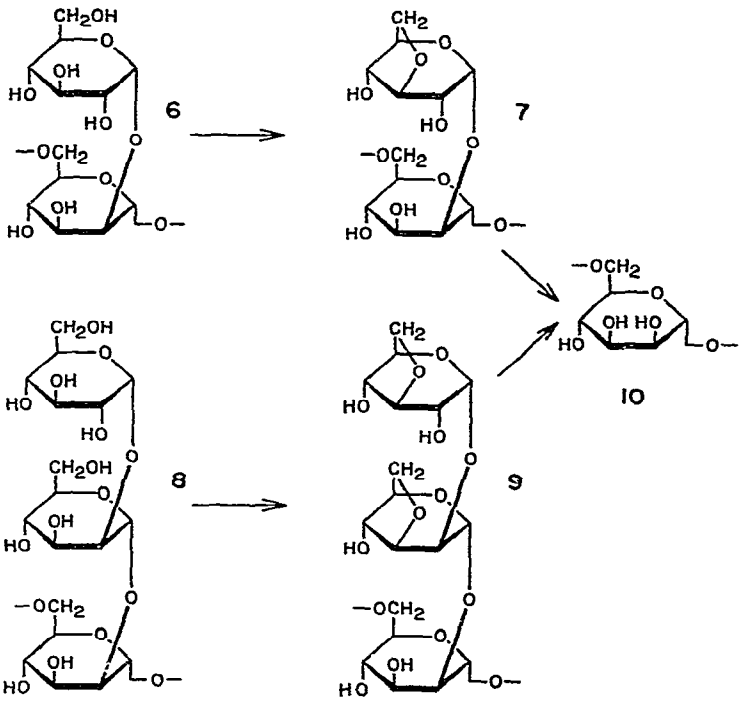

Fig. 2.

p-toluenesulfonation to give the 6-*O*-*p*-tolylsulfonyl acetate, and (5) formation of the 3,6-anhydro derivatives (7, 9) by treatment with alkali[19]. (For the sake of simplicity the degradation of the mannopyranose side-chain is not included in Fig. 2.) The conditions required for hydrolysis of the 3,6-anhydrohexose units were much more vigorous than those used by Haworth and co-workers[19], as preparation of the mannan main chain (**10**) required 0.33M sulfuric acid at 100°. The polymeric products were separated from 3,6-anhydro-D-glucose and 3,6-anhydro-D-mannose by precipitation with ethanol, and their p.m.r. spectra and yields following hydrolysis for 1 h and 5 h are presented in Fig. 1. After degradation for 1 h there was only a relatively small H-1 signal at τ 4.54, corresponding to (1→6)-linked α-D-mannopyranose residues[3] (Spectrum C), present, but after degradation for 5 h it was the predominant signal (Spectrum D). The origin of the τ 4.54 signal was confirmed by addition of sodium borate, which resulted in a downfield shift[2] of 10 Hz. The presence of a small signal (20% of the total) at τ 4.34 is probably due to the presence of some 3,6-anhydro-D-glucose residues, which were detected by paper chromatography following acid hydrolysis.

CONCLUSIONS

The L-rhamno-D-mannan of *C. ulmi* and D-gluco-D-mannan of *C. brunnea* contain α-(1→6)-D-mannopyranose main-chains, in common with the majority of

yeast D-mannans[3,6,7,21] and D-galacto-D-mannans[1,3,7] that have been investigated. Other yeast mannans contain main-chains having either alternating (1→3)- and (1→4)-linked β-D-mannopyranose residue[22] or "block type" structures having α-(1→3)- and (1→6)-linked D-mannopyranose units[8]. Heteropolymers of the L-arabino-D-xylo-D-mannan[4] or L-methylpentosyl-D-mannan[5] type contain α-(1→3)-linked D-mannopyranose main-chains.

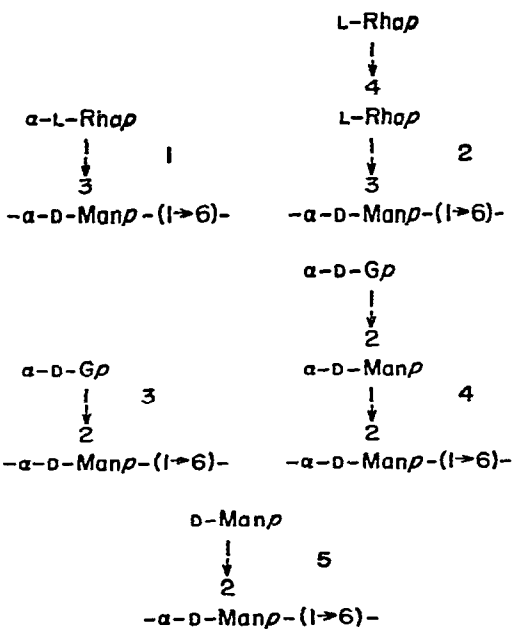

Chart 1.

The side chains of the L-rhamno-D-mannan and D-gluco-D-mannan differ from those of yeast polysaccharides.

EXPERIMENTAL

General. — P.m.r. spectra were obtained from 20% solutions of polysaccharides in D_2O at 70° with a 100 MHz Varian spectrometer. The chemical shifts are expressed as τ values, based on an external standard of tetramethylsilane.

Cultures of *Ceratocystis* spp. were obtained from the Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands. Cultural methods for producing cells, the methods of isolation of purified polysaccharides from cells, and their hydrolysis are similar to those described by Gorin and Spencer[1].

To test for possible heterogeneity of the *C. ulmi* L-rhamno-D-mannan and *C. brunnea* D-gluco-D-mannan, the polysaccharides were sedimented at concentrations of 0.15%, 0.30%, and 0.45% in 0.5% aqueous sodium chloride at 59,000 r.p.m. Both polysaccharides gave single peaks.

Paper chromatography was carried out with 40:11:19 (v/v/v) butyl alcohol–ethanol–water as solvent and *p*-anisidine hydrochloride and/or ammoniacal silver nitrate sprays when appropriate.

Isolation and estimation of L-*rhamnose and* D-*mannose in the* L-*rhamno*-D-*mannan of* C. ulmi. — The L-rhamno-D-mannan, $[\alpha]_D^{25}$ −10° (*c* 0.5, water), was hydrolyzed (M sulfuric acid for 18 h at 100°) and the resulting mixture chromatographed on a cellulose column with 9:1 (v/v) butyl alcohol–water as eluant. α-L-Rhamnose, m.p. and mixed m.p. 126–127° (from ethanol–ethyl acetate) and $[\alpha]_D^{25}$ −3(2 min) → +9° (*c* 0.7, water; equil.) was isolated initially, followed by D-mannose, m.p. and mixed m.p. 131° (from ethanol) and $[\alpha]_D^{25}$ +14° (*c* 0.4, water; equil.). Comparison of the areas of the H-1 and C–CH_3 signals in the p.m.r. spectrum of the L-rhamno-D-mannan (at τ 4.28–4.81 and τ 8.13 and τ 8.20, respectively) showed that rhamnosyl and mannosyl residues are present in the molar ratio 57:43.

Partial and complete Smith degradations of the L-*rhamno*-D-*mannan of* C. ulmi. — Based on a 57:43 ratio of rhamnose to mannose residues in the L-rhamno-D-mannan, it consumed 0.91, 0.92 and 0.92, mole of sodium periodate per mole of aldose residue with concomitant production of 0.39, 0.42, and 0.43 mole/mole of acid after 1, 2, and 3 days, respectively. The L-rhamno-D-mannan (0.50 g) was oxidized by sodium periodate and the resulting "polyaldehyde" successively reduced with sodium borohydride to a polyalcohol (0.30 g) and partially hydrolyzed to a mannan by the conditions described previously[4]. The mannan had $[\alpha]_D^{25}$ +73° (*c* 0.5, water), gave mannose only (paper chromatogram) on hydrolysis, and consumed 1.83 moles of sodium periodate per mole of mannose unit after 2 days with production of 0.83 mole/mole of acid. Its H-1 p.m.r. spectrum in D_2O contained a single signal at τ 4.57 (at 70°)[3], which was shifted downfield by 10 Hz on addition of sodium borate[2].

Complete hydrolysis of the polyalcohol (M sulfuric acid for 18 h at 100°) gave mannose and 4-deoxy-L-erythritol (paper chromatogram), the latter almost cochromatographing with glycerol. These components were resolved by g.l.c., as acetates, by using an 8 ft × 1/4 in. outside diameter copper tube packed with 2% LAC-1-R 296 on Chromosorb W at 180° with helium (40 lb.in^{-2}) as carrier gas. The g.l.c. unit was of conventional design with thermal-conductivity detectors. Peaks were obtained corresponding to the acetates of 4-deoxy-L-erythritol [retention time (r.t.) 21.5 min] and a barely detectable amount of glycerol (r.t. 7.2 min).

Methylation–fragmentation analysis of rhamnomannan from C. ulmi. — The L-rhamno-D-mannan (1.0 g) was partially methylated by the Haworth procedure[23] and the product completely methylated by the Kuhn method[24]. The fully methylated product (0.82 g), 0.8% solution in dry chloroform, showed no i.r. absorption for hydroxyl groups at *ca.* 3600 cm^{-1} (path length 5 mm). A portion of the product (0.50 g) was successively cleaved with refluxing 5% methanolic hydrogen chloride and 3 M sulfuric acid for 18 h at 100°. The free *O*-methylhexoses were fractionated on a cellulose column. 1000:50:1 (v/v/v) Benzene–ethanol–water eluted 2,3,4-tri-*O*-methyl-L-rhamnose (56 mg), $[\alpha]_D^{25}$ +21° (*c* 0.5, water), which was characterized as its *N*-phenylglycosylamine derivative[25], which had (from *n*-hexane) m.p. and mixed m.p.

121–123°. 500:50:1 (v/v/v) Benzene–ethanol–water eluted a di-*O*-methyl-L-rhamnose fraction (40 mg). It was converted into its methyl glycosides and examined by g.l.c. by using an 8 ft × 1/4 in. outside diameter column of 2% neopentylglycol succinate at 150°. Methyl 3,4-di-*O*-methyl-α,β-L-rhamnopyranosides (r.t. 16.8 min) could not be detected. Finally 250:50:1 (v/v/v) benzene–ethanol–water eluted 2,4-di-*O*-methyl-D-mannose (41 mg) having m.p. and mixed m.p. 124–126°[16] (from ethyl acetate) and $[\alpha]_D^{25}$ +13° (*c* 0.3, water; equil.).

Partial acetolysis of L-*rhamno*-D-*mannan of* C. ulmi. — The L-rhamno-D-mannan (1.0 g) was partially acetolyzed by the conditions of Lee and Ballou[26], and the resulting free sugars obtained on deacetylation with sodium methoxide in methanol were fractionated on a cellulose column. 9:1 (v/v) Butyl alcohol–water eluted rhamnose (100 mg) and mannose (79 mg). 9:1 (v/v) Acetone–water eluted a disaccharide fraction (338 mg) which gave mannose and rhamnose on hydrolysis and had $[\alpha]_D^{25}$ −65° (*c* 0.3, water). It consumed 0.85, 1.00, and 1.03 moles/mole of lead tetraacetate[14] after 2,5 and 15 min, respectively. The alditol obtained on reduction with sodium borohydride gave rhamnose and a hexitol on hydrolysis, and had $[\alpha]_D^{25}$ −34° (*c* 0.5, water).

Isolation and determination of D-*glucose and* D-*mannose from the* D-*gluco*-D-*mannan of* C. brunnea. — The D-gluco-D-mannan, $[\alpha]_D^{25}$ +93° (*c* 0.3, water) was hydrolyzed with M sulfuric acid for 18 h at 100° and the product chromatographed on a cellulose column by using 9:1 (v/v) butyl alcohol–water as eluant. D-Mannose, having m.p. and mixed m.p. 129–131° (from ethanol) and $[\alpha]_D^{25}$ +12° (*c* 0.3, water; equil.) was obtained, followed by D-glucose [m.p. and mixed m.p. 144–146° from methanol, and $[\alpha]_D^{25}$ +51° (*c* 0.3, water; equil.)].

A portion of the hydrolyzate was reduced with sodium borohydride and the product acetylated. The acetates were gas chromatographed on a column of 8 ft × 1/4 in. outside diameter packed with 2% LAC-1-R 296 on Chromosorb W (80–100 mesh) at 210°. D-Glucitol (r.t. 76.2 min) and D-mannitol derivatives (r.t. 64.2 min) were detected in a peak area ratio of 1:1.3. Under these conditions authentic samples of D-glucitol and D-mannitol acetates gave the same detector response.

Smith degradation of the D-*gluco*-D-*mannan of* C. brunnea. — The D-gluco-D-mannan consumed 1.47, 1.50, and 1.51 moles of sodium periodate per mole of anhydrohexose residues, with formation of 0.47, 0.52, and 0.53 mole per mole of acid after 1, 2, and 3 days, respectively. The D-gluco-D-mannan was converted into a polyalcohol by successive periodate oxidation and sodium borohydride reduction. Hydrolysis with M sulfuric acid for 18 h at 100° gave glycerol as the only detectable product on a paper chromatogram.

Methylation–fragmentation analysis of the D-*gluco*-D-*mannan of* C. brunnea. — The D-gluco-D-mannan (1.0 g) was converted into its fully methylated derivative (0.74 g) by the method outlined above for the L-rhamno-D-mannan. Half of the product was converted into a mixture of *O*-methyl-D-hexoses by successive treatment with refluxing 5% methanolic hydrogen chloride overnight and 3M sulfuric acid for 18 h at 100°. The mixture was fractionated by cellulose-column chromatography.

1000:50:1 (v/v/v) Benzene–ethanol–water eluted a tetra-*O*-methyl-D-hexose fraction (165 mg), from which 2,3,4,6-tetra-*O*-methyl-D-glucose[27] was isolated, having m.p. and mixed m.p. 90–92° (from *n*-hexane) and $[\alpha]_D^{25}$ +80° (*c* 0.3, water; equil.). G.l.c. of the t.m.s. derivatives[16] of the fraction showed that 2,3,4,6-tetra-*O*-methyl derivatives of D-glucose and D-mannose were present in an approximately 4:1 molar ratio. A tri-*O*-methyl-D-hexose fraction (40 mg) was obtained on elution with 500:50:1 (v/v/v) benzene–ethanol–water and a portion of this was converted into methyl glycosides. G.l.c. of these and their t.m.s. ethers[16] showed that the 2,3,4- and 3,4,6-tri-*O*-methyl derivatives of methyl α,β-D-mannopyranosides were present in an approximately 7.1:10 molar ratio. Elution of the column with 250:50:1 (v/v/v) benzene–ethanol–water gave 3,4-di-*O*-methyl-α-D-mannose (140 mg), which had m.p. and mixed m.p. 101–102°[28] (from ethyl acetate) and $[\alpha]_D^{25}$ +15 (2 min) → +4° (*c* 0.4, water; equil.).

Partial acetolysis of the D-*gluco*-D-*mannan of* C. brunnea. — The D-gluco-D-mannan (2.0 g) was partially acetolyzed[26] and following deacetylation of the mixed acetates, the free sugars were chromatographed on a cellulose column. 9:1 (v/v) Butyl alcohol–water eluted mannose (210 mg) and 7:1 (v/v) acetone–water eluted a disaccharide (0.50 g) having a paper chromatographic rate, relative to lactose (R_{Lact}) of 1.7. It has $[\alpha]_D^{25}$ +77° (*c* 0.6, water) and gave glucose and mannose on hydrolysis. It consumed 0.05, 0.07, and 0.10 mole/mole of lead tetraacetate after 2,5 and 15 min, respectively, corresponding to a 2-*O*-linked disaccharide[14]. The alditol obtained after sodium borohydride reduction had $[\alpha]_D^{25}$ +60° (*c* 0.3, water) and gave a glucose and a hexitol on hydrolysis.

Another disaccharide fraction (65 mg) having R_{Lact} 1.3 was obtained, but its p.m.r. spectrum in D_2O showed that it was a complex mixture.

A trisaccharide (225 mg) having R_{Lact} 0.3 and $[\alpha]_D^{25}$ +86° (*c* 0.6, water) was eluted with 4:1 (v/v) acetone–water. It gave mannose and a smaller quantity of glucose on hydrolysis (paper chromatogram) and it consumed 0.03, 0.05, and 0.13 mole/mole of lead tetraacetate after 2, 5, and 15 min, respectively, corresponding to a 2-*O*-linked reducing-unit. The alditol, produced by sodium borohydride reduction, was hydrolyzed giving apparently equal amounts of glucose and mannose together with a hexitol (paper chromatogram). The alditol was methylated by the Kuhn procedure[24] and converted into methyl *O*-methyl-α,β-D-hexopyranosides and an *O*-methyl-D-mannitol. G.l.c. analyses of the product and its t.m.s. derivatives[16] showed the presence of the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-mannose.

Isolation of the D-*mannan main-chain of the* D-*gluco*-D-*mannan of* C. brunnea *via the 3,6-anhydro-derivative.* — The D-gluco-D-mannan (2.0 g) was converted successively into the trityl derivative (2.95 g), the trityl acetate (3.43 g), and acetate (1.59 g) following detritylation, and a *p*-tolylsulfonyl acetate (1.82 g; S, 4.65%) by *p*-toluenesulfonation, according to the method summarized by Ingle and Whistler[20]. Part of the *p*-tolylsulfonyl acetate (0.83 g) in chloroform (20 ml) was treated with 0.1M methanolic sodium methoxide (10 ml) for 2 h. The resulting suspension was

evaporated and the residue shaken with potassium hydroxide (0.4 g) in water (20 ml). After 1 h the suspension dissolved and, after a further 4 h at room temperature, the solution was heated for 1 h at 80°. It was then deionized with Amberlite IR-120 (H^+ form) and Dowex-1 X8 (hydrogen carbonate form), filtered and evaporated to a small volume. Excess acetone was added and the resulting precipitate of 3,6-anhydro-derivative (0.32 g) was isolated. Hydrolysis gave mannose and 3,6-anhydro-D-glucose ($R_{Rhamnose}$ 1.2 on a paper chromatogram) and traces of glucose and 3,6-anhydro-D-mannose ($R_{Rhamnose}$ 1.0), which were detected as yellow spots with the *p*-anisidine hydrochloride spray.

In order to remove the side chains from the 3,6-anhydro-polysaccharide, portions (100 mg) were treated with 0.33M sulfuric acid (2 ml) for 1 h and 5 h at 100°. The hydrolyzates were deionized and polysaccharide was separated from 3,6-anhydro-D-mannose and 3,6-anhydro-D-glucose by ethanol precipitation. Yields after 1 h and 5 h runs: 40 mg and 33 mg, respectively. The p.m.r. spectra of these products are recorded in Fig. 1. The product, after hydrolysis for 5 h, had a main signal at τ 4.54, which was shifted downfield by 10 Hz on addition of sodium borate. Complete hydrolysis of the polysaccharide gave mannose and a trace of 3,6-anhydro-D-glucose.

Attempted partial enzymic and acid cleavages of the D-*gluco*-D-*mannan of* C. brunnea. — Solutions (1%) of D-gluco-D-mannan were treated at 25° with suitable amounts of enzyme preparations in order to remove β-D-glucopyranosyl side-chains. No glucose was formed by using crude α-amylase (Worthington Corp.), Rhozyme HP 150 hemicellulase (Rohm and Hass Co.), and α-D-glucosidase from *Saccharomyces fragilis* PRL 324, Macerozyme pectinase from a *Rhizopus* sp. and Pancellase, a crude cellulase from *Trichoderma viride* (both from All Japan Biochemicals Ltd.), Small amounts of glucose were formed by using Onozuka cellulase SS (a cruder form of Pancellase), an active dextranase from a *Penicillium* sp. (Worthington Corp.) and Meiselase P cellulase from *T. viride* (Meiji Seika Kaisha Ltd.). However, in the last 3 cases residual D-gluco-D-mannan was obtained, even after prolonged incubation over several days.

The D-gluco-D-mannan, as a 5% solution in 0.33M sulfuric acid was heated for 3 days at 100°. At intervals during this period aliquots were abstracted and residual polysaccharide obtained following precipitation by excess ethanol. On hydrolysis each polysaccharide preparation gave glucose and mannose.

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CONFORMATIONAL STUDIES ON CYCLOAMYLOSES*

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ABSTRACT

The "non-bonded" interaction energies have been computed for cyclohexaamylose, cycloheptaamylose, and cyclooctaamylose. From hydrogen-bond criteria the conformation having an angle of 119° at the bridge oxygen atom is preferred for cyclohexaamylose. It is also shown that cycloamyloses having fewer than six D-glucose residues could not be cyclized because of steric overlaps. Out of three possible cyclic compounds, cyclohexaamylose has the lowest energy. Cyclohexa-, -hepta-, and -octa-amyloses are stabilized by the formation of intramolecular hydrogen bonds between contiguous D-glucose residues. These intramolecular hydrogen bonds are stronger in the hepta- and octa-amyloses than in the hexaamylose.

INTRODUCTION

The cycloamyloses (Schardinger dextrins) are a group of homologous oligosaccharides, produced originally by the cultivation of *Bacillus macerans* on starch solutions. These crystalline dextrins are produced from starches in high yield through the use of bacteria-free, enzyme-containing filtrates of *B. macerans*. In the early stages of reaction, the α-dextrin is formed in maximum yield. After a few days, the β-dextrin preponderates. From chemical studies, Freudenberg *et al.*[1], showed that the Schardinger dextrins are cyclic molecules consisting of D-glucose residues, connected by the α-D-(1→4) (maltose type) linkage. French and coworkers[2,3] showed that the number of D-glucose residues in the α, β, and γ dextrins is six, seven, and eight, respectively. French recommended the names cyclohexaamylose, cycloheptaamylose, and cyclooctaamylose for these dextrins.

While there is evidence for the existence of cycloamyloses having 9, 10, 11, and 12 residues[4,5], cycloamyloses containing fewer than six D-glucose residues are not known to exist[6]. Of these cycloamyloses, the crystal structure of cyclohexaamylose[7] alone has been solved. X-ray structural analysis[7] and n.m.r. studies[8,9,10] on cycloamylose have established that all of the D-glucose residues in these cyclic

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molecules exist exclusively in the *C1* (D) conformation. The present paper describes an attempt to study theoretically the conformations of these molecules by using potential-energy calculations. This study incidentally throws some light on the question whether the failure of *B. macerans* to produce smaller rings may be a consequence of steric overlap or a reflection of enzyme specificity.

CALCULATION OF PARAMETERS FOR THE CYCLOAMYLOSES

The coordinates of a D-glucose residue in the *C1* (D) conformation were obtained from the bond lengths and angles reported by Hybl *et al.*[7] (Table I, set 1). In a helical amylose chain, the parameters characterizing the helix are: n, the number of residues per turn; and h, the unit translation parallel to the axis of the helix. The helical parameters were calculated[11] for different values of the angle τ at the bridge

TABLE I

BOND LENGTHS AND BOND ANGLES IN THE D-GLUCOPYRANOSE RING

Bond	*Length* (Å)		*Bond angle*	*Value (degrees)*	
	Set 1[a]	*Set 2*[b]		*Set 1*[a]	*Set 2*[b]
C-1–C-2	1.520	1.529	O-5–C-1–C-2	108.9	110.2
C-2–C-3	1.526	1.534	C-1–C-2–C-3	109.6	108.8
C-3–C-4	1.521	1.529	C-2–C-3–C-4	109.8	109.9
C-4–C-5	1.544	1.539	C-3–C-4–C-5	110.2	109.5
C-5–O-5	1.433	1.422	C-4–C-5–O-5	109.2	110.2
C-1–O-5	1.427	1.419	C-5–O-5–C-1	113.5	113.6
C-1–O-1	1.408	1.380	O-5–C-1–O-1	111.3	110.9
C-2–O-2	1.430	1.422	O-1–C-1–C-2	107.2	109.4
C-3–O-3	1.431	1.439	C-1–C-2–O-2	109.4	109.3
C-4–O-4	1.428	1.417	O-2–C-2–C-3	112.2	111.9
C-5–C-6	1.526	1.570	C-2–C-3–O-3	110.3	107.5
C-6–O-6	1.398	1.420	O-3–C-3–C-4	108.0	107.9
			C-3–C-4–O-4	105.5	107.3
			O-3–C-4–C-5	108.2	109.2
			C-4–C-5–C-6	110.1	111.9
			C-6–C-5–O-5	106.0	105.6
			C-5–C-6–O-6	111.3	109.5

[a]Set 1, data taken from Ref. 7. [b]Set 2, average values obtained from the data of Refs. 7, 13, and 14.

oxygen atom, for a limited range of (ϕ, ψ), the dihedral angles of rotation about the glycosidic bonds C-1–O and O–C-4', as in Fig. 1. [The notation (ϕ, ϕ') was used in the earlier papers[11,12]. In the present notation, $\psi = \phi' + 180°$]. The position corresponding to (ϕ,ψ) = (0°, 0°) was chosen as the one in which the C-1, O, and O-4 atoms of the first residue and C-4', O, and O-1' atoms of the second residue are in a plane, and the bonds C-1–H-1 and C-4'–H-4' are *cis* to the bonds O–C-4' and C-1–O, respectively. Since the cyclic conformation is analogous to one turn of the helix, the dihedral angles (ϕ, ψ) corresponding to $n = 6$ and $h = 0$ were obtained.

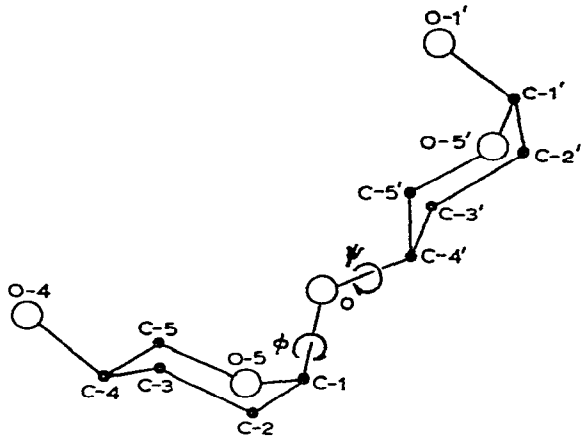

Fig. 1. Perspective view of a pair of α-D-glucose residues joined through a (1→4′) linkage. The directions of rotations ϕ and ψ are marked. When viewed from C-1 towards O, rotation of the primed (reducing) D-glucose residue as a whole in a clockwise direction about the C-1–O bond, with the unprimed (non-reducing) D-glucose residue being held stationary, gives an increase in the value of ϕ. Similarly, when viewed from O towards C-4′, rotation of the primed D-glucose residue in the clockwise direction about the O–C-4′ bond, with the unprimed D-glucose unit being held stationary, gives an increase of the value ψ.

These values are given in Table II. By using each set of (ϕ, ψ) between the adjacent residues, six D-glucose residues were generated, to form the cyclohexaamylose. For $\tau = 119°$, the dihedral angles (ϕ, ψ) correspond to ($-11°$, 9°). Since the X-ray crystal structure of cyclohexaamylose shows no variation of (ϕ, ψ) between successive residues, the change of (ϕ, ψ) at successive bridge oxygen-atoms was not considered. The $-CH_2OH$ groups in all of these residues were rotated about the C-5–C-6 bond and kept at the position of minimum energy. The favorable orientation was found to be *trans* with respect to the C-4–C-5 bond.

TABLE II

CONFORMATIONAL ANGLES (ϕ, ψ) FOR CYCLOHEXAAMYLOSE FOR DIFFERENT τ ANGLES

Angle at the bridge oxygen atom (*degrees*)	(ϕ, ψ) (*degrees*)
110	(−27, 20)
112	(−25, 18)
116	(−18, 14)
118	(−15, 10)
119	(−11, 9)
121	(0, 0)

The coordinates of the atoms of the D-glucose residues in cycloheptaamylose were also generated in the same way. However, $n = 7$ was found to be possible only for the value of $\tau = 110°$. Hence, the residues in the cycloheptaamylose were

generated with this value for the angle τ and the corresponding (ϕ, ψ) values, which are ($-10°$, 8°) in this case.

Even though it may be possible that cyclooctaamylose in solution exists in a dynamic state, in which various symmetrical or non-symmetrical conformers are rapidly interconvertible, an eight-fold symmetry is assumed for this molecule for the energy calculations. However, with the bond lengths and angles mentioned above, this assumption (that is, $n = 8$) proved to be inadmissible. Hence, the averages of the bond lengths and angles (Table I, Set 2) observed in various α sugars[7,13,14] were used to generate the cyclooctaamylose. As is seen in Table I, the two sets of parameters used differed slightly. When the angle τ was made equal to 110°, and (ϕ, ψ) were given the values of $-10°$ and 7°, respectively, it was possible to generate the cyclooctaamylose.

ENERGY CALCULATIONS

The energy of "non-bonded" interactions were calculated by using the Kitaigorodsky type of potential functions[15]:

$$V(i,j) = 3.5(8600\, e^{-13Z} - 0.04/Z^6) \qquad (1)$$

where $Z = v_{ij}/v_0$, v_{ij} being the distance between the interacting atoms i and j, and v_0 is the equilibrium distance between them. The values of v_0 used here were the same as described earlier[11].

The strain involved in deforming the angle C–O–C was estimated by using the expression:

$$V(\theta) = A\delta^2 \qquad (2)$$

where A is the equivalent force-constant, the value of which was chosen as 79.5 kcal. mole^{-1} (an average of several experimental reports)[16,17,18], and δ is the deviation of the angle (in radians) from the tetrahedral value.

DISCUSSION OF THE CONFORMATIONS OF CYCLOAMYLOSES

A. Cyclohexaamylose. — The energy of a residue in the cyclohexaamylose molecule as a function of the angle τ is shown in Fig. 2. (If this energy is multiplied by the total number of residues in the cycloamylose, the energy of the molecule can be obtained). Curve 1 in Fig. 2 includes only the non-bonded energy and the bond-angle deformation-energy involved in changing the angle τ. Curve 2 also includes the hydrogen-bond energy, as estimated from the curve of Lippincott and Schroeder[19]. It is seen from curve 1 that the energy curve has a broad minimum for values of τ between 116 and 119°. It appears, therefore, that it is difficult to choose the proper value for the angle τ from calculations of non-bonded energy alone. On the other hand, the hydrogen-bond search reveals that intramolecular hydrogen bonds of the O-2...O-3′ type between contiguous residues are possible for $\tau = 118$ and 119°. In fact, the formation of such O-2...O-3′ types of hydrogen bond between

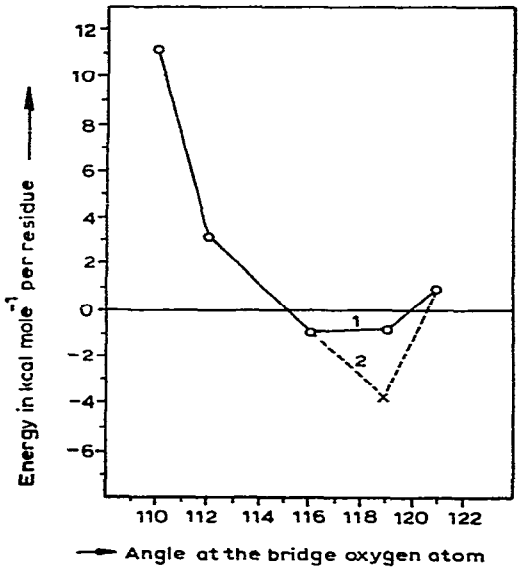

Fig. 2. Graphs plotted between the calculated energy (kcal.mole^{-1} per residue) and the angle at the bridge oxygen atom for cyclohexaamylose. Curve 1 gives non-bonded interaction energy and bond-angle deformation energy; curve 2 gives the same plus hydrogen-bond energy.

contiguous residues has been shown by n.m.r. studies[10]. In the conformation having $\tau = 118°$, the length of the hydrogen bond is only about 2.96 Å, which is fairly weak compared with the hydrogen-bond length of 2.85 Å obtained with $\tau = 119°$. In spite of the energy involved in increasing angle τ by 1°, it is found that there is a sharp minimum in curve 2 of Fig. 2 at 119°, when the hydrogen-bond energy is included. Therefore, the conformation having $\tau = 119°$ is to be preferred for cyclohexaamylose. Interestingly, the angle C–O–C is found to be 119° in the crystal structure of cyclohexaamylose[7].

B. Relative stability of the cycloamyloses. — Fig. 3 shows the variation of potential energy with the number of D-glucose residues in the cycloamylose. In curve 1 of Fig. 3 are included the "non-bonded" energy and the bond-angle strain-energy involved in changing the angle τ. Curve 2 includes the hydrogen-bond energy also. It is seen from this figure that cyclohexaamylose has the minimum energy, and the energy increases on either side. The increase in energy is drastic when the number of D-glucose residues is fewer than six. This is due to the fact that the diameter of a cycloamylose having five D-glucose residues or fewer cannot accommodate the $-CH_2OH$ group, which points approximately towards the interior. Hence, it appears that unfavorable steric interactions prohibit cyclization of cycloamyloses having fewer than six D-glucose residues. This conclusion confirms the earlier observations of French[6].

It is seen from Fig. 3 that the calculated "non-bonded" energies of cyclohepta- and cycloocta-amyloses are greater than that of cyclohexaamylose. This is because the distances between C-1 and H-1, and C-4′ and H-4′, of adjacent residues fall

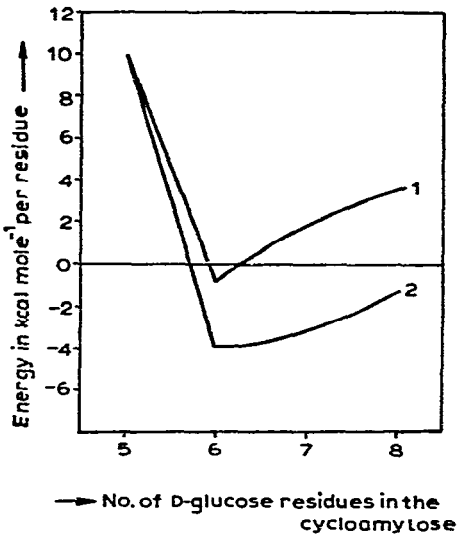

Fig. 3. Relationship between the calculated energy (kcal.mole^{-1} per residue) and the number of D-glucose residues in the cycloamyloses.

slightly short of the minimum limit permissible in cyclohepta- and cyclooctaamyloses. The energy difference between the cyclohexa-, -hepta-, and -octa-amyloses is reduced, however, when the hydrogen-bond energy is also included (curve 2 of Fig. 3). Hence, these molecules are stabilized mainly by the formation of strong, intramolecular, hydrogen-bonds of the O-2...O-3′ type between contiguous residues. This hydrogen bond in cyclohepta- and cyclooctaamylose has a length of about 2.7 Å, which indicates that it is stronger than that (2.85 Å) of cyclohexaamylose. In fact, the n.m.r. studies of Casu and coworkers[10] have indicated that the foregoing type of hydrogen bond between contiguous residues is stronger in cycloheptaamylose than in cyclohexaamylose. Fig. 3 shows that cyclohexaamylose has higher stability than cyclo-

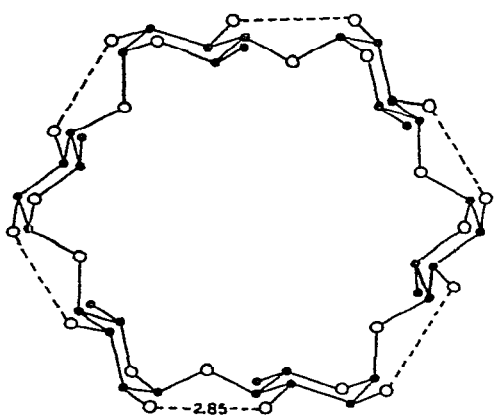

Fig. 4. Perspective view of the proposed conformation of the cyclohexaamylose molecule ($\phi = -11°$, $\psi = 9°$, and $\tau = 119°$). The O-2...O-3′ type of hydrogen-bond distances are marked.

hepta- or -octa-amylose. On the other hand, it has been stated from experimental studies[6] that cycloheptaamylose is more stable than cyclohexa- or cycloocta-amylose. This disagreement between theory and experiment might be due to the fact that ideal models have been considered in the present energy calculations. In solution it might be possible that some puckering in the macro-ring might take place, to relieve unfavorable conflicts between atoms C-1 and H-1, and C-4′ and H-4′, of the adjacent residues in cyclohepta- or -octa-amyloses. Such puckering might decrease the non-bonded energy and increase the stability of these molecules. The perspective views of the cycloamyloses are shown in Figs. 4, 5, and 6.

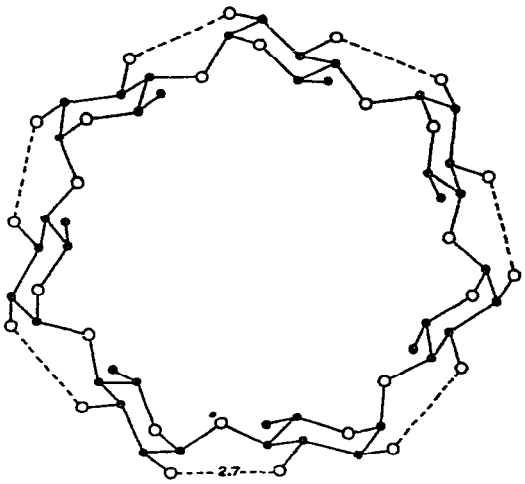

Fig. 5. Perspective view of the proposed conformation of the cycloheptaamylose molecule ($\phi = -10°$, $\psi = 8°$, and $\tau = 110°$. The O-2...O-3′ type of hydrogen-bond distances are marked.

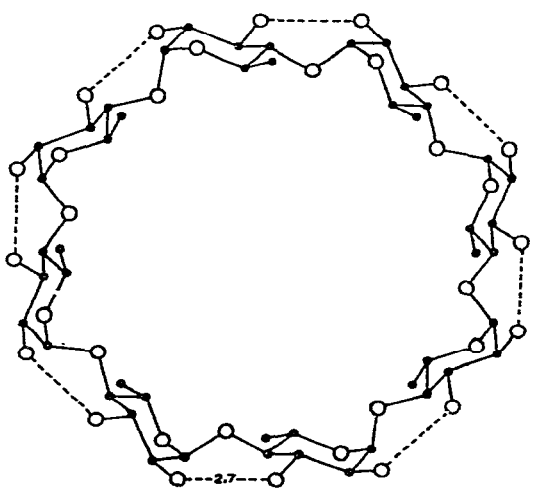

Fig. 6. Perspective view of the proposed conformation of the cyclooctaamylose molecule ($\phi = -10°$, $\psi = 7°$ and $\tau = 110°$). The O-2′...O-3′ type of hydrogen-bond distances are marked.

The higher cycloamyloses having $n = 9$, 10, and so on, could be obtained only by decreasing the angle τ. However, this decrease leads to serious steric conflicts between the C-1 and H-1, and C-4′ and H-4′ atoms of adjacent residues. As mentioned above, it might be possible to relieve such unfavorable interactions by introducing distortions in the macro-ring. Hence, it is most likely that the higher cycloamyloses will exist in puckered forms in solution.

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SOLUTION PROPERTIES OF ALGINATE

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ABSTRACT

The intrinsic viscosity at zero rate of shear was determined for several alginate samples ranging in weight-average molecular weight from about 1×10^5 to 2.7×10^6. The measurements were carried out in aqueous salt solutions of various strengths. The intrinsic viscosity was found to be linear in the reciprocal square root of the ionic strength, and its value at infinite ionic strength could be obtained with good accuracy by extrapolation. An acid-soluble fraction of alginate had the same intrinsic viscosity in hydrogen form at pH 1 as in sodium form at infinite ionic strength. The exponent a in the Mark–Houwink equation decreased with increasing salt concentration, but even at infinite ionic strength the value was very high ($a = 0.84$), suggesting that the discharged alginate molecule is very extended in water. Indications for a high degree of extension of uncharged alginate was also obtained from the high value (155 Å) of the Kuhn statistical segment-length at infinite ionic strength. The separation between solvent effects and chain rigidity in causing the extension was found difficult for theoretical reasons, but it is argued that the main cause of the extension is a high degree of chain rigidity. A comparison with results for amylosic and cellulosic chain polymers revealed a close similarity with the latter.

INTRODUCTION

Alginic acid consists of linear chains of (1→4)-linked β-D-mannuronic and α-L-guluronic acid residues in various proportions[1]. These residues are arranged in "blocks" of mannuronic or guluronic acid residues, which are linked by blocks in which the sequence of the two acid residues is predominantly alternating[2–5]. Except for fractions that are much enriched in the blocks having the alternating sequence[6,7], alginic acid is generally insoluble in water. Sodium alginate and most other alginates of monovalent metals are soluble in water and give solutions of high viscosity. Although alginate is manufactured mainly because of its ability to give highly viscous solutions, its hydrodynamic properties have not been extensively studied. In a recent paper[8], measurements of the intrinsic viscosity, molecular weight, and radius of gyration of a series of alginate samples in 0.1M aqueous salt solution were reported. At this salt concentration, alginate behaved like a very extended, flexible coil.

Whether the high degree of extension was due to mechanical inflexibility of the polymer backbone, to electrostatic expansion caused by the charged groups, or to expansion caused by a high degree of interaction between the water and the polymer segments, could not be decided. The object of the present paper is to study the effect of the addition of inorganic salt upon the viscosity; and from these data to extrapolate to infinite ionic strength, in order to obtain information about the extension of the alginate molecule in an effectively uncharged state. For comparison, measurements of the intrinsic viscosity of alginate fractions that are soluble in their acidic, uncharged form[6] are included. It will be shown that the "equivalent sphere" hydrodynamic theory of Flory[9] fails to describe the hydrodynamic properties of alginate. This failure makes it difficult to account quantitatively for the expansion due to the solvent–segment interaction at infinite ionic strength and thus to obtain the "unperturbed" dimensions of alginate. However, by using only the data for the samples having the highest molecular weights, and by assuming that the "equivalent sphere" model in this case is applicable, some parameters describing the stiffness of the alginate chain can be calculated.

