Carbohydrate Research
Elsevier Pubishing Company, Amsterdam
Printed in Belgium

REACTION OF AMMONIA WITH SOME ACETYLATED AND BENZOYLATED MONOSACCHARIDES

PART XI*. INTRAMOLECULAR MIGRATION OF *O*-BENZOYL GROUPS IN THE PARTIAL AMMONOLYSIS OF PENTA-*O*-BENZOYL-D-GLUCOSES

ALBERTO LEZEROVICH, EDUARDO G. GROS**, JORGE F. SPROVIERO, AND VENANCIO DEULOFEU

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Perú 222, Buenos Aires (Argentina)

(Received September 29th, 1966; in revised form, November 7th, 1966)

In the course of studies on the ammonolysis of benzoylated monosaccharides, it has been possible, by appropriate labeling of the benzoyl groups, to determine the individual amount of O→N migration of each such group and thus its contribution to the formation of the 1,1-bis(benzamido)-1-deoxyalditol isolated[2,3]. The results obtained for two benzoylated D-glucopyranoses and for penta-*O*-benzoyl-D-galactopyranose are shown in Table I.

TABLE I

APPARENT CONTRIBUTION (IN MOLE) OF EACH *O*-BENZOYL GROUP TO THE FORMATION OF THE *N*-BENZOYL GROUPS IN 1,1-BIS(BENZAMIDO)-1-DEOXYHEXITOLS

Benzoyl derivative	*Location of the benzoyl group*			
	O-2	*O-3*	*O-4*	*O-6*
Penta-*O*-benzoyl-D-glucopyranose[a]	0.12 ± 0.03	0.76 ± 0.02	0.82 ± 0.02	0.31 ± 0.02
2,3,4,6-Tetra-*O*-benzoyl-D-glucopyranose[a]	0.12 ± 0.03	0.80 ± 0.03	0.81 ± 0.02	0.27 ± 0.02
Penta-*O*-benzoyl-D-galactopyranose[b]	0.13 ± 0.01	0.62 ± 0.01	1.02 ± 0.02	0.18 ± 0.01

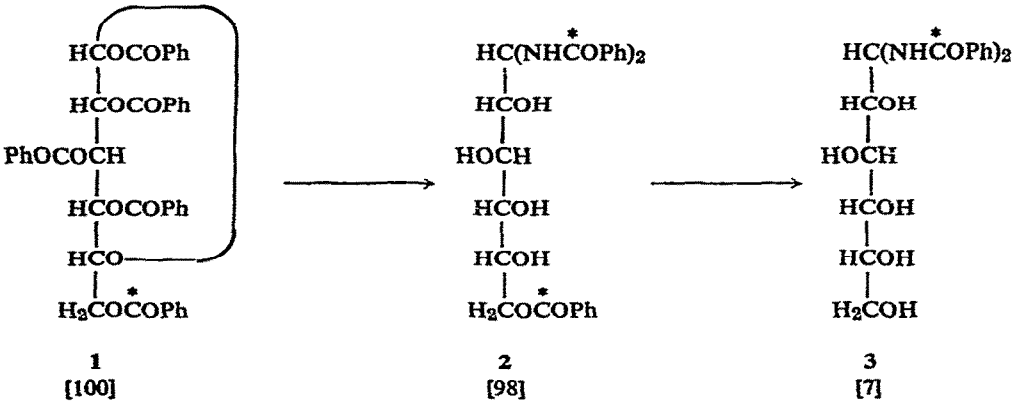
[a]See Ref. 2. [b]See Ref. 3.

Two points of interest arise from these results: the contribution of the 2-*O*-benzoyl group is the lowest (and is almost identical for the three compounds), and the contribution of the 4-*O*-benzoyl group increases on passing from D-glucose to D-galactose, this increase being made at the expense of the 3- and 6-*O*-benzoyl groups. In the latter case, the migration of the 4-*O*-benzoyl group is that of one per molecule, which is indicative that this benzoyl group is present in all molecules of the intermediate producing 1,1-bis(benzamido)-1-deoxy-D-galactitol.

There are not enough data to permit analysis of the several factors which deter-

*Part X, see Ref. 1.
**Research Member of the Consejo Nacional de Investigaciones Cientificas y Técnicas.

mine the amount of migration of each benzoyl group. In the case of the 6-*O*-benzoyl group, with which we shall be particularly concerned, one of the factors limiting its migration is its resistance to nucleophilic reagents, which, in the form of ammonia or an ammonia-like reagent, must play a role in its migration from the oxygen atom on C-6 to the nitrogen atom on C-1. This resistance is readily shown when lower concentrations of ammonia are employed or when methanol is replaced by an alcohol, such as isopropyl alcohol, having a higher pK. Under the latter conditions, 1,1-bis (benzamido)-6-*O*-benzoyl-1-deoxy-D-glucitol (**2**) was obtained from penta-*O*-benzoyl-*β*-D-glucopyranose (**1**), and 1,1-bis(benzamido)-6-*O*-benzoyl-1-deoxy-D-galactitol from penta-*O*-benzoyl-*α*-D-galactopyranose[4].



1 [100] **2** [98] **3** [7]

Fig. 1. Ammonolysis of compound 1*.

The production of a compound having a 6-*O*-benzoyl group afforded us the opportunity to determine, by the use of three differently labeled penta-*O*-benzoyl-*β*-D-glucopyranoses, whether the original 6-*O*-benzoyl group had been partially or wholly replaced by other identical groups during the process. If replacement occurs, the origin of any benzoyl group which might have migrated to O-6 can be ascertained, as well as the fate of the benzoyl group originally attached to that oxygen atom.

When 1,2,3,4-tetra-*O*-benzoyl-6-*O*-benzoyl(carbonyl-^{14}C)-*β*-D-glucopyranose(**1**) was ammonolyzed with 8% ammonia in isopropyl alcohol, 0.98 mole of the original 6-*O*-benzoyl group was present in the compound **2** isolated. The remaining 0.02 mole (a value within the error of the method of measurement employed) was probably lost in the reaction medium. Elimination of the 6-*O*-benzoyl group from **2**, by treatment with 16% methanolic ammonia, showed that 0.91 mole of the labeled benzoyl group remained at O-6, and that 0.07 mole had migrated and was present as benzamido group at C-1 (see Fig. 1).

An experiment with 1,2,3,6-tetra-*O*-benzoyl-4-*O*-benzoyl(carbonyl-^{14}C)-D-glucopyranose (**4**) established that the nonlabeled benzoyl group (0.09 mole) present

*Labeled carbon atoms are indicated with asterisks. Figures in brackets represent relative specific activity.

at O-6 in compound **2** was derived exclusively from the benzoyl group formerly located at O-4 in the ammonolyzed **1**. The results, summarized in Fig. 2, show that, in this case, 93% of the labeled 4-*O*-benzoyl group migrated to C-1 and O-6. Presumably, 0.07 mole was removed by hydrolysis.

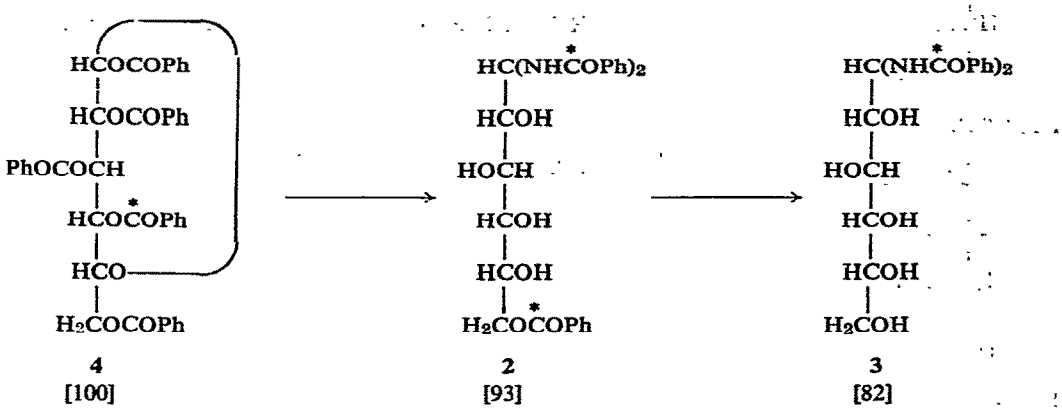


Fig. 2. Ammonolysis of compound 4*.

The two-step ammonolysis of 1,2,3-tri-*O*-benzoyl(carbonyl-^{14}C)-4,6-di-*O*-benzoyl-D-glucopyranose (**5**) (see Fig. 3) shows that there is no migration of a labeled benzoyl group to O-6 from O-2 and O-3, but that, within the limits of experimental error, the labeled benzoyl groups have migrated to the N on C-1 or have been split off into the solvent.

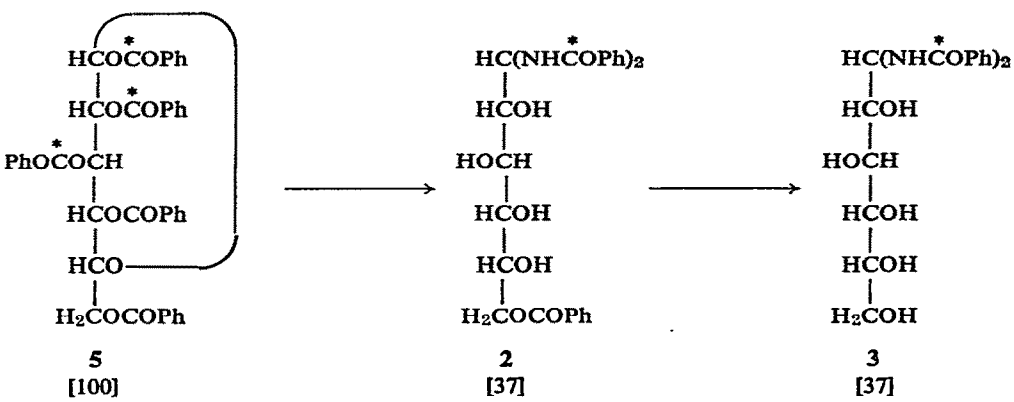


Fig. 3. Ammonolysis of compound 5*.

We have also applied the same reactions to an acyclic derivative, sodium 2-*O*-benzoyl(carbonyl-^{14}C)-3,4,5,6-tetra-*O*-benzoyl-D-glucitol 1-sulfonate (**6**). The results obtained (see Fig. 4) are quite different from those for the D-glucopyranoses, and show a very high capacity for intramolecular migration of the 2-*O*-benzoyl group. Not only

*Labeled carbon atoms are indicated with asteriks. Figures in brackets represent relative specific activity.

is a much larger migration to the N on C-1 especially noticeable, but, in this case, migration from O-2 to O-6 also occurred.

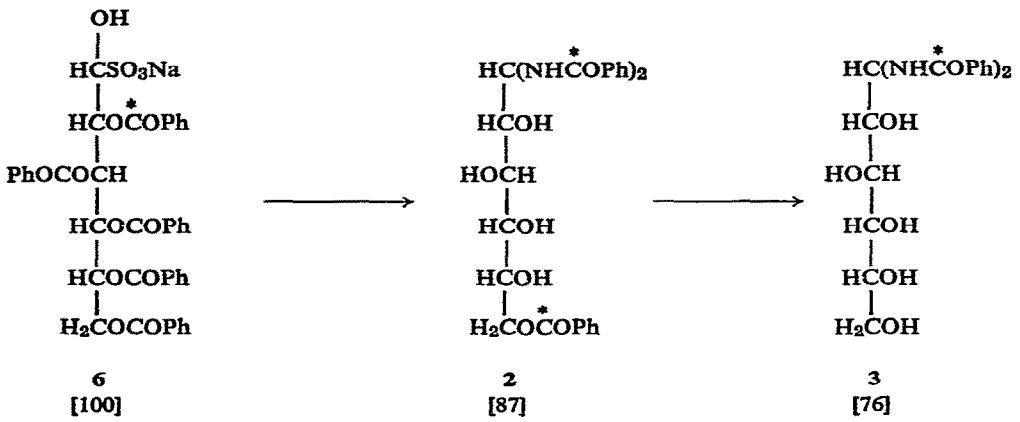


Fig. 4. Ammonolysis of compound 6*.

Obviously, the qualitative and quantitative differences in benzoyl migration between the ammonolysis of the penta-*O*-benzoyl D-glucopyranoses (**1**, **4**, and **5**) and the acyclic derivative (**6**) are the result of the different structures of the initial compounds and the dissimilar steps that the reaction must follow for each type of substance, although the same product, **2**, is obtained in each instance.

Important differences in the reaction steps are determined by the fact that, in the ammonolysis of **6**, the starting and final products are acyclic derivatives, whilst for **1**, **4**, and **5**, certain initial steps might take place while the cyclic structure is retained. It is also evident that, at some step in the last reaction, acyclic intermediates must be formed, but they should not be the same as those derived from **6**. These conditions together determine the variation in benzoyl migration to the N on C-1 and to O-6 from the other oxygen atoms.

The migration of benzoyl groups in acylated aldose derivatives under basic catalysis has been reported several times[5]. Thus, Josephson[6] described, and Brigl and Grüner[7] later studied, the treatment of methyl 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside with methanolic ammonia to produce methyl 2,6-di-*O*-benzoyl-β-D-glucopyranoside and, after a longer period of time, methyl 6-*O*-benzoyl-β-D-glucopyranoside. This is a typical example of migration, to O-6, of benzoyl groups located at other oxygen atoms of the benzoylated aldose, and it also shows the higher resistance to ammonolysis of the 6-*O*-benzoyl group.

This property of the 6-*O*-benzoyl group is characteristic of cyclic and acyclic derivatives; however, we do not feel that the analogy between the two reactions can be carried any further, because, in the previous example, (*a*) the compounds maintained

*Labeled carbon atoms are indicated with asterisks. Figures in brackets represent relative specific activity.

the cyclic structure throughout the whole reaction, and (*b*) it is impossible to know which benzoyl group or groups have migrated to O-6.

Other examples of known benzoyl migrations in the carbohydrate series have been summarized by Bonner[5], but most of them are not specifically related to our experiments.

EXPERIMENTAL

Radioactive compounds were recrystallized to constant activity, which was measured by the method previously described[2].

Preparation of the labeled benzoyl-D-glucose derivatives

1,2,3,4,6-Penta-O-benzoyl-β-D-glucopyranoses (**1**, **4**, and **5**). — Compounds **1**, **4**, and **5** were prepared as described by Gros *et al.*[2]

2-O-Benzoyl(carbonyl-^{14}C)-3,4,5,6-tetra-O-benzoyl-1-D-glucose diethyl dithioacetal. — The method of Brigl and Mühlschlegel[8] was adapted. To a solution of 3,4,5,6-tetra-*O*-benzoyl-D-glucose diethyl dithioacetal (11.7 g) in pyridine (50 ml) cooled to 0°, benzoyl(carbonyl-^{14}C) chloride (1475 ± 13 c.p.m./mM) (6.5 ml) was slowly added. The mixture was kept for 24 h at room temperature, and then poured onto ice-water, giving a yellow, gummy precipitate. The gum was washed with cold water until it solidified; the solid was filtered off and crystallized from ethanol. After recrystallization from the same solvent, the product (11.5 g) had m.p. 95–97° and $[\alpha]_D^{20}$ + 49.0° (*c* 1.0, chloroform); its activity (1493 ± 15 c.p.m./mM) was equivalent to 1.01 moles of labeled benzoyl group per mole. Lit.[8] m.p. 97–98°, $[\alpha]_D^{20}$ + 49.6°.

Sodium 2-O-benzoyl(carbonyl-^{14}C)-3,4,5,6-tetra-O-benzoyl-D-glucitol 1-sulfonate (**6**). — Demercaptalation was performed by the improved method of Weygand *et al.*[9] The previous compound (18 g) was added to a mixture of glacial acetic acid (575 ml), water (80 ml), and concentrated hydrochloric acid (28 ml), and the suspension was maintained at 50°, with stirring, until a clear solution was obtained. The mixture was allowed to cool to room temperature and, with constant stirring, a solution of bromine (7.2 ml) in acetic acid (235 ml) was slowly added, followed by crystalline sodium acetate (115 g). The almost colorless solution was poured onto ice-water, and the suspension was stirred for 2 h. The white solid was filtered off, washed with water, and immediately dissolved in ethanol (1.8 l); a small residue was filtered off, and the filtrate was mixed with a saturated solution of sodium hydrogen sulfite (1.8 l) and the suspension shaken for 20 h. The crystalline product thus obtained was filtered off, washed with ethanol, and dried. The compound (16 g) had m.p. 108–112°, with sintering from 95°; its activity (1442 ± 14 c.p.m./mM) was equivalent to 0.98 mole of labeled benzoyl group per mole. Lit.[8] m.p. 108–114°, with sintering from 103°.

Ammonolysis of the labeled benzoyl-D-glucose derivatives

Preparation of 1,1-bis(benzamido)-6-O-benzoyl-1-deoxy-D-glucitol (**2**). — The penta-*O*-benzoyl-D-glucopyranoses (**1**, **4**, and **5**) were ammonolyzed in isopropyl

alcohol–ammonia (8%, v/v), as already described[4], and **2** was isolated in 32% yield; recrystallized from ethanol, the compound had m.p. 209–210° (undepressed on admixture with authentic material) and was shown to be pure by paper chromatography[4]. For **6**, dissolution in isopropyl alcohol–ammonia occurred immediately; the solution was kept for 18 h at room temperature. After an inorganic precipitate had been filtered off, **2** was isolated as described above (60% yield).

*Preparation of 1,1-bis(benzamido)-1-deoxy-*D*-glucitol* (**3**). — In a typical experiment, compound **2** (1 g) was suspended in methanolic ammonia (18%, v/v) (35 ml) and shaken to dissolution, and the solution was kept for 18 h at room temperature. The solution was evaporated, and the residue was crystallized from the minimum volume of ethanol. Pure **3**, m.p. 206–207°, was obtained in all cases (95–97% yield).

ACKNOWLEDGMENT

We thank the Division Moléculas Marcadas, Comisión Nacional de Energía Atómica, for the use of its equipment for measurement of radioactivity.

SUMMARY

By the use of three differently labeled penta-*O*-benzoyl-D-glucopyranoses, it was possible to detect a partial, benzoyl migration from O-4 to O-6 during the ammonolysis of penta-*O*-benzoyl-D-glucopyranose in isopropyl alcohol. When the same reaction was applied to a penta-*O*-benzoyl-*aldehydo*-D-glucose derivative, a partial migration of a benzoyl group from O-2 to O-6 was also encountered.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PREPARATION AND REACTIONS OF SOME 1-AMINO-1-DEOXYHEPTITOLS

H. J. F. ANGUS* AND NELSON K. RICHTMYER

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014 (U.S.A.)

(Received November 4th, 1966)

In their studies on the condensation of nitromethane with hexoses, Sowden and Fischer[1] reduced 1-deoxy-1-nitro-D-*glycero*-D-*gulo*-heptitol with hydrogen and Raney nickel, and isolated 1-amino-1-deoxy-D-*glycero*-D-*gulo*-heptitol (**1**) as the crystalline *p*-toluenesulfonate. Later, Sowden and Schaffer[2] reduced 1-deoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol similarly, and isolated 1-amino-1-deoxy-D-*glycero*-D-*galacto*-heptitol (**2**) as the crystalline oxalate. More recently, McDonald[3] obtained a crystalline *N*-acetyl derivative of **2** after reducing the nitro compound in the presence of a palladium-on-carbon catalyst. Apparently, no other 1-aminoheptitols have been described previously, and neither the free bases nor the hydrochlorides have been obtained crystalline.

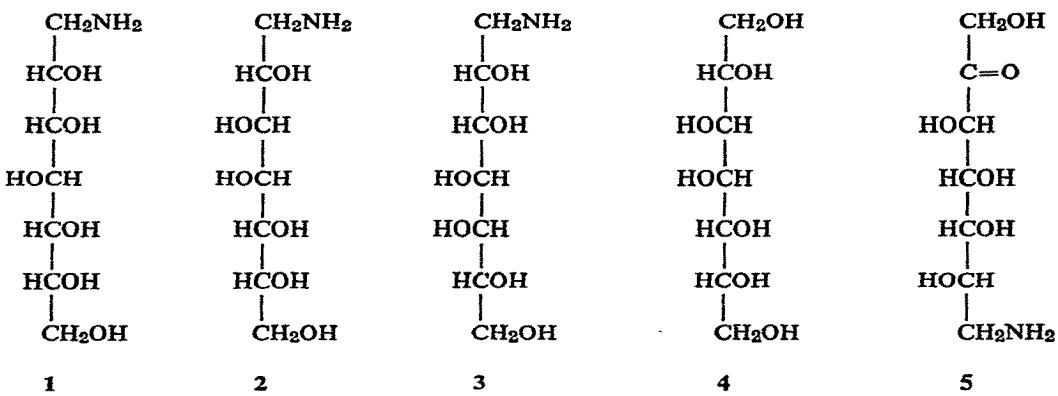
We have reduced D-*glycero*-D-*gulo*-heptose oxime[4] with hydrogen and platinum oxide to **1**, which was isolated as the crystalline hydrochloride. The reduction of 1-deoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol[2] with hydrogen and platinum oxide gave **2**, also isolated as its crystalline hydrochloride; removal of the hydrogen chloride by standard methods afforded crystalline **2**. This base was further characterized through its crystalline *N*-salicylidene derivative, as well as through the known *N*-acetyl derivative[3]. The reduction of 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol[5] with hydrogen and platinum oxide gave 1-amino-1-deoxy-D-*glycero*-L-*manno*-heptitol (**3**), also isolated as its crystalline hydrochloride.

The deamination of **2** with nitrous acid gave three products that could be readily separated on a column of Dowex 50W-X8 (Ba^{2+}). One of these was identified as perseitol (**4**; D-*glycero*-D-*galacto*-heptitol ≡ L-*glycero*-D-*manno*-heptitol), formed by replacement of the amino group by a hydroxyl group. The other two had mobilities on paper chromatograms that suggested that they might be anhydroheptitols, and the composition of one that crystallized (m.p. 162–163°) confirmed this speculation. Since the formation of an anhydroheptitol by deamination might involve an epoxide at C-1 and C-2, and its migration to C-2 and C-3, then 2,6-anhydro-D-*glycero*-D-*galacto*-heptitol would be one possible product; Sowden *et. al.*[6] have, however, described this compound (m.p. 142–144°), and it is obviously different from ours. So

*Fellow in the Visiting Program of the National Institutes of Health, September 1964 to October 1965; present address: Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire, Wales.

also are the two other anhydroheptitols known, namely, 2,6-anhydro-D-*glycero*-D-*gulo*-heptitol (m.p. 204–205°) and 2,6-anhydro-D-*glycero*-L-*manno*-heptitol (hemihydrate, m.p. 121–122°), described by Coxon and Fletcher[7]. Similar deamination of **1** with nitrous acid gave three substances that, from paper-chromatographic evidence only, are believed to be the expected *meso-glycero-gulo*-heptitol and two anhydroheptitols.

McDonald[3] has recently prepared 7-acetamido-7-deoxy-L-*galacto*-heptulose by the action of *Acetobacter suboxydans* on 1-acetamido-1-deoxy-D-*glycero*-D-*galacto*-heptitol (the *N*-acetyl derivative of **2**). Unaware of this work, we started our studies of the action of *A. suboxydans* on the unacetylated aminoheptitols **1**, **2**, and **3**. As would be expected from the Bertrand rule, as modified by Hann *et. al.*[8], both **1** and **2** were oxidized, whereas growth of the *A. suboxydans* in the medium containing **3** was not detected. The product from **2**, presumably 7-amino-7-deoxy-L-*galacto*-heptulose (**5**), has been obtained in crystalline form and will be further described at a later date. These 7-amino-7-deoxyheptuloses, when spotted on paper and sprayed with the orcinol–hydrochloric acid reagent and then heated, give a reddish orange color, in contrast to the blue or greenish blue colors characteristic of the corresponding heptuloses themselves.



EXPERIMENTAL

1-Amino-1-deoxy-D-glycero-D-gulo-*heptitol* (**1**) *hydrochloride*.—D-*glycero*-D-*gulo*-Heptose oxime was prepared by the following modification of the method described by Hockett and Chandler[4]. Anhydrous sodium carbonate (14.0 g; 0.13 mole) was added to a stirred solution of hydroxylamine hydrochloride (14.0 g; 0.20 mole) in 250 ml of absolute ethyl alcohol at 50–60°. A vigorous reaction ensued; this subsided in *ca.* 25 min. D-*glycero*-D-*gulo*-Heptose[9] (21.0 g; 0.10 mole) was added, and the mixture stirred for 45 min at 70–80° under reflux. The hot mixture was rapidly filtered and the solid was washed on the funnel with ethyl alcohol (100 ml). The combined filtrate and washings were concentrated *in vacuo* to a colorless syrup (28.0 g) consisting

of the heptose oxime, apparently contaminated with hydroxylamine hydrochloride. This crude syrup was dissolved in 3:1(v/v) ethyl alcohol–water (200 ml), acetic acid (50 ml) and platinum oxide (0.2 g) were added, and the mixture was shaken with hydrogen at atmospheric pressure for 48 h. The catalyst was removed by filtration, and the solution concentrated *in vacuo* to a syrup that was dissolved in water (100 ml). This solution was passed down a column (3 × 27 cm) of Amberlite IR-120 (H^+) ion-exchange resin. The column was then washed with water (750 ml), and eluted with 3N ammonia (1 l). The eluate was concentrated *in vacuo* to a syrup (17.9 g) that was dissolved in water (100 ml) and acidified with 2N hydrochloric acid (30 ml). The yellow solution was decolorized (Darco X), filtered, and concentrated to a mobile syrup to which pyridine (25 ml) was added. The solution was cooled, nucleated with crystals of the hydrochloride (obtained in a preliminary experiment), and kept for 4 days at − 10°. The resulting small prisms were filtered off, washed successively with cold pyridine, ethyl alcohol, and ether, and dried at 40° *in vacuo*; 12.7 g (51%, based on the D-*glycero*-D-*gulo*-heptose), m.p. 114–115°. Recrystallization was difficult, but was effected by dissolving the hydrochloride (12.7 g) in water (8 ml), adding pyridine (80 ml), and keeping the solution for 48 h at − 10°. The m.p. of the material thus obtained varied from 113–115° to 117–118° with different preparations. The compound appeared to crystallize initially as a pyridine solvate; spears, m.p. 92–94°. A sample having m.p. 113–115° and $[\alpha]_D^{20} - 4.7°$ (*c* 1.1, water) was analyzed.

Anal. Calc. for $C_7H_{18}ClNO_6$: C, 33.94; H, 7.33; Cl, 14.31; N, 5.66. Found: C, 34.17; H, 7.05; Cl, 14.31; N, 5.71.

1-Amino-1-deoxy-D-glycero-D-galacto-*heptitol* (**2**) *hydrochloride.* — To a solution of 1-deoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol[2] (6.0 g; 0.025 mole) in water (200 ml) were added acetic acid (5 ml) and platinum oxide (0.2 g). The mixture was reduced overnight with hydrogen at atmospheric pressure. The catalyst was removed by filtration, and the theoretical volume of hydrochloric acid (12.5 ml of 2N) was added to the filtrate. Evaporation of the solution yielded the hydrochloride of **2** (5.9 g; 96%) as clusters of tiny needles; after two recrystallizations from 2:1 (v/v) ethyl alcohol–water (4 ml/g), m.p. 184–185°, $[\alpha]_D^{20}$ −5.9° (*c* 1.0, water).

Anal. Calc. for $C_7H_{18}ClNO_6$: C, 33.94; H, 7.33; Cl, 14.31; N, 5.66. Found: C, 34.20; H, 7.30; Cl, 14.05; N, 5.57.

1-Amino-1-deoxy-D-glycero-D-galacto-*heptitol* (**2**). — A sample (5 g) of the hydrochloride of **2** was dissolved in water (100 ml) and the solution passed down a column (3 × 27 cm) of Amberlite IR-120 (H^+) ion-exchange resin. The column was washed with water (500 ml), and then eluted with 3N ammonia (500 ml). Concentration of the eluate gave an almost quantitative yield of **2** which, on recrystallization from 2:1 (v/v) ethyl alcohol–water, separated as fine needles; m.p. 181–184°, $[\alpha]_D^{20} + 0.7°$ (*c* 2.0, water).

Anal. Calc. for $C_7H_{17}NO_6$: C, 39.81; H, 8.11; N, 6.63. Found: C, 39.86; H, 8.24; N, 6.56.

1-Deoxy-1-(salicylidenamino)-D-glycero-D-galacto-*heptitol.* — To a solution of **2** (0.20 g) and sodium acetate trihydrate (0.22 g; 2 mol. equiv.) in water

(4 ml) was added a solution of salicylaldehyde (0.20 g; 2 mol. equiv.) in methanol (12 ml). Yellow prisms soon crystallized from the solution while it was kept for 3 h in the dark. The product was filtered off, and successively washed with 3:1 (v/v) methanol–water (20 ml) and ether; yield, 0.24 g (94%). After recrystallization from hot water (40 ml) by the addition of methanol (20 ml), it had m.p. 228–229°.

Anal. Calc. for $C_{14}H_{21}NO_7$: C, 53.33; H, 6.71; N, 4.44. Found: C, 53.46; H, 6.48; N, 4.32.

1-Acetamido-1-deoxy-D-glycero-D-galacto-*heptitol.* — A portion (5.0 g) of the hydrochloride of **2** was converted into the free base as described above, and the ammoniacal eluate was evaporated *in vacuo* to a crystalline residue. To this were added acetic anhydride (5 ml) and methanol (50 ml), and the mixture was heated for 30 min on a steam bath. Cooling and filtration gave the title compound (4.5 g; 88%). After recrystallization from 2:1 (v/v) ethyl alcohol–water (10 ml/g), the compound (very small prisms) had m.p. 199–200° and $[\alpha]_D^{20}$ −16.5 ± 1.5° (*c* 1.1, water), in good agreement with the lit. values[3] of 200° and −15.5°, respectively. Analyses for carbon, hydrogen, and nitrogen confirmed its composition.

1-Amino-1-deoxy-D-glycero-L-manno-*heptitol* (**3**) *hydrochloride.* — 1-Deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol monohydrate (6.5 g)[5] was reduced to the amino compound as described for **2**, and the resulting hydrochloride was recrystallized, in clusters of small plates from aqueous pyridine as described for the hydrochloride of **2**, giving 4.0 g (64%) of product which showed no evidence of forming a solvate with pyridine; m.p. 150–152°, and $[\alpha]_D^{20}$ −3.5° (*c* 1.1, water).

Anal. Calc. for $C_7H_{18}ClNO_6$: C, 33.94; H, 7.33; Cl, 14.31; N, 5.66. Found: C, 33.91; H, 7.05; Cl, 14.43; N, 5.50.

Deamination of 1-amino-1-deoxy-D-glycero-D-galacto-*heptitol* (**2**) *hydrochloride.* — The hydrochloride of **2** (1.24 g; 0.005 mole) was dissolved in a solution of acetic acid (3 ml) in water (25 ml). Sodium nitrite (1.03 g; 0.015 mole) was added with stirring, and the solution was kept overnight at room temperature. After deionization with Amberlite IR-120 (H^+) and Duolite A-4 (OH^-) resins, the solution was concentrated to a syrup. This was dissolved in water (10 ml), the solution was transferred to the top of a column (2.5 × 68 cm) of Dowex 50W-X8 (Ba^{2+}) resin, and elution was conducted with water. The eluate was collected in 4-ml fractions.

Fractions 33–50 contained a substance having the mobility of perseitol on paper chromatograms. The combined fractions were evaporated to a crystalline residue that was recrystallized twice from aqueous ethyl alcohol, to give 60 mg of product, m.p. 186–187° alone, m.p. 187–188° when mixed with authentic **4**.

Fractions 22–28 contained a substance having a mobility slightly less than that of rhamnose in 3:1:1 butyl alcohol–ethyl alcohol–water. These fractions were combined, and evaporated to a syrup which soon crystallized; recrystallization from aqueous ethyl alcohol yielded 30 mg of a compound having m.p. 162–163° and the composition of an anhydroheptitol.

Anal. Calc. for $C_7H_{14}O_6$: C, 43.29; H, 7.27. Found: C, 43.43; H, 7.06.

Fractions 12–18 contained a substance having practically the same R_F value as

the crystalline product isolated from fractions 22–28, but it has thus far resisted all attempts at crystallization; it is presumed to be a second anhydroheptitol.

ACKNOWLEDGMENT

The authors thank Dr. William C. Alford and his associates of the Section on Microanalytical Services and Instrumentation of this Laboratory of Chemistry for making the elementary analyses and determining the specific rotations.

SUMMARY

The hydrochlorides of 1-amino-1-deoxy-D-*glycero*-D-*gulo*-heptitol (**1**), 1-amino-1-deoxy-D-*glycero*-D-*galacto*-heptitol (**2**), and 1-amino-1-deoxy-D-*glycero*-L-*manno*-heptitol (**3**), as well as the free aminoheptitol **2**, have been obtained in crystalline form. Deamination of **2** with nitrous acid yielded three products: perseitol (**4**) and two compounds that appear to be anhydroheptitols. Deamination of **3** gave similar results. In agreement with the accepted rule, compounds **1** and **2** were oxidized by *Acetobacter suboxydans*, whereas compound **3** was not. The oxidation product from **2**, presumably 7-amino-7-deoxy-L-*galacto*-heptulose (**5**), has been obtained crystalline.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

POLYSACCHARIDES ELABORATED BY *Armillaria mellea* (TRICHOLOMATACEAE)

PART I. A WATER-SOLUBLE POLYSACCHARIDE FROM THE FRUIT BODIES

R. NEIL FRASER AND BENGT LINDBERG
Träkemiska Avdelningen, Svenska Träforskningsinstitutet and Institutionen för Organisk Kemi, Stockholms Universitet (Sweden)

(Received September 22nd, 1966)

INTRODUCTION

Investigations of the molecular structure of skeletal and reserve polysaccharides, and polysaccharides synthesised by enzyme systems of fungi have been generally restricted to moulds and yeast-like fungi[1]. Information in this field on the higher fungi of the Basidiomycetes group is concerned essentially with the skeletal polysaccharides of the fruit bodies, and only a few studies on other polysaccharides have been reported. Glucans, especially of the β-D-(1→3)-linked type, are widely distributed[2], and have been encountered in *Poria*[3] and *Polyporus*[4,5] species. Glucans having α-D-linkages have been found in *Polyporus giganteus*[4], *P. betulinus*[5], and *P. tumulosus*[6]. Some heteropolysaccharides have also been investigated. A xylomannan has been isolated from *P. tumulosus*[6], and a heterogalactan containing D-galactose, D-mannose, and L-fucose from *P. giganteus*[4]. This communication reports the isolation of, and the results of a structural investigation on, a water-soluble polysaccharide isolated from the fruit bodies of *Armillaria mellea.*

RESULTS AND DISCUSSION

The fruit bodies of *A. mellea* were harvested locally and stored in ethanol. After continuous extraction with methanol for seven days, they were air-dried, disintegrated, and extracted by ultrasonic treatment with cold water, and the water extract was processed to give Fraction 1. Extraction of the residue with 0.3M potassium hydroxide solution gave two further fractions: an alkali-soluble water-soluble Fraction 2, and an alkali-soluble, water-insoluble Fraction 3. The composition of the fractions is given in Table I.

Only Fractions 1 and 2 were further investigated. Fraction 1 was treated with the enzymes pepsin and trypsin, and deionised, and non-carbohydrate, acidic material was removed by using a DEAE-cellulose column in the acetate form. Fraction 2 was similarly treated, except that the enzymic degradation was omitted. The sugar composition of both samples was unchanged, except that ribose was not present in Fraction 1.

A solution of Fraction 1, when treated with cetyltrimethylammonium hydroxide (CTA–OH)[7] to pH 8, gave a precipitate which, after dissolution and processing, showed only glucose on hydrolysis. It had a low, positive optical rotation, was

TABLE I

COMPOSITION OF POLYSACCHARIDE FRACTIONS

Fraction	*Solubility in water*	*Glu*	*Gal*	*Man*	*Fuc*	*Xyl*	*Rib*	*Amino acids*
1	soluble	++[a]	++	++	++	(+)	+	+
2	soluble	++	+	+	+	+	—	—
3	insoluble	++	—	—	—	—	—	—

[a] ++, present in large proportion; +, present; (+), traces; —, absent.

difficultly soluble in water, and was, in all probability, a β-D-(1→3)-glucan. Further addition of CTA-OH, giving a strongly alkaline solution, gave no precipitate. Treatment with CTA-OH of a solution of the residue in boric acid precipitated a heteropolysaccharide composed of D-galactose, D-mannose, and L-fucose, leaving in solution a material which gave mainly glucose on hydrolysis, with traces of mannose and xylose. Fraction 2 gave a similar fractionation pattern.

The heteropolysaccharide from both fractions had $[\alpha]_{5780}$ +98° and a molar ratio of D-galactose:L-fucose:D-mannose of 6.5:2:1. The methylated polysaccharide ($[\alpha]_{5780}$ +92°; OCH_3, 41.6%; calc.: 43.8%) yielded, on hydrolysis, a mixture of methylated sugars which were separated by carbon-column chromatography, paper chromatography, and paper electrophoresis in borate buffer, to give the methyl ethers listed in Table II.

As can be seen from Table II, all the main sugars are present in the pyranosidic form. D-Mannose is present exclusively as terminal, non-reducing residues, and L-fucose is involved in both non-reducing end-groups, and as chain units linked through C-1 and C-3. D-Galactose is found as non-reducing end-groups, chain units linked through C-1 and C-6, and as branch points linked through C-1, C-2, and C-6. The small amounts of the last three sugars in Table II indicate either undermethylation or demethylation during hydrolysis, and are probably not structurally significant.

TABLE II

METHYL ETHERS FROM METHYLATED HYDROLYSED HETEROGALACTAN

Sugar	*Mole (%)*	*Retention time (T)*[10]	
		Column a	*Column* b
2,3,4,6-Me_4-Gal[a]	3.7	1.80	1.50, 1.60
2,3,4,6-Me_4-Man	9.6	1.41	1.27
2,3,4-Me_3-Fuc	9.2	0.72	
2,4-Me_2-Fuc	9.7	1.62, 2.22	
2,3,4-Me_3-Gal	43.2	7.3	2.60, 2.89
3,4-Me_2-Gal	19.2		
2,3-Me_2-Gal	2.7		
2(?)-Me-Fuc	0.7		
3-Me-Gal	1.5		

[a] 2,3,4,6-Me_4-Gal = 2,3,4,6-tetra-*O*-methyl-D-galactose, etc.

A stepwise, partial hydrolysis with acid was carried out with the isolation of fragments of low molecular weight between each step. The oligosaccharides obtained were separated by chromatography on thick filter-paper. Table III contains a list of the fragments found, and some of their constants. The determination of their structures was based on optical rotations, hydrolysis products before and after borohydride reduction, and comparison with authentic samples by paper chromatography in several solvent systems, and electrophoresis in germanate and sulphonated benzeneboronic acid buffers.

TABLE III

OLIGOSACCHARIDES FROM A PARTIAL HYDROLYSIS OF THE HETEROGALACTAN (1.5 g)

Oligosaccharide	*Yield, mg*	$[\alpha]_{5780}$,°	R_{Gal}			M_G	$R_{M/n}$[a]
			Solvent a	*Solvent* b	*Solvent* c		
3-*O*-α-D-mannosyl-L-fucose	41	+0.5	0.85	0.65		0.8 (buffer *b*)	
α-(1→6)-galactobiose	43	+141	0.46	0.16	0.44	1.40 (buffer *a*)	0.36
α-(1→6)-galactotriose	32	+147	0.36		0.21		0.36
α-(1→6)-galactotetraose	16		0.22		0.10		0.36
α-(1→6)-galactopentaose	9		0.12		0.05		0.35

[a]Solvent *c*; R_F for galactose, 0.36.

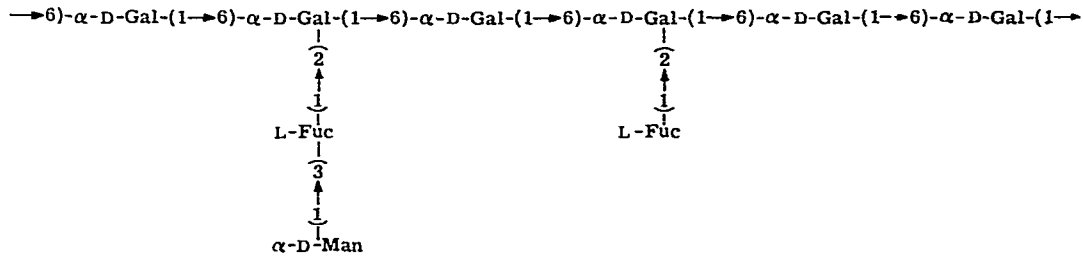
The disaccharide 3-*O*-α-D-mannopyranosyl-L-fucose had been obtained previously from the *Polyporus giganteus* heterogalactan[4], but the β-form was erronously assigned to the D-mannopyranosidic linkage. From the optical rotation of the disaccharide (*ca.* 0°), and the high, negative value for L-fucose at equilibrium (−76°), it may, however, be concluded that the D-mannopyranosidic linkage is in the α-form.

A sample of the polysaccharide was treated with sodium metaperiodate, and it was found to consume 1.58 moles of periodate and to release 0.71 moles of formic acid per anhydrohexose residue. The results are in reasonably good agreement with the values calculated for a polysaccharide having a galactose:mannose:fucose ratio of 6.5:1:2, and using the methylation results given in Table II, *viz.* 1.61 and 0.70, respectively. Fucose was the only reducing sugar found in the hydrolysate of the periodate-oxidised, borohydride-reduced polysaccharide.

On the basis of these results, it is possible to suggest the structure shown on p. 15 as being a possible repeating-unit for the *A. mellea* fruit-body heterogalactan.

This is the first reported isolation of a homologous series of α-D-(1→6)-linked oligosaccharides from a polysaccharide source. A polymer having such a backbone is also new. The polysaccharide isolated from the fruit bodies of *Polyporus giganteus*[4] has a superficial resemblance to the above polysaccharide, but has a backbone of β-D-(1→6)-linked galactose residues. The presence of L-fucose as a non-reducing end group is restricted to the *A. mellea* polysaccharide, whereas both share the structural unit 3-*O*-α-D-mannopyranosyl-L-fucose.

The structural studies give no information on the configuration of the L-fucopyranosidic residues. The optical rotation of the polysaccharide may be estimated from



the contributions of the different sugar residues. Such calculations, when compared with the observed value of the optical rotation, lend some support to the assumption that the L-fucopyranosidic residues are in the α-form.

Certain similarities in structure between the *A. mellea* heterogalactan and two polysaccharides now under investigation in this laboratory have been noticed. One of these was isolated from the mycelial growth of the same fungus, the other from the fruit bodies of *Polyporus pinicola*.

EXPERIMENTAL

General methods. — Evaporations were carried out under reduced pressure at temperatures not exceeding 40°. Paper chromatograms were run on Whatman No. 1 and 3 MM papers, using the following systems (v/v): (*a*) ethyl acetate–acetic acid–water (3:1:1); (*b*) ethyl acetate–pyridine–water (8:2:1); (*c*) ethyl acetate–pyridine–water (2:1:2, upper phase); (*d*) butyl alcohol–ethanol–water (10:3:5); (*e*) butanone–water–conc. ammonia (200:17:1). Electrophoretic analyses[8] were performed on Whatman No. 1 papers in one of the following electrolytes: (*a*) 0.05M germanate at 40°, pH 10.7; (*b*) 0.05M sulphonated benzeneboronic acid at 40°, pH 6.5; 0.1M borate at room temperature, pH 10. Components were detected with alkaline silver nitrate or *p*-anisidine hydrochloride. Sugar determinations were made by using g.l.c. of trimethylsilyl ethers[9]. G.l.c. was performed on a Perkin–Elmer Model 800 instrument, using the following columns : (*a*) 15% butane-1,4-diol succinate on Chromosorb W at 175°; (*b*) 15% polyphenol ether on Chromosorb W at 200°. The retention times of the methylated methyl glycosides are relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside[10]. Optical rotations were determined at room temperature (20–22°) in aqueous solutions, unless otherwise stated.

Extraction of the polysaccharides. — The fungus (100 l) was stored in ethanol immediately after harvesting. Before the extraction, it was squeezed as dry as possible and was then extracted continuously with hot methanol for seven days. After being air-dried, the material was disintegrated in a Turmix blender with water present, and the slurry was extracted twice with cold water with the aid of a Philip ultrasonic bath. Extracted polysaccharides were recovered from the concentrated solution by precipi-

tation with ethanol. The procedure was repeated with 0.3M potassium hydroxide, one fraction being precipitated on neutralisation of the extract with acetic acid, and a second on addition of ethanol. Table I indicates the composition of the fractions obtained.

Fraction 3 gave glucose plus traces of laminaribiose and laminaritriose on formolysis. It was soluble only in alkali, and had $[\alpha]_{5780}$ + 12°. It was concluded that this fraction was most likely to be a β-D-(1→3)-glucan, and it was not further investigated.

Fraction 1 (35 g) was dissolved in water (500 ml), and the pH was adjusted to 2 with 2M hydrochloric acid. Pepsin (1 g) in water (15 ml, pH 1.5) was added, and the digestion was maintained at 37° for 36 h. The pH was then adjusted to 7.5 with 2M potassium hydroxide, trypsin (1 g) in water (10 ml) was added, and the solution was kept for 48 h at 37°. After treatment on a steam bath for 2 h, the solution was dialysed for 48 h, concentrated, and added to ethanol. The precipitated polysaccharide (26 g) contained only traces of amino acids.

Enzyme-treated Fraction 1 in water (500 ml) was passed through Dowex 50 (H^+) and Dowex 2 (free base) columns, concentrated, and precipitated with ethanol to give deionised polysaccharide (19 g). Fraction 2 was not treated with enzymes, but was deionised to give deionised Fraction 2 (43 g).

Fractionation of the water-soluble polysaccharides. — Fraction 1 (19 g) was dissolved in water (1 l), and 0.25M cetyltrimethylammonium hydroxide (70 ml) was added until a red colour was obtained with phenolphthalein. The precipitate formed was treated with dilute acetic acid and ethanol to give a water-insoluble fraction (1A, 6.6 g). Further CTA-OH (300 ml + 5 ml of 0.1M NaOH) yielded a second precipitate, which was worked up to give fraction 1B (240 mg). The supernatant gave, on deionisation, concentration, and precipitation, a third fraction (1C, 10.5 g).

On formolysis, Fractions 1A and 1B gave only glucose, with traces of maltose and laminaribiose. They were not further investigated.

A glucose-free polysaccharide was obtained by gradual precipitation of a solution of Fraction 1C in 0.1M boric acid solution (500 ml) with 0.25M CTA-OH (Table IV). Fractions $1C_1$ and $1C_2$ (3.05 g) were combined. The polysaccharide had $[\alpha]_{5780}$ + 97° (*c*, 0.7), and the galactose:mannose:fucose ratio was 6.48:1:1.98.

Fraction 2 (43 g) was subjected to exactly the same fractionation procedure. The

TABLE IV

FRACTIONATION OF FRACTION 1C

Fraction	*0.25 M CTA-OH ml added*	*Weight (g)*	*Gal*	*Glu*	*Man*	*Fuc*	*Xyl*
$1C_1$	20	2.64	++[a]	(+)	++	++	—
$1C_2$	25	0.41	++	(+)	++	++	—
$1C_3$	50 + 5 ml of M NaOH	1.25	++	++	+	+	—
$1C_4$	residue	2.96	(+)	++	(+)	(+)	(+)

[a] ++, present in large proportion; +, present; (+), traces; —, absent.

heterogalactan was enriched in Fraction 2C (8.5 g) and essentially pure in Fraction $2C_2$ (0.94 g), which had $[\alpha]_{5780}$ +98° and a galactose:mannose:fucose ratio of 6.51:1:1.99. Fractions $1C_1$, $1C_2$, and $2C_2$ were combined and used in all investigations of the heterogalactan.

Methylation analysis. — The heterogalactan (1.03 g) was methylated by the method of Srivastava *et al.*[11] (one treatment being sufficient to obtain a chloroform-soluble product) and then permethylated by using silver oxide and methyl iodide. Three treatments were required to give a product (630 mg) with $[\alpha]_{5780}$ +92° (*c* 1.1, chloroform), and OCH_3, 41.6%. The product was treated for 1 h with 90% formic acid at 100°, and then hydrolysed with 0.25M sulphuric acid for 18 h at 100°. The hydrolysate was deionised, and concentrated to a syrup (540 mg).

The syrup was added to the top of a charcoal–Celite column (1:1, 24 × 4 cm), which was then washed with gradients of water to 30% ethanol. The separation was followed by thin-layer chromatography of the fractions, and appropriate tubes were combined to give four fractions (Table V). Mixed fractions (except for No. 4) were further fractionated on thick filter-paper by using solvent (*b*) for Fraction 1, and multiple development in solvent (*e*) for Fractions 2 and 3. Fraction 4 was shown to contain only the sugars listed in Table V by (*i*) demethylation to give fucose, galactose, and mannose, and (*ii*) g.l.c. of the mixture of methyl glycosides. The results and molar percentages are given in Table II.

TABLE V

FRACTIONS FROM THE HYDROLYSATE OF METHYLATED HETEROGALACTAN

Fraction no.	*Weight (mg)*	*Constituents*[a]
1	40	3-Me-Gal ++; 3,4-Me_2-Gal (+).
2	132	3,4-Me_2-Gal ++; 2,3-Me_2-Gal +; 2(?)-Me-Fuc (+).
3	201	2(?)-Me-Fuc (+); 2,3,4-Me_3-Gal ++; 2,4-Me_2-Fuc ++.
4	108	2,3,4-Me_3-Fuc ++; 2,3,4,6-Me_4-Man ++; 2,3,4,6-Me_4-Gal +.

[a]See Tables I and II for explanation of symbols.

The different ethers were characterised by demethylation with boron trichloride, by their reaction with triphenyltetrazolium chloride, by paper-chromatographic and -electrophoretic comparison with authentic samples, by g.l.c. of their methyl glycosides, and, for the major components, by preparation of crystalline derivatives as follows: 3-*O*-methyl-D-galactose, crystallised from acetone–methanol, had m.p. and mixed m.p. 145.5–146.5°; 3,4-di-*O*-methyl-D-galactose gave an anilide[4] with m.p. 148–149°; 2,4-di-*O*-methyl-L-fucose[4,12], crystallised from ethyl acetate, had $[\alpha]_{5780}$ −92° (*c* 0.3) and m.p. 135–136°: 2,3,4-tri-*O*-methyl-D-galactose gave an anilide with m.p. and mixed m.p. 162–164°.

Partial hydrolysis of the heterogalactan. — The polysaccharide (1.5 g) was given successive hydrolytic treatments for 1 h at 100°: first, twice with 0.05M hydrochloric acid; secondly, five times with 0.1M hydrochloric acid; and finally, once with 0.25M

hydrochloric acid. The hydrolysis mixture was neutralised after each treatment (Dowex 2, free base), and the part to be hydrolysed further was precipitated with ethanol. The fragments of low molecular weight were fractionated on thick filter-paper by using solvent system (*a*). The results are summarised in Table III. The fragments were characterised as follows: D-galactose, $[\alpha]_{5780}$ + 72°, as galactitol, m.p. and mixed m.p. 184–186°; D-mannose, $[\alpha]_{5780}$ + 19°, as methyl α-D-mannopyranoside, m.p. and mixed m.p. 193–194°; L-fucose, $[\alpha]_{5780}$ − 54°, as the phenylosazone, m.p. and mixed m.p. 177–178°; 3-*O*-α-D-mannopyranosyl-L-fucose, $[\alpha]_{5780}$ + 0.5° (*c* 1.0), gave equimolecular amounts of mannose and fucose on acid hydrolysis. It was chromatographically and electrophoretically indistinguishable from the mannosyl-fucose isolated from the heteropolysaccharide of *Polyporus giganteus*.

The galactose-containing oligosaccharides formed a homologous series. Each oligosaccharide gave only galactose on hydrolysis, and the lower members of the series when partially hydrolysed. The electrophoretic movement of the disaccharide in germanate buffer was the same as that of β-(1→6)-galactobiose.

Periodate oxidation. — A sample of the polysaccharide consumed 1.58 moles of periodate and released 0.71 moles of acid per anhydrohexose unit. The periodate consumption was followed spectrophotometrically[13], and the released acid was titrated potentiometrically with sodium hydroxide solution. After completion of the oxidation, sodium borohydride was added, and, on hydrolysis of the deionised residue, fucose was the only reducing sugar found in the hydrolysate.

ACKNOWLEDGMENT

The authors are indebted to *Cellulosaindustriens Stiftelse för teknisk och skoglig forskning samt utbildning* for financial support.

SUMMARY

The annual fruit bodies of *Armillaria mellea* have been examined for polysaccharide content, and a polymer containing D-galactose, D-mannose, and L-fucose residues, in the molar ratio of 6.5:1:2, was isolated. Subsequent investigation has shown that it has a backbone of α-(1→6)-linked D-galactopyranose residues, approximately every third of which is substituted in position 2 by either L-fucopyranosyl residues or by 3-*O*-α-D-mannopyranosyl-L-fucopyranosyl residues.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

POLYSACCHARIDES ELABORATED BY *Armillaria mellea* (TRICHOLOMATACEAE)

PART II. WATER-SOLUBLE MYCELIUM POLYSACCHARIDES

HANS O. BOUVENG, R. NEIL FRASER, AND BENGT LINDBERG

Träkemiska avdelningen, Svenska Träforskningsinstitutet and Institutionen för Organisk Kemi, Stockholms Universitet, Stockholm (Sweden)

(Received October 8th, 1966)

INTRODUCTION

In Part I[1] of this Series, studies of a polysaccharide isolated from the fruit bodies of *Armillaria mellea* were reported. The present paper describes studies of the water-soluble polysaccharides isolated from cultures of the same fungus, with D-glucitol as the carbon source.

ISOLATION AND FRACTIONATION OF POLYSACCHARIDES

The harvested mycelia were disintegrated and extracted with cold water under ultrasonic treatment, and the extract was processed for polysaccharide content. The material obtained was freed from salts by ion exchange, and from highly coloured, acidic, non-carbohydrate material with the aid of a DEAE-cellulose column in the acetate form. A hydrolysate of the purified material contained glucose, galactose, mannose, xylose, fucose, and another reducing sugar, which was shown to be 3-*O*-methyl-D-galactose.

The residue from the water extraction was treated with 0.3M potassium hydroxide, again using ultrasonic treatment. On neutralisation with acetic acid, a precipitate was obtained, and subsequent investigation suggested that it consisted almost entirely of a β-D-(1→3)-glucan. The supernatant solution was treated in the same manner as the water extract, and the resulting polysaccharide material gave the same hydrolysis pattern as the water-extracted material.

Various fractionation techniques were used in an attempt to separate the constituents of the polysaccharide mixtures, but little success was obtained. However, they did serve to indicate that the material was grossly heterogeneous. A partially successful fractionation was obtained by chromatography of the product on a DEAE-cellulose column in the borate form[2]; elution was effected with gradients of increasing concentration of sodium borate.

The fractionation patterns obtained from the water-soluble fraction and from the alkali-soluble, water-soluble fraction are given in Fig. 1. So that direct comparison could be made, the volumes of the columns, the eluants, and the fractions collected were the same in both separations.

Four fractions (A–D and A1–D1, respectively) were obtained from each separation and, as can be seen from Fig. 1, the patterns are similar. The eight fractions were

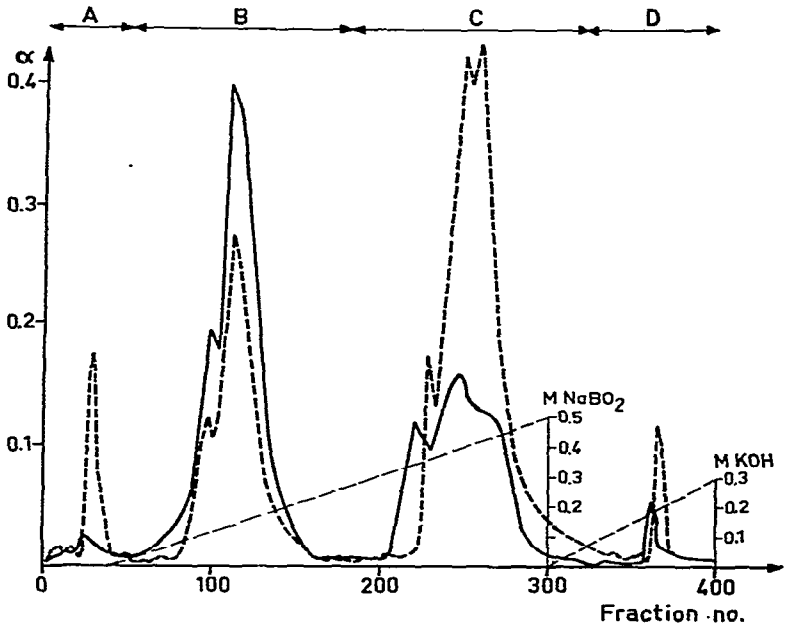


Fig. 1. Fractionation of water-soluble polysaccharides from *A. mellea* on DEAE-cellulose in the borate form.
Water-extracted polysaccharides, — — — —; alkali-extracted polysaccharides, ————.

hydrolysed, and the relative amounts of the sugars in the hydrolysates were estimated visually from paper chromatograms. The results are given in Table I. It can safely be assumed that A and A1 are equivalent, as are B and B1, C and C1, and D and D1. Only the second (B, B1) and third fractions (C, C1) were studied more closely. Fractions A and D probably consist essentially of a glycogen-type glucan and a β-D-(1→3)-glucan, respectively.

TABLE I

COMPOSITION OF POLYSACCHARIDE FRACTIONS FROM DEAE-CELLULOSE COLUMN

Fraction no.	*Gal*	*Glu*	*Man*	*Xyl*	*Fuc*	*3-O-Me-Gal*
A	—[a]	++	—	—	—	—
B	—	++	++	+	—	—
C	++	+	+	(+)	+	+
D	(+)	++	(+)	(+)	—	—
A1	—	++	—	—	—	—
B1	—	+	++	++	—	—
C1	++	+	++	(+)	+	+
D1	(+)	++	(+)	(+)	—	—

[a] ++, present in large proportions; +, present; (+), traces; —, absent.

THE XYLOMANNAN

On further investigation, Fraction B was found to consist of a glucan and a xylomannan, although the method used to demonstrate this, *viz.*, chromatography on a lightly loaded DEAE-cellulose column in the borate form (with a shallow salt gradient and small fraction volumes), could not be adapted to yield workable amounts of each polysaccharide. The mixture was treated first with α-amylase (from *Bacillus subtilis*), and secondly with a (1→3)-β-D-glucanase from Basidiomycete QM 806[3]; these degradations diminished the glucose content to approximately 4%. No release of xylose or mannose was detected.

The xylomannan had $[\alpha]_{5780}$ +71° and a molar ratio of mannose to xylose of 5:4. Hydrolysis of the fully methylated xylomannan ($[\alpha]_{5780}$ +68°; OCH_3, 41.2%, calc.: 43.0%) yielded the methyl ethers given in Table II. Their separation was achieved by the use of t.l.c. on silica gel, and by paper chromatography. The molar percentage of each sugar was calculated on a weight basis.

TABLE II

METHYL ETHERS FROM HYDROLYSATE OF METHYLATED XYLOMANNAN

Methyl ether	*Weight, mg*	*molar %*	*Retention time (T)*[4] *column a*	*column b*
2,3,4-Me_3-Xyl[a]	41.2	21.3	0.46, 0.56	0.45, 0.54
2,3,4,6-Me_4-Man	9.4	4.0	1.43	1.27
2,4,6-Me_3-Man	66.4	31.1	3.86	2.02
2,3-Me_2-Xyl	35.8	20.1	1.50, 1.79	0.64 0.75
2,6-Me_2-Man	41.7	20.7		3.12
mono-Me-Man	5.5	3.0		

[a]2,3,4-Me_3-Xyl = 2,3,4-Tri-*O*-methyl-D-xylose; etc.

A stepwise, partial hydrolysis with acid was performed, with the isolation of fragments of low molecular weight between each step, and the products were separated by using chromatography on thick filter-paper. Table III contains a list of the fragments found, and some of their properties. Both D-xylose and D-mannose were obtained, and crystalline derivatives were prepared. A small amount of residue was left after the series of partial hydrolyses had been performed. A sample had $[\alpha]_{5780}$ +79°, and yielded only mannose on hydrolysis.

The oligosaccharides were characterised as fully as possible by means of their optical rotations, their hydrolysis products both before and after reduction with borohydride, paper chromatographic comparisons with authentic samples, and their electrophoretic mobilities in germanate and borate buffers. From these results, coupled with those from the methylation analysis, the xylobiose was shown to have an α-(1→4)-linkage, this being the first reported isolation of this disaccharide[5] from a natural product. The existence of a homologous series of α-(1→3)-linked D-mannose

oligosaccharides was also demonstrated. Such a series has also been reported from the partial hydrolysis with acid of a periodate-oxidised phosphonomannan[6].

TABLE III

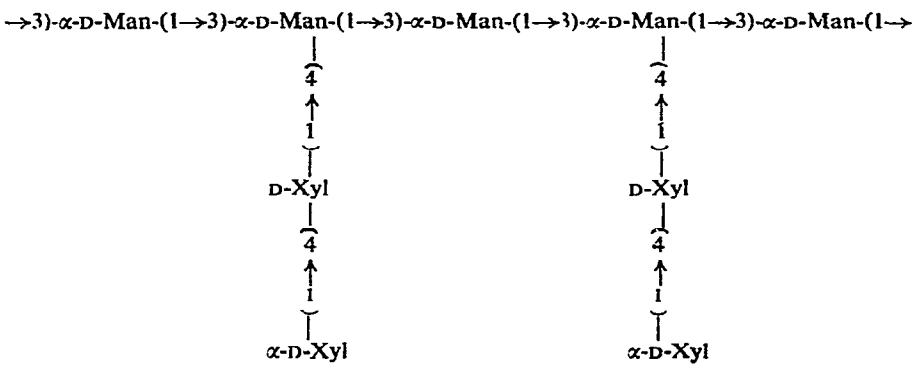
FRAGMENTS FROM PARTIAL ACID HYDROLYSIS OF THE XYLOMANNAN

Oligosaccharide	$[\alpha]_{5780}$, (°)	R_{Gal} *solvent a*	*solvent b*	*solvent c*	M_G	R_{Mn}[a]
α-(1→4)-xylobiose	+ 84	1.01	1.04		0.32 (buffer *a*)	
α-(1→3)-mannobiose	+ 64	0.59		0.67	0.67 (buffer *b*)	0.26
α-(1→3)-mannotriose		0.34		0.42		0.26
α-(1→3)-mannotetraose		0.20		0.27		0.25
α-(1→3)-mannopentaose		0.11		0.17		0.24
α-(1→3)-mannohexaose		0.06		0.10		0.25

[a] R_F for mannose, 0.33; solvent *c*.

A sample of the polysaccharide consumed 0.78 moles of periodate and released 0.23 moles of formic acid per anhydrosugar residue. These results are in reasonably good agreement with the figures of 0.72 and 0.25, respectively, calculated on the basis of the methylation analysis. Hydrolysis of the periodate-oxidised, borohydride-reduced polysaccharide, followed by paper chromatographic examination of the hydrolysate, indicated that mannose was the only reducing sugar present.

On the basis of these results, it is possible to suggest that the xylomannan has a backbone of α-(1→3)-linked D-mannopyranose residues, every second or third of which is substituted in the 4-position by a 4-*O*-D-xylopyranosyl-D-xylopyranose residue. The structural studies give no information on the configuration of the linkage by



which the xylobiose residue is attached to the mannan chain. A comparison of the optical rotation of the polysaccharide with an estimated value, calculated from the contributions of the different sugar residues, lends some support to the assumption that this linkage is in the β-D-configuration.

The isolation of a water-soluble xylomannan from the fruit bodies of *Polyporus tumulosus* has been reported[7]. The molar ratio of mannose:xylose is 3:1, but no

rotation was given. Unlike the xylomannan from *A. mellea*, this polymer forms a copper complex. A series of mannose oligosaccharides was obtained on partial hydrolysis. The linkage was reported to be (1→3), but no configuration was given. Work being carried out in the present laboratory has shown the presence of a fucoxylomannan in the fruit bodies of *Polyporus pinicola*[8].

THE HETEROGALACTAN

Further investigation of Fraction C indicated the presence of glucose-containing polymers. Treatment of the mixture with α-amylase and (1→3)-β-D-glucanase diminished the glycose content to below 2%. Glucose was the only sugar released. Re-examination of the enriched fraction on the borate DEAE-cellulose column, with shallow gradients and small fraction volumes, indicated the existence of two polymers. Small fractions were obtained that, on hydrolysis, gave only mannose. At no time were fractions obtained that gave only galactose, fucose, and 3-*O*-methylgalactose on hydrolysis. Mannose was always present, and visual inspection of chromatograms showed that, although the relative proportions of galactose, fucose, and 3-*O*-methylgalactose stayed relatively constant, the proportion of mannose varied. Various fractionation techniques were investigated for separating the mannan from the heterogalactan, but all were only partially successful, and none could be readily scaled up to give workable amounts of each fraction. The polysaccharide mixture was shown to have the following composition (percentage by weight); D-galactose, 42; D-mannose, 30; L-fucose, 13; and 3-*O*-methyl-D-galactose, 15. The four sugars were isolated after hydrolysis and characterised.

A sample of the polysaccharide mixture was fully methylated ($[\alpha]_{5780}$ +92°; OCH_3, 40.9%), and the methanolysis products were examined by g.l.c. A sample was hydrolysed, and the methylated sugars were examined by using paper chromatography. As a result of the complexity of the mixtures, a quantitative estimation of chromatograms was not attempted. The methyl ethers found are listed in Table IV.

TABLE IV

METHYL ETHERS FROM THE METHYLATED HETEROGALACTAN–MANNAN MIXTURE

Methyl ether	*Retention time (T)*[4]	
	column a	*column b*
2,3,4,6-Me_4-Gal[a]	1.80	1.52, 1.60
2,3,4,6-Me_4-Man	1.42	1.28
2,3,4-Me_3-Fuc	0.71	
2,3,4-Me_3-Gal	7.3	2.61, 2.89
2,3,4-and/or 3,4,6-Me_3-Man	3.10	1.64
2,4,6-Me_3-Man	3.86	2.06
2,4-Me_2-Fuc	1.62, 2.22	
3,4-Me_2-Gal		
3,4-Me_2-Man		
3-Me-Gal		

[a]2,3,4,6-Me_4-Gal = 2,3,4,6-tetra-*O*-methyl-D-galactose; etc.

A series of partial acid hydrolyses, with increasing concentrations of acid, were performed, with isolation of the fragments of low molecular weight between each step. The oligosaccharides obtained were separated by means of paper chromatography, and characterised as fully as possible by using optical rotation, hydrolysis before and after borohydride reduction, and comparison, using authentic samples, of mobilities in paper chromatography and in electrophoresis in germanate and sulphonated benzeneboronic acid buffers. Table V contains data on the oligosaccharides that were the main fragments isolated and characterised from the complex mixture of fragments of low molecular weight. Traces of mannose-containing oligomers were detected on paper chromatograms and electrophoretograms, but were present in such small proportions that it was not feasible to isolate them. No evidence was obtained for the existence of oligosaccharides containing mannose linked to either galactose or 3-*O*-methyl-galactose, nor for the existence of a 2-*O*-galactosyl-galactose.

TABLE V

FRAGMENTS FROM PARTIAL ACID HYDROLYSIS OF THE HETEROGALACTAN–MANNAN MIXTURE

Oligosaccharide	$[\alpha]_{5780}$, (°)	R_{Gal} *solvent a*	*solvent b*	*solvent c*	M_G	$R_{M/n}$[a]
3-*O*-α-D-Man*p*-L-fuc	+ 4.7	0.87	0.66		0.9 (buffer *b*)	
6-*O*-α-(3-*O*-Me-D-gal*p*)-D-gal	+ 124	0.72	0.30		1.13 (buffer *a*)	
α-(1→6)-Galactobiose	+ 140	0.46	0.16	0.44	1.40 (buffer *a*)	0.36
α-(1→6)-Galactotriose	+ 144	0.36		0.21		0.36
α-(1→6)-Galactotetraose		0.22		0.10		0.36
α-(1→6)-Galactopentaose		0.12		0.05		0.35

[a] R_F for galactose, 0.36; solvent *c*.

The polysaccharide mixture consumed 1.34 moles of periodate and released 0.45 moles of formic acid per anhydrohexose residue. Fucose and 3-*O*-methyl-galactose were the only reducing sugars found in the hydrolysate of the periodate-oxidised, borohydride-reduced material. From the methylation results, it is possible to predict that a small amount of mannose should have been periodate-resistant, but none could be detected in the hydrolysate.

From these results, and assuming that the mixture contains two distinct polysaccharides, *viz.*, a mannan and a heterogalactan, containing galactose, fucose, mannose, and 3-*O*-methylgalactose, it is possible to make some suggestions as to their molecular structures.

If it is assumed that fucose is the only sugar to which the mannose contained in the heterogalactan is linked, and if the figure of approximately one terminal to one chain-residue of fucose (as can be estimated from visual inspection of paper chromatograms of the hydrolysed, methylated mixture) is accepted, it is possible to calculate that the mixture contains about 23% of mannan and 77% of heterogalactan. The relative molar proportions of the sugars in the latter are then galactose:fucose: 3-*O*-methylgalactose:mannose, 6:2:2:1.

The evidence available for drawing any conclusions on the structure of the mannan is very slight, but it is possible that it has a highly branched structure containing (1→2)-, (1→3)-, and (1→6)-linkages, and is similar to the mannans isolated from *Saccharomyces cerevisiae*[1] and *S. rouxii*[10].

The heterogalactan appears to contain the following structural features, a backbone of α-(1→6)-linked D-galactopyranose residues, with the following as side chains linked through C-2 of the D-galactose residues: L-fucopyranose (1 part), 3-*O*-α-D-mannopyranosyl-L-fucose (1 part), and 6-*O*-(3-*O*-methyl-α-D-galactopyranosyl)-D-galactopyranose (2 parts). The configuration of the linkages at the point of attachment of these side chains is open to conjecture. Comparison with a heterogalactan isolated from the fruit bodies of the same fungus[1] suggests that the first two side-chains are linked by α-L linkages. All of the other D-galactose residues in the heterogalactan appear to be involved in α-linkages, so it is possible that the third side-chain is also attached to the backbone *via* an α-(1→2)-linkage.

From the results of the methylation analysis and the isolation of a series of α-(1→6)-linked oligosaccharides from the partial hydrolysis, it is reasonable to assign the 3-*O*-methyl-D-galactose to the position of a terminal, non-reducing, end group, and to reject any idea that it is part of the backbone. Further support for this conclusion comes from the failure to isolate a disaccharide having 3-*O*-methyl-D-galactose as the reducing residue.

The conclusions drawn on the structure of the two components are naturally open to discussion; it cannot, for example, be excluded that the heterogalactan is a mixture of two polysaccharides, *e.g.*, a mannofucogalactan and a 3-*O*-methylgalactogalactan, both polymers having the same backbone of α-(1→6)-linked D-galactose residues.

The coexistence of 3-*O*-methyl-D-galactose and L-fucose in a polysaccharide is unique, the former having been found naturally in only two, quite-unrelated species of plant. It is present to the extent of about 17% in slippery elm mucilage[11], in combination with D-galactose, L-rhamnose, and D-galacturonic acid. It is also present to the extent of 25% in a polysaccharide from *Sassafras albidum*[12], in combination with D-galactose, L-rhamnose, D-xylose, L-arabinose, and D-galacturonic acid.

EXPERIMENTAL

The general methods are as described in Part I[1].

Culture of the organism and isolation of the polysaccharides. — The culture solution consisted of the following, in g/l: D-glucitol, 80; KH_2PO_4, 2.5; $(NH_4)_2HPO_4$, 0.7; $(NH_4)_2SO_4$, 0.5; L-glutamic acid, 3.5; $MgSO_4 \cdot 7H_2O$, 2.0; NaCl, 0.1; $CaCl_2 \cdot 2H_2O$, 0.3; yeast extract, 0.5. The following solutions (1 ml/l) were also added: 0.3% of thiamine; a solution containing, in mg/100 ml, $CuSO_4 \cdot 5H_2O$, 4; $MnSO_4 \cdot 4H_2O$, 4; $ZnSO_4$, 17; ferric citrate, 27; and a solution containing, in mg/100 ml, H_3BO_3, 5; $(NH_4)_6Mo_7O_{27} \cdot 4H_2O$, 2. The pH of the solution was adjusted to 5.1 with aqueous potassium hydroxide. The inoculated solutions were kept for 4–5 weeks at 25°, the time of growth being governed by the rate of formation of the dark-brown colour of the

mature organism. The mycelia were harvested immediately this dark colour began to appear.

The mycelial growth was removed, and the culture solution was poured into acidified ethanol. A small amount of precipitate was obtained and processed to give Fraction 1. The growth was heated to 90° for 30 min, slurried in water with a Turmix blendor, and then twice extracted with water for 30 min, by using an ultrasonic bath (Philips L 368, 40 Kc.p.s.). After centrifugation, the water extract was worked up to give Fraction 2. The residue was similarly extracted with 0.3M potassium hydroxide, and yielded the water-soluble Fraction 3, and the alkali-soluble, water-insoluble Fraction 4. The results of the fractionation are summarised in Table VI.

TABLE VI

POLYSACCHARIDE FRACTIONS FROM *A. mellea* MYCELIA

Fraction no.	*Gal*	*Glu*	*Man*	*Fuc*	*Xyl*	*3-O-Me-Gal*
1	+[a]	(+)	++	(+)	+	(+)
2	++	++	++	++	+	+
3	++	++	++	+	++	+
4	−	++	+	−	+	−

[a]See Table I, for explanation of symbols.

Fraction 4 was not further investigated. Fractions 1 and 2 were combined to give Fraction 12. Fractions 12 and 3 were deionised [Dowex 50 (H^+) and Dowex 2 (free base)], but still contained dark-coloured, acidic, non-carbohydrate material. Fraction 12 was added to a column of DEAE-cellulose (AcO^-), and eluted successively with water, 0.1M and M potassium acetate, and 0.3M potassium hydroxide. Only the water-eluted fraction contained polysaccharides. Fraction 3 was treated in the same manner and gave a similar result.

The total weight of purified polysaccharide amounted to approximately 0.4 g/l of culture solution. The only sugar that was found to decrease in proportion during the purification steps was glucose.

Fractionation of the polysaccharides. — Fraction 12 (2.28 g) was placed on a DEAE-cellulose column (60 × 5 cm, borate form). The column was eluted with water (2 l), with gradients of sodium borate (0–0.1M, 2.5 l; and 0.1M–0.5M, 2.5 l), and with a gradient of potassium hydroxide (0–0.3M, 2.5 l). The fractionation was followed polarimetrically (Fig. 1). The compositions of the four fractions obtained (A,B,C, and D) are given in Table I. Fraction 3 (2.58 g) was subjected to the same treatment, yielding fractions A_1, B_1, C_1, and D_1.

Fractions B and B_1 were combined, as were fractions C and C_1. The other fractions were not further investigated.

Purification of the xylomannan. — The combined B-fractions had $[\alpha]_{5780}$ +95°, and gave glucose, mannose, and xylose on acid hydrolysis. Rechromatography of a small sample on a DEAE-cellulose column in the borate form, with a

shallow gradient of sodium borate, indicated that the fraction was a mixture containing a glucan (probably α-D linked) and a xylomannan.

The polysaccharide mixture (940 mg) in a phosphate buffer (0.02M, pH 6.9, 50 ml) was treated with α-amylase (*Bacillus subtilis*, 3 mg) for 24 h at 20°. As the polysaccharide fraction still contained glucan, the treatment was repeated. The residue, which still contained glucan, was given two treatments with (1→3)-β-D-glucanase in acetate buffer (0.025M, pH 4.2) for 24 h at 50°. Only glucose was released by these treatments. The residue (595 mg) had $[\alpha]_{5780}$ +71°, contained 4% of glucose, and had a mannose:xylose ratio of 5:4.

Methylation analysis of xylomannan. — The xylomannan (615 mg) was methylated by using the method of Srivastava[13] (one treatment being sufficient to obtain a chloroform-soluble product) and then permethylated by using silver oxide and methyl iodide (three treatments being required to give a product having $[\alpha]_{5780}$ + 68° and OCH_3, 41.2%). The methylated xylomannan was hydrolysed with 90% formic acid (20 ml) for 1 h at 100°, followed by 0.25M sulphuric acid (30 ml) for 18 h at 100°. The hydrolysate was deionised [Dowex 50 (H^+) and Dowex 2 (free base)], concentrated to a syrup, and spotted on silica-gel plates (Kieselgel HF_{2540}; thickness, 1 mm), which were then developed in benzene–ethanol (5:2). The following fractions were obtained: (1) mono-*O*-methyl-D-mannose, 14 mg; (2) 2,6-di-*O*-methyl-D-mannose, 64 mg; (3) 2,3-di-*O*-methyl-D-xylose + 2,4,6-tri-*O*-methyl-D-mannose, 162 mg; (4) 2,3,4,6-tetra-*O*-methyl-D-mannose, 15 mg; (5) 2,3,4-tri-*O*-methyl-D-xylose, 64 mg. Fraction C was separated into its components by paper chromatography in solvent *d*. All fractions were purified by filtration through Celite pads, and by ion exchange. Some properties of the methylated sugars are listed in Table II. They were characterised as follows:

2,6-Di-*O*-methyl-D-mannose, $[\alpha]_{5780}$ +11° (*c*, 0.4) was chromatographically indistinguishable from a partially characterised sample, obtained[8] from a polysaccharide from the fruit body of *Polyporus pinicola*. It gave mannose on demethylation, and gave no reaction with triphenyltetrazolium chloride; no trityl derivative was obtained, and it had zero mobility on electrophoresis in borate buffer.

2,3-Di-*O*-methyl-D-xylose[14], $[\alpha]_{5780}$ +23° (*c* 0.7), gave xylose on demethylation, and gave no reaction with triphenyltetrazolium chloride. The anilide[15] had m.p. 120–122° (recrystallised from ethyl acetate) and $[\alpha]_{5780}$ +178° (*c* 0.4, ethyl acetate). G.l.c. examination of the methyl glycoside mixture showed correct retention times[4].

2,4,6-Tri-*O*-methyl-D-mannose, $[\alpha]_{5780}$ +17° (*c* 0.4), gave only mannose on demethylation. The amide of the corresponding mannonic acid had m.p. 142°. The sugar gave no reaction with triphenyltetrazolium chloride, and no trityl derivative was obtained. The methyl glycoside was examined by g.l.c. and had the correct retention times[17].

2,3,4,6-Tetra-*O*-methyl-D-mannose. The sugar gave only mannose on demethylation. It was indistinguishable, by paper chromatography, from an authentic sample (solvent *d*), and the retention times of the methyl glycoside as examined by g.l.c. were those reported[4].

2,3,4-Tri-*O*-methyl-D-xylose, $[\alpha]_{5780}$ +20° (*c* 0.5), gave only xylose on demethylation. It was indistinguishable (paper chromatography) from an authentic sample (solvent *d*), and its methyl glycosides had the correct retention times on g.l.c. The anilide[18] had m.p. 101°.

Partial hydrolysis of the xylomannan with acid. — The polysaccharide (370 mg) was given successive hydrolytic treatments for 1 h at 100° (twice with 0.05M hydrochloric acid, twice with 0.1M hydrochloric acid, and finally with 0.25M hydrochloric acid). The hydrolysis mixture was neutralised (Dowex-2, free base) after each treatment, and the part to be hydrolysed further was precipitated with ethanol. The first treatments yielded xylose and xylobiose, and, as the acid strength increased, mannose and mannose-containing oligosaccharides appeared. The hydrolysates were combined, and fractionated by chromatography on thick paper in solvent *a*. A list of the fragments obtained and some of their properties are given in Table III. D-Xylose ($[\alpha]_{5780}$ +19°) gave the phenylosazone, m.p. and mixed m.p. 163°; D-mannose ($[\alpha]_{5780}$ +14°) gave methyl α-D-mannopyranoside, m.p. and mixed m.p. 192–194°.

4-*O*-α-D-Xylopyranosyl-D-xylose[5] ($[\alpha]_{5780}$ +84°) gave only xylose on hydrolysis. It gave a positive reaction with triphenyltetrazolium chloride. Its M_G value in germanate buffer was 0.32, compared with 0.0 for 2-*O*-methyl-D-xylose, 1.7 for 3-*O*-methyl-D-xylose, and 0.3 for 4-*O*-methyl-D-xylose.

The mannose-containing oligosaccharides formed a homologous series[6], and were obtained in yields of 14.0, 8.5, 6.3, and 8.2 mg, respectively. On examination in solvent system *c*, the mannopentaose was found to be contaminated with mannohexaose. The first member of the series, 3-*O*-α-D-mannopyranosyl-D-mannose[17], had $[\alpha]_{5780}$ +64°, and gave only mannose on hydrolysis. It had the same electrophoretic mobility as an authentic sample in both germanate and borate buffers. Partial hydrolysis of the higher members of the series resulted in formation of the lower homologues.

Periodate oxidation of the xylomannan. — Periodate oxidations were performed by using sodium metaperiodate. The periodate consumption was followed spectrophotometrically[19], and the released formic acid was titrated potentiometrically with sodium hydroxide solution. Analytical data were extrapolated to zero time. The solution was treated with sodium borohydride on completion of the oxidation, and mannose was the only reducing sugar detected after hydrolysis of the deionised concentrate.

Purification of the heterogalactan–mannan mixture. — Fraction C + C1 was treated twice with α-amylase and twice with β-D-(1→3)-glucanase, by using the conditions described for the xylomannan. Glucose was the only reducing sugar released. The glucose content of the resulting polysaccharide fraction was less than 2%. The fraction had $[\alpha]_{5780}$ +97°, and contained galactose, 42; mannose 30; fucose, 13; and 3-*O*-methyl-D-galactose, 15%.

The techniques of copper complexing with Fehling's solution, precipitation with barium hydroxide, and precipitation with boric acid—cetyltrimethylammonium hydroxide failed to resolve the mixture. A partial separation into a mannan and a

heterogalactan was obtained when a sample was chromatographed on a DEAE-cellulose column in the borate form, with a weak, salt gradient. The method was not practical for preparing larger amounts of the components.

Methylation of the mixture. — The polysaccharide mixture (200 mg) was methylated, by using the same method employed for the xylomannan, to give a product (97 mg) having $[\alpha]_{5780}$ +92° and OCH_3, 40.9%. The methanolysis products were examined by g.l.c., and the hydrolysis products were examined by paper chromatography in solvent systems *b*, *d*, and *e*. A list of the methyl ethers found is given in Table IV.

Partial hydrolysis of the mixture. — The polysaccharide mixture (375 mg) was subjected to a series of partial hydrolyses, with increasing strength of acid, by using the method described for the hydrolysis of the xylomannan. The fragments of low molecular weight were separated by chromatography on thick paper, and characterised as follows:

D-galactose, $[\alpha]_{5780}$ +70°, galactitol, m.p. and mixed m.p. 185–186°; D-mannose, $[\alpha]_{5780}$ +16°, methyl α-D-mannopyranoside, m.p. and mixed m.p. 193–194°; L-fucose, $[\alpha]_{5780}$ −55°, phenylosazone, m.p. and mixed m.p. 177–178°; 3-*O*-methyl-D-galactose, $[\alpha]_{5780}$ +110°, m.p. and mixed m.p. 145–146°; 3-*O*-α-D-mannopyranosyl-L-fucose, $[\alpha]_{5780}$ +1.2° (*c* 0.7), gave equimolecular amounts of mannose and fucose on hydrolysis, and only mannose after borohydride reduction and hydrolysis. It was indistinguishable from an authentic sample[20] on paper chromatography and on paper electrophoresis in sulphonated benzeneboronic acid buffer; 6-*O*-(3-*O*-methyl-α-D-galactopyranosyl)-D-galactose, $[\alpha]_{5780}$ +124° (*c* 2.1), gave equimolecular amounts of 3-*O*-methyl-D-galactose and galactose on hydrolysis. 3-*O*-Methyl-D-galactose was the only reducing sugar detected after borohydride reduction, followed by hydrolysis. The disaccharide had an M_G value of 1.13 in germanate buffer, consistent with either a (1→3)- or a (1→6)-linkage, of which the former is excluded by the results of the methylation analysis.

The galactose-containing oligosaccharides formed a homologous series. Some of their properties are listed in Table V. Hydrolysis of a sample of each resulted in the formation of the lower homologues.

ACKNOWLEDGMENTS

The authors are indebted to Miss Birgitta Svensson for skilful assistance, to Dr. E. T. Reese for the gift of β-D-(1→3)-glucanase, and to *Cellulosaindustriens stiftelse för teknisk och skoglig forskning samt utbildning* for financial support.

SUMMARY

Cultures of *Armillaria mellea*, with D-glucitol as the carbon source, have been examined for content of water-soluble polysaccharide. Of the complex mixture obtained, two components have been isolated and structurally examined. The first has

been shown to be a xylomannan having a backbone of α-(1→3)-linked D-mannopyranose residues, every second to third of which is substituted in the 4-position by a 4-*O*-α-D-xylopyranosyl-D-xylopyranose residue. The second polysaccharide contains D-galactose, D-mannose, L-fucose, and 3-*O*-methyl-D-galactose, in a molecular ratio of 6:1:2:2. Structural features include a main chain of α-(1→6)-linked D-galactopyranose residues, substituted in the 2-position by single L-fucopyranose residues or by 3-*O*-α-D-mannopyranosyl-L-fucopyranose and 6-*O*-(3-*O*-methyl-α-D-galactopyranosyl)-D-galactose residues.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

5-THIO-D-RIBOPYRANOSE

PART I*. THE FREE SUGAR AND THE METHYL GLYCOSIDES

C. J. CLAYTON AND N. A. HUGHES

Department of Organic Chemistry, School of Chemistry, University of Newcastle upon Tyne (Great Britain)

(Received September 24th, 1966)

INTRODUCTION

When a sugar contains a thiol group suitably placed so that there are alternative possibilities for the formation of a hemithioacetal ring or a hemiacetal ring, the former should be preferred, since thiols react more readily with aldehydes than do alcohols. In recent years[2], several thio-sugars of this type have been synthesised. Our own researches have involved 5-thio-D-ribose. D-Ribose is an exceptional sugar in that furanoid forms are more than usually favoured in furanose–pyranose equilibria. In the reaction with methanol, methyl α- and β-D-furanosides are the kinetic products and are still present in considerable amounts at equilibrium[3,4]. Although crystalline D-ribose has the β-pyranose form[5], its mutarotation is complex[6] due to the involvement of furanoid[7] and, possibly, *aldehydo* forms[8]. Accordingly, it seemed of interest to prepare 5-thio-D-ribose in order to examine and compare its properties with those of D-ribose, since the preference for the pyranoid form containing a sulphur atom in the ring was expected to offset the tendency to furanose formation.

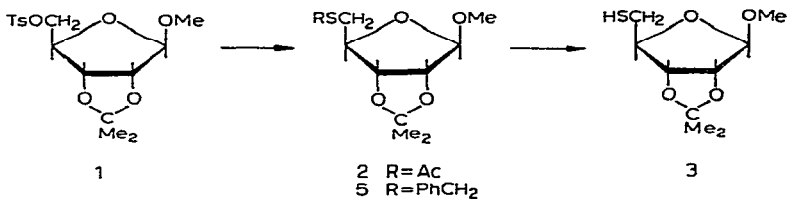
DISCUSSION

Displacement of the sulphonyloxy group of methyl 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside (**1**) occurred in nearly quantitative yield using potassium thiolacetate in hot *N*,*N*-dimethylformamide to give the thiolacetate (**2**). Saponification of the acetyl group, with ammonia or sodium methoxide, gave the free thiol (**3**) as a syrup that consumed the theoretical amount of iodine in undergoing oxidation to disulphide **4**. Thiol **3** underwent atmospheric oxidation too readily for other characterisation. Disulphide **4** has also been obtained by Ingles and Whistler[9]. They obtained the thiol (**3**) from the benzylthio compound (**5**), which was also prepared *via* sulphonate **1**.

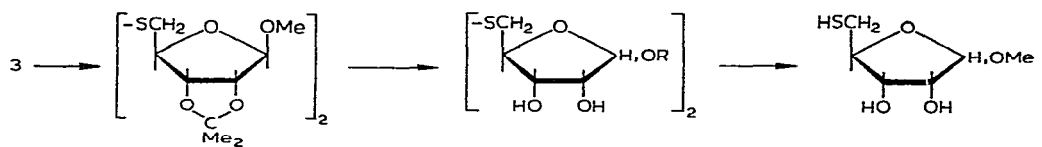
Hydrolysis of disulphide **4** with aqueous acid readily gave a reducing substance, presumably the di-sugar disulphide (**6**), which was not characterised further, but was treated with methanolic hydrogen chloride to give the crystalline di-$\beta\beta$-glycoside disulphide **7b** (β-D-configuration at each anomeric centre) as the major product.

*For a preliminary communication, see Ref. 1.

A brief treatment of compound **7b** with acetone containing sulphuric acid regenerated the di-*O*-isopropylidene derivative (**4**), and borohydride reduction of **7b** gave thiofuranoside **8b**. Unlike the β-D-ribofuranoside derivatives **2** and **4**, both of which have negative optical rotations as expected, the di-ββ-glycoside disulphide (**7b**) was remarkable in showing a positive rotation. Chromatographic examination of the mother



1 2 R=Ac 5 R=PhCH2 3



4 6 R=H 7 R=Me 8

[Configurations at anomeric centres: a, αα ; b, ββ ; c, αβ]

liquors from the Fischer reaction indicated the presence of two other products in addition to **7b**; these were probably the di-αα- and -αβ-glycoside disulphides (**7a**) and (**7c**), since borohydride reduction of the residue gave the thiol (**8b**), in addition to a second thiol (**8a**) of lower R_F value. The yields and R_F values of the disulphides were in the order: (**7b** > **7c** > **7a**). Methyl β-D-ribofuranoside has a higher R_F value than the α-D anomer in the solvent system used, and the α:β ratio in the Fischer reaction is approximately [4]1:3. The diglycosides (**7**) could be obtained directly from disulphide **4** by treatment with methanolic hydrogen chloride, but removal of the isopropylidene groups occurred very slowly under these conditions.

Hydrolysis of acetate **2** with aqueous acid gave only poor yields of 5-thio-D-ribose, probably due to the prolonged reaction times necessary to complete the removal of the *S*-acetyl group. Careful acidic hydrolysis of the 5-thioglycoside (**3**) readily gave the thio sugar. Initially, it was obtained as a syrup, but the absence of an SH band in its infrared spectrum indicated the pyranoid structure (**9**)[10]. After several months, the sugar crystallised, and samples were obtained having various melting points. Examination of these samples by polarimetry indicated that, in the main, they were different mixtures of α-D and β-D anomers (**9a**) and (**9b**), which had co-crystallised. Small samples of the reasonably pure anomers, however, were obtained on isolated occasions, the β-D-anomer (**9b**) {$[\alpha]_D$ − 37 (3 min) → +28°} being obtained in the earlier stages of a crystallisation, and the α-D anomer (**9a**) {$[\alpha]_D$ +127 (2 min) → +25°} in the later

stages. Attempts to obtain further quantities of the pure anomers, by seeding solutions of the sugar with these samples, failed. Although polarimetry was generally used to estimate the α:β ratio in the crystalline samples, infrared spectroscopy proved to be the most sensitive way of detecting small amounts of anomeric impurity; none of the spectra obtained showed SH bands. In these compounds and in derivatives mentioned later, it was assumed from considerations of rotations of known ribopyranose derivatives that dextrorotatory isomers were α-D anomers and laevorotatory isomers were β-D anomers.

The first mutarotation studies were performed in aqueous solutions. Smooth curves were obtained giving rise to straight line plots of "t against log $(r_t - r_\infty)$"

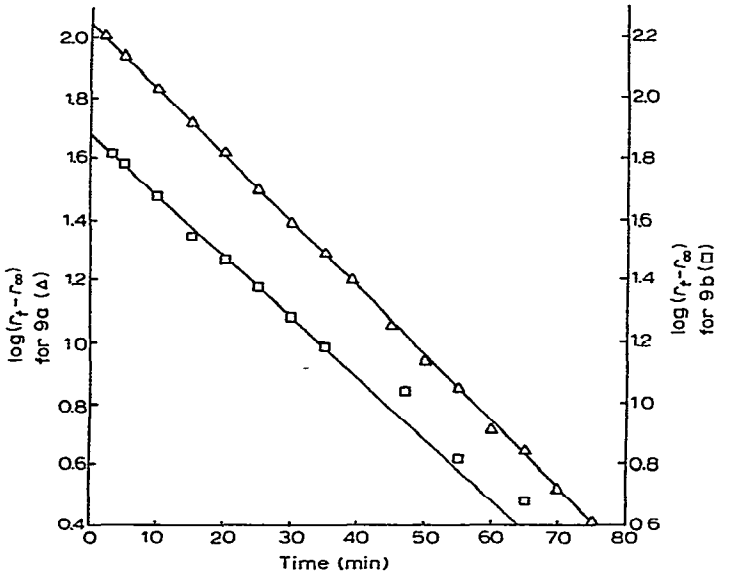
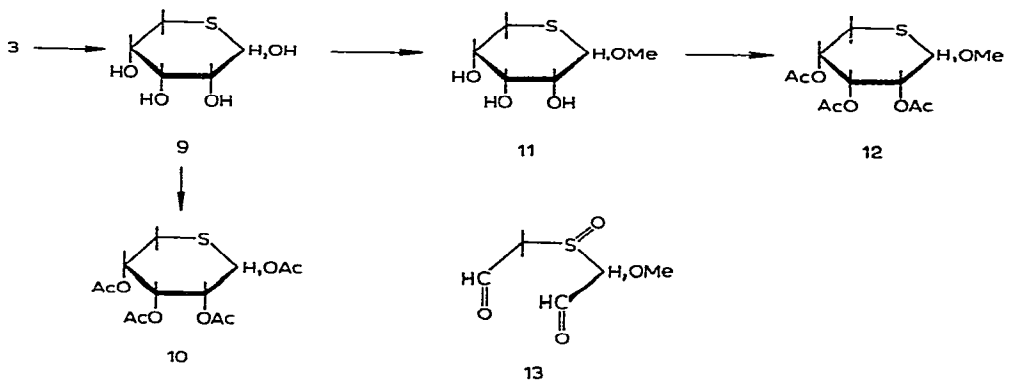


Fig. 1. Plots of $\log(r_t - r)$ against time for the mutarotation of 5-thio-α-D-ribopyranose (**9a**) (△ and 5-thio-β-D-ribopyranose (**9b**) (□) in water at 23° and 24°, respectively.

indicating an equilibrium involving two anomeric forms only[11]. This result, together with the absence of an SH band in the infrared spectrum, provides good evidence that, as expected, furanoid and *aldehydo* forms play no significant part in the mutarotation. For aqueous solutions, the mutarotation coefficients were not always reproducible, probably due to pH variations, but use of phthalate buffers[12] gave reproducible values. Schwarz and Yule[13] have already pointed out the remarkable sensitivity of the mutarotation half-life of 5-thio-α-D-xylopyranose to variations of pH in the regions near neutrality (>10 h at pH *ca.* 4.4, and *ca.* 10 min. at pH *ca.* 6.6), and the mutarotation half-lives of 5-thio-α-D-ribopyranose (**9a**) are closely similar (*ca.* 10 h at pH *ca.* 4.4, and *ca.* 7 min at pH *ca.* 6.6) at these pH values.

Acetylation of the syrupy thio-sugar **9** with acetic anhydride in pyridine gave a crystalline tetra-acetate; spectral evidence indicated the absence of *S*-acetyl groups, and, in view of its negative optical rotation, the β-D-pyranoid structure (**10b**) was

assigned. The syrupy α-D anomer (**10a**), obtained from the mother liquors by chromatography on silica gel, had a positive optical rotation and spectral characteristics similar to those of the β-D anomer (**10b**).



[Formulae 8–12 : a, α-D anomer; b, β-D anomer]

With refluxing, methanolic hydrogen chloride, thio sugar **9** readily gave a mixture of the methyl pyranosides (**11a**) and (**11b**). Initially, separation of these glycosides was troublesome, for, although crystallisation occurred readily, chromatography indicated that both anomers were co-crystallising; the same problem had already been encountered with the free sugar (**9**). Fortunately, a separation was readily achieved by chromatography on a basic ion-exchange resin[14], and both glycosides were obtained in crystalline form. Earlier attempts to separate the acetyl derivatives (**12**) by chromatography on silica gel were less satisfactory. Only the α-D tri-acetate (**12a**) could be obtained crystalline. A more direct preparation of glycoside **11b**, albeit in low yield, by methanolysis of thiol **3** has been reported[9]. In view of the constants given for the product, and the ease with which both anomers co-crystallise, the product was probably an αβ-mixture. Organic sulphides are oxidised by periodate to sulphoxides[15], and, in keeping with structures **11a** and **11b**, both glycosides consumed three equivalents of periodate and liberated one equivalent of acid. The periodate uptake was measured spectrophotometrically[16], or by the thiosulphate method[17], since "dialdehyde" **13** reacted with iodine; behaviour of this type has been noted on previous occasions[18]. The reaction of thio sugar **9** with methanolic hydrogen chloride at room temperature was examined by polarimetry and chromatography. The first glycoside to be detected was the α-D-pyranoside (**9a**) (0.17 h), followed closely by the β-D-pyranoside (**9b**) (0.5 h) and then the β-D-furanoside (**8b**) (1 h). The α-D-furanoside (**8a**) was only detected much later (22 h), at which time the free sugar (**9**) finally disappeared from the reaction mixture. Both furanosides (**8**) also eventually disappeared from the reaction mixture. The optical rotation of the solution showed an initial increase up to a maximum at *ca.* 20 h, and thereafter decreased smoothly to become constant after 500 h. This behaviour suggests the initial, rapid formation of the α-D-pyranoside (**11a**), which then equilibrates slowly to give increasing amounts of the

β-D-pyranoside (**11b**). The furanosides (**8**) are only minor products, in contrast to the Fischer reaction of D-ribose[3,4]. In its reaction with methanol, thio sugar **9** has again shown the expected preference for the pyranoside form containing a sulphur atom in the ring.

A comparison of the ease of hydrolysis of the ribopyranosides containing a sulphur or an oxygen atom in the ring was made by following the hydrolyses by chromatography. The results are given in the accompanying Table, together with the optical rotations of the glycosides. It is to be noted that, apart from having the same

TABLE I

HYDROLYSIS TIMES AND OPTICAL ROTATIONS OF METHYL D-RIBOPYRANOSIDES

Compound	*Hydrolysis time (h)*	$[\alpha]_D$	2*A*
Methyl α-D-ribopyranoside	14	+103[a]	+34,000
Methyl β-D-ribopyranoside	30	−106[a]	
Methyl 5-thio-α-D-ribopyranoside (**11a**)	1.0	+301	+98,000
Methyl 5-thio-β-D-ribopyranoside (**11b**)	3.0	−246	

[a]Ref. 19.

signs, the 2A values show little agreement. The sulphur-containing glycosides are hydrolysed approximately ten times faster than are their oxygen analogues. This behaviour closely parallels that observed[20] in the D-xylose and 5-thio-D-xylose series by Whistler and Van Es[20]. The sulphur-containing xylopyranosides were hydrolysed faster, by a factor of *ca.* 10, in the α-D series, and by a factor of *ca.* 14 in the β-D series. These authors[20] have discussed this rate enhancement in terms of the reaction mechanism.

It was hoped to modify suitable derivatives of thio sugar **9** by methylation to give sulphonium compounds. Mild treatment of the tetra-acetate (**10b**), the tri-acetate (**12a**), and the glycoside (**11b**) with methyl iodide left them unaffected; more drastic conditions merely brought about slow decomposition.

The methyl α- and β-D-ribopyranosides required for the hydrolysis experiments were obtained from the mixture of glycosides, formed in the Fischer reaction, by chromatography on a basic ion-exchange resin. It was noted that ribofuranosides and ribopyranosides could be distinguished by their behaviour on chromatograms with the periodate–Schiff reagent[21]. The furanosides gave intense, blue spots, whereas the pyranosides gave much weaker spots, often having white centres. In addition, ribopyranosides appeared as brown (iodine) spots on a white background when the excess of periodate was decomposed carefully with sulphur dioxide; the colour was cleared with more sulphur dioxide before applying the Schiff reagent. D-Ribose, 1,5-anhydroribitol, and the β-D-pyranoside (**11b**) behaved similarly. Barker and Shaw[22] have shown that D-ribose and methyl β-D-ribopyranoside form complexes with the periodate ion and have suggested that this is possible because of the *cis,cis*-triol system present in ribopyranose derivatives. The above behaviour is probably due

to this complex formation, the complexes being only slowly decomposed with sulphur dioxide.

EXPERIMENTAL

General methods. — Wherever possible, reactions were carried out under nitrogen, and a nitrogen bleed was used for evaporations under reduced pressure. Silica gel, Hopkin and Williams, M.F.C., and Merck, G. grade, respectively, were used for column and thin-layer chromatography; compounds were detected in t.l.c. by iodine vapour. Whatman No. 1 paper was used with ascending development in the system butyl alcohol–water (86:14, v/v) for paper chromatography. α-Glycols were detected by the periodate–Schiff reagent[21] or the periodate–benzidine reagent[23]. Reducing sugars were detected with aniline hydrogen phthalate[24] or silver nitrate[25] (sulphides also responded to this latter reagent, although much more slowly than reducing sugars). Sulphur compounds were detected with potassium iodoplatinate[26]. Thiols were revealed as purple spots by a 1% solution of sodium nitroprusside in dilute sodium carbonate; subsequent treatment with a solution of sodium cyanide (2 g), sodium hydrogen carbonate (2 g), and sodium carbonate (2 g) in water (50 ml) then revealed disulphides. Optical rotations were measured on a Bendix NPL Polarimeter Type 143A in 1-cm cells, or on a Bellingham and Stanley Optical Polarimeter in 10-cm cells.

Methyl 5-S-acetyl-2,3-O-isopropylidene-5-thio-β-D-ribofuranoside (**2**). — A solution of the sulphonate (**1**) (10.5 g) and potassium thiolacetate (4.0 g) in *N,N*-dimethylformamide (170 ml) was kept for 8 h at 100°. *N,N*-Dimethylformamide was distilled off under reduced pressure, and the semi-solid residue was partitioned between chloroform and water. Evaporation of the dried chloroform solution yielded a brown oil. Further purification by vacuum distillation gave thiolacetate **2** (7.1 g), b.p. 100°/0.07 mm., $[\alpha]_D^{20} - 49°$ (*c* 1.0, methanol), n_D^{20} 1.4780, ν_{max} 1689 cm^{-1} (SAc), λ_{max} 230 mμ (ε 4,000) (Found: C, 50.5; H, 6.8; S, 11.9. $C_{11}H_{18}O_5S$ calc.: C, 50.4; H, 6.9; S, 12.2%).

Bis(methyl 5-deoxy-2,3-O-isopropylidene-β-D-ribofuranoside) 5,5'-disulphide (**4**). — Sodium methoxide (0.4N, 0.3 ml) was added to a solution of the *S*-acetyl derivative **2** (279 mg) in methanol (2 ml). After 18 h, N sulphuric acid (0.1 ml) was added, followed by 0.5N sodium acetate (15 ml) and water (30 ml). The solution was then decolourised with 0.0845N iodine solution (11.8 ml; theory, 12.5 ml). During the titration, the product began to crystallise. A crystal of sodium thiosulphate was added to remove any excess of iodine, and the solution was evaporated (to 10 ml) and left at 0°. The disulphide (**4**) (220 mg) crystallised as fine needles, m.p. 76–78°, $[\alpha]_D^{20} - 108°$ (*c* 0.7, methanol), λ_{max} 251 mμ (ε 600) (lit.[9], m.p. 67°, $[\alpha]_D^{25} - 124°$) (Found: C, 49.5; H, 7.0; S, 14.4. $C_{18}H_{30}O_8S_2$ calc.: C, 49.3; H, 6.9; S, 14.6%).

Bis(methyl 5-deoxy-β-D-ribofuranoside) 5,5'-disulphide (**7b**). — Disulphide **4** (220 mg) was stirred and heated under reflux with acetic acid (1 ml), N sulphuric acid (1 ml), and water (8 ml) for 30 min. Paper chromatography then indicated a single reducing compound of R_F 0.16. The solution was passed through Dowex-1 (AcO^-, 2 ml)

and evaporated to a syrup. This was dissolved in methanol (10 ml), concentrated hydrochloric acid (0.2 ml) was added, and, after 30 min at room temperature, the solution was passed through Dowex-1 (AcO^-, 5 ml) and evaporated to dryness. Crystallisation of the residue from ethyl acetate gave disulphide **7b** (97 mg), m.p. 135–136°, $[\alpha]_D^{20}+52°$ (*c* 0.5, methanol), R_F 0.60 (Found: C, 40.2; H, 6.3; S, 17.75. $C_{12}H_{22}O_8S_2$ calc.: C, 40.2; H, 6,15; S, 17.9%).

Evaporation of the mother liquors left a syrupy residue (60 mg). Paper chromatography indicated the presence of a mixture of three non-reducing, glycol-containing disulphides having R_F values 0.60, 0.51, and 0.45, presumably the isomers **7a–c**.

A solution of the crystalline product (10 mg) in acetone (1 ml) containing concentrated sulphuric acid (0.01 ml) was left for 5 min at room temperature and then neutralised with solid sodium carbonate. Solids were filtered off, and the acetone was evaporated to leave a crystalline residue. Recrystallisation from aqueous methanol gave the *O*-isopropylidene derivative (**4**) (9 mg), m.p. and m.m.p. 76–78°.

Borohydride reductions. — Samples (5 mg) of the di-$\beta\beta$-glycoside **7b** and the mixed diglycosides **7** were dissolved in aqueous solutions (0.5 ml) of sodium borohydride (5 mg). After 30 min at room temperature, the solutions were neutralised with dilute acetic acid and passed through Dowex-50 (NH_4^+, 1 ml). The eluates were evaporated to dryness, and the residues were taken up in methanol (10 ml). The solutions were concentrated (1 ml) at atmospheric pressure. Examination of the residual solutions by paper chromatography indicated the presence of a single thiol (R_F 0.69) in the first experiment, and of two thiols (R_F 0.69 and 0.62) in the second.

*5-Thio-*D*-ribopyranose* (**9**). — Sodium methoxide (0.5N, 0.5 ml) was added to a solution of the *S*-acetyl derivative **2** (2.63 g) in methanol (10 ml). After 24 h at room temperature, the solution was evaporated, and the residual syrup was stirred with 0.1N sulphuric acid (170 ml) for 1 h at 80°. The cooled solution was passed through Dowex-1 (AcO^-, 30 ml), and evaporated to a syrup that crystallised after several months. Recrystallisation from ethanol gave **9** (1.00 g), R_F 0.30. The best sample of the α-D anomer (**9a**) had m.p. 160°, $[\alpha]_D^{23}+127°$ (2 min)→ $+25°$ (*c* 0.6, water), $k_1 + k_2$: 0.022 min^{-1} (water, 23°); 0.00043 min^{-1} (0.001M potassium hydrogen phthalate, pH *ca.* 4.4, 21°); 0.042 min^{-1} (0.005M potassium hydrogen phthalate, pH *ca.* 6.6, 21°) (Found: C, 36.4; H, 6.1; S, 19.2. $C_5H_{10}O_4S$ calc.: C, 36.1; H, 6.1; S, 19.3%). The best sample of the β-D anomer **9b** had m.p. 142–144°, $[\alpha]_D^{24}-37$ (3 min) → $+28°$ (*c* 1.12, water), $k_1 + k_2$: 0.020 min^{-1} (water, 24°). After repeated recrystallisation from propyl alcohol, it had m.p. 167–168° (Found: C, 36.3; H, 6.1; S, 19.2. $C_5H_{10}O_4S$ calc.: C, 36.1; H, 6.1; S, 19.3%).

Neither the i.r. spectrum of the syrupy product (as a capillary film) nor the spectra of crystalline products (as KBr discs) showed absorption in the region of 2560 cm^{-1} (SH). The following peaks (cm^{-1}) were usefully diagnostic of the anomeric forms: α, 960, 920, 870, 850, 810; β, 1010, 935, 910, 900. The syrupy product was generally sufficiently pure for the preparation of further derivatives.

*1,2,3,4-Tetra-*O*-acetyl-5-thio-*α-(**10a**) *and-*β-D*-ribopyranose* (**10b**) — A solution of thio sugar **9** (118 mg) in pyridine (2 ml) was treated with acetic anhydride (1 ml) and

kept for 15 h at 0°. Processing, in the usual manner, with chloroform gave a syrup that crystallised from ethanol to give the β-D anomer (37 mg), m.p. 123–124°, $[\alpha]_D^{23}$ −61° (*c* 1.0, methanol), ν_{max} 1748 cm^{-1} (OAc) (Found: C, 47.1; H, 5.3; S, 9.35. $C_{13}H_{18}O_8S$ calc.: C, 46.7; H, 5.4; S, 9.6%).

The residue (94 mg, $[\alpha]_D^{23}$ + 150°) from the mother liquors was chromatographed on silica gel, when a single peak was obtained. Earlier fractions, however, had successively increasing rotations. Later fractions gave syrupy material (53 mg) of constant rotation, $[\alpha]_D^{23}$ + 221° (*c* 0.77, methanol) ν_{max} 1748 cm^{-1} (OAc) (Found: S, 9.2. $C_{13}H_{18}O_8S$ calc.: 9.6%).

Polarimetric and chromatographic study of the reaction of 5-thio-D-ribopyranose (**9**) *with methanolic hydrogen chloride.* — The thio sugar (**9**, 20.2 mg, $[\alpha]_D^{23}$ *ca.* + 34 → + 28°) was dissolved in 1% methanolic hydrogen chloride (2 ml). A portion of the solution was placed in the polarimeter cell; the remainder was used for chromatographic studies in which samples were withdrawn, applied to the paper, and neutralised with ammonia fumes before being dried in a stream of nitrogen. Under these conditions, no oxidation of thiols to disulphides was observed.

Methyl 5-thio-α-(**11a**) *and -β-D-ribopyranosides* (**11b**). — After being heated under reflux for 1 h, a solution of the thio sugar (**9**, 2.06 g) in 1% methanolic hydrogen chloride (80 ml) was left at room temperature for 16 h. The solution was neutralised with Dowex-2 (CO_3^{2-}), and evaporated to a pale-yellow syrup (2.17 g) that crystallised from ethyl acetate to give the mixed glycosides **11** (1.30 g), m.p. 95–98°, $[\alpha]_D^{25}$ − 67° (lit.[9], m.p. 97°, $[\alpha]_D^{25}$ + 18.6°). Further recrystallisation did not improve the purity.

A portion (352 mg) of the mixed glycosides **11** was chromatographed on a column (29 × 1.2 cm) of Dowex-1 (HO^-) and eluted with water (10-ml fractions). Fractions 4–6 contained the chromatographically pure α-D-glycoside (120 mg), which, after recrystallisation from benzene, had m.p. 73°, $[\alpha]_D^{26}$ + 301° (*c* 0.9, methanol), R_F 0.50 (Found: C, 40.0; H, 6.5; S, 17.75. $C_6H_{12}O_4S$ calc.: C, 40.0; H, 6.7; S, 17.8%). Periodate uptake: 2.90 moles/mole; acid produced: 1.08 moles/mole (only slight overoxidation occurred). Fractions 8–15 contained the β-D-glycoside (227 mg), which crystallised from ethyl acetate in needles having m.p. 124°, $[\alpha]_D^{25}$ − 246° (*c* 1.1, methanol). R_F 0.60 (Found: C, 40.15; H, 6.45; S, 17.5. $C_6H_{12}O_4S$ calc.: C, 40.0; H, 6.7; S, 17.8%). Periodate uptake: 3.26 moles/mole; acid produced: 0.93 moles/mole (slow overoxidation occurred).

Separation of the methyl 5-thio-α-(**11a**) *and-β-D-ribopyranosides* (**11b**) *as their tri-acetates* (**12a** and **12b**). — A sample of the mixed glycosides **11** (180 mg) was acetylated in the usual manner with acetic anhydride (1 ml) in pyridine (3 ml) to give a syrupy mixture (299 mg) of the tri-acetates (**12**). The mixture was chromatographed on silica gel (40 g), and eluted with benzene–ether (97:3, 30-ml fractions). Two peaks were obtained, with a few, intermediate fractions. The first peak yielded the β-D- triacetate (**12b**, 163 mg), $[\alpha]_D^{25}$ − 78° (*c* 1.0, methanol), which did not crystallise; deacetylation of **12b** with ammonia, with crystallisation of the product from ethyl acetate, gave the β-D-glycoside (**11b**) (57 mg), m.p. and m.m.p. 123°. The second peak gave methyl 2,3,4-tri-*O*-acetyl-5-thio-α-D-ribopyranoside (**12a**, 52 mg) as lustrous plates

from aqueous methanol, m.p. 65–66°, $[\alpha]_D^{21} + 227°$ (*c* 0.8, methanol) (Found: C, 46.7; H, 6.2; S, 10.7. $C_{12}H_{18}O_7S$ calc.: C, 47.0; H, 5.9; S, 10.5%); deacetylation with ammonia, and crystallisation from benzene gave the α-D-glycoside (**11a**), m.p. and m.m.p. 73°.

Hydrolysis experiments. — The appropriate weight of glycoside was dissolved in 0.2N hydrochloric acid, so that the solution was 0.023M with respect to the glycoside. The solutions were kept at 80° in stoppered tubes. At selected times, samples were withdrawn, spotted on to paper chromatograms, neutralised with ammonia fumes, and dried in a stream of nitrogen. The times taken for the glycosides to disappear are shown in Table I.

Separation of methyl D-*ribosides on Dowex-1 (HO^-).* — A solution of D-ribose (800 mg) in 1% methanolic hydrogen chloride was kept at 70° in a sealed tube for 10 days. After neutralisation with silver carbonate, evaporation gave a pale-yellow syrup (880 mg).

A portion of the syrup (193 mg) was chromatographed on a column (29 × 1.2 cm) of Dowex-1 (HO^-) and eluted with water (10-ml fractions). Fraction 4 gave pure methyl α-D-ribopyranoside (15 mg), $[\alpha]_D^{22} + 106°$ (*c* 0.5, methanol) (lit.[19], $[\alpha]_D^{20} + 103.3°$), R_F 0.30. Fraction 5 contained a mixture (38 mg) of the α-D-pyranoside, the β-D-pyranoside and the α-D-furanoside. Fractions 6 and 7 contained a mixture (86 mg) of the β-D-pyranoside and the α-D-furanoside. From ethyl acetate, fractions 5, 6, and 7 yielded methyl β-D-ribopyranoside (53 mg), m.p. 78–79°, $[\alpha]_D^{22} - 106°$ (lit.[19], m.p. 78°, $[\alpha]_D^{20} - 106.5°$), R_F 0.42. Fractions 9–13 gave the crystalline β-D-furanoside (18 mg), m.p. 77° (lit.[27], m.p. 79–80°), R_F 0.38.

ACKNOWLEDGMENT

We thank the D.S.I.R. for a Research Studentship (to C.J.C.); and Imperial Chemical Industries Limited for financial assitance (to C.J.C).

SUMMARY

5-Thio-D-ribose has been synthesised from methyl 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside. Two crystalline forms of the sugar having different signs of optical rotation were obtained although, more commonly, they co-crystallised. The absence of an SH absorption in their infrared spectra indicates the dextrorotatory and laevorotatory forms to be the α-D-pyranose and β-D-pyranose isomers, respectively. Their mutarotation curves are simple, in contrast to that of β-D-ribopyranose, suggesting the absence of furanoid forms in the equilibria. Methyl 5-thio-α- and -β-D-ribopyranosides are the major products of the Fischer reaction of the sugar with methanolic hydrogen chloride. The two thiopyranosides undergo acidic hydrolysis more readily than the corresponding methyl α- and β-D-ribopyranosides.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SUBSTITUTED CEREBROSIDES

PART III. 1-*O*-(4-*O*-β-D-HEXOPYRANOSYL-β-D-GLUCOPYRANOSYL)DIHYDROCERAMIDES

H. M. FLOWERS
The Department of Organic Chemistry, The Weizmann Institute of Science, Rehovoth, Israel
(Received October 5th, 1966)

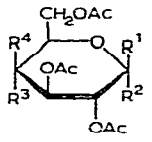
INTRODUCTION

The gangliosides and other complex glycosphingolipids containing more than one hexose residue generally include a 4-*O*-β-D-hexopyranosyl-β-D-hexopyranosyl moiety attached to ceramide[1–4]. The widely occurring cytolipin H has been recently characterized as (4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-ceramide[5]. Chemical synthesis of such naturally occurring disaccharide-lipids is of great interest *per se*, and may also provide a route to the synthesis of the more complex derivatives. Cytolipin H was recently synthesized by utilizing the disaccharide derivative hepta-*O*-acetyl-α-lactosyl bromide[6] (**1**). It was considered useful to devise an alternative approach by a stepwise process, first preparing a suitably protected cerebroside (monosaccharide–lipid), and then attaching to this the required second monosaccharide residue. This second step would thus provide a model for the synthesis of more-complex glycosphingolipids which could be visualized as arising from the attachment of various sugar moieties (of monosaccharides, disaccharides, etc.) to the cerebroside. A problem inherent in this approach is the known low reactivity of the secondary hydroxyl groups in hexopyranoses in respect to the formation of disaccharides[7], illustrated by the low yields generally obtained in the synthesis of such relatively simple compounds as lactose[8,9] and cellobiose[10–13]. In the preparation of disaccharide–lipids, it is obviously more convenient to use disaccharide derivatives when they are readily available (those of lactose, for example). However, where the disaccharide may be obtainable only with difficulty, it becomes practical to prepare the disaccharide–lipid by attachment of a monosaccharide residue to a monosaccharide–lipid, instead of first synthesizing the disaccharide in low yield and then attaching this to the lipid by a second glycosylation reaction.

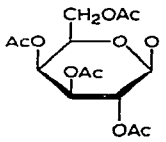
The feasibility of the stepwise approach has been shown by the recently reported preparation of a protected cerebroside having the primary (C-6) hydroxyl group free, and its reaction with acylglycosyl halides to give disaccharide-lipids[14]. There are indications that the secondary hydroxyl group on C-4 is the least reactive in many glycopyranosides[15], and so success of glycosylation reactions at C-4 would offer hope for similar reactions of other secondary hydroxyl groups of glycopyranosides, thus offering the means of synthesizing a variety of glycolipids. Such compounds have not yet been described, but might provide suitable models for comparison with natural products of unknown structure.

RESULTS AND DISCUSSION

Syrupy 2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (**2**) was prepared in good yield by reaction, under mild conditions, of 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose[16] with a solution of hydrogen bromide in acetic acid–chloroform. The product, although homogeneous on t.l.c., could not be induced to crystallize and was unstable. It gave the high dextrorotation and the n.m.r. spectrum to be expected of a tri-*O*-acetyl-α-D-glycopyranosyl halide. Reaction with anhydrous methanol in the presence of mercuric cyanide, followed by *p*-toluenesulfonylation, led to the isolation of the known methyl 2,3,6-tri-*O*-acetyl-4-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (**3**) in good yield. It is thus reasonable to presume that there was no migration of acetyl groups either in the preparation of the bromide or in its reaction with methanol to give the methyl β-D-glucopyranoside ester.



1 $R^1 = R^4 = H$; $R^2 = Br$; $R^3 =$



2 $R^1 = R^4 = H$; $R^2 = Br$; $R^3 = OH$

3 $R^1 = OCH_3$; $R^2 = R^4 = H$; $R^3 = OTs$

6 $R^1 = R^4 = H$; $R^2 = Br$; $R^3 = OAc$

7 $R^1 = R^3 = H$; $R^2 = Br$; $R^4 = OAc$

Reaction of the bromide with 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine* (**4**) gave, in good yield, the cerebroside ester (**5**) as a low-melting solid. The product was treated with tetra-*O*-acetylglycosyl halides (**6**,**7**), to give disaccharide esters. Reaction was only partial; after catalytic transesterification, the required dihexoside could be isolated by chromatography on silicic acid. The glucocerebroside obtained as a byproduct was shown to be identical with the known 1-*O*-β-D-glucopyranosyl-*N*-octadecanoyl-DL-dihydrosphingosine** (**8**)[14], thus confirming the β-D configuration of the glycosyl–dihydrosphingosine linkage.

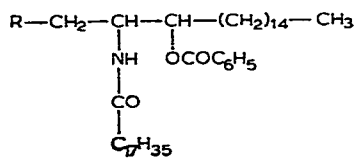
The dihexosides obtained were indistinguishable, on t.l.c. in two solvent systems, from authentic specimens of 1-*O*-(4-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-*N*-octadecanoyl-DL-dihydrosphingosine*** (**9**) and 1-*O*-(4-*O*-β-D-galacto-

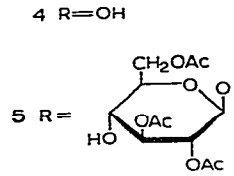
*N-(DL-*erythro*-3-Benzoyloxyoctadecan-2-yl)octadecanamide.

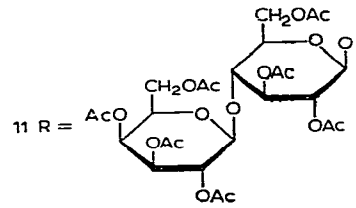
**N-[DL-*erythro*-1-(β-D-Glucopyranosyl)oxyoctadecan-2-yl]octadecanamide.

***N-[DL-*erythro*-1-(4-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxyoctadecan-2-yl]octadecanamide.

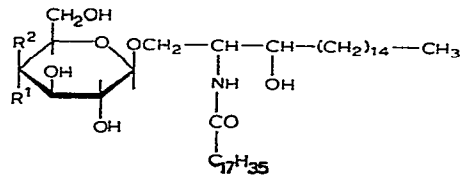
pyranosyl-β-D-glucopyranosyl)-*N*-octadecanoyl-DL-dihydrosphingosine* (**10**), respectively, prepared by the reaction of hepta-*O*-acetylcellobiosyl bromide or hepta-*O*-acetyllactosyl bromide on the ester (**5**). However, their m.p. and optical rotations

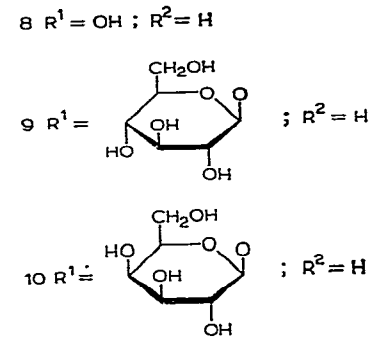






varied with the conditions of the second glycosylation reaction, and differed from those of the authentic specimens. It was found that more-vigorous reaction conditions,





with a large excess of the bromide, gave products containing large proportions of the corresponding α-D-linked disaccharides. Although the Koenigs–Knorr reaction in the presence of mercuric cyanide generally leads to the isolation of β-D-glycosides[14,17], cases have been reported of the isolation of α-D anomers from such reactions[18,19]. The mechanism generally proposed for the reaction of acylglycosyl halides with a hydroxyl group involves participation of the acyl substituent at C-2, facilitating the ionization of the halide, thus tending to favor the formation of β-D-glycosides (by rearside attack on C-1 in an S_N2 type of reaction) from the D-glucosyl or D-galactosyl derivatives[20]. It is possible that, in the more-drastic reaction conditions present at higher temperatures and in the presence of an excess of the (acidic) acylglycosyl

*N-[DL-*erythro*-1-(4-*O*-β-D-Galactopyranosyl-β-D-glucopyranosyl)oxyoctadecan-2-yl]octadecanamide.

halide, ionization of the halide, with formation of the carbonium ion which reacts without participation of the acyl substituent at C-2 (in an S_N1 type of reaction) is predominant, thus leading to a mixture of the α- and β-D-anomers.

EXPERIMENTAL

Melting points were taken between glass slides on a Fisher–Johns apparatus and are corrected. Rotations were determined, in semimicro tubes, with a Perkin–Elmer No. 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, with tetramethylsilane as the internal standard and deuteriochloroform as the solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used, and elution was stepwise, in order of increasing polarity of the eluents. The proportion of weight of substance (added to the column) to weight of adsorbent was 1 to 50–100. The fractions eluted were 2 ml/g of the column. "Silicic acid" refers to Bio-Rad silicic acid, used after a preliminary heating for 60 min at 120°. Thin-layer chromatograms were run on Kieselgel G (E. Merck, Darmstadt). Analyses were made in the Institute's Microanalytical Laboratory, under the direction of Mr. R. Heller.

2,3,6-Tri-O-acetyl-α-D-glucopyranosyl bromide (**2**). — To a solution of 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose[16] (2.0 g) in alcohol-free chloroform (10 ml) was added a solution of hydrogen bromide in glacial acetic acid (45% w/v, 3 ml). The clear solution was kept for 2 h at room temperature, additional chloroform and ice were added, and the chloroform extract was successively washed with a cold, saturated solution of sodium hydrogen carbonate (until the washings were no longer acid) and water, and dried with calcium chloride. Removal of the solvent *in vacuo* left a pale-yellow oil which was decolorized by dissolution in anhydrous ether and addition of a little carbon. After filtration, and evaporation of the ether *in vacuo*, a colorless syrup (1.5 g) remained, $[\alpha]_D^{20} + 186°$ (*c* 2.53 chloroform). T.l.c. in benzene–ether 2:1, R_F 0.25 (R_F of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, 0.63). N.m.r.: a comparison with the published spectrum of (**6**)[21] showed the following resemblances — a doublet at τ 3.40 (*J* 4 c.p.s.) attributable to H-1 split by H-2, a triplet at τ 4.50 (H-3), a quartet at τ 5.20 (H-2), and a complex group of signals at τ 5.66–5.85. The methyl protons at τ 7.91–7.93 (6) and τ 7.96 (3) were attributable to the 3 acetate groups, and the triplet at τ 4.86 (H-4) present in **6** had shifted upfield and was located at τ *ca.* 5.8. This is to be expected if the acetoxy group at C-4 has been replaced by a hydroxyl group. The oil was unstable and became discolored after a few days, even when preserved in a desiccator *in vacuo*, with sodium hydroxide as the desiccant.

Anal. Calc. for $C_{12}H_{17}BrO_8$: C, 39.04; H, 4.64; Br, 21.65. Found: C, 38.29; H, 4.78; Br, 21.17.

Methyl 2,3,6-tri-O-acetyl-4-O-p-tolylsulfonyl-β-D-glucopyranoside (**3**). — A solution of **2** (0.40 g) in 2:1 nitromethane–benzene (20 ml) containing anhydrous methanol (0.08 ml) and mercuric cyanide (0.35 g) was kept for 18 h at 50–60°. After concentration *in vacuo*, the residue was dried thoroughly and dissolved in pyridine

(1 ml). *p*-Toluenesulfonyl chloride (0.14 g) was added, and the solution was kept overnight at room temperature and then for 20 min at 60°. The solvent was removed by a stream of nitrogen, and the residue crystallized on addition of absolute alcohol and cooling; slender prisms, (0.25 g, 49% overall yield), m.p. 115–117°. Recrystallization (absolute alcohol) gave prisms, m.p. 116–117°, $[\alpha]_D^{25}$ − 42.0° (*c* 0.44, chloroform) [lit.[22] m.p. 116–117°, $[\alpha]_D$ − 43.8°].

Anal. Calc. for $C_{20}H_{26}O_{11}S$: C, 50.62; H, 5.52; S, 6.76. Found: C, 50.69; H, 5.40; S, 6.50.

3-O-*Benzoyl*-N-*octadecanoyl*-*1*-O-(*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosyl*)-DL-*dihydrosphingosine** (**5**). — To a stirred solution of **4** (0.67 g, 1 mmole) in an anhydrous mixture of nitromethane and benzene (2:1, 50 ml)[14] were added mercuric cyanide (0.25 g, 1 mmole) and then a solution of **2** (0.35 g, 0.95 mmole) in benzene (5 ml), dropwise over 1 h. The reaction was allowed to proceed overnight at 70° under anhydrous conditions. After being cooled, the mixture was diluted with benzene (30ml) and washed successively with a cold saturated solution of sodium hydrogen carbonate and water, dried with sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in a few ml of hexane and passed through a column of silica gel (50 g). Hexane (100 ml) eluted 0.62 g (64%) of a white solid which crystallized from cold methanol, giving 500 mg (52%) of m.p. 50–52°, $[\alpha]_D^{25}$ − 17.0° (*c* 1.33 chloroform). R_F 0.2 (t.l.c. with 2:1 benzene–ether).

Anal. Calc. for $C_{55}H_{93}NO_{12}$: C, 68.79; H, 9.76. Found: C, 68.89; H, 10.02.

3-O-*Benzoyl*-N-*octadecanoyl*-*1*-O-[*4*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosyl*]-DL-*dihydrosphingosine*** (**11**). — To a stirred solution of **4** (0.67 g, 1 mmole) in 2:1 nitromethane–benzene (50 ml) at 70° were added mercuric cyanide (0.25 g, 1 mmole) and hepta-*O*-acetyllactosyl bromide[23] (0.70 g, 1 mmole). The stirred solution was kept for 6 h at 70°, further portions of mercuric cyanide (0.12 g, 0.5 mmole) and hepta-*O*-acetyllactosyl bromide (0.35 g, 0.5 mole) were added, and the reaction was continued overnight at 70° under anhydrous conditions. The solution was processed as previously described, and the waxy residue left after removal of the organic solvent *in vacuo* was dissolved in a little hexane and purified by passage through a column of silica gel (50 g). Hexane (100 ml) eluted a product which crystallized from cold methanol: yield 0.87 g (67%), m.p. 68–70°, $[\alpha]_D^{24}$ − 9.2° (*c* 1.00, chloroform).

Anal. Calc. for $C_{69}H_{111}NO_{21}$: C, 64.21; H, 8.67. Found: C, 64.46; H, 8.88.

1-O-(*4*-O-β-D-*galactopyranosyl*-β-D-*glucopyranosyl*)-N-*octadecanoyl*-DL-*dihydrosphingosine**** (**10**). — Ester **11** (100 mg) dissolved, on slight warming, in anhydrous methanol (1 ml) containing a catalytic amount of sodium methoxide. After the solution

*N-[DL-*erythro*-3-Benzoyloxy-1-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)oxyoctadecan-2-yl]octadecanamide.

**N-{DL-*erythro*-3-Benzoyloxy-1-[4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]oxyoctadecan-2-yl} octadecanamide.

***N-[DL-*erythro*-1-(4-*O*-β-D-Galactopyranosyl-β-D-glucopyranosyl)oxyoctadecan-2-yl]octadecanamide.

had been kept overnight at room temperature, addition of cold water (1 ml) caused the formation of a bulky precipitate. This was separated by filtration, washed with water and acetone, and dried. Crystallization from 5:1 acetone–pyridine (8 ml) gave tiny needles, m.p. 243–244° (sintering at 180°), $[\alpha]_D^{22}$ −4.7° (*c* 1.70, pyridine).

Anal. Calc. for $C_{48}H_{93}NO_{13}$: C, 64.61; H, 10.51. Found: C, 64.50; H, 10.65.

Reaction of ester (**5**) *with 2,3,4,6-tetra-*O*-acetyl-α-*D*-glycopyranosyl bromides* (**6** or **7**). — (A) To a stirred solution of **5** (300 mg, 0.3 mmoles) in 2:1 nitromethane–benzene (20 ml) at 35° were added mercuric cyanide (75 mg, 0.3 mmoles) and **6** (123 mg, 0.3 mmoles). After 18 h, further portions of mercuric cyanide (25 mg) and **6** (40 mg) were added, and the reaction mixture was maintained for a total of 60 h at 35°. The solution was cooled, diluted with benzene, and processed in the usual way. The crude residue was dissolved in methanol and catalytically de-esterified as previously described. Cold water precipitated a solid which was separated by filtration, washed, dried, dissolved in a few ml of 19:1 chloroform–methanol, and purified by chromatography of the solution on silicic acid. Chloroform–methanol (19:1) eluted fractions weighing 130 mg (57%) identical[14] with **8** by m.p., optical rotation, and t.l.c. in two solvent systems. Chloroform–methanol (9:1) eluted fractions weighing 21 mg (6%). Recrystallization from methanol gave a product of m.p. 209–211°, $[\alpha]_D^{24}$ −3.0° (*c* 0.5, pyridine), indistinguishable, on t.l.c. in 20:5:0.1 chloroform–methanol–aqueous ammonia (R_F 0.36) and 65:25:4 chloroform–methanol–water (R_F 0.62), from an authentic specimen[24] of **9**.

Anal. Calc. for $C_{48}H_{93}NO_{13}$: C, 64.61; H, 10.51. Found: C, 64.44; H, 10.38.

(B) Reaction of **6** (320 mg) with **7** under similar conditions, followed by the usual isolation procedure, catalytic de-esterification, and chromatography on silicic acid, led to the isolation of 31 mg (9%) of a white powder which crystallized from methanol in tiny clusters of needles, m.p. 240–241°, $[\alpha]_D^{20}$ −5.6° (*c* 0.55, pyridine), indistinguishable from **10** on t.l.c. in 20:5:0.1 chloroform–methanol–aqueous ammonia (R_F 0.33) and 65:25:4 chloroform–methanol–water (R_F 0.57).

Anal. Calc. for $C_{48}H_{93}NO_{13}$: C, 64.61; H, 10.51; Found: C, 64.85; H, 10.36.

(C) To a stirred solution of **5** (300 mg) in nitromethane–benzene were added molecular proportions of mercuric cyanide and **6**, and the solution was kept at 70–80° for 96 h, further molecular proportions of mercuric cyanide and **6** being added after after 18, 42, and 66 h. After the isolation and catalytic deesterification procedures described previously, a solution in 19:1 chloroform–methanol was passed through a column of silicic acid. The first fractions eluted gave **8** (100 mg). Chloroform–methanol (9:1) eluted fractions totalling 44 mg (14%). Crystallization from methanol gave a product of m.p. 238–239°, $[\alpha]_D^{26}$ +16.2° (*c* 0.34, pyridine) which was indistinguishable from **9** on t.l.c. in the two solvent-systems used. However, the authentic specimen[24] has m.p. 210°, $[\alpha]_D$ −3.13°, whereas 1-*O*-[4-*O*-α-D-glucopyranosyl-β-D-glucopyranosyl]-*N*-octadecanoyl-DL-dihydrosphingosine shows[24] m.p. 244° and $[\alpha]_D$ +29.61°.

(D) By use of the same reaction conditions as in (C), but with replacement of of **6** by **7** a product was obtained (13% yield) which crystallized from methanol in

tiny needles, m.p. 242–243°, $[\alpha]_D^{25}$ +21° (*c* 0.76, pyridine). It was indistinguishable from **10** on t.l.c. in the two solvent-systems used.

SUMMARY

Condensation of 2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide with 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine afforded the dihydrocerebroside derivative 3-*O*-benzoyl-*N*-octadecanoyl-1-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-DL-dihydrosphingosine (**5**). Reaction of the same bromide with methanol, followed by *p*-toluenesulfonylation, gave a good yield of methyl 2,3,6-tri-*O*-acetyl-4-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (**3**).

Dihydroceramide dihexosides that are β-D-(1→4)-linked were prepared by reaction of **5** with 2,3,4,6-tetra-*O*-acetyl-α-D-gluco(or galacto)-pyranosyl bromide. The anomeric configuration of the interglycosidic linkage depended on the conditions of the reaction; under relatively mild conditions, β-cellobiosyldihydroceramide and β-lactosyldihydroceramide ("dihydrocytolipin H") resulted.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE GLYCOLIPIDS OF *Arthrobacter globiformis**

R. W. WALKER AND CHRISTINE P. BASTL**
Institute of Agricultural and Industrial Microbiology, Department of Environmental Sciences, University of Massachusetts, Amherst, Massachusetts 01002 (U.S.A.)

(Received July 5th, 1966; in revised form, November 1st, 1966)

INTRODUCTION

Complete structural studies of microbial glycolipids have only recently been made[1–5]. These compounds, which in some instances make up a major portion of bacterial lipids, are widely distributed. During the course of an investigation of the lipids of *Arthrobacter globiformis*, three glycolipids were found. This paper describes the characterization of these glycolipids.

EXPERIMENTAL

The bacterial cells were grown in a medium containing glucose and salts as previously described[6]. The freeze-dried cells were extracted with chloroform–methanol (2:1) and chloroform–methanol (7:1) containing 5.5% aqueous ammonium hydroxide (28% w/v). The total lipid fraction (4.0% of the cell dry-weight) was fractionated on a diethylaminoethylcellulose (DEAE) column[7].

The lipid (28 g) was applied to a 100 × 120 mm column of DEAE cellulose. The fraction containing the glycolipid (19.64 g; 68% of the lipid applied) was eluted with chloroform–methanol (7:1; 3 l). This material was deacylated[8], acidified, extracted with petroleum ether, neutralized, concentrated, deionized, and finally concentrated to a syrup.

Approximately 1 g of the syrup was placed on a column (20 × 80 mm) which was packed with 20 g of a 1:1 mixture of Celite 545 and acid-washed Norit A charcoal (Pfanstiehl, Waukegan, Illinois). The column was washed with water (100 ml), water containing 5% ethanol (100 ml), and water–ethanol (1:1; 200 ml). Glycerol and salts were eluted in the water fraction, glycosides A and B were eluted with water–5% ethanol, and glycoside C was eluted with water–ethanol (1:1). The fractions were further purified by preparative paper chromatography on Whatman paper 3MM developed with butyl alcohol–pyridine–water (6:4:3); the spots were located with the

*A contribution from the Agricultural Experiment Station, University of Massachusetts, Amherst. This investigation was supported in part by a grant (AI-06210) from the Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.
**Present address: Western Reserve Medical School, Cleveland, Ohio.

periodate–permanganate reagent[10]. Glycosides GA, GB, and GC were obtained and glycosides GB and GC were twice crystallized from ethanol.

The intact glycolipids were isolated and determined quantitatively by chromatography on DEAE cellulose and Florisil. Preparative t.l.c., performed on silica gel PF_{254} (E. Merck, Darmstadt, Germany), was used to purify fractions.

The following analytical methods were used: ester groups by the method of Rapport and Alonzo[11] using a tripalmitin standard, hexose by the anthrone procedure, galactose by galactose oxidase, glycerol by glycerol dehydrogenase (in the presence of 0.2M hydrazine[12] at pH 9.6), periodate oxidation by a micro-iodometric procedure[13], formaldehyde by a chromotropic acid procedure[14], and formic acid by titration of the ethylene glycol-quenched solution to pH 6.0.

The following chromatographic methods were used to establish identity or homogeneity: (a) the glycolipids LA, LB, and LC by t.l.c. (Adsorbosil-1, Applied Science Labs., State College, Pennsylvania), in chloroform–methanol (80:20) containing 2% pyridine and 2% water; (b) the products of hydrolysis (2N HCl for 3–4 h at 100°) of the glycosides on Whatman No. 1 paper in butyl alcohol–pyridine–water (6:4:3), 2-propanol–pyridine–water–acetic acid (8:8:4:1), and ethyl acetate–acetic acid–water (3:1:3, upper phase) at room temperature.

The glycosides were methylated with methyl iodide and silver oxide in *N,N*-dimethylformamide[15]. The methylated glycosides were hydrolyzed[16] in H_2SO_4, and methyl glycosides were prepared[17] and analyzed by g.l.c. with an Aerograph A-600C flame-detector gas-chromatograph (Varian Aerograph, Walnut Creek, California), equipped with a column (10 ft × 1/8 in.) packed with Celite (545-A.W.-80/100 mesh) coated with 20% poly (ethylene glycol succinate) at 194°.

RESULTS AND DISCUSSION

The analyses of the glycolipids are given in Table I. The calculated values of moles of carbohydrate and ester groups per mole of lipid were based on a molecular weight of 740 for LA and 914 for LB and LC (estimated from an average fatty acid chain length of 15.2 carbon atoms).

Acid hydrolysis of the glycosides showed the presence of only galactose and

TABLE I

ANALYSES OF LIPIDS LA, LB, AND LC

Lipids	*M.p.*[a] *degrees*	*Total lipid* (%)	*Carbohydrate*[b] (%)	*Galactose*[c]	*Mannose*[c]	*Ester groups*[c]
LA	157–159.5	11	26.1(25.2[d])	1.07	—	1.87
LB	194–196.5	2	35.3	—	1.89	2.04
LC	205.5–207	0.6	38.5	2.06	—	1.82

[a]Uncorr. [b]By anthrone. [c]Moles/mole, based on mol. wt. of 740 for LA, and 914 for LB and LC. [d]By galactose oxidase after hydrolysis.

glycerol in GA and GC, and mannose and glycerol in GB. All three glycosides rapidly gave a purple color with the periodate–Schiff reagent[18] indicating a C-1-substituted glycerol. All glycosides were nonreducing. Their R_F in butyl alcohol–pyridine–water (6:4:3) was: GA 0.34, GB 0.25, and GC 0.13. Glycosides A and C were completely hydrolyzed by β-galactosidase into galactose and glycerol (the hydrolysis was followed qualitatively by paper chromatography[19]). Glycoside B was completely hydrolyzed by a crude α-mannosidase prepared from jack bean[20] (the hydrolysis was also followed by paper chromatography[19]).

The results of the periodate oxidation (Table II) suggest an *O*-galactosyl-(1→1)-glycerol linkage in GA, an *O*-mannosyl-(1→3)-*O*-mannosyl-(1→1)-glycerol linkage in GB, and a *O*-galactosyl-(1→6)-*O*-galactosyl-(1→1)-glycerol linkage in GC. These conclusions were confirmed by the results of the methylation procedure. Methyl 2,3,4,6-tetra-*O*-methylgalactoside (rel. retention time 1.71)* was found in the methanolysis products of methylated, hydrolyzed GA, methyl 2,3,4,6-tetra-*O*-methylmannoside (rel. retention time 1.38) and methyl 2,4,6-tri-*O*-methylmannoside (rel. retention time 3.83) in GB, and methyl 2,3,4,6-tetra-*O*-methylgalactoside and methyl 2,3,4-tri-*O*-methylgalactoside (rel. retention time 2.28) in GC.

The fatty acid composition of each glycolipid as analyzed by g.l.c. of the methyl esters was found to be identical with that reported for the whole organism[6]: *i*-14:0**, 0.8%; 14:0, 0.6%; *a*-15:0, 66.8%; *i*-16:0, 10.6%; 16:0, 3.6%; *a*-17:0, 17.6%.

A. globiformis glycolipids are assumed to be diglycerides, the acyloxy groups being located at C-1 and C-2 of the glycerol moeity[22], but the exact locations have not been determined. Lennarz and Talamo[1], however, have shown by biosynthesis that, in *Micrococcus lysodeikticus*, the major glycolipid is in fact a dihexosyldiglyceride.

Glycoside B is identical with the mannosylmannosylglycerol isolated by Lennarz and Talamo[1] from *M. lysodeikticus*. Glycoside B crystallized as a monohydrate, has nearly identical optical rotation, melting point, and periodate oxidation reaction as Lennarz and Talamo's preparation. These authors found the parent lipid to constitute 7.5 to 15% of the total lipid and to be the major glycolipid. We have found glycolipid B to make up only 2% of the total lipid, the monogalactosylglycerol lipid being the major glycolipid.

Wickberg[19] and Carter *et al.*[8] have isolated galactosylglycerol-containing glycolipids from red algae and wheat flour respectively. Wickberg[23] has synthesized a series of *O*-α-D- and *O*-β-D-galactopyranosyl-(1→1)-glycerol derivatives. The *O*-β-D-galactopyranosyl-(1→1)-glycerol isolated in these studies had melting points of 139–141.5° and $[\alpha]_D$ + 3.77°. Our glycoside A, which did not crystallize spontaneously, had a slightly higher specific rotation ($[\alpha]_D^{27}$ + 7.5°).

Bacterial glycolipids, although apparently widely distributed[1,3,22,24,25], have not been studied in great detail. Extensive structural determinations have been reported

*Retention time of the α-D- anomer relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside[21].

***i* = *iso*, *a* = *anteiso*; Thus, *i*-C_{14} is 12-methyltridecanoic acid and *a*-C_{17} is 14-methylhexadecanoic acid.