EXPERIMENTAL

The alginate was prepared[1] from *Laminaria digitata* harvested at Tarva, August 29, 1961. In order to obtain a sample of very high intrinsic viscosity ($[\eta] = 55$ dl/g in 0.1M sodium chloride), precautions had to be taken to avoid acid[10], alkaline[11], and oxidative-reductive[12] depolymerization during the different extraction periods. The ratio[1] between mannuronic and guluronic acid residues in this alginate was 1.6. The alginate was degraded[8] at pH 4 to give samples of different molecular weights. An acid-soluble alginate fraction was prepared by fractional precipitation at pH 1.4 of some slightly degraded alginate samples[6] from *Ascophyllum nodosum*. The ratio between mannuronic and guluronic acid residues was 1.8. The sequence of the uronic acid residues in both the unfractionated[4] and in the acid-soluble fraction[7] has been investigated previously.

Viscosity measurements were made at 20.0° in a Zimm–Crothers Model A, low-shear, rotating cylinder viscometer[13]. The average shear stress was approximately 0.002 dyne/cm^2. The viscosities obtained at this shear stress were, within experimental error, equal to the viscosities obtained by extrapolation to zero shear-stress of data obtained in a capillary viscometer with varying hydrostatic pressure[14]. Intrinsic viscosities were obtained at all ionic strengths from "isoionic dilution" experiments. Of the counterions of sodium alginates, 60% were regarded as bound in ion-pairs to the alginate[15,16]. The remainder were assumed to be free, and the amount of sodium chloride that was added to an alginate solution was calculated according to this assumption. By dilution with a sodium chloride solution of the appropriate ionic strength, the amount of free sodium ions was kept constant at all concentrations of alginate. All plots of $\log(\eta_{sp}/c)$ against the alginate concentration (c) were linear as before[14], and $[\eta]$ was found from the intercept.

RESULTS AND DISCUSSION

Effect of ionic strength upon the intrinsic viscosity

A general property of polyelectrolytes seems to be that the intrinsic viscosity is very nearly a linear function in the reciprocal of the square root of the ionic strength, over a rather wide range of ionic strengths[17–20]. It is known that the viscosity of alginates depends strongly upon the ionic strength of the solution[14], but its exact relationship to the ionic strength has not yet been established.

The intrinsic viscosity of a sample of sodium alginate was determined at eight different concentrations of sodium chloride (Table I and Fig. 1a). An essentially linear plot of $[\eta]$ against $1/\sqrt{I}$ (I is the ionic strength in molar units) was obtained. Thus, it is possible to obtain, with good accuracy, the intrinsic viscosity at infinite ionic strength. Such extrapolations to infinite ionic strength are widely used for characterizing the properties of polyelectrolytes[18,20–22] in an effectively uncharged state, since the screening of the fixed charges on the polymer is thought to be so severe at infinite ionic strength that the mutual repulsion between them is essentially removed. The configurational properties should therefore become those of neutral polymer molecules. It is readily understood that remote, fixed charges, which must be separated, at least partly, by salt solution, cannot interact at infinite ionic strength. It may be questionable, however, whether the interaction between the charged groups

TABLE I

DEPENDENCE UPON IONIC STRENGTH (I) OF THE INTRINSIC VISCOSITY $[\eta]$ OF AN ALGINATE SAMPLE HAVING $M_w = 6.5 \times 10^5$

I	0.005	0.01	0.02	0.05	0.1	0.2	1.0	2.0	∞
$[\eta]$(*100 ml/g*)	29.5	22.0	18.7	14.5	13.0	12.0	10.2	9.5	9.0

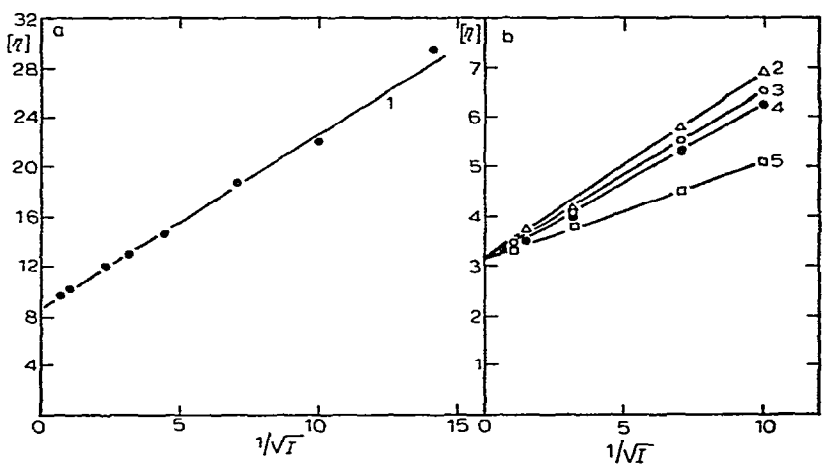

Fig. 1. Intrinsic viscosity of alginate as a function of the reciprocal of the square-root of the ionic strength. Curve 1, $M_w = 6.5 \times 10^5$. Curves 2–5, $M_w = 2.0 \times 10^5$. Curves 1 and 3, sodium chloride. Curve 2, lithium chloride. Curve 4, potassium chloride. Curve 5, magnesium sulphate.

on adjacent monomer units is removed in this way. It was therefore considered of interest to compare the intrinsic viscosities of alginates "neutralized" in different ways.

Studies of equilibrium dialysis[15] and potentiometric titration[16] showed that magnesium ions were bound more strongly to alginate than were monovalent ions. A comparison of the viscosity after addition of different salts of monovalent cations and of magnesium ions was therefore made, and the result is shown in Fig. 1b. At low salt concentrations, the intrinsic viscosity is markedly lower with magnesium sulfate present than with salts of monovalent cations. The difference between the three alkali metal salts is small but significant, with lithium chloride giving the highest and potassium chloride the lowest viscosity. In the range of ionic strengths shown, all of the salts gave a linear dependence in $1/\sqrt{I}$, and the curves tended to give very nearly the same intrinsic viscosity at infinite ionic strength. Since magnesium ions most probably form ion-pairs with the carboxyl groups of alginate to a much higher extent than do the alkali-metal ions, these results suggest that the viscosity at infinite ionic strength, based upon the results with sodium chloride, is representative of the uncharged alginate molecule.

More conclusive evidence was sought by comparing the viscosity of an acid-soluble alginate fraction[7] in acid and sodium form. Preliminary experiments with this fraction showed that, in solutions of ionic strength 0.1, the intrinsic viscosity was independent of pH between 10 and 4.6. Between pH 4.6 and pH 2.0, the intrinsic viscosity decreased continuously. No further decrease occurred down to pH 1.0. At this pH, the ionic strength could be increased up to M without affecting the viscosity. The alginate should therefore be fully discharged at pH 1, which is in accordance with the known dissociation constants of alginates[1], and this pH was chosen for comparison with sodium alginate. The result is shown in Table II, where the intrinsic viscosity at $I = 0.01$, 0.1, and 1.0 is given, together with the result from a linear extrapolation to infinite ionic strength. The intrinsic viscosity at infinite ionic strength is seen to differ insignificantly from that determined in 0.1M hydrochloric acid.

TABLE II

COMPARISON OF THE INTRINSIC VISCOSITIES OF ACID-SOLUBLE ALGINATE FRACTIONS IN THEIR SODIUM AND HYDROGEN FORMS

	Sodium alginate				*Alginic acid*
Ionic strength (I)	0.01	0.1	1.0	∞	0.1M HCl
First fraction[a]	11.1	6.3	4.85	4.2	4.15
Second fraction[a]	7.0	4.8	3.8	3.35	3.5

[a]See text, pp. 4 and 5.

It was observed that the intrinsic viscosity in 0.1M hydrochloric acid decreased when the solutions were stored at room temperature. After two days, the solution could be separated, by centrifugation at 28,000 g, into a dilute and a concentrated

phase. The dilute phase contained 60% of the alginate. This fraction (second fraction) was neutralized with sodium hydroxide, precipitated, and dried in the usual way[1]. Again, comparison of the viscosity of the sodium form at infinite ionic strength with that of the acid form revealed no significant difference, as seen in Table II.

The extrapolation to infinite ionic strength of data obtained with sodium chloride therefore seems to be a valid procedure for obtaining the intrinsic viscosity of the uncharged alginate molecule.

Dependence of the intrinsic viscosity upon molecular weight

Ten samples of alginate having different molecular weights were prepared by partial acid-degradation. The relationship between intrinsic viscosity in 0.1M sodium chloride and weight-average molecular weight of these samples was reported[8] to be

$$[\eta] = 2.0 \times 10^{-5} \times M_w^{1.0} \quad (1)$$

where $[\eta]$ is expressed in 100 ml/g. The intrinsic viscosity in 0.01, 0.1, and M sodium chloride was determined for each sample, and the results, together with the molecular weights calculated from equation (1), are shown in Table III. Plots of the intrinsic viscosity against $1/\sqrt{I}$ gave, in each case, linear curves having well-defined intercepts. The slopes of the curves depended very much upon the molecular weight and increased with it in proportion to its (1.36)th power. The graphical representation of these results, together with results obtained with other polyelectrolytes, will be published later. In Table III, only the values of the intrinsic viscosities obtained from the linear extrapolations to infinite ionic strength are given.

TABLE III

DEPENDENCE UPON IONIC STRENGTH OF THE INTRINSIC VISCOSITY OF ALGINATE SAMPLES OF DIFFERENT MOLECULAR WEIGHTS

$M_w \times 10^{-5}$	$[\eta]_{0.01}$	$[\eta]_{0.1}$	$[\eta]_{1.0}$	$[\eta]_\infty$
27.0	120	55	36	24.5
16.5	67	33	23	18.5
15.5	58	31	21	16.2
9.7	38.1	19.5	15.0	12.5
6.5	22.0	13.0	10.2	9.0
4.7	16.0	9.4	7.9	6.9
2.95	9.2	5.9	5.2	4.7
2.48	7.15	4.95	4.35	3.95
1.44	4.0	2.88	2.50	2.35
1.12	3.05	2.25	1.95	1.85

One important feature of the data in Table III is that the intrinsic viscosity at a given molecular weight is very high, even at infinite ionic strength. This may be seen clearly by comparing the present results with the intrinsic viscosity of native DNA. From a plot[23] of intrinsic viscosity against molecular weight for DNA, it is seen that an average value of the intrinsic viscosity (in 0.2M salt solution) of DNA of molecular

weight 2.7×10^6 is 21, whereas alginate of this molecular weight has $[\eta] = 24.5$ at infinite ionic strength. It is rather astonishing that alginate has as high an intrinsic viscosity as a stiff[24] double helix of the same molecular weight. The stiffness of the chains cannot, however, be directly compared in this way, since the mass per unit chain-length is different in the two substances, and is much higher for the double-stranded DNA than for alginate.

The most common way of expressing and comparing viscosimetric results is by means of the well-known Mark–Houwink equation (the modified Staudinger equation)

$$[\eta] = KM^a \tag{2}$$

The double logarithmic plot of $[\eta]$ versus M required for obtaining the indices K and a is shown in Fig. 2. The straight lines drawn in the figures follow the equations given in Table IV. The data for ionic strength of 1.0 and infinity are seen from Fig. 2 to deviate somewhat from straight lines. Such curvature has previously been observed for DNA molecules[23], and it has been explained theoretically by the hydrodynamic theory for worm-like, chain molecules[25]. For the present purpose, the effect seems too small to justify any detailed discussion in terms of such a model, and the fully drawn, straight lines will, in most cases, form the basis for the theoretical interpretations. The value of the exponent (a) is seen to decrease with increasing ionic strength. The decrease is not very marked, however, and even at infinite ionic strength, a is much higher than 0.5, the value common to Gaussian coils in θ-solvents[9]. According to all hydrodynamic theories, the high a-value found at infinite ionic strength must indicate that the uncharged alginate molecule is very extended in aqueous solution. The high degree of extension previously found[8] in 0.1M sodium chloride cannot, therefore, be due solely to high electrostatic expansion caused by the ionized carboxyl groups. The relative importance of charge effects in determining the extension of the molecules may be estimated by using a viscosity theory for calculating the molecular dimensions.

TABLE IV

MARK–HOUWINK EQUATIONS AT DIFFERENT IONIC STRENGTHS

$I = 0.01$	$[\eta] = 4.8 \times 10^{-6}.M_w^{1.15}$
$I = 0.1$	$[\eta] = 2.0 \times 10^{-5}.M_w^{1.0}$
$I = 1.0$	$[\eta] = 0.91 \times 10^{-4}.M_w^{0.87}$
$I = \infty$	$[\eta] = 1.2 \times 10^{-4}.M_w^{0.84}$

Calculation of molecular dimensions

The well-known "equivalent sphere" model of Flory[26,9] is widely used for calculating molecular dimensions for flexible chain molecules. The relevant equation is

$$[\eta] = \Phi \frac{(\overline{r^2})^{3/2}}{M} \tag{3}$$

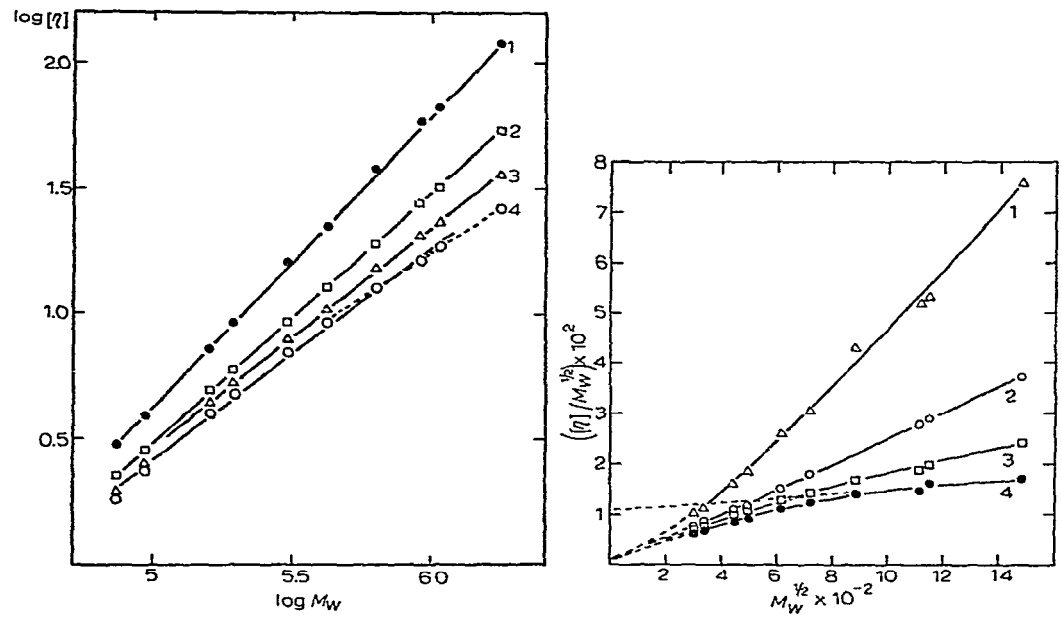

Fig. 2. Mark–Houwink plot for alginate at different ionic strengths. Curve 1, $I = 0.01$. Curve 2, $I = 0.1$. Curve 3, $I = 1.0$. Curve 4, $I = \infty$.

Fig. 3. Stockmayer–Fixman–Burchard plot for alginate at different ionic strengths. The curves are numbered as in Fig. 2.

where Φ is the Flory viscosity constant and $\overline{r^2}$ is the mean-square end-to-end distance. The constant, Φ, is found experimentally to have a typical value of 2.0×10^{21} for synthetic, flexible, neutral polymers in good solvents[27]. The theoretical value[28] of 2.86×10^{21} obtained by the Kirkwood and Riseman theory for Gaussian molecules having large hydrodynamic interactions (non-drained molecules) is found to agree well with experiments in poor solvents[27]. Recent experimentation seems to indicate that the values of Φ do not always fall within this range for polymers that are very extended in solution[17,22,29,30], and lower values are then usually obtained. Whether the extension in question is due to chain rigidity, goodness of the solvent, or to polyelectrolyte effects is not of critical importance. Bloomfield and Zimm[31] have incorporated all of these three effects into a single parameter ε, which, in their theory for non-drained molecules, is related to the exponent a in the Mark–Houwink equation by the relation

$$\varepsilon = \frac{(2a-1)}{3} \tag{4}$$

They calculated Φ as a function in ε and found it to decrease from 2.81×10^{21} for $\varepsilon = 0$ to 0.686×10^{21} for $\varepsilon = 0.5$, and tabulated $\Phi(\varepsilon)$ for different ε-values. This theory has been used for calculating molecular dimensions of native DNA samples[19] having very high molecular-weight. An extension of the same theory has also been used with success for estimation of chain rigidity of some vinyl polymers[32].

From the values of a given in Table III, the corresponding values of $\Phi(\varepsilon)$ were found to be 1.50, 1.43, 1.13, and 0.82 ($\times 10^{21}$) for alginate in solution of ionic strength ∞, 1.0, 0.1, and 0.01, respectively.

In Table V, the root-mean-square (r.m.s.) end-to-end distances are given for the sample of highest molecular weight. The calculation is made with $\Phi = 2.86 \times 10^{21}$, 2.0×10^{21}, and $\Phi(\varepsilon)$ as given above. In the Table, the Kuhn[33,34] statistical segment length, A_m, is also calculated from the relation

$$(\overline{r^2})^{1/2} = (A_m b_0 P)^{1/2} \quad (5)$$

where b_0 is the length of a monomer unit and P is the degree of polymerization. The A_m-values were obtained by using 198 as the weight and 5.15 Å as the length of a monomer unit[35], and 2.7×10^6 as the molecular weight of the polymer chain.

TABLE V

ROOT-MEAN-SQUARE END-TO-END DISTANCE, $(\overline{r^2})^{1/2}$, AND KUHN STATISTICAL SEGMENT LENGTH, A_m, FOR AN ALGINATE HAVING $M_w = 2.7 \times 10^6$

Ionic strength (I)	$(\overline{r^2})^{1/2}$ (Å), *assuming* Φ *equal to*			A_m (Å), *assuming* Φ *equal to*		
	2.86×10^{21}	2.0×10^{21}	$\Phi(\varepsilon)$	2.86×10^{21}	2.0×10^{21}	$\Phi(\varepsilon)$
	2900	3200	3300	120	145	155
1.0	3200	3600	4000	145	190	230
0.1[a]	3700	4300	5200	205	265	390
0.01	4900	5600	7500	350	455	825

[a]Light-scattering result for $I = 0.1$, $(\overline{r^2})_w^{1/2} = 5100$ Å, $A_m = 350$ Å.

By means of the previously determined radii of gyration for alginate in 0.1M sodium chloride[8], it is possible to test the validity of the dimensions calculated from intrinsic viscosity data at this salt concentration. The relationship between the z-average radius-of-gyration and the weight-average molecular weight obtained by light scattering was

$$(R_G)_z(\text{Å}) = 0.95 \times M_w^{0.54} \quad (M_w > 65.000) \quad (6)$$

A comparison of calculated and obtained dimensions is somewhat complicated by the fact that the alginate samples were polydisperse with respect to the molecular weight, the ratio[8] of the weight- to number-average molecular weight being 3.0. Information about the exact molecular weight distribution is lacking, but there is no reason to believe that the molecular weight distribution is not unimodal, and use was therefore made of the Schulz–Zimm relationship[36,37] for molecular weight distribution for converting the values for the z-average radius-of-gyration into weight averages. By the aid of the relationship[9]

$$(\overline{r^2})^{1/2} = 6^{1/2} R_G$$

the weight-average r.m.s. end-to-end distance and the statistical segment length, A_m, were calculated for the sample of highest molecular weight, with the result shown in Table V. It is seen that the light-scattering dimensions agree rather closely with those obtained by the Bloomfield–Zimm viscosity theory. In spite of the somewhat arbitrary way used to account for the polydispersity, it seems safe to conclude that the Bloomfield–Zimm theory gives the most correct molecular dimensions. This does not mean that the assumption of non-drainage is necessarily correct. If the comparison between dimensions obtained from light scattering and from viscosity measurements had been performed for a sample of very low molecular weight, even the Bloomfield–Zimm viscosity theory would have yielded molecular dimensions that are too low. As discussed later, this will be taken as evidence for the existence of partial free-drainage of alginate in 0.1M sodium chloride. With the Bloomfield-Zimm viscosity theory, the length of the Kuhn statistical segment decreases by a factor of more than 5 as the ionic strength increases from 0.01 to infinity, but even at infinite ionic strength, the value 155 Å is high compared with common values for vinyl polymers (*e.g.*, A_m = 19 Å for polyvinyl alcohol in water[34]) and also high compared with amylose in aqueous potassium chloride [38,39](A_m = 22 Å). The value is within the range reported for carboxymethylcellulose at infinite ionic strength [A_m = 335 Å (Ref. 40) and A_m = 110 Å (Ref. 22)]. Only for substances known to be extremely inflexible have higher values been reported (*e.g.*, A_m = 176 and 332 Å for two samples of cellulose trinitrate in acetone[29], and A_m = 540 Å for native DNA in 0.2M sodium chloride[25]). The result therefore strengthens the earlier conclusion that the uncharged alginate molecule has a very extended form in aqueous solution.

Determination of unperturbed dimensions

Having concluded, with a rather high degree of certainty, that the uncharged alginate molecule is very extended in aqueous solution, a much more difficult task remains, namely, to understand, at the molecular level, the reasons for this stiffness. The rigidity of a chain molecule is a function of the bond lengths, the bond angles, and the hindered rotation of the monomer units. The r.m.s. end-to-end distance may be calculated for the situation where all monomers are allowed to rotate freely around the fixed bond angles[9], and this type of calculation generally shows that the dimensions of polysaccharides are large compared with vinyl polymers[21,41], because of the size and inflexibility of the monomeric unit. The observed dimensions are always larger than those corresponding to free rotation, which may be due in part to hindrance to rotation and in part to solvent expansion (excluded volume effects). For estimation of chain rigidity, it is necessary, therefore, to separate these two effects.

For synthetic polymers, it is usually possible to find θ-solvents, *i.e.*, poor solvents that do not expand the polymer coil. The corresponding "unperturbed dimensions" can therefore be determined directly. The steric factor, σ, defined by the relation[41]

$$\sigma = (\overline{r_\theta^2})^{1/2}/(\overline{r_f^2})^{1/2} \qquad (7)$$

where subscripts θ and f refer to θ-conditions and free rotation, respectively, is then a measure of the hindrance to rotation. The steric factor has been determined for many vinyl polymers. It is found to depend somewhat on the bulkiness of the monomer and lies typically between 2 and 3 (Refs. 9 and 41). For polysaccharides, it is very difficult to find θ-solvents, and one then has to rely on a theory for solvent expansion, and extrapolate to a hypothetical θ-condition for obtaining the unperturbed dimensions.

Burchard[42] has recently reviewed the different extrapolation procedures for obtaining unperturbed dimensions from viscosity measurements in good solvents. The most frequently used method is due to Burchard[43], and to Stockmayer and Fixman[44]. It is based upon the Flory viscosity theory[9] which may be written

$$[\eta] = K_\theta M^{1/2} . \alpha^3 \tag{8}$$

$$K_\theta = \Phi(\overline{r_\theta^2}/M)^{3/2} \tag{9}$$

and α is the solvent expansion factor, depending on the molecular weight according to Fixman's theory[45]

$$\alpha^3 = 1 + C' . M^{1/2} \tag{10}$$

in which C' is a polymer–solvent interaction parameter, which is a constant for a given polymer–solvent system. Combination of equations (10) and (8) gave

$$[\eta]/M^{1/2} = K_\theta + C' . K_\theta . M^{1/2} \tag{11}$$

A plot of $[\eta]/M^{1/2}$ versus $M^{1/2}$ should yield straight lines if free draining is not operative, with an intercept, K_θ. The unperturbed dimensions may then be calculated from equation (9).

A graphical representation of the results for the four ionic strengths according to this theory is seen in Fig. 3. Only the points for ionic strength 0.1 lie on a straight line. For the lowest ionic strength, an upwards, and for the two highest ionic strengths, a downwards curvature is indicated. All of the lines seem to tend to a common intercept, with K_θ near to 1×10^{-3}.

Although such a value of K_θ is typical for vinyl polymers[41], there are reasons to believe that it is not the correct one for alginate. It has previously been suggested, from a comparison of light-scattering and viscosity results, that the alginate molecule is partially free drained in 0.1M salt[8]. By using these results in equation (2) and accounting for the polydispersity in the same way as before, Φ may be calculated as 1.0×10^{21} for the sample of the highest ($M_w = 2.7 \times 10^6$) and 0.35×10^{21} for the sample of the lowest molecular weight ($M_w = 11.2 \times 10^4$). Even though the exact value of Φ may be open to question because of the aforementioned uncertainties concerning the corrections for polydispersity, the rather pronounced variation of Φ with the molecular weight is indicative of partial free-drainage. A straight line in the Stockmayer–Fixman–Burchard plot seems, therefore, not to be conclusive evidence for the absence of drainage. The value of the intercept resulting from the straight-line

extrapolation of the results in 0.1M salt cannot then, in the author's opinion, be used together with any well-founded value of Φ in equation (9) for calculating unperturbed dimensions.

According to Flory[9] and others, the assumption of non drainage must hold in the limit of very high molecular weight, and retain its validity at progressively lower molecular weights as the polymer coil becomes less extended. An estimate of the unperturbed dimensions of alginate may, therefore, best be obtained by an extrapolation of the results obtained for the samples of the highest molecular weight at infinite ionic strength. The straight line (shown in Fig. 3) through the four upper points of the curve for infinite ionic strength yielded $K_\theta = 1.1 \times 10^{-2}$ as the intercept. The value of Φ used in equation (9) is commonly between 2 and 2.8×10^{21}, but since the Bloomfield–Zimm viscosity theory was shown to give the best fit with the results for alginate of high molecular weight, it is here preferred to use a value of Φ obtained with that theory. In Fig. 2, it is seen that the three upper points at infinite ionic strength lie below the fully drawn curve. A straight line through these three points had the slope 0.77. By using this figure as the exponent in the Mark–Houwink equation, the corresponding Φ-value is found[31] to be 1.75×10^{21}. The unperturbed r.m.s. end-to-end distance for the sample of $M_w = 2.7 \times 10^6$ was calculated to be 3020 Å, corresponding to a Kuhn-segment length of 130 Å.

These values are not very much smaller than those obtained with the Bloomfield–Zimm viscosity theory at infinite ionic strength, which suggests that the main cause of the extension of uncharged alginate in water is a high degree of chain rigidity. To get a quantitative expression for the hindrance to rotation of the monomers, a knowledge of the ring conformation of the uronate residues in alginate is required. This question is at present not absolutely clear[35,46], and it must be arbitrarily assumed that both the uronate residues exist in the *C1* ($^4C_1$) conformation. This makes the dimensions that may be calculated for the case of free rotation identical with those of a cellulosic chain. This calculation has been performed with the result[47]

$$(\overline{r_f^2})^{1/2} = 7.75 \times P^{1/2} \tag{12}$$

By using this relation, and comparison with the obtained unperturbed dimension, the steric factor, σ, was calculated to be 3.1.

It should be stressed that this value rests upon the assumption of non-drainage for the samples of high molecular-weight at infinite ionic strength, and the assumption that the Fixman relation for the expansion factor is valid for alginate in this range of molecular weights. The first assumption, apart from being intuitively easy to accept, agrees with experimental findings for carboxymethylcellulose[22] and sodium hyaluronate[30], and may therefore be considered to be fairly sound. The Fixman treatment of the excluded volume problem was based on a model that is not physically very realistic for the present system, and the value of the steric factor should, therefore, only be regarded as the best estimate at present, and it may be open to change when more realistic models are available.

With these reservations in mind, the results indicate that the restriction to rotation in the uncharged alginate molecule corresponds to the restriction in the most inflexible vinyl polymers. Whether this restriction is due solely to steric interactions between the atoms on adjacent rings, or is in part due to firmly attached solvent molecules, is not clear. The last possibility must generally be considered to be of importance for polar molecules in polar solvents[41], and needs investigation.

Comparison with other polysaccharides

It would be of interest to compare the results obtained here with those for other (1→4)-linked polysaccharides. The most extensively studied polysaccharides are cellulose and amylose, and their derivatives. A comparison with these substances is somewhat difficult, because contradictory views exist in the literature concerning their conformational and configurational properties in the unperturbed state[41,42,48–50,53].

This is due mostly to the lack of well-defined θ-solvents for cellulose and cellulose derivatives at normal temperatures (20–30°), with the resulting theoretical difficulties in estimating the unperturbed dimensions.

Except for some experiments at high temperature[51] (120–140°), cellulose and cellulose derivatives generally give higher values of the exponent, a, in the Mark–Houwink equation than do amylose and its derivatives[41], which in some cases yield figures equal or close to 0.5, the theoretical value for Gaussian coils in θ-solvents. In the work of Kurata and Stockmayer[41], a large value of a is attributed to high value of the expansion factor α; this assumption automatically yields small unperturbed dimensions as may be seen from equations (8) and (9). They concluded, therefore, that amylose and its derivatives showed somewhat larger σ-values than does the cellulosic chain. An alternative interpretation of the experimental results is possible, however, since for two polymers measured in a solvent giving the same expansion factor for both substances, the polymer having the largest a-value would have the largest unperturbed dimensions. If, therefore, no major difference exists in the solvation of the cellulosic and amylosic chain, the latter has to be the more flexible. Flory[54] and others have indeed suggested that the cellulosic chain is characterized by small values of the expansion factor in most solvents and that this chain is very stiff.

Because considerations concerning the solvation of the polysaccharides are of prime importance for estimation of their unperturbed dimensions, one cannot compare the value of the steric factor obtained for alginate with values reported for other polysaccharides, without a lengthy discussion of the many ways used to account for the solvent-expansion factor. This would be outside the scope of this paper, and a more direct way of comparing chain stiffness was sought.

Banks and Greenwood[49] determined the indices in the Mark–Houwink equation for amylose triacetate in solvent–precipitant mixtures and found that the exponent, a, equalled 0.5 near the precipitation point. This result is to be expected for a non-drained Gaussian coil according to the Flory θ-point theory. Cellulose triacetate, on the other hand, yielded $a = 0.83$ near the precipitation point[52], and

Banks and Greenwood favoured the view that the high a-values commonly reported for the cellulosic chain were a consequence of high chain-stiffness.

Alginate is precipitated by molar concentrations of monovalent salts[1], and the instability of the alginic acid fraction used in this work suggests that this sample was near the precipitation point in 0.1M hydrochloric acid solution. It is interesting to note, therefore, that the a-value reported here for uncharged alginate is the same as for cellulose triacetate at the precipitation point, and it is tempting to conclude that the same type of glycosidic linkage in alginate and cellulose is the reason for this.

This conclusion must only be regarded as tentative, since the use of the Flory θ-concept in these highly polar systems may be an oversimplification. In this theory, any specific interactions between solvent and polymer are not considered, and they may be of importance in the present system. The negative temperature coefficient of the viscosity, previously observed for alginate[1], disagrees with the normal behaviour of a solution near the precipitation point according to the Flory theory, and one way of explaining this is by assuming the existence of a specific, temperature-dependent solvation. It seems, therefore, that both a more systematic investigation of the extension of polysaccharides in chemically different precipitation mixtures, and a comparison of different polysaccharides in one particular solvent are needed to get a clearer picture of their solution properties. Investigations along these lines are currently being carried out in this laboratory.

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SYNTHESIS OF *N*-ACETYLNEURAMINIC ACID 8-METHYL ETHER

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ABSTRACT

N-Acetylneuraminic acid 8-methyl ether (**1**) has been synthesized. Benzylation of 2-methyl-4,5-(4,6-*O*-benzylidene-2-deoxy-β-D-mannopyrano)-2-oxazoline (**2**), followed by mild, acid hydrolysis, yielded 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose (**3**). Treatment of **3** with the potassium salt of di-*tert*-butyl oxaloacetate gave 6-*O*-benzyl-7,9-*O*-benzylidene-*N*-acetylneuraminic acid γ-lactone (**4**). Methylation of **4**, followed by removal of the benzyl and benzylidene groups and opening of the γ-lactone ring, then gave **1**. The inhibition by acid **1** of the hydrolysis of *p*-nitrophenyl *N*-acetyl-α-D-neuraminoside by *Vibrio cholerae* neuraminidase has been investigated.

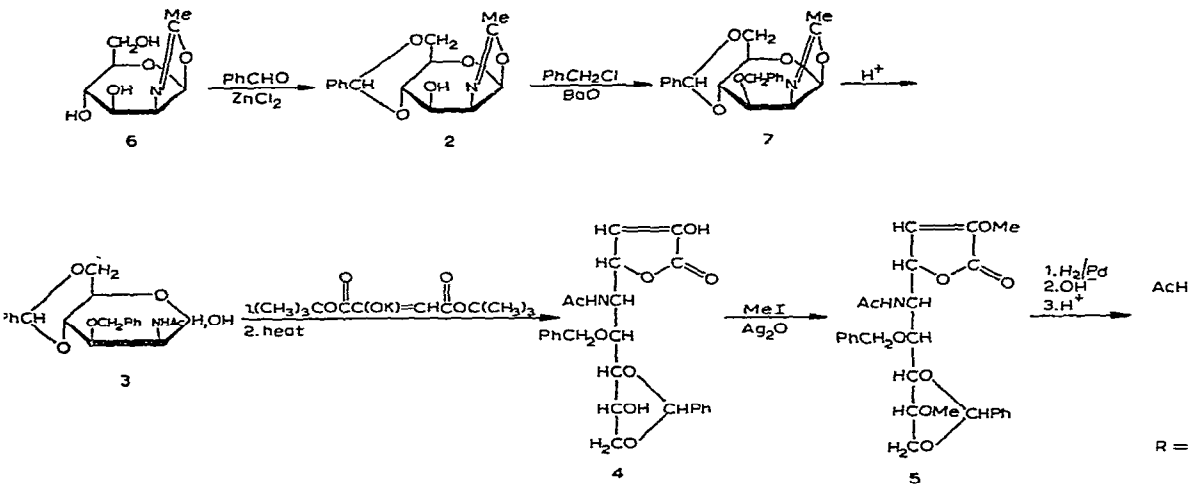
INTRODUCTION

The specific substitution of *N*-acetylneuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid, NANA) is of importance in connection with studies of the mode of bonding of the sialic acid moieties in biopolymers, and with elucidation of the structural features of NANA which determine its specific receptor properties. Previous papers of this series have dealt with the synthesis of the benzhydryl ester[1] of NANA, and the acylation[2], tritylation, and glycosylation of NANA[3]. We now describe the synthesis of the 6-*O*-benzyl-7,9-*O*-benzylidene derivative (**4**) of the γ-lactone of *N*-acetylneuraminic acid which is a key intermediate for the preparation of 8-*O*-substituted derivatives of NANA. From **4**, the 8-methyl ether (**1**) of NANA (an analogue of the 8-methyl ether of *N*-glycolylneuraminic acid which was isolated from *Asterias forbesi*[4]) has been prepared.

RESULTS AND DISCUSSION

The synthesis of **1** from 2-methyl-4,5-(2-deoxy-β-D-mannopyrano)-2-oxazoline (**6**) has been accomplished according to the following scheme (on p. 374). The key compound in this reaction series was 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose (**3**) obtained by conversion of the oxazoline **2** into the benzyl derivative **7** followed by mild treatment with acid to effect opening of the oxazoline ring.

The presence of the oxazoline ring in intermediates **2**, **6**, and **7** was demonstrated by the i.r. bands at 1650–1680 cm^{-1} (C=N) and by the absence of bands at 1510–



1570 cm^{-1} characteristic of secondary amides. The structure of the oxazoline **7** is also supported by the absence of OH absorption at 3200–3650 cm^{-1}. The i.r. spectrum of **3** shows bands of equal intensity at 1550 and 1665 cm^{-1} characteristic of the CONH grouping.

Condensation[5] of **3** with the potassium salt of di-*tert*-butyl oxaloacetate yielded a lactone (48%) which was not purified but was treated with methyl iodide–silver oxide in methanol to give the methylated lactone **5**. The benzyl and benzylidene groups were removed in a single step by hydrogenolysis over palladium–charcoal. Subsequent opening of the lactone ring under alkaline conditions, and treatment with cation-exchange resin gave the desired 8-*O*-methyl-NANA (**1**).

Besides elemental and functional-group analysis, the structure of **1** was confirmed as follows. The acid **1** gave a colour reaction typical for sialic acid with the Svennerholm[6] and Ehrlich[7] reagents. Esterification of **1** with methanol in the presence of a cation-exchange resin, followed by acetylation with acetic anhydride in pyridine, gave 2,4,7,9-tetra-*O*-acetyl-8-*O*-methyl-*N*-acetylneuraminic acid methyl ester (**8**). The n.m.r. spectrum of **8** showed the presence of one ether and one ester methyl group (singlets at 3.42 and 3.82 p.p.m.). Comparison of the n.m.r. spectra of **8** and of 2,4,7,8,9-penta-*O*-acetyl-*N*-acetylneuraminic acid methyl ester revealed similar patterns in the region of 2.20–3.40 and 3.80–6.00 p.p.m. (*cf.* Ref. 8), confirming the D-*glycero*-D-*galacto* configuration for **8** and for **1**.

Further evidence for the stereochemistry of **1** was obtained on investigation of its inhibitory activity during enzymic hydrolysis of *p*-nitrophenyl *N*-acetyl-α-D-neuraminoside[9] with neuraminidase from *Vibrio cholerae*. Both **1** and NANA are competitive inhibitors and exhibit similar affinity for the active site of the neuraminidase (K_i-values 3.6×10^{-3}M and 4.9×10^{-3}M, respectively). The fact that blocking of the OH group at C-8 in NANA does not affect the affinity of the monosaccharide for the active site of the enzyme suggests that this group does not participate in formation of the enzyme–inhibitor complex.

EXPERIMENTAL

General. — Melting points were determined on a Kofler apparatus and are corrected. Optical rotations were measured with a Hilger M-142 polarimeter. Evaporation was performed at 35–40° in *vacuo*. T.l.c. was carried out on KSK silica gel with 5% gypsum by using ether (*A*), chloroform–methanol [95:5 (*B*); and 8:2 (*C*)] or, propyl alcohol–water [7:3 (*D*)]. Detection was effected with conc. sulphuric acid and Svennerholm's reagent[6]. Paper chromatography was carried out on "Goznak" paper with propyl alcohol–butyl alcohol–0.1N hydrochloric acid [2:1:1 (*E*)] and butyl alcohol–pyridine–water [6:4:3 (*F*)]. Detection was effected with aniline hydrogen phthalate and Ehrlich's reagent. N.m.r. spectra were obtained with a JNM-4H-100 spectrometer with tetramethylsilane as internal reference and chloroform-*d* as solvent. I.r. spectra were recorded with a UR-10 spectrophotometer. Absorbance at 400 nm was measured with a SF-4A spectrophotometer.

*2-Methyl-4,5-(2-deoxy-β-*D*-mannopyrano)-2-oxazoline* (**6**). — This compound, obtained as described elsewhere[10] in 98,5% yield, had m.p. 169–170°, $[\alpha]_D^{20}$ +9 ±2° (*c* 1.0, pyridine); R_F 0.12, R_{GlcNAc} 1.79 (*B*); ν_{max} 1674 cm^{-1} (C=N).

*2-Methyl-4,5-(4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyrano)-2-oxazoline* (**2**). — A suspension of **6** (1.05 g) and zinc chloride (0.4 g) in benzaldehyde (50 ml) was stirred magnetically overnight at room temperature in an evacuated device kept dry with phosphoric anhydride. The mixture was poured into a mixture of saturated, aqueous potassium hydrogen carbonate and ice, stirred for 30 min, and, after filtration, extracted with chloroform (5 × 50 ml). The extract was washed with water, dried ($MgSO_4$), and concentrated, and benzaldehyde was removed from the residue at 70°/1 mm. A solution of the resulting syrup in chloroform was washed with aqueous potassium hydrogen carbonate, dried, and evaporated. The residue was treated with light petroleum, and the resulting solid was recrystallised from methanol–ether–light petroleum to give the title compound (0.98 g, 68.5%), m.p. 174–175°, $[\alpha]_D^{20}$ −22,6° (*c* 0.71, chloroform), R_F 0.31 (*B*), ν_{max} 1680 cm^{-1} (C=N) (Found: C, 61.73; H, 5.95; N, 4.77. $C_{15}H_{17}NO_5$ calc.: C, 61.85; H, 5.88; N, 4.88%).

*2-Methyl-4,5-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyrano)-2-oxazoline* (**7**). — To a stirred suspension of **2** (0.5 g) and barium oxide (0.5 g) in methyl sulphoxide (20 ml), benzyl chloride (10 ml) was added dropwise. After 3 h, more barium oxide (0.5 g) was added, and the mixture was stirred overnight. The precipitate was filtered off, and washed with chloroform, and the combined filtrates (containing benzyl chloride) were evaporated. The residue was twice recrystallised from methanol to give **7** as colorless needles (0.42 g, 64%), m.p. 169°, $[\alpha]_D^{20}$ −18 ±2° (*c* 1.5, chloroform); R_F 0.47 (*B*), 0.58 (*C*); ν_{max} 1678 cm^{-1} (C=N) (Found: C, 69,03; H, 5.94; N, 3.38. $C_{22}H_{23}NO_5$ calc.: C, 69.29; H, 6.03; N, 3.67%).

*2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*D*-mannopyranose* (**3**). — To a solution of **7** (0.3 g) in 80% aqueous acetone (5 ml), 0.1N hydrochloric acid (1 ml) was added. After 3 h at 20°, the mixture was evaporated by co-distillation with ethanol, and the residue was recrystallised from ethanol to give **3** (0.30 g, 96%),

m.p. (dec.) *ca.* 120°, $[\alpha]_D^{20}$ −20° (*c* 1.0, methanol), R_F 0.43 (*C*), ν_{max} 1540 and 1645 cm^{-1} (CONH) (Found: C, 65.98; H, 5.97; N, 3.26. $C_{22}H_{25}NO_6$ calc.: C, 66.17; H, 6.26; N, 3.50%).