TABLE II

ANALYSES OF GLYCOSIDES GA, GB AND GC

Glycosides	*M. p. degrees*[a]	$[\alpha]_D^{27}$ *degrees*[b]	*Galactose* (%)	*Mannose*	*Glycerol*	*Periodate consumed* moles/mole	*Formaldehyde produced*	*Formic acid produced*
GA	—	+7.5 (*c*, 2.7)	0.94	—	0.90	2.73	0.80	0.74
GB	129–134	+85 (*c*, 4.0)	—	2.0	0.99	2.94	0.96	1.03
GC	225–225.5	+16 (*c*, 1.9)	1.9	—	1.2	5.07	0.74	1.97

[a]Uncorr. [b]In water.

for the glycolipid carbohydrate moieties of only three organisms: 1-*O*-(β-D-galactofuranosyl)glycerol from *Bacteriodes symbiosus*[2]; *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→1)-glycerol and 1-*O*-(α-D-glucopyranosyl)glycerol from Types I[3] and XIV[4] *Diplococcus pneumoniae*; and *O*-α-D-mannosyl-(1→3)-*O*-α-D-mannosyl-(1→3)-glycerol from *Micrococcus lysodeikticus*[1].

Bacterial glycolipids, which may constitute a significant portion of the total lipid, have been found to be located in the membrane[22] and are mono- or dihexosyldiglycerides. The exact function of these compounds is unknown, but several theories have been advanced for their function: they may be involved in polysaccharide synthesis[1,3], or cell wall synthesis[26], or they may be structural components of membrane[3].

Cohen and Panos[26] have found elevated levels of diglucosyldiglyceride in membrane lipids of an L form derived from *Streptococcus pyogenes*, in contradistinction to the parent strain, where levels are lower. They postulated that the diglucosyldiglycerides are involved in wall synthesis, accumulation resulting from the inability of the L form to synthesize a cell wall.

ACKNOWLEDGMENTS

We gratefully acknowledge gifts of methylated hexoses from Dr. G. O. Aspinall, Edinburgh, Scotland, Dr. Arthur S. Perlin, Saskatoon, Saskatchevan, and Dr. J. K. N. Jones, Kingston, Ontario; a gift of methyl β-D-mannoside from Dr. D. F. Mowery, New Bedford, Mass.; advice from Dr. J. H. Nordin, Biochemistry Department, University of Massachusetts; and assistance from the University of Massachusetts Research Council.

SUMMARY

Three glycolipids were isolated from *Arthrobacter globiformis* 616. Their deacylation products were characterized as *O*-β-D-galactopyranosyl-(1→1)-glycerol, *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→1)-glycerol, and *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl-(1→1)-glycerol. The parent lipids comprise 11, 0.6, and 2% of the total lipids, respectively. The fatty acid content of each lipid was found to be the same as that of the total lipids.

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STUDIES ON URONIC ACID MATERIALS

PART XIX*. THE COMPOSITION OF THE GUM FROM *Acacia laeta* VAR. *hashab*

D. M. W. ANDERSON AND R. N. SMITH
Department of Chemistry, The University, Edinburgh 9 (Great Britain)

(Received August 17th, 1966; in revised form, November 17th, 1966)

INTRODUCTION

Some samples of gum arabic (*Acacia senegal*) tend to form mucilaginous gels, rather than the desired, viscous, aqueous solutions; this behaviour remains unexplained. The gel formation is, however, a property of only a small proportion of nodules[2,3], with the actual proportion determining the commercial acceptability of a particular sample. *Acacia laeta* (previously referred to, erroneously, as *A. lacta*[4]) differs[2-4] from other *Acacia* species in the extent to which its exudate forms gels at low concentrations. As suggested[4], it is necessary to ascertain how the properties and structure of *A. laeta* differ from those of other *Acacia* exudates[5].

There is a further reason for investigating this species. *Acacia laeta* R. Br. ex Benth. is a natural hybrid of *A. senegal* (L.) Willd. and *A. mellifera* (Vahl.) Benth., and it occurs in two varieties: *A. laeta* var. *hashab* (which resembles *A. senegal*) and *A. laeta* var. *mellifera* (which resembles *A. mellifera*). Intercomparisons of the properties of the exudates from these four species may, in due course, contribute to the present meagre knowledge of the mechanism of gum formation.

This paper deals with the composition of the gum from *A. laeta* var. *hashab*; preliminary experiments[3,6] had indicated a marked, inter-nodule variation, and also some close similarities to *A. senegal* gum. To give the desired basis for comparison, it became important to establish the possible range of variation and the average values for the composition and properties of the gum from *A. laeta* var. *hashab*.

EXPERIMENTAL

Origin of specimens. — Samples of the gum exudate from *Acacia laeta* var. *hashab* were collected by (the late) M. P. Vidal-Hall, formerly Gum Research Officer to the Republic of Sudan, from authenticated, numbered trees in El Ain Forest Reserve, near El Obeid. Twelve samples, each comprising a small number (5–8) of pale-brown nodules exuded from a single tree in response to tapping, were obtained as detailed in Table I.

One of the samples contained an unusually high number (14) of nodules.

*For Part XVIII, see Ref. 1.

TABLE I

ORIGIN OF SPECIMENS

Sample	*Tree number*	*Date of tapping*	*Number of days between tapping and collection*
1	124	6 Nov., 1960	40
2	285		40
3	251		40
4	133	15 Nov., 1960	40
5	133		82
6	251		82
7	84		40
8	84		56
9	84	13 Nov., 1962	71
10	90		40
11	90		56
12	90		71
N1–N6	124	6 Nov., 1960	40

Several of these were crushed to form Sample 1, and six of the nodules (designated N1–N6) were examined individually after purification; three further nodules (N7–N9) were examined individually in the native form.

Analytical methods. — The general analytical methods and chromatographic systems used have been described[7]. Gas–liquid partition chromatography (g.l.c.) was performed with a chromatograph (Model S3A, Gas Chromatography Ltd.) fitted with flame ionisation detectors. The carrier gas was nitrogen at 100 ml/min. The columns used were: (*A*) 15% by weight of poly(ethylene glycol adipate) on Celite (75 × 0.5 cm) at 160°; (*B*) 15% by weight of poly(butane-1,4-diol succinate) on Celite (120 × 0.5 cm) at 175°. Retention times (*T*) are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Purification of samples. — Each gum sample was ground to a coarse powder, and a portion (10 g) was dissolved in water (300 ml). The insoluble residue was removed by filtration, and the solution was dialysed against distilled water (4 l) for 24 h (this dialysate was examined as described below). Purification was then completed by electrodialysis[8]. In the first series of purifications, the solutions were concentrated and freeze-dried. On storage over a period of 3–6 months, these freeze-dried samples gradually became insoluble; this effect was most marked where the samples had been freeze-dried from fairly concentrated solutions. In subsequent purifications, therefore, the electrodialysed solutions were not concentrated before freeze-drying. In confirmation of earlier reports[2–4], solutions for which the concentration exceeded 5% (w/v) were liable, if allowed to stand for several days, to turn into insoluble, mucilaginous gels.

RESULTS

The free sugars in A. laeta *gum.* — The dialysate from the preliminary stage of

purification was concentrated, and examined chromatographically[7] in solvents *B* and *D*. Glucuronic acid, galactose, arabinose, and rhamnose were detected.

Analyses of the crude gum. — Analyses (*cf.* Ref. 5) were made on the crude gum samples. The following values are the average results: moisture, 14.9% (range, 13.2–15.6%); insoluble material, 1.1% (range, 0.53–2.05%); ash, 3.3% (range, 2.8–3.8%); $[\alpha]_D^{18}$ (*c* 2.0, M sodium chloride), −35° (range, −30° to −42°); limiting-viscosity number (in M sodium chloride), 21.3 cm^3g^{-1} (range, 19.1–23.4); N, 0.69% (range, 0.48–0.85%); OMe, 0.41% (range, 0.30–0.53%); uronic anhydride (by decarboxylation), 14% (range, 12–16%).

Analyses of electrodialysed samples. — Table II gives the data obtained for the purified, bulk samples from single trees (specimens 1–12), and for the purified, single nodules (specimens N1-N6, all from tree 124). Average values are also shown for specimens 1–12, for specimens N1–N6, and for all of the 18 specimens examined.

Viscosity characteristics of the gum. — The loss of solubility that occurred on storage of electrodialysed specimens precluded a reliable comparison of the viscosity of each specimen in the purified form. Fig. 1 gives the viscosity behaviour of the crude specimens dissolved in molar sodium chloride, an electrolyte concentration sufficient to swamp any variations in the viscosity arising from the natural cation content associated with the uronic acid groups in the gum. Checks with two of the samples confirmed earlier indications (*cf.* Ref. 9) that, for comparative purposes, there is little difference in the viscosity behaviour of crude and purified samples of gum, provided that an adequate concentration of salt is present.

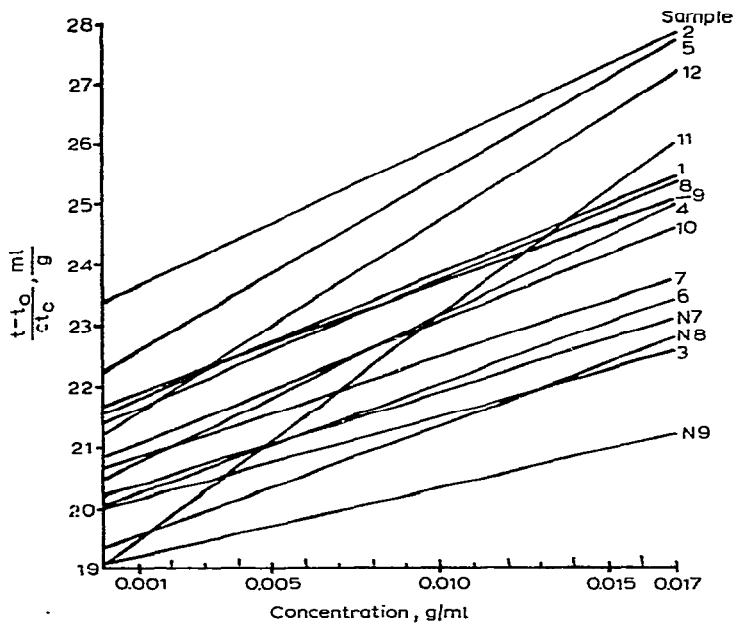


Fig. 1. The viscosity–concentration relationship for different specimens of the gum from *A. laeta* var. *hashab*.

TABLE II

ANALYTICAL DATA[a] FOR PURIFIED SPECIMENS OF GUM FROM *Acacia laeta* VAR. *hashab*

Specimen	*Nitrogen* (%)	*Methoxyl* (%)	*Reducing power*[b]	$[\alpha]_D^{18}$ (c 1.0, *water*)	*Neutralisation equivalent*[c]	*Uronic anhydride*[d], %	*Molar ratios of neutral sugars*		
							Gal	*Ara*	*Rha*
1	0.56	0.52	0.72	−32	1240	14.2	10	9.0	2.9
2	0.61	0.34	0.67	−36	1430	12.2	10	8.4	3.7
3	0.74	0.37	0.71	−30	1400	12.6	10	9.1	3.1
4	0.60	0.32	0.70	−39	1180	14.9	10	9.8	3.9
5	0.78	0.56	0.63	−41	1260	14.0	10	10.1	3.2
6	0.79	0.27	0.80	−36	1090	16.1	10	8.3	3.2
7	0.65	0.34	0.71	−42	1250	14.0	10	6.7	3.0
8	0.79	0.36	0.65	−31	1260	14.0	10	7.4	3.3
9	0.94	0.42	0.64	−41	1440	12.2	10	7.9	3.4
10	0.59	0.37	0.86	−29	1300	13.4	10	7.6	3.8
11	0.80	0.39	0.88	−32	1580	11.1	10	7.7	4.1
12	0.93	0.39	0.87	−30	1440	12.2	10	8.6	3.3
N1	0.54	0.52	0.69	−35	1210	14.5	10	8.4	2.5
N2	0.55	0.49	0.73	−32	1240	14.3	10	8.5	2.5
N3	0.47	0.54	0.71	−37	1230	14.3	10	8.3	2.3
N4	0.49	0.46	0.73	−39	1250	14.0	10	8.8	2.5
N5	0.56	0.50	0.70	−35	1320	13.4	10	8.5	2.6
N6	0.49	0.53	0.71	−34	1270	13.9	10	8.1	2.6
Av. 1–18	0.66	0.43	0.73	−35	1300	13.6	10	8.4	3.1
Av. 1–12	0.73	0.38	0.74	−35	1320	13.4	10	8.4	3.4
Av. N1–N6	0.52	0.51	0.71	−35	1250	14.1	10	8.4	2·5

[a] All data are corrected to an ash-free, dry-weight basis. [b] Expressed as apparent % of free pentose. [c] By direct titration with alkali to phenolphthalein end-point; potentiometric titration gave results in close agreement. [d] Calculated from neutralisation equivalent, assuming that all acidity arises from the uronic acid groups.

The sugar components of the gum (from complete hydrolysis). — Electrodialysed gum (sample 3, 5 g) was dissolved in sulphuric acid (2N, 500 ml) and heated under reflux for 12 h. The hydrolysate was cooled, neutralised (barium hydroxide and barium carbonate), centrifuged, and then treated with Amberlite IR-120 (H^+) resin. The product was fractionated on a column of Duolite A4 resin (20 × 2 cm) in the formate form. The neutral fraction was eluted with water (2 l); the acidic fraction was eluted with formic acid [5% (v/v), 2 l]. After evaporation, the neutral fraction gave a syrup (3.3 g); paper chromatography (solvent *B*) indicated galactose, arabinose (R_{Gal} 1.34), rhamnose (R_{Gal} 2.11) and, between the arabinose and rhamnose, a diffuse trace of material giving a pink colour with aniline hydrogen oxalate. The acidic fraction was taken to dryness several times from aqueous solution to eliminate formic acid; paper chromatography (solvent *D*) indicated the presence of D-glucuronic acid (R_{Gal} 1.18), D-glucurono-6,3-lactone (R_{Gal} 3.1), and 4-*O*-methyl-D-glucuronic acid (R_{Gal} 2.62).

The neutral fraction was chromatographed on a cellulose column (60 × 3 cm) with solvent *B*. Fractions (20 ml) were collected; every fifth fraction was concentrated and examined chromatographically (solvent *B*). The appropriate fractions were combined, and concentrated; the respective syrups of D-galactose, L-arabinose, and L-rhamnose crystallised on standing. After recrystallisation from water, the identities of the sugars were confirmed as follows:

Fraction (a): D-galactose monohydrate (900 mg); m.p. and mixed m.p. 118–119°; $[\alpha]_D^{18}$ + 80° (equilibrium, *c* 4.0, water). The sugar was characterised by oxidation to galactaric acid, m.p. 213–214° (lit.[10], 213°).

Fraction (b): L-arabinose (1040 mg); m.p. and mixed m.p. 159–160°; $[\alpha]_D^{18}$ + 105° (equilibrium, *c* 3.0, water); R_{Gal} 1.36 (solvent *B*). The X-ray diffraction pattern was identical to that of an authentic sample of L-arabinose. The phenylosazone had m.p. 164–165° (lit.[10], 165°).

Fraction (c): L-rhamnose monohydrate (275 mg); m.p. and mixed m.p. 93–94°; $[\alpha]_D^{18}$ + 9° (equilibrium, *c* 3.0, water); R_{Gal} 2.10 (solvent *B*). The X-ray diffraction pattern was identical to that of an authentic sample of L-rhamnose. The phenylosazone had m.p. 181–182° (lit.[10], 182°).

Partial acid hydrolyses: trace neutral sugars and aldobiouronic acids. — After trial partial hydrolyses with acid and with an acidic resin, the gum (sample 7, 30 g) was hydrolysed successively at 100° with sulphuric acid under the following, graded conditions: (*a*) 0.01N, 500 ml, 60 h; (*b*) 0.2N, 500 ml, 4 h; (*c*) 0.5N, 250 ml, 2 h; (*d*) 2N, 250 ml, 1 h; (*e*) 2N, 250 ml, 4 h; (*f*) 2N, 250 ml, 2 h. At each stage after (*a*), the hydrolysate was neutralised (barium carbonate), centrifuged, de-ionised (IR-120 resin), evaporated, and added to ethanol. The degraded gum was removed by centrifugation, and the centrifugate was evaporated to a syrup, which was separated into acidic and neutral fractions on a column (15 × 2.5 cm) of DEAE-Sephadex G-25 (formate form). The neutral fraction was eluted with water (1.5 l), and the acidic fraction with formic acid (3% v/v, 1.5 l).

When the sequence of graded hydrolyses was complete, the neutral fractions

obtained from each stage were combined, treated with charcoal, and evaporated to a syrup (2.6 g). Similarly, the acidic fractions were combined, evaporated to dryness several times to eliminate formic acid, treated with charcoal, and evaporated to a syrup (310 mg). A subsequent Paper will discuss the detailed examination of the degraded gums and of the oligosaccharides in the combined, neutral fractions. In this Paper, we report on the trace monosaccharides in the combined, neutral fractions, and on the identification of the aldobiouronic acids in the combined, acidic fractions.

The trace, neutral sugars travelled as a single band between arabinose and rhamnose (solvent *B*). When the band was re-chromatographed on Whatman No. 1 paper in solvent *C*, two fractions were obtained. Fraction (*a*) (6 mg) had R_{Gal} 1.28 (solvent *D*) and R_{Gal} 1.95 (solvent *C*); it was identical with xylose in both these solvents. After conversion into the methyl glycosides, and methylation[11], the product was examined by g.l.c. and by paper chromatography, together with the product obtained by similar treatment of authentic xylose. In each case, two major components were detected [*T* 0.41, 0.51 (column *A*); *T* 0.49, 0.60 (column *B*)], and, after hydrolysis, both products gave methylated, reducing sugars having R_G 0.83, 0.92, and 0.49 (minor component) in solvent *A*. This fraction was therefore tentatively identified as xylose. Fraction (*b*) (3.1 mg) had R_{Gal} 1.52 (solvent *D*) and R_{Gal} 2.32 (solvent *C*); it was identical with ribose in both these solvents, and also in the comparative tests outlined above for fraction (*a*).

Identification of the aldobiouronic acids. — The combined acidic fraction from the partial, acid hydrolysates was resolved into components *A* and *B* by chromatography on 3 MM paper in solvent *B*.

Component A: (189.5 mg) had R_{Gal} 0.20 (solvent *D*); $[\alpha]_D^{18}$ 0° (*c* 3.8, water); (lit.[7,12], − 5°; + 1.7°, − 3°, − 7.8°). The aldobiouronic acid was isolated as a chromatographically pure syrup. Hydrolysis gave only galactose and glucuronic acid. Reduction of the methyl ester methyl glycosides with potassium borohydride, followed by hydrolysis, gave only galactose and glucose; the glucose was isolated by chromatography on 3MM paper, (solvent *B*), and had $[\alpha]_D^{18}$ + 50° (equilibrium, *c* 0.25, water); the phenylosazone had m.p. 204–205° (lit.[10], 205°). The methanolysis product of the fully methylated aldobiouronic acid was examined by g.l.c. and found to contain the methyl glycosides of 2,3,4-tri-*O*-methyl-D-glucuronic acid [*T* 2.14, 2.66 (column *A*); *T* 2.41, 3.08 (column *B*)]; 2,3,4-tri-*O*-methyl-D-galactose [*T* 5.36 (column *A*); *T* 6.44 (column *B*)]; 2,3,5-tri-*O*-methyl-D-galactose (trace only) [*T* 3.27, 4.32 (column *A*); *T* 4.02, 5.37 (column *B*)]. Reduction of the methyl ester methyl glycosides (lithium aluminium hydride) gave a product, which, after hydrolysis, was examined in solvent *A*; components identical with 2,3,4-tri-*O*-methyl-D-glucose (R_{Gal} 0.86) and 2,3,4-tri-*O*-methyl-D-galactose (R_{Gal} 0.70) were revealed. Component *A* was thereby identified as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose.

Component B: (75 mg) had R_{Gal} 0.68 (solvent *D*); $[\alpha]_D^{18}$ + 4° (*c* 1, water) [lit., − 1°, + 6° (ref. 12); + 6° (ref. 7)]. The aldobiouronic acid was isolated as a chromatographically pure syrup. Hydrolysis gave only galactose and 4-*O*-methylglucuronic acid [(R_{Gal} 2.64 (solvent *D*)]. Methanolysis of the methylated[11] aldobiouronic acid gave the same

components as identified for component *A*. Methanolysis of the aldobiouronic acid, followed by potassium borohydride reduction and subsequent hydrolysis, gave only galactose and 4-*O*-methylglucose (solvent *D*). The 4-*O*-methylglucose was isolated by chromatography on 3 MM paper, and had $[\alpha]_D^{18}$ +55°; R_{Gal} 1.62 (solvent *D*), 2.63 (solvent *A*), and 2.23 (solvent *C*). The phenylosazone had m.p. 157° (lit.[13], 158–159°). Component *B* was therefore identified as 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

DISCUSSION

The exudate from *A. laeta* var. *hashab* gives a further interesting illustration of the occurrence of inter-nodule variation; by calculation, the data obtained give average values for the parameters expressing the composition and properties of the acidic gum polysaccharide. The variations from the average values for this species are similar in extent to those found for *A. seyal*[9], and more marked than those for *A. nilotica*[7].

Of the *Acacia* species studied to date, *A. laeta* and *A. seyal* are unusual in occurring in varieties distinguishable by their external, botanical characteristics. It will be of interest to discover the differences that occur in the constitution and properties of the gums exuded by these varieties. In *A. laeta*, for example, the assignment of a particular tree to the *hashab* variety or to the *mellifera* variety may be a botanical oversimplification; a range of variation between two extreme forms of the hybrid may exist. Studies of the exudates from *A. mellifera* and *A. laeta* var. *mellifera* are therefore necessary.

The specimens available gave the opportunity to ascertain that the variation between six, single nodules from one tree (samples N1–N6) is considerably less than the variation between "bulk" samples (*i.e.*, a mixture of a few nodules) from different, individual trees (samples 1–12). The same conclusion was reached[14] in similar experiments with single nodules of *A. senegal* gum. Although the only consistent variation in the present results involves an increase in the nitrogen content of the nodules with their period of formation relative to the date of tapping, the danger of drawing premature conclusions from the data must be avoided: the work involved in examining a statistically useful number of specimens appears at present to be prohibitive. The present results are sufficient, however, to indicate the direction that further experiments should pursue.

The variation in the limiting-viscosity numbers for the specimens (Fig. 1) suggests that significant variations in the molecular size of the gum may exist from sample to sample. The significance of the variation in slope of the viscosity plots (Fig. 1) is not clear; similar variations were reported[9] for *A. seyal* gum. The possibility that these variations in slope reflect changes in the typical molecular shape, or in molecular composition, will be investigated. The extent of the inter-nodule variations further suggests that attempts to effect fractionation (*cf.* Ref. 15) must be made prior to general structural studies, and studies of fine structure involving consecutive Smith degradations[16].

The analytical data presented do not indicate any unusual features for an *Acacia* species now that the presence of methoxyl groups has become expected[17]. Of the other *Acacia* species examined to date[5,7], *A. senegal*[5,16] most closely resembles *A. laeta* var. *hashab*. The trace components of the partial acid hydrolysates, identified as xylose and ribose, may have been artefacts formed during the hydrolysis procedure. If these sugars do exist in the gum, they are present in such small proportions that they are unlikely to have structural significance. Previous workers have reported traces of xylose in *A. sundra*[18], of xylose and fucose in *A. sieberiana*[19], and of ribose in *A. seyal*[9].

ACKNOWLEDGMENTS

We thank Sir Edmund Hirst, C.B.E., F.R.S., for his interest in these studies, and Samuel Jones Ltd. (London), Laing–National Ltd. (Manchester), and Rowntree Ltd. (York) for providing a maintenance grant (to R.N.S.)

SUMMARY

Inter-nodule variations in the composition and properties of the gum from *Acacia laeta* var. *hashab* have been investigated. Six nodules from one tree showed less variation than samples from other trees. Average values for the analytical parameters expressing the composition of the gum have been calculated from analytical data for eighteen samples. Of the other *Acacia* gums investigated to date, *A. senegal* is the most closely similar in composition.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

DEPOLYMERIZATION OF SOME POLYSACCHARIDES AND SYNTHETIC POLYMERS BY L-ASCORBIC ACID*

A. HERP, T. RICKARDS, G. MATSUMURA, L. B. JAKOSALEM, AND W. PIGMAN
Department of Biochemistry, New York Medical College, New York, New York 10029 (U.S.A.)
(Received July 26th, 1966; in revised form, November 10th, 1966)

INTRODUCTION

Previous reports from this and other laboratories have shown that hyaluronic acid, a polysaccharide widely distributed in mammalian connective tissue, can readily undergo depolymerization in the presence of oxygen or autoxidants normally present in living tissues, such as ascorbic acid, thiols, and metallic ions[1-4]. The name oxidative–reductive depolymerization (ORD) reaction was proposed for the general reaction, and some phases of the mechanism have been studied in this laboratory[5].

The reaction appears to involve the formation and attack of the polysaccharides by free radicals, and may have features in common with degradations produced by ionizing radiations or by other sources of free radicals, such as ultrasonic vibrations[6]. The literature on the degradation of hyaluronic acid and other glycosaminoglycans was reviewed recently[7]. Alginic acid[8], pectic acids[9], starch[3], and polymethacrylic acid[10], in addition to hyaluronic acid, were also degraded by autoxidants, but the reactions have not been as well studied.

The purpose of the present study was to investigate the specificity of the reaction carried out under comparable conditions. The effects of ascorbic acid and several other autoxidants on the viscosity of the solutions of some common acidic and neutral polysaccharides and synthetic polymers were compared.

EXPERIMENTAL

Polymethacrylic acid was prepared by u.v. irradiation of an aqueous solution of methacrylic acid (K & K Lab. Inc., Jamaica, N.Y.). Alginic acid (Kelacid), sodium alginate (Algin), and propylene glycol alginate (Kelcoloid HVF) were obtained from Kelco Co. (Clark, N.J.). The pectin preparations were supplied by Atlantic Gelatin Div. (Hollywood, Calif.). The methylcellulose used in this study was a commercial product (Methocel) of the Dow Chemical Company (Midland, Mich.). The copolymer of methyl vinyl ether and maleic anhydride was a product of General Aniline and Film Corp. (New York, N.Y.), sold under the trade name Gantrez An 169. Soluble

*This work was supported by Grant AM-04619 of the National Institute for Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

starch was obtained from Pfanstiehl Lab. Inc. (Waukegan, Ill.) Dextran (mol. wt. approx. 200,000), dextrin (bacteriological), and glycogen (shellfish) were purchased from Mann Research Lab. Inc. (New York, N.Y.). The hyaluronic acid used for oxygen, absorption studies was prepared according to our previously published method[11].

Experimental values of intrinsic viscosity for some of the polymers used in the current work are given in Fig. 1.

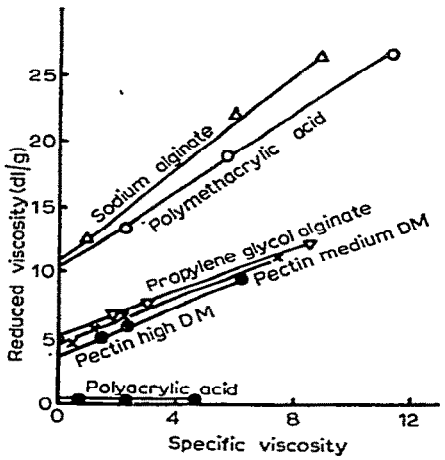


Fig. 1. Intrinsic viscosity of original polymers in 0.2M phosphate buffer, pH 7.3; temp. 30°.

For the study of the effect of autoxidants, the water-soluble polymers were dissolved in 0.3M sodium phosphate buffer, pH 7.3. The soluble starch was dissolved in boiling buffer. Dextrin and glycogen solutions were homogeneous. Methylcellulose was mixed with a small amount of hot water and stirred until the powder became thoroughly wetted. The slurry was then homogenized with phosphate buffer, pH 7.3, to a final concentration of 0.3M.

For the study of the effect of variations in pH on the viscosity of sodium alginate, propylene glycol alginate, and methylcellulose, sodium phosphate buffer (0.3M) of different pH values was used to dissolve the polymer.

Viscosities were measured with Cannon–Manning capillary glass viscometers (Cannon Instrument Co., State College, Pa.). Either size 100 (flow-time for buffer, *ca.* 60 sec) or size 50 (flow-time for buffer, *ca.* 200 sec) was employed, the latter type being used for less viscous polymers.

The following standard procedure was adopted: the polymers dissolved in 0.3M phosphate buffer (prepared as described above) were mixed with the autoxidant to give solutions with final concentrations of 0.33mM or 3.3mM autoxidant, in 0.2M buffer in all instances. Control solutions consisted of the same concentrations of polymer in 0.2M buffer in the absence of autoxidant. For viscosity measurements, 0.3 ml or 0.5 ml (according to the type of viscometer used) of the final reaction mixture and of the control solutions were each introduced into viscometers kept in a water-

bath at 30.00 ± 0.01°. The viscosities were measured at frequent time-intervals over a period of 2 h.

The depolymerization of the polymer produced by the autoxidant was calculated as % change in specific viscosity compared to that of the control solution. The viscosity of the latter remained virtually constant during the 2-h incubation period.

RESULTS

Fig. 2 shows the effect of several autoxidants on the extent of reduction of the viscosity of sodium alginate (Kelacid) solutions after 2 h. Of the three autoxidants

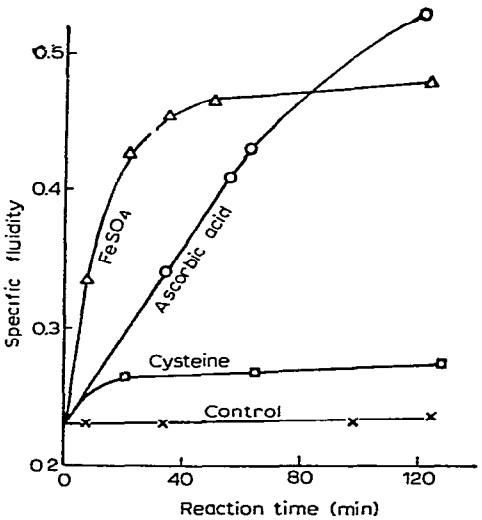


Fig. 2. Rate of increase in fluidity of sodium alginate (Kelacid) by different autoxidants. Concentration of sodium alginate: 0.66%; 0.2M phosphate buffer, pH 7.3. Concentration of autoxidant: 3.3mM; temp. 30°.

used, L-ascorbic acid was the most active and L-cysteine the least active depolymerizing agent. Even at 3.3mM concentration, the decrease produced by L-cysteine for a 0.2% solution of sodium alginate was only 28%; the decrease was barely noticeable at a concentration of 0.3mM. These results are comparable to those obtained in earlier work, when hyaluronic acid was used as substrate[2,12,13].

Fig. 2 also gives the rate of increase of specific fluidity ($1/\eta_{sp}$) of sodium alginate (Kelacid) solutions in the presence of the three autoxidants. Depolymerization by L-ascorbic acid resulted in a linear increase in specific fluidity with time during the first 60 min of incubation. For ferrous sulfate and L-cysteine, the reactions were practically complete within the first 20 min.

In Table I are summarized the data of the effects of L-ascorbic acid, at two concentrations, on the depolymerization of all the polymers included in this study. The results are expressed as percentage reduction from the initial specific viscosity, after

a 2-h incubation period at 30°. With the exception of starch, dextrin, and glycogen, all the polymers show losses in viscosity after exposure to the action of ascorbic acid. The higher concentration of ascorbic acid (3.3mM) generally produced the greater decrease in specific viscosity. The alginates and polymethacrylic acid were the most sensitive to autoxidation by ascorbic acid. For these two polymers, the decrease in viscosity after 2 h, even at a 0.3mM concentration of the autoxidant, was of the order of 85%. For propylene glycol alginate, the pectins, methylcellulose, and polyacrylic acid, the viscosity reduction ranged between 40 and 70%. Dextran and the copolymer of methyl vinyl ether and maleic anhydride showed a marked decrease in viscosity only when ascorbic acid was used at the 3.3mM concentration.

Fig. 3 shows the influence of pH at constant ionic strength on the depolymerization of 0.13% sodium alginate (Algin), 0.13% propylene glycol alginate, and 0.5% methylcellulose in the presence of ascorbic acid (3.3mM). For all three polymers tested, the degradation by ascorbic acid was highest in the pH range of 3.3 to 7.3. Outside these pHs, the extent of depolymerization generally declined. However, the pH optimum for maximal degrading effect is known to depend upon the nature of the autoxidant[14,15].

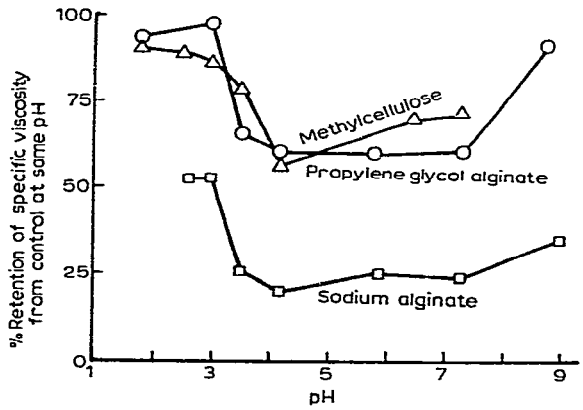


Fig. 3. Effect of pH on the viscosity of 0.66% sodium alginate (Algin), 0.133% propylene glyco alginate (Kelcoloid HVF) and 0.5% methylcellulose in the presence of 0.33mM ascorbic acid in 0.2M phosphate after 2 h at 30°.

In another experiment, two different pectin products, with methylation degrees of 75% and 83%, were tested with ascorbic acid (0.33mM and 3.3mM) at pH 7.3. As seen from Fig. 4, the two polymer species showed parallel slopes in their fluidity curves; the extent of degradation, at 2 h, of the two pectin types was almost identical (Table I).

DISCUSSION

In view of the practical importance which oxidative–reductive depolymerizations may have in biological phenomena, it was of interest to investigate the in-

TABLE I

EFFECTS OF L-ASCORBIC ACID ON THE SPECIFIC VISCOSITY OF SOME NATURAL AND SYNTHETIC POLYMERS

Polymer[a]	*Concentration* (%)	*Initial* η_{sp}	*Concentration of* L-*Ascorbic Acid* (mM)	
			0.3	*3.3*
			% Decrease in η_{sp} *after 2 h*	
Sodium alginate (Kelacid)	0.2	0.79	52	82
	0.4	1.64	49	78
	0.66	4.30	43	60
Sodium alginate (Algin)	0.50	0.60	82	85
	0.1	1.25	76	82
	0.2	5.88	77	86
	0.33	8.81	75	82
Propylene glycol alginate	0.05	0.24	54	72
	0.083	0.37	55	66
	0.2	1.17	40	76
	0.3	2.18	28	71
	0.66	7.52	29	68
Starch, soluble	0.9	0.16	—	0
Dextrin	0.9	0.27	1	2
Glycogen	0.9	0.16	—	0
Dextran	0.66	0.78	5	37
Hyaluronic acid	0.04	4.05	95	—
Methylcellulose	0.2	0.87	43	58
Copolymer of methyl vinyl ether and maleic anhydride	0.4	2.81	18	58
	0.66	3.84	21	43
Pectin (83% methylation)	0.26	1.51	48	50
	0.4	2.39	29	44
	0.66	6.20	42	46
Pectin (73% methylation)	0.27	1.80	47	48
	0.4	3.06	33	55
	0.66	8.48	38	56
Polyacrylic acid (sodium salt)	0.2	0.07	12	68
	0.73	0.25	30	54
	1.31	0.47	20	46
Polymethacrylic acid (sodium salt)	0.09	1.16	80	90
	0.17	2.24	84	85
	0.3	5.66	82	85
	0.42	11.18	85	91

[a] In 0.2M phosphate buffer, pH 7.3, 30°.

fluence of several autoxidants on the viscosity of several polysaccharides and synthetic polymers of different structures. Indeed, this reaction may have importance in the breakdown of deoxyribonucleic acid[16,17], in the role of hyaluronic acid in controlling

connective tissue permeability in normal and pathological conditions[18–20], as well as in the preparation, use, and stability of synthetic polymers[21–23].

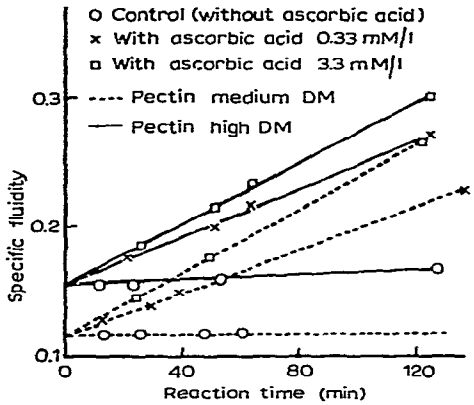
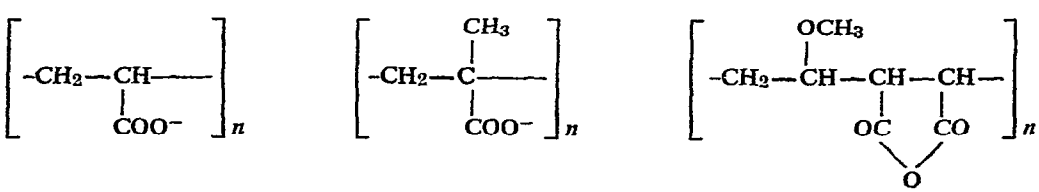


Fig. 4. Rate of increase in fluidity of pectin solutions [medium (75%) degree of methylation and high (83%) degree of methylation] in the presence of ascorbic acid.
Pectin concentration: 0.66%; 0.2M phosphate buffer, pH 7.3; temp. 30°.

Table I shows that, under the experimental conditions used in this study, L-ascorbic acid was a strong degrading agent of most of the natural and synthetic polymers tested. It markedly reduced the viscosity of all polymers listed, with the exception of starch, dextrin, and glycogen. The higher concentration of the autoxidant gave the greater decrease in specific viscosity. Earlier work from this laboratory has shown, however, that, as the concentration of autoxidant is increased, the extent of depolymerization passes through a maximum[5].

The relation between structure and extent of degradation of the polymers is difficult to interpret, since the intrinsic viscosities of the substances varied greatly. Of the two alginate preparations, the one with the higher intrinsic viscosity was degraded more extensively. In the case of alginates, the esterified preparation (propylene glycol alginate) was more resistant to degradation than the compounds with free carboxyl groups (sodium alginates). Pectins of various degrees of esterification showed no marked differences with respect to the extent of substrate degradation.

The polymethacrylate was attacked more extensively than the polyacrylate, but, in addition to a structural change, the polymethacrylate had much the greater intrinsic viscosity. The copolymer of methyl vinyl ether and maleic anhydride, as shown in the formulas, is structurally related to the acrylates. It was also depolymerized

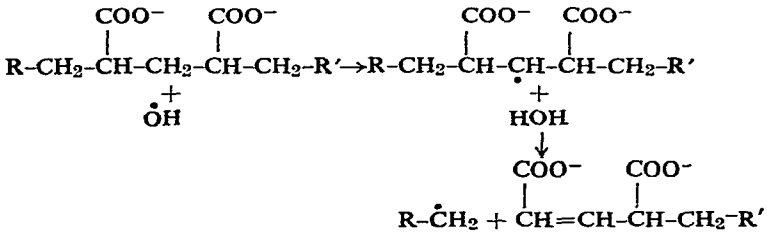


to about the same extent as polyacrylate. The substances which had no carboxyl or ester groups were starch, dextrin, glycogen, dextran, and methylcellulose. A soluble starch was used because of the difficulty of making true solutions of the unmodified starches at pH 7. The solutions did not sediment out at 18,000 *g*, with the exception of clear corn-starch dispersions. On the basis of viscosity changes the methylcellulose was degraded fairly extensively, the dextran less, and the starches not at all.

The presence of carboxyl groups or ester groups in the molecule appears generally to increase the ease of degradation of the polysaccharides with the exception of methylcellulose.

All the polysaccharides, except dextran, have (1→4)-linkages as the principal ones. In hyaluronic acid the hexosamine residue is linked to C-4 of the uronic acid residue. The dextran has mainly (1→6)-linkages, whereas the glycogen has both types. Obviously, the study of a wide variety of polysaccharides with different structural configurations is necessary before any definite correlations can be made between structure and susceptibility to depolymerization by this reaction. In the limited group here, no marked effect of linkage is evident.

The structure of polyacrylates is relatively simple, and a possible mechanism for the degradation, assuming the OH · free radical is the attacking agent[23,24], is as follows:



This scheme is essentially the reverse of the polymerization reaction, and is similar to that proposed for the depolymerizing effect of ionizing radiations[22,25]. It involves a preliminary abstraction of a hydrogen atom by a free radical and subsequent β-elimination. The presence of the carboxyl ion or ester would assist the elimination by its tendency to attract electrons from the neighboring C–C linkage.

If the polysaccharides are degraded by a similar mechanism, the attack would be at C-5 for (1→4)-linked materials, and a carboxyl group at C-6 might be expected to assist the elimination.

The striking difference in the slopes of the fluidity curves, obtained for the depolymerization of sodium alginate in the presence of ascorbic acid, ferrous ions or cysteine (Fig. 2), indicates that different mechanisms are probably involved. With hyaluronic acid, ferrous ions also appeared to act by a different mechanism than did ascorbic acid[13]. When the depolymerization reaction had apparently gone to completion, as evidenced by a plateau in the fluidity curve, addition of ferrous ions resulted in a second increase in fluidity, yielding the same type of curve[26].

These observations suggest that, for ferrous ions and cysteine, the reaction terminates because of consumption of the autoxidant. However, the situation is not clear; indeed, in earlier work, it was calculated that, for a 113μM hyaluronic acid solution (mol. wt. originally 3.5×10^6) only 8 random chain-breaks are required to produce an 83% reduction in intrinsic viscosity after a 2-h incubation with ferrous ions. This implies a random cleavage rather than an end-group attack. The more sustained action of ascorbic acid may result from the fact that its decomposition products are even more active than is ascorbic acid[27].

The autoxidation by reducing agents, such as ascorbic acid, ferrous ions, and cysteine, in aqueous media is generally considered to be a free radical-reaction[10,28]. The free radicals produced would in turn be responsible for the depolymerization effect. Consequently, the difference in rate of depolymerization of sodium alginate observed with various autoxidants is presumably due to differences in ability of autoxidizable compounds to produce or to inhibit free radicals.

Considerable evidence exists that the oxidative depolymerization is a true degradation[29,30]. Further work on the identification of breakdown products, resulting from the degradation of such polymers by autoxidants is currently under study in this laboratory in order to relate viscosity changes, oxygen absorptions, and molecular structures of different polysaccharides.

SUMMARY

The viscometric behavior of several naturally occurring polysaccharides and synthetic polymers was studied in the presence of L-ascorbic acid, L-cysteine, or ferrous ions. The viscosity of all the polyelectrolytes investigated decreased in the presence of autoxidants; neutral polysaccharides, with the exception of methylcellulose showed no changes in viscosity. A possible mechanism for the observed reduction in viscosity is discussed.

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THE ACID HYDROLYSIS OF GLYCOSIDES

III. HYDROLYSIS OF *O*-METHYLATED GLUCOSIDES AND DISACCHARIDES

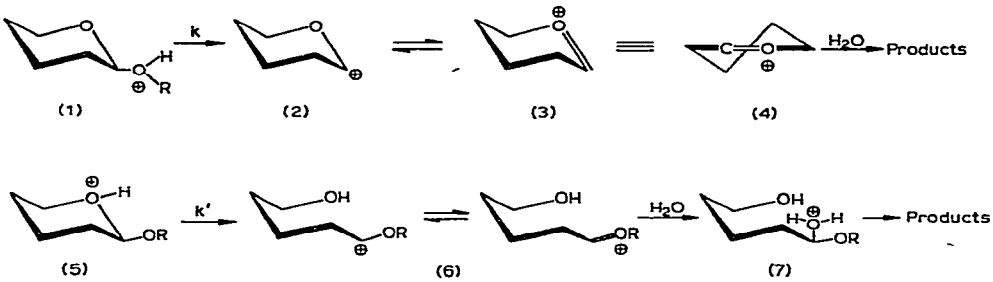
K. K. DE AND T. E. TIMELL
Cellulose Research Institute and Department of Forest Chemistry, State University College of Forestry Syracuse, N. Y. 13210 (U. S. A.)

(Received October 17th, 1966)

INTRODUCTION

Previous contributions in this series dealt with the effect of the aglycon[1] and of substituents at C-5[2] on the acid-catalyzed hydrolysis of the glycosidic bond. The present study is concerned with the influence of mono-*O*-methyl substituents on the rate of hydrolysis of methyl β-D-glucopyranoside and 6-*O*-β-D-glucopyranosyl-D-glucose (gentiobiose).

The currently accepted mechanism for the acid-catalyzed hydrolysis of glycosidic bonds involves a rapid, equilibrium-controlled protonation of the glycosidic oxygen atom to form a conjugate acid (**1**), that, in a slow, rate-determining, unimolecular heterolysis, decomposes to (**2**) and (**3**). As first pointed out by Edward[3], the latter will tend to assume a half-chair conformation (**4**). Alternatively, the ring oxygen atom might be protonated, giving (**5**), after which the ring is opened in a slow, rate-controlling step. The carbonium–oxonium ion (**6**) then adds water to give (**7**). Although the second mechanism cannot be entirely excluded, currently available evidence[4,5] is in favor of the first.



It was pointed out by Edward[3] that the transition of the chair to the half-chair conformation requires rotation about the C-2 to C-3 and the C-4 to C-5 bonds, and that any substitution in these positions should hinder this change, thus decreasing the rate of hydrolysis. It was the object of this investigation to educe more information on this point.

RESULTS

The 2-, 3-, 4-, and 6-*O*-methyl-D-glucoses were prepared by standard methods, and converted into the corresponding methyl β-D-glucopyranosides by a Koenigs–Knorr[6] condensation of the poly-*O*-acetylglycosyl bromides with methanol in the presence of silver carbonate. The poly-*O*-acetylglycosyl bromides of the 2-, 3-, 4-, and 6-*O*-methyl-D-glucoses were condensed in the same way with 1,2,3,4-tetra-*O*-acetyl-β-D-glucose, giving the corresponding mono-*O*-methyl derivatives of gentiobiose in good yields. The latter four compounds, which are new, could not be induced to crystallize, but were chromatographically pure and were characterized through their crystalline peracetates. Crystalline gentiobiose was synthesized in the same manner.

Hydrolyses were conducted at 60, 70, and 80° with 0.025M glycoside solutions in 0.5M aqueous sulfuric aid, and the extent of hydrolysis was determined polarimetrically, as described previously[1]. Rate coefficients, energies of activation, and entropies of activation at 60° are shown in Table I. Deviations from the mean were in the range of 1–2%. All reactions were of the first order, and $\ln k$ was, throughout, a linear function of 1/T.

TABLE I

RATE COEFFICIENTS AND KINETIC PARAMETERS FOR THE HYDROLYSIS OF METHYL MONO-*O*-METHYL-β-D-GLUCOPYRANOSIDES AND 6-*O*-(MONO-*O*-METHYL-β-D-GLUCOPYRANOSYL)-D-GLUCOSES IN 0.5M SULFURIC ACID

Compound	k × 10^6, sec^{-1}			E, *kcal* $mole^{-1}$	$\Delta S^{\ddagger}$ *at 60°, cal* deg^{-1} $mole^{-1}$
	60°	*70°*	*80°*		
Methyl mono-*O*-methyl-β-D-glucopyranosides					
2-*O*-Methyl-	1.19	5.22	20.8	33.4	+12.9
3-*O*-Methyl-	1.27	5.66	23.8	34.0	+41.9
4-*O*-Methyl-	1.15	4.97	21.2	33.8	+41.1
6-*O*-Methyl-[a]	0.84	3.88	16.1	34.9	+16.8
Unsubstituted[b]	1.38	6.25	24.1	32.5	+10.6
6-*O*-(Mono-*O*-methyl-β-D-glucopyranosyl)-D-glucoses					
2-*O*-Methyl-	1.56	6.69	26.7	33.1	+12.5
3-*O*-Methyl-	1.77	7.69	32.0	33.7	+14.5
4-*O*-Methyl-	1.48	7.04	28.3	34.4	+16.4
6-*O*-Methyl-	1.37	5.46	22.3	32.6	+10.9
Unsubstituted	1.87	8.25	32.9	33.3	+14.3

[a]From Ref. 2. [b]From Ref. 1.

DISCUSSION

Inspection of the rate coefficients in Table I shows that the effect of the *O*-methyl substituents is the same in the D-glucoside and in the gentiobiose series, indicating that the nature of the aglycon is of little consequence in this case. All of the mono-

O-methyl compounds are hydrolyzed more slowly than the corresponding unsubstituted sugars. If conformational factors only were involved, methoxyl groups at C-2 and C-3 would be expected to have the same influence on the rate of hydrolysis. The fact that the 2-*O*-methyl compounds are hydrolyzed somewhat more slowly than the 3-*O*-methyl derivatives could possibly be attributed to steric interference of the 2-*O*-methyl group in the protonation of the exocyclic oxygen atom.

The 4-*O*-methylated compounds are hydrolyzed slightly more slowly than the 2- and 3-methyl ethers; this was to be expected, as a substituent at C-4 is in opposition, not to a hydroxyl group, but to the (larger) hydroxymethyl group on C-5. Overend, Rees, and Sequeira[7] have noted a similar effect in comparing the acid hydrolysis of methyl 3-deoxy-α-D-*ribo*-hexopyranoside and methyl 4-deoxy-α-D-*xylo*-hexopyranoside. The ratio of the rate coefficients for methyl β-D-glucopyranoside and methyl 4-*O*-methyl-β-D-glucopyranoside found here was 1.23. Theander[8] has reported a ratio of 1.25 for the same sugars under similar conditions of hydrolysis.

In both series, the 6-*O*-methylated compounds were hydrolyzed at a rate considerably lower than those of the other *O*-methyl sugars. Similarly, it has been shown[9] that, in various methyl glycosides, a 6-sulfate group stabilizes the glycosidic linkages towards acids to an extent greater than does a sulfate group at C-3. The reason for this is difficult to ascertain at present. Steric, conformational factors alone cannot be invoked to explain the influence of substitution at C-5 on the rate of hydrolysis, because methyl 6-deoxy-α-D-glucopyranoside is hydrolyzed at the same rate as methyl α-D-xylopyranoside, and, for 6-*O*-substituted methyl β-D-glucopyranosides, the rate decreases in the order isopropyl > ethyl > methyl[2]. The possibility that both polar and steric effects are involved cannot be excluded.

The energies and entropies of activation were all within the range to be expected for acid-catalyzed hydrolysis of neutral glycopyranosides[1,2,7]. The fairly high, positive values for the entropies of activation indicate that a unimolecular reaction mechanism is involved.

The present results, which show that substitution with an *O*-methyl group at various positions in both methyl β-D-glucopyranoside and gentiobiose renders these compounds more stable towards acid, are in accordance with the predictions of Edward[3], and thus constitute additional evidence in favor of the cyclic, carbonium-ion mechanism. More information is needed, however, to explain how substituents at C-5 influence the rate of hydrolysis.

EXPERIMENTAL

All melting points are corrected. Specific rotations were measured at 25.0° ($c = 2.0$) with chloroform as the solvent for acetates, and water for all other compounds. The purity of noncrystalline products was checked by paper, thin-layer, and/or gas–liquid chromatography.

Synthesis of mono-O-methyl-D-glucoses. — *2-O-Methyl-D-glucose.* This compound was prepared by methylation of *N*-(3,4,6-tri-*O*-acetyl-D-glucopyranosyl)piperi-

dine[10], in an overall yield of 14% based on the D-glucose used. After recrystallization from ethanol, the compound had m.p. 158.5–159° and $[\alpha]_D$ +66°.

3-O-Methyl-D-glucose. This sugar was obtained, in an overall yield of 58%, via 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. Crystals of the α-D-pyranose form, obtained from methanol, had m.p. 165–166°, $[\alpha]_D$ +56°.

4-O-Methyl-D-glucose. Methyl 4-*O*-methyl-β-D-glucopyranoside was prepared by methylation of methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside according to the method of Bouveng, Lindberg, and Theander[11]. The product was recrystallized from ethyl acetate, yield 6.2%, m.p. 102–103°, $[\alpha]_D$ −19°. Acid hydrolysis gave amorphous 4-*O*-methyl-D-glucose, yield 60%, $[\alpha]_D$ +51°.

6-O-Methyl-D-glucose. This compound was prepared in an overall yield of 4.1% by methylation of 6-*O*-acetyl-1,2:3,5-di-*O*-methylene-α-D-glucofuranose. After crystallization from ethanol, the product had m.p. 141–143°, $[\alpha]_D$ +63°.

Synthesis of methyl mono-O-methyl-β-D-glucopyranosides. — Tetraacetates of the mono-*O*-methyl-β-D-glucoses were prepared by treatment with acetic anhydride in the presence of anhydrous sodium acetate. Tri-*O*-acetyl-mono-*O*-methyl-α-D-glucosyl bromides were obtained as described by Scheurer and Smith[12]. One of the poly-*O*-acetylglycosyl bromides, namely 3,4,6-tri-*O*-acetyl-2-*O*-methyl-D-glucosyl bromide was obtained crystalline for the first time. After recrystallization from ethyl ether, it had m.p. 85–86°, $[\alpha]_D$ +208°. The poly-*O*-acetylglycosyl bromides were condensed with anhydrous methanol in the presence of silver carbonate, iodine, and Drierite, and then acetate groups were removed by treatment with methanolic sodium methoxide. The yields and characteristics of intermediates and final products are summarized in Table II.

TABLE II

EXPERIMENTAL DATA FROM THE SYNTHESIS OF METHYL MONO-*O*-METHYL-β-D-GLUCOPYRANOSIDES

Compound	*Tetra-O-acetyl-mono-O-methyl-β-D-glucose*			*Methyl tri-O-acetyl-mono-O-methyl-β-D-gluco-pyranoside*			*Methyl mono-O-methyl-β-D-glucopyranoside*		
	Yield[a], %	*M.p.*, *degrees*	$[\alpha]_D$, *degrees*	*Yield*[b], %	*M.p.*, *degrees*	$[\alpha]_D$, *degrees*	*Yield*[d], %	*M.p.*, *degrees*	$[\alpha]_D$, *degrees*
2-*O*-Methyl-	56	107–108	+22	67	71–72	+5.4	49	96–97	−41
3-*O*-Methyl-	54	95–96	− 5	46	90–91	−31	66		−25
4-*O*-Methyl-	52	98–99	− 7					102–103	−19
6-*O*-Methyl-	57	90–91	+24		103–105[c]	−15[c]		133–135[c]	−28[c]

[a]Based on mono-*O*-methyl-D-glucose. [b]Based on tetraacetate. [c]From ref. 2. [d]Based on triacetate.

Synthesis of 6-O-(mono-O-methyl-β-D-glucopyranosyl)-D-glucoses[13,14]. — *6-O-(3,4,6-Tri-O-acetyl-2-O-methyl-β-D-glucopyranosyl)-1,2,3,4-tetra-O-acetyl-β-D-glucose.* 1,2,3,4-Tetra-*O*-acetyl-β-D-glucose (m.p. 127–128°, $[\alpha]_D$ + 10°) (5.7 g), silver carbonate (15 g), Drierite (50 g), and dry benzene (120 ml) were stirred for one h, and then iodine

(2 g) was added. 3,4,6-Tri-*O*-acetyl-2-*O*-methyl-D-glucosyl bromide (6.3 g), dissolved in benzene (30 ml), was added dropwise to the reaction mixture during 15 min. The mixture was stirred for 2 days in the dark at room temperature, and filtered through Celite, and the filtrate and washings were combined and concentrated to a syrup. The latter was dissolved in ethanol, treated with Darco-G60 charcoal, filtered, concentrated, and again dissolved in ethanol (250 ml). The solution was kept overnight at +4°, whereupon a crystalline product formed, yield (2.3 g (22%), m.p. 139–140°, $[\alpha]_D$ +6.2°.

6-O-(2-O-Methyl-β-D-glucopyranosyl)-D-glucose. A portion of the above compound (2.0 g) was dissolved in anhydrous methanol (30 ml) containing 0.1% of sodium methoxide. After one h at room temperature, some water was added, and sodium ions were removed with Amberlite IR-120 (H^+) ion-exchange resin. The solution was decolorized with charcoal and, after filtration and evaporation, gave a colorless syrup (0.9 g., 90%) that could not be induced to crystallize; it had $[\alpha]_D$ +3.8°.

The other three mono-*O*-methylgentiobioses were prepared in the same way. Their peracetates were all crystalline, but the free disaccharides were amorphous. The yields and characteristics of the intermediate and final compounds are presented in table III.

TABLE III

EXPERIMENTAL DATA FROM THE SYNTHESIS OF 6-*O*-(MONO-*O*-METHYL-β-D-GLUCOPYRANOSYL)-D-GLUCOSES

Compound	*6-O-(Tri-O-acetyl-mono-O-methyl-β-D-glucopyranosyl)-1,2,3,4-tetra O-acetyl-β-D-glucose*					*6-O-(mono-O-methyl-β-D-glucopyranosyl)-D-glucose*	
				Found			
	Yield[a], %	*M.p.*, *degrees*	$[\alpha]_D$, *degrees*	*Carbon*[b]	*Hydrogen*[c]	*Yield*, %	$[\alpha]_D$, *degrees*
2-*O*-Methyl-	22	139–140	+ 6.2	50.0	5.95	90	+ 3.8
3-*O*-Methyl-	18	191–192	−11.3	49.7	5.87	90	+ 8.1
4-*O*-Methyl-	24	159–160	−10.2	49.5	6.00	88	+18.2
6-*O*-Methyl-	45	201–202	− 1.0	49.3	5.93	80	+11.3

[a]Based on the poly-*O*-acetylglycosyl bromide. [b]Calc. for $C_{27}H_{38}O_{18}$: 49.85. [c]Calc., 5.85. [d]Based on the heptaacetate.

Synthesis of 6-O-β-D-glucopyranosyl-D-glucose. — Gentiobiose was synthesized in the usual way[13,14] by a Koenigs–Knorr[6] condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 1,2,3,4-tetra-*O*-acetyl-β-D-glucose, in 15% yield, based on the latter compound. Crystallization from aqueous ethanol gave a product having m.p. 190–192°, $[\alpha]_D$ +10.7°.

Kinetic Measurements. — Kinetic measurements were performed as described previously[1], except that a Perkin–Elmer photoelectric polarimeter Model 141 was used for following the hydrolysis. All rate-coefficients were determined by the Guggenheim method[15].

ACKNOWLEDGMENTS

The authors express their gratitude to Mr. M. D. Saunders and Mr. N. Roy for valuable help and discussions. This investigation was partly supported by a U. S. Public Health Service Research Grant No. 5 RO1 AMO 7193 from the National Institute of Arthritis and Metabolic Diseases, which is hereby gratefully acknowledged.

SUMMARY

Rate coefficients and kinetic parameters have been determined for the acid-catalyzed hydrolysis of 2-, 3-, 4-, and 6-*O*-methyl derivatives of methyl β-D-glucopyranoside and of gentiobiose. All of the monomethyl ethers were hydrolyzed somewhat more slowly than the unsubstituted compounds, the rate decreasing for both series in the order 3-*O*-methyl > 2-*O*-methyl > 4-*O*-methyl > 6-*O*-methyl. The results are in agreement with those to be expected if the unimolecular hydrolysis proceeds *via* a cyclic, carbonium ion; they thus offer additional evidence in favor of this mechanism.

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STUDIES ON ONUPHIC ACID

PART III. PRINCIPAL STRUCTURAL FEATURES

F. G. E. PAUTARD AND H. ZOLA
Astbury Department of Biophysics, The University, Leeds 2 (Great Britain)

(Received July 23rd, 1966; in revised form, October 29th, 1966)

INTRODUCTION

Previous communications[1,2] have established that onuphic acid, a macromolecule isolated from the tube of the polychaete *Hyalinoecia tubicola*, contains phosphate (in both the monoester and the diester forms), glucose, and smaller proportions of fucose. We now report on the structure of the macromolecule.

MATERIALS AND METHODS

Onuphic acid, used without decationisation, was prepared by extraction with N hydrochloric acid, as described previously[1].

Reducing sugar was determined by two standard methods[3,4]. Periodate oxidation was performed in phosphate buffer at pH 6.0 in dark bottles at 2°, using an onuphic acid concentration of 0.5% and periodate in great excess. Blanks containing periodate without onuphic acid, and onuphic acid without periodate, were used as controls. Periodate uptake was determined by the method of Fleury and Lange[5]. Reduction of the periodate-oxidised macromolecule by borohydride, with subsequent hydrolysis, was performed by the method of Abdel-Akher and Smith[6]. Gel filtration, chromatography, ultracentrifugation, and determinations of relative viscosity were performed by methods described elsewhere[1,2].

RESULTS

Determinations of reducing power showed that 1 g of onuphic acid was equivalent to 1.32 mg of free glucose by the alkaline copper method[3], and 1.51 mg of free glucose by the Prussian Blue method[4]. The rate of periodate oxidation of onuphic acid is shown in Fig 1. After the reaction was essentially complete (400 h), the total periodate consumption was 44.5×10^{-5} moles per g of onuphic acid, *i.e.*, approximately 1 mole of periodate per 10 gram-atoms of onuphic acid phosphorus. After partial dephosphorylation (with 50% of the initial phosphorus content remaining; see Pautard and Zola[2]), onuphic acid showed no increase in periodate consumption.

The product obtained from borohydride reduction of the periodate-oxidized onuphic acid was isolated by gel filtration on Sephadex G25, followed by lyophilization.

The acid hydrolysate of this material contained glucose and inorganic phosphate. Fucose and non-reducing, polyhydroxy compounds were not detected, but a reducing substance, having the chromatographic mobility of a disaccharide, was present as a minor component. Alkaline hydrolysis of the acid hydrolysate, with potassium hydroxide at room temperature for 4 h in the absence of oxygen, gave no new components.

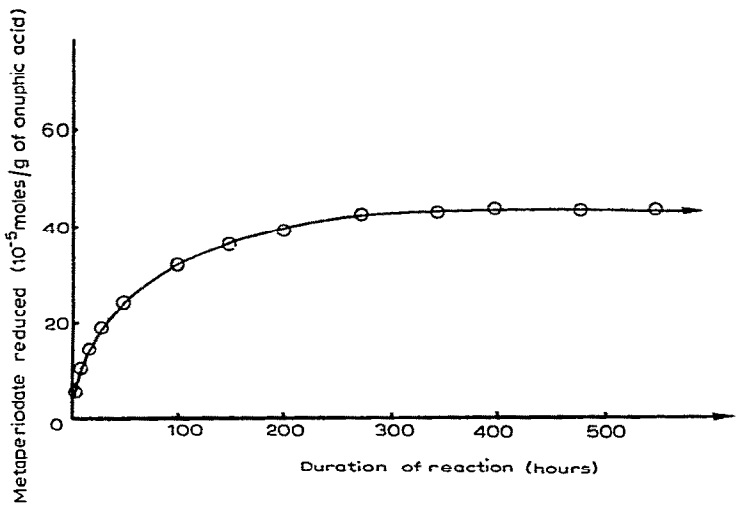


Fig. 1. Course of periodate oxidation of onuphic acid with time.

Treatment of onuphic acid with acid or alkali resulted in a reduction of viscosity as shown in Fig. 2. Partial acid- and alkali-hydrolysates were recovered after the viscosity–time curves had reached plateaux. Phosphodiesterase-hydrolysates were

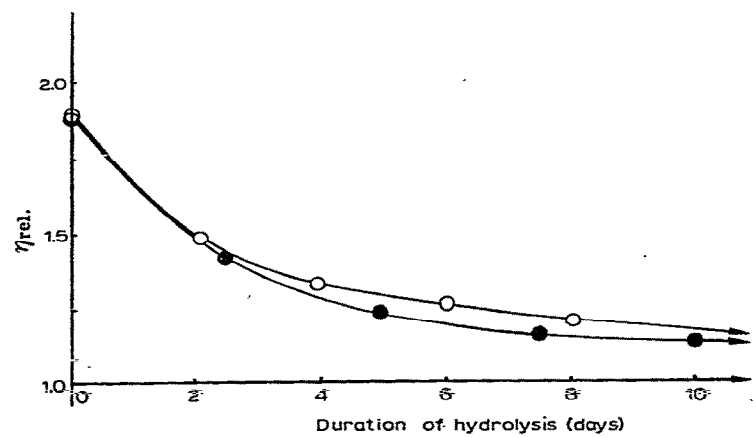


Fig. 2. Reduction of viscosity of onuphic acid during hydrolysis: O – O 2N hydrochloric acid at 25° (1% onuphic acid, HCl extract); ●–● 0.2N potassium hydroxide 25° (0.6% onuphic acid, HCl extract).

recovered similarly (see the previous communication[2] for the kinetics of phosphodiester hydrolysis). Each of these hydrolysates gave a single, broad, rapidly diffusing peak in the ultracentrifuge, in contrast to the sharp peak obtained from unhydrolysed onuphic acid[1]. Fractionation of these hydrolysates on Sephadex G25 gave, in each case, an excluded fraction, having high molecular weight, and a retarded fraction, having low molecular weight. The former contained no free or bound fucose, and the glucose–phosphate ratio was essentially the same as that in the parent molecule. The latter fractions contained buffer constituents and, in addition, fucose in the enzyme hydrolysate; fucose, glucose, and inorganic phosphate in the acid hydrolysate; and glucose and inorganic phosphate in the alkaline hydrolysate.

The fraction of high molecular weight from the acid hydrolysate was isolated by freeze-drying and redissolved in 2N hydrochloric acid to a concentration of 1%. The effect of further hydrolysis at 80° was observed by viscosity determination, and it was found that the initial rate of viscosity drop was comparable with that of the native onuphic acid, measured previously (see Fig. 2).

DISCUSSION

From previous studies of onuphic acid[1,2], we propose the following ratio of components: glucose (22):fucose (1):phosphomonoester (25): phosphodiester (3). Since partial degradation results in macromolecular fragments that show little change in the relative proportions of glucose and phosphate, it seems likely that the components are distributed evenly throughout the parent molecule. The presence of a single peak in the sedimentation patterns of partially hydrolysed products suggests that the labile linkages are evenly distributed. The apparently polydisperse nature of the patterns does not exclude the possibility that the ratios proposed above represent a true repeating unit, with a molecular weight of approximately 6,000, since such a small molecule would be unlikely to give a sharp peak. Although the shape of the viscosity–time graph for the acid hydrolysis of onuphic acid might indicate that the material isolated after treatment for 3 h represents an acid-stable core, the fact that the partially hydrolysed material can be further hydrolysed at the same inital rate, after concentration, suggests that this is not so.

Fucose is released rapidly from onuphic acid by mild acid[2] or by alkaline treatment, indicating its association with a labile linkage. The release of free fucose by a phosphodiesterase preparation, and the absence of the deoxyhexose from the residue of high molecular weight, suggests that the fucose may be bound to the rest of the macromolecule through phosphodiester linkages.

Some of the phosphodiester groups in onuphic acid may be present in the main chain, or may form long branches[2]. The fragments released by phosphodiesterase treatment are excluded by Sephadex G25, and thus have a molecular weight probably in excess of 5,000.

The bulk of onuphic acid consists of phosphorylated glucose residues, the low

reducing power indicating that the glycosidic positions are blocked, as in normal polysaccharides. The limited attack by periodate on the macromolecule indicates that either the 3 position, or both the 2 and 4 positions, are substituted on most of the glucose residues. Further, the failure of partial dephosphorylation to increase the reactivity towards periodate indicates not only that the blocking group is not phosphate, but also that the inter-saccharide linkage is probably through the 3 position, since if it were through 2 or 4 positions, the removal of phosphate from the other blocked position would expose an unsubstituted *vic*-diol. Similarly, the low consumption of periodate, and the failure of partial dephosphorylation to increase the reactivity indicate that the majority of the glucose residues are present as pyranosyl residues. The rapid degradation of the carbohydrate of onuphic acid by alkali[2] indicates that the 2 position is free, and supports the suggestion from the other evidence that the glucan is (1→3)-linked (see, for instance, Whistler and BeMiller)[7].

The phosphate ester groups are thus in positions 4 or 6, and the excess of phosphate groups over sugar residues indicates that in some residues both positions will be phosphorylated. However, phosphoryl migration during hydrolysis might make it difficult to identify the position of phosphorylation with any precision.

ACKNOWLEDGMENTS

This work was supported in part by grant DE-01346-03 from the U.S. National Institutes of Health; specimens of *Hyalinoecia* were purchased from a Scientific Investigation Grant administered by the Royal Society. One of us (H.Z.) is grateful for a grant from Unilever Ltd.

SUMMARY

The data from periodate oxidation and hydrolysis indicate that onuphic acid consists principally of chains of (1→3)-linked glucose residues, phosphorylated at positions 4 or 6. Partial degradation of the macromolecule results in fragments of similar composition, but of no characteristic size.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

ETHYLIDENE DERIVATIVES OF D-ERYTHROSE
I. 2,3-*O*-ETHYLIDENE-β-D-ERYTHROFURANOSE

J. W. VAN CLEVE AND C. E. RIST
Northern Regional Research Laboratory, Peoria, Illinois 61604 (U.S.A.)*

(Received September 1st, 1966; in revised form, November 7th, 1966)

When "dialdehyde starch"** is heated with sulfurous acid under optimum conditions of pressure, temperature, and concentration, D-erythrose and glyoxal are produced in good yield[1]. Isolation of each of these products from the sulfurous acid hydrolyzate has been undertaken previously, but with limited success[2]. Whereas glyoxal could readily be removed as its insoluble complex with barium bisulfite[3], an equally facile method for separating D-erythrose from the hydrolyzate was not apparent.

Since it is known[4] that D-erythrose can be acetalated with paraldehyde in aqueous acid, we attempted to ethylidenate hydrolyzates of "dialdehyde starch" by this method. The reaction was successful, but only when sulfuric acid was also added; the acidity provided by the sulfurous acid alone was evidently insufficient to catalyze the reaction. The ethylidene derivative of D-erythrose thus formed could be extracted with chloroform and subsequently isolated as a pure, crystalline compound.

It appeared likely that our ethylidene derivative was identical with the one reported by Ballou[4]. However, Ballou had not isolated the product of the acetalation reaction, nor had he defined its structure by unequivocal proof[5]. Consequently, we repeated Ballou's preparation, and isolated a product that crystallized and which proved to be identical with the ethylidenated D-erythrose prepared from hydrolyzates of "dialdehyde starch". We present herein a rigorous proof of structure for this new ethylidene derivative (**1**) of D-erythrose.

Characterization of 2,3-O-ethylidene-β-D-erythrofuranose

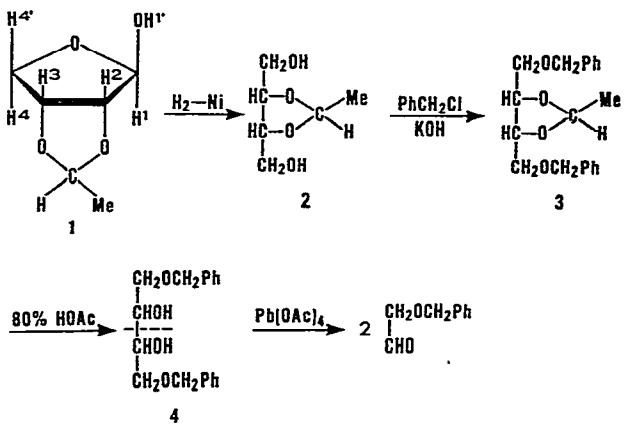
Rearrangement[4] of the readily available 2,4-*O*-ethylidene-D-erythrose with acid provided a convenient source of **1**, an easily crystallized substance which could be isolated pure by distillation *in vacuo* (no decomposition). In water, **1** showed a constant, negative rotation, and did not reduce boiling Fehling solution. These properties suggested that **1** is a nonreducing compound. On the other hand, it was subsequently found to give a silver mirror, albeit slowly, with Tollens' reagent; furthermore, it reacted with (2,4-dinitrophenyl)hydrazine, with some difficulty, to give the corresponding hydrazone in poor yield.

*This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.
**Periodate-oxidized starch.

Study of molecular models resolved these apparently contradictory properties. Assuming that, in accordance with Ballou's conclusions, **1** is 2,3-*O*-ethylidene-D-erythrose, hemiacetal formation would be expected to produce the α-D- and β-D-furanose anomers. However, only the β-D-furanose form is sterically feasible; in the α-D form, the anomeric hydroxyl group is sterically hindered by the ethylidene ring. This finding would explain the apparent lack of mutarotation of compound **1**, as well as the negative optical rotations of the crystalline esters that it readily afforded. N.m.r. spectra subsequently confirmed that compound **1**, a reducing sugar, does indeed exist exclusively in the β-D-furanose form.

Catalytic hydrogenation of **1** gave a syrupy, optically inactive mono-*O*-ethylidene-erythritol (**2**) that was characterized as its crystalline bis(*p*-nitrobenzoate). Attempts to prepare a trityl derivative of **2** failed, probably because of steric hindrance by the ethylidene ring. However, benzylation gave a di-*O*-benzyl-*O*-ethylidene-erythritol (**3**) in good yield; mild, acidic hydrolysis of this syrupy compound gave a crystalline di-*O*-benzylerythritol (**4**). Compound **4** consumed 1 molar equivalent of lead tetraacetate, the product of the reaction being 2-(benzyloxy)acetaldehyde, identified as its crystalline (2,4-dinitrophenyl)hydrazone.

From these experiments, the following deductions may be made: (1) the di-*O*-benzylerythritol (**4**), derived from **3**, contains an α-glycol group, and (2) of the two possible di-*O*-benzylerythritols having an α-glycol group, only the 1,4-diether could



provide 2-(benzyloxy)acetaldehyde on cleavage with lead tetraacetate. Hence, compound **4** is 1,4-di-*O*-benzylerythritol, compound **2** is 2,3-*O*-ethylidene-erythritol*, and compound **1** must, therefore, be 2,3-*O*-ethylidene-D-erythrose. The sequence of conversions is illustrated in the accompanying chart.

*Of the theoretically possible mono-*O*-ethylidene derivatives of D-erythritol (1,3-, 2,3-, and 3,4-), only the 2,3-derivative can be optically inactive.

The following interpretation of the n.m.r. spectrum of **1** was provided by R.U. Lemieux.

The spectrum required that the observed splitting of the H-1 signal should arise only from coupling with the hydroxyl hydrogen H-1′ (intensity for one -OH proton only), since the doublet collapsed to a singlet on deuteration. The first-order coupling between H-1 and H-2 must, therefore, be near zero; this is confirmed by the fact that the H-2 signal is observed as a doublet. The latter signal shows a spacing of 6.5 c.p.s., resulting from coupling with H-3; the same 6.5-c.p.s. spacing for the two main bands in the signal for H-3 confirms this assignment. The coupling of H-3 with H-4 and H-4′ is obscured by the weak chemical shift between the latter two hydrogens.

The fact that the coupling between H-1 and H-2 is almost zero requires that these hydrogens define a dihedral angle[6] of about 80–90°. Therefore, the compound is in the β-D configuration shown. The fact that H-1 is coupled with H-1′ requires that the compound be in the lactol ring form. The 6.5-c.p.s. spacing for H-2 in coupling with H-3 is in agreement with a dihedral angle of *ca.* 30 to 40° for these hydrogens, as would be expected on the basis of (a) the *erythro* configuration and (b) conformational analysis.

Identification of 2-(benzyloxy)acetaldehyde

The synthesis of 2-(benzyloxy)acetaldehyde was described 34 years ago, but the published details of its preparation[7,8] are somewhat sketchy, and the purity and identity of the (syrupy) intermediates in the synthesis were, for the most part, assumed but not proved.

More recently[9], a convenient synthesis of certain mixed acetals of glycolaldehyde, whose structures have been rigorously proved, has provided easy access to a variety of ethers and esters of glycolaldehyde. Thus, periodate oxidation of methyl pento- or hexo-pyranosides, followed by reduction, gives, respectively, the glycolyl methyl acetal or β-glyceryl methyl acetal of glycolaldehyde, in approximately 80% yield. Methylation of these acetals followed by hydrolysis[10] has given 2-(methoxy)-acetaldehyde (glycolaldehyde methyl ether).

One of these acetals, (*S*)-2-hydroxy-1-[2-hydroxy-1-(2-hydroxyethyl)ethoxy]-1-methoxyethane (glycolaldehyde β-glyceryl methyl acetal), obtained from methyl α-D-glucopyranoside[9], was benzylated. The resulting benzyl ether, obtained in 83–86% yield, was readily hydrolyzed by acid, to give the anticipated 2-(benzyloxy)acetaldehyde. This syrupy aldehyde gave a semicarbazone and a (2,4-dinitrophenyl)-hydrazone.

EXPERIMENTAL

General. — Evaporations were conducted under diminished pressure. All melting points are uncorrected.

2,3-O-Ethylidene-β-D-erythrofuranose (**1**). — (*a*) *By acidic rearrangement of 2,4-O-ethylidene-D-erythrose.* A solution of 2,4-*O*-ethylidene-D-erythrose[11] (5.35 g) in N

sulfuric acid (100 ml) was kept* for 3 days at 25°. Subsequent neutralization with excess barium carbonate, followed by filtration and evaporation, gave a syrup contaminated somewhat with barium salts. The syrup was dissolved in absolute ethanol (100 ml), and ether (400 ml) was added. The precipitated barium salts were removed by filtration, and the clear, salt-free filtrate was evaporated to a syrup**. This syrup was extracted thoroughly with benzene by digestion on a steam bath, and the combined extracts were evaporated. The resulting syrup, essentially free from D-erythrose, was dissolved, for the most part, by hot heptane. On concentrating, cooling, and seeding, the heptane solution deposited **1** as long needles, m.p. 66–67°; $[\alpha]_D^{25}$ −68° (*c* 2, water); −116° (*c* 1, benzene).

Anal. Calc. for $C_6H_{10}O_4$: C, 49.3; H, 6.9. Found: C, 49.1; H, 6.8.

A solution of **1** (0.068 g) in 0.1N sulfuric acid was steam-distilled. The recovery of liberated acetaldehyde, determined by the hydroxylamine hydrochloride method[12], was 93.5% of the theoretical.

(*b*) *By acetalation of* D-*erythrose in dilute acid.* To a solution of D-erythrose[13] (2.04 g) in N sulfuric acid (100 ml) was added paraldehyde (7 ml). The mixture was kept at room temperature and the change in optical rotation (1-dm tube) was: $[\alpha]_D^{25}$ −0,35° (15 min) → −0.95° (70 h, constant). The reaction mixture was neutralized with excess barium carbonate, filtered, and evaporated (bath, 45–50°). The resultant syrup was freed of barium salts by the method used in (*a*). After evaporation of solvent, the salt-free syrup was digested several times with benzene on the steam bath, and the combined extracts were evaporated, and distilled to give **1** as a syrup, yield 0.70 g (25%), b.p. 90–95° (bath)/2 mm, which, on seeding, rapidly crystallized with evolution of heat. The crystals had m.p. and mixed m.p. 65–67°.

(*c*) *By acetalation of a hydrolyzate of "dialdehyde starch".* A sample of moist, defatted corn starch (3.50 g, dry basis) was suspended in 0.5M periodic acid (100 ml) and kept for 23 h at room temperature. Titration of an aliquot of the aqueous phase of the reaction mixture with standard sodium arsenite solution according to the method of Fleury and Lange[14] revealed that 1.04 moles of periodate had been consumed per D-glucose residue. The reaction mixture was filtered, and the solid product, essentially 100% "dialdehyde starch", was repeatedly washed with water until the washings failed to liberate iodine from potassium iodide–aqueous acetic acid solution. Excess water was then drawn off, and the product was pressed out on a funnel by aspiration.

The moist "dialdehyde starch" was suspended in water (85 ml) in a stainless-steel, Parr*** hydrogenation bomb that was cooled in an ice–water bath. The sus-

*We found that the reaction proceeds at room temperature with N acid. Ballou, on the other hand, conducted the reaction in 0.1N acid at 60° in a sealed vessel[4].

**If allowed to stand, the syrup often became partially crystalline.

***Manufactured by the Parr Instrument Co., Moline, Illinois (U. S. A.). 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.

pension was saturated with sulfur dioxide, and the bomb was sealed and heated, without agitation, for 3 h at 100°.

To the colorless, almost clear hydrolyzate was added conc. sulfuric acid (4.9 g, diluted by addition to a little water, and cooled); the volume was made up to 100 ml, to give a solution approximately N with respect to sulfuric acid. Paraldehyde (7 ml) was then added, and was dissolved by agitation of the mixture.

After 65 h at room temperature $[\alpha]_D^{25}$ −0.17 (initial, → −0.68° (final), 1-dm tube], the mixture was extracted with four 200-ml portions of chloroform. The combined extracts were shaken with an excess of aqueous suspension of barium carbonate, the suspension was filtered, and the filtrate evaporated to dryness (bath, 40–50°). The dry, syrupy residue of barium salts was thoroughly extracted with benzene by digestion on a steam bath, and the combined extracts, after filtration, were evaporated to 100 ml. The concentrated benzene solution was shaken with two 100-ml portions of water, the aqueous extracts were combined and evaporated to dryness, and the resulting syrup was heated to 70° under diminished pressure (water pump). The syrup was cooled and seeded, and a few drops of heptane were added; crystallization occurred slowly. Three recrystallizations from hot heptane gave pure **1**, m.p. and mixed m.p. 65–67°; $[\alpha]_D^{25}$ −117° (*c* 0.4, benzene).

Nuclear magnetic resonance data. — The n.m.r. spectrum of **1** was determined at 60 Mc.p.s., first in dry chloroform, and then, after the chloroform solution had been washed with deuterium oxide.

2,3-O-Ethylidene-1-O-p-nitrobenzoyl-β-D-erythrofuranose. — To a solution of **1** (0.17 g) in anhydrous pyridine (5 ml) was added *p*-nitrobenzoyl chloride (0.39 g), and the mixture was heated for 30 min at 80–90° (bath). The mixture was cooled, and poured, with stirring, into saturated, aqueous sodium hydrogen carbonate (50 ml); this caused immediate separation of crystalline 2,3-*O*-ethylidene-1-*O*-*p*-nitrobenzoyl-β-D-erythrofuranose, yield 0.28 g (87%); recrystallized from hot heptane, it had m.p. 129° and $[\alpha]_D^{20}$ −66° (*c* 1, benzene).

Anal. Calc. for $C_{13}H_{13}NO_7$: C, 52.9; H, 4.4; N, 4.8. Found: C, 53.2; H, 4.4; N, 4.7.

1-O-Benzoyl-2,3-O-ethylidene-β-D-erythrofuranose. — To a solution of **1** (0.15 g) in anhydrous pyridine (5 ml) was added benzoyl chloride (0.5 ml), and the mixture was heated for 30 min at 80–90° (bath), cooled, and poured into saturated, aqueous sodium hydrogen carbonate (50 ml). After 1 h, the syrupy suspension was extracted thoroughly with chloroform, and the combined extracts were dried (anhydrous sodium sulfate) and evaporated. The resulting syrup was dissolved in the minimum volume of hot, aqueous ethanol (20%), and the solution cooled; the title compound separated in crystals, yield 0.12 g (64%). Recrystallized from the same solvent, it had m.p. 96–97°, $[\alpha]_D^{20}$ −85° (*c* 1, benzene).

Anal. Calc. for $C_{13}H_{14}O_5$: C, 62.4; H, 5.6. Found: C, 62.3; H, 5.9.

1-O-Acetyl-2,3-O-ethylidene-β-D-erythrofuranose. — To a solution of **1** (0.62 g) in anhydrous pyridine (20 ml) was added acetic anhydride (12 ml), and the mixture was kept for 68 h at room temperature. It was then poured into ice–water

(200 ml) containing sodium hydrogen carbonate (35 g). After 30 min, the product was extracted into chloroform, as in the preceding experiment. Crystallization and recrystallization (twice) from heptane gave the title compound, yield 0.24 g (30%), m.p. 58°, $[\alpha]_D^{25}$ − 135° (*c* 1, benzene).

Anal. Calc. for $C_8H_{12}O_5$: C, 51.1; H, 6.4; CH_3CO, 22.9. Found: C, 51.2; H, 6.4; CH_3CO, 23.3.

2,3-O-Ethylidene-D-erythrose (2,4-dinitrophenyl)hydrazone. — To a solution of **1** (0.076 g, 0.0005 mole plus 4% excess) in 95% ethanol (3 ml) was added (2,4-dinitrophenyl)hydrazine (0.099 g, 0.0005 mole). After 30 h under reflux, most of the (2,4-dinitrophenyl)hydrazine had reacted, as only a few red crystals of it were interspersed among the orange needles of the hydrazone formed on cooling. Recrystallization from boiling, 95% ethanol gave the pure, crystalline, substituted hydrazone; yield 0.62 g (38%), m.p. 151–152° (softening).

Anal. Calc. for $C_{12}H_{14}N_4O_7$: C, 44.2; H, 4.3; N, 17.2. Found: C, 43.9; H, 4.3; N, 17.5.

2,3-O-Ethylidene-erythritol (**2**). — A solution of **1** (1.71 g) in absolute ethanol (70 ml) was placed in a 300-ml Parr hydrogenation bomb, and a nickel catalyst* was added. Hydrogenation was conducted at 2,500 lb. $in.^{-2}$ for 6 h at 110°, with mechanical shaking. After removal of catalyst by filtration, and of solvent by evaporation, a syrup was obtained that was distilled *in vacuo*. The syrupy distillate of **2** had b.p. 115–120° (bath)/2 mm, n^{20} 1.463, and was optically inactive.

2,3-O-Ethylidene-1,4-di-O-(p-nitrobenzoyl)erythritol. — To a solution of distilled **2** (0.20 g) in anhydrous pyridine (7 ml) was added *p*-nitrobenzoyl chloride (0.76 g), and the mixture was heated for 45 min at 80–90° (bath). After being cooled, the mixture was poured, with stirring, into saturated, sodium hydrogen carbonate solution (25 ml). The crystalline product that separated had, after two recrystallizations from chloroform–heptane, m.p 130–131°.

Anal. Calc. for $C_{20}H_{18}N_2O_{10}$: C, 53.8; H, 4.1; N, 6.3. Found: C, 54.1; H, 4.1; N, 6.3.

1,4-Di-O-benzyl-2,3-O-ethylidene-erythritol (**3**). — To a solution of **2** (1.03 g) in benzyl chloride (250 ml) was added powdered potassium hydroxide (6 g), and the mixture was stirred vigorously for 5 h at 90–100° (bath). Removal of excess of benzyl chloride by steam distillation (100°) was followed by extraction of the alkaline mixture with chloroform. The chloroform extract was washed free of alkali with water, dried (anhydrous sodium sulfate), and evaporated to a syrup. Distillation *in vacuo* yielded **3**, b.p. 190–195° (bath)/2 mm, n^{25} 1.533.

Anal. Calc. for $C_{20}H_{24}O_4$: C, 73.1; H, 7.4. Found: C, 73.1; H, 7.4.

1,4-Di-O-benzylerythritol (**4**). — A solution of **3** (1.00 g) in 80% aqueous acetic acid (20 ml) was heated for 1.5 h under reflux. The solvent was removed by evaporation by azeotropic distillation (several times) with benzene. The resulting syrup was dissolved

*"Girdler G-49A Reduced and Stabilized Nickel Hydrogenation Catalyst", manufactured by Chemical Products Division, Chemetron Corp., Louisville, Kentucky (U. S. A.).

in the minimal volume of heptane, and the solution cooled in a refrigerator for several h, giving crystalline **4** which, on recrystallization from the same solvent, had m.p. 59–60°.

Anal. Calc. for $C_{18}H_{22}O_4$: C, 71.5; H, 7.3. Found: C, 71.2; H, 7.3.

To a solution of **4** (0.2 g) in benzene (30 ml) was added lead tetraacetate (0.26 g, 1 equivalent) and the mixture was heated for 1.5 h at reflux*. The mixture was cooled, diluted with benzene, and freed of precipitated lead diacetate by filtration, and the filtrate was evaporated to a syrup. To a solution of the latter in ethanol (30 ml) was added glacial acetic acid (3 ml) and, (2,4-dinitrophenyl)hydrazine (0.25 g, 1.05 equivalents). The mixture was refluxed for 1.5 h, cooled, and diluted with water until the solution became turbid. The product that crystallized, 2-(benzyloxy)acetaldehyde (2,4-dinitrophenyl)hydrazone, had, after recrystallization from 75% aqueous acetic acid**, m.p. and mixed m.p. 114–115° (softening).

Anal. Calc. for $C_{15}H_{14}N_4O_5$: C, 54.6; H, 4.3; N, 16.9. Found: C, 54.7; H, 4.3; N, 16.7.

(S)-2-Benzyloxy-1-[2-benzyloxy-1-(2-benzyloxyethyl)ethoxy]-1-methoxyethane. — To a suspension of (*S*)-2-hydroxy-1-[2-hydroxy-1-(2-hydroxyethyl)ethoxy]-1-methoxyethane[9] (6.6 g) in benzyl chloride (50 ml) was added powdered potassium hydroxide (20 g). The mixture was stirred vigorously for 3.5 h at 130–140° (bath).

After completion of the reaction, excess benzyl chloride was removed by steam distillation (100°). The rest of the reaction mixture was then extracted twice with benzene; the combined benzene extracts were shaken twice with water, and evaporated to give a rather viscous syrup. Distillation of this syrup gave a fraction, yield 14.8 g (86%) of b.p. 235—245° (bath)***/4 mm, n^{27} 1.541, and $[\alpha]_D^{20}$ − 3.6° (*c* 4, methanol).

Anal. Calc. for $C_{27}H_{32}O_5$: C, 74.3; H, 7.4. Found: C, 74.3; H, 7.5.

Hydrolysis of (S)-2-benzyloxy-1-[2-benzyloxy-1-(2-benzyloxyethyl)ethoxy]-1-methoxyethane. — A solution of the above tribenzyl ether (7.0 g) in 80% aqueous acetic acid (63 ml) was heated for 1 h at 70° (bath), during which time the solution became optically inactive. The aqueous acetic acid was removed by distillation (45–50°, bath), residual amounts being removed by azeotropic distillation with benzene. Fractional distillation of the remaining syrup gave a mobile, fragrant liquid (1.65 g), b.p. 95–105° (bath)/4 mm, which gave a strong Schiff test. The syrup was used, without further purification, for preparing derivatives.

To a solution of the syrup (0.16 g) in 95% ethanol (5 ml) was added (2,4-dinitrophenyl)hydrazine (0.20 g), and the mixture was boiled for 1.5 h under

*In a separate experiment, 1 mole of **4** consumed 1.02 moles of lead tetraacetate, as determined by the method of Hockett and McClenahan[15].

**The crude hydrazone was dissolved in glacial acetic acid (3 ml/g) at room temperature, and water (1 ml/g) was added. Recrystallization from hot, 75% aqueous acetic acid caused some debenzylation, and the product that crystallized on cooling was contaminated with glyoxal bis[(2,4-dinitrophenyl)-hydrazone], m.p. and mixed m.p. 311–312°. An analogous difficulty has been reported by others[10] in the preparation of the (*p*-nitrophenyl)hydrazone of 2-(methoxy)acetaldehyde.

***"Dow Corning 550 Fluid", supplied by Dow Corning Corp., Midland, Michigan (U. S. A.).

reflux. Upon refrigeration for several h, the seeded reaction mixture deposited a crystalline product. Two recrystallizations from boiling heptane gave 2-(benzyloxy)-acetaldehyde (2,4-dinitrophenyl)hydrazone, m.p. 115–116°.

Anal. Calc. for $C_{15}H_{14}N_4O_5$: C, 54.6; H, 4.3; N, 16.9. Found: C, 54.5; H, 4.4; N, 16.8.

Another sample of the distilled syrup (0.11 g) was dissolved in water (3 ml), and to the solution was added semicarbazide hydrochloride (0.1 g) and sodium acetate trihydrate (0.15 g). The mixture was boiled for 30 min under reflux, and allowed to cool. On being seeded, it deposited crystalline 2-(benzyloxy)acetaldehyde semicarbazone which, after recrystallization from hot water (3 ml), had m.p. 118–120° (lit.[7] m.p. 119°).

Anal. Calc. for $C_{10}H_{13}N_3O_2$: C, 57.9; H, 6.3; N, 20.3. Found: C, 58.2; H, 6.5; N 20.5.

ACKNOWLEDGMENT

We are indebted to Professor R. U. Lemieux of the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada, for the n.m.r. spectra and interpretations.

We thank the following personnel of this Laboratory for their assistance: Mr. C. H. VanEtten and his associates for the microanalyses, and Mr. J. E. Hodge for helpful discussion of the manuscript.

SUMMARY

The principal product of the acidic rearrangement of 2,4-*O*-ethylidene-D-erythrose, and of the ethylidenation of D-erythrose in aqueous acid, is found to be 2,3-*O*-ethylidene-D-erythrose. The structure of this previously reported[4] compound, which has been isolated in the crystalline β-D-furanose form, has been confirmed by unequivocal proof.

The ethylidene ring of this D-erythrose acetal has a profound effect on the properties anticipated for a reducing sugar. Reactions of the aldehyde group are hindered, and hemiacetal formation gives only the β-D-furanose form; an α-D anomeric hydroxyl group would be sterically hindered. No mutarotation is observed, and acylation gives derivatives of the β-D anomer only.

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Carbohyd. Res., 4 (1967) 82–90

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF 3-*O*-BENZYL-D-ERYTHROSE

J. W. VAN CLEVE AND C. E. RIST
Northern Regional Research Laboratory, Peoria, Illinois 61604 (U. S. A.)*

(Received September 1st, 1966; in revised form, November, 7th 1966)

During studies of the rearrangement of 2,4-*O*-ethylidene-D-erythrose in acid[1], we required 3-*O*-benzyl-D-erythrose as a reference compound. The synthesis of this previously unknown ether of D-erythrose and its characterization by crystalline derivatives are described.

The most accessible derivative of D-erythrose is 2,4-*O*-ethylidene-D-erythrose[2], and, because the 3-hydroxyl group is unsubstituted, it seemed a logical starting point for the synthesis. Protection of the exposed aldehyde group presented a problem, as glycosidation did not seem feasible. Any group chosen for protecting the aldehyde group had to be capable of withstanding the conditions of the subsequent benzylation reaction. We were able to solve this problem as follows.

Treatment of 2,4-*O*-ethylidene-D-erythrose with 1-methyl-1-phenylhydrazine readily gave the crystalline (2-methyl-2-phenyl)hydrazone in 85–90% yield, and benzylation of this substituted hydrazone by the method of Croon and Lindberg[3] gave the crystalline 3-benzyl ether in 90–95% yield. In a parallel experiment, we attempted by the same method to benzylate the (2,4-dinitrophenyl)hydrazone of 2,4-*O*-ethylidene-D-erythrose. The dark, tarry product failed to yield a crystalline compound. Apparently, both of the hydrazine hydrogen atoms of the hydrazone must be substituted, to prevent undesired side-reactions during the benzylation.

Attempts to prepare 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose from its (2-methyl-2-phenyl)hydrazone, by heating the latter with an aqueous suspension of benzaldehyde[4], were unsuccessful under either neutral or slightly acidic conditions. However, when 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose (2-methyl-2-phenyl)hydrazone was heated with aqueous acetaldehyde in a hydrogenation bomb at 80°, under the general conditions of Collatz and Neuberg[5], the reducing sugar, 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose, was readily obtained and separated from the reaction mixture. The oily byproduct, acetaldehyde (2-methyl-2-phenyl)hydrazone[6], was volatile with water vapor and could be completely removed, along with excess acetaldehyde, by evaporation of the aqueous mixture. The syrupy 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose that remained was further purified by distillation, and was characterized as the cryst-

*This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

alline (2,4-dinitrophenyl)hydrazone. Mild, acidic hydrolysis of the former gave syrupy 3-*O*-benzyl-D-erythrose of analytical purity. This reducing, benzylated sugar was characterized by the preparation of two crystalline derivatives, the (*p*-nitrophenyl)-hydrazone and the (*p*-bromophenyl)osazone.

EXPERIMENTAL

General. — Evaporations were conducted under diminished pressure. All melting points are uncorrected.

2,4-O-Ethylidene-D-erythrose (2,4-dinitrophenyl)hydrazone. — To a solution of 2,4-*O*-ethylidene-D-erythrose (0.29 g) in absolute ethanol (10 ml) was added 1 equivalent (0.39 g) of (2,4-dinitrophenyl)hydrazine, and the mixture was boiled for 2.5 h under reflux. Evaporation of the solvent gave a yellow, crystalline residue, which was recrystallized, first from hot water, and then twice from boiling 1:1 (v/v) benzene–heptane, to give canary-yellow crystals of the title compound, m.p. 167–168°.

Anal. Calc. for $C_{12}H_{14}N_4O_7$: C, 44.2; H, 4.3; N, 17.2. Found: C, 44.5; H, 4.4; N, 17.5.

2,4-O-Ethylidene-D-erythrose (2-methyl-2-phenyl)hydrazone. — To a solution of 2,4-*O*-ethylidene-D-erythrose[2] (1.17 g) in absolute ethanol (15 ml) was added 1-methyl-1-phenylhydrazine (1.47 g), and the mixture was boiled for 4 h under reflux. Water (15 ml) was added to the hot reaction mixture, which, on cooling, solidified to a crystalline mass. Filtration of the product, followed by washing with a little cold 50% ethanol, gave the title compound, yield 1.76 g (86%), m.p. 152–153°, $[\alpha]_D^{20} -9.1°$ (*c* 2, absolute ethanol).

Anal. Calc. for $C_{13}H_{18}N_2O_3$: C, 62.4; H, 7.3; N, 11.2. Found: C, 62.3; H, 7.2; N, 11.3.

3-O-Benzyl-2,4-O-ethylidene-D-erythrose (2-methyl-2-phenyl)hydrazone. — To a solution of 2,4-*O*-ethylidene-D-erythrose (2-methyl-2-phenyl)hydrazone (5.36 g) in *N*,*N*-dimethylformamide (110 ml) was added benzyl bromide (40 ml). Silver oxide (40 g) was then added in small portions during 1 h, with vigorous stirring, and stirring was continued for 18 h. The mixture was diluted with several volumes of chloroform, and insoluble silver salts were removed by filtration. The filtrate was shaken with three 500-ml portions of water, evaporated to a small volume, and the unreacted benzyl bromide removed by steam distillation (100°). The aqueous suspension remaining was extracted with chloroform, and the extract was evaporated to a syrup which was dissolved in the minimum volume of boiling, 50% ethanol, and the solution cooled, affording the crystalline title compound; yield 6.8 g (93%), m.p. 107–108°, $[\alpha]_D^{20} -30.1°$ (*c* 2, benzene).

Anal. Calc. for $C_{20}H_{24}N_2O_3$: C, 70.6; H, 7.1; N, 8.2. Found: C, 70.4; H, 6.9; N, 8.1.

3-O-Benzyl-2,4-O-ethylidene-D-erythrose. — The following preparation utilizes optimal conditions for the hydrazone-cleavage reaction, based on recovery of unchanged hydrazone, which were deduced from preliminary experiments.

To a chilled, 35% aqueous solution of acetaldehyde (160 ml) in a 300-ml Parr* hydrogenation bomb were added glacial acetic acid (6 drops) and 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose (2-methyl-2-phenyl)hydrazone (1.00 g), and the mixture was agitated for 6 h at 80°, and then cooled in ice. The contents of the bomb were quantitatively transferred to a 1-l distilling flask with the aid of chloroform and water, and the volume of the aqueous layer was made up to 500 ml. Distillation was conducted at 50–55° (bath) under diminished pressure until all of the chloroform and acetaldehyde had been removed and approximately 200 ml of aqueous distillate had been collected. The latter contained all of the acetaldehyde (2-methyl-2-phenyl)hydrazone, a water-immiscible oil formed in the reaction. The mixture remaining in the flask was kept for several h, and the small amount of unreacted hydrazone that crystallized was separated by filtration. The clear filtrate was evaporated to a syrup (*ca.* 0.3–0.4 g). The combined syrupy product from five such experiments was distilled *in vacuo*, to give the title compound as a syrup, yield 1.39 g (40%), b.p. 135–145° (bath)/14 mm. After redistillation, the product had b.p. 130–132° (bath)/3 mm, n^{20} 1.516, and $[\alpha]_D^{20}$ − 26.2° (*c* 2, benzene).

Anal. Calc. for $C_{13}H_{16}O_4$: C, 66.1; H, 6.8. Found: C, 65.3; H, 7.1.

3-O-Benzyl-2,4-O-ethylidene-D-erythrose (2,4-dinitrophenyl)hydrazone. — To a solution of once-distilled 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose (0.10 g) in absolute ethanol (5 ml) was added (2,4-dinitrophenyl)hydrazine (0.82 g, 95% of 1 equivalent). The mixture was boiled for 4 h under reflux, and then water was added to the hot reaction mixture until it became turbid. As the mixture cooled, an oil separated; this slowly crystallized on refrigeration for several h. Recrystallization from aqueous ethanol, and then from hot heptane, gave the title compound as canary-yellow crystals, m.p. 127–128° (softening).

Anal. Calc. for $C_{19}H_{20}N_4O_7$: C, 54.8; H, 4.8; N, 13.5. Found: C, 55.1; H, 4.9; N, 13.7.

3-O-Benzyl-D-erythrose. — A solution of twice-distilled 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose (0.21 g) in 80% aqueous acetic acid (10 ml) was heated for 5 h at 85–90° (bath) and then evaporated to dryness, and residual acetic acid and moisture were removed by codistillation with benzene. The resultant syrup was dissolved in benzene, filtered to remove a small amount of D-erythrose resulting from debenzylation, and the filtrate evaporated, to give the title compound as a syrup, yield 0.184 g (99%), $[\alpha]_D^{20}$ −21.7° (*c* 1.8, benzene; no mutarotation).

Anal. Calc. for $C_{11}H_{14}O_4$: C, 62.8; H, 6.7. Found: C, 63.2; H, 6.9.

The ether is readily soluble in benzene, ethyl acetate, acetone, methanol, or chloroform; it is sparingly soluble in water and in heptane. It gives a strongly positive Schiff test and a weakly positive Fehling test, and it forms a silver mirror immediately with Tollens' ammoniacal silver nitrate reagent.

*Manufactured by the Parr Instrument Co., Moline, Illinois (U. S. A.). 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.

3-O-Benzyl-D-erythrose (p-nitrophenyl)hydrazone. — A solution of 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose (0.22 g) in 80% aqueous acetic acid (10 ml) was heated for 7 h at 80° (bath). The solvent was removed as previously described, and to the syrupy product was added glacial acetic acid (0.25 ml), water (3 ml), 2-methoxyethanol (3 ml), and (*p*-nitrophenyl)hydrazine (0.61 g, 4 equivalents). The mixture was heated for 2 h at 85° (bath), and allowed to cool, and the resulting oily suspension was extracted with chloroform. The extract was successively shaken with 1% hydrochloric acid and an aqueous suspension of barium carbonate, and evaporated to a syrup that was extracted with hot water to yield, on cooling, a yellow, crystalline product. Recrystallization from hot 25% aqueous ethanol gave the pure, crystalline, substituted hydrazone, m.p. 132–133° (softening).

Anal. Calc. for $C_{17}H_{19}N_3O_5$: C, 59.1; H, 5.6; N, 12.2. Found: C, 59.1; H, 5.4; N, 12.3.

3-O-Benzyl-D-erythrose (p-bromophenyl)osazone. — This derivative was prepared according to the general procedure recommended by Applegarth *et al.*[7] for preparing (*p*-bromophenyl)osazones. To syrupy 3-*O*-benzyl-D-erythrose (0.11 g) were added glacial acetic acid (3 ml), sodium acetate trihydrate (0.75 g), freshly recrystallized (*p*-bromophenyl)hydrazine hydrochloride (0.41 g), and water (2 ml). The mixture was heated for 10 min on a steam bath and allowed to cool. The resultant tarry suspension was extracted with chloroform, and the chloroform extract was washed successively with three 100-ml portions of 1% hydrochloric acid, water, and an aqueous suspension of barium carbonate. Evaporation of the chloroform solution gave a syrup; this was dissolved in a little absolute ethanol, and water was added to incipient turbidity, whereupon crystallization occurred, giving the (*p*-bromophenyl)osazone, m.p. 73–75° (softening).

Anal. Calc. for $C_{23}H_{23}Br_2N_4O_2$: C, 50.5; H, 4.2; Br, 29.2; N, 10.2. Found: C, 50.3; H, 4.2; Br, 28.9; N, 9.9.

ACKNOWLEDGMENT

We thank Mr. C. H. VanEtten and his associates for the microanalyses.

SUMMARY

3-*O*-Benzyl-D-erythrose has been synthesized from 2,4-*O*-ethylidene-D-erythrose in four steps. The (2-methyl-2-phenyl)hydrazone, readily obtained in 85-90% yield, was benzylated in *N*,*N*-dimethylformamide with benzyl bromide and silver oxide, to give a 90–95% yield of 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose (2-methyl-2-phenyl)-hydrazone. The methylphenylhydrazine residue was removed by heating the substituted hydrazone with aqueous acetaldehyde in an autoclave at 80°, to give 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose in 40% yield. Mild, acidic hydrolysis of the latter gave, in quantitative yield, syrupy 3-*O*-benzyl-D-erythrose, characterized as the (*p*-nitrophenyl)hydrazone and as the (*p*-bromophenyl)osazone.

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Carbohyd. Res., 4 (1967) 91–95

Notes

Migration of the *p*-nitrobenzoyl group. An attempted preparation of 1,3-di-*O*-*p*-nitrobenzoyl-D-erythritol

Esterification is commonly used for protecting hydroxyl groups in sugars and alditols. Occasionally, this method has been applied for elucidation of structural problems[1], although care must be exercised, because, as is well known, acyl groups, especially acetyl groups, can migrate under alkaline conditions. Controlled migration has been used in the synthesis of partially methylated sugars[2]. Instances of the migration of aroyl groups are, on the other hand, rather uncommon, although verified cases of the migration of the benzoyl group, even under acidic conditions, are to be found in the literature[3].

We have extended to the di-*p*-nitrobenzoates of erythritol the study of the migration of aroyl ester groups under acidic conditions. Reduction of 2,4-*O*-ethylidene-D-erythrose to the corresponding erythritol derivative[4], followed by esterification with *p*-nitrobenzoyl chloride in pyridine, gave optically active 2,4-*O*-ethylidene-1,3-di-*O*-*p*-nitrobenzoyl-D-erythritol. This compound, on mild hydrolysis with acid, yielded, not the anticipated 1,3-diester, but the known 1,4-di-*O*-*p*-nitrobenzoylerythritol[5].

EXPERIMENTAL

2,4-O-Ethylidene-1,3-di-O-p-nitrobenzoyl-D-erythritol. — To a solution of 2,4-*O*-ethylidene-D-erythritol[4] (0.22 g) in anhydrous pyridine (7 ml) was added *p*-nitrobenzoyl chloride (0.76 g), and the solution was heated for 30 min at 80–90° (bath), cooled, and poured portionwise, with stirring, into saturated aqueous sodium hydrogen carbonate. The oil that separated crystallized rapidly. The crude crystals were thoroughly washed with water, and recrystallized twice from chloroform–heptane to give the title compound, m.p. 153°, $[\alpha]_D^{20} -99°$ (*c* 2.5, chloroform).

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Anal. Calc. for $C_{20}H_{18}N_2O_{10}$: C, 53.8; H, 4.1; N, 6.3. Found: C, 53.9; H, 4.1; N, 6.3.

This ester (0.25 g) was dissolved in 80% acetic acid (20 ml) and the solution was boiled under reflux. The hydrolysis was followed by observing the optical rotation (1-dm tube): $[\alpha]_D^{20}$ −1.10 (initial) → −0.16° (8 h of refluxing). The solution was cooled and after having been kept for several h deposited a crop of crystals; recrystallization from benzene, and then from absolute ethanol, gave 1,4-di-*O*-*p*-nitrobenzoyl erythritol m.p. and mixed m.p.[5] 200-202°.

Anal. Calc. for $C_{18}H_{16}N_2O_{10}$: C, 51.4; H, 3.8; N, 6.7. Found: C, 51.6; H, 3.8; N, 6.5.

*Northern Regional Research Laboratory** J. W. VAN CLEVE
Peoria, Illinois 61604 (U. S. A.) C. E. RIST

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(Received September 1st, 1966; in revised form, November 7th, 1966)

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Action of dilute sulfuric acid on D-fructose

The modern literature records no study of the action on D-fructose of dilute sulfuric acid, a reagent often utilized for the hydrolysis of polysaccharides. In 1886, Conrad and Guthzeit[1] found that D-fructose is decomposed into levulinic acid, formic acid and humin by heating with 10% hydrochloric acid or 10% sulfuric acid. Wohl[2] discovered 5-(hydroxymethyl)-2-furaldehyde in the decomposition products formed when D-fructose is warmed with dilute hydrochloric acid.

The products obtained by prolonged refluxing of D-fructose with 10% sulfuric acid are now shown to be a brownish black, water-insoluble product (*ca.* 11.4%) that separates during the process of refluxing, and nonvolatile, water-soluble products (*ca.* 69%) from which levulinic acid (2,4-dinitrophenyl)hydrazone could be isolated.

Anal. Calc. for $C_{20}H_{18}N_2O_{10}$: C, 53.8; H, 4.1; N, 6.3. Found: C, 53.9; H, 4.1; N, 6.3.

This ester (0.25 g) was dissolved in 80% acetic acid (20 ml) and the solution was boiled under reflux. The hydrolysis was followed by observing the optical rotation (1-dm tube): $[\alpha]_D^{20}$ −1.10 (initial) → −0.16° (8 h of refluxing). The solution was cooled and after having been kept for several h deposited a crop of crystals; recrystallization from benzene, and then from absolute ethanol, gave 1,4-di-*O*-*p*-nitrobenzoyl erythritol m.p. and mixed m.p.[5] 200-202°.

Anal. Calc. for $C_{18}H_{16}N_2O_{10}$: C, 51.4; H, 3.8; N, 6.7. Found: C, 51.6; H, 3.8; N, 6.5.

*Northern Regional Research Laboratory** J. W. VAN CLEVE
Peoria, Illinois 61604 (U. S. A.) C. E. RIST

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The products obtained by prolonged refluxing of D-fructose with 10% sulfuric acid are now shown to be a brownish black, water-insoluble product (*ca.* 11.4%) that separates during the process of refluxing, and nonvolatile, water-soluble products (*ca.* 69%) from which levulinic acid (2,4-dinitrophenyl)hydrazone could be isolated.

The rest of the products (*ca.* 19.6%) were volatile and contained 5.6% of acetone, determined according to Messinger and Huppert[3] and identified by its i.r. spectrum and by conversion into its (2,4-dinitrophenyl)hydrazone, and 9% of carbon dioxide, as determined by precipitation as barium carbonate.

EXPERIMENTAL

A solution of D-fructose (10.0 g) in 500 ml of 10% sulfuric acid in a flask connected to a distilling apparatus was boiled over a direct flame, and the volume of the solution was kept nearly constant by dropwise addition of distilled water. A test of the distillate with barium hydroxide solution showed that carbon dioxide was present, and the iodoform test was positive until the elapse of 35 h, when it was negative (and the volume of the distillate was *ca.* 10 l). The mixture in the distilling flask (500 ml) was filtered, giving a brownish black, amorphous substance (1.14 g, 11.4%), insoluble in ether, but soluble in alkali. Half of the filtrate (250 ml) was treated with barium carbonate, to neutralize the sulfuric acid present, and the neutral solution was evaporated. The residue (3.45 g, 69%) was partially soluble in ether, and it gave a negative Seliwanoff test, and positive Fehling, ammoniacal silver nitrate, and (2,4-dinitrophenyl)hydrazine tests. The other half of the filtrate (250 ml) was treated with (2,4-dinitrophenyl)hydrazine reagent* affording a heavy, yellow precipitate that was filtered off, and fractionally recrystallized to give a yellow, crystalline material (50 mg), m. p. and mixed m. p. with levulinic acid (2,4-dinitrophenyl)hydrazone, 206°.

Identification of acetone. — The distillate gave positive Legal and iodoform tests. It was therefore treated with (2,4-dinitrophenyl)hydrazine reagent*. The yellowish red precipitate that separated (0.6 g) was filtered off after 24 h, and the filtrate was extracted several times with benzene. The extract was washed with water until neutral, and evaporated to dryness under diminished pressure at 50°; yield, 0.1 g. The yellowish red precipitate and the benzene extract were combined, diluted with benzene, and the solution chromatographed on aluminum oxide, with petroleum ether (b.p. 60–80°) as the mobile phase. Two main bands separated, the lower of which was eluted with petroleum ether to give a yellow, crystalline material. Recrystallization from ethanol gave acetone (2,4-dinitrophenyl)hydrazone (30 mg), m. p. and mixed m.p. 128°. Its i.r. spectrum was identical with that of an authentic sample. The upper band, eluted with benzene, gave an orange mixture that is still under investigation.

Determination of acetone. — A solution of D-fructose (1.0 g) in 30 ml of 10% sulfuric acid was treated as before for 35 h. The volume of the distillate collected was 500 ml. In two separate determinations, the acetone content of the total solution was 5.62 and 5.52%.

*Prepared by dissolving 0.25 g of (2,4-dinitrophenyl)hydrazine in 50 ml of water and 42 ml of concentrated hydrochloric acid, by warming on the water bath, cooling, and diluting the clear solution to 250 ml with distilled water.

Determination of carbon dioxide. — In a repetition of the preceding experiment, the distillate was trapped in 150 ml of 0.1N barium hydroxide, and air, free from carbon dioxide, was passed through the distilling apparatus for 35 h. The baryta solution, which had become turbid, was then filtered quickly. The precipitate was dried to constant weight (0.392 g). The content of carbon dioxide was found to be 8.7%.

ACKNOWLEDGMENT

The author thanks Professor H. El Khadem for his interest and for the infrared measurements.

Faculty of Science,
Alexandria University,
Alexandria (U.A.R.)

EL S. AMIN

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(Received October 11th, 1966; in revised form, November 14th, 1966)

Carbohyd. Res., 4 (1967) 96–98

Complexes of amylose with halogen-substituted hydrocarbons : diffraction results for wet precipitates

Diffraction diagrams of the dried precipitates obtained by treatment of aqueous solutions of amylose with *tert*-butyl alcohol[1] or isopropyl alcohol[2] can readily be indexed. A simple, pseudohexagonal unit exists, which can be accounted for by a close-packed arrangement of helices having seven D-glucose residues per helix turn[1]. The structure of the helix is similar to that found in "V" amylose, but expanded or enlarged by one D-glucose residue per helix turn. Powder patterns of wet precipitates formed from various branched-chain alcohol molecules with amylose are indistinguishable among themselves[3], and are considerably more detailed[2–4]. Optical examination by microscope has shown that crystals of amylose with certain halogen-substituted hydrocarbons exhibit the same crystal habit as do the amylose–branched-chain alcohol adducts[5,6]. The following X-ray examination of the addition product of amylose with several halogen-substituted hydrocarbons was undertaken to characterize these substances more fully. Diffraction spectra of complexes containing bromine and chlorine

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Faculty of Science,
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EL S. AMIN

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atoms are of interest, as the presence of heavy atoms in a crystalline lattice offers the possibility of altering X-ray diffraction intensities, and, thus, bringing out lines that might otherwise be masked by background on the film.

Amylose complexes were prepared with the following molecules: fluorobenzene, 1,1,2,2-tetrachloroethane, 1,1,2,2-tetrabromoethane, bromoform, and *tert*-butyl alcohol. An excess of halogen-substituted hydrocarbon was added, with the minimum agitation, to a solution (3% by wt.) of soluble starch (Merck's Lintner) in a test tube; to prepare the *tert*-BuOH complex, one part by vol. of alcohol was used to three parts of soluble starch solution. Reagents were preheated (75–90°), and the test tube containing the mixed system was placed in a Dewar flask containing some water at the same temperature. The Dewar flask was stoppered, and the mixture was allowed to cool for 18 to 30 h. The test tube was then centrifuged, and the liquid decanted. Precipitates were washed once with complexing agent, centrifuged, and pressed between filter paper (to remove excess liquid) before insertion into thin-walled, glass capillaries for X-ray examination. Photographs were taken with a powder camera having a 57.3-mm radius; Cu, Fe, and CrKα radiation were used for bringing out the maximum detail.

Table I records the results obtained from the best photographs, that is, those having the sharpest diffraction lines and the minimum of radiation background. The

TABLE I

OBSERVED d-SPACINGS, IN Å, FOR WET COMPLEXES OF AMYLOSE

Line intensity	*Complexing molecule*				
	tert-*Butyl alcohol*	*Fluorobenzene*	*1,1,2,2-Tetrachloroethane*	*1,1,2,2-Tetrabromoethane*	*Bromoform*
w	3.55	3.54			
vw	3.65				
vw	3.86	3.86			
m	4.51	4.51	4.50	4.51	4.51
m	4.70	4.69	4.69	4.70	4.71
s	4.98[a]	4.91	4.98	4.96	4.97[a]
s		5.05	5.08	5.08	
w	5.32	5.32	5.32	5.30	
m	5.87	5.89	5.90	5.88	5.85
m	6.85	6.9[a]	6.9[a]	6.9[a]	6.9[a]
m	7.25				
w	8.10[a]				
m	9.20[a]	9.10[a]	9.23[a]		
m	10.3	10.2	10.3		
m	12.8		12.8	12.8	12.9
m				14.2	14.3
m				14.5	
vw			16	16	
w	19			19	

[a]Broad line.

powder lines broadened and shifted position if precipitates were exposed to the atmosphere; the precipitates did not pack compactly in the capillary tubes when excess mother liquor or complexing agent was present, and background then masked the coherent scattering on the films.

For the five precipitates, the correlation in intensity values and d-spacings of less than 7.0 Å is good; in addition, diffraction halos, which were too broad to be measured precisely, also matched each other. It follows, then, that the conformation and the packing arrangement of amylose complexed with the halogen-substituted hydrocarbons is essentially the same as the conformation and packing of the amylose helices that are found in the *tert*-butyl alcohol complex[1]. Three new diffraction maxima were observed for the complexes with the halogen-substituted hydrocarbons, at d-values of 14.2 Å, 14.5 Å, and 16 Å. These powder lines may be interpreted as arising from the scattering of halogen atoms; the presence of new diffraction maxima indicates that the complexing molecules are located at specific sites, instead of at random sites, inside the amylose helix.

Recognizing that changes in crystal structure necessarily occur with drying, we designed the method of sample preparation so as to obtain wet complexes that had undergone the minimum amount of dehydration. It was noted that the resolved doublet having d-spacings of 14.2 Å and 14.5 Å merged to a single, powder line at 14.3 Å when the 1,1,2,2-tetrabromoethane adduct was allowed to stand in air. The presence of the doublet requires that the unit cell for the complex, as originally formed while in contact with the mother liquor, be exceedingly large; possibly, one of the cell dimensions is as long as 60 Å. Neither the size of the unit found by Yamashita and Hirai[2], based on an electron-diffraction diagram of wet isopropyl alcohol–amylose, nor the cell proposed by Senti[4] from an analysis of fiber patterns of the moist amylose–*tert*-butyl alcohol complex, accounts for this observed doublet. The former cell shows pmm Laue symmetry for the {hk0} net, where a = 30.0 Å and b = 28.3 Å; and fiber data from *tert*-butyl alcohol–amylose were indexed in terms of monoclinic cell parameters of a = 33.7 Å, b = 7.92 Å (fiber axis), c = 19.4 Å, and $\beta = 130°$. The diverse results of the different diffraction studies of wet precipitates indicate that complicated changes in molecular packing accompany dehydration.

The molecular volume, on a per helix basis, calculated for a cell[2] having a = 30.0 Å, and b = 28.3 Å, with c, the fiber axis, assumed to be 7.92 Å, is 10% greater than the molecular volume for the partially dried, hexagonal product[1], where a = b = 14.9 Å. By contrast, the monoclinic cell reported for moist fibers of *tert*-butyl alcohol–amylose has a molecular volume that is 30% greater, on a per helix basis, than that of the hexagonal product[4]. Whereas the hexagonal dimensions of the partially dried precipitate can best be explained in terms of a 7_1 helix[1], the dimensions of the monoclinic cell imply that there are eight D-glucose residues per helix turn in the moist amylose species[4]. If this conclusion is correct, the sizable contraction in molecular volume that occurs during dehydration is a consequence, in part, of a diminution in the number of D-glucose residues, per helix turn in the carbohydrate structure, from eight to seven.

Support of this work by a grant from the Corn Industries Research Foundation, Inc., is gratefully acknowledged.

Department of Chemistry,
Arizona State University,
Tempe, Arizona 85281 (U. S. A.)

R. R. BUMB
B. ZASLOW

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(Received October 18th, 1966)

Synthesis of substituted phenyl β-D-xylopyranosides

In the present work, we have prepared a further[1] series (Table I) of tri-*O*-acetyl-β-D-xylopyranosides of substituted phenols. Catalytic deacetylation (sodium or barium methoxide)[2] yielded the corresponding β-D-xylopyranosides (Table II).

EXPERIMENTAL

All acetates were crystallized from ethanol or methanol. The purity of the products was tested by t.l.c. on Silica gel G (Merck) with acetic acid–water–ethyl acetate (1:1:3) for xylosides, and ethyl acetate–benzene (3:7) for the acetates; spray reagent, 5% sulfuric acid in ethanol (10 min at 120°).

TABLE I

ACETYLATED β-D-XYLOPYRANOSIDES

Aglycone group	*M.p.* (°)	$[\alpha]_D^{22}$, (°) (c 2, *chloroform*)	*Yield*, %	*Found*, %		*Formula*	*Calc.*, %		*Method*
				C	*H*		*C*	*H*	
2,3-Dimethylphenyl	118–119	−55.8	47	60.0	6.3				1
2,6-Dimethylphenyl	119–120	−55.6	32	59.8	6.4				1
2,4-Dimethylphenyl	110–111	−47.9	25	60.0	6.4	$C_{19}H_{24}O_8$	59.9	6.3	1
3,4-Dimethylphenyl	123–124	−42.2	60	59.9	6.4				1
3,5-Dimethylphenyl	134–135	−44.4	61	59.9	6.3				1
2,3,5-Trimethylphenyl	130–131	−47.2	46	60.8	6.6	$C_{20}H_{26}O_8$	60.9	6.6	1
3,4-Dichlorophenyl	136–137	−50.2	14	48.5	4.4				1
2,5-Dichlorophenyl	105–107	−58.2	14	48.4	4.3	$C_{17}H_{18}Cl_2O_8$	48.5	4.3	2
2,3-Dichlorophenyl	133–134	−84.4	27	48.5	4.4				2
2,4-Dichlorophenyl	107–108	−72.1	24	48.5	4.5				2
p-Bromophenyl	136–137	−44.3	51	47.3	4.5				1
m-Bromophenyl	108–109	−46.4	16	47.4	4.9	$C_{17}H_{19}BrO_8$	47.3	4.4	1
2,4-Dibromophenyl	136–137	−65.6	26	40.0	3.6	$C_{17}H_{18}Br_2O_8$	40.0	3.5	2
o-Aminophenyl	156–157	−48.9	75	55.7	5.8				3
p-Aminophenyl	154–155	−41.9[a]	70	55.4	5.8	$C_{17}H_{21}NO_8$	55.6	5.7	3
o-Acetamidophenyl	148–149	−49.8	70	55.6	5.6				4
p-Acetamidophenyl	173–174	−41.6	80	55.4	5.3	$C_{19}H_{23}NO_9$	55.7	5.6	4
m-Acetamidophenyl	160–161	−47.1	80	55.4	5.6				4
α-Naphthyl	159–160	−92.3[b]	35	62.6	5.6	$C_{12}H_{12}O_8$	62.7	5.5	1

[a] *c* 1; [b] *c* 0.7.

Method 1. — β-D-Xylose tetraacetate[3] (0.1 mole) and the appropriate phenol (0.4 mole) were fused together, and the melt was treated with a solution of toluene-*p*-sulphonic acid (0.6 g) in acetic acid–acetic anhydride (95:5; 50 ml). The mixture was heated under diminished pressure at 100° for 1 h. After cooling, the resulting syrup was dissolved in chloroform (200 ml), thoroughly washed with ice-cold N sodium hydroxide and water, dried (Na_2SO_4), and evaporated *in vacuo*.

TABLE II

β-D-XYLOPYRANOSIDES

Aglycone group	*M.p.* (°)	*Solvent crystallisation*[a]	$[\alpha]_D^{22}$, (°) (c 2, *methanol*)	*Yield*, (%)	*Found*, % *C*	*H*	*Formula*	*Calc.*, % *C*	*H*
2,3-Dimethylphenyl	160–161	a	−45.4	95	61.2	7.2			
2,6-Dimethylphenyl	145–146	b	−11.1	91	61.0	7.0			
2,4-Dimethylphenyl	127–128	b	−37.9	98	61.2	7.0	$C_{13}H_{18}O_5$	61.4	7.1
3,4-Dimethylphenyl	164–165	b	−38.0	73	61.2	7.2			
3,5-Dimethylphenyl	112–113	c	−40.6	75	61.3	7.2			
2,3,5-Trimethylphenyl	182–183	b	−79.4[b]	80	62.6	7.6	$C_{14}H_{20}O_5$	62.7	7.4
3,4-Dichlorophenyl	156–157	b	−44.0	73	44.7	4.2			
2,5-Dichlorophenyl	180–181	b	−52.4	72	44.8	4.3			
2,3-Dichlorophenyl	191–192	d	−57.1	83	44.8	4.3	$C_{11}H_{12}Cl_2O_5$	44.7	4.1
2,4-Dichlorophenyl	177–178	b	−49.5	83	44.5	4.2			
p-Bromophenyl	168–169	g	−31.9	45	43.3	4.5			
m-Bromophenyl	150–151	b	−37.8[a]	82	43.2	4.8	$C_{11}H_{13}BrO_5$	43.3	4.3
2,4-Dibromophenyl	195–196	d	−40.7	91	34.4	3.2	$C_{11}H_{12}Br_2O_5$	34.4	3.1
o-Aminophenyl	173–174	e	−68.7	70	54.6	6.1			
p-Aminophenyl	192–193	e	−41.7[c](water)	70	54.7	6.2	$C_{11}H_{15}NO_5$	54.8	6.2
o-Acetamidophenyl	157–158	e	−64.4	61	55.1	6.2			
p-Acetamidophenyl	174–175	f	−32.0[c](water)	62	55.2	6.1	$C_{13}H_{17}NO_6$	55.1	6.0
m-Acetamidophenyl	184–185	e	−39.1[c]	74	55.2	6.2			
α-Naphthyl	160–161	b	−81.7[a]	91	65.0	6.0	$C_{15}H_{16}O_5$	65.2	5.8

[a]a, ethyl acetate; b, water; c, ethanol–water; d, methanol–water; e, ethanol; f, methanol; g, butanone; [b]*c* 0.4; [c]*c* 1; [a]*c* 1.5.

Method 2. — To a solution of the phenol (0.1 mole) in acetone (200 ml), potassium hydroxide (0.1 mole) in methanol (100 ml), and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[4] (0.1 mole) were added. After 5 h at room temperature, the mixture was filtered and evaporated *in vacuo*. The residue was dissolved in chloroform (200 ml), washed with ice-cold N sodium hydroxide and water, dried (Na_2SO_4), and evaporated *in vacuo*.

Method 3. — Nitrophenyl tri-*O*-acetyl-β-D-xylopyranoside (10 g), suspended in methanol (300 ml), was hydrogenated[5] at atmospheric pressure over 10% palladium–barium sulfate (0.5 g). When hydrogen uptake had ceased, the solution was filtered and evaporated *in vacuo*, and the product was crystallised from ethanol.

Method 4. — Aminophenyl tri-*O*-acetyl-β-D-xylopyranoside (1 g) was kept for 12 h at room temperature in a mixture of pyridine (5 ml) and acetic anhydride (5 ml), and then poured into ice-water. The resulting solid was filtered off, thoroughly washed with water, and crystallised from ethanol.

ACKNOWLEDGMENT

We thank Prof. Dr. L. Massart for his interest in this work, and Miss J. De Lat for carrying out the microanalyses.

H.I.K.W., Lab. Anorg. Scheikunde A,
Ghent State University,
Ghent (Belgium)

C. K. DE BRUYNE
F. VAN WIJNENDAELE

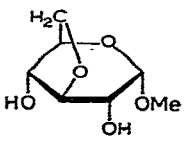
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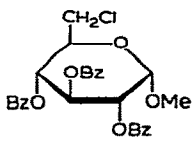
(Received September 16th, 1966)

Action of boron trichloride on methyl 3,6-anhydro-α-D-glucopyranoside and the furanoid analogue

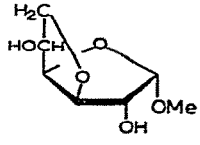
The tetrahydrofuran rings in 1,4:3,6-dianhydro-D-glucitol are cleaved under mild conditions with boron trichloride to give 1,6-dichloro-1,6-dideoxy-D-glucitol[1], and the possibility arises of using such rings as novel blocking groups in carbohydrate syntheses. In this connection, we have examined the action of the reagent on methyl 3,6-anhydro-α-D-glucopyranoside (**1**). Since anhydride **1** contains the sterically strained, bicyclo[3,2,1]octane ring-system {rearrangement into methyl 3,6-anhydro-α-D-glucofuranoside (**3**), having the bicyclo[3,3,0]octane ring-system, is caused[2] by traces of acid}, it was thought that a greater reactivity towards boron trichloride might be shown than by molecules containing less-strained tetrahydrofuran rings.



1



2



3

When a mixture of methyl 3,6-anhydro-α-D-glucopyranoside (**1**), dichloromethane, and boron trichloride was constituted at *ca.* −80° and then allowed to attain room temperature slowly (conditions under which methyl α-D-glucopyranoside is largely unaffected), several products were formed (t.l.c.), but little or no starting material survived. Benzoylation of the product mixture, followed by chromatography on alumina, gave methyl 2,3,4-tri-*O*-benzoyl-6-chloro-6-deoxy-α-D-glucopyranoside (**2**) as the main, crystalline product, together with a small proportion of a product *A* (m.p. 151–153°, $[\alpha]_D$ −6° in chloroform) isomeric with compound **2**. Because of the magnitude of the optical rotation, compound *A* probably has the β-D configuration but no evidence of ring size was obtained. The main component of the benzoate mixture was amorphous and methoxyl-free, and contained chlorine consistent with the presence of a substantial proportion of tetrabenzoylchlorodeoxyhexose.

Authentic compound **2** was prepared by treatment of methyl 6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside with lithium chloride in boiling *N*,*N*-dimethylformamide, followed by benzoylation.

Thus, it may be concluded that the anhydro ring in methyl 3,6-anhydro-α-D-glucopyranoside (**1**) is extensively cleaved by boron trichloride, and that considerable loss of the glycosidic methyl group occurs (*cf.* the results of Bonner *et al.*[3]).

For purposes of comparison, the action of boron trichloride on methyl 3,6-anhydro-α-D-glucofuranoside (**3**) was examined, and a more complex situation was encountered. Treatment with the reagent, followed by benzoylation, as for the pyranoside gave a mixture of esters which, by a combination of chromatography and frac-

tional crystallisation, gave methyl 3,6-anhydro-2,5-di-*O*-benzoyl-α- and -β-D-glucofuranoside. Additionally, a product identical with compound *A* described above was obtained. A substantial proportion of methoxyl-containing material could not be identified, and, as in the pyranoside reaction, a considerable proportion of chlorine-containing, methoxyl-free material was formed. Clearly, in the case of the methyl 3,6-anhydro-α-D-glucofuranoside–boron trichloride reaction, the cleavage of the anhydro-ring is not complete, and it is likely that the Lewis acid also catalyses anomerisation, although the intermediate formation of a glycosyl chloride, and its subsequent reaction with methanol during the isolation procedure (see Experimental), cannot be excluded.

The synthetic utility of the cleavage of tetrahydrofuran rings by boron trichloride is being further examined.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was performed on Kieselgel with detection by iodine vapour or vanillin–sulphuric acid[4]. Gas–liquid chromatograms (g.l.c.) were obtained with a Pye Argon instrument with β-ionization detection and a stationary phase of poly(ethylene glycol adipate). N.m.r. spectra were obtained with a Varian A-60 spectrometer by using *ca.* 20% solutions, with a 6% solution of tetramethylsilane in chloroform as external reference. Optical rotations were determined in chloroform, unless otherwise stated.

Action of boron trichloride on methyl 3,6-anhydro-α-D-glucopyranoside (**1**). — A mixture of the title compound[2] (3 g) and dichloromethane (25 ml) at *ca.* −80° was treated with boron trichloride (5 g) and then stored at room temperature for 15 h. Volatile material was removed under diminished pressure, and the residual borate esters were decomposed with methanol[1] to give a hygroscopic glass (3.01 g), $[\alpha]_D$ +60° (*c* 1.2, water), R_F 0.95, 0.61, 0.51 (main component), 0.32, 0.19, and 0.00 (t.l.c., ethyl acetate–methanol, 19:1); the starting material had R_F 0.67. A second treatment with boron trichloride apparently caused no further reaction. After trimethylsilylation[5] of the mixture, examination by g.l.c. at 140° revealed components having retention times of 23, 25, 61, and 73 min.

The foregoing mixture (2.45 g) was decolourised with charcoal, and a portion (1.5 g) of the product (2.19 g) was esterified with benzoyl chloride (3 ml) and pyridine (10 ml) to give a syrup (3.87 g) that was fractionated on a column (34 × 2 cm) of alumina as follows: 1 (benzene, 125 ml), unidentified syrup (20 mg); 2 (benzene, 125 ml), solid (1.04 g), which, on recrystallisation from ethanol, gave methyl 2,3,4-tri-*O*-benzoyl-6-chloro-6-deoxy-α-D-glucopyranoside (0.71 g), m.p. 136–138°, $[\alpha]_D$ +53° (*c* 0.5) (Found: C, 63.6; H, 4.7; Cl, 7.1. $C_{28}H_{25}ClO_8$ calc.: C, 64.1; H, 4.7; Cl, 6.8%); 3 (benzene, 25 ml), syrup (40 mg); 4 (benzene, 100 ml), solid (0.236 g), which, after two recrystallisations from ethanol, gave compound *A* (90 mg), m.p. 151–153°, $[\alpha]_D$ −6° (*c* 0.5), τ 6.30 (chloroform, methoxyl protons) (Found: C, 64.1; H, 4.9; Cl. 7.1%); 5 (benzene, 40 ml; benzene–ether, 9:1, 75 ml; benzene–ether, 1:1, 525 ml), semi-

crystalline material (1.48 g), $[\alpha]_D$ + 51° (*c* 0.7), methoxyl-free (n.m.r.) (Found: Cl, 6.7. $C_{34}H_{27}ClO_9$ calc.: 5.8%).

Methyl 2,3,4-tri-O-benzoyl-6-chloro-6-deoxy-α-D-glucopyranoside (**2**).—A solution of methyl 6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside[2] (7 g) in *N*,*N*-dimethylformamide (50 ml) containing anhydrous lithium chloride (2.6 g) was boiled under reflux, for 1 h. The cooled mixture was poured into aqueous sodium hydrogen carbonate and the solution was evaporated to dryness under diminished temperature and pressure. The residue was extracted with boiling ethyl acetate (500 ml). Decolourisation (charcoal) and concentration of the extract, with crystallisation of the residue (3.2 g) from methanol, yielded methyl 6-chloro-6-deoxy-α-D-glucopyranoside (1.47 g, 55%), m.p. 114–115°, $[\alpha]_D$ + 151° (*c* 0.3, methanol); lit.[6], m.p. 110–112° for the compound prepared by a different method.

Esterification of the foregoing product (1.18 g) with benzoyl chloride (2 ml) and pyridine (5 ml) gave compound **2** (1.1 g), m.p. 136–138° (from ethanol) alone or in admixture with the product from fraction 2 described above, $[\alpha]_D$ + 53° (*c* 0.5).

Action of boron trichloride on methyl 3,6-anhydro-α-D-glucofuranoside (**3**). — A mixture of the title compound[2] (1.64 g) and dichloromethane (30 ml) was treated with boron trichloride (10 ml), as for the pyranoside, to afford a syrup (1.77 g), $[\alpha]_D$ +38° (*c* 0.7, water), R_F 0.90, 0.86 (main component), 0.36, and 0.33–0.00 (streak) (t.l.c., ethyl acetate–methanol, 9:1), component retention times 23, 25, 62, and 73 min (g.l.c., after trimethylsilylation, 140°). Esterification with benzoyl chloride (3 ml) and pyridine (10 ml) gave a syrup (3.8 g), $[\alpha]_D$ + 37° (*c* 2.3), a portion (3.67 g) of which was fractionated on a column (33 × 2.5 cm) of alumina as follows (t.l.c. of individual fractions with carbon tetrachloride–ether, 9:1*, indicates main components): 1 (benzene, 100 ml), syrup (40 mg), R_F 0.98; 2 (benzene, 50 ml), syrup *A* (0.78 g), R_F 0.58*, 0.42*, and 0.35; 3 (benzene, 300 ml), syrup *B* (1.9 g), $[\alpha]_D$ + 55° (*c* 1.0), R_F 0.58, 0.38*, and 0.19, τ 6.85 and 6.70 (carbon tetrachloride, methoxyl protons); 4 (benzene, 200 ml, benzene–ether, 9:1, 150 ml), syrup (95 mg), R_F 0.38*, 0.19, and 0.12; 5 (benzene–ether, 9:1, 600 ml), glass *C* (0.72 g), $[\alpha]_D$ + 60° (c 1.0), R_F 0.12 and 0.07, methoxyl-free (n.m.r.) (Found: Cl, 3.7%). The infrared spectrum of glass *C* was similar to that of the corresponding methoxyl-free product from the pyranoside reaction.

Syrup *A* (0.78 g) was refractionated on a column (14 × 2.5 cm) of alumina by elution with benzene as follows: 1 (180 ml), syrup (0.43 g), R_F 0.58 and 0.42; 2 (60 ml), solid (41 mg), m.p. 136°, R_F 0.42, which, on recrystallisation from ethanol, gave compound *A*, m.p. 147–149°, identical (comparison of infrared spectra) with the minor, crystalline product isolated from the pyranoside reaction; 3 (250 ml), syrup (91 mg), R_F 0.42 and 0.35, $[\alpha]_D$ *ca.* − 40°.

Syrup *B* could be distilled, apparently without change, and the product (1.36 g, b.p. 194°/0.1 mm), on fractional crystallisation from ethanol, gave, as a first crop, methyl 3,6-anhydro-2,5-di-*O*-benzoyl-β-D-glucofuranoside (205 mg), m.p. 97–99°, $[\alpha]_D$ + 4° (*c* 3.0); lit.[7], m.p. 99° (Found: C, 66.1; H, 5.6. $C_{21}H_{20}O_7$ calc.: C, 65.6; H, 5.2%). The second crop (252 mg) had m.p. 74–84° and, after two recrystallisations from ethanol, gave methyl 3,6-anhydro-2,5-di-*O*-benzoyl-α-D-glucofuranoside, m.p.

83–88°, $[\alpha]_D$ + 108° (*c* 1.3), identical with the product obtained by benzoylation of methyl 3,6-anhydro-α-D-glucofuranoside (Found: C, 65.5; H, 5.2. $C_{21}H_{20}O_7$ calc.: C, 65.6; H, 5.2%).

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey, F.R.S., for his interest. This research was supported by a grant (DA-91-591-EUC-3530) from the United States Department of the Army.

Chemistry Department,
The University,
Birmingham (Great Britain)

M. A. BUKHARI
A. B. FOSTER*
J. M. WEBBER

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(Received October 7th, 1966)

*Present Address: Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London S. W. 3, Great Britain.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

METHYLATION STUDIES ON AMYLOSE POLYALCOHOL*

A. MISAKI** AND F. SMITH***

Department of Biochemistry, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota 55101 (U.S.A.)

(Received September 24th, 1966; in revised form, November 30th, 1966)

INTRODUCTION

It is generally accepted that amylose, the minor component of starch, is an essentially linear polysaccharide consisting of 1,000 or more (1→4)-linked D-glucose residues. However, there has been some dispute as to the presence of a few branches in the molecule of amylose[1]. Although direct chemical evidence has been lacking, the recent study by Kjølberg and Manners[2] has revealed that the anomalous linkages that prevent the complete degradation of amylose by β-amylase are probably α-D-(1→6)-glucosidic, inter-chain linkages, since the action of yeast isoamylase caused a significant increase in the β-amylolysis limit.

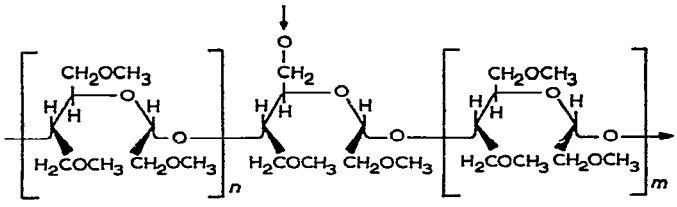
In previous communications[3,4], it was shown that the polyalcohols, derived from such polysaccharides as amylopectin and cellulose by periodate oxidation followed by reduction, are methylated readily. Analysis of the cleavage fragments derived from these methylated polyalcohols provides useful information about the structural features of the polysaccharides. In the present study, therefore, this technique has been applied to structural studies on corn and potato amylose, with the objective of obtaining evidence concerning the presence of (1→6)-linked branches in the amylose molecule.

RESULTS AND DISCUSSION

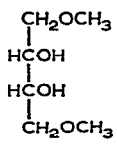
The amylose prepared either from corn or potato starch was highly purified by repeated fractionations with different reagents. It was subjected to prolonged periodate oxidation, and the "polyaldehyde" formed was reduced with sodium borohydride to the corresponding polyalcohol. Hydrolysis of the water-soluble, amylose polyalcohol yielded erythritol. The absence of D-glucose from the hydrolyzate revealed that no (1→3)-like linkages are present in the amylose molecule. Treatment of the amylose polyalcohol with methyl sulfate and sodium hydroxide, and then with methyl iodide and silver oxide in the presence of *N,N*-dimethylformamide, afforded the fully methylated amylose polyalcohol (**1**). Methanolysis

*Paper No. 6077, Scientific Journal Series, Minnesota Agricultural Experiment Station.
**Present address: The Microbiological Research Laboratory, Research and Development Division, Takeda Chemical Industry, Ltd., Osaka, Japan.
***Deceased.

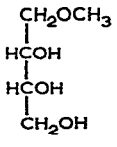
of **1** with 3% methanolic hydrogen chloride gave 1,4-di-*O*-methylerythritol (**2**) and a small proportion of a 1-*O*-methylerythritol (**3**), which were readily separated by paper chromatography. The 1,4-di-*O*-methylerythritol, which must have arisen from the (1→4)-linked D-glucose residues, was characterized as the crystalline 2,3-di-*O*-*p*-tolylsulfonyl derivative.



1



2



3

The isolation of a 1-*O*-methylerythritol (identified as its 2,3,4-tris-*p*-nitrobenzoate) may be significant in connection with the structure of amylose. If the amylose chain is branched by α-D-(1→6)-glucosidic linkages, hydrolysis of **1** will afford the optically active 1-*O*-methyl-D-erythritol (**3**), which arises from the branching D-glucose residues joined through C-1 ,C-4, and C-6, as revealed in the methylation study of amylopectin polyalcohol[3]. However, 1,4-di-*O*-methylerythritol (**2**) tends to undergo demethylation during methanolysis[3]. If it does, the resulting mono-*O*-methylerythritol will be a racemic form and be optically inactive, as shown in the previous study[4], since the methoxyl groups at C-1 and C-4 of **2** are sterically equivalent. A careful examination of the optical rotation of the tris-*p*-nitrobenzoate indicated that approximately 10% of the 1-*O*-methylerythritol, isolated either from the methylated corn or potato amylose polyalcohol, must have originated from α-D-(1→6)-linked, D-glucosidic interchain linkages, and 90% was probably formed by demethylation of 1,4-di-*O*-methylerythritol during hydrolysis.

It is possible to estimate the degree of branching in the parent amylose from the yield of **3**. Thus, the molar proportion of **3** derived from the methylated polyalcohol indicates that one (1→6)-linked branch occurs for approximately every 300 D-glucose residues in corn amylose and for every 400 D-glucose residues in potato amylose. The molar ratio of **2** to **3** also provides an estimate of the branching in amylose. However, this ratio may not be reliable, because, in the present study, the recovery of **2** was relatively low, owing to its high volatility[3].

The possibility that such branches in amylose may arise from contaminating amylopectin can be ruled out, since the degree of branching found in these highly purified amylose preparations would represent a 6% (potato) or 8% (corn) con-

tamination by amylopectin, assuming that the average chainlength of amylopectin is approximately 25. These observations support the view that the amylose molecule is an essentially linear chain having two or three (1→6)-linked branch points per molecule. It is of interest that the recent study by Greenwood[5] on the physical properties of amylose in solution suggests the occurrence of long-chain branching in some fractions of amylose.

EXPERIMENTAL

General. — All evaporations were conducted under diminished pressure at 35–40°, unless otherwise stated. Paper chromatography was performed by the descending method on Whatman No. 1 or 3 MM paper with the following solvent systems: (A) 4:1:5 butyl alcohol–ethanol–water and (B) butanone–water azeotrope. The chromatograms were sprayed with *p*-anisidine hydrochloride (for reducing sugars) or with ammoniacal silver nitrate.

Amylose preparation. — Corn starch (Matheson, Coleman and Bell) or potato starch (Mallinckrodt) was defatted by refluxing twice with methanol for 2 h. Amylose was prepared by fractionation of the defatted starch with thymol, in a nitrogen atmosphere, according to Street and Close[6]. In order to remove the last traces of amylopectin, the amylose preparation was further fractionated with thymol (twice) and Pentasol (thrice)[7], and was finally crystallized with 1-butanol (twice)[8]; the yield of purified amylose from corn starch and potato starch was 7.2% and 7.6%, respectively. The iodine-affinity of the corn and potato amylose was 18.9 and 19.0 mg of iodine per 100 mg of amylose, respectively, as measured by amperometric titration[9].

Periodate oxidation of amylose, and reduction of the product with sodium borohydride. — Amylose (2.5 g) from corn starch or potato starch was suspended in water (125 ml) to which 0.2N periodic acid (125 ml) was added, and oxidation was conducted in the dark at 5°, with continuous stirring. Aliquots were taken at suitable intervals, and the periodate consumption was determined by the arsenite method[10]. After 20 days (periodate consumption: 1.04 moles and 1.07 moles per mole of D-glucose residues of corn and potato amylose, respectively), the insoluble, amylose "polyaldehyde" (2.3 g) was separated by centrifugation, and washed with ice-cold water until all traces of periodate had been removed. The "polyaldehyde" so obtained was suspended in water (200 ml), and a solution of sodium borohydride (1 g in 10 ml of water) was added with stirring. The reaction mixture was kept overnight at 25°; additional borohydride (0.2 g) was then added, and the solution was kept for a further 5 h. The alkaline solution was neutralized (pH 7.0) in the cold, and the amylose polyalcohol was precipitated with ethanol, to give a white, gummy material.

A portion (75 mg) of corn or potato-amylose polyalcohol was hydrolyzed with N sulfuric acid (2 ml) for 1.5 h at 100°. The hydrolyzate was neutralized (barium carbonate) and filtered, and the filtrate was evaporated to a syrup (43 mg). Paper-chromatographic analysis (solvent A) revealed the presence of erythritol only, which was identified by conversion into tetra-*O*-*p*-tolylsulfonylerythritol in the

manner described previously[11], m.p. and mixed m.p. 165–166°. Neither glucose nor glycerol was detected on the chromatogram.

Methylation of amylose polyalcohol. — The corn-amylose polyalcohol (2 g), dissolved in 30% sodium hydroxide (20 ml), was methylated by dropwise addition of 30% sodium hydroxide (200 ml) and methyl sulfate (75 ml), with vigorous stirring during 3 h at 55°. Acetone was added periodically, to solubilize the partially methylated product. After the excess methyl sulfate had been destroyed by heating for 1 h at 90°, the methylated polyalcohol was extracted from the cooled reaction mixture with chloroform (3 × 300 ml). The combined extracts were washed with water, dried (magnesium sulfate), and evaporated to a syrup (1.87 g). In order to cleave any borate complexes and to facilitate complete methylation, the first methylation product was acetylated with pyridine (8 ml) and acetic anhydride (5 ml) at room temperature. After being kept overnight, the reaction mixture was poured into ice-cold water (500 ml), and the acetylated product was extracted with four 300-ml portions of chloroform. The extract was washed with water, and evaporated to a syrup (1.90 g), which was methylated in acetone solution with methyl sulfate and 30% sodium hydroxide as before. After isolation of the methylated product as before, it was treated with methyl iodide (15 ml) and silver oxide (10 g) in *N,N*-dimethylformamide (50 ml) according to Kuhn[12]. The reaction mixture was shaken for 24 h at room temperature and, after removal of salts by centrifugation, another portion of silver oxide (10 g) was added and the mixture was shaken for 24 h. The reaction mixture was extracted with chloroform (150 ml), and the extract washed with 5% potassium cyanide, and water. The extract was dried (magnesium sulfate), and concentrated to a syrup, yield 1.3 g (Found: OMe, 44.30%).

Potato-amylose polyalcohol (2.2 g) was methylated in the same way, yielding the corresponding methylated polyalcohol, yield 1.27 g (Found: OMe, 44.42%).

Methanolysis of the methylated amylose polyalcohol and identification of 1,4-di-O-methylerythritol and 1-O-methylerythritol. — A portion of the respective methylated amylose polyalcohol (100 mg) was treated with 3% methanolic hydrogen chloride (3 ml) in a sealed tube at 100°. The solution was neutralized (silver carbonate) and filtered, and the filtrate was concentrated to a syrup by an air current. Paper-chromatographic analysis (solvent B) showed that, in the methanolyzates from corn and potato amylose, two components were present, one corresponding to 1,4-di-*O*-methylerythritol (R_F 0.55; major) and the other (trace) to 1-*O*-methylerythritol (R_F 0.25). Methoxyacetaldehyde[3], which had arisen from C-1 and C-2 of all of the building units in the methylated amylose-polyalcohol, was lost during the concentration process.

In another experiment, the corn-amylose polyalcohol (195.0 mg) was methanolyzed with 3% methanolic hydrogen chloride (5 ml) in the same way. After evaporation of the neutralized reaction mixture, the syrupy residue (consisting of 1,4-di-*O*- and 1-*O*-methylerythritol) was subjected to chromatographic separation (solvent B) on Whatman 3 MM paper. The zone of each component was extracted with 50% aqueous methanol (50 ml), and the extract was evaporated to a syrup, which was then purified

by extraction with the minimum volume of 1:1 ether–methanol. This procedure yielded 1,4-di-*O*-methylerythritol (64.0 mg) and 1-*O*-methylerythritol (5.6 mg); the low yield of 1,4-di-*O*-methylerythritol may be due to its volatility[3,4].

Treatment of the syrupy 1,4-di-*O*-methylerythritol (37 mg) with *p*-toluenesulfonyl chloride (150 mg) in dry pyridine (3 ml) in the usual way[3] afforded crystalline 1,4-di-*O*-methyl-2,3-di-*O*-*p*-tolylsulfonylerythritol (55 mg), m.p. and mixed[3] m.p. 140° (after recrystallization from ethanol).

1-*O*-Methylerythritol (5.6 mg) was heated with *p*-nitrobenzoyl chloride (50 mg) in dry pyridine for 1 h at 80°, to give a 1-*O*-methyl-2,3,4-tri-*O*-(*p*-nitrobenzoyl)-erythritol (15.5 mg) as white crystals, m.p.[3,4] and mixed m.p. 162.5–164°, $[\alpha]_D^{25}$ +9.03° (*c* 3.1, chloroform). An authentic specimen of the tris-*p*-nitrobenzoate of 1-*O*-methyl-D-erythritol showed m.p. 162.5–164° and $[\alpha]_D^{25}$ +85° (chloroform)[13].

The methylated potato-amylose polyalcohol (193.1 mg) was methanolyzed, and the product fractionated, as described for corn-amylose polyalcohol, to give 1,4-di-*O*-methylerythritol (53.0 mg) and a 1-*O*-methylerythritol (3.0 mg); these were identified as the corresponding di-*p*-toluenesulfonate and tris-*p*-nitrobenzoate. The latter showed $[\alpha]_D^{25}$ +12.20° (*c* 3.0, chloroform).

Estimation of the degree of branching in amylose. — The low, but significant, optical activity of the tris-*p*-nitrobenzoate of the 1-*O*-methylerythritol isolated from both corn- and potato-amylose polyalcohol indicated that the mono-*O*-methylerythritol is a mixture of the D form (originating from the branch point) and the racemic form (arising from demethylation of 1,4-di-*O*-methylerythritol[4]). The approximate amounts of 1-*O*-methyl-D-erythritol are calculated, on the basis of the optical rotation of authentic 1-*O*-methyl-D-erythritol tris-*p*-nitrobenzoate ($[\alpha]_D^{25}$ +85°), to be 0.6 mg (5.6 mg × 9.0/85) for corn- and 0.4 mg (3.0 mg × 12.2/85) for potato-amylose polyalcohol, respectively. Thus, 195.0 mg (940 μmoles of monomer residues) of the methylated corn-amylose polyalcohol yielded 0.6 mg (3.4 μmoles) of 1-*O*-methyl-D-erythritol, which corresponds to one (1→6)-linked branch out of every 280 D-glucose residues. Similarly, the amount of 1-*O*-methyl-D-erythritol (2.3 μmoles) in the methylated potato-amylose polyalcohol (937 μmoles of monomer residues) indicates that a branch occurs for every 410 D-glucose residues of potato amylose.

ACKNOWLEDGMENTS

The authors thank the Corn Industries Research Foundation for financial support. Thanks are also due Dr. B. A. Lewis, University of Minnesota, for help in the preparation of the manuscript.

SUMMARY

Amylose (corn and potato) was subjected to prolonged periodate oxidation, and the resulting polyaldehyde was reduced with sodium borohydride to the corresponding polyalcohol. Methylation of the amylose polyalcohol, followed by methano-

lysis, gave 1,4-di-*O*-methylerythritol and a small proportion of 1-*O*-methyl-D-erythritol. These findings support the view that amylose from both sources is slightly branched by (1→6)-D-glucosidic linkages.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

DIMETHYLTHIOCARBAMATES OF SUGARS*

D. HORTON AND H. S. PRIHAR
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)
(Received December 12th, 1966)

INTRODUCTION

Alkylxanthate esters of suitably protected sugars[1], having a *cis* hydrogen atom vicinal to the alkylxanthate group, appear not to undergo the Chugaev elimination[2] to give *cis*-alkenes when they are pyrolyzed[1,3], unless there is a methylene[4] or methyl[5] group in the vicinal position. Instead, a rearrangement to an (alkylthio)carbonylthio derivative (Reaction 1) takes place in those derivatives that are sufficiently stable to



(1)

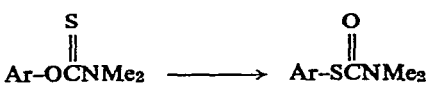
withstand the severe conditions of the reaction. This reaction is of potential utility for replacement of an oxygen atom in a sugar by sulfur, with (assumed) retention of configuration, and provides a direct route, through desulfurization, to deoxy sugars[6]. A serious limitation is that the drastic pyrolysis involved leads to extensive decomposition, even with very stable sugar derivatives.

Rearrangements related to Reaction 1 (*cf.* ref. 3) take place in the conversion of di-*O*-aryl thionocarbonates into *O*-aryl *S*-aryl thiocarbonates[7] (Reaction 2) and



(2)

in the conversion of *O*-aryl dialkylthiocarbamates into *S*-aryl dialkylthiocarbamates[8] (Reaction 3); the latter rearrangement takes place much more readily than the former.



(3)

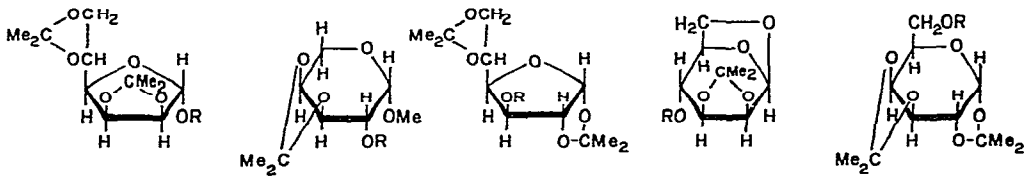
It was of interest, therefore, to prepare dialkylthiocarbamates of sugars.

*Supported, in part, by the Agricultural Research Service, U.S. Department of Agriculture, Grant No. 12-14-100-7208 (71) (O.S.U.R.F. Project 1827) administered by the Northern Utilization Research and Development Division, Peoria, Illinois. Funds for purchase of the n.m.r. spectrometer were provided by the National Science Foundation.

DISCUSSION

This paper describes the conversion of various sugar derivatives, each having a single, free, hydroxyl group, into the corresponding dimethylthiocarbamates. Examples are described that have the *O*-dimethylthiocarbamoyl substituent on a hemiacetal hydroxyl group, on a primary hydroxyl group, and on secondary hydroxyl groups at various ring positions. The protecting groups were selected as being stable to base, and with the expectation that they would confer stability at elevated temperatures on the sugar molecule.

Each of the derivatives (**1**, **2**, **3**, **4**, and **5**) was prepared from the appropriate protected sugars (**6**, **7**, **8**, **9**, or **10**, respectively) by conversion of the latter into their *O*-sodio derivatives, followed by treatment with dimethylthiocarbamoyl chloride. The sodio derivatives were prepared by treatment of the protected sugars **6**–**10**, in *N*,*N*-dimethylformamide, with sodium hydride; this general procedure[8] has been used as a step in the *O*-alkylation of sugar derivatives[9]. The products **1**–**5** crystallized when the reaction mixtures were poured into water.



1 R = OCNMe$_2$ (S) **2** R = OCNMe$_2$ (S) **3** R = OCNMe$_2$ (S) **4** R = OCNMe$_2$ (S) **5** R = OCNMe$_2$ (S)

6 R = H **7** R = H **8** R = H **9** R = H **10** R = H

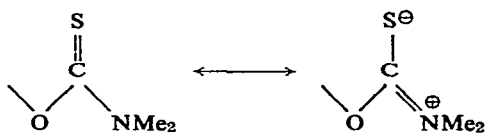
11 R = Ac **12** R = Ac **13** R = Ac **14** R = Ac **15** R = Ac

The i.r. spectra of substances **1**–**5** showed, in each case, a strong absorption at 6.5–6.6 μ (1540–1515 cm^{-1}). This absorption was assigned to the *O*-dimethylthiocarbamoyl group; it was absent from the spectra of the parent hydroxy compounds **6**–**10**. *S*-Dialkylthiocarbamoyl derivatives (*N*,*N*-dialkyldithiocarbamates) have been reported[10] to show strong absorption at 6.71–6.80 μ (1490–1470 cm^{-1}).

Substances **1**–**5** all showed strong absorption in their u.v. spectra at about 249 mμ (ε 13,000–19,000) and moderate absorption near 204 mμ (ε 4,000–9,000). Weak "end absorption" between 285 and 300 mμ was present, but there was no discernible maximum in this region. These absorptions appear to be characteristic of the *O*-dimethylthiocarbamoyl group. The n$\rightarrow\pi^*$ transition of the thiocarbonyl group is probably in the region of "end absorption". The u.v. spectrum of *N*,*N*-dimethyl-*O*-methylthionocarbamate has been reported[11] to show strong absorption at 247 mμ (ε ~10,000), weak (shoulder) absorption at 280 mμ, and moderate absorption below 210 mμ. Related absorption bands have been observed with *O*,*S*-dialkylxanthates[11,12]. The n$\rightarrow\pi^*$ transition in xanthates of optically active alcohols gives rise to a Cotton effect in the o.r.d. spectra of the xanthates, and the sign of this effect can be used for

configurational correlation with carbohydrate derivatives[12]. The dimethylthiocarbamates may similarly be useful in o.r.d. studies.

The n.m.r. spectra of substances **1–5**, measured at ~40° in chloroform-*d*, revealed that, in each case, the two *N*-methyl groups were nonequivalent; their signals were observed as sharp, three-proton singlets. This nonequivalence is analogous to the situation observed with *N*,*N*-dimethylacetamide and *N*,*N*-dimethylformamide[13,14]. Rotation about the bond between nitrogen and the thiocarbonyl group is hindered because of the partial double-bond character of this bond. When the samples were heated, the signals of the *N*-methyl groups showed broadening and partial collapse, but collapse was incomplete at the normal boiling point of the solvent. When hexachloroacetone was used as solvent, it was possible to observe, as the temperature was raised, progressive line-broadening, collapse to a broad singlet, and finally the formation of a sharp, six-proton singlet for the two *N*-methyl groups. Sharp signals for the individual *N*-methyl groups were observed at ~40° in hexachloroacetone. The



temperature at which the peaks collapsed to a broadened singlet (temperature of coalescence, T_c) was 80–90°, and a sharp, single line was observed above 110°. The reported values of T_c for *N*,*N*-dimethylacetamide and *N*,*N*-dimethylformamide are 52° and 99°, respectively[13].

The low-field portions of the n.m.r. spectra of substances **1–5** are shown in Fig. 1. Details of chemical shifts, and first-order interpretations of the spectra, are recorded in the experimental section for substances **1–5**, the parent hydroxyl derivatives **7** and **8**, and the acetates **12**, **13**, and **15**. Data have already been published[15] for the hydroxy derivatives **6** and **9** and their respective acetates **11** and **14**, and spectral details for substance **10** have also been reported[16]. Spectral details are also included in the experimental section for three analogs of substances **2**, **7**, and **12**, namely methyl 3,4-*O*-isopropylidene-2-*O*-(methylsulfonyl)-β-L-arabinopyranoside, its 2-*O*-(*p*-tolylsulfonyl) analog, and methyl 3,4-di-*O*-acetyl-2-*O*-(*p*-tolylsulfonyl)-β-L-arabinopyranoside.

Most noteworthy is the strong deshielding effect that the dimethylthiocarbamate group has on the hydrogen atoms attached to the carbon atom bearing the substituent. In Table I, the effect of the dimethylthiocarbamate group on an adjacent methine proton is compared with the effect of the acetoxy and the hydroxyl group in the same parent structure. In each case, the signal of the methine proton, in the dimethylthiocarbamates, is observed 0.5–0.8 p.p.m. to lower field than its position in the corresponding acetates. An accurate measure of the total downfield shifts of the methine signals, relative to the parent hydroxy compounds, was not obtained, because the appropriate methine signals in **8** and **9** were not sufficiently resolved to permit first-order analysis. Similarly, the H-6 methylene signals in **5**, **10**, and **15** were not well

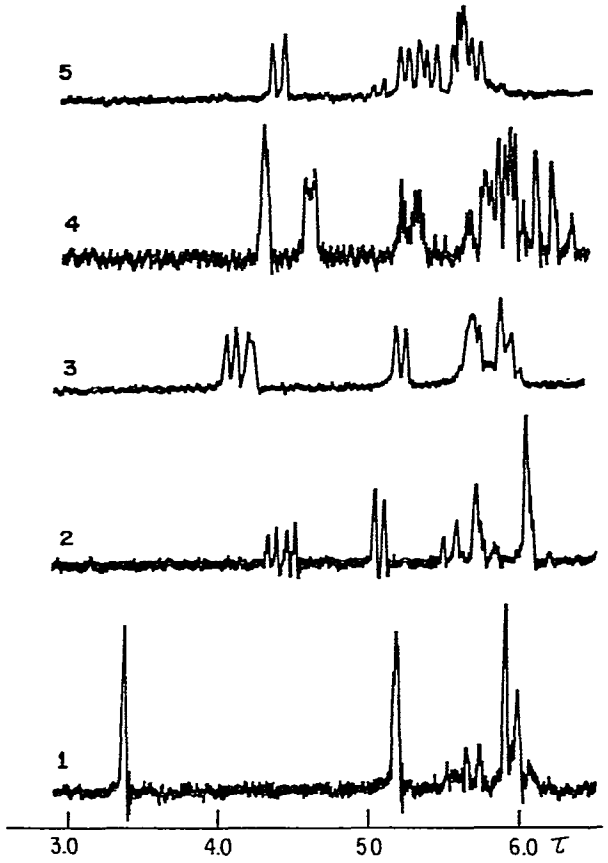


Fig. 1. The low-field portions of the n.m.r. spectra, measured in chloroform-*d* at 60 Mc.p.s., of 1-*O*-dimethylthiocarbamoyl-2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (**1**), methyl 2-*O*-dimethylthiocarbamoyl-3,4-*O*-isopropylidene-β-L-arabinopyranoside (**2**), 3-*O*-dimethylthiocarbamoyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**3**), 1,6-anhydro-4-*O*-dimethylthiocarbamoyl-2,3-*O*-isopropylidene-β-D-mannopyranose (**4**), and 6-*O*-dimethylthiocarbamoyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**).

resolved, but the spectra clearly showed that the major peaks in the H-5,6 multiplet occurred at lower field in **5** than in **15**.

In substance **2**, the H-2 signal is observed at considerably lower field than the H-1 signal, so that first-order $J_{1,2}$ and $J_{2,3}$ couplings are readily determined. The magnitude of $J_{2,3}$ (7.8 c.p.s.) clearly indicates that the compound does not adopt the *1C* (L) conformation, and the data are consistent with the *C1* (L) conformation, that is, with the C-1 and C-4 substituents axial and the C-2 and C-3 substituents equatorial. Methyl 3,4-di-*O*-acetyl-2-*O*-(*p*-tolylsulfonyl)-β-L-arabinopyranoside may also be deduced to exist in this conformation, because H-4 shows only small coupling with each H-5 proton, indicating[17] that H-4 is not antiparallel with one of the H-5 protons, as would be the case with the *1C* (L) conformation.

Comparison of the spectrum of **2** with those of the corresponding 2-methane-

TABLE I

RELATIVE EFFECT OF HYDROXY, ACETOXY, AND DIMETHYLTHIOCARBAMOYLOXY SUBSTITUENTS ON THE CHEMICAL SHIFT OF A METHINE PROTON[a]

Substance	*Substituent*	*Position of Substituent*	*Methine proton and chemical shift*		
			Methine proton	*Chemical shift, τ*	*Relative shift, p.p.m.*[b]
1	O(C=S)NMe$_2$	C-1	H-1	3.38	−0.50
2	O(C=S)NMe$_2$	C-2	H-2	4.42	−0.76
3	O(C=S)NMe$_2$	C-3	H-3	4.23	−0.55
4	O(C=S)NMe$_2$	C-4	H-4	4.32	−0.62
6	OH	C-1	H-1	4.63[c,d]	+0.75
7	OH	C-2	H-2	6.26	+1.08
8	OH	C-3	H-3	~5.8	~+1.0
9	OH	C-4	H-4	~5.9[c]	~+1.0
11	OAc	C-1	H-1	3.88[c]	0.00
12	OAc	C-2	H-2	5.18	0.00
13	OAc	C-3	H-3	4.78	0.00
14	OAc	C-4	H-4	4.94[c]	0.00

[a]Details of spectral measurements are given in the experimental section. [b]Relative to the shift of the methine proton in the corresponding acetate. [c]Data from ref. 15. [d]Corrected value.

sulfonate and 2-*p*-toluenesulfonate indicates that the signal of the methine proton (H-2), relative to its position in **2**, is 1.0 p.p.m. further upfield in the methanesulfonate, and >1.3 p.p.m. further upfield in the *p*-toluenesulfonate.

Rearrangements of the type given in Reaction **1** have been assumed to proceed with retention of configuration[1], presumably by an S_Ni type of process[3]. Direct evidence that this is so is afforded by examination of the n.m.r. spectrum of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-(methylxanthate)[1] and that of the pyrolytically rearranged product. The latter, formulated[1] as 1,2:5,6-di-*O*-isopropylidene-3-*S*-(methylthio)carbonyl-3-thio-α-D-glucofuranose, shows the H-2 signal as a sharp doublet, τ 5.38, close to the position observed with other 1,2-*O*-isopropylidene-α-D-glucofuranose derivatives[18]. The doublet spacing, 3.8 c.p.s., is the same as that of the H-1 signal, and indicates that $J_{2,3}$ is approximately equal to zero. From this, it may be concluded[18] that H-2 and H-3 are *trans*, and that the rearrangement does proceed with retention of configuration to give the product having the D-*gluco* configuration. Details of the spectra are given in the experimental section. It is noteworthy that, as with the dimethylthiocarbamates, the xanthate ester group causes strong deshielding of the adjacent methine hydrogen, in this case H-3.

The strong deshielding effect that the *O*-dimethylthiocarbamoyl (or xanthate) group has on an adjacent methine hydrogen atom suggests that such derivatives may have utility for configurational and conformational studies, when it is desired to observe, and interpret on a first-order basis, the signal of an individual methine hydrogen that is a part of a complex multiplet in the parent compound.

Details of the behavior of substances **1–5** on thermolysis and photolysis, together with o.r.d. and c.d. measurements, will be the subject of a separate report.

EXPERIMENTAL

General. — Evaporations were performed under diminished pressure. Melting points were determined with a Thomas–Hoover apparatus (Arthur H. Thomas Co., Philadelphia, Pennsylvania) and are uncorrected. Specific rotations were measured with a 2-dm tube. Infrared spectra were measured with a Perkin–Elmer Model 137 "Infracord" infrared spectrophotometer. Ultraviolet spectra were measured with a Bausch and Lomb "Spectronic 505" recording spectrophotometer. N.m.r. spectra were measured at 60 Mc.p.s. with a Varian A-60 n.m.r. spectrometer equipped with a Varian V-6040 variable-temperature probe. Unless otherwise stated, the solutions were ~10% in chloroform-*d*, and spectra were measured at the ambient probe temperature (~40°). Tetramethylsilane (τ = 10.00) was used as the internal standard. Spectra were analyzed on a first-order basis, and the coupling constants recorded are the measured line-spacings. Elemental analyses were determined by W. N. Rond. X-ray powder diffraction data give interplanar spacings, Å, for CuK_α radiation. The camera diameter was 114.59 mm. Relative intensities were estimated visually: s, strong; m, moderate; w, weak, v. very. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

Preparation of dimethylthiocarbamoyl chloride. — This substance was prepared, either from thiophosgene and two equivalents of dimethylamine, by the procedure of Lecher and Heuck[19], or by chlorination of bis(dimethylthiocarbamoyl) disulfide (Thiram)*, according to the procedure of Ritter and co-workers[20]. The latter procedure was more convenient and economical. The product was distilled, and the fraction having b.p. 90–95°/0.5 mm, which solidified on cooling, was used.

1-O-Dimethylthiocarbamoyl-2,3:5,6-di-O-isopropylidene-α-D-mannofuranose (**1**). — A solution of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose[21] (6.5 g) in dry *N,N*-dimethylformamide (150 ml) was treated with 1.2 g of a 51.8% sodium hydride dispersion in mineral oil** and the mixture was stirred in an atmosphere of dry nitrogen for 1 h, with initial cooling to maintain the temperature below 20°. To the mixture was added dimethylthiocarbamoyl chloride (3.2 g) portionwise with stirring. After 1.5 h, the mixture was poured into ice and water (200 ml), and the solid product that separated was filtered off and dried. Recrystallization from chloroform gave pure **1**; yield 4.4 g (51%), m.p. 96.5°, $[\alpha]_D^{21}$ +30 ±1° (*c* 1.6, chloroform); λ_{max}^{KBr} 6.47 (C=S), 7.17, 7.28 μ (CMe_2); λ_{max}^{EtOH} 249 (ε 19,000), 205 mμ (9,000); n.m.r. data: τ 3.38 (1-proton singlet, H-1), τ 5.18 (2-proton, narrow multiplet), 5.60 (1-proton quartet), and 5.88–6.16 (3- proton multiplet) (H-2,3,4,5,6,6'), τ 6.67, 6.92 (3-proton singlets, NMe_2), τ 8.51, 8.56, 8.65, 8.66 (3-proton singlets, CMe_2); X-ray powder diffraction data: 10.16 m, 9.03 m, 7.87 w, 7.20 w, 5.81 w, 5.11 m, 4.82 s (2), 4.56 s (1), 4.13 w, 3.95 s (3), 3.44 w.

*Supplied by the Pennsalt Manufacturing Co., Three Penn Center, Philadelphia, Pa.

**Metal Hydrides, Inc., Beverly, Massachusetts. Either sodium hydride or the equivalent amount of the dispersion in mineral oil could be used; the latter, being less hazardous, was more convenient to use.

Anal. Calc. for $C_{15}H_{25}NO_6S$: C, 51.85; H, 7.25; N, 4.03; S, 9.23. Found: C, 51.99; H, 7.34; N, 4.39; S, 9.44.

The two sharp singlets in the n.m.r. spectrum that were assigned to the NMe_2 group were observed as broadened singlets with a separation of 12.5 c.p.s. when the spectrum was measured at 68°; no change in the rest of the spectrum was observed at the higher temperature.

Methyl 2-O-dimethylthiocarbamoyl-3,4-O-isopropylidene-β-L-arabinopyranoside (**2**). — Methyl 3,4-*O*-isopropylidene-β-L-arabinopyranoside[22] (10 g) in *N,N*-dimethylformamide (150 ml) was treated with sodium hydride (1.2 g) and then with dimethylthiocarbamoyl chloride (6.2 g), by the procedure used in the foregoing experiment. The product that separated when the reaction mixture was poured into water was filtered off, dried, and recrystallized from methanol; yield 8.6 g (60%), m.p. 124–125°, $[\alpha]_D^{20}$ +204 ±1° (*c* 2, chloroform); λ_{max}^{KBr} 6.53 (C=S), 7.21 μ (CMe_2); λ_{max}^{EtOH} 249 (ε 15,000), 206 mμ (7,000); n.m.r. data: τ 4.42 (1-proton quartet, $J_{2,3}$ 7.8 c.p.s., H-2), τ 5.07 (1-proton doublet, $J_{1,2}$ 3.4 c.p.s., H-1), τ 5.44–5.90, 5.95–6.20 (multiplets, 4 protons, H-3,4,5,5'), τ 6.67 (6-proton singlet, OMe, NMe), τ 6.85 (3-proton singlet, NMe), τ 8.42, 8.65 (3-proton singlets, CMe_2); X-ray powder diffraction data: 9.02 m, 7.02 vs (1,1), 6.53 m, 5.79 vs (1,1), 5.34 w, 4.86 vs (2), 4.52 s, 4.33 w, 4.03 m, 3.91 vs (3), 3.62 m, 3.51 w, 3.37 m, 2.90 w, 2.79 m, 2.67 w, 2.50 s, 2.12 m.

Anal. Calc. for $C_{12}H_{21}NO_5S$: C, 49.46; H, 7.26; N, 4.81; S, 11.01. Found: C, 49.15; H, 6.92; N, 4.78; S, 10.71.

The sharp signals in the n.m.r. spectrum, at τ 6.67 and 6.85, appeared broadened in a spectrum measured at 60°.

3-O-Dimethylthiocarbamoyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**3**). — A solution of 1,2:5,6 di-*O*-isopropylidene-α-D-glucofuranose[23] (25 g) in *N,N*-dimethylformamide (200 ml) was treated with 56% sodium hydride dispersion (4.6 g) and then with dimethylthiocarbamoyl chloride (12.3 g) by the procedure used for the preparation of **1**. The crude product, yield 25.5 g (79%), was recrystallized from ethanol to give pure **3**, m.p. 105.5°, $[\alpha]_D^{22}$ −70 ±1° (*c* 2, chloroform); λ_{max}^{KBr} 6.57 (C=S), 7.20, 7.31 μ (CMe_2); λ_{max}^{EtOH} 249 (ε 14,000), 206 mμ (4,000); n.m.r. data: τ 4.12 (1-proton doublet, $J_{1,2}$ 3.8 c.p.s., H-1), τ 4.23 (1-proton broadened singlet, W_h 3.5 c.p.s., H-3), τ 5.23 (1-proton doublet, $J_{2,3}$ ~0 c.p.s., H-2), τ 5.59–6.06 (4-proton multiplet, H-4,5,6,6'), τ 6.61, 6.86 (3-proton, sharp singlets, NMe_2), τ 8.48, 8.59, 8.68 (3-, 3-, and 6-proton singlets, CMe_2); X-ray powder diffraction data: 8.39 s (3), 7.03 s (2), 6.03 vs (1,1), 5.28 m, 5.01 m, 4.62 m, 4.35 vs (1,1), 3.72 w, 3.21 vw.

Anal. Calc. for $C_{15}H_{25}NO_6S$: C, 51.85; H, 7.25; N, 4.03; S, 9.23. Found: C, 52.00; H, 7.38; N, 4.01; S, 9.27.

1,6-Anhydro-4-O-dimethylthiocarbamoyl-2,3-O-isopropylidene-β-D-mannopyranose (**4**). — A solution of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[24] (3 g) in *N,N*-dimethylformamide (100 ml) was treated with sodium hydride (0.4 g) and then with dimethylthiocarbamoyl chloride (2.4 g) by the procedure used for the preparation of **1**. The crude product, yield 2.4 g (56%), was recrystallized from ethanol to give pure **4**, m.p. 135°, $[\alpha]_D^{20}$ −64.5 ±0.5° (*c* 2, chloroform); λ_{max}^{KBr} 6.55 (C=S),

7.25 μ (CMe_2); λ_{max}^{EtOH} 249 (ε 13,000), 207 mμ (ε 4,500); n.m.r. data: τ 4.32 (1-proton broadened singlet, W_h 2.8 c.p.s., H-4), τ 4.62 (1-proton broadened doublet, W_h 5.5 c.p.s., H-1), τ 5.28 (1-proton multiplet, $J_{2,3}$ ~6 c.p.s., H-2), τ 5.60–6.36 (4-proton multiplet, H-3,5,6,6'), τ 6.62, 6.82 (3-proton sharp singlets, NMe_2), τ 8.43, 8.66 (3-proton singlets, CMe_2); X-ray powder diffraction data: 9.26 s (2,2), 6.32 w, 5.44 s (1), 4.90 m, 4.25 s (2,2), 3.94 w, 3.76 w, 3.52 m, 3.29 w, 3.14 w, 2.85 vw, 2.75 vw, 2.41 w.

Anal. Calc. for $C_{12}H_{19}NO_5S$: C, 49.81; H, 6.62; N, 4.84; S, 11.08. Found: C, 49.99; H, 6.69; N, 4.88; S, 11.44.

The appearance of the H-1 and H-2 signals in the n.m.r. spectrum was indicative of long-range coupling[25], $J_{1,3}$ ~1.3 c.p.s.

6-O-Dimethylthiocarbamoyl-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**5**). — A solution of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[26] (6 g) in dry *N,N*-dimethylformamide (150 ml) was treated with sodium hydride (0.6 g) and then with dimethylthiocarbamoyl chloride (3 g) by the procedure used for the preparation of **1**. The crude product was recrystallized from ethanol, yield 2.5 g, m.p. 88–89°, $[\alpha]_D^{20}$ −47.3 ±0.5° (*c* 2, chloroform); λ_{max}^{KBr} 6.56 (C=S), 7.25 μ (CMe_2); λ_{max}^{EtOH} 248 (ε 15,000), 205 mμ (7,000); n.m.r. data: τ 4.42 (1-proton doublet, $J_{1,2}$ 5.0 c.p.s., H-1), τ 5.02–5.48 (3-proton multiplet, H-2,3,4), τ 5.55–5.90 (3-proton multiplet, H-5,6,6'), τ 6.60, 6.82 (3-proton sharp singlets, NMe_2), τ 8.47, 8.52, 8.64, (3-, 3-, and 6-proton singlets, CMe_2); X-ray powder diffraction data: 9.56 w, 8.38 w, 7.41 m, 6.71 vw, 6.19 m, 5.47 s (1), 4.95 s (2), 4.62 m, 4.30 s (3), 3.87 w, 3.62 w, 3.49 m, 3.30 w, 3.07 w, 2.95 w, 2.78 w, 2.67 w, 2.46 w, 2.20 w.

Anal. Calc. for $C_{15}H_{25}NO_6S$: C, 51.85; H, 7.25; N, 4.03; S, 9.23. Found: C, 51.67; H, 7.37; N, 4.02; S, 9.10.

Variable-temperature n.m.r. studies in hexachloroacetone. — (a) *1-O-Dimethylthiocarbamoyl-2,3:5,6-di-O-isopropylidene-α-D-mannofuranose* (**1**). The spectrum of **1**, measured at 40°, gave the following data: τ 3.44 (1-proton singlet, H-1), τ 5.24 (2-proton narrow multiplet), 5.68 (1-proton multiplet), and 5.92–6.18 (3-proton multiplet) (H-2,3,4,5,6,6'), τ 6.68, 6.88 (3-proton sharp singlets, NMe_2), τ 8.55, 8.65, 8.69 (3-, 3-, 6-proton singlets, CMe_2). As the temperature was raised, the signals of the NMe groups broadened and moved closer together. Two broad peaks were still discernible at 70°, but, at 80°, only one, broadened peak was observed. At higher temperatures, the single peak became progressively narrower, and was observed as a sharp, 6-proton singlet at temperatures above 110°. No change in the appearance of the other signals was observed over the temperature range studied.

(b) *Methyl 2-O-dimethylthiocarbamoyl-3,4-O-isopropylidene-β-L-arabinopyranoside* (**2**). The spectrum of **2**, measured at 40°, gave the following data: τ 4.61 (1-proton quartet, H-2), τ 5.16 (1-proton doublet, H-1), τ 5.58–6.26 (multiplets, 4 protons, H-3,4,5,5'), τ 6.70 (6-proton sharp singlet, OMe, NMe), 6.84 (3-proton sharp singlet, NMe), τ 8.54, 8.74 (3-proton singlets, CMe_2). As the temperature was raised, the signals at τ 6.70 and 6.84 became broader and moved together, and at the higher temperatures, a sharp singlet at τ 6.70 could be observed at slightly lower field than the broadened peaks. At 90°, a sharp, 3-proton singlet (τ 6.70, OMe) and a broad,

6-proton singlet (τ 6.80, NMe) were observed. At 120°, both signals were observed as sharp singlets. The appearance of the other signals in the spectrum was not changed as the temperature was varied.

(c) *3-O-Dimethylthiocarbamoyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose* (**3**). The spectrum of **3**, measured at 40°, gave the following data: τ 4.24 (1-proton doublet, H-1), τ 4.35 (1-proton broadened singlet, H-3), τ 5.39 (1-proton doublet, H-2), τ 5.70–6.16 (4-proton multiplet, H-4,5,6,6'), τ 6.63, 6.83 (3-proton sharp singlets, NMe_2), τ 8.50, 8.62, 8.70 (3-, 3-, and 6-proton singlets, CMe_2). The spectrum showed little change as the temperature was raised, except that the signals at τ 6.63 and 6.83 broadened and merged. At 90°, a broadened singlet was observed that became a sharp, 6-proton singlet, τ 6.82, at 110°.

Spectral data for reference compounds. — (a) *Methyl 3,4-O-isopropylidene-β-L-arabinopyranoside*[22] (**7**) *and its 2-acetate*[22] (**12**). *For* **7**: τ 5.31 (1-proton doublet, $J_{1,2}$ 3.2 c.p.s., H-1), τ 5.70–5.92 (2-proton multiplet, H-3,4), τ 6.10 (2-proton multiplet, H-5,5'), τ 6.26 (1-proton multiplet, multiplicity diminished by deuteration, H-2), τ 6.60 (3-proton singlet, OMe), τ 6.98 (1-proton doublet, J 6 c.p.s. disappears on deuteration, OH), τ 8.45, 8.61 (3-proton singlets, CMe_2).

For **12**: τ 5.18 (1-proton multiplet, H-2), τ 5.29 (1 proton, H-1), τ 5.65–5.90 (2-proton multiplet, H-3,4), τ 6.03-6.20 (2-proton broadened singlet, H-5,5'), τ 6.67 (3-proton singlet, OMe), τ 7.93 (3-proton singlet, OAc), τ 8.52, 8.68 (3-proton singlets, CMe_2).

(b) *1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose*[23] (**8**) *and its 3-acetate*[27] (**13**). *For* **8**: τ 4.06 (1-proton doublet, $J_{1,2}$ 3.8 c.p.s., H-1)[28], τ 5.46 (1-proton doublet, $J_{2,3}$ ~0 c.p.s., H-2)[28], τ 5.60-6.07 (5-proton multiplet, H-3,4,5,6,6')[28], τ 6.74 (1-proton singlet, disappears on deuteration, OH), τ 8.50, 8.55, 8.61, 8.67 (3-proton singlets, (CMe_2).

For **13**: τ 4.17 (1-proton doublet, $J_{1,2}$ 3.8 c.p.s., H-1), τ 4.78 (1-proton broadened doublet, W_h 4 c.p.s., H-3), τ 5.55 (1-proton doublet, $J_{2,3}$ ~ 0 c.p.s., H-2), τ 5.71–6.10 (4-proton multiplet, H-4,5,6,6'), τ 7.94 (3-proton singlet, OAc), τ 8.51, 8.61, 8.71 (3-, 3-, 6-proton singlets, CMe_2)

(c) *6-O-Acetyl-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose*[29] (**15**): τ 4.45 (1-proton doublet, $J_{1,2}$ 5.0 c.p.s., H-1), τ 5.38 (1-proton quartet, $J_{2,3}$ 2.3 c.p.s., $J_{3,4}$ 7.5 c.p.s., H-3), τ 5.57–6.03 (5-proton multiplet, H-2,4,5,6,6'), τ 7.92 (3-proton singlet, OAc)[16], τ 8.47, 8.55, 8.65 (3-, 3-, 6-proton singlets, CMe_2).

(d) *Methyl 3,4-O-isopropylidene-2-O-(methylsulfonyl)-β-L-arabinopyranoside*[30]: τ 5.13 (1-proton doublet, $J_{1,2}$ 3.2 c.p.s., H-1), τ 5.43 (1-proton multiplet, H-2), τ 5.60–5.82, 5.95–6.22 (multiplets, 2, 2 protons, H-3,4,5,5'), τ 6.57 (3-proton singlet, OMe), τ 6.83 (3-proton singlet, SO_2Me), τ 8.43, 8.63 (3-proton singlets, CMe_2).

(e) *Methyl 3,4-O-isopropylidene-2-O-(p-tolylsulfonyl)-β-L-arabinopyranoside*[22]: τ 2.17, 2.68 (2-proton multiplets, aryl), τ 5.17 (1-proton multiplet, W_h 4 c.p.s., H-1), τ 5.69–5.91, 6.03–6.14 (multiplets, 3, 2 protons, H-2,3,4,5,5'), τ 6.65 (3-proton singlet, OMe), τ 7.59 (3-proton singlet, C-Me of Ts), τ 8.75, 8.86 (3-proton singlets, CMe_2),

(f) *Methyl 3,4-di-O-acetyl-2-O-(p-tolylsulfonyl)-β-L-arabinopyranoside*[22]: τ 2.19,

2.65 (2-proton multiplets, aryl), τ 4.61–4.87 (2-proton multiplet, H-3,4), τ 5.15 (1-proton narrow multiplet, H-1), τ ~5.27 (1-proton broad multiplet, H-2), τ 6.10, 6.42 [2 protons, AB of ABX system, $J_{5,5'}$ ~12 c.p.s., $J_{4,5}$ and $J_{4,5'}$ < 2 c.p.s., H-5,5', indicative[17] of the *Cl* (L) conformation], τ 6.69 (3-proton singlet, OMe), τ 7.58 (3-proton singlet, C-Me of Ts), τ 7.93, 8.25 (3-proton singlets, acetyls).

(g) *1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose 3-(methylxanthate)*[1]: τ 4.11 (2-proton multiplet, W_h 5 c.p.s., H-1,3), τ 5.37 (1-proton doublet, $J_{1,2}$ 3.7 c.p.s., $J_{2,3}$ ~0 c.p.s., H-2), τ 5.62–6.24 (4-proton multiplet, H-4,5,6,6'), τ 7.43 (3-proton singlet, SMe), τ 8.47, 8.59, 8.69 (3-, 3-, 6-proton singlets, CMe_2). The product had λ_{max}^{EtOH} 358 (ε 175), 279 (23,000), 226 mμ (19,500).

(h) *1,2:5,6-Di-O-isopropylidene-3-S-(methylthio)carbonyl-3-thio-α-D-glucofuranose*[1]: τ 4.22 (1-proton doublet, $J_{1,2}$ 3.8 c.p.s., H-1), τ 5.38 (1-proton doublet, $J_{2,3}$ ~0 c.p.s., H-2), τ 5.62–6.42 (5-proton multiplet, H-3,4,5,6,6'), τ 7.57 (3-proton singlet, SMe), τ 8.49, 8.60, 8.68 (3-, 3-, 6-proton singlets, CMe_2). The product had λ_{max}^{EtOH} 247 mμ (ε 10,000).

ACKNOWLEDGMENTS

The authors thank Professor M. S. Newman and Dr. H. A. Karnes for valued counsel, and J. S. Jewell and R. H. Bell for n.m.r. measurements.

SUMMARY

A series of protected sugars having one hydroxyl group free have been converted into their *O*-dimethylthiocarbamoyl derivatives. The latter show characteristic spectral absorptions in the i.r. at 6.5–6.6 μ and in the u.v. near 249 mμ (ε 13,000–19,000) and 205 mμ (ε 4,000–9,000). The *N*-methyl groups of the dimethylthiocarbamates are nonequivalent by n.m.r. spectroscopy at room temperature, but become equivalent at temperatures above 110°. Conversion of a hydroxyl substituent into its dimethylthiocarbamate (or xanthate) causes strong deshielding of the proton(s) on the carbon atom bearing the hydroxyl substituent. The 3-*S*-(methylthio)carbonyl-3-thio derivative formed by pyrolytic rearrangement of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-(methylxanthate) is shown by n.m.r. spectroscopy to have the D-*gluco* configuration, verifying the assumption that the rearrangement proceeds with retention of configuration.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

4,6-ANHYDRO DERIVATIVES OF L-*xylo*-HEXULOFURANOSE

L. HOUGH AND B. A. OTTER*
The School of Chemistry, The University, Bristol (Great Britain)
(Received October 20th, 1966)

INTRODUCTION

Under stereospecific conditions, primary alkyl- and aryl-sulphonyl groups in carbohydrate structures can undergo intramolecular displacement by an oxygen anion to form an oxetan ring. For example, treatment of 1,2-*O*-isopropylidene-5-*O*-tosyl**-α-D-xylofuranose with methanolic sodium methoxide at room temperature affords 3,5-anhydro-1,2-*O*-isopropylidene-α-D-xylofuranose[1]. Essentially similar conditions have been used to prepare oxetans from primary sulphonate esters of DL-xylitol[2], D-glucitol[3,4], and from several pentofuranosyl pyrimidine nucleosides[5–8]. More recently, it has been shown that a secondary tosyl group can undergo intramolecular displacement by a primary oxygen anion. Thus, 1-(3,5-anhydro-2-deoxy-β-D-*threo*-pentofuranosyl)adenine was obtained as the minor product of the reaction of 1-(2-deoxy-3-*O*-tosyl-β-D-*threo*-pentofuranosyl)adenine with sodium ethoxide in ethanol[9]. Carbohydrates having a free hydroxyl group in a *trans* relationship to an epoxide ring can undergo base-catalysed, reversible, oxide-ring migrations to form oxetans. In this way, oxetan derivatives of D-glucose[10], L-idose[10], and D-xylose[11] have been prepared.

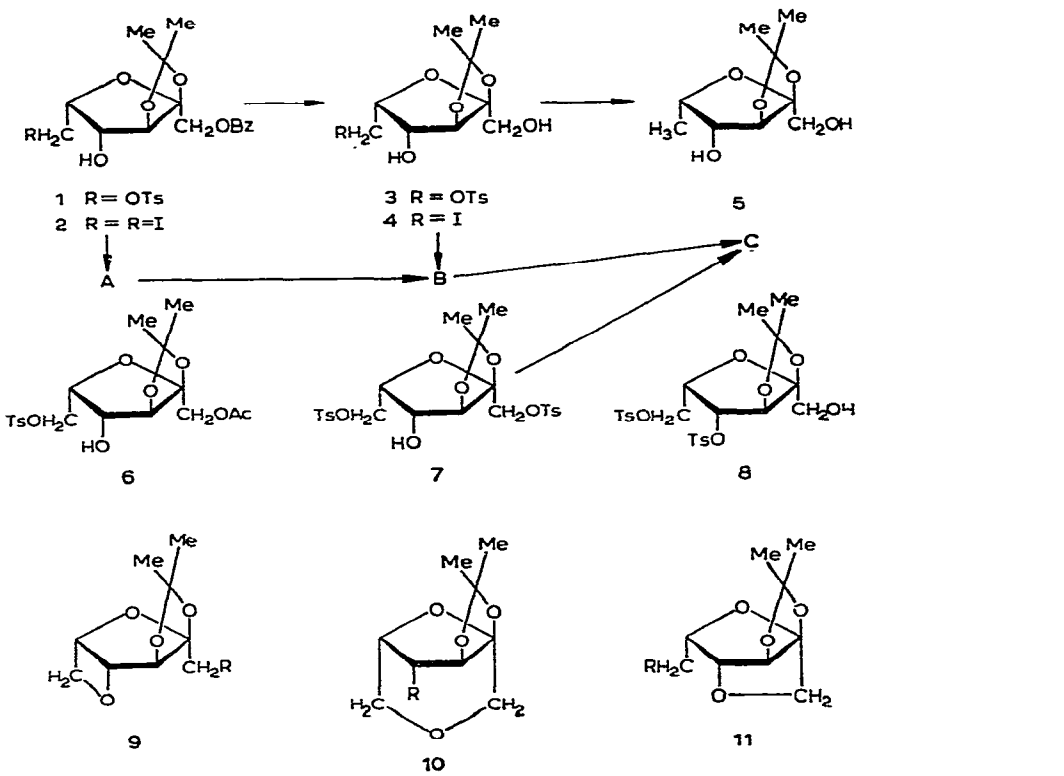
In a recent paper[12], we prepared carbohydrate oxetans without the use of strongly alkaline reagents. Thus, treatment of the 5-deoxy-5-iodo or 5-*O*-tosyl derivatives of 1,2-*O*-isopropylidene-α-D-xylofuranose with silver fluoride in cold pyridine afforded 3,5-anhydro-1,2-*O*-isopropylidene-α-D-xylofuranose in good yield. Similarly, methyl 4,6-*O*-benzylidene-2-*O*-tosyl-α-D-glucopyranoside gave methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside when treated with silver fluoride[13]. These mild reactions may find some application to compounds bearing alkali-labile substituents. We now report on the extension of this reaction to the preparation of a series of anhydro compounds in the L-*xylo*-hexulofuranose series.

RESULTS AND DISCUSSION

Treatment of either 1-*O*-benzoyl-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-

*Present Address: Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, New York, N. Y. 10021, U. S. A.
**Tosyl = toluene-*p*-sulphonyl.

hexulofuranose[12] (**1**) or the 6-deoxy-6-iodo analogue[12] (**2**) with anhydrous silver fluoride in pyridine at room temperature afforded a high yield of a crystalline *O*-benzoyl-*O*-isopropylidenehexulose anhydride (*A*). Catalytic debenzoylation of **1** and **2** with sodium methoxide in methanol gave compounds **3** and **4**, respectively, both of which reacted with silver fluoride to give a hydroxy-2,3-*O*-isopropylidenehexulose anhydride (*B*), which was also prepared by debenzoylation of *A*; it readily formed a toluene-*p*-sulphonate (*C*). The similarity of the n.m.r. spectra of these anhydro compounds showed that they had the same basic ring structure, and, by analogy with our previous results[12], they were tentatively assigned the 4,6-anhydro structure (**9**). However, the possibility existed that the anhydro compounds (*A*–*C*) were derivatives of the 1,6-anhydro compound (**10**). Such compounds could be formed directly from **3** and **4**, but, in the case of compounds **1** and **2**, a benzoyl migration would be necessary. The remaining possibility, the 1,4-anhydro system (**11**), was not considered until it was found that the physical constants of the anhydro compounds *B* and *C* were in close agreement with those reported by Tokuyama[14,15] and his co-workers for compounds claimed to be 1,4-anhydro-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**11**, R = OH) and the toluene-*p*-sulphonate (**11**, R = OTs), respectively. An actual comparison of compound *C* with a sample of Tokuyama's compound prepared from the 1,6-disulphonate (**7**) confirmed that these compounds were identical, and suggested that Tokuyama's formulation (**11**, R = OTs) was incorrect.



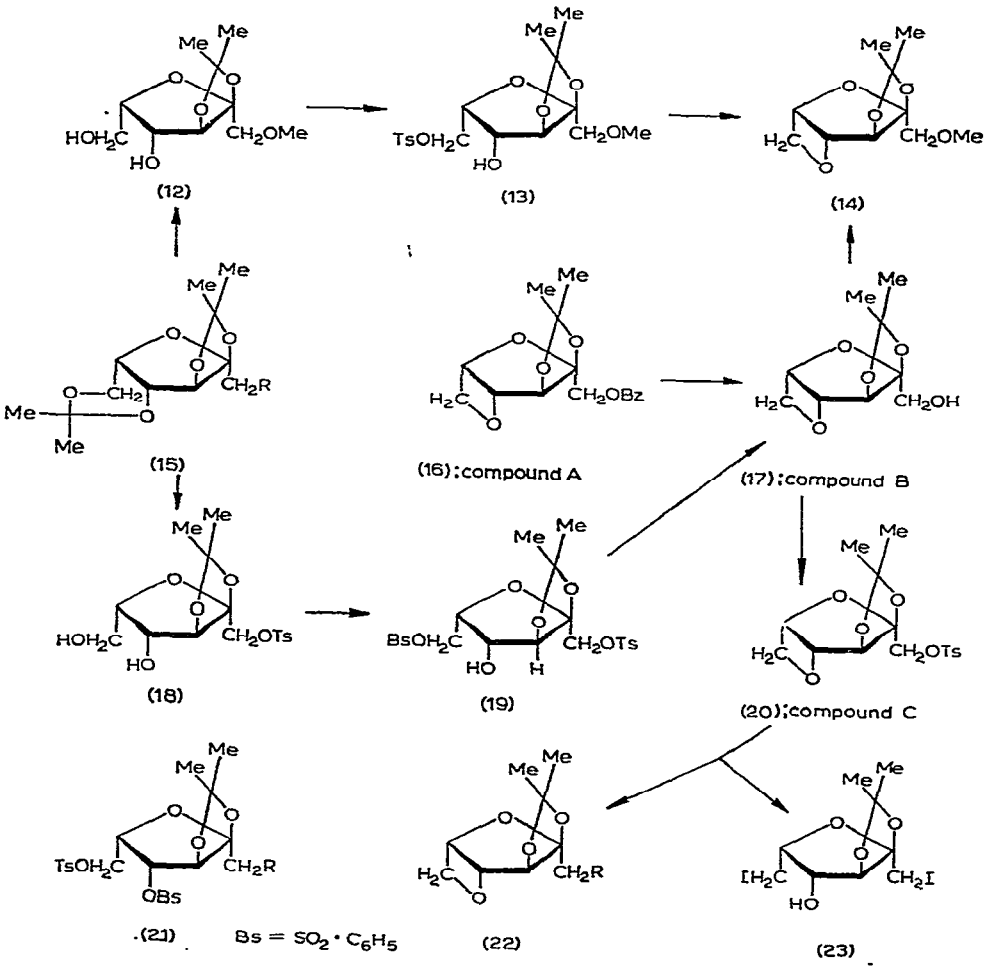
Tokuyama *et al.*[14] reported that treatment of either 2,3-*O*-isopropylidene-1,6-di-*O*-tosyl-α-L-*xylo*-hexulofuranose (**7**) or the 4,6-di(toluene-*p*-sulphonate) (**8**) with potassium hydroxide in warm ethanol afforded the 1,4-anhydro compound (**11**, R = OTs). This compound was also obtained when **7** was refluxed in methanol containing potassium cyanide, but, when **7** was treated with sodium hydroxide in liquid ammonia, desulphonylation occurred, and it was suggested that the 1,4-anhydro compound (**11**, R = OH) was formed[15]. It was considered that these preparative procedures afforded sufficient proof of the 1,4-anhydro structure, and no supporting evidence was given. A further complication arose when it was found that the melting point and specific rotation of the 6-toluene-*p*-sulphonate (**3**) differed from the values reported by Tokuyama[14] for material prepared by a different route. It was therefore necessary to confirm the structure of compound **3**, and to determine the structure of the anhydro compounds *A*–*C*, the possible structures for which were **9**, **10**, and **11**.

When compound **3** was heated with sodium iodide in butanone, the tosyl group was rapidly replaced, and the iodo compound **4** was isolated in 80% yield. Reduction of **4** with hydrogen in the presence of Raney nickel afforded a crystalline product, the physical constants of which were in good agreement with those reported by Müller and Reichstein[16] for 6-deoxy-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**5**). Tokuyama[14] claimed that **3** was formed when the 1-acetate (**6**) was heated with an excess of potassium hydroxide in ethanol. Treatment of our material **3** under these conditions afforded the anhydro compound *B* in 74% yield. These reactions, which prove that the structures assigned to compounds **3**, **4**, and their precursors are correct, indicate that Tokuyama's compounds are incorrectly formulated as **3** and **6**.

Since compounds **1**–**4** have the leaving group located at C-6, 1,4-anhydrides cannot be formed directly, and the reaction would involve such molecular rearrangements as benzoyl and anhydro-ring migrations. The first method used to investigate the anhydro ring in compounds *A*–*C* was designed so as to prevent migrations involving C-1, by having an alkali-stable group at this position. Selective hydrolysis of the known 2,3:4,6-di-*O*-isopropylidene-1-*O*-methyl-α-L-*xylo*-hexulofuranose (**15**, R = OMe) with 50% acetic acid at 35° afforded a 70% yield of 2,3-*O*-isopropylidene-1-*O*-methyl-α-L-*xylo*-hexulofuranose (**12**). Esterification of **12** in pyridine with one equivalent of tosyl chloride, followed by ring closure of the resulting 6-sulphonate (**13**) with sodium methoxide in methanol, yielded a syrupy anhydro compound, the only possible structure for which is 4,6-anhydro-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**14**). Compound **14** was shown to be identical with the product obtained by methylation of anhydro compound *B* with silver oxide and methyl iodide. These reactions prove that anhydro compound *B* is 4,6-anhydro-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**17**), and that compound *A*, from which *B* was derived, is the 1-*O*-benzoyloxetan (**16**).

The second approach was based on Tokuyama's preparation of anhydro compound *C* from the 1,6-di(toluene-*p*-sulphonate) (**7**)[14]. Although inspection of a molecular model of **7** indicates that formation of the highly strained 1,4-anhydro compound (**11**, R = OTs) is unlikely, in theory, either a 4,6- or a 1,4-anhydro compound could

be formed, depending on which of the two tosyl groups is displaced by the C-4 oxygen anion. To determine which of the two sulphonyl groups is displaced in this reaction, a 1,6-di-*O*-sulphonyl compound having dissimilar sulphonyloxy groups was prepared, namely, 6-*O*-benzenesulphonyl-2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuran-



ose (**19**) from **15** (R = OTs) as outlined. Treatment of disulphonate **19** under the conditions described by Tokuyama for ring closure of **7** afforded an 85% yield of the same anhydro compound *C* as was formed from **7**. This result shows that the 6-*O*-benzenesulphonyl group had been displaced, and that the anhydro compound *C* was therefore 4,6-anhydro-2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose (**20**) and not the 1,4-anhydro compound (**11**, R = OTs).

The reactions undergone by anhydro compound *C* are consistent with the oxetan structure. Tokuyama reported[14] that compound *C* reacted with liquid ammonia at 100°, and with aniline at 160°, to give 1,6-diamino-1,6-dideoxy- and 1,6-dideoxy-2,3-*O*-isopropylidene-1,6-bis(phenylamino)-α-L-*xylo*-hexulofuranose, respectively.

Compound *C* underwent ring opening and desulphonylation when refluxed with sodium benzylthiolate in methanol to give 6-*S*-benzyl-2,3-*O*-isopropylidene-6-thio-α-L-*xylo*-hexulofuranose[17]. Searles[18] has shown that oxetan rings are opened by a variety of nucleophiles such as amines and thiols. These reactions take place much less readily than with epoxides, but the reaction conditions used by Tokuyama were severe enough to ensure ring cleavage and, in two cases, replacement of the 1-*O*-tosyl group.

The 1-*O*-tosyl group of oxetan **20** did not undergo nucleophilic displacement easily. This lack of reactivity is characteristic of C-1 sulphonated hexuloses[19], and is attributed to the electronic effects of the two neighbouring C-2 oxygen atoms. When oxetan **20** was refluxed with sodium iodide in butanone, replacement of the 1-*O*-tosyl group proceeded only to a minor extent. However, with sodium iodide in *N,N*-dimethylformamide, most of the starting material had reacted after 22 h at 140°, and two crystalline compounds were obtained after fractionation of the crude product on silica gel. The minor product was shown to be a di-iodo compound, and was, presumably, the 1,6-di-iodo derivative (**23**) formed by attack of iodide ion at C-6 of the oxetan ring of (**22**, R = I); a similar reaction was observed by Horwitz[20] with a nucleoside oxetan. Thus, treatment of 1-(3,5-anhydro-2-deoxy-β-D-*threo*-pentofuranosyl)-thymine with sodium iodide in butanone, containing a trace of acetic acid to facilitate ring-opening by protonation of the initially formed anion, afforded 1-(2,5-dideoxy-5-iodo-β-D-*threo*-pentofuranosyl)thymine. The major product from the reaction of oxetan **20** with sodium iodide in *N,N*-dimethylformamide was the expected 4,6-anhydro-1-deoxy-1-iodo-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**22**, R = I). Reduction of this compound with hydrogen in the presence of Raney nickel then afforded the 1-deoxyoxetan (**22**, R = H). The n.m.r. spectrum of a solution of **22** (R = H) in deuterochloroform showed, in addition to the isopropylidene signals, a sharp singlet at τ 8.14 assignable to a *C*-methyl group. A comparison of the three alternative structures (**9**, **10**, and **11**) for the original anhydro compound *C* shows that only with **9** (R = OTs) and **11** (R = OTs) could replacement of a toluene-*p*-sulphonate group with iodide ion, followed by reduction, give a *C*-methyl derivative. A *C*-methyl derivative derived in this way from C-6 of **11** (R = OTs) would give a doublet in the n.m.r. spectrum due to the coupling (6–7 c.p.s.) with the adjacent hydrogen atom on C-5, whereas a *C*-methyl group derived from C-1 of **9** (R = OTs) would not be coupled, and would, therefore, give rise to a singlet. Since the n.m.r. spectra showed that the conversion of **20** into **22** (R = H) was not accompanied by structural rearrangement, the methyl singlet observed in the spectrum of the 1-deoxy derivative (**22**, R = H) provides conclusive evidence that the anhydro compound *C* has the 4,6-oxetan structure (**20**). Therefore, all of the anhydro compounds reported by Tokuyama[14,15,17] must be revised accordingly. The n.m.r. spectra of the oxetan derivatives referred to in this paper will be discussed in detail elsewhere.

The formation of 4,6-anhydro compounds from some of the precursors described by Tokuyama appears to involve either anhydro-ring or tosyl group migrations. For example, treatment of the known 2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose (**18**) with sodium hydroxide in liquid ammonia for prolonged periods

yielded[15] the anhydro compound *B*, which we have now shown to be 4,6-anhydro-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**17**). This reaction could involve either an inter- or intra-molecular migration of a tosyl group to give the 6-sulphonate (**3**), which would then afford the oxetan (**17**). Although intramolecular migrations of tosyl groups have not been reported, Cope and Shen[21] have demonstrated the occurrence of an intermolecular tosyl migration in the preparation of 1,4:2,5:3,6-trianhydro-D-mannitol from 1,4:3,6-dianhydro-5-*O*-tosyl-D-mannitol. Alternatively, the formation of oxetan **17** from the 1-toluene-*p*-sulphonate (**18**) could involve the formation of a 1,6-anhydro intermediate (**10**, R=OH), which could then undergo an anhydro ring migration by attack of the C-4 oxygen anion on C-6.

Tokuyama's report that anhydro compound *C* [now shown to be the 1-*O*-tosyloxetan (**20**)] was formed from the 4,6-disulphonate (**8**) is of interest in view of the report of Helferich and Burgdorf[22] that 1,2-*O*-isopropylidene-3,5-di-*O*-methanesulphonyl-α-D-xylofuranose reacts with base to give 3,5-anhydro-1,2-*O*-isopropylidene-α-D-xylofuranose. If such a reaction took place with the L-*xylo*-hexulofuranose compound (**8**), both tosyl groups would be lost, and the oxetan (**17**), rather than the 1-*O*-tosyloxetan (**20**), would be formed. If substantiated, this reaction could involve a tosyl group migration (either inter- or intra-molecular) from C-4 to C-1 to give the 1,6-di(toluene-*p*-sulphonate) (**7**), which would then afford the 1-*O*-tosyloxetan (**20**). To test the possibility of such a migration during the formation of **20** from **8**, 4-*O*-benzenesulphonyl-1-*O*-benzoyl-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose (**21**, R=OBz) was prepared by benzenesulphonylation of **1**. If a migration takes place, the product resulting from the interaction of the mixed sulphonate (**21**, R=OBz) with base would be 4,6-anhydro-1-*O*-benzenesulphonyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose. When the 1-benzoate (**21**, R=OBz) was treated with potassium hydroxide in aqueous ethanol, either for 0.5 h at room temperature, or for 4 h at 50°, the debenzoylated compound (**21**, R=OH) was the major product, although chromatography indicated the presence of a minor component in the reaction mixture. The minor component was isolated by preparative thin-layer chromatography, but, although a crystalline compound was obtained, it was not completely pure. The n.m.r. spectrum (deuterochloroform) showed the presence of five aromatic protons and the absence of an aromatic methyl signal, thus indicating that the tosyl group, but not the benzenesulphonyl group, had been lost. However, this spectrum bore no resemblance to that of 4,6-anhydro-2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose (**20**), and the compound was clearly not an oxetan. The most likely structure for this compound is the 1,6-anhydride (**10**, R=OBs). The n.m.r. spectrum is consistent with this structure, but no supporting chemical evidence is at present available. This result, which is clearly at variance with that of Tokuyama and co-workers, suggests that their starting material could not have been the 4,6-di(toluene-*p*-sulphonate) **8**. Final conclusions cannot be made until Tokuyama's work has been repeated, and structural proofs of the precursors carried out.

EXPERIMENTAL

Concentrations were carried out under reduced pressure. Melting points were determined on a Kofler microstage apparatus, and are uncorrected. Thin-layer chromatography was performed on silica gel G (Merck) with the indicated solvent system. The separated materials were detected by spraying with 5%, ethanolic sulphuric acid, followed by heating for *ca.* 10 min at 110–115°. Optical rotations were measured on a Zeiss Polarimeter in 10-cm tubes of 1- or 10-ml capacity. Chloroform solutions were used unless otherwise stated. Gas–liquid chromatography (g.l.c.) was performed on an F and M Biochemical gas chromatograph Model 400, equipped with a 6′ column packed with 3.1% S.E. 30 on Diatoport S, with nitrogen as carrier gas. N.m.r. spectra were measured on a Varian A-60 spectrometer with tetramethylsilane as internal standard. Light petroleum (b.p. 80–100°) was used unless otherwise stated. Pyridine was dried by three distillations from phosphoric oxide, and stored over potassium hydroxide. Methanol was dried by the iodine–magnesium method. Butanone was dried over potassium carbonate, and purified by distillation from sodium iodide.

*2,3-O-Isopropylidene-6-O-tosyl-α-*L-xylo-*hexulofuranose* (3). — A solution of 1-*O*-benzoyl-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose[12] (1) (2.0 g) in dry methanol (30 ml) was treated at room temperature with sodium methoxide (0.5 ml of a 0.1N solution in methanol). After 18 h, the solution was concentrated to a syrup which spontaneously crystallised. After two recrystallisations from ethyl acetate–petroleum, the product was obtained as fine needles (1.5 g, 96%), m.p. 91–92°, $[\alpha]_D^{24}+10.3°$ (*c* 2.1, acetone) (Found: C, 51.5; H, 6.10; S, 8.5. $C_{16}H_{22}O_8$ S calc.: C, 51.3; H, 5.9; S, 8.55%). The melting point and specific rotation differed from those reported by Tokuyama[15], namely, m.p. 105° and $[\alpha]_D^{22}+15.1°$(*c* 1.075, acetone).

*6-Deoxy-6-iodo-2,3-*O-*isopropylidene-α-*L-xylo-*hexulofuranose* (4). — (*i*) Debenzoylation of 1-*O*-benzoyl-6-deoxy-6-iodo-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose[12] (2) (2.0 g) with sodium methoxide as described above, with recrystallisation of the product from ethyl acetate–petroleum, afforded fine needles (1.3 g, 87%), in two crops, m.p. 118–121°. An analytical sample, obtained by sublimation at 115° (bath) 0.25 mm, had m.p. 120–121°, $[\alpha]_D^{24}+46.1°$ (*c* 3.1) (Found: C, 32.8; H, 4.65; I, 38.3. $C_9H_{15}IO_5$ calc.: C, 32.7; H, 4.6; I, 38.4%).

(*ii*) A solution of 2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose (3) (500 mg) in butanone (5 ml) containing sodium iodide (500 mg) was refluxed for 15 h. The sodium toluene-*p*-sulphonate was filtered off, and the filtrate concentrated to dryness. The residue was partitioned between water (10 ml) and chloroform (10 ml), and the chloroform layer was washed with water, dried (Na_2SO_4), and concentrated to a colourless syrup, which crystallised on standing. Sublimation of this solid at 110° (bath)/0.25 mm afforded pure material (350 mg, 80%), m.p. and mixed m.p. 120–121°. The i.r. spectrum was identical with that of material prepared as above.

*6-Deoxy-2,3-*O-*isopropylidene-α-*L-xylo-*hexulofuranose* (5). — 6-Deoxy-6-iodo-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (4) (300 mg) in methanol (10 ml)

containing Raney nickel (100 mg) and sodium carbonate (100 mg) was hydrogenated at room temperature and pressure for 4 h. The catalyst was filtered off, and the filtrate evaporated to dryness. A filtered solution of the residue in chloroform (10 ml) was washed with water (5 ml), dried (Na_2SO_4), and evaporated to a syrup (180 mg, 97%), which crystallised on standing. Pure material, obtained by sublimation at 80° (bath)/0.5 mm, had m.p. 60–63°, $[\alpha]_D^{22}+16.7°$ (*c* 2, ethanol). Müller and Reichstein[16] reported m.p. 62–63° and $[\alpha]_D^{21}+17.9°$ (*c* 2.8, ethanol).

*4,6-Anhydro-1-O-benzoyl-2,3-O-isopropylidene-α-*L*-xylo-hexulofuranose* (*A*, **16**). — (*i*) A solution of 1-*O*-benzoyl-2,3-*O*-isopropylidene-6-deoxy-6-iodo-α-L-*xylo*-hexulofuranose[12] (**2**) (3.0 g) in dry pyridine (25 ml) was shaken at room temperature with anhydrous silver fluoride (3 g). T.l.c. (ether–petroleum, 1:1) showed that the reaction was complete within 2.5 h, and that one major and three minor products had been formed. The black reaction mixture was diluted with ether (50 ml), the supernatant was decanted, and the residue was shaken with ether (2 × 50 ml). The combined ether–pyridine solution was decolourised by passage through a small column of silica gel. The colourless filtrate, ether and washings, were evaporated at 40° to give a syrup that slowly crystallised (2.0 g, 94%), m.p. 40–57°. Analytically pure material was obtained by distillation of a sample at 110° (bath)/0.25 mm. The distillate crystallised spontaneously and had m.p. 55–57°, $[\alpha]_D^{24}-21.7°$ (*c* 2.3) (Found: C, 62.65; H, 6.1. $C_{16}H_{18}O_6$ calc.: C, 62.7; H, 5.9%).

(*ii*) A solution of 1-*O*-benzoyl-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose[12] (**1**) (2.0 g) in pyridine (20 ml) was shaken at room temperature with anhydrous silver fluoride (2.0 g). The reaction proceeded more slowly than for the corresponding 6-deoxy-6-iodo compound (**3**) and was complete after 9 h. The reaction mixture was processed as described above to give crude material (1.2 g, 94%), m.p. 40–54°. Pure material obtained as above had m.p. and mixed m.p. 55–57°.

*4,6-Anhydro-2,3-O-isopropylidene-α-*L*-xylo-hexulofuranose* (*B*, **17**). — (*i*) 4,6-Anhydro-1-*O*-benzoyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**16**) (1.0 g) was dissolved in dry methanol (25 ml) and treated at room temperature with sodium methoxide (2.0 ml of a 0.1N solution in methanol) for 14 h, and the solution was then concentrated to dryness. A solution of the solid residue in acetone was filtered and concentrated. Most of the methyl benzoate was removed in high vacuum at 40° after several hours. The solid was finally purified by sublimation at 85–90° (bath)/0.3 mm (yield: 550 mg, 83%). This compound did not have a sharp melting point, but softened from 80° (with sublimation) and finally melted near 95°; $[\alpha]_D^{24}-33.0°$ (*c* 1.5) (Found: C, 53.4; H, 6.8. $C_9H_{14}O_5$ calc.: C, 53.5; H, 7.0%). Tokuyama[15] reported m.p. 92° and $[\alpha]_D^{24.5}-34.5°$.

(*ii*) A solution of 2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose (**3**) (200 mg) in 0.5N ethanolic potassium hydroxide (2 ml) was maintained for 2 h at 50°. The reaction mixture was cooled and filtered from the precipitated sodium toluene-*p*-sulphonate, and the filtrate and washings were evaporated to dryness. The residue was partitioned between water (5 ml) and chloroform (3 × 5 ml). The combined chloroform extracts were washed with water (5 ml), dried and evaporated to a crystalline

residue (80 mg, 74%). Sublimation at 85–90° (bath)/0.3 mm gave pure material, which had an i.r. spectrum identical with that of material prepared above.

(*iii*) A solution of 6-deoxy-6-iodo-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**4**) (700 mg) in pyridine (8 ml) was shaken with anhydrous silver fluoride (700 mg) for 7 h. The dark reaction mixture was passed through a small column of silica gel, and the column was washed with pyridine until the effluent was colourless. The pyridine solution was evaporated to dryness at <40°. The dark residue was triturated with ethanol, some residual silver salts were removed by filtration, and the filtrate was evaporated to dryness. Sublimation afforded pure material (250 mg, 58%), identical with that prepared above. Similarly, a 50% yield of pure compound **17** was obtained from the reaction between anhydrous silver fluoride and 2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose (**3**).

2,3-O-*Isopropylidene-1*-O-*methyl*-α-L-xylo-*hexulofuranose* (**12**). — A solution of 2,3:4,6-di-*O*-isopropylidene-1-*O*-methyl-α-L-*xylo*-hexulofuranose[23] (**15**, R = OMe) (5.0 g) in 50% acetic acid (50 ml) was maintained for 6 h at 35°. The solution was neutralised with an aqueous solution of sodium carbonate, and the solution extracted with chloroform (3 × 40 ml). The combined chloroform extracts were washed with water, dried (Na_2SO_4), and evaporated to a colourless syrup (3.3 g, 77%), b.p. 140° (bath)/1 mm. (Found: C, 50.9; H, 7.9. $C_{10}H_{18}O_6$ calc.: C, 51.3; H, 7.7%).

2,3-O-*Isopropylidene-1*-O-*methyl-6*-O-*tosyl*-α-L-xylo-*hexulofuranose* (**13**). — A solution of tosyl chloride (1.3 g) in pyridine (10 ml) was added to a cooled solution (−5°) of 2,3-*O*-isopropylidene-1-*O*-methyl-α-L-*xylo*-hexulofuranose (**12**) (1.5 g) in pyridine (10 ml) over a period of 2 h. The solution was then warmed to room temperature and kept overnight. Concentration of the reaction mixture afforded a thin syrup that contained one major and two minor components (t.l.c.). Chloroform (40 ml) was added to the syrup, and the solution was washed sequentially with N hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated to a syrup (2.3 g), which contained the 6-toluene-*p*-sulphonate (**13**). Pure material (1.8 g, 70%) was obtained by chromatography of the crude syrup on silica gel with benzene–methanol (10:1). Crystallisation of the pure syrup occurred only after storage for several weeks at room temperature. Recrystallisation from benzene–petroleum (3:2) afforded needles, which, although analytically pure, reverted gradually to a syrup, and melted over a wide range (40–50°), $[\alpha]_D^{20}$ +25.3° (*c* 3.4) (Found: C, 52.7; H, 6.1. $C_{17}H_{24}O_8S$ calc.: C, 52.6; H, 6.2%).

4,6-Anhydro-2,3-O-*isopropylidene-1*-O-*methyl*-α-L-xylo-*hexulofuranose* (**14**). — (*i*) Syrupy 2,3-*O*-isopropylidene-1 *O*-methyl-6-*O*-tosyl-α-L-*xylo*-hexulofuranose (**13**) (3.0 g) was dissolved in dry methanol (30 ml) containing sodium (360 mg). The solution was kept at room temperature for 30 h; t.l.c. (benzene–methanol, 8:1) then showed complete reaction, with the formation of one main component together with one minor component, which corresponded in R_F to 2,3-*O*-isopropylidene-1-*O*-methyl-α-L-*xylo*-hexulofuranose (**12**). The reaction mixture was evaporated to dryness, and the syrupy residue partitioned between water (100 ml) and chloroform (2 × 30 ml). The chloroform extracts were washed with water (50 ml), dried (Na_2SO_4), and

evaporated to afford a pale-yellow syrup that was further purified by chromatography on silica gel with benzene–ether (8:1) The appropriate fractions containing the main component were combined and evaporated to a colourless syrup, which, on distillation at 100° (bath)/0.25 mm, gave analytically pure material, $[\alpha]_D^{24} -26.8°$ (*c* 4.5) (Found: C, 55.5; H, 7.5. $C_{10}H_{16}O_5$ calc.: C, 55.4; H, 7.5%).

(*ii*) To a stirred solution of 4,6-anhydro-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**17**) (200 mg) in refluxing methyl iodide (2.0 ml) was added freshly prepared silver oxide (1.0 g) in five equal portions at half-hourly intervals. More methyl iodide (1.0 ml) was then added, and the heating continued for a further 3 h. The cooled reaction mixture was diluted with acetone (25 ml) and then filtered. The residue was washed with hot acetone, and the combined filtrate and washings were concentrated to a pale-yellow syrup (193 mg, 90%), b.p. 100° (bath)/0.25 mm. This product had an i.r. spectrum identical with that of **14** as prepared above, and had the same retention time (8 min) at 130° in g.l.c.

2,3:4,6-Di-O-*isopropylidene-1*-O-*tosyl*-α-L-xylo-*hexulofuranose* (**15**, R = OTs). — Esterification of 2,3:4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulofuranose[24] (10.0 g) with tosyl chloride in pyridine, in the usual manner, afforded compound **15** (R = OTs) (12.0 g, 76%), which, after two recrystallisations from aqueous ethanol and one from petroleum, had m.p. 65–74°, $[\alpha]_D^{23} +3.0°$ (*c* 4). The melting point, which was not changed on repeated recrystallisation, differed from those previously recorded, namely, m.p. 102–103°, $[\alpha]_D^{22} +4.2°$ (*c* 5)[25]; and m.p. 101–102°, $[\alpha]_D^{22} +2.8°$ (*c* 5)[26] (Found: C, 55.1; H, 6.3; S, 7.7. $C_{19}H_{26}O_8S$ calc.: C, 55.1; H, 6.3; S, 7.7%).

2,3-O-*Isopropylidene-1*-O-*tosyl*-α-L-xylo-*hexulofuranose* (**18**). — Treatment of 2,3:4,6-di-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose (**15**, R = OTs) with 50% acetic acid for 7 h at 35°, with isolation of the product as described for the corresponding 1-methyl ether (**12**), afforded (**18**) as colourless needles, m.p. 106–108°, $[\alpha]_D^{23} +16.3°$ (*c* 3) (Found: C, 51.2; H, 5.9; S, 8.45. $C_{16}H_{22}O_8S$ calc.: C, 51.3; H, 5.9; S, 8.55%). Sullivan[25] reported m.p. 120°, $[\alpha]_D^{28} +15.7°$ (*c* 3) for this compound.

6-O-*Benzenesulphonyl-2,3*-O-*isopropylidene-1*-O-*tosyl*-α-L-xylo-*hexulofuranose* (**19**). — A solution of 2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose (**18**) (5.0 g) in dry pyridine (40 ml) was cooled to −5°, and a solution of benzenesulphonyl chloride (1.9 ml, 1.1 mol.) in pyridine (10 ml) was added dropwise over a period of 1 h. The reaction mixture was kept at room temperature for 16 h, and then tipped into cold water (200 ml). The crystalline product was removed by filtration, and recrystallised from hot methanol to give needles (5.5 g, 80%) of the 6-benzenesulphonate. Recrystallisation from ethyl acetate–petroleum afforded pure material (5.0 g, 73%), m.p. 143–145°, $[\alpha]_D^{24} +15.5°$ (*c* 5.8) (Found: C, 51.5; H, 5.2; S, 12.4. $C_{22}H_{26}O_{10}S_2$ calc.: C, 51.35; H, 5.1; S, 12.5%).

4,6-Anhydro-2,3-O-*isopropylidene-1*-O-*tosyl*-α-L-xylo-*hexulofuranose* (*C*, **20**). — (*i*) A solution of tosyl chloride (600 mg) in pyridine was added dropwise to a cooled (5°) solution of 4,6-anhydro-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**17**) (420 mg). The solution was kept at room temperature for 16 h, and then poured into cold water (75 ml). The crystalline product was filtered off and recrystallised from hot

methanol to give needles of compound *C* (600 mg, 81%), m.p. 114–115°, $[\alpha]_D^{24}$ —11.2° (*c* 4, acetone) (Found: C, 53.8; H, 5.7; S, 9.0. $C_{16}H_{20}O_7S$ calc.: C, 53.8; H, 5.7; S, 8.9%). Tokuyama[15] reported m.p. 115°, $[\alpha]_D^{24}$ —11.6 (*c* 1.04, acetone) for this compound.

(*ii*) 6-*O*-Benzenesulphonyl-2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose (1.0 g) was dissolved in 0.5N potassium hydroxide in ethanol (10 ml), and the solution maintained at 50° for 1 h. The solution was filtered, and chloroform (40 ml) was added. The solution was washed with water (2 × 50 ml), dried (Na_2SO_4), and concentrated to afford a solid residue that crystallised from methanol as needles, m.p. and mixed m.p. 113–115°, $[\alpha]_D^{23}$ —11.2° (*c* 4.0, acetone). The i.r. spectrum and retention time in g.l.c. (40 min at 180°) were identical with those of material prepared as above and from the 1,6-di(toluene-*p*-sulphonate) (7) by Tokuyama's procedure.

*4,6-Anhydro-1-deoxy-1-iodo-2,3-*O*-isopropylidene-α-*L*-xylo-hexulofuranose* (**22**, R = I). — 4,6-Anhydro-2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose (**20**) (2.0 g) was heated in *N*,*N*-dimethylformamide (50 ml) containing sodium iodide (3.0 g) for 22 h at 140°. T.l.c. (benzene–ether, 2:1) showed that most of the starting material had reacted, and that one major and one minor product had been formed. The cooled solution was evaporated at 70° until most of the solvent had been removed. The dark, syrupy residue was partitioned between chloroform (50 ml) and dilute sodium thiosulphate solution (50 ml); the aqueous layer was extracted with fresh chloroform (50 ml), and the combined extracts were washed with water (2 × 50 ml). The solution was dried (Na_2SO_4), and concentrated to a dark syrup. This was placed on a column of silica gel (2.5 × 50 cm), and elution with benzene–ether (4:1) gave fractions containing the minor component in the 150–200-ml portion of the effluent. Concentration of this fraction afforded a white solid (60 mg), which was purified by vacuum sublimation to give needles of 1,6-dideoxy-1,6-diiodo-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**23**), m.p. 137–139° (Found: C, 24.8; H, 3.3; I, 56.8. $C_9H_{14}O_4I_2$ calc.: C, 24.55; H, 3.2; I, 57.6%).

Concentration of the 220–370-ml portion of the effluent gave the major component as a syrup (630 mg, 36%), which readily crystallised as needles from petroleum, m.p. 60–62°, $[\alpha]_D^{24}$ —35.7° (*c* 3.0) (Found: C, 34.5; H, 4.1; I, 40.6. $C_9H_{13}IO_4$ calc.: C, 34.6; H, 4.2; I, 40.7%).

*4,6-Anhydro-1-deoxy-2,3-*O*-isopropylidene-α-*L*-xylo-hexulofuranose* (**22**, R = H). — A solution of 4,6-anhydro-1-deoxy-1-iodo-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**22**, R = I) (190 mg) in methanol (10 ml) containing Raney nickel (100 mg) and sodium carbonate (100 mg) was hydrogenated at room temperature and pressure for 2.5 h. The catalyst was removed by filtration, and the combined filtrate and washings were evaporated at 30°. The semi-solid residue, consisting of the product and sodium carbonate, was partitioned between water (5 ml) and chloroform (2 × 5 ml). The chloroform solution was washed with water (5 ml), dried (Na_2SO_4), and concentrated to afford a colourless, mobile syrup (100 mg, 89%), which was homogeneous by g.l.c. (retention time, 5 min at 130°), but which failed to crystallise. An analytical sample was obtained by distillation at 60° (bath)/0.1 mm; $[\alpha]_D^{24}$ —18.5° (*c* 2.25) (Found: C, 57.95; H, 7.6. $C_9H_{14}O_4$ calc.: C, 58.05; H, 7.6%).

4-O-Benzenesulphonyl-1-O-benzoyl-2,3-O-isopropylidene-6-O-tosyl-α-L-xylo-hexulofuranose (**21**, R = OBz). Benzenesulphonyl chloride (0.73 g) was added to a solution of 1-*O*-benzoyl-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose (1 g) in pyridine (10 ml), and the solution was kept at room temperature. After 16 h, t.l.c. (benzene–ether, 2:1) showed that the reaction had proceeded only to a minor extent. Benzenesulphonyl chloride (0.35 g; total, 3 equivalents) was added, and the solution was maintained at 40° for 48 h. The reaction mixture was evaporated at 40° to a dark syrup, from which the last traces of pyridine were removed by co-distillation with dry toluene. The product was separated from a small amount of starting material by chromatography on silica gel (benzene-ether, 2:1), and was obtained as a colourless syrup (0.78 g, 60%).

Action of ethanolic potassium hydroxide on compound **21**. — (*i*) The syrupy 4-benzenesulphonate (**21**, R = OBz) (250 mg) was added to a solution of potassium hydroxide (25 mg) in ethanol (3 ml) at room temperature. Reaction was complete after 0.5 h, as shown by t.l.c. (benzene–ether, 2:1) which showed one main product, together with a small proportion of a minor product. The reaction mixture was diluted with chloroform (10 ml), the solution was washed with water (2 × 5 ml), dried (Na_2SO_4), and evaporated to a crystalline residue. Two recrystallisations from aqueous ethanol afforded needles (140 mg), 69%) of 4-*O*-benzenesulphonyl-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose (**21**, R = OH), m.p. 163–164°, $[\alpha]_D^{22}+17.6°$ (Found: C, 50.3; H, 5.0. $C_{21}H_{26}O_{10}S_2$ calc.: C, 50.2; H, 5.1%).

(*ii*) When the above experiment was repeated at 50°, the minor product was formed more quickly, but, after 4 h, a large proportion of **21** (R = OH) remained. The reaction mixture was filtered to remove some precipitated sodium sulphonate, and processed as described above. The final chloroform solution was evaporated to a small volume, and fractionated by preparative t.l.c. The zone containing the minor component was removed and extracted with hot acetone. Concentration afforded a syrup (20 mg), which was contaminated with methyl benzoate. The syrup was crystallised from aqueous ethanol, but was not obtained analytically pure. The n.m.r. spectrum of this compound indicated that it was not an oxetan, but was probably 1,6-anhydro-4-*O*-benzenesulphonyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**10**, R = OBs).

ACKNOWLEDGMENTS

We thank the Science Research Council for an award (to B.A.O.), and Conrad Cone for his advice and assistance.

SUMMARY

Crystalline 4,6-anhydro-1-*O*-benzoyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose (**16**) has been prepared by treatment of either 1-*O*-benzoyl-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose or the corresponding 6-deoxy-6-iodo compound with anhydrous silver fluoride in pyridine. In a similar manner, 4,6-anhydro-2,3-*O*-

isopropylidene-α-L-*xylo*-hexulofuranose (**17**) was prepared from the 6-*O*-tosyl and 6-deoxy-6-iodo derivatives of 2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose. The oxetan **17**, also prepared by debenzoylation of **16**, formed a toluene-*p*-sulphonate (**20**), which was shown to be identical with a compound previously, but incorrectly, reported to be 1,4-anhydro-2,3-*O*-isopropylidene-6-*O*-tosyl-α-L-*xylo*-hexulofuranose. The oxetan structure of the anhydro compounds **16**, **17**, and **20** was proved by three methods. Methylation of **17** afforded a syrupy compound that was identical with 4,6-anhydro-2,3-*O*-isopropylidene-1-*O*-methyl-α-L-*xylo*-hexulofuranose prepared by an unambiguous route. Treatment of 6-*O*-benzenesulphonyl-2,3-*O*-isopropylidene-1-*O*-tosyl-α-L-*xylo*-hexulofuranose with base afforded the anhydro compound **20**, thus showing that the tosyl group of **20** was located at C-1. Replacement of this tosyl group with iodide ion, followed by reduction, afforded a *C*-methyl derivative, the n.m.r. spectrum of which provided unambiguous evidence that the oxetan structure was correct.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

ANION-EXCHANGE SEPARATIONS OF ALDOBIOURONIC ACIDS IN BORATE MEDIUM

OLOF SAMUELSON AND LEIF WICTORIN
Department of Engineering Chemistry, Chalmers Tekniska Högskola, Göteborg (Sweden)
(Received November 3rd, 1966)

INTRODUCTION

Various chromatographic methods are available for the isolation of aldobiouronic acids from plant materials. Some of these are based upon chromatography on cellulose[1], or on carbon–Celite[2], whereas others are based upon the application of anion-exchange resins[3]. A combination of ion-exchange chromatography with other methods has given the most satisfactory results in analyses of plant materials[4]. Sodium acetate and acetic acid have been applied as eluants in recent work on ion-exchange chromatography[5]. Since, in some systems containing hydroxy acids, better resolution has been obtained with sodium tetraborate[6,7], an investigation of this eluant in separations of aldobiouronic acids was deemed to be of interest.

EXPERIMENTAL

Materials. — 4-*O*-(α-D-Galactopyranosyluronic acid)-D-xylose was obtained from birch (*Betula verrucosa*) by acid hydrolysis of a xylan fraction[8], and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-lyxose was prepared from 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose by epimerization[4]. The other acids have been described earlier[5,9].

Anion-exchange columns and analysing system. — All experiments were carried out with a strongly basic resin (Dowex-1, X-8) fractionated to obtain a particle diameter of 23–25 μ. The resin was used in its borate form. The jacketed column, which, unless otherwise mentioned, had an inner diameter of 3 mm and a length of 870 mm, was conditioned with the eluant to be used in the subsequent elution. The eluant was boiled under reflux before being fed into the column, which was kept at 30° by means of circulating water.

In the chromatograms shown, the loading of simple uronic acids was 0.50–0.95 mg, and that of aldobiouronic acids, 0.26–1.51 mg. Before the column was loaded with D-glucofuranurono-6→3-lactone, the lactone ring was opened by keeping the solution at pH 8 for about 5 h. Unless otherwise mentioned, the elution was performed at a flow rate of 8.45 ml. $cm^{-2}.min^{-1}$.

The eluate was monitored continuously[5]. The main components were a peristaltic pump (Technicon Instruments Co., Ltd., Chertsey), and a three-channel absorptio-

meter (LKB-Produkter, Stockholm). The reactions used were oxidation with chromic acid, and reaction with carbazole. In the dichromate method, the green colour formed in the reduction to Cr^{III} was determined at 633 mμ. In the carbazole method, the colour was measured at 531 mμ. Both methods were used simultaneously, except in the experiment shown in Fig. 3, where only the carbazole method was used.

RESULTS AND DISCUSSION

In a large number of runs, each with one uronic acid, the elution volumes of the peaks were determined. From these values, the volume distribution coefficients (D_v) were calculated as usual[10]. The results are given in Table I. Some values obtained in runs with acetic acid are included for comparison. It is seen that, in the borate medium, the separation factors, *i.e.*, the ratios between the distribution coefficients, are favourable with most uronic and aldobiouronic acids studied.

As shown in Table I, the distribution coefficients of all uronic and aldobiouronic acids decrease very rapidly with increased concentration of eluant. This behaviour is typical of hydroxy acids that form borate complexes, and thus appear as multivalent ions[11].

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS (D_v) OF VARIOUS URONIC AND ALDOBIOURONIC ACIDS IN SODIUM TETRABORATE, AND IN ACETIC ACID

Uronic acid	*D_v in various media*				
	Borate, 0.04M	0.05M	0.06M	0.12M	*Acetic acid,* M
2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-lyxose	23.7	15.1			8.6
2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose	30.2	18.3	12.9	4.2	6.1
4-*O*-Methyl-D-glucuronic acid	31.3	20.3	15.2		18.4
2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose	44.3	26.8			3.8
Cellobiouronic acid	49.5	30.8			12.0
2-*O*-(α-D-Glucopyranosyluronic acid)-D-xylose		40.1			8.3
4-*O*-(α-D-Galactopyranosyluronic acid)-D-xylose		57.2	38.2	9.2	5.7
6-*O*-(β-D-Glucopyranosyluronic acid)-D-galactose				32.3	7.2
D-Galacturonic acid				40.8	11.0
D-Glucuronic acid				58.5	22.7

Knowledge about the borate complexes of hydroxy acids is very limited, but certain correlations between the position on the chromatogram and the structure of the acids are evident. One rule, deducible from theoretical considerations, suggests that

the distribution coefficients of aldonic acids should increase with an increased number of hydroxyl groups. By analogy, the early appearance of the two methylated aldobiouronic acids on the chromatogram was expected, as well as the observation that 4-*O*-methyl-D-glucuronic acid appears much earlier than D-glucuronic acid.

In a study of the elution of various disaccharides from a borate column, Khym *et al.*[12] observed that those having a (1→6)-glycosidic linkage were held much more strongly than those having (1→4)-linkages. A theoretical explanation of the exceptionally large distribution coefficients observed with the former group was also presented. It could, therefore, be anticipated that 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose should exhibit a much larger distribution coefficient than the other aldobiouronic acids studied. This is confirmed by the results given in Table I. It is likely that extended studies with other aldobiouronic acids would reveal important correlations between structure, complex formation, and elution behaviour.

In practical applications, it is desirable to use a high concentration of eluant to effect a rapid elution. With most species, the separation factors are less favorable than at low concentration, and the separation of some aldobiouronic acids is not satisfactory. A separation in 0.12M tetraborate of a mixture of three aldobiouronic acids together with D-glucuronic acid is shown in Fig. 1. It is seen that all species are well

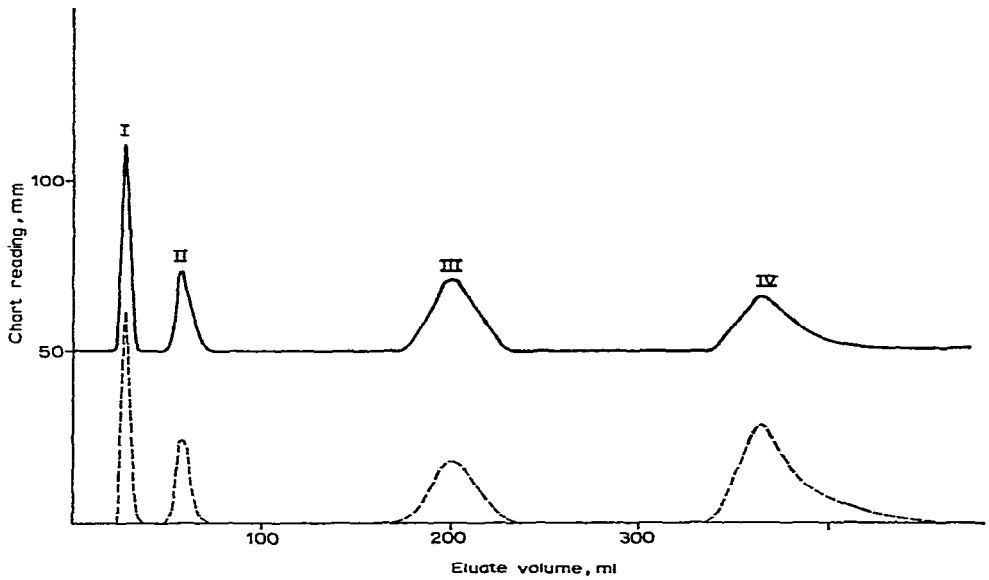


Fig. 1. Separation of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (I), 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose (II), 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (III), and D-glucuronic acid (IV). Analyzed by the carbazole method (— — —), and by the dichromate method (———). Eluant, 0.12M sodium tetraborate.

separated, but that those which appear late in the chromatogram give broad elution curves. This has been observed also in the elution of other types of hydroxy acids in borate medium[6].

A more difficult separation is demonstrated in Fig. 2. To obtain an improved separation of the aldobiouronic acids which appear first in the eluate, the borate concentration was lowered in this experiment. During the first period of elution, the eluant concentration was 0.05M, but, after these species were eluted, the concentration was increased to 0.12M to make it possible to complete the elution within a reasonable

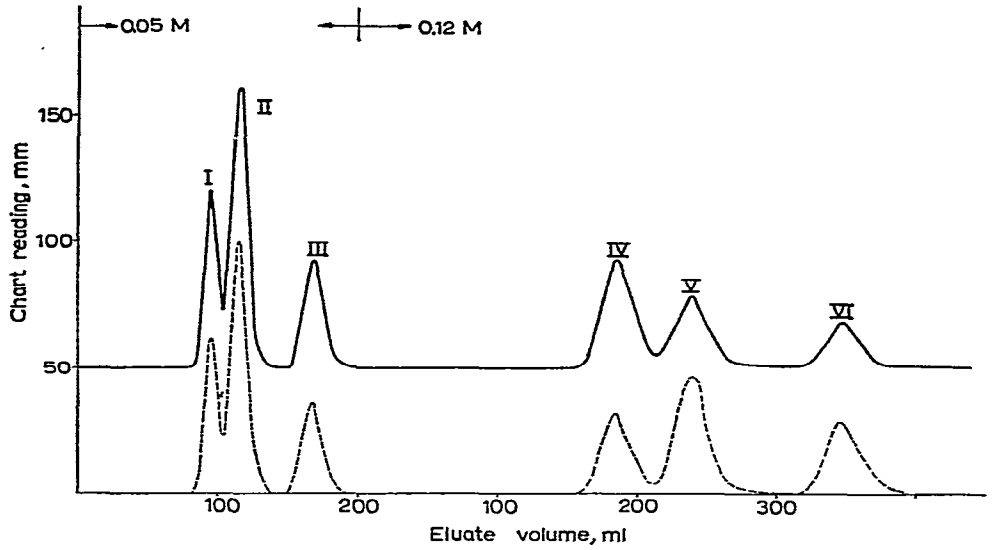


Fig. 2. Separation of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-lyxose (I), 2-*O*-(4-*O* methyl-α-D-glucopyranosyluronic acid)-D-xylose (II), 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose (III), 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (IV), D-galacturonic acid (V), and D-glucuronic acid (VI). Analyzed by the carbazole method (— — —), and by the dichromate method (———). Stepwise elution with 0.05M and 0.12M sodium tetraborate.

time. The first three aldobiouronic acids were eluted in about 6 h, whereas 18 h had elapsed before the last band, D-glucuronic acid, was completely eluted.

A study of the influence of the flow rate upon the chromatographic separations showed that a lowering of the flow rate resulted in only a slight sharpening of the elution curves (Fig. 3). The results indicate that the equilibrium between the external solution and the resin is attained at high rates. The pressure drop in the column was about 20 atmospheres in the runs at the higher rate of flow. A further increase in flow rate led to mechanical troubles.

The results given above show that the method is very time-consuming when galacturonic and glucuronic acids are present in the solution to be analyzed. It is worth mentioning that these two uronic acids have previously been studied in borate medium by Hallén[13]. To speed up the elution, this author used a borate solution containing hydrogen carbonate. Use of the latter can, however, give rise to various complications with the types of compound of interest in analyses of plant materials. In chromatographic separations of other acids, elution at high temperature is advantageous, but cannot be recommended when uronic acids are involved[14]. As can be

seen from Table I, another disadvantage of borate elution is that a 4-*O*-methylglucuronic acid has almost the same position on the chromatograms as one of the aldobiouronic acids. A simple group separation of aldobiouronic acids from hexuronic acids can be made by ion-exchange chromatography in sodium acetate medium[15], and it is, therefore, recommended that this separation be made before the aldobiouronic acids are analyzed by other methods. All common hexuronic acids can be well separated by a combination of elution with sodium acetate and acetic acid[5], and these separations are more rapid than those in borate medium.

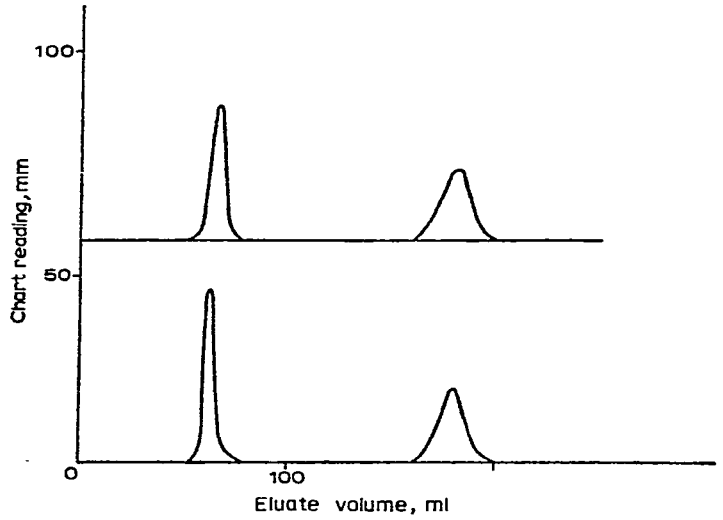


Fig. 3. Influence of the flow rate upon the separation of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (I) and 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose (II). Upper diagram, 8.45 ml. $cm^{-2}.min^{-1}$; lower diagram, 4.23 $ml.cm^{-2}.min^{-1}$. Analyzed by the carbazole method. Eluant, 0.06M sodium tetraborate. Column dimensions, 3 × 675 mm.

Generally speaking, the separation factors of most aldobiouronic acids are more favorable in sodium tetraborate than in sodium acetate and acetic acid. Several aldobiouronic acids can, however, also be separated in these media. Since some acids, which show serious overlapping in acetate, are well separated in acetic acid, and *vice versa*, separations with a combination of these eluants can compete with those in borate medium. With complicated mixtures of aldobiouronic acids, especially those containing only minor proportions of some acids and large proportions of others, a complete resolution can be obtained only with difficulty, even if both acetate and acetic acid are employed as eluants. An example of interest in wood chemistry is the separation of small amounts of 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose from large amounts of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. As can be seen from Table I, the separation factor is about 3.1 in 0.05M tetraborate, and 2.2 in 0.12M solution. In M acetic acid, the separation factor is only 1.07, and, in 0.02M sodium acetate, it is 1.25. Therefore, with this mixture, acetic acid cannot be employed, and borate is much more satisfactory than sodium acetate.

Elution with borate is also useful in controlling the purity of aldobiouronic acids isolated in other chromatographic procedures. It is also worth mentioning that, when dealing with very complicated mixtures of acids of various types, *e.g.*, from hydrolysis of wood pulps, a combination of all three eluants, acetate, acetic acid, and sodium tetraborate, may give improved resolution. The characteristic positions of the peaks on the chromatograms in borate medium are reproducible with deviations from the mean of 2% or less, and are also useful for identification purposes. It is therefore believed that ion-exchange chromatography of aldobiouronic acids in borate medium will be a valuable complement to other chromatographic methods.

ACKNOWLEDGMENT

The financial support of the Swedish Technical Research Council is gratefully acknowledged.

SUMMARY

Mixtures containing several aldobiouronic acids have been separated by anion-exchange chromatography with sodium tetraborate as eluant. With most species, the separation factors were more favorable than those obtained with sodium acetate and acetic acid.

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DÉPLACEMENT NUCLÉOPHILE D'UN ESTER *p*-TOLUÈNESULFONIQUE *exo* SUR PENTOFURANOSE. NOUVELLE MÉTHODE D'ACCÈS AU 3-DÉSOXY-D-*érythro*-PENTOSE*

JACQUES DEFAYE ET JEAN HILDESHEIM
Institut de Chimie des Substances Naturelles, C.N.R.S., 91-Gif-sur-Yvette (France)

(Reçu le 20 novembre 1966)

Dans le cadre de nos travaux sur les 2,5-anhydrides de sucres, nous avons été amenés à envisager l'utilisation de quantités importantes de 3-désoxy-D-*érythro*-pentose.

On connaît, par ailleurs, l'intérêt de ce dérivé trouvé comme composant de la *cordycépine* (3-désoxy-adénosine)[1], métabolite de *Cordyceps militaris* et agent cytostatique[2].

Diverses méthodes[3–7] permettent d'accéder au 3-désoxy-D-*érythro*-pentose. Elles sont dans l'ensemble fondées sur l'introduction, par ouverture d'un époxyde, d'un substituant facilement éliminable par action d'un agent réducteur. Elles présentent par conséquent l'inconvénient de conduire à des mélanges d'isomères dont la séparation est malaisée. La méthode de Walton et coll.[6] qui conduit essentiellement au méthyl 3-désoxy-*β*-D-*érythro*-pentofuranoside par hydrogénation en présence de nickel du méthyl 2,3-anhydro-*β*-D-ribofuranoside est actuellement la meilleure voie d'accès au 3-désoxy-D-*érythro*-pentose, mais le nombre d'étapes est élevé et le rendement final peu important. La méthode introduite par Szabó et Szabó[7] et qui consiste à partir du 3-désoxy-1,2-*O*-isopropylidène-D-*ribo*-hexofuranose constituerait une voie d'accès aisée, cependant ce dernier composé, malgré les améliorations récentes de Murray et Prokop[8] et de Brown et Jones[9], reste encore difficilement accessible.

Dans le présent travail, nous avons tenté d'accéder au 3-désoxy-D-*érythro*-pentose en partant d'un dérivé aisément accessible, le 1,2-*O*-isopropylidène-5-*O*-triphénylméthyl-D-xylofuranose (**1**)[10]. On pouvait penser que des réactions de substitution ou de déplacement sur l'hydroxyle à C-3 seraient susceptibles de conduire à des dérivés halogénés ou soufrés facilement clivables par hydrogénation en dérivés désoxy correspondants.

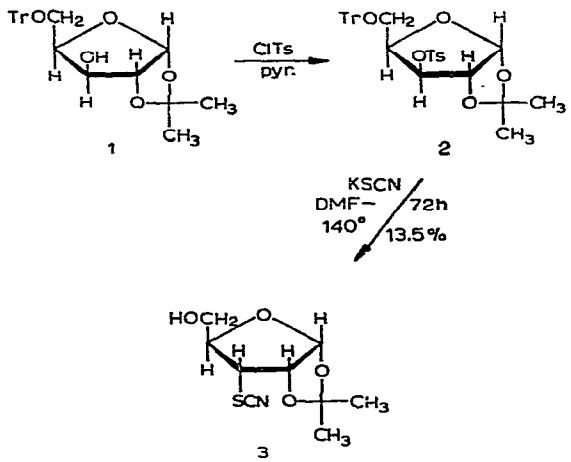
Le composé (**1**) présentait d'autre part l'intérêt de permettre une étude comparative avec les dérivés correspondants du glucofuranose qui ont fait l'objet de nombreux travaux.

Un premier essai nous a conduits sans résultat à tenter sur cette substance (**1**) l'action du triphénylphosphite methiodure (réactif de Rydon)[11]. L'inertie du com-

*Ce travail a fait l'objet d'une communication orale aux Journées de Chimie Organique d'Orsay, 22–24 septembre 1966. Il constitue une partie du mémoire présenté par M. J. Hildesheim en vue d'obtenir le diplôme d'Études Supérieures (Sciences Physiques).

posé (1) à ce réactif peut être attribuée à l'encombrement stérique du groupement isopropylidène; Kochetkov[12,13] a, de même, rapporté l'insuccès d'essais similaires tentés sur le 1,2:5,6-di-*O*-isopropylidène-D-glucofuranose.

Brimacombe[14] a récemment suggéré un accès possible aux désoxy sucres par l'intermédiaire d'un dérivé *oxo*. On sait que des méthodes récentes permettent d'oxyder aisément l'hydroxyle en position 3 d'un cycle furanose[15,16]. La réduction d'une hydrazone correspondante devrait permettre un accès aisé de l'alkane. Cependant, travaillant à titre de substance témoin sur l'hydrazone ou la *p*-tolylsulfonylhydrazone du 1,2:5,6-di-*O*-isopropylidène-D-*ribo*-3-hexulofuranose, nous n'avons pu, en utilisant respectivement les méthodes adaptées de la réaction de Wolff–Kishner[17,18] dans le cas de l'hydrazone, ou la réaction de Caglioti[19] dans le cas de la *p*-tolylsulfonyl hydrazone, obtenir aucune réduction des dérivés correspondants. Dans ce cas, également, l'encombrement des groupements isopropylidènes est probablement de nature à empêcher l'approche de la base[20].



L'insuccès rencontré avec le 1,2:5,6-di-*O*-isopropylidène-D-*ribo*-3-hexulofuranose ne nous a pas incités à poursuivre ce travail sur le dérivé *oxo* en C-3 du xylose.

Nous nous sommes alors tournés vers les possibilités offertes par les réactions de déplacement en partant du 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-5-*O*-triphénylméthyl-D-xylofuranose (2). Dans le cas du 1,2:5,6-di-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-glucofuranose, des réactions de ce type n'ont généralement pas abouti[21–23]. Le seul exemple connu est le déplacement par l'hydrazine, qui conduit en bon rendement au 3-désoxy-3-hydrazino-1,2:5,6-di-*O*-isopropylidène-D-glucofuranose[24]. Un essai préliminaire de réduction du groupe *p*-tolylsulfonyle dans l'ester (2) nous a conduits au 1,2-*O*-isopropylidène-5-*O*-triphénylméthyl-D-xylofuranose (1), ce qui montre bien que le *p*-toluènesulfonate en C-3 chez ce composé a une réactivité analogue, du fait de l'encombrement stérique, à celle de son homologue dans le 1,2:5,6-di-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-glucofuranose. Rappelons que l'action de

l'hydrure de lithium et d'aluminium sur le dérivé du glucofuranose conduit à une détosylation simple[26].

Pour effectuer les déplacements du *p*-toluènesulfonate (2), nous avons choisi des solvants aprotoniques polaires classiques : *N,N*-diméthylformamide (DMF), diméthylsulfoxyde (DMSO) et hexaméthylphosphortriamide (HMPT). En ce qui concerne les nucléophiles, notre choix s'est porté sur les molécules hautement polarisables du type RS^-, susceptibles, en cas de déplacement du tosylate, de fournir un radical clivable par le nickel Raney.

Le tableau I résume les premiers résultats obtenus. Dans tous les cas, le mélange réactionnel brut a été traité, après extraction, par le nickel Raney et soumis à une hydrolyse acide, de façon à cliver le groupement isopropylidène.

TABLEAU I

ESSAIS DE DÉPLACEMENT PAR DES NUCLÉOPHILES DU TYPE $^-$SR DU GROUPE *p*-TOLUÉNESULFONATE EN C-3 DU 1,2-*O*-ISOPROPYLIDÈNE-3-*O*-*p*-TOLYLSULFONYL-5-*O*-TRIPHÉNYLMÉTHYL-D-XYLOFURANOSE (2)

	Nucléophile	*Solvant*	*Température degrés*	*Temps h*	*Rendt. en 3-désoxy-D-érythro-pentose %*
1	KSCN	DMF[a]	140 ± 2	21	10 à 15
2				63	25 à 30
3			146 ± 2	87	Décomposition
4		DMF[a]–HMPT[b] 1:1	100 ± 2	50	Traces
5		DMSO[c]	22	42	0
6			70 ± 2	20	0
7			140 ± 2	63	10
8	$PhCH_2SNa$	MeOH	Ébull.	24	Détosylation
9	PhCOSNa	EtOH	Ébull.	63	Détosylation

[a]*N,N*-Diméthylformamide. [b]Hexaméthylphosphortriamide. [c]Diméthylsulfoxide.

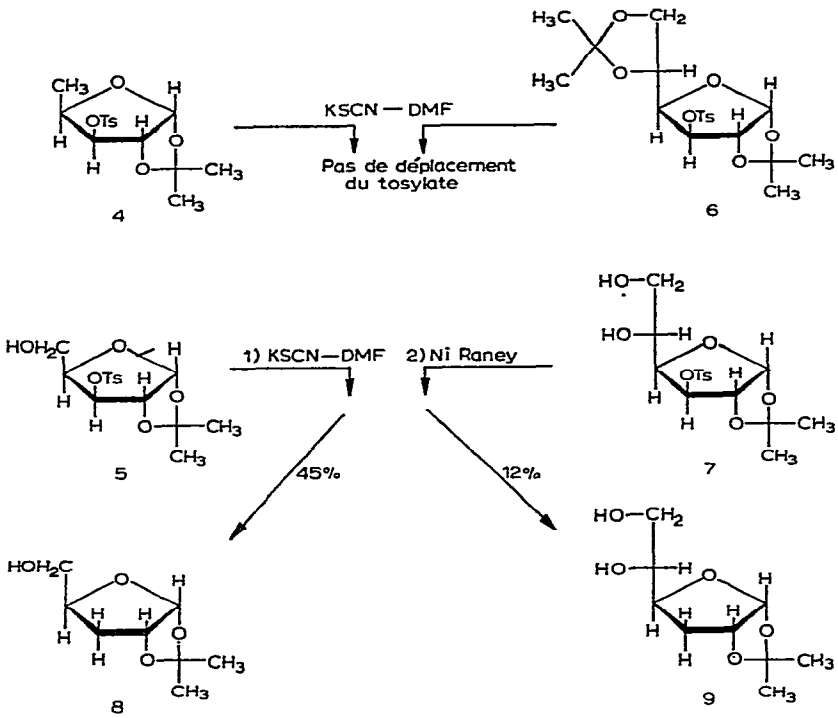
Ces résultats montrent d'une part la supériorité — dans ce cas — de la *N,N*-diméthylformamide sur les deux autres solvants, d'autre part, l'importance des conditions de chauffage.

Pour améliorer le rendement de la réaction en 3-désoxy-D-*érythro*-pentose, il était nécessaire d'en connaître le mécanisme. Nous avons par conséquent repris les conditions de l'essai 2 (tableau I) qui nous avait fourni le rendement maximal en 3-désoxy-D-*érythro*-pentose. Nous avons constaté que le traitement de 2 par le thiocyanate de potassium dans la *N,N*-diméthylformamide à 140° pendant 63 h provoquait la formation d'une importante quantité de triphénylcarbinol (25 à 30%). La scission du groupe triphénylméthyle sur le composé (2) n'est pas due, ainsi que l'a montré un essai témoin effectué en absence de thiocyanate de potassium, à la présence de l'agent nucléophile. Ce résultat suggère néanmoins que le déplacement du tosylate en C-3 dans le composé (2) est essentiellement lié à l'hydrolyse du groupe triphénylméthyle en C-5.

Une chromatographie sur colonne de gel de silice du produit brut de la réaction a permis de séparer, à côté de composé de départ inchangé et de triphénylcarbinol,

une substance cristalline (rendement 12,5%) que nous avons identifié au 3-désoxy-1,2-*O*-isopropylidène-3-thiocyano-D-ribofuranose (3) de la façon suivante :

Le spectre infra-rouge montre une bande hydroxyle à 3500 cm^{-1} et un pic à 2140 cm^{-1} attribuable à un groupement thiocyanate[26]. On ne relève pas de vibrations attribuables à un groupe *p*-tolylsulfonyle ou triphénylméthyle.



Le spectre de masse montre un pic à m/e 216. On sait que les dérivés isopropylidènes de sucres donnent fréquemment un ion à M-15[27]. On remarque également un pic à m/e 173 correspondant au départ de $[CH_3\ CO]^+$ et confirmant la présence d'un groupement *O*-isopropylidène. Un pic à m/e 200 attribuable au départ de $[CH_2OH]^+$ exocyclique suggère enfin que nous sommes en présence d'un 3-désoxy-1,2-*O*-isopropylidène-3-thiocyanopentofuranose. L'analyse élémentaire est également en accord avec cette structure.

Le spectre de résonnance magnétique nucléaire (Fig. 1) montre, outre le doublet à 88 c.p.s. dû au groupement isopropylidène et un singulet d'intensité 1 à 128 c.p.s. attribuable à l'hydroxyle, un doublet de doublets à 220 c.p.s. attribuable à l'hydrogène en C-3* couplé avec les hydrogènes en C-2 et C-4. Les constantes de couplage $J_{2,3}$ 5 c.p.s. et $J_{3,4}$ 10 c.p.s. indiquent respectivement une position *cis* pour les hydrogènes

*Ce déplacement chimique correspond sensiblement aux valeurs données par Mathias[28] pour des thiocyanates d'alcoyle.

en C-2 et C-3 et *trans* pour les hydrogènes en C-3 et C-4. On remarque également à 256 c.p.s. un doublet de quadruplets attribuable à l'hydrogène en C-4 couplé avec le méthylène en C-5 et l'hydrogène en C-3 ($J_{3,4}$ 10 c.p.s. et $J_{4,5}$ 5 c.p.s.).

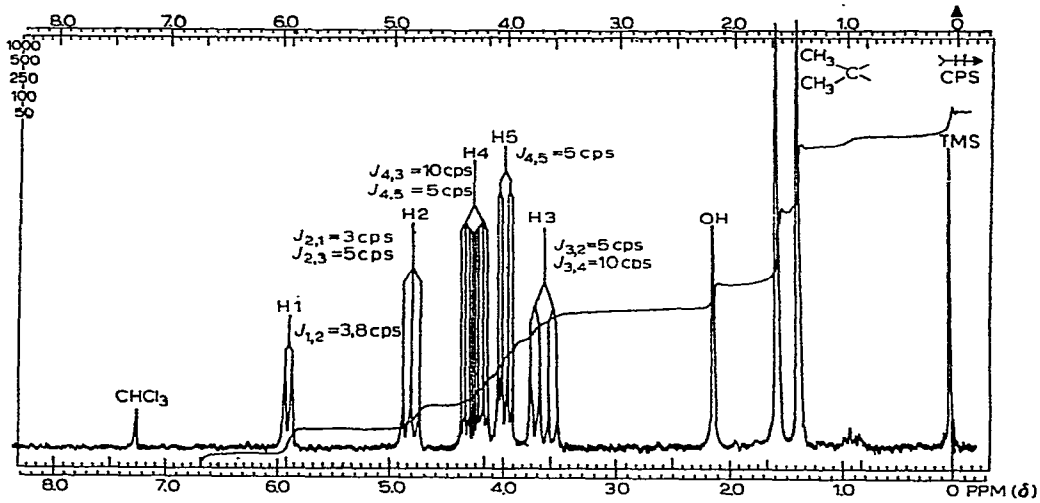


Fig. 1. Spectre de n.m.r. du 3-désoxy-1,2-*O*-isopropylidène-3-thiocyano-D-ribofuranose (3) mesuré dans $CDCl_3$ à la fréquence de 60 Mc.

Étant donné que le groupement isopropylidène est resté inchangé et que nous avons conservé la forme furanose, comme le montre la spectrométrie de masse, il est peu probable que la configuration du carbone en position 4 ait varié comparativement au produit de départ (2). La position *trans* des hydrogènes en C-3 et C-4 confirme par conséquent que nous nous trouvons en présence du composé 3.

L'isolement de ce composé, bien qu'en faible rendement, comme produit essentiel de la réaction du thiocyanate de potassium sur 2 suggère donc que l'ensemble du 3-désoxy-D-*érythro*-pentose isolé dans l'essai 2 (tableau I) passe au moins par le composé intermédiaire (3). Nous n'avons pu mettre en évidence de dérivé tritylé correspondant.

On sait que le déplacement sans assistance d'un ester sulfonique en C-3 sur cycle furanoïde est un phénomène particulièrement rare. Ainsi, Jamieson et Brown[23] n'ont pu déplacer le sulfonate du 1,2:5,6-di-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-glucofuranose (6) par le benzylmercaptan. Un agent nucléophile puissant, l'azothydrate de sodium, est également sans effet[21].

Hughes et Speakman[29] ont réussi, récemment, le déplacement d'un ester *p*-tolylsulfonique en 3 *endo* sur cycle furanose dans le 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-ribofuranose, ce qui s'explique par le fait que le déplacement des esters sulfoniques est considérablement facilité[30] par leur position *endo* sur le cycle, du fait que l'attaque nucléophile a la possibilité d'intervenir par le côté le moins encombré de la molécule. En revanche, les mêmes auteurs[29] n'ont pu déplacer par l'ion benzoate

dans la *N*-methyl-2-pyrrolidone le *p*-toluènesulfonate *exo* en C-3 du 1,2-*O*-isopropylidène-3,5-di-*O*-*p*-tolylsulfonyl-D-xylofuranose, dans les conditions qui avaient permis le déplacement sur le dérivé ribofuranose correspondant.

Gramera, Ingle et Whistler[31] ont obtenu l'élimination d'un *p*-toluène sulfonate *exo* en C-5 du glucofuranose et montré que cette élimination était aidée par l'assistance de l'hydroxyle en C-3 resté libre.

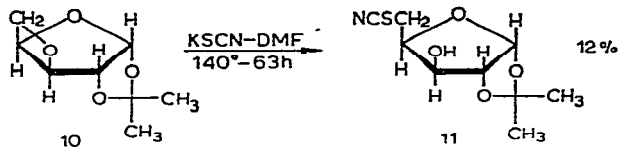
Dans le cas qui nous occupe ici, il était par conséquent important de préciser si un hydroxyle en C-5 libre sur le 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-xylofuranose (**5**) était susceptible de faciliter le déplacement du *p*-toluènesulfonate en C-3 par l'ion SCN^-.

Nous avons, par conséquent, tenté successivement le déplacement d'un ester *p*-toluènesulfonique en C-3 sur le 5-désoxy-1,2-*O*-isopropylidène-3-*O*-*p*-tolylsufonyl-D-xylofuranose (**4**) et sur **5**. Nous avons étendu nos essais à deux composés de stéréochimie voisine, le 1,2:5,6-di-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-glucofuranose (**6**)[32] et le 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-glucofuranose (**7**)[33].

Par traitement de **4** par le thiocyanate de potassium dans les conditions qui ont permis le déplacement du sulfonate en C-3 sur **2**, nous n'avons pu obtenir aucune formation du dérivé thiocyanate recherché. Opérant dans des conditions identiques sur **6**, aucun déplacement du groupe sulfonyle n'est intervenu. Ces résultats confirment par conséquent que le déplacement d'un groupe *p*-toluènesulfonate en C-3 *exo* sur le xylofuranose ou le glucofuranose est improbable sans participation.

Nous avons alors répété nos essais sur **5** et sur **7**. Le premier composé a été préparé par hydrolyse ménagée du radical triphénylméthyle du composé **2**. L'action du thiocyanate de potassium dans la *N*,*N*-diméthylformamide sur le composé **5** a conduit au dérivé thiocyanate attendu, qui après désulfurisation par le nickel Raney a permis d'obtenir le 3-désoxy-1,2-*O*-isopropylidène-D-*érythro*-pentose (**8**) avec un rendement de 45%. Opérant dans des conditions identiques sur le composé **7**, nous avons pu obtenir le 3-désoxy-1,2-*O*-isopropylidène-D-*ribo*-hexofuranose (**9**) avec un rendement de 12%. Ces résultats rendent donc incontestable l'influence de l'hydroxyle en C-5 libre dans le composé **5** et des hydroxyles en C-5 ou C-6 dans le composé **7**.

Une possibilité d'assistance par l'hydroxyle en C-5 dans l'élimination du groupe *p*-toluènesulfonate en C-3 dans le composé **5** consisterait à passer par l'intermédiaire d'un oxétane (**10**).

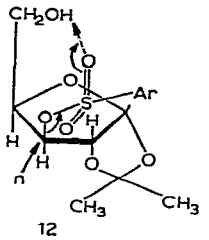


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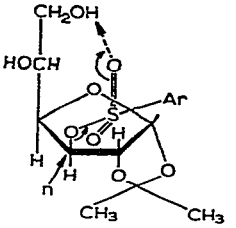
La formation d'un oxétane dans le cas de **5** est, pour des raisons stériques, tout à fait improbable, car elle devrait se faire selon un mécanisme SNi, inusité dans les conditions utilisées. Néanmoins, afin d'éliminer toute possibilité de déplacement du groupe sulfonate en C-3 par un intermédiaire de ce type, nous avons tenté l'ouverture

d'un tel cycle sur le 3,5-anhydro-1,2-*O*-isopropylidène-D-xylofuranose (**10**)[10]. Ce composé s'est montré très résistant à l'attaque par le thiocyanate de potassium dans la *N*,*N*-diméthylformamide à 140°. Après 63 h, nous avons pu isoler avec un rendement de 12% le 5-désoxy-1,2-*O*-isopropylidène-5-thiocyano-D-xylofuranose (**11**), le produit restant étant le composé de départ inchangé. Ce résultat rend par conséquent improbable toute possibilité d'obtention du thiocyanate en C-3 (**3**) par l'intermédiaire d'un oxétane.

Nos résultats ne nous paraissent donc interprétables qu'en faisant intervenir la possibilité d'une liaison hydrogène entre l'hydroxyle en C-5 d'une part et l'un des oxygènes du groupement sulfonyle (**12**). L'établissement d'une telle liaison aurait pour effet, de par sa nature électrophile, de favoriser un déplacement des charges négatives



12



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sur le sulfonate, permettant ainsi le développement d'un état de transition plus propice à l'attaque nucléophile sur C-3. Une hypothèse analogue est avancée par Henbest et Lovell dans une autre série[34].

La réactivité plus faible du sulfonate dans **7**, qui, dans des conditions similaires de traitement ne produit qu'un rendement inférieur au tiers en dérivé thiocyanate, peut être imputable au fait que l'hydroxyle porté par le carbone asymétrique en 5 est situé en *trans* par rapport au groupe *p*-tolylsulfonyle en C-3. Les possibilités de liaison hydrogène sont de ce fait plus difficiles avec cet hydroxyle. Une telle liaison peut néanmoins intervenir avec l'hydroxyle primaire en C-6 plus éloigné (**13**) avec pour conséquence une moindre assistance au déplacement que dans le dérivé du xylose correspondant.

Tout en apportant un exemple de participation d'un hydroxyle dans le déplacement d'un ester *p*-toluènesulfonique *exo* sur cycle furanoïde, ce travail ouvre une nouvelle voie d'accès au 3-désoxy-D-*érythro*-pentose par déplacement nucléophile du groupe *p*-toluènesulfonate du 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-xylofuranose (**5**) par le thiocyanate de potassium*.

PARTIE EXPÉRIMENTALE

Les spectres de n.m.r. ont été mesurés dans $CDCl_3$ à la fréquence de 60 Mc

*Les rendements obtenus par cette méthode sont de l'ordre de 25% calculés par rapport au D-xylose de départ.

avec un spectrophotomètre Varian A-60 par M^me^ L. Alais. Les déplacements chimiques sont mesurés à partir de la raie du tétraméthylsilane prise comme zéro de référence et exprimés en c.p.s. ou en δ. Les micro-analyses ont été exécutées sous la direction de M^lle^ Müller au Laboratoire Central de Microanalyse du CNRS. Les pouvoirs rotatoires ont été déterminés à l'aide du polarimètre «Quick» de Roussel et Jouan. Les points de fusion ont été mesurés sous microscope, sur platine de Leitz et sont corrigés. L'évaporation des solvants a été menée sous pression réduite avec évaporateur rotatif «Büchi».

p-*Tolylsulfonylhydrazone du 1,2:5,6-di*-O-*isopropylidène*-D-ribo-*3-hexulofuranose*. — Le 1,2:5,6-di-*O*-isopropylidène-D-*ribo*-3-hexulofuranose[15] (258 mg, 1 mmole) dissous dans le méthanol absolu (3 ml) est additionné de *p*-tolylsulfonylhydrazine recristallisée (340 mg, 1,8 mmole) et porté à reflux pendant 45 min. Après élimination du solvant sous pression réduite, le résidu réactionnel est dissous dans le benzène en présence d'un peu d'éther. Par trituration, la *p*-tolylsulfonylhydrazone cristallise. Après deux recristallisations, on obtient 250 mg de cristaux (50%); p.f. 179,5–181°, $[\alpha]_D^{21} + 230°$ (*c* 0,8, méthanol).

Anal. Calc. pour $C_{19}H_{26}N_2O_7S$: C, 53,51; H, 6,15; N, 6,57; Trouvé : C, 53,31; H, 6,12; N, 6,52.

Hydrazone du 1,2:5,6-di-O-*isopropylidène*-D-ribo-*3-hexulofuranose*. — Le 1,2;5,6-di-*O*-isopropylidène-D-*ribo*-3-hexulofuranose[15] (258 mg, 1 mmole) dissous dans l'éthanol absolu (3 ml) est additionné goutte à goutte d'un équivalent d'hydrazine à 96% dissous dans l'éthanol absolu (2 ml). Après une ébullition de 5 min, on laisse reposer 72 h à température ordinaire. Le solvant, évaporé sous pression réduite, laisse un résidu qui est distillé en tube à boules. On recueille 190 mg (70%) d'un sirop visqueux très hygroscopique. p. éb.$_{0,02}$ 99°; n_D^{18} 1,4845; $[\alpha]_D^{20} + 394°$ (*c* 0,54, méthanol).

Anal. Calc. pour $C_{12}H_{20}N_2O_5$: C, 52,94; H, 7,40; N, 10,29. Trouvé : C, 52,55; H, 7,08; N, 9,98.

1,2-O-*Isopropylidène-3*-O-p-*tolylsulfonyl-5*-O-*triphénylméthyl*-D-*xylofuranose* (**2**). — Le 1,2-*O*-isopropylidène-5-*O*-triphénylméthyl-D-xylofuranose[10] (1 g) dissous dans la pyridine anhydre (5 ml) est additionné de chlorure de *p*-toluènesulfonyle recristallisé (480 mg). Après 48 h à température du laboratoire, on ajoute quelques cristaux de glace et on laisse reposer 30 min. La solution est alors extraite deux fois par 250 ml de chloroforme. Les extraits chloroformiques réunis et lavés successivement par des solutions glacées d'hydrogénosulfate de potassium à 5%, d'hydrogénocarbonate de sodium et enfin par l'eau, sont séchés sur sulfate de sodium anhydre. Après concentration sous pression réduite, le sirop brun obtenu est dissous dans très peu de benzène et étendu d'hexane jusqu'à début de trouble. Après trituration, le produit cristallise. p. f. 128°; $[\alpha]_D^{21} - 51,3°$ (*c* 1,5 chloroforme).

Anal. Calc. pour $C_{34}H_{34}O_7S$: C, 69,61; H, 5,84; S, 5,46. Trouvé : C, 69,71; H, 5,56; S. 5,28.

Action du thiocyanate de potassium sur le composé **2** (*Essai 2 du tableau I*). — Dans un ballon de 50 ml muni d'un réfrigérant et d'une garde à chlorure de calcium, on place le 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-5-*O*-triphénylméthyl-D-xylo-

furanose (**2**) (586 mg, 1 mmole) dissous dans la *N,N*-diméthylformamide sèche (8 ml). Après addition de thiocyanate de potassium (776 mg, 8 mmoles), le mélange réactionnel est porté à 140° avec agitation magnétique. Après 63 h de chauffage, la solution brune obtenue est versée dans 300 ml de chloroforme et lavée quatre fois par 50 ml d'eau saturée de chlorure de sodium. Après séchage de la solution chloroformique sur sulfate de sodium, le chloroforme est évaporé sous pression réduite et la *N,N*-diméthylformamide résiduelle chassée sous vide poussé à 60° pendant 3 h. Le résidu brun montre alors une bande à 2140–2160 cm^{-1} dans le spectre infrarouge[26].

La désulfurisation est effectuée par le nickel Raney dans l'éthanol à reflux pendant une nuit à raison de 500 mg de catalyseur pour 100 mg de substance dissous dans 15 ml d'alcool. Après réaction, le nickel est éliminé par filtration sur Célite et la solution claire obtenue est évaporée sous pression réduite. Le produit obtenu est étudié par chromatographie sur couche mince de gel de silice dans le mélange benzène–méthanol (92:8) avec détermination à l'aide du Chromoscan «Joyce» par rapport à un témoin après révélation par l'acide sulfurique. On peut observer alors la présence de 20 à 30% de 3-désoxy-1,2-*O*-isopropylidène-D-*érythro*-pentofuranose dans le sirop.

L'hydrolyse du groupement isopropylidène s'effectue en présence d'acide chlorhydrique 0,05N à reflux pendant 2,5 h à raison de 4 ml d'acide pour 100 mg de sirop. L'acide chlorhydrique est éliminé par le carbonate d'argent. Le sirop obtenu après concentration contient de 25 à 30% de 3-désoxy-D-*érythro*-pentose, l'autre composant étant le xylose. Ces composés ont été déterminés par chromatographie sur papier dans deux systèmes de solvants (butanol–acide acétique–eau 4:1:5 et pyridine–acétate d'éthyle–eau 1:2:2) et électrophorèse sur papier (tampon borate de soude 0.1M). Les valeurs respectives des taches sont obtenues après révélation par le phtalate acide d'aniline, par rapport à un témoin, à l'aide du Chromoscan «Joyce».

Isolement du 3-désoxy-1,2-O-isopropylidène-3-thiocyano-D-ribofuranose (**3**). — Le composé **2** (586 mg, 1 mmole) est traité par le thiocyanate de potassium dans la *N,N*-diméthylformamide comme décrit dans l'essai précédent. Après réaction et élimination de la *N,N*-diméthylformamide, le résidu brun est chromatographié sur colonne de gel de silice (15 g). Le composé de départ (**2**) inchangé est élué par un mélange benzène–éther (9:1) ainsi que le triphénylcarbinol. Par élution à l'éther on obtient 30 mg (13,5%) de cristaux incolores; p.f. 101,5–102,5°; $[\alpha]_D^{24} + 50°$ (*c*, 0.5, chloroforme)

Anal. Calc. pour $C_9H_{13}NO_4S$: C, 46,75; H 5,67; N, 6,06; S, 13,85. Trouvé : C, 46,47; H, 5,55; N, 6,06; S, 13,61.

5-Désoxy-1,2-O-isopropylidène-5-thiocyano-D-xylofuranose (**11**). — Le 3,5-anhydro-1,2-*O*-isopropylidène-D-xylofuranose[10] (**10**) (700 mg) dissous dans la *N,N*-diméthylformamide sèche (15 ml) est additionné de thiocyanate de potassium (3.15 g) et porté à 140° pendant 63 h avec agitation magnétique. L'extraction est faite comme précédemment et la *N,N*-diméthylformamide résiduelle ainsi que l'oxétane de départ (**10**) sont éliminés par chauffage à 40° sous vide poussé pendant 3 h. Par chromatographie du résidu sur colonne de gel de silice (25 g), on isole 80 mg (12%) qui cristallisent spontanément dans l'éther; p.f. 108–110°; $[\alpha]_D^{22} - 16°$ (*c* 0,4, méthanol).

Anal. Calc. pour $C_9H_{13}NO_4S$: C, 46,75; H, 5,62; N, 6,06; S, 13,85. Trouvé : C, 46,51; H, 5,61; N, 5,98; S, 13,52.

Le spectre infrarouge de ce composé montre un pic à 2140 cm^{-1} attribuable à un groupement thiocyanate[26].

Le spectre de n.m.r. mesuré dans $CDCl_3$ montre, outre le doublet à δ 1,4 p.p.m. dû au groupement isopropylidène et un singulet à 2,6 p.p.m. attribuable à un hydroxyle, un doublet d'intensité 2 à 3,22 p.p.m. attribuable au groupement CH_2SCN[29].

1,2-O-Isopropylidène-3-O-p-tolylsulfonyl-D-xylofuranose (**5**). — Le composé **2** (586 mg) dissous dans 30 ml de méthanol contenant 0,16% d'acide chlorhydrique (densité 1.18) est soumis à une agitation magnétique à température ordinaire. La réaction est suivie par chromatographie sur couche mince de gel de silice. Après 8 h d'agitation à température du laboratoire, suivies d'un séjour d'une nuit à 0°, il ne reste plus de produit de départ. L'acide chlorhydrique est éliminé par le carbonate de plomb et la solution, filtrée, est concentrée sous pression réduite. Une chromatographie sur colonne de gel de silice permet d'éliminer le triphénylcarbinol et son ester méthylique qui sont élués par le benzène. Le méthanol élue alors une huile incolore qui cristallise dans un mélange éther–éther de pétrole; p. f. 87–88°; $[\alpha]_D^{24} - 15°$ (*c* 0,8, méthanol).

Anal. Calc. pour $C_{15}H_{20}O_7S$: C, 52,32; H, 5,86; S, 9,29. Trouvé : C, 52,42; H, 5,71; S, 9,52.

Ce composé a été obtenu par d'autres auteurs[35] par une autre méthode; les constantes n'en sont pas décrites.

5-Désoxy-1,2-O-isopropylidène-3-O-p-tolylsulfonyl-D-xylofuranose (**4**). — Le 5-désoxy-1,2-*O*-isopropylidène-D-xylofuranose[36] (2,480 g) dissous dans la pyridine anhydre (10 ml) est additionné de chlorure de *p*-toluènesulfonyle (3,26 g, 1,2 équivalent). Après 24 h à température ordinaire, on extrait par le chloroforme de la manière habituelle. On obtient ainsi une huile jaune n'ayant pas tendance à cristalliser. Une séparation sur colonne de gel de silice (120 g) fournit 3,210 g (69%) d'une substance incolore éluée par un mélange benzène–éther (3:1) et homogène par chromatographie sur couche mince de gel de silice (solvant: benzène–méthanol 98:2); n_D^{21} 1,4993; $[\alpha]_D^{21}$ − 17.6° (*c* 0.51, chloroforme).

Anal. Calc. pour $C_{15}H_{20}O_6S$: C, 54,87; H, 6,14. S, 9,74. Trouvé : C, 54,89; H, 5,88; S, 9,68.

3-Désoxy-1,2-O-isopropylidène-D-érythro-pentofuranose (**8**) — Le 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-xylofuranose (**5**) (1,890 g) dissous dans la *N,N*-diméthylformamide (40 ml) est additionné de thiocyanate de potassium (4,45 g) et porté à 140° pendant 63 h avec agitation magnétique. Après refroidissement, la solution est reprise par le chloroforme (500 ml) et lavée trois fois par 100 ml d'eau saturée de chlorure de sodium. La solution chloroformique est alors séchée, concentrée sous pression réduite et la *N,N*-diméthylformamide résiduelle chassée sous vide poussé. Le résidu est repris dans l'éthanol et traité par 10 g de nickel Raney fraîchement préparé, pendant une nuit à reflux. Après élimination du nickel par filtration sur Célite, la solution obtenue est évaporée sous pression réduite. Le sirop résiduel est alors chromatographié sur colonne

de 30 g de gel de silice équilibré dans l'éther. Le même solvant élue 423 mg (45%) de produit cristallin; p.f. 73–75°; $[\alpha]_D^{24} - 6°$ (*c* 0,5, méthanol). Szabó et Szabó[7] ont rapporté p.f. 74–76°; $[\alpha]_D - 3°$ (chloroforme).

Anal. Calc. pour $C_8H_{14}O_4$: C, 55,15; H, 8,1. Trouvé : C, 55,05; H, 8,21.

REMERCIEMENTS

Ce travail a bénéficié d'une aide matérielle de la Délégation Générale à la Recherche Scientifique et Technique auprès du Premier Ministre, Convention n° 66.00.308.

Nous remerçions M. le Professeur E. Lederer de l'intérêt qu'il a porté à cette étude. Nous remerçions M. Z. Welwart d'avoir bien voulu en discuter certains aspects.

RÉSUMÉ

Le déplacement du groupe *p*-toluènesulfonate du 1,2-*O*-isopropylidène-3-*O*-*p*-tolylsulfonyl-D-xylofuranose (**5**) par l'ion NCS^- dans la *N*,*N*-diméthylformamide conduit au 3-désoxy-1,2-*O*-isopropylidène-3-thiocyano-D-ribofuranose (**3**). Ce déplacement est favorisé par l'assistance de l'hydroxyle en C-5; le mécanisme en est discuté. La désulfurisation du thiocyanate (**3**) conduit, après clivage du groupement isopropylidène, au 3-désoxy-D-*érythro*-pentose.

SUMMARY

The displacement of the *p*-tolylsulfonyloxy group from 1,2-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-D-xylofuranose (**5**) by the CNS^- ion in *N*,*N*-dimethylformamide gave 3-deoxy-1,2-*O*-isopropylidene-3-thiocyano-D-ribofuranose (**3**). This displacement was favored by assistance of the hydroxyl group at C-5; the mechanism of the reaction is discussed. Desulfurization of compound (**3**) followed by removal of the isopropylidene group gave 3-deoxy-D-*erythro* pentose.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF SOME ESTERS AND LACTONES OF ALDONIC ACIDS

W. J. HUMPHLETT
Research Laboratories, Eastman Kodak Company, Rochester, New York 14650 (U. S. A.)

(Received September 6th, 1966; in revised form, December 23rd, 1966)

INTRODUCTION

Characterization of the methyl esters of a few aldonic acids, namely, D[1]- and L-arabinonic[2], D- and L-galactonic[3], and D-erythronic acids[4], has been reported in the literature. Among the limiting factors encountered in the synthesis of esters of aldonic acids is the nature of the reactants. Variation occurs among aldonic acids in the ease with which they form methyl or ethyl esters. Little has been reported concerning the preparation of their higher alkyl esters. When higher alcohols are employed, lactone formation is often favored over esterification. Alkyl esters of fully acetylated or benzoylated aldonic acids are readily formed, without dependence upon the reactant aldonic acid or alcohol.

RESULTS AND DISCUSSION

Addition of hydrogen chloride in methanol to potassium D-arabinonate afforded methyl D-arabinonate almost quantitatively[1]; however, ethanolic hydrogen chloride gave mostly D-arabinono-1,4-lactone. Gas–liquid chromatography of the trimethylsilylated[5] reaction product indicated yields of the ethyl ester and lactone of 11 and 65%, respectively.

Addition of hydrogen chloride to potassium D-arabinonate and benzyl, butyl, isobutyl, isopropyl, or propyl alcohol resulted in isolation of the lactone only. This use of isopropyl alcohol furnishes an effective route to D-arabinono-1,4-lactone (89% yield). By an earlier procedure[1], the lactone has been prepared by conversion of potassium D-arabinonate into the calcium salt, followed by treatment with oxalic acid in water[1]. The lactone has also been prepared by the reaction of D-arabinonic acid and ethanolic hydrogen chloride under pressure[6].

Addition of hydrogen chloride to isopropyl tetra-*O*-acetyl-D-arabinonate in isopropyl alcohol yielded the deacetylated lactone (60%), instead of the isopropyl ester. However, addition of hydrogen chloride to the lactone or its triacetate in methanol produced the methyl ester (70–79%). Treatment of the lactone in ethanol by a reported[7] method gave the ethyl ester in a yield of only 16%. Higher conversion into the ethyl ester (57%, as determined by g.l.c.) occurred when a solution of D-arabinonic acid in ethanol containing 0.5% of hydrogen chloride was refluxed for 24 h; the lactone was formed also (18%).

The solubility of the equilibrium products appears to be a limiting factor. In the corresponding alcohol at 23°, D-arabinono-1,4-lactone is 38, 27, 7, 5, and 2 times as soluble as methyl, ethyl, propyl, isopropyl, and butyl D-arabinonate, respectively. The methyl ester has been isolated after dissolving the lactone in methanol without a catalyst[7]. Agitation of the lactone and ethanol without a catalyst yielded ethyl D-arabinonate (51%). The isopropyl ester could not be similarly obtained. However, a solution of the lactone and sulfuric acid, in a volume of the respective alcohol favoring separation of the ester, afforded butyl, isopropyl, and propyl D-arabinonate. L-Arabinose was oxidized with bromine[2], and the resulting crude lactone was treated with the corresponding alcohols containing sulfuric acid, to provide enantiomorphs of the above esters.

Methyl D-mannonate was obtained from D-mannono-1,4-lactone by a similar procedure. By treatment of the lactone with hydrogen chloride and the appropriate alcohol, ethyl[8] (but not butyl, isopropyl, and methyl) D-mannonate could be obtained as reported, the latter having been described without analyses[9]. Ethyl L-mannonate has been isolated on recrystallization of the lactone from ethanol[10].