6-O-Benzyl-7,9-O-benzylidene-8-O-methyl-N-acetylneuraminic acid γ-lactone (**5**). — A suspension of **3** (0.2 g) and the potassium salt of di-*tert*-butyl oxaloacetate[5] (0.15 g) in 1:1 methanol–*p*-dioxane (10 ml) was stirred for 5 days at room temperature, filtered, and, after deionisation with Amberlite IR-120 (H^+), evaporated to dryness. The residue was repeatedly washed with ether, dissolved in *p*-dioxane (10 ml) and heated for 15–20 min at 100° until no more gas was evolved. The solution was decolorised with charcoal and evaporated to dryness to give lactone **4** (0.115 g, 48%), R_F 0.53 (*C*).

Lactone **4** (0.10 g) and freshly prepared silver oxide (3 g) were stirred in dry methanol (3 ml) and methyl iodide (10 ml) for 3 h at 40–45° and then overnight at 20°. After filtration, the precipitate was washed with boiling chloroform (3 × 40 ml), and the combined filtrates were evaporated to dryness. The residue was treated as described above to give the title compound (0.11 g, 92%) as a colourless solid, $[\alpha]_D^{20}$ −4.4° (*c* 1.3, methanol), R_F 0.80 (*D*) (Found: C, 66.01; H, 6.24; N, 2.81. $C_{28}H_{33}NO_8$ calc.: C, 65.74; H, 6.50; N, 2.74%).

N-*Acetylneuraminic acid 8-methyl ether* (**1**). — Lactone **5** (0.10 g) was hydrogenated over 5% palladium-on-carbon (3 g) in methanol (10 ml) for 2 days at room temperature. The catalyst was filtered off and washed with methanol (3 × 50 ml), and the combined filtrates were evaporated. A solution of the residue in 50% aqueous methanol was brought to pH 9 with N sodium hydroxide, kept overnight at 0°, deionized with Amberlite IR-120 (H^+), decolourized with charcoal, and freeze-dried to give **1** (0.05 g, 83%) as a colourless powder, $[\alpha]_D^{20}$ −28° (*c* 1.0, water), R_{NANA} 0.27 (*D*), 0.39 (*E*) (Found: C, 44.16; H, 6.57; N, 4.12; OCH_3, 9.41. $C_{12}H_{21}NO_9$ calc.: C, 44.58; H, 6.55; N, 4.33; OCH_3, 9.60%).

Determination of inhibition activity of NANA and 8-O-methyl-NANA (**1**). — The enzymic hydrolysis of *p*-nitrophenyl *N*-acetyl-α-D-neuraminoside was carried out as described elsewhere[9] in 0.1M acetate buffer (pH 5.6) containing 0.9% of sodium chloride and 0.1% of calcium chloride with 0.3 mg/ml of neuraminidase (from *Vibrio cholerae*, N. V. Philips–Duphar, Holland) at substrate concentrations 0.58, 1.17, 1.75, 2.30, and 3.50 mM at 37°. The substrate was incubated in the presence of NANA or **1** (1.10 mM). *p*-Nitrophenol was determined[11] in incubated and control mixtures by spectrophotometry at 400 nm. The initial rate of hydrolysis was determined by extrapolation to zero time[12]. A plot[13] of $1/V$ against $1/S$ gave, in all the experiments, equal V_{max}-values (53 μmoles/min) and K_i-values of 3.6×10^{-3}M and 4.9×10^{-3}M for **1** and NANA, respectively.

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SYNTHESIS AND REACTIONS OF GLYCOSYL METHYL- AND BENZYL-XANTHATES: A FACILE SYNTHESIS OF 1-THIOGLYCOSIDES

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ABSTRACT

Treatment of acetylated glycosyl bromides of D-galactose, D-glucuronic acid, and D-xylose with potassium methyl- and benzyl-xanthates in alcohol affords the corresponding glycosyl methyl- and benzyl-xanthates respectively. The same condensation in acetone yields the corresponding 1-thioglycosides and diglycosyl sulfides instead of glycosyl xanthates. The glycosyl xanthates, on heating with sodium iodide in acetone, give the corresponding 1-thioglycosides in high yield. The mechanism of decomposition of glycosyl xanthates is discussed in detail.

INTRODUCTION

As one of the important intermediates for the preparation of acetylated derivative of 1-thio sugars, glycosyl xanthates have attracted the attention of many investigators[1]. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl ethylxanthate (**2c**) was first synthesized by Schneider and coworkers[2] by treating 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**) with potassium ethylxanthate in hot ethanol to give **2c**, which was then transformed into sodium 1-thio-D-glucose with sodium methoxide. Ethylxanthates of D-mannose[3], D-glucuronic acid[4], 2-amino-2-deoxy-D-glucose[5], D-arabinose,[6] D-ribose[3], and D-xylose[7] have been synthesized by the same procedure. In the previous paper[8] it was shown that the course of the reaction of **1** with potassium methyl- and benzyl-xanthate could be altered by changing the temperature and the medium. Condensation of **1** with potassium methyl- or benzyl-xanthates in alcohol at room temperature gave the expected 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl methyl- or benzyl-xanthates (**2a** and **2b**), respectively, in good yield. On the other hand, bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) sulfide (**4**) and the corresponding methyl or benzyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**3a** and **3b**) were obtained when **1** was treated with these potassium xanthates in boiling acetone. With other potassium xanthates, such as potassium ethylxanthate, **1** reacted normally in acetone and gave **2c** under the same conditions. Recently, an analogous reaction of aryl and alkyl xanthates with tertiary amines, to give mixed sulfides, was reported[9].

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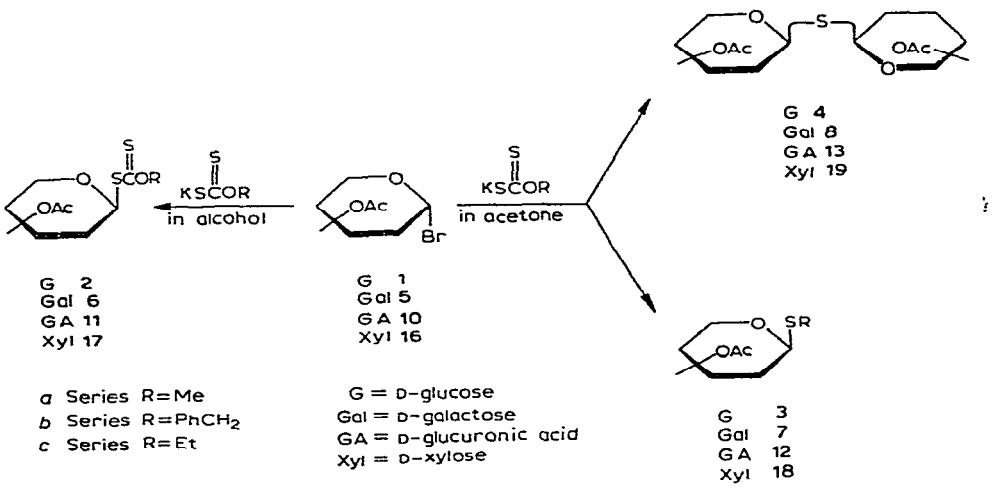
In this paper, the reactions of acetylated glycosyl bromides of D-galactose, D-glucuronic acid, and D-xylose with potassium methyl- and benzyl-xanthates are described and the mechanism of decomposition of glycosyl xanthates is also discussed.

RESULTS AND DISCUSSION

As expected, the unusual reactions noted with D-glucose were also observed when acetylated glycosyl bromides of the sugars described above were condensed in boiling acetone with both potassium methyl- or benzyl-xanthate. In alcohol at low temperature the corresponding glycosyl xanthates were obtained. The D-galactose derivatives showed some differences in reactivity from the D-glucose derivatives. Treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) with potassium ethylxanthate in boiling acetone gave bis(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) sulfide (**8**) in small proportion (10%), together with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl ethylxanthate (**6c**). Condensation of **5** with potassium methylxanthate in alcohol at room temperature gave the acetylated galactosyl methylxanthate (**6a**) in good yield, whereas in boiling acetone **8** and methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**7a**) were obtained in 48 and 40% yield, respectively. Treatment of **5** with potassium benzylxanthate in alcohol under the same conditions gave the acetylated galactosyl benzylxanthate **6b** in 75% yield, and in acetone **8** and the acetylated benzyl 1-thio-galactoside **7b** were obtained in 43 and 27% yield, respectively. Compound **8** was also synthesized, by the route used by Schneider and Wrede[10] for the D-glucose derivative, from **5** and potassium sulfide; the product was deacetylated to give crystalline bis(β-D-galactopyranosyl) sulfide (**9**). The reactivities of the D-glucuronate and D-xylose derivatives resembled those of the D-glucose derivatives. Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[11] (**10**) was treated with potassium methyl- or benzyl-xanthate in acetone under reflux to give, bis[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate] sulfide (**13**) in 40% yield, and the corresponding acetylated methyl or benzyl thioglucosiduronate (**12a** and **12b**) in 10 and 12% yield, respectively. Similarly, methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl methylxanthate)uronate (**11a**) was obtained by condensation of **10** with potassium methylxanthate in hot methanol. Compound **13** was also prepared from **10** and potassium sulfide, and was deacetylated with methanolic ammonia to give bis(β-D-glucopyranosyluronamide) sulfide (**14**), which was subsequently desulfurized with Raney nickel to give 1,5-anhydro-L-gulonamide (**15**). The reaction of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (**16**) with potassium methyl- or benzylxanthate in hot acetone also gave bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl) sulfide (**19**) and the corresponding acetylated 1-thioxylosides (**18a** and **18b**), in fairly good yield. When the reaction was conducted in ethanol 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl benzylxanthate (**17b**) was obtained. The corresponding methylxanthate of D-xylose failed to crystallize. Compound **19** was also prepared from **16** and was deacetylated to give bis(β-D-xylopyranosyl) sulfide (**20**). The 1-thioglycosides described above were prepared from the sodium salts of the corresponding 1-thio sugars and methyl

iodide or benzyl chloride, after the method of Černý[12] or from the acetylated glycosyl bromides and potassium mercaptides. The results described suggest that this "anomalous" reaction is common to potassium methyl- and benzyl-xanthates. It is already known that **1** and 1.2 molar equivalents of potassium methylxanthate, on heating in acetone for 5 min, give the thioglucoside **3a** and sulfide **4** in 25 and 56% yield,



respectively[8]. Prior addition of 0.5 molar equivalent of **2a** to this reaction system increased the yields of **3a** and **4** to 48 and 75%, respectively (based on the amount of **1**). This result indicated that almost half of the added **2a** reacted with **1** to give **4** and the rest was degraded concurrently to **3a**. Consequently, it is evident that reaction of an acetylated glycosyl bromide with potassium methyl- or benzyl-xanthate in acetone gives initially the glycosyl xanthate which, in part, reactes with more bromide to give the diglycosyl sulfide on one hand, and in part decomposes to the 1-thioglycoside. As described above, **2a** liberated carbonyl sulfide and gave **3a** in high yield when it was heated for a short period in acetone in the presence of potassium methylxanthate. Since potassium methylxanthate was mostly recovered after the reaction, it seemed that this salt was not involved directly in the reaction, but played a role in elevating the dynamic polarization of the solvent. Therefore an acetone-soluble salt (sodium iodide) was examined in place of potassium methylxanthate, and the expected result was observed; heating **2a** in acetone containing 10% of sodium iodide gave **3a** in high yield. The transformation of **2a** into **3a** by refluxing in acetone alone was very slow (~20 h). Pyridine and nitrobenzene were also found to be effective for this purpose, but in benzene (a non-polar solvent) no change of **2a** was observed after heating for 30 h. These facts showed that a polar solvent is essential for elimination of carbonyl sulfide from the glycosyl xanthate.

It was also observed that ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside was obtained in 70–80% yield by refluxing the corresponding xanthate for 8 h in acetone containing 10% of sodium iodide. The rate of conversion of the ethyl-xanthate was very slow compared with that of the methyl- or benzyl-xanthates.

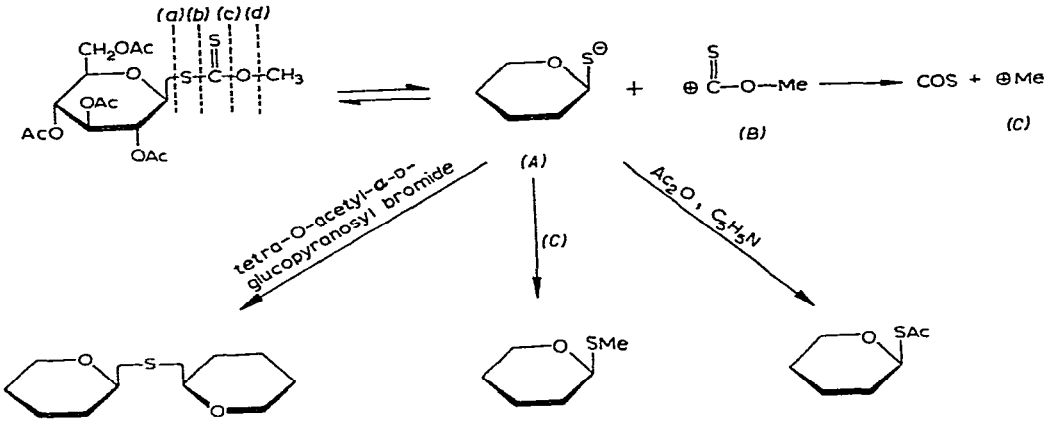
The pyrolysis of these glycosyl xanthates was also examined. On heating, **2a** melted and evolved gas at 140–150°. The evolution of gas ceased after 1 h. The residue was crystallized from alcohol and was identified as the 1-thioglycoside **3a**. The same pyrolytic transformation gave satisfactory yields with the methyl- and benzyl-xanthates of the other sugars, but the glycosyl ethylxanthates failed to give 1-thioglycosides on pyrolysis. The pyrolysis of glycosyl xanthates was thus considered to be analogous to the removal of carbonyl sulfide in polar solvents, that is, the molten glycosyl xanthate itself plays the role of a polar solvent. The effects of solvents, temperature, reaction periods, and yield, on the transformation of the acetylated D-glucosyl methylxanthate **2a** into the methyl 1-thio-D-glucoside **3a** are summarized in Table I.

TABLE I

TRANSFORMATION OF 2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSYL METHYLXANTHATE INTO METHYL 2,3,4,6-TETRA-*O*-ACETYL-1-THIO-β-D-GLUCOPYRANOSIDE

Solvent	*Temperature (degrees)*	*Reaction time (min)*	*Yield (%)*
KS(C=S)OR in acetone	56	5	89
NaI in acetone	56	5	90
Pyridine	90–95	5–10	87
Nitrobenzene	90–95	5–10	90
Acetone	56	1200	93
Thermal decomposition	140–150	60	93
Benzene	80	1800	0

As one part of the investigation for elucidating the mechanism of elimination of carbonyl sulfide, a cross-over reaction between two kinds of glycosyl xanthates was effected. Equimolar quantities of the acetylated D-glucosyl methylxanthate **2a** and the acetylated D-xylosyl benzylxanthate **17b**, dissolved in acetone containing 10% of sodium iodide, were kept at room temperature. One min later, two additional glycosyl xanthates, the acetylated D-xylosyl methylxanthate **17a** and the acetylated D-glucosyl benzylxanthate **2b**, were detected by t.l.c., together with **2a** and **17b**. Thereafter, the reaction mixture was refluxed for 5 min and the four thioglycosides could be isolated almost in equimolar proportions. In this experiment, the observation that interchange of methyl and benzyl groups takes place upon dissolving these xanthates in a polar solvent indicates some kind of dissociation of the glycosyl xanthates. Various possibilities are shown below. Dissociation at (*a*) can be ruled out because the anomeric configuration remained unchanged throughout the reaction. The possibility of dissociation at (*c*) was discounted because D-glucosyl methyl- or benzyl-xanthate can be recrystallized unchanged from alcohol. The possibility of dissociation at (*d*) was excluded because rearrangement of the glycosyl xanthate into a glycosyl dithiocarbonate was not observed upon dissolution in a polar solvent. Accordingly, the conversion of the glycosyl xanthates is proposed to be as follows: for example, **2a** is



dissociated at (*b*) into the 1-thio-D-glucose anion (*A*) and methoxythiocarbonyl cation (*B*), and the latter is irreversibly decomposed further into carbonyl sulfide and a methyl cation (*C*). The reaction of *A* with *C* would give **3a**, with **1** would give **4**, and with *B* would lead to the interchange of xanthate groups, as demonstrated in the cross-over reaction. The fact that **2a** gave 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose[2] in high yield when **2a** was dissolved in a mixture of pyridine and acetic anhydride supports this mechanism. The difference in reactivity between glycosyl methyl- and benzyl-xanthates and glycosyl ethyl- and the other alkyl-xanthates can be explained by the concept of the electron-releasing effect of the *O*-alkyl group[13] in the xanthate part. The greater ability of methyl and benzyl groups to release electrons, compared with that of ethyl and other alkyl groups, is well known[14].

A number of preparative method for 1-thioglycosides have been described: (*a*) direct acid-catalyzed glycosidation, (*b*) preparation from dithioacetals, (*c*) synthesis from glycosyl halides and salts of thiols, and (*d*) alkylation of 1-thioaldoses. For the synthesis of 1-thio-β-D-glycopyranosides on a preparative scale the methods are essentially restricted to (*c*) and (*d*). As summarized in Table II, the process reported here *via* glycosyl xanthates gives excellent yields. The isolation of intermediary glycosyl xanthates is not required (see experimental section).

EXPERIMENTAL

Evaporations were normally conducted under diminished pressure below 40°. Melting points are uncorrected. The solvent systems used for t.l.c., on Silica Gel GF (E. Merck, Darmstadt, Germany), were 4:1 benzene–ethyl ether (v/v) and 3:1 ethyl ether–light petroleum, unless otherwise stated. The detection of glycosyl xanthates was effected with u.v. light (short, 2537 Å) and other compounds were indicated by spraying the plates with aqueous sulfuric acid (10% v/v) and heating the plates at 140°. New compounds had i.r. and u.v. spectra consistent with their assigned structures. Satisfactory elemental analysis were obtained for compounds prepared in this work and previously reported in the literature.

TABLE II

1-THIOGLYCOSIDES FROM XANTHATES BY ELIMINATION OF CARBONYL SULFIDE

Sugar	*R*	*Reaction time (min)*	*Yield (%)*
D-Glucose	methyl	5	93
	ethyl	600	84
	benzyl	5	86
D-Galactose	methyl	5	73
	ethyl	300	70
	benzyl	5	86
D-Xylose	methyl	5	61
	ethyl	480	90
	benzyl	5	90
D-Glucuronic acid	methyl	5	70
	ethyl	180	69
	benzyl	5	69

Bis(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl) sulfide (**8**) *and methyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside* (**7a**). — 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) (3.0 g) was added in one portion to acetone (15 ml) containing potassium methylxanthate (1.5 g) and the solution was refluxed for 3 min. After cooling, the mixture was poured into water (100 ml), and then extracted with chloroform (30 ml). The organic layer was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was triturated with ethyl ether (20 ml) and the insoluble, crude **8** was collected by filtration. Recrystallization of **8** from methanol gave 1.2 g (48%) of pure material, m.p. 201–202°, $[\alpha]_D^{20} -35.5°$ (*c* 1.0, chloroform). Compound **8** was also obtained by treatment of **1** with potassium sulfide according to the direction given by Schneider[10], m.p. and mixed m.p. 201–202°.

Anal. Calc. for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.62. Found: C, 48.22; H, 5.63; S, 4.80.

To the mother liquor from **8**, petroleum ether was added until a slight turbidity persisted. After refrigeration overnight analytically pure **7a** was obtained after two recrystallizations from ethanol; yield, 1.0 g (40%), m.p. 110–111°, $[\alpha]_D^{20} +4.3°$ (*c* 1, chloroform). Lit.[12], m.p. 110–111°, $[\alpha]_D +4°$ (*c* 2, chloroform).

Benzyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (**7b**) *and* **8**. — Compounds **7b** and **8** were obtained by treatment of **5** (3.0 g) with potassium benzylxanthate (1.8 g) in boiling acetone according to the procedure described above, yield, 1.1 g (43%) of **8** and 0.9 g (27%) of **7b**. Compound **7b** had m.p. 97°, $[\alpha]_D^{20} -78.0°$ (*c* 1.0, chloroform). Lit.[12], m.p. 97–99°, $[\alpha]_D -76°$ (*c* 2, chloroform).

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl ethylxanthate (**6c**). — Compound **5** (3.0 g) and potassium ethylxanthate (1.1 g) were dissolved in ethanol (30 ml) and the solution was stirred for 1 h at room temperature. The mixture was poured into water (100 ml), and kept at 0°. The precipitated deposits were filtered off and recrystallized from ethanol; yield, 2.8 g (83%), m.p. 79–80°, $[\alpha]_D^{20}+51.6°$ (*c* 1.0, chloroform).

Anal. Calc. for $C_{17}H_{24}O_{10}S_2$: C, 45.09; H, 5.35; S, 14.17. Found: C, 45.16; H, 5.37; S, 13.89.

Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (**7c**), **6c**, *and* **8**. — Componds **7c** and **8** were obtained by treatment of **5** (3.0 g) with potassium ethylxanthate (1.3 g) in boiling acetone (30 ml) for 1 h as described above; yield, 0.3 g (10%) of **8** and 1.3 g (50%) of **7c**. Compound **7c** had m.p. 75°, $[\alpha]_D^{20}-8.0°$ (c.1.0, chloroform). Lit.[12], m.p. 75°, $[\alpha]_D^{20}-7.8°$ (*c* 2.5, chloroform).

Reaction for only 3 min according to the procedure described above gave **6c** and **8**; yield, 0.3 g (10%) of **8** and 1.6 g (50%) of **6c**.

Bis(β-D-galactopyranosyl) sulfide (**9**). — A solution of **8** (2.0 g) in methanol (30 ml) saturated with ammonia at 0° was kept overnight at room temperature. The solution was evaporated to a crystalline residue. Recrystallization from ethanol–water gave colorless crystals; yield, 1.0 g (95%), m.p. 229°, $[\alpha]_D^{20}-40.0°$ (*c* 1.0, water).

Anal. Calc. for $C_{12}H_{22}O_{10}S$: C, 40.22; H, 6.19; S, 8.95. Found: C, 40.46; H, 6.42; S, 8.78.

Bis[methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate] sulfide (**13**) *and methyl (methyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside)uronate* (**12a**). — Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (**10**) (4.0 g) was added portionwise with stirring to a solution of potassium methylxanthate (2.0 g) in hot acetone (50 ml). The solution was refluxed for 3 min and treated according to the p ocedure described for the D-galactose derivatives. Recrystallization of **13** from hot methanol gave needles; yield, 1.5 g (45%), m.p. 236° (dec.), $[\alpha]_D^{20}-54.1°$ (*c* 1.0, chloroform). Alternatively, **13** was prepared by treatment of potassium sulfide with **10** according to the direction of Schneider[10]. The m.p. and mixed m.p. were 236–237° (dec.).

Anal. Calc. for $C_{26}H_{34}O_{18}S$: C, 46.85; H, 5.14; S, 4.81. Found: C, 46.93; H, 5.09; S, 4.76.

A solution of **12a** in ethyl ether was evaporated under vaccum to a thick syrup and the residue was dissolved in a small amount of ethanol. Compound **12a** crystallized gradually on cooling; yield, 0.3 g (10%), m.p. 118–119°, $[\alpha]_D^{20}-27.7°$ (*c* 1.2, chloroform).

Anal. Calc. for $C_{14}H_{20}O_9S$: C, 46.15; H, 5.53; S. 8.80. Found: C, 46.19; H, 5.67; S, 9.02.

Methyl (benzyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside)uronate (**12b**) *and* **13**. — Compound **10** (3.7 g) and potassium benzylxanthate (2.5 g) were treated in hot acetone (50 ml) according to the procedure described above; yield, 1.1 g (35%) of **13**, and 0.45 g (12%) of **12b**, m.p. 117°, $[\alpha]_D^{20}-106°$ (*c* 1.2, chloroform).

Compound **12b** was also prepared by treatment of **10** with potassium α-toluene-

thioxide in hot methanol according to the direction of Helferich[15]; m.p. and mixed m.p. 117–118°.

Anal. Calc. for $C_{20}H_{24}O_9S$: C, 54.54; H, 5.49; S, 7.28. Found: C, 54.64; H, 5.57; S, 7.26.

Bis(β-D-glucopyranosyluronamide) sulfide (**14**). — A solution of **13** (1 g) in methanol (30 ml) was saturated with ammonia at 0° and kept for 2 h at room temperature. The solution was concentrated under vaccum to give white crystals. Recrystallization from ethanol–water gave pure material; yield, 0.5 g (86%), m.p. 297.5–299°, $[\alpha]_D^{20} -127°$ (*c* 1.0, water).

Anal. Calc. for $C_{12}H_{20}O_{10}N_2S$: C, 37.50; H, 5.25; N, 7.29; S, 8.34. Found: C, 37.53; H, 5.35; N, 7.15; S. 8.34.

1,5-Anhydro-L-gulonamide (**15**). — Compound **14** (0.9 g) was suspended in water (50 ml) and refluxed for 2 h with freshly prepared Raney nickel (9.0 g). The mixture was filtered and the filtrate was evaporated. Crystallization from methanol gave pure **15**; yield, 0.6 g (68%), m.p. 173–174°, $[\alpha]_D^{20} +14.7°$ (*c* 1.0, water).

Anal. Calc. for $C_6H_{11}O_5N$: C, 40.68; H, 6.26; N, 7.91. Found: C, 40.80; H, 6.06; N, 8.17.

Bis(2,3,4-tri-O-acetyl-β-D-xylopyranosyl) sulfide (**19**) *and methyl 2,3,4-tri-O-acetyl-1-thio-β-D-xylopyranoside* (**18a**). — 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (**16**) (5.0 g) and potassium methylxanthate (2.5 g) were heated in acetone (50 ml) according to the procedure described for the D-galactose derivatives; yield, 1.9 g (47%) of **19** (obtained after recrystallization from methanol), m.p. 163–164°, $[\alpha]_D^{20} -144°$ (*c* 1.0, chloroform). Treatment of potassium sulfide with **16** according to the direction of Schneider[10] also gave **19**; m.p. and mixed m.p. 163–164°.

Evaporation of the mother liquor gave crystalline residues from which pure **18a** (1.8 g, 45%) was obtained after recrystallization from ethanol; m.p. 88–89°, $[\alpha]_D^{20} -74.0°$ (*c* 1.0, chloroform). Lit.[16], m.p. 90°, $[\alpha]_D -71.5°$ (*c* 1.2, chloroform).

Benzyl 2,3,4-tri-O-acetyl-1-thio-β-D-xylopyranoside (**18b**) *and* **19**. — **16** (5.0 g) and potassium benzylxanthate (3.0 g) were heated in acetone (25 ml) according to the procedure described for the D-galactose derivatives; yield, 1.7 g (42%) of **19** and 1.5 g (30%) of **18b**, m.p. 88°, $[\alpha]_D^{20} -125.1°$ (*c* 3.48, chloroform). Lit.[16], m.p. 88°, $[\alpha]_D -124.9°$ (*c* 0.54, chloroform).

Bis(β-D-xylopyranosyl) sulfide (**20**). — A solution of **19** (1.0 g) in methanol (20 ml) was saturated with ammonia at 0°, kept overnight at room temperature, and evaporated. The residual white powder was recrystallized from ethanol–water to give colorless needles; yield, 0.54 g (99%), m.p. 217–218°, $[\alpha]_D^{20} -103.0°$ (*c* 1.0, water).

Anal. Calc. for $C_{10}H_{18}O_8S$: C, 40.26; H, 6.08; S, 10.75. Found: C, 40.22; H, 5.85; S, 10.74.

Reaction of 2,3,4,6-treta-O-acetyl-α-D-glucopyranosyl bromide (**1**) *and potassium methylxanthate*[8]. — Potassium methylxanthate (0.80 g) was added to **1** (2.00 g) in warm acetone (10 ml) and the solution was refluxed for 5 min. After cooling, the mixture was triturated with ethyl ether (10 ml) and the insoluble material was filtered off. Recrystallization from methanol gave pure bis(2,3,4,6-tetra-*O*-acetyl-β-D-

glucopyranosyl) sulfide (**4**); yield, 0.90 g (56%), m.p. 175–176°, $[\alpha]_D^{20}$ −35.5° (*c* 1.0, chloroform).

The filtrate was concentrated to a syrup and the residue was dissolved in small amount of ethanol. Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**3a**) crystallized gradually on cooling; yield, 0.45 g (25%), m.p. 96°, $[\alpha]_D^{20}$ −12.0° (*c* 1.0, chloroform).

Reaction of **1**, *potassium methylxanthate, and 2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl methylxanthate* (**2a**). — Potassium methylxanthate (0.80 g) was added to a mixture of **1** (2.00 g) and **2a** (1.00 g) in warm acetone (10 ml), and the solution was refluxed for 5 min. Separation by the foregoing procedure gave **4** and **3a**; yield, 1.25 g (74% based on **1**) of **4** and 0.88 g (48% based on **1**) of **3a**.

Reaction of **2a** *and potassium methylxanthate.* — A solution of **2a** (1.00 g) and potassium methylxanthate in acetone (10 ml) was refluxed for 5 min and the mixture was poured into water (100 ml). The precipitate was filtered off and recrystallized from ethanol to give pure **3a**; yield, 0.77 g (89%).

Formation of **3a** *from* **2a** *by elimination of carbonyl sulfide.* — *A.* Compound **2a** (1.00 g) was heated in a flask on an oil bath for 1 h at 140–150° until the evolution of carbonyl sulfide ceased. The residue was dissolved in a small amount of ethanol and **3a** crystallized on cooling; yield, 0.80 g (93%).

B. A solution of **2a** (1.00 g) in pyridine (2 ml) was heated for 5 min on a steam bath. Carbonyl sulfide was evolved. The solution was poured into water (50 ml) and kept in a refrigerator. The precipitated **3a** was collected and recrystallized from warm ethanol; yield, 0.75 g (87%).

C. A solution of **2a** (1.00 g) in nitrobenzene (1 ml) was heated for 5 min on a steam bath. T.l.c. of the solution revealed only one spot (of **3a**). The nitrobenzene was removed by coevaporation with water, to give a crystalline mass. Recrystallization from ethanol gave pure **3a**; yield, 0.78 g (90%).

D. A solution of **2a** (1.00 g) in acetone (5 ml) was refluxed for 20 h, and then evaporated. The residue was recrystallized from ethanol to give pure **3a**; yield, 0.80 g (93%).

Benzyl 2,3,4,6-tetra-O-*acetyl-1-thio-β*-D-*glucopyranoside* (**3b**) *from 2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl benzylxanthate* (**2b**) *by elimination of carbonyl sulfide.* — A solution of **2b** (2.0 g) in pyridine (4 ml) was heated for 5 min on a steam bath. The mixture was poured into water (100 ml) and the resulting crystalline precipitate was recrystallized from ethanol to give 1.5 g (86%) of **3b**, m.p. 100–101°, $[\alpha]_D^{20}$ −92.8° (*c* 1.0, chloroform).

Ethyl 2,3,4,6-tetra-O-*acetyl-1-thio-β*-D-*glucopyranoside* (**3c**) *from 2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl ethylxanthate* (**2c**) *by elimination of carbonyl sulfide.* — A solution of **2c** (5.0 g) (see ref. 2) in acetone (30 ml) containing 10% of sodium iodide was refluxed for 5 h. The mixture was poured into water (100 ml). A crystalline precipitate formed gradually at room temperature. Recrystallization from ethanol gave 3.8 g (84%) of pure **3c**; m.p. 82°, $[\alpha]_D^{20}$ −25.5° (*c* 1.0, chloroform). Lit.[17], m.p. 81–82°, $[\alpha]_D$ −24.4° (*c* 1.16, chloroform).

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl benzylxanthate (**6b**). — A solution of **5** (3.0 g) and potassium benzylxanthate (1.8 g) in ethanol (30 ml) was stirred for 1 h at room temperature. The mixture was then poured into water (100 ml), and kept in a refrigerator. The precipitate formed was recrystallized from ethanol to give 2.8 g (75%) of **6b**; m.p. 106–107°, $[\alpha]_D^{20}$ +32.0° (*c* 1.5, chloroform).

Anal. Calc. for $C_{22}H_{26}O_{10}S_2$: C, 51.35; H, 5.09; S, 12.46. Found: C, 51.35; H, 5.14; S, 12.40.

Formation of **7b** *from* **6b** *by elimination of carbonyl sulfide.* — A solution of **6b** (2.0 g) in pyridine (2 ml) was heated for 5 min on a steam bath. The solution was poured into water (100 ml) and kept in a refrigerator. The precipitates formed was recrystallized from ethanol to give pure **7b**; yield, 1.5 g (86%), m.p. 97°, $[\alpha]_D^{20}$ −78.0 (*c* 1.0, chloroform).

Formation of **7c** *from* **6c** *by elimination of carbonyl sulfide.* — A solution of **6c** (1.0 g) in acetone (10 ml) containing sodium iodide (0.5 g) was refluxed for 5 h. The mixture was poured into water (100 ml), and then extracted with chloroform (20 ml). The solvent was evaporated and the residue were recrystallized from ethanol to give 0.6 g (70%) of **7c**; m.p. 75°, $[\alpha]_D^{20}$ −8.0° (*c* 1.0, chloroform).

Formation of **7a** *from* **5**. — Compound **5** (3.0 g) was added to a stirred solution of potassium methylxanthate (1.1 g) in ethanol (30 ml). After for 1 h at room temperature the solvent was evaporated. The residue was dissolved in acetone (30 ml) containing potassium methylxanthate (1.0 g) and the solution was refluxed for 5 min. After cooling, the mixture was poured into water (100 ml) and then extracted with chloroform (30 ml). The extract was evaporated and the residue was recrystallized from ethanol to give 2.0 g (73%) of **7a**, m.p. 110–111°, $[\alpha]_D^{20}$ +4.0° (*c* 1.0, chloroform).

2,3,4-Tri-O-acetyl-β-D-xylopyranosyl benzylxanthate (**17b**). — Compound **16** (2.0 g) and potassium benzylxanthate (1.4 g) were treated with ethanol (30 ml) at room temperature according to the procedure described for the D-galactose derivative **6b**; yield, 1.8 g (69%), m.p. 88°, $[\alpha]_D^{20}$ +19.2° (*c* 1.3, chloroform).

Anal. Calc. for $C_{19}H_{22}O_8S_2$: C, 51.51; H, 5.01; S, 14.47. Found: C, 51.65; H, 4.92; S, 14.02.

Compound **18b** *from* **17b** *by elimination of carbonyl sulfide.* — Compound **17b** (1.0 g) was treated with pyridine (2 ml) according to the elimination procedure already described; yield, 0.8 g (90%), m.p. 88° $[\alpha]_D^{20}$ −92.5° (*c* 1.0, chloroform).

Ethyl 2,3,4-tri-O-acetyl-1-thio-β-D-xylopyranoside (**18c**) *from 2,3,4-tri-O-acetyl-β-D-xylopyranosyl ethylxanthate* (**17c**) *by elimination of carbonyl sulfide.* — Compound **17c** (2.0 g), which was prepared by the procedure adapted from Gehrke[7], was treated with acetone (20 ml) containing 10% of sodium iodide for 5 h; yield, 1.2 g (90%), m.p. 100–101°, $[\alpha]_D^{20}$ −77.0° (*c* 1.0, chloroform). Lit.[7], m.p. 101°, $[\alpha]_D$ −83.5°.

Compound **18a** *from* **16**. — Compound **16** (5.0 g) and potassium methylxanthate (2.2 g) were allowed to react in ethanol (50 ml) for 1 h at room temperature with subsequent treatment with acetone according to the procedure described for the D-

galactose derivative **7a**; yield, 2.75 g (61%), m.p. 88–89°, $[\alpha]_D^{20}$ −74.0° (*c* 1.0, chloroform).

Methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl methylxanthate)uronate (**11a**). — A solution of **10** (2.0 g) in hot methanol (15 ml) was mixed at 50° with methanol (15 ml) containing potassium methylxanthate (0.8 g). The mixture was refluxed for 2 min and then poured into water (100 ml), and kept in a refrigerator. The resultant precipitate was filtered off and recrystallized from ethanol to give 1.3 g (61%) of **11a**; m.p. 116.5°, $[\alpha]_D^{20}$ +47.8° (*c* 1.0, chloroform).

Anal. Calc. for $C_{15}H_{20}O_{10}S_2$: C, 42.45; H, 4.75; S, 15.11. Found: C, 42.27; H, 4.81; S, 15.17.

Methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl benzylxanthate)uronate (**11b**). — Compound **10** (2.0 g) and potassium benzylxanthate (1.2 g) were treated in methanol (30 ml) at 50° according to the procedure described above; yield, 1.1 g (44%), m.p. 128–129.5°, $[\alpha]_D^{20}$ +37.0° (*c* 1.0, chloroform).

Anal. Calc. for $C_{21}H_{24}O_{10}S_2$: C, 50.39; H, 4.83; S, 12.81. Found: C, 50.65; H. 4.89; S, 13.06.

Compound **12a** *from* **11a** *by elimination of carbonyl sulfide.* — A solution of **11a** (1.0 g) in pyridine (4 ml) was heated for 10 min at 60°. The product was poured into water (30 ml) and kept in a refrigerator. The precipitate was filtered off and recrystallized from ethanol to give pure **12a**; yield, 0.60 g (70%), m.p. 118–119°, $[\alpha]_D^{20}$ −27.7° (*c* 1.2, chloroform).

Compound **12b** *from* **11b** *by elimination of carbonyl sulfide.* — Compound **11b** (1.0 g) was treated in pyridine (4 ml) according to the procedure described above; yield, 0.55 g (69%), m.p. 117°, $[\alpha]_D^{20}$ −106.0° (*c* 1.0, chloroform).

Methyl (ethyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside)uronate (**12c**) *from methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl ethylxanthate)uronate* (**11c**) *by elimination of carbonyl sulfide.* — Compound **11c** (3.0 g, see ref. 4) was treated with acetone (30 ml) containing 10% of sodium iodide, for 3 h to give 1.8 g (69%) of **12c**, m.p. 107–108°, $[\alpha]_D^{20}$ −37.3° (*c*. 10, chloroform). Lit.[15], m.p. 106–107°, $[\alpha]_D^{26}$ −38.0° (*c* 1, chloroform).

Cross-over experiment in the carbonyl sulfide elimination. — A mixture of 438 mg (1 mmole) of **2a** and 442 mg (1 mmole) of **17b** was dissolved in acetone (10 ml). Sodium iodide (1 g) was added and the reaction mixture was examined by t.l.c. Four glycosyl xanthates were detected by u.v. light and were identified as **2a**, **2b**, **17a**, and **17b**. The mixture was refluxed for 5 min, poured into water (100 ml), and then extracted with chloroform (20 ml × 2). The extract was washed with water, dried over sodium sulfate, and evaporated to 10 ml. Examination of this solution by t.l.c. showed the presence of four components detected with 10% sulfuric acid, which were identified as **18b** (R_F 0.55), **18a** (R_F 0.50), **3b** (R_F 0.45), and **3a** (R_F 0.40), respectively. The zones of **18b** and **3b**, which have a benzyl moiety, were detected in u.v. light as dark areas on a fluorescent background in preparative t.l.c. (3:7 ethyl ether–benzene). Pure samples of **18b** (58 mg), **18a** (52 mg), **3b** (69 mg), and **3a** (57 mg)

were obtained by preparative separation of a portion of the mixture. The molar ratio of these 1-thioglycopyranosides was **18b:18a:3b:3a** = 1.00:1.15:1.05:1.00.

1-Thio-2,3,4,6-tetra-O-*acetyl-1*-S-*acetyl-β*-D-*glucopyranose from* **2a**. — Compound **2b** (1.0 g) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) and the mixture was heated for 5 min on a steam bath. The mixture was poured into water (50 ml) and kept in a refrigerator. The resultant precipitate was recrystallized from ethanol to give 0.78 g (84%) of pure material, m.p. 121°, $[\alpha]_D^{20}$ +9.5° (*c* 1.0, chloroform). Lit.[2], m.p. 120°, $[\alpha]_D^{22}$ +10.17° (*c* 2.95, chloroform).

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UNSATURATED NITRO SUGARS. PREPARATION OF 4-*O*-ACETYL-1,2-DIDEOXY-3,5-*O*-ETHYLIDENE-1-NITRO-D-*erythro*-PENT-1-ENITOL*

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ABSTRACT

The title compound was prepared from D-glucose in 10–25% overall yield in a 5-step sequence. Condensation of nitromethane with 2,4-*O*-ethylidene-D-erythrose according to the Sowden–Fischer procedure gave an approximately equimolar mixture of 1-deoxy-3,5-*O*-ethylidene-1-nitro-D-ribitol and the corresponding D-arabinitol derivative, which were separated by chromatography on silica gel. The mixture of nitrodiols was acetylated to the corresponding diacetates, which were not separated. Attempts were unsuccessful at dehydroacetoxylation of the latter by refluxing in benzene solution with sodium hydrogen carbonate or carbonate bases. However, dehydroacetoxylation occurred during chromatography of the diacetates on silica gel, and this proved to be a satisfactory method for preparing 4-*O*-acetyl-1,2-dideoxy-3,5-*O*-ethylidene-1-nitro-D-*erythro*-pent-1-enitol. New data are given for several previously known intermediate compounds prepared in this study.

RESULTS AND DISCUSSION

4-*O*-Acetyl-1,2-dideoxy-3,5-*O*-ethylidene-1-nitro-D-*erythro*-pent-1-enitol (**7**), a possible precursor in the synthesis of a naturally occurring glucosinolate currently under investigation in this laboratory, was prepared by conversion of 2,4-*O*-ethylidene-D-erythrose (**3b**) into the homologous deoxy nitro alditol *via* the Sowden–Fischer[1] aldehyde/nitromethane condensation reaction and the sequence outlined[2–7] in Scheme I. Yield data are summarized in Table I.

D-Glucose was converted into 4,6-*O*-ethylidene-D-glucose (**2a**) according to published procedures[4,6,7] by treatment with paraldehyde§. The crystalline triol **2a** was characterized as its amorphous triacetate, 1,2,3-tri-*O*-acetyl-4,6-*O*-ethylidene-D-glucopyranose (**2b**), which was obtained as a mixture of α- and β-isomers[8].

*A preliminary account of part of this work has been given: K. D. Carlson, C. R. Smith, Jr., and I. A. Wolff, *Abstracts Papers Amer. Chem. Soc. Meeting*, 156 (1968) Carb 18.
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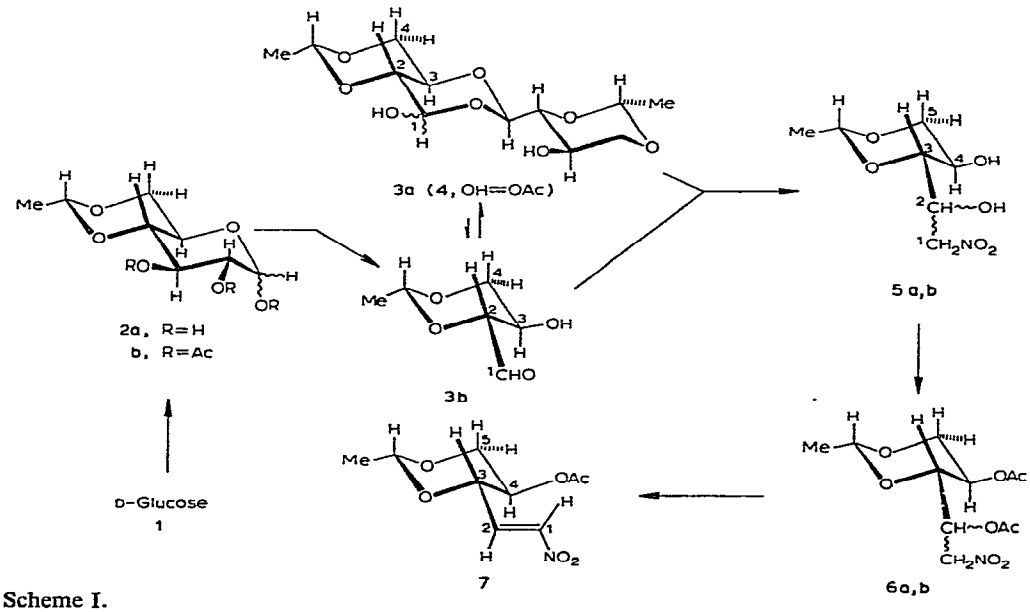
‡This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.
§We assume that the methyl group has an equatorial orientation since n.m.r. and t.l.c. data indicate the formation of one isomer. This assumption is consistent with the conditions of preparation and thermodynamic considerations regarding isomer stability.



Scheme I.

TABLE I

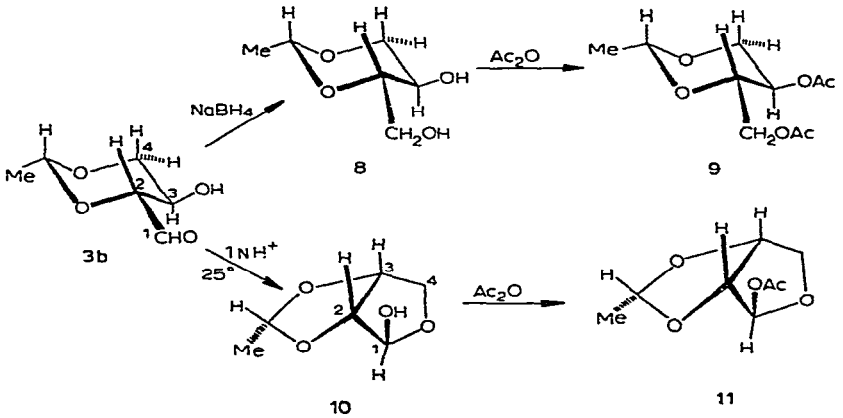
SUMMARY OF YIELD DATA

Compound	*Yield range* (%)	*Overall yield* (%)	
		Low	*High*
2a	60–80	60	80
3a	87	52	70
5	68–77	35	54
6	72–87[a]	25	47
7	43–55	11	26

[a]Crude yield; all others are purified yields.

Periodate oxidation[4,7] of **2a** gave 2,4-*O*-ethylidene-D-erythrose (**3**) as an amorphous solid, which was homogeneous by t.l.c. The lack of significant aldehyde carbonyl absorption in the i.r. spectrum indicated that the compound exists preferentially as the dimeric cyclic acetal, bis(2,4-*O*-ethylidene-D-erythrose) 1,1′:1,3′-cyclic acetal (**3a**), as first reported by Schaffer[5,9]. Direct evidence for this dimeric structure was obtained from the 60-MHz n.m.r. spectrum (chloroform-*d*) of **3a**, which showed signals of two magnetically different *O*-ethylidene methyl groups as a pair of doublets (J_{H,CH_3} 5 Hz) at τ 8.65 and 8.67, in the ratio 1:1. The dimer diacetate, 1,3′-di-*O*-acetyl-[bis(2,4-*O*-ethylidene-D-erythrose)-1,1′:1′,3-cyclic acetal)] (**4**), obtained in 55% yield upon acetylation of **3a**, similarly exhibits two overlapping three-proton doublets of equal intensity at τ 8.65 and 8.69 for the *O*-ethylidene methyl groups. Three-proton singlets of equal intensity were observed at τ 7.86 and 8.00 for the two acetate methyl groups.

2,4-*O*-Ethylidene-D-erythrose (**3**) was further characterized (Scheme II) by: (*1*) quantitative sodium borohydride reduction to 1,3-*O*-ethylidene-L-erythritol* [(**8**), refs. 6 and 10] and (*2*) acid-catalyzed rearrangement to 2,3-*O*-ethylidene-β-D-



Scheme II.

erythrofuranose[7,11] (**10**). Acetylation gave the corresponding acetates **9** (ref. 10) and **11** (ref. 11). Although **10** is probably the product of kinetic control[9], *endo*-2-methyl-1,3-dioxolans reportedly are more stable than their *exo*-isomers[12,13]. Our assignment of the *endo*-methyl configuration to **10** and **11** is discussed in the following paper[8].

Treatment of an alcoholic solution of 2,4-*O*-ethylidene-D-erythrose (**3**) and nitromethane with sodium alkoxide at 10° gave a bromine-red syrup in 68–77% yield, which according to t.l.c. analysis consisted of an approximately equimolar mixture of 1-deoxy-3,5-*O*-ethylidene-1-nitro-D-ribitol and 1-deoxy-3,5-*O*-ethylidene-1-nitro-D-arabinitol (**5a, b**). The mixture of nitrodiols was freed of several unidentified contaminants, and each was obtained pure in small amounts, by repeated chromatography on silica gel**. The diols are normally pale-yellow syrups, which may or may not crystal-

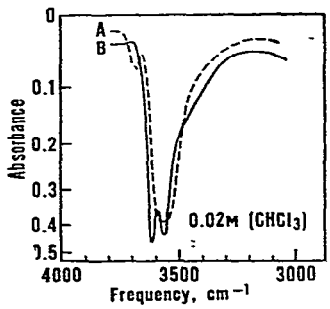
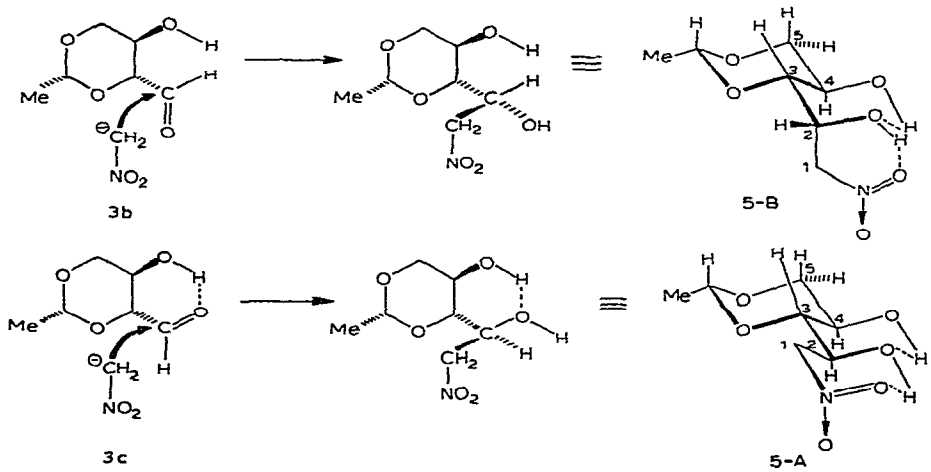


Fig. 1. I.r. spectrum of nitrodiol 5A and 5B showing hydroxyl absorptions in dilute chloroform solution.

*Or, 2,4-*O*-ethylidene-D-erythritol.

**This is a tedious separation. The corresponding *O*-benzylidene derivatives reportedly[3] are easily separated and crystallized owing to a marked difference in solubilities in chloroform and ether, and they are thus preferred if sizeable quantities of pure isomers are desired.

lize and which gradually darken in color (yellow) at room temperature. The two isomers are easily distinguished by their characteristic i.r. absorptions between 10 and 12 μm and by the nature of their OH absorptions in dilute solution (Fig. 1). Isomer **A**, which we believe to be the ribitol derivative, shows only broad, bonded-OH absorption (3589–3533 cm^{-1}). Isomer **B**, assigned the arabinitol structure, exhibits both free (3617 cm^{-1}) and bonded (3567 cm^{-1}) OH bands (Fig. 1). These patterns do not change on dilution (<20 mM) and are therefore indicative of intramolecular association as illustrated by **5-A** and **5-B** (Scheme III). The "axial" nitromethyl group at C-2 of the six-membered ring formed by the hydrogen bond between the C-4



Scheme III.

hydroxyl proton and the C-2 hydroxyl oxygen atom of **5-B** should destabilize this completely hydrogen-bonded form relative to **5-A** (equatorial nitromethyl group), and therefore the arabinitol derivative should show more free OH absorption and/or more intermolecular hydrogen bonding, as is observed for isomer **B** (3300–3500 cm^{-1}, Fig. 1). Greater chromatographic mobility of the ribitol derivative could be expected if internal hydrogen bonding is more important[14] as in **5-A**. In accord with this expectation, isomer **A** is eluted first from a silica-gel column and has a higher t.l.c. mobility (R_F) than isomer **B**.

Cram's rule[15,16] of steric control of asymmetric induction predicts that the arabinitol derivative **5-B** should be formed preponderantly from the reaction of nitromethane with 2,4-*O*-ethylidene-D-erythrose. However, polarization of the carbonyl group by weak hydroxyl–carbonyl bonding[17] (Scheme III) should enhance the proportion of **3c** relative to **3b** at equilibrium, lower the transition-state energy for addition of the nucleophile to the carbonyl group, and thus raise the proportion of **5-A** in the product mixture. Indeed, the proportion of ribitol isomer (**5-A**) in the product mixture is about 60%, based on weights of the two isomers obtained from chromatographic separations.

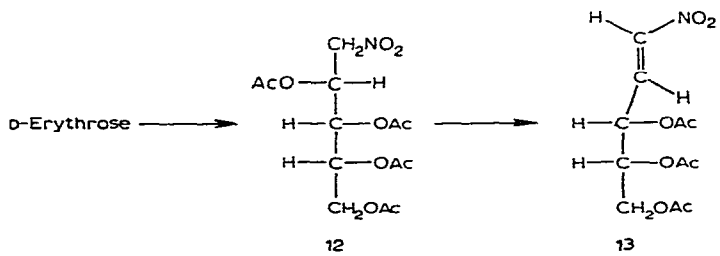
Acid-catalyzed acetylation of the nitrodiol mixture **5** apparently gives a mixture

of the corresponding nitro diacetates, 2,4-di-*O*-acetyl-1-deoxy-3,5-*O*-ethylidene-1-nitro-D-ribitol and 2,4-di-*O*-acetyl-1-deoxy-3,5-*O*-ethylidene-1-nitro-D-arabinitol (**6a, b**). T.l.c. analysis of the product mixtures generally gave ill-defined chromatograms, the nature of which apparently depends upon, and is quite sensitive to, the activity of the silica gel. Crystallization of material from the product mixture could not be induced. Neither of the diacetates has been isolated pure, but their presence in mixtures with the nitroolefin **7** was detected by n.m.r. analysis.

Attempts to prepare the nitroolefin **7** by refluxing the nitro acetates (**6**) in benzene in the presence of potassium or sodium hydrogen carbonate[2,3] failed to give satisfactory results. Other bases and solvent systems also failed in that the olefin was not formed at a rate significantly greater than its destruction by subsequent side-reactions. Addition of nucleophiles and anions to nitroolefins is well known[18–25], and Nielsen and Archibald[26] showed that 1,3-dinitroalkanes, resulting from Michael addition of a nitroalkane α-carbanion to the corresponding nitroolefin, may yield cyclic nitronic esters. These products could account for the unidentified, highly polar materials and for the difficulties encountered in our work.

The nitroolefin **7** was obtained in satisfactory yields (43–64%) from the crude nitro diacetate mixture (**6**) by repeated chromatography on silica gel. Others[24c,25] have reported dehydroacetoxylation of β-acetoxynitroalkanes on silica gel. The overall yield of **7** from D-glucose is 11–26%. Best results were obtained when intermediates in the 5-step synthesis were freed of contaminating byproducts before proceeding to the next step.

For comparative purposes, and as a possible alternative route to the nitropentene skeleton, 3,4,5-tri-*O*-acetyl-1,2-dideoxy-1-nitro-D-*erythro*-pent-1-enitol[2,3] (**13**) was prepared from D-erythrose *via* 2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-nitro-D-arabinitol (**12**) (Scheme IV).



12 13

Scheme IV.

Pertinent bands in the i.r. spectra of the two olefins (**7** and **13**) are at 3.3, 6.01, and 10.42 μm (*trans*-disubstituted double bond) and 6.55 and 7.40 μm (olefinic nitro group)[19,25,27]. N.m.r. spectra of the two olefins are in accord with the assigned structures[8].

The dehydroacetoxylations of both **6** and **12** appear to be stereospecific, since no evidence was found for formation of the *cis* nitroolefins. However, it is not known whether a *cis* olefin would isomerize on the catalyst surface (sodium carbonate, silica gel) to the *trans* olefin in these heterogeneous reactions. Consequently, we do not know

whether *cis* or *trans* elimination occurs. The formation of either olefin (**7** and **13**) can be monitored by the appearance of the bands at 6.55 and 10.42 μm ($CH{=}CH{-}NO_2$) and by the disappearance of the band at 6.45 μm ($-CH_2NO_2$).

Significantly, the acyclic olefin **13** is not formed on silica gel chromatography of tetraacetate **12**. However, stirring a solution of **12** in benzene with alumina resulted in partial dehydroacetoxylation (apparent equilibrium mixture of **12** and **13** by i.r. was approximately 30:70).

The reasons for the marked differences in the dehydroacetoxylation reactions of the nitro acetates **6** and **12** are not obvious. A study of the reaction of **12** with potassium carbonate in refluxing benzene was carried out by observing the 6.45 μm ($-CH_2NO_2$ band of **12**) and 6.55 μm ($=CHNO_2$ band of **13**) bands in the i.r. spectrum with time. Fig. 2 shows that the nitroolefin concentration reaches a maximum and then begins to decrease as the olefin is consumed by side reactions.

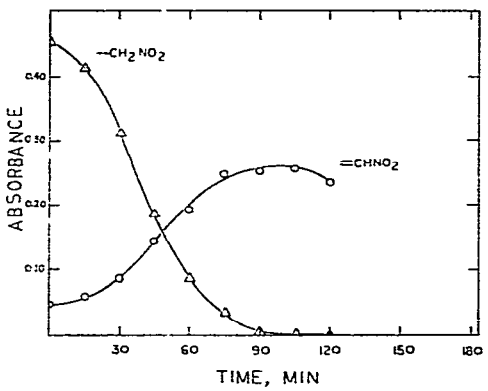


Fig. 2. Reaction of **12** with potassium carbonate (70±1°). Plot of absorbance of saturated ($A_{CH_2NO_2}$ and olefinic nitro groups ($A_{=CHNO_2}$) as a function of time (**12**→**13**).

Sodium carbonate was less reactive than potassium carbonate in promoting dehydroacetoxylation of **12**, although the former catalyst gave better (76 *vs.* 62%) yields of olefin **13** than did potassium carbonate. In our hands sodium hydrogen carbonate was much less effective than either carbonate base. Plots of $\log A_t/A_0$ *vs.* time were not linear and the reaction rate slowly increased with time. The rate increase may result either from an increase in effective surface area or surface activity of the carbonate base in this heterogeneous system, or from catalysis by accumulating sodium acetate produced in the reaction. Although a similar formal study was not made of the formation of **7** from **6**, it was apparent from our data that side reactions consumed **7** at a rate comparable to its formation in the presence of carbonate base.

EXPERIMENTAL

General. — Melting points are uncorrected. T.l.c. was performed on Silica Gel G (0.25 mm) with 73:18:9 chloroform–ethyl ether–methanol for the free sugars (solvent *A*) and 88:12 carbon tetrachloride–methanol for the acetates (solvent

B). The plates were developed by spraying with aqueous sulfuric–chromic acid solution, followed by charring at 150°. I.r. spectra were recorded on Perkin–Elmer* spectrophotometers (Models 137 and 337) on dilute solutions (10%) of the compounds in carbon tetrachloride or chloroform, or as thin films between sodium chloride windows. Optical rotations were measured with a visual polarimater (Franz Schmidt and Haensch, Berlin, Germany) equipped with a 1.0-dm tube. N.m.r. spectra (60 and 100 MHz) were recorded on Varian Associates spectrometers (Models A-60 and HA-100) with the compounds in solutions of carbon tetrachloride, chloroform-*d*, deuterium oxide, or benzene-d_6. Chemical shifts were measured relative to internal tetramethylsilane (τ 10.00). All peaks assigned to hydroxyl groups were confirmed by deuterium oxide exchange. Satisfactory analyses were obtained for all previously reported compounds.

4,6-O-Ethylidene-D-glucose (**2a**). — The procedure followed is that of Barker and MacDonald[6]. From a mixture of 1 ml of concentrated sulfuric acid, 135 ml (~1 mole) of paraldehyde, and 180 g (1 mole) of D-glucose (dextrose, Difco Laboratories) was obtained 126.6 g (62%) of material. Recrystallization from absolute ethanol gave 90.9 g (45%) of **2a**, m.p. 180–182° (lit[6]. m.p. 179–181°), $[\alpha]_D^{25}$ −2.0° (*c* 2, water); R_F 0.15 (solvent *A*); n.m.r. data: τ 8.70 (3-proton doublet, ethylidene methyl, *J* 5 Hz). A second experiment with reactant quantities and reaction time doubled gave 165 g of **2a** (77%).

1,2,3-Tri-O-acetyl-4,6-O-ethylidene-D-glucopyranose (**2b**). — A mixture of 10 ml of pyridine, 2.07 g (10 mmoles) of **2a**, and 6 ml of acetic anhydride was heated for 7 h on a steam bath. Excess acetic anhydride was decomposed by stirring the reaction mixture with ice-cold 10% hydrochloric acid. The product was taken up in ether (3 × 25-ml portions). The combined ether extract was washed, dried, and evaporated to leave 2.09 g (63%) of syrup, which was dissolved in the minimum amount of ether–pentane and kept for 4 days at −10°. Deposition of a syrup occurred, the solvent was decanted, and the syrup was dried for 24 h at 57° (0.3 mm). The resultant glass was scraped with a spatula to give 1.39 g (42%) of amorphous **2b**, m.p. 35–36°, $[\alpha]_D^{25}$ +66.0° (*c* 2, chloroform); R_F 0.75 (solvent *B*); n.m.r. data[8] ($CDCl_3$): τ 8.69 (3-proton doublet, *J* 5 Hz, ethylidene methyl), τ 7.87, 7.96, 8.02 (3-proton singlets, OAc), τ 3.79 (1-proton doublet, $J_{1,2}$ 3.8 Hz, H-1 of α-anomer, major product), τ 4.30 ($J_{1,2}$ 7.5 Hz, H-1 of β-anomer, minor component).

Anal. Calc. for $C_{14}H_{20}O_9$: C, 50.60; H, 6.07. Found: C, 50.90; H, 6.39.

2,4-O-Ethylidene-D-erythrose (**3**). — 4,6-*O*-Ethylidene-D-glucose (**2a**) was oxidized according to the procedure of Rappoport and Hassid[4] by the addition of an aqueous slurry of 203 g (0.95 mole) of sodium metaperiodate to 100 g (0.48 mole) of **2a** and 80.0 g (0.95 mole) of sodium hydrogen carbonate in 600 ml of water. The amorphous product (**3**, 60.5 g, 87%) failed to show significant aldehyde carbonyl absorption in the i.r., showed a single spot on t.l.c., and had a wide melting-range,

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m.p. 65–80° (for the L enantiomorph lit.[4] m.p. 73–79°); $[\alpha]_D^{24}$ −37.8° (*c* 3.12, water) (lit.[6] $[\alpha]_D^{20}$ −36.8°); R_F 0.36 (solvent *A*).

1,3'-Di-O-acetyl-[bis(2,4-O-ethylidene-D-erythrose)]-1,1':1',3-cyclic acetal (**4**). — A mixture of 3.19 g (21.8 mmoles) of **3**, 20 ml of pyridine, and 10 ml of acetic anhydride was stirred for 45 min at 25° and then heated for 1 h on a steam bath. The deep reddish brown mixture was cooled, diluted 5:1 with water, and then extracted with 3 × 25-ml portions of ether. The combined ether extract was washed thoroughly with water and then dried. Evaporation of the ether left 2.24 g (55%) of a syrup, which was dissolved in equal volumes (15 ml) of ether and pentane. Slow evaporation of solvent under a stream of nitrogen resulted in the formation of a few crystals, whereupon the solution was stored for 16 h at 10°. The crystals were removed by filtration (624 mg) and recrystallized from ether–pentane to give 515 mg of **4**, m.p. 176.0–176.5° (lit.[5] m.p. 171.5–172°), $[\alpha]_D^{25}$ −47.4° (*c* 0.8, chloroform); R_F 0.8 (solvent *B*); n.m.r. data ($CDCl_3$): τ 8.65, 8.69 (3-proton doublets, *J* 5 Hz, ethylidene methyl-groups), τ 5.22-5.32 (overlapping 1-proton quartets, ethylidene methine protons), τ 7.86, 8.00 (3-proton singlets, OAc), τ 4.1 (1-proton multiplet, H-1, $J_{1,2} \simeq 7.5$ Hz). Calc. mol. wt., 376. Found, 361 (vapor-pressure osmometer).

1,3-O-Ethylidene-L-erythritol (**8**). — Ten g (68.5 mmoles) of **3** in 50 ml of absolute ethanol was quantitatively reduced with 1.30 g (34.3 mmoles) of sodium borohydride as described by Barker and MacDonald[6]. Product **8** was recrystallized from chloroform–hexane, m.p. 98.5–100.5° (lit.[6] m.p. 99.5–100.5°), $[\alpha]_D^{25}$ −51.1° (*c* 0.67, water); R_F 0.36 (solvent *A*); n.m.r. data ($CDCl_3$): τ 8.70 (3-proton doublet, *J* 5 Hz, ethylidene methyl), τ 5.33 (1-proton quartet, *J* 5 Hz, ethylidene methine), τ 7.69 (1-proton triplet, $J_{1-OH,H-1}$ 6 Hz, C-1 hydroxyl), τ 7.35 (1-proton doublet, $J_{3-OH,H-3}$ 5 Hz, C-3 hydroxyl).

2,4-Di-O-acetyl-1,3-O-ethylidene-L-erythritol (**9**). — Acetylation of **8** was performed in the usual manner with 840 mg (5.67 mmoles) of **8**, 5 ml of acetic anhydride, and 10 ml of pyridine. The sweet-smelling, oily product was distilled at 0.3 torr (100–110°) to give 400 mg of colorless oil (**8**); R_F 0.68 (carbon tetrachloride–methanol, 95:5); λ_{max}^{film} 5.72 μm (C=O), 8.0–8.2 μm (C–O–, acetate), and 8.6–9.5 μm (C–O–, acetal); n.m.r. data[8] (CCl_4): τ 8.74 (3-proton doublet, ethylidene methyl), τ 5.41 (1-proton quartet, ethylidene methine), τ 8.00, 8.03 (3-proton singlets, OAc).

2,3-O-Ethylidene-β-D-erythrofuranose (**10**). — The procedure followed is that of Van Cleve and Rist[11]. From 4.63 g (31.7 mmoles) of **3** in 75 ml of M sulfuric acid (closed flask, 4 days at 25°) was isolated 1.21 g (27%) of **10**, recrystallized from heptane–benzene as long, white needles, m.p. 64–66° (lit.[11] m.p. 66–67°); $[\alpha]_D^{25}$ −66.4° (*c* 2, water); R_F 0.66 (solvent *A*); n.m.r. data are given elsewhere[8].

1-O-Acetyl-2,3-O-ethylidene-β-D-erythrofuranose (**11**). — A mixture of 111.5 mg of **10**, 0.5 ml of pyridine, and 2 ml of acetic anhydride was set aside for 5 days at 25°, after which most of the pyridine and excess acetic anhydride were evaporated at 50° in a stream of nitrogen. The residue (106 mg, 74%) was chromatographed on 5 g of Adsorbosil (silica gel, Applied Science Laboratories) with ether as the eluant; 84 mg of **11** was recovered in fraction 1, m.p. 53.5–54.5° (lit.[11] m.p. 58°); R_F 0.66 (solvent *B*);

$\lambda_{max}^{CCl_4}$ 5.72 μm (C=O), 8.0–8.3 μm (C–O–, acetate), 8.7–9.0 μm (C–O–, acetal), 9.55, 9.82, 10.0, 10.4, 10.95, and 11.45 μm (fingerprint region); n.m.r. data are given elsewhere[8].

*1-Deoxy-3,5-O-ethylidene-1-nitro-*D*-ribitol* (**5a**) *and* -D-*arabinitol* (**5b**). — The following conditions were explored for reaction of nitromethane with 2,4-*O*-ethylidene-D-erythrose[2–4,6] (**3**). In six experiments the molar ratio of reactants [sodium methoxide (or ethoxide)/2,4-*O*-ethylidene-D-erythrose/nitromethane] was varied within the limits 1.0–1.9/1.0–1.2/1.2–10.8, respectively, and reaction times were varied from 5 to 24 h. No significant differences in crude yield (68–77%) or product quality were observed. The reaction product was always a deep bromine-red oil, which failed to crystallize, and consisted of two major components (**5a, b**) and several minor components (by t.l.c.).

In a representative experiment a mixture of 10.04 g (69 mmoles) of **3**, 4.75 g (78 mmoles) of nitromethane, and 50 ml of absolute ethanol was added to a cooled (5–10°) and stirred mixture of 1.50 g of sodium in 100 ml of absolute ethanol (65.3 mmoles of sodium ethoxide). The initially formed, white salt dissolved in 20 min and was replaced by a pale-yellow salt and a red solution during 5 h at 25°. The pH was adjusted to 6 with glacial acetic acid, and the solution was evaporated under diminished pressure (20 mm, 45°) to give 11.0 g (77%) of a deep-red oil. T.l.c. analysis showed two major components (**5a, b**) and five minor contaminants. The product was chromatographed on 50 g of methanol-washed Adsorbosil (chloroform–carbon tetrachloride and chloroform elution) to give 8.63 g (60%) of nitrodiol mixture, R_F 0.48, 0.59 (solvent *A*). Repeated chromatography of small quantities of this material on 10 g of Adsorbosil and elution with 1% methanol–chloroform separated the isomers; thus 425 mg of mixture gave 227 mg isomer **A** and 163 mg isomer **B**; 631 mg of mixture gave 387 mg of **A** and 221 mg of **B**.

Isomer **A** (*ca.* 60%): m.p. 82.5–84°, $[\alpha]_D^{25}$ −44.5° (*c* 1.9, chloroform); R_F 0.59 (solvent *A*); λ_{max}^{film} 2.88 (OH), 6.45 and 7.25 (C–NO_2), 10.30, 10.65, 11.10, 11.43, 11.90 μm; n.m.r. data (acetone-d_6): τ 8.79 (3-proton doublet, ethylidene methyl), τ 5.33 (1-proton quartet, ethylidene methine).

Isomer **B** (*ca.* 40%): m.p. 86–88°, $[\alpha]_D^{25}$ −6.3° (*c* 1.3, chloroform); R_F 0.48 (solvent *B*): λ_{max}^{film} 2.88 (OH), 6.45 and 7.25 (C–NO_2), 10.32, 10.65, 11.10, 11.45, 11.90 μm; n.m.r. data (acetone-d_6): τ 8.78 (3-proton doublet, ethylidene methyl), τ 5.33 (1-proton quartet, ethylidene methine).

Anal. Calc. for $C_7H_{13}NO_6$: C, 40.58; H, 6.32; N, 6.76. Found, for isomer **A**: C, 40.60; H, 7.03; N, 6.50. For isomer **B**: C, 40.79; H, 6.43; N, 6.83.

*2,4-*Di-O-*acetyl-1-deoxy-3,5-*O-*ethylidene-1-nitro-*D*-ribitol* (**6a**) *and* -D-*arabinitol* (**6b**). — To 1.23 g (5.92 mmoles) of a mixture of **5a, b** in a few ml of ether was added 5 ml of acetic anhydride containing a drop of concentrated sulfuric acid. The ether was removed in a stream of nitrogen while the mixture was warmed on a steam bath (1 h). Heating and evaporation with portions of benzene were continued for 2 h. Excess acetic anhydride was decomposed by addition of methanol to the cooled reaction mixture. Acetic acid was neutralized with solid sodium hydrogen carbonate, after

which the benzene solution was filtered and then evaporated to give 1.49 g (72%) of a reddish syrup; λ_{max}^{film} 5.72 (C=O), 6.45 μm (C–NO_2), no OH or olefinic absorptions; t.l.c., ill-defined spots and streaking; n.m.r. data (CCl_4): τ 8.69 (3-proton doublet, ethylidene methyl), τ 4.78 (1-proton quartet, ethylidene methine), τ 7.89 and 7.97 (3-proton singlets, OAc).

4-O-Acetyl-1,2-dideoxy-3,5-O-ethylidene-1-nitro-D-erythro-*pent-1-enitol* (**7**). — The acetylation product (1.12 g) from above was chromatographed in two parts on Adsorbosil with mixtures of ether–pentane. Fractions were collected and monitored by t.l.c. and i.r. analysis. Fractions rich in nitroolefin (408 mg, 40%) were combined and rechromatographed. Fractions containing only nitroolefin were combined to give 276 mg (27%) of a pale-yellow syrup, which subsequently crystallized, **7**, m.p. 63–65°, $[\alpha]_D^{25}$ −92.8° (*c* 3, benzene); R_F 0.69 (solvent *B*); λ_{max}^{film} 5.72 (C=O), 3.3, 6.01, 10.42 (–CH=CH–, *trans*), 6.55 μm (=CH–NO_2); n.m.r. data are reported elsewhere[8].

Anal. Calc. for $C_9H_{13}NO_6$: C, 46.75; H, 5.67; N, 6.06. Found: C, 47.16; H, 5.70; N, 6.03.

2,3,4,5-Tetra-O-*acetyl-1-deoxy-1-nitro*-D-*arabinitol* (**12**). — Without isolation of intermediates, this nitro tetraacetate was prepared from **3** in poor yield[3]. From 20.05 g (138 mmoles) of 2,4-*O*-ethylidene-D-erythrose (**3**) was obtained 23.88 g (50%) of red, syrupy tetraacetate (**12** and, presumably, the ribitol isomer). The syrup was dissolved in chloroform and hexane was added to turbidity. The solution deposited 1.19 g (2.5% from **3**) of crystals during several weeks at −10°. Two crystallizations from chloroform–hexane gave 516 mg of 2,3,4,5-tetraacetoxy-1-nitropentane, m.p. 111–112° (lit.[3] m.p. 64–65°, ribitol isomer; m.p. 123–125°, arabinitol isomer **12**), presumably a mixture of isomers. The tetraacetate was chromatographed on Adsorbosil and recovery was nearly quantitative, demonstrating that dehydroacetoxylation of **12** does not occur on the column. Crystalline material isolated from the chromatographic fractions had m.p. of 123–127°, 125–128°, and 132–135° and positive specific rotations, data which are consistent with the arabinitol derivative[3] **12**. No material was isolated having m.p. <110°. The i.r., n.m.r., and t.l.c. data on the different fractions were identical; $\lambda_{max}^{CHCl_3}$ 5.72 (C=O), 6.45 μm (C–NO_2); $[\alpha]_D^{25}$ +16.5° (*c* 1.4, benzene); R_F 0.65 (solvent *B*); n.m.r. data are reported elsewhere[8].

3,4,5-Tri-O-*acetyl-1,2-dideoxy-1-nitro*-D-erythro-*pent-1-enitol* (**13**). — Dehydroacetoxylation of **12** was carried out with three bases.

(*a*) *Sodium hydrogen carbonate.* A mixture of 45 mg of **12**, 100 mg of sodium hydrogen carbonate, and 4 ml of benzene was refluxed for 2.5 h. I.r. analysis of samples removed at 45-min intervals showed essentially no reaction.

(*b*) *Potassium carbonate.* To the reaction mixture from (*a*) above was added 75 mg of potassium carbonate. The reaction mixture was refluxed for 4 h. I.r. monitoring of the 6.45 μm (C–NO_2) and 6.55 μm (=C–NO_2) bands at intervals indicated that dehydroacetoxylation was complete in 2 h. Decomposition of the olefin occurred at longer reflux times. The yield of **13** after chromatography of the product on Adsorbosil was 23 mg (62%).

(*c*) *Sodium carbonate.* A mixture of 100.4 mg of **12** in 2 ml benzene and 107 mg

of sodium carbonate was refluxed for 7 h before dehydroacetoxylation was complete; yield 77 mg (93%, crude), after chromatography on Adsorbosil, 63 mg (76%).

The reactions with the two carbonate bases were repeated under identical conditions for comparison of relative reactivities of base. The tetraacetate (**12**, 75.0 mg) was dissolved in each of two 5.00-ml portions of benzene (43 mM in **12**). The solutions were heated to 70.0±1.0°, and 119.0 mg of potassium carbonate was added to one solution while 91.0 mg of sodium carbonate was added to the second. Aliquots (0.25 ml) were removed at 15-min (K_2CO_3) and 60-min (Na_2CO_3) intervals, and the absorbances of the –C=O ($A_{C=O}$), $-CH_2NO_2$ ($A_{CH_2NO_2}$), and $=CHNO_2$ ($A_{=CHNO_2}$) groups were determined and normalized ($A_{C=O} = 1.000$). The results were plotted as in Fig. 2 and as log A_t/A_0 versus time, where A_t/A_0 is the ratio of $A_{CH_2NO_2}$ at times t and zero. The latter plots were not linear, the rate of olefin formation increasing with time.

Physical data for **13**: m.p. 61–62° (lit.[3] 63–65°), $[\alpha]_D^{25}$ +3.4° (*c* 1.8, benzene); R_F 0.67 (solvent *B*); λ_{max}^{film} 5.72 (C=O), 3.3, 6.01, 10.42 (–C=C–, *trans*), and 6.55 μm (=CNO_2); n.m.r. data are given elsewhere[8].

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N.M.R. SPECTRA AND BENZENE-INDUCED SOLVENT SHIFTS. ACETYLATED CARBOHYDRATES SUBSTITUTED WITH 1,3-*O*-ETHYLIDENE OR NITRO GROUPS*

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ABSTRACT

The n.m.r. spectra of six acetylated sugar derivatives are presented and compared on the basis of structural similarities due to the presence of *O*-ethylidene, nitro, or nitroolefinic groups. Definite patterns are observed in the chemical shifts of related protons on 1,3-dioxane rings. Chemical shifs of methyl and methine protons of *O*-ethylidene groups are quite insensitive to structural variations. Benzene-induced shifts relative to carbon tetrachloride or chloroform-*d* are presented. Shielding values (Δ) associated with *O*-acetyl and *O*-ethylidene groups are discussed, and a structure is postulated for the benzene–2-methyl-1,3-dioxane collision complex.

INTRODUCTION

In the preceding paper[1] we described the preparation of 4-*O*-acetyl-1,2-dideoxy-3,5-*O*-ethylidene-1-nitro-D-*erythro*-pent-1-enitol (**3**). Compounds prepared in the study (**1–6**) were characterized by analysis of their n.m.r. spectra. Limited information is available on the n.m.r. spectra of *O*-ethylidene derivatives[2–4] (2-methyl-1,3-dioxanes or 2-methyl-1,3-dioxolans) although the literature on other *O*-alkylidene or *O*-arylidene derivatives is rather extensive, if not detailed[5–13]. Similarly, n.m.r. data for nitroalkanes and nitroalkenes are limited to a few reports[14–17].

In this paper we comment upon some of the features of the n.m.r. spectra of **1–6**, and relate our observations on the chemical shift displacements induced by benzene-d_6 relative to carbon tetrachloride or chloroform-*d*. To identify the compounds, unequivocally, names are given in the text based on accepted carbohydrate nomenclature. However, it should be noted that numbering of the *O*-ethylidene compounds, **1–4** (Scheme 1), is not based on carbohydrate rules. This numbering system that is used in the accompanying tables, figures, and discussion, permits ready

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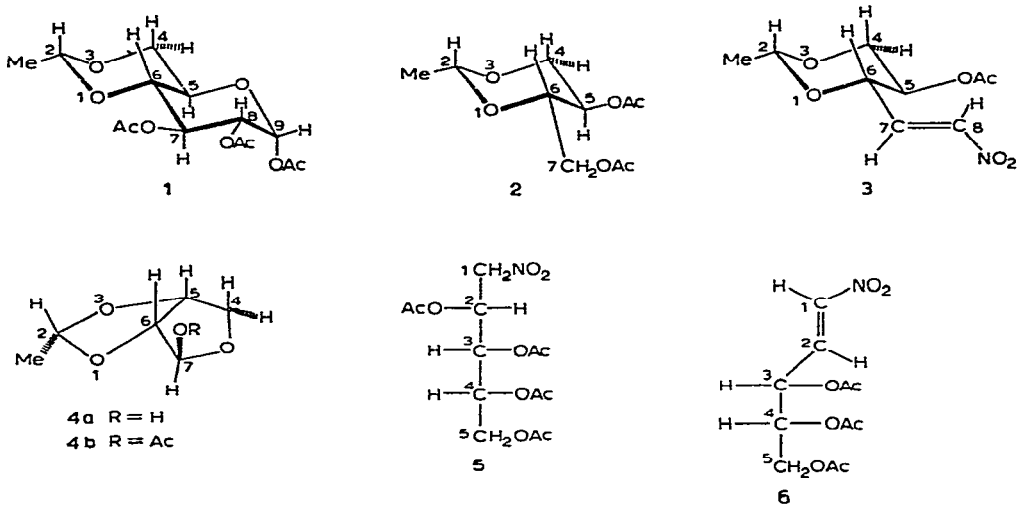
reference to related protons in different molecules that occupy similar or identical chemical and magnetic environments.



Scheme 1.

RESULTS AND DISCUSSION

The n.m.r. parameters for **1–6** are given in Tables I–III. In general, most of the protons resonate at slightly lower field in chloroform-*d* relative to carbon tetrachloride. Much larger solvent shifts, usually but not always upfield, are observed in benzene-d_6 and these are considered below.

Several generalizations can be made from the data in Tables I and II. It is apparent that the 1,3-dioxanes (**1–3**) exist in rigid chair conformations; for example the large values for $J_{4a,5}$ and $J_{5,6}$ (as well as $J_{6,7}$ and $J_{7,8}$ in **1**), and the chemical-shift difference between H-4a and H-4e (0.64–1.16 p.p.m.) in the dioxanes are consistent only with noninverting six-membered rings[18–22]. The *O*-ethylidene methyl- and methine-proton signals observed for seven derivatives (including **1–4**) are relatively insensitive to molecular structure, appearing as high-field doublets (τ 8.69 $\pm$0.03) and low-field quartets (τ 5.33 $\pm$0.04), respectively, in carbon tetrachloride and chloroform-*d* (τ 8.79 $\pm$0.03 and 5.68 $\pm$0.11 in benzene-d_6)*. The chemical shifts of the C-4 protons of **1–3** also are relatively insensitive to structural features [H-4a, τ 6.62 $\pm$0.06 (CCl_4, $CDCl_3$), τ 6.94 $\pm$0.11 (C_6D_6); H-4e, τ 5.83 $\pm$0.07 (CCl_4, $CDCl_3$), τ 5.97 $\pm$0.09 (C_6D_6)]. Finally, in compounds **1–3** the following characteristic patterns are observed: H-4a resonates as a high-field 1:2:1 triplet (since $J_{4a,4e} \simeq J_{4a,5}$), H-4e as a low-field doublet of doublets, and H-5 as a sextet (since $J_{4a,5} \simeq J_{5,6}$).

The anomeric proton (H-9e) of 1,2,3-tri-*O*-acetyl-4,6-*O*-ethylidene-α-D-

*We assume that the methyl group has an equatorial configuration, an assumption which is consistent with considerations of the relative stabilities of axial and equatorial methyl groups on the 1,3-dioxane ring[4b,20].