Although methyl D-galactonate has been obtained by treatment of the lactone in methanol with catalysts of hydrogen chloride or methyl sulfate[3], no ester was isolated when ethanol was used. Treatment of potassium D-galactonate with ethanolic hydrogen chloride gave the lactone. Potassium D-ribonate, D-*glycero*-D-*gulo*-heptonate, D-lyxonate, and D-erythronate in methanolic hydrogen chloride followed a similar course. Earlier work also failed to yield the corresponding methyl ester from the reaction of L-ribono-1,4-lactone in this medium[7]. Similar use of isopropyl alcohol provides a recommended route to D-lyxono- and D-erythrono-1,4-lactone (72–78%). D-Lyxono-1,4-lactone and D-*glycero*-D-*gulo*-heptono-1,4-lactone have been prepared previously by treatment of the aldonic acid hydrazide with nitrous anhydride[11]. Methyl[4] and butyl D-erythronate[12] and syrupy methyl[13] and crystalline ethyl D-gluconate[14] have been reported earlier.

Most of the known esters of fully acetylated aldonic acids have been methyl esters, prepared by using diazomethane[15]. Among others reported have been the ethyl esters of acetylated D-galactonic[16], D-gluconic, and DL-xylonic acids[17]. In the present study, esters were prepared without complication in 80–90% yields from a variety of alcohols and acetylated D-arabinoyl[16], L-arabinoyl[18], D-lyxonyl, D-ribonyl[18], D-gluconyl[19], D-galactonyl[16], D-*glycero*-D-*gulo*-heptonyl[20], and tri-*O*-benzoyl-L-erythronyl chloride. Certain esters, which were obtained as liquids, were distilled under diminished pressure without decomposition. Tetra-*O*-acetyl-D-lyxonyl chloride and hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptonyl chloride, which resisted crystallization, were similarly purified. Intermediate amides distilled were L-erythronamide and tetra-*O*-acetyl-D-lyxonamide[21].

The data for the new compounds obtained appear in Table I.

Preferred synthetic methods developed in this study are described in the experimental section.

EXPERIMENTAL

General. — Melting points, determined in capillary tubes, are corrected; boiling points are uncorrected. Vacuum distillation was conducted with a stirred, oil-jacketed, 100-ml Hickman still and an oil pump connected to a two-stage diffusion, glass ejector pump. Known substances were identified by mixed melting points and by infrared spectra.

Infrared spectra. — I. r. spectra were recorded for potassium bromide disks in a Baird Atomic Spectrophotometer, Model NK-1, having sodium chloride optics. The esters showed λ_{max} 3.0–3.5 μ (s, OH), indicating hydrogen bonding. Ethyl, propyl, and butyl D-arabinonate and methyl and ethyl D-mannonate had, in addition, sharp λ_{max} 2.9 μ (OH), indicating weaker hydrogen-bonding. Carbonyl absorption (strong) appeared at 5.8 μ for methyl D-galactonate; at 5.78 μ for ethyl and propyl, and at 5.75 μ for butyl D-arabinonate; and at 5.7 μ for ethyl and 5.67 μ for butyl tetra-*O*-acetyl-D-arabinonate. Esters showed variable strong bands for the -O- group, but consistent peaks at 8.0–8.2 μ (vs, -O-) when acetylated. Methyl esters absorbed at 7.3 μ (s, *sym*-CH_3); when acetylated, they showed maxima at 7.0 μ (w) and 7.3 μ (s). Lactones had λ_{max} 5.63–5.68 μ (s, C=O). When acetylated, they showed λ_{max} 5.58 μ (strained lactone C=O) and 5.7 μ (C=O).

Preparation of potassium aldonates. — A modified method[1,20] of oxidation of D-glucose provided potassium D-arabinonate. Precipitation of the salt from aqueous methanol prevented further degradation.

Oxygen was added through two dispersion tubes to a rapidly stirred solution of potassium hydroxide (672 g) in water (1.44 l) and methanol (6 l). Simultaneous addition of D-glucose (720 g, 4 moles) in water (1.44 l) was made over 7.75 h. The temperature was maintained at 30–35°. Crystallization began after 3 h. When the addition of D-glucose was complete, introduction of oxygen was continued for 0.5 h. [The rate of addition of oxygen caused a decrease of pressure from an initial cylinder pressure of 1700 to a final pressure of 1500 $lb.in^{-2}$ (224-ft^3 cylinder).] Air was then introduced for 48 h. Methanol was added periodically to replace that lost by evaporation. Filtration afforded potassium D-arabinonate (620 g, 76%). Recrystallization, from 4 volumes of water by adding 8 volumes of methanol, furnished pure product (92% recovery), m.p. 219° (dec.).

Anal. Calc. for $C_5H_9KO_6$: C, 29.4; H, 4.4. Found: C, 29.6; H, 4.6.

In similar preparations of potassium D-lyxonate (68%) from D-galactose, and of potassium L-erythronate (64%) from L-arabinose, an equal volume of methanol was added after the addition of air. Stirring for 24 h then resulted in crystallization of product suitable for synthetic use.

Other potassium aldonates were obtained by combining methanolic solutions of potassium hydroxide and the appropriate lactones.

Preparation of aldono-1,4-lactones. — The preparation of L-erythrono-1,4-lactone illustrates the general procedure. Into a stirred suspension of potassium L-erythronate (173 g, 1.05 mole) in isopropyl alcohol (700 ml) was passed hydrogen

TABLE I

PROPERTIES AND ANALYSES OF ESTERS AND LACTONES OF ALDONIC ACIDS

Compound	*Recrystallization solvent*	*M.p., degrees*[a]	*B.p., degrees/microns Hg*	n_D^{25} [b]	*Formula*	$[\alpha]_D^{25}$ *degrees*[c]	*Calc., %* C	H	*Found, %* C	H
Benzyl tri-*O*-benzoyl-L-erythronate			205/2	1.5716	$C_{32}H_{26}O_8$	−14.8	71.4	4.9	71.3	5.1
Bis-*O*-(penta-*O*-acetyl-D-gluconyl)ethylene glycol	EtOH	83–84			$C_{34}H_{46}O_{24}$	+16.2	48.7	5.5	48.6	5.8
Bis-*O*-(tetra-*O*-acetyl-D-arabinoyl)ethylene glycol	MeOH	106			$C_{28}H_{38}O_{20}$	+33.2	48.4	5.5	48.2	5.6
Butyl D-arabinonate	BuOH	104–105			$C_9H_{18}O_6$	−3.0	48.6	8.2	48.5	8.5
Butyl DL-arabinonate	BuOH	99–100				0			48.8	8.1
Butyl L-arabinonate	BuOH	104–105				+3.5			48.7	8.2
Butyl tetra-*O*-acetyl-D-arabinonate			111/4	1.4410	$C_{17}H_{26}O_{10}$	+29.3	52.3	6.7	52.4	7.0
Ethyl D-arabinonate	EtOH	132–133			$C_7H_{14}O_6$	0	43.3	7.3	43.5	7.4
Ethyl DL-arabinonate	EtOH	112				0			43.1	7.3
Ethyl hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptonate	EtOH–pentane	91			$C_{21}H_{30}O_{14}$	+16.0	49.8	6.0	49.7	5.7
Ethyl tetra-*O*-acetyl-D-arabinonate	EtOH	111			$C_{15}H_{22}O_{10}$	+34.0	49.7	6.1	49.5	6.2
Ethyl tetra-*O*-acetyl-DL-arabinonate	EtOH	93–94				0			49.5	6.3
Ethyl tetra-*O*-acetyl-L-arabinonate	EtOH	111				−33.7			49.8	6.3
Ethyl tetra-*O*-acetyl-D-lyxonate			97/1	1.4418		+14.0			49.6	6.1
Ethyl tetra-*O*-acetyl-D-ribonate	iso-PrOH	58				−13.5			49.9	6.4
Ethyl tri-*O*-benzoyl-L-erythronate			182/5	1.5528	$C_{27}H_{24}O_8$	−16.3	68.3	5.1	68.5	5.4
Hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptonyl chloride			126/8[d]	1.4585	$C_{19}H_{25}ClO_{13}$[e]	+23.5	46.0	5.1	45.8	5.1
Isobutyl penta-*O*-acetyl-D-galactonate	EtOH	111			$C_{20}H_{30}O_{12}$	+22.0	51.9	6.5	51.8	6.4
Isobutyl tetra-*O*-acetyl-D-arabinonate	Et_2O–pentane	40			$C_{17}H_{26}O_{10}$	+27.7	52.3	6.7	52.3	6.7
Isopropyl D-arabinonate	iso-PrOH	142–143			$C_8H_{16}O_6$	−3.8	46.2	7.8	46.3	7.5
Isopropyl DL-arabinonate	iso-PrOH	121				0			46.0	7.7
Isopropyl L-arabinonate	iso-PrOH	142–143				+4.2			46.4	7.9
Isopropyl hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptonate			133/3[f]	1.4480	$C_{22}H_{32}O_{14}$	+16.7	50.8	6.2	50.7	6.5
Isopropyl penta-*O*-acetyl-D-galactonate	EtOH	99–100			$C_{20}H_{28}O_{12}$	+24.2	50.9	6.3	50.7	6.0
Isopropyl penta-*O*-acetyl-D-gluconate	EtOH	95				+11.5			51.0	6.5
Isopropyl tetra-*O*-acetyl-D-arabinonate	EtOH–pentane	73			$C_{16}H_{24}O_{10}$	+28.5	51.1	6.4	51.2	6.6

TABLE I *(contd.)*

Compound	*Recrystallization solvent*	*M.p., degrees*[a]	*B.p., degrees/microns Hg*	n_D^{25} [b]	*Formula*	$[\alpha]_D^{25}$, *degrees*[c]	*Calc.,* % *C*	*H*	*Found* % *C*	*H*
Isopropyl tetra-*O*-acetyl-D-ribonate			102/1	1.4369		−10.0			50.8	6.5
Isopropyl tri-*O*-benzoyl-L-erythronate			180/3	1.5458	$C_{28}H_{26}O_8$	−21.0	68.6	5.3	68.8	5.1
Methyl DL-arabinonate	MeOH	141			$C_6H_{12}O_6$	0	40.0	6.7	40.0	6.8
Methyl D-mannonate	MeOH	134–135[d]			$C_7H_{14}O_7$	+5.0	40.0	6.7	40.1	6.6
Methyl *O*-(penta-*O*-acetyl-D-gluconyl)-glycolate	EtOH	62–63			$C_{19}H_{26}O_4$	+15.3	47.7	5.5	47.9	5.7
Methyl *O*-(tetra-*O*-acetyl-D-arabinoyl)-glycolate	MeOH	66–67			$C_{16}H_{22}O_{12}$	+31.2	47.3	5.5	47.5	5.5
Methyl tetra-*O*-acetyl-DL-arabinonate	EtOH	116			$C_{14}H_{20}O_{10}$	0	48.3	5.8	48.2	5.9
Methyl tetra-*O*-acetyl-D-lyxonate			92/1	1.4431		+15.3			48.3	6.0
Methyl tri-*O*-benzoyl-L-erythronate			189/1	1.5583	$C_{26}H_{22}O_8$	−18.0	67.6	4.8	67.8	5.0
Propyl D-arabinonate	PrOH	123–124			$C_8H_{16}O_6$	−3.3	46.2	7.8	46.5	8.1
Propyl DL-arabinonate	PrOH	105–106				0			45.9	8.0
Propyl L-arabinonate	PrOH	123–124				+3.3	46.2	7.8	46.1	7.9
Propyl hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptonate			132/3	1.4500	$C_{22}H_{32}O_{14}$	+13.7	50.8	6.2	50.9	6.3
Propyl penta-*O*-acetyl-D-galactonate	EtOH	114			$C_{19}H_{28}O_{12}$	+14.8	50.9	6.3	50.7	6.1
Propyl penta-*O*-acetyl-D-gluconate	EtOH	82				+12.5			50.8	6.2
Propyl tetra-*O*-acetyl-D-arabinonate	Et_2O-pentane	44			$C_{16}H_{24}O_{10}$	+29.2	51.1	6.4	51.0	6.6
Propyl tetra-*O*-acetyl-D-ribonate			100/1[h]	1.4395		−17.3			51.3	6.5
Tetra-*O*-acetyl-D-lyxonamide			141/1[i]	1.4643	$C_{13}H_{19}NO_9$[j]	+18.7	46.9	5.8	46.8	5.9
Tetra-*O*-acetyl-D-lyxonyl chloride			99/2	1.4511	$C_{13}H_{17}ClO_9$	+16.5	44.3	4.9	43.9	5.2
2,3,5-Tri-*O*-acetyl-D-arabinono-1,4-lactone	EtOH	81[k]			$C_{11}H_{14}O_8$	+53.2	48.2	5.2	48.4	5.0
2,3,5-Tri-*O*-acetyl-DL-arabinono-1,4-lactone	EtOH	60				0			48.0	5.1
2,3,5-Tri-*O*-acetyl-L-arabinono-1,4-lactone	EtOH	81[l]				−53.0			48.0	5.3
Tri-*O*-benzoyl-L-erythronyl chloride	Et_2O-pentane	94			$C_{25}H_{21}ClO_4$[m]	+1.5	64.3	4.1	64.3	4.3

[a]After recrystallization from the solvent indicated. [b]After redistillation at the boiling point and pressure indicated. [c]For acetylated and benzoylated derivatives, $c = 2$, in chloroform; all others were determined in water. [d]Ref. 20 reports a syrup suitable for use. [e]Calc.: Cl, 7.1. Found: Cl, 6.9. [f]Crystallized, m.p. 64°. [g]Ref. 9 gives m.p. 155°. [h]Crystallized, m.p. 37°. [i]Ref. 21 reports a syrup suitable for use. [j]Calc.: N, 4.2. Found: N, 4.3. [k]G. B. ROBINS AND F. W. UPSON [*J. Am. Chem Soc.*, 62 (1940) 1074] gave m.p. 68–69°. [l]C. PAAL AND M. KINSCHER [*Ber.*, 44 (1911) 3548] reported m.p. 52–54°. [m]Calc.: Cl, 7.6. Found: Cl, 7.4.

chloride during 20 min. The mixture was cooled, and hydrogen chloride was again passed in. The mixture was heated to boiling, and the precipitated potassium chloride was collected, and rinsed with isopropyl alcohol. Crystallization of the product occurred when the filtrate was concentrated *in vacuo* to 200 ml. The mixture was kept overnight at 5°, and the crystals (90 g) were collected; the filtrate yielded a second crop (6.4 g). Recrystallized from isopropyl alcohol, the lactone (89 g, 72%) had m.p. 100–101° (lit.[22] m.p. 103–104°).

*2,3,5-Tri-*O*-acetyl-*D-, L-, *and* DL*-arabinono-1,4-lactone.* — The enantiomorphs, obtained as described, showed properties at variance with those reported (Table I). Anhydrous zinc chloride (8.8 g) was dissolved in acetic anhydride (100 ml) by stirring for 2 h at 0°. The respective lactone (14.8 g, 0.1 mole) was added, and the colorless mixture was stirred for 2 h at 5° and overnight at room temperature. Acetic anhydride was evaporated off *in vacuo* with warming, and 200 g of ice was added to the resulting syrup. Crystallization occurred during several h. After recrystallization, the yields were 76–79%.

The solubility of lactones. — On agitation of an excess of the lactone in 100 ml of the alcohol for 24 h at 23°, methyl, ethyl, benzyl, isopropyl, propyl, and butyl alcohol dissolved 66.0, 16.6, 5.4, 5.0, 4.7, and 3.1 g, respectively, of D-arabinono-1,4-lactone. Methyl, ethyl, and isopropyl alcohol dissolved 1.8, 0.3, and 0.1 g, respectively, of D-mannono-1,4-lactone. Methyl alcohol dissolved 23.2, 5.6, and 1.0 g of D-lyxono-, D-galactono, and D-*glycero*-D-*gulo*-heptono-1,4-lactone, respectively, and 1.4 g of D-glucono-1,5-lactone. Ethyl alcohol dissolved 1.1 g of D-galactono-1,4-lactone.

Preparation of esters of aldonic acids. — *(a) From potassium* D*-arabinonate.* Treatment of this salt and methanol with hydrogen chloride as described[1] afforded methyl D-arabinonate (98%); it was recrystallized from methanol, with concentration *in vacuo* (92% recovery), m.p. 143° (*lit.*[1] m.p. 140°). Concentration by boiling (600 g sample in methanol) gave only 53% recovery and syrup.

For the preparation of the ethyl ester, the method was inferior, and it failed for the higher esters.

(b) From aldono-1,4-lactones. Ethyl D-arabinonate was obtained by shaking a suspension of D-arabinono-1,4-lactone (5 g, 0.034 mole) in ethanol (25 ml) for 24 h, yielding 3.3 g (51%) of the ester, m.p. 131–132° (lit.[7] m.p. 126.5°).

Propyl D-arabinonate was prepared by a general procedure. A solution of D-arabinono-1,4-lactone (60.5 g, 0.41 mole) in propyl alcohol (750 ml) containing sulfuric acid (2 ml) was concentrated to 585 ml by boiling, and the mixture was kept overnight at 5°. The flaky mass was collected (70 g), and a second crop (7.4 g) was obtained. The product was recrystallized from propyl alcohol; yield, 56.5 g (75%). Similar treatment of the lactone with the corresponding alcohol afforded isopropyl (9%), butyl (39%), and ethyl (56%) D-arabinonate. Unreacted lactone could be further treated; *e.g.*, repeated concentration of the acidic filtrates, followed by crystallization, gave a 69% yield of isopropyl D-arabinonate. Methyl D-mannonate was similarly prepared (46%, in two crops).

(c) From fully acetylated or benzoylated aldonyl chlorides. The aldonyl chlorides

required were prepared by the thionyl chloride method[18]. The synthesis of isopropyl tetra-*O*-acetyl-D-gluconate provides an example. To a solution of tetra-*O*-acetyl-D-gluconyl chloride[19] (12.4 g, 0.029 mole) and 5 ml of isopropyl alcohol in dichloromethane (125 ml) was added pyridine (8 ml). The ensuing reaction was exothermic. After 2 h, the solution was extracted with an excess of 10% hydrochloric acid, and the combined, aqueous phases were extracted with dichloromethane. The organic phases were combined, extracted with water, and dried over anhydrous sodium sulfate. Filtration, and concentration *in vacuo*, provided a solid (12.3 g). Recrystallization gave the pure ester (11.6 g, 89%).

Equivalent amounts of ethylene glycol were used for preparing its esters.

Liquid esters were purified by distillation

(*d*) *Racemic esters.* Recrystallization together of equal amounts of the enantiomorphs furnished optically inactive isomers.

The solubility of esters. — The corresponding alcohol (100 ml) at 23° dissolved 1.7, 0.7, 0.7, 0.9, and 1.5 g of methyl, ethyl, propyl, isopropyl, and butyl D-arabinonate, and 1.1 and 0.3 g of methyl and ethyl D-mannonate, respectively.

ACKNOWLEDGMENTS

Assistance by the following colleagues is gratefully acknowledged: Messrs. B. C. Branner and D. S. Cox, for high-vacuum distillations; Miss T. J. Davis, for i. r. spectra and their interpretation; and Mr. G. F. McNaughton, of Distillation Products Industries, for g.l.c. analyses.

SUMMARY

The formation of esters of aldonic acids by treatment with an alcohol in the presence of an acid depends on the nature of the reagents and on the solubility of the products. The formation of aldonolactones is often a favored, competing reaction. Fully acetylated or benzoylated aldonic esters are formed without these restrictions. Among the products, liquid esters and certain of their acid chloride and amide intermediates are distillable. Some effective methods of synthesis of esters and lactones are described, based on the results of this investigation.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

IMMUNOCHEMICAL STUDIES ON BLOOD GROUPS. XXXVI. THE NATURE OF THE UNSATURATED RESIDUE IN SOME OLIGOSACCHARIDES PRODUCED BY THE ACTION OF SODIUM HYDROXIDE–SODIUM BOROHYDRIDE ON BLOOD-GROUP A, B, AND H SUBSTANCES*

KENNETH O. LLOYD AND ELVIN A. KABAT

Departments of Microbiology and Neurology, College of Physicians and Surgeons, Columbia University and the Neurological Institute, Presbyterian Hospital, New York, N. Y. 10032 (U. S. A.)

(Received December 14th, 1966; in revised form, January 9th, 1967)

Many of the oligosaccharides resulting from the degradation of blood-group A, B, and H substances with sodium hydroxide–sodium borohydride[1] are terminated at their reduced ends by an unsaturated alditol residue, that of a 3-hexene-1,2,5,6-tetrol (R)[2–4]. This residue is believed to be formed from a D-galactopyranose residue that, in the intact polysaccharide, is present as a branch point such after peeling of the main chains down to this point, alkaline elimination of oligosaccharide side chains leads to its unsaturation[3].

A sample of the free hexenetetrol isolated from the degradation products was found to be a mixture of the *trans*-D-*threo* and *trans-erythro* forms of the unsaturated alditol[3]. It has now been found, by hydroxylation and identification of the hexitols formed, that, in the unsaturated oligosaccharides also, the tetrol occurs in both of these forms, and that the samples are really pairs of oligosaccharides, isomeric at the unsaturated residue. The position of attachment of the oligosaccharide chains to the hexenetetrol residue, which has not previously been studied, must be C-2 or C-6 thereof, as C-3 and C-4 are not available, and C-5 would entail the presence of a furanose form of the original D-galactose residue. In the lowest member of this series of oligosaccharides (GNAc*p*-R), the linkage has been shown to be β-D-(1→6).

MATERIALS AND METHODS

Analytical methods for nitrogen, 2-amino-2-deoxyhexose, 2-acetamido-2-deoxyhexose, and quantitative oxidation by periodate, as well as other general methods, have been described previously[1,3,5].

Paper chromatography was conducted by the descending method, with S and S 589 Green Label paper. Whatman Chromedia cellulose was used for thin-layer chromatography. The solvents used were 6:4:3 butyl alcohol–pyridine–water (solvent 1), 6:1:1 butyl alcohol–pyridine–water (solvent 2), and 10:4:3 ethyl acetate–pyridine–

*Aided by grants from the National Science Foundation (G-18727 and GB-3675), the Office of Naval Research (Nonr 266-13), and the General Research Support grant of the National Institutes of Health, U. S. Public Health Service.

water (solvent 3). Sugars and alditols were detected with alkaline silver reagent[6] or with periodate–benzidine[7]. Hexitols were identified by thin-layer chromatography (t.l.c.) on calcium sulfate plates, by elution with 1:1:3 benzene–acetic acid–methanol as described by Schwane and Nakon[8]; iditol, mannitol, and glucitol had R_F 0.50, 0.42, and 0.29, respectively. Alditol acetates were separated by gas–liquid chromatography (g.l.c.) on an ECNSS-M (ethylene glycol succinate combined with a cyanoethylsilicone) column, according to the technique of Sawardeker *et al.*[9] An F and M 810 R gas chromatograph was used; the columns were 6 ft × 1/8 in, the operating temperature was 200°, and the nitrogen flow-rate was 30 ml/min. Five of the six hexitol acetates could be completely separated in 30 min (see Fig. 1A); however, mannitol hexaacetate and talitol hexaacetate are not separated under these conditions, although the mannitol derivative has a slightly shorter elution time. By lowering the column temperature to 175°, the two compounds could be almost completely resolved; mannitol hexaacetate is then eluted in 37 min, and talitol hexaacetate in 40 min.

D-Glucitol, D-mannitol, and galactitol were prepared by reduction of the corresponding aldoses with sodium borohydride[10]. L-Iditol was kindly provided by Dr. G. Ashwell, and allitol and D-talitol were obtained from the collection of the late Professor J. C. Sowden. Their hexaacetates were prepared by treatment with acetic anhydride–pyridine[11]: galactitol hexaacetate (m.p. 171–172°), D-glucitol hexaacetate (m.p. 100°), D-mannitol hexaacetate (m.p. 126°), and allitol hexaacetate (m.p. 59°). D-Talitol and L-iditol were available in only small amounts, and, without isolation from the reaction mixtures, their acetates were used directly for chromatography. *trans*-3-Hexene-*erythro*-1,2,5,6-tetrol was a gift from Dr. E. F. L. J. Anet, and the D-*threo* isomer from Dr. R. S. Tipson. The unsaturated oligosaccharides were those obtained from human and hog blood-group A, B, and H substances, described previously[2–4]. The disaccharide (β-D-GNAc*p*-R) was prepared in amounts larger than had been isolated before (see the experimental section).

EXPERIMENTAL

Isolation and purification of the O-(*2-acetamido-2-deoxy-β-*D-*glucopyranosyl*)-(*1→6*)-*3-hexene-1,2,5,6-tetrol* [β-D-*GNAc*p-(*1→6*)-*R*]. — The degradation of 40 g of hog-mucin, blood-group A and H substance with sodium hydroxide–sodium borohydride as described previously[1], and fractionation of the resulting dialyzates[3] (performed by Dr. M. E. Kaplan), yielded 1.9 g of material enriched in the title disaccharide. The mixture was further fractionated by preparative paper-chromatography in solvent 2, and 193 mg of material having R_{Gal} 0.85 in this solvent was isolated. Gel filtration on a Biogel P2 column[3] removed a small proportion of another component, probably *O*-α-L-fucopyranosyl-(1→2)-D-galactitol (R_{Gal} 0.69). The compound was finally purified on a charcoal column, by elution with an ethanol gradient; it was eluted as a single peak, and 119 mg of syrupy product was isolated. The compound gave a positive test for unsaturation with potassium permanganate, and with bromine. Its optical rotatory dispersion (o.r.d.) spectrum showed a strong,

negative Cotton effect at 220 mμ ($[m]_{trough} - [m]_{3000} = -2100$), indicating a β-D-linkage in the disaccharide[4,12]. Analysis showed: N, 3.7; 2-amino-2-deoxyhexose; 43*, and 2-acetamido-2-deoxyhexose, 59% (Calc.: 4.0, 51.0, and 63.0%, respectively). Only 2-amino-2-deoxyglucose could be detected by paper chromatography of a hydrolyzed sample (the hexenetetrol is difficult to detect after hydrolysis[3]).

Hydroxylation of authentic trans-*3-hexene*-D-threo-*1,2,5,6-tetrol.* — A solution of the tetrol (74 mg) in 2.0 ml of water was treated with 75 μl of 30% hydrogen peroxide and a small crystal of osmium tetraoxide. After 3 h, osmic acid was removed on Dowex-1 (OAc^-). The product crystallized on standing, and recrystallization from 80% ethanol gave D-mannitol (26 mg), m.p. and mixed m.p. 166–167° (lit.[13] 166–167°).

Examination of the mother liquor by paper chromatography in solvent 3 showed that it contained two major components, mannitol and iditol. These were separated by preparative paper-chromatography in the same solvent, and 7.7 mg more of D-mannitol (total yield, 33.8 mg, 37%) and 11.4 mg (13%) of syrupy D-iditol were isolated.

The hydroxylation was repeated on another 75 mg of the tetrol, and 10.0 mg more of D-iditol was isolated by preparative paper-chromatography. Gel filtration through a Biogel P2 column gave 20.6 mg of product. The syrupy D-iditol did not crystallize, but acetylation[11] with acetic anhydride and pyridine gave 32.4 mg (8%) of a crystalline hexaacetate (m.p. 122–123°, lit.[13] 121–122°).

Hydroxylation of authentic trans-*3-hexene*-erythro-*1,2,5,6-tetrol.* — A solution of the tetrol (30 mg) in 1.0 ml of water was treated with 35 μl of 30% hydrogen peroxide and a small crystal of osmium tetraoxide. The product was isolated as above, and its major component had the same R_F as that of glucitol. There was a minor component at R_{Gal} 1.37. The syrupy product crystallized on standing, and recrystallization from aqueous ethanol gave DL-glucitol (8.2 mg, 22%), m.p. 134–136° (lit.[13] 136–138°).

Hydroxylation of authentic hexenetetrols, and identification of the products by chromatography. — The tetrol (*ca.* 2 mg) was dissolved in 50 μl of water, and 5 μl of 10% hydrogen peroxide and a tiny crystal of osmium tetraoxide were added. After 3 h, the osmic acid was removed as before. The product was dissolved in 100 μl of water, and a small portion of the solution was examined by paper chromatography in solvent 3 and by thin-layer chromatography. The results are shown in Table I. The remainder of the solution was evaporated to dryness, and the resulting syrup was acetylated by heating it with 150 μl of 1:1 acetic anhydride–pyridine for 2 h in a sealed tube at 100°. The products were examined directly by g.l.c. The results are shown in Table I, and typical chromatograms of the hexitol hexaacetates from the pure, authentic, D-*threo* and *erythro* isomers are shown in Fig. 1 (B and D).

*Analyses for oligo- and poly-saccharides containing 2-acetamido-2-deoxyhexose often give low results, because of the resistance of 2-amino-2-deoxyhexosyl linkages to acid hydrolysis.

TABLE I

HEXITOLS FORMED BY HYDROXYLATION OF AUTHENTIC HEXENETETROLS AND UNSATURATED OLIGOSACCHARIDES

Sample	*Structure*[a]	*Hexitols identified*			*Ratio of* D-threo[b] : erythro *isomers in the sample*
		By paper chromatography	*By t.l.c.*	*By g.l.c.*	
trans-3-Hexene-D-*threo*-1,2,5,6-tetrol	**2a**	mannitol and iditol	mannitol[c]	mannitol and iditol	
trans-3-Hexene-*erythro*-1,2,5,6-tetrol	**1a**	glucitol	glucitol	glucitol	
Hog, R_{Gal} 0.85 (β-D-GNAc*p*-R)	**1** and **2b**	[d]	mannitol[c] and glucitol	mannitol, iditol, and glucitol	1.3
Hog HR_L 0.75 (H-active tetrasaccharide)	**1** and **2c**			mannitol, iditol, and glucitol	0.85
ADR_L 0.52 I (A-active pentasaccharide)	**1** and **2d**			mannitol, iditol, and glucitol	1.10
ADR_L 0.52 II (A-active pentasaccharide)	**1** and **2d**			mannitol, iditol, and glucitol	2.00

[a]See Fig. 3.
[b]Determined from the ratio of the areas of the mannitol and iditol: glucitol peaks in g.l.c.
[c]The periodate–benzidine reagent is not sensitive enough to demonstrate the small proportion of iditol in the mixture.
[d]Paper chromatography with this solvent resolves mannitol from iditol, but is not suitable when all three hexitols are present.

Hydroxylation of the unsaturated oligosaccharides, and identification of the hexitols by gas–liquid chromatography. — The oligosaccharide (*ca.* 1 mg) was dissolved in 50 μl of water, and hydroxylated with 10 μl of 2% hydrogen peroxide and a tiny crystal of osmium tetraoxide. The product was hydrolyzed in 2N hydrochloric acid (150 μl) for 2 h at 100°, and the hydrolyzate was neutralized with silver carbonate. The solution was passed through a column of Dowex-50 (H^+) (*ca.* 0.5 ml) and Dowex-1 (OH^-) (*ca.* 1 ml) to remove reducing sugars; the absence of reducing sugars in the eluate was confirmed by paper chromatography. The hexitols were identified by g.l.c. of their hexaacetates as described in the previous section. Table I gives the results for the three oligosaccharides examined, and a typical chromatogram (of H-active tetraaccharide, HR_L 0.75) is shown in Fig. 1C.

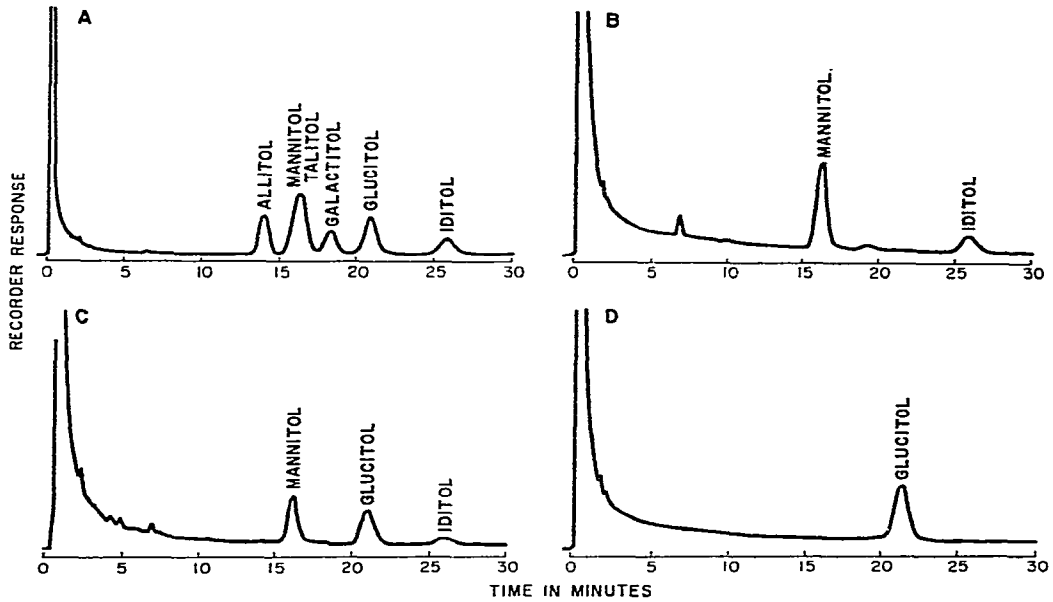


Fig. 1. Gas–liquid chromatograms of hexitol hexaacetate standards (A); hexitol hexaacetates from hydroxylation of *trans*-3-hexene-D-*threo*-1,2,5,6-tetrol (B), H-tetrasaccharide, HR_L 0.75 (C), and *trans*-3-hexene-*erythro*-1,2,5,6-tetrol (D). The samples were chromatographed on an ECNSS-M column at 200°. Each sample was also examined on the same column at 175°, in order to resolve mannitol from talitol.

Hydroxylation of β-D-GNAcp-(1→6)-R. — The unsaturated disaccharide (27 mg) was dissolved in 1.0 ml of water, and a small crystal of osmium tetraoxide and 35 μl of 10% hydrogen peroxide were added. The mixture was kept for 3 h at room temperature, and then an excess of Dowex-1 (OAc^-) was added. The following day, the resin was filtered off and the filtrate was evaporated to dryness. Examination of the product by paper chromatography in solvent 2 showed one main component (R_{Gal} 0.21), with minor components of R_{Gal} 0.33 and R_{Gal} 0.85 (starting material). Preparative paper-chromatography in solvent 1 gave 14.2 mg of material having R_{Gal} 0.72, (R_{Gal} 0.21 in solvent 2). It was eluted as a single peak from a column of Biogel P2, to

give 10.6 mg of syrupy product (β-GNAc-hexitols). Analysis showed: N, 3.5; 2-amino-2-deoxyhexose, 41*; and 2-acetamido-2-deoxyhexose, 53.5% (Calc.: 3.6, 46.8, and 57.3%, respectively). Only 2-amino-2-deoxyglucose and a mixture of hexitols could be detected by hydrolysis followed by paper chromatography. The hexitols were identified as iditol, mannitol, and glucitol (see Table I) by t.l.c., and by g.l.c. of their hexaacetates.

Periodate oxidation of β-D-GNAcp-(1→6)-R and its hydroxylated derivative. — Quantitative, periodate-oxidation data for β-D-GNAc*p*-(1→6)-R and β-D-GNAc*p*-(1→6)-hexitols are shown in Fig. 2. β-D-GNAc*p*-(1→6)-R is rapidly overoxidized, and the curve for periodate uptake does not shown a clear inflection point. The formation of 1 mole of formaldehyde per mole indicates that the tetrol is substituted on either C-2 of C-6. The oxidation of the hydroxylated product proceeds stoichiometrically: 5 moles of periodate are reduced per mole, and 3 moles of formic acid and 1 mole of formaldehyde are formed.

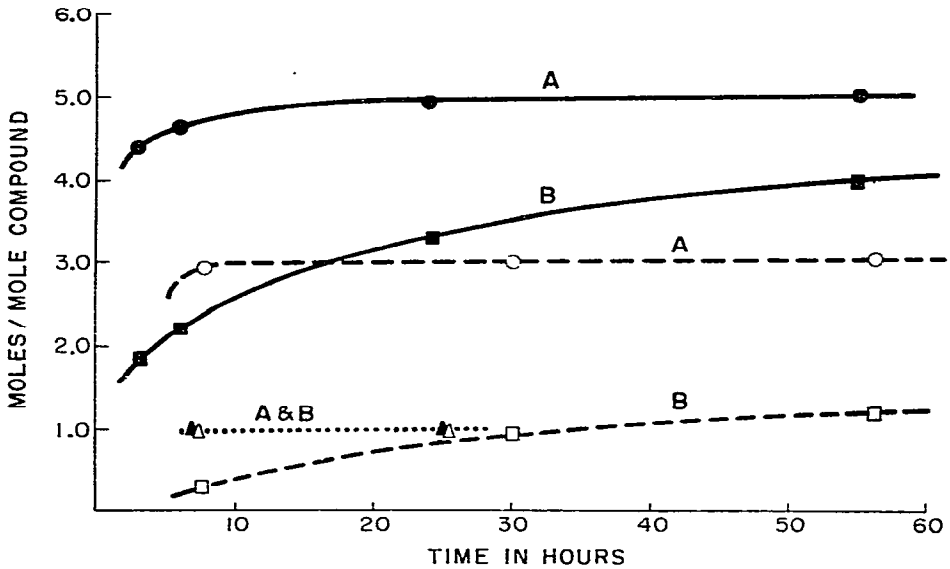


Fig. 2. Quantitative periodate oxidation of β-D-GNAc*p*-R (B) and hydroxylated β-D-GNAc*p*-R(A) Periodate uptake ————, formic acid release — — — —, formaldehyde . . . . . .

In addition, 1 mg of β-D-GNAc*p*-(1→6)-hexitols was treated with an excess of periodate for 24 h. Periodate and iodate ions were then removed with barium chloride, and an excess of sodium borohydride was added. After 2 h, the solution was deionized; the product was hydrolyzed with 1N hydrochloric acid for 2 h at 100°, and the solution was neutralized with silver carbonate. Thin-layer chromatography on cellulose (solvent 3) showed three products: glycerol (R_{Gal} 3.3), ethylene glycol (R_{Gal} 4.5), and another product (R_{Gal} 0.1). The latter compound is, presumably, 2-amino-2-deoxyglyceraldehyde, as it was also found by t.l.c., together with glycerol (but not ethylene

*See preceding footnote.

glycol), after methyl 2-acetamido-2-deoxy-β-D-glucopyranoside had been treated under identical conditions.

RESULTS AND DISCUSSION

Oxidation of carbon–carbon double bonds with hydrogen peroxide and osmium tetraoxide usually proceeds selectively, with *cis*-hydroxylation[14]. The 3-hexenetetrol residues found in the oligosaccharides produced by the degradation of blood-group substances with sodium hydroxide–sodium borohydride would thus be expected to be converted into the corresponding hexitol residues by this reaction. The identity of the hexitols formed would depend on the configuration (a) about the double bond, and (b) of the hydroxyl groups on C-2 and C-5.

The specificity of the reaction, under the conditions used here, was confirmed by the hydroxylation of two model compounds: *trans*-3-hexene-*erythro*-1,2,5,6-tetrol (recently synthesized by Anet[15]) and *trans*-3-hexene-D-*threo*-1,2,5,6-tetrol (prepared by Tipson and Cohen[16]). Oxidation of the latter gave crystalline D-mannitol (35%) and crystalline D-iditol acetate (8%), as would be expected for *cis*-addition (see Fig. 3). *trans*-Hydroxylation would have given D- and L-talitol. The only other product that could be detected was a trace component (R_{Gal} 1.37 in solvent 1) which was probably not a hexitol. It is interesting to note that D-mannitol is formed selectively over D-iditol. Hydroxylation of the *erythro* isomer gave DL-glucitol (22%) as the only product isolated; a trace of the compound of R_{Gal} 1.37 was also formed. Again, this is the expected result (see Fig. 3; note that D-gulitol ≡ L-glucitol), as *trans*-addition would have produced D-allitol and galactitol. Paper chromatography and t.l.c. on calcium sulfate confirmed these results (see Table I). The hexitols were also identified by g.l.c. of their hexaacetates on an ECNSS column[9]. From the *trans-erythro* isomer, only one peak, having the same elution time as that of glucitol, was detected (see Fig. 1D). The *trans*-D-*threo* compound gave two peaks, corresponding to mannitol and iditol; again, the preponderance of mannitol was demonstrated (see Fig. 1B).

The development of this technique for the identification of small amounts of hexitols by gas chromatography permitted application of the hydroxylation method to the unsaturated oligosaccharides from blood-group substances. Following hydroxylation, oligosaccharide samples were hydrolyzed, and free reducing sugars were removed by absorption on ion-exchange resins. The resulting hexitols were converted into their hexaacetates, and these were identified by g.l.c. under conditions that permitted the separation of all six possible hexitols. Four oligosaccharides were examined, and, in each case, mannitol, glucitol, and iditol were identified (Table I and Fig. 1C). This showed that each of the oligosaccharide samples was, in fact, a mixture of two oligosaccharides, one terminated by the 3-hexene-*erythro*- and the other by the 3-hexene-D-*threo*-tetrol (see Fig. 3). The two samples of A-active pentasaccharide (AR_L 0.52) that were examined (see Table I) were two fractions from a Biogel P2 column; it had been thought that the sample had been partially separated into two components[3]. However, we later realized that this apparent separation was an arti-

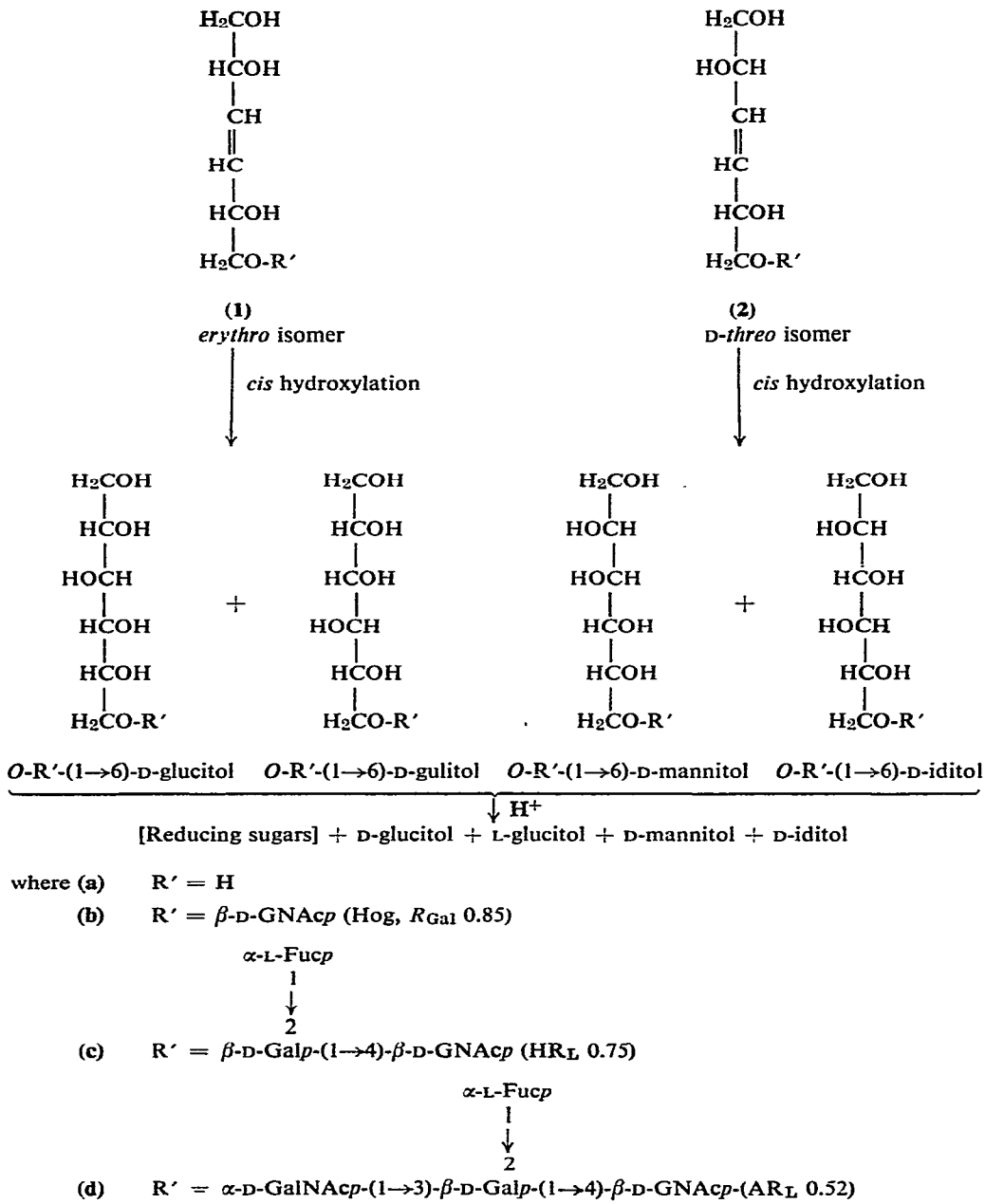


Fig. 3. Scheme for the hydroxylation and hydrolysis of oligosaccharides containing *trans*-3-hexene-1,2,5,6-tetrol residues.

fact attributable to the method used for assaying the column fractions. In this method, components are detected by their ability to reduce periodate as determined spectrophotometrically; however, the periodate-oxidized oligosaccharide absorbed ultra-

violet light, and in the peak tubes in which the absorption due to the IO_4^- ions was normally lowered, the values actually rose. Because of this phenomenon, the single peak appeared to be split into two components. Although the two fractions differed in the ratios of *erythro*: D-*threo* isomers present (see Table I), this partial fractionation was not observed when the experiment was repeated.

In addition to establishing the configuration about C-2, the results show that, in all of these compounds, the arrangement at the double bond is *trans*. Hydroxylation of the two isomers of *cis*-3-hexenetetrol would have given rise to a different set of hexitols (allitol, galactitol, and talitol). The formation of both the D-*threo* and *erythro* isomers of the hexenetetrol means that an epimerization step is involved in its formation from D-galactose; this could involve the classical Lobry de Bruyn–Alberda van Ekenstein transformation, or could occur by the borohydride reduction of a 2-keto intermediate. The latter mechanism is the more likely, as D-galactose is reduced quantitatively, without epimerization, to galactitol by 1% sodium borohydride in 2N sodium hydroxide solution*.

Hydroxylation of the reduced disaccharide (β-GNAc-R) was conducted on a larger scale, and the mixture of hexitol-containing disaccharides was isolated by chromatography (48% yield). Three hexitols, namely, glucitol, mannitol, and iditol, were detected after hydrolysis of the sample (see Table I), and the product is in fact a mixture of four reduced disaccharides (see Fig. 3). Quantitative, periodate oxidation of the original β-D-GNAc*p*-(1→6)-R (see Fig. 2) did not show whether the linkage is to the C-2 or the C-6 position of the hexenetetrol, as both structures would produce 1 mole of formaldehyde per mole. However, oxidation of the hydroxylated product showed that the linkage is (1→6) (see Fig. 2; by theory: 5 moles of IO_4^-, 3 moles of HCO_2H, and 1 mole of HCHO per mole of compound). This was confirmed by the chromatographic identification of glycerol and ethylene glycol in a sample that had been oxidized, and the product reduced with borohydride and then hydrolyzed. The ethylene glycol arises from C-5 and C-6 of the hexitols. Had the linkage been (1→2), only 4 moles of periodate would have been consumed per mole, and 2 moles of formic acid produced; also, no ethylene glycol, but an extra mole of glycerol, would have been formed (from C-1, C-2, and C-3 of the hexitols). Although the linkage was determined in this disaccharide only, it seems probable that, in the other unsaturated oligosaccharides also, the linkage is to C-6 of the hexenetetrol. As the hexenetetrol residue is probably formed from D-galactose, it is curious that no oligosaccharides containing *O*-glycosyl-(1→6)-D-galactose residues have been isolated by partial hydrolysis with acid. However, Yosizawa[17] isolated β-D-GNAc*p*-(1→6)-D-Gal and β-D-GNAc*p*-(1→3)-[β-D-GNAc*p*-(1→6)]-D-Gal by hydrazinolysis of hog blood-group substance followed by partial hydrolysis with acid; this technique has not yet been applied to human blood-group substances.

It is known that blood-group substances have two types of determinants[18], containing β-D-Gal*p*-(1→3)-D-GNAc*p* (Type I) and β-D-Gal*p*-(1→4)-D-GNAc*p*1

*K. O. Lloyd and E. A. Kabat, unpublished data.

(Type 2) sequences, respectively. However, after degradation with sodium hydroxide–sodium borohydride, all of the oligosaccharides thus far isolated that are terminated by a hexenetetrol residue have structures of Type 2 only. Although substituents on C-3 of 2-acetamido-2-deoxy-D-glucose are more alkali-labile than those on C-4, the peeling reaction in the formation of the unsaturated oligosaccharides has been stabilized at one residue before the 2-acetamido-2-deoxyhexose residue in question has been reached. A possible explanation suggested[3] for this result is that the chains of Type 1 are linked to the main chains at such positions that they are eliminated as the peeling reaction proceeds along the chains. It is possible that chains of Type 1 are linked to a position on the D-galactose residue that is alkali-labile and that gives rise to the unsaturated tetrol. Once liberated, these chains would be further degraded, from the reducing end, and this behavior would account for the formation of some of the smaller oligosaccharides isolated. The chains of Type 2, being linked to a relatively alkali-stable position (C-6) of the internal D-galactose residues, are isolated intact. This extensive destruction of Type-1 chains seriously limits the usefulness of alkaline borohydride in elucidating the structure of blood-group substances.

It is interesting to compare the oligosaccharides isolated by the degradation of blood-group substances by sodium hydroxide–sodium borohydride with (a) those formed from porcine submaxillary mucin under similar conditions, and (b) those derived from blood-group substances by hydrolysis with triethylamine. By treating blood-group substances with 2.5% of triethylamine in 50% aqueous methanol at 50°, Morgan and co-workers[19–21] have isolated, from A, B, H, and Le^a substances, mono-*O*-L-fucosyl-oligosaccharides having structures corresponding to those of the mono-*O*-L-fucopyranosyl-oligosaccharides isolated in our Laboratory, except that they are one unit shorter. Table II shows the structure of H-active oligosaccharides produced by the two methods. In the unsaturated oligosaccharides, the oligosaccharide chain is probably linked to the C-6 position of the hexenetetrol (see earlier) and, therefore, to the C-6 position of a D-galactose residue in the intact polysaccharide chain. Because substituents on this position are relatively stable to alkali[22], it appears that, when the peeling reaction reaches this residue, the sugar residue is reduced to the corresponding alditol residue before elimination of this substituent can occur to any appreciable extent. Peeling past this residue is thus largely prevented in the presence of sodium hydroxide–sodium borohydride. In the presence of triethylamine, however, the substituent on this position would eventually be eliminated, liberating oligosaccharides terminated by 2-acetamido-2-deoxy-D-glucose residues. These oligosaccharides may have either C-3 or C-4 of the 2-acetamido-2-deoxyhexose substituted (Type 1 or Type 2 determinants), and as the latter are actually isolated, the C-4 linkage must be sufficiently stable to alkali to stop substantially the peeling reaction at this point*.

*We have recently treated hog-mucin blood-group (A+H) substance with sodium hydroxide alone (Lloyd and Kabat, unpublished data), and have isolated a reducing, H-active trisaccharide that probably has the same structure as the trisaccharide described by Rege *et al.* (see Table II). This result again shows that the presence or absence of reducing agent is important in determining the positions (in the chains) at which the peeling reaction stops.

TABLE II

COMPARISON OF THE STRUCTURES OF SOME OF THE H-ACTIVE OLIGOSACCHARIDES PRODUCED BY THE ALKALINE HYDROLYSIS OF BLOOD-GROUP SUBSTANCES AND HOG SUBMAXILLARY MUCIN

Source of material, and degradation method	*Structure of H-active oligosaccharides*[a]	*References*
Human and hog blood-group substance ($NaOH–NaBH_4$)	α-L-Fuc*p* 1 ↓ 2 β-D-Gal*p*-(1→4)-β-D-GNAc*p*-R	3
Human blood-group substance (triethylamine)	α-L-Fuc*p* 1 ↓ 2 β-D-Gal*p*-(1→4)-D-GNAc*p*	20
Hog submaxillary mucin ($NaOH–NaBH_4$, or $KOH–NaBH_4$)	α-L-Fuc*p* 1 ↓ 2 β-D-Gal*p*-(1→4)-2-acetamido-2-deoxy-D-galactitol	23, 24

[a]Abbreviation used: Fuc*p*, fucopyranose; Gal*p*, galactopyranose; D-GNAc*p*, 2-acetamido-2-deoxy-D-glucopyranose; R, a 3-hexene-1,2,5,6-tetrol residue.

In contrast to the oligosaccharides, terminated by hexenetetrol and galactitol, that are formed from blood-group substances by the action of sodium hydroxide–sodium borohydride, Katzman and Eylar[23] and Carlson[24] isolated fragments terminated by 2-acetamido-2-deoxy-D-galactitol by treating hog submaxillary mucin with sodium hydroxide–sodium borohydride or potassium hydroxide–sodium borohydride. Unlike the oligosaccharides from blood-group substances, some of these oligosaccharides contained sialic acid. The structure of an H-active, reduced trisaccharide from this material is shown in Table II. As Carlson[24] found no reduced monosaccharides other than 2-acetamido-2-deoxy-D-galactitol in the degradation mixture, it appears that the 2-acetamido-2-deoxy-D-galactosyl residue is attached directly to the peptide portion of the intact mucin. There is evidence[25] that, in blood-group substances, also, at least some of the oligosaccharide chains are linked through 2-acetamido-2-deoxy-D-galactose residues to a peptide backbone. The difference in the patterns of the oligosaccharides isolated from the two sources may probably by explained by two properties: (a) in blood-group substances, the oligosaccharide chains are longer and more complex than those in hog submaxillary mucin, and (b) in hog submaxillary mucin, the oligosaccharides freed, by elimination, from the peptide portion are terminated by a reducing 2-acetamido-2-deoxy-D-galactose residue that is substituted on C-4. This is a position relatively stable to alkali[22], and reduction to the alditol residue would probably occur before further alkaline degradation took place. In a blood-group substance, however, the terminal 2-acetamido-2-deoxy-D-galactose residue (of chains that are

linked to the protein moiety through this sugar) is probably substituted on C-3, because the only oligosaccharide containing 2-acetamido-2-deoxy-D-galactose that has been isolated by partial hydrolysis with acid (apart from the A-active oligosaccharides) is[18] *O*-β-D-GNAc*p*-(1→3)-*O*-β-D-Gal*p*-(1→3)-D-GalNAc*p*. Substituents at C-3 are readily eliminated, and peeling of the chain should occur until alkali-stable linkages are reached. These differences are not due to differences between hog and human materials, since identical oligosaccharides have been isolated from human-cyst and hog-stomach blood-group substances[3]. Ovine submaxillary mucin appears to have very short oligosaccharide chains consisting only of *O*-(α-*N*-acetylneuraminyl)-(1→6)-2-acetamido-2-deoxy-D-galactose residues[26].

SUMMARY

Treatment of blood-group substances with sodium hydroxide–sodium borohydride gives a series of oligosaccharides, many of which are terminated by an unsaturated alditol residue, that of a 3-hexene-1,2,5,6-tetrol (R). *cis*-Hydroxylation of authentic samples of the *trans-erythro* and D-*threo* forms of this alditol with hydrogen peroxide and osmium tetraoxide gave the expected products, namely, DL-glucitol from the former, and D-mannitol and D-iditol from the latter. Three of the unsaturated oligosaccharides from blood-group substances, when hydroxylated under the same conditions and the product then hydrolyzed, gave mixtures of these three hexitols. The hexitols were identified by gas chromatography of their hexaacetates under conditions that permitted the separation of all six possible hexitol hexaacetates. Each oligosaccharide sample, is, therefore, a mixture of two oligosaccharides, one terminated by a *trans*-3-hexene-D-*threo*- and the other by a *trans*-3-hexene-*erythro*-tetrol residue.

Periodate oxidation of the mixture of hexitol-containing disaccharides obtained by hydroxylation of β-D-GNAc*p*-(1→6)-R showed that the 2-acetamido-2-deoxyhexose residue is linked to C-6 of the hexenetetrol.

The structures of the oligosaccharides obtained from blood-group substances by degradation with sodium hydroxide–sodium borohydride are compared with those from hog submaxillary mucin and with those formed by the hydrolysis of blood-group substances with triethylamine.

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Notes

The acid hydrolysis of glycosides IV. Hydrolysis of aldobiouronic acids

It has long been known that aldobiouronic acids are more stable towards acids than are the corresponding neutral disaccharides, and that this is why they are often obtained in high yields on partial, acid hydrolysis of acidic polysaccharides. Few attempts have, however, been made to assess, quantitatively, the stabilizing effect exerted by the uronic group. The present investigation is concerned with the synthesis and acid-catalyzed hydrolysis of two aldobiouronic acids and their neutral analogs.

RESULTS AND DISCUSSION

In previous investigations in this series, it was found that the nature of the aglycon has little influence on the rate of hydrolysis of neutral glycosides[1], but that this is not the case with glycosiduronic acids[2]. In an attempt to obtain a comparison between two closely related aldobiouronic acids, 6-*O*-(β-D-glucopyranosyluronic acid)-D-glucose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose were synthesized, together with their neutral analogs, gentiobiose and 6-*O*-(β-D-glucopyranosyl)-D-galactose. Rate coefficients, energies of activation, and entropies of activation for their hydrolysis were determined in 0.5M sulfuric acid solution at 60, 70, and 80°, the hydrolysis being followed polarimetrically. The results are shown in Table I.

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TABLE I

RATE COEFFICIENTS AND KINETIC PARAMETERS FOR THE HYDROLYSIS OF TWO ALDOBIOURONIC ACIDS AND TWO DISACCHARIDES IN 0.5M SULFURIC ACID

Compound	$k \times 10^6$, sec^{-1}			E (*kcal* $mole^{-1}$)	$\Delta S^{\neq}$ *at* 60° (*cal* deg^{-1} $mole^{-1}$)
	60°	70°	80°		
6-*O*-β-D-Glucopyranosyl-D-glucose	1.87	8.25	32.88	33.3	+14.3
6-*O*-(β-D-Glucopyranosyluronic acid)-D-glucose	0.33	1.37	4.65	30.8	+ 2.5
6-*O*-β-D-Glucopyranosyl-D-galactose	1.67	7.81	31.67	34.0	+15.6
6-*O*-(β-D-Glucopyranosyluronic acid)-D-galactose	0.17	0.66	2.53	31.7	+ 3.9

It was noted previously[2] that, on acid hydrolysis, glycosiduronic acids generally have a somewhat lower energy of activation than do the corresponding glycosides, and that the entropy of activation of the former is invariably much lower than that of the latter. This has now been observed for the present compounds also, indicating that aldobiouronic acids and glycosiduronic acids are hydrolyzed by the same mechanism. The difference in the entropy of activation of the acidic and the neutral disaccharides would suggest that these two types of compound are hydrolyzed by different mechanisms, but the nature of this difference remains unknown.

Both of the aldobiouronic acids were hydrolyzed at a rate considerably lower than that of their neutral analogs. Whistler and Richards[3], whose results were recently re-evaluated by Meller[4], compared the acid-catalyzed hydrolysis of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-D-xylitol, a comparison that did not take into account the fact that the reducing disaccharide and the alditol are hydrolyzed at somewhat different rates[5]. In a stricter comparison, Johansson, Lindberg, and Theander[6] determined the rate coefficients for the acid hydrolysis of cellobiose and cellobiouronic acid. The ratios between the rate coefficients for the neutral disaccharides and for the corresponding aldobiouronic acids obtained in the previous and the present investigations are summarized in Table II.

TABLE II

RATIO OF HYDROLYTIC RATE COEFFICIENTS OF VARIOUS NEUTRAL DISACCHARIDES AND CORRESPONDING ALDOBIOURONIC ACIDS

Aldobiouronic acid	*Molarity of* H_2SO_4	*Temp., degrees*	*Ratio*	*References*
2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose	0.53	95	17.6	3
			16.0	4
4-*O*-(β-D-Glucopyranosyluronic acid)-D-glucose	1.00	80	37.4	6
		90	35.3	
6-*O*-(β-D-Glucopyranosyluronic acid)-D-glucose	0.50	80	7.1	
6-*O*-(β-D-Glucopyranosyluronic acid)-D-galactose	0.50	80	12.5	

The effect of the uronic acid group on the rate of hydrolysis is largest for the (1→4)-linked aldobiouronic acid (cellobiouronic acid), only half of this for the (1→2)-linked aldobiouronic acid, and least for the two (1→6)-linked compounds. The five times greater stability of cellobiouronic acid as compared to gentiobiouronic acid is especially noteworthy, for these two aldobiouronic acids differ only in the point of attachment of the glycosiduronic acid linkage. It is evident that the nature of this linkage has a considerable influence on the magnitude of the stabilizing effect exerted by the carboxyl group on C-5.

The difference in relative stability between the two (1→6)-linked aldobiouronic acids studied here is interesting. Substitution of an axial for an equatorial hydroxyl group on C-4 in the reducing moiety of these compounds results in a decreased rate of hydrolysis. It has been found[2,7] that polar groups in the aglycon of glycosiduronic acids, but not of glycosides, have a profound influence on their stability towards acids. In the present study, the two neutral disaccharides were hydrolyzed at approximately the same rate, whereas the two aldobiouronic acids differed considerably in their rate of hydrolysis. It is conceivable that the spatial orientation of a hydroxyl group on C-4 could influence the hydrolysis of a bond involving the adjacent hydroxymethyl group on C-5, but it is not clear why this would require the presence of a carboxyl group on C-5'.

EXPERIMENTAL

The general experimental conditions were the same as those in a previous investigation[8].

6-O-β-D-Glucopyranosyl-D-glucose. — The synthesis of this compound[9,10] has been described[8]. The compound had m.p. 190–192°, $[\alpha]_D$ +10.7° (water).

6-O-β-D-Glucopyranosyl-D-galactose. — 1,2:3,4-Di-*O*-isopropylidene-α-D-galactose, $[\alpha]_D$ −50° (*c* 4.0, chloroform), was obtained by acetonation of D-galactose, in 11% yield with anhydrous copper sulfate[11,12] as catalyst, and in 81% yield when anhydrous zinc chloride[13,14] was used. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide m.p. 87–88° and $[\alpha]_D$ +196° (*c* 1.0, chloroform), was prepared in a yield of 80% by the method of Bárczai–Martos and Kőrsöy[15]. Koenigs–Knorr condensation (silver carbonate) of the two compounds gave 6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactose[16,17], yield 54%, m.p. 142–143°, and $[\alpha]_D$ −58.5° (*c* 3.0, chloroform); lit[16b] m.p. 140°. Successive treatments in the usual way with methanolic sodium methoxide and with Amberlite IR-120 (H^+) ion exchange resin afforded 6-*O*-β-D-glucopyranosyl-D-galactose, yield 60%. The amorphous but chromatographically pure disaccharide had $[\alpha]_D$ +37° (*c* 2.0, water); lit.[16b] m.p. 128–130°, $[\alpha]_D^{25}$ +34.2→+14.7° (*c* 1.6, water); lit.[16a] $[\alpha]_D^{18}$ +1.6→+13.9° (water).

6-O-(β-D-Glucopyranosyluronic acid)-D-glucose. — Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[18], m.p. 102–103° and $[\alpha]_D$ +210° (*c* 1.5, chloroform) was condensed in a Koenigs–Knorr reaction (silver carbonate) with 1,2,3,4-tetra-*O*-acetyl-β-D-glucose[20] [m.p. 127–128°, $[\alpha]_D$ +12° (*c* 6.0, chloroform)]

to give 6-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-1,2,3,4-tetra-*O*-acetyl-D-glucose[19,21], yield 13%, m.p. 199–201°, $[\alpha]_D$ −10.6° (*c* 3.7, chloroform). Removal of the acetate groups in the usual way afforded amorphous 6-*O*-β-D-glucopyranosyluronic acid)-D-glucose[19], $[\alpha]_D$ −23° (*c* 2.0, water).

6-O-(β-D-Glucopyranosyluronic acid)-D-galactose. — Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate was condensed in the usual way with 1,2:3,4-di-*O*-isopropylidene-α-D-galactose, to give 6-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-1,2:3,4-di-*O*-isopropylidene-α-D-galactose[19,22], yield 55%, m.p. 114–115°, $[\alpha]_D$ −65° (*c* 4.5, chloroform); lit.[19b] m.p. 121°, $[\alpha]_D^{24}$ −66.3°° (*c* 1.36, chloroform). Crystalline 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, obtained in the usual way in 70% yield , had $[\alpha]_D$ −8.7° (*c* 2.0, water).

Kinetic measurements. — Hydrolyses were conducted with 0.025M solutions of the sugars in 0.5M sulfuric acid, as described previously[1,8]. Rate coefficients were computed by using the measured, initial rotation of each compound, and also from its calculated, final rotation.

ACKNOWLEDGMENT

This investigation was partly supported by a U. S. Public Health Service Research Grant No. RO1 AMO7193 from the National Institute of Arthritis and Metabolic Diseases, which is hereby gratefully acknowledged.

Cellulose Research Institute and
Department of Forest Chemistry,
State University College of Forestry,
Syracuse, N. Y. 13210 (U.S.A)

K. K. DE
T. E. TIMELL

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(Received October 17th, 1966)

Carbohyd. Res., 4 (1967) 177–181

An improved preparation of the capsular polysaccharide from *Diplococcus pneumoniae*

Antigenic capsular polysaccharides from *D. pneumoniae* have been isolated from the culture broth following growth and lysis of the organisms[1]. Improved methods[2] consisted of (*a*) grinding the organisms prior to lysis, and (*b*) high-speed stirring of a suspension of cells with 1,1,1-trichloro-2,2,2-trifluoroethane[3]. The former method yields an extract contaminated with protein and C-substance, and the latter employs rather dilute suspensions (10^8 cells/ml), making it impractical for large quantities.

We now report a method that conveniently yields the capsular material virtually free from C-substance and proteins.

D. pneumoniae Type 31 was serially mouse-passaged, and sustained on fresh blood–agar plates. The organisms were then grown in aqueous brain-heart infusion. The growth was followed by turbidity measurements against a sterile-medium blank at 530 mμ. When growth reached a maximum, lysis was found to be rapid, but it was subsequently learned, in our work with Type 13, that the addition of a small proportion of catalase[4] (129 Σ units/ml*) prevented lysis** and increased the permissible number of cells per ml (see Fig. 1). The culture was killed, at the appropriate time, with formaldehyde (10 ml of 40% formalin per liter of culture), and, after 24 h, it was collected on a centrifuge. The residual mixture, still containing formalin, was heated for 45 min at 60°, and the medium was washed out completely with 0.9% saline, until the supernatant liquor was free from protein (Folin), approximately seven washes (each of four times the pellet volume) usually being sufficient. The cells thus obtained were resistant to shearing with glass beads for 11 h at 200 strokes per min, very little antigen being released.

The cells from 7.5 liters of medium were then suspended in 125 ml of sodium acetate buffer (of ionic strength 0.1) at pH 6.0, sonicated for 10 min at 0°, and the

*Sigma Chemical Company, 3500 DeKalb Street, St. Louis, Missouri, U.S.A.
**The build-up of hydrogen peroxide in the culture fluid has also been prevented by the addition of cysteine: L. B. HOLT, *J. Gen. Microbiol.*, 27 (1962) 327.

18 G. N. BOLLENBACK, J. W. LONG, D. G. BENJAMIN, AND J. A. LINDQUIST, *J. Am. Chem. Soc.*, 77 (1955) 3310.
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(Received October 17th, 1966)

Carbohyd. Res., 4 (1967) 177–181

An improved preparation of the capsular polysaccharide from *Diplococcus pneumoniae*

Antigenic capsular polysaccharides from *D. pneumoniae* have been isolated from the culture broth following growth and lysis of the organisms[1]. Improved methods[2] consisted of (*a*) grinding the organisms prior to lysis, and (*b*) high-speed stirring of a suspension of cells with 1,1,1-trichloro-2,2,2-trifluoroethane[3]. The former method yields an extract contaminated with protein and C-substance, and the latter employs rather dilute suspensions (10^8 cells/ml), making it impractical for large quantities.

We now report a method that conveniently yields the capsular material virtually free from C-substance and proteins.

D. pneumoniae Type 31 was serially mouse-passaged, and sustained on fresh blood–agar plates. The organisms were then grown in aqueous brain-heart infusion. The growth was followed by turbidity measurements against a sterile-medium blank at 530 mμ. When growth reached a maximum, lysis was found to be rapid, but it was subsequently learned, in our work with Type 13, that the addition of a small proportion of catalase[4] (129 Σ units/ml*) prevented lysis** and increased the permissible number of cells per ml (see Fig. 1). The culture was killed, at the appropriate time, with formaldehyde (10 ml of 40% formalin per liter of culture), and, after 24 h, it was collected on a centrifuge. The residual mixture, still containing formalin, was heated for 45 min at 60°, and the medium was washed out completely with 0.9% saline, until the supernatant liquor was free from protein (Folin), approximately seven washes (each of four times the pellet volume) usually being sufficient. The cells thus obtained were resistant to shearing with glass beads for 11 h at 200 strokes per min, very little antigen being released.

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residue collected on the centrifuge; this procedure was repeated twice. The clear, supernatant liquors were combined and treated with 1,1,1-trichloro-2,2,2-trifluoroethane[3], but little or no protein was present, as evidenced by (*a*) the fact that little

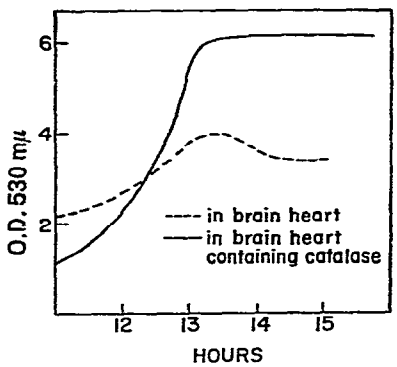


Fig. 1. Growth of *D. pneumoniae* Type 13 in brain-heart infusion, with and without catalase.

formation of gel took place, and (*b*) analysis, the turbidity of the sonicate (1 ml), 30% sulfosalicylic acid (0.2 ml), and water (2 ml) being measured[5] against a water blank at 600 mμ. The polysaccharide was concentrated *in vacuo*, dialyzed, and lyophilized. It showed a single peak in the Spinco ultracentrifuge in sodium acetate buffer (ionic strength, 0.1) at pH 6.0 (see Fig. 2). Only when a concentrated solution



Fig. 2. Sedimentation of crude S-31 polysaccharide (0.5%) in sodium acetate buffer (ionic strength 0.1 pH 6.0) at 50,740 rpm for *ca.* 100 min.

(1%) was used in immunoelectrophoresis in phosphate buffer (ionic strength, 0.1) at pH 7.0 at constant current (20 ma) for 5 h did the material reveal the presence of C-substance (see Fig. 3). Quantitative estimation, by dilution titration *versus* horse anti-Pn-III serum containing antibody to C-substance*, showed that the crude sample did not contain more than 0.2–0.5% of C-substance by weight. This could be removed by precipitation with alcohol.

In a preliminary experiment, an extract from lysed cells, highly contaminated with C-substance, was shown to be resolvable upon chromatography on DEAE-cellulose by elution with gradient sodium chloride solution, the C-substance being collected before the S-31 polysaccharide. Our present preparation was checked by elu-

*A generous gift from Professor M. Heidelberger.

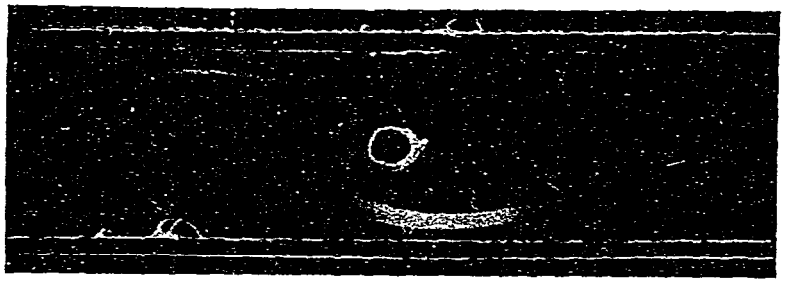


Fig. 3. Immunoelectrophoresis of crude S-31 polysaccharide (1.0%). Top trough: horse anti-Pn-III serum containing antibody to C-substance. Bottom trough: rabbit anti-Pn-31 serum.

tion of 45 mg off a column (20 × 2.5 cm) of DEAE-Sephadex A-50 with a gradient of 0→2M sodium chloride in sodium acetate buffer (ionic strength, 0.05) at pH 6.0 (see Fig. 4), and by elution of 22 mg off a column (28 × 3.7 cm) of Sephadex G-200 with physiological saline as the eluant (see Fig. 5). Carbohydrates were analyzed by the

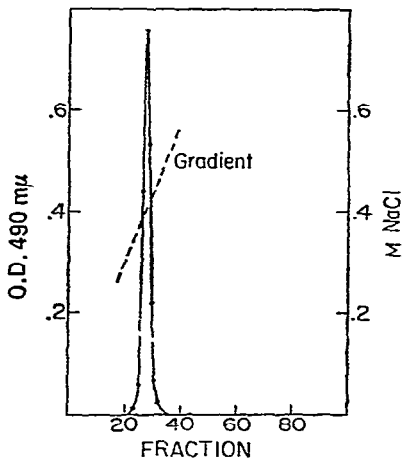


Fig. 4. Elution of S-31 polysaccharide off a column of DEAE-Sephadex A-50.

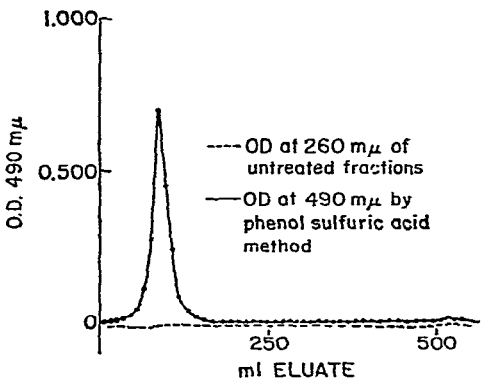


Fig. 5. Elution of S-31 polysaccharide off a column of Sephadex G-200.

phenol–sulfuric acid method[6]. Neither experiment revealed the presence of more than one component. The data of Fig. 5 also show that the preparation is free from nucleic acids.

Figure 6 gives the sedimentation of the purified polysaccharide at 50,740 rpm in the Spinco ultracentrifuge in the buffer indicated.

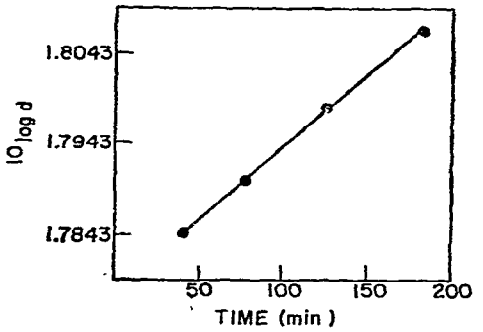


Fig. 6. Sedimentation of the S-31 polysaccharide boundary at 50,740 rpm in sodium acetate buffer (ionic strength, 0.1) at pH 6.0.

Thus prepared, the polysaccharide was obtained in a yield of 30 mg per liter of culture; $[\alpha]_D^{20} -19°$ (*c* 0.5, water).

ACKNOWLEDGMENTS

This work was supported by grants from the Department of Health, Education, and Welfare, U. S. Public Health Service (Grants Nos. 1-S01-FR-05358-04 and 5R01AI05830-03AIA), while one of us (C.P.J.G.) was the holder of Career Development Award USPHS GM 25720-01.

Departments of Biochemistry and Microbiology, Yale University School of Medicine, New Haven, Connecticut 06510 (U. S. A.)

C. P. J. GLAUDEMANS
H. P. TREFFERS

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(Received December 21st, 1966)

Carbohyd. Res., 4 (1967) 181–184

Correlation between the periodate consumption of, and the percentage of D-glucose and maltose in, industrial D-glucose syrup

This study deals with a correlation between the D-glucose and maltose contents of industrial D-glucose syrup (from rice starch) of high dextrose equivalent (D. E.) and the periodate consumption of the syrup.

The D-glucose and maltose contents were estimated after elution from a carbon–Celite column[1] with water and 7% aqueous ethanol, respectively. However, when subjected to paper chromatography, the eluted maltose showed spots of higher oligosaccharides, and better results were obtained by carrying out the separation on paper chromatograms. The sugar spots (after being revealed by spraying with 10% ammonium molybdate) were cut off from the sheet and heated with a solution of ammonium molybdate in dilute sulfuric acid[2]; the molybdenum blue produced was then measured colorimetrically.

The results (see Table I) obtained by these two methods were concordant, except for a slightly higher maltose content found by the first method (attributed to its contamination with higher oligosaccharides). From Table I, it may also be seen

TABLE I

PERCENTAGE OF D-GLUCOSE AND MALTOSE FOUND IN SEVEN D-GLUCOSE SYRUPS, AND THEIR VALUES CALCULATED FROM THE PERIODATE CONSUMPTION BY USING THE GRAPH IN FIG. 1

Sample No.	*Method*	*D-Glucose (%)*		*Maltose (%)*		*Periodate consumed per equivalent weight 180*
		Found	*Calc.*	*Found*	*Calc.*	
1	a	21.7	23.0	23.4	21.0	2.80
	b	23.5		21.9		
2	a	25.4	24.9	19.6	20.0	2.85
	b	24.7		17.8		
3	a	42.3	40.0	9.06	9.0	3.37
	b	42.6		6.0		
4	a	45.3	44.0	7.0	6.0	3.50
	b	46.2		4.1		
5	a	29.2	28.0	25.4	18.0	2.96
	b	29.9		20.9		
6	a	46.4	47.0	9.7	4.0	3.60
	b	46.5		6.3		
7	a	38.2	35.0	16.4	13.0	3.20
	b	37.5		13.5		

that, in these D-glucose syrups of high D. E., the proportion of maltose decreased when the D-glucose content was high (in more fully hydrolyzed samples), a fact noticeable in previous work[3] on corn-starch D-glucose syrups.

It is obvious that the periodate consumption of a sample of D-glucose syrup is influenced to a much greater extent by the percentage of D-glucose (which consumes

5 moles of periodate per mole) and of maltose (which consumes 2.5 molecules per hexose residue of equivalent weight 342/2 = 171, or 2.6 molecules per equivalent weight 180) than by the higher oligosaccharides and polysaccharides (which consume from 2.3 to 1.1 moles of oxidant per equivalent weight 180). We have therefore correlated on a graph (see Fig. 1) the percentage of D-glucose and maltose in D-glucose

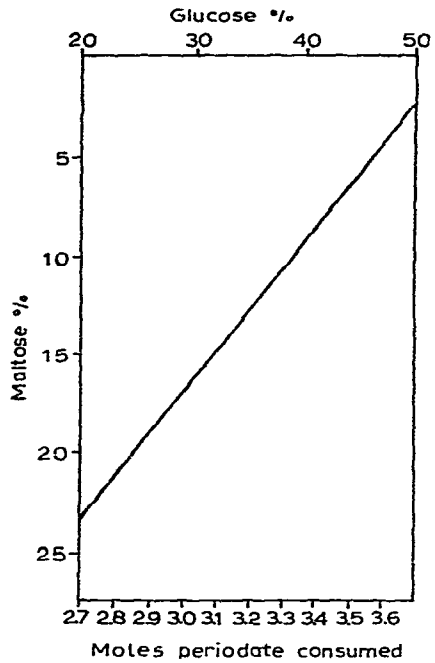


Fig. 1. Correlation between the number of moles of periodate consumed per equivalent weight 180 and the percentage of D-glucose and maltose in industrial D-glucose syrup.

syrup with the periodate consumption of the syrup. Table I shows good agreement between the percentage of D-glucose and maltose found in the samples and the values calculated from their periodate consumption by using the graph. As expected, when the periodate consumed was high (in more fully hydrolyzed samples), the content of D-glucose was high and that of the maltose was low; and when the periodate consumed was low (in less fully hydrolyzed samples), the D-glucose content was low and the maltose content high.

The percentage of higher oligosaccharides determined, after separation by multiple-development chromatography (see Table II), in two of these rice-starch D-glucose syrups and the percentage of D-glucose and maltose given in Table I do not differ much from the value given by Patterson and Buchan[4] for corn-starch D-glucose syrup manufactured in Great Britain, suggesting that the above relation would probably hold for corn-starch D-glucose syrups.

TABLE II

PERCENTAGE OF HIGHER OLIGOSACCHARIDES IN TWO D-GLUCOSE SYRUPS

Component	*Syrup No. 1*	*Syrup No. 2*
Maltotriose	12.6	16.7
Maltotetraose	5.4	11.5
Maltopentaose	3.6	4.7
Maltohexaose	2.7	2.2

EXPERIMENTAL

The industrial D-glucose syrup was manufactured locally by the acid hydrolysis of rice starch by a procedure that give a D. E. of 40–50.

Estimation of D-*glucose syrup*

(*a*) *After separation on a carbon–Celite column.* — A solution of D-glucose syrup [20 mg (dry wt.)/10 ml of water] was allowed to flow down a column of 1:1 carbon–Celite[1], with water as the eluent. After 100 ml had been collected with water 7:93 ethanol–water was used as eluent, and 100 ml was collected. Each mean of duplicate analyses on 5 ml portions of aqueous and aqueous ethanol eluates by the Somogyi copper micro-method[5] is given in Table I.

(*b*) *After separation on paper chromatograms.* — Spots containing 4 μl of a dilute solution of D-glucose syrup [10 g (dry wt.)/100 ml of water] were applied with a micropipette and syringe to Whatman No. 1 filter paper (33 cm long and 22 cm wide); and 3 spots of standard D-glucose solution and 3 spots of standard maltose solution were placed separately on the same sheet as controls. The chromatograms were run in duplicate (one on each side of the trough) with the upper layer of 40:25:25:10 butyl alcohol–pyridine–water–benzene for 48 h; they were then dried, sprayed with 10% ammonium molybdate solution[6], and heated for 10 min at 100°. Care was taken not to leave the chromatograms exposed to strong light, as they then acquire a blue coloration. Circles (2 cm in diameter) were cut around each revealed spot, and each circle was placed in a test tube containing 6 ml of ammonium molybdate–sulfuric acid reagent[2]. Each tube was heated in a briskly boiling water-bath for 10 min with occasional shaking, and kept for 5 min at room temperature. A blank was prepared by cutting a circle of the same size from a blank part of the chromatogram paper and subjecting it to the same treatment. The weights of D-glucose and maltose in D-glucose syrup (see Table I) were determined by comparing their absorbance with standard curves.

Periodate oxidation of D-*glucose syrup.* — A solution of D-glucose syrup [2 mg (dry wt.)/1 ml of water] was treated with 10 ml of 0.01N sodium metaperiodate and 0.5 ml of acetate buffer (pH 5.2; 50 ml of N sodium acetate/10 ml of N hydrochloric acid, made up to 250 ml). After 24 h at room temperature, 2 ml of 5% aqueous sodium hydrogen carbonate solution was added, followed by 1 ml of 20% aqueous potassium

iodide solution and 12 ml of 0.005N sodium arsenite. After 15 min, the solution was titrated against 0.005N iodine. The moles of periodate consumed per equivalent weight 180 are given in Table I.

Estimation of higher oligosaccharides. — A dilute solution of D-glucose syrup [1.5 mg (dry wt.)/10 μl of water] was evenly distributed at the origin of a Whatman filter-paper chromatogram, except for 5 cm on each side, where a spot of another portion of the same batch of D-glucose syrup was placed. The developing solvent was 30:46:24 pyridine–butyl alcohol–water. The chromatograms were run for 24 h, dried, redeveloped with the same solvent for 24 h, and dried. This procedure was repeated once more, and then strips (4 cm wide) were cut on each side of the chromatogram along its whole length; the strips were sprayed with 3,5-dinitrosalicylic acid[7], and dried at 105°. The positions of the bands of D-glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and higher saccharides were thus revealed, and these bands were then marked on the unsprayed part of the chromatogram; the sugar bands were cut out, and eluted with water to a volume of 15 ml each. The maltotriose and higher oligosaccharides were hydrolyzed with N sulfuric acid (1 ml) for 2.5 h at 100°, and the solutions neutralized by adding N sodium carbonate (1 ml). The reducing sugars were determined volumetrically by the method of Hagedorn and Jensen[8] (see Table II).

Chemistry Department, Faculty of Science,
Alexandria University,
Alexandria, Egypt (U.A.R.)

H. EL KHADEM
Z. M. EL-SHAFEI
M. S. HEKAL

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(Received October 3rd, 1966; in revised form, October 26th, 1966)

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF 2-*O*-α-L-FUCOPYRANOSYL-L-FUCOPYRANOSE

H. M. FLOWERS, A. LEVY, and N. SHARON

Departments of Organic Chemistry and Biophysics, The Weizmann Institute of Science, Rehovoth (Israel)

(Received October 31st, 1966)

INTRODUCTION

Many oligosaccharides containing L-fucosyl residues have been obtained from natural sources[1]. These include three L-fucosyl-L-fucose disaccharides isolated from partial acetolyzates of the seaweed polysaccharide fucoidin[2], and several oligosaccharides containing L-fucose together with other sugars. Some of the latter were found in human milk, and others were prepared by the partial hydrolysis of blood-group substances where they serve as antigenic determinants[3]. However, no oligosaccharides containing L-fucosyl residues have been synthesized as yet. In this communication, we describe the chemical synthesis of the disaccharide 2-*O*-α-L-fucopyranosyl-L-fucopyranose[2].

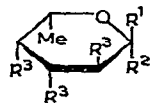
RESULTS AND DISCUSSION

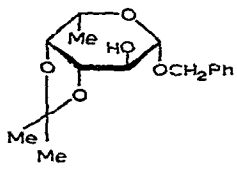
Acetylation of L-fucose (6-deoxy-L-galactose) with acetic anhydride and sodium acetate[4] gave a syrup which was treated with a solution of hydrogen bromide in acetic acid to yield 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide (**1**), a relatively unstable oil which was practically pure. The optical rotation and n.m.r. spectrum[5] were in agreement with the α-L configuration. Treatment of the bromide **1** with benzyl alcohol in the presence of silver oxide gave the crystalline benzyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside (**2**); the assignment of configuration at C-1 was based on optical rotation and n.m.r. spectrum. The overall yield of **2** from L-fucose was 34%. Catalytic transesterification afforded benzyl β-L-fucopyranoside (**3**), a syrup which was converted into benzyl 3,4-*O*-isopropylidene-β-L-fucopyranoside (**4**) by treatment with acetone and sulfuric acid[6]. The latter compound could not be induced to crystallize, but gave satisfactory elementary analysis and n.m.r. spectrum. Condensation of compound **1** with **4** in a nitromethane–benzene solution in the presence of mercuric cyanide[7] gave a mixture from which the disaccharide benzyl 3,4-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-L-fucopyranoside (**5**) was isolated. This product was crystalline and was obtained in a yield of 20%, based on **4**. Examination of the reaction mixture failed to indicate the presence of the other possible anomer (β-L-linked) of the disaccharide. The assignment of the α-L configuration to the interglycosidic linkage in **5** is based not only on a highly negative optical rotation, consis-

tent with the values reported for the rotations of α-L-fucopyranosides[2,8], but also on the presence of a doublet at τ 4.45, J 3.5 c.p.s.[9], absent in the n.m.r. spectrum of compound **2**.

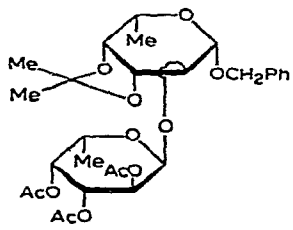
The Koenigs–Knorr reaction, modified by use of mercuric cyanide in nitromethane solution, leads generally to the formation of β-D-(or α-L-) linked disaccharides[10], and involves inversion of the configuration at C-1 of the reacting acylglycosyl halide. However, in some cases, α-D-linked disaccharides have been isolated from the

	R^1	R^2	R^3
1	Br	H	OAc
2	H	OCH_2Ph	OAc
3	H	OCH_2Ph	OH

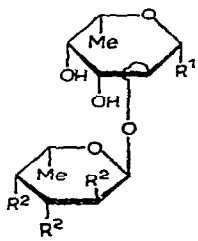




4



5



6 $R^1 = OCH_2Ph$; $R^2 = OAc$
7 $R^1 = OCH_2Ph$; $R^2 = OH$
8 $R^1 = R^2 = OH$

reaction mixture[11,12]. To explain these variations in the anomeric configuration of the interglycosidic linkage of the disaccharide formed, it has been postulated[12] that a mixture of anomers may result, the nature of the compound isolated depending upon the method of separation (*e.g.* by crystallization). However, it has not always been possible to show the presence of both anomers in the reaction mixture. A mechanism of reaction of acylglycosyl halides involving participation of the acetoxy group at C-2 to stabilize the carbonium ion formed on separation of the halogen atom, with subsequent rear-side attack of the hydroxyl function of the aglycon to give, finally, inversion of the configuration at the anomeric center[13], cannot apply when there is preservation of the configuration. In the case described in the present paper, a 2-α-L-linked disaccharide was prepared in good yield (bearing in mind the known low reactivity of the secondary hydroxyl group of glycopyranosides[14]). In a similar reaction, 2-*O*-α-D-galactopyranosyl-D-glucose has been synthesized by condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose in the presence of mercuric cyanide in nitromethane solution[11].

Deacetonation of the disaccharide **5** under mild acidic conditions gave crystalline benzyl 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-L-fucopyranoside (**6**), in good yield. Its n.m.r. spectrum was in full agreement with the structure indicated. Catalytic transesterification of **6** yielded benzyl 2-*O*-(α-L-fucopyranosyl)-β-L-fucopyranoside (**7**), which was converted into the desired 2-*O*-α-L-fucopyranosyl-L-fucopyranose (**8**) by catalytic hydrogenolysis. Both **7** and **8**, obtained in good yield, were crystalline and gave satisfactory elementary analyses. Compound **8** gave a single spot on paper chromatograms in three solvent systems, and its rate of migration was identical with that of a sample of 2-*O*-α-L-fucopyranosyl-L-fucose isolated from fucoidin and kindly supplied by Dr. R. H. Côté. The optical rotation and m.p. of the synthetic product were identical with those given for the natural product[2].

EXPERIMENTAL

Melting points were observed on substance between glass slides with a Fisher–Johns apparatus and are corrected. Rotations were determined in semimicro tubes with a Perkin-Elmer No. 141 polarimeter or a Bendix polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, with tetramethylsilane as internal standard and deuterochloroform as solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used and elution was stepwise, in order of increasing polarity of eluents. The proportion of weight of substance to weight of adsorbent added to the column was 1 to 50–100. Thin-layer chromatograms were performed on Kieselgel G (E. Merck, Darmstadt). Analyses were carried out in the Institute's Microanalytical Laboratory under the direction of Mr. R. Heller. The i.r. spectra of all compounds were recorded with a Perkin-Elmer Infracord Spectrometer, either in chloroform solution (when soluble) or as discs pressed in KBr, and were found to agree with the structures proposed.

*2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl bromide* (**1**). — L-Fucose (Pfanstiehl) (10 g) was added in small portions to a boiling solution of anhydrous sodium acetate (5 g) in acetic anhydride (70 ml). After addition of L-fucose, the reaction mixture was allowed to cool, and was then poured with stirring into water (100 ml). The mixture was neutralized with dry sodium hydrogen carbonate and extracted twice with chloroform. The combined chloroform extracts were washed with water until neutral, and were dried with calcium chloride. The chloroform was evaporated *in vacuo*, leaving a syrupy residue (20 g, 99%), $[\alpha]_D^{25}$ −34° (*c* 2.02, chloroform). This product migrated as a single spot on t.l.c. (benzene–methanol 9:1 v/v).

Anal. Calc. for $C_{14}H_{20}O_9$: C, 50.60; H, 6.02. Found: C, 50.80; H, 6.09.

To the syrup (20 g) was added a solution of hydrogen bromide in glacial acetic acid (45% w/v, 50 ml). The clear solution was kept for 1.5 h at room temperature. It was then poured into ice-water and the resulting mixture was extracted with chloroform. The extract was washed successively with cold water, a cold saturated solution of sodium hydrogen carbonate, and finally with water until neutral. It was dried with calcium chloride and evaporated *in vacuo*, to yield a syrup (20 g, 94%), $[\alpha]_D^{28}$ −160°

(*c* 2.26, chloroform). Examination of this product by t.l.c. (benzene–ether 1:1 v/v) showed that the compound was not completely pure; n.m.r. data: τ 3.35 (low-field doublet[5], *J* 3.5 c.p.s., H-1); τ 7.84 (3H, axial OAc); τ 7.92 and 8.00 (6H, two equatorial OAc); τ 8.80 (doublet, *J* 6.5. c.p.s., 3H, CH-CH_3). No attempts to purify the bromide further were made, because of its instability.

*Benzyl 2,3,4-tri-*O*-acetyl-β-*L*-fucopyranoside* (**2**). — To a solution of the crude bromide (20 g) in benzene (30 ml) were added freshly distilled benzyl alcohol (30 ml) and silver oxide (10 g). The mixture was heated with shaking for 0.5 h at 35–40°. The temperature was then raised to 85°, and the mixture was kept at this temperature, with continued shaking, for 15 min, and then cooled. After removal of insoluble silver salts by filtration, the solution was evaporated, and the remaining benzyl alcohol was steam-distilled. The syrupy residue was dried *in vacuo*, and addition of ethanol gave a crystalline material, m.p. 136–9° (8.1 g, 37%) (single spot on t.l.c. in benzene–methanol 9:1); after recrystallization from ethanol, m.p. 141–141.5°, $[\alpha]_D^{25}$ +41° (*c* 2.08, chloroform); n.m.r. data: τ 2.70 (5H, C_6H_5); τ 5.51 (doublet, *J* 8 c.p.s., H-1); τ 7.82 (3H, axial OAc); τ 8.00 and 8.02 (6H, two equatorial OAc); τ 8.74 (doublet, *J* 6.5 c.p.s., 3H, CH-CH_3).

Anal. Calc. for $C_{19}H_{24}O_8$: C, 60.00; H, 6.31. Found: C, 59.88; H, 6.23.

*Benzyl β-*L*-fucopyranoside* (**3**). — To a solution of **2** (10.8 g) in methanol (50 ml) was added a solution of sodium methoxide in methanol (M, 3.5 ml). The mixture was kept overnight at room temperature, and was then neutralized with dilute sulfuric acid, filtered, and evaporated to dryness. The residue was dissolved in methanol, filtered, and again evaporated to dryness. The resulting syrup (7.1 g, 98%) gave two spots on t.l.c. (benzene–methanol 2:1 v/v), a major one with R_F 0.52 and a minor one with R_F 0.30 (identical with L-fucose). A portion of the crude syrup was dissolved in chloroform, and the solution was chromatographed on silica gel. Chloroform–methanol (4:1 v/v) eluted fractions which gave a single spot on t.l.c. (benzene–methanol 2:1 v/v, R_F 0.52) $[\alpha]_D^{25}$ +46° (*c* 2.24, chloroform); n.m.r. data: τ 2.73 (5H, C_6H_5); τ 8.80 (doublet, *J* 6.5 c.p.s., 3H, CH-CH_3).

*Benzyl 3,4-*O*-isopropylidene-β-*L*-fucopyranoside* (**4**). — A solution of crude **3** (7.5. g) in acetone (500 ml) containing conc. sulfuric acid (3 ml) was stirred for 5.5 h at room temperature. It was neutralized by addition to an aqueous solution of potassium hydrogen carbonate. The insoluble salts were removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was extracted with chloroform, and the solution concentrated *in vacuo*. Examination of the resulting syrup by t.l.c. (benzene–methanol 9:1 v/v) revealed the presence of two components in different proportions. The syrup was fractionated on a silica gel column. Elution with benzene–ether 5:1 gave a syrupy product (0.8 g), identified as 1,2:3,4-di-*O*-isopropylidene-L-fucose by n.m.r., which was not investigated further. Elution with benzene–ether 4:1 gave 6.2 g (72%) of the desired product **4**, as a syrup which could not be crystallized, $[\alpha]_D^{25}$ +3° (*c* 2.07, chloroform); n.m.r. data: τ 2.70 (5H, C_6H_5); τ 8.58 (doublet, *J* 6.5 c.p.s., 3H, CH-CH_3); τ 8.48 and 8.65 [6H, $C(CH_3)_2$].

Anal. Calc. for $C_{16}H_{22}O_5$: C, 65.30; H, 7.48. Found: C, 65.23; H, 7.06.

Benzyl 3,4-O-isopropylidene-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-L-fucopyranoside (**5**). — A stirred solution of **4** (1.10 g, 3.75 mmoles) in nitromethane–benzene (1:1, 60 ml) was boiled, to ensure complete dehydration, until approx. 20 ml of the solvent mixture had distilled, and then cooled to 60°. Mercuric cyanide (0.85 g, 3.3 mmoles) and the bromide (**1**, 1.31 g, 3.3 mmoles) were added, and the reaction was continued for a total of 3 days at 60–70°, a further addition of mercuric cyanide (1.5 mmole) and **1** (1.5 mmole) being made after 20 h.

After being cooled, the mixture was diluted with benzene (50 ml) and washed successively with a cold saturated solution of sodium hydrogen carbonate and with water, then dried with sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in benzene, and the solution was passed through a column of silica gel. Benzene–ether 9:1 eluted fractions which showed two spots on t.l.c. in benzene–methanol 9:1 having R_F 0.84 and R_F 0.60 (the latter corresponding to **4**). The fractions were combined and crystallized from ether–benzene to give needles (0.43 g, 20%), m.p. 173–175°. A portion was recrystallized from methanol as needles, m.p. 176–178°, $[\alpha]_D^{25} -92.6°$ (*c* 0.85, chloroform); n.m.r. data: τ 2.70 (5H, C_6H_5); τ 4.48 (doublet, *J* 3.5 c.p.s., H, α-L-fucosyl residue); τ 5.65 (doublet, *J* 8 c.p.s., H, β-L-fucosyl residue); τ 7.85 (3H, axial OAc); τ 8.03 and 8.13 (6H, two equatorial OAc); τ 8.44 and 8.62 [6H, $C(CH_3)_2$]; τ 8.58 and 8.88 (doublet, *J* 6.5 c.p.s., 6H, 2 CH-CH_3).

Anal. Calc. for $C_{28}H_{38}O_{12}$: C, 59.35; H, 6.76. Found: C, 59.59; H, 6.67.

The mother liquors from the first crystallization showed the presence of two compounds on t.l.c. (R_F 0.84 and R_F 0.60). In another experiment, they were separated by chromatography on silica gel G, with benzene–ether 3:1 as eluent. The product with R_F 0.84 could be crystallized to give an additional 2% yield of **5**, whereas the syrup with R_F 0.60 (unchanged **4**) was recovered in a yield of approximately 60%. There was no indication of a second isomer of **5** in the mother liquors.

Benzyl 2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-L-fucopyranoside (**6**). — A portion of **5** (0.43 g) was dissolved in acetone (10 ml) and 1% sulfuric acid (w/v, 5 ml) was added. The clear solution was kept for 20 h at room temperature (approx. 28°), and then poured into a cold dilute solution of sodium carbonate (50 ml). A precipitate was obtained which was extracted with chloroform (3 × 30 ml). The combined chloroform extracts were washed once with water, dried with calcium chloride, and concentrated *in vacuo*. The addition of ether to the residue gave 0.305 g (76%), m.p. 78–80°. Recrystallization from acetone–ether produced prisms, m.p. 78–80°, $[\alpha]_D^{30} -55.5°$ (*c* 1.12, chloroform); n.m.r. data: τ 2.70 (5H, C_6H_5); τ 4.45 (doublet, *J* 3.5 c.p.s., H-1, α-L-fucosyl residue); τ 5.26 (doublet, *J* 9 c.p.s., H, β-L-fucosyl residue); τ 7.86 (3H, axial OAc); τ 8.01 (3H); τ 8.15 (3H, equatorial OAc); τ 8.68 and 8.88 (2 doublets, *J* 6.5 c.p.s., 2 CH-CH_3).

Anal. Calc. for $C_{25}H_{34}O_{12}$: C, 57.05; H, 6.50. Found: C, 56.78; H, 6.65.

Benzyl 2-O-(α-L-fucopyranosyl)-β-L-fucopyranoside (**7**). — A portion of **6** (0.29 g) was dissolved, with slight warming, in anhydrous methanol containing a catalytic amount of sodium methoxide. The solution was kept overnight at room temperature, and a crystalline precipitate was slowly deposited. The suspension was

cooled for a short time to 5°, and the precipitate was separated by filtration and washed with cold methanol. Yield: 0.16 g (73%) of needles, m.p. 242–244°. Recrystallization from absolute alcohol did not raise the m.p., $[\alpha]_D^{28}$ −81.8° (*c* 0.74, 95% ethanol).

Anal. Calc. for $C_{19}H_{28}O_9$: C, 56.97; H, 7.05. Found: C, 57.20; H, 6.90.

2-O-α-L-Fucopyranosyl-L-fucopyranose (**8**). — A portion of **7** (0.09 g) was dissolved in ethanol (95% v/v, 50 ml) and hydrogenolyzed in the presence of a palladium-on-charcoal (10%) catalyst for 24 h at 50 lb/in^2. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo*. The resulting syrup was dissolved in a small amount of ethanol, and the solution was filtered to remove traces of remaining charcoal. Addition of ethyl acetate precipitated 0.045 g (65%) of fine needles, m.p. 189–192° (dec.), $[\alpha]_D^{30}$ −158° (5 min) → −163° (20 h) (*c* 0.60, water).

Anal. Calc. for $C_{12}H_{22}O_9$: C, 46.45; H, 7.15. Found: C, 46.06; H, 7.08.

The product was homogeneous, as shown by chromatography on Whatman No. 1 paper (descending) in 3 solvent systems: butanol–acetic acid–water 4:1:5, R_{Fuc} 0.77; butanol–ethanol–water 4:1:1, R_{Fuc} 0.74; and butanol–pyridine–water 3:1:1, R_{Fuc} 0.79.

ACKNOWLEDGMENTS

This investigation was supported by a research grant (AI-03528) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service. We wish to thank Dr. R. H. Côté for a sample of 2-*O*-α-L-fucopyranosyl-L-fucose.

SUMMARY

Acetylation of L-fucose (6-deoxy-L-galactose) with acetic anhydride and sodium acetate, followed by treatment of the resulting syrup with hydrogen bromide in acetic acid, gave 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide (**1**). Treatment of this compound with benzyl alcohol in the presence of silver oxide gave the crystalline benzyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside (**2**). Catalytic de-esterification afforded benzyl β-L-fucopyranoside (**3**), which was converted into benzyl 3,4-*O*-isopropylidene-β-L-fucopyranoside (**4**). The Koenigs–Knorr reaction of compound **1** with **4** in the presence of mercuric cyanide afforded the crystalline disaccharide, benzyl 3,4-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-L-fucopyranoside (**5**). Removal of the protecting groups gave crystalline 2-*O*-α-L-fucopyranosyl-L-fucopyranose (**8**), identical with a disaccharide previously isolated from fucoidin.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

REAKTIONEN ENOLISCHER ZUCKERDERIVATE

TEIL IV*. DIE MARKIERUNG VON ENOLISCHEN ZUCKERDERIVATEN UND ACETYLIERTEN ZUCKERN MIT TRITIUM

JOCHEN LEHMANN
Chemisches Laboratorium der Universität Freiburg i. B. (Deutschland)
(Eingegangen den 7. November, 1966)

EINFÜHRUNG

Von Helferich und Himmen[1] wurde 1928 festgestellt, daß käufliches rohes Silberfluorid in Pyridin sich besonders gut zur Eliminierung von Jodwasserstoff oder Bromwasserstoff aus den entsprechenden 6-halogenierten Glucosidderivaten eignet. Mit Hilfe dieser Reaktion lassen sich in guter Ausbeute enolische Zuckerderivate darstellen, die zur Synthese spezifisch markierter Zucker verwendet werden können[2]. Bei dem Versuch, aus Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-6-jod-α-D-glucopyranosid-*5-t* (**1**), das durch Jodierung nach einer Methode von Raymond und Schroeder[3] aus Methyl-α-D-glucopyranosid-*5-t*[2] ohne Tritiumverlust gewonnen wurde, Jodwasserstoff-*t* mit Silberfluorid in Pyridin zu eliminieren, wurde festgestellt, daß im Reaktionsprodukt Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid(**2**) noch 17% der in **1** fixierten Tritiumaktivität enthalten waren[4]. Dieses Ergebnis ließ den Schluß zu, daß während der Eliminierungs-reaktion eine Umgruppierung von Wasserstoffatomen erfolgte. Die Beweglichkeit oder Austauschbarkeit von Wasserstoff spielt bei der Verwendung tritiummarkierter Verbindungen zur Aufklärung biochemischer Eliminierungs- und Umlagerungs-reaktionen[5] eine große Rolle. Es ist zu erwarten, daß Wasserstoffatome, die an einer fixierten enolischen Doppelbindung stehen, in Bezug auf ihre Bindungsfestigkeit eine Sonderstellung gegenüber solchen an einer isolierten C–C-Doppelbindung einnehmen. Eine Beeinflussung durch geeignete Katalysatoren, die am Enolsauerstoff angreifen können, ist möglich.

DISKUSSION

Die Tatsache, daß bei der Eliminierung von Jodwasserstoff aus 5-tritiertem **1** ein Teil des Tritiums im Reaktionsprodukt **2** verbleibt, ließ es angebracht erscheinen, den Mechanismus dieser Reaktion näher zu untersuchen. Zunächst lag die Annahme nahe, die Reaktion könnte teilweise unter α-Eliminierung verlaufen, wobei das Tritium in 5-Stellung schließlich nach der 6-Stellung wandert. Diese Annahme erwies sich aber als nicht haltbar, da bei Verwendung von 6-tritiertem **1** vollständige Reten-

*Teil III : J. LEHMANN UND H. FRIEBOLIN, *Carbohyd. Res.*, 2 (1966) 499.

tion von Tritium in **2** festgestellt wurde[4]. Eine andere Erklärung könnte durch einen nachträglichen Wasserstoffaustausch in **2** mit dem aus **1**-*5*-*t* quantitativ abgespaltenen Tritium gegeben werden. Die Retention von Tritium bei Verwendung von **1**-*6*-*t* wäre teilweise durch einen sehr hohen Isotopieeffekt zu deuten. Wie im folgenden gezeigt wird, findet der Austausch in **2** an zwei voneinander unabhängigen Stellen, und zwar vorwiegend an Acetylgruppen* und in untergeordnetem Maße an der enolischen Doppelbindung statt. Im Falle **1**-*5*-*t* ist der Effekt der Rücktritierung sehr groß, da die Acetylgruppen den größten Teil des wieder eingebauten Tritiums aufnehmen, während im Falle **1**-*6*-*t* sich ein Austausch nur an der enolischen Doppelbindung abspielt, der aufgrund eines hohen Isotopieeffekts und des geringen Angebots an Wasserstoffionen (maximal ein Mol pro Mol Substrat) nur äußerst gering sein kann.

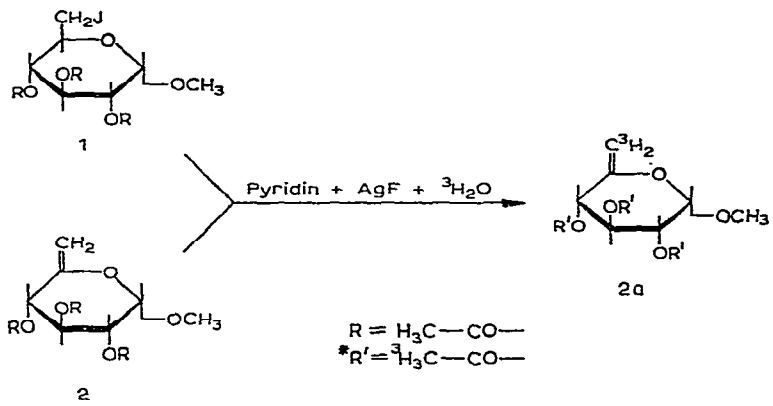


Abb. 1

Die scheinbare Retention von Tritium bei der Dehydrohalogenierung von **1**-*5*-*t* beruht tatsächlich auf einer Rücktritierung des Reaktionsprodukts **2** durch die während der Reaktion eliminierten Tritiumionen. Führt man nämlich die Eliminierungsreaktion mit unmarkiertem **1** in Pyridin durch, dem eine Spur ^3H_2O hoher spezifischer Aktivität zugesetzt ist, werden 15–20% der im Pyridin vorhandenen Tritiumaktivität im kristallinen Reaktionsprodukt **2** fixiert. Naturgemäß schwanken die Tritiumausbeuten beträchtlich, da es sich um eine heterogene Reaktion handelt und käufliches Silberfluorid keine definierte Verbindung ist. Die Bedingungen können daher nicht immer genau reproduziert werden. Eine ähnliche Markierung durch Tritiumaustausch erfolgt, wenn unmarkiertes **2** mit Silberfluorid in Pyridin–^3H_2O geschüttelt wird.

Aus einer Reihe von qualitativen Untersuchungen** kann zusammenfassend gefolgert werden : Der Einbau von Tritium in Verbindung **2** wird durch technisches Silberfluorid katalysiert. In Pyridin–^3H_2O oder Pyridin–^3HCl allein erfolgt kein Tritiumaustausch, ebensowenig bei Verwendung von Silberacetat oder Silbernitrat.

*Es konnte nicht geklärt werden, welche oder wieviel Acetylgruppen mit Tritium markiert werden.
**Die verschiedenen Gemischkombinationen werden nach 20-stündigem Schütteln dünnschichtchromatographisch analysiert.

Auch verliefen Versuche in anderen Lösungsmitteln, z.B. *N,N*-Dimethylformamid, negativ. Vermutlich sind Silberfluorid und mit Spuren Wasser entstehendes, hydratisiertes Silberoxid zusammen mit Pyridin für die Katalyse des Tritiumaustausches verantwortlich. Diese Annahme wird durch die Tatsache unterstützt, daß frisch gefälltes Silberoxid in Pyridin–^3H_2O eine, wenn auch geringe, katalytische Aktivität aufweist. Reines kristallines Silberfluorid in absolut trockenem Medium bewirkt eine Veränderung des Substrats und ist daher für den Zweck der Markierung ungeeignet. Das nach der Tritierung durch Extraktion mit Äther isolierte **2a** (Verbindung **2a** bedeutet **2** nach der Tritierung, also mit vollem Tritiumgehalt; im Gegensatz zu **2b**, in dem die tritierten Acetylgruppen durch unmarkierte ersetzt sind)* enthält das Tritium fest gebunden. Nach wiederholter Chromatographie auf Papier mit 1-Butanol–Propionsäure–Wasser oder auf Kieselgel mit Benzol–Methanol kann kein Aktivitätsverlust beobachtet werden, ebensowenig bei wiederholter Kristallisation aus Methanol.

Bei der katalytischen Entacetylierung von **2a** erhält man sirupöses Methyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid (**3**), das nur noch etwa 1% des in **2a** enthaltenen Tritiums aufweist. Auch diese Restaktivität bleibt nach Papier- oder Dünnschichtchromatographie erhalten. Nur durch längere Behandlung mit einem großen Überschuß wässriger Alkalilauge erfolgt quantitativer Verlust des Tritiums aus **3**. Durch Addition von Natriumbisulfit kann **3** ohne Aktivitätsverlust in das kristalline Natriumsalz der Methyl-6-desoxy-α-D-glucopyranosid-6-sulfonsäure[6] und dieses wiederum ohne Aktivitätsverlust nach Säurehydrolyse in das kristalline Natriumsalz der freien 6-Desoxy-α-D-glucopyranose-6-sulfonsäure[7] umgewandelt werden (Tabelle I).

TABELLE I

UMWANDLUNGSPRODUKTE AUS MARKIERTEM METHYL-2,3,4-TRI-*O*-ACETYL-6-DESOXY-α-D-*xylo*-HEX-5-ENOPYRANOSID (**2a**) MIT ZUGEHÖRIGEN TRITIUMAKTIVITÄTEN

Umwandlungsprodukte	*Aktivität in cpm/mMol*
Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid (**2a**)	1.26×10^8
Methyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid **3**[a]	1.12×10^6
Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid (**2b**[b])	1.10×10^6
Methyl-6-desoxy-α-D-glucopyranosid-6-sulfonsäure (Natriumsalz)	1.14×10^6
6-Desoxy-α-D-glucopyranose-6-sulfonsäure (Natriumsalz)	1.08×10^6

[a]Papierchromatographisch gereinigt.
[b]Durch Acetylierung von **3** gewonnen.

Verseift man **2a** mit wässriger Natronlauge, so tauscht ein Teil des Tritiums mit den Protonen des Wassers aus. Der Hauptanteil an Tritium findet sich jedoch in der abgespaltenen Essigsäure (Tabelle II). Da vermutet wurde, daß die mit wässrigem

*Alle im experimentellen Teil unter 8, 10, 11, 12 beschriebenen Umsetzungen wurden sowohl mit dem aus **1** wie dem aus **2** hergestellten **2a** durchgeführt. Dadurch konnte gezeigt werden, daß die Tritiumverteilung in **2a** unabhängig vom Ausgangsprodukt ist.

Alkali austauschbare Aktivität in 6-Stellung von **2a**, also in β-Stellung der enolischen Doppelbindung lokalisiert ist, wurde **2a** nach einer Methode von Pappo *et al.*[8] mit Osmiumtetroxid und Natriumperjodat in Wasser und Äther unter Spaltung der Doppelbindung abgebaut. Der aus C-Atom 6 von **2a** gebildete Formaldehyd, als Dimedonderivat isoliert, enthielt etwa 1.5% der Aktivität von **2a**. Die Aktivität, die nach der katalytischen Entacetylierung von **2a** in **3** gefunden wird, liegt bei etwa 0.9%. Diese Differenz deutet darauf hin, daß bei der katalytischen Entacetylierung ein Teil des in 6-Stellung gebundenen Tritiums mit dem Lösungsmittel (Methanol), selbst unter milden Bedingungen austauscht. In neutraler und schwach saurer Lösung findet kein Tritiumverlust aus **3** mehr statt.

Um eine ausschließliche Markierung am C-Atom 6 von **3** zu beweisen, wurde dieses nach papierchromatographischer Reinigung von eventuell noch anhaftendem, unvollständig entacetylierten Produkten mit unmarkiertem Acetanhydrid in Pyridin reacetyliert und mit unmarkiertem **2** verdünnt. Nach Abbau mit Osmiumtetroxid und Natriumperjodat enthält der gewonnene Dimedon–Formaldehyd–Komplex fast dieselbe Tritiumaktivität wie das eingesetzte Produkt **2b*** (Tabelle II). Daraus folgt, daß in **2** bei der Behandlung mit Silberfluorid und Pyridin–^3H_2O ein starker Austausch der Acetyl-Wasserstoff-Atome neben einem geringeren Austausch der Wasserstoffatome in β-Stellung der enolischen Doppelbindung erfolgt. Wasserstoffatome an normalen C–C-Doppelbindungen, wie beispielsweise in β-Pinen oder Cholesterinacetat, werden unter den beschriebenen Reaktionsbedingungen nicht ausgetauscht.

Wie **2** konnten auch Tri-*O*-acetyl-D-glucal, Methyl-6-desoxy-2,3,4-tri-*O*-methyl-α-D-*xylo*-hex-5-enopyranosid[2], Methyl-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosid und Methyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosid markiert werden. Vollacetylierte Pentosen und Hexosen werden zwar markiert, aber bei längerer Behandlung (20 Stunden) tritt teilweise Entacetylierung am anomeren C-Atom und Acetylwanderung ein. Jedoch genügt eine ein- bis zwei-stündige Behandlung für eine deutliche Markierung, wobei die eingesetzte Substanz chemisch nicht verändert wird, wenn sie keine sehr labile glykosidische Bindung enthält. Eine solche Ausnahme ist Penta-*O*-acetyl-β-D-mannopyranose, die nach einstündiger Behandlung bereits teilweise in eine zweite Substanz umgewandelt wird, bei der es sich aufgrund der dünnschichtchromatographischen Analyse wahrscheinlich um das konformativ weit stabilere α-D-Anomere handelt.

Wahrscheinlich hat Silberfluorid in Pyridin einen starken Effekt auf das glykosidische sowie das Ringsauerstoffatom. Dieser Einfluß hängt sicher eng mit einer Labilisierung der C–H-Bindungen sowohl an enolischen Doppelbindungen als auch an der Acetylgruppe zusammen, die dem anomeren Zentrum benachbart ist. Eine änliche spezifische Reaktivität der Acetylgruppe in 2-Stellung von Penta-*O*-acetyl-D-glucose ist bei der Behandlung mit Phosphorpentachlorid in Tetrachlorkohlenstoff zu beobachten[10]. Hier wird ausschließlich diese Acetylgruppe perchloriert.

*Die gegenüber **2b** erhöhte Tritiumaktivität im Formaldehyd-Derivat ist wahrscheinlich auf Überoxidation des Formaldehyds zurückzuführen. Hierbei macht sich ein Isotopieeffekt bemerkbar[9].

TABELLE II

VERTEILUNG DER TRITIUMAKTIVITÄT

Gemessene Verbindung	*Erfaßte C-Atome im Zucker*	*Erfaßte Acetyl-gruppen*	*cpm/mMol*
Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid (**2a**)	1–6	alle	1.26×10^8
Natriumacetat aus **2a**	—	alle	1.11×10^8
Methyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid (**3**) aus **2a** durch katalytische Verseifung	1–6	keine	1.12×10^{6a}
Formaldehyd aus **2a**	6	keine	2.10×10^{6a}
Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid (**2b**) aus **3** und nach Verdünnung mit **2**	1–6	keine	7.40×10^4
Formaldehyd aus **2b**	6	keine	9.20×10^4
Methyl-tetra-*O*-acetyl-α-D-glucopyranosid (**4**)	1–6	alle	1.28×10^7
Methyl-α-D-glucopyranosid aus **4** durch katalytische Entacetylierung	1–6	keine	0
Natriumacetat aus **4**	—	alle	1.21×10^7

[a]Der große Unterschied im Tritiumgehalt von Formaldehyd aus **2a** gegenüber dem in **3** ist auf eilweisen Verlust von Tritium am C-6 während der katalytischen Entacetylierung zurückzuführen.

Die Markierung von Acetylgruppen in Zuckeracetaten kann auch zur quantitativen Analyse von acetylierten Zuckergemischen verwendet werden. Am Beispiel des Gemisches Penta-*O*-acetyl-β-D-glucopyranose und Tetra-*O*-acetyl-β-D-xylose konnte gezeigt werden, daß der Tritiumeinbau, wenigstens für das genannte Beispiel, stöchiometrisch erfolgt.

TABELLE III

QUANTITATIVE BESTIMMUNG EINES GEMISCHES VON PENTA-*O*-ACETYL-β-D-GLUCOPYRANOSE (**5**) UND TETRA-*O*-ACETYL-β-D-XYLOPYRANOSE (**6**) NACH TRITIERUNG MIT SILBERFLUORID–PYRIDIN–^3H_2O

	5	**6**	*Molverhältnis*	*Aktivitäten-verhältnis*
Eingewogene Menge, mMol	0.197	0.137	1.44	
Tritiumaktivität nach chromatographischer Trennung; gemessen: integrierte Peaks in cm^2	4.56	3.07		1.49
Tritiumaktivität mit Hilfe der Isotopenverdünnungsmethode gemessen cpm	16.8×10^4	12.0×10^4		1.40

Die hier beschriebene Methode zur Markierung von enolischen Zuckerderivaten und acetylierten Zuckern bedient sich mit ^3H_2O einer der billigsten tritierten Substanzen. Durch Verwendung eines nicht protischen Lösungsmittels wird eine Verdünnung des tritierten Wassers vermieden. Die Anwendung der Methode ist

sehr einfach und bedarf keines besonderen Aufwands. Die Möglichkeit der Direktmarkierung ist besonders im Fall der enolischen Zuckerderivate von Bedeutung, da verlustreiche Synthesen, ausgehend von markierten, einfachen Zuckern, vermieden werden.

Kenntnisse über den Wasserstoff–Tritium–Austausch sind vor allem bei der Aufklärung biochemischer Vorgänge unter Verwendung tritiummarkierter Substrate von großer Bedeutung[5]. Es wird hier gezeigt, daß Protonen in β-Stellung einer fixierten enolischen Doppelbindung bei geeigneter Katalyse zum Austausch befähigt sind.

EXPERIMENTELLER TEIL

Allgemeine Methoden. (*1*) *Papierchromatographie.* — Papierchromatogramme wurden absteigend auf Whatman Nr. 1 — Chromatographiepapier in 1-Butanol–Propionsäure–Wasser (142:71:100) angefertigt. Zur Entwicklung enolischer Zuckerderivate wird das Chromatogramm mit einer Lösung von Kaliumpermanganat (5% in Wasser) besprüht. Im Bereich der Flecken erfolgt sofort Reduktion zu Braunstein. Nach zwei Minuten wird Permanganat mit Wasser ausgewaschen und das Chromatogramm getrocknet. Tritiummarkierte Substanzen werden mit Hilfe eines fensterlosen Isotopenzählgerätes (Modell 380 der Packard Instrument Company) lokalisiert.

(*2*) *Dünnschichtchromatographie.* — Zur Anfertigung von Dünnschichtchromatogrammen wurden " Eastman Chromagram Sheets " verwendet. Als Laufmittel dienen entweder Benzol–Methanol (9:1) oder Äther–Petroläther-50–70° (1:1). Entwicklung erfolgt mit Joddampf. Tritiummarkierte Substanzen werden wie unter (*1*) beschrieben lokalisiert. Tritierungsgemische (Substrat oder Substratgemisch–Silberfluorid–Pyridin–^3H_2O) werden mit einer feinen Pipette in möglichst schmalem Strich auf eine 5-cm breite Platte aufgetragen, sodaß an den Rändern mindestens je 0.5 cm frei bleiben. Man läßt die Platten etwa 0.5 Stunden in einem gut belüfteten Abzug trocknen. Chromatographie soll vor allem bei vollacetylierten Zuckern mit kleinem R_F Wert in Äther–Petroläther dreimal mit der gleichen Platte wiederholt werden, um gute Trennungen zu gewährleisten. Silbersalze, die einen großen Teil der Radioaktivität enthalten, bleiben an der Startlinie zurück. Die Radioaktivität an der Startlinie nimmt im Gegensatz zu der im Substrat fixierten bei längerem Aufbewahren langsam ab. Bei Verwendung von Benzol–Methanol findet starker Austausch zwischen dem Lösungsmittelgemisch und dem mit anorganischer Substanz assoziierten Tritium statt. Die im Substrat fixierte Aktivität bleibt auch hier unverändert. Ein ungefähres Maß für die in das Substrat eingebaute Aktivität ergibt ein Vergleich der Aktivität an der Startlinie mit der an die Substanz gebundenen Aktivität. Bei Verwendung des gleichen Reagenzgemisches [siehe (*4*)] und bei der Verwendung eines aprotischen Lösungsmittels wie Äther–Petroläther bleibt die Startlinienaktivität etwa konstant. Die Messung sollte immer mit frisch angefertigten Chromatogrammen erfolgen.

(*3*) *Quantitative Bestimmung der Tritiumaktivität.* — Eine quantitative Bestimmung der in Substanzen fixierten Tritiumaktivität kann entweder mit Hilfe

eines fensterlosen Isotopenzählgeräts [siehe (*1*)] direkt auf Chromatogrammen erfolgen oder nach Auflösen einer abgewogenen oder pipettierten Menge der zu messenden Substanz in Methanol (5 ml) und Scintillationsflüssigkeit (10 ml) (Basis Toluol) in einem Scintillationszählgerät (720 Series, Liquid Scintillation System der Nuclear Chicago Company).

(*4*) *Reagenzgemisch für die Markierung mit Tritium.* — Über Natriumhydroxid getrocknetes und destilliertes Pyridin (200 ml) wird mit ^3H_2O (0.2 ml, spezifische Aktivität etwa 1–4 curie/ml) vermischt und als Standardlösung verwendet. Die Lösung wird im Dunkeln bei 0° aufbewahrt. Zur Markierung mischt man 1 mMol der zu markierenden Substanz mit Silberfluorid (Th. Schuchhardt GmbH) (0.5 g), das über Phosphorpentoxid getrocknet und pulverisiert wurde, sowie Pyridin–^3H_2O (3 ml Standardlösung).

(*5*) *Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-*D*-*xylo*-hex-5-enopyranosid-5-t* (**2a**) *aus Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-6-jod-α-*D*-glucopyranosid* (**1**). — Verbindung **1** (4.3 g) wird mit dem Markierungsgemisch (5 g AgF und 30 ml Pyridin–^3H_2O) 20 Stunden in der Dunkelheit bei Zimmertemperatur geschüttelt. Die Reaktionsmischung gießt man langsam unter starkem Rühren in Äther (500 ml) ein. Zur Klärung wird etwas Aktivkohle (5–10 g) zugegeben und nach 30 Minuten Rühren filtriert. Die klare Lösung wird unter vermindertem Druck eingedampft. Der sirupöse Rückstand kristallisiert aus 80%igem Methanol. Nach zweimaligem Umkristallisieren bleibt die spezifische Aktivität konstant. (1.26×10^8 cpm/mMol). Die Ausbeuten an reinem Produkt liegen zwischen 60 und 70% der Theorie. Die Ausbeuten an Tritium pro Mol **2a** bezogen auf eingesetztes Pyridin–^3H_2O, bei 15–20%.

Bei der Markierung von kleineren Mengen (0.1 mMol) wird das Reaktionsgemisch dünnschichtchromatographisch wie unter (*2*) beschrieben gereinigt, die Substanz **2a** eluiert und mit unmarkiertem Produkt **2** cokristallisiert.

(*6*) *Direkte Markierung von Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-*D*-xylo-hex-5-enopyranosid* (**2**)[1]. — Die Behandlung von **2** (3.2 g) mit Markierungsgemisch (5 g AgF und 30 ml Pyridin–^3H_2O) und Aufarbeitung nach den unter (*5*) beschriebenen Bedingungen ergibt wie dort Verbindung **2a** (vgl. Fußnote*, Seite 198). Die Ausbeuten an reiner Substanz und an eingebautem Tritium liegen ebenfalls innerhalb der unter (*5*) angegebenen Grenzen.

(*7*) *Direkte Markierung von Methyl-tetra-*O*-acetyl-α-*D*-glucopyranosid* (**4**). — Markierung und Aufarbeitung von **4** (10 mMol) erfolgt wie unter (*5*) beschrieben. Nach Umkristallisation aus Äthanol erhält man markiertes **4** mit einer Ausbeute von 85% der Theorie. Die Tritiumausbeute beträgt 16%.

(*8*) *Entacetylierung von* **2a** *im wässrigen Medium.* — Verbindung **2a** (0.243 g; 1.26×10^8 cpm/mMol) werden in 2N Natronlauge (20 ml) bei Zimmertemperatur gerührt. Nach etwa 3 Stunden hat sich **2a** vollständig gelöst. Nach insgesamt 20 Stunden wird unter vermindertem Druck zur Trockne eingedampft. Die Tritiumaktivität im abdestillierten Wasser beträgt auf 20 ml Wasser berechnet 6.25×10^5 cpm und auf mMol **2a** bezogen 8.15×10^5 cpm.

Der trockene Rückstand wird unter Kühlung mit 2N Schwefelsäure (21 ml) angesäuert

und die freigesetzte Essigsäure abdestilliert. Das Destillat wird mit 0.1N Natronlauge genau titriert (Verbrauch 92% der Theorie). Die Natriumacetatlösung wird zur Trockne eingedampft, der Rückstand mit wenig absolutem Äthanol ausgewaschen und die Tritiumaktivität in einer über Phosphorpentoxid getrockneten, abgewogenen Menge Natriumacetat bestimmt. Umgerechnet auf drei Acetylgruppen pro Mol **2a** ergibt sich eine Aktivität von 1.11×10^8 cpm. Ausgangsprodukt **2a**: 1.26×10^8 cpm/mMol; alkalilabiles Tritium: 8.15×10^5 cpm/mMol; Acetyl-*t*: 1.11×10^8 cpm/3 mMol.

(*9*) *Entacetylierung von* **4** *im wässrigen Medium.* — Verbindung **4** (0.380 g; 1.28×10^7 cpm/mMol) wird wie unter (*8*) beschrieben verseift und die Tritiumaktivität im Wasser und im Natriumacetat bestimmt und auf eingesetztes Produkt umgerechnet. Ausgangsprodukt **4**: 1.28×10^7 cpm/mMol; alkalilabiles Tritium: 3×10^3 cpm/mMol)*; Acetyl-*t* : 1.21×10^7 cpm/4 mMol.

(*10*) *Katalytische Entacetylierung von* **2a** *und Reacetylierung von* **3** *zu* **2b**. — Verbindung **2a** (0.5 g; 1.26×10^8 cpm/mMol) wird mit 0.01N Natriummethylat-Lösung in absolutem Methanol (3.5 ml) 30 Minuten geschüttelt. Dann wird das Natriummethylat durch Zugabe eines Tropfens Wasser und einiger Körnchen Trockeneis desaktiviert. Die Lösung wird unter vermindertem Druck zum Sirup **3** eingedampft und über Phosphorpentoxid getrocknet. Ausbeute 0.260 g entsprechend 95% der Theorie; Tritiumaktivität: 1.9×10^6 cpm/mMol.

Zur Reindarstellung wird ~~eine~~ 10%ige Lösung von **3** auf Papier aufgetragen und chromatographiert. Auf einem Probestreifen wird die Verteilung der Radioaktivität bestimmt und anschließend durch Besprühen mit Kaliumpermanganat-Lösung die Substanz lokalisiert [siehe (*1*)]. Während die Analyse der Radioaktivität neben einer scharfen Zone A noch Substanzen mit höherem R_F Wert und geringerer Aktivität aufweist, zeigt die Entwicklung mit Kaliumpermanganat ein einziges Produkt, dessen Lage mit Zone A übereinstimmt. Bei den schneller wandernden markierten Substanzen handelt es sich wahrscheinlich um Spuren von unvollkommen entacetyliertem **2a**, die auf Grund der relativ hohen Tritiumaktivität in den Acetylgruppen noch erfaßt werden können. Elution der ausgeschnittenen Zone A mit Wasser bei Zimmertemperatur und lyophilisieren der Eluate ergibt **3** (0.22 g Sirup). Das durch Behandlung mit Acetanhydrid (2 ml) und Pyridin (2 ml) und Aufarbeitung in gewohnter Weise in kristallines **2b** mit einer Aktivität von 1.1×10^6 cpm/mMol umgewandelt wird.

(*11*) *Abbau von* **2a** *mit Osmiumtetroxid und Natriumperjodat* [8]. — Verbindung **2a** (1.02 g; 1.26×10^8 cpm/mMol) und Osmiumtetroxid (0.065 g) werden zu einem kräftig gerührten Gemisch von Äther und Wasser (je 15 ml) gegeben. Während die Reaktionsmischung auf 24–26° gehalten wird, gibt man in kleinen Portionen fein pulverisiertes Natrium-meta-perjodat (2.3 g) zu. Danach wird noch zwei Stunden weitergerührt, die wässrige Phase wird abgetrennt und im Hochvakuum lyophilisiert. Wasser und Formaldehyd werden in einer mit flüssigem Stickstoff gekühlten Falle aufgefangen. Aus dem aufgetauten Lyophilisat wird Formaldehyd als Dimedon-

*Wert liegt innerhalb der Backgroundaktivität.

Derivat[11] abgeschieden. (Ausbeute 0.25 g). Die schlechte Ausbeute an Formaldehyd ist auf ungenügenden Abbau von **2a** zurückzuführen, von dem noch 0.4 g unverändert aus der ätherischen Phase isoliert werden konnten. Nach Umkristallisieren aus Äthanol–Wasser zeigte das Dimedon–Formaldehyd-Derivat eine Aktivität von 2.1×10^6 cpm/mMol.

(*12*) *Abbau von* **2b** *mit Osmiumtetroxid und Natriumperjodat.* — Verbindung **2b** (0.52 g; 7.4×10^4 cpm/mMol) wird wie unter (*11*) beschrieben in Äther–Wasser (je 10 ml) mit Osmiumtetroxid (0.038 g) und Natrium-meta-perjodat (1.2 g) abgebaut. Die Ausbeute an Dimedon–Formaldehyd-Derivat beträgt 0.11 g. Die Tritiumaktivität im Formaldehyd-Derivat beträgt 9.2×10^4 cpm/mMol.

(*13*) *Markierung von Penta-*O*-acetyl-β-*D*-glucopyranose* (**5**) *und Tetra-*O*-acetyl-β-*D*-xylopyranose* (**6**) *im Gemisch.* — Verbindung **5** (0.0767 g; 0.197 mMol) und **6** (0.0435 g; 0.137 mMol) werden eine Stunde mit Silberfluorid (0.15 g) und Pyridin–^3H_2O (1 ml) kräftig geschüttelt. Vom Reaktionsgemisch wird, wie unter (*2*) beschrieben, ein Dünnschichtchromatogramm angefertigt. Mit dem Zählgerät werden zwei Zonen Z (**5**) und Z (**6**) registriert. Die zugehörigen Peaks werden planimetriert. Z (**5**): 4.56 cm^2; Z (**6**): 3.07 cm^2; Molverhältnis **5**/**6**: 1.44; Aktivitäten-Verhältnis Z (**5**)/Z(**6**): 1.49.

Vom aufgeschüttelten Reaktionsgemisch werden 0.1 ml entnommen und zu einer Lösung von unmarkiertem **5** (3 g) in Chloroform (50 ml) gegeben und kräftig durchgeschüttelt. Man wäscht dreimal mit je 100 ml Wasser aus, trocknet über Calciumchlorid, filtriert und dampft die Lösung zur Trockne ein. Der kristalline Rückstand wird aus Äthanol bis zur konstanten spezifischen Aktivität umkristallisiert (etwa dreimal). Eine abgewogene Menge (zwischen 0.15 und 0.05 g) wird im Scintillationszählgerät [siehe (*3*)] auf Tritiumaktivität untersucht. Die Zählrate in cpm wird auf die ursprüngliche Menge von 3 g **5** umgerechnet. Auf gleiche Weise werden 0.1 ml des Reaktionsgemisches mit unmarkiertem **6** (3 g) vermischt, wie oben beschrieben aufgearbeitet und die Tritiumaktivität, bezogen auf 3 g **6** bestimmt. Aktivität für **5**: 16.8×10^4 cpm; Aktivität für **6**: 12.0×10^4 cpm; Molverhältnis **5**/**6**: 1.44; Aktivitäten-Verhältnis **5**/**6**: 1.40.

DANK

Der Deutschen Forschungsgemeinschaft danke ich für ein Habilitanden-Stipendium und eine Sachbeihilfe.

ZUSAMMENFASSUNG

Es wird gezeigt, daß enolische Zuckerderivate wie beispielsweise Methyl-2,3,4-tri-*O*-acetyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid mit einem Gemisch aus käuflichem Silberfluorid und Pyridin, dem eine Spur ^3H_2O zugesetzt wurde, mit Tritium markiert werden können. Die Markierung erfolgt in β-Stellung der enolischen Doppelbindung, d.h. am C-Atom 6 des erwähnten Substrats, und an den Acetyl-

gruppen. Es konnte nicht geklärt werden, an welchen Acetylgruppen Markierung erfolgt. Diese neue Markierungsmethode scheint allgemein auf enolische Zuckerderivate und acetylierte Zucker anwendbar zu sein. Die Anwendungsmöglichkeiten der Methode für die quantitative Bestimmung von acetylierten Zuckergemischen werden diskutiert.

SUMMARY

A mixture of commercial silver fluoride and pyridine with a trace of water-*t* was shown to be a good labelling reagent for such enolic sugar derivatives as methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-*xylo*-hex-5-enopyranoside. Labelling takes place at the β-position of the enolic double bond (position 6 in the case quoted), and in acetyl groups. It could not be established which acetyl groups undergo labelling. This new labelling technique seems to be generally applicable to enolic sugar derivatives and acetylated sugars. The potential usefulness of the technique for quantitative determinations of sugar acetates is discussed.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE REACTION OF MALONALDEHYDE WITH PERIODATE, AND ITS APPLICATION TO THE DETERMINATION OF VICINAL DIOLS IN DEOXY SUGAR DERIVATIVES*

MRS. PATRICIA SZABÓ AND L. SZABÓ
Institut de Biochimie, Faculté des Sciences, Orsay (France)
(Received November 21st, 1966)

INTRODUCTION

Besides their well-known reaction of cleaving vicinal diols, periodic acid and its salts also oxidise several other classes of compounds, the latter group of reactions being generally described as non-Malapradian or "overoxidation" reactions. These are mostly encountered during the periodate oxidation of such compounds as deoxy sugars, methyl and benzyl ethers of carbohydrates, and certain di- and polysaccharides. In these cases, they render the quantitative estimation of vicinal-diol groups uncertain, if not impossible. As the quantitative estimation of vicinal-diol groups by means of periodate oxidation is one of the most convenient methods that can be used for structural studies in the carbohydrate field, we have investigated the behaviour of free "malonaldehyde"** and that of its postulated[1,2] oxidation product, "hydroxymalonaldehyde" (tartronaldehyde) (compounds that are generally considered to be responsible for the "overoxidation" of deoxy sugars), towards periodate, with the aim of finding conditions in which this "overoxidation" could either be stopped, or would occur by consuming stoichiometric amounts of the oxidant, and thereby extend the use of periodate titrations to deoxy sugars and their derivatives.

RESULTS AND DISCUSSION

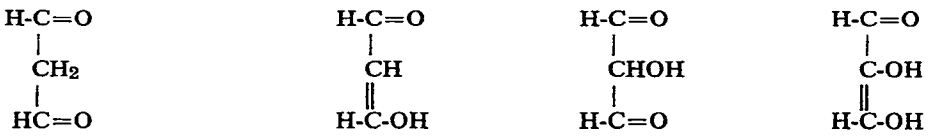
The reaction of malonaldehyde with periodate has been studied previously. One group[2] of authors suggested that this compound reduces three molar equivalents of periodate with the concomitant formation of three molar equivalents of formic acid, whereas another[1] found an uptake of four molar equivalents of the oxidant with the formation of two molar equivalents of formic acid and one molar equivalent of carbon dioxide. In the latter case, the malonaldehyde solution was obtained by hydrolysis of the appropriate acetal, and, in the former, its formation from a deoxy sugar was postulated as a result of diol cleavage; in neither case were the stoichiometric amounts of products formed. The behaviour of crystalline malonaldehyde,

*For preliminary notes, see *Compt. Rend.*, 262C (1966) 513, 1103.
The term "malonaldehyde" refers to an equilibrium mixture of unknown composition of malonaldehyde (1**) and of β-hydroxyacrolein (**2**).

although it can be easily prepared[3], has not been investigated, probably because of the extreme instability of this compound.

It is generally admitted that the periodate oxidation of active methylene groups, both in acyclic[2,4] and cyclic[5] compounds, proceeds by an initial hydroxylation stage, in which one of the "active" hydrogen atoms is replaced by a hydroxyl group. The mechanism of this reaction is not known with certainty, although it has been suggested[6] that in the case of malonaldehyde the hydrated enol form (β-hydroxyacrolein hydrate) undergoes a concerted attack by one molecule of periodate and two molecules of water, the result being a reaction similar in nature to that of glycol cleavage proposed by Buist and Bunton[7].

The product of hydroxylation of malonaldehyde, by the mechanism proposed[6], would be hydroxymalonaldehyde or tartronaldehyde (**3**), a tautomeric form of triose reductone (**4**)[8].



1 **2** **3** **4**

It has been suggested[1,9] that compound **4** should be oxidised by periodate to yield two molar equivalents of formic acid and one molar equivalent of carbon dioxide. However, when Ahlborg[10] oxidised crystalline **4** with periodic acid in dilute sulphuric acid solution, he found that an almost stoichiometric amount of iodine (one atom equivalent per mole of substrate) was set free. In a moderately dilute, aqueous solution of periodic acid, compound **4** was also found[11] to liberate iodine immediately, to reduce two molar equivalents of the oxidant, and to yield one molar equivalent of both formic and glyoxylic acids.

Although the isolation of glyoxylic acid (as its *p*-nitrophenylhydrazone) in high yield may seem surprising (in view of the reports[2, 12–14] that this acid is rapidly oxidised by periodate, and forms equimolar amounts of formic acid and carbon dioxide), the appearance of free iodine during the periodate oxidation of compounds having either an active hydrogen atom[15] or an enediol structure[10,16] has been frequently observed, and has sometimes been considered as a proof for the transient formation of such structures[17].

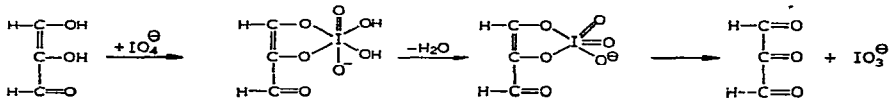
The formation of free iodine during periodate oxidations implies that further reduction of iodate, formed during the main reaction, takes place. Indeed, it has been shown that, in acid solution, iodate is fairly easily reduced by such compounds as triose reductone[10], dihydroxyfumaric[16], tartronic[2], or even formic acids[18]; such oxidations are strongly dependent on the concentration of iodate[2,16]. We have examined this reaction with triose reductone as substrate, and found that, whereas iodine was invariably set free from both sodium periodate and sodium iodate if the concentration of the reductone was greater than 10^{-3} M, no iodine was liberated at lower

concentrations of substrate, even in the presence of relatively large proportions of the oxidant.

The above-mentioned reaction of iodate with triose reductone is not only a function of the concentration of the reagents, it is also dependent on the pH of the solution. Thus, iodine is set free from iodate by triose reductone (even at very great dilution of the substrate) if the pH of the solution is lower than about 3. A similar reaction is observed with dihydroxyfumaric or L-ascorbic acids, which also have a free ene-diol structure. Thus, if triose reductone or other compounds of a similar nature are expected to appear during periodate oxidation (as is the case with aldohexofuranosides, aldoheptopyranosides, and certain 1,2-, 1,3-, or 1,4-linked disaccharides), the concentration of the substrate must be kept low, and the pH of the solution above 3, if the reduction of iodate and the appearance of free iodine are to be avoided.

The observation[10,11] that iodine is liberated during the periodate oxidation of triose reductone is therefore probably explained by the fact that limited amounts of free periodic acid were used to oxidise a relatively concentrated solution of this compound. Under these conditions, the iodic acid formed by the initial reduction of periodic acid is further reduced, perhaps to iodite[19], which could then react with any remaining periodic acid to yield free iodine. If this were the case, the presence of glyoxylic acid in the mixture would be explained by the lack of adequate amounts of periodic acid for its oxidation.

With these results in mind, the course of the oxidation of triose reductone by periodate was re-examined. Triose reductone is an acid of medium strength, having a pK_a value[20] of 1.17×10^{-5}; it can be titrated with strong bases in the presence of phenolphthalein. It is also an α,β-unsaturated aldehyde, absorbing strongly in the region of 270–290 mμ[21]. Finally, it is a vicinal diol and, as such, should react with periodate. If the oxidation occurs according to the diol-cleaving mechanism proposed by Buist and Bunton[7], the reaction of triose reductone (**4**) with one molar equivalent of periodate should yield mesoxalaldehyde (**5**):

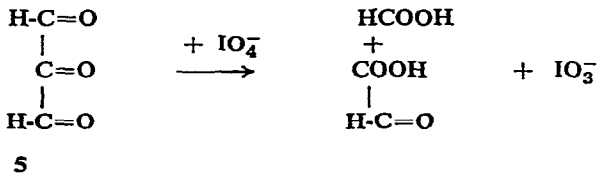


4 **5**

That this reaction occurs is indicated by the fact that, if a dilute solution of triose reductone is treated with one molar equivalent of sodium periodate, the solution that, before oxidation, required for neutralisation one molar equivalent of base, does not contain (within experimental error) any titratable acid. The strong ultraviolet absorption of the original solution also disappears, and this indicates the destruction of the unsaturated aldehyde. These observations can be most readily explained by the formation of the neutral compound, mesoxalaldehyde (**5**), which is known[22] to be formed from triose reductone with other oxidising agents. Like other vicinal tricarbonyl compounds (*e.g.* mesoxalic acid, pentane-2,3,4-trione, ninhydrin, alloxan), mesoxal-

aldehyde forms a stable hydrate[23] in which the two carbonyl groups are not conjugated; its absorption will therefore be weak (essentially that of the independent carbonyl groups) and not detectable at the high dilutions used.