TABLE I

CHEMICAL SHIFTS FOR 1,3-*O*-ETHYLIDENE DERIVATIVES[a]

Compound	*Solvent*	*H-2*	*H-4*[b]	*H-4*[c]	*H-5*	*H-6*	*H-7*	*H-8*	*H-9*	*Ethyl. methyl*	*Acetate methyl*
1	C_6D_6	5.70q	6.85t	6.11q	6.07st	6.79t	4.20t	4.84dd	3.52d	8.78d	8.31, 8.36, 8.50
	$CDCl_3$	5.36	6.55	5.91	6.16	6.58	4.57	5.01	3.79	8.69	7.87, 7.96, 8.02
	Δ[d]	0.34	0.30	0.20	−0.09	0.21	−0.37	−0.17	−0.27	0.09	0.52, 0.43, 0.37 (±0.07)
2	C_6D_6	5.64q	6.87t	5.87q	5.14st	6.49o	5.79, 5.87			8.76d	8.32, 8.37
	CCl_4	5.37	6.70	5.87	5.32	6.36	5.87, 5.95			8.72	7.99, 8.01
	Δ[e]	0.27	0.17	0.0	−0.18	0.13	−0.08, −0.08			0.04	0.36, 0.34 (±0.03)
3	C_6D_6	5.88q	7.10t	5.94dd	5.56st	6.45dd	3.10s	3.10s		8.86d	8.58
	$CDCl_3$	5.25	6.58	5.72	5.36	5.70	2.84	2.84		8.62	7.92
	CCl_4	5.31	6.65	5.80	5.46	5.76	2.92	2.92		8.68	7.98
	Δ[d]	0.63	0.52	0.22	0.20	0.75	0.26	0.26		0.24	0.66
	Δ[e]	0.57	0.45	0.14	0.10	0.69	0.18	0.18		0.18	0.60
4a	CCl_4	5.11q	6.07d	6.07d	5.40dt	5.67d	4.71s			8.69d	
4b	C_6D_6	5.26	6.39d	6.03dd	5.80dd	5.74d	3.56s			8.76d	8.42
	CCl_4	5.09	6.17	5.98	5.38	5.60	3.99			8.68	8.04
	Δ[e]	0.17	0.22	0.05	0.42	0.14	−0.43			0.08	0.38

[a]In τ, at 100 MHz; s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, o = octet, st = sextet, t = triplet, q = quartet. [b]Axial (*exo* in 4). [c]Equatorial (*endo* in 4). [d]$\Delta = (\tau_{C_6D_6} - \tau_{CDCl_3})$. [e]$\Delta = (\tau_{C_6D_6} - \tau_{CCl_4})$.

TABLE II

COUPLING CONSTANTS (J) FOR 1,3-*O*-ETHYLIDENE DERIVATIVES[a]

Compound	J_{2,CH_3}	$J_{4_a,4_e}$	$J_{4_a,5}$	$J_{4_e,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$
1	5.0	−9.8	9.5	4.8	9.5	9.8	10.0	3.8
2	5.0	−10.0	10.0	5.0	10.0	3.0[b] 5.0		
3	5.0	−10.5	9.5	5.0	9.5	1.0–1.5	[c]	
4a	5.0	−10.0[d]	2.0[e]	2.0[f]	6.0	0.0		
4b	5.0	−10.0[d]	3.5[e]	0.0[f]	6.0	0.0		

[a]Observed splittings in Hz, at 100 MHz. Protons are numbered according to Scheme 1. [b]$J_{6,7}$ and $J_{6,7'}$. [c]Not observably coupled. [d]$J_{exo,endo}$. [e]$J_{exo,5}$. [f]$J_{endo,5}$.

glucopyranose (**1**) resonates as a doublet at τ 3.79 ($CDCl_3$). The low-field position and the small splitting ($J_{8,9}$ 3.8 Hz, Table II), in conjunction with the low-field axial *O*-acetyl methyl at τ 7.87, are consistent with the α-configuration[18c,19]. The β-isomer was detected as a contaminant in the n.m.r. sample of **1** by the appearance of a weak doublet at τ 4.30 ($J_{8,9}$ 7.5 Hz, axial anomeric proton)[18c].

The spectrum of 2,4-*O*-acetyl-1,3-*O*-ethylidene-L-erythritol (**2**) is shown in Fig. 1. Many of the features noted above are readily apparent; for instance the ethylidene methyl doublet and methine quartet, H-4a triplet, and H-5a sextet. Note

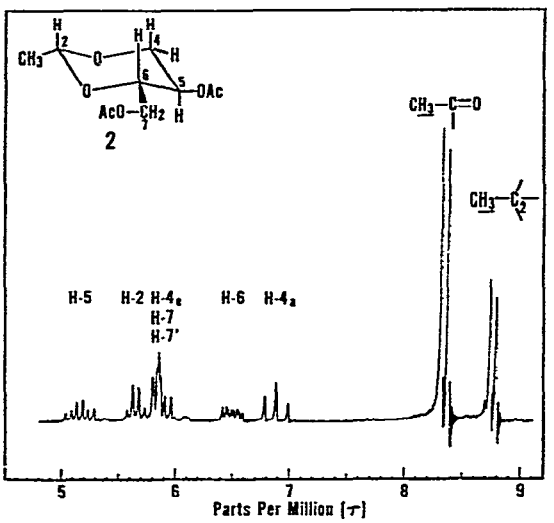


Fig. 1. N. m.r. spectrum of 2,4-di-*O*-acetyl-1,3-*O*-ethylidene-L-erythritol (100 MHz, C_6D_6).

that H-6 appears as an octet because of unequal spin-coupling with the C-7 methylene protons ($J_{6,7}$ 3.0 Hz, $J_{6,7'}$ 5.0 Hz). The magnitude of these coupling constants indicates preference for an approximately gauche relationship between H-6 and each of the C-7 protons in the favored rotational conformation.

TABLE III

N.M.R. PARAMETERS FOR ACETYLATED NITRO SUGARS[a]

Compound	*Solvent*	*Chemical shift, τ*							
		H-1	*H-1'*	*H-2*	*H-3*	*H-4*	*H-5*	*H-5'*	*Acetate methyls*[b]
5	C_6D_6	5.90	5.98	4.16	4.80	4.88	6.07	6.14	8.25, 8.32, 8.39, 8.46
	$CDCl_3$	5.48dd	5.56dd	4.14sp	4.67dd	4.93o	5.79dd	5.86dd	7.89, 7.94, 7.95, 7.98
	Δ[c]	0.42	0.42	0.02	0.17	−0.09	0.28	0.28	0.47, 0.42, 0.41, 0.37 (±0.07)
6	C_6D_6	3.29q		3.15q	4.46dt	4.87dt	5.97q	6.08q	8.36, 8.40, 8.45
	CCl_4	2.96		2.90	4.32	4.86	5.79	5.89	7.90, 7.95, 7.99
	Δ[c]	0.33		0.25	0.14	0.01	0.18	0.19	0.50, 0.44, 0.41 (±0.03)
	Coupling constants, J (Hz)[d]								
	$J_{1,1'}$	$J_{1,2}$	$J_{1',2}$	$J_{1,3}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{4,5'}$	$J_{5,5'}$
5	−13.5	3.3	9.3		2.5	9.0	2.5	4.5	−12.5
6		13.4		−0.6	5.1	4.4	4.2	6.0	−12.1

[a]100 MHz; dd = doublet of doublets, dt = doublet of triplets, o = octet, sp = septet, q = quartet. [b]Solvent shifts are averages of all possible combinatons of chemical shifts. [c]$\Delta = [\tau_{C_6D_6} - \tau_{CDCl_3 \text{ (or } CCl_4)}]$, p.p.m. [d]Obtained from ABX approximations where appropriate, C_6D_6.

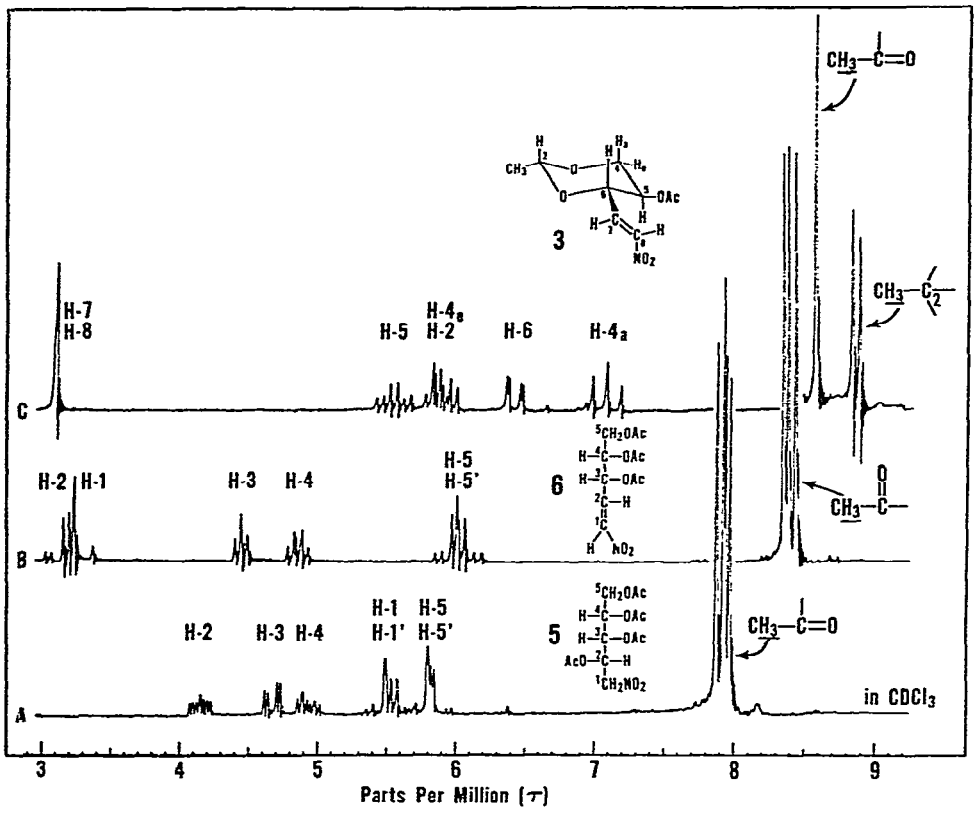


Fig. 2. N.m.r. spectra of nitro acetates: A, $CDCl_3$; B and C, C_6D_6 (100 MHz).

The spectrum of 4-*O*-acetyl-1,2-dideoxy-3,5-*O*-ethylidene-1-nitro-D-*erythro*-pent-1-enitol (3) is shown in Fig. 2C. The olefinic protons, although *trans* and therefore subject to strong spin–spin coupling, are not observably coupled in any of the three solvents since they have identical chemical shifts. The small vicinal coupling between H-6 and H-7 ($J_{6,7}$ 1.5 Hz, Table II) and the lack of allylic coupling between H-6 and H-8 suggest that H-6 preferentially lies near to, or in the plane of, the double bond; that is, perpendicular to the π-orbitals on the time average[23–25]. The solvent dependency of allylic coupling-constants has been interpreted as due to changes in rotamer populations[26], and allylic coupling-constants have been observed to diminish as rotamer populations were changed by substitution of bulky groups on the allylic carbon atom[23]. In 3 no difference was observed in the olefinic-proton region of the spectrum in any of the three solvents, although formation of a complex between 3 and benzene might have been expected to alter the equilibrium distribution of rotamers and thus alter the spectrum. These observations are consistent with restricted rotation of the olefinic group as a result of steric interactions with the dioxane ring, or electronic interactions (dipole–dipole) with the neighboring acetate group, or both.

The spectrum of the acyclic nitroolefin, 3,4,5-tri-*O*-acetyl-1,2-dideoxy-1-nitro-

D-*erythro*-pent-1-enitol (**6**, Fig. 2B) in benzene-d_6, shows a significantly different pattern for olefinic protons. H-1 and H-2 are not magnetically equivalent in this compound and are observably coupled ($J_{1,2}$ 13.4 Hz, Table III). Furthermore, the vicinal coupling-constant ($J_{2,3}$ 5.1 Hz) is about four times larger in **6** than in **3**, and the allylic coupling-constant ($J_{1,3}$ −0.6 Hz, negative sign assumed[23–26]) is readily discernible on an expanded scale (sweep width of 50 Hz). The allylic coupling-constant is about one-half the magnitude of values reported for rigid steroidal systems[24] in which the allylic hydrogen and the π-orbitals of the double bond are eclipsed. Thus, there is significant allylic coupling of H-1 and H-3 through the π-system of the double bond of **6**. It is noteworthy that $J_{1,3} \simeq J_{2,3} \simeq 0$ for **6** in carbon tetrachloride. The spectrum consists of a distorted AB quartet due to coupling between the magnetically nonequivalent H-1 and H-2. Apparently the two nitroolefins (**3** and **6**) adopt closely related rotational forms (relative to the double bond and H-3) in carbon tetrachloride, but different conformations in benzene-d_6. In other words, rotamer populations for **6** are solvent-dependent, and formation of a stronger complex with benzene-d_6 is suggested for this olefin.

The three acetate methyl resonances of **6** are clearly observed. The C-5 methylene protons are part of an ABX pattern with H-4 and are nonequivalent in the two solvents used. Signals for H-3 and H-4 are multiplets simplified by line-overlap to six-line patterns. The magnitude of $J_{3,4}$ (4.4 Hz) is indicative of a favored conformation in which H-3 and H-4 are gauche[27].

Analysis of the spectrum of 2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-nitro-D-arabinitol (**5**) in chloroform-*d* (Fig. 2A) is straightforward. Both groups of methylene protons at C-1 and C-5 are magnetically nonequivalent, giving rise to ABX patterns with H-2 and H-4, respectively. These last two protons are also coupled to H-3, and H-2 resonates as the low-field septet (octet, middle lines overlap) and H-4 as a distorted doublet of triplets (octet, lines 2,3 and 6,7 overlap). The large coupling between H-3 and H-4 ($J_{3,4}$ 9.3 Hz) indicates that the molecule favors a conformation in which H-3 and H-4 are antiperiplanar, whereas the small coupling between H-2 and H-3 ($J_{2,3}$ 2.5 Hz) signifies that these protons favor a gauche or synclinal arrangement[27]. The spectrum of **5** in benzene-d_6 is considerably more difficult to analyze because of overlap between signals for H-3 and H-4 and between signals for the two methylene groups.

The 100-MHz n.m.r. spectra of the erythrofuranose derivatives (**4a, b**) are shown in Fig. 3. Some n.m.r. data for 2,3-*O*-ethylidene-β-D-erythrofuranose (**4a**) have been reported by Van Cleve and Rist[28]. The β-configuration at the anomeric center is shown by the appearance of the H-7 signal as a singlet in both **4a** and **4b** ($J_{6,7}$ 0 Hz, and the dihedral angle between H-6 and H-7 is about 90°) and confirmed by the doublets for H-6 due to coupling only with H-5. In the alcohol (**4a**), H-5 is coupled equally with the magnetically equivalent H-4_{exo} and H-4_{endo} ($J_{5,4exo} = J_{5,4endo} = 2.0$ Hz) as indicated by the doublet of triplets at τ 5.40. Appropriately the C-4 methylene protons resonate as a two-proton doublet. These observations imply that each five-membered ring is twisted from a planar conformation[29] so that the

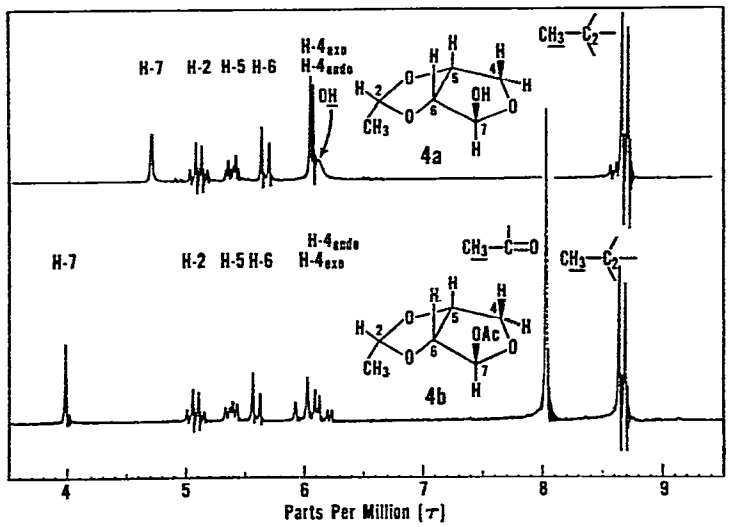


Fig. 3. N.m.r. spectra of β-D-erythrofuranose derivatives (100 MHz, CCl_4).

C-5–H-5 bond bisects the geminal H–C–H angle at* C-4. In the acetate **4b** however, H-5 (doublet of doublets at τ 5.38) is coupled only to H-4_{exo} (doublet of doublets at τ 6.17). Thus the degree of twist and hence the conformation of the acetate must differ significantly from the alcohol.

The *endo* assigment of the methyl group of **4** is based largely on the work of Baggett and coworkers[10] who established from n.m.r. data the *endo*-phenyl configuration for 2,3-*O*-benzylidene-β-D-erythrofuranose, which they isolated from acid-catalyzed rearrangement of 2,4-*O*-benzylidene-D-erythrose. We assume that the same stereospecificity obtains in the rearrangement[10] of 2,4-*O*-ethylidene-D-erythrose to give **4a**. The configuration could be established unequivocally if the *exo*-methyl isomer of **4** were available[4c,7,10]. However, the benzene-induced shifts of the signals of the ethylidene methyl and methine protons are consistent with the *endo* assignment (*vida infra*).

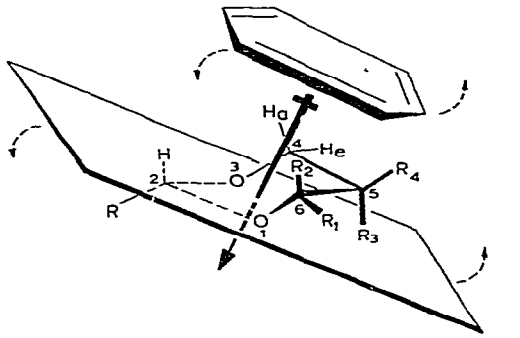
Solvent effects and benzene-induced shifts. — A considerable body of literature has been compiled in the past few years concerning the solvent shifts of proton resonances induced by benzene (relative to carbon tetrachloride or chloroform) and the significance of these shifts as applied to structural, stereochemical, and conformational problems[30–39]. The polarizable electron-cloud of benzene is considered to interact with polar sites in solute molecules *via* an induced dipole–dipole mechanism[30] involving transient complexes in which the benzene ring is as far from the negative end of the local (or molecular) dipole as possible.

Shielding data [$\Delta = \Delta^{C_6D_6}_{CCl_4(CDCl_3)} = \tau_{C_6D_6} - \tau_{CCl_4(CDCl_3)}$] for **1–6** are listed in Tables I and III. Shielding values derived from spectra obtained in chloroform-*d* are

*Alternative explanations, where $\varphi_{5,4exo} \neq \varphi_{5,4endo}$ and $J_{5,4exo} = J_{5,4endo} = 2.0$ Hz, are difficult to visualize.

generally about 0.05–0.10 p.p.m. larger than those derived from carbon tetrachloride; that is, chloroform deshields a proton relative to carbon tetrachloride[38].

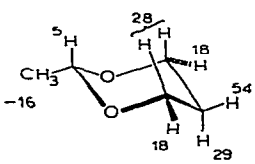
Anderson[4b] and Cookson, Crabb, and Vary[22] have commented on benzene-induced shifts in various alkyl substituted 1,3-dioxanes of the type shown in Scheme 2,



R is: *t*-Bu, iso-Pr, Et, Me

R_1, R_2, R_3, R_4 may be: H, Me, or Et

including several 2-methyl-1,3-dioxanes (**7–9**, numbers are $\Delta \cdot 10^2$, p.p.m.). Anderson[4b] concluded that benzene and 1,3-dioxanes interact to give specific, but weak, 1:1 complexes, but he did not decide whether the benzene molecule lies "above" (as in



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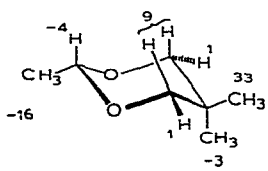

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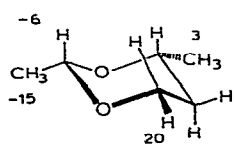

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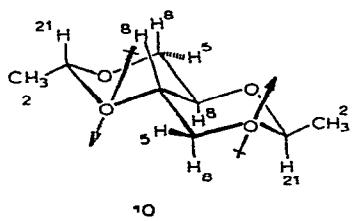
Scheme 2) or "below" the solute molecule. We conclude from Anderson's data[4b] and our own that a collision complex of the geometry represented in Scheme II is useful for predicting and understanding solvent shifts in 1,3-dioxanes. We assume that the electron cloud of benzene interacts with the positive end of the dipole so that the plane of the aromatic ring preferably is perpendicular to the molecular dipole moment[30].

In Scheme 2 we have drawn a reference plane parallel to the aromatic ring and passing through the oxygen atoms of the dioxane-ring. Any shift of the benzene ring is reflected by a corresponding shift of the reference plane. In the parent molecules (R_1–R_4 = H) any proton above the plane is shielded, protons below the plane are deshielded, and protons in or near the plane suffer small shielding or deshielding effects. Thus, R is strongly deshielded and H-2 weakly deshielded (or shielded), whereas protons at C-4, C-5, and C-6 are strongly shielded (R_1–R_4 = H, Scheme 2; see **7**)[4b]. As R_1–R_4 are changed from hydrogen to methyl (*e.g.*, **7–9**) the shielding of R and H-2 are not greatly affected, but protons at positions 4–6 are less strongly shielded than in the parent dioxanes[4b]. That is, there appears to be a moderate steric

effect arising from a greater mean separation of the benzene and dioxane molecules in the collision complex as R_1–R_4 are made bulkier*. On the other hand, the steric bulk of R has little effect on the shielding values for any of the protons. From Anderson's data[4b] then, we can define an order of shielding values for protons of the parent (R = alkyl, R_1–R_4 = H) and alkyl-substituted dioxanes (R, R_1–R_4 = alkyl) as follows: $\Delta R_4 > \Delta R_2 > \Delta R_1 \geqslant \Delta R_3$. It appears that the model will serve well for those dioxanes substituted with nonpolar groups (H, alkyl).

In **1–3** additional sites for formation of a complex with benzene are afforded by the polar substituents. A pattern of shielding similar to that outlined above is followed (H-4a $\geqslant$ H-6a > H-4e > H-5a), except that the ethylidene methyl group is weakly shielded (0.04–0.18 p.p.m.) and H-2 is strongly shielded (0.27–0.57 p.p.m.). The implication is that the shielding cone of benzene lies nearer to the ethylidene carbon atom in these molecules. The strength of this shielding, which is also reflected by other protons in **1–3**, depends strongly upon the substituent at C-6. Association of benzene with substituents in the side chain thus modifies shielding values associated with the 1,3-dioxane ring.

Evidence for a shift in position and plane of the benzene ring in the interacting complex, when additional dipoles are introduced, is given by the shielding parameters ($\Delta^{C_6D_6}_{CDCl_3}$) associated with 1,3:2,4-di-*O*-ethylidene-erythritol (**10**, numbers are $\Delta \cdot 10^2$, p.p.m.). The molecule has a center of symmetry and the dipoles in the two halves of



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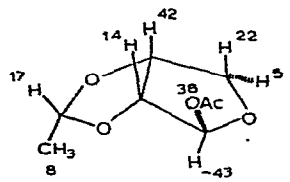
the molecule are parallel and opposite in orientation. The most strongly shielded protons now are the ethylidene methines (H-2, H-2′, $\Delta \cdot 10^2 = 21$ p.p.m.). The benzene molecules that interact on opposite sides of the molecule with the two dipoles are forced away from the positive dipole termini toward the ethylidene carbons by the negative dipole termini in the other ring. Since there is solvent–solute interaction on both sides of the hypothetical plane (passing approximately through the four oxygen atoms) all protons are shielded.

From the shielding parameters for 1-*O*-acetyl-2,3-*O*-ethylidene-β-D-erythro-furanose (**4b**; numbers are $\Delta \cdot 10^2$, p.p.m.) it is apparent that benzene associates with the molecule from the "top" (convex) side. Only the anomeric proton is deshielded, due to specific association of benzene with the acetate carbonyl group[37]. The shielding values for the *exo* (0.22) and *endo* (0.05 p.p.m.) C-4 methylene protons lend credence to the configurational assignment at the ethylidene carbon; that is, the *exo*

*For an alternative interpretation, see reference 4*b*.

methine proton is shielded more strongly than the *endo* methyl protons (0.17 versus 0.08 p.p.m.).



4b

The ready solubility of polyacetates, such as **5** and **6**, in benzene indicates significant solute-solvent interaction associated with the carbonyl group. Shielding values of 0.25–0.45 p.p.m. have been reported for acetate methyl protons[37–40], and comparable values are found for **1–6** (Tables I and III). The presence of a nitro group (**3, 5, 6**) leads to somewhat larger shielding values (0.40–0.60 p.p.m.) for the acetate methyl protons. Strong association between benzene and the nitro group[41] is also evidenced by the greater shielding of the C-1 methylene protons of **5** (versus the C-5 methylene protons), the olefinic protons of **6**, and the ring protons of **3** (relative to **1** and **2**).

In rigid systems, methine protons of secondary acetates usually are deshielded in benzene relative to inert solvents ($\Delta = -0.10$ to -0.27 p.p.m.)[37,39,40]. Similar values are recorded in Table I for the appropriate methine protons of **1**, **2**, and **4** ($\Delta = -0.17$ to -0.43 p.p.m.). Deshielding of these methine protons may result from (*i*) proximity of the proton to the deshielding region of the complexed benzene molecule(s) or (*ii*) a preferred conformation in which eclipsing of the proton and the acetate carbonyl occurs through an orientation effect within the benzene–solute collision complex. The second explanation seems most plausible, since steric and electronic (dipole–dipole) interactions between neighboring acetate groups are likely to restrict rotation of the *O*-acetyl groups in these rigid ring systems. In acyclic systems such as **5** and **6**, rotational restrictions may be less pronounced and averaged shielding values for the methine protons might be expected to be more positive. The shielding data of Table III show that the acetate methine protons of **5–6** may be strongly shielded, weakly deshielded, or affected little by solvents. These results suggest time-averaged shielding values resulting from the various rotational forms of the *O*-acetyl groups.

Culvenor[42] has discussed the so-called "acylation shifts" of carbinol methine protons that occur upon acetylation of alcohols. These shifts are attributed to carbonyl deshielding, and their magnitude is understandable in terms of rotational forms of the *O*-acetyl group[42]. Populations of these rotamers may be influenced by the geometry of the benzene–solute complex.

EXPERIMENTAL

Compounds **1–6** were prepared as described elsewhere[1]. 1,3:2,4-Di-*O*-ethylidene erythritol (**10**) was prepared by mixing equimolar amounts (3.4 mmoles) of 1,3-*O*-

ethylidene-L-erythritol[1] and paraldehyde with a trace of sulfuric acid. The mixture was left in a closed flask for 2 days at 25°. The acid was neutralized with sodium methoxide in methanol, the solvent evaporated, and the organic residue extracted with chloroform. Evaporation of the chloroform and sublimation of the residue gave 18 mg of white, waxy solid, m.p. 86–89°. The 100-MHz n.m.r. spectrum of **10** in chloroform-*d* (benzene-d_6) showed at 2-proton quartet at τ 5.29 (τ 5.50) for the ethylidene methine protons, a six-proton doublet at τ 8.76 (τ 8.78) for the two ethylidene methyl groups, a two-proton multiplet centered at τ 5.99 (τ 6.04) due to the axial protons of the two methylene groups, and a four-proton multiplet centered at τ 6.56 (τ 6.64) due to the remaining protons. The symmetry features of **10** are exemplified by the simplicity of the n.m.r. spectrum.

N.m.r. spectra were obtained with a Varian Associates HA-100 instrument*, and chemical shifts (τ) are given relative to internal tetramethylsilane standard. Spectra were obtained in carbon tetrachloride, chloroform-*d*, and benzene-d_6 on 0.2–0.4M solutions at an operating temperature of 31° and a sweep width of 50–250 Hz. Chemical shifts and coupling constants were determined either from first-order analyses or from appropriate ABX approximations[43]. Calculated and observed parameters agreed reasonably well with one another. Proton assignments are based on expected chemical shifts and the interrelation of spin–spin coupling constants as determined by double resonance techniques.

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SYNTHESIS OF CENTOSE AND TWO ISOMERIC D-GLUCOSE TRISACCHARIDES

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ABSTRACT

Condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 1,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose in acetonitrile containing mercuric cyanide and mercuric bromide, gave the hendecaacetates of *O*-α-D-glucopyranosyl-(1→4)-*O*-[α-D-glucopyranosyl-(1→2)]-β-D-glucopyranose (β-centose) (21.3%) and the β-D-(1→2)-linked isomer (14.4%). The main condensation product (18.4%) obtained from the anomerically unsubstituted β-maltose heptaacetate was identified tentatively as the hendecaacetate of *O*-α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl α-D-glucopyranoside.

INTRODUCTION

Centose, a reducing trisaccharide isolated from honey[1], has been identified as *O*-α-D-glucopyranosyl-(1→4)-*O*-[α-D-glucopyranosyl-(1→2)]-D-glucose[2] (**1**). Following the preparation[3] of 1,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (**2**), the synthesis of the hendecaacetate of **1** has been investigated by condensation of **2** and tetra-*O*-acetyl-α-D-glucopyranosyl bromide under conditions which result in the formation of 2-*O*-glycosyl-D-glucopyranose acetates from the anomeric 1,3,4,6-tetra-*O*-acetyl-D-glucopyranoses and acetylated glycosyl bromides[4,5].

DISCUSSION

Condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose and tetra-*O*-acetyl-α-D-glucopyranosyl bromide in acetonitrile containing mercuric cyanide and mercuric bromide occurs almost completely, and leads[6] to the formation of approximately equal quantities of the α-octaacetates of kojibiose (2-*O*-α-D-glucopyranosyl-D-glucose) and sophorose (2-*O*-β-D-glucopyranosyl-D-glucose)[5]. Treatment of the heptaacetate **2** with tetra-*O*-acetyl-α-D-glucopyranosyl bromide under similar conditions resulted in incomplete conversion into the two isomeric trisaccharide acetates containing an α- and a β-D-(1→2) glycosidic bond, respectively. Even under the most satisfactory conditions attained, the heptaacetate **2** and the trisaccharide products were all present in the reaction mixture in approximately equal proportions, indicating that only *ca.* 50% of **2** had undergone the desired condensation. Control

experiments with 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose confirmed the markedly higher reactivity of HO-2 in this compound.

In the separation by t.l.c. of the anomeric peracetates of D-glucose, maltose, and cellobiose, Tate and Bishop[7] have found that, for each pair, the β-acetate is slightly more mobile than the α-anomer. The same order of separation is obtained[3] with acetone–benzene, and the observation has been extended to the anomeric octa-acetates of kojibiose and sophorose. However, the reverse behaviour has been noted[8] for the heptaacetates of 2-*O*-α-L-rhamnopyranosyl-D-galactopyranose, so that there is no reliable rule at present.

For octa-*O*-acetyl-D-glucopyranosyl-D-glucopyranoses, the presence of an α-interglycose linkage confers a higher chromatographic mobility, at least with the (1→2)- and (1→4)-linked compounds. Thus, the octaacetate of β-maltose is more mobile than that of β-cellobiose[7], and octa-*O*-acetyl-β-kojibiose is more mobile than octa-*O*-acetyl-β-sophorose. By analogy, the more-mobile reaction product **3** obtained in the present study was tentatively considered to be the β-hendecaacetate of **1**, and the slightly less-mobile product **4** to be the β-hendecaacetate of the β-D-(1→2)-linked isomer **5**.

The trisaccharide acetate **3** failed to crystallize, but, after purification by t.l.c., **4** was obtained crystalline. Initially, when hepta-acetate **2** of low m.p. was employed, a crystalline product **6** was obtained, which was slightly less mobile than, but only partially separable from, **3** during t.l.c. The compound was subsequently identified as a non-reducing trisaccharide acetate which was formed by condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (**7**), present as an impurity in **2**. This was confirmed by employing authentic **7** for the condensation.

The i.r. spectra of **3**, **4**, and **6** showed slight differences in the 900–1240 cm^{-1} region, but were almost identical over the range 400–4000 cm^{-1}. In the n.m.r. spectra of **3** and **4** in chloroform-*d*, the signals for H-1 appeared as doublets at τ 4.34 ($J_{1,2}$ 7.8 Hz) and 4.27 ($J_{1,2}$ 7.0 Hz), respectively, characteristic[3,9] of the β-D configuration at C-1. By contrast, in **6**, in which a glycosyloxy instead of an acetoxyl residue occurs at C-1 of the maltose unit, the signal for H-1 occurs further upfield, and could not be identified with certainty.

Compounds **3**, **4**, and **6** were deacetylated to give the trisaccharides **3a**, **4a**, and **6a**. The identification, in each case, of residual trisaccharide after treatment of **3a**, **4a**, and **6a** with *ca.* 0.1N hydrochloric acid in boiling *ca.* 25% (v/v) aqueous methanol for 1 h eliminated the possibility of orthoester linkages in any of the compounds[10]. The three products formed from each trisaccharide were isolated and identified as crystalline peracetates. In addition to β-D-glucopyranose pentaacetate and octa-*O*-acetyl-β-maltose, octa-*O*-acetyl-β-kojibiose was obtained from **3a**, octa-*O*-acetyl-β-sophorose from **4a**, and α,β-trehalose octaacetate from **6a**.

The rates of enzyme-catalyzed hydrolysis of **3a**, **4a**, and **6a** by amylo- and β-D-glucosidase were monitored by t.l.c., and compared with those of kojibiose, maltose, and sophorose controls. Although the amyloglucosidase employed in the

present study rapidly hydrolysed maltose over a wide pH range (2.0–8.3), hydrolysis of kojibiose was only observed below pH 5.5, and did not occur under the conditions previously described[2]. Under the conditions of the present study, maltose was completely hydrolyzed within 2 h, whereas residual kojibiose was still apparent after 48 h (Table I). In addition, hydrolysis of sophorose occurred at approximately the same rate as that of kojibiose, particularly during the first 24 h, so that α- and β-D-(1→2) glycosidic bonds could not be reliably differentiated. Despite these findings, differences in the rates of amyloglucosidase-catalyzed hydrolysis of **3a**, **4a**, and **6a** were observed. Thus, glycosidation of maltose at C-2 retarded the rate of hydrolysis of the α-D-(1→4) glycosidic bond in both **3a** and **4a**, but, contrary to expectation, the effect was very much more pronounced in the α- than in the β-D-(1→2)-linked isomer. After enzyme treatment for 4 h, analysis revealed only a trace of residual **4a** whose identity as the β-D glycosidically bonded isomer was confirmed by the identification of sophorose as the disaccharide formed. Unhydrolyzed **3a**, on the other hand, was still clearly apparent after a reaction time of 48 h, and at no stage was the intermediate disaccharide (identical with kojibiose by chromatography) present as more than a minor constituent of the reaction mixture, indicating that the resistance to enzymic hydrolysis of the α-D-(1→4) glycosidic bond in **3a** is of approximately the same order as that of the α-D-(1→2) bond in kojibiose. Compound **6a** was completely hydrolyzed to D-glucose and a disaccharide within 2 h. The disaccharide was inseparable from maltose or kojibiose by t.l.c., but could be distinguished from these compounds, not only by its intermediate rate of hydrolysis (Table I), but also by its negative reaction towards aniline hydrogen phthalate[11].

TABLE I

AMYLOGLUCOSIDASE-CATALYZED HYDROLYSIS OF D-GLUCOSE OLIGOSACCHARIDES IN *ca.* 1.2M ACETATE BUFFER (pH 3.6) AT 36°

Compound	*Product formed*	*Time (h)*				
		2	*4*	*9*	*24*	*48*
3a		+++	+++	+++	++	+
	Kojibiose	trace	trace	+	+	trace
	D-Glucose	trace	trace	+	++	+++
4a		+	trace	−	−	−
	Sophorose	++	+++	++	++	+
	D-Glucose	+	++	++	++	+++
6a		−	−	−	−	−
	Disaccharide	+++	++	+	trace	−
	D-Glucose	++	++	+++	+++	+++
Kojibiose		+++	+++	+++	++	+
	D-Glucose	−	−	trace	++	+++
Maltose		−				
	D-Glucose	+++				
Sophorose		+++	+++	+++	++	++
	D-Glucose	−	−	trace	++	++

In experiments with β-D-glucosidase, significant hydrolysis of both maltose and kojibiose was apparent after 9 h and 24 h, respectively (Table II), but enzyme specificity is maintained during at least a 4-h reaction period (Table II), and the hydrolysis of **4a** to D-glucose and a disaccharide identical with maltose by chromatography accords with the structure **5** proposed for this trisaccharide. Similarly, the complete resistance of both **3a** and **6a** to hydrolysis by β-D-glucosidase during 4 h is in agreement with the formulation of **3a** as **1**, and indicates that **6a** also does not posess a terminal β-D-glucosyl residue.

TABLE II

β-GLUCOSIDASE-CATALYZED HYDROLYSIS OF D-GLUCOSE OLIGOSACCHARIDES IN *ca.* 1.2M ACETATE BUFFER (pH 3.6) AT 36°

Compound	*Product formed*	*Time (h)*			
		2	*4*	*9*	*24*
3a		+++	+++	+++	++
	Disaccharide	−	−	trace	+
	D-Glucose	−	−	+	++
4a		+	trace	−	−
	Maltose	++	++	++	+
	D-Glucose	+	++	++	+++
6a		+++	+++	+++	++
	Disaccharide	−	−	trace	+
	D-Glucose	−	−	trace	++
Kojibiose		+++	+++	+++	++
	D-Glucose	−	−	trace	+
Maltose		+++	+++	++	+
	D-Glucose	−	−	+	+++
Sophorose		−			
	D-Glucose	+++			

In extending Klyne's rule[12] to calculation of the optical rotations of peracetates of D-glucopyranose trisaccharides, the value for the aglycone has been replaced by that of the corresponding anomeric form of penta-*O*-acetyl-D-glucopyranose. The molecular rotation of the trisaccharide acetate is thus considered to be equal to the sum of the molecular rotations of the D-glucopyranose pentaacetate[13] and the two relevant methyl D-glucopyranoside tetraacetates[14]. Calculated by this procedure, the optical rotations of the β-hendecaacetates of **1** and **5** are in good agreement with the observed values for **3** and **4**, respectively (Table III). Similarly, of the calculated values for the four possible isomeric structures for the non-reducing trisaccharide hendecaacetate **6**, that for the peracetate of *O*-α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl α-D-glucopyranoside (**8**) is in closest agreement with the observed value (Table III). This conclusion is supported by the results of treatment of the free sugar (**6a**) with amylo- and β-D-glucosidase (Tables I and II), which are also best accommodated by structure **8**. However, the general usefulness and validity of the

current method of calculating optical rotation remain to be established. Previous authors[14–16] have calculated the molecular rotations of non-reducing di- and trisaccharide peracetates as the sum of the values of the acetylated methyl glycosides corresponding to the monosaccharide, and mono- plus di-saccharide components, respectively. In the latter case, and in the case of higher oligosaccharides, a potential advantage of the current method of calculation is that only the rotational values of derivatives corresponding to the monosaccharide components are employed, and these are usually accurately known under the required conditions. The method is not intended for the accurate calculation of optical rotations, but as a simple means of differentiating between the anomeric forms of glycosidic bonds whose positions within the oligosaccharide molecule are known. For example, differentiation of the three isomeric trehalose octaacetates is possible, although the calculated optical rotations agree less satisfactorily with the observed values[17] than do those obtained by the method of Staněk[14]. Both methods of calculation are, however, empirical.

TABLE III

OPTICAL ROTATIONS OF D-GLUCOSE TRISACCHARIDE HENDECAACETATES IN CHLOROFORM

Compound	*Observed* $[\alpha]_D$ *(degrees)*	*Hendecaacetate*	*Calculated* $[\alpha]_D$ *(degrees)*
Penta-*O*-acetyl-α-D-glucopyranose	+102[13]	*O*-α-D-Glucopyranosyl-(1→4)-*O*-[α-D-glucopyranosyl-(1→2)]-β-D-glucopyranose	+99.4
Penta-*O*-acetyl-β-D-glucopyranose	+4[13]	*O*-α-D-Glucopyranosyl-(1→4)-*O*-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranose	+43.7
Methyl tetra-*O*-acetyl-α-D-glucopyranoside	+130.5[14]	*O*-α-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl α-D-glucopyranoside	+139.0
Methyl tetra-*O*-acetyl-β-D-glucopyranoside	−18.2[14]	*O*-α-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl β-D-glucopyranoside	+83.3
3	+103.0	*O*-α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl α-D-glucopyranoside	+99.4
4	+46.5	*O*-α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl β-D-glucopyranoside	+43.7
6	+101.8		

As none of the free sugars (**3a**, **4a**, and **6a**) has so far been obtained crystalline, characterization has only been attempted in the case of **3a**, in view of the fact that its structure (**1**) has been assigned to centose[2]. The synthetic material had $[\alpha]_D^{15}$ +142.3° (*cf.* +140° for the natural product[2]). Assuming the same anomeric equilibrium in water as for D-glucose, and employing the values $[\alpha]_D$ +52.7° for D-glucose, and $[\alpha]_D$ +158.9° for methyl α-D-glucopyranoside[14], the calculated $[\alpha]_D$ for structure **1** is +141.2°. A sample of natural centose was, unfortunately, not available for direct comparison with the synthetic product.

EXPERIMENTAL

General. — Thin-layer chromatography (t.l.c.) of oligosaccharide acetates was performed on Merck Kieselgel G with 24% (v/v) acetone in benzene, and sulphuric

acid as spray reagent. T.l.c. of free sugars was performed on Merck Kieselgel DC-Fertigplatten with 7:1:2 propyl alcohol–ethyl acetate–water and detection with aniline hydrogen phthalate[11] and sulphuric acid. Paper chromatography and paper electrophoresis of sugars were performed as described elsewhere[2], but, for paper chromatography, the upper phase of the solvent system (*A*) 5:1:3:3 butyl alcohol–benzene–pyridine–water was also employed. General experimental methods in respect of preparative t.l.c. and the determination of m.ps., and i.r. and n.m.r. spectra were the same as in a previous investigation[3].

Hendeca-O-*acetyl*-O-α-D-*glucopyranosyl*-(*1→4*)-O-[α-D-*glucopyranosyl*-(*1→2*)]-β-D-*glucopyranose* (*β-centose hendecaacetate*) (**3**). — 1,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-maltose[3] (**2**; 0.38 g) was dissolved in a solution of mercuric cyanide (0.08 g) and mercuric bromide (0.11 g) in acetonitrile (1.8 ml) at room temperature, and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.40 g) was added. After 15 h at 20°, the solvent was evaporated, and the residue was treated with chloroform (40 ml) and filtered. The filtrate was washed with M potassium bromide (3 × 10 ml) and water (2 × 20 ml), and evaporated to dryness. The residue (0.68 g) was fractionated by preparative t.l.c. (≯20 mg mixture per plate). The more-mobile product (**3**; 123 mg, 21.3%), although homogeneous by t.l.c., failed to crystallise. The compound had $[\alpha]_D^{15}$ +103.0° (*c* 2.51, chloroform); ν_{max}^{KBr} 1755s, 1438w, 1372m, 1235s, 1168w, 1140w, 1075m (shoulder), 1040s, 945w, 913w, 602w cm^{-1}; n.m.r. data: τ 4.34 (doublet, $J_{1,2}$ 7.8 Hz, H-1); 4.47–6.42 (multiplets, 20 protons); 7.90, 7.91, 7.99, 8.00, 8.01 (singlets, 33 protons, 11 acetyl groups).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.7; H, 5.6; acetyl, 49.0. Found: C, 49.4; H, 5.5; acetyl, 48.2.

Hendeca-O-*acetyl*-O-α-D-*glucopyranosyl*-(*1→4*)-O-[β-D-*glucopyranosyl*-(*1→2*)]-β-D-*glucopyranose* (**4**). — The less-mobile condensation product, separated by t.l.c. as described above, crystallized from ethanol to yield **4** (83 mg, 14.4%), m.p. 195–196°, $[\alpha]_D^{25}$ +46.5° (*c* 2.49, chloroform); ν_{max}^{KBr} 1755s, 1438w, 1372m, 1235s, 1170w, 1130w (shoulder), 1075m (shoulder), 1040s, 960w, 910w, 605w cm^{-1}; n.m.r. data: τ 4.27 (doublet, $J_{1,2}$ 7.0 Hz, H-1); 4.49–6.42 (multiplets, 20 protons); 7.89, 7.91, 7.99, 8.00, 8.01 (singlets, 33 protons, 11 acetyl groups).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.7; H, 5.6; acetyl, 49.0. Found: C, 49.6; H, 5.5; acetyl, 49.2.

Hendeca-O-*acetyl*-O-α-D-*glucopyranosyl*-(*1→4*)-β-D-*glucopyranosyl* α-D-*glucopyranoside* (**6**). — 2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-maltose[18] (**7**; 0.38 g) was dissolved with gentle heating in a solution of mercuric cyanide (0.08 g) and mercuric bromide (0.11 g) in acetonitrile (1.8 ml), and, after cooling to 30°, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.40 g) was added. Further treatment prior to purification by t.l.c. was as described for **3**. Examination of the reaction mixture by t.l.c. revealed the presence of at least three closely moving, condensation products, of which the most mobile (**6**) preponderated. The product **6** (0.67 g) was isolated by preparative t.l.c. (20 plates). After several recrystallizations from ethanol, material was obtained having m.p. 195–197°, $[\alpha]_D^{27}$ +101.8° (*c* 2.16, chloroform); ν_{max}^{KBr} 1755s,

1438w, 1372m, 1240s, 1215s (shoulder), 1170w, 1132w, 1036s, 940w, 900w, 602 cm^{-1}; n.m.r. data: τ 4.55–6.17 (multiplets, 21 protons); 7.85, 7.90, 7.98, 8.00 (singlets, 33 protons, 11 acetyl groups).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.7; H, 5.6; acetyl, 49.0. Found: C, 49.5; H, 5.6; acetyl, 48.7.

Deacetylation and enzyme treatments. — The trisaccharides (**3a**, **4a**, and **6a**) were obtained from the acetates (**3**, **4**, and **6**, respectively) as follows. Sodium methoxide (2.34%, 0.035 ml) was added to a suspension of the acetate (3.0 mg) in absolute methanol (0.1 ml). (Compound **6** required brief warming at 35° to promote initial dissolution.) On addition of glacial acetic acid (0.015 ml) after 20 min at room temperature, a clear, colourless solution was obtained. Controls of kojibiose, maltose, and sophorose octa-acetates were similarly deacetylated. The methanol was evaporated, and the residue was dissolved in a solution (suspension in the case of amyloglucosidase) of the enzyme (6.0 mg) in water (0.2 ml), and incubated at 36°. Reactions with amyloglucosidase (fungal, Koch–Light Laboratories) and β-D-glucosidase (almond, Seravac Laboratories) were monitored by t.l.c. and by paper chromatography in solvent *A*. The results are summarized in Tables I and II.

Acid-catalyzed hydrolysis. — The trisaccharide acetate (50 mg) was dissolved by gently heating in methanolic sodium methoxide (0.47%, 0.5 ml). After 20 min at room temperature, aqueous hydrochloric acid (*ca.* 0.15N, 1.5 ml) was added, and the solution was boiled under reflux for 1 h, cooled, neutralized with methyl di-*n*-octylamine[19], and evaporated to dryness. The residue was boiled for 3 min in acetic anhydride (0.6 ml) containing anhydrous sodium acetate (30 mg). The mixture was cooled, diluted with water (10 ml), left for 2 h at room temperature, and extracted with chloroform (2 × 1 ml). The combined chloroform extracts were washed with water (2 × 4 ml), the solvent was evaporated, and the residue was separated by t.l.c. (2 plates, 10% acetone in benzene). The acetylated material was resolved into 4 components (I–IV in order of decreasing mobility), the respective yields for each trisaccharide acetate being as follows. Compound **3**: 20.01, 10.26, 7.78, and 9.93 mg; compound **4**: 19.23, 10.57, 8.48, and 11.33 mg; compound **6**: 18.71, 9.51, 8.66, and 12.17 mg. All of the components crystallized from ethanol except IV from compound **3**. In each case, I was identified as β-D-glucopyranose pentaacetate, m.p. and mixed m.p. 131–132°; and II was identified as octa-*O*-acetyl-β-maltose[3], m.p. and mixed m.p. 158–159°. Product III from compound **3** had m.p. 117–118°, undepressed on admixture with β-kojibiose octaacetate[20]; and IV was identical with **3** by i.r. spectrometry and t.l.c. Product III from compound **4** had m.p. 191–192°, undepressed on admixture with β-sophorose octaacetate[5], and IV had m.p. 195–196° alone and in admixture with **4**. Product III from compound **6** had m.p. 140-141° alone and in admixture with α,β-trehalose octaacetate[21]; IV had m.p. 195–197°, undepressed on admixture with **6**. The identifications were supported in all cases by comparison with authentic compounds by i.r. spectrometry and t.l.c.

Preparation and characterization of **3a** (*centose*). — Compound **3** (130 mg) in absolute methanol (1.5 ml) was treated with sodium methoxide (2.34% 0.25 ml).

After 20 min at room temperature, the pH was adjusted to 7.0 with aqueous hydrochloric acid, and the solution was desalted (Pleuger Chromatodesalter), evaporated to near dryness, diluted with 95% aqueous ethanol (1.0 ml), and filtered. The precipitate of **3a**, which formed on prolonged standing, contained only slight traces of monosaccharide (D-glucose) and disaccharide contaminants, the latter being distinct from sophorose, but inseparable from either kojibiose or maltose by t.l.c. Compound **3a** had paper-chromatographic and paper-electrophoretic properties consistent with those reported for centose[2], and had $[\alpha]_D^{15}$ +142.3° (*c* 0.59, water; lit.[2] $[\alpha]_D^{27}$ +140° (water); ν_{max}^{KBr} 3420s, 2935m, 1635w, 1420w, 1375w, 1265w, 1200w, 1150m, 1080s, 1040s, 920w, 850w, 770w, 575m cm^{-1}.

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.9; H, 6.3. Found: C, 42.5; H, 6.4.

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PROTEIN–CARBOHYDRATE INTERACTION.

PART XXII. A CHEMICALLY-SYNTHESIZED D-MANNAN AND THE INTERACTION OF SOME SYNTHETIC D-MANNANS WITH CONCANAVALIN A

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ABSTRACT

1,6-Anhydro-β-D-mannopyranose was polymerized in the presence of chloroacetic acid. A high-molecular-weight fraction insoluble in 80% ethanol was isolated and studied. Periodate-oxidation studies showed the polymer to contain 42% of (1→6)-like linkages, 36% of (1→4)- or (1→2)-like linkages, and 22% (1→3)-like linkages. End-group analysis gave $\overline{d.p.} = 113$. The synthetic D-mannan reacted vigorously with concanavalin A, indicating that it was a highly branched polymer containing multiple α-D-mannopyranosyl residues at chain ends. A synthetic D-mannan obtained by the polymerization of D-mannose in the presence of phosphorous acid gave a much weaker reaction with concanavalin A, and two synthetic, linear polymers failed, as expected, to form a precipitate with this plant protein.

INTRODUCTION

The chemical synthesis of polysaccharides has stimulated a good deal of interest, especially during the past ten years[1]. Recently, methods have been developed for the stereospecific polymerization of sugar derivatives to give polymers of defined linkage-type[2–6].

In view of our interest in protein–carbohydrate interaction[7–11] we have prepared a synthetic D-mannan and have investigated its interaction with concanavalin A. This plant protein forms a precipitate with biopolymers containing terminal, non-reducing α-D-glucopyranosyl, α-D-mannopyranosyl, and β-D-fructopyranosyl residues. We have suggested that concanavalin A, although more limited in its specificity compared with certain anticarbohydrate antibodies, can be considered as belonging to this class of "protein reagents", all of which may be used for the detection and preliminary characterization of various structural features present in complex carbohydrates. (See Heidelberger[12] for a review.)

This report describes the acid-catalyzed, addition polymerization of 1,6-anhydro-β-D-mannopyranose and the isolation of a high-molecular-weight D-mannan

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**This work was done while the author was an Established Investigator of the American Heart Association. To whom inquiries regarding this paper should be sent.

for structural characterization. The interaction of concanavalin A with the described D-mannan, as well as with synthetic D-mannans prepared by several other procedures, was also studied.

MATERIALS AND METHODS

1,6-Anhydro-β-D-mannopyranose was prepared from the corresponding 2,3-isopropylidene acetal according to the procedure of Knauf *et al.*[13]; m.p. 209–210°, $[\alpha]_D^{20}$ −127° (*c* 1, water). Lit.[13] m.p. 210–211°; $[\alpha]_D$ −127° (*c* 1.5, water).

Three samples of synthetic D-mannans were the gifts of Dr. Peter T. Mora[14], Dr. Conrad Schuerch, and Professor P. S. O'Colla[15]. Chloroacetic acid was a product of Distillation Products Industries, Rochester, New York. Concanavalin A was prepared according to the method of Agrawal and Goldstein[16]. Glycerolde hydrogenase was purchased from Worthington Biochemical Corporation, Freehold, New Jersey.

EXPERIMENTAL

Polymerization of 1,6-anhydro-β-D-mannopyranose. — Vacuum-dried 1,6-anhydro-β-D-mannopyranose (4.0 g) and chloroacetic acid (95 mg) were finely powdered in a dry mortar. The resulting mixture was distributed equally in 8 test tubes and the tubes were immediately sealed. The tubes were heated in a glycerol bath (126–129°) for 5.3 h, to give a yellow glass that fractured upon cooling. The tubes were opened and water (5 ml) and solid sodium hydrogen carbonate (25 mg) were added to each tube. After 2 h with occasional stirring the yellow solutions from the 8 tubes were combined and dialyzed overnight against distilled water as follows: 1 liter of water (15 h, dialyzate was brownish yellow); 1 liter of water (8 h, dialyzate was light brownish-yellow); 1 liter of water (39 h, dialyzate was deep yellow); 1 liter of water (7 h, dialyzate was colorless). The 4 dialyzates were concentrated individually, the weights of the residue in each case being (*1*) 1.82 g, (*2*) 0.399 g, (*3*) 0.941 g, and (*4*) 0.068 g.

The indiffusible polymer solution from the dialysis sac was transferred to a beaker and the volume adjusted with water to 100 ml. Absolute ethanol (567 ml) was added dropwise with stirring, making the solution 85% with respect to ethanol. The resulting, turbid solution was centrifuged and the residue was washed successively, twice each with 85% ethanol, absolute ethanol, ether, and finally with petroleum ether (b.p. 30–60°) discarding the supernatant liquid each time. The residue was dried *in vacuo* to give a brown solid (polymer I, 1.219 g). Estimation of carbohydrate by the phenol–sulfuric acid procedure[17] revealed polymer I to contain 23.5% of moisture. The net yield was 0.933 g (26%).

Polymer I (0.90 g) was dissolved in water (50 ml) and absolute ethanol (200 ml) was added dropwise with stirring, thus bringing the concentration of ethanol to 80%. The turbid solution was centrifuged and the residue (polymer II) washed successively with 80% ethanol, abs. ethanol, acetone, ether, and petroleum ether, and dried *in vacuo* over calcium chloride. Polymer II (0.726 mg) was obtained as a yellowish-brown powder (corrected for 12.9% water content by the phenol–sulfuric acid method[17]).

Paper-chromatographic analysis of polymers and dialyzates. — The four dialyzates from polymer I as well as polymers I and II were chromatographed [10:4:3 (v/v) ethyl acetate–pyridine–water] against D-mannose. Sugars were visualized using the silver nitrate–sodium hydroxide reagent[18]. Dialyzates I and II had components migrating with the mobility of mannose and higher saccharides, dialyzate III had only higher saccharides. Dialyzate IV, as well as polymers I and II, were immobile in this solvent system.

Periodate oxidation of polymer II. — To a solution of polymer II (132 mg) in cold water (10 ml) was added ice-cold 0.2M sodium periodate solution (25 ml) and the volume was adjusted to 50 ml. A control solution (without polymer) was prepared similarly. Both blank and reaction solutions were kept at 5° in the dark. Periodically, aliquots were removed and titrated for periodate consumption and formic acid liberation by using standard thiosulfate solution to titrate the iodine liberated upon addition of potassium iodide. On a dry-weight basis, the periodate consumption and formic acid liberation per D-mannosyl residue was 1.20 moles/mole and 0.42 moles/mole, respectively, after 69 h (constant).

Reaction of synthetic D-mannan with Fehling solution. — Addition of Fehling solution to an aqueous solution of the synthetic D-mannan (10 mg of polymer II in 1 ml of water) gave an immediate precipitate.

End-group analysis[19] of polymer II. — To a solution of polymer II (26.7 mg, dry weight) in water (1 ml) was added sodium borohydride (30 mg) and the reaction was allowed to proceed for 49 h at 23°. The reaction mixture was adjusted to pH 5.5 (acetic acid), cooled to 5° and cold 1.0M sodium metaperiodate solution (2.5 ml) was added. The reaction volume was adjusted to 25 ml and the reaction flask was kept at 5° in the dark. A control solution containing all components except polymer II was prepared similarly. At various time intervals, aliquots (2 ml) were removed and analyzed for formaldehyde[20] giving the following results.

Time:	(h)	2	24	46	120	169	
HCHO:	(μg)	6.25	37.5	63.1	78.1	78.1	(2.6 μmoles)

When polymer II (26.7 mg, 165 μmoles) was oxidized directly with sodium metaperiodate (no prior reduction with borohydride) and analyzed for liberation of formaldehyde as above, there was obtained 34.3 μg (1.14 μmoles) of formaldehyde after 169 h (constant).

If it is assumed that the difference in the amount of formaldehyde produced upon periodate oxidation of borohydride-reduced polymer II and native polymer II represents the proportion of formaldehyde liberated from the reducing-end alditol residue, then the $\overline{\text{d.p.}}$ can be calculated from the formula[19]:

$$\text{d.p.} = \frac{Y(30 \times n)}{162X},$$

in which Y = wt (g) of polymer; X = wt (g) of HCHO (mol wt 30); n = moles of

HCHO liberated from the reducing end-group ($n = 2$ for a C-3 or a C-4 linked end-group, $n = 1$ for a C-2, C-5 or C-6 linked end-group; 162 = wt (g) of monomeric residue.

Since the nature of the end-group linkage is unknown, it was further assumed that $n = 1$; and thus $\overline{\text{d.p.}} = 113$.

Studies on periodate-oxidized, reduced polymer II. — Polymer II (45 mg, dry weight) was oxidized with 0.1N sodium metaperiodate for 69 h at 5° as above. The reaction mixture was neutralized with barium carbonate, filtered and the filtrate and washings were concentrated. The residue was dissolved in water (5 ml) and the solution added to a solution of sodium borohydride (97 mg) in water (1 ml). After 4.5 h, an additional portion of sodium borohydride (100 mg) was added and the reaction mixture was kept for 20 h. The reaction mixture was neutralized (acetic acid), treated with Amberlite IR-120 (H^+), and filtered. The filtrate was evaporated and anhydrous methanol was evaporated several times from the residue to remove methyl borate.

A solution of the residue in 1.25N sulfuric acid (3 ml) was heated for 3 h on a boiling water bath. The reaction mixture was cooled, treated with Amberlite IR-45 anion-exchange resin, and filtered into a 25-ml volumetric flask. The washings from the resin were added and the volume was adjusted to 25 ml. Paper-chromatographic analysis of the hydrolyzate [4:5:1 (v/v) butyl alcohol–ethanol–water] showed the presence of glycerol, erythritol, and mannose (alkaline silver nitrate spray-reagent[18]).

Mannose in the hydrolyzate was determined by quantitative paper chromatography (48 h in above solvent system) with the phenol–sulfuric acid method[17]. Found: 9.68 mg.

Erythritol was determined, after elution from the same chromatogram, by the method of Lambert and Neish[20]. Found: 5.46 mg.

Glycerol was determined by an enzymic procedure with glycerol dehydrogenase[21]. Found: 10.5 mg.

Precipitation reaction between concanavalin A and the synthetic D-*mannan.* — A quantitative precipitation study was conducted between concanavalin A (36.9 μg N)

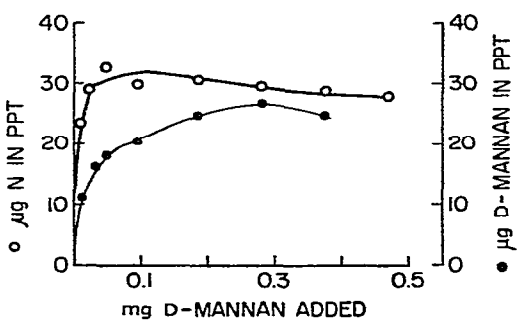

Fig. 1. Quantitative precipitation curve of synthetic D-mannan (polymer II) with concanavalin A. The total amount of synthetic D-mannan in the precipitate is also illustrated. Concanavalin A, 36.9 μg N.

and increasing quantities of polymer II (0.01–0.5 mg), as described previously[9]. The carbohydrate content at each stage of the precipitation curve was also determined[9] and the results are presented in Fig. 1.

By the procedure of So and Goldstein[9], the solubility of the concanavalin A–polymer II D-mannan was determined to be 0.3 μg N/ml.

Interaction of the synthetic D-mannan prepared by Mora *et al.*[14] with concanavalin A (48.3 μg N) was studied. The precipitation curve is presented in Fig. 2.

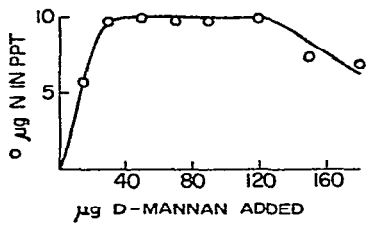

Fig. 2. Quantitative precipitation curve of synthetic D-mannan (Mora[14]) with concanavalin A (48.3 μg N).

The linear D-mannan from Dr. Schuerch was tested at levels of 40, 100, 150, 200, 300, and 500 μg against concanavalin A (40 μg N and 120 μg N) and failed to form a precipitate. The synthetic D-mannan prepared by O'Colla and Lee[15] also failed to react with concanavalin A (5, 15, 25, 50, 250, and 500 μg D-mannan against 40 μg and 120 μg of concanavalin A nitrogen, respectively).

DISCUSSION

Addition polymerization of 1,6-anhydro-β-D-mannopyranose in the presence of chloroacetic acid[22] gave rise to a yellow glass from which a fraction insoluble in 80% ethanol was isolated in 20% yield and selected for study. The polymer was non-dialyzable, immobile on paper chromatograms, and precipitated readily upon addition of Fehling solution. It consumed 1.20 moles of periodate and liberated 0.42 molar proportions of formic acid per D-mannosyl residue. These results correspond to the presence of 42% (1→6)-like linkages, 36% (1→4)- or (1→2)-like linkages, and 22% (1→3)-like linkages. Borohydride reduction of the periodate-oxidized D-mannan followed by complete acid hydrolysis afforded glycerol (53 mole %), erythritol (21 mole %), and D-mannose (26 mole %).

Molecular-weight determination was conducted by an end-group procedure[19] which involved determining the quantity of formaldehyde liberated upon periodate oxidation of the native and the borohydride-reduced polymer. This method gave $\overline{\text{d.p.}}$ 113, assuming that the reducing-end D-mannosyl residue liberated one mole of formaldehyde [i.e. $n = 1$ (ref. 19)].

In this connection it is noteworthy that the native polymer liberated 1 mole of formaldehyde for every 148 D-mannosyl residues, suggesting the presence of D-mannofuranosyl residues unsubstituted at C-5 and C-6. Such residues were reported

for a D-glucan synthesized by Mora *et al.*[23–25] and analyzed by Dutton and Unrau[26]. Dutton and Unrau have also demonstrated the presence of furanose residues in a synthetic D-mannan[27].

These results point to a complex, randomly-polymerized structure containing a wide variety of glycosidic linkages. The presence of a substantial proportion of periodate-resistant D-mannose residues indicates the existence of (1→3)- or multiply-linked [e.g. (1→2) and (1→4)] mannosyl residues. The high proportion of glycerol attests to the presence of a substantial number of (1→6)-linked residues and/or terminal D-mannopyranosyl residues.

Although periodate-oxidation studies give no information concerning the branching frequency of the polymer, it is probable that this synthetic D-mannan is a highly branched structure; in fact its interaction with concanavalin A is completely consistent with such a suggestion.

The interaction of concanavalin A with the synthetic D-mannan (polymer II) is presented in Fig. 1. The curve is quite typical of polysaccharides reactive with concanavalin A, rising sharply to a maximum (48 μg of D-mannan added); at this point, 90% of the concanavalin A added was found in the precipitate. The solubility of the precipitate (0.3 μg N) is characteristic of highly branched α-D-mannans[11]. This interaction of polymer II with concanavalin A is strong evidence for the presence of many terminal, nonreducing α-D-mannopyranosyl residues.

On the other hand, the D-mannan obtained by polymerization of D-mannose in the presence of phosphorous acid[14] gave a much weaker reaction with concanavalin A (Fig. 2) precipitating only 21% of the protein at the point of maximum precipitation. In this example there are apparently far fewer sites available for interaction with concanavalin A. Mora *et al.*[14] commented that this polymer appears to be less branched than their D-glucan, and attribute this difference to the lower temperature and shorter heating cycle employed in its preparation.

The (1→6)-α-D-mannopyranan[28] ($[\alpha]_D$ +122° in methyl sulfoxide, prepared by Schuerch by the same procedure[3,4] he employed in the synthesis of an (1→6)-α-D-glucopyranan) gave, as expected, no reaction with concanavalin A; nor did the synthetic D-mannan prepared by O'Colla and Lee[15] by the melt polymerization of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose in the presence of zinc chloride. (Chemical investigation showed this polymer to be mainly an α-D-(1→6)-linked polymer having a $\bar{P}_n$ of 10.) These results confirm the linear nature of these latter two polymers.

Diffusion studies on agar gel with the synthetic D-mannan (polymer II) described in this investigation have already been presented[8]. The relatively broad precipitation band formed when concanavalin A and polymer II interacted indicate the synthetic D-mannan to be quite polydisperse. Furthermore, the closeness of the precipitation band to the well containing concanavalin A, relative to the position of a precipitation band (very sharp) between yeast D-mannan (from *Saccharomyces cerevisiae*) and concanavalin A, also indicates the synthetic polymer to be a mixture of low-molecular-weight species; end-group analysis as performed in this study supports this view.

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GLYCOSYL ESTERS OF AMINO ACIDS

PART II*. SYNTHESIS OF 2,3,4,6-TETRA-*O*-ACETYL-1-*O*-(2-ACYL-D- AND L-AMINOACYL)-β-D-GLUCOPYRANOSES. STUDIES OF THE RACEMIZATION OF THE AMINO ACID MOIETY

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ABSTRACT

Syntheses of acetylated 1-*O*-(2-acyl-D- and L-aminoacyl)-β-D-glucopyranoses by (*a*) the dicyclohexylcarbodiimide and (*b*) the silver salt method are described, and factors affecting the racemization of the amino acid moiety during the syntheses are investigated. In glucosyl esters prepared by method (*a*), the amino acid moiety undergoes racemization when acetyl is used as the *N*-protecting group, whereas with phthalyl and benzyloxycarbonyl groups configuration is retained; evidence for the racemization *via* the oxazolone formation is given. By method (*b*), the synthesis proceeds with 80–85% retention of configuration, regardless of the nature of the *N*-protecting group. In general, the asymmetric centre of the amino acid aglycon group contributes negligibly to the rotatory power of the whole glucosyl ester molecule. The synthesis of the benzylated 1-*O*-(*N*-acetyl-L-alanyl)-α- and -β-D-glucopyranose is reported.

INTRODUCTION

In Part I of this series[1], we described the synthesis of some 1-*O*-(2-acetamidoacyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoses (*i.e.*, compounds having an ester-bonded amino acid as the aglycon group) by using (*a*) the dicyclohexylcarbodiimide (DCC) and (*b*) the silver salt method. Racemic amino acid derivatives were used, and, after purification, the products were obtained as crystalline β-D anomers by both methods of preparation.

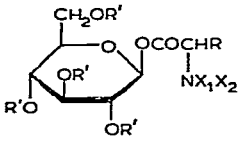
It seemed of interest to examine to what extent the asymmetric centre of the amino acid aglycon affects the properties of the whole D-glucosyl ester molecule. It was first necessary to discover whether, under the conditions used in methods (*a*) and (*b*), the amino acid component retains its configuration or undergoes racemization, and we now report on these studies.

RESULTS AND DISCUSSION

Starting with *N*-acyl derivatives of D- and L-amino acids, the corresponding acetylated D-glucosyl esters **1–5** (Table I) were synthesized by methods (*a*) and (*b*);

*Part I: Ref. 1.

the reaction conditions and the isolation procedure were the same as those described[1] for the DL analogues. The n.m.r. spectra of the products revealed, in all cases, a doublet (7 Hz) at τ 4.10–4.20 characteristic of the β-D configuration. The chromatographic behaviour and i.r. spectra of a particular D-glucosyl ester were identical, regardless of the method of preparation and of the configuration of the starting amino-acid derivative.



1–6

1–5 R′=Ac ; 6 R′=PhCH$_2$

1	R = Me ;	X_1 = H; X_2 = Ac
2	R = $(CH_2)_2$ SMe;	X_1 = H; X_2 = Ac
3	R = $PhCH_2$;	X_1 = H; X_2 = Ac
4	R = Me ;	X_1 = X_2 = Phthaloyl
5	R = Me ;	X_1 = H ; X_2 = $PhCH_2OCO$
6	R = Me ;	X_1 = H ; X_2 = Ac

The data (Table I) for melting points and optical rotations of D-glucosyl esters **1**–**3** of *N*-acetylamino acids show that the influence of the configuration of the starting amino acid component is rather small; this is most evident with the products obtained by the DCC method. The fact that the D-glucosyl esters prepared[1] from the corresponding DL-amino acids have the same constants indicates that the amino acid component underwent racemization. In method (*b*), there are some differences in physical constants between the products obtained from D- and L-amino acid derivatives; this suggests that either the asymmetric centre in the aglycon group makes a negligible contribution to the properties of the whole molecule, or that the amino acid moiety underwent a partial racemization.

In order to clarify this point, each of the four samples of D-glucosyl ester **1** was subjected to acid and/or alkaline hydrolysis, and the resulting alanine or *N*-acetylalanine was checked for optical purity (Table II). Control hydrolyses performed with *N*-acetyl derivatives of D- and L-alanine ethyl esters showed that no racemization of the amino acid took place. Both samples of ester **1** prepared by the DCC method gave essentially inactive alanine and *N*-acetylalanine, whereas the products of the silver salt method yielded the corresponding isomer with 80–85% retention of configuration.

The racemization of an acylamino acid can occur either by base-catalyzed proton abstraction from the asymmetric centre[2] or through the formation and racemization of an intermediate oxazolone[3]. In control experiments with *N*-acetyl derivatives of L-alanine and L-methionine, it was established that the catalytic amount of triethylamine used in method (*a*) is insufficient to cause proton abstraction. The formation of oxazolones is also favoured by basic conditions and takes place particularly readily with acetylamino acids; in addition, DCC is an excellent reagent for their preparation[3]. Thus, it is very likely that in the synthesis of esters **1**–**3** by the DCC method the rate of formation and racemization of the intermediate oxazolones is much larger than the rate of esterification of the sugar component. The omission of the base, which would slow down oxazolone formation, is not feasible because triethylamine has been shown[1] to be essential for a successful synthesis of these compounds.

TABLE I

DATA FOR 2,3,4,6-TETRA-*O*-ACETYL-1-*O*-(2-ACYLAMINOACYL)-β-D-GLUCOPYRANOSES

Configuration of the starting amino acid	D-*Glucosyl esters*							
	Compound	*Acylaminoacyl group*	*DCC method* (a)			*Ag-salt method* (b)		
			Yield (%)	*M. p.* (*degrees*)	$[\alpha]_D^{22a}$ (*degrees*)	*Yield* (%)	*M. p.* (*degrees*)	$[\alpha]_D^{22a}$ (*degrees*)
L-	1	Acetylalanyl	42	136–137	+8.7	35	138–140	−5.0
D-			40	136–138	+8.8	30	150–151	+15.0
L-	2	Acetylmethionyl	38	100–102	+3.6	15	140–141	+3.8
D-			36	101–103	+3.7	13	126–127	+2.6
L-	3	Acetylphenylalanyl	25	121–123	+10.0	20	138–140	+11.0
D-			23	122–123	+10.5	18	135–136	+9.2
L-	4	Phthaloylalanyl	48	92–94	+24.0	40	93–95	+22.3
D-			50	141–143	+24.5	38	142–144	+23.8
L-	5	Benzyloxycarbonylalanyl	55	133–134	−4.1	—	—	—
D-			54	106–108	+8.3	—	—	—

[a]Determined in chloroform (*c* 1–2).

TABLE II

OPTICAL ROTATIONS OF ALANINE AND *N*-ACETYLALANINE OBTAINED BY THE HYDROLYSIS OF ACETYLATED* AND BENZYLATED** 1-*O*-(2-ACYLAMINOACYL)-β-D-GLUCOPYRANOSES

Configuration of the starting amino acid	D-*Glucosyl ester hydrolysed*			*Hydrolysis product* $[\alpha]_D^{22}$ *(degrees)*	
	Compound	*Acylaminoacyl group*	*Method of preparation*	*Alanine*[a]	N-*Acetylalanine*[b]
L-	1*	Acetylalanyl	Ag	+13.0	−52.0
D-				−13.5	+55.3
L-	1*	Acetylalanyl	DCC	+0.5	+4.0
D-				−0.8	+2.5
L-	4*	Phthaloylalanyl	Ag	+13.5	—
D-				−13.5	—
L-	4*	Phthaloylalanyl	DCC	+13.3	−54.5[c]
D-				−12.6	—
L-	5*	Benzyloxy-	DCC	+13.0	−53.5[c]
D-		carbonylalanyl		−13.1	—
L-	6**	Acetylalanyl	Ag	—	−60.0

[a]Determined in 5N HCl (*c* 1–2); lit.[6] $[\alpha]_D$ +14.5° for the L-isomer. [b]Determined in H_2O (*c* 1–2); lit.[6] $[\alpha]_D$ −66.2° for the L-isomer. [c]Obtained by acetylation of alanine originating from acid hydrolysate.

To determine the effect of the *N*-substituent on the racemization of the amino acid aglycon, the amino acid component was protected with phthalyl and benzyloxycarbonyl groups which preclude formation of oxazolones. In the presence of DCC, both optical isomers of *N*-phthaloyl- and *N*-benzyloxycarbonyl-alanine reacted smoothly with the acetylated sugar moiety, giving the corresponding D-glucosyl esters **4** and **5**. Hydrolysis of the products afforded alanine which, in all cases, was more than 80% optically pure (Table II). For comparison, ester **4** was also prepared from the D- and L-amino acids by the silver salt method; the products were identical to those synthesized by the DCC method (Tables I and II). It follows that, under conditions which do not allow oxazolone formation, the C-1 esterification of acetylated D-glucose by the DCC method proceeds without racemization of the aglycon. Thus, the principles of the racemization mechanism valid for amino acid coupling are also applicable in this nonpeptide-forming reaction.

A convenient route to the free glycosyl esters seemed to involve protection of the sugar moiety with inert benzyl groups. However, condensation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose and *N*-acetylalanine failed with method (*a*) (*cf.* Refs. 4 and 5). 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride reacted with the silver salt of *N*-acetylalanine to give the D-glucosyl ester **6** as an anomeric mixture in which the β-D anomer preponderated, and from which both anomers were obtained in crystalline form.

Alkaline hydrolysis of the β-D anomer of **6** gave *N*-acetylalanine with 90% retention of optical activity. This is a higher percentage of retention than is ever observed with the corresponding acetyl derivatives. However, at present, it is not

possible to say whether the acetylated D-glucose moiety has any influence on this loss of activity.

The optical rotations of the β anomers of D-glucosyl esters differing only in the configuration of the amino acid aglycon group are very similar. Thus, at the D line, the asymmetric centre of the amino acid aglycon group contributes negligibly to the rotatory power of the whole D-glucosyl ester molecule.

EXPERIMENTAL

General. — Melting points are uncorrected. Solvent evaporation was performed in a rotatory evaporator *in vacuo* at $<40°$. Column chromatography was conducted on silica gel (0.05–0.2 mm, Merck). Thin-layer chromatography (t.l.c.) was performed on Silica Gel G (Merck) with 5:1:1 ether–acetone–light petroleum (*A*), 5:1 benzene–methanol (*B*), 65:25:15 butyl alcohol–acetic acid–water (*C*), 1:1 benzene–ethyl acetate (*D*). The spots were detected by spraying with a 10% (v/v) solution of sulphuric acid in water and subsequent heating, with ninhydrin, or with Bromocresol Green (0.1% in ethanol+1 drop of morpholine). I.r. spectra were determined on a Perkin–Elmer Model 137 spectrometer; n.m.r. spectra were obtained with a Varian A-60A spectrometer, using tetramethylsilane as the internal standard. *N*-Phthaloyl-D- and -L-alanine were prepared by the procedure of Sheehan *et al.*[7]. Silver salts of *N*-phthaloyl-D- and -L-alanine were obtained by the general procedure[8] for substituted silver benzoates. After one recrystallization (2:1 ethanol–water), the silver salts were analytically pure.

Preparation of 2,3,4,6-tetra-O-acetyl-1-O-(2-acylaminoacyl)-β-D-glucopyranoses (**1–5**). — Starting with optically active amino acid derivatives, these compounds were prepared[1] by (*a*) the DCC and (*b*) the silver salt methods. Compounds **1–3** synthesized from the corresponding *N*-acetyl-D- and L-amino acid derivatives by both methods gave, in all cases, microanalytical values (C, H, N) within the experimental error.

The synthesis of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-phthalyl-D- and -L-alanyl)-β-D-glucopyranoses (**4**) by method (*b*) was accomplished after 1h; the reaction mixture was fractionated on a column of silica gel by using solvent (*B*), and the product was crystallized from methanol.

Anal. Calc. for $C_{25}H_{27}NO_{13}$: C, 54.65; H, 4.95; N, 2.55. Found: C, 54.75; H, 5.00; N, 2.39 for L diastereomer by method (*b*); C, 54.86; H, 5.13; N, 2.60 for D diastereomer by method (*b*); N, 2.40 for L diastereomer by method (*a*); N, 2.70 for D diastereomer by method (*a*).

2,3,4,6-Tetra-*O*-acetyl-1-*O*-(*N*-benzyloxycarbonyl-D- and -L-alanyl)-β-D-glucopyranoses (**5**) were synthesized by method (*a*) on a 2-mmole scale by using dichloromethane as the solvent. After the removal of *N,N'*-dicyclohexylurea (80%) and evaporation of the solvent, the oily residue was chromatographed on a column of silica gel by using solvent (*D*). The D-glucosyl ester emerged first, and was crystallised from ether–light petroleum.

Anal. Calc. for $C_{25}H_{31}NO_{13}$: C, 54.25; H, 5.65; N, 2.53. Found: C, 54.22; H, 5.39; N, 2.65 for D diastereomer; C, 54.00; H, 5.39; N, 2.78 for L diastereomer.

2,3,4,6-Tetra-O-benzyl-1-O-(N-acetyl-L-alanyl)-β- and -α-D-glucopyranose (**6**). — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride[5,9] (1.22 g) and the silver salt of *N*-acetyl-L-alanine (502 mg) in dry benzene (60 ml) were refluxed with mechanical stirring for 9 h. The precipitate was centrifuged off, and washed with benzene, and the combined supernatants were evaporated *in vacuo*. The solid residue was chromatographed on a column of silica gel with 7:1 and then 1:1 benzene–ethyl acetate. The D-glucosyl ester **6**, contaminated with some tetra-*O*-benzyl-D-glucose, emerged in the later fractions; after fractionation on a second column with solvent (*D*), the product **6** was obtained as a semicrystalline solid (300 mg, 22%) which gave one elongated spot on t.l.c. in solvent (*D*). It was recrystallized from benzene–light petroleum to yield the β anomer of **6** as a gelatinous precipitate (170 mg), m.p. 134–135°, $[\alpha]_D^{22}$ +2° (*c* 1.0, chloroform); i.r. data: ν_{max}^{KBr} 3270 (NH), 1760 (C=O), 1660 and 1570 (amides I and II), 1210 cm^{-1} (C–O–C); n.m.r. data ($CDCl_3$): τ 4.32 (1-proton doublet, $J_{1,2}$ 7.0 Hz, axial H-1).

Anal. Calc. for $C_{39}H_{43}NO_8$: C, 71.65; H, 6.63; N, 2.14. Found: C, 71.79; H, 6.76; N, 2.28.

The mother liquors were evaporated *in vacuo* to a syrup which was fractionated on a column of silica gel with solvent (*D*); the α anomer of **6** emerged in earlier fractions as an oil which was obtained from benzene–light petroleum as a flocculent precipitate (50 mg), m.p. 45–46°, $[\alpha]_D^{22}$ +60° (*c* 1.0, chloroform); n.m.r. data: τ 3.60 (1-proton doublet, $J_{1,2}$ 2.7 Hz, equatorial H-1).

Anal. Found: C, 71.57; H, 6.89; N, 2.12.

Hydrolysis of D-glucosyl esters. — (*a*) *Acidic hydrolysis of compounds* **1**, **4**, *and* **5**. A suspension of 1 mmole of the appropriate ester in 10 ml of 5N hydrochloric acid was refluxed for 1 h, the dark solution was treated with charcoal, and the filtrate evaporated to dryness. A solution of the residue in water (25 ml) was passed through a column of Dowex-50 X8 (H^+), and the amino acid was eluted with 2N ammonium hydroxide. After evaporation of the eluate, the residue was crystallised from water–ethanol to constant optical rotation.

(*b*) *Basic hydrolysis of compounds* **1** *and* **6**. A solution of the ester **1** (1 mmole) in dry acetone (10 ml) was shaken with 2 ml of N sodium hydroxide at room temperature, the progress of hydrolysis (2–3 h) being followed by t.l.c. (solvent *C*). The solution was treated with charcoal, the filtrate evaporated to dryness, and the residue was dissolved in water (25 ml) and passed through a column of Dowex-2 X8 (Cl^-). *N*-Acetylalanine was eluted with N hydrochloric acid and crystallized from ethyl acetate to constant optical rotation. In the case of **6**, an equivalent amount of N sodium hydroxide was used; after the removal of the solvent, the residue was taken up in water–chloroform, and the aqueous layer was extracted with chloroform and then passed through a column of Dowex-50 X8 (H^+). The acidic effluent was evaporated to dryness, and the *N*-acetylalanine was crystallized.

Optical rotation data are recorded in Table II.

ACKNOWLEDGMENT

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Note

Quantitative determination with periodate of compounds subject to non-Malapradian oxidation
Part IV[1]. 2-Ketoses

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It has long been known that, in the conditions usually employed, hex-2-uloses show fractional consumption of periodate[2–9]. It has been shown[9] that this is due to the fact that these sugars are oxidised by two different routes to give products which behave differently towards periodate. Cleavage of a 2-ketose between C-1 and C-2 leads to the formation of glyoxylic acid which is further oxidised[6,8,10,11] by one equivalent of periodate, whereas cleavage between C-2 and C-3 gives glycolic acid which is practically unaffected by periodate[12]. Thus, the amount of periodate reduced by a hexulose varies between four and five molar equivalents, depending on the proportions of the sugar oxidised by each route. It has also been reported[5] that three hept-2-uloses (D-*gluco*-, D-*manno*-, and L-*galacto*-) are likewise oxidised by the two pathways, reducing between five and six molar equivalents of periodate; it was noted[5] that less glycolic acid was formed from these sugars than from the hexuloses. To our knowledge, the periodate oxidation of octuloses has not been studied.

It is evident that 2-ketoses should reduce a whole number of equivalents of periodate if oxidised in conditions where neither glyoxylic nor glycolic acids are attacked by the oxidant. It has been shown[13] that glyoxylic acid is not oxidised by periodate in 0.1N sulphuric acid at 0° ("cold acid" method[14]); it has now been established that the same is true for glycolic acid. A number of 2-ketoses were therefore oxidised in these conditions; as would be expected, the hexuloses reduced exactly four, the heptuloses five, and the octuloses six molar equivalents of periodate (Table I). In each case, the relative importance of each of the two pathways in the oxidation of the sugar was assessed by determination of the amounts of formaldehyde, glyoxylic acid, and glycolic acid formed (Table I).