If a solution of triose reductone is treated with two molar equivalents of periodate (or if a further molar equivalent of periodate is added to the above solution), the disappearance of the ultraviolet absorption is accompanied by the formation of two molar equivalents of titratable acid; this is in agreement with the following reaction:



S

Applying the cleavage to the hydrated form of the tricarbonyl compound, the mechanism is again in agreement with that proposed. Both of these reactions occur, for practical purposes, instantaneously, which also indicates that the normal, glycol-cleavage reaction is operative. Addition of a third molar equivalent of periodate (which is reduced more slowly than the first two molar equivalents), or of an excess of periodate, does not change the amount of titratable acid present in the solution; however, the mixture now also contains one molar equivalent of carbon dioxide. This result is in agreement with the observed cleavage of glyoxylic acid by periodate[2,12–14].

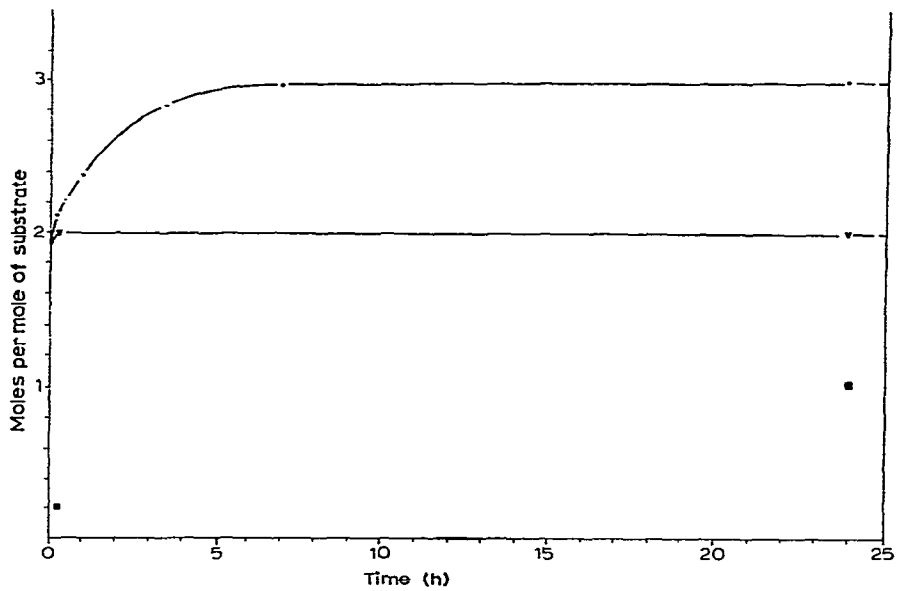
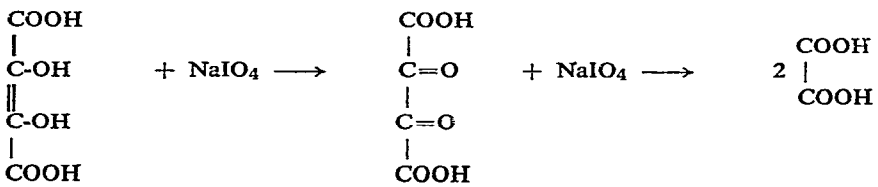


Fig. 1. Oxidation of triose reductone (5.8×10^{-4}M) with an excess of sodium metaperiodate (4.8×10^{-3}M) in unbuffered aqueous solution at room temperature. Moles of periodate reduced (—.—), moles of titratable acid produced (▼), and moles of carbon dioxide formed (■), per mole of substrate.

It is also significant that the reduction of the third mole of periodate is appreciably slower than that of the first two moles, indicating that another type of reaction is taking place (Fig. 1). These experiments establish beyond doubt that the overall oxidation of triose reductone (at a concentration of 6×10^{-4}M) is relatively rapid, stoichiometric quantities of periodate being consumed, and quantitative amounts of formic acid and carbon dioxide being formed.

The reaction sequence postulated for triose reductone is corroborated by the behaviour of dihydroxyfumaric acid under the same conditions. The behaviour of this compound towards oxidising agents has been investigated previously[24], and it has been shown that it can be oxidised with one molar equivalent of iodine to the corresponding oxoacid hydrate, the oxidation being accompanied by the disappearance of the strong absorption band of the ene-diol at 290 mμ. Addition of one molar equivalent of periodate to an aqueous solution of dihydroxyfumaric acid brings about the same changes in optical properties. Moreover, the reduction of periodate is not accompanied by any increase in the acidity of the solution. In the presence of an excess of periodate, two molar equivalents of the oxidant are reduced, the reaction being now accompanied by the appearance of two molar equivalents of titratable acid; two molar equivalents of oxalic acid are then present in the solution, no iodine is set free. The reaction sequence is as follows:



The reaction has been described earlier[16] as proceeding, in more concentrated solution, with the concomitant liberation of iodine; this is no doubt due to the secondary reactions discussed above.

It follows from these results that, if periodate oxidation of malonaldehyde does proceed exclusively by hydroxylation of C-2, the total consumption of periodate

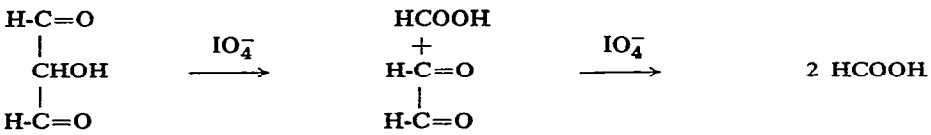
TABLE I

OXIDATION OF MALONALDEHYDE WITH SODIUM METAPERIODATE IN UNBUFFERED SOLUTIONS AT ROOM TEMPERATURE (AS DESCRIBED IN EXPERIMENTAL)

Time, hours	*Moles of IO_4^- reduced per mole of substrate*	*Moles of titratable acid formed per mole of substrate*	*Moles of carbon dioxide formed per mole of substrate*
65	2.92	1.73	0.6
112	3.70	1.85	0.7
160	4.10	1.93	0.85
257	4.76	1.98	——
303	4.85	2.00	0.9
400	5.09	2.00	0.9

per mole of malonaldehyde should be four molar equivalents; the concomitant formation of two molar equivalents of formic acid and of one molar equivalent of carbon dioxide should also be observed and, as in the case of malonic acid[2], the rate-determining step should be the "hydroxylation" step. However, when we oxidised malonaldehyde in the same way as triose reductone, we found that stoichiometric amounts of periodate were not reduced, nor did the reduction of periodate stop when the expected amount of acid had been formed and the production of carbon dioxide had ceased (Table I), whereas excellent results for quantitative analysis were obtained with crystalline triose reductone. It follows that the reason for observing unexpected results must either be connected with the primary reaction between periodate and malonaldehyde, or it must be assumed that the total amount of "hydroxymalonaldehyde" formed does not react with periodate in the tautomeric triose reductone form.

As to the second point, the mechanism of oxidation proposed by Foster and his colleagues[6] leads to the formation of tartronaldehyde (3), *i.e.*, the keto form of triose reductone. This compound could[2], by classical diol-cleavage, give rise to three molar equivalents of formic acid and no carbon dioxide, with the concomitant reduction of only two molar equivalents of periodate (instead of three for the enolic form):



3

The results of periodate oxidation will thus depend on the ratio K/K' (K = rate of enolisation; K' = rate of oxidation of the keto-form) in as much as, for practical purposes, the oxidation of the enolic form is instantaneous. Triose reductone (4) has been described[8] as the enol form of tartronaldehyde (3), but neither free 3, nor its rate of enolisation seem to be known. When crystalline triose reductone is dissolved in water, an acidic solution is obtained; this can be titrated with standard base, the end point is stable, and the quantity of base necessary for the neutralisation is that which would be required if all of the compound were in the enol form. When triose reductone was titrated with standard iodine in acid (0.02N H_2SO_4), "neutral" (the solution's own pH), or weakly alkaline (saturated sodium hydrogen carbonate) solutions, the amount of iodine reduced was found to be 97, 98, and 96% of that calculated for two atomic equivalents per mole of substrate. This behaviour is quite different from that (described in detail below) of malonaldehyde, which, on titration with base shows a fading end-point, and, upon titration with iodine, indicates an enol content of only 70–80% of the theoretical value. These results were taken to indicate that, for the purposes of periodate titrations, an aqueous solution of triose reductone may be considered as reacting entirely in the enolic, *i.e.*, triose reductone (4) form. The results of periodate titrations reported above are in complete agreement with this conclusion. Nevertheless,

it is possible that enolisation of tartronaldehyde, as formed from deoxy sugars on periodate oxidation, is slower than its reaction with periodate, and that this may be one source of error in the estimation of vicinal diol groups in deoxy sugars.

As to the first point, it has been reported[3,25] that malonaldehyde exists in solution mainly in the enolic form (**2**); it is, however, not necessarily in this form when first formed from deoxy sugars. The possibility of oxidation of the two molecular species by different routes and at different rates thus again exists. As this may also explain the anomalous and variable results obtained during periodate oxidation of compounds yielding malonaldehyde, the rate of appearance of β-hydroxyacrolein (**2**) after periodate oxidation was determined. It is known[25] that **2**, possessing a conjugated double-bond system, absorbs strongly in the region of 240–270 mμ, depending on the pH of the solution. On the other hand, the dialdehyde form (**1**), which has only two weak, isolated chromophores, is not expected to show any significant absorption in the same region (*cf.* the spectrum of 2,2,5,5-tetramethylcyclohexane-1,3-dione as compared with that of 5,5-dimethylcyclohexane-1,3-dione[26]). Therefore it should be possible to follow the transformation of the dialdehyde into the enolic form by measuring the increase of the extinction at the appropriate wavelength between 240 and 270 mμ. The following experiments were carried out. Firstly, crystalline 3-deoxy-*erythro*-pentitol[27] was treated with an excess of periodate in unbuffered olution, and the rate of formation of malonaldehyde determined by the thiobarbi

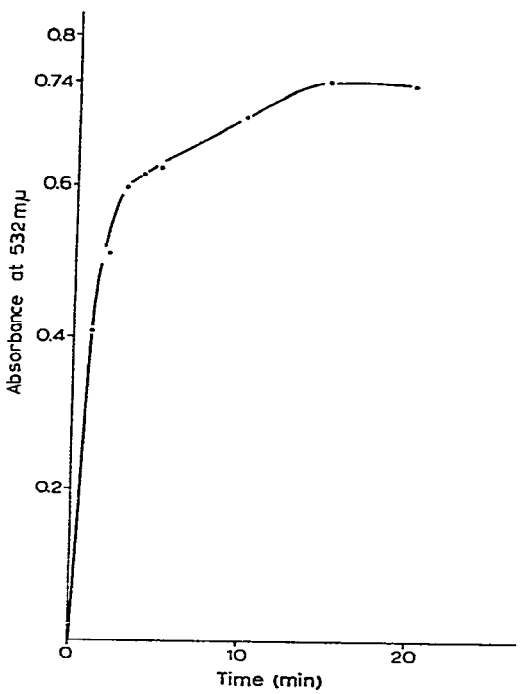


Fig. 2. Rate of production of malonaldehyde from 3-deoxy-*erythro*-pentitol by periodate oxidation (see Experimental).

turate method[28]. The results indicated that about 80% of the theoretical amount of malonaldehyde is present after 3 minutes at room temperature, and that about 15 minutes are required for complete reaction. In a similar oxidation, stopped after 3 minutes by the addition of ethylene glycol, the ultraviolet absorption spectrum of the solution was determined (with an equimolecular solution of periodate treated with ethylene glycol as a blank, in order to eliminate the absorption of the iodate.) The extinction maximum of the malonaldehyde, in the conditions of the reaction, was thus found to be at 250 mμ; it can be seen from Fig. 3 that the spectrum of malonaldehyde

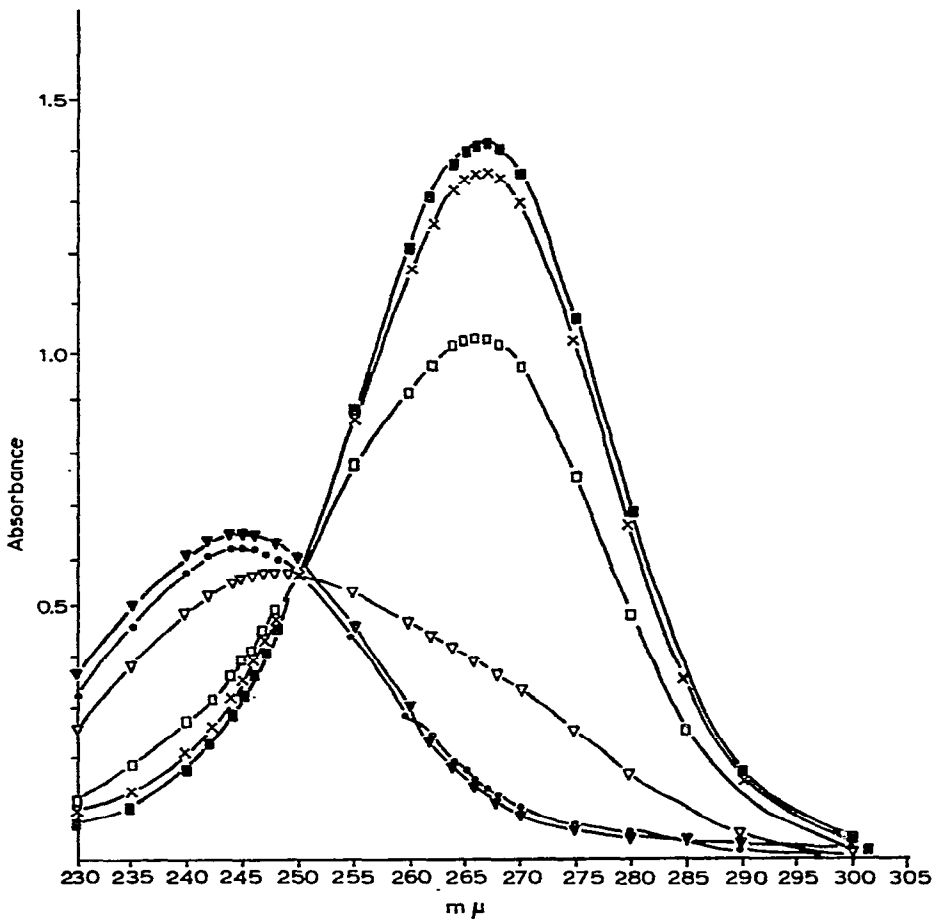


Fig. 3. Absorption spectra of malonaldehyde (5.6×10^{-5}M) [prepared from triethoxypropene by method (*b*)] at various pH values; 0.6 (▼); 3.0 (●); 4.05 (▽); 5.0 (□); 5.8 (×); 6.82 (■). (The spectra at pH values of 7.75, 8.92, 10.0, and 11.6 are similar to that at pH 6.82).

varies considerably with the pH, particularly in the region where the oxidation experiment is carried out. Finally, 3-deoxy-*erythro*-pentitol was oxidised under similar conditions, the reaction stopped at three minutes by addition of ethylene glycol, and the increase of the absorbance measured at 250 mμ as a function of time. The results

given in Fig. 4 show that the rearrangement of malonaldehyde (**1**) to β-hydroxyacrolein (**2**) is very rapid. Two minutes after the addition of ethylene glycol, the absorbance of the solution indicates that about 80% of the enol present in the final, equilibrium mixture is already formed, the maximal value being attained within fifteen minutes. As can be seen from Fig. 2, no measurable amount of malonaldehyde is destroyed by periodate during the first 30 minutes of the reaction, and it is therefore clear that the isomerisation of **1** to **2** is much faster than its reaction with periodate. It follows that in the pH region where most periodate oxidations are carried out, it is the equilibrium mixture which is

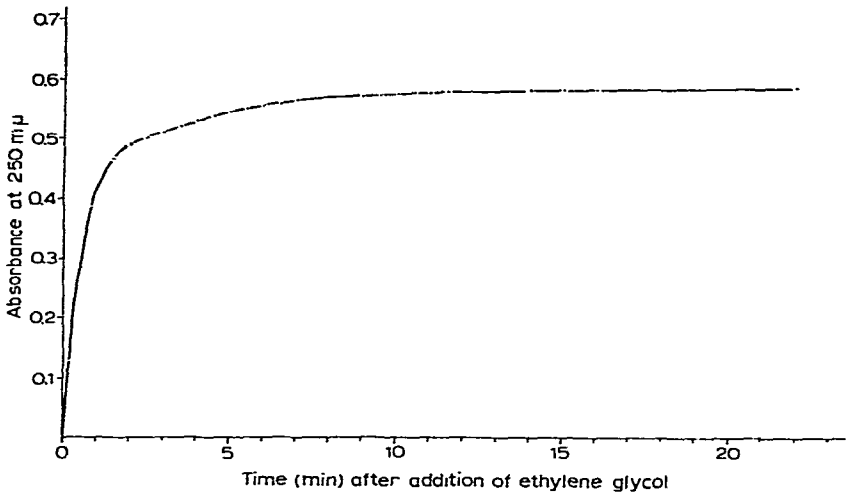


Fig. 4. Variation of the absorbance at 250 mμ of a malonaldehyde solution formed by periodate oxidation of 3-deoxy-*erythro*-pentitol as a function of time.

submitted to the oxidation. The actual composition of this mixture is not known, but the equilibrium presumably lies far to the side of the enol form.

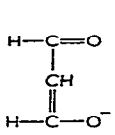
Fleury and his colleagues[1] observed that when 1,1,3,3-tetraethoxypropane was hydrolysed with acid, the quantity of malonaldehyde formed, as estimated both by acidimetry and iodometry, was only about 80% of that expected, irrespective of the length of the hydrolysis. We found that when a very carefully distilled sample of β-ethoxyacrolein diethyl acetal (1,3,3-triethoxyprop-1-ene) was hydrolysed with water[3], the hydrolysis (easily followed without disturbing the keto-enol equilibrium by measuring the conductivity of the solution) was complete within three hours at room temperature. Titration of such solutions with standard base showed a rapidly fading end-point, and indicated that not more than about 84% of the expected acidity had developed; increased times of hydrolysis did not alter this figure. If, in the above titration, after the first fading end-point had been attained, further amounts of base were added so as to maintain a pH of about 9 until no more base was consumed, the final figure indicated a yield of about 93%; this process required about twelve days. Approximately the same value (95%) was obtained when the acid solution, formed by hydrolysis, was treated with an excess of base, and the solution back-titrated after

seven hours; the value rose to 98% when the exposure to the base was maintained for three days. Colorimetric determinations of the malonaldehyde content of the hydrolysis mixtures also gave consistently higher figures than those indicated by direct acidimetry, although because of the relatively large inherent error (± 3%) of the method, it is less reliable for the estimation of small differences. Fleury *et al.*[1] interpreted their results as being due to some unidentified reaction of the starting material or to the decomposition of the free malonaldehyde. They therefore calculated the periodate consumption, production of acid, and evolution of carbon dioxide on the basis of the quantity of malonaldehyde as determined by titration, and then obtained analytical figures in fairly good agreement with the equation:

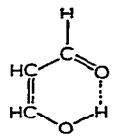
$$OHC\text{–}CH_2\text{–}CHO + 4\ IO_4^- = 2\ HCOOH + CO_2$$

However, in view of the above observations, it would appear that malonaldehyde solutions, as obtained by hydrolysis of appropriate acetals, contain two different forms of malonaldehyde: the enolic form, which can be titrated with acid or iodine and which reacts with periodate according to the equation given by Fleury *et al.*, and another form which does not. The most obvious conclusion is that the form which is not acidic and does not follow the above equation is the dialdehydo form, and therefore attempts were made to estimate the amount of this tautomer by independent methods.

Investigation of the variations of the ultraviolet spectra of equilibrium mixtures obtained by acid hydrolysis of 1,1,3,3-tetraethoxypropane have led to the conlusion[25] that malonaldehyde exists in aqueous solution essentially as the mono-enol, forming the ion (**6**) in weakly alkaline solution, and the hydrogen-bonded, six-membered chelate (**7**) in weakly acid media.



6



7

The question of the importance of the contribution of the dialdehydo and conjugated acid forms was, however, left open, although the absorption band observed at 350 mμ in weakly alkaline solutions was attributed[25] to the "independent carbonyl absorption". As widely different values (200 and 1000) have been reported for the extinction coefficient for this band, its behaviour at different pH values was examined. When 1,3,3-triethoxyprop-1-ene was hydrolysed with N sulphuric acid for about 3 hours at room temperature, the solution showed only one peak (λ_{max} 245 mμ, ε 1.366×10^4 at pH 0.4), as found by previous authors[25]. Such a solution is stable for at least one week at room temperature, as judged by the thiobarbiturate test and by the optical density at 350 mμ. Fleury *et al.*[1] also found that 0.1 molar aqueous solutions of malonaldehyde, N with respect to sulphuric or hydrochloric acid, are stable for over 15 days at 4°. A similarly stable solution is obtained when the solution is made slightly alkaline

(pH 7–8). Neither of these solutions shows an absorption maximum at 350 mμ comparable to those found by previous authors[25] (ε 8.3, 61.5, and 69 for solutions of pH 0.4, 7.15, and 9.5, respectively). However, if the pH of the hydrolysate is adjusted to 3–5, the absorption at 350 mμ increases fairly rapidly (Fig. 5); it should be noted that the highest value recorded (ε 932, after 3 weeks) does not represent the final stage of the reaction. As a corollary, the malonaldehyde content of this solution, as estimated by

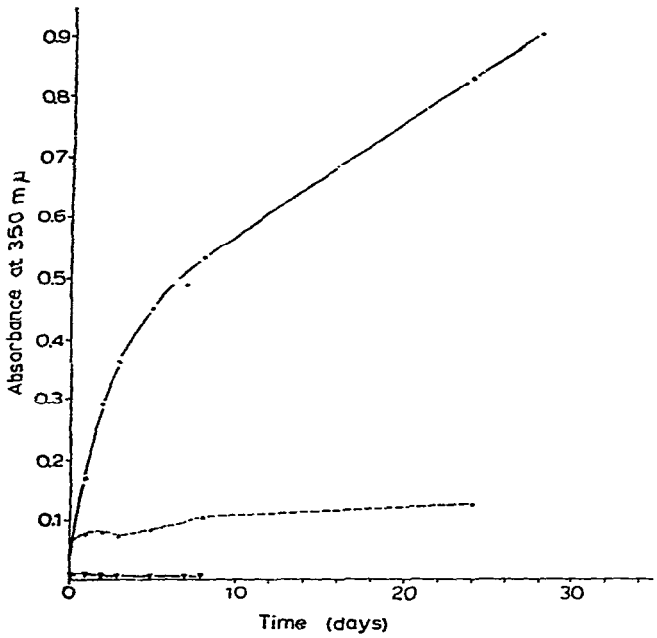


Fig. 5. Variation of the absorbance at 350 mμ of 9.6×10^{-4}M malonaldehyde solutions [prepared from triethoxypropene by method (*b*)] as a function of time; (▼) pH 0.5 (ionic strength N with respect to SO_4^{2-}); (——) pH 4.75 and (– – –) 7.15 (ionic strengths 0.5N with respect to SO_4^{2-}).

the thiobarbiturate method, also decreases (*ca.* 50% after 3 weeks). These results indicate that the absorption band at 350 mμ is not due to "independent carbonyl absorption", and therefore it cannot be used for the estimation of the dialdehydo form which may be present in equilibrium mixtures of malonaldehyde; they also suggest that one reason for the anomalous behaviour of deoxy sugars towards periodate could be that malonaldehyde undergoes a secondary reaction fairly readily at the pH values where periodate oxidations are normally carried out.

The rate of reaction of malonaldehyde with periodate was next investigated. Malonaldehyde, obtained by hydrolysis of 1,3,3,-triethoxyprop-1-ene in water, was treated with a large excess of periodate, and its rate of disappearance measured by the thiobarbiturate reaction as a function of time. It was found (Fig. 6) that the reaction is quite fast above pH 4, but rather slow in acid conditions. Fleury and Courtois[4] have observed a similar effect of pH on the rate of oxidation of malonic acid, and Huebner *et al.*[2] also noted that this acid was oxidised more rapidly with sodium periodate than

with periodic acid. However, as can be seen from Fig. 6, the coloration given in the thiobarbiturate reaction does not reach zero even after long periods of time, but remains constant at a low value representing 2–3% of the amount of malonaldehyde

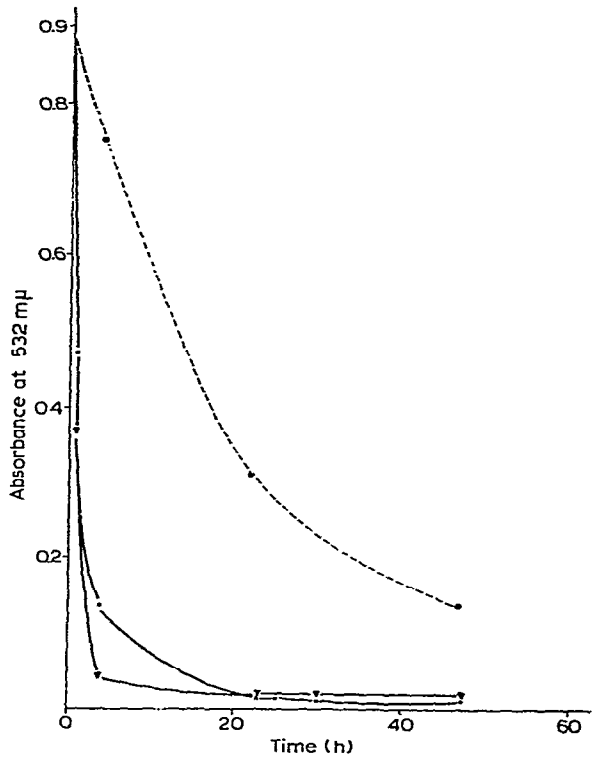


Fig. 6. Rate of disappearance of malonaldehyde (4.2×10^{-4}M) [prepared from triethoxypropene by method (*a*)] in the presence of a large excess of sodium metaperiodate (2×10^{-2}M), as measured by the thiobarbituric acid method; pH 2.5 (----); pH 4.7 (—·—); pH 7.4 (▼).

theoretically present. Further investigation of this phenomenon revealed that it also occurs when deoxy sugars or derivatives are oxidised with periodate. As a corollary, the uptake of periodate by deoxy sugars under the same conditions is also non-stoichiometric and, as in the case of malonaldehyde, the reduction of periodate does not cease when the expected amounts of acid and carbon dioxide are formed (Table II). These observations indicated that it would probably be quite difficult to find conditions in which the periodate oxidation of malonaldehyde, and hence that of deoxy sugars, would occur by controlled reactions proceeding at a reasonable speed. It was decided, therefore, to seek conditions in which the oxidation of malonaldehyde could be arrested whilst glycol cleavage would proceed normally.

As can be seen from Fig. 6, the rate of periodate oxidation of malonaldehyde is relatively low in acid solution. It is also known[29] that the temperature coefficient of the oxidation of "active" methylene groups is quite considerable. It was therefore decided to make use of both of these effects when attempting to suppress the attack

of periodate on malonaldehyde. When periodate oxidation of deoxy sugars and related compounds was effected in 0.1N sulphuric acid solution at about 2–4°, the quantity of periodate reduced corresponded exactly to the number of vicinal diol groups in the molecule, and the malonaldehyde set free as a result of diol cleavage was stable

TABLE II

PERIODATE OXIDATIONS OF DEOXY SUGARS AT VARYING pH VALUES AT ROOM TEMPERATURE

Conditions	*Time, h*	*Moles of sodium metaperiodate reduced per mole of*		
		2-Deoxy-D-erythro-*pentose* (5.96×10^{-4}M)	*2-Deoxy*-D-arabino-*hexose* (6.01×10^{-4}M)	*3-Deoxy*-D-xylo-*hexitol* (6.04×10^{-4}M)
$NaIO_4$	0.25	1.56	1.47	2.94
6.52×10^{-3}M	0.5	1.68	1.83	3.06
in	1	1.90	1.86	3.14
unbuffered	3.5	2.26	2.22	3.42
solution	6	2.46	2.69	3.61
	24	3.35	3.89	4.58
	52	4.23	4.84	5.21
	123.5	4.98	5.80	5.85
	149	5.19	6.07	6.01
	173.5	5.32	6.23	6.14
	221	5.47[a]	6.43[b]	6.29[c]
	287.5	5.62	6.70	6.47
	336	5.71	6.81	6.58
	461	5.76	6.90	6.81
		(6.01×10^{-4}M)	(6.0×10^{-4}M)	(6.006×10^{-4}M)
$NaIO_4$	52	5.63	6.85	—
6.67×10^{-3}M	66.5	—	—	6.26
in 0.05M	142.5	—	—	6.31
veronal	148.5	6.67	7.9	—
buffer	239	7.5	8.63	6.33
(pH 8.5)	330.5	—	—	6.43
			(6.04×10^{-4}M)	(6.0×10^{-4}M)
$NaIO_4$	0.25		1.89	2.65
6.62×10^{-3}M	0.5		2.55	2.84
in 0.1N	1		2.80	2.90
H_2SO_4	1.5		2.82	2.92
(pH 1.5)	2		2.84	2.92
	4		2.88	3.00
	5		2.92	3.00
	6		2.90	3.00
	7.5		2.92	3.00
	28		3.00	3.10
	50		3.19	3.27
	122		3.80	3.89

[a]Mequiv. of titratable acid present, 2.92; [b]3.87; [c]2.85 (1.0 mequiv. of CO_2).

for at least seven days, and could be estimated by means of the thiobarbiturate reaction[28]. The compounds tested, the amount of periodate reduced, and the quantity of malonaldehyde formed are, for each case, given in Table III.

TABLE III

PERIODATE OXIDATION OF DEOXY SUGARS AND RELATED COMPOUNDS IN 0.1N H_2SO_4 AT 4° (SEE EXPERIMENTAL)

Substrate	*Moles of IO_4^- reduced per mole of substrate*		*Moles of malonaldehyde formed per mole of substrate. Calc. 1*	*Approx. time for complete glycol cleavage (h)*
	Calc.	*Found*		
2-Deoxy-D-*erythro*-pentose (2-deoxy-D-ribose)	2	1.9	0.91	6
2-Deoxy-D-*arabino*-hexose (2-deoxy-D-glucose)	3	2.9	0.96	20
3-Deoxy-D-*ribo*-hexose[a] (3-deoxy-D-glucose)	3	3.1	0.99	75
3-Deoxy-D-*arabino*-hexose[a] (3-deoxy-D-mannose)	3	3.0	1.0	85
2,6-Dideoxy-D-*ribo*-hexose (Digitoxose)	2	2.0	1.0	2
3-Deoxy-*erythro*-pentitol (3-deoxyribitol)	2	2.1	0.97	6
3-Deoxy-D-*xylo*-hexitol (3-deoxy-D-galactitol)	3	2.9	1.0	20
3,6-Dideoxy-D-*ribo*-hexitol	2	2.0	0.90	50

[a]Gifts of Professor D. H. Murray[39].

The method cannot be used for titrating phosphorylated deoxy sugars, since, under the conditions described, these give anomalous results. The reason for this is now under investigation.

The absence of reaction between malonaldehyde and periodate may be related to the fact that, in the presence of strong acid, the malonaldehyde can add a proton to form a conjugate acid; it has been shown that triose reductone[30] and the analogous glutaconaldehyde[31] form the corresponding protonated species, and that 5,5-dimethylcyclohexane-1,3-dione (dimedon) and 2-hydroxydimedon give crystalline hydrochlorides[32]. It is not surprising that the conjugate acid of malonaldehyde should not undergo the same reactions with periodate as does the neutral molecule.

It should be emphasised that the method described here has many advantages over any previously reported. It can be used for the quantitative estimation of vicinal diol groups in deoxy sugars and related compounds that yield malonaldehyde. Its use is not limited to those compounds that react rapidly with periodate (*cf.* Ref. 28), as the malonaldehyde formed is stable over long periods of time. In addition, under the conditions used for the glycol cleavage, the malonaldehyde can be estimated quantitatively by the colorimetric method[28], but there is no need to use standards, since the malonaldehyde reacts quantitatively with thiobarbituric acid to form a dye having a well-defined composition whose molar extinction coefficient is now known[33].

EXPERIMENTAL

Estimation of periodate by the acid thiosulphate method[34]. — Samples of the oxidation mixture (4 ml for solutions 6.6×10^{-3}M, and 5 ml for solutions 6×10^{-3}M with respect to sodium metaperiodate) were acidified with 2N H_2SO_4 (5 ml); a 30% solution of KI (5 ml) was added, and the liberated iodine was titrated immediately (25 ml precision burette) with 0.01N sodium thiosulphate (standardised against KIO_3). Soluble starch indicator was added when the solution was pale yellow. The volumes of oxidation mixture were chosen to give a relatively large difference between the consumption of thiosulphate by periodate and by iodate, as the precision of the estimation is a function of this difference, Blank solutions were run with all experiments.

Estimation of carbon dioxide[35] *and acidity*[36]. — Solutions (250 ml) containing substrate (6×10^{-4}M) and periodate ($4.8–6.6 \times 10^{-3}$M, depending on the nature of the substrate) in hermetically closed systems were kept in the dark. At appropriate intervals, the oxidation was stopped by addition of ethylene glycol. Carbon dioxide was estimated by sweeping it from the solution with a stream of CO_2-free nitrogen into an excess of baryta of known concentration (*ca.* 0.4N). The remaining base was then titrated (phenolphthalein) with 0.1N HCl. Preliminary trials with tartaric acid proved this method of estimation to be accurate. It was also shown that no formic acid was removed from the solution under these conditions. The acid content of the solution freed from CO_2 was estimated by titration (phenolphthalein) with 0.1N NaOH.

Preparation of solutions of free malonaldehyde by hydrolysis of 1,3,3-triethoxyprop-1-ene (triethoxypropene). (a) Hydrolysis in water. — For these experiments, triethoxypropene[37] was purified by several distillations under diminished pressure. The compound (1–1.2 mmoles) was shaken for 2 h with water (10 ml); appropriate dilutions were then made for each experiment.

(*b*) *Hydrolysis in* N *sulphuric acid.* — Triethoxypropene (0.192 mmole), purified by preparative gas–liquid chromatography [Aerograph Autoprep 700; column, silicone SE 30, 30% on Chromosorb W, 45/60, 20′/$\frac{3}{8}$″; temperature injector, 300°; column, 205°; detector, 185°; vector gas, He], was shaken for 3 h with N H_2SO_4 (50 ml), and the volume of the solution was then adjusted to 100 ml with N H_2SO_4. For measurements of the 350-mμ peak, the pH of samples of this solution was adjusted either with acid or base, and the volume then completed to give suitable dilutions. For spectral measurements, solutions approximately 5×10^{-5}M were used for the range 220–300 mμ, and 1×10^{-3}M for 300–400 mμ.

Estimation of malonaldehyde with thiobarbituric acid[28]. — The oxidation mixture (the corresponding blank solution was treated similarly at the same time) (1 ml, *ca.* 6×10^{-4}M in substrate) was added to the arsenite solution (1 ml), and when all of the iodine formed had disappeared (or even later if the estimation was not done immediately; in which case, the solutions were kept in the cold room), water (2 ml) was added. To an aliquot of this solution (1 ml) was added 0.3% thiobarbituric acid

reagent (2 ml), the mixture was heated for 20 min in a boiling-water bath (the tubes were fitted with teardrop condensers), cooled in water at room temperature, and diluted 1:10 with 0.01N HCl (final concentration of malonaldehyde, *ca.* 5×10^{-6}M; pH 2), and the absorbance was read at 532 mμ against the blank solution. Under these conditions, the molar extinction coefficient of the malonaldehyde–thiobarbiturate adduct was found to be 1.53×10^5 when the malonaldehyde was generated from 3-deoxy-D-*xylo*-hexitol or digitoxose by periodate oxidation (in 0.1N H_2SO_4 at 4°, as described below), and 1.45×10^5 with the malonaldehyde prepared by acid hydrolysis of triethoxypropene (method *b*). As the error involved in weighing the oily triethoxypropene can be considered to be greater than that in weighing the crystalline deoxy sugars, the figure of 1.53×10^5 has been used to calculate the yields of malonaldehyde in all of the experimental results given. The reproducibility of the above method was estimated to be about ±3%. Kuhn and Lutz found molar extinction coefficients of 1.35×10^5 at 532 mμ in water, and 1.53×10^5 at 543 mμ in cyclohexanone, for the isolated pigment[33].

*Studies on malonaldehyde generated from 3-deoxy-*erythro*-pentitol (3-deoxy-ribitol).* — (*a*) The deoxypentitol (1.978 mg, 0.0145 mmole) was dissolved in water (10 ml), an aqueous solution of sodium metaperiodate (10 ml containing 65.56 mg of $NaIO_4$) was added, and the solution was made up to 25 ml with water. A blank solution containing the same amount of periodate was prepared first. Malonaldehyde was estimated at given time intervals with thiobarbituric acid, as described above. The results are shown in Fig. 2.

(*b*) Solutions similar to those in (*a*) were prepared, and, 3 min after the addition of periodate to the sugar, aliquots of the solutions were treated with ethylene glycol to convert the periodate into iodate. The ultraviolet absorption curve of the sugar-containing solution was determined by reading it against the iodate blank-solution in cells having a 1-mm light path. The absorption maximum was found to be at 248–250 mμ.

(*c*) The experiment was carried out as in (*b*), and the change of the absorbance at 250 mμ with time was observed. The results are shown in Fig. 4.

Oxidation of malonaldehyde with sodium metaperiodate in unbuffered solutions at room temperature (Table I). — Aqueous solutions (250 ml) of malonaldehyde (6×10^{-4}M), prepared from triethoxypropene by method (*a*), were oxidised with sodium metaperiodate (4.8×10^{-3}M) in the dark, and the uptake of periodate was determined by the acid thiosulphate method. A series of similar solutions was prepared simultaneously for the estimation of carbon dioxide and acid production according to the method described above.

Oxidation of deoxy sugars and related compounds yielding malonaldehyde in 0.1N H_2SO_4 at 4° (Table III). — The compound to be titrated (0.6 mmole) was weighed into a 100-ml volumetric flask. All subsequent operations, except for the thiosulphate titrations, were carried out in the cold room (2–4°). Stock solutions, namely 0.1N H_2SO_4 and aqueous sodium metaperiodate dissolved in 0.1N H_2SO_4 (containing 0.66 mmole of periodate/10 ml), were precooled; as were the sampling

pipettes and the receiving flasks. The compound was dissolved in the cold 0.1N H_2SO_4 (*ca.* 80 ml), the acidic sodium metaperiodate (10 ml) added, and the volume of the solution adjusted to 100 ml with 0.1N H_2SO_4 (the final concentrations were 6×10^{-4}M substrate, and 6.6×10^{-3}M sodium metaperiodate). At intervals, suitable aliquots were removed, 2N H_2SO_4 and KI were added immediately, and the solution was titrated with thiosulphate at room temperature. A blank solution, containing periodate, but no sugar, was prepared similarly to determine the initial amount of periodate present. The error in these titrations was estimated to be about $\pm 3\%$. In later experiments, the blank was determined by taking an aliquot of the oxidation mixture, destroying the excess of periodate with ethylene glycol, and titrating the mixture by the acid thiosulphate method after it had stood for about 1 h in the dark at room temperature. The blank was determined from the mean of three such titrations. This method was found to be more accurate than that using a separate blank, the error in the titration being estimated at about $\pm 1.5\%$. It was still necessary to prepare a suitable blank solution of periodate for use in the determination of malonaldehyde. In addition, the solutions were kept in a bath of melting ice at 0° to avoid the effect of small variations of temperature of the cold room; these were found to have a marked influence on the rates of periodate uptake.

3-Deoxy-D-xylo-*hexitol* (*3-deoxy*-D-*galactitol*). — A solution of sodium borohydride (2 g) in water (50 ml) was added dropwise to a stirred solution of 3-deoxy-D-*xylo*-hexose (*ca.* 9.5 g obtained by acid hydrolysis of 3-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose[38]) in water (100 ml). The mixture was left for 24 h at room temperature, and then acidified [Amberlite IR-120 (H^+)]. The resin was filtered off and washed with water, and the filtrate and washings were neutralised by addition of a strongly basic, ion-exchange resin [IRA-400 (OH^-)]. The resin was filtered off and washed thoroughly with water, and the resulting solution was concentrated to dryness *in vacuo* giving 2.6 g of oily hexitol that was benzoylated as below. The washed Amberlite IRA-400 was then suspended in water, and stirred, and acetic acid was added until the aqueous supernatant was faintly acid. The resin was filtered off and washed with water, and the resulting solution concentrated to dryness *in vacuo*. The residue was dissolved in methanol, and the methanol removed *in vacuo*. The process was repeated several times to remove borate. The residue was then crystallised from ethanol. After one recrystallisation from ethanol, the 3-deoxyhexitol (2 g) had m.p. 90–93°, $[\alpha]_D^{21}$ + 40.2° (*c* 2.07, water).

Anal. Calc. for $C_6H_{14}O_5$: C, 43.37; H, 8.43; O 48.19. Found: C, 43.24; H, 8.46; O, 48.10.

The compound gives a single spot when chromatographed in several solvents systems, and shows no reducing power. With benzoyl chloride and pyridine, in the usual manner, the oily hexitol (2.6 g) gave the pentabenzoate (6.6 g), m.p. 104–106° (from ethanol), unchanged by further recrystallisations; $[\alpha]_D^{21}$ + 50.2° (*c* 2.14, chloroform).

Anal. Calc. for $C_{41}H_{34}O_{10}$: C, 71.72; H, 4.96. Found: C, 71.62; H, 4.92.

SUMMARY

In dilute solution and at pH 3 to 5, triose reductone reduces three molar equivalents of periodate to iodate, and forms two molar equivalents of formic acid and one molar equivalent of carbon dioxide; iodine is not liberated in the process. Other ene-diols react similarly. At higher concentrations or lower pH values, iodine is liberated in all cases. Conditions in which malonaldehyde would react with stoichiometric amounts of periodate could not be found in the pH range of 1 to 8. It is shown that enolisation of malonaldehyde is faster than its reaction with periodate, and it was observed that malonaldehyde undergoes an unidentified chemical reaction at those pH values (3 to 5) where most periodate oxidations are carried out. At 4° in 0.1N sulphuric acid, malonaldehyde does not react with periodate over long periods of time, whereas glycol cleavage proceeds normally; therefore vicinal diol groups of deoxy sugars, deoxy sugar alcohols, and similar compounds can be titrated quantitatively in these conditions. The malonaldehyde formed can be estimated simultaneously by a colorimetric method, no standard being required. Syntheses of 3-deoxy-D-*xylo*-hexitol and of its pentabenzoate are described.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

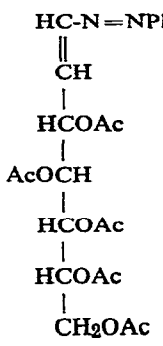
SUBSTITUTED ARYLAZOETHYLENES FROM ALDOSE ARYLHYDRAZONES

H. EL KHADEM, M. L. WOLFROM, Z. M. EL SHAFEI, AND S. H. EL ASHRY

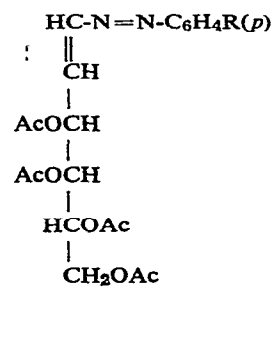
Chemistry Department, Faculty of Science, Alexandria University, Alexandria, (U.A.R.) and Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U.S.A.)

(Received December 7th, 1966; in revised form, January 17th, 1967)

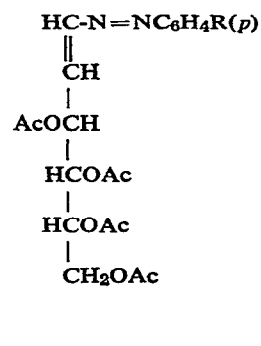
It has been shown[1,2] that the acetylated forms of the acyclic phenylhydrazones of D-glucose, D-mannose, and D-galactose readily eliminate acetic acid to yield 1-phenylazo-*trans*-1-hexenetetrol tetraacetates when boiled with aqueous ethanol. We have extended this reaction to a heptose derivative, namely D-*glycero*-D-*gulo*-heptose phenylhydrazone[3], which afforded the corresponding 1-phenylazo-*trans*-1-heptene-D-*gluco*-3,4,5,6,7-pentol pentaacetate (D-*gluco*-3,4,5,6,7-pentaacetoxy-1-phenylazo-*trans*-1-heptene) (**1**) in crystalline form. Like the hexose phenyl derivatives, **1** showed three strong absorption bands, at 230, 305 and 430 mμ in the u.v. spectrum, and in the i.r. region, showed the ester band at 1740 cm^{-1} and no C=N band. We have also prepared a number of crystalline 1-arylazo-*trans*-1-hexene-D-*lyxo*-3,4,5,6-tetrol tetraacetates (D-*lyxo*-3,4,5,6-tetraacetoxy-1-arylazo-*trans*-1-hexenes) (**2b–e**) and 1-arylazo-*trans*-1-hexene-D-*arabino*-3,4,5,6-tetrol tetraacetates (D-*arabino*-3,4,5,6-tetraacetoxy-1-arylazo-*trans*-1-hexenes) (**3a–c**) having Me, Cl, Br, or I in the *para* position of the benzene ring, from the corresponding penta-*O*-acetyl-*aldehydo*-D-galactose arylhydrazones and penta-*O*-acetyl-*aldehydo*-D-mannose arylhydrazones, respectively. The hydrazone acetates from D-galactose were obtained in crystalline form, but on heating with ethanol during crystallization, those from D-mannose were rapidly converted into the azoethylene compounds. The *p*-substituted arylhydrazone acetates from D-mannose,



1



2a, R=H
2b, R=Me
2c, R=Cl
2d, R=Br
2e, R=I

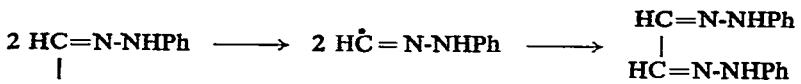


3a, R=Me
3b, R=Cl
3c, R=I

like the phenyl derivative[1], could not, therefore, be obtained crystalline. The u.v. absorption spectra of the 1-arylazo-*trans*-1-hexene-3,4,5,6-tetrol tetraacetates were, as expected, identical with those of the corresponding D-*arabino* derivatives. The *p*-tolyl, *p*-chlorophenyl, and *p*-bromophenyl derivatives showed absorption maxima at 235, 315, and 430 mμ. The *p*-iodophenyl derivatives showed these absorptions at 238, 327, and 430 mμ. The i.r. spectra were characterized by strong ester bands at 1740–1750 cm^{-1} and by the absence of the C=N bands present in the spectra of the hydrazones and their acetates.

The assignment of a *trans* configuration to the olefinic bond has been made[1,2] on the basis of n.m.r. data. The compounds herein reported are given this assignment by analogy, and by the similarity of their u.v. and i.r. spectra to those of previously reported analogs. The u.v. spectra of the previously reported[1] 1-phenyl- [and (*p*-bromophenyl)]-azo-*trans*-1-hexene-D-*arabino*-3,4,5,6-tetrol tetraacetate and 1-phenylazo-*trans*-1-hexene-D-*lyxo*-3,4,5,6-tetrol tetraacetate are herein recorded in greater detail than before.

In an attempt to prepare azoethylene derivatives from free hydrazones, D-galactose phenylhydrazone and D-arabinose phenylhydrazone were heated with methanolic or ethanolic pyridine in an autoclave at 120–130°. Both hydrazones were decomposed and gave glyoxal bis(phenylhydrazone)[4]. It appears probable that fission between C-1 and C-2 of the saccharide chain occurred, giving free radicals that combined to afford glyoxal bis(phenylhydrazone).



EXPERIMENTAL

General methods. — Infrared absorption spectra were measured with a Unicam SP 200 spectrophotometer, and ultraviolet absorption spectra with a Zeiss PMQ II spectrophotometer and a Unicam SP 800 spectrophotometer. Analyses were performed by W. N. Rond.

1-*Phenylazo*-trans-1-*heptene*-D-gluco-*3,4,5,6,7-pentol pentaacetate* (**1**). — A solution of D-*glycero*-D-*gulo*-heptose phenylhydrazone[3] (0.6 g) in dry pyridine (5 ml) was treated with acetic anhydride (7 ml), and the mixture was kept for 2 days at room temperature, whereupon it was poured with stirring onto crushed ice. The resultant syrup was washed repeatedly with water and, on attempted crystallization from ethanol–water, the acetate was converted into compound **1**; yield 0.3 g, m.p. 118–119°; $[\alpha]_D^{20}$ + 85° (*c* 0.7, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc for $C_{23}H_{28}N_2O_{10}$: C, 56.09; H, 5.73; N, 5.69. Found: C, 56.03; H, 5.80; N, 5.82.

1-p-*Tolylazo*-trans-*1-hexene*-D-lyxo-*3,4,5,6-tetrol tetraacetate* (**2b**). — D-Galactose *p*-tolylhydrazone (2 g) was acetylated with acetic anhydride and pyridine in the usual manner, and the acetate, after one crystallization from methanol, was dissolved in ethanol (10 ml) and pyridine (3 ml), water was added to opalescence, and the solution

was boiled gently for 10 min under reflux. A red oil separated during this process. The mixture was kept overnight in the cold, and diluted with water; the syrup that separated was removed by decantation, and crystallized as yellow needles from ethanol–water; yield, 0.3 g. of **2b**, m.p. 94–95°; $[\alpha]_D^{20}$ − 54.5° (*c* 0.2, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc. for $C_{21}H_{26}N_2O_8$: C, 58.06; H, 6.03; N, 6.45. Found: C, 57.75; H, 6.16; N, 6.40.

*Penta-O-acetyl-*aldehydo-D-*galactose* (p-*chlorophenyl)hydrazone.* — A solution of D-galactose (*p*-chlorophenyl)hydrazone (2 g) in dry pyridine (15 ml) was treated with acetic anhydride (16 ml). After 2 days, the solution was poured onto crushed ice. The acetate that separated was collected, and was crystallized as needles from ethanol; yield 2 g, m.p. 167–170°; $[\alpha]_D^{20}$ + 71° (*c* 0.17, ethanol); ν_{max}^{KBr} 1600 (C=N) and 1750 cm^{-1} (OAc).

Anal. Calc. for $C_{22}H_{27}ClN_2O_{10}$: C, 51.40; H, 5.29; N, 5.44. Found: C, 51.07; H, 5.46; N, 5.59.

1-(p-*Chlorophenyl)azo-*trans-*1-hexene-*D-lyxo-*3,4,5,6-tetrol tetraacetate* (**2c**). — Penta-*O*-acetyl-*aldehydo*-D-galactose (*p*-chlorophenyl)hydrazone (1 g) was dissolved in ethanol (10 ml) and pyridine (3 ml), and treated as for the *p*-tolyl derivative. Compound **2c** separated, and was crystallized as yellow needles from ethanol–water; yield 0.8 g, m.p. 101–103°; $[\alpha]_D^{20}$ − 15.7° (*c* 0.35, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc. for $C_{20}H_{23}ClN_2O_8$: C, 52.81; H, 5.10; N, 6.16. Found: C, 52.89; H, 5.10; N, 6.24.

*Penta-O-acetyl-*aldehydo-D-*galactose* (p-*bromophenyl)hydrazone.* — A solution of D-galactose (*p*-bromophenyl)hydrazone (2 g) in dry pyridine (15 ml) was treated with acetic anhydride (16 ml) as for the *p*-chloro derivative. The acetate that separated was collected, and crystallized as plates from ethanol; yield 3 g, m.p. 184–185° (decomp.); $[\alpha]_D^{20}$ + 77° (*c* 0.13, ethanol); ν_{max}^{KBr} 1595 (C=N) and 1748 cm^{-1} (OAc).

Anal. Calc. for $C_{22}H_{27}BrN_2O_{10}$: C, 47.24; H, 4.86; N, 5.01. Found: C, 47.52; H, 4.76; N, 5.01.

1-(p-*Bromophenyl)azo-*trans-*1-hexene-*D-lyxo-*3,4,5,6-tetrol tetraacetate* (**2d**). — Penta-*O*-acetyl-*aldehydo*-D-galactose (*p*-bromophenyl)hydrazone (1 g) was dissolved in ethanol (10 ml) and pyridine (3 ml), and treated as for the *p*-tolyl derivative. Compound **2d** separated, and was crystallized as yellow needles from ethanol–water; yield 0.9 g, m.p. 115°; $[\alpha]_D^{20}$ − 29° (*c* 0.38, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc. for $C_{20}H_{23}BrN_2O_8$: C, 48.11; H, 4.64; N, 5.61. Found: C, 48.01; H, 4.48; N, 5.67.

D-*Galactose* (p-*iodophenyl)hydrazone.* — A hot solution of D-galactose (7 g) in water (14 ml) was treated with (*p*-iodophenyl)hydrazine (9 g) in ethanol (18 ml), and the mixture was heated for 10 min on the water-bath. After 24 h, the resulting crystals were separated, washed with ethanol, and crystallized as prismatic needles from ethanol; yield 10 g, m.p. 182–184°; $[\alpha]_D^{20}$ + 17° (*c* 0.24, methanol).

Anal. Calc. for $C_{12}H_{17}IN_2O_5$: C, 36.38; H, 4.33; N, 7.07. Found: C, 36.60; H, 4.58; N, 7.29.

Penta-O-acetyl-aldehydo-D-*galactose* (p-*iodophenyl*)*hydrazone*. — A solution of D-galactose (*p*-iodophenyl)hydrazone (3 g) in dry pyridine (20 ml) was treated with acetic anhydride (21 ml) as for the *p*-chloro derivative. The acetate that separated was collected, and crystallized as plates from ethanol; yield 3.5 g, m.p. 172–174°; $[\alpha]_D^{20}$ +106° (*c* 0.9, ethanol); ν_{max}^{KBr} 1600 (C=N) and 1750 cm^{-1} (OAc).

Anal. Calc. for $C_{22}H_{27}IN_2O_{10}$: C, 43.58; H, 4.49; N, 4.62. Found: C, 43.71; H, 4.65; N, 4.83.

1-(p-*Iodophenyl*)*azo*-trans-*1-hexene*-D-lyxo-*3,4,5,6-tetrol tetraacetate* (**2e**). — Penta-*O*-acetyl-*aldehydo*-D-galactose (*p*-iodophenyl)hydrazone (1.5 g) in ethanol (10 ml) and pyridine (3ml) was treated as for the *p*-tolyl derivative. Compound **2e** separated, and was crystallized as yellow needles from ethanol–water; yield 1.1 g, m.p. 125°; $[\alpha]_D^{20}$ −36.4° (*c* 0.6, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc. for $C_{20}H_{23}IN_2O_8$: C, 43.97; H, 4.24; N, 5.13. Found: C, 44.05; H, 4.23; N, 5.04.

1-p-*Tolylazo*-trans-*1-hexene*-D-arabino-*3,4,5,6-tetrol tetraacetate* (**3a**). — A suspension of D-mannose *p*-tolylhydrazone (2 g) in dry pyridine (20 ml) was treated with acetic anhydride (16 ml), and the mixture was kept for 2 days at room temperature, whereupon it was filtered and the filtrate was poured onto crushed ice. The resultant, syrupy acetate was washed with water; on attempted crystallization from ethanol–water, it was converted into **3a**, which separated as yellow needles; yield 1.5 g, m.p. 146°; $[\alpha]_D^{20}$ − 21.5° (*c* 1.0, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc. for $C_{21}H_{26}N_2O_8$: C, 58.06; H, 6.03; N, 6.45. Found: C, 58.22; H, 6.11; N, 6.72.

1-p-(*Chlorophenyl*)*azo*-trans-*1-hexene*-D-arabino-*3,4,5,6-tetrol tetraacetate* (**3b**). — A suspension of D-mannose (*p*-chlorophenyl)hydrazone (2 g) in dry pyridine (20 ml) was treated with acetic anhydride (16 ml) as for the *p*-tolyl derivative. Upon crystallization of the acetate from ethanol–water, compound **3b** separated as yellow needles; yield 0.5 g, m.p. 136–137°; $[\alpha]_D^{20}$ − 11° (*c* 0.18, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc. for $C_{20}H_{23}ClN_2O_8$: C, 52.81; H, 5.10; N, 6.16. Found: C, 52.79; H, 4.91; N, 6.34.

D-*Mannose* (p-*iodophenyl*)*hydrazone*. — A hot solution of D-mannose (6 g) in water (12 ml) was treated with (*p*-iodophenyl)hydrazine (7.8 g) in ethanol (15 ml), and the mixture was heated for 10 min on the water bath and cooled. The resulting hydrazone was separated, and crystallized as plates from ethanol; m.p. 213–216° (decomp.); ν_{max}^{KBr} 1595 cm^{-1} (C=N).

Anal. Calc. for $C_{12}H_{17}IN_2O_5$: C, 36.38; H, 4.33; N, 7.07. Found: C, 36.63; H, 4.41; N, 7.06.

1-(p-*Iodophenyl*)*azo*-trans-*1-hexene*-D-arabino-*3,4,5,6-tetrol tetraacetate* (**3c**). — A suspension of D-mannose (*p*-iodophenyl)hydrazone (3 g) in dry pyridine (25 ml) was treated with acetic anhydride (21 ml) as for the *p*-tolyl derivative. On attempted crystallization from ethanol–water of the acetate that separated, it was converted into compound **3c**, which separated as yellow needles; m.p. 158°; $[\alpha]_D^{20}$ − 31.4° (*c* 0.35, methanol); ν_{max}^{KBr} 1740 cm^{-1} (OAc).

Anal. Calc. for $C_{20}H_{23}IN_2O_8$: C, 43.97; H, 4.24; N, 5.13. Found: C, 43.96; H, 4.11; N, 5.44.

Glyoxal bis(phenylhydrazone). — (*a*) A solution of D-galactose phenylhydrazone (3 g) in 60 ml of methanol (or 60 ml of 5% pyridine in ethanol) was heated in an autoclave for 30 min at 120–130°, and the solution was then concentrated. The product that separated crystallized from methanol as yellow needles, and was identified as glyoxal bis(phenylhydrazone)[4] by mixed m.p. and comparative i.r. and u.v. spectra.

Anal. Calc. for $C_{14}H_{14}N_4$: C, 70.59; H, 5.88; N, 23.53. Found: C, 70.41; H, 6.08; N, 23.13.

(*b*) D-Arabinose phenylhydrazone (2 g), treated as above, also afforded glyoxal bis(phenylhydrazone), which was identified by mixed m.p. and comparative i.r. and u.v. spectra.

Ultraviolet spectra. — The u.v. spectra of all the aryl *trans*-1-hexene derivatives prepared from the aldose arylhydrazones showed λ_{max}^{EtOH} 204–5 ($\log_{10}\varepsilon$ 3.97–4.14), 224–30 (shoulder) (3.93–4.04), 230–35 (4.01–4.11), 234–41 (shoulder) (3.95–4.05), 303–15 (4.31–4.49), and 425–30 mμ (2.52–2.75). No shoulders were found for the two *p*-iodophenyl derivatives, and two of their absorption maxima were at 237–242 ($\log_{10}\varepsilon$ 4.08) and 327 mμ (4.41). The λ_{min}^{EtOH} values were 214–218 ($\log_{10}\varepsilon$ 3.74–3.91), 247–258 (3.52–3.90), and 370–386 mμ (2.05–2.57). The range of data includes not only the u.v. spectra for the compounds reported herein, but also those for 1-phenylazo [and 1-(*p*-bromophenyl)azo]-*trans*-1-hexene-D-*arabino*-3,4,5,6-tetrol tetraacetate and 1-phenylazo-*trans*-1-hexene-D-*lyxo*-3,4,5,6-tetrol tetraacetate, previously reported[1] in less detail.

SUMMARY

A number of 1-arylazo-*trans*-1-hexene-D-*lyxo*- and -D-*arabino*-3,4,5,6-tetrol tetraacetates having Me, Cl, Br, or I in the *para* position of the benzene ring were prepared from the corresponding D-galactose and D-mannose arylhydrazone acetates. The reaction was also extended to a heptose derivative. Aldose phenylhydrazones afforded glyoxal bis(phenylhydrazone) when heated in methanolic solution at 120–130°.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE USE OF 6-*O*-METHYLAMYLOSE AS A SUBSTRATE FOR AMYLASES*

C. EDWIN WEILL AND MARTIN BRATT
Department of Chemistry, Rutgers, The State University, Newark, N. J. 07102 (U. S. A.)

(Received December 8th, 1966; in revised form, January 20th, 1967)

INTRODUCTION

Two routes have been used repeatedly in the study of the mechanism of enzyme action. In one, the enzyme protein is itself studied, in an effort to determine which amino acid residues are important in the binding and catalytic processes. The other is concerned with the nature of those groups in the substrate that seem to be critical to the binding and catalysis.

Many advances have been made in the investigation of both routes in respect of the hydrolysis of the peptide linkage. Studies have also been made on the importance of substrate groups for glycosidases, that class of enzyme that catalyzes the hydrolysis of simple glycosides, including disaccharides. Gottschalk[1] reviewed much of the early work on the glycosidases. The importance of various amino acid residues in one amylase, namely β-amylase, has been studied[2-4]. There has, however, been a scarcity of information on the nature of the groups, in the substrates, that are important for those enzymes that catalyze the hydrolysis of polysaccharides. At least part of the difficulty arises from the problem of obtaining substrates containing the α-D-(1→4)-glycopyranosyl linkage. Certainly, it would be valuable to study the action of amylases on substrates in which one or more of the D-glucose residues is modified in structure or configuration.

Because natural substrates of this sort are not available, such investigations must use synthetic polymers containing this type of linkage. Two routes are available for the preparation of such polymers. One is the completely synthetic path, starting from monomers. Because the synthesis of the α-D-(1→4)-glucosidic linkage is so difficult by the usual chemical means, it offers little promise. However, the synthesis of modified D-glucosaccharides by enzymic techniques is a definite possibility. It has been under investigation in this laboratory for some time, and is the subject of another communication[5].

The other route to synthetic polymers is the alteration of a fraction of the glycosyl residues in a natural polymer. A partially methylated amylose was first used in amylase studies by Husemann and Lindemann[6], but no information was given as

*Presented, in part, at the 150th National Meeting of the American Chemical Society, Atlantic City, New Jersey, September 12th, 1965.

to the location of the methyl ether groups. An outline of methods for the preparation of amylose modified at specific positions on each D-glucose residue was then published by Bines and Whelan[7] as a premilinary note. A more detailed method of preparing such modified amyloses was described by Sanella and Whistler[8]. The basic techniques of these authors were used in this laboratory to prepare amylose molecules in which a fraction of the primary hydroxyl groups had been methylated to produce material that will be called 6-*O*-methylamylose*.

The action of several amylases on this 6-*O*-methylamylose has been studied, to determine the effect of modification of the substrate on the action of the enzyme.

EXPERIMENTAL

Materials. — Corn amylose (A. E. Staley Co.), 3-*O*-methyl-D-glucose (Calbiochem), crystalline, sweet-potato β-amylase and hog-pancreatic amylase (Worthington Biochemicals Corp.), *Bacillus subtilis* amylase (Enzyme Development Corp.), and the *Aspergillus niger* enzyme preparation called glucamylase (Clinton Corn Processing Co.) were commercial samples. The 2-*O*-methyl-D-glucose[9], 6-*O*-methyl-D-glucose[10], cyclohexaamylose[11], isomaltose[12], and potato phosphorylase[13] were prepared by methods in the literature. The potato phosphorylase contained 10 units per ml. Cyclodextrin transglucosylase was furnished by Prof. M. Wheeler. The 2,3-di-*O*-benzoylamylose[8] had the following analysis.

Anal. Calc. for $(C_6H_8O_5.C_{14}H_{10}O_2)_n$: benzoyl[14], 56.7. Found: 56.8.

Methylation of 2,3-di-O-benzoylamylose. — 2,3-Di-*O*-benzoylamylose was methylated at room temperature, in the dark, in a centrifuge bottle under exclusion of moisture. The methyl iodide–silver oxide reagent was used, with efficient stirring, in *N*,*N*-dimethylformamide, a reagent which dissolves the amylose ester. After many trials, conditions were found that afforded products suitable for enzyme studies. These were: 3 ml of methyl iodide, 2 g of silver oxide, and 15 ml of *N*,*N*-dimethylformamide per g of 2,3-di-*O*-benzoylamylose; reaction time 5 h (preparation 6), 3 h (preparation 7). Larger proportions of methyl iodide and silver oxide, or longer reaction times, led to final products that were heterogeneous.

To terminate the reaction, the mixture was centrifuged at 9,000 r.p.m. for 10 min at 15°. The sediment was washed with *N*,*N*-dimethylformamide, and the washings and supernatant liquor were combined and poured into a large excess of methanol with vigorous stirring. The precipitate was stirred several times with methanol in a Waring Blendor, and dried for 16 h in a vacuum oven at 60°; it consisted of methylated 2,3-di-*O*-benzoylamylose contaminated with silver salts.

De-esterification was effected with 0.2M methanolic sodium methoxide (133 ml per g of ester). After 48 h, the mixture was neutralized, saturated sodium chloride

*The term 6-*O*-methylamylose has been used to designate these molecules, even though only a fraction of the primary hydroxyl groups have been methylated. The extent of methylation is shown by the degree of substitution (D. S.). Thus, a D. S. of 0.5 means that half of the primary hydroxyl groups have been methylated.

was added, and the solution was warmed. The precipitate was collected, and suspended in a solution of potassium cyanide (5 g per 100 ml). The solution was dialyzed for 4 days to remove silver salts. After concentration, the final product (*ca.* 80% yield) was dried in a vacuum oven (preparation 6: OCH_3, 7.65%; D.S., 0.414) or lyophilized (preparation 7: OCH_3, 4.12%; D.S., 0.219). The infrared spectra of these materials (KBr pellets) showed no carbonyl absorption.

Hydrolysis of 6-O-methylamylose. — The products were hydrolyzed with N hydrochloric acid (6 h at 100°). The hydrolyzate was neutralized by extraction with a 5% solution of methyldioctylamine in chloroform, concentrated to a small volume, and examined by t.l.c. (silica gel G; 45:35:20 ethanol–toluene–ethyl ether, with detection by spraying the dried plates with 5% ethanolic sulfuric acid and then heating for 30 min at 105°). An intense spot for glucose (R_F 0.32) and a strong spot for 6-*O*-methylglucose (R_F 0.44) were found for both preparations; the relative intensities were consistent with the respective D.S. values of 0.4 and 0.2. Neither 2-*O*-methylglucose, 3-*O*-methylglucose, nor any polymethylated glucose was detected in the hydrolyzates.

The hydrolyzate of preparation 6 (D.S. 0.4) was tritylated[15], and the product was subjected to g.l.c. in an Aerograph Model 1520 gas chromatograph (Wilkens Instrument and Research Co.) with dual columns (5 ft × 0.318 cm) of 5% silicone gum rubber on 60–80 mesh Chromosorb W, and helium as the carrier gas (flow rate, *ca.* 33 ml per min). Samples were injected at 200°, with the dual thermal-conductivity detector set at 275°. The initial temperature of the column was 140°, with a programmed temperature increase of 2° per min. Recordings were made at a chart speed of 0.5 in per min. As overlapping peaks were found for D-glucose (16.5 and 20.3 min) and for 6-*O*-methyl-D-glucose (15.5 and 16.5 min), corresponding to the anomers o each, the D.S. value for preparation 6 was checked as follows. A mixture of D-glucose and 6-*O*-methyl-D-glucose simulating D.S. 0.40 was subjected to the same acid treatment, deacidification, concentration, and tritylation, and was then subjected to g.l.c. Comparison of the areas under the curves for this mixture and for the hydrolyzate gave a D.S. of 0.44 for the latter, in agreement with the methoxyl analysis.

The hydrolyzate of preparation 7 (D.S. 0.2), which was available in larger amount, was subjected to preparative chromatography (Whatman 3MM paper; 6:4:3 butanol–pyridine–water, descending, 30 h). Guide strips were treated with silver nitrate reagent[16] to locate the 6-*O*-methyl-D-glucose and D-glucose. Zones were excised and eluted, and the extracts were concentrated to a fixed volume. Aliquots were examined for purity (t.l.c.) and for reducing sugar[17,18]. The D.S. value obtained was 0.204 ± 0.04.

Enzyme studies. — For the enzyme studies, amylose and 6-*O*-methylamylose were ground with 1.67M potassium hydroxide (1.0 ml per 100 mg of sample). After partial dilution, the pH was adjusted to the desired value by addition of a weak acid; dilution was completed with buffer solution of the correct pH. After addition of the enzyme, the final concentrations were: methylated amylose (D.S. 0.4), 0.5 mg per ml; methylated amylose (D.S. 0.2), 1.0 mg per ml; and amylose, 1.0 mg per ml.

All digestions with amylases were conducted at 25°. The solutions were 0.05M with respect to acetate at pH 4.8 (β-amylase), and 0.10M with respect to acetate at pH 5.7 (*Bacillus subtilis* enzyme). Digestions with pancreatic amylase were conducted at pH 7.0 with phosphate and sodium chloride[19]. The mold-enzyme preparation was used at pH 4.5 in a solution which was 0.10M with respect to acetate. The concentrations of the enzymes are shown in the appropriate Tables.

Aliquots of the enzyme reaction mixtures were analyzed for reducing sugars[18]. The residual solution was boiled (to inactivate the enzyme), concentrated, and examined by t.l.c. (45:35:20 ethanol–toluene–ether). The digest obtained with the mold glucamylase preparation was subjected also to chromatography on Whatman 3MM paper (4:1:1 butyl alcohol–ethanol–water).

Tests with potato phosphorylase were conducted at 36° at pH 7.0 in 0.05M citrate buffer. The concentration of inorganic phosphate was 0.02M. The proportion of phosphorylase was 0.33 units[13] per mg of substrate. Aliquots of incubated reaction mixture were mixed with an equal volume of 5% aqueous trichloroacetic acid to precipitate the protein, and inorganic phosphate was determined for a measured portion of the supernatant solution by a slight modification of the method of Lowry and Lopez[20]. The concentrations of L-ascorbic acid and molybdate were doubled, to remove the interference otherwise caused by the citrate buffer.

The coupling of D-glucose to 6-*O*-methyl-D-glucose with cyclodextrin transglucosylase was conducted for 48 h at room temperature[21]. The reaction mixture was then heated, concentrated, and chromatographed on Whatman 3MM paper (6:4:3 butyl alcohol–pyridine–water). Unreacted 6-*O*-methyl-D-glucose and the transfer products were removed by excision. The disaccharide fraction was subjected to a second chromatographic separation with the same solvent mixture, to obtain pure *O*-α-D-glucopyranosyl-(1→4)-6-*O*-methyl-D-glucopyranose. This was then subjected to the action of the glucamylase preparation.

Ionophoresis of the presumed disaccharide from the mold glucamylase digest was conducted in a Model E 800-2B Paper Electrophoresis chamber (Warner Chilcott, Laboratory Instrument Division) by using Kieselguhr G on glass, and borate buffer[22] at 70 milliamps and 500 volts. The spots were located as in t.l.c.

RESULTS

The 6-*O*-methylamyloses of D.S. 0.2 and D.S. 0.4 were tested as possible substrates for crystalline, sweet-potato β-amylase; no reducing sugar was detected in the solution, even after prolonged incubation at 25°. Potato phosphorylase (another exo-enzyme) was allowed to act on the sample having D.S. 0.4 in the presence of inorganic phosphate. There was no decrease in inorganic phosphate in the solution, showing that this 6-*O*-methylamylose was not a substrate for the enzyme.

On the other hand, certain enzymes capable of catalyzing the hydrolysis of interior linkages did effect the degradation of these substrates. These included two crystalline enzymes, namely, the amylase from *Bacillus subtilis* and that from hog pancreas.

The crystalline, *Bacillus subtilis* enzyme caused slow and incomplete hydrolysis of the 6-*O*-methylamylose of D.S. 0.4; even after prolonged incubation, the hydrolysis, calculated as percent of maltose liberated, was less than 10%. A very small proportion of maltose was the only small fragment detected in the hydrolyzate by t.l.c. The *Bacillus subtilis* enzyme caused much more extensive hydrolysis of the 6-*O*-methylamylose of D.S. 0.2. A high concentration of the enzyme produced reducing sugars (calculated as maltose) equivalent to about 40% of the theoretical value in 30 min, but only an additional 10% during the next 24 h (see Table I). Chromatography revealed the presence of maltose, but no monosaccharide, in the hydrolyzate.

TABLE I

THE ACTIVITY OF *B. subtilis* AMYLASE ON 6-*O*-METHYLAMYLOSE (D.S. 0.2) AND AMYLOSE

Substrate	*Enzyme (mg/mg of substrate)*	*Time (h)*	*Yield of maltose (% of the theoterical)*
6-*O*-Methylamylose	0.2	0.5	39
		1.0	42
		2.0	44
		4.0	45
		24.0	49
Amylose	0.002	0.5	36
		1.0	49
		2.0	56
		24.0	64
	0.02	0.5	62
		1.0	65
		2.0	67
		24.0	68
	0.2	0.5	70
		1.0	73
		2.0	75
		24.0	79

Pancreatic amylase is an *alpha* type of enzyme that usually yields a higher percentage of D-glucose and maltose than do the bacterial amylases. The composition of a digest of waxy-maize starch with pancreatic amylase has been described by Whelan[19]. When the crystalline, pancreatic enzyme was incubated for 24 h with the product having D.S. 0.4, there was 14% hydrolysis (calculated as maltose). Examination of the hydrolyzate by t.l.c. showed some maltose and a smear of higher saccharides, but glucose was not detected.

Pancreatic amylase caused much more extensive hydrolysis of the 6-*O*-methylamylose of D.S. 0.2 (see Table II). An examination of the 22-h hydrolyzate by t.l.c. showed a significant amount of maltose, but no fraction having an R_F greater

than that of maltose. Fractions that moved more slowly than maltose were present to such an extent that individual spots were not differentiable.

TABLE II

THE ACTIVITY OF PANCREATIC AMYLASE ON 6-*O*-METHYLAMYLOSE (D.S. 0.2) AND AMYLOSE

Substrate	*Enzyme (mg/mg of substrate)*	*Time, (h)*	*Yield of maltose (% of the theoretical)*
6-*O*-Methylamylose	15×10^{-5}	0.5	19
		1.0	26
		2.0	37
		4.0	47
		22.0	55
Amylose	15×10^{-5}	0.5	75
		1.0	85
	3×10^{-5}	0.5	27
		1.0	47
		2.0	72
		4.0	82
		22.0	84

Although both of the above endoamylases caused significant hydrolysis of the 6-*O*-methylamylose of D.S. 0.2, there was no evidence of the cleavage of any 6-*O*-methyl-D-glucosyl bond; at least, 6-*O*-methylglucose was not detected as a hydrolysis product. Tests were therefore continued with amyloglucosidase (glucamylase), an enzyme that degrades amylose almost quantitatively to D-glucose, and that has been shown by Pazur and Ando[23] to be an exo-enzyme (as is β-amylase). Such enzymes are usually obtained from *Aspergillus niger* or from *Rhizopus* molds. A crude, enzyme preparation was used; it was a mixture of *alpha*-type amylase, at least one amyloglucosidase, and a transglucosylase that can form isomaltose. The crude mixture was used because it seemed possible that such a mixture of enzymes would cause extensive hydrolysis of the modified amyloses, and might liberate some 6-*O*-methyl-D-glucose. The results obtained with this glucamylase mixture, calculated as the percent of the theoretical amount of D-glucose, are shown in Table III.

Several of the glucamylase digests of the modified amyloses were examined by both t.l.c. and paper chromatography. Glucose was found in the hydrolyzates of both the D.S. 0.4 and D.S. 0.2 substrates. For the latter, it was present in such a large concentration that isolation of any of the slower-moving products was difficult. In contrast, 6-*O*-methylglucose was not detected in any of the hydrolyzates.

The t.l.c. plates and the paper chromatograms showed a component having an R_F greater than that of maltose but lower than that of glucose. This component was not isomaltose, which has an R_F smaller than that of maltose with both of the solvent systems that were used. An area containing this fraction was extracted from

the paper. One sample of the concentrated extract was subjected to ionophoresis on Kieselguhr G. It had an M_G value of 0.27, compared with 0.37 for maltose and 0.67 for isomaltose. This low value suggested that the product was not the product of a 1→6 tranfer reaction. Another portion was subjected to acid hydrolysis, and the hydrolyzate was chromatographed. Glucose and 6-*O*-methylglucose were both present, suggesting that the component was a disaccharide of these two hexoses. The most likely possibilities are: *O*-α-D-glucopyranoyl-(1→4)-6-*O*-methyl-D-glucopyranose (**1**) and *O*-(6-*O*-methyl-α-D-glucopyranosyl)-(1→4)-D-glucopyranose.

TABLE III

ACTIVITY OF "GLUCAMYLASE" ON 6-*O*-METHYLAMYLOSE AND AMYLOSE

Substrate	*Enzyme (mg/mg of substrate)*	*Time, (h)*	*Yield of* D-*glucose (% of the theoretical)*
6-*O*-Methylamylose, D.S. 0.4	0.4	0.25	5.8
		0.5	7.6
		1.0	14
		2.0	18
		5.0	24
		24.0	31
6-*O*-Methylamylose, D.S. 0.2	0.16	0.5	20
		1.0	34
		2.0	49
		4.0	51
		22.0	62
		46.0	63
Amylose	0.16	0.5	35
		1.0	63
		2.0	80
		4.0	83
		46.0	93
	0.4	0.25	33
		0.5	64
		1.0	86
		2.0	91
		4.0	93

It has not yet been possible to isolate a sufficient amount of this disaccharide to permit making a positive identification. It is, however, possible to state that structure **1** is not resistant to the glucamylase mixture and, hence, would not be expected to accumulate in the digest. The disaccharide **1** was prepared by coupling D-glucose to 6-*O*-methyl-D-glucose, using the techniques described by Wheeler *et al.*[21]. Pure **1** was separated from the cyclodextrin transglucosylase reaction mixture by paper chromatography. This pure **1** was completely hydrolyzed to D-glucose and 6-*O*-methyl-D-glucose by the mold-glucamylase preparation, thus demonstrating that it would not have accumulated in the hydrolyzates (see Table III).

DISCUSSION

The failure of β-amylase and potato phosphorylase to cause any measurable degradation of either of the 6-*O*-methylamyloses suggests that D-glucosyl groups at or near the nonreducing ends of these molecules must have been methylated. It has been known for some time that the action of either enzyme on amylopectin is confined to the outer branches, and ceases at or before the branch points of that substrate. It is interesting that the relatively small methyl group has the same nullifying effect as the bulky, natural (1→6)-branch formed by substitution by D-glucosyl groups; this result could not have been anticipated.

In contrast, partial hydrolysis of the 6-*O*-methylamyloses by crystalline pancreatic and bacterial endo-amylases was observed. Such α-amylases are known to cleave branched (as well as unbranched) amylopolysaccharides into small fragments by hydrolyzing (1→4)-linkages in various parts of the molecule. Evidently, the methylation of the primary hydroxyl group is no more of a hindrance to the cleavage of interior links than is the branch in the amylopectin molecule. The very low degree of hydrolysis observed in the sample having D.S. 0.4, the failure of the hydrolysis of the sample having D.S. 0.2 to attain the degree of hydrolysis obtained with amylose, and the absence of detectable amounts of D-glucose in the hydrolyzates of the methylated molecules, all suggest that these enzymes do not cleave the substrate in the immediate vicinity of the 6-*O*-methyl-D-glucose residue. The degree of substitution, even in the sample having D.S. 0.2, is higher than the number of (1→6)-linkages in amylopectin. This degree of substitution is sufficiently high that an early encounter would be made with one of the 6-*O*-methyl-D-glucose residues.

As regards the action of the mold "glucamylase" preparation (which is a mixture of an α-amylase, amyloglucosidase, and a transglucosylase), the absence of 6-*O*-methyl-D-glucose from the hydrolyzate seems highly significant. This finding indicates that the 6-*O*-methyl-D-glucosyl bond is resistant to cleavage by all of the enzymes in the preparation. Normally, all of the D-glucosidic linkages in starch are hydrolyzed by such mold preparations, affording an almost quantitative yield of D-glucose molecules. The capacity of the mold amyloglucosidase preparation to hydrolyze *O*-α-D-glucopyranosyl-(1→4)-6-*O*-methyl-D-glucopyranose is confirmatory evidence that the essential points of resistance to hydrolysis in 6-*O*-methylamylose are the 6-*O*-methyl-D-glucosyl bonds to neighboring D-glucose residues.

SUMMARY

A type of chemically modified amylose, in which a fraction of the primary hydroxyl groups is specifically methylated, has been prepared by the following sequence of reactions: amylose → 6-*O*-tritylamylose → 2,3-di-*O*-benzoyl-6-*O*-tritylamylose → 2,3-di-*O*-benzoylamylose → 2,3-di-*O*-benzoyl-6-*O*-methylamylose → 6-*O*-methylamylose.

Two such preparations of 6-*O*-methylamylose, having D.S. 0.2 and D.S. 0.4,

were examined as model substrates for a number of starch-degrading enzymes. Neither was detectably attacked by sweet-potato β-amylase or potato phosphorylase. Some hydrolysis (much greater for the 6-*O*-methylamylose of D.S. 0.2 than for that of D.S. 0.4) did occur with crystalline α-amylase preparations from hog pancreas and *Bacillus subtilis*. Relatively extensive hydrolysis was effected by an *Aspergillus niger* enzyme preparation containing both α-amylase and amyloglucosidase.

Products of the action of the pancreatic and *Bacillus subtilis* amylases included maltose and higher saccharides, but neither D-glucose nor 6-*O*-methyl-D-glucose was detected. Products obtained with the *A. niger* enzyme preparation included D-glucose and a saccharide tentatively identified as 4-*O*-(6-*O*-methyl-α-D-glucopyranosyl)-D-glucose; 6-*O*-methyl-D-glucose was not detected.

The failure of the exo-enzymes to effect any hydrolysis, and the extent of hydrolysis and the nature of the products formed by the two crystalline α-amylases, demonstrate that hydrolysis is blocked in the vicinity of the methylated primary hydroxyl groups. The results suggest that those limitations on amylase action normally attributed to the bulky (1→6)-D-glucose branch are also imposed by a small group, namely, the methyl group.

ACKNOWLEDGMENT

This work was supported by Grant GM 08927 of the Research Grants Branch of the National Institute of General Medical Science.

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Carbohydrate Research
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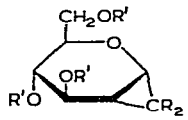
THE ADDITION OF DICHLOROMETHYLENE TO UNSATURATED SUGARS*

J. S. BRIMACOMBE, M. E. EVANS, E. J. FORBES, A. B. FOSTER, AND J. M. WEBBER
Chemistry Department, The University, Birmingham 15 (Great Britain)

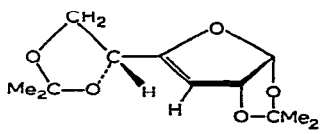
(Received October 17th, 1966; in revised form, December 17th, 1966)

Recent publications indicate a renewed interest in unsaturated sugars (reviewed by Ferrier[1]) as synthetic intermediates, and new and improved methods of preparation have been reported[2]. As far as we are aware, there has been no report of the addition of carbenes to unsaturated sugars, and we now report on some exploratory reactions.

The reaction of dichloromethylene (generated[3] by the action of sodium methoxide on ethyl trichloroacetate) with 3,4,6-tri-*O*-methyl-D-glucal[4,5b] was conveniently monitored by gas–liquid chromatography (g.l.c.). The adduct *A*, obtained in 82% yield, appeared to be homogeneous by g.l.c., suggesting that the carbene addition was essentially stereospecific. Although no direct experimental evidence for the configuration of adduct *A* was obtained, an analogy may be drawn with the epoxidation of glycals[5] and 3-substituted cyclohexanes[6] by perbenzoic acid. Addition of oxygen occurs *trans* to a non-hydroxylic substituent that is vicinal to the double bond, and the structure 1,5-anhydro-2-deoxy-1,2-*C*-(dichloromethylene)-3,4,6-tri-*O*-methyl-D-*glycero*-D-*ido*-hexitol (**1**) is tentatively assigned to adduct *A*.



1 R=Cl ; R'=Me
2 R=Cl ; R'=H
3 R=Cl ; R'=Bz
4 R=H ; R'=Me



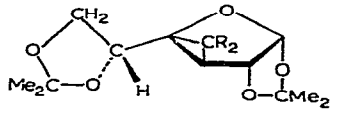
5

Treatment of compound **1** with boron trichloride[7] at *ca.* −70° resulted in smooth demethylation to afford syrupy 1,5-anhydro-2-deoxy-1,2-*C*-(dichloromethylene)-D-*glycero*-D-*ido*-hexitol (**2**), characterised as the tribenzoate (**3**). Reduction of adduct **1** with lithium aluminium hydride in boiling ether gave 1,5-anhydro-2-deoxy-3,4,6-tri-*O*-methyl-1,2-*C*-methylene-D-*glycero*-D-*ido*-hexitol (**4**).

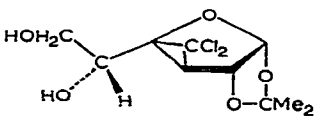
Treatment of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose[8,9] (**5**) with a ten-fold excess of dichloromethylene gave, in good yield, a single, crystalline adduct (*B*), as evidenced by thin-layer chromatography and n.m.r. spectro-

*A preliminary report of this work has appeared: *Abstracts Papers Am. Chem. Soc. Meeting*, 150 (1965) 26D.

scopy. Stereoselective, if not stereospecific, addition of dichloromethylene to the double bond from the *exo*-side of the trioxabicyclo[3.3.0]octene ring system to give compound **6** would be expected by analogy with the hydroboration[10] and hydrogenation[9] of olefin **5**. Nucleophilic substitution[11] and addition[12] reactions with reagents having high steric requirements are found to be virtually stereospecific for fused-ring systems related to that in compound **5**, and proceed only when attack is possible from the *exo*-side.



6 R = Cl
7 R = H



8

Reduction of adduct **6** with lithium aluminium hydride gave 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3,4-*C*-methylene-α-D-galactofuranose (**7**), whilst graded hydrolysis, with acid in aqueous *p*-dioxane, afforded crystalline 3-deoxy-3,4-*C*-(dichloromethylene)-1,2-*O*-isopropylidene-α-D-galactofuranose (**8**). The graded hydrolysis was conveniently monitored by observing the change in pattern of the signals for the isopropylidene Me protons[13] in the n.m.r. spectrum. Attempts to remove both isopropylidene groups from compound **6** gave a mixture containing two, major, reducing components, which, at concentrations up to 10 mg/ml, showed no inhibitory action to growth of *Staphylococcus pyogenes, Streptococcus pyogenes, Klebsiella aerogenes, and Escherichia coli.*

EXPERIMENTAL

Thin-layer chromatography was performed on Kieselgel, and detection was effected with iodine vapour. Gas–liquid chromatography was performed on poly(ethylene glycol adipate)–Celite at 140° or 150° by using a Pye-Argon instrument with β-ionisation detection. N.m.r. spectra were generally measured on *ca.* 10% solutions in *N*,*N*-dimethylformamide, with a 6% solution of tetramethylsilane in chloroform as external reference, by using a Varian A-60 spectrometer operating under normal conditions.

*Addition of dichloromethylene to 3,4,6-tri-*O*-methyl-*D*-glucal.* — A vigorously stirred slurry of sodium methoxide (15 g) and 3,4,6-tri-*O*-methyl-D-glucal (13 g, b.p. 60–64° (bath)/0.2–0.3 mm, lit.[5b], 48–52°/0.01 mm) in dry hexane (200 ml) was treated at 0–4° with ethyl trichloroacetate (23.6 g), and was then stirred overnight at ambient temperature. Water (100 ml) was added, and when all of the sodium methoxide had been destroyed, the organic layer was separated. The aqueous layer was extracted with light petroleum (b.p. 60–80°) (2 × 150 ml), the combined organic layers were dried ($MgSO_4$), and the solvents removed. Analysis of the residue by g.l.c. revealed

the presence of two components, one of which had the same retention time as the starting material. Two further treatments were required to remove all of the starting material, and the residue was finally distilled to give 1,5-anhydro-2-deoxy-1,2-*C*-(dichloromethylene)-3,4,6-tri-*O*-methyl-D-*glycero*-D-*ido*-hexitol (**1**) (15.4 g, 82%), b.p. 84–90°/0.1 mm, $[\alpha]_D$ + 51° (*c* 4, chloroform) (Found: C, 44.35; H, 5.6; Cl, 25.95. $C_{10}H_{16}Cl_2O_4$ calc: C, 44.3; H, 5.9; Cl, 26.2%).

1,5-Anhydro-3,4,6-tri-O-*benzoyl-2-deoxy-1,2*-C-(*dichloromethylene*)-D-glycero-D-ido-*hexitol* (**3**). — A solution of compound **1** (3 g) in dichloromethane (8 ml) was cooled to *ca.* − 70°, and then treated[7] with an excess of boron trichloride. The residue was decolourised with charcoal in boiling methanol, solids were filtered off, and the filtrate was concentrated to a syrup (*ca.* 2 g) that failed to crystallise. T.l.c. (benzene–methanol, 9:1) demonstrated the presence of a single, major component, and the absence of starting material.

A portion (0.3 g) of the syrup was treated with benzoyl chloride in pyridine, in the usual manner, to give compound **3** (0.39 g), m.p. 184–185° (from ethanol–light petroleum, b.p. 60–80°), $[\alpha]_D$ + 51° (*c* 1.2, chloroform) (Found: C, 62.2; H, 4.05; Cl, 12.9. $C_{28}H_{22}Cl_2O_7$ calc.: C, 62.1; H, 4.05; Cl, 13.1%).

1,5-Anhydro-2-deoxy-3,4,6-tri-O-*methyl-1,2*-C-*methylene*-D-glycero-D-ido-*hexitol* (**4**). — A solution of adduct **1** (5 g) in dry ether (100 ml) was added to a suspension of lithium aluminium hydride (4 g) in dry ether (50 ml), and the mixture was heated under reflux for 1 week. Ethyl acetate and water were added to decompose the excess of reagent, solids were filtered off, the organic layer was separated and dried ($MgSO_4$), and the solvents were removed. Examination of the residue by g.l.c. showed the presence of two components, one of which had the same retention time as the starting material. The residue was subjected to a second treatment, whereupon g.l.c. showed the presence of a single component. Distillation of the residue afforded compound **4** (2.2 g, 59%), b.p. 85–90°(bath)/0.1 mm, $[\alpha]_D$ + 44.5° (*c* 0.9, chloroform) (Found: C, 59.7; H, 9.1. $C_{10}H_{18}O_4$ calc.: C, 59.45; H, 8.9%).

Addition of dichloromethylene to 3-deoxy-1,2:5,6-di-O-*isopropylidene*-α-D-erythro-*hex-3-enofuranose*. — The unsaturated sugar (4.6 g, m.p. 46–48°, prepared by the method of Weygand and Wolz, who gave[9] m.p. 51°) was treated with a ten-fold excess of dichloromethylene, essentially as described previously. The resulting, solid residue was stored *in vacuo* to remove traces of ethyl trichloroacetate. T.l.c. (hexane–ethanol, 9:1) revealed the presence of a major component, together with a minor proportion of a second component. Crystallisation from ethanol–hexane gave 3-deoxy-3,4-*C*-(dichloromethylene)-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**6**) (4.9 g, 80%), m.p. 72–74°. After recrystallisation from ethanol, the product had m.p. 78–79°, $[\alpha]_D$ +28° (*c* 0.85, chloroform) (Found: C, 48.3; H, 5.6; C, 21.7. $C_{13}H_{18}Cl_2O_5$ calc.: C, 48.1; H, 5.5; Cl, 21.85%). N.m.r. data: τ 8.78, 8.84, 8,95 (singlets; relative intensities, 1:1:2; two CMe_2 groups).

3-Deoxy-1,2:5,6-di-O-*isopropylidene-3,4*-C-*methylene*-α-D-*galactofuranose* (**7**). — Compound **6** (0.8 g) was reduced with lithium aluminium hydride (1 g) by the procedure described herein. Distillation of the residue gave compound **7** (0.3 g, 48%),

b.p. 105–110° (bath)/0.25 mm, $[\alpha]_D$ + 47.5° (*c* 1.05, chloroform) (Found: C, 60.8; H, 8.0. $C_{13}H_{20}O_5$ calc.: C, 61.0; H, 7.8%).

3-Deoxy-3,4-C-(dichloromethylene)-1,2-O-isopropylidene-α-D-galactofuranose (**8**). — A solution of compound **6** (0.11 g) in a mixture of *p*-dioxane–methanol–10N sulphuric acid (3 ml, 6:3:1) was kept at room temperature, and the removal of the *O*-isopropylidene group was followed by n.m.r. spectroscopy[13]. This was complete after 45 min, whereupon water was added, and the solution was neutralised with Amberlite IRA-400 (CO_3^{2-}), and filtered. Removal of the solvents, with recrystallisation of the residue from ethanol–hexane, gave compound **8** (25 mg), m.p. 102–103°, $[\alpha]_{5461}$ +47° (*c* 1, chloroform) (Found: C, 42.0; H, 4.9. $C_{10}H_{14}Cl_2O_5$ calc.: C, 42.15; H, 4.9%).

When compound **6** (0.5 g) was treated with *p*-dioxane–methanol–10N sulphuric acid (3 ml, 6:3:1) for 5 days, and processed as described above, the residue was shown by paper chromatography (butyl alcohol–ethanol–water, 4:1:5) to contain two, major, reducing components (R_F 0.80, 0.88), a minor reducing component (R_F 0.65), and a small proportion of compound **8**. The residue was partitioned between water (200 ml) and chloroform (50 ml) to remove compound **8**, and the aqueous layer was concentrated to a syrup (0.13 g) that contained the same reducing components (paper chromatography). The crude hydrolysate was inactive in tests for antibacterial activity (see Discussion)

ACKNOWLEDGMENTS

We thank Professor M. Stacey, C.B.E., F.R.S., for his interest, and Mr. E. Chelton for the biological tests. The award of a maintenance grant (to M. E. E.) by the Science Research Council is acknowledged.

SUMMARY

Addition of dichloromethylene to 3,4,6-tri-*O*-methyl-D-glucal gave a single product that was tentatively identified, by stereochemical considerations, as 1,5-anhydro-2-deoxy-1,2-*C*-(dichloromethylene)-3,4,6-tri-*O*-methyl-D-*glycero*-D-*ido*-heptitol(**1**), and was characterised, after demethylation, as the tribenzoate **3**. 3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose reacted with dichloromethylene to give 3-deoxy-3,4-*C*-(dichloromethylene)-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**6**), which was then converted by mild hydrolysis with acid into the 1,2-*O*-isopropylidene derivative (**8**).

Reduction of compound **1** and **6** with lithium aluminium hydride gave the corresponding cyclopropyl derivatives, **4** and **7**, respectively.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
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CONVERSION OF BENZYL 2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSIDE INTO THE D-GALACTOSE ANALOG*

PAUL H. GROSS**, FRANÇOISE DU BOIS, AND ROGER W. JEANLOZ

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and the Massachusetts General Hospital, Boston, Massachusetts 02114 (U.S.A.)

(Received January 18th, 1967)

INTRODUCTION

2-Amino-2-deoxy-D-galactose (D-galactosamine), a constituent of a great number of biological products[1], is a compound difficult to prepare, in pure state, by isolation from natural products, and consequently, synthetic procedures of preparation have been devised[2,3]. Recently, conversion of 2-amino-2-deoxy-D-glucose derivatives into 2-amino-2-deoxy-D-galactose derivatives by change of configuration at C-4 of a 2-(benzyloxycarbonyl)amino derivative[4] has been accomplished. Since derivatives of 2-amino-2-deoxy-D-galactose exist in Nature as *N*-acetyl derivatives, change of configuration at C-4 was studied on an *N*-acetyl derivative of 2-amino-2-deoxy-D-glucose. The benzyl α-D-glucoside was selected, because of the ease of removal of the aglycon under mild, hydrogenolytic conditions, and because of the possible use of this method for interconversion of benzyl glycosides of disaccharides that contain 2-amino-2-deoxy-D-glucose. Synthesis of oligosaccharides containing 2-amino-2-deoxy-D-galactose is of importance for further synthesis of gangliosides and of substances possessing blood group-A activity. In addition, the resulting benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[5] is a starting material for synthesis of the D-*galacto* analog of muramic acid [2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose], by analogy with the procedure described by Flowers and Jeanloz[6].

DISCUSSION

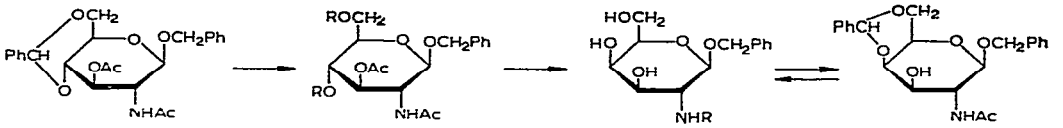
Brendel, Gross, and Zimmerman[4] have shown that the (methylsulfonyl)oxy group at C-4 of benzyl 3-*O*-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-4,6-di-*O*-

*Amino sugars L. This is publication No. 425 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. This investigation was supported by research grants from the National Institute of Allergy and Immunology, National Institutes of Health, United States Public Health Service (Grant AI-04282-03 and 06692-01) and the National Science Foundation (Grant G-2312). A preliminary communication was presented at the Winter Meeting of the American Chemical Society, Phoenix, Arizona, January, 1966.

**Present address: Department of Chemistry, University of the Pacific, Stockton, California 95204.

(methylsulfonyl)-α-D-glucopyranoside can be exchanged by an acetate group, presumably through an S_N2 reaction. The exchange took place with Walden inversion, but without neighboring-group participation, and the resulting benzyl 3,4,6-tri-*O*-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-galactopyranoside was obtained in 53% yield.

In order to apply this procedure to *N*-acetyl derivatives, optically pure benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**2**), obtained[7] from the 4,6-*O*-benzylidene derivative **1**, was transformed into the dimethanesulfonate **3**. Acetolysis of **3** was attempted under anhydrous conditions, as described for the 2-(benzyloxycarboxyl)amino derivative[4], but the yield of galactose derivative obtained was low (10–15%). Addition of water, however, markedly increased the yield. After *O*-deacetylation, benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**4**) was obtained from **3** in 47% yield.



1 **2** R = H; **3** R = Ms **4** R = Ac; **5** R = H·HCl; **6** R = $OCOCH_2Ph$ **7**

In an alternative approach, catalytic hydrogenation of benzyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-galactopyranoside[4,8] (**6**) gave benzyl 2-amino-2-deoxy-α-D-galactopyranoside hydrochloride (**5**), which was *N*-acetylated to give **4**. Treatment of **4** with benzaldehyde gave benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**7**). Compounds **4** and **7** have been obtained[5] directly by glycosidation of 2-amino-2-deoxy-D-galactose. Small differences between the constants previously reported and those reported here may be due to small contamination with the β-D anomer of the products obtained by glycosidation.

EXPERIMENTAL

General.—Melting points were taken on a hot stage equipped with a microscope, and correspond to "corrected melting point". Specific rotations were determined on a Perkin–Elmer polarimeter, Model No. 200; the chloroform used was A. R. grade and contained approximately 0.75% of ethanol. Chromatograms were made with the flowing method on "Silica Gel Davison", from the Davison Co., Baltimore, Maryland (grade 950, 60–200 mesh), which was used without pretreatment. The proportion of weight of substance to weight of absorbent was 1 to 50–100. The proportion of weight of substance (in grams) to volume of fraction of eluent (in milliliters) was 1 to 100. The ratio of diameter to length of the column was 1 to 20. The homogeneity of all compounds, except **5**, was tested by t.l.c. on silica gel G (Merck), with chloroform and a proportion of methanol sufficient to raise the R_F value to

0.2–0.5. Evaporations were conducted *in vacuo*, with the temperature of the outside bath kept below 45°. Amounts of volatile solvent smaller than 20 ml were evaporated under a stream of dry nitrogen. The microanalyses were done by Dr. M. Manser, Zürich, Switzerland.

Benzyl 2-acetamido-3-O-acetyl-2-deoxy-α-D-glucopyranoside (**2**). — Compound **1** (2.2 g, 5 mmoles) was dissolved in glacial acetic acid (50 ml). Water (20 ml) was slowly added, at 90°, to the stirred solution, at such a rate that no permanent precipitation occurred. After 1 h, the solvent was evaporated, and the residue was treated first with water and then with toluene, each treatment being followed by evaporation. The residue, which was dry and free from acetic acid, was obtained in crystalline form by treatment with ether, and it was recrystallized from ethyl acetate–2-isopropoxypropane, to give 1.6 g (90%) of prismatic needles, m.p. 119–121°, $[\alpha]_D^{25}$ + 132° (*c* 1, pyridine).

Anal. Calc. for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.56; N, 3.96. Found: C, 57.68; H, 6.47; N, 4.04.

Benzyl 2-acetamido-3-O-acetyl-4,6-di-O-(methylsulfonyl)-α-D-glucopyranoside (**3**) — A solution of **2** (3.53 g, 10 mmoles) in dry pyridine (20 ml) was cooled to − 10°, with exclusion of moisture. Freshly distilled methanesulfonyl chloride (2 ml) was slowly added, with stirring, in a closed system. The mixture was kept for 10 h at 0°, and then poured onto ice. The resulting precipitate crystallized, and it was filtered off, dried, and recrystallized from 2-propanol–2-isopropoxypropane–ether to give 4.5 g (88%) of fine needles, m.p. 147–8°, $[\alpha]_D^{25}$ + 107° (*c* 1, pyridine).

Anal. Calc. for $C_{19}H_{27}NO_{11}S_2$: C, 44.78; H, 5.34; N, 2.75; S, 12.58. Found: C, 44.91; H, 5.48; N, 2.69; S, 12.54.

Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**4**). — *A. From* **3**. A mixture of compound **3** (1 g, 2 mmoles), glacial acetic acid (7 ml), water (0.36 ml), and dry potassium acetate (7 g) was heated for 24 h at 130–135°. Acetic anhydride (5 ml) was added, and heating was continued for an additional hour. The solution was poured onto a mixture of potassium carbonate (20 g), ice (100 g), and ether (200 ml), and was stirred for 2 h. The ether solution was separated, the aqueous layer was extracted twice with ether, and the ether extracts were washed with water. The ether extracts were combined, dried with sodium sulfate, and evaporated. The residual syrup (750 mg) was dissolved in abs. methanol (20 ml), the solution was treated at − 10° with 0.5M barium methoxide in methanol (1 ml), and kept overnight at 0°. Silica gel "Davison" (10 g) and 40 ml of toluene were added, and the mixture was evaporated *in vacuo*. The residue was added to the top of a 30-cm long column of silica gel "Davison" (50 g), and elution was performed with chloroform containing increasing proportions of methanol. The 9:1 chloroform–methanol mixture eluted some impurities, and the 4:1 mixture eluted 320 mg of product which was recrystallised from 2-propanol–ethyl acetate–ether to give 295 mg (47%) of small, prismatic needles, m.p. 202–3°, $[\alpha]_D^{25}$ + 211° (*c* 1, water). The m.p. of the mixture with the material described in the next paragraph showed no depression. Flowers and Shapiro[5] reported m.p. 207–9°, $[\alpha]_D^{23}$ + 204° (*c* 0.98, water).

B. From **5**. A solution of **5** (0.92 g, 3 mmoles) in water (3 ml) and methanol (30 ml) was treated with Dowex-1 (4.5 mequiv., OH^-) and acetic anhydride (3 ml). After being stirred at room temperature for 1 h, the mixture was filtered through a 10-cm long column of Dowex-1 (9 mequiv., OH^-). The column was washed with 1:1 methanol–water, and the eluate was immediately treated with acetic anhydride (3 ml). After 1 h at room temperature, the solution was filtered again through the same column. The column was washed, and the eluants were evaporated. The residue was dried by addition, followed by evaporation, first of water, and then of ethanol–toluene. It was crystallized from isopropyl alcohol–ethyl acetate–ether, to give 0.87 g (93%), m.p. 208–9°, $[\alpha]_D^{25}$ +230° (*c* 1, water).

Anal. Calc. for $C_{15}H_{21}NO_6$: C, 57.86; H, 6.80; N, 4.50; 0. 30.84. Found: C, 57.67; H, 6.84; N, 4.56; O, 30.91.

C. From **7**. Treatment of **7** with acetic acid–water, under conditions similar to those used for the preparation of **2**, gave a product having m.p. 208–9°, $[\alpha]_D^{25}$ +225° (*c* 1, water), and showing no depression of the m.p. in admixture with the products described above.

Benzyl 2-amino-2-deoxy-α-D-galactopyranoside hydrochloride (**5**). — Compound **6** (2 g, 5 mmoles) was dissolved in methanol (100 ml), conc. hydrochloric acid (0.5 ml) was added, and the solution was hydrogenated in the presence of 10% palladium-on-charcoal catalyst (0.3 g) under a pressure of 3–4 atmospheres; the catalyst was wetted with water before being introduced into the mixture. After 140 min, the catalyst was filtered off on a glass funnel having a fritted disk, the filtrate was evaporated, and the residue was recrystallized from isopropyl alcohol to give 1.2 g (80%) of needles, m.p. 176–8°, $[\alpha]_D^{25}$ +126° (*c* 1, water).

Anal. Calc. for $C_{13}H_{20}ClNO_5$: C, 51.07; H, 6.60; Cl, 11.60; N, 4.58; O, 26.17. Found: C, 51.05; H, 6.60, Cl, 11.70; N, 4.67; O, 26.36.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (**7**). — A mixture of **4** (6.2 g, 20 mmoles, dried for 5 h at 100° *in vacuo*), zinc chloride (5 g), and benzaldehyde (100 ml) was shaken overnight. Ice, 2-isopropoxypropane, and a few ml of dil. ammonium chloride (to prevent precipitation of zinc hydroxide) were added. The precipitate was filtered off, washed with water and a small amount of 2-propanol, and crystallized from acetone–isopropyl alcohol to give a product having m.p. 240–3°, $[\alpha]_D^{25}$ +186° (*c* 1, pyridine). Flowers and Shapiro[5] reported m.p. 246–247°, $[\alpha]_D^{28}$ +219° (*c* 2.12, pyridine).

Anal. Calc. for $C_{22}H_{25}NO_6$: C, 66.16; H, 6.31; N, 3.51; O, 24.03. Found: C, 66.11; H, 6.22; N, 3.60; O, 23.95.

ACKNOWLEDGMENTS

The authors thank Chas. Pfizer and Company for a gift of 2-acetamido-2-deoxy-D-glucose, and Miss Marjorie Rascher for technical assistance.

SUMMARY

Acetolysis of benzyl 2-acetamido-3-*O*-acetyl-4,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside gave, after *O*-deacetylation, benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside in 47% yield.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

NEUARTIGE ISOMERISIERUNGEN VON ZUCKERDERIVATEN

TEIL V[1]. TRENNUNG UND IDENTIFIZIERUNG DER ISOMERISIERUNGSPRODUKTE AUS 1,1,2,3,4,5-HEXA-*O*-ACETYL-6-*O*-*p*-TOLUOLSULFONYL-*al*-D-GALAKTOSE

FRITZ MICHEEL UND ERICH MATZKE
Organisch-Chemisches Institut der Universität Münster/Westf. (Deutschland)

(Eingegangen den 9. Dezember, 1966)

In der III. Mitteilung[2] konnten F. Micheel und R. Böhm mit Hilfe chromatographischer Verfahren zeigen, daß bei der Isomerisierung von 2,3,4,5-Tetra-*O*-acetyl-6-*O*-*p*-toluolsulfonyl-*al*-D-galaktose mit Zinkchlorid und Essigsäureanhydrid neben Hepta-*O*-acetyl-*al*-D,L-galaktose als Hauptprodukt wahrscheinlich die Hepta-acetate aller möglichen isomeren Aldosehexosen gebildet werden: durch Chromatographie an Cellulose-Säulen wurden mehrere Fraktionen gewonnen, deren R_F-Werte denen von Glucose and Allose, bzw. Gulose und Mannose, bzw. Altrose, Talose und Idose entsprachen. Darüber hinaus gelang es, Glucose durch Vergärung und Mannose durch Bildung des schwerlöslichen Mannosephenylhydrazons nachzuweisen. Der Mechanismus dieser Isomerisierungsreaktion wurde mit Hilfe von D-Galaktose-*1*-^{14}C aufgeklärt[3].

Um auch die Bildung der übrigen Hexosen präparativ nachzuweisen, haben wir die Isomerisierung von 1,1,2,3,4,5-Hexa-*O*-acetyl-6-*O*-*p*-toluolsulfonyl-*al*-D-galaktose, die nach F. Micheel und R. Böhm als Zwischenprodukt bei der Isomerisierung von 2,3,4,5-Tetra-*O*-acetyl-6-*O*-*p*-toluolsulfonyl-*al*-D-galaktose entsteht, genauer untersucht. Hierbei wurde gegenüber der früheren Vorschrift[2] die doppelte Menge Essigsäureanhydrid angewandt. Dadurch gelang es, die Bildung von 1,1,2,3,4,5-Hexa-*O*-acetyl-6-chlor-6-desoxy-*al*-D-galaktose, die ebenfalls als Nebenprodukt auftritt, von 42 auf 17% herabzudrücken. Durch Chromatographie des Isomerisierungsgemisches an Kieselgel-Säulen mit Cyclohexan–Benzol–Pyridin (10:9:1) gelang es, das Gemisch der Hexose-*al*-acetate, der 6-Chlor-Verbindung und der weiteren Isomerisierungsprodukte mit einer Ausbeute von 44% als farblosen Sirup abzutrennen. Durch Verseifung dieses Gemisches der Hepta-*O*-acetyl-*al*-hexosen mit Natriummethylat[4] wurde das Gemisch der freien isomeren Hexosen in einer Ausbeute von *ca.* 90% erhalten. Diese wurden an Cellulose-Säulen mit 1-Butanol–Pyridin–Wasser (3:1:1) aufgetrennt. Eine Übersicht über die Trennung gibt die folgende Tabelle I.

Bei einer genaueren Untersuchung der so erhaltenen Fraktionen ließ sich die Fraktion *d* in mehrere Komponenten zerlegen: durch Chromatographie an Cellulose-Säulen mit wassergesättigtem Butanon[5] wurden zunächst zwei Hexosen erhalten (d_1 und d_2), deren R_F-Werte identisch sind mit denen von Altrose und Talose. Durch Wiederholung dieser Trennung mit einer größeren Menge der Substanz *d* ließ sich mit sehr geringer Ausbeute eine weitere Komponente (d_3) isolieren, deren R_F-Wert identisch

ist mit dem von Idose. Eine weitere Auftrennung der Fraktionen *b* und *c* gelang dagegen nicht.

TABELLE I

SÄULENCHROMATOGRAPHISCHE TRENNUNG VON 152.8 MG ISOMERISIERUNGSGEMISCH AUS 469 MG DER HEPTA-*O*-ACETYL-*al*-HEXOSEN

Fraktion (Bezeichnung)	R_{Man}[a]	*Menge (mg)*	$[\alpha]_D$,°
e	1.95	22.8	+72
d	1.21	18.8	+11
d + *c*	1.20/1.00	2.6	
c	1.00	17.2	+ 9
c + *b*	1.00/0.88	1.2	
b	0.88	6.3	± 0
b + *a*	0.88/0.70	3.6	
a	0.70	59.5	−21
		132.0 (86%)	

[a] R_{Man}: R_F-Werte der Hexosen bezogen auf R_F (Mannose).

Zur Identifizierung der so erhaltenen Hexosen ist deren Reaktion mit Phenylhydrazin-hydrochlorid und Natriumacetat in wässriger Lösung besonders geeignet. Die dabei entstehenden Phenylosazone liefern charakteristische IR-Spektren[6]. Darüber hinaus lassen sie sich als Boratkomplexe auf Grund ihrer z.T. stark verschiedenen R_F-Werte papierchromatographisch gut charakterisieren[7]: die Fraktion *e* liefert mit Phenylhydrazin farbloses kristallines 6-Chlor-6-desoxy-D-galaktose-phenylhydrazon. Bei der Fraktion d_3 konnte es sich nach dem chromatographischen Befund nur um Idose handeln. Ihr amorphes Reaktionsprodukt mit Phenylhydrazin verhält sich chromatographisch wie L-Sorbose-phenylosazon. Aus dem Drehwert von d_3 (+ 33°) folgt, daß zu 81% die L-Form der Idose vorliegt.

Die optisch inaktive, nach dem Chromatogramm aus D,L-Talose bestehende Fraktion d_2 bildet ein Osazon, dessen IR-Spektrum identisch ist mit dem eines Osazons, das aus der Fraktion *a* des Hexose-Gemisches gewonnen wurde. Diese Fraktion enthält aber nur D- und L-Galaktose. Darüber hinaus stimmt das Methylphenylhydrazon von d_2 überein mit authentischem Talose-methylphenylhydrazon[8].

Die Fraktion d_1, die vorwiegend aus D-Altrose besteht, liefert das Osazon nach mehrfachem Umkristallisieren aus Äthanol–Wasser als schwer lösliche D,L-Verbindung. Das IR-Spektrum ist identisch mit einem aus der Fraktion *b* gewonnenen Osazon, das aus Allose stammt. Das IR-Spektrum des Benzylphenylhydrazons von d_1 stimmt mit dem entsprechenden D-Altrose-Derivat überein[9]. Damit ist bewiesen, daß die Fraktion d_1 aus Altrose besteht. Aus den spez. Drehwerten von d_1 und D-Altrose (+ 16 bzw. + 35°) ergibt sich der Gehalt an D-Altrose zu 74%.

Die Fraktion *c* liefert bereits bei Raumtemperatur farbloses Mannosephenylhydrazon. Aus der Mutterlauge entsteht beim Erhitzen ein Osazongemisch, das

vermutlich aus Gulose-phenylosazon und Mannose-phenylosazon besteht. Versuche, diese Osazone durch fraktionierte Kristallisation aus verschiedenen Lösungsmitteln zu trennen, schlugen fehl. Im Phenylhydrazon der Fraktion *c* liegt, wie aus dem spez. Drehwert (+ 17.8°) und dem entsprechenden Wert des D-Mannose-phenylhydrazons (+ 26.6°) folgt, zu 83% das Derivat der D-Form vor.

Die optisch inaktive Fraktion *b* liefert ein Osazon, das im Chromatogramm zwei Komponenten zeigt. Danach liegt ein Gemisch von etwa gleichen Mengen Allose-phenylosazon und Glucose-phenylosazon vor. Durch Umkristallisieren aus Aceton und Pyridin–Wasser konnte reines Glucose-phenylosazon als kristalline D,L-Form erhalten werden. Aus der Mutterlauge konnte durch Behandeln mit Äthanol–Wasser Allose-phenylosazon isoliert werden, das nur noch eine Spur Glucose-phenylosazon enthielt. Die Fraktion *b* enthält also etwa gleiche Mengen von D- und L-Glucose sowie von D- und L-Allose.

Die Fraktion *a* war shon durch die Arbeiten von F. Micheel und R. Böhm[2] als Gemisch von D- und L-Galaktose identifiziert worden. Durch das IR-Spektrum und das Chromatogramm ihres Osazons wurde dieses Ergebnis bestätigt.

Insgesamt ergibt sich folgende Zusammensetzung des Reaktionsgemisches bei der totalen Isomerisierung von 1,1,2,3,4,5-Hexa-*O*-acetyl-6-*O*-*p*-toluolsulfonyl-*al*-D-galaktose: den Hauptanteil macht mit 30% L-Galaktose aus. D-Galaktose und 6-Chlor-6-desoxy-D-galaktose werden zu je 17% erhalten. In beträchtlichen Mengen entstehen außerdem D-Mannose und D-Altrose (11 bzw. 7%), während L-Mannose, L-Altrose sowie die D- und L-Formen der Talose, Glucose and Allose zu je 1–3% vorkommen.

BESCHREIBUNG DER VERSUCHE

Isomerisierung von 1,1,2,3,4,5-Hexa-O-*acetyl-6*-O-p-*toluolsulfonyl*-al-D-*galaktose*. — Die Lösung von 1.5 g wasserfreiem Zinkchlorid und 6 g rohem D-Galaktose-Derivat in 180 cm³ Essigsäureanhydrid wird 7 Stdn. unter Ausschluß von Feuchtigkeit auf dem Dampfbad erhitzt. Das ausgeschiedene Zink-*p*-toluolsulfonat (1.8 g, 89%) wird abgesaugt und das Filtrat in 2 l Eiswasser eingerührt. Nach 12 Stunden wird mit Chloroform extrahiert und die Chloroformphase wie üblich gereinigt. Nach dem Trocknen mit Natriumsulfat wird im Vak. zur Trockne gedampft: 5.5 g dunkelbraune amorphe Substanz.

Reinigung des Isomerisierungsproduktes. — Das Rohprodukt (10.9 g), gewonnen in zwei Ansätzen aus 12 g D-Galaktose-Derivat, wird durch Chromatographie an einer Kieselgelsäule (40 × 3.5 cm) mit Cyclohexan–Benzol–Pyridin (10:9:1) gereinigt. Das Eluat wird durch Dünnschichtchromatographie an Kieselgel mit dem gleichen Laufmittel untersucht. Die Fraktionen mit R_F-Werten zwischen 0.18 und 0.27 werden vereinigt und ergeben 4.3 g eines farblosen Sirups (44%).

Verseifung des Isomerisierungsproduktes. — Die Lösung von 4.3 g gereinigten Isomerisierungsproduktes in 200 cm³ abs. Methanol wird mit 11 cm³ N-Natriummethylat-Lösung versetzt und über Nacht bei Raumtemperatur aufbewahrt. Zur Ent-

fernung der Salze wird mit 10 g Amberlite IR-120 geschüttelt, filtriert und im Vak. eingedampft. Der Rückstand wird im Wasser aufgenommen, durch Schütteln mit Aktivkohle entfärbt und erneut eingedampft. 1.4 g (89%) farbl. Sirup.

Trennung des Hexosegemisches. — Das Verseifungsprodukt (152.8 mg) wird an einer Cellulosesäule (55 × 3.5 cm) mit 1-Butanol–Pyridin–Wasser (3:1:1) getrennt. Es werden Fraktionen von je 20 ccm aufgefangen. Die einzelnen Fraktionen werden chromatographisch untersucht (absteigende Papierchromatographie an Whatman Nr. 1-Papier, 1-Butanol–Pyridin–Wasser (3:1:1). Nach der Aufarbeitung ergibt sich die in Tab. 1 dargestellte Verteilung.

Trennung der Fraktion d. — Ein Gemisch (1.096 g) der Fraktionen *c* und *d* wird an einer Cellulose-Säule mit Butanon–Wasser (gesättigt) getrennt. Es ergibt sich folgende Verteilung:

Fraktion (Bezeichnung)	d_3	d_2	$d_2 + d_1$	d_1	$d_1 + c$
Menge (mg)	11	147	98	258	189

Umsetzung der Hexosen mit Phenylhydrazin. — Alle Osazone wurden nach der Vorschrift von E. Fischer[10] mit Phenylhydrazin-hydrochlorid und Natriumacetat dargestellt. Zu Vergleichszwecken wurden die Osazone von Glucose, Sorbose, Galaktose und Altrose dargestellt. Die IR-Spektren sind identisch mit denen von W. Otting[6].

(*a*) Osazon der Fraktion d_3 (Idose): aus 11 mg d_3 entsteht ein amorphes Produkt, das in heißer Methylcellosolve gelöst und bis zur Trübung mit Wasser versetzt wurde. Ausb. 1.6 mg einer gefärbten Substanz.

(*b*) Osazon der Fraktion d_2 (Talose): Ausb. 47 mg (47% aus 50 mg d_2). Es wird aus Äthanol–Wasser, Methylcellosolve–Wasser und Äthanol umkristallisiert. Schmp. 196°.

(*c*) Osazon der Fraktion d_1 (Altrose): Ausb. 28 mg (34% aus 42 mg d_1). Es wird zweimal aus Äthanol–Wasser umkristallisiert. Schmp. 169°.

(*d*) Umsetzung der Fraktion *c* (Mannose + Glucose) mit Phenylhydrazin: es kristallisiert sofort Mannose-phenylhydrazon. Ausb. 53 mg (40%). Schmp. 176°; $[\alpha]_D$ + 17.8° (*c* 1, Pyridin). Nach dem Umkristallisieren aus Äthanol: Schmp. 184°.

Die Mutterlauge liefert nach zweistündigem Erhitzen einen gelben Niederschlag (14.5 mg, Schmp. 175°), der weder mit Mannose-phenylosazon noch Gulose-phenylosazon übereinstimmt. Nach der Extraktion mit Aceton wird der Rückstand aus Methylcellosolve–Wasser und Pyridin–Wasser umkristallisiert. Ausb. 3.6 mg; Schmp. 182–187°. Der IR-spektroskopische Vergleich zeigt, daß es sich wahrscheinlich um Mannose-phenylosazon handelt, das durch Gulose-phenylosazon verunreinigt ist.

(*e*) Osazone der Fraktion *b* (Gulose + Allose): Ausb. 26 mg (9% aus 140 mg *b*). Das Gemisch wird in heißem Aceton gelöst. Beim Erkalten kristallisiert Glucose-phenylosazon aus. Es wird zweimal aus Pyridin–Wasser umkristallisiert. Ausb. 5.1 mg. Schmp. 199°. Aus der Aceton-Lösung wird nach dem Eindampfen und zweimaligen Umkristallisieren aus Äthanol–Wasser 1.0 mg Allose-phenyl-osazon gewonnen. Schmp. 168°.

(*f*) Osazon der Fraktion *a* (Galaktose): Ausb. 13 mg (24% aus 27 mg *a*). Nach dem Umkristallisieren aus Äthanol: Schmp. 198°.

Talose-methylphenylhydrazon. — Darstellung nach *loc. cit.* 8. Ausb: (*1*) 2 mg aus 10 mg D-Talose; Schmp. 150°; (*2*) 3.6 mg aus 18.6 mg d_2; Schmp. 151°.

Altrose-benzylphenylhydrazon. — Darstellung nach *loc. cit.* 9. Ausb.: (*1*) 23.2 mg aus 36.1 mg D-Altrose; Schmp. 149° (*2*) 12 mg aus 23.7 mg d_1; Schmp. 146°.

Chromatographie der Osazone. — Abweichend von der Vorschrift von Arreguín[7] wurde Whatman-Nr. 1-Papier benutzt, das mit Borax-Lösung imprägniert wurde. Während mit dem von Arreguín angegebenen Laufmittel Dioxan–*n*-Hexan–Wasser–Benzol (4:5:4:2) bei einer Laufzeit von 48 Stdn. noch keine Trennung von Sorbose-phenylosazon, Glucose-phenylosazon und Galaktose-phenylosazon erreicht wurde, ließen sich nach Änderung des Mischungsverhältnisses auf (11:10:5:4) Altrose-phenylosazon und Galaktose-phenylosazon voneinander und von Glucose-phenylosazon und Sorbose-phenylosazon nicht trennen.

DANK

Wir danken dem Fonds der Chemie sowie dem Landesamt für Forschung (NRW) für Mittel, die bei dieser Arbeit Verwendung fanden.

ZUSAMMENFASSUNG

Aus dem Gemisch der isomeren Hexosen, die sich bei der kationischen Isomerisierung von 1,1,2,3,4,5-Hexa-*O*-acetyl-6-*O*-*p*-toluolsulfonyl-*al*-D-galaktose bilden, lassen sich auf Grund des chromatographischen und IR-spektroskopischen Verhaltens ihrer Hydrazone bzw. Osazone zahlreiche Hexosen identifizieren: die D- und L-Formen von Galaktose, Mannose, Altrose, Talose, Gulose und Allose. Idose und Gulose können in Spuren nachgewiesen werden.

SUMMARY

On the basis of the chromatographic behaviour and infrared spectra of the derived hydrazones and osazones, the D- and L-forms of galactose, mannose, altrose, talose, gulose and allose have been identified in the mixture of isomeric hexoses obtained by cationic isomerisation of 1,1,2,3,4,5-hexa-*O*-acetyl-6-*O*-toluene-*p*-sulphonyl-*aldehydo*-D-galactose. Idose and gulose were present in trace proportions.

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Notes

Condensation of nitroethanol and D-ribose 5-phosphate A novel route to sedoheptulose 7-phosphate

The base-catalyzed condensation of 2-nitroethanol with aldoses in methanol has been applied with some success as a key step in the synthesis of several ketoses[1]. The first chemical synthesis of D-*altro*-heptulose 7-phosphate (sedoheptulose 7-phosphate) by an analogous method, in aqueous solution, was described in an earlier communication[2]. This compound, which is unavailable commercially, is a very important metabolite[3], and has been prepared by lengthy, enzymic methods[4,5] and isolated in a partially purified state. In the earlier report, C, H, and P analyses of the sedoheptulose 7-phosphate were compatible with a monohydrated structure[2]. However, a subsequent barium analysis of 27.37% was low. We now describe the preparation of a sedoheptulose 7-phosphate which gives a correct analysis for the nonhydrated structure, and describe further characteristics of the synthesis. The latter is significant, because of the likelihood that the procedure will open a new synthetic route to rare or unknown ketose phosphates.

EXPERIMENTAL AND RESULTS

Product analysis. — Orcinol assay[6] was used as a rapid, semiquantitative probe for heptulose phosphate. An enzymic assay for sedoheptulose 7-phosphate was also employed[7]; with the exception of transaldolase, all of the enzymes required for this analysis are available from CalBiochem, Los Angeles, California. We thank Dr. B. L. Horecker for a generous gift of transaldolase.

Kinetics of the reaction. — A reaction was conducted exactly as described earlier[2], but on one-tenth of the scale. Over a 24-h period, several small aliquots were removed, and centrifuged to remove a small amount of white solid. As the reaction progressed, the amount of this white solid diminished. After centrifugation, which required about 5 min, a 0.25-ml aliquot of the supernatant liquor was treated with 2.5 ml of cold, reagent-grade acetone. After 70 min, the precipitated salts were collected by centrifugation, and the pellet was treated with 0.18 ml of cold, 4N hydrobromic acid. Part of each solution was analyzed by the orcinol method. The remainder was stored (−20°), and later neutralized and diluted to 5.0 ml. Aliquots were then assayed by the standard enzymic procedure. The results are shown in Figure 1, from which it is evident that the yield of sedoheptulose 7-phosphate decreases with time. Although the data are not shown, orcinol analyses revealed no decrease with time in the total yield of heptulose phosphate.

Preparation of sedoheptulose 7-phosphate. — Barium D-ribose 5-phosphate

trihydrate (6.05 g; Nutritional Biochemicals Corp.) was dissolved in 130 ml of cold 0.01N hydrobromic acid, the solution was treated with 66 ml of cold 0.52N sodium sulfate (17.2 mmoles), and the barium sulfate was removed by centrifugation. The precipitate was washed once with 12 ml of cold 0.01N hydrobromic acid and the

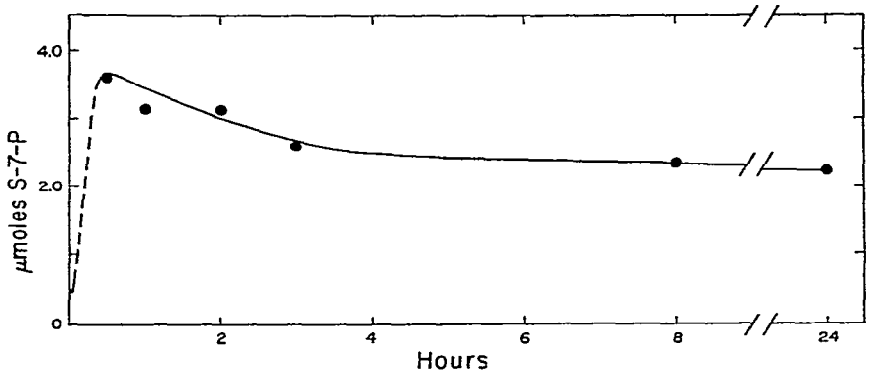


Fig. 1. Course of the synthesis of sedoheptulose 7-phosphate (S-7-P).

washing was combined with the original, supernatant liquor. At this stage, orcinol assay revealed the presence of 11.1 mmoles of the sodium salt of a pentose derivative, presumably, the sodium salt of D-ribose 5-phosphate. Cold N sodium hydroxide (66 mequivalents) and water were then added to bring the total volume to 380 ml, and cold nitroethanol (Columbia Organic Chemicals Co., 13.5 ml) was added dropwise, with stirring at 0°. After 50 min, the solution was centrifuged in glass cups, for 10 min at 1500 *g*, and the supernatant liquor was immediately treated with 10 volumes of cold acetone.

Earlier results, based on orcinol assay, had suggested that cold, 4N hydrobromic acid was superior to cold sulfuric acid for the desired decomposition of the intermediate salts (N. W. Rokke, unpublished observation). To obtain additional, quantitative data, the following experiment was performed. One h after precipitation, six 25-ml portions of the suspension in acetone were removed, and centrifuged at 2° to collect the precipitated salts. To each precipitate, 3.5 ml of the specified concentration of either cold sulfuric acid or cold hydrobromic acid was added dropwise, to compare these acids as decomposing agents. The results (see Table I) confirmed the suitability of 4N hydrobromic acid for the decomposition. At the highest concentration of both acids, the resultant solutions were discolored, suggesting extensive decomposition to undesired products.

The remainder of the product was collected from the acetone-treated reaction mixture (*ca.* 4.2 l) in two batches, by centrifuging for 10 min at 4500 *g* (2°) in a Servall HG-4 rotor having polypropylene cups. The supernatant liquor was decanted from the thick, syrupy, amorphous salts, and, to the salts, 525 ml of cold 4N hydrobromic acid was slowly added. Evolution of nitrous oxide occurred. Barium bromide dihydrate (6 g, 18 mmoles) was then added to the solution, and a small amount of precipitate was removed by filtration at 0°. The pH of the ice-cold filtrate was

adjusted to 7.7 by adding solid lithium hydroxide, and the barium salts were precipitated by adding 5 volumes of cold ethanol. After 1 h, the precipitate was collected by centrifugation with the HG-4 rotor. Most of the alcohol and water in the salts was then removed *in vacuo* at 25° over sulfuric acid as the desiccant. The fairly dry residue was dissolved in 100 ml of 0.01N hydrobromic acid, and 26 mmoles of sodium sulfate was added. The precipitated barium sulfate was washed, and the washing plus solution was subjected to column chromatography.

A column of Dowex-1 (X8, Cl^-, 200–400 mesh) resin (5 × 84 cm) was converted into the formate form by treatment with 2N sodium formate preadjusted to pH 4.1 with formic acid, and was then washed with deionized water.

The sample was loaded on the column, and was eluted at room temperature, by a gradient process, with 6 l of 0.04N formic acid containing 0.1N sodium formate (pH 4.1) and 6 l of water in a mixing bottle. The elution rate was maintained at 1.2–1.9 ml/min and 22-ml fractions were collected. Analysis by orcinol suggested the occurrence of fractionation into two major, overlapping peaks containing heptulose phosphate, eluted in the range of 9.46–11.62 l. D-Ribose phosphate did not begin to emerge until *ca.* 12 l of effluent had been collected. The fractions containing heptulose phosphate were pooled into: fraction *a*, 9.46–10.25 l (leading peak); fraction *b*, 10.25–10.79 l (overlapping zone); and fraction *c*, 10.79–11.62 l (second peak). Barium bromide dihydrate (3 g) was added to each fraction, and the pH was adjusted to 7.7 with solid lithium hydroxide. Ethanol was added at room temperature until very slight turbidity appeared (*ca.* 1.5 vol of ethanol). In the hope of crystallizing the products the light suspensions were stored at −20°. More precipitate appeared in each fraction, but in all cases it was amorphous. The precipitates were collected, and dried *in vacuo* overnight at 25° over sulfuric acid.

TABLE I

EFFECT OF COLD MINERAL ACIDS ON THE DECOMPOSITION OF NITROALCOHOL SALT TO SEDOHEPTULOSE 7-PHOSPHATE

Sulfuric acid	*Sedoheptulose 7-phosphate (μmoles) in aliquot analyzed*[a]	*Hydrobromic acid*	*Sedoheptulose 7-phosphate (μmoles) in aliquot analyzed*[a]
16N	0.0125	8.4N	0.0000
8N	0.0295	6.7N	0.0322
4N	0.0264	1.7N	0.0311

[a]After treatment with acid, a 0.2-ml aliquot was diluted, neutralized with NaOH, and the volume brought to 5.0 ml with water. An aliquot (0.25 ml) was then analyzed by the enzyme assay described.

Characterization of sedoheptulose 7-phosphate. — Table II presents evidence that fraction *c* contained sedoheptulose 7-phosphate, as would be expected[2]. After treatment with orcinol[6] and cysteine–sulfuric acid[8], the product, which was of high purity, gave molar extinction coefficients identical with those given by a sample of sedoheptulosan monohydrate kindly furnished by Dr. N. K. Richtmyer. For the

TABLE II

ELEMENTAL AND ENZYMIC ANALYSES OF PRODUCTS

Fraction	*Yield of heptulose phosphate*[a], *mg*	*Sedoheptulose 7-phosphate by transaldolase assay, %*	*Elemental analysis*[b] *%C*	*%H*	*%Ba*	*%P*
a	460 (10%)	0				
b	441 (9%)	17				
c	474 (10%)	95	19.45	2.95	32.16	7.09

[a]Percentage yields are based on the content of D-ribose 5-(sodium phosphate) in the reaction mixture, as assayed with orcinol. This assay is nonspecific and, consequently, apparent yields are minimal. [b]The theoretical values for heptulose monophosphate are C, 19.78; H, 3.06; Ba, 32.10; P, 7.28.

cysteine–sulfuric acid treatment, Beer's law is obeyed at fairly low concentrations ($< 2.5 \times 10^{-4}$M) of the sugar phosphate.

Figure 2 shows the optical rotary dispersion spectrum of sedoheptulose 7-phosphate dissolved in 0.3N hydrobromic acid. The specific rotation $[\alpha]^{22}_{5890}$ was +4.65°

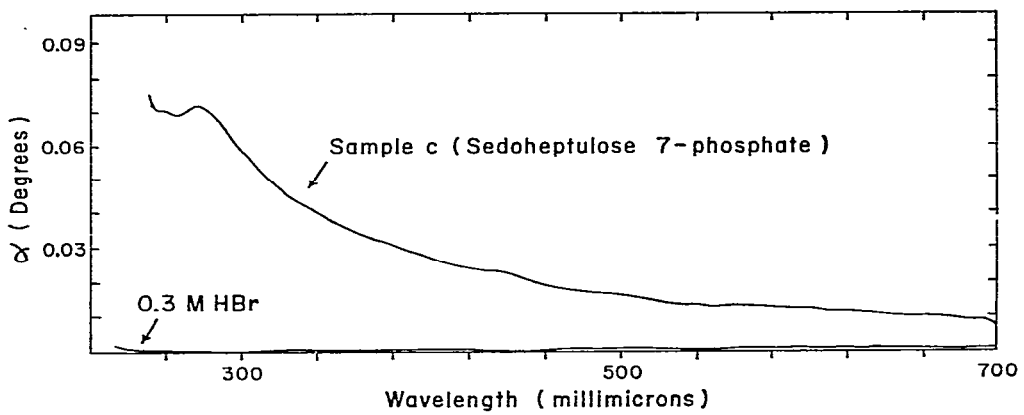


Fig. 2. Optical rotatory dispersion spectrum of sedoheptulose 7-phosphate (Ba salt) obtained with a Durrum–Jasco ORD/UV-5 optical rotatory dispersion spectropolarimeter at 22°; (*c* 2.58, 0.3N hydrobromic acid; *l* = 1 cm).

DISCUSSION

The condensation of 2-nitroethanol with D-ribose 5-phosphate occurs rapidly in the presence of 0.17M alkali in the cold. The intermediate nitroalcohol salts may be readily isolated in crude form, and smoothly decomposed to the heptulose phosphates with cold hydrobromic acid. In addition to smoother decomposition, use of hydrobromic acid should have another advantage over sulfuric acid in syntheses of sugar phosphates by this route because most bromides are quite soluble in aqueous alcohol or aqueous acetone. In contrast, in the presence of a large excess of sulfate ion, the alkaline-earth salts of sugar phosphates cannot be selectively precipitated prior to chromatography. To increase the sugar phosphate/anion ratio and ensure good chromatographic separation on the anion exchanger, precipitation is essential.

Besides sedoheptulose 7-phosphate, one of the major products (fraction *a*) is probably the epimer, D-*allo*-heptulose 7-phosphate[2], as would be expected. As the condensation time is increased, the yield of sedoheptulose 7-phosphate decreases, but the yield of total heptulose phosphate remains constant; this may reflect reversal of the condensation, and base-catalyzed isomerization of D-ribose 5-phosphate, analogous to that which occurs with free sugars under mild conditions[9]. Such isomerization might result in the ultimate production of isomeric heptulose phosphates. Indeed, three peaks containing heptulose phosphate were observed after a considerably longer condensation-time[2]. It seems unlikely that cold, dilute alkali catalyzes migration of the phosphate group[10]. However, acid-catalyzed rearrangements are known to occur[10], emphasizing the importance in the present method of avoiding prolonged storage after generation of the products with cold hydrobromic acid.

In the earlier work[2], where a condensation time of 24 h was used, a minimum yield of 28% of sedoheptulose 7-phosphate was obtained in the crude mixture prior to chromatography. In the present work, the final product, which was essentially pure, was recovered in a yield of approximately 10%. However, in the present work, crystallization of products was attempted. The addition of more alcohol during the final precipitation might have increased the recovery considerably. It seems likely that the present method should routinely result in 10–25% yields of sedoheptulose 7-phosphate of high purity. The biological methods available[4,5], which give comparable yields, are relatively lengthy, although separation of the sedoheptulose 7-phosphate takes less time.

The availability of essentially pure sedoheptulose 7-phosphate permitted determination of its specific rotation, and of molar extinction coefficients after treatment with orcinol or cysteine–sulfuric acid. The extinction coefficients are identical with those for sedoheptulosan monohydrate, proving that the sugar is a reliable standard for the sugar phosphate.

ACKNOWLEDGMENTS

This work was supported, in part, by the Medical and Biological Research Fund of Washington State University and by Research Grants RG-5904 and GM-09039 from the National Institutes of Health. B. A. M. is a Research Career Development Awardee (1-K3-A1-5268) of the National Institutes of Health.

This manuscript was completed, and partially typed, at the Department of Microbiology, University of Illinois. The senior author thanks L. L. Campbell for the many courtesies then extended to him.

Department of Chemistry,
Washington State University,
Pullman, Washington (U. S. A.)

B. A. McFADDEN
L. L. BARDEN
N. W. ROKKE*
M. UYEDA
T. J. SIEK**

*Present address: Department of Chemistry, Purdue University, Lafayette, Indiana.
**Present address: Department of Food Science, Oregon State University, Corvallis, Oregon.

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(Received October 3rd, 1966; in revised form, December 27th, 1966)

Carbohyd. Res., 4 (1967) 254–259

Confirmation of a cellulose-like structure for the glucan sulphate of *Buccinum undatum* L.

Hunt and Jevons[1] recently described the isolation and characterization of a glucan sulphate from the hypobranchial mucin of the whelk *Buccinum undatum* L. The main glycosidic linkages of this mucopolysaccharide were thought to be β-D-(1→4). The polysaccharide would thus be similar to cellulose were it not for the sulphation of the glucose residues. Evidence for this came from studies of the desulphated polysaccharide by infrared spectroscopy, partial hydrolysis with acid, and enzymic degradation. In the present study by X-ray diffraction, further weight has been added to the earlier deductions of a cellulose-like structure for the desulphated glucan.

For purposes of comparison, X-ray diffraction patterns of both the desulphated polysaccharide and of a powdered viscose rayon were prepared. Preliminary examination of the polysaccharide showed the presence of spurious, sharp diffractions possibly arising from a contaminant produced during the desulphation process. Treatment of the polysaccharide with 10% EDTA for 36 h eliminated most of these additional diffractions. The X-ray diagrams are shown in Fig. 1.

Rings 1 and 3, which are nearly the same in both diffraction diagrams, are reflections due to the regular, side-to-side packing of cellulose chains, *i.e.*, *101* and *002*,

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For purposes of comparison, X-ray diffraction patterns of both the desulphated polysaccharide and of a powdered viscose rayon were prepared. Preliminary examination of the polysaccharide showed the presence of spurious, sharp diffractions possibly arising from a contaminant produced during the desulphation process. Treatment of the polysaccharide with 10% EDTA for 36 h eliminated most of these additional diffractions. The X-ray diagrams are shown in Fig. 1.

Rings 1 and 3, which are nearly the same in both diffraction diagrams, are reflections due to the regular, side-to-side packing of cellulose chains, *i.e.*, *101* and *002*,

respectively. Ring 2 consists of two main contributions; one of these arises from the side-to-side packing only, *10$\bar{1}$*, whereas the other is *021* having a component due to chain-length repeats. In contrast to rings 1 and 3, ring 2 appears stronger in the viscose diagram *A*; this indicates a greater number of planes giving rise to the *021* reflection,

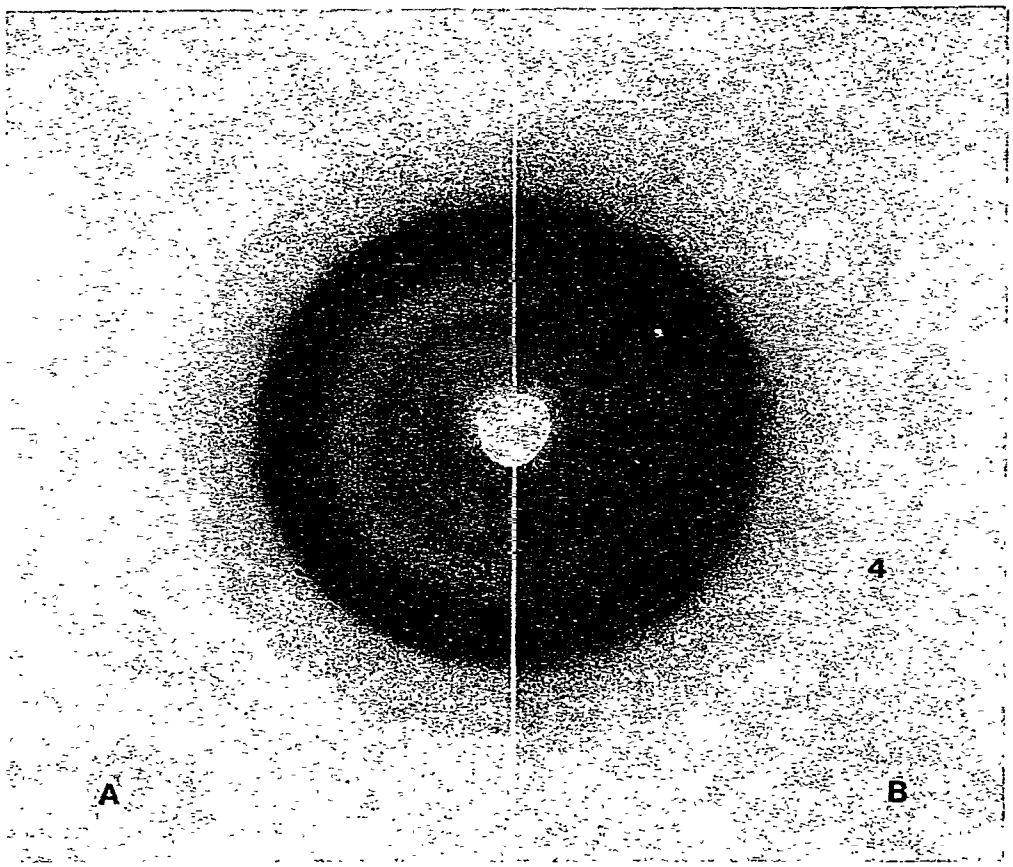


Fig. 1. X-ray powder diagrams of *A*, viscose rayon; *B*, desulphated polysaccharide from hypobranchial mucin of *Buccinum undatum*.

and hence a greater chain-length. Outer lines in the diagrams are largely influenced by chain-length characteristics, and are much less well-defined in the desulphated polysaccharide *B*. However, in the case of ring 4, there are contributions from different planes, some of which [(*312*), (*013*), (*311*)] lie more nearly parallel to the chain axis.

The lack of definition in chain-length diffractions may arise in part from occasional branching or occasional (1→3)-linkage[1]. The absorption at 790 cm^{-1} in the infrared spectrum of the desulphated polysaccharide may be associated with such branching, as may also the insolubility in cuprammonium solution[1]. The apparent, short length of extended chains in crystalline regions may be due to extensive folding or other changes in conformation, since, unlike the viscose rayon used for comparison in *A*, the polysaccharide chains in *B* would not be drawn out during preparation. Nevertheless, in thin flakes of the desulphated polysaccharide precipitate, there are conspicuous, positively birefringent, fibrous regions, and preliminary X-ray tests on these show that typical fibre diagrams should be obtained. Detailed comparisons of oriented viscose and oriented polysaccharide seem possible, and would provide better information about the chain-length characteristics in the latter.