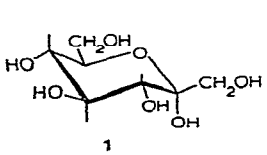
It is interesting to note that no glycolic acid could be detected in the oxidation mixtures of three of the five heptuloses (D- and L-*gluco*- and D-*manno*-) and of one of the two octuloses (D-*glycero*-L-*gluco*-) studied. That no cleavage had occurred between C-2 and C-3 in these compounds was confirmed by the fact that, in each case, two molar equivalents of formaldehyde and one of glyoxylic acid were found amongst the reaction products (these results differ from those previously reported[5] for D-*gluco*- and D-*manno*-heptuloses). None of these compounds show mutarotation; they must

TABLE I

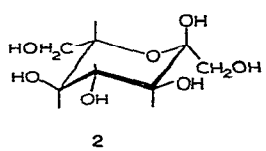
PERIODATE OXIDATION OF 2-ULOSES

2-Ketose	*Molar equivalents of*			
	IO_4 reduced	*Formaldehyde formed*	*Glyoxylic acid formed*	*Glycolic acid formed*
D-Fructose	3.97	1.54	0.51	0.53
L-Sorbose	3.98	1.66	0.71	0.31
D-Tagatose	3.97	1.79		0.20
D-*manno*-Heptulose	4.99	2.02	0.99	0
D-*gluco*-Heptulose	4.85	1.92	0.97	0
L-*gluco*-Heptulose	4.86	1.97	1.04	0
L-*allo*-Heptulose	4.84	1.63	0.62	0.36
L-*galacto*-Heptulose	4.85	1.75	0.80	0.25
D-*glycero*-L-*gluco*-Octulose	5.88	1.98	1.00	0
D-*glycero*-D-*gulo*-Octulose	5.96	1.77	0.84	0.21

therefore be oxidised in a given conformation. It has been shown by n.m.r. studies[15] that D-*gluco*-heptulose exists in the α-pyranoid form and has the *C1* (D) conformation **1**; it can be expected that D-*manno*-heptulose and D-*glycero*-L-*gluco*-octulose (homomorphous with L-*gluco*-heptulose **2**) will also exist as α-pyranoses in the *C1* (D) and *1C* (D) conformations (**3** and **4**, respectively).



1



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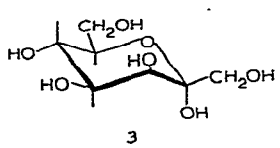

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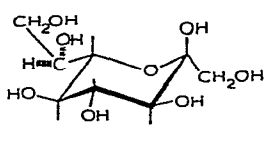

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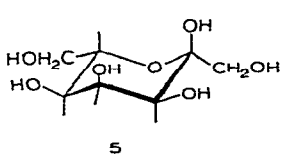

5

It is not unexpected that preferential cleavage of the bond between C-1 and C-2 should occur in structures **1**–**4**; the *trans* diaxial relationship of the hydroxyl groups on C-2 and C-3 of D-*manno*-heptulose is known to be unfavorable for rapid glycol cleavage, and for D- and L-*gluco*-heptuloses and D-*glycero*-L-*gluco*-octulose it is reasonable to find that the vicinal diol having an exocyclic primary hydroxyl group should be cleaved first. On the other hand, L-*allo*-heptulose, which is reported to show no mutarotation, gives some glycolic acid on periodate oxidation. This heptulose most likely exists in the conformation **5** having the same steric arrangement about C-1, C-2, and C-3 as has L-*gluco*-heptulose. The absence of exclusive cleavage between

C-1 and C-2 is probably due to the known destabilising effect of 1,3-diaxial hydroxyl groups (C-2 and C-4).

The hexuloses, the fifth heptulose (L-*galacto*-heptulose), and the second octulose all show mutarotation. This takes place more rapidly than does the reaction of glycol cleavage, so that it is not possible to draw any conclusions as to the form or forms in which these sugars are oxidised.

EXPERIMENTAL

Periodate oxidations were carried out at 0° in 0.1N sulphuric acid (0.6mM in substrate and 6.6mM in sodium periodate) as described previously[14]. The 2-ketose to be oxidised was dissolved in 0.1N sulphuric acid at 0° for 48 to 72 h before the addition of periodate, in order to allow mutarotation to go to completion. Formaldehyde was determined with chromotropic acid[16], glyoxylic acid with periodate[13], and glycolic acid with 2,7-dihydroxynaphthalene[17].

No reduction of periodate by glycolic acid could be detected either in the conditions described above or when the pH of the oxidation mixture was adjusted to 5 with sodium acetate, and the solution was kept at room temperature.

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We thank Dr. Nelson K. Richtmyer for gifts of D-*gluco*-, L-*gluco*-, L-*allo*-, and L-*galacto*-heptuloses and of D-*glycero*-D-*gulo*- and D-*glycero*-L-*gluco*-octuloses (the latter, described in the literature[18,19] as a syrup, has now been obtained in crystalline form by Dr. Richtmyer[20]), and Dr. H. H. Haas for D-tagatose.

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Note

An improved synthesis of 1,2-*O*-isopropylidene-β-L-idofuranose*

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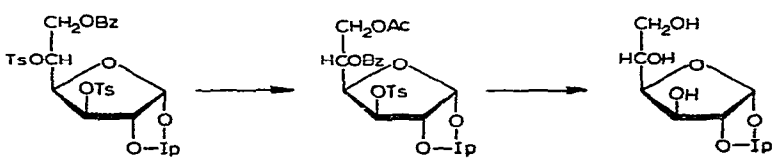
(Received October 20th, 1969)

One of the syntheses of L-idose from D-glucose derivatives is based on inversion of the configuration at C-5, either by intramolecular displacement of a 5-*p*-toluenesulfonate group in an appropriate D-glucose derivative *via* a 5,6-anhydrosugar intermediate[2], or by nucleophilic displacement of the 5-*p*-toluenesulfonate group by the acetate ion[3,4]. In all cases, derivatives of 1,2-*O*-isopropylidene-α-D-glucofuranose were used as starting material. The selective tosylation of the exocyclic hydroxyl group at C-5[2,3,5] or protection of the endocyclic hydroxyl group at C-3[2,4] prior to tosylation was essential, since desulfonylation of endocyclic *p*-tolylsulfonyl derivatives of 1,2-*O*-isopropylidene-α-D-glucofuranose was difficult and proceeded with low yield[2,6–10].

Meyer and Reichstein[2] found that treatment of 5,6-anhydro-1,2-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-β-L-idofuranose with sodium amalgam in 80% methanol afforded, in an extremely low yield, a desulfonylated product which was difficult to free from sulfur. Karrer and Brettcher[8] reported that the reductive desulfonylation of 1,2-*O*-isopropylidene-3,5-di-*O*-*p*-tolylsulfonyl-α-D-xylofuranose with lithium aluminium hydride gave less than 35% yield of the desulfonylated product, 5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose.

Since we found that the reduction of 1,2-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-α-D-glucofuranose derivatives with sodium in liquid ammonia afforded the desulfonylated product in good yields[11], we attempted the synthesis of an L-idose derivative from the readily accessible 6-*O*-benzoyl-1,2-*O*-isopropylidene-3,5-di-*O*-*p*-tolylsulfonyl-α-D-glucofuranose *via* selective nucleophilic displacement of the 5-*p*-toluenesulfonate group by the acetate ion[5,6]. The use of Dowex-1 (X-10, AcO^-) resin[12],



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instead of potassium acetate, considerably improved the yield of the displacement reaction. Desulfonylation, as well as removal of the 6-*O*-benzoyl and 5-*O*-acetyl groups was accomplished by reduction with sodium in liquid ammonia.

EXPERIMENTAL

General. — All melting points are uncorrected. The specific rotations were determined on a Cary 60 spectropolarimeter with 10.0 mm cells. I.r. spectra were recorded on a Perkin–Elmer Model 337 grating spectrophotometer, and n.m.r. spectra on a Varian HR-100 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in p.p.m. (δ).

6-O-Acetyl-5-O-benzoyl-1,2-O-isopropylidene-3-O-p-tolylsulfonyl-β-L-idofuranose (**2**). — Dowex 1 (X-10, 200–400 mesh, AcO^-) ion-exchange resin (64 g) was suspended in acetic anhydride (240 ml) and 6-*O*-benzoyl-1,2-*O*-isopropylidene-3,5-di-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (**1**, 10.0 g) was added. The reaction mixture was treated for 12 h at reflux, the resin was filtered off, and the filtrate evaporated to dryness *in vacuo*. The crystalline residue was recrystallized from abs. ethanol to yield 7.1 g (86%), m.p. 125.5–126.5°, $[\alpha]_D^{27}$ −8.3° (*c* 1.0, chloroform); i.r. data (chloroform): *v* 3010 and 1610 (aromatic CH), 1740 and 1275 (acetate and benzoate), and 1383 and 1177 cm^{-1} (*p*-toluenesulfonate); n.m.r. data in chloroform-*d*: δ 5.96 (1-proton, doublet, $J_{1,2}$ 4.0 Hz, H-1), 5.54 (1-proton, quartet, $J_{4,5}$ 7.2, $J_{5,6}$ 5.8 Hz, H-5), 5.03 (1-proton, doublet, $J_{3,4}$ 3.0 Hz, H-3), 4.78 (1-proton, doublet, $J_{1,2}$ 4.0 Hz, H-2), 4.54 (1-proton, quartet, $J_{3,4}$ 3.0, $J_{4,5}$ 7.2 Hz, H-4), 4.30 (1-proton, quartet, $J_{5,6}$ 3.3, $J_{6,6'}$ 12.5 Hz, H-6), 4.10 (1-proton, quartet, $J_{5,6'}$ 5.8, $J_{6,6'}$ 12.5 Hz, H'-6), 2.44 (3-protons, singlet, Me group from *p*-toluenesulfonate), 2.02 (3-protons, singlet, Me group from acetate), and 1.52 and 1.30 (6-protons, two singlets, Me groups from isopropylidene group).

Anal. Calc. for $C_{25}H_{28}O_{10}S$: C, 57.68; H, 5.42; S, 6.16. Found: C, 57.46; H, 5.35; S, 6.09.

1,2-O-Isopropylidene-β-L-idofuranose (**3**). — Compound **2** (5.2 g, 0.02 mole) was dissolved in tetrahydrofuran (20 ml), and the solution was slowly added (30 min) to a stirred solution of sodium (2.8 g, 0.12 mole) in liquid ammonia (100 ml). After the reaction mixture had been stirred for 2 h at −80° and 1 h at room temperature, ammonium chloride and water were added, and the ammonia was evaporated at room temperature. The residual solution was diluted with ethanol and saturated with carbon dioxide. The precipitate of sodium hydrogen carbonate was filtered off, washed several times with small portions of ethanol, and the combined filtrates were evaporated to dryness *in vacuo*. The dry residue was dissolved in boiling chloroform (*ca.* 200 ml), and the undissolved material was filtered off. The solution was evaporated to dryness, and a brownish, oily residue (4.18 g) was chromatographed on silica gel ("Merck", grain size <0.08 mm). Elution with 8:11:1 chloroform–acetone–methanol afforded 2.07 g which, after recrystallization from acetone–hexane, gave 1.46 g (66%), m.p. 116–117°.

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Note

Nucleophilic displacement reactions in carbohydrates. Part XIII[1]. A synthesis of benzyl 5,6-acetylepimino-5,6-dideoxy-2,3-*O*-isopropylidene-β-L-gulofuranoside

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The adduct formed by iodine azide and 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuran-5-enose (**1**) has been shown[2] by X-ray crystallographic analysis to be 6-azido-5,6-dideoxy-5-iodo-1,2-*O*-isopropylidene-β-L-idofuranose (**2**). The addition can be said to exhibit [I-C(5)]-regiospecificity[3], or, in more familiar terms, an *anti*-Markovnikov addition has occurred. Since the addition of iodine azide to a number of terminal, unsaturated sugars was planned, it was desirable to establish a convenient chemical proof of structure of the adducts formed. It should be possible[4] to convert the adducts (*e.g.*, **2**) into 5,6-epimino derivatives (e.g., **3**; R = H or Ac) by treatment with lithium aluminium hydride and *N*-acetylation. Provided that other terminal, unsaturated sugars exhibit the same regiospecificity towards iodine azide as **1**, identification of the derived epimino derivative will establish the stereochemistry of the adduct at C-5. No 5,6-epimino sugars were known at the instigation of this work, but syntheses of *N*-substituted derivatives having the D-*gluco*-[5], L-*altro*-[6], L-*ido*-[6,7], L-*talo*-[7], and L-*manno*-[7] configurations have been described recently. In view of projected studies on the addition of iodine azide to the unsaturated sugar **4**, we sought to prepare benzyl 5,6-acetylepimino-5,6-dideoxy-2,3-*O*-isopropylidene-β-L-gulofuranose (**11**). A synthesis of this epimine was achieved by procedures essentially similar to those already described[5,6].

Monomethanesulphonylation of the diol[8] **5** gave, principally, benzyl 2,3-*O*-isopropylidene-6-*O*-methanesulphonyl-α-D-mannofuranoside (**6**), whose structure was established by its conversion into 6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranose (**7**) following desulphonyloxylation with lithium aluminium hydride, and hydrogenolysis of the glycosidic substituent. The identity of compound **7** was readily established by comparison of its physical and spectroscopic properties with those of the L enantiomer[9]. The sulphonic ester group of **6** was smoothly displaced with azide ion in *N*,*N*-dimethylformamide to give benzyl 6-azido-6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranoside (**8**), which was esterified to give the 5-methanesulphonate (**9**).

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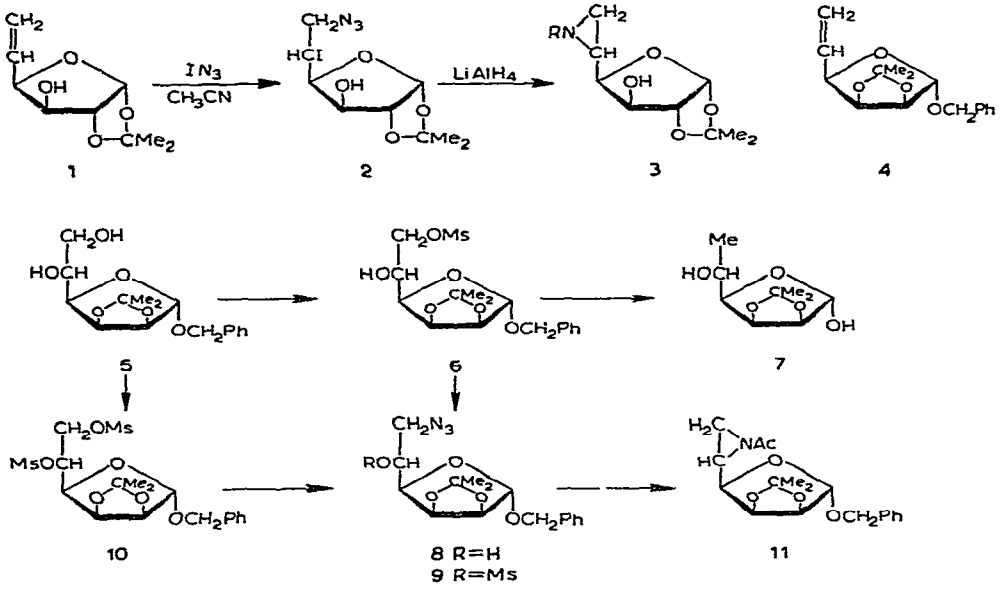

Subsequently, it was found that the primary sulphonic ester group of benzyl 2,3-*O*-isopropylidene-5,6-di-*O*-methanesulphonyl-α-D-mannofuranoside (**10**) could be selectively displaced with sodium azide in *N*,*N*-dimethylformamide, thereby affording a more direct route to **9**.

Treatment of **9** with lithium aluminium hydride at room temperature gave, after *N*-acetylation of the product, compound **11**. The n.m.r. spectrum of **11** supported the general structure assigned, but, more significantly, the i.r. spectrum exhibited a prominent absorption at *ca.* 1700 cm^{-1} characteristic[10] of the *N*-acetylepimino group, whereas absorptions attributable to a NH group were absent from the spectrum.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) and column chromatography on silica gel were conducted by using mixtures of toluene and acetone as the irrigant. Infrared spectra were recorded with a Perkin–Elmer Infracord spectrometer, and n.m.r. spectra were measured on *ca.* 10% solutions in deuteriochloroform with a Perkin–Elmer R-10 spectrometer. Light petroleum refers to the fraction having b.p. 40–60°.

Benzyl 2,3-O-isopropylidene-6-O-methanesulphonyl-α-D-mannofuranoside (**6**). — Methanesulphonyl chloride (1.15 ml) was added to a solution of the diol[8] **5** (3.1 g) in dry pyridine (50 ml), and the mixture was kept overnight at room temperature. Work up, in the usual way, gave the methanesulphonate **6** (3.5 g, 90%), m.p. 92–93° (from ether–light petroleum), $[\alpha]_D$ +69° (*c* 1, chloroform) (Found: C, 52.5; H, 5.8; S, 8.2. $C_{17}H_{24}O_8S$ calc.: C, 52.6; H, 6.2; S, 8.2%). N.m.r. data: τ *ca.* 2.70 (singlet, 5 aromatic protons), 4.92 (singlet, H-1), 5.45 (AB quartet, *J* 12 Hz, benzyl methylene protons), 6.96 (singlet, 3 protons, OMs), 8.54 and 8.68 (singlets, each 3 protons, CMe_2).

Benzyl 2,3-O-isopropylidene-5,6-di-O-methanesulphonyl-α-D-mannofuranoside (**10**). — A solution of the diol **5** (10 g) in dry pyridine (100 ml) was treated with methanesulphonyl chloride (15 ml) overnight at room temperature, whereupon the reaction mixture was worked up in the usual way to give the disulphonate **10** (14.6 g, 97%), m.p. 107–108° (from aqueous methanol), $[\alpha]_D$ +48° (*c* 1, chloroform) (Found: C, 45.9; H, 5.6; S, 13.8. $C_{18}H_{26}O_{10}S_2$ calc.: C, 45.9; H, 5.6; S, 13.85%). N.m.r. data: τ *ca.* 2.70 (singlet, 5 aromatic protons); 4.92 (singlet, H-1); 5.45 (AB quartet, *J* 12 Hz, benzyl methylene protons); 6.89, 6.95 (singlets, each 3 protons, OMs); 8.54 and 8.70 (singlets, each 3 protons, CMe_2).

6-Deoxy-2,3-O-isopropylidene-α-D-mannofuranose (**7**). — A solution of sulphonate **6** (1.7 g) in dry ether (50 ml) containing lithium aluminium hydride (1 g) was heated under gentle reflux for 18 h, whereafter ethyl acetate and water were added to decompose the excess of reagent. Solid material was filtered off, the solvents were removed, and the major component (1.1 g) of the product mixture was separated by chromatography on silica gel. A portion (0.51 g) of this component in methanol (25 ml) containing 10% palladised charcoal (0.3 g) was shaken overnight at room temperature with hydrogen at a pressure slightly greater than one atmosphere. Removal of the catalyst and solvent left a thick syrup which crystallised on standing. Recrystallisation from ether–light petroleum gave the acetal **7** (0.2 g), m.p. 90–91°, $[\alpha]_D$ −8° (*c* 1, water), whose i.r. spectrum was indistinguishable from that of the L enantiomer[9], m.p. 90°, $[\alpha]_D$ +6.4° (*c* 1, water).

Benzyl 6-azido-6-deoxy-2,3-O-isopropylidene-α-D-mannofuranoside (**8**). — A solution of sulphonate **6** (2 g) in *N,N*-dimethylformamide (20 ml) containing sodium azide (2 g) was heated for 4 h at 70°, whereupon t.l.c. showed that all of the starting material had reacted. Water (30 ml) was added, and the solution was extracted with chloroform (2 × 50 ml), which was washed thoroughly with water and dried ($MgSO_4$). Removal of the solvent and distillation of the residue yielded the azide **8** (1.5 g, 87%), b.p. 140–150°/0.1 mm, $[\alpha]_D$ +67° (*c* 1.4, chloroform), ν_{max} 2100 cm^{-1} (N_3) (Found: C, 56.7; H, 6.6; N, 12.1. $C_{16}H_{21}N_3O_5$ calc.: C, 57.3; H, 6.3; H, 12.5%). N.m.r. data: τ *ca.* 2.70 (singlet, 5 aromatic protons), 4.93 (singlet, H-1), 5.40 (AB quartet, *J* 12 Hz, benzyl methylene protons), 8.55 and 8.69 (singlets, each 3 protons, CMe_2).

Benzyl 6-azido-6-deoxy-2,3-O-isopropylidene-5-O-methanesulphonyl-α-D-mannofuranoside (**9**). — (*a*) The azide **8** (0.1 g) in dry pyridine (5 ml) was treated with a slight excess of methanesulphonyl chloride for 3 h at room temperature, and the solution was then processed in the usual way. Compound **9** (0.12 g, 97%) had m.p. 73–74° (from ether–light petroleum), $[\alpha]_D$ +49.5° (*c* 1, chloroform), ν_{max} 2100 cm^{-1} (N_3) (Found: C, 49.05; H, 5.8; N, 9.7; S, 8.1. $C_{17}H_{23}N_3O_7S$ calc.: C, 49.4; H, 5.6; N, 10.2; S, 7.7%). N.m.r. data: τ *ca.* 2.70 (singlet, 5 aromatic protons), 4.94 (singlet, H-1), 5.45 (AB quartet, *J* 12 Hz, benzyl methylene protons), 6.95 (singlet, 3 protons, OMs), 8.56 and 8.72 (singlets, each 3 protons, CMe_2).

(*b*) A solution of the disulphonate **10** (3 g) in *N,N*-dimethylformamide (30 ml) containing sodium azide (0.48 g) was heated overnight at 85–90°, and the solution was processed essentially as described in the previous azide-exchange reaction.

Recrystallisation from ether–light petroleum gave compound **9** (2.6 g, 98%), m 73–74°, $[\alpha]_D$ +51° (*c* 1, chloroform), which could not be distinguished (i.r. and n.m spectroscopy, mixed m.p.) from the product obtained in (*a*).

Benzyl 5,6-acetylepimino-5,6-dideoxy-2,3-O-isopropylidene-β-L-gulofuranosi (**11**). — A solution of **9** (2 g) in ether (50 ml) containing lithium aluminium hydri (*ca.* 1 g) was stirred for 16 h at room temperature, whereupon the excess of reage was decomposed by adding ethyl acetate and water. Removal of solids and the solver left a semi-crystalline material (1.2 g, ~85%) which failed to recrystallise sat factorily. A portion of crude epimine (0.14 g) was dissolved in dry methanol (6 m acetic anhydride (2 ml) was added, and the solution was left for 2 h at room temper ture. Removal of the solvents (aided by co-distillation with toluene) afforded a thi syrup which was chromatographed on silica gel to give the acetylepimine **11** (0.14 87%), m.p. 61–62° (from chloroform–light petroleum), ν_{max} 1710 cm^{-1} (NAc $[\alpha]_D$ +26 ±2° (*c* 0.6, chloroform) (Found: C, 65.2; H, 7.2; N, 3.6. $C_{18}H_{23}NO_5$ calc C, 64.85; H, 6.95; N, 4.2%). N.m.r. data: τ *ca.* 2.70 (singlet, 5 aromatic protons 4.85 (singlet, H-1), 5.43 (AB quartet, *J* 12 Hz, benzyl methylene protons), 7.7 (singlet, 3 protons, NAc), 8.52 and 8.70 (singlets, each 3 protons, CMe_2).

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Note

The identity of madurose with 3-*O*-methyl-D-galactose

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Madurose has been the term used[1] to refer to an unknown sugar found in the whole-cell hydrolyzates of certain filamentous bacteria known as actinomycetes. These include members of the genera *Actinobifida, Actinomadura, Dermatophilus, Microbispora, Planomonospora, Spirillospora,* and *Streptosporangium.* The presence of this compound in cells has been found to have taxonomic significance[2].

Previously[3], it has been reported that the sugar, which gives a brown color upon reaction with aniline phthalate, is nonfermentable and migrates just ahead of arabinose in many paper-chromatographic systems. It is not present in the hydrolyzates of purified cell walls of the above organisms[4], and is not considered part of the murein. Mild hydrolytic conditions are required to release the madurose-containing polysaccharide from whole cells. Extraction with neutral solvents is ineffective.

We have isolated the sugar from a strain of *Actinomadura madurae* by acid hydrolysis of the whole cells, followed by purification by ion-exchange and preparative paper chromatography. The per(trimethylsilyl) ether derivative has been purified by preparative gas chromatography. The mass spectrum of this derivative showed small but real peaks at 467 (M—Me) and 435 (M—Me and MeOH) mass units which suggested an *O*-methylhexose. An examination of the entire mass spectrum, in the light of the excellent identification scheme given by Petersson and Samuelson[5] for the determination of the number and position of methyl groups in methylated aldohexoses, indicated that madurose was a 3-*O*-methylhexose. Finally, comparison of madurose with authentic 3-*O*-methyl-D-galactose[6] by paper and gas chromatography showed the compounds to have identical characteristics. The D configuration was established by the use of D-galactose oxidase[7]. Thus, we wish to report the addition of actinomycetes to the list of natural sources of 3-*O*-methyl-D-galactose. These have included slippery elm mucilage[8] and sassafras polysaccharide[9,10]. As far as we know, this is the first time that this sugar has been isolated from a microorganism.

EXPERIMENTAL

Fermentation. — *Actinomadura madurae* N15 grown on yeast-extract-dextrose agar slants [Difco yeast extract (10 g), cerelose (crude dextrose) (10 g), agar (15 g), and

tap water (1000 ml), pH 7.2] at 28° for 2 weeks was used as inoculum for two 250-ml Erlenmeyer flasks containing 50 ml of the just described medium without agar (YD). After 6 days of continuous shaking at 215 r.p.m. at 28° (rotary-action shaker, Model V, New Brunswick Scientific Co., Inc., New Brunswick, N.J.), the resultant growth was used at a concentration of 5% to inoculate 20 more flasks of the same type. After shaking for 2 days, this growth was used to inoculate 20 2-liter Erlenmeyer flasks containing 200 ml of YD. After agitation on a reciprocal shaker at 60 strokes per minute (model T-R, New Brunswick Scientific Co.) for 3 days, the wet cells, which were harvested and washed by filtration, were hydrolyzed with 0.5M sulfuric acid for 3h, at 97°.

Isolation and purification. — The hydrolyzate was partially neutralized to pH 5.5 with solid barium hydroxide, the resulting suspension centrifuged, and the supernatant lyophilized. Aqueous solutions of the dried residue were passed successively through columns of Amberlite IR-120 (H^+) and IR-45 (OH^-), and the effluent was lyophilized. Aqueous solutions of this material were spotted on Whatman No. 3MM paper, and the chromatograms were developed by descending chromatography in either water-saturated butyl alcohol or 5:3:3:4 butyl alcohol–pyridine–water–toluene for 72 or 24 h, respectively. The various sugars present were detected at the edges of the paper with the aniline phthalate reagent. The bands of madurose were cut out, and eluted with distilled water, and the eluates were lyophilized.

The residues were treated for 10–30 min in closed vials with an excess of "Sil-Prep" reagent (Applied Science Labs., Inc., P.O. Box 440, State College, Pa.), and then the solutions were diluted with water and extracted with cyclohexane. The concentrated cyclohexane solutions were chromatographed directly on a column (6-foot × 0.25 inch) of 10% SE 30 on Diatoport W, 60-80 mesh, in an F and M Model 700 dual-column gas chromatograph equipped with a thermal conductivity detector. For analytical gas chromatography, the conditions were as follows: He, 46 ml per min; column temperature, 190° isothermal; injection port temperature, 260°; detector temperature and voltage, 266° and 150 mV. The chromatograms of madurose and authentic 3-*O*-methyl-D-galactose are reported in Fig. 1. For comparison, authentic samples of the various sugars were kept in aqueous solution at room temperature for 3 days prior to "Sil-Prep" treatment.

For the preparation of a 1-mg sample of the per(trimethyl)silyl derivative of madurose for mass spectroscopy, the column was programmed at 4° per min starting at 140°; the other conditions remained the same. A solution of the crude material (about 3 mg) in 100 μl of solvent was injected, and up to 1 mg of purified product was collected at the exit port with a glass-capillary tube (117 × 1.3 O.D. mm). Only the two major, overlapping, peaks were collected at 20.2 min. Ribitol, which was present as a contaminant, and was identified by comparison with an authentic sample both by gas and paper chromatography, was well separated from madurose; it had a retention time of 18.8 min. Mass spectroscopy was performed with a Hitachi-Perkin Elmer RMU-7 using an ionization potential of 70 eV, and chamber and inlet temperatures of 200° and 155°, respectively.

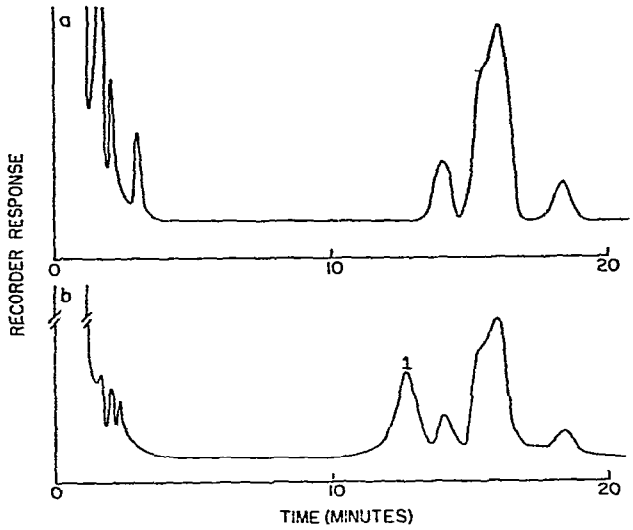

Fig. 1. Gas chromatograms of per(trimethylsilyl) ether derivatives of equilibrated sugar samples: (a) authentic 3-*O*-methyl-D-galactose; (b) madurose (1, ribitol contaminant).

Samples of D-galactose, authentic 3-*O*-methyl-D-galactose, and madurose were treated with a commercial D-galactose oxidase preparation (Galactostat Reagent, Worthington Biochemical Corp., Freehold, N.J., U.S.A.) according to the directions

TABLE I

MOBILITY OF MADUROSE AND OTHER SUGARS ON PAPER CHROMATOGRAMS IN VARIOUS SOLVENTS[a]

Sugars	*Solvent systems*[b]		
	A	*B*	*C*
Madurose	0.72	0.73	0.43
3-*O*-Methyl-D-galactose	0.72	0.73	0.43
6-*O*-Methyl-D-galactose	0.76	0.85	0.52
D-Galactose	0.27	0.37	0.18
D-Glucose	0.34	0.40	0.19
D-Allose	0.41	0.48	–
D-Mannose	0.47	0.52	0.28
L-Arabinose	0.58	0.63	0.43
D-Altrose	0.72	0.63	–
L-Fucose	0.80	0.86	–
2-Deoxy-D-galactose	0.83	0.88	–
2-Deoxy-D-glucose	0.99	0.95	–
3-*O*-Methyl-D-glucose	1.06	0.97	–
L-Rhamnose	1.26	1.14	1.00

[a]Mobility relative to D-ribose; descending paper chromatography on Whatman No. 1 paper. [b]A, Butyl alcohol–pyridine–water–toluene (5:3:3:4, upper phase), 48 h at 25°; B, ethyl acetate–acetic acid–water (3:1:3, upper phase), 12 h at 18°; C, sodium borate–saturated butanone–water azeotrope, 41 h at 25° (Ref. 13).

of the manufacturer. The chromogen was oxidized, in all 3 cases, to compounds having a broad peak of absorption at approximately 429 nm, thereby establishing the D configuration for madurose.

From our own results and those of others[11,12], it would seem that the gas-chromatographic comparison of per(trimethylsilyl) ethers of sugars equilibrated in aqueous solution should serve as a valuable "fingerprint" for identification. For example, equilibrated 3-*O*-methyl-D-glucose yielded, using the same conditions as those reported for Fig. 1, three peaks having retention times (relative to D-glucose, the asterisk indicating a major peak) of 0.58*, 0.69, and 0.86*, 6-*O*-methyl-D-galactose, peaks at 0.54*, 0.66*, and 0.79* (spacing and peak area clearly resembling the published data[12]) and D-galactose, peaks at 0.75, 0.90* and 1.08*. Madurose and 3-*O*-methyl-D-galactose had peaks at 0.59, 0.66* (shoulder), 0.67* and 0.77.

A comparison of madurose with other sugars by paper chromatography in several solvent systems is given in Table I.

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The authors wish to thank Drs. B. Lindberg and J. K. N. Jones for a sample of authentic 3-*O*-methyl-D-galactose, and Dr. H. S. Isbell for authentic allose and altrose. The technical assistance of Mrs. P. F. Gregorowicz and Mr. E. Martin is gratefully acknowledged. This work was supported by a National Science Foundation grant (GB-7675).

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Note

Further considerations of the kinetics of acid hydrolysis of cellotriose

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Recent experimental work[1] has shown that the hydrolysis rate $[k_3^{(2)}]$ of the glucosidic bond near the non-reducing end of cellotriose is 1.53 times greater than that near the reducing end $[k_3^{(1)}]$ for hydrolysis in 50% and 5% sulphuric acid. Further, both rate constants are significantly smaller than that for cellobiose (k_2) under the same conditions. Consequently, acid-catalyzed hydrolysis of cellotriose proceeds according to Case I (ref. 2), and

$$e^{k_2 t}(1-\alpha) = B(e^{-Ct}-1)+1 \simeq -BCt+1 \text{ for small values of } t, \quad (1)$$

where $C = k_3^{(1)}+k_3^{(2)}-k_2$

$B = (C-k_2)/2C$

α = degree of bond splitting in the hydrolysis of cellotriose.

Since $k_3^{(2)} = 1.53k_3^{(1)}$, equation (*1*) can be written as

$$e^{k_2 t}(1-\alpha) = (k_2-1.265k_3^{(1)})t+1. \quad (2)$$

For small values of t, both the measured[2] and corrected[3,4] values of α are nearly identical, which implies that side reactions are negligible in the early periods of the hydrolysis of cellotriose. Under these conditions, the plotted values of $e^{k_2 t}(1-\alpha)$ *vs.* t lie on a straight line, and $k_3^{(2)}$ can be estimated from the slope of the line. As shown in the following tabulated data, cellobiose is hydrolyzed 1.4 times faster than the glucosidic bond near the non-reducing end of the cellotriose molecule.

Hydrolysis conditions	$k_2 \times 10^6$ (sec^{-1})	$k_3^{(1)} \times 10^6$ (sec^{-1})	$k_3^{(2)} \times 10^6$ (sec^{-1})	$k_2/k_3^{(2)}$
65% H_2SO_4, 6°[a]	1.35	0.630	0.963	1.4
18°[b]	11.10	5.185	7.950	1.4
51% H_2SO_4, 18°[b]	1.56	0.733	1.117	1.4
30°[b]	11.33	5.290	8.080	1.4

[a]Ref. 5. [b]Ref. 3.

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Note

A sulfenyl iodide in the mixture formed by oxidation of a sugar xanthate with iodine

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Oxidation of xanthates by iodine gives dithiobis(thioformates) according to the equation

$$2RO(C=S)SNa+I_2 \rightarrow [RO(C=S)S]_2+2NaI$$

This coupling reaction with iodine and with other oxidants has been employed for over a century to prepare products having industrial utility. There have been numerous reports of these oxidative reactions[1], but the mechanism of the reaction represented by the equation has not been investigated.

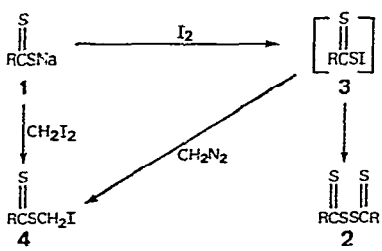
Our work with carbohydrate xanthates and the corresponding dithiobis(thioformates)[2] suggested that these systems might be appropriate for studying the coupling reaction, since the carbohydrate dithiobis(thioformates) are usually crystalline and are readily characterized. The present report proves that a carbohydrate sulfenyl iodide is formed during the oxidation of a sugar xanthate by iodine.

When a concentrated solution of 1,2:5,6-di-*O*-isopropylidene 3-*O*-(*S*-sodium dithiocarbonyl)-α-D-glucofuranose (**1**) was treated with a concentrated solution of iodine, t.l.c. of the mixture showed one major spot — the known dithiobis(thioformate) (**2**), and two minor spots, a slower-migrating one identified as 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, and a faster-migrating one. The latter component, formulated as 3-*O*-(*S*-iododithiocarbonyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**3**), was easily observed on t.l.c. because of its brown color on the white background of the plate. On spraying the t.l.c. plate with a solution of starch, the brown spot turned immediately blue, indicating the presence of iodine. However, during attempts to remove the component from the plate it lost iodine and became transformed into **2**. Previously[3] we showed that **2** reacts very readily with chlorine to give the corresponding chloromethylsulfenyl chloride, which on standing gives acidic gases. The possibility of formation of a similar product with the oxidation with iodine was rejected since pure **2** did not react with iodine to give **3**. The structure proposed for **3** was proved through a more stable derivative prepared by reaction of **3** with diazomethane.

Although **2** remained intact on treatment with diazomethane under neutral

conditions, **3** gave 3-*O*-[(iodomethylthio)thiocarbonyl]-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**4**), which was identified by elemental analysis, u.v., i.r., and n.m.r. spectroscopy, and by its synthesis by a different route. The insertion of a methylene group by reaction of a sulfenyl chloride derivative with diazomethane is known[4]. The u.v. spectrum of **4** showed absorption maxima at 235 and 290 nm, whereas the known 1,2:5,6-di-*O*-isopropylidene-3-*O*-[(methylthio)thiocarbonyl]-α-D-glucofuranose absorbed at 230 and 280 nm. The latter formed when **2**, in the presence of alkali, reacted with diazomethane. The n.mr. spectrum of **4** in carbon disulfide showed a singlet for the methylenic ester group somewhat overlapping the doublet of the H-2 signal that was centered at τ 5.5. Irradiation of H-1 resulted in a sharpening of the singlet. The independent synthesis of **4** was done by treating **1** with diiodomethane. T.l.c. of the reaction mixture revealed two main components, identified as **4** and bis(1,2:5,6-di-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose)dithiomethylene. Compound **4** is unstable and darkens when kept overnight at room temperature; after a few days, t.l.c. showed several components, one of which was identified as 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. The decomposition is accelerated by exposure to heat and u.v. light.

When bis(methyl 4,6-*O*-benzylidene-α-D-glucopyranoside) 2,2′-dithiobis(thioformate) (**5**) was prepared from the corresponding xanthate (**6**) and iodine, it decomposed with loss of the benzylidene group when kept in methanol for 3 h at 25°. However, when **5** was prepared from **6** and sodium chlorite, it underwent no change during several h in methanol. The difference in behavior of the two preparations might be due to the presence of small amounts of sulfenyl iodide in the former preparation. The sulfenyl iodide intermediate would decompose with liberation of hydrogen iodide, which would cause hydrolysis of the benzylidene group.



R = 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-*O*-

EXPERIMENTAL

Melting points were determined with a Fisher–Johns* apparatus and are uncorrected. Optical rotations were measured in a 1-dm tube with a Rudolph polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 137 or 621 spectrophotometer having silver chloride optics as Nujol mulls or films, and the u.v. spectra were recorded with a Perkin–Elmer Model 202 spectrophotometer. N.m.r. spectra

*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.

were recorded by a Varian HA-100 spectrometer with tetramethylsilane as internal reference standard (τ = 10.00). For t.l.c. Silica Gel G was used as the adsorbent, 9:1 (v/v) carbon disulfide–ethyl acetate as the solvent, and 19:1 (v/v) methanol–sulfuric acid as the spray reagent. Diazomethane was prepared from 1-methyl 1-nitrosourea[5].

Bis(1,2:5,6-di-O-isopropylidene-α-D-glucofuranose) 3,3'-[dithiobis(thioformate)] (2). — *A. With iodine.* 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (1 g) in methyl sulfoxide (0.5 ml) and carbon disulfide (1 ml) was treated with 5M sodium hydroxide solution (1 ml) for 5 min. The solution was cooled to 5°, neutralized with acetic acid (5M), and the xanthate thus produced was crosslinked by iodine solution (0.38M). A syrupy, dark precipitate formed upon crosslinking. The color was apparently not caused by free iodine since it could not be removed by washing with water or with an aqueous solution of sodium iodide. However, when the syrup was dissolved in ether and washed with an aqueous solution of sodium thiosulfate the dark color turned to bright yellow. The ether solution was washed with water, dried ($MgSO_4$), and evaporated to a heavy syrup, which subsequently crystallized to give **2**.

B. With sodium chlorite. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (1 g) in methyl sulfoxide (0.5 ml) and carbon disulfide (1 ml) was treated with 5M sodium hydroxide solution (1 ml) for 5 min. The mixture was cooled to 0°, sodium chlorite (0.5 g) in water (2 ml) was added, followed by 5M acetic acid until the solution became neutral. T.l.c. of an ether extract showed **2**, together with traces of the starting sugar. Evaporation of the ether gave a heavy syrup which was crystallized from ethanol to give pure **2**. This product was stable during 3 h in methanolic solution, whereas **2** prepared with iodine partly decomposed upon like treatment.

3-O-[(Iodomethylthio)thiocarbonyl]-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**4**). — *A.* The dark syrup obtained by crosslinking the xanthate with iodine (see part *A* of the previous section) was dissolved in ether, cooled to 5°, and treated with an excess of a cold, ethereal solution of diazomethane. The solution turned bright yellow. After about 15 min, **4** was isolated by preparative t.l.c. with ether as eluent; yields 1–2% (based on 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose).