However, the diffraction diagrams demonstrate that the dominant structure is that of hydrated or regenerated cellulose, *i.e.*[2], cellulose II. This confirmation of a cellulose-like structure raises questions concerning the biosynthesis of the polysaccharide sulphate. As β-(1→4)-linked polymers of D-glucose become increasingly insoluble with increasing chain-length, it seems likely that the sulphation precedes, or closely accompanies, formation of the glycosidic bonds.

Department of Biological Chemistry, S. HUNT
University of Manchester,
Manchester (Great Britain)
The Astbury Department of Biophysics, K. M. RUDALL
The University,
Leeds 2 (Great Britain)

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(Received November 26th, 1966)

Benzyl β-L-arabinopyranoside

The directions recommended[1] in *Methods in Carbohydrate Chemistry* for the preparation of benzyl β-D-arabinopyranoside consist of shaking overnight at room temperature a mixture of D-arabinose (1 part) and benzyl alcohol (5 parts) that has been saturated with hydrogen chloride. The product is said to crystallise in the reaction mixture, being isolated in 91% yield after dilution with ether. In our hands, however, the product (from the L-sugar) repeatedly failed to crystallise under these conditions, and we have now shown that the best yields (*ca.* 78%) are obtained with smaller concentrations (*ca.* 50% saturation) of hydrogen chloride. The effects of variation of concentration of hydrogen chloride may be seen in Table I. The high yields recorded

TABLE I

THE EFFECT OF CONCENTRATION OF HYDROGEN CHLORIDE ON THE YIELD OF BENZYL β-L-ARABINOPYRANOSIDE

Expt.[a]	*BA*[b] *(ml)*	*BA satd. (18°)*[c] *with HCl (ml)*	*Yield (%)*[d]	*M.p.*[e] (°)
1	–	10	9 (23)	161–166
2	2	8	40 (49)	169–171
3	3	7	68 (79)	171.5–172.5
4	4	6	68 (86)	168.5–170
5	5	5	78 (89)	171.5–172
6	6	4	73 (82)	171–171.5
7	8	2	30 (52)	164–167

[a]Each experiment was carried out on L-arabinose (2 g) for 24 h at 18°. Crystalline product formed except in Expt. 1. [b]Benzyl alcohol. [c]Benzyl alcohol increases in volume by about 16% on saturation with hydrogen chloride at 18°. [d]Yield of crude product in parentheses. [e]Benzyl β-L-arabinoside has m.p. 172–173° (corr.), $[\alpha]_D^{20}$ +215.2° (*c* 1, water)[4].

in the literature[1–3] are probably attributable to the difficulty (in a large-scale preparation) of effectively saturating the viscous mixture of arabinose and benzyl alcohol.

EXPERIMENTAL

Melting points are not corrected.

Benzyl β-L-arabinopyranoside. — Benzyl alcohol (125 ml, freshly distilled) was saturated (during cooling) with dry hydrogen chloride, allowed to attain 18°, and then added to a mixture of L-arabinose (50 g) and benzyl alcohol (125 ml). The mixture was shaken vigorously for 3.5 h at room temperature, whereupon the sugar gradually dissolved, and, later, the product began to crystallise. After 24 h, dry ether (500 ml) was added, and subsequent cooling (3°, 4 h) gave a product (71.7 g, 90%), m.p. 156–164°, after collection and washing with ether (2 × 250 ml). One recrystallisa-

tion from ethanol (approximately 17 parts) gave benzyl β-L-arabinopyranoside (59.7 g, 75%), m.p. 171–172.5°, $[\alpha]_D^{29}$ +208.6° (*c* 0.4, water).

The experiments recorded in Table I were performed by essentially the above method, with appropriate adjustments in the concentration of hydrogen chloride.

ACKNOWLEDGMENTS

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Laboratories of the
Medical Research Council of Ireland,
Trinity College, Dublin 2 (Ireland)

JOAN E. MCCORMICK

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Received January 4th, 1967)

Carbohyd. Res., 4 (1967) 262–263

Nucleosides and related substances
Part VI*. The synthesis of 9-α-D-ribofuranosyladenine (α-adenosine)

Naturally occurring nucleosides and nucleic acid-nucleosides generally have the aglycon and the hydroxyl group at C-2 of the carbohydrate moiety in *trans* relationship. Only a few nucleosides having the *cis* relationship (α-nucleosides) have been found, for example, in spongonucleosides[1], in certain vitamins[2], and in yeast ribonucleic acid[3]. α-Nucleosides are of increasing interest from the chemical and biological point of view, and their chemical synthesis has recently been investigated[4].

The present paper describes a new synthesis of 9-α-D-ribofuranosyladenine (α-adenosine). Condensation of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide with 6-benzamidopurine was carried out in *N*,*N*-dimethylformamide containing phosphorus pentaoxide, and α-adenosine (4–5%) was successfully isolated from the reaction

*For Part V, see Ref. 7

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The present paper describes a new synthesis of 9-α-D-ribofuranosyladenine (α-adenosine). Condensation of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide with 6-benzamidopurine was carried out in *N*,*N*-dimethylformamide containing phosphorus pentaoxide, and α-adenosine (4–5%) was successfully isolated from the reaction

*For Part V, see Ref. 7

mixture. In the n.m.r. spectrum of α-adenosine, the H-1′ signal appeared at δ 6.38 (doublet, $J_{1',2'}$ 4.8 c.p.s.), whereas that of 9-β-D-ribofuranosyladenine (adenosine) appeared at δ 6.02 (doublet, $J_{1',2'}$ 5.9 c.p.s.). Other peaks in the spectrum were similar to those of adenosine, with a slight difference of the chemical shift of the H-8 signal. The H-2 signal of both of the anomers appeared at δ 8.05, but the H-8 signal of α-adenosine appeared at δ 8.32, and that of adenosine at δ 8.25. Similar observations were also made with the picrates: the H-1′ signal of α-adenosine picrate and adenosine picrate appeared at δ 6.36 (doublet, $J_{1',2'}$ 5.3 c.p.s.) and δ 5.99 (doublet, $J_{1',2'}$ 5.5 c.p.s.), respectively. The infrared spectrum of α-adenosine picrate had essentially the same absorption peaks as that of adenosine picrate, but a slight difference was found in the 810–850 cm^{-1} region, which may be due to the C–H-deformation vibration at the anomeric carbon atom[5]. The position of substitution (N-9) of the adenine residue in α-adenosine was established on the basis of the ultraviolet absorption characteristics in different solvents[6]. The α-D configuration was supported by the plain, positive, o.r.d. curve. All of these data support the structure of α-adenosine for the substance showing $R_{adenine}$ 0.47.

In addition to the method described in the experimental part, two other procedures have been tested for the synthesis of α-adenosine. Firstly, the reaction of 2,3,5-tri-*O*-acetyl-D-ribofuranose or 2,3,5-tri-*O*-benzoyl-β-D-ribofuranose with 6-benzamidopurine, when performed in *N*,*N*-dimethylformamide containing phosphorus pentaoxide according to the procedure reported previously[7], yielded adenosine and α-adenosine in yields of 10 and 1–2%, respectively. Secondly, the fusion reaction of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose with 6-benzamidopurine in the presence of zinc chloride[8] gave only a trace of α-adenosine (detected chromatographically), adenosine being the main product.

The mechanism of the formation of α-adenosine in the Koenigs–Knorr reaction described above is still unknown, but it is of interest to note that Shimizu and Miyaki[9] isolated 3-*N*-alkylpurines as intermediates in the formation of 9-*N*-alkylpurines by the reaction of purines with alkyl halides, and the same observation was also made with 9-*N*-ribosylpurines. A similar mechanism might apply in the formation of α-adenosine in the present work. This is partly supported by the fact that the reaction of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide with theophylline gave neither 7-α-D- nor 9-α-D-ribofuranosyltheophylline. It is also of interest that no nucleoside was detected when 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide was used in place of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide in this procedure.

EXPERIMENTAL

N.m.r. spectra were recorded at 60 Mc.p.s. with a Varian A-60 spectrometer at its normal operating temperature. Chemical shifts are given on the δ scale in parts per million (p.p.m.) downfield displacement from sodium 2,2-dimethyl-2-silapentane-5-sulphonate (δ 0.00, internal standard for solutions in deuterium oxide), or from tetramethylsilane (δ 0.00, internal standard for solutions in dimethyl sulphoxide-d_6).

Infrared spectra were measured with a Shimadzu AR-6 spectrophotometer (sodium chloride optics) by using Nujol or KBr pellets. O.r.d. curves were recorded for aqueous solutions with a JASCO automatic, recording spectropolarimeter. Paper chromatography was carried out on Toyo Roshi No. 51 and No. 50 filter papers by the descending technique, with butyl alcohol–water (86:14) as developing solvent, and detection with ultraviolet light. Mobilities are expressed as $R_{adenine}$ values.

*Reaction of 2,3,5-tri-*O*-acetyl-*D*-ribofuranosyl bromide*[10] (**1**) *with 6-benzamidopurine* (**2**). — Compounds **1** (2.0 g, 5.8 mmoles) and **2** (1.5 g, 6.2 mmoles) were dissolved in 30 ml of *N,N*-dimethylformamide. To the solution was added 4.0 g of phosphorus pentaoxide, with stirring. After storage for 12 h at 60–70°, with exclusion of moisture, the reaction mixture was poured into *ca.* 50 ml of ice–water with vigorous stirring, and then extracted with chloroform (3 × 30 ml). The aqueous layer was submitted to chromatography on a column of Dowex-50 (H^+). The column was then washed with a large volume of water, and eluted with N ammonium hydroxide. Fractions that showed ultraviolet absorption were combined, and concentrated *in vacuo* to produce an amorphous powder that was found by paper chromatography to contain adenine and two compounds showing $R_{adenine}$ 0.55 and 0.47, respectively. In preparative paper chromatography (elution for one week), adenine was allowed to run off the sheet, and the remaining, u.v.-absorbing zones were cut out and eluted with water. Concentration of the combined extracts of each zone afforded the corresponding nucleosides.

The nucleoside showing $R_{adenine}$ 0.47 (0.08 g, 5%) had $[\alpha]_D^{17}$ + 16° (*c* 0.7, water); $\lambda_{max}^{H_2O}$ 259 mμ, $\lambda_{max}^{0.1N\ HCl}$ 257 mμ, $\lambda_{max}^{0.1N\ NaOH}$ 260 mμ; o.r.d. (*c* 1.0, water) at 17°, $[\phi]_{300}$ + 320, $[\phi]_{400}$ + 120, $[\phi]_{500}$ + 75, $[\phi]_{600}$ + 60, $[\phi]_{700}$ + 50°; ν_{max}^{Nujol} 3100–3400 (OH, NH), 1675 (−C=N−C=), 1610, 1510 (−C=N−, −C=C−), 1630 ($-NH_2$), 1010–1140 (C−O−C, C−O), 920, and 813 cm^{-1}; n.m.r. δ^{D_2O} 8.05 (H-2,1 proton), 8.32 (H-8,1 proton), 6.38 (1-proton doublet, $J_{1',2'}$ 4.8 c.p.s., H-1'), 3.85–5.00 (broad, 5-proton multiplet). Crystallization of the nucleoside was unsuccessful, but the syrupy product was crystallized as the picrate. Two recrystallizations from hot water gave fine needles, m.p. 210–213° (decomp.); ν_{max}^{Nujol} 3000–3500 (OH, NH), 1550 (NO_2), 750, and 845 cm^{-1}.

Anal. Calc. for $C_{16}H_{16}N_8O_{11}$: C, 38.72; H, 3.25; N, 22.58%. Found: C, 38.76; H, 3.51; N, 22.63%.

Reported data[4] for α-adenosine: m.p. 201°; $[\alpha]_D$ + 24° (*c* 0.65, water); $\lambda_{max}^{H_2O}$ 259 mμ, $\lambda_{max}^{acid\ solutions}$ 257 mμ; picrate, m.p. 190° (decomp.).

Adenosine was isolated from the zone showing $R_{adenine}$ 0.55. After two recrystallizations from water, the product (0.51 g, 34%) had m.p. 233–234°, $[\alpha]_D^{17}$ − 60.4° (*c* 1.0, water); $\lambda_{max}^{H_2O}$ 259 mμ (ε 14.4 × 10^3), $\lambda_{max}^{0.1N\ HCl}$ 257 mμ (ε 13.2 × 10^3).

From the chloroform layer obtained above, adenosine (4%, m.p. 233°) was isolated after treatment with methanolic ammonia, and α-adenosine was also detected by paper chromatography.

*Reaction of 2,3,5-tri-*O*-benzoyl-*D*-ribofuranosyl bromide* (**3**) *with 6-benzamidopurine* (**2**). — The reaction of **3** with **2** was performed according to the procedure

described above, and an anomeric mixture of 9-D-ribofuranosyladenines was found to be present, mainly in the aqueous layer. Adenosine, m.p. and mixed m.p. 231–232°, (21% yield); α-adenosine (4%) was isolated as a syrup. The infrared spectrum of the syrup was identical with that of α-adenosine obtained above.

Reaction of 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide (**1**) *with theophylline* (**4**). — Condensation of **1** with **4**, in a manner similar to that described above, afforded only 7-β-D-ribofuranosyltheophylline (16%), m.p. 191–192°, $[\alpha]_D^{21}$ +10° (*c* 0.5, water); $\lambda_{max}^{H_2O}$ 272 mμ (ε 8.7 × 10^3).

Anal. Calc. for $C_{12}H_{16}N_4O_6 \cdot H_2O$: C, 43.60; H, 5.49; N, 16.96. Found: C, 43.34; H, 5.48; N, 17.34. The water of crystallization was lost on drying for 2 h at 100° *in vacuo*.

Laboratory of Biochemistry,
Department of Agricultural Chemistry,
Kyoto University, Kyoto (Japan)

KONOSHIN ONODERA
SHIGEHIRO HIRANO
FUMIYA MASUDA

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(Received August 24th, 1966; in revised form, December 7th, 1966)

The preparation of D-galactose-*6-t* and some of its derivatives by using galactose oxidase

During an investigation of the catabolism of the galactan in the snail *Helix aspersa*, radioactive galactan and α-D-galactopyranosyl phosphate were required. This note describes the preparation of galactan-*6-t* and α-D-galactopyranosyl-*6-t* phosphate, and the extension of the method as a general one for the introduction of tritium into D-galactose and some of its derivatives.

The enzyme galactose oxidase oxidizes the primary alcohol group at C-6 of D-galactose (and many of its derivatives of biological importance, including polysaccharides and α-D-galactopyranosyl phosphate) to the aldehyde[1]. In the absence of another reducible group, the aldehyde can be reduced with sodium borohydride-*t* to give a compound specifically tritiated at C-6 of the terminal D-galactose residue.

In order to avoid reduction of C-1, methyl α-D-galactopyranoside was used as the starting material for the preparation of D-galactose-*6-t*. Similarly, since the galactan from *Helix aspersa* has approximately one reducing group per two hundred residues, it was first reduced with sodium borohydride in order to avoid tritiation at C-1 of this residue.

In order to confirm the location of the tritium, the hydrogen atoms at C-6 were isolated, where possible, by conversion of the tritiated D-galactose derivative into D-galactose-*6-t*, followed by periodate oxidation and isolation of the formaldehyde–dimedone (5,5-dimethylcylohexane-1,3-dione) complex. The specific activity of the dimedone complex was, in each case, between 94 and 99% of that of the D-galactose (Table I).

Snail galactan, a multi-branched, β-D-(1→3)-linked polymer[2], had not previously been used as a substrate for galactose oxidase. It was a good substrate for the enzyme, and had K*m*. at 25°, 1.1×10^{-2}M, calculated as molarity of galactose residues.

EXPERIMENTAL

Radioactivity was measured as previously described[3].

Incorporation of tritium onto C-6 of D-*galactose derivatives.* — The D-galactose derivative (100 μmoles) was incubated with galactose oxidase (Sigma London, Ltd.) and catalase (5 mg, Koch–Light Ltd.) in 0.25M sodium phosphate buffer (pH 7, 2 ml) for the time stated (Table I). The indicated quantity of sodium borohydride-*t* (53 μcuries/mmole) was added, and the solution was left for 15 min at room temperature, diluted with water (10 ml), and deionized with Amberlite IR-120 (H^+) followed by Amberlite IRA-400 (CO_3^{2-}). The solution was evaporated to dryness *in vacuo*, and chromatographed on Whatman No. 3MM paper with butyl alcohol–ethanol–water (4:1:5, upper layer) as solvent to ensure complete removal of protein and salts, and complete equilibration of replaceable hydrogen atoms. The product was eluted and either crystallized directly, or after addition of carrier, or converted directly into D-galactose-*6-t*.

TABLE I

Compound	*Galactose oxidase (units)*	*Incubation time (h)*	*Sodium borohydride-t (mg)*	*Specific activity (μcuries/mmole)*	*Percentage of total tritium at C-6 of derived D-galactose*
Methyl α-D-galactopyranoside	40	3	1.5	250[a]	98
Melibiitol (6-*O*-α-D-galactopyranosyl-D-glucitol)	40	3	1.5	83[a]	94
o-Nitrophenyl β-D-galactopyranoside	100	5	2.3	75	99
α-D-Galactopyranosyl phosphate	80	4.5	0.9	90	94
Galactan *(Helix aspersa)*[b]	80	3	3.4	94[c]	97
2,5-Anhydro-D-galactitol	11	2	0.5	2000	—

[a]Converted into D-galactose-*6-t* without reisolation of starting material. [b]500 mg (3.1 mmoles of D-galactose residues) used. [c]Specific activity per D-galactose residue.

Galactan-6-t. — Galactan (500 mg, isolated from the albumen gland[4] of *H. aspersa*) in water (2 ml) was treated with sodium borohydride (10 mg) in water (1 ml). After 20 min at room temperature, N hydrochloric acid (0.1 ml) was added to decompose excess of borohydride, followed by N sodium hydroxide (0.1 ml). Galactose oxidase (80 units) and catalase (10 mg) were added in 0.5M sodium phosphate buffer (pH 7, 2 ml), and incubated for 3 h at 37°. Sodium borohydride-*t* (3.4 mg) was added in water (1 ml), and the mixture was allowed to stand at room temperature for 15 min. The solution was heated to 100° for 3 min (to decompose excess of sodium borohydride-*t*, and denature the protein), and the mixture was dialysed against three changes of distilled water at 2°. The dialysed solution (14 ml) was centrifuged, and freeze-dried to give galactan-*6-t*. Yield, 450 mg; 90%; 94 μcuries/mmole.

α-D-*Galactopyranosyl*-6-t *phosphate dipotassium salt*. — The general method for galactose derivatives was used with the following modifications. No buffer was used, but the solution of α-D-galactopyranosyl phosphate dipotassium salt (Boehringer & Soehne) was adjusted to pH 7.0 by the addition of Amberlite IR-120 (H^+). The solution was not deionized after reduction with borohydride-*t*, but protein was removed by heating to 100° for 5 min and centrifuging, before chromatography on Whatman No. 3MM paper with butyl alcohol–acetic acid–water (4:1:5, upper layer) as solvent. On elution from the paper, the D-galactosyl-*6-t* phosphate was immediately titrated to pH 8.0 with 0.1N potassium hydroxide. The solution was evaporated *in vacuo* to 0.3 ml, and ethanol was added to induce crystallization at 0°.

D-*Galactose*-6-t. — The D-galactoside-*6-t* was hydrolysed with 2N sulphuric acid for 14 h at 100°. (In the case of D-galactosyl-*6-t* phosphate, 0.1N sulphuric acid for 5 min at 100° was used.) The solution was deionized, and chromatographed on Whatman No. 3MM paper with butyl alcohol–ethanol–water (4:1:5, upper layer) as solvent, and the area corresponding to galactose was eluted. The product was either

crystallized after addition of D-galactose carrier, or used directly to determine the proportion of label at C-6.

Degradation[5] *of* D-*galactose*-6-t. — Periodic acid (0.2 ml, 50% w/w) was added to D-galactose-*6-t* and carrier D-galactose (total, 18 mg, 100 μmoles) dissolved in water (1ml), and the solution was left in the dark at room temperature for 4 h. It was then poured into a solution (20 ml) containing dimedone (100 mg), Na_2HPO_4 (93 mg), and $NaH_2PO_4.2H_2O$ (70 mg), and left overnight. The formaldehyde–dimedone complex was twice recrystallized from methanol, and had m.p. 186–187°. The percentage recovery of the tritium contained in the original solution is recorded in Table I.

Department of Physiology and Biochemistry, J. E. G. BARNETT
University of Southampton (Great Britain)

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(Received November 22nd, 1966)

Carbohyd. Res., 4 (1967) 267–269

Preliminary communications

Equilibria between furanoses and pyranoses

Although of fundamental importance in carbohydrate chemistry, the composition of the equilibrium mixture formed when a reducing sugar is dissolved in water is not quantitatively known; in particular, there are no data available on the proportion of furanoid and pyranoid forms present. Until the advent of n.m.r. spectroscopy, there was no method known capable of providing such data. Recently, Rudrum and Shaw[1] and Lemieux and Stevens[2] determined the composition of an aqueous solution of D-ribose. We have found that, in the aqueous (D_2O) solution of many aldoses, the p.m.r. signals of each of the four isomeric forms are recognizable, identification often being possible on comparison with the spectra of suitable model compounds. Integration of the signals gives the proportions of the four forms. In no case were more than four anomeric signals detected, but the data do not unambiguously exclude the possibility that one of the signals is due to a hydrated, *aldehydo* form.

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Department of Physiology and Biochemistry, J. E. G. BARNETT
University of Southampton (Great Britain)

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Because of their importance, we now list in Table I the data so far obtained. For comparison, the results on the composition of the aqueous solution of D-ribose[1,2]

TABLE I

COMPOSITION[a] (IN %) OF AQUEOUS SOLUTIONS OF ALDOSES AT 40°

Sugar	*Furanose*		*Pyranose*	
	α-	β-	α-	β-
D-Allose	5	7	16	71
D-Altrose	20	13	27	40
D-Gulose		22		78
D-Talose	20	11	40	29
D-Arabinose		3	63	34
D-Ribose[1,2]	6	18	20	56
3-Deoxy-D-*ribo*-hexose	6	26	26	40

[a] Experimental error in the measurements was $< \pm 2\%$.

are also included. It is intended to publish full details of the spectra; they were all obtained with a Varian A-60 spectrometer on 15–20% solutions in deuterium oxide at 40°. No signals corresponding to furanoses could be detected in the spectra of equilibrium solutions of D-glucose, D-mannose, D-galactose, D-xylose, D-lyxose, and 2-deoxy-D-*arabino*-hexose. It is of particular interest to note the high proportion of furanoid forms in the solution of 3-deoxy-D-*ribo*-hexose, as compared to that of D-glucose. Apparently, the most unfavorable interaction in the furanoses is the one between the side chain and the oxygen atom on C-3 when these are *cis* to each other. No sugar having this arrangement of groups appears to afford substantial proportions of the furanoid forms in its equilibrium solution in water.

School of Chemistry,
The University of New South Wales,
Kensington, N. S. W.
(Australia)

S. J. ANGYAL
V. A. PICKLES

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(Received December 21st, 1966)

Carbohyd. Res., 4 (1967) 269–270

The relative signs of proton–proton spin-coupling constants in saturated and unsaturated carbohydrate derivatives*

Although there have been many investigations[1] aimed at establishing the relative signs of proton–proton spin-coupling constants for a variety of different compounds, no comparative investigations[2] have so far been reported for carbohydrate derivatives**. Such information is of importance for two reasons. First, it is often necessary to know the signs of coupling constants before a spectral analysis is checked by a computer program. Second, it is possible that the signs of certain coupling constants may be dependent upon the relative stereochemistry and molecular environment of the nuclei involved+.

We now report the results of a preliminary investigation of the relative signs of carbohydrate ^1H–^1H coupling constants. The latter are summarized in Table I. All experiments were performed with a Varian HA-100 n.m.r. spectrometer operating in the frequency-sweep mode, either by the strong-field " spin-decoupling " method[6] or by the weak-field " spin-tickling " technique[7].

A detailed description of these experiments will be published in due course. Briefly, if, in any mutually coupled three-spin system (such as an ABX system), irradiation of a *high*-field coupling[6] or *transition*[7] in the A multiplet causes an appropriate perturbation in the *high*-field region of the B resonance, the signs of $J_{A,X}$ and $J_{B,X}$ are the same; if, on the other hand, the *low*-field region of the B resonance is perturbed, the signs of $J_{A,X}$ and $J_{B,X}$ are opposite. Such an experiment gives only the *relative* signs of the coupling constants, but these may now be placed with some confidence on an *absolute* basis by reference to the work of Buckingham and McLauchlan[8], and the signs in Table I have been normalized on that basis.

Although most of the couplings have the expected signs, there are several comparisons that are of interest. For the " saturated " derivatives **7**, **9**, and **10**, the *geminal* couplings are negative and the *vicinal* couplings are positive, which is as expected on the McConnell extension[9] of the Dirac–Van Vleck vector model[10]. However, this model predicts a negative sign for the long-range couplings across four bonds, whereas a positive sign is found for the H-4–H-6 coupling of derivative **2** and for the H-2–H-4 coupling of **8**.***

The epithio derivative **4** shows a negative *geminal* and a positive *vicinal* coupling constant, and in this respect is similar to derivatives **2**, **7**, **9**, and **10**. In contrast to this

*Reported, in part, at the 152nd National Meeting of the American Chemical Society, New York City, New York, September 11–16, 1966.

**Relative signs of couplings have been determined for some 1,3-dioxolane derivatives[2a], a streptose derivative[2b], and some unsaturated sugar derivatives[2c].

***We have found that the long-range coupling $J_{1,3}$ (0.6 Hz) in 3,4,6-tri-*O*-acetyl-1-*O*-benzoyl-2-deoxy-2-iodo-α-D-mannopyranose is negative in sign. Should this difference in sign between $J_{e,a}$ and $J_{e,e}$ prove to be general, this will clearly facilitate some conformational assignments.

+Signs have been determined for vicinal[3], vinylic and allylic[4], and geminal[5] couplings.

behavior, the epoxide derivative **3** has both *geminal* and *vicinal* couplings positive[1a], and at the same time it shows a *negative* long-range coupling. This pattern of signs is the same as that of the unsaturated derivatives **1**, **5**, and **6**, and is in marked contrast

TABLE I

SUMMARY OF THE RELATIVE SIGNS[a] OF PROTON–PROTON COUPLING CONSTANTS OF CARBOHYDRATE DERIVATIVES

Derivative	*Signs and Magnitudes of Coupling Constants, Hz*
3-O-Acetyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose (**1**)[b]	$J_{5,6}$ +10.0; $J_{5,6'}$ +17.4; $J_{6,6'}$ +2.5
1,6-Anhydro-β-D-idopyranose triacetate (**2**)[c]	$J_{4,5}$ +4.3; $J_{4,6}$ +1.1; $J_{5,6}$ +4.3
5,6-Anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose (**3**)[b,d]	$J_{5,6}$ +4.2; $J_{5,6'}$ +2.8; $J_{6,6'}$ +4.85; $J_{4,6'}$ −0.3
5,6-Dideoxy-5,6-epithio-1,2-*O*-isopropylidene-β-L-idofuranose (**4**)[b]	$J_{5,6}$ +6.55; $J_{5,6'}$ +5.3; $J_{6,6'}$ −1.45
5,6-Dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose (**5**)[b,e]	$J_{4,5}$ +4.8; $J_{5,6}$ +10.0; $J_{5,6'}$ +17.3; $J_{6,6'}$ +2.5; $J_{4,6}$ −1.6; $J_{4,6'}$ −1.4
D-Glucal triacetate (**6**)[f]	$J_{1,2}$ +6.4; $J_{2,3}$ +3.2; $J_{1,3}$ −1.3
Methyl tetra-*O*-acetyl-β-D-glucopyranoside (**7**)[f]	$J_{5,6}$ +4.85; $J_{5,6'}$ +2.4; $J_{6,6'}$ −12.3
β-D-Ribopyranose tetraacetate (**8**)[f]	$J_{3,4}$ +3.2; $J_{2,3}$ +3.2; $J_{2,4}$ +0.8
1,3,4,6-Tetra-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranose (**9**)[f]	$J_{1,2e}$ +2.4; $J_{1,2a}$ +9.8; $J_{2e,2a}$ −12.4; $J_{2e,3}$ +5.0; $J_{2a,3}$ +11.0
β-D-Xylopyranose tetraacetate (**10**)[f]	$J_{4,5e}$ +4.9; $J_{4,5a}$ +8.5; $J_{5e,5a}$ −11.8

[a]The signs have been made to be coincident with the absolute sign determinations of Buckingham and McLauchlan (ref. 8). [b]Measured in chloroform-*d* solution. [c]Measured in benzene-d_6. [d]This compound was studied in both chloroform and acetone solutions. The magnitudes of the couplings were solvent-dependent. [e]The values of $J_{6,6'}$ is an average value. It is relevant to note that the apparent magnitude of this coupling differed slightly between the two H-6 multiplets; compare ref. 12. [f]Measured in acetone-d_6 solution.

to the signs shown by derivatives **2**, **4**, **7**, and **10**. It is evident from this behavior that the mechanism of spin-coupling between protons associated with an epoxide substituent is different from that of other systems which are also nominally sp^3-hybridized, and thus calculations based on the Karplus approach are probably invalid for epoxide substituents[11].

It is planned to extend these determinations of relative signs to other groups that are commonly found in carbohydrate derivatives.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge financial support from the National Research Council of Canada and the National Cancer Institute of Canada.

Department of Chemistry,
The University of British Columbia,
Vancouver 8, B. C. (Canada)

L. D. HALL
J. F. MANVILLE

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(Received December 8th, 1966; in revised form, January 23rd, 1967)

Carbohyd. Res., 4 (1967) 271–273

Mass spectrometry of trimethylsilyl ethers of carbohydrates

Although partial hydrolysis is a widely applied method in the structural elucidation of polysaccharides, there is a need for small-scale and rapid procedures for establishing the structures of the oligosaccharides produced. In view of the results obtained with methyl ethers of disaccharides[1], the use of mass spectrometry for this purpose seemed promising.

In spite of recent improvements[2], the exhaustive methylation of oligosaccharides is a complex and time-consuming operation. We were thus attracted to the use of the readily available trimethylsilyl ethers of sugars, since these compounds are volatile and have already found wide application in gas–liquid chromatography[3].

We now report on the mass spectra of trimethylsilyl ethers of D-glucose, D-galactose, D-mannose, L-arabinose, L-rhamnose, and their methyl glycosides, which can be considered as models for reducing and non-reducing residues of oligosacchar-

ACKNOWLEDGMENTS

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Department of Chemistry,
The University of British Columbia,
Vancouver 8, B. C. (Canada)

L. D. HALL
J. F. MANVILLE

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We now report on the mass spectra of trimethylsilyl ethers of D-glucose, D-galactose, D-mannose, L-arabinose, L-rhamnose, and their methyl glycosides, which can be considered as models for reducing and non-reducing residues of oligosacchar-

ides. The peak of the molecular ion is absent from the mass spectra of these compounds. The ion M-15, probably resulting from the loss of a methyl group from a trimethylsilyl residue[4], has the highest m/e value. The corresponding peak has low intensity. The peaks of the M-105 and M-195 ions, arising from the M-15 ion by splitting of one (90 m.u.) or two (180 m.u.) trimethylsilanol molecules, are more intense.

The most intense peaks in all of the spectra are those at m/e 204 and 217, corresponding to the ions $(Me_3SiO\text{-}CH=CH\text{-}OSiMe_3)^+$ and $Me_3SiO\text{-}CH=CH\text{-}CH=\overset{+}{O}SiMe_3$, respectively, which are analogues of the ions having m/e 88 and 101 in mass spectra of methyl ethers of monosaccharides[5].

The peak at m/e 191 in the mass spectra of the trimethylsilyl ethers of monosaccharides also has a high intensity. The corresponding ion $(Me_3SiO\text{-}CH=\overset{+}{O}SiMe_3)$ probably arises as a result of a rearrangement analogous to that leading to the J_1-ion (m/e 75) for methylated methyl glycosides[5]. In agreement with such an assumption is the fact that the peak of the J_1-ion in the spectra of trimethylsilyl ethers of methyl glycosides is shifted to m/e 133, corresponding to $Me_3SiO\text{-}CH=\overset{+}{O}Me$.

The ion $[Me_3SiO\text{-}CH=C(OSiMe_3)CH=\overset{+}{O}SiMe_3]$ having m/e 305 is an

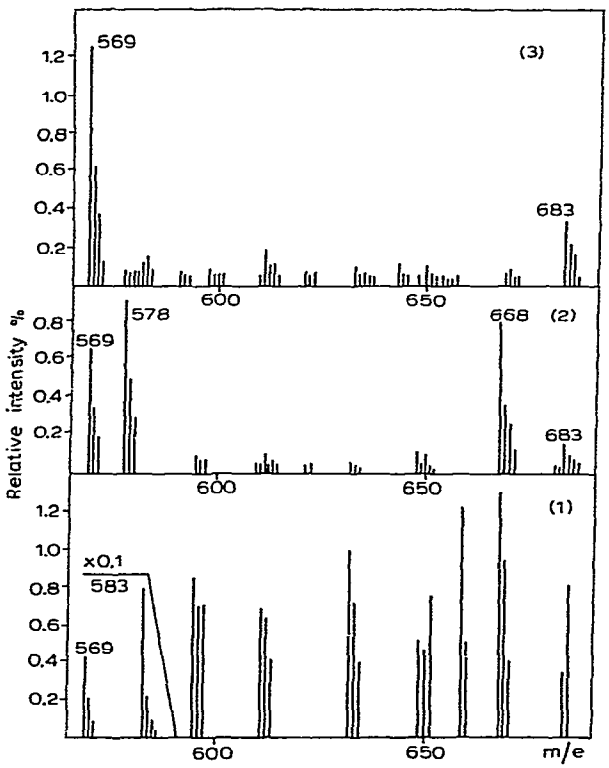


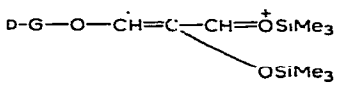
Fig. 1. Partial mass spectra of the trimethylsilyl ethers of 6-*O*-β-D-glucopyranosyl-D-galactose (1), cellobiose (2), and laminaribiose (3).

analogue of the ion B_2 (m/e 131) in the spectra of methyl ethers[5], and appears for all the compounds studied.

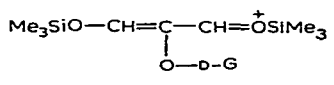
The presence of relatively intense peaks at m/e 147, 103, and 73 is also characteristic of the mass spectra of trimethylsilyl ethers of the monosaccharides and methyl glycosides, the following structures being the most probable for these ions (*cf.* ref. 4):

$Me_3Si\text{-}\overset{+}{O}=SiMe_2$ m/e 147 $\quad$ $CH_2=\overset{+}{O}\text{-}SiMe_3$ m/e 103 $\quad$ $^{+}SiMe_3$ m/e 73

The mass spectra of the disaccharides 6-*O*-*β*-D-glucopyranosyl-D-galactose (**1**), cellobiose (**2**), laminaribiose (**3**) (see Fig. 1), maltose, and lactose were also studied. There are characteristic differences between the mass spectra of disaccharides having different types of linkage. The peak at m/e 583 is present in the mass spectrum of the (1→6)-linked disaccharide (**1**), whereas that at m/e 683 is characteristic for disaccharides **2** and **3**. The mass spectrum of **2** can be distinguished from that of **3** by the presence of peaks at m/e 668 and 578. A structure analogous to that of the ion at m/e 305 can be assigned to the ion having m/e 683:

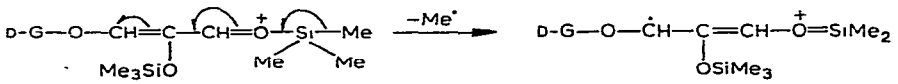


m/e 683 (from 2)



m/e 683 (from 3)

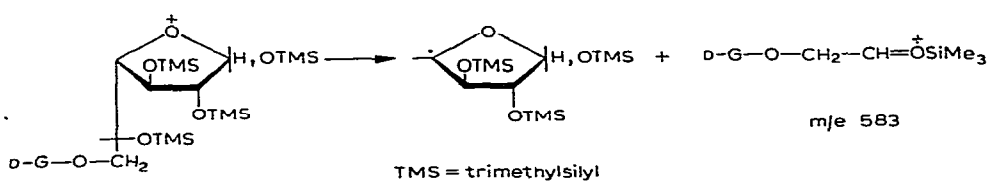
Loss of one methyl group from the ion having m/e 683 results in formation of the ion having m/e 668:



m/e 683 (from 2) $\quad$ m/e 668 (from 2)

D-G = 2,3,4,6-tetra-*O*-(trimethylsilyl)-D-glucopyranosyl.

The subsequent loss of a trimethylsilanol molecule from this ion gives the ion having m/e 578 (metastable peak at m/e 501). The symmetrical structure of the ion having m/e 683 (from **3**) prevents the loss of a methyl radical, and results in the absence of the peaks at m/e 668 and 578, and the metastable peak at m/e 501, from the mass spectrum of the trimethylsilyl ether of laminaribiose. The ion having m/e 583 probably arises from the furanoid form of disaccharide **1**:

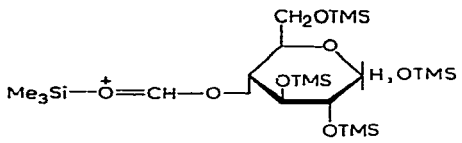


m/e 583

TMS = trimethylsilyl

The formation of this ion from **2** and **3** is evidently impossible.

The ion corresponding to the peak at m/e 569 might be an analogue of the ions having m/e 191 and 133 (see above).



m/e 569

This peak is present in the mass spectra of all of the disaccharides. However, its intensity depends to a large extent on the type of linkage. For example, the relative intensities are 4, 1.2, and 0.6% in the mass spectra of **1**, **3**, and **2**, respectively. The intensity decreases with an increase in the spatial compression of the charged fragment $Me_3SiO^+=CH\text{-}O\text{-}$ by the neighbouring groups; the charged group is less hindered in position 6 than in positions 3 or 4. Thus, mass spectra may be used for detecting the types of linkage in disaccharides.

The configuration of the glycosidic bond has no significant influence on the fragmentation pattern, as is evident from the mass spectra of the trimethylsilyl ethers of cellobiose and maltose. However, the fragmentation pattern depends on the configuration of the monosaccharide residues involved, these differences being more pronounced for trimethylsilyl ethers than for methyl ethers. For example, there are the relatively intense peaks at m/e 611, m/e 521, and m/e 445 (metastable peak), corresponding to the process 611→521, in the mass spectrum of the trimethylsilyl ether of lactose, whereas these peaks are absent from the mass spectra of the maltose and cellobiose ethers. This result provides additional possibilities for the use of mass spectrometry in the study of the structures of oligosaccharides.

The authors thank Dr. V. N. Bochkarev and Mr. A. Sulima for assistance in the measurement of mass spectra.

Institute for Chemistry of Natural Products,
U. S. S. R. Academy of Sciences,
Moscow (U. S. S. R.)

O. S. CHIZHOV
N. V. MOLODTSOV
N. K. KOCHETKOV

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(Received November 14th, 1966; in revised form, January 20th, 1967)

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

AN EXTRACELLULAR POLYSACCHARIDE FROM *Arthrobacter viscosus**

I. R. SIDDIQUI
Food Research Institute, Canada Department of Agriculture, Ottawa (Canada)
(Received November 23rd, 1966)

INTRODUCTION

The extracellular polysaccharide from *Arthrobacter viscosus* NRRL B-1973 was isolated, purified, and characterized by Jeanes *et al.*[1]. The polysaccharide contains D-glucose, D-galactose, and D-mannuronic acid in the proportions of 1:1:1, and approximately 25% of *O*-acetyl groups. Fragmentation[2] analysis of the polysaccharide yielded a glucopyranosyl-D-galactose, a (mannopyranosyluronic acid)-D-glucose, and a (mannopyranosyluronic acid)-D-glucopyranosyl-D-galactose.

The acidic polysaccharide of *Arthrobacter viscosus* NRRL B-1973 was placed at our disposal through the kindness of Dr. F. R. Senti and Dr. Allene Jeanes. The purpose of the present investigation was three fold, namely, to investigate the homogeneity of the polysaccharide, to confirm that mannuronic acid was the only hexuronic acid present, and to elucidate the nature of the glycosidic linkages by methylation studies.

RESULTS AND DISCUSSION

A purified, deacetylated[3] sample of the polysaccharide showed a single, symmetrical peak on boundary electrophoresis[4] (Fig. 1). The sedimentation[5] patterns (Fig. 2) indicated that the polymer existed as a soft gel, and was not monomolecularly

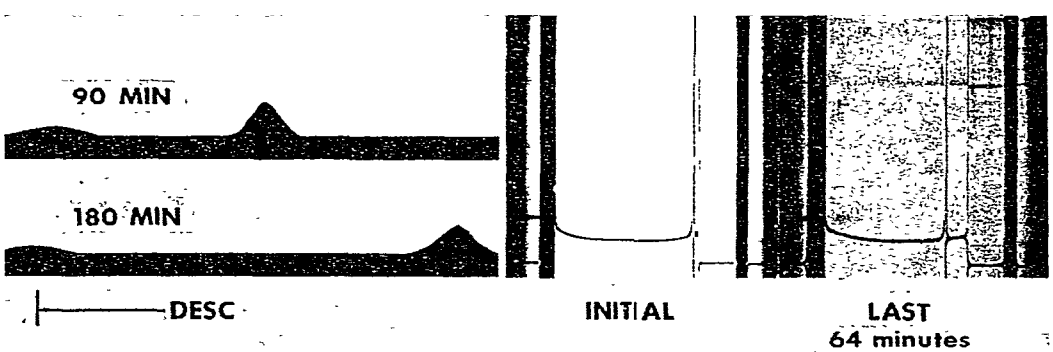


Fig. 1 Fig. 2

*Publication No. 49 of the Food Research Institute.

dispersed. On the basis of the electrophoretic results, the material appeared to be a chemically homogeneous polysaccharide.

It has become customary in recent years to find more than one hexuronic acid in complex, acidic polysaccharides. Although the four, common, hexuronic acids, namely, glucuronic, galacturonic, mannuronic, and guluronic acids, are difficult to separate by paper chromatography, after reduction to the corresponding aldonolactones, they can readily be separated either by paper chromatography or by gas–liquid chromatography after trimethylsilation[6]. Application of this technique to the present problem showed that mannuronic acid was the only hexuronic acid present.

The polysaccharide was methylated, and the fully methylated product was subjected to methanolysis and hydrolysis. The *O*-methyl sugars were quantitatively determined by gas–liquid chromatography[7] of their methyl glycosides giving an overall ratio of di- to tri-*O*-methylhexosides of 0.09:1. The ratio between the two tri-*O*-methylhexosides was approximately 1:1.

The methylated uronic acid fraction contained mainly 2,3-di-*O*-methylmannuronic acid, together with small proportions of a methylated aldobiouronic and, probably, a methylated di-mannuronic acid. The conclusion that some of the uronic acid was attached to glucose was provided by the isolation of small amounts of the methylated aldobiouronic acid, which gave a 2,3-di-*O*-methylmannuronic acid and a 2,3,6-tri-*O*-methylglucose on acid hydrolysis, and a 2,3-di-*O*-methylmannose and a 2,3,6-tri-*O*-methylglucose after reduction and hydrolysis. Hydrolysis, before and after reduction, of the methylated di-mannuronic acid yielded a 2,3-di-*O*-methylmannuronic acid and a 2,3-di-*O*-methylmannose. This suggests that some of the uronic acid residues occupy adjacent positions.

Approximately 0.1 mol. of a di-*O*-methyl sugar fraction, which was composed of comparable proportions of 2,3- and 2,6-di-*O*-methylglucose, and 2,3- and 2,6-di-*O*-methylgalactose, was not considered to be of any structural significance. These products presumably arose by demethylation of 2,3,6-tri-*O*-methylglucose and the galactose analogue.

Analytical data for the major methylated components are summarized as follows:

Component	*Molar ratio*	*Mode of linkage*
2,3,6-Tri-*O*-methyl-D-glucose	1	→4)-D-G*p*-(1→
2,3,6-Tri-*O*-methyl-D-galactose	1	→4)-D-Gal*p*-(1→
2,3-Di-*O*-methyl-D-mannuronic acid	1	→4)-D-Man*p*A-(1→

All of these sugars were characterized by the isolation of crystalline derivatives. The proportion of glucose:galactose:mannuronic acid, calculated from the proportion of methylated sugars, was in excellent agreement with that (1:1:1.14) found[1] for the original polysaccharide.

The methylation analysis showed that the molecule has a linear structure

consisting of the repeating units *O*-(β-D-mannopyranosyluronic acid)-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-galactose and/or *O*-(β-D-mannofuranosyluronic acid)-(1→5)-*O*-β-D-glucopyranosyl-(1→4)-D-galactose. In view of some recent work by Sloneker *et al.*[8], the latter possibility is excluded. The negative, specific, optical rotations (− 50°) for the unmethylated polysaccharide, and (− 27.9°) for the methylated polysaccharide, indicate that the majority of the glycosidic bonds are of the β-D type.

The present data do not permit any further deduction. Fragmentation studies to isolate oligosaccharides with and without mannuronic acid will reveal the detailed structure of this polysaccharide.

EXPERIMENTAL

Paper chromatography was performed by the descending method on Whatman No. 1 and 3 MM papers with the organic phases of (*A*) butyl alcohol–acetic acid–water (4:1:5); (*B*) butyl alcohol–ethanol–water (40:11:19); (*C*) butanone, saturated with water containing 2% of ammonia; (*D*) ethyl acetate–pyridine–water (8:2:1). Sugars, as their borate complexes, were separated by electrophoresis[9] in 0.2M buffer (pH 10), by using Whatman No. 3 MM paper at 800 volts for 2–3 h. Chromatograms were sprayed with (*A*) aniline hydrogen phthalate for reducing sugars[10]; and (*B*) alkaline silver nitrate for non-reducing sugars[11]. Evaporations were carried out at 35° with a rotary evaporator. Melting points are corrected, and rotations are equilibrium values.

Analysis of the acidic polysaccharide. — The acidic polysaccharide supplied to us had N, 0.76; P, 0.70; sulphated ash, 20.6; and *O*-acetyl, 25%. Electrophoresis (Spinco Model H apparatus) of a purified, deacetylated[3] sample of the polysaccharide as a 0.25% solution in 0.5M borate buffer (pH 9.2) showed a single, symmetrical peak (Fig. 1). The sedimentation[5] pattern of a 0.3% solution in borate buffer (pH 9.2; ionic strength, 0.2; rotational speed, 59780 rev./min) is shown in Fig. 2.

Analysis of the polysaccharide for mannuronic acid. — The acidic polysaccharide (500 mg) was hydrolysed with 2N sulphuric acid (15 ml) for 4 h at 100°. The sugars were recovered, and separated by paper chromatography, and a portion of the hexuronic acid fraction (*ca.* 20 mg) was dissolved in water and converted into the corresponding aldonolactone by the procedure of Perry *et al.*[6]. Examination of the aldonolactone by paper chromatography (solvent *B*, spray reagent *B*) showed the presence of only a mannonolactone.

Methylation of the acidic polysaccharide. — The polysaccharide (6 g) in water (120 ml) was methylated by adding 30% sodium hydroxide (320 ml) and methyl sulphate (120 ml). The reagents were added at such a rate that the reaction mixture retained a near-neutral pH for the first few hours. The additions took place over several hours; after which the solution was stirred for 24 h. After three such additions during 3 days, a further five additions of 30% sodium hydroxide (100 ml) and methyl sulphate (35 ml) were made during five days. The reaction mixture was then cooled, and acidified with glacial acetic acid, and the solution was extracted with chloroform (5 × 500 ml). The dried ($MgSO_4$) extract was filtered and evaporated to a syrup.

The chloroform-insoluble residue (1.5 g), recovered from the aqueous phase after dialysis and freeze-drying, was suspended in water (20 ml) and, after nine additions of 30% sodium hydroxide (100 ml) and methyl sulphate (35 ml), recovered in the usual way. The total product (3.8 g) recovered by chloroform extraction was dissolved in chloroform (65 ml) to which methyl iodide (135 ml) was added, followed by silver oxide (21 g) in seven equal portions during 24 h. After eight further methylations by Purdie's method, using methyl iodide (125 ml) and silver oxide (10 g) per methylation, a product (3.6 g) was recovered that showed a methoxyl content of 44.7%; $[\alpha]_D^{27}$ − 27.9° (*c* 3.0, chloroform); and no hydroxyl absorption in its infrared spectrum.

Methanolysis and hydrolysis of the methylated polysaccharide. — The methylated polysaccharide (3.0 g) was dissolved in 3.5% methanolic hydrogen chloride (150 ml), and the solution was refluxed for 24 h, neutralized with silver carbonate, filtered, and then evaporated to a syrup that was hydrolysed with N sulphuric acid (125 ml) for 38 h at 100°. The hydrolysate was neutralized with barium carbonate, filtered, and evaporated to a syrup (2.67 g).

A solution of the mixture of acidic and neutral sugars (2 g) in water (100 ml) was stirred for 24 h with Rexyn RG-50 (CO_3^{2-}; 100 ml). The slurry was then made into a column, and washed with water (1.5 l) to elute neutral, methyl sugars (1.13 g), and with N ammonium carbonate (1 l) to elute the methylated uronic acid fraction (570 mg). The latter was recovered by removing most of the ammonium carbonate *in vacuo* at 65–70° and the remainder by passing the solution over Rexyn AG-3.

Examination and identification of neutral methyl sugars. — A portion (10 mg) of the neutral, methyl sugars was refluxed with 2% methanolic hydrogen chloride for 8 h, and then neutralized with silver carbonate, and the resulting methyl glycosides were examined[7] by g.l.c. using a 4-ft column of 15% polyphenyl ether on Chromosorb W at 200°, and an argon flow-rate of 100 ml/min. The areas under the peaks gave an overall, quantitative ratio of di-*O*-methylhexosides to tri-*O*-methylhexosides of 0.09:1. The molar ratio of individual tri-*O*-methylhexosides was determined on an 8-ft column of 3% Carbowax 20M on 60/80 Chromosorb W, at 145° with a nitrogen flow-rate of 40 ml/min. The ratio of methyl 2,3,6-tri-*O*-methyl-D-glucoside to methyl 2,3,6-tri-*O*-methyl-D-galactoside was 1:0.92.

The remaining mixture of neutral sugars was separated on sheets of Whatman No. 1 paper by using solvent *C*. The results of a typical separation using sugar mixture (250 mg) and 8 sheets of paper were as follows:

Component No.	*Component*	*Yield (mg)*
1	2,3,6-Tri-*O*-methyl-D-galactose	99
2	2,3,6-Tri-*O*-methyl-D-glucose	101
3	Di-*O*-methyl fraction	20

Identification of 2,3,6-tri-O-methyl-D-galactose. — The syrup showed an R_F value (0.48, solvent *C*) and colour reaction identical with those of an authentic sample

of 2,3,6-tri-*O*-methyl-D-galactose. Demethylation with boron trichloride[13] showed galactose to be the parent sugar. The syrup (60 mg) in water (2 ml) was oxidized with an excess of bromine in the presence of barium carbonate (60 mg) for 70 h. Bromine was removed by aeration, and the acidified solution was extracted with chloroform for 2 days. The chloroform extract was dried and concentrated, and the residue was distilled [140–160° (bath)/0.01 mm] to yield a syrup (28 mg). Crystallization of this syrup from ethanol–light petroleum gave 2,3,6-tri-*O*-methyl-D-galactono-1,4-lactone, m.p. and mixed m.p. 100–101°; $[\alpha]_D^{26}$ −26 ±2° (*c* 1.05, water); lit.[12], m.p. 101°; $[\alpha]_D$ −28° (water).

*Identification of 2,3,6-tri-*O*-methyl-D-glucose.* — The syrupy fraction showed an R_F value (0.56, solvent *C*) and colour reaction identical with those of authentic 2,3,6-tri-*O*-methyl-D-glucose. Demethylation[13] with boron trichloride produced only glucose. Crystallization from isopropyl ether gave 2,3,6-tri-*O*-methyl-D-glucose, m.p. 104–106° which was raised to 112–115° after several recrystallizations from isopropyl ether; $[\alpha]_D^{24}$ +70.3 ±1° (c 1.45, water); lit.[14], m.p. 121–123°, $[\alpha]_D$ +70°. The X-ray diffraction pattern of the crystals was identical with that of the authentic compound.

A portion of the above material (8 mg) in dry pyridine (0.5 ml) was treated with freshly crystallized *p*-nitrobenzoyl chloride (20 mg) for 1 h at 60–65°, and then overnight at room temperature. Isolation in the usual way[15] gave the di-*p*-nitrobenzoate, m.p. 191–192°, $[\alpha]_D^{27}$ −33.5 ±2° (*c* 0.84, chloroform); lit.[15], m.p. 190–191°, $[\alpha]_D^{25}$ −34° (*c* 1.0, chloroform).

*Examination of the di-*O*-methyl sugar fraction.* — The di-*O*-methyl fraction, on demethylation[13], gave glucose and galactose. Paper chromatography (solvent *C*) revealed two components, whereas paper electrophoresis revealed at least 4 components (M_G values 0.13, 0.21, 0.34, and 0.57), none of them being a major one. There were not sufficient amounts of any of these materials (which were presumably demethylation products, and hence of no structural significance) for complete identification.

Examination of the methylated uronic acid fraction. — The methylated uronic acid fraction (565 mg) was separated on ten sheets (18 × 21 in) of Whatman No. 1 paper by using solvent *A*. The three fractions (R_F 0.63, 0.46, and 0.32) were isolated and examined further.

Fraction 1. — A portion (2 mg) of Fraction 1 (16 mg, R_F 0.63) was hydrolysed with 2N sulphuric acid for 6 h at 100°. Paper chromatographic examination of the hydrolysate in solvents *A* and *C* showed the presence of 2,3,6-tri-*O*-methylglucose and 2,3-di-*O*-methylmannuronic acid (R_F 0.46). The remainder of the fraction was then converted into the methyl ester methyl glycoside by refluxing with 2% methanolic hydrogen chloride for 8 h. After reduction with lithium aluminium hydride[16] in tetrahydrofuran, the product was recovered, and hydrolysed with N sulphuric acid. Paper chromatography (solvent *C*) showed two components with R_F values corresponding to those of 2,3,6-tri-*O*-methylglucose and 2,3-di-*O*-methylmannose. Demethylation[13] of the syrup yielded both glucose and mannose. The fraction therefore was a methylated aldobiouronic acid.

Fraction 2. — Fraction 2 (440 mg, R_F 0.46) was converted into the methyl ester methyl glycoside, which was dissolved in tetrahydrofuran–ether (1:1), and reduced with an excess of lithium aluminum hydride[15]. The reduced product was recovered in the usual way, and subjected to hydrolysis with N sulphuric acid for 6 h at 100°. Paper chromatography (solvent *C*) and paper electrophoresis showed one spot having R_F and M_G values identical to those of 2,3-di-*O*-methylmannose. Demethylation of the sugar yielded mannose.

A portion of the reduced product (30 mg), when treated with freshly crystallized *p*-nitrobenzoyl chloride (80 mg) in dry pyridine (1 ml) in the usual way, gave the tri-*p*-nitrobenzoate, m.p. 194–195° (from ethyl acetate–methanol), $[\alpha]_D^{26}$ + 63.5 ±1° (*c* 1.0, chloroform). Reported values[17] for 2,3-di-*O*-methyl-1,4,6-tri-*O*-*p*-nitrobenzoyl-D-mannose are m.p. 194°; $[\alpha]_D$ + 65° (chloroform).

Fraction 3. — A portion (5 mg) of fraction 3 (28 mg, R_F 0.32) was hydrolysed with 2N sulphuric acid for 8 h at 100°. Paper electrophoretic and chromatographic examination (solvents *A* and *C*) of the hydrolysate failed to show any neutral sugars. The two components detected were the unhydrolysed fraction and 2,3-di-*O*-methylmannuronic acid. Reduction[16] and hydrolysis, as described for earlier fractions, produced only 2,3-di-*O*-methyl-D-mannose. Fraction 3 was, presumably, a di-mannuronic acid composed of two residues of 2,3-di-*O*-methylmannuronic acid.

ACKNOWLEDGMENTS

The author thanks Drs. F. R. Senti and Allene Jeanes for the polysaccharide, Dr. V. R. Harwalkar for the sedimentation analysis, and Mr. A. F. Peterkin for the boundary electrophoresis.

SUMMARY

The electrophoretic pattern indicated that the acidic polysaccharide of *Arthrobacter viscosus* was homogeneous. Methylation studies showed that the polysaccharide produced 2,3,6-tri-*O*-methyl-D-glucose (1 mole), 2,3,6-tri-*O*-methyl-D-galactose (1 mole), and 2,3-di-*O*-methyl-D-mannuronic acid (1 mole). Evidence is also presented for the presence of a small proportion of a methylated dimannuronic acid in the methylated cleavage products. The general structural features of the polysaccharide are discussed.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

AN EXTRACELLULAR POLYSACCHARIDE FROM *Xanthomonas campestris**

I. R. SIDDIQUI
Food Research Institute, Canada Department of Agriculture, Ottawa (Canada)

(Received, November 23rd, 1966)

INTRODUCTION

The polysaccharide produced by *Xanthomonas campestris* NRRL B-1459 has been a subject for publications on production[1], properties[2], composition[3], and structure[4]. The closely related polysaccharides of *Xanthomonas oryzae*[5] and *Xanthomonas phaseoli*[6] have also been investigated. These polysaccharides are similar in composition in that all contain D-glucose, D-mannose, and D-glucuronic acid as major constituents, with different amounts of pyruvic acid substituent[7] attached through an acetal linkage. In addition, the polysaccharide from *X. campestris* has been shown to contain *O*-acetyl groups[2].

The polysaccharide of *Xanthomonas campestris* was placed at our disposal through the kindness of Dr. F. R. Senti and Dr. Allene Jeanes. The present study was designed to establish the homogeneity of the polysaccharide, and to elucidate the nature of the glycosidic bonds between the component sugars by using methylation analysis.

RESULTS AND DISCUSSION

A purified, deacetylated[2] sample of the polysaccharide showed a single, symmetrical peak on boundary electrophoresis[8] (Fig. 1). The sedimentation[9] pattern (Fig. 2) indicated that the polymer existed as a soft gel, and was not monomolecularly dispersed. On the basis of the electrophoretic results, this material appeared to be a chemically homogeneous polysaccharide.

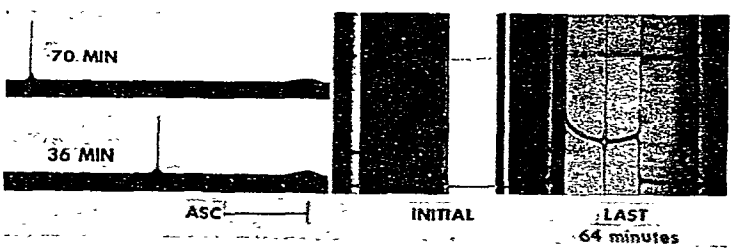


Fig. 1 Fig. 2

*Publication No. 50 of the Food Research Institute.

The polysaccharide was methylated first by the Haworth method, then by the modified Freudenberg method[10], and finally by the Purdie method. Methanolysis and hydrolysis of the methylated polysaccharide was followed by gas–liquid chromatography and preparative paper chromatography. The *O*-methyl sugars were quantitatively determined, in a portion of the hydrolysate, by gas–liquid chromatography[11] of their methyl glycosides. The overall, quantitative ratios between di-, tri-, and tetra-*O*-methylhexosides were 3:4:3. The ratios between the individual components, which were obtained by paper chromatography and gas–liquid chromatography, were in excellent agreement.

Analytical data for the major components are summarized as follows:

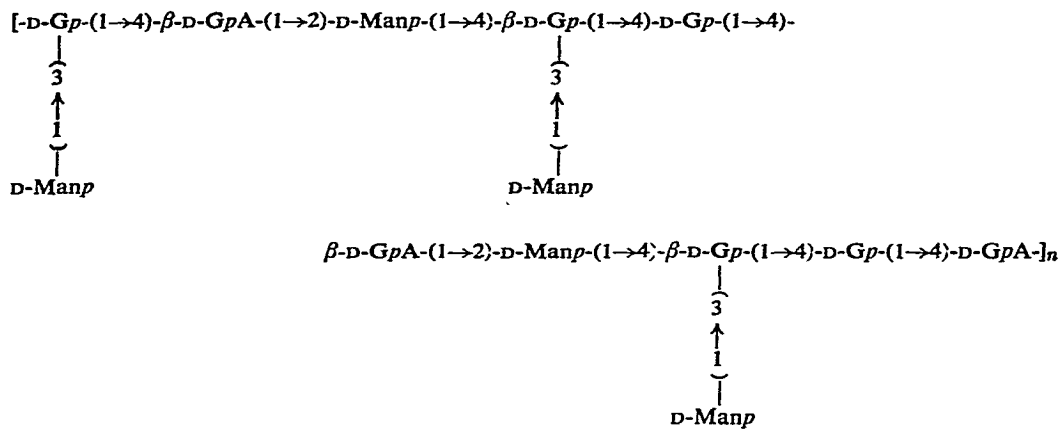
Component No.	*Component*	*Molar ratio*	*Mode of linkage*
1	2,3,4,6-tetra-*O*-methyl-D-mannose	3	D-Man*p*-(1→
2	2,3,6-tri-*O*-methyl-D-glucose	2	→4)-D-G*p*-(1→
3	3,4,6-tri-*O*-methyl-D-mannose	2	→2)-D-Man*p*-(1→
4	2,3-di-*O*-methyl-D-glucose	3	→4,6)-D-G*p*-(1→
5	2,3-di-*O*-methyl-D-glucuronic acid	3	→4)-D-G*p*A-(1→

All of the above sugars were obtained either in crystalline form, or were characterized as crystalline derivatives. In addition to these major components, 0.3 mol. of a 2,3-di-*O*-methylmannose, 0.5 mol. of an approximately equimolar mixture of a 3,4-di-*O*-methylmannose and a 2,3-di-*O*-methylglucose, and 0.5 mol. of a mixture of a 2- and a a 3-*O*-methylglucose, recognized only by paper chromatography and electrophoresis, were detected. The overall ratios of glucuronic acid:glucose:mannose, calculated from the present results, are 2:3.8:3.7.

The methylation analysis showed that the molecule has a highly branched structure. Thus, of the 14 sugar residues that make up an average repeating-unit of the polysaccharide, there are 3 terminal, non-reducing end-groups made up of mannose residues. There are 3 residues of glucose at which branching (through 3 and 4 positions) occurs in the polysaccharide. The 7 non-terminal residues consist of two (1→4)-linked glucose residues, two (1→2)-linked mannose residues, and three (1→4)-linked glucuronic acid residues. The remaining (14th) sugar residue of the repeating unit appears to be the terminal, D-glucose side-chain reported by Sloneker *et al.*[4] to carry a 4,6-*O*-(1-carboxyethylidene) substituent. This residue would be expected to remain intact through the methylation procedures and thus give rise to 2,3-di-*O*-methyl-D-glucose (0.25 mol. isolated), or, through steric effects, to the incompletely methylated 2- and 3-*O*-methyl-D-glucoses (0.5 mol. isolated).

The low optical rotations (*ca.* 0° and + 7.5°, respectively) for the unmethylated and methylated polysaccharides strongly indicated that the glycosidic bonds were predominantly of the β-D-type.

On the basis of these methylation results, and some of those of Sloneker *et al.* that established the sequence of certain sugar residues[4], the following structure is proposed for the repeating unit of the polysaccharide, based on 13 sugar residues.



Differences between this structure and that of Sloneker *et al.*[4] are, on the whole, minor. One significant variation is between the periodate data (especially formic acid) calculated from this formula, and the figures reported by Sloneker. Qualitatively, however, this repeating unit would account for the 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose that was isolated from the methylated and unmethylated polysaccharides. Isolation of an aldobiouronic acid consisting of all three sugars, with a D-glucose residue as the reducing end-group, is also consistent with the present formulation, as is the isolation of 2-*O*-β-D-glucopyranosyl-D-erythritol, which is indicative of the fact that a major portion of the D-glucose residues occur in pairs linked[4] β-(1→4).

The configuration of the glycosidic linkages are the same in both cases. The structural variations are based mainly on the quantitative differences between glucuronic acid and terminal mannose residues, as obtained by the two different techniques.

EXPERIMENTAL

Paper chromatography was performed by the descending method on Whatman No. 1 and 3 MM papers by using the organic phases of: (*A*) butyl alcohol–acetic acid–water (4:1:5); (*B*) butanone, saturated with water containing 2% of ammonia; (*C*) ethyl acetate–pyridine–water (8:2:1). Paper electrophoresis[12] was performed on Whatman No. 3 MM paper with 0.2M borate buffer (pH 10), at 800 volts for 2–3 h. Paper chromatograms and ionophoretograms were sprayed with (*A*) aniline hydrogen phthalate[13]; (*B*) naphthoresorcinol[14]. Evaporations were carried out at 35° with a rotary evaporator. Melting points are corrected, and rotations are equilibrium values.

Analysis of the acidic polysaccharide. — The polysaccharide had the following analysis: N, 0.44; P, 0.14; chloride, 0.25; sulphated ash, 12.0, and *O*-acetyl, *ca.* 5.0%. Electrophoretic examination[8] (Spinco Model H) of a purified, deacetylated[7] sample of the polysaccharide, as a 0.25% solution in 0.5M borate buffer (pH 9.2), showed a single, symmetrical peak (Fig. 1). The sedimentation[9] pattern of a 0.3% solution in

borate buffer (pH 9.2; ionic strength, 0.2; rotational speed, 59,780 rev./min) is shown in Fig. 2.

Methylation of the acidic polysaccharide. — The polysaccharide (6 g) dispersed in water (120 ml) was methylated by adding 30% sodium hydroxide (320 ml) and methyl sulphate (120 ml). The reagents were added at such a rate that the reaction mixture retained a near-neutral pH for the first few hours. The additions took place over several hours, and the solution was then stirred for 24 h. After 3 such additions during 3 days, a further five additions of 30% sodium hydroxide (100 ml) and methyl sulphate (35 ml) were made during 5 days. The reaction mixture was then cooled, and acidified with acetic acid, and the product was recovered by dialysis and freeze-drying. The freeze-dried material was dissolved in 30% sodium hydroxide (500 ml), and methylated by the dropwise addition of methyl sulphate (175 ml) during 5 h at 50–55°. After addition was complete, the reaction mixture was stirred overnight at room temperature. This process was repeated three times with 30% sodium hydroxide (150 ml) and methyl sulphate (55 ml) during 3 days, and 5 times with 30% sodium hydroxide (100 ml) and methyl sulphate (35 ml) during 5 days. The reaction mixture was then neutralized with sulphuric acid, under cooling, and the methylated product that could not be extracted with chloroform was recovered by dialysis and freeze-drying; yield, 6.1 g.

A thoroughly dried portion (4.7 g) of the partially methylated polysaccharide was treated with sodium and methyl iodide in liquid ammonia at −40°, according to the modified Freudenberg method[10]. After seven additions of the methylating agents (reaction time: sodium, 1 h; methyl iodide, 1.5 h) had been made, the ammonia was allowed to evaporate under anhydrous conditions, the last traces being removed *in vacuo* at 60°. After dialysis, the freeze-dried product showed OMe, 32.8%. A repetition of the methylation procedure gave the methylated product (3.4 g). This product was dissolved in chloroform (50 ml), and methyl iodide (120 ml) and silver oxide (20 g) were added in four equal portions, and the solution was refluxed and stirred overnight. Four further methylations with methyl iodide (100 ml) and silver oxide (10 g) gave a product (3.15 g) that had OMe, 40.9%; $[\alpha]_D^{26}$ +7.5° (*c* 4.2, chloroform).

Methanolysis and hydrolysis of the methylated polysaccharide. — The methylated polysaccharide (3 g) was dissolved in 3.5 % methanolic hydrogen chloride (150 ml), and the solution was refluxed for 24 h. After neutralization (silver carbonate), filtration, and evaporation, the syrupy glycosides were hydrolysed with N sulphuric acid for 38 h at 100°. The solution was neutralized with barium carbonate, filtered, and evaporated to a syrup (2.33 g).

A solution of the mixture of acidic and neutral sugars (2 g) in water (100 ml) was stirred for 24 h with Rexyn RG-50, (CO_3^{2-}, 100 ml). The slurry was then made into a column and washed with water (1 litre) to recover the neutral sugars (1.58 g). Elution with N ammonium carbonate (1 litre) gave the acidic sugars (495 mg). The latter were recovered by removing most of the ammonium carbonate by distillation *in vacuo* at 65–70° and the remainder by passing the solution over Rexyn AG-3.

Examination and identification of neutral sugars. — A portion (10 mg) of the

neutral methyl sugars was refluxed with 2% methanolic hydrogen chloride for 8 h, the acid was neutralized with silver carbonate, and the resulting methyl glycosides were examined[11] by g.l.c. with a 4-ft. column of 15% polyphenyl ether on Chromosorb W at 200° and an argon flow-rate of 100 ml/min. The areas under the peaks gave the overall, quantitative ratios of di-, tri-, and tetra-*O*-methylhexosides to be 3:4:3. Molar ratios of major, individual components were determined, with the exception of the di-*O*-methylhexoside fraction, on an 8-ft. column of 3% Carbowax 20M on 60/80 Chromosorb W, at 150° and a nitrogen flow-rate of 40 ml/min. The ratio for the latter fraction was evaluated from the combination of g.l.c. data and the actual weights of the fractions isolated. These ratios, converted into the nearest whole number, were 2,3,4,6-tetra-*O*-methyl-D-mannose (3); 2,3,6-tri-*O*-methyl-D-glucose (2); 3,4,6-tri-*O*-methyl-D-mannose (2); 2,6-di-*O*-methyl-D-glucose (3). In addition to these major components, 0.5 mol. of an approximately equimolar mixture of 2,3-di-*O*-methylglucose and 3,4-di-*O*-methylmannose, 0.3 mol. of 2,3-di-*O*-methylmannose, and 0.5 mol. of a monomethyl fraction (composed of an approximately equimolar mixture of 2- and 3-*O*-methylglucose) were also detected.

The remaining mixture of neutral sugars was separated on sheets of Whatman No. 1 paper, by using solvent *B*. The results of a typical separation, using sugar mixture (250 mg) and 6 sheets of paper, were as follows:

Component No.	*Component*	*Yield (mg)*
1	2,3,4,6-tetra-*O*-methyl-D-mannose	67
2	2,3,6-tri-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-mannose	84
3	2,3-di-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-mannose	10
4	2,6-di-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-mannose	61
5	2- and 3-*O*-methyl-D-glucose	9

Fraction 1. The syrup (50 mg) in methanol (3 ml) containing aniline (25 mg) was refluxed for 5 h. Evaporation of the solvent, with recrystallization of the product from ether, gave 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine, m.p. 144–145°, $[\alpha]_D^{26}$ −7.5 ± 1° (*c* 1.5, methanol); lit.[15], m.p. 144–145°, $[\alpha]_D$ −7.5° in methanol.

Fraction 2. This fraction, on demethylation[16], gave both glucose and mannose. Paper electrophoresis of the original mixture separated the sugars into a complexing fraction (*x*, 39 mg) and a non-complexing fraction (*y*, 38 mg).

Demethylation of Fraction *x* produced mannose. Paper chromatography (solvent *B*) showed a single component having R_F and M_G values, and colour reaction, identical with those of 3,4,6-tri-*O*-methyl-D-mannose. The syrup (33 mg) crystallized on seeding with an authentic specimen and, on recrystallization from ether, in the cold, gave 3,4,6-tri-*O*-methyl-D-mannose, m.p. and mixed m.p. 102–103°, $[\alpha]_D^{27}$ +35 ± 1° (*c* 1.1, methanol); lit.[17], m.p. 101–102°; $[\alpha]_D$ +36° (methanol).

The syrupy fraction *y* had an R_F value (0.56, solvent *B*) and colour reaction (*A*) identical with those of 2,3,6-tri-*O*-methyl-D-glucose. Demethylation[16] with boron trichloride gave glucose. Crystallization from isopropyl ether gave 2,3,6-tri-*O*-methyl-D-glucose, m.p. 104–106° (raised to 112–115° after several recrystallizations), $[\alpha]_D^{24}$ + 71 ± 1° (*c* 1.69, water); lit.[18], m.p. 121–123°, $[\alpha]_D$ + 70°. X-ray diffraction patterns of the product and 2,3,6-tri-*O*-methyl-D-glucose were identical.

A portion of the above material (10 mg) in dry pyridine (0.5 ml) was treated with freshly crystallized *p*-nitrobenzoyl chloride (25 mg), in the usual way, to give the di-*p*-nitrobenzoate[19], m.p. and mixed m.p. 191–192°.

Fraction 3. This fraction, on demethylation[16], produced glucose and mannose. Paper chromatography and paper electrophoresis showed that it was composed of a mixture of 2,3-di-*O*-methylglucose and 3,4-di-*O*-methylmannose, in an approximate ratio of 1:1.

Fraction 4. This fraction, on demethylation[16], produced glucose, with traces of mannose. The syrupy fraction showed $[\alpha]_D^{24}$ + 60° (*c* 3.2, water); R_F 0.17 (solvent *B*) and M_G 0.18 (elongated spot). The syrupy di-*O*-methyl fraction (50 mg) was kept in 2% methanolic hydrogen chloride for 24 h at room temperature. The solution was neutralized (silver carbonate), filtered, and evaporated to a syrup. Paper chromatography (solvent *B*, spray reagent *B*) showed 3 components having R_F values of 0.2, 0.59, and 0.72. The component (4 mg) having R_F 0.2 was a reducing component (spray *A*) and, on demethylation, gave mannose. Its paper chromatographic and ionophoretic behavior (M_G 0.14) strongly suggested that it was 2,3-di-*O*-methylmannose.

The components 2 and 3, which were non-reducing, were hydrolysed with 0.5N sulphuric acid for 5 h at 100°. After neutralisation, paper electrophoresis showed one spot (M_G 0.18) in each case.

The syrup (18 mg), corresponding to the component having R_F 0.72, was dissolved in dry pyridine (7 ml), and freshly crystallized *p*-phenylazobenzoyl chloride (450 mg) was added. The mixture was kept for 70 h at 48°. Isolation, in the usual way[20], gave 2,6-di-*O*-methyl-1,3,4-tri-*O*-*p*-phenylazobenzoyl-D-glucose, m.p. 210–211°, $[\alpha]_D^{26}$ − 336° (*c* 1.5, chloroform); lit.[20], m.p. 210–211°, $[\alpha]_D^{25}$ − 333° (chloroform).

Fraction 5. Paper chromatography (solvent *B*) showed that the component had R_F 0.06, which corresponded to a mono-*O*-methyl sugar; demethylation[16] gave glucose. Paper electrophoresis revealed two components having M_G values of 0.25 and 0.85, corresponding to 2-*O*-methyl-D-glucose and 3-*O*-methyl-D-glucose, respectively. Scarcity of material precluded further investigation.

Acidic Components. The methylated uronic acid fraction was separated on eight sheets of Whatman No. 1 paper by using solvent *A*. The two fractions (R_F 0.62 and 0.48) were recovered in a ratio of 1:3.2.

A portion (2 mg) of Fraction 1 (R_F 0.62) was hydrolysed with 2N sulphuric acid for 6 h at 100°. Paper chromatography (solvents *A* and *B*) and paper electrophoresis of the hydrolysate showed the presence of 2,3-di-*O*-methyl-D-glucuronic acid and 3,4,6-tri-*O*-methyl-D-mannose. The remainder of the fraction was converted into its

methyl ester methyl glycoside by refluxing with 2% methanolic hydrogen chloride for 12 h. A portion (50 mg) of the methyl ester methyl glycoside was kept in methanolic ammonia for 48 h. Evaporation of the solvent, with crystallization of the syrup from acetone and ether in the cold, gave a small yield of crystals having m.p. 250°. Another portion (25 mg) of the methyl ester methyl glycoside was reduced with lithium aluminum hydride[21] in tetrahydrofuran. The product thus recovered was hydrolysed with N sulphuric acid. Paper chromatography (solvent *B*) and electrophoresis of the hydrolysate showed two components having R_F and M_G values corresponding to those of a 2,3-di-*O*-methylglucose and a 3,4,6-tri-*O*-methylmannose.

The syrupy Fraction 2 (R_F 0.48) was treated with methanolic hydrogen chloride as described above, and a portion of the product (50 mg) was treated with freshly distilled phenylhydrazine (25 mg) in ethanol (3 ml). The solution, after being heated for 20 min at 80°, was freed from the solvent, and the residue was heated for 8 h at 110°. The reaction mixture was extracted under reflux with ether. Upon evaporation of the solvent, a crystalline product was formed; this was recrystallized from acetone and light petroleum to give the phenylhydrazide of methyl 2,3-di-*O*-methyl-D-glucosiduronic acid, m.p. and mixed m.p. 218–220°; lit.[22], m.p. 219–220°.

ACKNOWLEDGMENTS

I thank Drs. F. R. Senti and Allene Jeanes for providing the polysaccharide, and Dr. V. R. Harwalkar for the sedimentation analysis. Boundary electrophoresis was performed by Mr. A. F. Peterkin.

SUMMARY

Electrophoretic results indicated that the acidic polysaccharide of *Xanthomonas campestris* was homogeneous. Methylation studies showed that the polysaccharide had a highly branched structure. Hydrolysis of the methylated polysaccharide gave 2,3,4,6-tetra-*O*-methyl-D-mannose (3 moles); 2,3,6-tri-*O*-methyl-D-glucose (2 moles); 3,4,6-tri-*O*-methyl-D-mannose (2 moles); 2,3-di-*O*-methyl-D-glucuronic acid (3 moles); 2,6-di-*O*-methyl-D-glucose (3 moles). The significance of these structural features is discussed.

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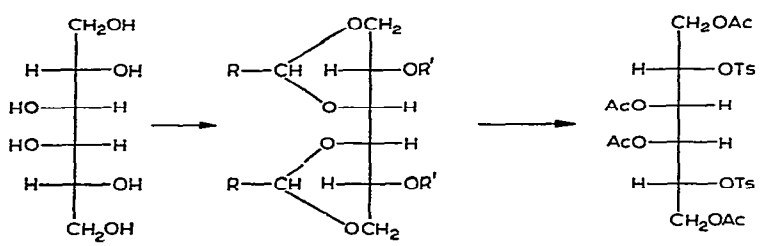
PREPARATION AND CHARACTERIZATION OF 1,3:4,6-DI-*O*-CHLOROETHYLIDENEGALACTITOL

H. B. SINCLAIR AND WILLIAM J. WHEADON
Northern Regional Research Laboratory, Peoria, Illinois 61604 (U.S.A.)*

(Received November 15th, 1966; in revised form January 23rd, 1967)

Although a few examples have been reported of the synthesis of chloro- or bromoethylidene acetals of carbohydrates[1], no investigations have been conducted on the structures of these acetals. When chloroacetaldehyde diethyl acetal was allowed to react with galactitol (dulcitol) in concentrated hydrochloric acid, a crystalline product was easily isolated in moderate yield. This paper describes the preparation and characterization of this product as 1,3:4,6-di-*O*-chloroethylidenegalactitol (**1**).

When galactitol was suspended in chloroacetaldehyde diethyl acetal and concentrated hydrochloric acid added, a slightly exothermic reaction occurred and the galactitol was rapidly dissolved. After about 18 h, the reaction mixture was diluted with water and, usually within 30 min, a fine powder slowly separated from solution. Recrystallization from 95% ethanol gave rather stout needles of compound **1** having m.p. 214–219°. Compound **1** gave analytical data corresponding to $C_{10}H_{16}Cl_2O_6$, and its i.r. spectrum revealed bands assigned to hydroxyl (3330 cm^{-1}), acetal (1140 cm^{-1}), and C-Cl[2] (775 cm^{-1}). Since treatment of **1** with excess *p*-toluenesulfonyl chloride in anhydrous pyridine gave a di-*O*-*p*-tolylsulfonyl derivative (**2**), two hydroxyl groups must be present. The corresponding diacetate (**3**) and dibenzoate (**4**) were prepared similarly. Although the combined data of chemical analysis, i.r. and u.v. spectra, and esterification reactions established that **1** was a di-*O*-chloroethylidene galactitol, they did not reveal the positions of the acetal groups.



1 R = $ClCH_2$, R' = H
2 R = $ClCH_2$, R' = Ts
3 R = $ClCH_2$, R' = Ac
4 R = $ClCH_2$, R' = Bz
5 R = Ph, R' = H
6 R = Ph, R' = Ts

7

*This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

When the di-*O*-chloroethylidene-di-*O*-*p*-tolylsulfonylgalactitol (**2**) was hydrolyzed with acid, no identifiable product was isolable. Ethanolic sulfuric acid (2.5N) did not hydrolyze the acetal **2** even after 16 h at reflux; it was recovered unaltered. Attempts to hydrolyze compound **2** with acid of increased strength were unsuccessful. Although a reaction did occur in 12N sulfuric acid, it resulted in a reddish-brown, intractable syrup.

The stability of **2** was further shown by its failure to be cleaved under the acetolysis procedure of Ness, Hann, and Hudson[3], which is usually employed to cleave the acid-stable methylene acetals. Even when the acetolysis mixture was kept on a steam bath for several h, unchanged **2** was recovered. By increasing the sulfuric acid concentration from 1% to about 7% and by heating the acetolysis mixture briefly under reflux, a product was isolated, in moderate yield, whose analysis was correct for a tetra-*O*-acetyl-di-*O*-*p*-tolylsulfonylgalactitol; it melted at 161–162° and had acetate and sulfonate bands in its i.r. spectrum.

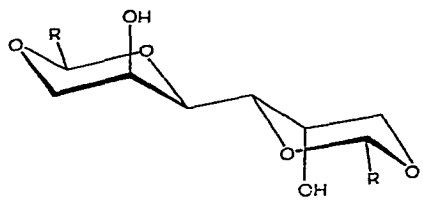
Matheson and Angyal[4] reported that acetolysis of 1,3:4,6-di-*O*-benzylidene-2,5-di-*O*-*p*-tolylsulfonylgalactitol gives 1,3,4,6-tetra-*O*-acetyl-2,5-di-*O*-*p*-tolylsulfonylgalactitol (**7**), m.p. 169°. The difference in m.p. between our product and that of Matheson and Angyal was large enough to make the identity of the two products questionable, and we consequently undertook to prepare substance **7** by Matheson and Angyal's procedure.

1,3:4,6-Di-*O*-benzylidenegalactitol (**5**) and its 2,5-di-*p*-toluenesulfonate (**6**) were prepared according to the directions of Haskins, Hann, and Hudson[5]. These compounds were revealed to be mixtures when examined by t.l.c. Even after several recrystallizations, neither the m.p. nor melting ranges attained the values reported by Haskins *et al.*, and the chromatographic patterns were unchanged. However, analyses and i.r. and u.v. spectroscopy indicated that the structural units of a di-*O*-benzylidenegalactitol (or a di-*O*-benzylidene-di-*O*-*p*-tolylsulfonylgalactitol) were present. Considering the evidence presented by Haskins *et al.*[5] on the structure of at least the major product in the di-*O*-benzylidenegalactitol preparation, we assumed that the mixture was chiefly 1,3:4,6-di-*O*-benzylidenegalactitol containing small proportions of other di-*O*-benzylidenegalactitols. Acetolysis of **6** by Matheson and Angyal's procedure gave 1,3,4,6-tetra-*O*-acetyl-2,5-di-*O*-*p*-tolylsulfonylgalactitol (**7**), which melted at 161–163°.

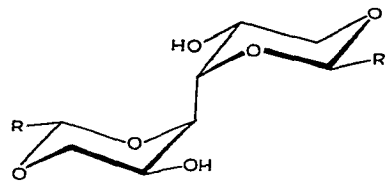
When the tetra-*O*-acetyl-di-*O*-*p*-tolylsulfonylgalactitols, prepared by acetolysis from the two di-*O*-*p*-tolylsulfonyl acetals **2** and **6**, were compared by i.r. (3800–200 cm^{-1}) and u.v. (230–400 mμ) spectroscopy, and by t.l.c. and mixed m.p., the products were identical.

Identification of 1,3,4,6-tetra-*O*-acetyl-2,5-di-*O*-*p*-tolylsulfonylgalactitol showed that the acetal substituents in **1** were either 1,3:4,6 or 1,6:3,4. The second structure seems unlikely, as it requires a 9- and 5-membered acetal, whereas the 1,3:4,6 structure involves two 6-membered rings. Although the evidence does not exclude the 1,6:3,4 structure, this seems unlikely in view of the experience with cyclic acetals[6]; and we consider that a 1,3:4,6-diacetal **1** is the correct structural assignment.

Two conformers, **8** and **9**, can be written for the 1,3:4,6-diacetals of galactitol, in which the 1,3-dioxane rings adopt a chair conformation and the group attached



8



9

to the acetal carbon atom assumes an equatorial position. By focusing attention on the galactitol chain, **8** is derived from an *anti*-carbon chain and **9**, from an *anti*-arrangement of hydroxyl groups.

If the 1,3-nonbonded interactions are considered alone, we would consider **8** to be favored, chiefly because of the C-3–C-4, H-1–H-6, and two acetal-hydrogen axial interactions in **9**. But both the ring oxygen–hydroxyl dipolar interactions and hydrogen bonding would, in our opinion, favor **9**. In particular, the steric arrangement for hydrogen bonding between the 2-OH and 4-O (acetal) and the 5-OH and 3-O (acetal) would not only be favored, but also result in an extremely compact structure. The energy lowering of these hydrogen bonds would, we believe, be sufficient to override the axial interactions. For these reasons, we prefer to depict 1,3:4,6-di-*O*-chloroethylidenegalactitol as **9**.

EXPERIMENTAL

T.l.c. was carried out on silica gel G with air-equilibrated plates of 0.25 mm thickness and the solvent systems specified. The spots were detected by spraying the plates with 5% ethanolic sulfuric acid and heating until the products charred. I.r. spectra were measured with a Perkin–Elmer Model 21 or 621 spectrophotometer*, in solution or KBr pellets as specified. U.v. spectra were measured with a Cary Model 14 spectrophotometer. Melting points were measured on a Mel-Temp apparatus in capillary tubes. All analytical samples were dried at 78° and 0.1–1 mm vacuum.

1,3:4,6-Di-O-chloroethylidenegalactitol (**1**). — To 32.0 g of galactitol slurried in 60 g of freshly distilled chloroacetaldehyde diethyl acetal was added with swirling 100 ml of concentrated hydrochloric acid; a slightly exothermic reaction occurred on addition of the acid. The flask was stoppered and shaken mechanically until the galactitol had dissolved (about 1 h). After having been kept overnight at room temperature, the reaction mixture was poured into 500 ml of water and stirred gently. Over a period of 4 h a fine, granular powder separated, which was removed by filtration and washed on a funnel with 100 ml of water. After brief drying in air, the powder was dried over

*The mention of firm names or trade products does not imply that they are endorsed by the U. S. Department of Agriculture over similar or unmentioned products.

phosphorus pentaoxide; yield 10.58 g, m.p. 209–215°. Recrystallization of 10.0 g from 300 ml of 95% ethanol gave 8.68 g of compound **1**, m.p. 214–219°; λ_{max}^{KBr} 3330 (OH), 1140 (acetal), 760 μ (C-Cl); R_F 0.6 (40:1 v/v chloroform–isopropyl alcohol, tailing reduced by adding a few drops of acetic acid).

Anal. Calc. for $C_{10}H_{16}Cl_2O_6$: C, 39.62; H, 5.32; Cl, 23.39. Found: C, 39.45; H, 5.35; Cl, 22.82, 23.13.

If the molar ratio of acetal to galactitol was below 2.0, the yield was very low; if the ratio was greater than 3.0, the yield was not improved, but isolation was more difficult because of a small amount of oil that separated.

1,3:4,6-Di-O-chloroethylidene-2,5-di-O-p-tolylsulfonylgalactitol (**2**). — To 5.0 g of compound **1** dissolved in 75 ml of anhydrous pyridine and cooled in an ice-water bath was added 8.65 g of *p*-toluenesulfonyl chloride in small portions over a period of 1–1.5 h. After 65 h at room temperature, the reaction product was isolated by slow addition of water (*ca.* four 1-ml portions) with cooling and agitation and final addition of about 400 ml of ice and water. The resultant solid was separated by filtration and dried in a vacuum desiccator over sodium hydroxide and sulfuric acid to yield 9.5 g of compound **2**, m.p. 223–224° (decomp.). The melting point depended upon the rate of heating and the temperature of insertion; melting points as low as 216° were found; and the m.p. of the product, mixed with starting compound, was depressed by about 8–11°.

An analytical sample was prepared by recrystallizing 2.0 g from a mixture of 90 ml of 95% ethanol and 100 ml of *N,N*-dimethylformamide; recovery 1.77 g of **2**, m.p. 223–224° (decomp.); λ_{max}^{KBr} 1360, 1180 (SO_3), 1150 (acetal), 879 (aryl), 760 μ (C-Cl); λ_{max}^{MeCN} 257 (shoulder) (ε 951), 262 (1168), 267 (shoulder) (1068), 274 mμ (951); R_F ~0.6 (10:1 v/v benzene–ethyl acetate).

Anal. Calc. for $C_{24}H_{28}Cl_2O_{10}S_2$: C, 47.14; H, 4.61; Cl, 11.59; S, 10.48. Found: C, 46.62; H, 4.54; Cl, 11.19; S, 10.25.

2,5-Di-O-acetyl-1,3:4,6-di-O-chloroethylidenegalactitol (**3**). — To 0.86 g of compound **1** dissolved in 10 ml anhydrous pyridine was added 2 ml of acetic anhydride. After 48 h at room temperature the pyridine solution (containing some crystalline material) was added to ice (*ca.* 150 ml). The solid (0.93 g) was separated by filtration and recrystallized from 450 ml of 95% ethanol to give compound **3**, m.p. 249–251°; λ_{max}^{KBr} 1740 (C=O), 1220 (C-O, ester), 1140 (acetal), 752 μ (C-Cl); R_F 0.6 (30:1 v/v chloroform–acetic acid).

Anal. Calc. for $C_{14}H_{20}Cl_2O_8$: C, 43.43; H, 5.20. Found: C, 43.44; H, 5.20.

2,5-Di-O-benzoyl-1,3:4,6-di-O-chloroethylidenegalactitol (**4**). — To 1 g of compound **1** dissolved in 15 ml of anhydrous pyridine was added 0.75 ml of benzoyl chloride. After 48 h at room temperature, the reaction mixture was filtered to separate a solid. This solid (0.34 g) was recrystallized from 90 ml of 95% ethanol to give compound **4** m.p. 221–222.5°; λ_{max}^{KBr} 1722, 1700 (C=O, doublet), 1260 (C-O ester), 1138 (acetal), 738 (C-Cl), 700 μ (aryl); λ_{max} in 95% ethanol, 275 (ε 947), 282 mμ (773); R_F 0.7 (30:1 v/v benzene–ethyl acetate).

Anal. Calc. for $C_{24}H_{24}Cl_2O_8$: C, 56.37; H, 4.73. Found: C, 56.76, H, 4.84.

1,3,4,6-Tetra-O-acetyl-2,5-di-O-p-tolylsulfonylgalactitol (7). — (*a*) *From compound* **2**. To a mixture of 10 ml of acetic anhydride, 5 ml of glacial acetic acid and 1 ml of concentrated sulfuric acid was added 876 mg of compound **2**, and the suspension was stirred for 3.5 h at room temperature. (In a separate experiment, when the acetolysis was terminated at this point by adding ice, unaltered starting compound **2** was recovered.) The mixture was rapidly heated to reflux and maintained for a total heating time of 0.5 h; the solution progressively turned brown, dark brown, reddish brown and reddish black. The hot reaction mixture was immediately poured into a flask on to about 75 ml of ice containing a small amount of sodium acetate. A reddish-brown syrup separated that clung to the walls of the flask. After 0.5 h the aqueous solution was decanted from the syrup, which was then dissolved in 50 ml of absolute ethanol by boiling, treating with decolorizing charcoal (*ca.* 5 min) and filtering while hot. On being cooled to room temperature, the brown filtrate crystallized slowly; the crystals were separated by filtration after storage for 1 h at −5°. After the tan crystals had been washed with 5 ml of absolute ethanol, they were redissolved in 15 ml of boiling absolute ethanol, and the solution was cooled overnight at −5°. The fine, matted needless of compound **7**, 173 mg, m.p. 158–160°, were separated by filtration.

An analytical sample was prepared by recrystallizing 148 mg from 20 ml of abs. ethanol; the recovery was 140 mg of compound **7**, m.p. 161–162°; λ_{max}^{KBr} 1748, 1734 (C=O, doublet), 1352, 1189 (SO_3), 880 μ (aryl); λ_{max} in 95% ethanol: 258 (shoulder) (ε 1020), 263 (1250), 266 (shoulder) (1200), 273 mμ (1090); R_F 0.4 (5:1 v/v benzene–ethyl acetate).

Anal. Calc. for $C_{28}H_{34}O_{14}S_2$: C, 51.05; H, 5.20. Found: C, 50.80; H, 5.46.

(*b*) *From compound* **6**. Compound **6** was acetolyzed according to the procedure of Matheson and Angyal[4], and the resultant compound **7**, after recrystallization from 95% ethanol, melted at 161–163°. This product **7** was compared with compound **7** from part (*a*) by i.r. spectrophotometry, both in solution and in KBr, by u.v. spectrophotometry, and by t.l.c.; the two were identical. The m.p. of a mixture of compound **7** from part (*a*) and part (*b*) was undepressed.

1,3:4,6-Di-O-benzylidenegalactitol (**5**). — The compound was prepared according to the directions of Haskins *et al.*[5]. The m.p. was 205–215°, and examination by t.l.c. (20:1 v/v carbon tetrachloride–methanol) revealed four spots, $R_F \sim$ 0, 0.2, 0.4 and 0.9. Several crystallizations did not sharpen the melting range, raise the m.p., or change the t.l.c. pattern. The once-recrystallized compound was employed for subsequent reactions. The i.r. and u.v. spectra were consistent with the assigned structure.

1,3:4,6-Di-O-benzylidene-2,5-di-O-p-tolylsulfonylgalactitol (**6**). — Compound **6** was prepared according to the directions of Haskins *et al.*[5]. Its m.p. (212–213°; decomp.) and i.r. and u.v. spectra were consistent with the assigned structure.

ACKNOWLEDGMENTS

We are indebted to Mrs. Clara McGrew, Mrs. Bonita Heaton, and Mrs. Lila P. Burgess for the microchemical analyses.

SUMMARY

When galactitol was allowed to react with chloroacetaldehyde diethyl acetal in concentrated hydrochloric acid, a di-*O*-chloroethylidenegalactitol was isolated. This product, on treatment with excess *p*-toluenesulfonyl chloride in pyridine, gave an extremely acid-stable di-*O*-chloroethylidene-di-*O*-*p*-tolylsulfonylgalactitol, which on acetolysis in boiling acetic anhydride–acetic acid containing sulfuric acid, was cleaved to give 1,3,4,6-tetra-*O*-acetyl-2,5-di-*O*-*p*-tolylsulfonylgalactitol in moderate yield. The identification of this tetra-*O*-acetyl-di-*O*-*p*-tolylsulfonylgalactitol supports the structure of 1,3:4,6-di-*O*-chloroethylidenegalactitol for the original diacetal.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

STUDIES ON URONIC ACID MATERIALS

PART XX*. THE VISCOSITY–MOLECULAR WEIGHT RELATIONSHIP FOR *Acacia* GUMS

D. M. W. ANDERSON AND S. RAHMAN**
Department of Chemistry, The University, Edinburgh 9 (Great Britain)
Received December 6th, 1966)

INTRODUCTION

Since the classical investigations of Kern[2], there have been significant advances in the understanding of polyelectrolyte behaviour, particularly of those synthetic polymers in which structure, and distributions of molecular weight can be controlled. Corresponding advances have not been made with natural polymers, such as plant gums. In particular, studies of molecular weight in the genus *Acacia* appear to have been confined to *A. cyanophylla*[3] gum and commercial samples of *A. senegal* gum[4].

The observation[5] that molecular weight fractions can be obtained from *A. senegal* gum by fractional precipitation with sodium sulphate led to a light-scattering study[4] of a specimen of that gum and three fractions obtained from it. The viscosity characteristics of these four materials have now been studied, and, in consequence, the constants involved in the Mark–Houwink[6] modification of Staudinger's viscosity–molecular weight relationship can be calculated.

In view of the indication[7] that the exudates from some *Acacia* species differ extensively from *A. senegal* gum in composition and properties, it was necessary to test whether the Staudinger constants for *A. senegal* gum would be valid for other *Acacia* species.

Weight-average molecular weights for specimens of *A. arabica* gum and *A. nubica* gum have therefore been determined by light-scattering. These particular species were selected as test cases for the following reasons: (*a*) their limiting-viscosity numbers are[7,8] lower than the values typical of *A. senegal* gum; (*b*) preliminary experiments[8] with molecular-sieve chromatography[9,10] indicate that *A. arabica* gum has a higher molecular weight than *A. senegal* gum; (*c*) structural studies of the gums from *A. arabica*[8] and *A. nubica*[11] indicate significant differences in structure from that of *A. senegal* gum.

EXPERIMENTAL AND RESULTS

The origin of the gum specimens from *A. arabica* and *A. nubica* have been given[7], and the fractionation procedure[5] with sodium sulphate has been described. Light-

*For Part XIX, see Ref. 1.
**Present address: Department of Chemistry, Chittagong College, East Pakistan.

scattering data have been published[4] for the specimen of *A. senegal* gum, and for the particular set of fractions used in the present study. The gum, and its fractions, were in the fully neutralised, sodium salt, form.

Viscosity measurements. — These were made at 25.00 ± 0.01° in suspended-level, Ubbelohde, dilution viscometers having solvent flow-times greater than 170 sec, and negligible, kinetic energy correction. The data obtained for *A. senegal* gum and its fractions, as sodium salts in M sodium chloride, are given in Table I; preliminary

TABLE I

VISCOSITY DATA FOR *A. senegal* GUM AND ITS FRACTIONS IN MOLAR SODIUM CHLORIDE SOLUTION AT 25.00°

Fraction 1		*Fraction 2*		*Fraction 3*		*Whole gum*	
Conc × 10^3 (*g/ml*)	$\frac{\eta Sp}{c}$ (*ml/g*)	*Conc* × 10^3 (*g/ml*)	$\frac{\eta Sp}{c}$ (*ml/g*)	*Conc* × 10^3 (*g/ml*)	$\frac{\eta Sp}{c}$ (*ml/g*)	*Conc* × 10^3 (*g/ml*)	$\frac{\eta Sp}{c}$ (*ml/g*)
17.83	38.4	19.63	14.6	18.81	14.0	19.99	20.5
12.74	31.9	14.02	13.8	13.45	13.5	14.29	19.4
8.91	30.3	9.82	13.1	9.41	13.3	10.00	18.5
5.94	28.3			6.27	13.1	6.67	17.7
By graphical extrapolation:							
lim ($c \to 0$)	25.4	lim ($c \to 0$)	12.9	lim ($c \to 0$)	12.5	lim ($c \to 0$)	16.4

experiments[12] had shown that the limiting-viscosity number was constant for concentrations of sodium chloride ranging from 2–8% w/v. Independent investigations, under comparable conditions to those described above, established limiting-viscosity numbers (ml/g) of 9.8 for *A. nubica*[7] gum, and 12.5 (ref. 7) and 9.9 (ref. 8) for different batches of the *A. arabica* gum (a large, bulk sample from many trees).

Calculation of the Staudinger constants for A. senegal *gum.* — The values of K' and a (following Flory's symbols[13]) in the Mark–Houwink[6] equation,

$$[\eta] = K' M_w^a \tag{1}$$

were found from the plot of log $[\eta]$ *versus* log M_w for each fraction and for the whole gum (Fig. 1). The values deduced are $K' = 1.3 \times 10^{-2}$ and $a = 0.54$.

The experimental data were also used to obtain the additional parameters given by the Flory–Fox[14] equation (2), where K and the molecular expansion factor (α)

$$[\eta] = K M_w^{\frac{1}{2}} \alpha^3 \tag{2}$$

$$K = \phi \left[\frac{(\bar{r}_o)^2}{M_w} \right]^{1.5} \tag{3}$$

$$\alpha = \frac{(\bar{r}^2)^{\frac{1}{2}}}{[(\bar{r}_o)^2]^{\frac{1}{2}}} \tag{4}$$

are given by equations 3 and 4. For equation 4, $(\bar{r}^2)^{\frac{1}{2}}$ is the root-mean-square, end-to-end distance of the molecule, and $[(\bar{r}_o)^2]^{\frac{1}{2}}$ its unperturbed dimension; ϕ is a universal constant, equal to 2.1×10^{23} when $[\eta]$ is expressed in ml/g. The expressions of Kurata

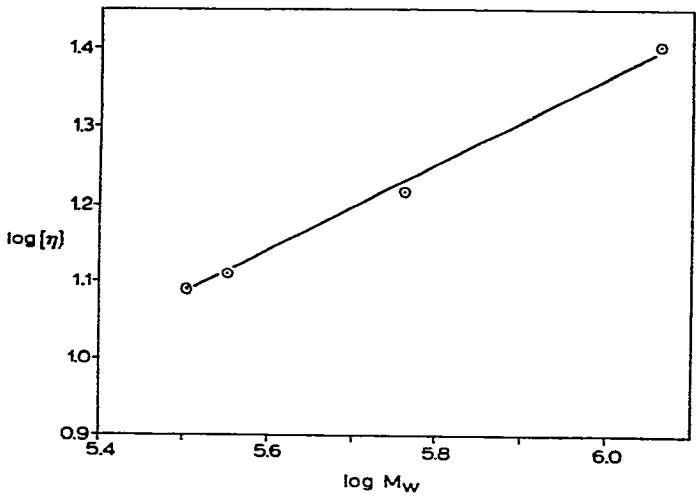


Fig. 1. Log $[\eta]$ *versus* log M_w for *Acacia senegal* gum and its fractions (in the sodium salt form).

and Stockmayer[15], and Stockmayer and Fixman[16], provide a graphical method of evaluating K from equation 5.

$$[\eta] = KM_w^{\frac{1}{2}} + 0.51\ \phi\ BM \tag{5}$$

By plotting $[\eta]M_w^{-\frac{1}{2}}$ *versus* $M_w^{\frac{1}{2}}$, a value of 1.96×10^{-2} was found for K by extrapolation, and substitution in equation 2 gave $\alpha = 1.042$. Values for $[(\bar{r}_o)^2]^{\frac{1}{2}}$ were then calculated from equation (3), and the theoretical values for $(\bar{r}^2)^{\frac{1}{2}}$ from equation (4) are compared in Table II with those determined experimentally.

TABLE II

M_w, $[\eta]$, $[(\bar{r}_o)^2]^{\frac{1}{2}}$, $(\bar{r}^2)^{\frac{1}{2}}$ FOR GUM FRACTIONS FROM *Acacia senegal*, AS THE SODIUM SALTS IN MOLAR SODIUM CHLORIDE

		Fractions			*Whole Gum*
		1	*2*	*3*	
$M_w \times 10^{-5}$		11.85	3.56	3.20	5.80
$[\eta]$ (ml/g)		25.4	12.9	12.5	16.4
$[(\bar{r}_o)^2]^{\frac{1}{2}}$ (Å),	theory[a]	494	271	257	345
$(\bar{r}^2)^{\frac{1}{2}}$ (Å),	theory[b]	514	282	267	360
	experimental	930	635	460	745
$\phi \times 10^{-23}$	theory	2.1	2.1	2.1	2.1
	experimental[c]	0.34	0.18	0.41	0.23

[a]Calculated from equation 3; [b]calculated from equation 4; [c]deduced from the experimental value for $(\bar{r}^2)^{\frac{1}{2}}$ by means of equations 2, 3, and 4.

Light-scattering measurements. — These were made (through the courtesy of Dr. G. Stainsby) in the Procter Department, Leeds University. The photometer, and the experimental procedure used to determine the values of M_w for *A. senegal* gum and its fractions, have been described[4]. The same experimental conditions were used to determine molecular weights for the gums from *A. arabica* and *A. nubica* in the form of their sodium salts in M sodium chloride. The plots of the reciprocal, reduced intensity-of-scatter (G_θ^{-1}) *versus* $\sin^2(\theta/2)$ for *A. arabica* gum are given in Fig. 2; Fig. 3 gives the corresponding data for *A. nubica* gum. Table III compares the data derived from these plots with those obtained previously[4] for *A. senegal* gum.

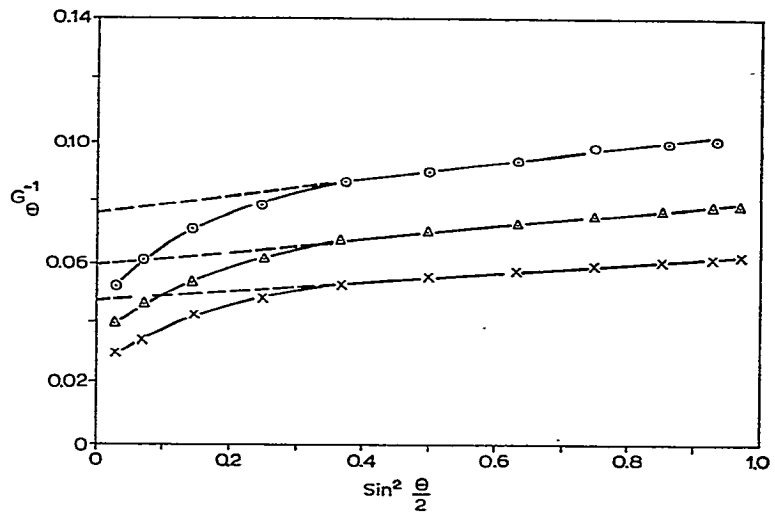


Fig. 2. The reciprocal, reduced intensity-of-scatter (G_θ^{-1}) *versus* $\sin^2(\theta/2)$ for *A. arabica* gum as the sodium salt in M sodium chloride.

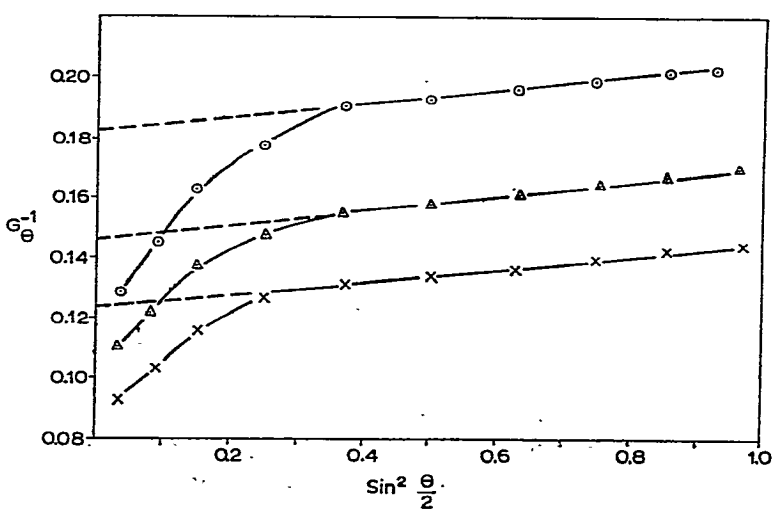


Fig. 3. The reciprocal, reduced intensity-of-scatter (G_θ^{-1}) *versus* $\sin^2(\theta/2)$ for *A. nubica* gum as the sodium salt in M sodium chloride.

DISCUSSION

The data obtained for *A. senegal* gum obey the Mark–Houwink relationship, and the value obtained for the modified Staudinger constant a falls within the usual range[13]. Although a linear plot of log $[\eta]$ *versus* log M_w (Fig. 1) was obtained from the four points available, two of these points are not widely separated. In any fractional

TABLE III

DATA FOR GUMS FROM *A. senegal*, *A. arabica*, AND *A. nubica*, AS SODIUM SALTS IN MOLAR SODIUM CHLORIDE

Acacia senegal			Acacia arabica			Acacia nubica		
Conc × 10^4 g/ml	Z	M_w × 10^{-5}	*Conc* × 10^4 g/ml	Z	M_w × 10^{-5}	*Conc* × 10^4 g/ml	Z	M_w × 10^{-5}
8.39	1.47	5.42	11.0	1.21	18.6	9.5	1.12	8.42
6.29	1.41	6.22	8.8	1.23	19.2	7.6	1.12	8.87
4.72	1.43	5.77	7.0	1.25	18.3	6.1	1.10	8.85
Mean Values:								
Z	1.44			1.23			1.11	
$M_w \times 10^{-5}$	5.80			18.9			8.71	
$(\bar{r}^2, \mathring{i})$, Å	739			550			340	
$[\eta]$, ml/g	16.4			9.9 (ref. 8)			9.8 (ref. 7)	
				12.5 (ref. 7)				

precipitation procedure, the number of fractions isolated is arbitrary[5], and it is intended to repeat the fractional precipitation procedure to obtain, for the present specimen of gum, an increased number of fractions separated as widely as possible within the molecular weight range. Although analyses have established[17] that the sample used in the present study is not atypical in terms of empirical composition, *A. senegal* is known[18] to be very variable botanically, and the values obtained for K' and a may not hold accurately for all specimens of *A. senegal* gum. Fractionation experiments with other specimens of the gum are therefore also necessary.

Further factors also require consideration. The equations relating M_w to $[\eta]$ presume that the different fractions involved are identical chemically, and differ only in molecular size. The composition of three fractions isolated from *A. senegal* gum in a separate experiment has been investigated[5]. Although small variations in chemical composition were detectable, particularly for the ratio of galactose to arabinose, the periodate oxidation data indicated that there are unlikely to be extensive differences in the general pattern of branching. In the fractionations of *A. senegal* gum that have been effected with sodium sulphate, a correlation has been noted[5,12] between the nitrogen content and the limiting-viscosity number of each fraction. The nitrogen content is known to arise from proteinaceous material. Although aggregation of the polysaccharide by the protein appears to be unlikely during viscosity determinations when M sodium chloride is used as solvent[5], further examination of the effect is desirable.

Caution must also be exercised in the application of these constants to degraded forms of the gum. It is clearly established (*cf.* refs. 19, 20, 5, and references cited therein) that the extent of the degradation that occurs under mild conditions, such as autohydrolysis, is much greater than would be expected to result from the removal of the available, labile residues at the periphery of what is now believed to be a relatively highly branched molecule[21]. Some deep-seated form of decomposition affecting the branched "core" (a term preferable[5] to "main chain") of the polymer must accompany modifications at its periphery. The values of K' and a for the whole gum may, therefore, not be valid for any of its degraded forms. Studies of the changes in size and shape of gum molecules during degradative processes are necessary, and molecular-sieve chromatography[5,9,10] may facilitate these.

The experimental values for $(\bar{r}^2)$ and ϕ differ markedly from the theoretical values (Table II) obtained from relationships developed for essentially linear macromolecules. These differences support the view that the polysaccharide entities in *A. senegal* gum are more highly branched[21] than previously believed. Veis and Eggenberger[22] also obtained higher experimental values for $(\bar{r}^2)^{\frac{1}{2}}$ than were predicted from theoretical relationships.

In conclusion, the data in Table III indicate that values of the modified Staudinger constants for *A. senegal* will not generally be applicable to other *Acacia* species: although less viscous, the gums from *A. nubica* and *A. arabica* are of higher molecular weight than *A. senegal*. These observations are supported by the light-scattering data for Z and $(\bar{r}^2)^{\frac{1}{2}}$, which indicate that the gum molecules in *A. nubica* and *A. arabica* are more compact and more highly branched (therefore tending to be even more spherical) than in *A. senegal*. These results are further supported by the results of chromatographic experiments with molecular sieves, and structural studies, currently in progress[8].

In Figs. 2 and 3, linear extrapolations have been used, consistent with the treatment previously adopted[4] (in preference to that of Benoit, Holtzer, and Doty[23]) for the corresponding data for *A. senegal* gum.

ACKNOWLEDGMENTS

We thank Professor Sir Edmund Hirst, C.B.E., F.R.S., for his interest in these studies, and the Director of Public Instructions, East Pakistan, for financial support (to S.R.). We gratefully acknowledge the helpful advice and kind co-operation of Dr. G. Stainsby, Leeds University, who placed his light-scattering apparatus at our disposal for these experiments.

SUMMARY

The relationship between the viscosity and molecular weight of a sample of *Acacia senegal* gum has been investigated, and values for the modified Staudinger constants have been calculated. Reasons for exercising caution in the use of these

constants are discussed. Determinations of the molecular weight of the gums from *Acacia nubica* and *Acacia arabica* have shown that the Staudinger constants for *Acacia senegal* gum will not be of general applicability within the *Acacia* genus.

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Carbohydrate Research
Elsevier Pubishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF BENZYL 6-*O*-BENZOYL-3,4-*O*-ISOPROPYLIDENE-β-D-GALACTOPYRANOSIDE AND OF 2-*O*-α-L-FUCOPYRANOSYL-D-GALACTOSE

A. LEVY, H. M. FLOWERS, AND N. SHARON

Departments of Biophysics and Organic Chemistry, The Weizmannn Institute of Science, Rehovoth (Israel).

(Received December 28th, 1966)

INTRODUCTION

The *O*-L-fucosyl-(1→2)-D-galactose sequence appears to be of wide distribution in Nature. The disaccharide 2-*O*-α-L-fucopyranosyl-D-galactose (**1**) has been isolated from milk oligosaccharides[1], and from blood-group substances[2] where it serves as an important antigenic determinant[2-4]. More recently, the trisaccharide *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-galactitol was isolated from porcine submaxillary glycoproteins, and it has been suggested that the above fucosylgalactose linkage is a very general one that may be present in a wide variety of glycoproteins[5]. The synthesis of the disaccharide **1** has therefore been undertaken, by using the techniques applied for the preparation of the related 2-*O*-α-L-fucopyranosyl-L-fucose[6].

RESULTS AND DISCUSSION

Treatment of benzyl β-D-galactopyranoside[7] with acetone and sulfuric acid[8] afforded the crystalline benzyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**2**) in good yield. A crystalline isomeric compound, to which the structure benzyl 4,6-*O*-isopropylidene-β-D-galactopyranoside (**3**) has been assigned, was obtained as a by-product. The structure of **2** was established as follows. Treatment with trityl chloride gave the amorphous 6-*O*-trityl derivative (**4**); under the same conditions, compound **3** did not react with trityl chloride. Acetylation of compound **2** gave the crystalline benzyl 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**5**); mild acid treatment of **5** converted it into crystalline benzyl 2,6-di-*O*-acetyl-β-D-galactopyranoside (**6**) which did not appear to have axial acetate groups (n.m.r.) and which consumed one mole of periodate per mole of **6**.

Treatment of **2** with slightly more than one equivalent of benzoyl chloride in pyridine gave a mixture from which crystalline benzyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**7**), crystalline benzyl 6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**8**), and unchanged **2** could be isolated. Compound **8** was obtained in 28% yield from **2**. The location of the benzoyloxy group at C-6 was established by deacetonation to yield benzyl 6-*O*-benzoyl-β-D-galactopyranoside (**9**) and by comparison of the product with benzyl 2-*O*-benzoyl-β-D-

galactopyranoside (**10**), which was obtained from benzyl 2-*O*-benzoyl-3,4-*O*-isopropylidene-6-*O*-trityl-β-D-galactopyranoside. Compounds **9** and **10** were both crystalline, but differed in m.p., optical rotation, and rate of migration on t.l.c. Their i.r. spectra showed absorptions for benzoate and alcoholic hydroxyl groups, but differed in their fine details of their patterns.

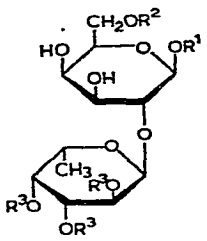
Condensation of compound **8** with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide, under conditions previously described[6], followed by chromatography on silica gel, afforded a mixture containing the desired benzyl 6-*O*-benzoyl-3,4-isopropylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside. The n.m.r. spectrum of this mixture showed the presence of benzyl, benzoate, and isopropylidene groups in approximately equimolar proportions. However, when the five aromatic protons of the benzyl group were used as reference, an excess of acetate protons (11 instead of 9) and *C*-methyl protons (3.5 instead of 3) over that to be expected from the structure of the desired disaccharide was observed in the n.m.r. spectrum. This probably indicates the presence of 2,3,4-tri-*O*-acetyl-L-fucose in the mixture. Attempts to purify the above disaccharide were unsuccessful. The mixture was therefore treated with a dilute solution of sulfuric acid in aqueous acetone in order to remove the isopropylidene group. Fractionation of this mixture on a column of silica gel gave syrupy benzyl 6-*O*-benzoyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**11**). Acetylation of **11** with acetic anhydride in pyridine led to the addition of two acetate groups to the molecule, one apparently axial and the other equatorial[9], as shown by n.m.r. spectroscopy. The high negative optical rotation of compound **11** and of its acetylation product strongly suggests that the interglycosidic linkage has the α-L configuration. This is analogous to the α-L-anomeric configuration of the disaccharides described in our recent paper on the synthesis of 2-*O*-α-L-fucopyranosyl-L-fucose[6].

Catalytic deacylation of **11** gave crystalline benzyl 2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranoside (**12**) in 54% yield from **11**. No crystalline material having a different anomeric configuration could be isolated from the mother liquor of **12**; thus there was no evidence for the presence of a disaccharide having a β-L-interglycosidic linkage. Catalytic hydrogenolysis of **12** in presence of palladium–charcoal in ethanol afforded the desired disaccharide **1**. The synthetic compound was identical with a sample of the natural product[1] in its optical rotation, i.r. spectrum, and rate of migration on t.l.c. and on paper chromatography in three solvent systems.

EXPERIMENTAL

Melting points were taken between glass slides on a Fisher–Johns apparatus and were corrected. Rotations were determined in semimicro tubes with a Perkin–Elmer No. 141 polarimeter or a Bendix polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer with tetramethylsilane as internal standard and chloroform-*d* as solvent, except where otherwise specified. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used and elution was stepwise, in order of increasing polarity of eluents.

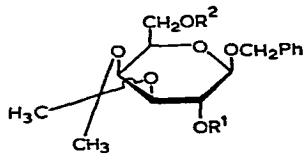
The proportion of weight of substance to weight of adsorbent added to the column was 1 to 50–100. Thin-layer chromatograms were run on Kieselgel G (E. Merck, Darmstadt). Analyses were performed in the Institute's Microanalytical Laboratory



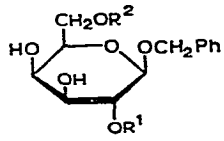
1 $R^1 = R^2 = R^3 = H$

11 $R^1 = CH_2Ph$; $R^2 = Bz$
$R^3 = Ac$

12 $R^1 = CH_2Ph$; $R^2 = R^3 = H$



2 $R^1 = R^2 = H$
4 $R^1 = H$; $R^2 = Tr$
5 $R^1 = R^2 = Ac$
7 $R^1 = R^2 = Bz$
8 $R^1 = H$; $R^2 = Bz$



6 $R^1 = R^2 = Ac$
9 $R^1 = H$; $R^2 = Bz$
10 $R^1 = Bz$ $R^2 = H$

under the direction of Mr. R. Heller. The i.r. spectra of all compounds prepared were examined on a Perkin–Elmer No. 137 infrared spectrophotometer, either in chloroform solution (when soluble), or as discs pressed in KBr, and were found to be in agreement with the structures given.

Benzyl 3,4-O-isopropylidene-β-D-galactopyranoside (**2**). — A solution of benzyl β-D-galactopyranoside[7] (4 g) in acetone (400 ml) containing conc. sulfuric acid (2 ml) was shaken for 5 h at room temperature and then was poured into a saturated aqueous solution of potassium hydrogen carbonate (60 ml). The insoluble salts were removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was extracted with chloroform, and the extract was concentrated *in vacuo*. The resulting syrup was fractionated by chromatography on silica gel. Elution with ether gave a syrupy product (0.4 g), apparently di-*O*-isopropylidene-D-galactose (n.m.r.), which was not investigated further, followed by fractions which crystallized on concentration *in vacuo*. The crystalline fractions were combined and recrystallized from acetone–petroleum ether, giving the title product. Yield 1.9 g (42%), m.p. 118–119°; $[\alpha]_D^{25}$ −6.0° (*c* 0.87, chloroform); n.m.r. data: τ 2.68 (5H, C_6H_5), τ 8.49 and τ 8.66 (6H, C-Me_2).

Anal. Calc. for $C_{16}H_{22}O_6$: C, 61.93; H, 7.09. Found: C, 61.81; H, 7.08.

Elution with 2:1 ethyl acetate–acetone afforded a second crystalline product (**3**), which was recrytallized from acetone–petroleum ether. Yield 0.33 g (7%), m.p. 124.5–125.5°, $[\alpha]_D^{25}$ −66.0° (*c* 1.03, chloroform); n.m.r. data: τ 2.68 (5H, C_6H_5), τ 8.55 (6H, C-Me_2).

Anal. Calc. for $C_{16}H_{22}O_6$: C, 61.93; H, 7.09. Found: C, 61.80; H, 6.97.

Benzyl 3,4-O-isopropylidene-6-O-trityl-β-D-galactopyranoside (**4**).—To a solution of **2** (0.5 g) in pyridine (5 ml) was added chlorotriphenylmethane (0.5 g). The solution was kept at room temperature for 24 h, and then poured into ice–water. The resulting mixture was extracted with chloroform, and the extract was washed twice with water. The chloroform solution was dried with calcium chloride and evaporated *in vacuo*. The resulting syrup was dissolved in benzene and chromatographed on a column of silica gel. Benzene–ether (10:1) eluted fractions which crystallized upon removal of the solvent (0.25 g, triphenylmethanol). Benzene–ether (5:1) eluted the title product which could not be crystallized (0.45 g, 50%); $[\alpha]_D^{26}$ −27° (*c* 0.74, chloroform). The product was homogeneous on t.l.c. in 9:1 benzene–methanol (R_F 0.69) and in 2:1 ether–ethyl acetate (R_F 0.71).

Anal. Calc. for $C_{35}H_{36}O_6$: C, 76.06; H, 6.57. Found: C, 76.87; H, 6.53.

The n.m.r. spectrum was in agreement with the structure given. Under the same conditions, compound **3** did not react with chlorotriphenylmethane, indicating the absence of a free primary hydroxyl group in this compound.

Benzyl 2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranoside (**5**). — Acetic anhydride (7 ml) was added to a solution of **2** (3.9 g) in pyridine (15 ml). The solution was kept overnight at room temperature, and then evaporated *in vacuo*. The syrup obtained was crystallized from acetone–petroleum ether. Yield 4.3 g (87%), m.p. 86–87°. A portion was recrystallized from acetone–petroleum ether, giving fine needles, m.p. 87–88°, $[\alpha]_D^{25}$ −4.4° (*c* 1.07, chloroform); n.m.r. data: τ 2.70 (5H, C_6H_5). τ 7.90, and τ 7.94 (6H, 2 equatorial OAc), τ 8.42 and τ 8.67 (6H, C-Me_2).

Anal. Calc. for $C_{20}H_{26}O_8$: C, 60.91; H, 6.60. Found: C, 60.96; H, 6.51.

Benzyl 2,6-di-O-acetyl-β-D-galactopyranoside (**6**). — A mixture of **5** (8.3 g) and Amberlite IRC 50(H^+) (6 g) in water (1000 ml) was boiled under reflux for 4 h. The resin was removed by filtration, and evaporation of the filtrate afforded crystalline material which was recrystallized from benzene; yield 3.7 g (50%), m.p. 115.5–116.5°, $[\alpha]_D^{25}$ −36° (*c* 1.00, chloroform); n.m.r. data: τ 2.70 (5H, C_6H_5), τ 7.90 and τ 7.92 (6H, 2 equatorial OAc)[9].

Anal. Calc. for $C_{17}H_{22}O_8$: C, 57.62; H, 6.27. Found: C, 57.40; H, 6.31.

On periodate oxidation[10] at 25°, compound **6** consumed 0.91 mole and 1.1 mole periodate per mole in 2 and 3.5 h, respectively, thus indicating the presence of 2 vicinal hydroxyl groups. After 22 h, 1.25 mole of periodate were consumed, apparently due to over-oxidation.

Benzyl 6-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranoside (**8**). — To a cooled solution of **2** (2.4 g, 7.74 mmoles) in pyridine (15 ml), benzoyl chloride (1 ml, 8.57 mmoles) was added, and the mixture was kept overnight at room temperature. It was then poured into ice–water, and the resulting mixture was extracted twice with chloroform. The extract was dried with calcium chloride and concentrated *in vacuo*. The residue was dissolved in benzene and passed through a column of silica gel. Benzene–ether (10:1) eluted fractions which crystallized on removal of the solvent. Recrystallization from benzene–petroleum ether gave 0.82 g (20%) of rosettes, m.p.

118–118.5°, $[\alpha]_D^{25}$ −18.5° (*c* 1.92, chloroform); the n.m.r. spectrum agreed with the structure benzyl 2,6-di-*O*-benzoyl-3,4-isopropylidene-β-D-galactopyranoside (**7**).

Anal. Calc. for $C_{30}H_{30}O_8$: C, 69.49; H. 5.79. Found: C, 69.63; H, 5.99.

Benzene–ether (5:1) eluted the title product **8**, which was crystallized from acetone–petroleum ether. Yield 0.93 g (29%), m.p. 114–114.5°, $[\alpha]_D^{25}$ −9.9° (*c* 1.59, chloroform).

Anal. Calc. for $C_{23}H_{26}O_7$: C, 66.66; H, 6.28. Found: C, 66.92; H, 6.48.

The starting material **2** (0.5 g, 21%) was recovered by elution with ethyl acetate–acetone (2:1).

Benzyl 6-O-benzoyl-β-D-galactopyranoside (**9**). — Acetic acid (15 ml, 50% v/v) was added to **8** (110 mg), and the solution was boiled for 1 h under reflux. Evaporation of the solution *in vacuo* gave **9** (88 mg, 87%), which migrated as a single spot on t.l.c. in 4:1 benzene–methanol (R_F 0.34). The product was crystallized from alcohol; m.p. 92–94°, $[\alpha]_D^{25}$ −22.5° (*c* 0.87, methanol). Repeated analysis of the product failed to give satisfactory agreement with the calculated values. λ_{max}^{KBr} 2.92, 3.03 (alcoholic OH), 5.85, 7.82 (benzoate), 13.3, 14.0, 14.3 mμ (phenyl).

N.m.r. data in methyl sulfoxide: τ 1.9−2.5 broad peaks, (5H, benzoate), τ 2.7 (5H, benzyl C_6H_5).

Benzyl 2-O-benzoyl-β-D-galactopyranoside (**10**). — Benzoyl chloride (0.2 ml) was added to a cooled solution of **4** (0.44 g) in pyridine (5 ml), and the mixture was kept for 24 h at room temperature. It was then poured into ice–water, and the resulting mixture was extracted twice with chloroform. The extract was dried with calcium chloride and concentrated *in vacuo*. The syrupy residue was dissolved in benzene and chromatographed on silica gel. Benzene–ether (10:1) eluted a syrupy product (0.3 g) which moved as a single spot on t.l.c. in 9:1 benzene–methanol (R_F 0.77).

Acetic acid (5 ml, 50% v/v) was added to a portion of the above syrup (60 mg), and the solution was boiled under reflux for 1 h. Evaporation of the solvent *in vacuo* yielded a solid residue which was extracted with benzene to remove triphenylmethanol. After drying, the residue weighed 16 mg (30% based on **4**), m.p. 174–176°. Recrystallization from acetone–petroleum ether gave the pure material, m.p. 175.5–176.5°, $[\alpha]_D^{25}$ −41° (*c* 0.95, methanol). On t.l.c. in 4:1 benzene–methanol, the product was homogeneous (R_F 0.30, different from that of **9**), λ_{max}^{KBr} 3.0 (alcoholic OH), 5.82, 7.80 (benzoate), 13.5, 14.0, 14.3 mμ (phenyl).

Anal. Calc. for $C_{20}H_{22}O_7$: C, 64.17; H, 5.88. Found: 63.74; H, 5.88.

Benzyl 6-O-benzoyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**11**). — A stirred solution of **8** (0.98 g, 2.4 mmoles) in 1:1 nitromethane–benzene (60 ml) was evaporated until *ca.* 20 ml of the solvent mixture had distilled, and then cooled to 60°. Mercuric cyanide (0.51 g, 2.0 mmoles) and 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[6] (0.71 g, 2.0 mmoles) were added, and the reaction mixture was kept for a total of 3 days at 60–70°, further additions of mercuric cyanide (2.0 mmoles) and of the bromide (2.0 mmoles) being made after 20 h and 44 h.

After cooling, the mixture was diluted with benzene (50 ml) and washed successively with a cold, saturated solution of sodium hydrogen carbonate and with water, dried with sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in

benzene and passed through a column of silica gel. Benzene–ether (9:1) eluted material which could not be crystallized. T.l.c. in 9:1 benzene–methanol showed the presence of a major component (R_F 0.85) and a minor one (R_F 0.70). Earlier fractions eluted from the column (0.76 g) were free from the slower-moving component.

The n.m.r. spectrum of the apparently homogeneous fractions (R_F 0.85) showed the following peaks: τ 2.74 (5H, benzyl C_6H_5) τ 2.0 and 2.5 (5H, Bz), τ 7.86–8.10 (11H, OAc), τ 8.50, 8.68 (6H, C-Me_2); doublets at τ 8.82, J 7 c.p.s. (approx. 1.5H, CH-Me) and at τ 9.16, J 7 c.p.s. (approx. 2H, CH-Me). The later fractions containing material having R_F 0.85 and R_F 0.70 showed relatively more acetate protons, but the isopropylidene, benzyl, and benzoate groups were still present.

The mixture obtained by pooling all the fractions containing material having R_F 0.85 (1.7 g) was dissolved in acetone (25 ml). After addition of an aqueous solution of sulfuric acid (1% w/v, 18 ml), the solution was stirred for 30 min at 50–60°. The cooled solution was neutralized with an aqueous solution of sodium carbonate (10% w/v) and extracted with chloroform (3 × 30 ml). The combined chloroform extracts were washed twice with water and dried with anhydrous calcium chloride. Removal of the solvent left a syrup (1.60 g), which was dissolved in benzene and chromatographed on silica gel. Benzene–ether (3:1) eluted a total of 0.6 g of material shown by t.l.c. and n.m.r. to contain compounds in which the isopropylidene group was still intact. Benzene–ether (1:1) eluted fractions weighing 0.45 g, and showing on t.l.c. in 9:1 benzene–methanol R_F 0.2. The fractions eluted with 3:1 benzene–ether were treated again with acetone–sulfuric acid, affording an additional quantity (0.15 g) of the substance having R_F 0.2. The product (total weight 0.60 g, overall yield of 41% from **8**) was essentially homogeneous on t.l.c., but could not be induced to crystallize; $[\alpha]_D^{25}$ −61.0° (*c* 1.0, chloroform); n.m.r. data: τ 2.0, 2.5 (5H, benzoate), τ 2.74 (5H, benzyl C_6H_5), τ 7.85–8.02 (9H, OAc), and a doublet at τ 9.20, J 7.0 c.p.s. (3H, CH-Me). Acetylation of a portion of this product with acetic anhydride and pyridine gave a syrup, $[\alpha]_{26}^{D}$ −106.3° (*c* 0.80, chloroform). Comparison of the n.m.r. spectrum of this compound with that of **11** revealed an additional peak at τ 7.85 (3H, axial OAc); the peak at τ 8.02 (equatorial OAc) was accentuated. Integration of the acetate peaks gave a total of 15 protons, by using the benzyl protons as reference.

Benzyl 2-O-α-L-fucopyranosyl-β-D-galactopyranoside (**12**). — A portion of **11** (0.55 g) was dissolved in anhydrous methanol (3 ml) containing a catalytic amount of sodium methoxide. The clear solution was kept overnight at room temperature, neutralized with a drop of aqueous acetic acid (25% w/v), and concentrated *in vacuo* to a syrup. The syrup was dissolved in a small volume of water and passed through a column of Dowex-50 (H^+). Evaporation of the emerging aqueous solution afforded a syrup which crystallized on the addition of absolute alcohol. Yield 0.20 g (54%), m.p. 196–199°. Recrystallization from ethanol gave needles, m.p. 205–207°, $[\alpha]_D^{25}$ −97.8° (*c* 0.92, water); n.m.r. data in D_2O: τ 2.70 (5H, C_6H_5); doublets at τ 4.9, J 3.0 c.p.s. (H, α-L-linked disaccharide); τ 9.20, J 6.5 c.p.s. (3H, CH-Me).

Anal. Calc. for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 54.48; H, 7.10.

2-O-α-L-Fucopyranosyl-D-galactose (**1**). — A portion of **12** (0.080 g) was dissolved

in alcohol (95% v/v, 50 ml) and hydrogenolyzed in the presence of palladium–charcoal (10%) at 50 lb/in^2 for 24 h. The catalyst was removed by filtration, and the filtrate concentrated *in vacuo*. Addition of absolute alcohol (1 ml) gave an amorphous solid (0.045 g). The alcoholic solution was evaporated to a syrup (0.030 g). The solid and syrup were examined separately by t.l.c. (4:5:3 butanol–acetone–water) and were found to be homogeneous and chemically identical.

On paper chromatography (descending), $R_{lactose}$ 1.58 (10:4:3 ethyl acetate–pyridine–water), 1.83 (25:6:25 butanol–acetic acid–water), 2.3 (4:1:1 butanol–ethanol–water), indistinguishable from the $R_{lactose}$ of an authentic specimen[1]; $[\alpha]_D^{25}$ −56.7° (*c* 1.20, water) (lit.[1] −56.5°).

Anal. Calc. for $C_{12}H_{22}O_{10}\cdot H_2O$: C, 41.86; H, 7.32. Found: C, 41.49; H, 6.96.

On paper chromatograms, in three solvent systems, as well as on t.l.c., **1** migrated at the same speed as an authentic specimen of 2-O-α-L-fucopyranosyl-D-galactose[1].

ACKNOWLEDGMENTS

This investigation was supported by a research grant (AI-03528) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service. We wish to thank Prof. R. Kuhn and Dr. A. Gauhe for a gift of 2-O-α-L-fucopyranosyl-D-galactose.

SUMMARY

Benzyl β-D-galactopyranoside was converted into the crystalline 3,4-O-isopropylidene derivative (**2**), which was selectively benzoylated to give crystalline benzyl 6-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranoside (**8**). The structure of **8** was established by various reactions, which included the preparation of benzyl 6-O-benzoyl-β-D-galactopyranoside (**9**) and of benzyl 2-O-benzoyl-β-D-galactopyranoside (**10**). Condensation of **8** with 2,3,4-tri-O-acetyl-α-L-fucopyranosyl bromide, followed by removal of the isopropylidene benzoyl and acetate groups, afforded crystalline benzyl 2-O-α-L-fucopyranosyl-β-D-galactopyranoside (**12**). Catalytic hydrogenolysis of **12** gave the disaccharide 2-O-α-L-fucopyranosyl-D-galactose (**1**), which was identical with the natural product.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF SOLABIOSE (3-*O*-β-D-GLUCOPYRANOSYL-D-GALACTOSE) AND 3-*O*-(β-D-GLUCOPYRANOSYLURONIC ACID)-D-GALACTOSE

H. M. FLOWERS
The Department of Organic Chemistry, The Weizmann Institute of Science, Rehovoth (Israel)
(Received January 3rd, 1967)

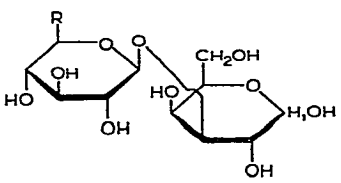
INTRODUCTION

The disaccharide solabiose was isolated from partial acid hydrolyzates of the alkaloid glycoside solamine and characterized as 3-*O*-β-D-glucopyranosyl-D-galactose[1] (**1**). It was later prepared from the extracellular polysaccharide of phytopathogenic *Xanthomas spp*[2]. The corresponding uronic acid derivative, 3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (**2**), has been recently prepared from chondroitin 4-sulfate, where the glucosyluronogalactose sequence has been shown to play an important part in the linkage of the polysaccharide to protein[3]. The same disaccharide was also isolated from partial acid hydrolyzates of the capsular polysaccharide[4] of *E. coli* K12. As part of a program concerned with the study of glycosides and oligosaccharides of D-galactose, it was considered of interest to synthesize chemically these two disaccharides.

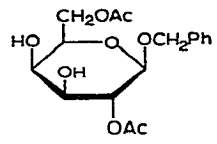
RESULTS AND DISCUSSION

Treatment of benzyl 2,6-di-*O*-acetyl-β-D-galactopyranoside[5] (**3**) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide[6] (**4**) in nitromethane–benzene solution in the presence of mercuric cyanide afforded benzyl 2,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**5**) in 34% yield. A similar treatment of **3** with (methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate) bromide[7] (**6**) gave the corresponding methyl uronate (**7**). The selective substitution of the D-glucopyranosyl residue and of the D-glucopyranosyluronic residue at C-3 of the galactopyranose ring in **5** and **7**, respectively, (rather than at C-4 which is also available for substitution), is to be expected owing to the considerably lower reactivity of the hydroxyl group at C-4 compared with that of C-3 of the galactopyranose moiety[8]. Acetylation of **5** and **7** and comparison of their n.m.r. spectra with those of the acetylated products showed that an axial acetate group had, in each case, been added on acetylation[9]. The n.m.r. spectra were consistent with the *C1* conformation of the galactopyranoside ring, and with all the acetate groups being in equatorial conformation in compounds **5** and **7**; an additional (axial) acetate group, added on acetylation, can only be located at C-4 in this conformation. These results confirm structures **5** and **7**, if it is assumed that migration of the acetate group from C-2 to C-3 of the galactopyranoside ring has not occurred.

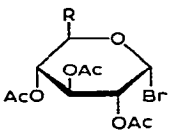
Catalytic deacetylation of **5** afforded benzyl 3-*O*-β-D-glucopyranosyl-β-D-galactopyranoside (**8**), while the uronate **7** gave benzyl 3-*O*-(methyl β-D-glucopyranosyluronate)-β-D-galactopyranoside (**9**). The free uronic acid, benzyl 3-*O*-(β-D-glucopyranosyluronic acid)-β-D-galactopyranoside (**10**), was prepared by catalytic deacetylation of **7**, followed directly by alkaline saponification, without isolation of the intermediate uronic ester (**9**).



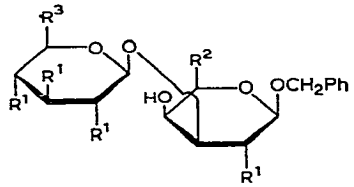
1 R = CH_2OH
2 R = CO_2H
11 R = CO_2Me



(3)



4 R = CH_2OAc
6 R = CO_2Me



5 R^1 = OAc ; R^2 = R^3 = CH_2OAc
7 R^1 = OAc ; R^2 = CH_2OAc ; R^3 = CO_2Me
8 R^1 = OH ; R^2 = R^3 = CH_2OH
9 R^1 = OH ; R^2 = CH_2OH ; R^3 = CO_2Me
10 R^1 = OH ; R^2 = CH_2OH ; R^3 = CO_2H

The structure of the glycoside **8** and the absence of acetate migration during the reaction of compounds **3** and **4** were confirmed by periodate oxidation. The periodate consumption of **8** (2 moles per mole of disaccharide) was equal to that required theoretically for the glucopyranoside moiety. Hence, the galactopyranoside moiety could only be substituted at C-3, since any other point of substitution would give a larger periodate consumption (3 or 4 moles per mole of disaccharide). Since the uronate **7** was converted into the same glycoside **8** by reduction of the carboxymethyl group at C-6 to hydroxymethyl and cleavage of the acetate groups (achieved by treatment with lithium aluminium hydride in tetrahydrofuran solution), the structure of **7** was also indirectly confirmed.

Catalytic hydrogenolysis of the benzyl glycosides (**8**, **9**, and **10**) gave the corresponding disaccharides (**1**, **2**, and **11**). The disaccharide **1** had the same rate of migration as a specimen of the natural product[1] by t.l.c. in two solvent systems and by paper chromatography in three solvent systems. Their melting points and optical rotations were essentially identical. The chromatographic mobility and optical rotation of the uronic disaccharide (**2**) were in very good agreement with the published values[2].

EXPERIMENTAL

Melting points were observed between glass slides on a Fisher–Johns apparatus, and were corrected. Rotations were determined in semimicro tubes with a Perkin–Elmer No. 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, tetramethylsilane as internal standard, and chloroform-*d* as solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used and elution was stepwise, in order of increasing polarity of the eluents. The proportion of weight of substance to weight of absorbent added to the column was 1 to 50–100. The fractions eluted were 2 ml/g wt. of the column. Thin-layer chromatograms were performed on Kieselgel G (E. Merck, Darmstadt). *R* values refer to the substance indicated in subscript. Analyses were made in the Institute's Microanalytical Laboratory under the direction of Mr. R. Heller.

Benzyl 2,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**5**). — A stirred solution of benzyl 2,6-di-*O*-acetyl-β-D-galactopyranoside[5] (**3**, 0.83 g, 2.3 mmoles) in 1:1 nitromethane–benzene (60 ml) was evaporated until *ca.* 20 ml of the solvent mixture had distilled, and cooled to 60°. Mercuric cyanide (0.63 g, 2.5 mmoles) and tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4**, 0.66 g, 1.6 mmoles) were added and the reaction continued overnight at 60°. T.l.c. in ether–ethyl acetate (9:1) showed that the bromide (**4**) had reacted completely. A further portion (0.33 g, 0.8 mmoles) of the bromide (**4**) was now added and the reaction continued for 24 h. Examination of the mixture by t.l.c. in benzene–methanol (4:1) showed that a small quantity of unchanged **3** remained. Further portions of mercuric cyanide (0.3 g) and of **4** (0.33 g) were added, and the reaction continued for 24 h at 60°.

After being cooled, the mixture was diluted with benzene (50 ml) and washed successively with a cold, saturated solution of sodium hydrogen carbonate and water, dried with sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in benzene and chromatographed on silica gel. Benzene–ether (3:1) eluted fractions (200 mg) which did not crystallize. Benzene–ether (1:1) eluted fractions which crystallized spontaneously on evaporation of the solvent (total yield 1.0 g). Recrystallization from ether gave needles (0.54 g, 34%, m.p. 193–195°). The m.p. was unaffected by recrystallization from acetone–ether; $[\alpha]_D^{25}$ −35.2° (*c* 1.05, chloroform); n.m.r. data : τ 2.73 (5H, C_6H_5) and τ 7.87–8.02 (18H, six OAc groups).

Anal. Calc. for $C_{31}H_{40}O_{17}$: C, 54.38; H, 5.89. Found: C, 54.17; H, 5.96.

Acetylation of a portion of this product (**5**, 90 mg) in acetic anhydride–pyridine and evaporation of the solution gave a syrup (95 mg) which could not be crystallized. On t.l.c. in benzene–methanol (4:1), it was homogeneous (R_5 1.4); $[\alpha]_D^{25}$ −23.3° (*c* 1.65, chloroform); n.m.r. data: τ 2.73 (5H, C_6H_5), τ 7.83 and τ 7.88–8.00 (21H, a total of 7 OAc groups).

Benzyl 3-O-β-D-glucopyranosyl-β-D-galactopyranoside (**8**). — (i) A solution of **5** (0.25 g) in methanol (1 ml), containing a catalytic amount of sodium methoxide, was kept overnight at room temperature, and then for 1 h at 5°. The crystalline

precipitate which deposited was separated by filtration, washed with a little cold methanol, and recrystallized from alcohol to give 0.12 g (74%) of fine needles, m.p. 200–202°; $[\alpha]_D^{25}$ −20.7° (*c* 0.90, water).

Anal: Calc. for $C_{19}H_{28}O_{11} \cdot H_2O$: C, 50.66; H, 6.66. Found: C, 50.94; H, 6.94.

(ii) A solution of **7** (100 mg) in anhydrous tetrahydrofuran (10 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (200 mg) in anhydrous tetrahydrofuran (20 ml) cooled to 5–10°. The reaction mixture was warmed slowly to the b.p. and boiled for 1 h under reflux. After cooling, ethyl acetate (1 ml) was added dropwise, followed by acetic acid–water (25% v/v, 5 ml). An amorphous precipitate was separated by filtration and the clear filtrate was concentrated to a few ml and passed through a column of Dowex-50 (H^+); the column was washed with water and concentration of the aqueous solution *in vacuo* afforded a syrup (60 mg). On addition of alcohol, the product crystallized; m.p. 200–202°, which was not depressed by admixture with the product described under (i); $[\alpha]_D^{25}$ −20.0° (*c* 0.80, water). The product was indistinguishable from **8** on t.l.c. in chloroform–methanol–water (65:25:4) (R_F 0.1) and in butanol–acetone–water (4:5:3) ($R_{lactose}$ 2.0). Periodate oxidation[10] of compound **8** showed the consumption of 2.0 moles of periodate per mole of disaccharide during 18 h at 25°; prolongation of the reaction for 3 days gave an overoxidation corresponding to an additional 0.3 mole.

3-O-β-D-Glucopyranosyl-D-galactose (**1**). — A solution of **8** (0.10 g) in ethanol 95% (50 ml), containing palladium–charcoal (10%) catalyst, was hydrogenolyzed at 50 lb/in^2 for 24 h. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo* to give a syrup. Addition of abs. ethanol gave needles, m.p. 203–205°, $[\alpha]_D^{22}$ +40.0° (*c* 0.80, water) [Kuhn *et al.*[1] reported m.p. 200°, $[\alpha]_D$ +40.7° (water)]; t.l.c. in butanol–acetone–water (4:5:3) and in butanol–pyridine–water (3:2:1): $R_{lactose}$ 1.0 (indistinguishable from an authentic specimen of **1**). Chromatography on Whatman paper No. 1 (descending) in butanol–pyridine–water (3:1:1), $R_{lactose}$ 1.36; in ethyl acetate–acetic acid–formic acid–water (18:3:1:4), R_{gal} 0.62; and in ethyl acetate–acetic acid–water[2] (9:2:2), $R_{lactose}$ 1.14. The product was indistinguishable from an authentic specimen in all three solvent systems.

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.42. Found: C, 41.90; H, 6.15.

Benzyl 2,6-di-O-acetyl-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-β-D-galactopyranoside (**7**). — Methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate) bromide (**6**, 1.4 molar equivalents total) was added in three separate portions to a solution of **3** (1.44 g) in nitromethane–benzene (1:1) in the presence of mercuric cyanide as described for **5**. The mixture was processed in the usual way, dissolved in benzene, and chromatographed on silica gel. Benzene–ether (1:1) eluted fractions which crystallized on concentration *in vacuo* and addition of ether to the resulting syrup; yield 0.70 g (26%), m.p. 210–212°. Recrystallization from methanol gave needles (0.56 g), m.p. 212–214°, $[\alpha]_D^{30}$ −57.6° (*c* 0.85, chloroform); n.m.r. data: τ 2.74 (5H, C_6H_5), τ 6.28 (3H, CO_2CH_3), τ 7.88 (3H, equatorial OAc group) and τ 7.95–8.0 (12H, 4 equatorial OAc groups).

Anal. Calc. for $C_{30}H_{38}O_{17}$: C, 53.37; H, 5.71. Found: C, 53.61; H, 5.79.

A portion of **7** (70 mg) was acetylated with acetic anhydride in pyridine. The solution was concentrated *in vacuo* to a syrup. Addition of ether gave needles, m.p. 154–155°, $[\alpha]_D^{30}$ −43.3° (*c* 0.90, chloroform); n.m.r. data: τ 2.74 (5H, C_6H_5), τ 6.26 (3H, CO_2CH_3), τ 7.83 (3H, axial OAc group), τ 7.90–9.0 (15H, five equatorial OAc groups).

*Benzyl 3-*O*-(methyl β-*D*-glucopyranosyluronate)-β-*D*-galactopyranoside* (**9**). — A solution of **7** (0.25 g) in abs. methanol (2 ml), containing a catalytic amount of sodium methoxide, was kept overnight at room temperature. An amorphous precipitate was formed, but was dissolved on acidification with a drop of acetic acid. The solution was evaporated to give a syrup and dissolved in the minimum volume of warm water. On cooling, a white crystalline product precipitated. Yield 0.15 g (88%), m.p. 202–204°. Recrystallization from alcohol gave needles, m.p. 205–207°, $[\alpha]_D^{22}$ −38.5° (*c* 1.00, water).

Anal. Calc. for $C_{20}H_{28}O_{12}$: C, 52.17, H, 6.13. Found: C, 51.91; H, 6.25.

*Benzyl 3-*O*-(β-*D*-glucopyranosyluronic acid)-β-*D*-galactopyranoside* (**10**). — A solution of **7** (200 mg) in methanol (2 ml) containing a catalytic amount of sodium methoxide was kept overnight at room temperature. A few drops of an aqueous solution of 2N sodium hydroxide were added, and the progress of the reaction was followed by t.l.c. in butanol–acetone–water (4:5:3). The initial single spot corresponding to the ester (R_F 0.40) rapidly disappeared with the concomitant appearance of a slower-migrating spot (R_F 0.1). After 30 min, complete saponification of the ester had been achieved. The solution was neutralized with acetic acid, concentrated to a syrup, and redissolved in water. After passage through Dowex-50 (H^+), the solution was passed through Sephadex G-25 to remove any inorganic salts present. The eluant from the gel filtration was tested for uronic acid with carbazole[11], and the requisite fractions were pooled and lyophilized. The residue (110 mg, 82%) crystallized from alcohol as prisms, m.p. 160–162° (dec.), $[\alpha]_D^{25}$ −41.3° (*c* 1.00, water).

Anal. Calc. for $C_{19}H_{26}O_{12}$: C, 51.11; H, 5.87. Found: C, 51.08; H, 6.07.

*3-*O*-(β-*D*-Glucopyranosyluronic acid)-*D*-galactose* (**2**). — A solution of **10** (100 mg) in ethanol (95% v/v), containing a drop of acetic acid and palladium–charcoal (10%) catalyst, was hydrogenolyzed for 24 h at 50 lb/in^2 at room temperature. The filtered solution was concentrated *in vacuo* to a glass (75 mg); t.l.c. in butanol–pyridine–water (3:2:1), R_{10} 0.5; paper chromatography in ethyl acetate–acetic acid–formic acid–water (18:3:1:4), R_{gal} 0.40 (Rodén and Armand[3] reported R_{gal} 0.40), $R_{lactose}$ 1.30; $[\alpha]_D^{20}$ +10.0° (*c* 0.80, water).

Anal. Calc. for $C_{12}H_{20}O_{12}$: C, 40.45; H, 5.66. Found: C, 40.08; H, 5.85.

*3-*O*-β-*D*-(Methylglucopyranosyluronate)-*D*-galactose* (**11**). — Compound **9** (30 mg) was hydrogenolyzed as described for **2**. The resulting product was a syrup; $[\alpha]_D^{25}$ −5.5° (*c* 0.55, water); t.l.c. in butanol–pyridine–water (3:2:1), R_9 0.33, R_2 1.90; paper chromatography in ethyl acetate-acetic acid–formic acid–water (18:3:1:4), R_{gal} 0.89, $R_{lactose}$ 2.9, R_2 2.3.

Anal. Calc. for $C_{13}H_{22}O_{12}$: C, 42.16; H, 5.99. Found: C, 42.58; H, 6.15.

ACKNOWLEDGMENTS

The author is indebted to Dr. I. Löw and Professor R. Kuhn for the gift of a sample of solabiose.

SUMMARY

The reaction of benzyl 2,6-di-*O*-acetyl-β-D-galactopyranoside (**3**) with acylglycosyl halides (**4**, **6**),followed by removal of protecting groups, afforded the disaccharide 3-*O*-β-D-glucopyranosyl-D-galactose (solabiose, **1**) and 3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (**2**). The structures of the disaccharides were confirmed by n.m.r. spectral data and periodate oxidation of intermediates.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PROPERTIES OF CYCLODEXTRINS

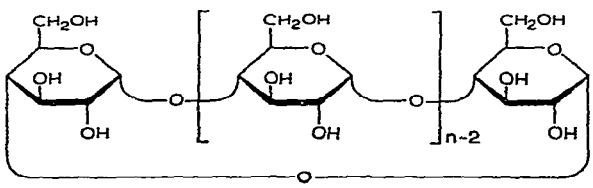
PART I. SOLUBILITY MEASUREMENTS

N. WIEDENHOF AND J. N. J. J. LAMMERS
Laboratory of General Chemistry, Technological University, Eindhoven (Netherlands)

(Received December 21st, 1966)

INTRODUCTION

Cyclodextrins (Schardinger dextrins, cycloamyloses, CD) are cyclic, homologous oligosaccharides (**1**; α-CD, $n = 6$; β-CD, $n = 7$) containing rings of α-(1→4)-linked D-glucose residues. The different, homologous cyclodextrins are obtained simultaneously from the breakdown of starch by the action of an amylase produced by *Bacillus macerans*. They are especially important in that they can form inclusion compounds.



(1)

In order to discuss the properties of the inclusion compounds, it is necessary to have a clear understanding of the identity (as determined by physical properties) of the inclusion compound and of its components. In this respect, it is remarkable that the recorded values of the solubility of α-CD in water differ appreciably (Table I).

TABLE I

SURVEY OF THE VALUES OF THE SOLUBILITY OF α-CD

Solubility of α-CD in water	*Reference*	*Method*
12.0 g/100 ml	1, 2, 5	polarimetric[a]
14.5 g/100 ml	3,5	polarimetric[b]
10 g/100 g. sol.	4	weight of residue after evaporation[c]
16.0 g/100 ml	5 (6)	?

[a]The temperature was not recorded. In ref. 2, the value 12.0 g/100 ml is reported together with values obtained from ref. 3. In ref. 3, however, 14.5 g/100 ml is given for the solubility of α-CD in water. [b]Room temperature. [c]At 20°.

For these determinations, only Freudenberg *et al.*[4] reported the temperature, but the value obtained for the solubility of α-CD is still insufficiently defined because the water content of the solid phase was not considered.

In this publication, measurements of the refractive indices of saturated solutions of α- and β-CD in water are reported and discussed. Values of the solubility of a number of hydrates of α-CD in water are given. In future publications, we hope to report other physical properties of α-CD, β-CD, and a number of their inclusion compounds.

EXPERIMENTAL

Preparation of cyclodextrins. — Cyclodextrins were prepared according to French's method[7] and purified as follows: α-CD was recrystallised twice from propyl alcohol–water (60:40), and an aqueous solution of the product (which contained propyl alcohol) was concentrated in a rotary evaporator at 40° *in vacuo*; this procedure was repeated three times, and α-CD was then recrystallised three times from water. β-CD was recrystallised three times from water. The samples were ground in a mortar, dried *in vacuo* for 24 h at 80°, and stored over silica gel. They still contained 2.5% of water (*cf.* Table III), and were very hygroscopic.

Analysis. — The purity of the CD samples was assessed as follows: (*a*) By t.l.c. on microplates[8]. The improved method involved a blank run with developing solvent (butyl alcohol–glacial acetic acid–water–pyridine–*N*,*N*-dimethylformamide, 6:3:1:2:4) and, after the chromatoplate had been dried on a hot plate, the sample was applied. The chromatoplate was then developed twice with the same solvent (drying after each development) and finally sprayed with dichromate solution[8]. (*b*) By ascending paper chromatography with Whatman No. 1 paper, ethyl acetate–propyl alcohol–water (2:6:5) as irrigant, and a 1% alcoholic solution of iodine as indicator. (*c*) By column chromatography with Whatman, Standard-grade, cellulose powder and butyl alcohol–ethanol–water (42:29:29). The optical rotation of the eluate was measured continuously by using a Bendix automatic polarimeter. The details of this method will be described elsewhere[13].

The percentage of β-CD detectable in mixtures of α- and β-CD are: paper chromatography, 10%; t.l.c., 8%; column chromatography, 0.5%. The results of these purity tests are listed in Table III. The percentages of α-CD detectable in mixtures of α- and β-CD are: paper chromatography, 2%; t.l.c., 3%; column chromatography, 0.3%.

The water content of the CD samples was measured by g.l.c., with Porapak P (100–120 mesh) as the stationary phase[12]. A solution of the sample in *N*,*N*-dimethylformamide [dehydrated in a column containing molecular sieve 4A (Union Carbide)] was applied to the column; further details of this procedure will be published elsewhere.

Determination of the refractive indices of aqueous solutions of CD. — The solubility measurements of α-CD were carried out by measuring (Model A Zeiss Abbé-refractometer) the refractive indices of solutions of CD in water. The refractive

index is a sensitive and linear function of the concentration in aqueous solution. All concentrations are given in weight %, and all solutions were equilibrated in a thermostat (±0.1°). The refractive index of our twice-distilled water was n_D^{20} 1.3326 ±0.0001.

Because of the low solubility of β-CD in water, there is only a small difference in refractive index between water and a saturated, aqueous solution of β-CD. A Bausch and Lomb precision refractometer (Type 33-45-03-01) was therefore used for these solutions. The refractive index of our twice-distilled water, measured on this instrument, was n_D^{20} 1.33299 ±0.00003.

RESULTS AND DISCUSSION

All measurements were carried out with identically prepared samples of α-CD containing a small proportion of water (Table III). In Table II, the weight % of α-CD,

TABLE II

VALUES OF WEIGHT % OF α-CD AND n_D^{20}

α-CD (sample F)	*20.0°*	*α-CD (sample I)*	*20.0°*
weight (%)	n_D^{20}	*weight (%)*	n_D^{20}
0.000	1.3326	0.000	1.3326
0.900	1.3339	1.460	1.3346
1.232	1.3343	1.990	1.3356
1.909	1.3354	3.140	1.3373
2.483	1.3362	3.930	1.3382
3.044	1.3370	4.880	1.3396
3.266	1.3372	4.930	1.3397
4.295	1.3388	5.190	1.3403
5.032	1.3399	5.480	1.3405
5.881	1.3412	7.000	1.3429
7.042	1.3428	7.420	1.3435
7.444	1.3434	7.620	1.3440
		9.170	1.3462

and the corresponding refractive indices, are given at 20.0° for sample *F* and sample *I*. The data in Table II are plotted in Fig. 1. From these data, the relation 1 can be computed (see Appendix) in which x = weight % of α-CD containing about 2.5% of

$$n_D^{20} = 0.00148\,x + n_{D\text{water}}^{20} \quad (1)$$

water (*cf.* Table III). All other samples of α-CD gave values of n_D^{20} and x according to this formula. Assuming that the water content of the α-CD sample is 2.5 ±0.3% (w/w), the concentration (c) of water-free α-CD in % (w/w), expressed as a function of the refractive index, may be derived from formula 2. The refractive indices of saturated

$$c = (n_D^{20} - n_{D\text{water}}^{20})\,(659 \pm 10) \quad (2)$$

solutions in contact with solid CD, and of different samples of α-CD in water, at 20.0°

showed different values. The time required to obtain a solution of constant refractive index was, in most cases, approximately 6 days when the solutions were shaken inter-

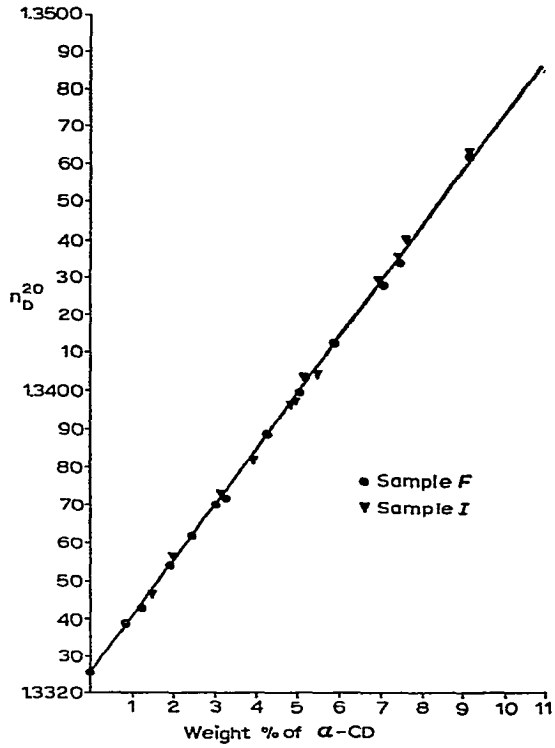


Fig. 1 — Refractive index of solutions of α-CD *versus* weight percent of α-CD.

TABLE III

DATA ON SAMPLES OF α-CD

Sample[a]	n_D^{20}	*Water (%, w/w); ±0.3*	*Solubility (g of hydrated α-CD/100 g of solution); ±0.1*	*Solubility (g of α-CD/100 g of solution)*	*Modification*
D	1.3459	—[b]	9.1	—	I
F	1.3458	2.2	9.0	8.80 ± 0.13	
C	1.3472	2.5	10.0	9.75 ± 0 14	
I	1.3474	—	10.1	—	II
A	1.3482	2.5	10.6	10.33 ± 0.15	
G	1.3481	—	10.6	—	
H	1.3481	—	10.6	—	III

[a]All samples were shown to be homogeneous by paper chromatography and t.l.c., and by column chromatography for *A*, *C*, and *F*. [b]Not measured, because insufficient product was available.

mittently by hand, or 3 days when the solutions were shaken continuously. No effect on the solubility was found by varying the excess amount of crystals.

In some cases, after an interval, the refractive index suddenly decreased, and this suggest the formation of a different, less soluble, and more stable modification. Table III shows the n_D^{20} of saturated solutions of various samples of α-CD in water. The corresponding values of the solubility are computed as indicated in the Appendix.

From the solubility measurements alone, it would appear that α-CD crystallises in at least three, different modifications (I, II, and III) differing only very slightly in stability. In this connection, it is interesting to observe that Hess[9] found a number of different X-ray powder diagrams for the compounds that he obtained by crystallising α-CD from water and from aqueous ethanol. Recently, Casu[10] reported four hydrated modifications of α-CD. It is not known whether any of the three modifications postulated here is isomorphic with any of those found by Hess[9] or by Casu[10], or with the structures of inclusion compounds formed by α-CD. It will be difficult to ascertain this identity or non-identity, because the preparation of metastable modifications is generally a matter of chance.

The solubility of sample *I* in water at various temperatures was measured in the same way as described above, *viz.*, by plotting graphs of the weight % of α-CD and the corresponding refractive indices at various temperatures. The solubilities (Table IV)

TABLE IV

SOLUBILITY OF α-CD (SAMPLE *I*) AT DIFFERENT TEMPERATURES

Temp. (°)	18	22	25	30
Solubility[a]	9.2	10.1	11.1	13.4

[a]Hydrated α-CD (g) per 100 g of solution.

were read from these graphs. As it is not certain whether, at the various temperatures, the solution is in equilibrium with the same modification of solid α-CD, no conclusion can be drawn with respect to the enthalpy of solution.

Crystallisation from a supersaturated solution of α-CD is a very slow process, and sterilisation of the solution would be necessary in order to avoid mould growth at 20°. The stability of α-CD towards sterilisation conditions is unknown. A 16% (w/w) aqueous solution of α-CD at 20° did not crystallise even after 20 days. Consequently, an approach to equilibrium from this direction at 20° was not further attempted.

The refractive index at 20.0° of saturated, aqueous solutions of three samples of β-CD, obtained from different batches, appeared to be identical (1.33506 ±0.00003), within experimental error. No effect of varying the excess amount of crystals on the refractive index of the solution was found, and from this it may be concluded that only one modification of β-CD (hydrate) has been obtained. This is in striking contrast with the number of α-CD hydrates found.

APPENDIX

In accordance with the experimental data shown in Fig. 1, we have assumed that the relationship between refractive index and weight % of α-CD in water is a linear one. Let the two variables be y and x, respectively, then the estimated linear equation is $\hat{y} = \hat{p}x + \hat{q}$, in which $\hat{p}$, $\hat{q}$, and $\hat{y}$ are estimates of p, q, and y, the true values; $\hat{p}$ and $\hat{q}$ were computed by the method of least squares, and had values of 0.0014774 and 1.332514, respectively.

Estimation of the uncertainty in p *and* q. — To compute the error in $\hat{p}$ and $\hat{q}$, the estimated regression equation, $\hat{\underline{y}} = \underline{a} + \underline{b}(x-\bar{x})$, is used. The true regression equation is $\eta = \alpha + \beta(x-\bar{x})$. The statistical model is $\underline{y} = \eta + \underline{e}$, in which $\hat{\underline{y}}$ = estimated η; underlined symbols = random variables; $\bar{x}$ = mean value of $x_i = \Sigma x_i/n$; n = number of observations; Σ = the summation operation, $\sum_{i=1}^{n}$ over $i = 1, \ldots . n$.

For a simple solution of the error, it is assumed that x has been determined accurately, and that there is a relative uncertainty in y.

As can be seen, $\hat{\underline{p}} = \underline{b}$; and $\hat{\underline{q}} = \text{a} - \underline{b}\bar{x}$.

The confidence interval of $\hat{\underline{p}}$ is $\hat{\underline{p}} \pm t_{n-2}(\tfrac{1}{2}\alpha)\underline{s}_p$,

and of $\hat{\underline{q}}$ is $\hat{\underline{q}} \pm t_{n-2}(\tfrac{1}{2}\alpha)\underline{s}_q$,

in which t_{n-2} is the Student variable at n–2 degrees of freedom; $\underline{s}_p^2$ and $\underline{s}_q^2$ are the estimators of var $\hat{\underline{p}}$ and var $\hat{\underline{q}}$, respectively; and α is the level of significance (in this case, 0.05).

Now, $\text{var}\ \hat{\underline{p}} = \text{var}\ \underline{b} = \dfrac{\sigma^2}{\Sigma(x_i-\bar{x})^2}$

$$\text{var}\ \hat{\underline{q}} = \text{var}\ (\underline{a}-\underline{b}\bar{x}) = \sigma^2\left(\frac{1}{n} + \frac{\bar{x}^2}{\sum(x_i-\bar{x})^2}\right)$$

in which $\sigma^2 = \text{var}\ \underline{e} = \text{var}\ \underline{y}$.

s^2 is an estimate of σ^2

$$s^2 = \frac{1}{n-2}\left[\sum(y_i-\bar{y})^2 - \frac{\{\sum(x_i-\bar{x})(y_i-\bar{y})\}^2}{\sum(x_i-\bar{x})^2}\right] = 1.32\times10^{-8} (n=25).$$

Hence, $s_{\hat{p}} = \sqrt{\dfrac{s^2}{\Sigma(x_i-\bar{x})^2}} = 9.67\times10^{-6}$

$$s_{\hat{q}} = \sqrt{s^2\left\{\frac{1}{n} + \frac{\bar{x}^2}{\Sigma(x_i-\bar{x})^2}\right\}} = 4.83\times10^{-5}$$

$t_{n-2}(\tfrac{1}{2}\alpha) = 2.074$ (ref. 11).

Our results are: $p = 0.00148 \pm 0.00002$, with $\alpha = 5\%$;

$q = 1.3525 \pm 0.0001$

Estimation of the uncertainty in the solubility of α-CD. — In the estimated regression equation $\underline{\hat{y}} = \underline{a} + \underline{b}(\underline{x} - \bar{x})$, a new observation is made, *viz.*, $\bar{y}'$, the mean value of m observations of the refractive index. Insertion of $\bar{y}'$, for $\underline{\hat{y}}$, and solution of the equation for x, gives

$$\underline{\hat{x}} = \bar{x} + \frac{\bar{y}' - \underline{a}}{\underline{b}}$$

The expected value of $\bar{y}'$ is η, and the true value of $\underline{\hat{x}}$ is ξ, therefore

$$\xi = \bar{x} + \frac{\eta - \alpha}{\beta}$$

The confidence limits of ξ are ξ_1 and ξ_2, the upper and the lower confidence limit, respectively. According to Brownlee[11], the following approximate equations may be used:

$$\xi_2 = \bar{x} + \frac{\bar{y}' - a}{b} - \frac{t_{n-2}(\frac{1}{2}\alpha) \cdot \underline{s}}{|b|} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{y}' - a)^2}{b^2 \Sigma (x_i - \bar{x})^2}}$$

$$\xi_1 = \bar{x} + \frac{\bar{y}' - a}{b} + \frac{t_{n-2}(\frac{1}{2}\alpha) \cdot \underline{s}}{|b|} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{y}' - a)^2}{b^2 \Sigma (x_i - \bar{x})^2}}$$

$$\text{because } 0 < \frac{\{t_{n-2}(\frac{1}{2}\alpha)\}^2 \cdot \underline{s}^2}{b^2 \Sigma (x_i - \bar{x})^2} = 1.845 \times 10^{-3} \ll 1.$$

Then the confidence interval of ξ is $\underline{\hat{x}} - \Delta\underline{\hat{x}} < \xi < \underline{\hat{x}} + \Delta\underline{\hat{x}}$.
In Table V, x is inserted for ξ.

TABLE V

COMPUTATION OF THE SOLUBILITY OF α-CD IN WATER, AND THE ERROR IN THE SOLUBILITY

Sample	*Refractive index* $(\bar{y}')^a$	m[b]	*Weight (%) of the saturated solution computed* (x)	*Error of* x $(\Delta x)^c$
C	1.3472	5	9.95	0.11
D	1.3459	5	9.07	0.10
F	1.3458	5	9.00	0.10
G	1.3481	5	10.56	0.12
H	1.3481	5	10.56	0.12
A	1.3482	5	10.63	0.12
I	1.3474	5	10.09	0.11

[a] $\bar{y}'$ is the mean value of the refractive index measurements; [b] m is the number of observations of the refractive index; [c] In determining Δx, it is assumed that the level of significance is 5%.

SUMMARY

By using the refractive index (n_D^{20}) of aqueous solutions of α-cyclodextrin (α-Schardinger dextrin, α-CD), an accurate and reasonably quick method has been

developed for determining the concentrations of this compound, provided no foreign substances are present. A linear relationship exists between n_D^{20} and the concentration (c) of α-CD (on a water-free basis) expressed in % weight of solution in accordance with the equation, $c = (n_D^{20} - n_{D\,water}^{20})\ (659 \pm 10)$ at 20°.

The solubility of α-CD in water at 20° has been computed by measuring n_D^{20} of saturated solutions of this compound. For identically prepared batches of α-CD, different values of n_D^{20} have been found and, accordingly, the existence of at least three different modifications has to be postulated.

For β-CD, within experimental error, only one value for the refractive index of the saturated solution at 20° has been found, and thus only one modification of β-CD has been encountered.

Techniques have been developed for determining the content of β-CD in samples of α-CD, and *vice versa*.

The solid samples of α-CD, which were used in the solubility measurements and which have been extensively dried, still contain about 2.5% of water.

ACKNOWLEDGMENTS

We thank Mr. W. Brekelmans for technical assistance and the improvement of the micro t.l.c. method, Mr. N. J. D. van der Meijden and Mr. A. J. G. van Diemen for preparing the CD. The authors are grateful to Prof. dr. C. L. van Panthaleon Baron van Eck for his interest, and to Dr. A. J. Bosch for his advice with respect to the mathematical solution of the problem.

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STRUCTURE OF THE POLYSACCHARIDE FROM TAMARIND KERNEL

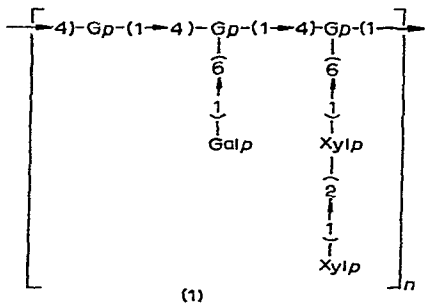
H. C. SRIVASTAVA AND P. P. SINGH
Ahmedabad Textile Industry's Research Association, Ahmedabad-9 (India)

(Received December 19th, 1966; in revised form, January 26th, 1967)

INTRODUCTION

The seed kerneis of the tamarind tree (*Tamarindus indica*) yield a water-soluble polysaccharide that is known in the literature by various names such as Tamarind Seed Polysaccharide, Pectin, Jellose, Polyose, Tikernose, and *Tamarindus* Amyloid. In this paper, however, it will be referred to as Tamarind Kernel Polysaccharide (TKP).

TKP contains[1,2] glucose, xylose, and galactose in molar ratios of 3:2:1, and, based on the results of methylation analysis, structure **1** was proposed[3], amongst other

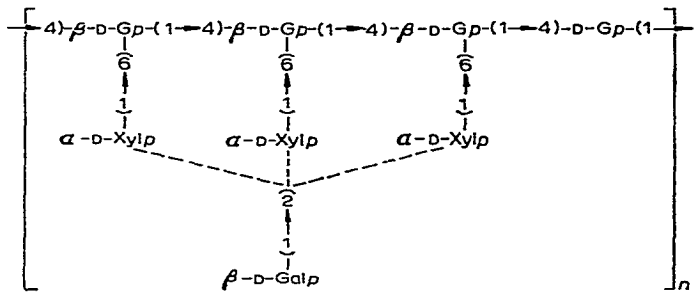


(1)

possibilities, for the repeating unit. According to this structure, the repeating unit is made up of three glucose residues, two of which are linked through C-6 branches to D-galactose and a (1→2)-linked xylobiose unit, respectively. The authors[3] also pointed out that some of the glucose residues may be in the side chain. Without reference to, but in close agreement with, the results of White and Rao[3], Khan and Mukherjee[4] published methylation data on TKP, and proposed a similar structure. Savur[5], on the other hand, claimed to have isolated a xylan from tamarind-seed kernels. On the basis of methylation analysis and periodate oxidation, the xylan was shown to have a backbone of (1→4)-linked D-xylose residues with occasional branchings at C-3.

These discrepancies in the proposed structures of TKP prompted us to undertake a detailed study of the polysaccharide. Whilst this study was in progress, Kooiman[6] published data on the fragmentation analysis of TKP. Kooiman's preparation ("*Tamarindus* Amyloid") was a homogeneous polysaccharide composed of D-glucose,

D-xylose, and D-galactose residues in molar ratios of 4:2.99:1.33. Partial hydrolysis with acid yielded a degraded polymer composed mainly of D-glucose residues, and having an X-ray diffraction diagram similar to that of cellohexaose and cellulose II. Enzymic hydrolysis of the acid-degraded polymer with heated *Myrothecium* cellulase produced cellobiose. The same disaccharide was obtained on acetolysis of the polysaccharide acetate. These results proved that the main chain of the polysaccharide was composed of (1→4)-linked D-glucose residues. The enzyme preparation "Luizym" hydrolyzed TKP to D-galactose, D-glucose, and 6-*O*-α-D-xylopyranosyl-D-glucopyranose, whereas *Myrothecium* cellulase yielded a number of oligosaccharides, the major components being a heptaose composed of D-glucose and D-xylose (4:3), an octaose composed of D-glucose, D-xylose, and D-galactose (4:3:1), and a nonaose composed of the same sugars in the proportions of 4:3:2. Partial hydrolysis of the polysaccharide with acid produced 2-*O*-β-D-galactopyranosyl-D-xylopyranose. Based on these results, Kooiman proposed structure 2 for TKP. According to this, the



(2)

repeating unit has a backbone of four (1→4)-linked D-glucose residues; two of these residues carry single D-xylose residues at C-6, one has a 2-*O*-β-D-galactopyranosyl-D-xylopyranose unit at C-6, and the fourth D-glucose residue is unsubstituted.

The present study deals with enzymic hydrolysis, acid hydrolysis, acetolysis, methylation analysis, and periodate oxidation of TKP. Based on these results, a structure for the repeating unit is proposed.

RESULTS AND DISCUSSION

Tamarind kernels were ground to give a cream- or buff-coloured powder from which the crude polysaccharide was obtained by extraction with hot water. The crude polysaccharide was fractionated by complexing with Fehling's solution or with barium hydroxide. The polysaccharide purified by either of the fractionation procedures was found to be homogeneous in the ultracentrifuge (Fig. 1) and by zone electrophoresis. It had $[\alpha]_D$ +75° (water), and, on acid hydrolysis, it gave D-glucose, D-xylose, D-galactose, and L-arabinose in molar ratios of 8:4:2:1, as determined by the phenol–sulphuric acid method[7]. Hydrolysis of the fully methylated polysaccharide, followed by remethylation and examination by gas–liquid chromatography, showed the presence

of fully methylated methyl glycosides of glucopyranose, xylopyranose, galactopyranose, and arabinofuranose in the proportions of 7.8:4:2:1.2 (Fig. 2). Considering the number of treatments given to the polysaccharide during the methylation analysis, the results on the proportions of sugar constituents are in good agreement with the results obtained by direct hydrolysis and determination of the free sugars.

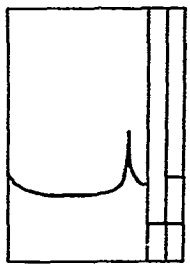


Fig. 1. Sedimentation of TKP purified *via* copper complex; 0.1% in 0.1M sodium tetraborate; at 42,040 r.p.m., for 60 min at 20.8°.

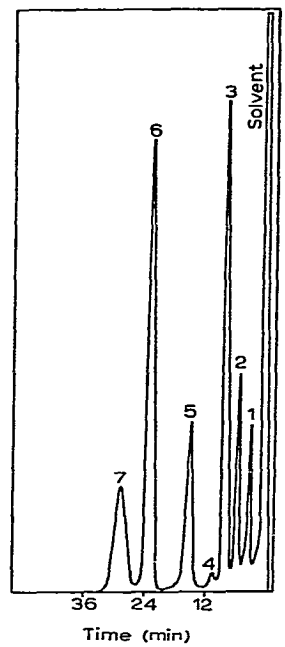


Fig. 2. Gas–liquid chromatogram (column *2*) of methylated fragments obtained by hydrolysis of methylated TKP, followed by remethylation. (*1*) Methyl 2,3,4-tri-*O*-methyl-β-D-xyloside, (*2*) methyl 2,3,5-tri-*O*-methyl-β-L-arabinoside (*3*) methyl 2,3,4-tri-*O*-methyl-α-D-xyloside, (*4*) methyl 2,3,5-tri-*O*-methyl-α-L-arabinoside, (*5*) methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside, (*6*) methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside, and (*7*) methyl 2,3,4,6-tetra-*O*-methyl-α,β-D-galactoside.

It is rather surprising that the two detailed investigations[3,6] carried out earlier do not mention arabinose as a component of this polysaccharide. Arabinose was easily detected in our polysaccharide on acid hydrolysis. In fact, when TKP was subjected to graded hydrolysis with 0.01N sulphuric acid at 95°, arabinose was the first sugar to be released, as shown by paper chromatography. In the literature[8–11], there have been conflicting reports about the presence of arabinose in the polysaccharide. These investigations, however, did not deal with the structure of TKP in any detail. Recently, Macmillan *et al.*[12], in agreement with our results, have reported that TKP contains arabinose besides glucose, xylose, and galactose. These authors have carried out extensive studies on the fractionation of TKP and have shown that the polysaccharide, obtained after repeated fractionations by various procedures, gives arabinose on acid hydrolysis. Apart from arabinose, the ratios of glucose, xylose, and galactose as determined by us (4:2:1) are different from those obtained by White and Rao[3] (3:2:1) and by Kooiman[6] (4:3:1).

The polysaccharide could not be completely methylated by the usual alkylation procedures. Complete methylation was achieved, however, by a modified procedure employing dimethyl sulfoxide, methyl sulfate, and solid sodium hydroxide[13]. The methyl-

ated polysaccharide, after fractionation, was hydrolyzed with sulfuric acid under conditions of least degradation and demethylation[14]. The methylated fragments were separated on a cellulose column, and, where necessary, further purification was achieved by paper chromatography. The mixture of 2,3,6-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose, which moved as a single spot on paper and remained unresolved on a cellulose column, was separated into its components by paper electrophoresis. Table I shows the molar ratios of methylated sugars obtained on methylation of TKP followed by acid hydrolysis of the polysaccharide methyl ether. Also included in Table I are results from previous studies on the methylation of TKP, as well as the calculated molar ratios of methylated sugars based on the structure proposed by Kooiman[6].

TABLE I

SUGARS OBTAINED ON HYDROLYSIS OF METHYLATED TKP

	Molar ratios obtained by			
Sugar	*White and Rao*[3]	*Khan and Mukherjee*[4]	*Kooiman*[a]	*Present study*
2,3,4-Tri-*O*-methyl-D-xylose	1.06	1.12	2	3
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.07	1.1	1	2
3,4-Di-*O*-methyl-D-xylose	1.02	1.03	1	1
2,3,6-Tri-*O*-methyl-D-glucose	0.86	1.01	1	2
2,3-Di-*O*-methyl-D-glucose	1.96	1.89	3	6
2,3,5-Tri-*O*-methyl-L-arabinose	—	—	—	1

[a]Calculated on the basis of the structure (2) proposed by Kooiman[6].

It will be observed that White and Rao[3] and Khan and Mukherjee[4] reported the same molar ratios of methyl ethers of glucose, galactose, and xylose, which are quite different from what would be expected for a polysaccharide having structure **2** as proposed by Kooiman[6]. Thus, structure **2** requires a ratio of tri-*O*-methyl- to di-*O*-methyl-xylose of 2:1, and that of di-*O*-methyl- to tri-*O*-methyl-glucose of 3:1, as against the corresponding ratios of 1:1 and 2:1 found by White and Rao[3] and by Khan and Mukherjee[4]. Our results, in respect of methyl ethers of glucose, galactose, and xylose, are in general agreement with structure **2**, except for a higher molar ratio of tri-*O*-methyl- to di-*O*-methyl-D-xylose. Furthermore, 2,3,5-tri-*O*-methyl-L-arabinose was not reported by the previous workers.

The general picture of TKP that emerges from the methylation analysis is that of a highly branched polymer having a backbone of (1→4)-linked D-glucose residues and to which are attached branches terminated by D-galactose, D-xylose, and L-arabinose residues. The highly branched character of the polysaccharide probably explains its resistance to complete methylation by the usual alkylation procedures.

Acetolysis of TKP produced a mixture of oligo- and mono-saccharides that was resolved into its components by a combination of carbon-column and paper chromatography. Two oligosaccharides (*A* and *B*) were obtained in this manner. Hydrolysis of TKP by an enzyme preparation 'Hemicellulase' produced another two oligosacchar-

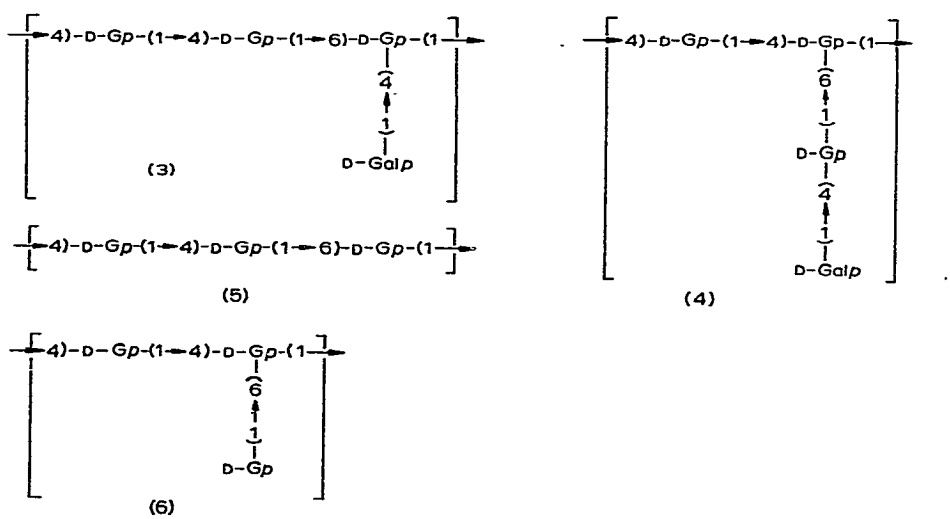
ides (*C* and *D*) in addition to monosaccharides. Based on classical techniques for structural determination of oligosaccharides, the disaccharides *A*, *B*, *C* and *D* were identified as 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose), 2-*O*-β-D-galactopyranosyl-D-xylopyranose, 4-*O*-β-D-galactopyranosyl-D-glucose (lactose) and 6-*O*-α-D-xylopyranosyl-D-glucopyranose, respectively.

Isolation of lactose shows that some D-galactose residues are linked directly to D-glucose residues through β-(1→4)-linkages. If one lactose residue is present per repeating unit of the polymer, the theoretical yield would be 15%; as this disaccharide was obtained in 14% yield, one of the two D-galactose residues in the repeating unit is linked to D-glucose. That the other D-galactose residue is linked to D-xylose through a β-(1→2)-linkage follows from the isolation of 2-*O*-β-D-galactopyranosyl-D-xylose, which constitutes one of the branches across the glucan backbone.

Isolation of disaccharide *D* in 40% yield shows that the majority, if not all, of the D-xylose residues are linked to the D-glucose backbone through α-(1→6)-linkages. The theoretical yield of this disaccharide, if all of the D-xylose residues in the polysaccharide were linked to D-glucose, would be 55%.

Methylation data support the results of the fragmentation analysis. Thus, all of the D-galactose residues in the polymer are present as non-reducing, end groups, as demonstrated by the isolation of 2,3,4,6-tetra-*O*-methyl-D-galactose as the only methylated galactose fragment. The identification of 3,4-di-*O*-methyl-D-xylose is in accord with the postulate that 2-*O*-β-D-galactopyranosyl-D-xylose units occur as branches in the molecule. That only half of the D-galactose residues are linked to D-xylose residues is indicated by the isolation of 2 moles of tetra-*O*-methyl-D-galactose as against only one mole of 3,4-di-*O*-methyl-D-xylose.

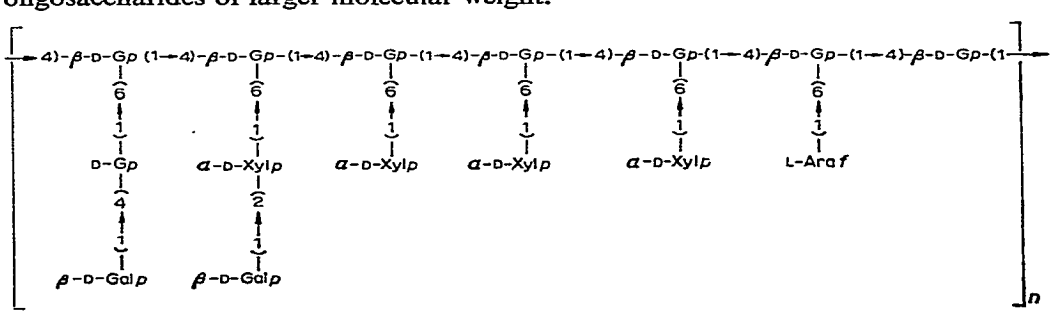
The lactose unit can be fitted into the polymer structure either by envisaging some (1→6)-linkages in the glucan backbone of principally β-D-(1→4)-linked residues



(structure **3**), or by assuming that the lactose unit occurs as a branch on a backbone composed exclusively of β-(1→4)-linked D-glucose residues (structure **4**). The possibility of some D-glucose residues being in the side chain was mentioned by White and Rao[3], but was discounted by Kooiman[6]. Supporting evidence in favour of structure **4** was gathered in the following manner. TKP, on mild hydrolysis with acid, gave an ethanol-insoluble, degraded polysaccharide composed of D-glucose and D-xylose residues, the latter being present in only trace amounts. The degraded polymer was methylated and subsequently hydrolysed to give 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, and 2,3,4-tri-*O*-methyl-D-xylose. No other methylated sugar was detected on paper chromatograms. Since there was no galactose present in the degraded polysaccharide, the D-Gal*p*-(1→4)-D-G*p* bond must have been cleaved during acid hydrolysis of TKP. However, a D-G*p*-(1→6)-D-G*p* bond will survive, since this linkage is more stable than the (1→4)-glucosidic bond[15]. Thus, partial hydrolysis of polysaccharides having structures **3** and **4** should give rise to fragments **5** and **6**, respectively. Methylation and hydrolysis of a polymer having structure **5** should yield 2,3,4-tri-*O*-methyl-D-glucose in addition to other methylated sugars, whereas similar treatment of a polymer having structure **6** will not result in any 2,3,4-tri-*O*-methyl-D-glucose. Thus, the absence of 2,3,4-tri-*O*-methyl-D-glucose in the hydrolysate of the methyl ether of degraded TKP favours structure **4** wherein the lactose unit forms a side chain. However, the configuration of the linkage by which the lactose unit is linked to the D-glucose residue is not known. These results confirm that the main chain of the polysaccharide is built up of (1→4)-linked D-glucose residues, the linkage being of the β-D-type as shown by the isolation of cellobiose by acetolysis of the polymer.

The position of the arabinofuranose residues with respect to other substituents along the polymer backbone is obscure. In order to explain the high molar ratio of 2,3-di-*O*-methyl-D-glucose in the hydrolysate of TKP methyl ether, it could be assumed that L-arabinose residues are linked directly to D-glucose residues of the main chain. The configuration of the linkage is not yet known.

In the absence of any information on the sequential distribution of substituents along the main chain, structure **7** is one of the many that may be written for TKP on the available evidence. Assignment of a more definitive structure requires isolation of oligosaccharides of larger molecular weight.



(7)

In a polysaccharide having structure 7, all of the sugar residues should be attacked by periodate consuming 1.33 moles of the oxidant with the liberation of 0.33 mole of formic acid per sugar residue. In fact, none of the sugar residues survived periodate oxidation; 1.35 moles of the oxidant were consumed with the liberation of 0.34 mole of formic acid. The oxopolysaccharide, on Smith degradation[16], yielded glycerol, erythritol, and glyceraldehyde in molar ratios of 2.5:8:1. The theoretical molar ratio for these products based on structure 7 would be 3:8:1 (glyceraldehyde arising from 2-*O*-substituted D-xylose residues). The sequence of oxidation, reduction, and hydrolysis (Smith degradation) of TKP is given in Fig. 3.

Further investigations on the constitution of TKP should relate to the sequential arrangement of branches, *i.e.*, D-xylopyranose, L-arabinofuranose, lactose, and 2-*O*-β-D-galactopyranosyl-D-xylopyranose residues, along the D-glucose backbone, and to the configuration of the linkages between the branches of lactose and L-arabinofuranose residues and the backbone.

EXPERIMENTAL

All evaporations were performed under diminished pressure. Paper chromatograms were run by the descending method with the following solvent systems (v/v): (*A*) butyl alcohol–pyridine–water (6:4:3); (*B*) pyridine–ethyl acetate–water (2:5:5); (*C*) butanone–water azeotrope containing ammonia; (*D*) benzene–ethanol–water–ammonia (200:47:14:1). Sugars on paper were detected by the following reagents: (*a*) *p*-anisidine hydrochloride[17]; (*b*) silver nitrate–alcoholic sodium hydroxide[18]; (*c*) periodate–benzidine[19]. R_F, R_G, and R_C represent rates of movement of sugars on paper chromatograms in relation to the solvent front, D-glucose, and cellobiose, respectively. Paper electrophoresis was performed in 0.1M borate buffer at 600 volts for 3 h. M_G represents the rate of movement of sugars on a pherogram in relation to that of D-glucose.

Gas–liquid chromatography (g.l.c.) was carried out on a Pye Argon chromatograph by using 4-ft columns of: (*1*) Apiezon M, 20% on Celite 545 at 150°; flow rate, 40 ml/min; and (*2*) poly(butane-1,4-diol succinate), 20% on Celite 545 at 175°; flow rate, 60 ml/min.

Acid hydrolysis, unless mentioned otherwise, was performed with N sulfuric acid for 10–12 h at 100°, and sugars were assayed by the phenol–sulfuric acid method[7]. Methyl ethers of sugars were demethylated with boron trichloride[20]. The reducing residue in disaccharides was determined by reducing the disaccharide with sodium borohydride, followed by hydrolysis and examination of the hydrolysate by paper chromatography (spray reagent *a*). Melting points are corrected, and specific rotations are equilibrium values unless stated otherwise.

Purification of the polysaccharide.—(*i*) *Fractionation* via *copper complex*. Crude, defatted, tamarind-kernel powder (10 g) was stirred with water (1 litre) in a blender. The suspension was then heated with stirring on a boiling-water bath for 1 h, cooled, and centrifuged to remove the suspended matter (2.2 g). To the supernatant, Fehling's

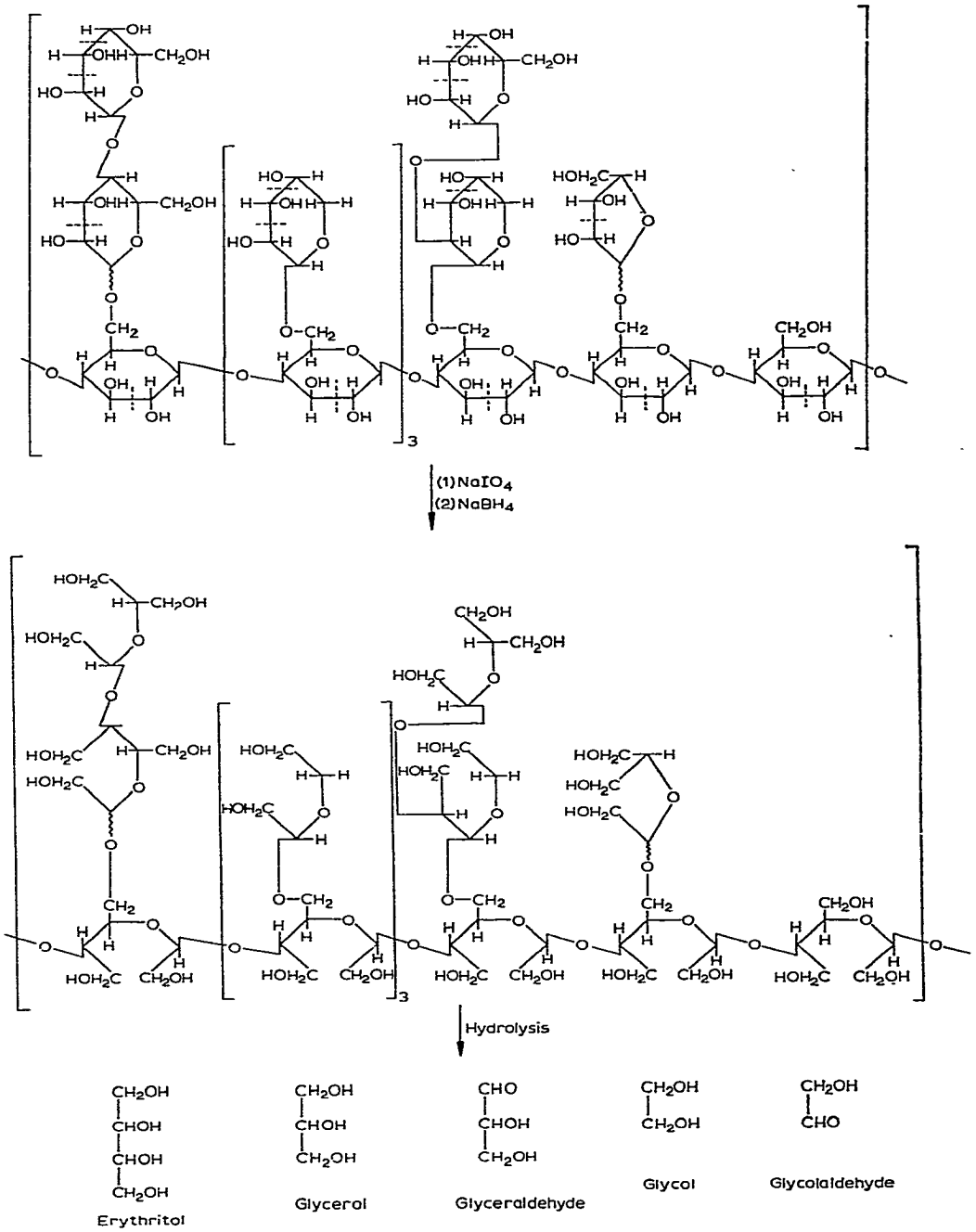


Fig. 3. Smith degradation of TKP.

solution (25 ml, diluted to 50 ml with water) was added dropwise with constant stirring. The complex was filtered off on a muslin cloth, washed with dilute Fehling's solution, and squeezed to remove excess of the reagent. A suspension of the complex in water (500 ml) was cooled in an ice–salt bath, and acidified with cold 0.2N hydrochloric acid. The resulting solution was poured into ethanol (1.5 l) to regenerate the polysaccharide which was dried by the usual technique of solvent exchange; yield 6 g, $[\alpha]_D$ +55.5° (*c* 0.5, water). The product obtained above was fractionated once more with Fehling's solution to yield a polysaccharide material (4.2 g), $[\alpha]_D$ +75° (*c* 0.5, water).

(*ii*) *Fractionation* via *barium complex.* Defatted, tamarind-kernel powder (10 g) was extracted with hot water, as described earlier. The insoluble impurities (2.2 g) were removed by centrifugation, and saturated, aqueous barium hydroxide (25 ml) was added to the supernatant with constant stirring. The barium complex of the polysaccharide, which separated as a gelatinous mass, was isolated by centrifugation and suspended in water. The suspension was cooled in an ice–salt bath, and the complex was decomposed by cold 0.2N hydrochloric acid. The resulting solution was poured into ethanol to precipitate the polysaccharide which was dried by solvent exchange. The material (5.5 g), $[\alpha]_D$ +58.3° (*c* 0.5, water), was fractionated once more by the above procedure to give the pure polysaccharide (3.8 g), $[\alpha]_D$ +75° (*c* 0.5 water).

The work reported herein was performed on the polysaccharide fractionated twice with Fehling's solution. For this purpose, 100 g of crude, tamarind-kernel powder was fractionated to afford 40 g of the pure polysaccharide.

Homogeneity of the polysaccharide. — The polysaccharide, purified by either of the fractionation methods described above, was shown to be homogeneous by zone electrophoresis in borate buffer. When a 0.1% solution of the polysaccharide in 0.1M sodium tetraborate was centrifuged at 42,040 r.p.m., in a Spinco Model E analytical centrifuge, a single, symmetrical peak (Fig. 1) indicated that the polysaccharide was homogeneous.

Composition of the polysaccharide. — The polysaccharide samples (100 mg each, obtained by the two fractionation procedures) were hydrolysed separately with N sulfuric acid (15 ml) for 16 h at 100°. Paper-chromatographic examination of the hydrolysates revealed glucose, xylose, galactose, and arabinose. The molar ratios of glucose, xylose, galactose, and arabinose in both the samples were 8:4:2:1, as determined by the phenol–sulfuric acid method[7].

Fully methylated TKP (50 mg) was hydrolysed, and the hydrolysate was remethylated[21] with *N,N*-dimethylformamide, barium oxide, and methyl iodide. The reaction mixture was poured into chloroform, and the chloroform extract was evaporated. The resulting syrup, when examined by g.l.c. on column *2*, showed the presence of fully methylated methyl glycosides of L-arabinofuranose, D-xylopyranose, D-glucopyranose, and D-galactopyranose in ratios of 1.2:2:4:7.8, as estimated from peak areas (Fig. 2).

Methylation of TKP. — (*a*) TKP (6.5 g) was methylated twice by the Haworth procedure[22] [dimethyl sulfate (60 ml) and sodium hydroxide (40%, 180 ml)] to yield

partly methylated polysaccharide (5.4 g) that was further methylated by Kuhn's procedure[21] with *N,N*-dimethylformamide (80 ml), methyl iodide (50 ml), and barium oxide–barium hydroxide (15:1; 20 g), to give a product (4.5 g) having a methoxyl value of 32.5%. Five Purdie methylations[23] did not increase the methoxyl content. However, when it was given a Kuhn methylation[21] with *N,N*-dimethylformamide (60 ml), methyl iodide (30 ml), and silver oxide (20 g), the methoxyl content of the product (3.5 g) was raised to 39.5%. The polysaccharide was incompletely methylated as shown by a pronounced hydroxyl-absorption in the infrared spectrum.

(*b*) TKP (5 g) was methylated three times by Haworth's procedure[22], followed by four Purdie methylations[23], to yield a product (4.2 g) having a methoxyl content of 37.2%. Further methylations with the Purdie reagents[23] did not increase the methoxyl content.

(*c*) Partly methylated TKP (1.05 g) from experiment (*b*) was further methylated by suspending it in liquid ammonia (20 ml) and adding sodium (0.2 g) and an equivalent amount of methyl iodide[24]. Processing of the reaction mixture in the usual manner afforded a product (1.0 g) having a methoxyl value of 38.5%.

(*d*) Partly methylated TKP (1.15 g) from (*b*) was dissolved in dimethyl sulfoxide, and methylation was performed by using barium oxide–barium hydroxide and methyl sulfate[25]. The product (0.9 g) had a methoxyl value of 39.5%.

(*e*) TKP (1.2 g) was methylated with methyl sulfoxide–sodium hydroxide–dimethyl sulfate; details of this method have already been published[13]. The product (1.4 g) had a methoxyl content of 41.5%.

(*f*) The combined, partly methylated polysaccharide from Experiments (*a*) (3.2 g; OCH_3, 39.5%) and (*b*) (2 g; OCH_3, 37.2%) was dissolved in dimethyl sulfoxide (100 ml), and sodium hydroxide pellets (30 g) and dimethyl sulfate (15 ml) were added in small amounts during a period of 5 h with constant, vigorous stirring[13]. The resulting mixture was stirred overnight and then heated for 1 h at 95° to decompose excess of dimethyl sulfate. Water was added to dissolve the residual sodium hydroxide, and the cooled solution was partly neutralized with cold, 10N sulfuric acid. After the neutralization had been completed with dilute acetic acid, the reaction mixture was filtered, and the filtrate was extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated to yield the methylated polysaccharide (5.33 g) (Found : OMe, 43.0. Calc.: 43.6%). The i.r. spectrum had no hydroxyl absorption.

Fractionation of methylated TKP. — Methylated TKP (5.2 g) from Experiment (*f*) was dissolved in acetone (100 ml), and ether (50 ml) was added to remove any inorganic impurities. Fractionation was then continued by adding increasing amounts of light petroleum (b.p. 40–60°). A total of seven fractions was recovered. The yields, specific rotations, and methoxyl values of different fractions are given in Table II. On the basis of their physical constants, Fractions III, IV, and VI were combined, and, after hydrolysis, the products were identified, whilst quantitative studies were carried out on Fraction V.

TABLE II

FRACTIONATION OF METHYLATED TKP

Fraction	*Weight (g)*	$[\alpha]_D$ *(chloroform),°*	OCH_3 *(%)*
I	0.333	+22.8	40.7
II	0.311	+29.3	42.8
III	1.773	+29.5	43.5
IV	0.919	+32.0	43.5
V	0.593	+25.0	43.6
VI	0.38	+23.4	43.6
VII	0.914	+ 5.7	35.7

Hydrolysis of methylated TKP, and separation of the methylated sugars. — Methylated TKP (2.8 g) was hydrolysed with sulfuric acid according to the method of Croon *et al.*[14]. The neutralized hydrolysate was deionised, and evaporated to a thick syrup. The mixture (1.6 g) was placed on a cellulose column and developed with solvent *C*. Fractions (10 ml) were examined by paper chromatography. Appropriate fractions were combined and, wherever necessary, further purification of the methylated sugars was performed on Whatman No. 3 papers.

Identification of methylated sugars. — *2,3,5-Tri-*O*-methyl-*L*-arabinose.* The syrup, $[\alpha]_D$ −33.5° (*c* 0.4, water), had the mobility of 2,3,5-tri-*O*-methyl-L-arabinose on paper in solvents *C* and *D*. Demethylation showed that it was a derivative of arabinose. The methylated sugar was treated with methanolic hydrogen chloride and then examined by g.l.c. with columns *1* and *2*. The resulting two peaks had the same retention times as those of an authentic sample of methyl 2,3,5-tri-*O*-methyl-α,β-L-arabinoside.

*2,3,4-Tri-*O*-methyl-*D*-xylose.* The syrupy product, $[\alpha]_D$ +20° (*c* 1, chloroform), which had the chromatographic mobility of 2,3,4-tri-*O*-methyl-D-xylose, crystallized on nucleation, and, on recrystallization from ether–light petroleum (b.p. 40–60°), afforded crystals of 2,3,4-tri-*O*-methyl-D-xylose, m.p. and mixed m.p. 91°, $[\alpha]_D$ +57.2 → +23.4° (*c* 0.7, chloroform)[26]. The syrupy product was boiled under reflux with ethanolic aniline to furnish crystals of 2,3,4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine, m.p. and mixed m.p. 101–102°.

*2,3,4,6-Tetra-*O*-methyl-*D*-galactose.* The syrupy product, $[\alpha]_D$ +110.5° (*c* 0.5, water), had the same mobility on paper in solvents *C* and *D* as an authentic sample of 2,3,4,6-tetra-*O*-methyl-D-galactose, and, on demethylation, gave essentially galactose. With boiling, ethanolic aniline, it afforded crystals of 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m.p. and mixed m.p. 196°, $[\alpha]_D$ −87.5° (*c* 0.7, acetone)[27].

*2,3,6-Tri-*O*-methyl-*D*-glucose and 3,4-Di-*O*-methyl-*D*-xylose.* The syrupy product, although apparently homogeneous on paper chromatograms, showed three constituents (M_G 0.0, 0.33, and 0.66) when examined by paper electrophoresis.

The mixture was resolved into its components by large-scale, paper electrophoresis. The different zones were cut out, and extracted exhaustively with water. The extracts were treated with Amberlite IR-120 and evaporated to dryness, followed

by repeated evaporations with methanol. The residue was then taken up in water and further deionised by passing through cation- and anion-exchange resins.

Component *A*, $[\alpha]_D$ +65° (*c* 1, methanol), was demethylated and found to be a derivative of glucose. The syrupy product was dissolved in pyridine and treated with *p*-nitrobenzoyl chloride to afford 2,3,6-tri-*O*-methyl-1,4-di-*O*-*p*-nitrobenzoyl-D-glucose, m.p. and mixed m.p. 190–192°, $[\alpha]_D$ −33.5° (*c* 0.5, acetone)[28].

Component *B*, $[\alpha]_D$ +19.5° (*c* 0.25, methanol), produced only xylose on demethylation, and was chromatographically indistinguishable from an authentic sample of 3,4-di-*O*-methyl-D-xylose. Oxidation of the syrupy product with bromine water in the presence of a little barium carbonate, followed by lactonization, produced crystals of 3,4-di-*O*-methyl-D-xylonolactone. Recrystallization from ether yielded crystals[29] having m.p. and mixed m.p. 66–67°.

2,3-Di-O-methyl-D-glucose. The syrup, $[\alpha]_D$ +45.5° (*c* 1, water), which was chromatographically indistinguishable from 2,3-di-*O*-methyl-D-glucose, produced glucose on demethylation. The syrup was treated with 3% methanolic hydrogen chloride, and the resulting glycoside, on crystallization from ether–light petroleum (b.p. 40–60°), afforded methyl 2,3-di-*O*-methyl-α-D-glucoside, m.p. and mixed m.p. 85°, $[\alpha]_D$ +144° (*c* 1, water)[30].

Determination of sugar ratios in the methylated TKP hydrolysate. — Methylated TKP (Fraction V) was hydrolysed according to the method of Croon *et al.*[14]. The neutralized hydrolysate was concentrated, and the sugar components were resolved on Whatman No. 3 paper in solvent *C*. The appropriate zones were cut out, and eluted with methanol. After filtration and evaporation of the extracts, the individual components were re-extracted with water. The extracts were filtered, and evaporated to constant weights.

The mixture of 2,3,6-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-glucose was resolved into its components by paper electrophoresis. The molar ratios of different methylated sugars are given in Table I.

Acetolyses. — Four 1-g samples of TKP were shaken separately with acetic anhydride (5 ml), acetic acid (5.6 ml), and concentrated sulfuric acid (0.28 ml) for four days. The acetolysates were mixed, and centrifuged to remove the insoluble matter. The supernatant was poured onto crushed ice, and the mixture was kept for 1 h with occasional stirring and then extracted with chloroform. The extract was washed with aqueous sodium hydrogen carbonate and water, and then dried (Na_2SO_4). Removal of the solvent gave a syrup (0.9 g), which was deacetylated (sodium methoxide)[31], and chromatographed in solvents *A* and *B* to reveal the presence of a mixture of oligo- and mono-saccharides. The oligosaccharides were isolated by a combination of charcoal-column and paper chromatography. Two disaccharides, *A* and *B*, having R_G 0.48 and 0.68 (solvent *A*), were obtained as syrups in yields of 58 and 42 mg, respectively.

Cellobiose. Disaccharide *A*, $[\alpha]_D$ +34° (*c* 0.5, water), on acid hydrolysis, produced only glucose. On paper, it had the mobility of cellobiose in different solvents. The syrup (50 mg) was acetylated with acetic anhydride (5 ml) and anhydrous sodium

acetate (500 mg) by heating on a boiling-water bath for 8 h. Processing of the reaction mixture in the usual way produced octa-*O*-acetyl-*β*-cellobiose, m.p. and mixed m.p. 188–189°.

2-O-β-D-Galactopyranosyl-D-xylopyranose. Disaccharide *B*, $[\alpha]_D$ +32.5° (*c* 0.5, water), R_G 0.68 in solvent *A*, upon acid hydrolysis, produced galactose and xylose in equimolar proportions. Xylose was found to be the reducing end of the disaccharide.

The disaccharide (28 mg) was given, successively, one Kuhn[21] and two Purdie methylations[23] to afford a fully methylated disaccharide (no hydroxyl absorption in the i.r. spectrum). Acid hydrolysis of the methyl ether produced 2,3,4,6-tetra-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-D-xylose as identified by paper chromatography and ionophoresis.

Enzymic hydrolysis. — A 1% aqueous solution of TKP was hydrolyzed in a cellophane tube with the enzyme "Hemicellulase" (Nutritional Biochemicals Corporation) (10% of the weight of the polysaccharide) for 51 days at 40°. The apparatus was essentially the same as described by Perila and Bishop[32]. The water outside the cellophane tube was changed every three days. The course of hydrolysis was checked by concentrating the dialysate, and examining it by paper chromatography. Similar fractions were combined and further resolved on Whatman No. 3 papers in solvent *A*. Two disaccharides, *C* and *D*, were obtained in 14 and 44% yields, respectively, based on the weight of the dry polysaccharide.

Lactose. Disaccharide *C*, R_C 0.8 (solvent *A*), had the same mobility on paper as lactose. Acid hydrolysis of *C*, before and after reduction with sodium borohydride, revealed that it was constituted of glucose and galactose residues, the former constituting the reducing end. The disaccharide had a D.P. of 1.78 as determined by the method of Timell[33]. By this method, an authentic sample of lactose gave a D.P. of 1.8. The syrup crystallized from aqueous ethanol to give crystals of lactose, m.p. and mixed m.p. 200–201°, $[\alpha]_D$ +90.5 → +49.5° (*c* 1, water). The i.r. spectrum of the crystalline sugar was identical with that of an authentic sample of lactose. Treatment of the sugar with phenylhydrazine and acetic acid in the usual way afforded crystals of lactose phenylosazone, m.p. and mixed m.p. 208–211°.

The disaccharide (80 mg) was dissolved in dimethyl sulfoxide (2 ml) and methylated[34] by adding barium oxide (200 mg) and methyl iodide (1 ml). The resulting product was found to be incompletely methylated (OH absorption in the i.r. spectrum). Methylation was therefore completed by three treatments with the Purdie reagents[23]. Hydrolysis of the fully methylated disaccharide gave 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-glucose. The former was identified as the aniline derivative, m.p. and mixed m.p. 196°, $[\alpha]_D$ −87° (acetone), and the latter as 2,3,6-tri-*O*-methyl-D-glucose 1,4-di-*p*-nitrobenzoate, m.p. and mixed m.p. 190°, $[\alpha]_D$ −33° (acetone).

6-O-α-D-Xylopyranosyl-D-glucopyranose. The syrupy disaccharide *D*, having $[\alpha]_D$ +122° (*c* 1, water) and R_C 1.22 in solvent *A*, produced equimolar amounts of glucose and xylose on acid hydrolysis. The D.P. of the sugar was 2 as determined by Timell's method[33]. Hydrolysis of the disaccharide, before and after reduction, and

paper chromatography of the hydrolysates revealed that glucose was the reducing residue.

The disaccharide (60 mg) was dissolved in *N*,*N*-dimethylformamide (2 ml) and methylated with barium oxide–barium hydroxide octahydrate (15:1, 300 mg) and methyl iodide (2 ml). The product, which was incompletely methylated, showed, after one Purdie methylation[23], complete absence of hydroxyl absorption in the i.r. spectrum. Hydrolysis of the disaccharide methyl ether produced 2,3,4-tri-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose, which were isolated by paper chromatography.

Treatment of 2,3,4-tri-*O*-methyl-D-glucose with boiling, ethanolic aniline for 3 h gave, on slow evaporation of the solvent in a desiccator, a product that on recrystallization from ether furnished *N*-phenyl 2,3,4-tri-*O*-methyl-D-glucosylamine, m.p. 144–145° (lit.[35], m.p. 145–146°).

2,3,4-Tri-*O*-methyl-D-xylose was also treated with ethanolic aniline, as described above, to afford the "anilide", m.p. and mixed m.p. 100–101°.

Partial Hydrolysis of TKP. — To a solution of TKP (20 g) in water (2 l), 2N sulfuric acid (500 ml) was added, and the mixture was then heated on a boiling-water bath for 3 h. The hydrolysate was neutralized ($BaCO_3$) and, after concentration to a small volume, was poured into ethanol. The precipitated polysaccharide (2.5 g) had $[\alpha]_D$ +33.5° (*c* 1, 0.5N sodium hydroxide) and produced glucose and xylose, in a molar ratio of 15:1, on acid hydrolysis. Galactose and arabinose were not detected in the hydrolysate.

Degraded TKP (1 g) was dissolved in dimethyl sulfoxide, and methylated[34] with barium oxide (6 g) and methyl iodide (10 ml). The partly methylated product was given three Purdie methylations[23], and methylation was then complete as indicated by the absence of hydroxyl absorption in the infrared spectrum; yield, 950 mg. The methylated product was fractionated by acetone–light petroleum to afford four fractions. On hydrolysis, all of the fractions produced 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,di-*O*-methyl-D-glucose. No 2,3,4-tri-*O*-methyl-D-glucose could be detected chromatographically.

The mixture of methylated sugars was treated with methanolic hydrogen chloride, and the resulting methylated glycosides were examined by g.l.c. using columns *1* and *2*. No methyl 2,3,4-tri-*O*-methyl-D-glucoside was detected.

Periodate Oxidation. — TKP (1 g) was oxidised with 0.5M sodium metaperiodate (500 ml) at 10° in the dark. The determinations[36,37] of the periodate consumed and formic acid liberated per mole of sugar residue (152; calculated on the basis of molar ratios of glucose, xylose, galactose, and arabinose as 8:4:2:1) are given in Table III.

When the oxidation of TKP was complete, the solution was treated with barium carbonate and filtered, and the filtrate was reduced with sodium borohydride (1 g) for 6 h. The solution was acidified with acetic acid, passed through IR-120 resin, evaporated to dryness, the residue distilled repeatedly with methanol. The residue

was hydrolysed with N sulfuric acid, and the hydrolysate was neutralized ($BaCO_3$) and filtered The filtrate was deionised with Amberlite IR-120 and IR-45 resins, and concentrated. Examination of the product by paper chromatography revealed glycerol, erythritol, and glyceraldehyde; no sugars were detected.

TABLE III

PERIODATE OXIDATION OF TKP

Time (h)	12	25	75	96	121	145	169
Consumption of periodate (moles/mole)	1.05	1.22	1.26	1.30	1.33	1.35	1.35
Liberation of formic acid (mole/mole)	0.30	0.312	0.32	0.328	0.337	0.34	0.34

The ratio of glycerol:erythritol:glyceraldehyde in the oxidised, reduced, hydrolysed product from TKP was determined in the following manner; the hydrolysate was divided into two portions *A* and *B*.

Portion *A* was oxidized with bromine water in the presence of barium carbonate for 48 h in the dark. Bromine was removed by aeration, and the solution was filtered. The filtrate was treated with Amberlite IR-120 and IR-45 resins, and concentrated. The mixture of glycerol and erythritol was resolved on Whatman No. 1 paper in solvent *A*. The respective zones containing glycerol and erythritol were cut out, eluted with water, and estimated by chromotropic acid[38]; erythritol–glycerol ratio, 1:0.32.

Portion *B* was reduced with sodium borohydride, neutralized with acetic acid, deionized with Amberlite IR-120 resin, and evaporated to dryness. Boric acid was removed from the residue by repeated distillations with methanol. The residue was dissolved in water, and the solution was deionised with Amberlite IR-120 and IR-45 resins, and concentrated. The amounts of erythritol and glycerol, determined as described earlier, were in the ratio 1:0.43.

Glycerol arising from glyceraldehyde $= 0.43 - 0.32 = 0.11$

$$\text{Proportion of glyceraldehyde} = \frac{0.11 \times 90}{92} = 0.108$$

Thus, the ratios of erythritol:glycerol:glyceraldehyde are 1:0.32:0.11 or 8:2.5:0.88.

ACKNOWLEDGMENTS

The authors thank Dr. G. A. Adams, National Research Council of Canada, Ottawa, for carrying out sedimentation studies on the polysaccharide. One of us (P.P.S.) thanks the Council of Scientific and Industrial Research, India, for the award of a Fellowship.

SUMMARY

Tamarind-kernel powder, on fractionation, afforded a homogeneous polysaccharide composed of D-glucose, D-xylose, D-galactose, and L-arabinose in the molar ratios of 8:4:2:1. Whereas acetolysis of the polysaccharide afforded 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose) and 2-*O*-β-D-galactopyranosyl-D-xylopyranose, hydrolysis with an enzyme preparation, "Hemicellulase", produced 4-*O*-β-D-galactopyranosyl-D-glucose (lactose) and 6-*O*-α-D-xylopyranosyl-D-glucopyranose. Methylation of the polysaccharide, followed by hydrolysis, yielded the following methylated sugars: 2,3,5-tri-*O*-methyl-L-arabinose (1 mole); 2,3,4-tri-*O*-methyl-D-xylose (3 moles); 2,3,4,6-tetra-*O*-methyl-D-galactose (2 moles); 3,4-di-*O*-methyl-D-xylopyranose (1 mole); 2,3,6-tri-*O*-methyl-D-glucopyranose (2 moles); and 2,3-di-*O*-methyl-D-glucopyranose (6 moles). On oxidation of the polysaccharide with sodium metaperiodate, 1.35 moles of oxidant were consumed and 0.34 mole of formic acid was liberated. On reduction with sodium borohydride, followed by acid hydrolysis, the oxopolysaccharide gave erythritol, glycerol, and glyceraldehyde in molar ratios of 8:2.5:1.

Partial hydrolysis of the polysaccharide with acid produced a degraded polymer composed of glucose and a small proportion of xylose. The degraded polymer on methylation and hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-glucose; 2,3,4-tri-*O*-methyl-D-xylose; 2,3,6-tri-*O*-methyl-D-glucose; and 2,3-di-*O*-methyl-D-glucose.

On the basis of the above results, a structure for the polysaccharide of tamarind kernel is proposed.

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Carbohydrate Research
Elsevier Pubishing Company, Amsterdam
Printed in Belgium

MODE OF DECOMPOSITION OF CHLORO- AND FLUORO-FORMATES OF SOME CARBOHYDRATES AND RELATED COMPOUNDS[1]

N. BAGGETT, K. W. BUCK*, A. B. FOSTER*, R. JEFFERIS, AND J. M. WEBBER
Chemistry Department, The University, Birmingham 15 (Great Britain)
(Received January 9th, 1967)

INTRODUCTION

Chloroformates and fluoroformates of simple aliphatic, primary and secondary alcohols may be readily decarboxylated to yield alkyl chlorides[2] and fluorides[3]. However, Welch and Kent[4] reported that they were unable to effect thermal or catalytic decarboxylation of the fluoroformates of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose and 1,2-*O*-isopropylideneglycerol. No experimental details were given. We have found that 6-*O*-fluoroformyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactose and related fluoroformates do react under the thermal and catalytic conditions employed for the decarboxylation of alkyl fluoroformates, to yield dialkyl carbonates, but not fluorides.

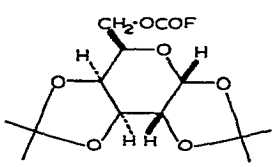
RESULTS AND DISCUSSION

Chloroformates are readily prepared by reaction of the appropriate alcohols with an excess of phosgene, and may be converted into the analogous fluoroformates by treatment with thallous fluoride[3]. We have confirmed the observation[4] that high yields are obtained in the latter reaction when methyl cyanide is employed as the reaction solvent, and the extent of reaction may be conveniently followed by means of infrared spectroscopy, since the C=O absorptions for chloroformates and fluoroformates occur at *ca.* 1780 and *ca.* 1830 cm^{-1}, respectively.

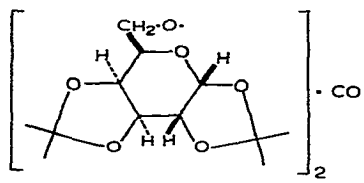
When a pyridine solution of 6-*O*-fluoroformyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**1**) was stored at 110–120° until reaction was complete, the apparent decarboxylation (see Experimental) was only *ca.* 50%. The predominant, if not the exclusive, reaction product was bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactose) 6,6′-carbonate (**2**); no trace of 6-deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-D-galactose[4] could be detected. Authentic bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactose) 6,6′-carbonate was obtained by reaction of phosgene with 2 mol. of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose in the presence of pyridine. Authentic samples of the other dialkyl

*Present addresses: K.W.B., Biochemistry Department, Imperial College, South Kensington, London S.W.7; A.B.F., Chemistry Department, Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London S.W.3. (Great Britain).

carbonates mentioned below were prepared by similar means. Decomposition of 6-*O*-fluoroformyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactose in the presence of an excess of pyridine, or in *N*,*N*-dimethylformamide containing potassium fluoride and pyridine, also gave the dialkyl carbonate as the sole product. The dialkyl carbonate was also the only product detected when 6-*O*-fluoroformyl-1,2:3,4-di-*O*-isopropylidene-DL-xylitol was decomposed in pyridine.

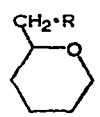


(1)

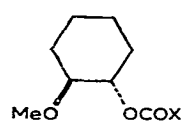


(2)

A more-detailed study of the reaction was then undertaken by using the haloformates of the model compound, 2-(hydroxymethyl)tetrahydropyran. Addition of pyridine to the chloroformate (**3**) resulted in an immediate exothermic reaction and, ultimately, 78% decarboxylation on completion of the reaction at 110–120°. 2-(Chloromethyl)tetrahydropyran (**4**) was formed, and the i.r. absorption at *ca.* 1750 cm^{-1} in the crude product, together with chromatographic data, indicated that bis(tetrahydropyran-2-methyl) carbonate was also formed. On the other hand, the fluoroformate (**5**) was unaffected by pyridine at room temperature, but decomposition appeared to be complete (53% decarboxylation) after 14 h at 110–120°; bis(tetrahydropyran-2-methyl) carbonate was the sole, detected product. In pyridine at 110–120°, the formation of a pyridinium complex from 2-(fluoroformyloxymethyl)-tetrahydropyran must occur slowly, since, after 2.5 h, a considerable proportion of undecomposed fluoroformate, but no 2-(fluoromethyl)tetrahydropyran (**6**), could be detected by g.l.c. [the authentic compound is readily obtained by treatment of 2-(toluene-*p*-sulphonyloxymethyl)tetrahydropyran with potassium fluoride in ethane-1,2-diol]; bis(tetrahydropyran-2-methyl) carbonate was also present in the product mixture. Pyridine was not essential for the formation of dialkyl carbonate, since thermal decomposition of 2-(fluoroformyloxymethyl)tetrahydropyran, although slow, gave bis(tetrahydropyran-2-methyl) carbonate.



3 R = OCOCl
4 R = Cl
5 R = OCOF
6 R = F



7 X = Cl
8 X = F

In all of the above cases where decomposition of fluoroformates in pyridine yielded a dialkyl carbonate, but no alkyl fluoride, the grouping F·CO·O·C–C·O is present and, in order to assess the role of the vicinal oxygen atom in the reaction, the decomposition of the chloroformate and fluoroformate of cyclohexanemethanol was

examined. As with the other chloroformates, cyclohexanemethyl chloroformate reacted immediately on contact with pyridine at room temperature, although elevation of temperature to 110–120° was necessary to complete the decomposition (89% decarboxylation). Chloromethylcyclohexane was subsequently isolated, and evidence was obtained for the formation of bis(cyclohexanemethyl) carbonate. The fluoroformate of cyclohexanemethanol did not react with pyridine at room temperature, and, after 17 h at 110–120°, only 38% apparent decarboxylation had occurred; the reaction was incomplete, since the product had absorption at *ca.* 1820 cm^{-1} (O·CO·F). The absorption at *ca.* 1750 cm^{-1} indicated the presence of bis(cyclohexanemethyl)-carbonate, and g.l.c. revealed the presence of cyclohexanemethyl fluoride. Thus, the oxygen atom which is vicinal to the fluoroformate group in 2-(fluoroformyloxymethyl)-tetrahydropyran is not solely responsible for the formation of carbonate when the compound is decomposed in pyridine.

In extending the investigation to secondary haloformates, *trans*-2-methoxycyclohexanol was selected as a model compound. In pyridine at *ca.* 100°, the chloroformate (7) had undergone 83% decarboxylation after 22 h. The principal reaction product was *cis,trans*-2-methoxycyclohexyl chloride, in which the *cis,trans*-ratio was 34:1 as established by g.l.c.; t.l.c. and i.r. spectroscopy indicated that bis(2-methoxycyclohexyl) carbonate was also formed. The occurrence of decarboxylation in pyridine with preponderant inversion of configuration parallels the results of other workers[5], as did thermal decomposition (140°; *cis,trans*-ratio, 1:28), and decarboxylation in the presence of boron trifluoride-etherate in boiling hexane (*cis,trans*-ratio, 1:9).

When *trans*-2-methoxycyclohexyl fluoroformate (8) was treated with pyridine at 90°, apparent decarboxylation was slow (21% after 24 h). Fractional distillation of the product mixture at this stage gave starting material and bis(*trans*-2-methoxycyclohexyl) carbonate.

The formation of carbonates in the decomposition of haloformates in pyridine, which, apparently, has not hitherto been recognised, is likely to occur generally, although the proportion will vary and may be very small in some cases, for example, ethyl chloro- and fluoro-formates and cyclohexyl chloroformate, which underwent *ca.* 100% decarboxylation. Cyclohexyl fluoroformate in pyridine at 110–120° underwent 52% decarboxylation during 27 h, and in a parallel experiment, where quinoline was used, the i.r. spectrum of the product had absorption at *ca.* 1750 cm^{-1} typical of dialkyl carbonate.

The above examples of formation of dialkyl carbonates must involve intermolecular reactions. An apparently analogous, intramolecular reaction was observed when *trans*-1,2-bis(fluoroformyloxy)cyclohexane was decomposed in quinoline for 22 h at 160°. The preponderant product was the cyclic carbonate of cyclohexane-*trans*-1,2-diol, and this was identified by comparison with the authentic compound[6].

Cyclohexa-1,3-diene (subsequently isolated and characterised as the tetracyanoethylene adduct) and a second, volatile compound (*X*) were also formed in small proportions in the decomposition of *trans*-1,2-bis(fluoroformyloxy)cyclohexane. Compound *X*, which could not be isolated in the pure state, decomposed on storage, evolving

hydrogen fluoride and affording cyclohexa-1,3-diene. A comparison may be made with the decomposition of *trans*-1,2-bis(chloroformyloxy)cyclohexane[2] in pyridine at 110–130° which afforded *trans*-1,2-dichlorocyclohexane (18%) and 1-chlorocyclohexene (17%). Cyclohexa-1,3-diene was not detected, and, in view of the above results, the significant proportion of unidentified, high-boiling material could be, at least in part, the cyclic carbonate of cyclohexane-*trans*-1,2-diol.

A study of the mechanism of dialkyl carbonate formation is in hand.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was performed on Kieselgel, and detection effected with vanillin–sulphuric acid[7] or iodine vapour. Gas–liquid chromatography (g.l.c.) was performed on (*a*) a Pye-Argon instrument (*β*-ionization detection) with a column packing of poly(ethylene glycol adipate), and a gas pressure of 8 lb/sq.in; (*b*) a Perkin–Elmer instrument (Katharometer detection) with a column packing of silicone gum–Celite.

Preparation of chloroformates. — (*a*) A solution of 1,2:3,4-di-*O*-isopropylidene-DL-xylitol[8] (48 g) in quinoline (40 ml, 1.5 mol.) and ether (180 ml) was added dropwise to a stirred, ice-cold, 20% solution of phosgene (25 g) in ether. After the solution had been stirred overnight at room temperature, the excess of phosgene was removed at ~12 mm, and the remaining ether solution was poured into water (250 ml). The ethereal layer was washed successively with ice-cold, 10% hydrochloric acid (150 ml), 5% aqueous potassium carbonate, and water. Concentration of the dried ($MgSO_4$) extract, with distillation of the residue, gave 5-*O*-chloroformyl-1,2:3,4-di-*O*-isopropylidene-DL-xylitol (28 g, 50%), b.p. 120°/0.9 mm, ν_{max} 1780 (C=O) and 686 cm^{-1} (C–Cl) (Found: C, 48.6; H, 6.2; Cl, 12.0. $C_{13}H_{19}ClO_7$ calc.: C, 48.9; H, 6.5; Cl, 12.1%).

The following compounds were prepared in an essentially similar manner from the appropriate alcohols. 2-(Chloroformyloxymethyl)tetrahydropyran (87%, b.p. 100–101°/17 mm, ν_{max} 1778 and 685 cm^{-1} (Found: C, 47.3; H, 6.2; Cl, 19.6. $C_7H_{11}ClO_3$ calc.: C, 47.1; H, 6.2; Cl, 19.9%); reaction of the chloroformate with aniline, or of 2-(tetrahydropyran)methanol with phenyl isocyanate, gave the carbanilate, m.p. 86–87° (Found: C, 66.4; H, 7.2; N, 5.9. $C_{13}H_{17}NO_3$ calc.: C, 66.4; H, 7.3; N, 5.6%). (Chloroformyloxymethyl)cyclohexane (84%), b.p. 76–77°/10 mm (Found: Cl, 20.1. $C_8H_{13}ClO_2$ calc.: Cl, 20.1%); reaction with aniline gave the carbanilate, m.p. 81.5–82.5° [from light petroleum (b.p. 60–80°)], as did the reaction of cyclohexanemethanol with phenyl isocyanate (Found: C, 72.2; H, 8.3; N, 5.9. $C_{14}H_{19}NO_2$ calc.: C, 72.1; H, 8.2; N, 6.0%). *trans*-2-Methoxycyclohexyl chloroformate (44%), b.p. 106°/~20 mm. (Found: C, 50.0; H, 6.7; Cl, 18.6. $C_8H_{13}ClO_3$ calc.: C, 49.9; H, 6.8; Cl, 18.4%); reaction of the chloroformate with aniline, or of *trans*-2-methoxycyclohexanol with phenyl isocyanate, gave the carbanilate, m.p. 67–69° (Found: C, 67.3; H, 7.5; $C_{14}H_{19}NO_3$ calc.: C, 67.4; H, 7.7%).

Preparation of fluoroformates. — (*a*) A mixture of 5-*O*-chloroformyl-1,2:3,4-di-*O*-isopropylidene-DL-xylitol (22.5 g) and methyl cyanide (32 ml) was stirred and

boiled under reflux in the presence of thallous fluoride (37 g) for 62 h (in subsequent experiments, the course of reaction was followed by i.r. spectroscopy and shown to be essentially complete in 3 h). The filtered solution was concentrated, and the residue distilled to yield 5-*O*-fluoroformyl-1,2:3,4-di-*O*-isopropylidene-DL-xylitol (15 g, 70%), b.p. 95°/0.9 mm, which crystallized on storage, m.p. 41–42° (from acetone) and ν_{max} 1830 cm^{-1} (C=O) (Found: C, 52.1; H, 6.8; F, 7.1. $C_{13}H_{19}FO_7$ calc.: C, 51.8; H, 6.8; F, 6.8%).

By essentially the above method (but with reaction times of 2–3 h), the relevant chloroformates were converted into the following compounds: 6-*O*-fluoroformyl-1,2:5,6-di-*O*-isopropylidene-α-D-galactose (80%), m.p. 72° (from acetone), $[\alpha]_D^{30}$ −51° (*c* 1.0, chloroform), ν_{max} 1825 cm^{-1}; lit.[4], m.p. 74° and $[\alpha]_D^{21}$ −50° (chloroform); 2-(fluoroformyloxymethyl)tetrahydropyran (70%), b.p. 73–74°/17 mm, ν_{max} 1828 cm^{-1} (Found: C, 51.8; H, 6.8; F, 12.3. $C_7H_{11}FO_3$ calc.: C, 51.9; H, 6.8; F, 11.7%), characterised as the carbanilate, m.p. 86–87°; *trans*-2-methoxycyclohexyl fluoroformate (92%), b.p. 86–88°/~20 mm, ν_{max} 1825 cm^{-1} (Found: C, 54.45; H, 7.3. $C_8H_{13}FO_3$ calc.: C, 54.55; H, 7.4%) (carbanilate, m.p. 67–69°); (fluoroformyloxymethyl)cyclohexane (84%), b.p. 82–84°/30 mm, ν_{max} 1826 cm^{-1} (Found: C, 59.9; H, 8.2; F, 11.4. $C_8H_{13}FO_2$ calc.: C, 60.0; H, 8.2; F, 11.9%).

(*b*) A stirred mixture of *trans*-1,2-bis(chloroformyloxy)cyclohexane (30 g) and methyl cyanide (150 ml) was boiled under reflux for 27 h in the presence of thallous fluoride (87.5 g, 3 mol.). The product was extracted with ether, the extract was concentrated, and the residue was distilled to yield, as the minor fraction, *trans*-1,2-bis(fluoroformyloxy)cyclohexane (3 g), b.p. 71°/4 mm (Found: C, 46.6; H, 4.6; F, 18.7. $C_8H_{10}F_2O_4$ calc.: C, 46.2; H, 4.8; F, 18.3%). Reaction with aniline gave the dicarbanilate[2], m.p. 214°. The major (unidentified) fraction, b.p. 120°/4 mm, crystallized on storage, and had m.p. 74–74.5° (from aqueous methanol) and ν_{max} 1750 cm^{-1} (C=O) (Found: C, 59.1; H, 6.8. $C_{14}H_{20}O_6$ calc.: C, 59.2; H, 7.0%). The molecular weight determined by micro-osmometry was 233, and saponification gave cyclohexane-*trans*-1,2-diol, m.p. 103–104°.

When a mixture of *trans*-1,2-bis(chloroformyloxy)cyclohexane (37 g), thallous fluoride (140 g), and methyl cyanide (50 ml) was boiled under reflux for 72 h, the difluoroformate (27.4 g, 86%), b.p. 48.5°/0.2 mm, was isolated. In subsequent experiments, the reaction course was followed by i.r. spectroscopy and shown to be complete in 3 h.

Preparation of alkyl fluorides. — A mixture of 2-(toluene-*p*-sulphonyloxymethyl)-tetrahydropyran (10 g), anhydrous potassium fluoride (10 g), and ethane-1,2-diol (60 ml) was stirred for 2 h at 130°. The cooled mixture was diluted with water (150 ml), and extracted with ether (2 × 100 ml). The combined extracts were washed with water (200 ml), dried ($MgSO_4$), and concentrated, and the residue was distilled to give 2-(fluoromethyl)tetrahydropyran (1.8 g, 40%), b.p. 121–123° (Found: C, 61.3; H, 9.2; F, 16.7. $C_6H_{11}FO$ calc.: C, 61.0; H, 9.3; F, 16.1%). Similarly, cyclohexanemethyl toluene-*p*-sulphonate (m.p. 82–82.5°) was converted into cyclohexanemethyl fluoride (56%), b.p. 116–118°, which was freed from olefinic material by preparative g.l.c.

(Wilkens Autoprep, silicone gum–Celite, 120°) (Found: C, 72.2; H, 11.3; F, 16.7. $C_7H_{13}F$ calc.: C, 72.4; H, 11.2; F, 16.4%).

Preparation of dialkyl carbonates. — A solution of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose (5 g) in dry pyridine (30 ml) was added dropwise to a solution of phosgene (1.5 g, 1.1 mol.) in dry hexane (100 ml). After being stirred for 3.5 h at room temperature, the mixture was poured into ice-water (700 ml) and extracted with benzene (3 × 100 ml). The combined extracts were washed quickly, and successively, with ice-cold, N hydrochloric acid (400 ml), 10% aqueous cadmium chloride, 5% aqueous sodium hydrogen carbonate, and water. The dried ($MgSO_4$) extract was decolourised with charcoal, and concentrated, and the residue was recrystallised from methanol to yield bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose) 6,6′-carbonate (3 g, 37%), m.p. 134–135°, $[\alpha]_D^{30}$ −73° (*c* 1.0, chloroform), ν_{max} 1738 cm^{-1} (C=O) (Found: C, 55.2; H, 7.2. $C_{25}H_{38}O_3$ calc.: C, 54.9; H, 7.0%).

Similarly, the following compounds were prepared from the respective alcohols: bis(1,2:3,4-di-*O*-isopropylidene-DL-xylitol) 5,5′-carbonate, b.p. 204–206°/0.5 mm, ν_{max} 1760 cm^{-1} (Found: C, 56.2; H, 7.8. $C_{23}H_{38}O_{11}$ calc.: C, 56.3; H, 7.8%); bis-(tetrahydropyran-2-methyl) carbonate, b.p. 132°/0.3 mm, ν_{max} 1747 cm^{-1} (Found: C, 60.3; H, 8.6. $C_{13}H_{22}O_5$ calc.: C, 60.4; H, 8.6%); bis(cyclohexanemethyl) carbonate (54%), b.p. 112°/0.25 mm, ν_{max} 1750 cm^{-1} (Found: C, 71.3; H, 10.3. $C_{15}H_{26}O_3$ calc.: C, 71.0; H, 10.3%; bis(*trans*-2-methoxycyclohexyl) carbonate (56%), b.p. 144°/0.5 mm, ν_{max} 1750 cm^{-1} (Found, C, 63.1; H, 9.2. $C_{15}H_{26}O_5$ calc.: C, 62.9; H, 9.15%).

Decomposition of haloformates. — Each haloformate was decomposed in a stream of nitrogen that was subsequently passed through Drechsel bottles containing aqueous barium hydroxide. The barium carbonate formed was collected, washed, dried, and weighed, and the amount was used to calculate the percentage of apparent decarboxylation. It must be emphasised that carbon dioxide is not necessarily the sole, gaseous product evolved.

(*a*) *6-O-Fluoroformyl-1,2:3,4-di-O-isopropylidene-α-D-galactose.* A mixture of the fluoroformate (1.77 g) and pyridine (5 ml, 1.5 mol.) was heated for 15 h at 110–120° (50% decarboxylation). A solution of the reaction products in ether (100 ml) was washed successively with water, 10% aqueous cadmium chloride, 5% aqueous sodium hydrogen carbonate, and water. Evaporation of the dried ($MgSO_4$) ethereal solution, with recrystallisation of the residue from methanol, gave bis(1,2:3,4-di-*O*-isopropylidene-α-D-galactose) 6,6′-carbonate, m.p. and mixed m.p. 134–135°. Examination of the mother liquors by t.l.c. (benzene–ether, 3:1) revealed a single component having mobility indistinguishable from that of the above dialkyl carbonate; no 6-deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-D-galactose[4] could be detected.

When the fluoroformate (3 g) was decomposed in pyridine (150 ml) for 15 h at 110–120°, the only detectable materials in the product mixture were the dialkyl carbonate and unchanged fluoroformate. A similar result was obtained when the fluoroformate was decomposed by boiling a solution in *N,N*-dimethylformamide containing pyridine and anhydrous potassium fluoride.

(*b*) *5-O-Fluoroformyl-1,2:3,4-di-O-isopropylidene-DL-xylitol.* A mixture of the

fluoroformate (1.23 g) and pyridine (4 ml) was heated for 18 h at 110–120° (52% decarboxylation). The product mixture was processed essentially as in (*a*) to give a crude product (ν_{max} 1760 cm^{-1}) that contained a single component having a mobility in t.l.c. identical to that of bis(1,2:3,4-di-*O*-isopropylidene-DL-xylitol) 5,5′-carbonate. A portion (*ca.* 300 mg) of the product was saponified by treatment with a mixture of N sodium hydroxide (10 ml) and ethanol (10 ml) for 2 h at 60°. The mixture was extracted with chloroform (60 ml), and the extract was dried ($MgSO_4$) and concentrated to yield a product having a mobility in t.l.c. identical to that of 1,2:3,4-di-*O*-isopropylidene-DL-xylitol; the product gave[9] a *p*-phenylazobenzoate, m.p. 110–111° alone or in admixture with the ester prepared from 1,2:3,4-di-*O*-isopropylidene-DL-xylitol (Found: C, 65.2; H, 6.2; N, 6.3. $C_{24}H_{28}N_2O_6$ calc.: C, 65.5; H, 6.4; N, 6.4%).

(*c*) *2-(Chloroformyloxymethyl)tetrahydropyran.* On addition of pyridine (3 ml) to the chloroformate (1.46 g) at room temperature, smooth decomposition occurred and was complete after storage for 18 h at 110–120° (78% decarboxylation). The reaction mixture was processed as in (*a*), after the ether extract had been washed with dilute hydrochloric acid, to give a product, having ν_{max} 1750 (C=O) and 740 cm^{-1} (C-Cl), which contained components having mobilities identical to those of bis(tetrahydropyran-2-methyl) carbonate (t.l.c.) and 2-(chloromethyl)tetrahydropyran [g.l.c., (*b*)]. In a larger-scale experiment, fractional distillation of the product mixture gave 2-(chloromethyl)tetrahydropyran (70%), b.p. 165° (Found: C, 53.2; H, 7.4; Cl, 26.1. $C_6H_{11}ClO$ calc.: C, 53.5; H, 8.2; Cl, 26.4%).

(*d*) *2-(Fluoroformyloxymethyl)tetrahydropyran.* No evolution of gas occurred when a mixture of the fluoroformate (1.52 g) and pyridine (3 ml) was stored at room temperature, and decomposition was complete (53% decarboxylation) after 14 h at 110–120°. The product, isolated essentially as described in (*c*), had ν_{max} at 1750 cm^{-1}, and contained a single component having a mobility (t.l.c.) identical to that of bis-(tetrahydropyran-2-methyl) carbonate. No 2-(fluoromethyl)tetrahydropyran could be detected by g.l.c. (*b*). In a subsequent experiment, distillation of the product gave bis-(tetrahydropyran-2-methyl) carbonate (58%), b.p. 116°/0.05 mm.

(*e*) trans-*2-Methoxycyclohexyl chloroformate.* A mixture of the chloroformate (2.19 g) and pyridine (6.8 ml) was heated at *ca.* 100°, and the extent of decomposition, expressed as percentage of decarboxylation, was as follows: 45 min, 66%; 5.5 h, 81%; 22 h, 83%. The product was isolated as in (*c*) and distilled to give *cis,trans*-2-methoxycyclohexyl chloride (0.685 g, 40%), b.p. 80–90°/~20 mm. (Found: C, 56.8; H, 9.0; Cl, 23.6. $C_7H_{13}ClO$ calc.: C, 56.6; H, 8.8; Cl, 23.85%); examination by g.l.c. [(*a*), 126°] revealed two peaks in the ratio of 34:1, with the faster component preponderating. The slower component had a retention distance identical to that of *trans*-2-methoxycyclohexyl chloride (for preparation, see below).

A higher-boiling fraction (b.p. 90–200°/20 mm) from the above mixture had ν_{max} 1750 cm^{-1} (C=O) and a mobility (t.l.c., benzene–methanol, 9:1) identical to that of bis(*trans*-2-methoxycyclohexyl) carbonate.

(*f*) trans-*2-Methoxycyclohexyl fluoroformate.* When a mixture of the fluoroformate (1.0 g) and pyridine (4.0 ml) was heated at 90°, the decomposition, expressed

as percentage of decarboxylation, was as follows: 4 h, 4%; 21 h, 18%; 24 h, 21%. The product was isolated as in (*c*) and distilled to yield starting material (0.24 g), b.p. 86°/~20 mm, and bis(*trans*-2-methoxycyclohexyl) carbonate.

(*g*) (*Chloroformyloxymethyl)cyclohexane.* When the chloroformate (7 g) was treated with pyridine (11 ml), an exothermic reaction occurred which was completed by elevation of the temperature to 110–120° for 24 h (89% decarboxylation). The cooled reaction mixture was extracted with ether (150 ml) as in (*c*), and the product was distilled to give (chloromethyl)cyclohexane (4 g, 74%), b.p. 168–170° (Found: C, 63.4; H, 9.8; Cl, 26.8. $C_6H_{11}Cl$ calc.: C, 63.4; H, 9.6; Cl, 26.8%).

The still residue was shown by g.l.c. (*b*) and i.r. spectroscopy to be a mixture of (chloromethyl)cyclohexane and bis(cyclohexylmethyl) carbonate.

(*h*) (*Fluoroformyloxy)cyclohexane.* No reaction occurred when the fluoroformate (1.4 g) was treated with pyridine (3.5 ml) at room temperature. Gas evolution began at 85–90° and was complete after 24 h at 110–120° (38% decarboxylation). The products were extracted with ether as in (*c*), and examination by g.l.c. (*b*) and i.r. spectroscopy revealed the presence of starting material, bis(cyclohexylmethyl) carbonate, and (fluoromethyl)cyclohexane.

(*i*) trans-*1,2-Bis(fluoroformyloxy)cyclohexane.* A mixture of the difluoroformate (6.5 g) and quinoline (2.6 g) was stored for 22 h at 160° in a stream of nitrogen. The material (1.5 ml) in the effluent gas stream was trapped at liquid air temperature and shown by g.l.c. (silicon gum–Celite) to contain cyclohexa-1,3-diene [characterised as the tetracyanoethylene adduct, m.p. 268° (from ethanol)] and a component (*X*) of higher retention time. On storage, hydrogen fluoride was evolved from the mixture, and the proportion of component *X* diminished.

The residual, non-volatile material was extracted with ether (100 ml), and the extract was washed with 3.5N hydrochloric acid (2 × 40 ml), dried ($MgSO_4$), and evaporated. Recrystallisation of the residue (1.3 g, 30%) from aqueous methanol gave the cyclic carbonate of cyclohexane-*trans*-1,2-diol, m.p. 54–55° alone or in admixture with the authentic compound[6], ν_{max} 1810 cm^{-1} (C=O) [Found: C, 59.1; H, 7.0: mol.wt. (micro-osometry), 143. $C_7H_{10}O_3$ calc.: C, 59.2; H, 7.0%; mol.wt., 142]. Saponification of the cyclic carbonate gave cyclohexane-*trans*-1,2-diol (63%), m.p. 102–103°.

trans-*2-Methoxycyclohexyl chloride. trans*-2-Chlorocyclohexanol[10] (10 g), on methylation with methyl iodide (40 g) and silver oxide (30 g) in the conventional manner, gave the title compound (5.27 g) (isolated by fractional distillation through a 10-cm Vigreux column), b.p. 106°/~15 mm (Found: C, 56.2; H, 8.5; Cl, 23.2. $C_7H_{13}ClO$ calc.: C, 56.6; H, 8.8; Cl, 23.8%). Examination of the product by g.l.c. (*a*) revealed *ca.* 2% of unidentified impurities.

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey, C.B.E., F.R.S., for his interest, Dr. H. Paulsen for micro-osmometric measurements, and Dr. W. M. Doane for a

sample of the cyclic carbonate of cyclohexane-*trans*-1,2-diol. The work was supported in part by a grant from the Sugar Research Foundation.

SUMMARY

It has been established that thermal and catalytic decomposition of certain alkyl haloformates affords dialkyl carbonates in addition to alkyl halides. Pyridine(or quinoline)-catalysed decomposition of ethyl and cyclohexyl chloroformate and ethyl fluoroformate affords high yields of alkyl halides, but cyclohexyl fluoroformate yields a significant proportion of dialkyl carbonate. In compounds containing the structural unit O·C-C·O·CO·Hal, chloroformates yield alkyl chlorides and dialkyl carbonates, whereas with fluoroformates, dialkyl carbonates are the sole products. The haloformates of the following compounds have been examined: cyclohexanemethanol, 2-(hydroxymethyl)tetrahydropyran, 1,2:3,4-di-*O*-isopropylidene-α-D-galactose, 1,2:3,4-di-*O*-isopropylidene-DL-xylitol, and *trans*-2-methoxycyclohexanol.

Quinoline-catalysed decomposition of *trans*-1,2-bis(fluoroformyloxy)cyclohexane affords mainly the cyclic carbonate of cyclohexane-*trans*-1,2-diol, together with small proportions of cyclohexa-1,3-diene and an unidentified, volatile product.

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Notes

Pyridine-catalysed decarboxylation of *cis*- and *trans*-4-*tert*-butylcyclohexyl chloroformate

As part of a programme concerned with the reactions of carbohydrate haloformates[1,2], the influence of conformation on the mode of decarboxylation of chloroformates was briefly examined in model compounds derived from *cis*- and *trans*-4-*tert*-butylcyclohexanol. It was anticipated that, as in other investigations[3], the bulk of the *tert*-butyl group would dominate the conformational equilibrium by its high preference for an equatorial position, so that the substituted oxygen function would be equatorial in the *trans*-series (**1**), and essentially axial in the *cis*-isomer (**2**).

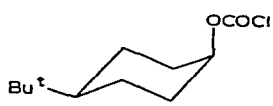
A convenient route to *cis*-4-*tert*-butylcyclohexanol involved treatment of the toluene-*p*-sulphonate of the *trans*-alcohol with sodium benzoate in boiling *N*,*N*-dimethylformamide[4]; this afforded uncontaminated *cis*-benzoate in good yield, and saponification subsequently gave the *cis*-alcohol. The *trans*-alcohol was readily obtained by reduction of 4-*tert*-butylcyclohexanone with lithium aluminium hydride–aluminium chloride[5].

Reactions of the *cis*- and *trans*-4-*tert*-butylcyclohexanols with phosgene in ether containing quinoline occurred smoothly to yield the liquid chloroformates, which were characterised by conversion into carbanilates by treatment with aniline. The authentic carbanilates were obtained by the reaction of the *cis*- and *trans*-alcohols with phenyl isocyanate.

When a mixture of *trans*-4-*tert*-butylcyclohexyl chloroformate (**1**) and pyridine was stored at 110–120°, decarboxylation was rapid and almost complete (96%). The identities and proportions of the products were established by analytical and preparative g.l.c., and were as follows: 4-*tert*-butylcyclohexene, 40%; *cis*-4-*tert*-butylcyclohexyl chloride, 60%; *trans*-chloride, trace; the *cis*- and *trans*-chlorides were



(1)



(2)

stable under the conditions of reaction. The preponderance of the chloride having inverted configuration parallels the behaviour of (+)-1-methylheptyl chloroformate under similar reaction conditions[6].

Pyridine-catalysed decarboxylation of *cis*-4-*tert*-butylcyclohexyl chloroformate (**2**) gave 4-*tert*-butylcyclohexene, 60%; *trans*-4-*tert*-butylcyclohexyl chloride, 35%; *cis*-chloride, 5%. Again, for the chloride products, preponderant inversion of configuration occurred, and, although a somewhat higher proportion of the olefin resulted from the decarboxylation of the *cis*-chloroformate than from the *trans*-

isomer, the difference is not significant. Presumably, the olefin arises by a carbonium ion mechanism, whilst the chlorides are formed by an intramolecular displacement[7]. In neither decarboxylation was dialkyl carbonate detected (see preceding paper[2]).

Decarboxylation of *cis*-4-*tert*-butylcyclohexyl chloroformate (**2**) at 150°, in the absence of pyridine, gave olefin, 30%; *trans*-chloride, 5%; and *cis*-chloride, 65%. The preponderant retention of configuration during formation of chloride by thermal decarboxylation accords with the findings of other workers[6].

EXPERIMENTAL

Analytical gas–liquid chromatography was performed with a Pye-Argon instrument (β-ionization detection) and a column packing of poly(ethylene glycol adipate).

Preparation of cis- *and* trans-*4*-tert-*butylcyclohexanols.* — The *trans*-isomer, m.p. 81–82°, was obtained by reduction of 4-*tert*-butylcyclohexanone[5].

A solution of *trans*-4-*tert*-butylcyclohexyl toluene-*p*-sulphonate (65 g, m.p. 89–90°) in *N*,*N*-dimethylformamide (1.5 l) was stirred and boiled under reflux for 10 h in the presence of sodium benzoate (160 g). The cooled mixture was diluted with water, and extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), and concentrated to give *cis*-4-*tert*-butylcyclohexyl benzoate (40 g, 74%), m.p. 117–118°. Saponification of the benzoate gave the *cis*-alcohol (16 g, 66%), uncontaminated by the *trans*-isomer.

The *cis*- and *trans*-4-*tert*-butylcyclohexanols were readily converted into the carbanilates by reaction with phenyl isocyanate: *cis*-carbanilate, m.p. 90–91° [from ethanol–light petroleum (b.p. 60–80°)] (Found: C, 74.5; H, 9.3; N, 5.1. $C_{17}H_{25}NO_2$ calc.: C, 74.2; H, 9.1; N, 5.1%); *trans*-carbanilate, m.p. 165° (Found: C, 74.1; H, 9.1; N, 5.1%).

Preparation of chloroformates. — A solution of *trans*-4-*tert*-butylcyclohexanol (20 g) in ether (50 ml) and quinoline (48 g) was added to an ice-cold, 20% solution of phosgene (23 g) in ether. After the solution had been stirred overnight at room temperature, water was added, and the ether layer was separated and washed with 3N hydrochloric acid. Concentration of the dried ($MgSO_4$) extract, with distillation of the residue, gave *trans*-4-*tert*-butylcyclohexyl chloroformate (19.6 g, 71%), b.p.78°/0.2–0.3 mm, ν_{max} 1780 (C=O) and 693 cm^{-1} (C–Cl) (Found: C, 60.6; H, 8.4; Cl, 16.4. $C_{11}H_{19}ClO_2$ calc.: C, 60.4; H, 8.7; Cl, 16.2%).

Reaction of the chloroformate with an excess of aniline gave a carbanilate, m.p. 164°, which was identical with the compound described above.

cis-4-*tert*-Butylcyclohexyl chloroformate (70%), b.p. 102°/12 mm, ν_{max} 1778 and 695 cm^{-1}, was prepared in a similar manner (Found: C, 60.8; H, 8.8; Cl, 16.5%). Reaction with aniline gave a carbanilate, m.p. 90–91°, which was identical with the authentic compound described above.

Decarboxylation of the chloroformates. — (*a*) When pyridine (5 ml) was added to *trans*-4-*tert*-butylcyclohexyl chloroformate (1.76 g), a white solid was formed, but

little carbon dioxide was evolved. At 110–120°, gas evolution was rapid, and 96% decarboxylation occurred (determined as described in the preceding paper).

In a subsequent experiment, the chloroformate (11 g) and pyridine (10 ml) were heated at 110–120°. After 30 min, the carbonyl band at 1780 cm^{-1} was extinct. The cooled mixture was extracted with ether (100 ml), washed with 2N hydrochloric acid and 10% aqueous cadmium chloride, and dried ($MgSO_4$). Examination by g.l.c. (75°) revealed, in order of increasing retention time, 4-*tert*-butylcyclohexene (40%), *cis*-(60%) and *trans*-4-*tert*-butylcyclohexyl chloride (trace).

The mixture was fractionated by preparative g.l.c. [Wilkens Autoprep 600, poly-(ethylene glycol adipate), 135°] to give 4-*tert*-butylcyclohexene, ν_{max} 1660 cm^{-1} (C=C) (Found: C, 86.7; H, 13.1. $C_{10}H_{18}$ calc.: C, 86.9; H, 13.0%); and *cis,trans*-4-*tert*-butylcyclohexyl chloride (Found: C, 68.6; H, 10.9; Cl, 20.0. $C_{10}H_{19}Cl$ calc.: C, 68.8; H, 11.0; Cl, 20.0%). The major component was identified as the *cis*-isomer, since it was readily dehydrohalogenated with 20% sodium hydroxide in 50% aqueous methanol.

(*b*) Decarboxylation of the *cis*-chloroformate as in (*a*) gave 4-*tert*-butylcyclohexene (60%), *trans*-4-*tert*-butylcyclohexyl chloride (35%), and *cis*-chloride (5%).

When the *cis*-chloroformate was heated at 150° in the absence of solvent, the carbonyl absorption was extinct after 5 h, and the products were as follows: olefin 30%, *trans*-chloride 5%, and *cis*-chloride 65%.

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey, C.B.E., F.R.S., for his interest. The research was supported by a grant from the Sugar Research Foundation.

Chemistry Department, A. B. FOSTER*
The University, R. JEFFERIS
Birmingham 15 (Great Britain) J. M. WEBBER

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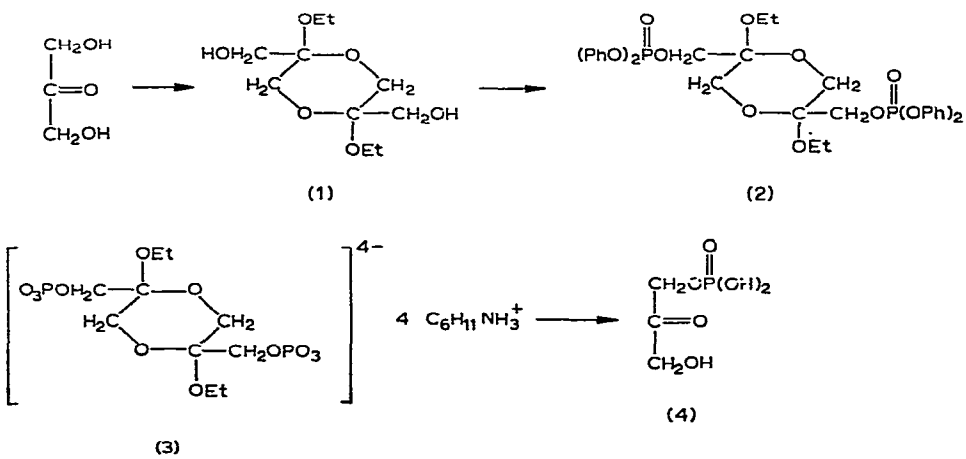
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(Received January 17th, 1967)

*Present address: Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London S. W. 3.

A synthesis of dihydroxyacetone phosphate from dihydroxyacetone

"Dihydroxyacetone phosphate" [1,3-dihydroxy-2-propanone 1-(dihydrogen phosphate)], an intermediate in glycolysis and photosynthesis, has previously been prepared by the dismutation of D-fructose 1,6-diphosphate[1]. More recently, Ballou and Fischer[2] reported its synthesis from 3-chloro-1,2-propanediol in nine steps, with an overall yield of 15%. In the method described in the present paper, 1,3-dihydroxy-2-propanone was converted into dihydroxyacetone phosphate in four steps, with a 27% overall yield. The cyclic acetal, 2,5-diethoxy-*p*-dioxane-2,5-dimethanol (**1**) (or its dimethoxy analog[3]) was esterified with phenyl phosphorochloridate in pyridine to 2,5-diethoxy-*p*-dioxane-2,5-dimethanol *O*-2^1,*O*-5^1-bis(diphenyl phosphate) (**2**). Hydrogenolysis, followed by neutralization with cyclohexylamine, led to isolation of 2,5-diethoxy-*p*-dioxane-2,5-dimethanol *O*-2^1-bis(dicyclohexylammonium phosphate) (**3**). Removal of the cyclohexylamine with anion-exchange resin released the acid, and heating of the aqueous solution (pH 1.0) for 4 h at 65° gave an 82% yield of dihydroxyacetone phosphate (**4**) (from the salt). Paper-chromatographic examination of the hydrolyzate indicated complete disappearance of the acetal. The dihydroxyacetone phosphate was assayed spectrophotometrically by using the concurrent oxidation of reduced adenine nicotinamide dinucleotide (NADH) in the presence of a glycerol phosphate[4,5].



Dihydroxyacetone phosphate was condensed with glycolaldehyde in the presence of aldolase to yield a D-*threo*-pentulose phosphate which, on dephosphorylation, gave D-*threo*-pentulose[6].

EXPERIMENTAL

2,5-Diethoxy-p-*dioxane-2,5-dimethanol* (cis *or* trans) (**1**). — The method of Fischer and Baer[3] was used for preparing compound **1**. The product, recrystallized from ethyl acetate–heptane instead of ethyl acetate, was obtained in a yield of 53%. The dimethoxy analog, similarly prepared, had m.p. 131–132° and mol. wt. (Rast, in camphor) of 211. Calc: 208.

2,5-Diethoxy-p-*dioxane-2,5-dimethanol* O-2[1], O-5[1]-*bis*(*diphenyl phosphate*) (**2**). — Phenyl phosphorochloridate (40 g) was slowly added at 0° to a solution of compound **1** (10.0 g) in pyridine (50 ml). After 5 h at room temperature, ether (500 ml) was added, and the solution was successively washed with water (2 × 250 ml), dilute hydrochloric acid (2 × 250 ml), dilute aqueous sodium hydrogen carbonate (250 ml), and water. The ether layer was then dried (magnesium sulfate) and concentrated to a syrup, which crystallized from a large volume of light petroleum (b.p. 40–60°). The yield was 23.2 g (78%), m.p. 78°.

Anal. Calc. for $C_{34}H_{38}O_{12}P_2$: C, 58.3; H, 5.5; P, 8.8. Found: C, 58.7; H, 6.0; P, 8.6.

The 2,5-dimethoxy analog had m.p. 124°.

Anal. Calc. for $C_{32}H_{34}O_{12}P_2$: C, 57.1; H, 5.1; P, 9.2. Found: C, 56.9; H, 5.2; P, 9.2.

2,5-Diethoxy-p-*dioxane-2,5-dimethanol* O-2[1],O-5[1]-*bis*(*dicyclohexylammonium phosphate*) (**3**). — A solution of compound **2** (2.0 g) in dry ethanol (50 ml) was shaken with hydrogen and platinic oxide (Adams catalyst) (200 mg) at room temperature and pressure. After 2.5 h, the hydrogen uptake was 1.2 times the theoretical. The catalyst was then removed by centrifuging, and a 10% solution of cyclohexylamine in ethanol (20 ml) was added. The mixture was cooled to 0°, and, 24 h later, the crystalline cyclohexylammonium salt **3** was filtered off and dried. The yield was 1.8 g (79%), m.p. 201° (dec.).

Anal. Calc. for $C_{34}H_{74}N_4O_{12}P_2$: P, 7.8. Found: 7.9.

Paper chromatography of the salt with a 13:5:2 propyl alcohol–ammonia–water system, indicated only a single substance, whose movement relative to inorganic phosphate was 0.68. The paper had been pre-washed with 2N acetic acid and 0.2% ethylenedinitrilotetraacetic acid. Phosphate was detected with the acidic molybdate reagent[7].

The 2,5-dimethoxy analog (79% yield) had m.p. 196–200°.

Anal. Calc. for $C_{32}H_{70}N_4O_{12}P_2$: C, 50.1; H, 9.2. Found: C, 50.4; H, 9.2.

1,3-Dihydroxy-2-propanone 1-(*dihydrogen phosphate*) (**4**). — A solution of **3** (0.20 g) in water (5.0 ml) was mixed with Dowex-50 (H^+) resin, and then filtered from the resin and heated for 4 h at 65°. Paper chromatography of the autohydrolyzate in 13:5:2 propanol–ammonia–water indicated the appearance of a new substance, whose movement relative to inorganic phosphate was 0.44. After 4 h of autohydrolysis, compound **4** and inorganic phosphate were the only substances detected with the acidic molybdate reagent.

Conversion into dihydroxyacetone phosphate was measured quantitatively by the reaction of the latter with NADH in the presence of α-glycerophosphate dehydrogenase. The following were combined in a 1-cm spectrophotometer cell: 0.05M phosphate buffer of pH 7.5 (1.0 ml), a solution (0.2 ml) containing 1.75 mg of NADH per ml of phosphate buffer, the sample of autohydrolyzate diluted 200-fold (1.0 ml), and distilled water to a final volume of 2.9 ml. The absorbance at 340 mμ was read, and then α-glycerophosphate dehydrogenase (0.1 ml) was added and the change in absorbance measured. (The α-glycerophosphate dehydrogenase, obtained from Sigma Chemical Company, was an ammonium sulfate suspension containing 10 mg of enzyme per ml of ammonium sulfate solution; 1 mg of enzyme reduced 50–100 μmoles of 1,3-dihydroxy-2-propanone phosphate per min at 25° and pH 7.4. This reagent was diluted ten-fold before use.) Data on the course of hydrolysis at 55° and 65° are shown in Table I.

TABLE I

HYDROLYSIS OF 2,5-DIETHOXY-*p*-DIOXANE-2,5-DIMETHANOL O-2[1],O-5[1]-BIS(DICYCLOHEXYLAMMONIUM PHOSPHATE) (3)

Wt. of cyclohexylammonium salt 3 *(g) in 5.0 ml*	*pH of solution*	*Temp. of reaction, degrees*	*Time of heating (h)* 0		2.0		4.0	
			%DHAP[a]	*%P*$_i$[b]	*%DHAP*[a]	*%P*$_i$[b]	*%DHAP*[a]	*%P*$_i$[b]
0.100	1.5	55	2	0.0	12	0.6	57	0.9
0.100	1.5	65	6	0.5	65	1.9	74	3.1
0.200	1.0	65	10	0.6	77	2.4	82	4.1

[a]DHAP = 1,3-dihydroxy-2-propanone 1-(dihydrogen phosphate) (4). [b]P_i = inorganic phosphate.

The cyclohexylammonium salt of 1,3-dihydroxy-2-propanone 1-(dihydrogen phosphate) (m.p. 73–75°), prepared from the dimethyl analog of 3, turned yellow on being kept; however, a freshly prepared sample and an authentic specimen had identical i.r. spectra, and they were not distinguishable by paper chromatography.

Condensation of 1,3-dihydroxy-2-propanone 1-(dihydrogen phosphate) with glycolaldehyde. — A neutral solution of 1,3-dihydroxy-2-propanone 1-(dihydrogen phosphate) (from 50 mg of the dimethyl analog of 3), glycolaldehyde (5 mg), and aldolase suspension (0.1 ml) (Nutritional Biochemical Corporation) were combined in water (10 ml). After being kept for 24 h at 37°, the solution was heated to 80°, and the denatured protein was removed. The filtrate was then adjusted to pH 4.5 and treated with acid phosphatase, and after 48 h at 37°, heated to 80°, cooled, filtered, and deionized. Paper-chromatographic examination (40:11:19 v/v 2-methyl-2-propanol–ethanol–water) of the residue obtained on concentration of the solution revealed the presence of a pentulose, identified by (*a*) the identity of its rate of movement with that of an authentic sample[5] and (*b*) the blue color[8] produced on reaction with the orcinol–trichloroacetic acid reagent. Blank experiments in which

(*a*) aldolase or (*b*) 1,3-dihydroxy-2-propanone 1-(dihydrogen phosphate) were omitted gave no pentulose.

Department of Chemistry, R. L. COLBRAN
Queen's University, J. K. N. JONES
Kingston, Ontario (Canada)

Department of Agricultural Chemistry, N. K. MATHESON
University of Sydney (Australia) IRENE ROZEMA

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(Received January 9th, 1967)

Carbohyd. Res., 4 (1967) 355–358

A copper reagent for the determination of hexuronic acids and certain ketohexoses*

Various solutions of copper acetate have been proposed for the assay of reducing monosaccharides in the presence of reducing oligosaccharides[1-5]. It was noticed that the presence of high concentrations of ions in these reagents, lactate and citrate in particular, may suppress the rate of reduction of Cu^{2+} by aldohexoses. In addition, the rate of reoxidation of Cu^{+} is relatively fast under the acid conditions employed for these assays. These factors are responsible for marked deviations in the reproducibility of the standard absorption curves for reducing sugars obtained with the acid–copper reagents.

We have now observed that with a modified acid solution, in which the concentration of free Cu^{2+} was decreased and the salt concentration appreciably elevated, aldoses reacted very poorly, whereas hexuronic acids could be sensitively determined. Optimal concentrations of reactants and conditions for a selective assay of hexuronic acids were worked out and are described below.

The present method is useful for the determination of hexuronic acids and certain ketoses in the presence of other sugars, aldoses in particular. It may be employed for a specific measurement of hexuronic acids at the reducing ends of oligosaccharides such as those appearing in enzymic digests of acidic polysaccharides. The reagent could also be helpful for differentiating between hexuronidase and other glycosidase activities that cleave a heteropolysaccharide.

METHOD

Reagents. — *Copper solution* (*A*). Dissolve by boiling and vigorous stirring 280 g of anhydrous sodium sulphate and 40 g of sodium chloride in 720 ml of water. Complete dissolution will occur when 100 ml of 2M acetate buffer (pH 5.1) and 65 ml of 0.32M cupric sulphate are added to the salt solution at about 60°. The pH of the reagent obtained should be 4.8, and, if necessary, the pH is carefully adjusted to this value by dropwise addition of 10N hydrochloric acid or sodium hydroxide. The volume is then completed with water to one liter. The reagent, stored at 37° in a well-stoppered brown bottle, is stable for at least two months.

Arsenomolybdate reagent (*B*) is prepared according to Nelson's method[6-7].

Standard sugar solution (*C*). A 0.01M stock solution of hexuronic acid (or other sugar) is prepared in 0.05% benzoic acid and kept at 4°.

Procedure—To a 0.5-ml sample containing 0.02–0.50 μmole of hexuronic acid in standard test-tubes, add 1.5 ml of reagent *A*. Mix, cover with glass marbles, and immerse in a boiling-water bath for exactly 10 min. Cool in an ice bath without

*Supported by Research Grant FG-IS-141 from the U. S. Department of Agriculture. Taken in part from a Ph. D. Thesis (by Y. Milner) to be submitted to The Hebrew University.

shaking. Add 1.0 ml of reagent *B*. Shake gently and add 2.0 ml of water. If the colour intensity is too high, water can be added to 10 ml without impairing the proportionality of the absorption. Read the absorbance at 600 mμ against a reagent blank. Typical, standard curves are shown in Fig. 1.

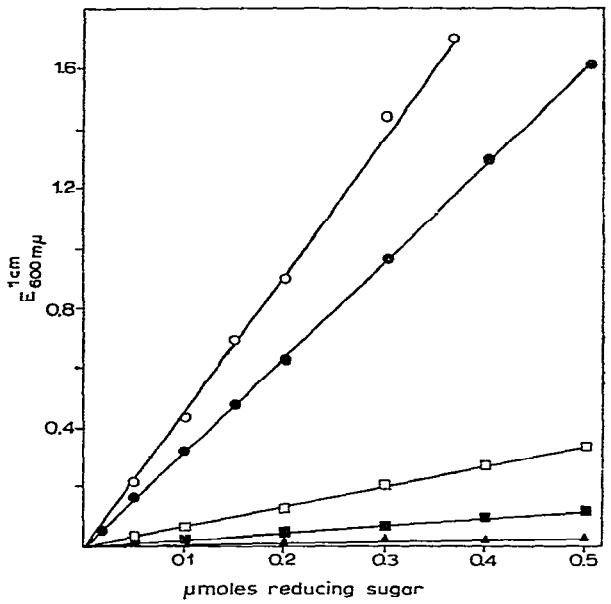


Fig. 1. *Standard absorption curves for various sugars.* Values recorded are average readings obtained from triplicate determinations (±3% deviation). Cells of 1-cm light path were used in the Zeiss Model PMQ II spectrophotometer. ○, glucuronic acid; ●, galacturonic acid; □, fructose; ■, xylose; ▲, glucose.

Comments. — The relative response of various sugars is given in Table I. The reaction is performed in 15.6 mM copper ions. Increasing their concentration will enhance the response of ketoses, whereas decreasing the amount of copper in the reagent will retard the rate of reduction by hexuronic acids. It could be established that the present procedure measures a difference between rates of reduction of Cu^{2+} in the acid solution. Oxidation of hexuronic acids is practically complete within 10 min. With several ketoses, such as D-*xylo*-5-hexulosonic acid and D-*threo*-2,5-hexodiulose (Table I), a faster reaction with complicated kinetics occurs, probably due to sugar degradation. The best selectivity and sensitivity of response to hexuronic acids is maintained at pH 4.6 to 5.0. Copper hydroxide will tend to precipitate in the reagent if the pH is not properly adjusted and is above 5.2. A very high concentration of salt is obligatory for suppressing the reoxidation of Cu^{+} and thus affording better reproducibility.

Absorption may be read with similar accuracy between 540 and 710 mμ, and even with higher sensitivity at the longer wavelengths. Reading at 600 mμ was selected for routine analysis as it is convenient for use with most laboratory colorimeters.

TABLE I

RELATIVE REACTION[a] OF DIFFERENT SUGARS IN THE ACID COPPER REAGENT

Sugar	*Per cent of reaction*	*Sugar*	*Per cent of reaction*
D-Glucuronic acid	100	D-Fructose	13
D-Galacturonic acid	75	L-Sorbose	22
D-Mannuronic acid	97	D-*threo*-2,5-Hexodiulose	355
D-Glucose	0.8	D-*arabino*-Hexulosonic acid	57
D-Galactose	0.2	D-*xylo*-5-Hexulosonic acid	670
D-Mannose	0.6	DL-Glyceraldehyde	91
L-Rhamnose	0.2	Pyruvic acid	13
D-Ribose	1.3	D-Gluconic acid	0
D-Xylose	3.0	Lactose	0.6
L-Arabinose	3.0	Cellobiose	0.6
L-Lyxose	8.5	Maltose	0.8
D-Glucose 6-phosphate	6.0	Melibiose	0.7
2-Amino-2-deoxy D-glucose	8.0	Oligogalacturonides[b]	75
2-Acetamido-2-deoxy-D-glucose	2.0		

[a]All values are compared on the basis of response elicited by 1.0 μmole of sugar in the standard assay procedure described in this communication.
[b]Obtained from a digestion of pectic acid by polygalacturonases obtained from *Saccharymyces fragilis*[8,9] and *Aspergillus niger*[10,11]. Also applicable to pectin digests obtained with pectolytic enzymes from several other moulds (Y. Milner, unpublished studies).

When the sample of sugar to be analyzed contains a strong buffer of pH > 5.0, it should be adjusted to pH 4.8 with acetic acid before addition of reagent *A*. If the solution examined contains a component, such as citrate, which chelates copper, it should be titrated with an equimolar amount of cupric sulphate prior to the application of copper reagent *A*.

Department of Biological Chemistry,
The Hebrew University of Jerusalem,
Jerusalem (Israel)

YORAM MILNER
GAD AVIGAD

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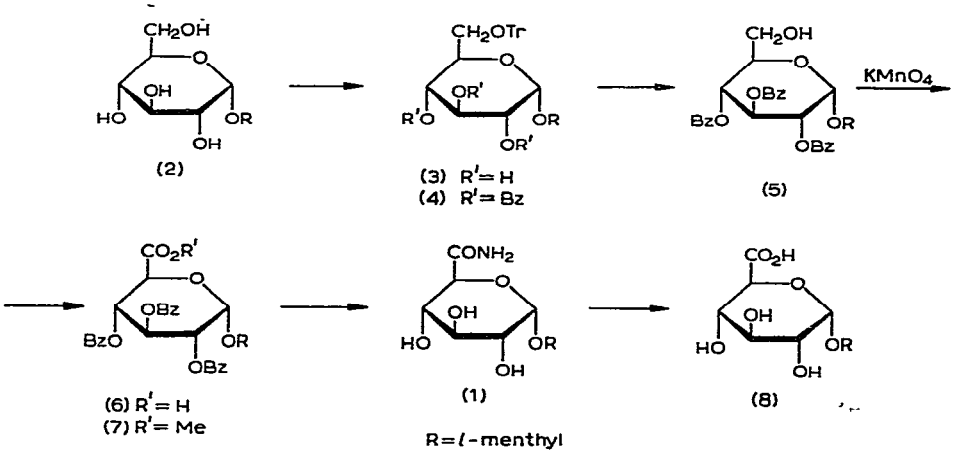
(Received January 26th, 1967)

Preliminary communication

An approach to the selective degradation of polysaccharide chains

The selective fragmentation of polymer chains, followed by elucidation of the structure of the resulting oligomers and assignment of the sequence of monomer units in the polymer, is the most general and expedient approach for the study of the primary structure of such biopolymers as polypeptides, proteins, and soluble ribonucleic acids. In considering the application of this principle to structural studies of polysaccharides, serious difficulties are encountered because of the more complex structures of these polymers. Noteworthy amongst these is the lack of selective methods for the partial degradation of polysaccharide chains (excluding the selective hydrolysis of furanosidic linkages[1], and some special cases of enzymic hydrolysis[2,3]). We now describe preliminary experiments on the selective splitting of hexuronidic linkages by conversion of the uronic acid residues into amides, followed by Hofmann degradation of the amides to give 5-aminopentopyranoses. The latter compounds, which are structurally analogous to glycosylamines[4], are hydrolysed by acid under very mild conditions.

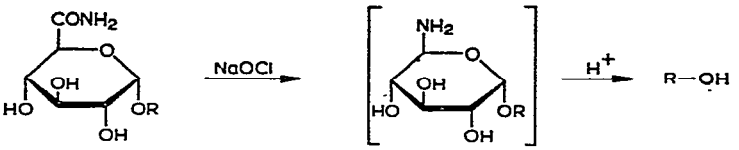
Menthyl α-D-glucopyranosiduronamide (**1**) was chosen as a model compound and was synthesised as follows:



Menthyl α-D-glucopyranoside[5] (**2**) was treated with chlorotriphenylmethane and then with benzoyl chloride in pyridine, and the product (**4**) was detritylated with 80% acetic acid (1 h, 100°). The resulting menthyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (**5**) was oxidized by potassium permanganate in acetic acid[6] to give acid **6**. The methyl ester (**7**), obtained by treatment of **6** with boiling, 0.5%, methanolic hydrogen chloride for 6 h, on treatment with ethanolic ammonia (20–24 h), gave amide **1**, m.p. 194–195° (from benzene–ethanol), $[\alpha]_D^{18}$ −61.7° (*c* 0.81, chloroform). The i.r. spectrum of **1** showed the presence of an α-D-glycosidic bond (850 cm^{-1}) and an

amide grouping (3430 and 1670 cm^{-1}). The n.m.r. spectrum contained a doublet at τ 4.6 (J 3 c.p.s., equatorial H-1) and signals at τ 9.1–9.3 (CH_3 group of the menthyl residue). (−)-Menthol and D-glucurono-6,3-lactone were identified after acidic hydrolysis. Treatment of **1** with 0.1N potassium carbonate gave menthyl α-D-glucopyranosiduronic acid[7,8] (**8**).

Amide cleavage was expected to proceed according to the scheme:



A typical experiment was performed as follows. To an ethanolic solution of amide **1** a ten-fold excess of 0.5N sodium hypochlorite was added at 0°. The mixture was heated to boiling point, cooled, neutralized with carbon dioxide, and evaporated to dryness. The residue (the structure of which is being investigated) was dissolved in 0.125N hydrochloric acid, kept overnight at room temperature, and then extracted with chloroform. The extract contained (−)-menthol and menthyl α-D-glucopyranosiduronic acid (**8**), which were separated by chromatography on silica gel; yield of (−)-menthol, 74%.

Thus, the glycuronosidic bond may be split under conditions where glycosidic bonds are stable. In an application of the method to polysaccharides, it was found that an acidic xylan from white birch [9], after conversion into the amide, treatment with hypochlorite, and mild acidic hydrolysis, underwent a 30–40% relative decrease in content of uronic acid. The method therefore has potential value in the structural determination of polysaccharides containing uronic acid residues.

N. D. Zelinsky Institute of Organic Chemistry,
Academy of Sciences of the U.S.S.R.,
Moscow (U.S.S.R.)

N. K. KOCHETKOV
O. S. CHIZHOV
A. F. SVIRIDOV

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(Received December 30th, 1966; in revised form, February 13th, 1967)

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

REACTION OF AMMONIA WITH ACYLATED ALDOHEPTOSES

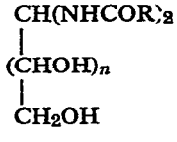
DERIVATIVES OF D-*glycero*-D-*gulo*-HEPTOSE, D-*glycero*-L-*manno*-HEPTOSE, AND D-*glycero*-D-*galacto*-HEPTOSE

JORGE O. DEFERRARI AND ROSA M. DE LEDERKREMER

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Perú 222, Buenos Aires (Argentina)

(Received September 16th, 1966; in revised form, January 31st, 1967)

The action of ammonia on acylated monosaccharides has been shown[1-7] to give the 1,1-bis(acylamido)-1-deoxyalditols (**1**) as principal products, except with penta-*O*-acetyl-*aldehydo*-D-glucose[8] and penta-*O*-acetyl-β-D-glucopyranose[9], which yield *N*-acetyl-D-glucofuranosylamine as the main product. Acylated disaccharides give the corresponding free disaccharides in high yield, together with smaller proportions of the 1,1-bis(acylamido)-1-deoxyaldobiitols and the *N*-acylaldobiosylamines[10-15]. In this paper, we describe the reaction of methanolic ammonia (16%) with the hexa-*O*-acetyl and hexa-*O*-benzoyl derivatives of D-*glycero*-D-*gulo*-heptose, D-*glycero*-L-*manno*-heptose, and D-*glycero*-D-*galacto*-heptose.



1

2 $n = 5$, R = Me

3 $n = 5$, R = Ph

All of the hexa-*O*-acetylheptoses gave the 1,1-bis(acetamido)-1-deoxy-D-heptitols (**2**), as crystalline products, from which the corresponding hexaacetates were obtained. Ammonolysis of the hexa-*O*-benzoylheptoses produced the expected 1,1-bis(benzamido)-1-deoxy-D-heptitols (**3**) as the main products. The yields of 1,1-bis(acylamido)-1-deoxy-D-heptitols (**2**, **3**) were, in general, higher than those from the acylated hexose derivatives, the values being between 35 and 60% (see Table I).

Hexa-*O*-benzoyl-D-*glycero*-L-*manno*-heptose reacted with ammonia to give 1,1-bis(benzamido)-1-deoxy-D-*glycero*-L-*manno*-heptitol in 35% yield, and *N*-benzoyl-D-*glycero*-L-*manno*-heptosylamine was isolated in 2.5% yield. It is interesting to note that, on ammonolysis of the configurationally related benzoates of D-mannose and L-rhamnose, the *N*-benzoyl-D-mannopyranosylamine and *N*-benzoyl-L-rhamnopyranosylamine were both obtained in low yield, together with the principal product. By analogy with the hexose derivatives, the *N*-benzoyl-D-*glycero*-L-*manno*-heptosylamine should have a pyranoid structure; the high negative rotation of this compound would indicate its β-D configuration. Acetylation of this substance afforded penta-*O*-

TABLE I

YIELDS OF 1,1-BIS(ACYLAMIDO)-1-DEOXY ALDITOLS FROM THE CORRESPONDING ACYLATED ALDOPYRANOSES

Starting material	*Product obtained*	*Yield, %*
Hexa-*O*-acetyl-D-*glycero*-β-D-*gulo*-heptose	1,1-bis(acetamido)-1-deoxy-D-*glycero*-D-*gulo*-heptitol (4)	49.0
Hexa-*O*-benzoyl-D-*glycero*-β-D-*gulo*-heptose	1,1-bis(benzamido)-1-deoxy-D-*glycero*-D-*gulo*-heptitol (5)	60.4
Hexa-*O*-acetyl-D-*glycero*-α-L-*manno*-heptose	1,1-bis(acetamido)-1-deoxy-D-*glycero*-L-*manno*-heptitol (6)	49.8
Hexa-*O*-benzoyl-D-*glycero*-L-*manno*-heptose(mixture of the anomers)	1,1-bis(benzamido)-1-deoxy-D-*glycero*-L-*manno*-heptitol (7)	34.5
	N-benzoyl-D-*glycero*-L-*manno*-heptosylamine (8)	2.5
Hexa-*O*-acetyl-D-*glycero*-α-D-*galacto*-heptose	1,1-bis(acetamido)-1-deoxy-D-*glycero*-D-*galacto*-heptitol (9)	43.3
Hexa-*O*-benzoyl-D-*glycero*-α-D-*galacto*-heptose	1,1-bis(benzamido)-1-deoxy-D-*glycero*-D-*galacto*-heptitol (10)	42.5
Penta-*O*-acetyl-β-D-glucose	*N*-acetyl-D-glucofuranosylamine	8.0[a]
Penta-*O*-benzoyl-α-D-glucose	1,1-bis(benzamido)-1-deoxy-D-glucitol	21.2[b]
Penta-*O*-acetyl-β-D-mannose	1,1-bis(acetamido)-1-deoxy-D-mannitol	26.5[c]
Penta-*O*-benzoyl-β-D-mannose	1,1-bis(benzamido)-1-deoxy-D-mannitol	20.8[c]
	N-benzoyl-D-mannopyranosylamine	8.0[c]
Penta-*O*-acetyl-α-D-galactose	1,1-bis(acetamido)-1-deoxy-D-galactitol	26.5[d]
Penta-*O*-benzoyl-α-D-galactose	1,1-bis(benzamido)-1-deoxy-D-galactitol	35.0[d]
Tetra-*O*-acetyl-L-rhamnose (mixture of the anomers)	1,1-bis(acetamido)-1-deoxy-L-rhamnitol	38.6[e]
Tetra-*O*-benzoyl-α-L-rhamnose	1,1-bis(benzamido)-1-deoxy-L-rhamnitol	18.6[e]
	N-benzoyl-L-rhamnopyranosylamine	1.84[e]

[a]See ref. 9. [b]See ref. 2 [c]See ref. 3 [d]See ref. 4 [e]See ref. 5.

acetyl-*N*-benzoyl-D-*glycero*-L-*manno*-heptosylamine. The *N*-acylheptosylamine probably was formed also, in very low yield, as a by-product on ammonolysis of the acyl derivatives of D-*glycero*-D-*gulo*-heptose and D-*glycero*-D-*galacto*-heptose. In fact, paper chromatography of the mother liquors, after crystallization of the 1,1-bis(acylamido)-1-deoxy-D-heptitol, showed the presence of the corresponding aldoheptose and another nonreducing compound whose relative mobility suggested that it was the *N*-acylheptosylamine.

The results of the ammonolysis of the *O*-acyl derivatives of aldoheptoses illustrate the generality of this reaction in the monosaccharide field.

EXPERIMENTAL

General. — Ammonolysis of the hexa-*O*-acylheptoses was effected by suspending them, in the proportion of 1 g to 30 ml, in methanolic ammonia (16%) and shaking to dissolution. After 24 h at room temperature, the solution was evaporated under diminished pressure below 60°. The 1,1-bis(acylamido)-1-deoxy-D-heptitols usually crystallized spontaneously. Descending paper-chromatography was performed on

Whatman No. 1 paper, with the following solvents: A, 5:1:4 (v/v) butyl alcohol–ethanol–water (top layer), and B, 10:4:4 butyl alcohol–ethanol–water. Spray reagents used were: (a) silver nitrate–sodium methoxide[16] and (b) aniline hydrogen phthalate[17]. D-Glucose was used as the standard. Melting points are uncorrected.

1,1-Bis(acetamido)-1-deoxy-D-glycero-D-gulo-*heptitol* (**4**)*. — Hexa-*O*-acetyl-D-*glycero*-β-D-*gulo*-heptose[18] (10 g) was ammonolyzed; the solution was evaporated, and the remaining syrup, dried in a vacuum desiccator, was extracted 5 times at 40° with a total of 180 ml of ethyl acetate to remove acetamide. The residue was dissolved in 65 ml of boiling 90% ethanol and was kept overnight at 5°. The crystalline precipitate was filtered off and recrystallized from the same solvent, to give **4** as white needles, yield, 2.72 g, m.p. 194–195°; $[\alpha]_D^{29}$ +7.3° (*c* 1.6, water). From the mother liquors, a further 0.57 g of **4** was obtained (total yield 49%).

Anal. Calc. for $C_{11}H_{22}N_2O_8$: C, 42.57; H, 7.15; N, 9.02. Found: C, 42.75; H, 7.00; N, 8.91.

Hexa-O-*acetyl-1,1-bis(acetamido)-1-deoxy*-D-glycero-D-gulo-*heptitol**. — Substance **4** (600 mg) was dissolved by heating with a 1:1 mixture of pyridine and acetic anhydride (20 ml). After 24 h at room temperature, the solution was poured into 120 ml of ice–water and extracted with chloroform. The extract was successively washed with 2N sulfuric acid, water, sodium hydrogen carbonate solution, and water, and dried (sodium sulfate). Solvent removal yielded 0.89 g of material. After three recrystallizations from 1:2 ethanol–ether, white needles, m.p. 109–110°, $[\alpha]_D^{29}$ +1.1° (*c* 1, chloroform), were obtained.

Anal. Calc. for $C_{23}H_{34}N_2O_{14}$: C, 49.10; H, 6.09; N, 4.98. Found: C, 48.78; H, 6.10; N, 4.67.