Anal. Calc. for $C_{14}H_{21}O_6S_2I$: S, 13.4; I, 26.6. Found: S, 13.0; I, 25.0.

B. Compound **4** was also prepared from the corresponding xanthate and diiodomethane. The xanthation was carried out as before and the mixture was neutralized and treated with diiodomethane (0.5 ml for every 1 g of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose). After about 15 min, t.l.c. of an ethereal extract showed two main components. Isolation of the components from preparative t.l.c. plates gave **4**, together with bis(1,2:5,6-di-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose)dithiomethylene (m.p. 112–113° from hexane).

Anal. Calc. for $C_{27}H_{40}O_{12}S_4$: C, 47.3; H, 5.8; S, 18.6; mol. wt., 684. Found: C, 46.9; H, 6.0; S, 18.8; mol. wt., 654 (Rast in camphor).

Reaction of **2** *with diazomethane.* — No reaction took place (t.l.c. and u.v.) when diazomethane was added to **2** dissolved in ether. However, when a drop of alkali was added to the mixture, t.l.c. revealed the presence of a compound having a R_F value higher than that of **2**; the product was isolated by preparative t.l.c. and

shown (i.r. and u.v. spectra, and t.l.c.) to be the known 1,2:5,6-di-*O*-isopropylidene-3-*O*-[(methylthio)thiocarbonyl]-α-D-glucofuranose. The latter had been previously prepared[7] by reaction of the corresponding xanthate with iodomethane.

Bis(methyl 4,6-O-benzylidene-α-D-glucopyranoside) 2,2'-[dithiobis(thioformate)] (**5**). — *A. With iodine.* Compound **5** was prepared by oxidation of the corresponding xanthate with iodine according to a procedure described earlier[6]. The product, which contained mostly the 2,2' isomer (about 85%), was kept at 25° in methanol. The hydrolysis of the benzylidene group was monitored by t.l.c. and verified by i.r. spectroscopy. In *p*-dioxane the hydrolysis was much slower and in ether it was undetectable.

B. With sodium chlorite. Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (1 g) in 3 ml of *p*-dioxane was treated with 5M sodium hydroxide (1 ml) and carbon disulfide (1 ml). Sodium chlorite (0.5 g) in water (2 ml) was added and the mixture was kept for about 10 min while an exothermic reaction took place. The product was then extracted with ether and the extract washed with water to give, besides the starting sugar, one major spot on t.l.c. which corresponded to methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-thionocarbonate and a minor spot corresponding to **5**. The structure of the thionocarbonate was verified by u.v. spectra (λ_{max}^{MeOH} 238 nm).

The xanthation reaction was repeated under slightly different conditions to give mostly **5**. Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (1 g) in *p*-dioxane (3 ml) was treated with 5M sodium hydroxide (1 ml) and carbon disulfide (1 ml). After 5 min at 25° the mixture was cooled to 0° and then treated with sodium chlorite (0.5 g in 2 ml of water). The reaction mixture was brought to pH about 4 with 5M acetic acid. Thorough mixing and slow addition of the acid during neutralization was found to be essential. After 10 min the mixture was extracted with ether and washed with water as above. The product thus formed (**5**) was stable (t.l.c.) on standing in methanol for 3 h.

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Note

The isolation of both a *talo*-heptulose and an *allo*-heptulose from the avocado. Some new paper-chromatographic data on heptuloses

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(Received December 19th, 1969)

In 1960, Charlson and Richtmyer[1] reported the isolation of a *talo*-heptulose from the avocado. They were unable to induce crystallization in their small sample by inoculation with synthetic D-*talo*-heptulose, but proof of its structure as a *talo*-heptulose seemed reliable, because, upon degradation with oxygen in cold alkaline solution, it yielded a lactone that was indistinguishable from D-talonolactone on paper chromatograms; the D configuration was tentatively assigned to the *talo*-heptulose, because all other naturally occurring higher-carbon ketoses then known (as well as those since discovered) belong to the D configurational series. In 1966, Begbie and Richtmyer[2] reported the isolation of crystalline D-*allo*-heptulose from primula roots.

Having new supplies of both L-*allo*- and D-*talo*-heptulose available for comparisons, we decided to try to confirm, by gas–liquid chromatography (g.l.c.), the presence of a *talo*-heptulose in the sample (X) isolated[1] from the avocado. The data obtained from studies with four different columns (see the Experimental section) demonstrated conclusively that the avocado sample did indeed contain a *talo*-heptulose; moreover, it also contained an *allo*-heptulose, as well as small proportions of other constituents, presumably polyhydric alcohols.

Because we knew that the *allo*- and *talo*-heptuloses are not readily separated from each other by paper chromatography, we tested a number of solvent systems to see if one could be found that would separate them. One system (described in the Experimental section), composed of butanone, acetic acid, and saturated aqueous boric acid, cleanly separated the two heptuloses in 64 hours; and, when the avocado sample (X) was treated similarly on a paper chromatogram, two spots, respectively having the same mobilities as L-*allo*-heptulose and D-*talo*-heptulose, were visibilized with an orcinol spray that gives a characteristic color with these heptuloses. During the course of our search, we measured the mobilities, in five solvent systems**, of the six heptuloses that were available to us, as well as that of sedoheptulosan

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**For the mobilities of some heptuloses in other solvent systems, see Ref. 3.

(2,7 anhydro-β-D-*altro*-heptulopyranose) compared to that of D-*manno*-heptulose (see Table I).

TABLE I

MOBILITIES OF SOME HEPTULOSES IN DIFFERENT SOLVENT-SYSTEMS

Heptulose	*Solvent system*				
	1	*2*	*3*	*4*	*5*
L-*galacto*-	0.90	0.91	0.95	0.97	1.21
D-*gluco*-	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
D-*manno*-	1.01	0.97	1.04	1.00	1.42
D-*altro*-	1.17	1.03	1.26	1.15	1.54
L-*allo*-	1.23	1.17	1.38	1.28	1.58
D-*talo*-	1.28	1.17	1.48	1.32	2.05
D-*talo* / L-*allo*-	1.04	1.02	1.07	1.03	1.24
Sedoheptulosan / D-*manno*-Heptulose	1.00	1.10	1.06	1.36	1.47

In summary, the presence of a *talo*-heptulose in the avocado has been confirmed both by g.l.c. and by paper chromatography, and the presence of an *allo*-heptulose in a plant has been shown for the second time. It is likely that these avocado heptuloses belong to the D configurational series.

EXPERIMENTAL

General. — G.l.c. was performed with an F & M Model 5750 Research Gas Chromatograph equipped with a flame-ionization detector; the columns were 200 × 0.5 cm, and the flow rate was 75 ml of helium/min. The crystalline heptuloses were first dissolved in water, and the solution was kept overnight to equilibrate and then freeze-dried (so as to be similar to the sample from the avocado). The per(trimethylsilyl (TMS) derivatives were prepared by adding pyridine and Regisil [bis(trimethylsilyl)trifluoroacetamide]* to the lyophilized compound, and letting the mixture stand overnight. Details of the individual columns are as follows. Column A (copper) was packed with 15% by weight of 2,2-dimethyl-1,3-propanediol (neopentyl glycol) succinate polyester on Chromosorb W (80–100 mesh); column B (copper), with 10% by weight of neopentyl glycol sebacate polyester on Chromosorb W (80–100 mesh); and column C (stainless steel), with 1% by weight of SE-30 (a methylsilicone polymer) on Gaschrom P (80–100 mesh). For columns A, B, and C, the column temperature

*Tri-Sil Z [*N*-(trimethylsilyl)imidazole in pyridine solution] was also tried, but it was unsatisfactory, because it gave "ghost peaks" when injected into the polyester columns; these peaks were probably due to products formed by cleavage of some of the polyester linkages under the influence of the basic, imidazole derivative.

was 180°. Column D (stainless steel) was packed with 10% by weight of SE-30 on Chromosorb W (80–100 mesh); the column temperature was 205°.

In the following discussion, retention times (r.t.) of the TMS derivatives are all relative to that of the tetra-TMS ether of TMS α-D-glucoside.

Results with column A. — The TMS derivative of D-*talo*-heptulose showed two peaks having r.t. 1.30 and 1.53; that of L-*allo*-heptulose showed a single peak having r.t. 1.34; and a mixture of the two showed peaks having r.t. 1.32 (obviously a combination of the 1.30 and 1.34 peaks) and 1.52. The TMS derivative of the avocado syrup (X) showed peaks having r.t. 1.33 and 1.54; the latter r.t. clearly indicates the presence of *talo*-heptulose in the mixture.

Results with column B. — The TMS derivative of D-*talo*-heptulose again showed two peaks, having r.t. 1.31 and 1.56; that of L-*allo*-heptulose showed a single peak having r.t. 1.42; and a mixture of the two showed all three peaks (though with considerable overlapping) having r.t. 1.34 (as a shoulder), 1.42, and 1.58. The TMS derivative of the avocado syrup (X) showed the same three peaks having r.t. 1.34 (as a shoulder), 1.41, and 1.59.

Results with column C. — The TMS derivative of D-*talo*-heptulose here showed three peaks having r.t. 1.32, 1.64, and 1.89; that of L-*allo*-heptulose now showed two peaks having r.t. 1.62 and 1.81; and a mixture of the two showed distinct peaks having r.t. 1.33, 1.64, and 1.81 followed by a slight shoulder undoubtedly derived from the peak having r.t. 1.89 (D-*talo*-heptulose). The TMS derivative of the avocado syrup (X) showed an almost identical pattern, with peaks having r.t. 1.31, 1.66, 1.81, and a shoulder having a slightly higher r.t.

Results with column D. — The TMS derivative of D-*talo*-heptulose again showed three peaks, having r.t. 1.29, 1.54, and 1.60, the last being a shoulder; that of L-*allo*-heptulose showed two distinct peaks having r.t. 1.54 and 1.75; and a mixture of the two showed four peaks having r.t. 1.29, 1.56, 1.62 (shoulder), and 1.76. The TMS derivative of the avocado syrup (X) showed an almost identical pattern of four peaks having r.t. 1.29, 1.57, 1.63 (shoulder), and 1.77.

Paper chromatography.— Paper chromatography was performed on Whatman No. 1 filter paper by the descending method at room temperature. The solvent systems used were (*1*), 6:4:3 (v/v) butyl alcohol–pyridine–water; (*2*), 40:11:19 (v/v) butyl alcohol–ethyl alcohol–water; (*3*), 72:20:23 (v/v) ethyl acetate–pyridine–water (upper phase); (*4*) 18:3:1:4 (v/v) ethyl acetate–acetic acid–formic acid–water; and (*5*) 9:1:1 (v/v) butanone–acetic acid–saturated aqueous boric acid. The spray reagent consisted of 2% of orcinol and 3% of concentrated hydrochloric acid in butyl alcohol, and the chromatograms were heated for 3 min at 100–110°; all of the sugars mentioned gave a blue color, except D-*manno*-heptulose, which gave a greenish blue color. Chromatograms developed with solvent (*5*) were preferably sprayed several times with methanol and allowed to dry each time in a hood (to remove the boric acid as methyl borate*)

*This procedure was suggested by Dr. C. P. J. Glaudemans of this laboratory.

before being sprayed with the orcinol–hydrochloric acid reagent; otherwise, it was necessary to spray and heat several times with the latter reagent to bring out the color.

Each of the other heptuloses was compared directly with D-*gluco*-heptulose as the standard. D-*talo*-Heptulose was also compared directly with L-*allo*-heptulose, and sedoheptulosan directly with D-*manno*-heptulose. The values recorded in Table I are the averages obtained from six spots on each chromatogram, and from chromatograms developed for various lengths of time; thus, in solvent (*1*), the chromatograms were developed for 24 and 40 h; solvent (*2*), 24 and 47 h; solvent (*3*) 16, 24, and 40 h; solvent (*4*), 17, 25 and, 41 h; and solvent (*5*) 24 h (except for the D-*talo*- *versus* L-*allo*-heptulose, for which the chromatograms were developed for 16, 24, 40, and 64 h). In addition to the solvent systems listed in Table I, two others were tried; in 4:1:1 (v/v) butyl alcohol–ethyl alcohol–water (24, 72, and 144 h), the *talo*-:*allo*-heptulose ratio was 0.97, and in 12:5:4 (v/v) ethyl acetate–pyridine–4% aqueous boric acid (16 and 24 h), the ratio was 1.05.

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Note

Preparation of trialcohols and some of their derivatives from nucleosides

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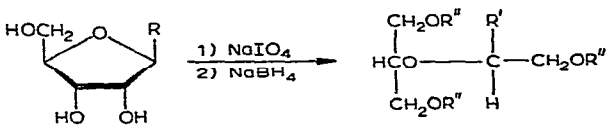
The nucleoside trialcohols have been known for at least a decade, and studies have been made of some of their chemical characteristics[1]. However, they have not been obtained as analytically pure compounds and therefore could not be completely characterized nor their potential biological properties be studied. For example, they may be potential inhibitors of enzymes of the nucleic acid metabolism, or can be used as reference compounds in the periodate oxidation–reduction of the terminal end of RNA[2]. The preparation, purification, and chemical characterization of the nucleoside trialcohols derived from adenosine, uridine, and cytidine, are the subjects of this report.



1 R = uracil-1-yl
2 R = adenin-1-yl
3 R = cytosin-1-yl
4 R′ = uracil-1-yl; R″ = H
5 R′ = uracil-1-yl; R″ = *p*-nitrobenzoyl
6 R′ = adenin-1-yl; R″ = H
7 R′ = adenin-1-yl; R″ = COPh
8 R′ = cytosin-1-yl; R″ = H
9 R′ = cytosin-1-yl; R″ = COPh

Each nucleoside trialcohol was isolated by a different procedure. The uracilyl derivative **4** was purified by application of ion-exchange chromatography. The adeninyl derivative (**6**) was converted into a picrate salt, which was purified by recrystallization. Regeneration of pure **6** was accomplished with an anion-exchange resin in the carbonate form[3]. In contrast to **6**, it was not possible to form a stable, crystalline picrate of the cytosinyl derivative (**8**). The general methodology, useful in the isolation of **4**, gave only very small amounts of **8**. Therefore, the crude nucleoside (**8**) was isolated as the organic soluble acetate and was washed free of salts. Removal of the acetate groups and column chromatography yielded pure **8**. In no case was it possible to achieve crystallization of any of the nucleoside trialcohols from common solvents. They were finally obtained as hygroscopic powders after lyophilization, and it was necessary to further dry these powders under high vacuum at elevated temperature.

The nucleoside trialcohols gave highly characteristic crystalline derivatives. Compounds **6** and **8** gave tribenzoates after purification *via* the picrate salts. Benzoylation of **4** did not give a crystalline derivative; therefore, a tri-*p*-nitrobenzoate (**5**) was characterized.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage and are corrected. Elementary analyses were determined by the Baron Consulting Co., Orange, Connecticut. T.l.c. was performed on Brinkmann F_{254} silica gel plates, and spots were located with a Mineralight lamp. The following solvent systems were used: (*A*) 5% aqueous disodium hydrogen phosphate; (*B*) 86:14 butyl alcohol–water; (*C*) 6:3:1 2-propanol–conc. ammonium hydroxide–water; (*D*) 9:1 chloroform–methanol; and (*E*) 9:1 ethyl acetate–methanol (all proportions v/v). Evaporations were performed *in vacuo* in a rotary evaporator with a bath temperature of 40–50°.

1-(1,3-Dihydroxy-2-propyl)-1-uracil-1-yl-1(R),*2-ethanediol*(**4**). — To a mixture of uridine (**1**, 2.5 g) and water (45 ml) was added sodium periodate (2.45 g) in small portions, while maintaining the temperature at 20–25° with an ice-bath. The reaction mixture was kept in a refrigerator, overnight, and poured into abs. ethanol (150 ml). After stirring for 15 min, the salt was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in water (500 ml) and the solution was added, dropwise, to a stirred solution, protected from light[4], containing sodium borohydride (2 g) dissolved in water (50 ml). After the solution had been kept for an additional 2 h in the dark, the pH was adjusted to 7 with Amberlite IR-120 (H^+) resin. The resin was removed by filtration, and the water was evaporated to yield a residue which was dissolved in water. The solution was passed through an Amberlite IRC-50 (H^+) column, which was washed with water. The water was evaporated, and four 100-ml portions of methanol were added and evaporated to remove boric acid as methyl borate. The syrupy residue was dissolved in 30% aqueous methanol (20 ml), and the solution was applied to the top of a column of Dowex 1-X2 (OH^-, 200-400 mesh, 32 × 2.5 cm) which had been equilibrated with the same solvent[5]. The column was washed with 60% aqueous methanol (1200 ml), 90% aqueous methanol (2600 ml), and water (400 ml), and the product was eluted with 0.1M ammonium hydrogen carbonate solution. Fractions (8.5 ml) were collected, and the contents of tubes 97–110 were combined and evaporated to dryness. The residue was dissolved in water and lyophilized. The white solid was extremely hygroscopic and became gummy in a few minutes. Further drying in a drying pistol over phosporus pentoxide for 18 h at 100° gave 1.4 g, $[\alpha]_D^{23}+49\pm1°$ (*c* 1.7, water); $\lambda_{max}^{H_2O}$ 262 nm (ε 10,050) $\lambda_{min}^{H_2O}$ 230 nm (ε 1,930); i.r. data: 3350 (broad OH, C=NH), 1680 (-NHCO- of pyrimidinone), 1458 (uracil ring), 1112–1040 (plateau C-O, C-O-C), 812, and 782–760 cm^{-1} (pyrimidine CH); t.l.c.: R_F 0.75 (*A*), 0.24 (*B*) and 0.61 (*C*).

Anal. Calc. for $C_9H_{14}N_2O_6$: C, 43.90; H, 5.73; N, 11.38. Found: C, 43.88; H, 5.78; N, 11.40.

*Tri-*p*-nitrobenzoate* (**5**) *of* **4**. — Compound **4** (118 mg) was treated with

p-nitrobenzoyl chloride in pyridine for 25 h, and the mixture was finally heated on a steam-bath for 35 min. After the usual work-up, a syrup was obtained which solidified after standing for several h. The solid was triturated with methanol–chloroform, filtered off, and recrystallized from 1:1 acetone–methanol (12 ml). The tan-colored rosettes (252 mg) had m.p. 186–188°; i.r. data: 3480, 3060 (C=N–H), 1755, 1725 (*para*-benzenoid substitution), 1710 (benzoate C=O), (-NHCO- of pyrimidinone), 1600 (Ph and pyrimidine ring), 1520 (NO_2), 1345 (aromatic C–N), 1320 (NO_2), 1265 (benzoate C–O–C), 1102, 1092, 1078 (C–O, C–O–C), and 814 cm^{-1} (*para*-disubstituted Ph C–H); t.l.c.: R_F 0.70 (*D*) and (*E*).

Anal. Calc. for $C_{30}H_{23}N_5O_{15}$: C, 51.95; H, 3.34; N, 10.10. Found: C, 52.53; H, 3.42; N, 9.82.

1-(1,3-Dihydroxy-2-propyl)-1-(adenin-1-yl)-1(R),*2-ethanediol* (**6**). — Adenosine (**2**, 5.34 g) was suspended in water (80 ml), and sodium periodate (4.4 g) was added in small portions as described for **1**. The mixture was kept for 1.5 h at room temperature and then reduced as described for the preparation of **4**. After neutralization and filtration, the filtrate was evaporated to a small volume and adjusted with water to 250 ml.

A 100-ml aliquot from this solution was evaporated to dryness. Warm methanol (100 ml) was added to dissolve the residue, the solution was cooled to room temperature, 10% methanolic picric acid (200 ml) was added, and the solution was kept overnight at 0°. The yellow crystals (1.45 g) of the picrate were filtered off. A portion of the picrate (0.7 g) was recrystallized from methanol as tiny platelets (0.55 g), subliming at temperatures above 170° to form tiny needles, which decomposed and melted slowly with continued heating; $[\alpha]_D^{24}$ +43.0 ±0.4° (*c* 1.41, *N*,*N*-dimethylformamide); i.r. data: 3310–3110 (broad OH, NH), 1690 (protonated adenine ring), 1600 (Ph and purine ring), 1560, 1540 (NO_2, asymmetrical stretching), 1358 (aromatic C–N stretching), 1315 (NO_2, symmetrical stretching), 1115, and 1075–1040 cm^{-1} (C–O, C–O–C).

Anal. Calc. for $C_{16}H_{18}N_8O_{11}$: C, 38.54; H, 3.64; N, 22.50 Found: C, 38.56; H, 3.63; N, 22.80.

A sample of the picrate (760 mg) was suspended in water (350 ml). Bio-Rad AG1-X8 (CO_3^{2-}) ion-exchange resin was added in small portions with stirring until the yellow color had disappeared from the solution[3]. After an additional 1.5 h of stirring, the resin was removed by filtration. Evaporation of the water yielded a white foam, whereas lyophilization of an aqueous solution gave a white powder (345 mg), which was thoroughly dried *in vacuo* for 24 h at 65°; $[\alpha]_D^{23}$ +58.9 ±0.5° (*c* 1.63, water); $\lambda_{max}^{H_2O}$ 260 nm (ε 14,000); i.r. data: 3340 (broad OH, NH), 1638, 1592, 1570, 1475 (NH_2–C=N, purine ring), 1112, and 1040 cm^{-1} (plateau C–O); t.l.c.: R_F 0.88 (A), 0.29 (B), and 0.48 (C).

Anal. Calc. for $C_{10}H_{15}N_5O_4$: C, 44.61; H, 5.62; N, 26.01. Found: C, 44.45; H, 5.37; N, 26.09.

Tribenzoate (**7**) *of* **6** .— Compound **6** obtained from 0.7 g of the picrate was benzoylated with an excess of benzoyl chloride in pyridine. The viscous syrup,

obtained after the usual work-up, was dissolved in hot ethanol, decolorized with Norit A, and the volume was condensed to about 25 ml. To this solution was added 10% ethanolic picric acid (20 ml), and the mixture was heated at reflux for 1 h[6], and kept for 24 h at room temperature. The yellow crystals of the picrate were filtered off and recrystallized from methanol to yield 198 mg, m.p. 147-152°, with prior softening starting about 135°; i.r. data: 3080–3020 (NH), 1715 (benzoate C = O), 1690 (protonated adenine), 1605, 1578 (Ph and purine ring), 1545 (NO_2), 1360 (aromatic C–N), 1312 (NO_2), 1265 (benzoate C–O–C), 1105, 1093, 1065 (C–O, C–O–C), and 708 cm^{-1} (monosubstituted Ph).

Anal. Calc. for $C_{37}H_{30}N_8O_{14}$: C, 54.82; H, 3.73; N, 13.82. Found: C, 54.09; H, 3.72; N, 14.10.

To a solution of the picrate (188 mg) in acetone (40 ml) and water (10 ml) was added Bio-Rad AG1-X8 (CO_3^{2-}) resin in small portions until the yellow color had disappeared and the mixture was stirred for 1 h[7]. The resin was filtered off, and the solution was evaporated to a syrup, which was dried by several additions of ethanol followed by evaporation. The product was crystallized from methanol as clusters of needles (50 mg), m.p. 145–145.5°; i.r. data: 3280, 3080 (NH), 1715 (benzoate C=O), 1665 (NH_2–C = N), 1598, 1570 (Ph and purine ring), 1265 (benzoate C–O–C), 1115, 1082, 1065 (C–O, C–O–C), and 704 cm^{-1} (monosubstituted Ph); t.l.c.: R_F 0.60 (*D*) and 0.51 (*E*).

Anal. Calc. for $C_{31}H_{27}N_5O_7$: C, 64.01; H, 4.68; N, 12.03. Found: C, 64.37; H, 4.79; N, 12.21.

1-(1,3-Dihydroxy-2-propyl)-1-(cytosin-1-yl)-1(R),2-ethanediol (**8**). — Cytidine (**3**, 2.43 g) was dissolved in water (40 ml) and treated with sodium periodate (2.64 g) and sodium borohydride in the same manner as described for the preparation of **6**. After neutralization, filtration, and evaporation, three additions of ethanol and one of dry pyridine to the residue were followed by evaporation. The white solid was suspended in dry pyridine (50 ml) by trituration and treated with acetic anhydride (25 ml) for 26 h. After work-up, a syrup (1.91 g) was obtained; i.r. data: 3200–3000 (NH), 1740 (acetate C=O), 1660, 1620 (pyrimidine ring), 1230 (acetate C–O–C), 1125–1088, and 1050 cm^{-1} (C–O). The blocking groups were removed with methanolic ammonia (130 ml) that had been previously saturated at 0°. The residue was dissolved in a small amount of water and applied to a column of Dowex-1 X2 (OH^-, 200–400 mesh, 30 × 2 cm) resin[5]. The column was eluted with water and 12-ml fractions were collected. Fractions 26-40, which showed strong absorption at 270 nm, were combined and evaporated to a hard, clear syrup. Its solution in water was lyophilized, and the white powder was dried under high vacuum for 16 h at 65°, then for 1.5 h at 100° to give a hard, white hygroscopic glass (0.615 g); $[\alpha]_D^{22}$ +62 ±1° (*c* 1.6, water); $\lambda_{max}^{H_2O}$ 217 nm (ε 8,320), $\lambda_{min}^{H_2O}$ 250 nm (ε 5,600); i.r. data: 3340–3180 (broad OH, NH), 1675 (NH_2–C=N), 1635, 1610 (pyrimidine ring), 1115, 1060–1030 (C–O, C–O–C), and 778 cm^{-1} (pyrimidine CH); t.l.c.: R_F 0.78 (*A*), 0.13 (*B*), and 0.50 (*C*).

Anal. Calc. for $C_9H_{15}N_3O_5$:C, 44.08; H, 6.18; N, 17.13. Found; C, 44.25; H, 6.21; N, 17.06.

Tribenzoate (**9**) *of* **8**. — Compound **8** (96 mg) was benzoylated in a manner similar to the preparation of **7**. The gummy residue was dissolved in hot ethanol (5 ml). A solution of 5% ethanolic picric acid (10 ml) was added, and the mixture was heated at reflux for 1 h[6]. The picrate crystallized slowly overnight. Recrystallization from methanol gave fine, yellow needles (100 mg), m.p. 105–118°, and after recrystallization from methanol (10 ml) 76 mg, m.p. 105–107° (moistening at 102°). The elementary analysis and i.r. spectrum indicated a solvate containing 2 moles of methanol; i.r. data: 3380, 3060 (OH, NH), 1720 (benzoate C=O), 1680 (protonated cytosine), 1604, 1575 (Ph and pyrimidine ring), 1535 (NO_2), 1318 (NO_2), 1265 (benzoate C–O–C), 1115, 1100, 1078, 1070 (C–O, C–O–C), and 710 cm^{-1} (monosubstituted Ph).

Anal. Calc. for $C_{36}H_{30}N_6O_{15}\cdot 2\ CH_3OH$: C, 53.65; H, 4.47; N, 9.88. Found: C, 53.83; H, 4.22; N, 10.28.

The picrate (69 mg) was dissolved in 80% aqueous acetone (88 ml) and **9** was regenerated as described for the preparation of **7**. The product was crystallized and recrystallized from ethanol, giving clusters of large, feathery needles, m.p. 191–191.5°; i.r. data: 3280 (NH), 1720 (benzoate C=O), 1660 (NH_2–C=N), 1625, 1600sh (Ph and pyrimidine ring), 1265 (benzoate C–O–C), 795, 782 (pyrimidine CH), and 705 cm^{-1} (monosubstituted Ph); t.l.c.: R_F 0.35 (*D*) and 0.28 (*E*).

Anal. Calc. for $C_{30}H_{27}N_3O_8$: C, 64.62; H, 4.89; N, 7.54. Found: C, 64.18; H, 4.95; N, 7.33.

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Preliminary communication

Saccharide oxadiazoles

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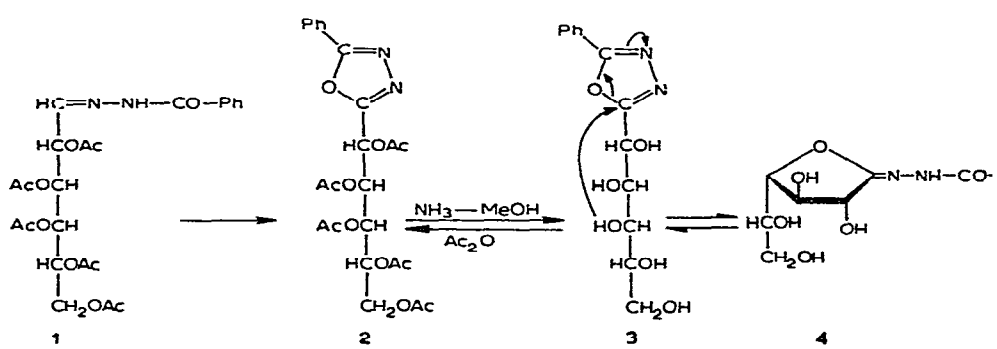
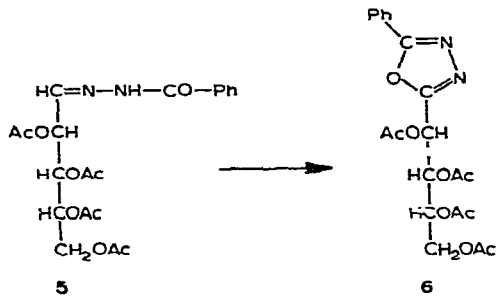
When oxidized with a mixture of iodine and mercuric oxide, the acetates of saccharide bis(benzoylhydrazones) undergo cyclization to substituted 1-amino-1,2,3-triazoles[1, 2]. We have subjected saccharide mono(benzoylhydrazone) acetates to the action of these oxidants, and have obtained a new type of derivative, a saccharide oxadiazole. This type constitutes a significant addition to the biologically important class of nitrogen heterocyles linked to saccharides, and the reaction is a simple route for the synthesis of 1,3,4-oxadiazoles in general.

Treatment of a solution of penta-*O*-acetyl-*aldehydo*-D-galactose benzoylhydrazone (**1**) (1 g) in ether (100 ml) with iodine (1 g), mercuric oxide (1.5 g), and magnesium oxide (1.5 g) for 24 h at room temperature afforded, after filtration, and evaporation of the solvent, a crystalline product (**2**), m.p. 82°, $[\alpha]_D^{22}$ +53.9° (*c* 1, ethanol), giving analytical data corresponding to the formula $C_{23}H_{26}N_2O_{11}$, which contains two hydrogen atoms less than the starting hydrazone acetate (**1**). The product (**2**) possessed five *O*-acetyl groups[3], and showed only one carbonyl band, at 1750 cm^{-1} (OAc), instead of the amide band (1670 cm^{-1}) and ester band (1760 cm^{-1}) shown by **1**. Its n.m.r. spectrum showed, in addition to the saccharide-chain protons[4], five acetyl protons and no imino protons. Accordingly, the structure of 2-(penta-*O*-acetyl-D-*galacto*-pentitol-1-yl)-5-phenyl-1,3,4-oxadiazole was assigned to **2**.



1 2 3 4

Deacetylation of 2 with methanolic ammonia at room temperature led to rearrangement of the oxadiazole (3) first produced, to the iminolactone form, probably the 1,4-lactone (4). It gave analyses corresponding to $C_{13}H_{16}N_2O_6$, had m.p. 234°, $[\alpha]_D^{22}$ +78° (*c* 1, pyridine), showed an amide band at 1650 cm^{-1}, an imino proton at δ 10.4 p.p.m., and gave back the oxadiazole acetate 2 upon acetylation.



5 6

Tetra-*O*-acetyl-*aldehydo*-D-arabinose benzoylhydrazone (5) gave, on oxidation with iodine and mercuric oxide, the oxadiazole 6, m.p. 105°, $[\alpha]_D^{22}$ −15.5° (c 1, ethanol); ν_{max}^{Kbr} 1740 cm^{-1} (OAc).

To test the applicability of this oxidation to non-saccharide hydrazones, we similarly prepared the known[5] 2,5-diphenyl-1,3,4-oxadiazole by oxidation of benzaldehyde benzoylhydrazone, and 1-phenyl-5-(*p*-methoxyphenyl)-1,3,4-oxadiazole by oxidation of either benzaldehyde (*p*-methoxybenzoyl)hydrazone or anisaldehyde benzoylhydrazone.

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Book review

Biokhimiya. Uglevody. Uspekhi v Izuchenii Stroeniya i Metabolisma (Biochemistry. Carbohydrates. Advances in Studies on Structure and Metabolism), by B. N. STEPANENKO, edited by B. L. KRETOVICH (General Editor of Series on Biological Chemistry) and D. A. LEVANDNAYA. Published by the Institute of Scientific and Technical Information, Academy of Science of USSR, Moscow, 1968, 300 pp., 18 Tables, 14×21.5 cm, cloth-back, 1,800 copies published, Rubles 1.35 (approximately $1.25 or 10s).

This book is a rather concise progress-report on the chemistry, biochemistry, and physiology of monosaccharides, oligosaccharides, and polysaccharides. It is a well-documented monograph (over 1,000 references up to the middle of 1965, and a few references for 1966 and 1967) that consists of three parts (8 chapters) and covers advances of the last ten to fifteen years.

It seems almost unbelievable that such a wide and diverse scope of published material could have so successfully been incorporated into a relatively modest volume that nevertheless offers an abundance of structural and mechanistic detail. As indicated by the author in the Introduction (6 pages, 45 general references on carbohydrates), the monograph is primarily designed to fill a long-standing gap for the Soviet professional reader.

Part I consists of 3 chapters (135 pages, 452 references) that describe the chemistry and metabolism of monosaccharides. Chapter 1 (43 pages) discusses absolute configuration (historical background) and conformations (determination by X-ray studies, and by i.r. and n.m.r. spectroscopy; and the Reeves and the Isbell–Tipson systems for naming conformers). Chapter 2 (42 pages) is devoted to new monosaccharides and their derivatives: hexoses, deoxy- and dideoxy-hexoses, heptoses, and higher sugars (occurrence in Nature, structure, and properties); branched-chain sugars (occurrence in antibiotics); mono- and di-amino sugars and derivatives; occurrence in Nature, structure, and physiological properties of uronic acids, sialic acids, and muramic acid; 1-thioaldoses and 1-thioglycosides, and their occurrence in Nature, synthesis, and properties; and synthesis and reactions of sugars containing a sulfur or nitrogen atom in the ring. Chapter 3 (51 pages) is concerned with certain aspects of the metabolism of monosaccharides. It covers phosphoric esters of sugars (fermentations, transaldolase and transketolase reactions, occurrence in Nature, and applications); glycosyl esters of nucleoside phosphates and pyrophosphates and their role in the metabolism of sugars (occurrence in Nature, classification, synthesis, reactions, and physiological functions); other nucleotides; the biosynthesis of trioses, tetroses, pentoses, hexoses, deoxyhexoses, and amino sugars); photosynthesis (the

Calvin cycle); and the metabolism of monosaccharides (the pentose phosphate cycle, and the Entner–Doudoroff, D-glucuronic acid–D-xylulose, and pyruvate pathways).

Part II describes the chemistry and physiology of oligosaccharides in 2 chapters (28 pages, 115 references). Chapter 4 (11 pages) discusses the conformation and structure of oligosaccharides from milk, and of oligofructosides and D-galactosyl-sucroses; chapter 5 (16 pages) covers the biosynthesis of oligosaccharides by trans-glycosylation and certain reactions involving enzymic hydrolysis (transfer of α- and β-D-glucosyl, β-D-fructofuranosyl, and D-galactosyl), and synthesis of α,α-trehalose, sucrose, lactose, and polymers.

Part III (2 chapters, 119 pages, 457 references) is devoted to a description of polysaccharides. Chapter 6 (42 pages) is concerned with homopolysaccharides (homoglucans): starch (heterogeneity, fractionation, amylose, amylopectins, and new starches); glycogens (structure and biological significance); dextrans; cellulose; β-D-(1→3)-glucans; and homopolysaccharides containing nitrogen (chitin, colominic acid, and Vi-antigen). Chapter 7 (46 pages) describes heteropolysaccharides and mixed biopolymers containing carbohydrate moieties: galactomannans; glucomannans; mucopolysaccharides (acidic glucosaminoglucans, hyaluronic acid, and heparin); and mixed biopolymers containing sugars teichoic acids, mucopeptides, glycoproteins from cells, glands, and blood, and glycolipids. Chapter 8 (30 pages) reports on certain aspects of the metabolism of homo- and hetero-polysaccharides: metabolism of polyglucans of the starch and glycogen type (enzymic degradation); and biosynthesis of polysaccharides (glycogens, cellulose, heteropolysaccharides, and mixed biopolymers).

The book concludes with a Subject Index and a Table of Contents; an Author Index is not provided. There is a tendency to adhere to the Rules of Carbohydrate Nomenclature [*J. Org. Chem.*, 28 (1963) 281]; however, several deviations were noted.

The monograph is scientifically sound, and the formulas are adequate. The book is written by a specialist who is actively involved in the field, as is evidenced from references to about sixty of his publications (in Parts I and II). The monograph also cites the work of his colleagues, for example, that by Kochetkov and coworkers (p. 110) in which the authors explained the specificity of glycosyl esters of nucleoside pyrophosphates as being due to hydrogen bonding between the heterocyclic ring and the sugar moiety to give a twisted conformation that facilitates contact with an enzyme and thus promotes the biological reaction.

In general, this book uses perspective and conformational formulas [for example the interesting arrow-type of representations of the *CI* (D) conformation on p. 22], and it contains a number of valuable schemes and tables; for example, Table 16 (p. 258) shows the function of different enzymes in the metabolism of starches. The book does not, however, mention the cardiac glycosides, which are important; it contains very little about dicarbonyl sugars; and there are some minor misspellings and printer's errors (for example, on pp. 30 and 35). It is obviously desirable that, in connection with discussion of the concept of absolute configuration (Part I, chapter 1), the term chirality should now find a place in the Russian chemical vocabulary.

The book is a valuable companion volume to one on carbohydrates by Kochetkov and coworkers [*Carbohyd. Res.*, 8 (1968) 123], now in the process of translation into English; it would be useful to those who cannot read Russian were this book, also, to be translated, because it would be helpful to many carbohydrate chemists, biochemists, and medicinal chemists.

Washington, D. C. ALEXANDER J. FATIADI

Carbohyd. Res., 13 (1970) 472–474

CORRIGENDA

Carbohyd. Res., 12 (1970)

page 84, line 14, should read:
adverse pH of 10.5, porcine, pancreatic alpha-amylase appeared to have multichain

page 84, line 20, should read:
same. From the values of the ratio $\overline{DP}_w/\overline{DP}_n$ under these conditions (Table I) and

page 87, line 7, should read:
enzyme–substrate complex would be slower than in a purely aqueous system: multiple-

PROFESSOR MELVILLE L. WOLFROM

Melville Lawrence Wolfrom was born on April 2, 1900. To commemorate the seventieth anniversary of his birth the Editors of *Carbohydrate Research* were preparing to dedicate the April, 1970, issue as a special Wolfrom Honor Issue, to be presented to Professor Wolfrom at a reception for him, his former students, and his colleagues at The Ohio State University. This reception was to have been a highlight of the celebrations for the centennial year of The Ohio State University, the institution where Professor Wolfrom began his scientific career as an undergraduate student and where he had served for over four decades on the faculty of the Department of Chemistry. His title of Regents' Professor was a special distinction conferred in 1965 by the University in recognition of his outstanding contributions to science.

On June 20th, 1969, after a very brief illness, Professor Wolfrom died. At that time, he was still at the peak of his scientific activity. His many friends and colleagues regret not having the opportunity of joining him on his seventieth birthday to offer a tribute to his life's work, and so this issue of *Carbohydrate Research* is dedicated to the memory of Professor Wolfrom, to acknowledge his enormous contributions to carbohydrate chemistry.

During his career in research, there was scarcely a facet of carbohydrate chemistry where Professor Wolfrom did not make important contributions, and details of all of his work and achievements will be recorded elsewhere. His early mentors, including the distinguished pioneers W. L. Evans, C. S. Hudson, and P. A. Levene, helped to form his interest in carbohydrate chemistry, with emphasis on organic chemistry but with broad extension to physicochemical, biochemical, and technological aspects. He had a prodigious capacity for work, and a facility for organization of research that accounts for the enormous productivity of his laboratory. At the same time, he was extremely meticulous in attention to details, and was especially insistent on historical accuracy in scientific publication. Many honors came his way for his research work, including the 1952 Honor Award of the Carbohydrate Division of the American Chemical Society, and the 1968 Spencer Award for contributions to agricultural chemistry. In 1950 he was elected to the National Academy of Sciences of the U.S.A. Beyond his own research contributions, his influence has extended across the length and breadth of the whole field of carbohydrate chemistry as a result of his work as Editor of *Advances in Carbohydrate Chemistry*, and because of his vigorous leadership in the development of sound carbohydrate nomenclature. In 1967 he received the Austin Patterson Award for his services to chemical documentation. His support and encouragement as a member of the Editorial Advisory Board of *Carbohydrate Research* has been an important factor in the establishment and growth of this journal.

Besides the contributions recorded in Professor Wolfrom's own research papers and in his editorial and nomenclatural work, his influence on carbohydrate chemistry has been carried around the world by the many people who worked with him. Almost

a hundred students received the Ph.D. degree under his immediate direction. Many students worked in his laboratory as postdoctoral fellows, and others studied with him for the M.S. degree. The papers in this issue of *Carbohydrate Research* are all original research contributions from former students of Professor Wolfrom's who have continued work independently in the field of carbohydrate chemistry; these articles have come not only from the North American continent but also from former associates now established in South America, Africa, Asia, and Europe. The topics of research range from physicochemical considerations of carbohydrates to their biological aspects, and from technology to medical applications; especially emphasized are the areas of synthetic organic transformations of carbohydrates and the structures of natural products containing carbohydrates.

Many former students of Professor Wolfrom's, not contributing papers to this Memorial Issue but wishing to join in this dedication, are listed at the end of the issue. Because of limitations of space, it was impossible to include papers from many other scientific colleagues and friends of Professor Wolfrom who might have wished to contribute articles to this issue, but their names are likewise added to the dedication.

D. HORTON