1,1-Bis(benzamido)-1-deoxy-D-glycero-D-gulo-*heptitol* (**5**)*. — Hexa-*O*-benzoyl-D-*glycero*-β-D-*gulo*-heptose[19] (12 g) was ammonolyzed, and the solution was evaporated at room temperature until crystals appeared. There resulted 3.60 g of white needles, m.p. 200–203°, and from the mother liquors a further 0.17 g was obtained by evaporation (total yield 60.4%). Two recrystallizations from water afforded needles, m.p. 209–210°, $[\alpha]_D^{29}$ −11.2° (*c* 1.2, pyridine).

Anal. Calc. for $C_{21}H_{26}N_2O_8$: C, 58.11; H, 6.04; N, 6.45. Found: C, 57.95; H, 6.07; N, 6.34.

The methanolic mother liquors were dried and the benzamide was extracted with ethyl acetate. Paper chromatography showed, with solvent A and spray reagent (a), the presence of **5** (R_F 0.67), D-*glycero*-β-D-*gulo*-heptose (R_F 0.09), and another nonreducing compound (R_F 0.52). With spray reagent (b), only D-*glycero*-β-D-*gulo*-heptose was detected.

Hexa-O-*acetyl-1,1-bis(benzamido)-1-deoxy*-D-glycero-D-gulo-*heptitol**. — Compound **5** (0.43 g) was dissolved with heating in 10 ml of 1:1 pyridine–acetic anhydride. After 24 h at room temperature, the solution was poured into 50 ml of ice–water; the oil obtained crystallized readily, to give 0.60 g of white needles, m.p. 170–172°.

*This compound was first prepared by Dr. Hector Rosenzvit.

Recrystallized once from benzene–petroleum ether and twice from 50% ethanol, the substance had m.p. 172–173°, $[\alpha]_D^{20} -17.0°$ (*c* 1.3, chloroform).

Anal. Calc. for $C_{33}H_{38}N_2O_{14}$: C, 57.72; H, 5.57; N, 4.08. Found: C, 57.96; H, 5.54; N. 3.98.

1,1-Bis(acetamido)-1-deoxy-D-glycero-L-manno-*heptitol* (**6**). — Hexa-*O*-acetyl-D-*glycero*-α-L-*manno*-heptose[19] (2 g) was ammonolyzed, and the solution was evaporated until crystals appeared. After having been cooled at 5°, the suspension was filtered, to give 0.51 g of **6**, m.p. 211–212°. The mother liquors were evaporated to dryness, and the residue was extracted with hot ethyl acetate to dissolve acetamide. The dried syrup was dissolved in hot ethanol, and a further 0.18 g of **6** was obtained (total yield 49.8%). Recrystallization from 80% ethanol afforded white needles, m.p. 213–214°, $[\alpha]_D^{28} +10.2°$ (*c* 1, water).

Anal. Calc. for $C_{11}H_{22}N_2O_8$: C, 42.57; H, 7.15; N, 9.02. Found: C, 42.67; H, 7.15; N, 9.00

Paper chromatography of the mother liquors with solvent B showed, with spray reagent (a), the presence of **6** (R_G 1.07), D-*glycero*-L-*manno*-heptose (R_G 0.95), and a faint nonreducing spot having R_G 0.70. With spray reagent (b), only D-*glycero*-L-*manno*-heptose was detected.

Hexa-O-*acetyl-1,1-bis(acetamido)-1-deoxy*-D-glycero-L-manno-*heptitol*. — Substance **6** (600 mg) was dissolved with heating in 20 ml of 1:1 pyridine–acetic anhydride. After 24 h at room temperature, the solution was evaporated in a desiccator over sulfuric acid and potassium hydroxide. The residue crystallized from benzene as white needles, yield, 0.85 g, m.p. 170–171°. After two recrystallizations from the same solvent and one from water, the m.p. did not change; $[\alpha]_D^{28} +15.8°$ (*c* 1, chloroform).

Anal. Calc. for $C_{23}H_{34}N_2O_{14}$: C, 49.10; H, 6.09; N, 4.98. Found: C, 48.98; H, 6.16; N, 4.90.

1,1-Bis(benzamido)-1-deoxy-D-glycero-L-manno-*heptitol* (**7**). — A mixture of the anomers of hexa-*O*-benzoyl-D-*glycero*-L-*manno*-heptose (5 g) was ammonolyzed, and the solution was evaporated to dryness. The solid residue crystallized from ethanol, yield 0.72 g (34.5%), m.p. 208–211°. Recrystallization from 80% ethanol afforded white needles, m.p. 211–212°, $[\alpha]_D^{29} -8.7°$ (*c* 0.5, pyridine).

Anal. Calc. for $C_{21}H_{26}N_2O_8$: C, 58.11; H, 6.04; N, 6.45. Found: C, 58.16; H, 5.92; N, 6.47.

Hexa-O-*acetyl-1,1-bis(benzamido)-1-deoxy*-D-glycero-L-manno-*heptitol*. — Compound **7** (0.43 g) was acetylated with pyridine–acetic anhydride, to give 0.70 g of crystals, m.p. 110–113°. Recrystallization from 5:1 benzene–petroleum ether afforded needles, m.p. 117–118°, $[\alpha]_D^{28} +4.7°$ (*c* 1, chloroform).

Anal. Calc. for $C_{33}H_{38}N_2O_{14}$: C, 57.72; H, 5.57; N, 4.08. Found: C, 57.66; H, 5.44; N, 4.10.

N-*Benzoyl*-D-glycero-L-manno-*heptosylamine* (**8**). — The methanolic mother liquors from the preparation of **7** were evaporated to dryness, and the residue was suspended in methanol. After filtration, 0.21 g of a solid was obtained. Paper chro-

matography with solvent A showed, with spray reagent (a), the presence of a main component (R_F 0.40) and **7** (R_F 0.64). The crude product was suspended in water and filtered; 0.05 g of **7** was obtained. From the aqueous mother liquors, after evaporation and subsequent recrystallization from 1:1 pyridine–ethanol, 0.06 g (2.5%) of **8** was obtained as long prisms which, after two recrystallizations from 80% methanol, melted at 211–213°, $[\alpha]_D^{15}$ −103° (*c* 0.5, water).

Anal. Calc. for $C_{14}H_{19}NO_7$: C, 53.67; H, 6.11; N, 4.47. Found: C, 53.75; H, 6.13; N, 5.34.

Penta-O-*acetyl*-N-*benzoyl*-D-glycero-L-manno-*heptosylamine*. — Compound **8** (0.02 g) was dissolved by boiling with 1 ml of 1:1 pyridine–acetic anhydride. After 24 h at room temperature, the solution was evaporated in a desiccator over sulfuric acid and potassium hydroxide. The residue crystallized from ethanol, yield 0.025 g. Recrystallization from the same solvent gave needles, m.p. 169–170°, $[\alpha]_D^{20}$ −28.7° (*c* 0.8, chloroform).

Anal. Calc. for $C_{24}H_{29}NO_{12}$: C, 55.06; H, 5.58; Found: C, 55.21; H, 5.80.

1,1-Bis(acetamido)-1-deoxy-D-glycero-D-galacto-*heptitol* (**9**). — Hexa-*O*-acetyl-D-*glycero*-α-D-*galacto*-heptose[20] (2 g) was ammonolyzed, the solution was evaporated, and the residue was crystallized from ethanol. There resulted 0.53 g of **9** as needles, m.p. 225–227°. From the mother liquors, after evaporation and drying followed by extraction of acetamide with hot ethyl acetate, another 0.05 g of **9** was obtained (total yield 43.3%). Recrystallized three times from 80% ethanol, the product had m.p. 226–227°, $[\alpha]_D^{28}$ +11° (*c* 1.1, water).

Anal. Calc. for $C_{11}H_{22}N_2O_8$: C, 42.57; H, 7.15; N, 9.02. Found: C, 42.47; H, 6.98; N, 9.14.

Paper chromatography of the mother liquors with solvent B and spray reagent (a) showed the presence of **9** (R_G 0.73), D-*glycero*-D-*galacto*-heptose (R_G 0.63), and another nonreducing spot (R_G 1.34).

Hexa-O-*acetyl-1,1-bis(acetamido)-1-deoxy*-D-glycero-D-galacto-*heptitol*. — Compound **9** (0.20 g) was acetylated with 1:1 pyridine–acetic anhydride (6.8 ml) to give 0.25 g of crystals, m.p. 178–180°. Recrystallization from benzene gave needles, m.p. 185–186°, $[\alpha]_D^{25}$ −24.6° (*c* 1, chloroform).

Anal. Calc. for $C_{23}H_{34}N_2O_{14}$: C, 49.10; H, 6.09; N, 4.98. Found: C, 49.00; H, 6.00; N, 4.70.

1,1-Bis(benzamido)-1-deoxy-D-glycero-D-galacto-*heptitol* (**10**). — Hexa-*O*-benzoyl-D-*glycero*-α-D-*galacto*-heptose[20] (4 g) was ammonolyzed, the solution was evaporated to dryness, and the crystalline residue was suspended in methanol. The suspension was filtered, and the crystals were washed with the same solvent; there resulted 0.80 g of needles, m.p. 198–200°; and from the mother liquors, another 0.085 g was obtained (total yield 42.5%). Recrystallized three times from water, the product had m.p. 225–226°, $[\alpha]_D^{28}$ −1.3° (*c* 1, pyridine).

Anal. Calc. for $C_{21}H_{26}N_2O_8$: C, 58.11; H, 6.04; N, 6.45. Found: C, 57.97; H, 6.32; N, 6.45.

Paper chromatography of the mother liquors with solvent B showed, with

spray reagent (a), spots having R_F 0.73 (corresponding to **10**), R_F 0.13 (corresponding to D-*glycero*-D-*galacto*-heptose), and two faint, nonreducing spots having R_F 0.63 and 0.23, which were not identified.

Hexa-O-acetyl-1,1-bis(benzamido)-1-deoxy-D-glycero-D-galacto-*heptitol.* — Compound **10** (0.32 g) was acetylated in the usual way with pyridine–acetic anhydride. After evaporating the solution to dryness in a desiccator over sulfuric acid and potassium hydroxide, there resulted 0.50 g of crystals, m.p. 110–115°. Recrystallized three times from 50% ethanol, the product had m.p. 119–120°, $[\alpha]_D^{25}$ −49.0° (*c* 1.3, chloroform).

Anal. Calc. for $C_{33}H_{38}N_2O_{14}$: C, 57.72; H, 5.57; N, 4.08. Found: C, 57.92; H, 5.70; N, 4.34.

SUMMARY

The reaction of methanolic ammonia with the hexa-*O*-acetyl and hexa-*O*-benzoyl derivatives of D-*glycero*-D-*gulo*-heptose, D-*glycero*-L-*manno*-heptose, and D-*glycero*-D-*galacto*-heptose affords the 1,1-bis(acylamido)-1-deoxy-D-heptitols (**2**, **3**), from which the acetates have been prepared. The yields of **2** and **3** are in general higher than those of the corresponding hexose derivatives.

On ammonolysis of hexa-*O*-benzoyl-D-*glycero*-L-*manno*-heptose, an *N*-benzoyl-D-*glycero*-L-*manno*-heptosylamine is formed, together with the main product.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PROPERTIES OF THE GLUCOAMYLASE FROM *Rhizopus delemar*

JOHN H. PAZUR AND SHIGETAKA OKADA*

Department of Biochemistry, Pennsylvania State University, University Park, Pennsylvania (U.S.A.) and Department of Biochemistry and Nutrition, University of Nebraska, Lincoln, Nebraska (U.S.A.)

(Received June 30th, 1966; in revised form, February 6th, 1967)

INTRODUCTION

Because of their ability to convert starch almost quantitatively into D-glucose, the glucoamylases [α-D-(1→4)-glucan glucohydrolases, E. C. 3:2:1:3] are of increasing importance in the commercial production of crystalline D-glucose and in the manufacture of corn syrups of high D-glucose content[1]. Since the enzymes of this group have a broad, substrate specificity[2], they are also useful for the determination of the types of glucosidic bonds in such heteropolymers as the teichoic acids[3], glycoproteins[4], and glycolipids[5]. Of the glucoamylases from fungal sources, that from *Aspergillus niger* has been most extensively investigated[6-8]. In our laboratory, the glucoamylase from *Rhizopus delemar*[9] has now been obtained in a high degree of purity. The purification procedure utilized filtration and chromatography on ion-exchange resins. The physical properties, the action pattern, and the carbohydrate content of the purified enzyme have been determined and compared with those of the glucoamylase from *A. niger*. The ability of the enzyme from each of the two organisms to catalyze reversion reactions has also been investigated.

RESULTS AND DISCUSSION

The glucoamylase from *Rhizopus delemar* has been purified by successive filtration and chromatography on Duolite A-2 resin, Sephadex G-50 gel, and Amberlite XE-64 resin**. The results of electrophoretic studies (Table I) and ultracentrifugation experiments (Fig. 1) indicated a molecular homogeneity in the purified sample. The purified glucoamylase migrated as a single band on electrophoresis at pH 5.6 or 7.6. Furthermore, as noted in Table I, the protein and the enzymic activity of the preparation were distributed in an identical fashion on the electrophoretic strips. The electrophoretic mobility of the *R. delemar* enzyme was markedly different from that of the major isoenzyme of the glucoamylase from *A. niger*. Thus, on electrophoresis at pH 5.6, the glucoamylase from *R. delemar* migrated 3 cm from the origin towards the

*Present address: The Osaka Municipal Technical Research Institute, Osaka, Japan.
**Duolite-A2 is a product of Diamond Alkali Company, Redwood City, California; Sephadex G-50 is a product of Pharmacia, Uppsala, Sweden; and Amberlite XE-64 is a product of Rohm and Haas Co., Philadelphia, Pennsylvania.

negative electrode, whereas glucoamylase I from *A. niger* remained at the origin. On electrophoresis at pH 7.6, the former enzyme remained at the origin, whereas the latter enzyme migrated 5 cm towards the positive electrode. The glucoamylase from *R. delemar* sedimented as a single component on density-gradient centrifugation (Fig. 1). As noted in Fig. 1, the glucoamylases from both organisms sediment at

TABLE I

ANALYTICAL DATA ON SAMPLES EXTRACTED FROM PAPER-ELECTROPHORETIC STRIPS (pH 5.6) OF THE GLUCOAMYLASE FROM *R. delemar*. THE SAMPLE NUMBERS INDICATE THE DISTANCE (IN CM) FROM THE ORIGIN (O) TO THE NEGATIVE (—) OR POSITIVE (+) ELECTRODE

Sample	*Activity units per ml*	*Protein, μg/ml*	*Carbohydrate, μg/ml*	*Hexosamine, μg/ml*
−5 to −4	0.2	4.1	0.0	0.1
−4 to −3	4.1	9.5	0.9	0.3
−3 to −2	24.1	58.0	5.4	1.4
−2 to −1	3.2	10.2	1.0	0.2
−1 to 0	0.2	2.2	0.2	0.1
0 to +1	0.1	0.9	0.0	0.0
+1 to +2	0.1	0.9	0.0	0.0

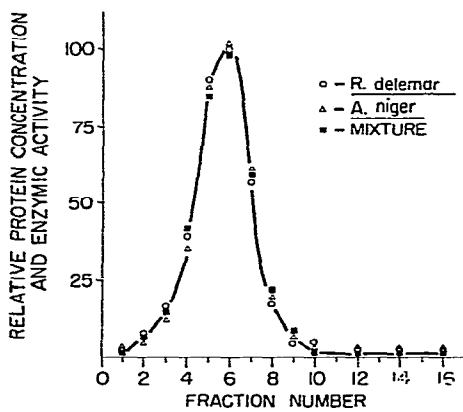


Fig. 1. Density-gradient centrifugation patterns of glucoamylases.

identical rates. This sedimentation behavior[10] indicates that the two enzymes are of the same molecular weight[2], approximately 100,000.

alpha-Amylase, which was detected in the crude enzyme sample by a sensitive, paper-chromatographic method described in the experimental section, was not present in the purified glucoamylase. The *alpha*-amylase was separated from the glucoamylase in the chromatographic steps that employed Sephadex G-50 or Amberlite XE-64. The recently discovered glucanosyltransferase[11] was also removed from the glucoamylase in the purification process, primarily by adsorption on Duolite A-2.

The glucoamylase from *R. delemar*, like that from *A. niger*[12], contains sugar

residues in its structure. The total carbohydrate in the *R. delemar* enzyme is 13%, representing approximately 80 sugar residues per molecule of enzyme. The total carbohydrate content of the glucoamylase from *R. delemar* and that of glucoamylase I from *A. niger*[12] are the same. However, whereas the latter enzyme contains D-mannose, D-glucose, and D-galactose, the enzyme from *R. delemar* contains primarily D-mannose and 2-amino-2-deoxy-D-glucose. These sugars have been identified in acid hydrolyzates of the enzymes by paper-chromatographic and enzymic procedures. The hexosamine is, in all likelihood, in the *N*-acetyl form in the native enzyme. The acid hydrolyzates of the enzymes also contained reducing compounds that, on paper chromatograms, migrated at rates characteristic of oligosaccharides, indicating that the carbohydrate residues are attached as polymeric segments to the polypeptide chain of the enzymes.

The evidence for a chemical linkage of the carbohydrate to the polypeptide chain in the *R. delemar* glucoamylase is indirect at this time. Fig. 2 shows that the carbohydrate in the enzyme preparation sediments, on ultracentrifugation, at a rate identical with that of the protein. It is also noted in Fig. 2 that the enzymic activities in the

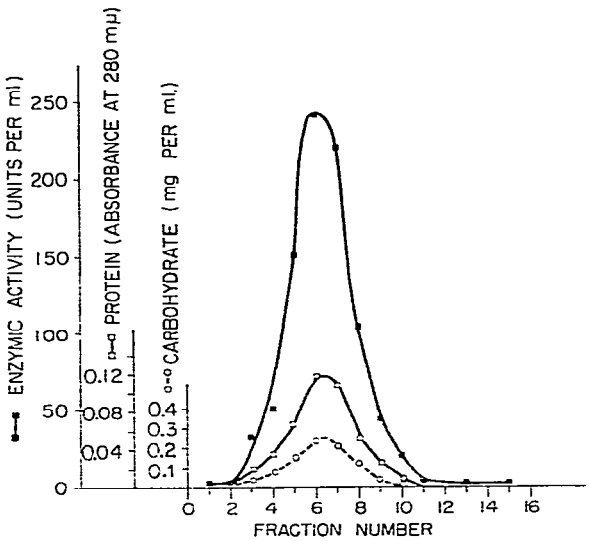


Fig. 2. Concentration of protein, carbohydrate, and enzymic activity in fractions obtained by density-gradient centrifugation of the glucoamylase from *R. delemar*.

various fractions correspond to the concentrations of protein. On paper electrophoresis, the protein and the carbohydrate in the purified glucoamylase migrated at the same rates (Table I). This electrophoretic behavior strongly suggests that the carbohydrate is linked to the protein of the enzyme. Similar results have been reported for glucose oxidase, which has been shown to possess a glycoprotein structure[13]. The data in Table I also show that a hexosamine comprises about 25% of the total carbohydrate in the *R. delemar* glucoamylase. Several lines of evidence indicate that the hexosamine is 2-amino-2-deoxy-D-glucose. Since the latter is not present in glucoamylases from

other sources, the detection of this compound in a glucoamylase sample may well serve as a qualitative test for the origin of the enzyme preparation.

The action patterns of glucoamylases from *R. delemar* and *A. niger* on starch and D-glucosyl-oligosaccharides were similar. In both cases, D-glucose was the only detectable hydrolysis product. An "oligosaccharide map" for the action of the glucoamylase from *R. delemar* on malto-oligosaccharides-*1*-^{14}C (Fig. 3) is similar to

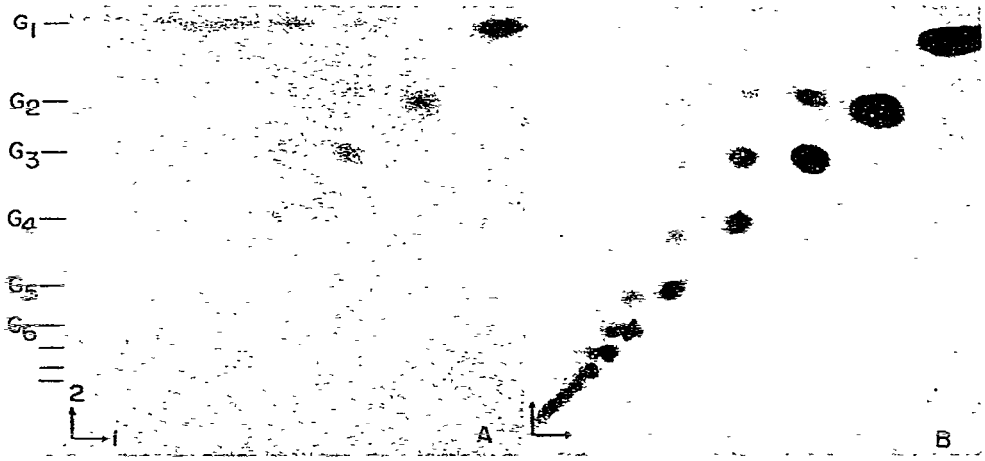


Fig. 3. Oligosaccharide map for the action of glucoamylase from *R. delemar* on malto-oligosaccharides-*1*-^{14}C; (A) reducing products, (B) radioactive products; 1, first direction of chromatography, 2, second direction of chromatography; G_1 = D-glucose, G_2 = maltose, G_3 = maltotriose, G_4 = maltotetraose, G_5 = maltopentaose, and G_6 = maltohexaose.

the recently published map for the enzyme from *A. niger*[11]. Apparently, the glucoamylase from *R. delemar*, like that from *A. niger*[7,14], acts by a multi-chain mechanism on malto-oligosaccharides and, presumably, on starch. Both enzymes hydrolyze the α-D-(1→6) linkage in isomaltose, but at a rate much less than that for the α-D-(1→4) linkage of maltose[14]. Both enzymes catalyze reversion reactions when incubated with D-glucose. The rate of the reversion reactions with the glucoamylase from *R. delemar* was approximately twice that for the enzyme from *A. niger* (Fig. 4). The latter observation is an important factor that must be considered when enzymes are selected for use in the production of crystalline D-glucose from starch.

EXPERIMENTAL

Purification of the glucoamylase from Rhizopus delemar. — A glucoamylase preparation from *Rhizopus delemar* was obtained from the Miles Chemical Company, Elkhart, Indiana. Glucoamylase activity was determined quantitatively by an arsenomolybdate reducing-sugar method[15], and qualitatively, by a paper-chromatographic method[14]. In the former method, one part of the enzyme solution was mixed with three parts of a 0.6% starch solution buffered to pH 5.2 with 0.02M acetate buffer at 30°. After 10 min, aliquots (1 ml) of the digest were removed and each was immediately

added to 1 ml of alkaline copper reagent. The reducing values of the aliquot and of a starch blank were determined, and the increase in reducing value was used as a measure of the production of D-glucose. A unit of glucoamylase activity is defined as that amount of enzyme which liberates 1 mg of D-glucose from starch in 60 min under the conditions of the assay. In the qualitative method, aliquots (0.1 ml) of the enzyme solu-

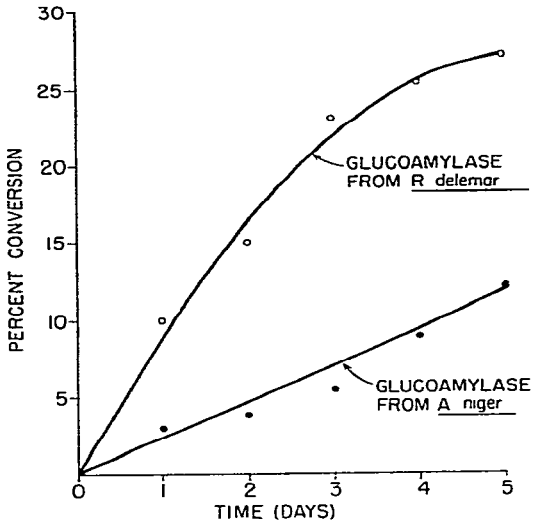


Fig. 4. Rate of synthesis of oligosaccharides from D-glucose by glucoamylases.

tion were incubated with an equal volume of 2% maltose or soluble starch solution for various periods of time. The nature, and the rate of production, of hydrolytic products from these substrates was determined by paper chromatography. The appearance of D-glucose from both substrates is indicative of glucoamylase activity. The appearance of malto-oligosaccharides (maltose, maltotriose, etc.) in the starch digests is indicative of *alpha*-amylase activity, and the appearance of other reducing oligosaccharides (isomaltose, *O*-isomaltosyl-D-glucose, etc.) in the maltose digests is indicative of transferase activity.

The glucoamylase preparation (5 g) was shaken with 50 ml of distilled water for 0.5 h, and the resulting mixture was filtered to remove the insoluble residue. An optical density measurement at 280 mμ on an aliquot of this filtrate, diluted 250-fold with distilled water, was 0.227. The protein concentration, measured on 0.5 ml of the diluted sample by the Lowry method[16], was 0.05 mg per ml. Another sample of the 10% glucoamylase solution was diluted 1000-fold and assayed for activity on the starch substrate. The enzymic activity of the concentrated solution was found to be 1,050 units per ml. The specific activity was 84 units per mg of protein. The 10% glucoamylase solution (25 ml) was passed through a column containing 40 g of Duolite A-2 resin, pre-equilibrated with 0.05M sodium acetate of pH 5. Samples of 15 ml were collected, and assayed for glucoamylase activity. Most of the activity was found in tubes 4 and 5, and the contents of these tubes were filtered through a column

(2.8 × 30 cm) of coarse Sephadex G-50. The column was washed with distilled water, and twenty-five 15-ml fractions were collected from the column. These fractions were assayed for glucoamylase and *alpha*-amylase activities by the paper-chromatographic method. The glucoamylase was found in fractions 3 to 5, but it was still contaminated with *alpha*-amylase. The sample was then introduced onto a column (2.6 × 19 cm) of Amberlite XE-64 resin, pre-equilibrated with 0.05M sodium acetate buffer of pH 5.0. This column was washed with an additional 100 ml of 0.05M sodium acetate buffer and 300 ml of M sodium acetate buffer of pH 5.0. Fractions of 10 ml of eluate were collected during the washing process. Assays of these fractions showed that *alpha*-amylase was in fractions 4 to 6, and glucoamylase in fractions 26 to 30. The samples from tubes 26, 27, and 28 were combined and analyzed for glucoamylase activity and protein content. The total glucoamylase activity in this sample was 96 units per ml, and the protein content was 0.31 mg per ml. The specific activity of the purified sample was 310 units per mg of protein. The combined sample was dialyzed, and concentrated ten-fold *in vacuo* at 2°; this sample, or a dilution thereof, was used as the enzyme source for the subsequent experiments.

Glucoamylase from Aspergillus niger. — *Aspergillus niger* produces two isoenzymes of glucoamylase, which were separated by chromatography on DEAE-cellulose as previously described[7]. The isoenzyme having the faster electrophoretic mobility, designated[17] glucoamylase I, was used in the comparative studies. The solution of glucoamylase I used in the current experiments contained 0.3% of protein. Appropriate dilutions of this sample were prepared for various experiments.

Electrophoresis. — Samples (0.01 ml) of the purified enzymes (30 μg of total protein), or mixtures of enzymes, were applied to paper strips buffered to pH 7.6 with 0.1M potassium phosphate buffer. Electrophoresis was performed with a Spinco Model R paper-electrophoresis unit, for 16 h at 160 volts and a current of 15 ma, and also at pH 5.6 in 0.1M phosphate buffer under similar conditions. The enzyme activity on the finished strips was detected by spraying the strips with 2% starch solution and incubating at room temperature for 5 min, and then spraying them with a solution of *o*-tolidine followed by a solution of glucose oxidase and peroxidase[18]. Glucoamylase activity on the paper strips appeared as a blue band. The protein on the paper strips was located by staining with Light Green SF dye[19].

Twenty-four samples of 0.01 ml of the purified glucoamylase from *R. delemar* were also subjected to paper electrophoresis at pH 5.6 as described above. The finished strips were cut into sections 1 cm wide. The corresponding sections of the 24 strips were combined, and extracted with 6 ml of water. The protein content of each extract was determined by measurements of absorbance at 280 mμ. Glucoamylase activity was measured by incubating 0.5 ml of each extract with 1.5 ml of a 0.6% starch solution for 10 min, and subsequently measuring the increase in reducing-sugar content[15]. The carbohydrate in the sample was measured by an orcinol method[20] on a 2-ml aliquot of the extract. The hexosamine was measured by a standard procedure[21] on the remaining 2.5 ml of the extract. These data are presented in Table I.

Density-gradient centrifugation. — Samples of 0.3 ml (900 μg protein) of each

of the purified glucoamylases, and of a mixture of equal parts of the two enzymes, were placed as a layer on the top of a glycerol gradient[13] (5 to 40%). The samples were centrifuged in a swinging bucket SW39L and Spinco Model L ultracentrifuge for 24 h at 35,000 rpm. At the end of this time, the tube contents were fractionated into 0.3-ml samples by means of a density-gradient fractionater. Each fraction was analyzed for protein by the absorbance at 280 mμ, and for enzyme activity by measuring the production of D-glucose from starch under the conditions of the assay procedure. The results of the analysis were expressed on a relative basis, the sample of highest enzymic activity or protein concentration being assigned an arbitrary value of 100. These results are presented in Fig. 1.

Carbohydrates in glucoamylase from R. delemar. — To a sample of 0.2 ml of glucoamylase (total protein, 600 μg) were added 0.8 ml of water and 8.5 ml of orcinol–sulfuric acid reagent[20]. The sample was heated for 15 min at 80°, and the absorbance of the resulting solution was measured at 505 mμ. The average absorbance value for a number of determinations was 0.170, corresponding to 80 μg of carbohydrate, calculated from a standard curve for D-mannose. A 0.1-ml sample (300 μg of total protein) of the enzyme solution was mixed with 0.1 ml of N hydrochloric acid and heated at 100°. After 0.25, 0.5, 1, and 2 h, aliquots (0.01 ml) of the hydrolyzate were removed and examined for the appearance of reducing sugars by paper chromatography with the solvents 6:4:3 (v/v) butyl alcohol–pyridine–water, and 4:1:1 (v/v) butyl alcohol–ethanol–water. Reducing compounds in the hydrolyzate were detected in the paper chromatograms by a silver nitrate method[22], and amino sugars were detected by the 2,4-pentanedione–*p*-dimethylaminobenzaldehyde reagent[23]. The monosaccharides present in the acid hydrolyzate of the enzyme had R_F values in both solvents identical with those of D-mannose and 2-amino-2-deoxy-D-glucose, and gave with the spray reagents color reactions typical of these monosaccharides. Other reducing compounds were also detected in the hydrolyzate, and these had R_F values characteristic of oligosaccharides.

The density-gradient, centrifugation method[13] was used to determine whether the carbohydrate moiety sedimented at the same rate as the protein moiety. The fractions from the density-gradient tubes were analyzed for total carbohydrate[20], in addition to the protein and enzymic activity. The results of one such experiment are presented in Fig. 2.

Action of glucoamylase on D-*glucosyl oligosaccharides and on starch.* — In order to compare the action pattern of the two glucoamylases on malto-oligosaccharides, a recently published method of "oligosaccharide mapping" was employed[11]. In this procedure, malto-oligosaccharides-*1*-^{14}C were separated in one direction on a paper chromatogram (30 × 30 cm) in the solvent system 6:4:3 (v/v) butyl alcohol–pyridine–water. A dilute solution of the glucoamylase (the standard glucoamylase solution diluted 1 to 2,000) was sprayed on the area of the chromatogram on which the oligosaccharides were separated. The sprayed chromatogram was then incubated at room temperature for 15 min, and the products of enzyme action on each of the oligosaccharides were separated by chromatography in a second direction. The labeled, hydrolysis

products were detected by autoradiography, and the reducing products were detected by a silver nitrate reagent[22]. A typical map of oligosaccharides for the glucoamylase from *R. delemar* is reproduced in Fig. 3.

Samples of 0.1 ml of glucoamylases from *R. delemar* and *A. niger* (total activity, 3 units) were incubated with 1 mg of pure maltose in 0.1 ml of buffer of pH 5. Aliquots of 0.01 ml were, at various time-intervals up to 24 h, analyzed for reaction products by paper chromatography. The reducing products in the aliquots were detected by the silver nitrate reagent. In a parallel series of experiments, isomaltose was used as the substrate, and the enzyme activity was increased 30-fold. The rates of production of D-glucose and other oligosaccharides from these substrates were noted.

Action of glucoamylases on D-*glucose.* — The rates at which the glucoamylases from the two organisms catalyze reversion reactions were measured by use of D-glucose *1-*^{14}*C*. The labeled substrate facilitates the measurement of the reversion products. A sample of 0.1 ml of 60% D-glucose-*1-*^{14}*C* (total activity, 8×10^5 disintegrations/min) was mixed with 0.1 ml of the enzyme solution, which contained 50 units of glucoamylase from *A. niger* or from *R. delemar*. The digests were incubated at 55°, and 0.01-ml aliquots were removed for analysis after incubation periods of 1 to 5 days. The reaction products in the aliquots were separated by paper chromatography in the solvent system 6:4:3 (v/v) butyl alcohol–pyridine–water. The positions of the radioactive products were located on the paper by autoradiography. On the basis of R_F values, three oligosaccharides were tentatively identified as nigerose, isomaltose, and isomaltotriose. These oligosaccharides are typical reversion-products with other oligosaccharide hydrolases[24,25]. The radioactivities of the oligosaccharides were measured directly on the paper by use of a thin-walled, Geiger–Müller tube and a suitable scaler. From the radioactivity of the products and the initial radioactivity in the labeled D-glucose, the rates of conversion of D-glucose into isomaltose by the two enzymes were calculated. These data are presented in Fig. 4.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Corn Industries Research Foundation, Washington, D.C. (U.S.A.), and from the Agricultural Research Service, U. S. Department of Agriculture, Grant. No. 12-14-100-7204 (71), administered by the Northern Utilization Research and Development Division, Peoria, Illinois, U. S. A. The work is published, with the approval of the Director, as Paper No. 1932 Journal Series, Nebraska Agricultural Experiment Station.

SUMMARY

The glucoamylase from *Rhizopus delemar* was purified by filtration and chromatography on Sephadex gel and ion-exchange resins. The purified enzyme is shown to be a glycoprotein containing residues of D-mannose and 2-amino-2-deoxy-D-glucose in its structure. The action pattern of the glucoamylase from *R. delemar* on

starch and malto-oligosaccharides is similar to that for the glucoamylase from *A. niger*. The two enzymes are of the same molecular size, but differ markedly in their electrophoretic properties. Both enzymes catalyze reversion reactions at a low rate; the rate for the glucoamylase from *R. delemar* is significantly higher than that for the glucoamylase from *A. niger*.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

CATALYTIC HYDROGENOLYSIS OF SACCHARIDES

PART I. QUALITATIVE AND QUANTITATIVE METHODS FOR THE IDENTIFICATION AND DETERMINATION OF THE REACTION PRODUCTS

G. VAN LING, C. RUITERMAN, AND J. C. VLUGTER
Department of Chemical Engineering, Technological University "Twente", Enschede (Netherlands)
(Received October 25th, 1966; in revised form, January 23rd, 1967)

INTRODUCTION

High pressure, catalytic hydrogenation and hydrogenolysis of saccharides, which can be considered as interesting base materials for the production of polyhydric alcohols, have been studied by several investigators[1-5]. The process has been applied[6] to produce a mixture of polyhydric alcohols for subsequent nitration to give a substitute for nitroglycerine. Similar work has been reported[7], and Korf [8] found that CuO–CeO_2–SiO_2 (95:5:100, w/w) was the most selective catalyst for the conversion of sucrose into glycerol. The hydrogenation experiments were carried out batchwise in rotating autoclaves, and the standard conditions are listed in Table I; the course of the reaction temperature and pressure changes are indicated in Fig. 1. By fractional

TABLE I

REACTION CONDITIONS IN THE STANDARD, AUTOCLAVE EXPERIMENT FOR THE CATALYTIC HYDROGENOLYSIS OF SUCROSE

Sucrose	100 g	Initial pressure of H_2	200 atm.
Methanol–water (75:25, w/w)	150 g	Reaction time	30 min
Catalyst, CuO–CeO_2–SiO_2	10 g	Reaction temperature	215°
Calcium hydroxide	1 g	Autoclave volume	500 ml

distillation of the reaction product, Korf obtained about 45% of glycerol. In continuing this work, we hoped to develop a better understanding of the complex reaction mechanism and of the kinetics of the various chemical reactions that take place simultaneously and consecutively, in order to establish optimal reaction conditions. For these studies, reliable and reproducible analytical methods for the determination of all intermediates and final reaction components are required.

DISCUSSION AND EXPERIMENTAL

Qualitative paper chromatography of the reaction products. — Many solvent systems and spray reagents have been described [9-11] as suitable for the paper chromatography of carbohydrates of low molecular weight. In the present work, the best

separations of the reaction products were achieved with butyl alcohol–ethanol–water (4:1.1:1.9)[10].

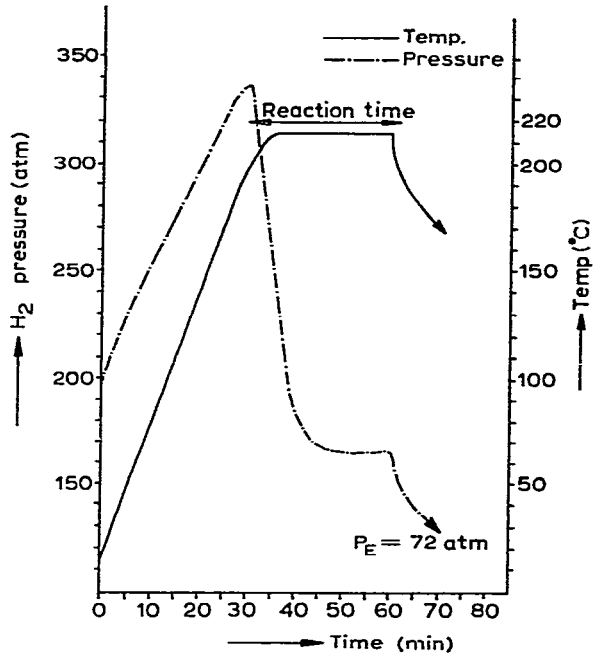


Fig. 1. Hydrogen pressure and reaction temperature as a function of the reaction time for catalytic hydrogenolysis of sucrose under standard, autoclave conditions.

Chromatography was performed at room temperature on Whatman No. 1 paper by the descending technique; the elution time was approximately 20 hours. For the simultaneous detection of saccharides and polyhydric alcohols, anisaldehyde–sulfuric acid–ethanol[12] was originally used; saccharides give, in general, violet spots, whereas polyhydric alcohols, with the exception of glycols, give pale, violet-blue spots. A disadvantage of the reagent is that charring of the paper occurs because of the presence of sulfuric acid. This could be prevented by replacing the sulfuric acid by benzenesulfonic acid. With other acids, the spray reagent is inactive. The composition of the modified reagent was anisaldehyde–benzenesulfonic acid–ethanol (1:2:20, w/w). Development of the spots was effected by heating for 15 min at 85°.

The paper chromatogram of the reaction product of the standard, autoclave experiment showed, besides traces of unreacted sucrose, the presence of hexitols, pentitols, tetritols, glycerol, and unknown compounds (red spots) having R_F values between 0.70 and 0.90. The unknown compounds are probably furan derivatives.

Quantitative analysis of the reaction products by gas–liquid chromatography. — Quantitative g.l.c. analyses were required for the determination of reaction products ranging from volatile alcohols to the high-boiling polyhydric alcohols. It was also important to have accurate analyses for water, since water is consumed during the hydrolysis step, and formed in the destructive hydrogenation of the polyhydric alcohols.

Because of the marked difference in volatility of the individual compounds, it was not possible to analyse the reaction product in one operation. A preliminary, fractional distillation was therefore performed to give the following fractions: (*a*) methanol–water (b.p. 95°); (*b*) water–glycol (b.p. 125°/10 mm); (*c*) glycerol–hexitol (b.p. > 125°/10 mm).

G.l.c. was performed with an F & M Model 720 chromatograph, provided with a dual-column system, temperature programming, and independent heating of the detector and injection blocks. The detector was a double, thermal-conductivity cell.

(*a*) *Methanol–water fraction.* G.l.c. of highly polar compounds (alcohols and water) is complicated by the phenomenon of tailing[13]. Deactivation of such supports as diatomaceous earth with hexamethyldisilazane (HMDS) or trimethylchlorosilane[14], or use of very inactive supports, such as Teflon[15], enables tailing to be suppressed, and water may then be determined[16].

The methanol–water fraction (*ca.* 90% methanol) was analysed with a 25-cm column of 20% diglycerol on Chromosorb W (60–80 mesh; HMDS). The conditions are listed in Table II, and the chromatogram is shown in Fig. 2.

TABLE II

CONDITION OF THE G.L.C. ANALYSES

	Methanol–water	*Water–glycol*	*Glycerol–hexitol*
Analytical column	25 × 0.4 cm Cu-tube (HMDS)	1 m × 4 mm Cu-tube (HMDS)	3 m × 4 mm S.S.-tube (HMDS)
Compensating column	—	—	1.5 m S.S.tube (HMDS)
Column filling	20% diglycerol/ Chromosorb 60–80 (HMDS)	15% diglycerol/ Chromosorb 60–80 (HMDS)	20% silicone rubber/ Chromosorb 60–80 (HMDS)
Column temperature (°)	60–80	70–135	125–280
Programming (°/min)	10	5	7.5
Temp. of injection block (°)	210	210	285
Temp. of detector (°)	320	320	320
Flow rate of H_2 (l/h)	3.0	3.0	6.0 and 3.6
Bridge current (mamp)	150	150	150
Injected amount (μl)	5	5	25

Alcohols may be detected in the methanol–water fraction by using a 12-m column of 10% benzyldiphenyl on Chromosorb W (60–80 mesh; HMDS), at 80°. With a flame-ionisation detector (F & M Model 1609), the water signal can be suppressed. In an analysed, methanol–water fraction, less than 0.1% of isopropyl alcohol was present.

(*b*) *Water–glycol fraction.* Similar difficulties to those above were encountered in the quantitative determination of water in this fraction. G.l.c. of glycols has been reported by several investigators[17]. The water–glycol fraction contains *ca.* 60% of water, 20% of ethylene glycol, 20% of propane-1,2-diol, and small percentages of

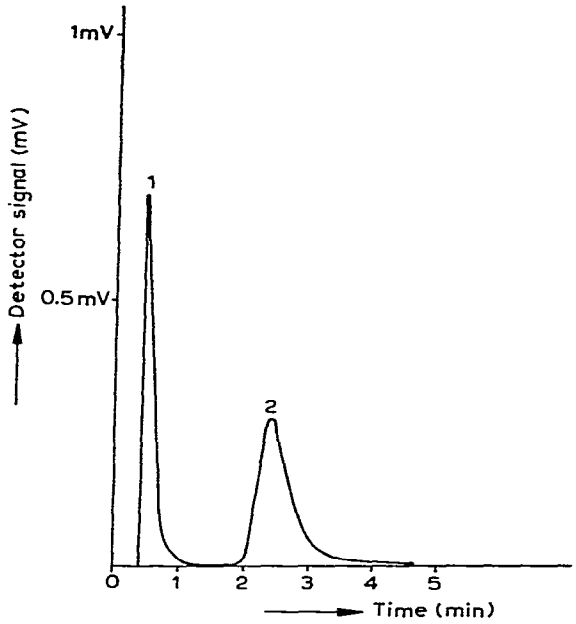


Fig. 2. G.l.c. of the methanol–water fraction.
1, methanol; 2, water.

butane-2,3-diol and methanol. Analysis was effected on a 1-m column of 15% diglycerol on Chromosorb W (60–80 mesh; HMDS). The conditions are given in Table II, and the chromatogram is shown in Fig. 3.

(*c*) *Glycerol–hexitol fraction.* This mixture is not sufficiently volatile to allow direct analysis by g.l.c., and must be converted into more volatile derivatives (ethers or acetates)[18]. Acetylation of the glycerol–hexitol fraction, followed by g.l.c., gave the best results. The following procedure was used for the acetylation. A portion (2.5 g) of the fraction was treated for 2 h with a boiling mixture of acetic anhydride and pyridine (50 ml; 1:2). Most of the acetic anhydride and pyridine was subsequently removed at 45°/15 mm, since these compounds caused tailing during g.l.c. The residue was then dissolved in anhydrous chloroform, and analysed rapidly so that no crystallisation of the hexa-acetates occurred.

Two liquid phases were investigated : (*i*) *Versamid 900* (polar), usable to 350°; and (*ii*) *Silicone gum rubber (methyl) GE-SE-30* (non-polar), usable to 400°. A good separation of the acetates was effected on a 3-m stainless-steel column (HMDS) of 20% Versamid 900 on Chromosorb W (60–80 mesh; HMDS). The isomeric pairs, erythritol–threitol, arabinitol–xylitol, and glucitol–mannitol, were separable by this system. The column, however, aged rapidly resulting in a change and a reduction of the retention volumes. The use of such a column is therefore limited to qualitative analysis.

The more stable, non-polar column (*ii*) gave better quantitative results, although the selectivity was less. The analyses were performed with a 3-m column of 20%

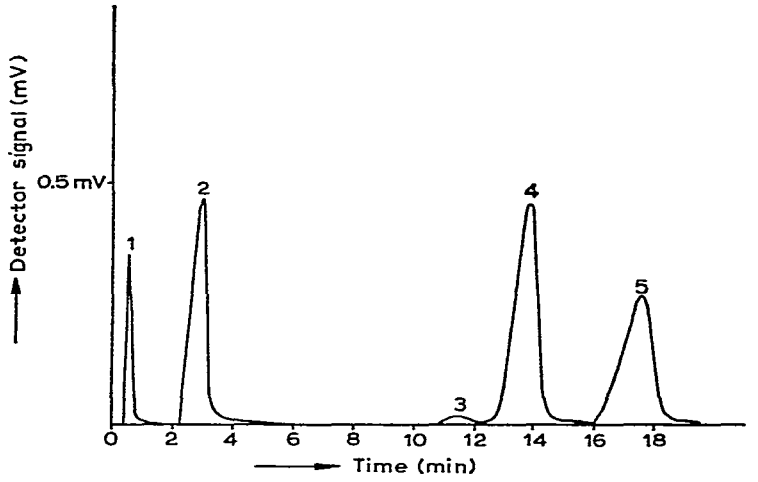


Fig. 3. G.l.c. of the water–glycol fraction.
1, methanol; 2, water; 3, butane-2,3-diol; 4, propane-1,2-diol; 5, ethylene glycol.

silicone gum rubber on Chromosorb W (60–80 mesh; HMDS). The conditions are listed in Table II, and the chromatogram is shown in Fig. 4.

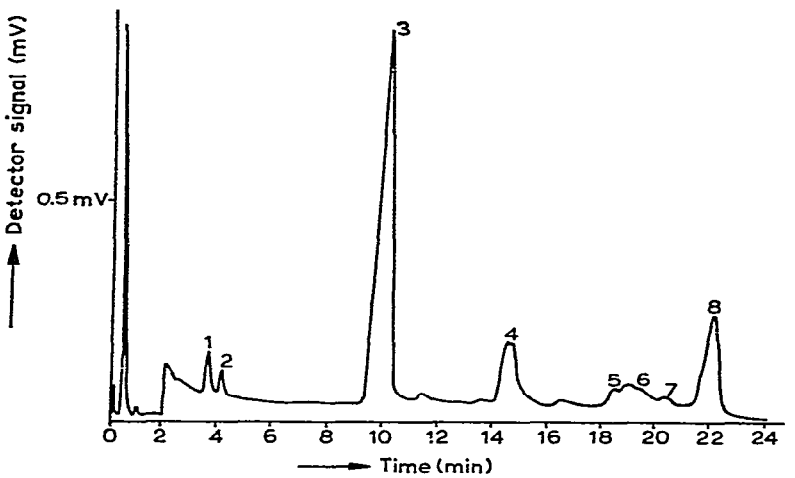


Fig. 4. G.l.c. of the glycerol–hexitol fraction (as acetates).
1, ethylene glycol; 2, propane-1,2-diol; 3, glycerol; 4, tetritols; 5, pentitols; 6, methyl α- and β-D-glucopyranosides; 7, dehydrated hexitols; 8, hexitols.

Because of the high temperature of the column, decomposition of the acetates might be expected. However, a synthetic, acetylated mixture of glycerol, erythritol, and glucitol gave only three peaks, and it is concluded that no decomposition occurs.

Calculations. — The analytical methods described above permit a detailed and reliable analysis of the mixture of reaction products. After measurement of the peak

areas with a planimeter, the composition of the sample can be calculated with the aid of relative, calibration factors[19]. A detailed analysis of the reaction products of the standard, autoclave experiment is shown in Table III; the relative errors are < 5%.

TABLE III

DETAILED ANALYSIS OF THE REACTION PRODUCT FORMED DURING CATALYTIC HYDROGENOLYSIS OF SUCROSE

Components	*Weight (%)*	*Components*	*Weight (%)*
Ethylene glycol	10.0	Methyl D-glucopyranosides	6.5
Propane-1,2-diol	13.2	Dehydrated hexitols	1.6
Butane-2,3-diol	0.4	Hexitols	17.5
Glycerol	35.1	Unknown	2.2
Tetritols	7.7	Lost product	3.2
Pentitols	2.6	Total	100.0

SUMMARY

The numerous, chemical reactions (hydrolysis, cracking, hydrogenation), occurring during the catalytic hydrogenolvsis of saccharides, result in a multicomponent mixture, consisting mainly of polyhydric alcohols. These alcohols, in general, have high boiling points and a poor thermostability. Separation of the individual components by high-vacuum distillation requires a relatively high temperature that causes dehydration, condensation, and charring. Qualitative paper chromatographic and quantitative gas–liquid chromatographic methods of analysis have therefore been developed; these provide useful tools for further studies on reaction mechanisms and reaction kinetics of the hydrogenolysis of saccharides.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE CARBOHYDRATE COMPONENT OF THE DOUM-PALM KERNEL: CHARACTERIZATION AND IDENTIFICATION OF A MANNAN

H. EL KHADEM AND M. A. E. SALLAM
Faculty of Science, Alexandria University, Alexandria (U. A. R.)
(Received February 23rd, 1967)

The African doum-palm (*Hyphaene thebaica*) has been cultivated since time immemorial in Egypt, where it was thought to be the favorite tree of the baboon Thout, the science God. Its kernels were used for the manufacture of buttons, but have recently been replaced by synthetic materials.

Maymone, Battaglini, and Tiberio[1] studied the constituents of the kernel, and found a mannose in its acid hydrolyzate.

In this work, we have isolated a mannan from the doum kernel, and have found it to be a true mannan as defined by Aspinall[2] (*i. e.*, containing 95% or more of a mannose). The mannan was isolated by two methods. The first method involved acetylation of the ground kernels, followed by regeneration of the mannan by deacetylation; this method yielded about 16% of mannan. A higher yield was obtained by extracting the ground kernels with 20% aqueous sodium hydroxide and precipitating the mannan as its copper complex by adding Fehling solution; the yield by this procedure amounted to 19%. In both cases, the precipitated mannan was exceedingly insoluble, even in 20% sodium hydroxide (in which it had initially dissolved), and consisted of 95% of D-mannose and 5% of D-galactose.

A completely methylated product containing 45.4% of methoxyl [theoretical value for $(C_9H_{16}O_5)_x$, 45.6%] was obtained by simultaneous deacetylation and methylation of the acetylated mannan with methyl sulfate and alkali, followed by a further methylation with methyl iodide and silver oxide in *N,N*-dimethylformamide[3]. Methanolysis of this product yielded, on fractionation, a methylated methyl mannoside containing 52.26% of methoxyl (theoretical value for a methyl tri-*O*-methylhexoside, 52.55%). Acid hydrolysis of the methanolysis product yielded a 2,3,6-tri-*O*-methylmannose and some 2,3,4,6-tetra-*O*-methylmannose, both identified as their "anilides". The isolation of a 2,3,6-tri-*O*-methylmannose, together with the results of periodate oxidation (in which one mole of oxidant was consumed per sugar residue), clearly suggested a (1→4)-mannopyranoside linkage.

The negative optical rotation of the mannan in formic acid, $[\alpha]_D^{20}$ −28.7°, and of the mannan acetate in chloroform, $[\alpha]_D^{20}$ −17.2°, indicated that the majority of the linkages in the polysaccharides are in the β-D form. The degree of polymerization, determined by the reducing power before hydrolysis, was 23; whereas, determined by titrating the formic acid liberated during periodate oxidation, it amounted to 14. These values are quite comparable with those of the ivory-nut mannan[4], which has a

degree of polymerization[4,5] ranging from 10 to 21, and an optical rotation of $[\alpha]_D^{20}$ −28° (in formic acid).

EXPERIMENTAL

Crude material. — The doum kernels used in this investigation were first freed of the brown seed-coat by filing, and were then ground. They were defatted with petroleum ether (b.p. 50–70°), washed successively with alcohol and water, and dried. Combustion analysis gave the following results: C, 40.5; H, 6.9; N, 1.2; ash, 1.7%.

Identification and determination of D-*mannose in the doum kernel hydrolyzate.* — The kernels (5 g) were treated overnight with 70% sulfuric acid (25 ml) at room temperature, and the mixture was diluted with 13 vols of water, and heated for 7 h on a steam-bath. The hydrolyzate was freed of sulfate ions (with barium hydroxide and barium carbonate), filtered, and evaporated. Paper chromatography (4:1:5 butyl alcohol–ethanol–water) revealed (with acetonic silver nitrate[6]) a major spot corresponding to a mannose and a very faint spot for a glucose. D-Mannose was identified by conversion into the phenylhydrazone, m.p. and mixed m.p. 186–189°. D-Mannose was determined (by the method of Jendrassik and Polgar[7]) by heating the dry, ground kernels (100 mg) with 0.5N hydrochloric acid (10 ml) for various times in a series of sealed tubes at 100°. Each tube was cooled, and the contents were filtered, neutralized with 0.5N sodium carbonate, and diluted to 10 ml with water. For each determination, 1 ml of this solution was diluted with 10 ml of water. The results were:

Time (h)	0	1	2	4	6	8	10
Reducing power (as mannose), %	0	27.29	33.62	39.14	43.55	45.41	45.41

Isolation of the mannan: (*a*) *By acetylation and deacetylation.* — The dry kernels (100 g) were acetylated with a mixture of acetic acid (195 ml) containing chlorine and acetic anhydride (300 ml) containing sulfur dioxide (equivalent to the chlorine). The mixture was stirred for 8 h at 65° and then filtered from unreacted material, and the filtrate was poured into cold water (10 vols). The precipitate was filtered off, washed successively with water and alcohol, and dried. The acetylated mannan was extracted from the residue with acetone (3 × 250 ml), the extract was concentrated, and the acetate was precipitated with ice-cold water (10 vols); yield, 60 g. The unreacted material from the acetylation was reacetylated in the same way, affording a further 4 g. The acetate was purified by reprecipitation from 2:1 (v/v) chloroform–acetone by petroleum ether (b.p. 50–70°); recovery 80%. The purified acetate was a white powder, insoluble in water and alcohol, $[\alpha]_D^{25}$ −17.2° (*c* 1.9, chloroform); Found: OAc, 39.7%. The purified acetate (5 g) was deacetylated by dissolving it in boiling acetone (100 ml) and shaking the solution with 30% aqueous potassium hydroxide (100 ml) at room temperature. The aqueous layer was diluted with water, acidified with acetic acid, and treated with ethanol (1.0 l). The precipitated mannan was centrifuged off, dialyzed against frequent changes of distilled water until

free from inorganic matter, and dried, affording 1.5 g of mannan containing no ash and having $[\alpha]_D^{20}$ −31.5° (90% formic acid).

(*b*) *Extraction of the mannan with alkali.* — The defatted kernels (50 g) were treated with 20% aqueous sodium hydroxide (1 liter) and the mixture was stirred for 7 h under nitrogen atmosphere at room temperature, and then for 1 h at 65°. The mixture was filtered, the residue was washed with 20% sodium hydroxide (500 ml), and the combined filtrate and washings were diluted with water (1.5 l). The mannan was precipitated from the solution as its insoluble, copper complex, with Fehling reagent (500 ml of Fehling solution A and 500 ml of Fehling solution B). A little acetone was added, to induce rapid settling of the precipitate; this was collected by centrifugation, and washed with Fehling reagent (200 ml) and then with water until it started to swell. The copper complex was decomposed by stirring it with 1% methanolic hydrogen chloride until the precipitate had been converted into a white suspension; this was centrifuged, and the precipitate was washed successively with methanol, methanol containing ammonia (to remove traces of hydrochloric acid), methanol, and ether (yield, 5 g). Upon repeating the alkali extraction on the residue from the doum kernels, another 4.5 g of mannan was obtained. The mannan (total yield, 19%) had $[\alpha]_D^{20}$ −28.75° (90% formic acid), and contained 0.32% of ash.

Reducing power of the mannan before and after hydrolysis. — This determination was performed by the method of Jendrassik and Polgar[7]; the reducing power of the unhydrolyzed sample was determined directly on 10 mg of mannan suspended in warm water (10 ml). For the hydrolyzed polysaccharide, portions (10 mg in 10 ml of 0.5N hydrochloric acid) were treated as for the doum kernel. The results were as follows:

Time (h)	0	1	2	4	6	8	10
Reducing power of the mannan, %							
by method *a*	4.562	76.6	85.94	96.42	98.42	98.42	98.42
by method *b*	4.872	67.32	76.93	92.93	96.16	96.16	96.16

Paper chromatography of the hydrolyzate, with the solvent mixture already described, revealed a spot corresponding to a mannose and a faint spot corresponding to a galactose.

Determination of the sugar components of the hydrolyzate. — The hydrolyzate from 0.5 g of mannan was dissolved in 1.2 ml of water, and applied to a paper chromatogram along the length of the line of origin. The chromatograms were developed for 48 h with the solvent mixture already described, and then dried. Strips (3 cm wide) were cut on the right and left side, together with a strip (1 cm wide) from the middle of the chromatogram; the positions of the sugar spots were marked, and cut off the unsprayed portions of the chromatograms; these were eluted with water to a volume of 25 ml. The aldoses were estimated by the method of Jendrassik and Polgar[7]. The ratio of D-mannose to D-galactose was 17:1.

Oxidation with periodate. (*a*) *Uptake of periodate.* — The mannan (20 mg) was suspended in water (10 ml), and treated with 2 ml of sodium metaperiodate (0.6417 g

in 10 ml of water) followed by 0.5 ml of acetate buffer (pH 5.2). After 30 h, the mixture was diluted to 1 l, and the excess periodate was estimated in 25-ml samples by titrating against standard arsenite solution; periodate consumed, 1.08 mole per mannose residue (equivalent weight, 162).

(*b*) *End-group assay.* — The mannan (0.1 g) was oxidized with sodium metaperiodate at room temperature. Portions (5 ml) of the solution were withdrawn, treated with ethylene glycol (1 ml), and allowed to stand for 1 h at room temperature; the formic acid liberated by oxidation of the end group was titrated against 0.1N sodium hydroxide, with Methyl Red as indicator. Three moles of formic acid per 13.1 mannose residues were liberated from the mannan obtained by method *a*, and 3 moles per 14.7 mannose residues from the mannan prepared by method *b*.

Methylation. — The pure mannan acetate (30 g) in acetone (200 ml) was simultaneously deacetylated and methylated at 55° by the gradual addition of 30% aqueous sodium hydroxide (320 ml) and methyl sulfate (120 ml) under an atmosphere of nitrogen. When all of the reagents had been added (2 h), the mixture was heated for 30 min at 100°. The solution was then cooled in ice, made almost neutral (faintly alkaline) with 5N sulphuric acid, and then made neutral with excess carbon dioxide. The filtrate was concentrated under diminished pressure to a small volume, and the sodium sulfate was precipitated with alcohol. The partially methylated mannan (OMe, 39.1%) was isolated from the filtrate by concentration followed by extraction with chloroform; it was dissolved in freshly distilled *N,N*-dimethylformamide (100 ml) and shaken with methyl iodide (40 ml) and dry silver oxide (40 g) for 12 h at room temperature. The precipitate was removed by centrifugation, and washed with *N,N*-dimethylformamide (50 ml) and chloroform (2 × 50 ml). The combined solution and washings were then washed successively with 14% aqueous potassium cyanide (50 ml) and water (3 × 100 ml), and concentrated to 100 ml, and the material was fractionated into four fractions by the gradual addition of petroleum ether (b.p. 50–70°).

Fraction	*Petroleum ether, ml*	*Weight, g*	*OMe, %*[a]
I[b] (brownish red)	200	1.50	39.3
II (pale yellow)	700	3.00	42.1
III (whitish yellow)	1400	0.08	43.3
IV (whitish yellow)	3000	0.04	45.4

[a]Calc. for $(C_9H_{16}O_5)_x$: OMe, 45.6%. [b]Contained inorganic material.

Methanolysis of the methylated mannan. — Fractions III and IV of the methylated manan were heated with 1% methanolic hydrogen chloride for 3 h at 100°, and the solution was neutralized (silver carbonate–Amberlite IR-120). The solution was evaporated, and the product was distilled at 110°/0.3 mm Hg, giving methyl 2,3,6-tri-

O-methyl-D-mannoside as a pale-yellow liquid, n_D^{30} 1.461; $[\alpha]_D^{25}$ +45.83° (*c* 0.24, methanol) (lit.[8], n_D^{20} 1.4595).

Anal. Calc. for $C_{10}H_{20}O_6$: OMe, 52.55. Found OMe, 52.26.

Hydrolysis of the methanolysis product, and identification of the methylated sugars. — The crude methanolysis product (0.4 g) was hydrolyzed in a sealed tube, with 0.5N hydrochloric acid for 10 h at 100°; the solution was neutralized as above, and evaporated to dryness. Upon refluxing the hydrolyzate with alcoholic aniline, and subsequent fractional recrystallization of the product from petroleum ether (b.p. 50–70°), 2,3,6-tri-*O*-methyl-D-mannose "anilide" was obtained; m.p. 123–125° (lit.[9] m.p. 127°); a small amount of 2,3,4,6-tetra-*O*-methyl-D-mannose "anilide" was also obtained, m.p. 140° (lit. m.p.[9] 141°; m.p.[8] 142–143°).

SUMMARY

A mannan composed of 95% of D-mannose residues and 5% of D-galactose residues has been isolated from the kernels of the doum palm (*Hyphaene thebaica*). It consists of 14-23 D-mannopyranose residues linked by β-D-(1→4)-linkages.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

EXTENSION OF SUGAR CHAINS THROUGH ACETYLENIC INTERMEDIATES

III. 1-HEPTYNE-L-*gluco*- AND L-*manno*-3,4,5,6,7-PENTOL FROM 2,3,4,5-TETRA-*O*-BENZOYL-*aldehydo*-L-ARABINOSE*

J. L. GODMAN, D. HORTON**, AND J. M. J. TRONCHET
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)

(Received February 20th, 1967)

Previous reports in this series[1,2] described the ethynylation of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-L-arabinose to give a mixture of 4,5:6,7-di-*O*-isopropylidene-1-heptyne-L-*gluco*-3,4,5,6,7-pentol and the L-*manno* epimer, separable by preparative g.l.c.[1,2] or by fractional recrystallization[2] of the derived 3-(3,5-dinitrobenzoates) (**5** and **8**, respectively). This method of chain extension, by ethynylation of *aldehydo* sugars, has been applied[1] with other substituted *aldehydo* sugars. In each case, cyclic acetal substituents were employed as protecting groups to prevent the aldehyde group from undergoing intramolecular cyclization.

The purpose of the present investigation was to determine if ethynylation of a fully acylated *aldehydo* sugar could be used as a practical method for chain extension. Fully acylated *aldehydo* sugars are generally more accessible than are *aldehydo* sugars having cyclic acetal substituents as protecting groups. However, since acyloxy groups are susceptible to attack by organometallic reagents, it was not known if selective attack at the aldehyde group could be effected.

In this report, it is shown that ethynylation of an aldehyde group in the presence of benzoyloxy substituents is feasible as a preparative procedure for extension of a sugar chain. Ethynylation of *aldehydo*-L-arabinose tetrabenzoate (**1**) gave a mixture of 3-epimeric pentol derivatives (**2**), separated as the perbenzoates (**3** and **6**). The latter, and the derived pentols (**4** and **7**) were characterized on a crystalline basis. Preparation of the pentols **4** and **7**, and of the pentabenzoates **3** and **6**, by an alternative, stereochemically definitive route, provided independent confirmation of structures assigned to the products prepared from **1**, and served to identify the epimers specifically.

3-*O*-(3,5-Dinitrobenzoyl)-4,5:6,7-di-*O*-isopropylidene-1-heptyne-L-*gluco*-3,4,5,6,7-pentol (**5**) and its L-*manno* epimer (**8**), whose configurations have been determined unequivocally by degradation to known, crystalline derivatives[2], were used to prepare

*Supported by the National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Maryland 20014; Grant No. GM-11976-03 (The Ohio State University Research Foundation Project 1820). Funds for purchase of the n.m.r. spectrometer were provided by the National Science Foundation, Washington, D. C.
**To whom inquiries should be addressed.

authentic samples of 1-heptyne-L-*gluco*-3,4,5,6,7-pentol* (**4**) and its L-*manno* epimer* (**7**), and also the corresponding pentabenzoates **3** and **6**. The substituted derivatives **5** and **8** were treated with aqueous acetic acid for 1.5 h at 100° to remove the *O*-isopropylidene groups, and subsequent removal of the 3,5-dinitrobenzoyl group with a catalytic amount of sodium methoxide in methanol (Zemplén deacylation) gave the free pentols **4** and **7**. Benzoylation of **4** and **7** gave the corresponding pentabenzoates **3** and **6**. The pentabenzoates **3** and **6** could be reconverted into the respective pentols by Zemplén deacylation.

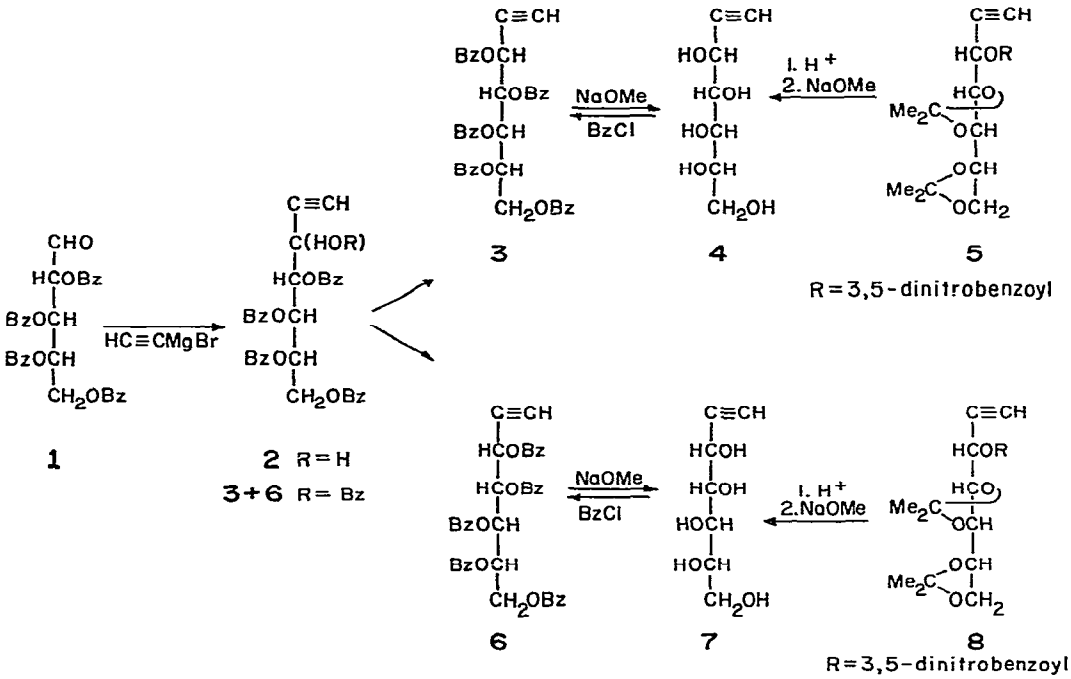
Spectroscopic evidence provided full support for the gross structures assigned to the perbenzoates **3** and **6**. A terminal acetylene function was indicated by the i.r. spectra, and the n.m.r. spectra showed a 1-proton, narrow doublet at τ 7.40 in **3** and at τ 7.51 in **6**, characteristic[2] of an acetylenic hydrogen, long-range coupled to a hydrogen atom four bonds distant ($J_{1,3} \sim 1.2$ Hz). Integration of the signals in the aryl region showed the presence of 5 benzoyl groups. A 2-proton, 8-peak multiplet, the AB portion of an ABX system, was observed near τ 5.3, and was assigned to the C-7 methylene group. The remaining signals, total integral 4 protons, observed in the region τ 3.4–4.3, were assigned to H-3, 4, 5, and 6, but the complexity of the multiplets observed made analysis in detailed stereochemical terms difficult. The two pentabenzoates had sharp melting points, gave different X-ray powder diffraction patterns, and showed different fine-structure in their n.m.r. and i.r. spectra. There was no evidence to indicate any interconversion of **3** and **6**, or other configurational change, during their preparation from **5** and **8**. A chromatographic method for effecting a clear separation of **3** and **6** was not found.

The free pentols (**4** and **7**) were both obtained crystalline. They showed absorptions characteristic of the acetylene group in their i.r. spectra, and both gave satisfactory elemental analyses. The pure L-*gluco* epimer (**4**) melted sharply at 114–116°, and the pure L-*manno* epimer (**7**) melted sharply at 122–123°. The two pentols were not separable by paper chromatography or electrophoresis in a number of different systems, but sharp separations were readily achieved by g.l.c. of the trimethylsilyl ethers, and it was possible to detect as little as 1% of the one 3-epimer in admixture with the other. Substances **4** and **7** were found to be completely homogeneous by this procedure. This result indicates that the starting materials for their preparation (**3** and **6**, or **5** and **8**) were epimerically pure and that no epimerization had occurred during their conversion into the respective pentols

Since it could be argued that the pentols **4** and **7** might undergo epimerization at C-3 under acid hydrolytic conditions, by allylic-type assistance in the generation of a carbonium ion at C-3, an experiment was performed to determine the configurational stability and gross structural stability of the pentols to aqueous acid. The separate pentols **4** and **7** were heated in N sulfuric acid for 1 h at 100°. The recovered

*Compounds **4** and **7** are strictly named as 6-heptyne-D-*gulo*- and L-*manno*-1,2,3,4,5-pentol, respectively. In this paper, and in the preceding one[2], the alkyne terminus is considered to be C-1, so that configurational relationships are readily apparent between the acetylenic derivatives, their precursors, and their degradation products.

products, analyzed by g.l.c. of their trimethylsilyl ethers, were epimerically pure and essentially unchanged. No epimerization at C-3 was noted, even after longer times of treatment. These observations, together with the conversions used in the preparation of **4** and **7** from **5** and **8**, are of consequence in connection with the use of acetylenic sugar



derivatives in synthesis; they indicate that the acetylenic alcohol system in such structures as **4** and **7** is stable to conditions of glycoside hydrolysis, hydrolysis of *O*-isopropylidene groups, and the Zemplén deacylation procedure.

2,3,4,5-Tetra-*O*-benzoyl-*aldehydo*-L-arabinose (**1**) was prepared from 2,3,4,5-tetra-*O*-benzoyl-L-arabinose diethyl dithioacetal by way of 1,1-di-*O*-acetyl-2,3,4,5-tetra-*O*-benzoyl-*aldehydo*-L-arabinose aldehydrol, essentially by the procedure of Zinner and coworkers[3,4] for the D-enantiomorph. N.m.r. spectral data for **1** and its precursors are recorded in the Experimental section. Ethynylation of the *aldehydo* sugar derivative **1** gave, in high yield, the mixed, 3-epimeric, ethynylated products (**2**). Benzoylation of this mixture gave a high yield of a mixture of the pentabenzoates **3** and **6**, in a ratio of about 1:2. Separation of this mixture was difficult, and isolation of pure samples of the 3-epimeric pentabenzoates **3** and **6** was achieved only at the expense of yield. Preliminary fractionation by silicate extrusion-chromatography, followed by fractional recrystallization, gave pure samples of the pentabenzoates **3** and **6** that were identical in all respects with the corresponding samples prepared for **5** and **8**, and saponification of the products gave the respective pentols **4** and **7**, identical with the authentic products.

Since fully acylated *aldehydo* sugars are readily prepared from aldose dialkyl

dithioacetals, the results of the present study increase the potential of the ethynylation reaction for synthesis in the carbohydrate field.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover melting-point apparatus (Arthur H. Thomas Co., Philadelphia, Pennsylvania) and are uncorrected. Specific rotations were determined in a 2-dm polarimeter tube. I.r. spectra were measured with a Perkin–Elmer Model 137 "Infracord" infrared spectrometer. N.m.r. spectra were measured at 60 MHz with a Varian A-60 n.m.r. spectrometer. Chemical shifts are given on the τ scale, and spectra were measured at *ca.* 40° with solutions (5–15%) in chloroform-*d*, with tetramethylsilane (τ 10.00) as the internal standard. Microanalyses were determined by W.N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. The camera diameter was 114.59 mm. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities. Paper chromatography was performed on Whatman No. 1 paper by downward irrigation with either (A) 3:1:1 butyl alcohol–ethanol–water, or (B) 5:5:3:1 pyridine–ethyl acetate–water–acetic acid. Paper electrophoresis was performed on Whatman No. 3 paper by the enclosed-strip technique[5] with either borate buffer[6] (pH 9.2) or molybdate buffer[7](pH 5). Components were detected with alkaline silver nitrate[8]. T.l.c. was performed with Silica Gel G (E. Merck, Darmstadt, Germany) activated at 110° as the adsorbent, 99:1 benzene–methanol as the developer, and sulfuric acid as the indicator. G.l.c. analyses were made with an Aerograph Autoprep Model 705 gas chromatograph (Wilkens Instrument and Research, Inc., Walnut Creek, California) equipped with a flame-ionization detector. Nitrogen was used as the carrier gas.

1-Heptyne-L-gluco-*3,4,5,6,7-pentol* (**4**). — A solution of 3-*O*-(3,5-dinitrobenzoyl)-4,5:6,7-di-*O*-isopropylidene-1-heptyne-L-*gluco*-3,4,5,6,7-pentol[2] (**5**, 163 mg) in 60% aqueous acetic acid (7 ml) was heated for 1.5 h at 100°. The solution was evaporated, and toluene was twice added to and evaporated from the residue, to yield 3-*O*-(3,5-dinitrobenzoyl)-1-heptyne-L-*gluco*-3,4,5,6,7-pentol as a white solid. To a suspension of the latter in dry methanol (5 ml) was added a catalytic amount of sodium methoxide in dry methanol (5 ml). Complete dissolution was achieved rapidly. The solution was kept for 2.5 h at room temperature, neutralized with solid carbon dioxide, and evaporated to dryness, and the residue was stirred with water (15 ml) for 15 min at room temperature. The resultant suspension was washed with two 25-ml portions of chloroform, and the combined chloroform solutions were extracted once with water (10 ml). The aqueous layers were combined, and stirred with a large excess of Amberlite IRC-50 (H^+) cation-exchange resin for 15 min. Evaporation of the solution gave a colorless, chromatographically homogeneous syrup (papergram), in essentially quantitative yield, which solidified on trituration with acetone. Recrystallization from acetone–ethanol gave the pentol **3** as fine granules, yield 33 mg (49%), m.p. 114–116°; R_F 0.30, R_G 1.59

(solvent A), R_G 1.49 (solvent B), M_G 0.85 (borate), $M_{Mannitol}$ 1.07 (molybdate); X-ray powder diffraction data : 7.95 m, 5.27 w, 4.96 m, 4.80 s (2,2), 4,26 s (3,3), 3.98 w, 3.66 s (3,3), 3.58 w, 3.46 w, 3.24 s, (2,2), 2.96 m, 2.66 w, 2.54 vw, 2.46 w, 2.36 s (1), 2.24 vw, and 2.13 w.

Anal. Calc. for $C_7H_{12}O_5$: C, 47.72; H, 6.87. Found: C, 47.75; H, 7.11.

1-Heptyne-L-manno-*3,4,5,6,7-pentol* (7). — 3-*O*-(3,5-Dinitrobenzoyl)-4,5,6,7-di-*O*-isopropylidene-1-heptyne-L-*manno*-3,4,5,6,7-pentol (8, 367 mg) was heated with aqueous acetic acid to give 3-*O*-(3,5-dinitrobenzoyl)-1-heptyne-L-*manno*-3,4,5,6,7-pentol, and the latter was saponified by the general procedure described for the L-*gluco* epimer. The resultant pentol (7), obtained as a chromatographically homogeneous syrup (papergram), yield 140 mg (97%), solidified on trituration with acetone. Recrystallization from acetone gave 7 as fine granules, yield 50 mg (35%). A second recrystallization from acetone gave 38 mg of 7, m.p. 122–123°; R_F 0.32, R_G 1.67 (solvent A), R_G 1.58 (solvent B), M_G 0.85 (borate), $M_{Mannitol}$ 1.07 (molybdate); λ_{max}^{KBr} ~2.9 (OH), 3.07 (C≡CH), 4.74 μ (C≡C); X-ray powder diffraction data: 8.78 m (4), 6.61 vs (1), 5.04 w, 4.83 w, 4.65 m, 4.40 s (2,2), 4.32 s (2,2), 4.03 m, 3.73 w, 3.49 w, 3.41 m, 3.23 w, 3.10 m, 2.99 vw, 2.88 w, 2.73 s (3), 2.62 w, 2.49 m, 2.40 w, 2.33 vw, and 2.21 m.

Anal. Calc. for $C_7H_{12}O_5$: C, 47.72; H, 6.87. Found : C, 47.41; H, 6.87.

The L-*manno* pentol 7 and the L-*gluco* pentol 4 in admixture were not clearly distinguishable by paper chromatography, either in solvent A or in solvent B. No separation was effected by electrophoresis in borate or molybdate buffers. The homogeneity and epimeric purity of the pentols 4 and 7 was verified by g.l.c. of their trimethylsilyl ethers, in which system a clear separation was achieved.

Gas–liquid chromatography of the trimethylsilyl ethers of 1-heptyne-L-gluco (*and* L-manno)-*3,4,5,6,7-pentol* (4 *and* 7). — Trimethylsilyl ethers were prepared by shaking the sample with 10–20 times its weight of a mixture of chlorotrimethylsilane and hexamethyldisilazane in pyridine (TRI-SIL, Pierce Chemical Company, Rockford, Illinois). The prepared samples were kept for at least 5 min before injection onto the column. A stainless-steel column (10 ft × 1/4 in) packed with 5% STAP stationary phase (Varian Aerograph, Walnut Creek, California) on Chromosorb W (AW-DMCS) (Varian Aerograph, Walnut Creek, California) was used, with nitrogen as the carrier gas. At temperatures in the range of 140–150°, with a carrier-gas flowrate in the range of 90–120 ml/min, the trimethylsilyl ether of the L-*manno* pentol 7 emerged as a single, sharp peak having a retention time in the range of 6.3–9.6 min. Under identical conditions, the trimethylsilyl ether of the L-*gluco* pentol 4 emerged approximately 1 min later than the L-*manno* derivative, and likewise gave a sharp, single peak. Analysis of prepared mixtures of 4 and 7, with integration of g.l.c. peak areas, indicated that quantitative determination of 4 and 7 in admixture could be achieved with a precision of ±1% or less.

Action of dilute acid on the pentols 4 *and* 7. — A solution of the L-*manno* pentol 7 (1 mg) was heated with N sulfuric acid (1 ml) for 1 h at 100°. The solution was neutralized with barium carbonate, and evaporated, and the product was trimethylsilylated.

G.l.c. revealed the presence of a single component having the retention time of the trimethylsilyl ether of **7**, and no component corresponding to the trimethylsilyl ether of **4** was detected.

The L-*gluco* pentol **4** was treated similarly with sulfuric acid. G.l.c. of the trimethylsilylated product revealed a major component having the same retention time as the trimethylsilyl ether of **4**, together with a minor component (~5%) having approximately twice the retention time. No component having the retention time of the trimethylsilyl derivative of **7** was detected.

No interconversion of the pentols **4** and **7** was observed, even after treatment with N sulfuric acid for 12 h at 100°.

3,4,5,6,7-Penta-O-*benzoyl-1-heptyne*-L-gluco-*3,4,5,6,7-pentol* (**3**). — To a solution of **4** (21.4 mg) in dry pyridine (3 ml) was added benzoyl chloride (0.10 ml), and the solution was stirred for 9 h at room temperature. A few drops of water were added, and after 15 min, the solution was poured into saturated aqueous sodium hydrogen carbonate (50 ml). The oily solution was extracted portionwise with a total of 80 ml of chloroform, and the combined extracts were washed with water (30 ml). The dried (magnesium sulfate) organic layer was evaporated to dryness, and toluene was twice added to, and evaporated from, the residue. The resulting syrup crystallized on trituration with ethanol, yield 52 mg (61%). Recrystallization from ethanol gave pure **3** as spherical clusters, yield 39 mg (46%), m.p. 122.5–123.5°, $[\alpha]_D^{18}$ −47.4 ±3° (*c* 0.56, chloroform); R_F 0.71 (t.l.c.); λ_{max}^{KBr} 3.15 (C≡CH), 4.78 (C≡C), 5.81 μ (benzoate); n.m.r. data: τ 1.69–2.25 (broad double peak) and 2.25–2.94 (broad multiplet) (25 protons, aryl), τ 3.43–3.63 (1-proton multiplet) and 3.72–4.23 (3-proton multiplet) (H-3, 4,5,6), τ 5.06 (1-proton quartet, $J_{6,7}$ 3.5 Hz, $J_{7,7'}$ 12.5 Hz, H-7), τ 5.40 (1-proton, quartet, $J_{6,7'}$ 5.5 Hz, H-7'), τ 7.40 (1-proton doublet, $J_{1,3}$ 1.2 Hz, H-1); X-ray powder diffraction data: 10.26 m, 9.32 w, 8.36 m, 7.36 vw, 6.62 m, 6.33 w, 6.03 w, 5.44 w, 5.19 m, 4.85 m, 4.39 vs (1), 4.22 s (2), 4.09 w, 3.93 s (3,3), 3.82 vvw, 3.71 vw, 3.58 w, 3.44 w, 3.28 s (3,3), and 3.18 s (3,3).

Anal. Calc. for $C_{42}H_{32}O_{10}$: C, 72.40; H, 4.62. Found: C, 72.42; H, 4.41.

3,4,5,6,7-Penta-O-*benzoyl-1-heptyne*-L-manno-*3,4,5,6,7-pentol* (**6**). — The L-*manno* pentol **7** (29 mg) was benzoylated by the procedure described for the L-*gluco* epimer **4**. The product **6** was obtained as a syrup that, on trituration with ethanol, crystallized as spherical clusters. Recrystallization from ethanol gave pure **6**, yield 52 mg (46%), m.p. 131–132.5°, $[\alpha]_D^{18}$ −8 ±4° (*c* 0.8, chloroform); R_F 0.72 (t.l.c.); λ_{max}^{KBr} 3.07 (C≡CH), 4.72 (C≡C), 5.80 μ (benzoate); n.m.r. data: τ 1.67–2.32 (broad single peak) and τ 2.32–3.00 (broad multiplet) (25 protons, aryl), τ 3.60–3.78 (1-proton multiplet) and 3.80–4.30 (3-proton multiplet) (H-3,4,5,6), τ 5.10 (1-proton quartet, $J_{6,7}$ 3.5 Hz, $J_{7,7'}$ 12.0 Hz, H-7), τ 5.47 (1-proton quartet, $J_{6,7'}$ 5.5 Hz, H-7'), τ 7.51 (1-proton doublet, $J_{1,3}$ ~1 Hz, H-1); X-ray powder diffraction data: 14.67 w, 9.19 s (2), 7.58 m, 6.78 vw, 5.81 m, 5.34 w, 4.90 vs (1), 4.71 s (3,3), 4.38 w, 4.09 m, 3.93 vw, 3.77 s (3,3), 3.45 vw, 3.29 vw, and 3.13 m.

Anal. Calc. for $C_{42}H_{32}O_{10}$: C, 72.40; H, 4.62. Found: C, 71.97; H, 4.64.

The n.m.r. spectra of **3** and **6** showed differences in the shape of the H-3,4,5,6

multiplets, in addition to the difference in appearance of the lower-field group of aryl-proton signals, but first-order analysis of the H-3,4,5,6 multiplets was not possible at 60 MHz.

2,3,4,5-Tetra-O-benzoyl-L-arabinose diethyl dithioacetal. — L-Arabinose diethyl dithioacetal[9] (30 g) in pyridine (300 ml) was treated with benzoyl chloride (80 ml) in pyridine (100 ml) at −10 to 0°. After 13 h at 0°, the solution was poured into 3.5 l of ice and water. After 14 h, the syrupy product was purified, and crystallized from methanol, as described[3] for the D enantiomorph, yield 65 g (83%), m.p. 67–69° (lit.[3] m.p. 69.5° for the D enantiomorph); λ_{max}^{KBr} 5.81 (OBz), 6.23, 6.32, 6.73, 6.92 μ (aryl); n.m.r. data: τ 1.80–2.10, 2.45–2.75 (multiplets, 20 protons, Ph), τ 3.51 (1-proton quartet, $J_{2,3}$ 3.8 Hz, $J_{3,4}$ 6.0 Hz, H-3), τ 3.92–4.20 (2-proton multiplet, H-2, H-4), τ 5.03 (1-proton quartet, $J_{4,5}$ 3.5 Hz, $J_{5,5'}$ 12 Hz, H-5), τ 5.38 (1-proton quartet, $J_{4,5'}$ 6.5 Hz, H-5'), τ 5.68 (1-proton doublet, $J_{1,2}$ 6.9 Hz, H-1), τ 3.28 (4-proton quartet, J 7.5 Hz, CH_2 of Et), τ 8.81 (6-proton triplet, CH_3 of Et).

Anal. Calc. for $C_{37}H_{36}O_8S_2$: C, 66.05; H, 5.39; S, 9.53. Found: C, 65.95; H, 5.25; S, 9.27.

The first-order $J_{1,2}$, $J_{4,5}$, and $J_{4,5'}$ couplings derived from the n.m.r. spectrum indicated that the quartet at τ 3.51 was not the H-2 or the H-4 signal. The observed couplings of hydrogen atoms on the chain were in agreement with a planar, zig-zag backbone of carbon atoms (largest groups antiparallel) as the favored conformation (most populated rotamer state), as has been noted[10] with other acyclic sugar derivatives. The occurrence of the H-1 signal at higher field than the signals of all other hydrogen atoms on the carbon chain is noteworthy.

1,1-Di-O-acetyl-2,3,4,5-tetra-O-benzoyl-aldehydo-L-*arabinose aldehydrol.* — 2,3,4,5-Tetra-*O*-benzoyl-L-arabinose diethyl dithioacetal (35 g) was dissolved in a mixture of acetic anhydride (400 ml) and sulfuric acid (20 ml) that had been cooled to −10°. The reaction mixture was treated as described by Zinner and co-workers[4] for the D enantiomorph. Crystallization from methanol gave the product, yield 22.1 g (63%), m.p. 128.5–129.5° (lit.[4] m.p. 130° for the D enantiomorph); λ_{max}^{KBr} 5.65 (OAc), 5.77 (OBz), 6.23, 6.32, 6.72, 6.91 μ (aryl); n.m.r. data: τ 1.81–2.15, 2.31–2.67 (20 protons, aryl), τ 2.80 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1), τ 3.73–4.30 (3-proton multiplet, H-2,3,4), τ 5.10 (1-proton quartet, $J_{4,5}$ 3Hz, $J_{5,5'}$ 12.5 Hz, H-5) τ 5.47 (1-proton quartet, $J_{4,5'}$ 6 Hz, H-5'), τ 7.97, 8.09 (3-proton singlets, OAc).

Anal. Calc. for $C_{37}H_{32}O_{12}$: C, 66.46; H, 4.82. Found: C, 66.67; H, 4.89.

2,3,4,5-Tetra-O-benzoyl-aldehydo-L-*arabinose* (**1**). — A solution of 1,1-di-*O*-acetyl-2,3,4,5-tetra-*O*-benzoyl-*aldehydo*-L-arabinose aldehydrol (22.1 g) in a mixture of *p*-dioxane (600 ml) and 15% perchloric acid (150 ml) was heated for 2 h at 100°, according to the general procedure of Zinner and co-workers[4]. The solution was poured into ice and water (1.5 l), and the product was extracted with dichloromethane. The extract was washed 3 times with water, dried (magnesium sulfate), and evaporated. The product was dried under vacuum for 24 h at 78° to give **1** as an amorphous solid, yield 13.65 g (73%), λ_{max}^{KBr} 5.82 (CHO, OBz), 6.29, 6.77, 6.95 μ (aryl); n.m.r. data: τ 0.30 (1-proton singlet, H-1), τ 1.82–2.12, 2.38–2.72 (20 protons, aryl), τ 3.78–4.23

(3-proton multiplet, H-2,3,4), τ 5.03 (1-proton quartet, $J_{4,5}$ ~3 Hz, $J_{5,5'}$ 12 Hz, H-5), τ 5.38 (1-proton quartet, $J_{4,5'}$ ~5 Hz).

Anal. Calc. for $C_{32}H_{26}O_9$: C, 69.96; H, 4.63. Found: C, 69.39; H, 4.76.

The product gave a positive Schiff test. In common with other (saturated) *aldehydo* sugars that have been examined[1,11], the signal of the aldehyde proton is observed as a singlet, indicating that $J_{1,2}$ is ~0 Hz.

*3,4,5,6,7-Penta-*O*-benzoyl-1-heptyne-*L-gluco *(and* L-manno*)-3,4,5,6,7-pentol* (**3** *and* **6**) *by ethynylation of 2,3,4,5-tetra-*O*-benzoyl-*aldehydo-L-*arabinose* (**1**). — The freshly prepared *aldehydo* sugar **1** (12.85 g) was treated with ethynylmagnesium bromide (prepared from 1 g of magnesium), essentially by the procedure described previously[2], except that addition of **1** (in 200 ml of tetrahydrofuran) was rapid, to give syrupy 4,5,6,7-tetra-*O*-benzoyl-1-heptyne-L-*gluco* (and L-*manno*)-3,4,5,6,7-pentol (**2**), yield 11.2 g (83%), λ_{max}^{film} 2.92 (OH), 3.07 (C≡CH), 4.72 (C≡C), ~5.8 μ (benzoate). This product (2.1 g) was treated with benzoyl chloride (2.1 ml) and pyridine, according to the general procedure used for the benzoylation of the pentols **4** and **7**. The product (**3** and **6**) was obtained as a dark syrup, yield 2.1 g (85%); λ_{max}^{film} 3.09 (C≡CH), 4.73 (C≡C), ~5.8 μ (benzoate), OH absent. The n.m.r. spectrum of the crude product indicated that it contained **3** and **6** in the ratio of approximately 1:2. T.l.c. indicated a principal component having R_F ~0.7 (**3** and **6**), together with 7 minor or trace components.

Separation of the mixed, benzoylated pentols **3** *and* **6**. — A sample (2.35 g) of the crude mixture from the preceding experiment was dissolved in benzene, and applied to a column (25 × 7.5 cm) of 1:1 Magnesol–Celite*. The column was developed with 1:100 *tert*-butyl alcohol–benzene (850 ml), and extruded by the procedure of Thompson[12], and zones were detected by streaking with 1% potassium permanganate in 10% aqueous sodium hydroxide. A major zone, centered 13 cm from the origin of the chromatogram, was detected. The portion of the column that was 12–14 cm from the origin was excised, and extracted with acetone, and the extract was evaporated, to give a colorless syrup, yield 1.15 g. A solution of the latter in ethanol (~20 ml) crystallized after 2 days at room temperature, yield 465 mg, m.p. 129–138°. Recrystallization of this product from ethanol gave the pure L-*manno* derivative **6**, m.p. 132–133°. This product was indistinguishable from **6**, prepared through the route **8**→**7**→**6**, by mixed m.p., X-ray powder diffraction pattern, elemental analysis, specific rotation, and comparative i.r. and n.m.r. spectra.

The mother liquors from the crystallization of **6** were refrigerated for 3 weeks. A second crop of crystals, yield 311 mg, m.p. 114–122°, was obtained. This product was recrystallized three times from ethanol, to give 133 mg of the pure L-*gluco* derivative **3**, m.p. 122.5–123.5°. The product was indistinguishable from **3**, prepared by the route **5**→**4**→**3**, by mixed m.p., X-ray powder diffraction pattern, elemental analysis, specific rotation, and comparative i.r. and n.m.r. spectra.

*Magnesol was obtained from the Westvaco Chemical Division of the Food, Machinery, and Chemical Corporation, South Charleston, West Virginia; Celite 545 was obtained from the Johns-Manville Co., New York, N. Y.

Conversion of the benzoylated pentols **3** *and* **6** *into the free pentols* **4** *and* **7**. — The benzoylated, L-*manno* pentol **6** (150 mg), obtained by separation of the mixture of **3** and **6**, was dissolved in dry methanol (5 ml), and a catalytic amount of sodium methoxide in dry methanol (5 ml) was added. After 2.5 h, the saponified product was isolated as described in the preparation of **7** from **8**. The syrupy, chromatographically homogeneous product **6**, yield 43 mg (80%), was crystallized from acetone to give **6** as fine granules, m.p. 122–122.5°. The product was indistinguishable, by X-ray powder diffraction pattern, from **6** prepared from **7**, and g.l.c. of the trimethylsilyl ether of the product showed a single peak having the same retention time as that of a reference sample.

Saponification of the benzoylated L-*gluco* pentol **3** (10 mg) from the epimeric mixture, by the procedure used for **6**, gave the L-*gluco* pentol **4**, indistinguishable by papergram, and by g.l.c. of the trimethylsilyl ether, from an authentic sample of **4** prepared from **5**.

SUMMARY

Ethynylation of 2,3,4,5-tetra-*O*-benzoyl-*aldehydo*-L-arabinose (**1**) gave a product (**2**), which was converted by benzoylation into a separable mixture of 3,4,5,6,7-penta-*O*-benzoyl-1-heptyne-L-*gluco*-3,4,5,6,7-pentol (**3**) and the L-*manno* epimer (**6**). Saponification of **3** and **6** gave 1-heptyne-L-*gluco*-3,4,5,6,7-pentol (**4**) and the L-*manno* epimer (**7**). The structures assigned to **3** and **4**, and to **6** and **7**, were verified by conversion of the known 3-*O*-(3,5-dinitrobenzoyl)-4,5:6,7-di-*O*-isopropylidene-1-heptyne-L-*gluco*-3,4,5,6,7-pentol (**5**) into **4** and thence into **3**, and by conversion of **8**, the known L-*manno* epimer of **5**, into **7** and thence into **6**. The 1-heptyne-3,4,5,6,7-pentols **4** and **7**, obtained crystalline, were separable by g.l.c. as their trimethylsilyl ethers, and were not decomposed or epimerized by treatment with N sulfuric acid for 1 h at 100°. The conversions demonstrate that ethynylation of a free aldehyde group in the presence of benzoyloxy groups is a feasible synthetic procedure for chain-extension reactions with carbohydrate derivatives. The terminal acetylene function on a polyhydroxyalkyl chain is shown to be stable under normal conditions of catalytic transesterification (Zemplén deacylation) and acid hydrolysis of acetals or glycosides.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF METHYL D-GALACTURONATE FROM D-GALACTOSE OR D-GALACTURONIC ACID: PREPARATION OF METHYL-^{14}C D-GALACTURONATE USING METHYL-^{14}C IODIDE

R. H. SHAH AND F. LOEWUS
Department of Biology, State University of New York at Buffalo, Buffalo, New York 14214 (U. S. A.)
(Received December 10th, 1966; in revised form, February 13th, 1967)

Although tracer studies have provided evidence for direct utilization of D-galacturonic acid (**1**) or its methyl ester (**2**) as galactosyluronic acid residues in pectic substance of detached ripening strawberries[1], they did not provide information on the fate of the methyl carbon of **2**. To investigate this aspect, a method was sought that would permit the synthesis of methyl-^{14}C-labeled **2** of high specific radioactivity. Preparative methods have been reported previously[2,3], but the procedures described did not meet the needs of a synthesis involving the use of a ^{14}C-labeled methylating agent where high radiochemical yield, efficient use of a ^{14}C-labeled reagent, and simple recovery of product were additional considerations.

This paper describes two synthetic routes to **2**, one from D-galactose *via* benzyl 2,3-di-*O*-benzyl-*β*-D-galactopyranosiduronic acid (**4**), the other from **1** *via* 1,2:3,4-di-*O*-benzylidene-D-galactopyranuronic acid (**7**). The sodium salt of **4** or **7**, dissolved in *N*,*N*-dimethylformamide, reacted smoothly with methyl iodide to form the corresponding methyl ester **6** or **9**. Hydrogenolysis of **6** or **9** gave **2** in good yield. When a slight excess of **5** was methylated with methyl-^{14}C iodide, the final product, methyl-^{14}C-labeled **2**, accounted for nearly all ^{14}C present and had a specific radioactivity equivalent to that of the methylating agent.

RESULTS AND DISCUSSION

In the first synthetic sequence, benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranoside (**3**)[4-8] was oxidized with oxygen in the presence of platinum-on-carbon[9] to give the uronic acid derivative **4**. Reaction of the sodium salt **5** of **4** with 30% excess of methyl iodide in *N*,*N*-dimethylformamide for 7 h at 75° gave the methyl ester derivative **6** as a syrup in 93% yield. The infrared spectrum showed peaks at 3550 cm^{-1} for a hydroxyl group and at 1725 cm^{-1} for an ester carbonyl group. In one experiment, the syrup crystallized from chloroform–hexane as a waxy solid that had a carbon, hydrogen, and methoxyl content corresponding to the expected structure **6**. Benzyl groups were removed from **6** by hydrogenolysis at room temperature and atmospheric pressure in the presence of palladium-on-carbon catalyst. The product, **2**, was obtained as a syrup, which showed on paper chromatography in benzene–butyl alcohol–pyridine–water (1:5:5:3, v/v) a single spot (R_G 1.52) and on thin-layer chromatography on cellulose a single spot corresponding to that of authentic methyl D-galacturonate[3] (**2**).

In the preparation of methyl-^{14}C-labeled **2**, a 20% excess of **5** over that of methyl-^{14}C iodide was employed to insure quantitative conversion of the labeled reagent into ester. Otherwise, reaction conditions were similar to those used in experiments with unlabeled methyl iodide. Thin-layer chromatography of the labeled product **2** showed a major radioactive spot which corresponded to that of authentic **2**. In addition, a second, minor, radioactive spot that moved faster than **2** was detected. This product had not been detected in runs made with unlabeled methyl iodide. The nature of the labeled methyl group in this minor constituent appeared to be similar to that in **2**, since treatment of the developed thin-layer plate with ammonia vapor removed all radioactivity from both spots. The possibility exists that dehydration occurred through elimination of water from C-4–C-5 during methylation. Further efforts are under way to determine the chemical nature of this minor product.

An alternate sequence for the synthesis of **2** from **1** was also investigated with unlabeled methyl iodide and shown to be a practical method for the preparation of methyl-^{14}C-labeled **2** starting from the uronic acid. Zinner and Thielebeule[10] have reported a synthesis of 1,2:3,4-di-*O*-benzylidene-D-galactopyranuronic acid (**7**) for which they listed a melting point of 212°. In the synthesis reported in this paper, D-galacturonic acid monohydrate was employed instead of anhydrous[10] **1**. The infrared spectrum of the product showed peaks for aromatic groups and a carbonyl. It had a broad melting point range of 190° to 200° that could not be sharpened by additional crystallization. When converted into its sodium salt (**8**) with one equivalent of sodium hydrogen carbonate, there was a shift in the infrared spectrum from 1725 cm^{-1} to 1620 cm^{-1}. Methylation with 2 equivalents of methyl iodide in *N,N*-dimethylformamide gave the methyl ester (**9**) in near quantitative yield. The presence of two carbonyl peaks in the infrared, one near 1740 cm^{-1}, the other near 1760 cm^{-1}, suggested that more than one isomer of **9** were present. Theoretically, four isomers of **7**, **8**, and **9** are possible, due to two asymmetric centers created in the formation of the dibenzylidene derivative **7**. In the course of one run, syrupy methyl ester **9** partially crystallized from methanol–petroleum ether. The crystalline product gave correct elemental analysis for **9**; it had a melting point of 148–150° and its specific rotation was −126°. Apparently the crystalline ester **9** described here is different from the one prepared by Zinner and Thielebeule[10], who reported m.p. 165° and $[\alpha]_D^{18}$ −168°. Following removal of this crystalline ester, the mother liquors were evaporated to a syrup. Attempts to recover additional crystals from this syrup were unsuccessful; however, after purification by chromatography on silica gel, a syrup was recovered that corresponded to **9**.

Gas chromatography of the crystalline ester gave a single peak from a six foot column packed with silicone polymer-coated particles. The syrup from which this crystalline ester was recovered gave four peaks; based on the retention time of hexakis-*O*-trimethylsilyl-*myo*-inositol as unity, their retention times were 1.78, 1.82, 1.88, and 1.92. Addition of a small amount of crystalline **9** to the syrup enhanced the first peak.

Hydrogenolysis of **9** in the presence of palladium-on-carbon catalyst gave syrupy ester **2** which showed, by t.l.c. on cellulose, a single spot having the same R_F value as

authentic methyl D-galacturonate[3]. The syrupy **2** was crystallized from methanol–dioxane. The melting point (141–144°) and the specific rotation, +86.2° (10 minutes) → +30.5° (equilibrium), agreed well with the reported values[2,3]. Thin-layer chromatography gave a major spot corresponding to authentic **2**. In addition, a faint spot moving slightly ahead of **2** was also observed.

EXPERIMENTAL

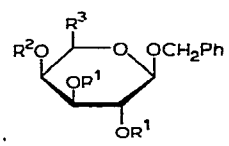
All melting points were determined on a Fisher–Johns melting-point apparatus. Melting points reported in this paper have been corrected. Infrared spectra were determined on a Perkin–Elmer Model 137 "Infracord" spectrophotometer. Optical rotations were determined with a Perkin–Elmer Model 141 Polarimeter. Thin-layer chromatography was performed on cellulose powder MN-300 from Brinkmann Instruments, Inc. Unless otherwise noted, thin-layer plates were developed in butyl alcohol–acetic acid–water (3:1:1, v/v) and sprayed with silver nitrate in acetone followed by potassium hydroxide in methanol[11]. Radioactive areas on the plates were detected with a Packard Model 7201 radiochromatogram scanner. Column chromatography was performed with silica gel, grade 923 or 950, from Will Scientific Corp. Methyl-^{14}C iodide was purchased from New England Nuclear Corp. Elementary analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. For gas chromatography a Packard gas chromatograph, Model 7821, equipped with a flame ionization detector and temperature programmer was used. Separations were made on a six foot, coiled, glass column packed with 3% silicone polymer, JXR, on Gas Chrom Q, 100–200 mesh (Applied Science Labs., State College, Pa.). Solutions of the samples in chloroform were injected into a stream of nitrogen (60 cc per min) and programmed from 140 to 240° at 3° per min. *myo*-Inositol was injected as a solution of its hexakis-*O*-trimethylsilyl derivative[12] in heptane.

*Benzyl 2,3-di-*O*-benzyl-β-*D*-galactopyranosiduronic acid* (**4**). — A modification of a method previously used to oxidize benzyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-glucopyranoside[9] was employed. To a suspension of benzyl 2,3-di-*O*-benzyl-β-D-galactopyranoside[4–8] (**3**) (1.9 g) and 10% platinum-on-carbon catalyst (1.7 g) in water (275 ml) was added 2.5% aqueous sodium hydrogen carbonate (5 ml). A continuous stream of oxygen (35 l/h) was passed through the suspension for 10 h, while the temperature was maintained at 95°. Additional portions of 2.5% aqueous sodium hydrogen carbonate were added as necessary to maintain the pH between 7–8 (total, 20.9 ml). After 4.5 h, a second portion of platinum-on-carbon catalyst (1.7 g) was added. After 10 h, no further change in pH was observed, and the suspension was filtered through Hyflo Super Cel and washed with water. The combined filtrates were evaporated to dryness, and the residue was extracted with chloroform. After the chloroform extract had been evaporated to dryness under reduced pressure, the residue was dissolved in acetone (100 ml) and water (50 ml), and adjusted to pH 2 with N HCl. Further removal of the solvent, extraction with acetone, and evaporation to a syrup at reduced pressure gave a product that crystallized from chloroform–heptane;

yield, 1.33 g (67%); m.p. 139–144°. Two recrystallizations from the same solvent gave 0.87 g (44%), m.p. 150–152°; $[\alpha]_D^{25}$ −33.2° (*c* 0.72, chloroform); ν_{max}^{KBr}: 3700 (weak, OH), 1720 (C=O), 1500 (weak), 1450, 750, 730, and 690 (aromatic) cm^{-1}.

Anal. Calc. for $C_{27}H_{28}O_7$: C, 69.82; H, 6.08. Found: C, 69.56; H, 5.99.

Sodium (benzyl 2,3-di-O-benzyl-β-D-galactopyranosid)uronate (**5**). — To a solution of **4** (0.107 g, 0.23 mmoles) in acetone (5 ml) was added powdered sodium hydrogen carbonate (0.019 g, 0.23 mmoles) and water (5 ml). After 1 h at 25°, the clear solution was evaporated and dried by successive evaporations with acetone (10 ml), acetone–benzene (1:1 v/v; 10 ml), and benzene (10 ml). The residue was triturated with heptane and filtered to give **5**, yield 0.097 g (87%); $[\alpha]_D^{25}$ −16.3° (*c* 0.75, chloroform); ν_{max}^{KBr}: 3500 (OH), 1610 (COO^-), 1490, 1440, 725, and 690 (aromatic) cm^{-1}.



(3) R^1 = CH_2Ph; R^2 = H; R^3 = CH_2OH
(4) R^1 = CH_2Ph; R^2 = H; R^3 = COOH
(5) R^1 = CH_2Ph; R^2 = H; R^3 = COONa
(6) R^1 = CH_2Ph; R^2 = H; R^3 = COOMe

Methyl (benzyl 2,3-di-O-benzyl-β-D-galactopyranosid)uronate (**6**). — Methyl iodide (20 µl; 31% excess) was added to a solution of **5** (0.119 g; 0.25 mmole) in *N,N*-dimethylformamide (0.25 ml). This solution was heated for 7 h at 75°. The solvent was removed by evaporation with xylene (2 × 3 ml). The residue was extracted with chloroform (3 ml), and the extract was added to a column (1 cm diameter) of silica gel (10 g) packed with chloroform. The column was developed with chloroform. Compound **6** appeared in fractions eluted between 100–140 ml. Removal of chloroform from these fractions gave a syrup; yield, 0.109 g (93%); ν_{max}^{film}: 3550 (OH), 1725 (C=O), 1490, 1440, 1430, 740, 725, and 685 (aromatic) cm^{-1}. In a similar run, the syrup crystallized from chloroform–hexane to give a waxy solid, m.p. 110–111°; $[\alpha]_D^{25}$ −28.4° (*c* 0.63, chloroform).

Anal. Calc. for $C_{28}H_{30}O_7$: C, 70.28; H, 6.32; OCH_3, 6.49. Found: C, 69.80; H, 6.27; OCH_3, 6.87.

For the synthesis of methyl-^{14}C labeled **6**, a 20% excess of **5** was employed. Methyl-^{14}C iodide (0.2 mmole) had 5 mc per mmole of ^{14}C. Radiochemical conversion into **6** was quantitative.

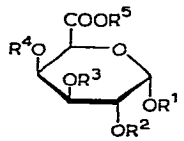
1,2:3,4-Di-O-benzylidene-D-galactopyranuronic acid (**7**)[10]. — **1**-Monohydrate was converted into **7**, using the procedure of Zinner and Thielebeule[10], in 33% yield; m.p. 199–201° (mainly at 198–201°) [lit.[10] m.p. 212°]; ν_{max}^{KBr} 3000–2500 (dimeric COOH), 1725 (C=O), 750 and 690 (aromatic) cm^{-1}.

Anal. Calc. for $C_{20}H_{18}O_7$: C, 64.85; H, 4.90. Found: C, 64.66; H, 5.03.

Sodium 1,2:3,4-di-O-benzylidene-D-galactopyranuronate (**8**). — Sodium hydrogen carbonate (2.74 g, 32.6 mmoles) was added to a solution of **7** (12.08 g, 32.6 mmoles) in acetone (200 ml) and water (200 ml). After 13.5 h, the solvents were removed and the residue was dissolved in water (125 ml). A small amount of insoluble matter was removed by filtration and the filtrate was evaporated to dryness. The

salt was dried over phosphorus pentoxide for several h at 0.05 mm; yield, 10.5 g (82%) ν_{max}^{KBr}: 1620 (COO^-), 760 and 700 (aromatic) cm^{-1}.

Methyl 1,2:3,4-di-O-benzylidene-D-galactopyranuronate (**9**). — To a suspension of the sodium salt **8** (4.98 g, 12.7 mmoles) in *N,N*-dimethylformamide (25 ml) was added methyl iodide (1.58 ml; 25.4 mmoles); the dissolution of the salt was immediate. The solution was heated in a water bath for 3.5 h at 95°. The solvent was removed by evaporation at 60° under reduced pressure, and finally by evaporation with xylene (4 × 25 ml). The residue was extracted with chloroform (15 ml) and then the extract was poured onto a column (2 cm diameter) of silica gel (50 g) packed in chloroform. The column was eluted with chloroform and the fractions eluted between 50–200 ml were combined. Removal of the solvent afforded a syrup; yield, 4.60 g (94%); ν_{max}^{film}: 1760 and 1740 (C=O), 750 and 690 (aromatic) cm^{-1}.



(7) $R^1R^2 = R^3R^4 =$ >CHPh, $R^5 = H$
(8) $R^1R^2 = R^3R^4 =$ >CHPh, $R^5 = Na$
(9) $R^1R^2 = R^3R^4 =$ >CHPh, $R^5 = Me$

Crystallization of the syrup from methanol–petroleum ether gave colorless crystals (1.55 g, 32%), m.p. 143–146°. An analytical sample, m.p. 148–150° (lit.[10] m.p. 165°), was obtained in another experiment by recrystallization from methanol; $[\alpha]_D^{25}$ −126° (*c* 1.06, chloroform) {lit.[10] $[\alpha]_D^{18}$ −168° (chloroform)}; ν_{max}^{KBr} 1760 (C=O), 760 and 700 (aromatic) cm^{-1}.

Anal. Calc. for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24; OCH_3, 8.07. Found: C, 65.50; H, 5.21; OCH_3, 8.15.

A second crop of crystals, m.p. 110–125°, was obtained from the mother liquor; g.l.c. showed it to be a mixture of 4 isomers; yield, 0.65 g (13%).

The mother liquor, after the removal of the second crop, was evaporated to a syrup which could not be induced to crystallize further; yield, 2.07 g (43%). In another experiment, this syrup was chromatographed on silica gel to obtain the analytical sample; ν_{max}^{film} 1760 and 1740 (C=O), 760 and 700 (aromatic) cm^{-1}.

Anal. Calc. for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24; OCH_3, 8.07. Found: C, 65.37; H, 5.35; OCH_3, 8.30.

In gas chromatography, the crystalline isomer (m.p. 148–150°) gave rise to a single peak, while the syrupy product gave 4 peaks, the first of which corresponded to that of crystalline **9**.

Methyl D-galacturonate (**2**) *from methyl (benzyl 2,3-di-O-benzyl-β-D-galactopyranosid)uronate* (**6**). — A solution of **6** (0.109 g) in absolute ethanol (125 ml) was hydrogenated slightly above atmospheric pressure, in the presence of 10% palladium–on–carbon catalyst (150 mg) until hydrogen uptake ceased. After removal of the catalyst and the solvent, a pale yellow syrup (**2**) remained in nearly quantitative yield; ν_{max}^{film}: 3500 (OH), 1750 (ester C=O) cm^{-1}. Paper chromatography on Whatman no. 1

paper with benzene–butyl alcohol–pyridine–water (1:5:5:3, v/v) gave a single spot (R_G 1.52). Thin-layer chromatography also gave a single spot, corresponding to authentic[3] **2**. Compound **2** (3.3 mg) was saponified with 0.03N sodium hydroxide (0.7 ml) for 2 h at 45°, and then neutralized by passage through a column of Dowex-50 (H^+) ion-exchange resin. The column was flushed with water, and the combined eluates were evaporated to dryness. Thin-layer chromatography of the residue showed a single spot with an R_F corresponding to that of authentic **1**. The saponification product and **1** also had the same electrophoretic mobility on paper (0.1M formate buffer, pH 3.8, 2500 volts, 1.25 h, Whatman No. 1 paper).

When methyl-^{14}C labeled **6** (0.10 g) was hydrogenated under the conditions described above, the yield of labeled ester was quantitative. Thin-layer chromatography showed the presence of a major radioactive spot corresponding to **2** and of a minor radioactive spot near the solvent front. When the thin-layer plate was exposed to ammonia vapor for a brief period and then re-scanned for radioactivity, no ^{14}C was found to be present on the plate.

(*b*) *From methyl 1,2:3,4-di-*O*-benzylidene-*D*-galactopyranuronate* (**9**). — A solution of syrupy **9** (0.842 g), shown to be a mixture of four isomers by g.l.c., in absolute ethanol (100 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% palladium–on–carbon catalyst (400 mg) until hydrogen uptake stopped. The catalyst was removed by filtration through Celite. Removal of the solvent under reduced pressure gave a nearly colorless syrup (0.52 g) which showed a single spot, having the same R_F value as authentic **2**, by t.l.c. on cellulose; the saponification product was identical with authentic **1**.

The syrup was crystallized from methanol–dioxane (1:3.5, v/v). The colorless crystals were collected by filtration and washed successively with dioxane–anhydrous ether (1:1, v/v) and anhydrous ether; yield, 0.134 g (29.3%); m.p. 141–144° [Morell and Link[2] reported a m.p. of 146–148°]; $[\alpha]_D^{25}$ +86.2° (10 min)→+30.5° (equilibrium, 2 days; *c* 1.08, methanol) [lit.[2] $[\alpha]_D^{25}$ +75.5° (3 min) →+38° (equilibrium, 90 min; methanol). Jansen and Jang[3] reported $[\alpha]_D^{25}$ +94° (initial) →+34° (equilibrium, 3 days; methanol)]; ν_{max}^{KBr} 3450 (OH), 1750 (ester C=O) cm^{-1}.

An additional crop of crystals (0.098 g; 21.4%), m.p. 129–131°, was obtained from the mother liquor, by crystallization of the residue from methanol–dioxane–anhydrous ether (1:6:6, v/v). The infrared spectrum of the second crop was superimposable on that of the first crop.

A nearly colorless syrup was obtained by evaporation of the mother liquor, which could not be crystallized further. Thin-layer chromatography of each of the two crops of crystals and of the residual syrup revealed a major spot identical with that of authentic **2**; in each case, a trace of a constituent moving slightly faster than **2** was observed.

ACKNOWLEDGMENTS

The authors thank the National Institutes of General Medicine, National

Institutes of Health, United States Public Health Service for a Grant (No. GM 12422) in support of this work. The authors are indebted to Dr. M. Schwartz for use of a polarimeter.

SUMMARY

Methyl D-galacturonate was prepared by two different routes; one from D-galactose by way of benzyl 2,3-di-*O*-benzyl-*β*-D-galactopyranosiduronic acid, and the other from D-galacturonic acid *via* its 1,2:3,4-di-*O*-benzylidene derivative. The first of these methods was employed for the preparation of methyl-^{14}C D-galacturonate, the radiochemical yield being nearly quantitative. Ester formation was achieved by reaction of the sodium salt of either D-galacturonic acid derivative (**5** or **8**) with methyl iodide in *N*,*N*-dimethylformamide. Benzyl and benzylidene groups were removed from the esters (**6** and **9**) by hydrogenolysis in ethanol solution. Either of the above methods gave methyl D-galacturonate chromatographically indistinguishable from authentic ester **2**.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

A 3,4-EPIMINOPYRANOSIDE

A. D. BARFORD AND A. C. RICHARDSON
Department of Chemistry, The University, Reading (Great Britain)
(Received February 3rd, 1967)

INTRODUCTION

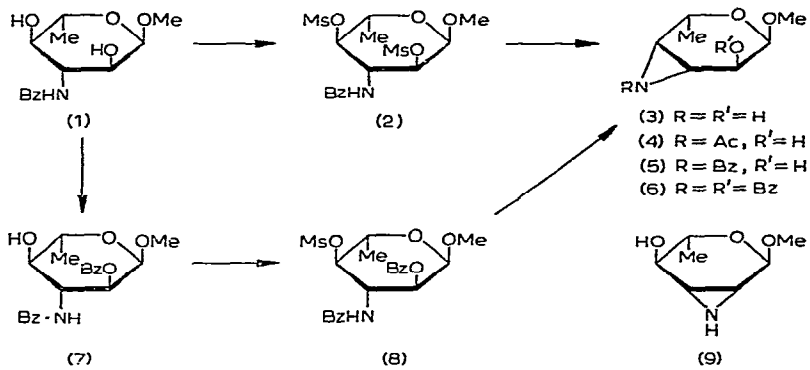
Carbohydrate epimines (aziridines) are of chemical interest because of their potential as synthetic intermediates. For example, they undergo ring-opening reactions with a variety of nucleophiles such as azide[1], chloride, bromide, and iodide[2] to give azido-amines and halo-amines, respectively. In contrast to the corresponding epoxides (oxiranes), they are exceptionally stable in strongly alkaline media and towards lithium aluminium hydride[3]. They may also be of pharmaceutical interest in cancer chemotherapy, since they may behave as *in vivo* alkylating agents.

The synthesis of 2,3-epiminopyranosides has been reported from methyl 4,6-*O*-benzylidene-D-hexopyranoside derivatives bearing a benzamido group *trans* to a methanesulphonyloxy substituent[3,4]. Treatment of such compounds with either lithium aluminium hydride or sodium methoxide gave the corresponding 2,3-epiminopyranoside, although oxazoline formation was a competing reaction when methoxide was used. Another method involves reduction of a compound containing an azide substituent situated *trans* to a vicinal sulphonyloxy group with either hydrazine–Raney nickel[5] or lithium aluminium hydride[6]. We have previously reported[3] that the method using reduction of benzamido-methanesulphonates with lithium aluminium hydride arose from an observation that treatment of methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside 2,4-dimethanesulphonate with this hydride gave an unexpected product suspected of being an epiminopyranoside. The object of this communication is to show that this supposition was correct and that the product was methyl 3,4,6-trideoxy-3,4-epimino-α-L-galactopyranoside (**3**).

RESULTS AND DISCUSSION

Methyl 3-amino-3,6-dideoxy-α-L-glucopyranoside[7] was converted into the *N*-benzoyl derivative (**1**) in high yield by treatment with benzoic anhydride in ethanol. Methanesulphonylation of **1** yielded the 2,4-dimethanesulphonate (**2**), which, upon reduction with lithium aluminium hydride in tetrahydrofuran, gave a weakly basic (pK_a 5.5), highly crystalline amine ($C_7H_{13}NO_3$). When treated with the appropriate acid anhydride in ethanol, the amine gave crystalline mono-*N*-benzoyl and mono-*N*-acetyl derivatives, both of which lacked N-H stretching or deformation absorptions (Amide II) in their infrared spectra, showing that the original amine was

secondary. In view of these results and those obtained from a study of epimine formation from methyl 4,6-*O*-benzylidene-α-D-hexopyranosides having a 2,3-*trans* arrangement of benzamido and methanesulphonyloxy substituents[3,4], it was considered that the product was either methyl 3,4,6-trideoxy-3,4-epimino-α-L-galactopyranoside (**3**) or methyl 2,3,6-trideoxy-2,3-epimino-α-L-mannopyranoside (**9**). Since it had already been established that the 4-sulphonyloxy substituent of methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside 2,4-dimethanesulphonate was considerably more reactive than that at C-2 towards nucleophilic displacement by the carbonyl group of the neighbouring acetamido group[7], structure **3** for the epimine was considered more likely.



The p.m.r. spectra of some 2,3-epiminopyranosides and their *N*-acetyl derivatives have been studied[8], and it has been noted that the chemical shifts of the protons present at the points of fusion of the aziridine and pyranoside rings occur in the range τ 6.9–7.7, well away from other ring-protons, and that no coupling occurs between these protons and vicinal *trans*-protons. The p.m.r. spectrum of our epimine, determined at 100 Mc.p.s. in deuterochloroform containing a little deuterium oxide, showed that all resonances due to non-identical protons were well separated, and it was therefore amenable to first-order analysis (Table I). The lowest-field doublet at τ 5.47 was assigned to H-1 because of the anticipated deshielding effect of the anomeric grouping. The coupling of this doublet ($J_{1,2}$ 4.7 c.p.s.) was identical with the splitting of another doublet at τ 6.27 suggesting that this signal must be due to H-2. Furthermore, the chemical shift of this doublet was not within the range expected[8] for a proton attached to the epimine ring, suggesting that C-2 was not part of the epimine ring, and that the epimine was therefore the 3,4-isomer (**3**). The two ring-protons of the epimine ring were, in fact, readily recognisable as a doublet (H-3) and a quartet (H-4) at τ 7.72 and 7.85, respectively. The apparent absence of coupling between the *trans*-hydrogens at C-2 and C-3 is consistent with the previous work done on the p.m.r. of epimines[8]. The structure of the epimine was further substantiated by conversion into the *N*,*O*-dibenzoyl derivative (**6**). The p.m.r. spectrum of **6** (Table I) likewise showed two doublets at τ 4.66 and 5.02, and their slight asymmetry indicated that they were mutually coupled. It is well established that the signal of a

TABLE I

CHEMICAL SHIFTS (τ-VALUES) AND FIRST-ORDER COUPLING CONSTANTS (C.P.S.)

Compound	*Solvent*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*-OMe*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
Epimine 3[a]	$CDCl_3$-D_2O	5.47 (d)[c]	6.27 (d)	7.72 (d)	7.85 (q)	5.93 (o)	8.72 (d)	6.59 (s)	4.7	0	6.0	2.0	6.3
N,O-Dibenzoyl-epimine 6[a]	$CDCl_3$	5.02 (d)	4.66 (d)	7.02	7.02	5.74 (o)	8.56 (d)	6.57 (s)	4.5	0	—	1.7	6.2
N-Benzoyl-epimine 5[b]	$CDCl_3$-D_2O	5.29 (d)	5.89 (d)	7.25 (d)	7.07 (q)	5.80 (o)	8.62 (d)	6.54 (s)	4.5	0	6.5	2.0	6.4
N-Acetyl-epimine 4[b]	$CDCl_3$-D_2O	5.37 (d)	6.17 (d)	7.18 (d)	7.36 (q)	6.05 (o)	8.64 (d)	6.53 (s)	4.5	0	7.0	2.0	6.5
Glucoside 2-benzoate 7[b]	$CDCl_3$-D_2O	5.07 (d)	4.79 (q)	5.33 (c.m.)	5.33 (c.m.)	6.18 (c.m.)	8.69	6.58 (d)	3.5	10.5	—	—	6.0

[a]At 100 Mc.p.s.; [b]At 60 Mc.p.s; [c] s = singlet, d = doublet, q = quartet, o = octet, c.m. = complex multiplet.

ring proton attached to the same carbon atom as a benzoyloxy group occurs to low field of most other ring protons because of the deshielding effect of the ester group[9,10], and consequently the doublet at τ 4.66 is assigned to H-2. In this case, the signals due to H-3 and H-4 were overlapping and not amenable to first-order analysis. A complete list of chemical shifts and coupling constants of these spectra and those of the *N*-benzoyl (**5**) and *N*-acetyl (**4**) derivatives are given in Table I, together with the first-order coupling constants. It is clear that these results are in accord with the 3,4-epimine (**3**), but quite inconsistent with the 2,3-epimine (**9**).

Further evidence for the structure of the epimine was provided by a more definitive, synthetic route from methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside (**1**). Selective benzoylation studies of certain methyl hexopyranosides have shown that, in all cases, the 4-hydroxyl group is the least reactive due to steric hindrance from the substituent at C-5, and that in the glucopyranoside the 2-hydroxyl group is the most reactive[10]. It was anticipated that the presence of a large 3-substituent on a glucopyranoside ring would further hinder the 4-hydroxyl towards benzoylation, so that selective 2-*O*-benzoylation of (**1**) should be readily achieved. Treatment of the 3-benzamido derivative (**1**) with one molecular proportion of benzoyl chloride afforded a crystalline monobenzoate in 54% yield. In the p.m.r. spectrum of the ester (Table I), the H-1 doublet was readily recognised at τ 5.07, with a splitting of *ca.* 3.5 c.p.s. A lower-field quartet was also present at τ 4.79, with splittings of *ca.* 10.5 and 3.5 c.p.s., and the asymmetries of these two signals showed them to be mutually coupled. Hence the quartet must be due to H-2, which has been strongly deshielded, and consequently the benzoyloxy substituent must be at C-2 as in **7**. Similar results have been achieved using methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside, and the location of the benzoate group at C-2 has been achieved by p.m.r. studies and by chemical means[11]. The monobenzoate afforded a methanesulphonate (**8**), which could be prepared directly from **1**, in 67% yield, by treatment with 1 molecular proportion of benzoyl chloride in pyridine followed by an excess of methanesulphonyl chloride. The monosulphonate, unlike the related disulphonate (**2**), is capable of forming only the 3,4-epimine (**3**), and, on treatment with lithium aluminium hydride, gave the same epimine as isolated from the disulphonate, thus proving the structure of the epimine.

EXPERIMENTAL

Concentrations were performed under reduced pressure. Melting points were determined on a Kofler microscope stage and are uncorrected. Optical rotations were determined to an accuracy better than ± 0.002° with a Bendix-N.P.L. Automatic Polarimeter Type 143C by using a 2-cm cell at 21 ± 2°. Thin-layer chromatography was performed on microscope slides at room temperature on silica gel (Whatman Chromedia SG41) with either (*i*) chloroform–methanol (5:1) or (*ii*) ether as mobile phase. The separated materials were detected by spraying the dried chromatogram with a 10% v/v solution of sulphuric acid in ethanol and heating on a hot-plate. Light petroleum (b.p. 40–60°) was used throughout. All products gave a single spot on

chromatograms, and their infrared spectra were in accord with the structures assigned. Pyridine was dried over sodium hydroxide pellets and used after decantation.

Methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside (**1**). — Benzoic anhydride (180 mg) was added to a suspension of methyl 3-amino-3,6-dideoxy-α-L-glucopyranoside[7] (100 mg) in ethanol (1 ml). A clear solution was first obtained, but the product soon crystallised. After 5 min, light petroleum (5 ml) was added, and the product filtered off. Recrystallisation from ethanol–light petroleum gave the *N*-benzoyl derivative (130 mg, 82%), m.p. 264–265°, $[\alpha]_D$ −148° (*c* 0.8, *N,N*-dimethylformamide). (Found: C, 60.0; H, 6.8; N, 5.35. $C_{14}H_{19}NO_5$ calc.: C, 59.8; H, 6.8; N, 5.0%).

Methyl 3-benzamido-3,6-dideoxy-2,4-di-O-methanesulphonyl-α-L-glucopyranoside (**2**). — Methanesulphonyl chloride (0.5 ml) was added to a solution of methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside (520 mg) in pyridine (6 ml). The reaction mixture was initially cooled under running tap-water and then left in the ice-box for 16 h. Decomposition of the reaction mixture by the addition of water afforded a crystalline product, recrystallisation of which from acetone–ether gave disulphonate **2** (710 mg, 86%), m.p. 195–196°, $[\alpha]_D$ −84° (*c* 0.64, *N,N*-dimethylformamide) (Found: C, 44.0; H, 5.15; N, 3.4; S, 14.55. $C_{16}H_{23}NO_9S_2$ calc.: C, 43.95; H, 5.25; N, 3.2; S, 14.65%).

Methyl 3-benzamido-2-O-benzoyl-3,6-dideoxy-α-L-glucopyranoside (**7**). — Benzoyl chloride (0.46 ml, 1.1 mol.) was added to a suspension of methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside (1 g) in pyridine (6 ml). The resulting solution was cooled under running tap-water and left at room temperature for 50 min. Addition of water caused separation of an oil that crystallised on the addition of a little ethanol. Recrystallisation from ethanol–light petroleum gave the dibenzoyl derivative (740 mg, 54%), m.p. 197–197.5°, $[\alpha]_D$ −143° (*c* 1.14, chloroform) (Found: C, 65.35; H, 5.95; N, 3.7. $C_{21}H_{23}NO_5$ calc.: C, 65.45; H, 5.95; N, 3.65%).

Methyl 3-benzamido-2-O-benzoyl-3,6-dideoxy-4-O-methanesulphonyl-α-L-glucopyranoside (**8**). — (*a*) Methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside (5.9 g) was treated as above with benzoyl chloride. After 1 h at room temperature, methanesulphonyl chloride (4 ml) was added, and the flask was again cooled under running tap-water. After a further 1.5 h, ice-water was added, and the crystalline product (6.5 g, 67%) was filtered off. Recrystallisation from ethanol gave sulphonate **8**, m.p. 169–171°, $[\alpha]_D$ −143° (*c* 0.95, chloroform) (Found: C, 56.85; H, 5.65; N, 3.5; $C_{22}H_{25}NO_8S$ calc.: C, 57.0; H, 5.4; N, 3.0%).

(*b*) To a solution of methyl 3-benzamido-2-*O*-benzoyl-3,6-dideoxy-α-L-glucopyranoside (50 mg) in pyridine (1 ml) was added methanesulphonyl chloride (0.04 ml). The mixture was kept for 45 min at room temperature, ice–water was then added, and the product (35 mg, 53%) was filtered off. Recrystallisation from ethanol gave the sulphonate **8**, m.p. 169–170°, $[\alpha]_D$ −143° (*c* 0.42, chloroform).

The infrared spectra of the two samples were identical.

Methyl 3,4,6-trideoxy-3,4-epimino-α-L-galactopyranoside (**3**). — (*a*) *From methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside 2,4-dimethanesulphonate* (**2**). Lithium aluminium hydride (1 g) was added to a suspension of **2** (1.2 g) in tetrahydrofuran

(50 ml), and the mixture was heated under reflux for 30 min. After being cooled, the excess of lithium aluminium hydride was decomposed by the dropwise addition of a saturated, aqueous solution of Rochelle salt. When the decomposition was complete, chloroform was added to the mixture which was then filtered through a pad of "Hyflo Supercel". The insoluble residue was then boiled twice with chloroform (50 ml) and filtered as before. The combined extracts were concentrated to a crystalline residue, recrystallisation of which from ethanol–ether afforded compound **3** (220 mg, 50%). A further recrystallisation from ethanol–ether gave the analytical sample, m.p. 174–176°, $[\alpha]_D$ −62° (*c* 1, water) [Found: C, 52.8; H, 8.3; N, 8.75%; mol. wt., 164 (titration). $C_7H_{13}NO_3$ calc.: C, 52.9; H, 8.15; N, 8.8%; mol. wt., 159].

(*b*) *From methyl 3-benzamido-2-O-benzoyl-3,6-dideoxy-4-O-methanesulphonyl-α-L-glucopyranoside* (**8**). Lithium aluminium hydride (1 g) was added to a solution of **8** (1g) in tetrahydrofuran (15 ml) and heated under reflux for 40 min. The mixture was processed as described above. Recrystallisation from ethanol–light petroleum yielded the epimine (225 mg, 66%), m.p. 173–175°, $[\alpha]_D$ −65° (*c* 0.55, water).

The infrared spectra of the two samples were identical.

Methyl 3,4-benzoylepimino-3,4,6-trideoxy-α-L-galactopyranoside (**5**). — Benzoic anhydride (250 mg) was added to a solution of methyl 3,4,6-trideoxy-3,4-epimino-α-L-galactopyranoside (140 mg) in ethanol (0.4 ml), and the solution was kept at room temperature for 10 min. On the addition of light petroleum, the *N*-benzoyl derivative (190 mg, 82%) separated. Recrystallisation from ethanol–light petroleum gave needles, m.p. 126.5–127.5, $[\alpha]_D$ −234° (*c* 0.8, chloroform) (Found: C, 63.75; H, 6.5; N, 5.45. $C_{14}H_{17}NO_4$ calc.: C, 63.9; H, 6.45; N, 5.3%).

Methyl 2-O-benzoyl-3,4-benzoylepimino-3,4,6-trideoxy-α-L-galactopyranoside (**6**). — To a solution of methyl 3,4,6-trideoxy-3,4-epimino-α-L-galactopyranoside (112 mg) in pyridine (3 ml) was added benzoyl chloride (0.18 ml). The solution was kept for 2 h at room temperature and then poured into water to give the crystalline dibenzoyl derivative (180 mg, 70%). Recrystallisation from ethanol–ether gave needles, m.p. 148.5–149.5, $[\alpha]_D$ −169° (*c* 0.57, chloroform) (Found: C, 69.1; H, 5.6; N, 3.95. $C_{21}H_{21}NO_5$ calc.: C, 68.7; H, 5.7; N, 3.8%).

Methyl 3,4-acetylepimino-3,4,6-trideoxy-α-L-galactopyranoside (**4**). — To a solution of methyl 3,4,6-trideoxy-3,4-epimino-α-L-galactopyranoside (100 mg) in ethanol (1 ml) was added acetic anhydride (0.1 ml). After 10 min, water was added, followed by an excess of solid sodium hydrogen carbonate. The resulting solution was then continuously extracted with chloroform overnight, and the extract was concentrated to a syrup that slowly crystallised. Recrystallisation from carbon tetrachloride–ether–light petroleum gave crystals of the *N*-acetyl derivative (67 mg, 53%) m.p. 79–82°, $[\alpha]_D$ −162° (*c* 0.44, chloroform) (Found: C, 54.25; H, 7.25; N, 6.95. $C_9H_{15}NO_4$ calc.: C, 53.75; H, 7.45; N, 6.95%).

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from CIBA Limited. Dr. L. D. Hall is thanked for the 100 Mc.p.s. p.m.r. spectra.

SUMMARY

The reduction of methyl 3-benzamido-3,6-dideoxy-2,4-di-*O*-methanesulphonyl-α-L-glucopyranoside with lithium aluminium hydride gave methyl 3,4,6-trideoxy-3,4-epimino-α-L-glucopyranoside. Its structure was proved by application of p.m.r. spectroscopy to the epimine and its derivatives, and by its formation from methyl 3-benzamido-2-*O*-benzoyl-3,6-dideoxy-4-*O*-methanesulphonyl-α-L-glucopyranoside, prepared by selective monobenzoylation of methyl 3-benzamido-3,6-dideoxy-α-L-glucopyranoside followed by methanesulphonylation.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SELECTIVE ACYLATION OF METHYL 3-ACETAMIDO-3,6-DIDEOXY-α-L-GLUCOPYRANOSIDE: ITS CONVERSION INTO THE L-*galacto* AND L-*manno* ANALOGUES

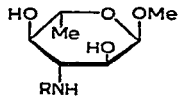
A. C. RICHARDSON
Department of Chemistry, The University, Reading (Great Britain)

(Received February 3rd, 1967)

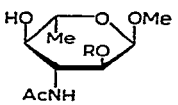
INTRODUCTION

Interest in 3-amino-3,6-dideoxyhexoses has been stimulated because of their isolation from microbiological sources. For example, the D-*manno* isomer (mycosamine) and the di-*N*-methyl-D-*gluco* isomer (mycaminose) occur quite widely as components of antibiotics[1], and the L-*galacto* isomer has recently been identified as a component of the cell walls of the plant pathogen *Xanthomonas campestris*[2]. At the outset of this study, most methods for synthesis of these sugars were comparatively tedious, employing either 3-amino-3-deoxyhexopyranosides (*e.g.*, Ref. 3) or epoxides of 6-deoxyhexopyranosides (*e.g.*, Ref. 4) as starting materials. The synthesis of methyl 3-amino-3,6-dideoxy-α-L-glucopyranoside (**1**) has been described in an overall yield of 31% from methyl 6-deoxy-α-L-mannopyranoside by the Baer–Fischer nitromethane synthesis[5], and the ready availability of this glycoside makes it a very attractive intermediate for conversion into other 3-amino-3,6-dideoxyhexose derivatives. Its conversion into the corresponding taloside has already been described by the solvolysis of the derived *N*-acetyl 2,4-dimethanesulphonate (**7**), in which inversion of configuration at C-2 and C-4 occurs by virtue of participation by the neighbouring acetamido substituent[5]. It was anticipated that if the hydroxyl groups of the *N*-acetylglucoside **2** could be selectively sulphonylated, selective inversion could then be brought about at either C-2 or C-4 to give the corresponding L-*manno* and L-*galacto* isomers, respectively. Recent work[6] on selective benzoylation of certain hexopyranosides has shown that, in all cases, including the 6-deoxyglycosides, the hydroxyl group at C-4 is the least reactive because of steric hindrance from the 5-substituent, and that for the glucopyranosides, the C-2 hydroxyl group is the most reactive secondary hydroxyl group[6]. It was considered that the presence of a large substituent at C-3 would further hinder the 4-position and give rise to greater stereoselectivity, and hence methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside should undergo selective reaction with benzoyl chloride and toluene-*p*-sulphonyl chloride at C-2 to provide suitable intermediates for the synthesis of the L-*manno* and L-*galacto* isomers. At the conclusion of this work, a communication by Čapek *et al.*[7] described very similar work on the partial acetylation and methanesulphonylation of **2**, and the partial deacetylation of its 2,4-diacetate. They found that esterification occurred predominantly at C-2 when the acid chloride was used, but that acetic anhydride was less specific and a mixture of

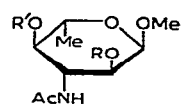
two monoacetates was formed. These authors used the derived monoesters to invert the configuration at either C-2 or C-4. Recently, a correlation between intramolecular hydrogen-bonding and enhanced rate of acylation of hydroxyl groups has been observed when acid chlorides in pyridine are used, but not with acid anhydrides[8]. This may explain the greater reactivity of the 2-hydroxyl group in α-D-glucopyranosides, which is able to hydrogen bond to the anomeric methoxyl group.* In agreement with this, the dimolar benzoylation of methyl β-D-glucopyranoside shows low selectivity.



(1) R = H
(2) R = Ac



(3) R = Bz
(4) R = Ts



(5) R = Bz, R' = Ms
(6) R = Ts, R' = Bz
(7) R = R' = Ms

DISCUSSION AND RESULTS

The reaction of methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside (**2**) with approximately one equivalent of benzoyl chloride in pyridine gave a monobenzoate (**3**) as the major product, which was isolated in 72% yield by direct crystallisation. The derived methanesulphonate (**5**) could be prepared from the monobenzoate in high yield, or by the addition of an excess of methanesulphonyl chloride to the benzoylation reaction mixture after benzoylation was complete. Single, secondary, benzoyloxy groups attached to pyranoside rings may be readily located by the use of p.m.r. spectroscopy, since the ring proton immediately adjacent to the benzoyloxy group is strongly deshielded and normally occurs downfield of all other ring protons, and hence is easily recognised[6]. The 100 Mc.p.s. p.m.r. spectrum (Table I) of the monobenzoate **3** showed a doublet at τ 5.16 (J 3.4 c.p.s.), due to the anomeric proton, partly overlapped with a downfield quartet at τ 5.06 (J 3.4 and 10.5 c.p.s.). The observed multiplicity and coupling constants of the downfield signal showed it to be due to H-2, and this was further confirmed by the asymmetry of the H-1 doublet and the quartet, which showed them to be mutually coupled. The signal due to H-4 would be expected to be a triplet (*ax-ax*, *ax-ax*, coupling), and it was recognised at higher field (τ 6.77). The H-3 signal was observed as a complex multiplet at τ 5.55, which collapsed into a triplet when the amide proton was exchanged with deuterium. The p.m.r. spectrum of the methanesulphonate (**5**) (Table I) also confirmed the presence of a 2-*O*-benzoyl group, since the low-field H-1 doublet (τ 5.07, J 3.0) and the H-2 quartet (τ 4.93, J, 3.0, 10.7) were readily recognised, and their asymmetry again showed them to be mutually coupled.

The methanesulphonate **5** underwent solvolysis with inversion of configuration at C-4, together with loss of the 2-*O*-benzoyl group, when heated with sodium acetate in aqueous 2-methoxyethanol. The resulting product, methyl 3-acetamido-3,6-dideoxy-

*This suggestion was first made by Dr. R. J. Ferrier in a private discussion.

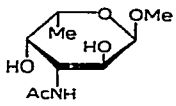
TABLE I

CHEMICAL SHIFTS (τ VALUES) AND FIRST-ORDER COUPLING CONSTANTS

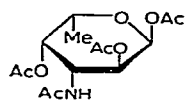
Compound	*Solvent*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*OMe*	*N-Ac*	*MeSO₂*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
3	100 Mc.p.s. $CDCl_3$-D_2O	5.16 (d)[a]	5.06 (q)	5.55 (t)	6.77 (t)	6.28 (o)	8.72 (d)	6.68 (s)	8.15 (s)	—	3.4	10.5	10.1	9.5	6.2
5	100 Mc.p.s. $CDCl_3$	5.07 (d)	4.93 (q)	5.21 (q)	5.59 (t)	6.05 (o)	8.65 (d)	6.66 (s)	8.12 (s)	6.94 (s)	3.0	10.7	9.5	9.0	6.3
6	60 Mc.p.s. $(CD_3)_2CO$		5.25 (c.m.)		4.98 (q)	6.02 (o)	8.86 (d)	6.63 (s)	8.49 (s)	*Ar-Me* 7.56 (s)	—	—	10.2	9.2	6.2

[a] s = singlet, d, = doublet, t = triplet, q = quartet, o = octet, c.m. = complex multiplet.

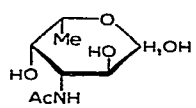
α-L-galactopyranoside (**8**), was isolated in *ca.* 60% yield. Acetolysis of the galactoside gave crystalline 3-acetamido-1,2,4-tri-*O*-acetyl-3,6-dideoxy-α-L-galactopyranoside (**9**), the anomeric configuration being assigned on the basis of optical rotation (−124°). De-*O*-acetylation afforded 3-acetamido-3,6-dideoxy-L-galactose (**10**). Comparison of the physical properties and i.r. spectrum of this monosaccharide with those of the sugar isolated from *Xanthomonas campestris*[2] showed them to be enantiomers. The synthesis of the L-enantiomorph confirms the structure of the natural sugar, which was proposed on the basis of chemical degradations. The synthesis of galactopyranoside **8** has been similarly achieved by Čapek *et al.*[7] using selective acetylation with acetyl chloride, but isolation of the 2-acetate required column chromatography and fractional crystallisation, whereas the 2-benzoate is isolable by direct crystallisation n much higher yield.



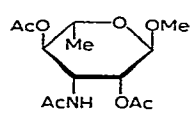
(8)



(9)



(10)



(11)

Reaction of **2** with one equivalent of toluene-*p*-sulphonyl chloride gave a monosulphonate (**4**) in 55% yield by direct crystallisation, together with a small proportion (4%) of the disulphonate. The monosulphonate gave a crystalline benzoate, (**6**) the p.m.r. spectrum (Table I) of which, in acetone-d_6, had a low-field quartet at τ 4.98 (*J* 10.2, 9.2), the coupling constants of which suggested *ax-ax*, *ax-ax* coupling. As before, this must be the H-4 signal, and hence the benzoate must be situated at C-4, showing that the sulphonate residue is present at C-2, as anticipated. This was confirmed by solvolysis of sulphonate **4** with sodium acetate in 2-methoxyethanol, which gave methyl 3-acetamido-3,6-dideoxy-α-L-mannopyranoside, isolated as the triacetate (**11**) in 36% yield. This product was shown by its infrared spectrum to be enantiomorphous with that derived from mycosamine. Čapek *et al.*[7] have described a similar series of reactions using selective methanesulphonylation.

EXPERIMENTAL

For general notes on experimental procedure, see preceding paper.

Methyl 3-acetamido-2-O-benzoyl-3,6-dideoxy-α-L-glucopyranoside (**3**). — Methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside (2 g) was suspended in dry pyridine (15 ml), and benzoyl chloride (1.15 ml, 1.1 mol.) was added. The reaction mixture was immediately cooled in ice–water and then kept for 30 min at room temperature. T.l.c. (chloroform–methanol, 9:1) revealed the monobenzoate (major product), and small proportions of dibenzoate and starting material. The mixture was then evaporated to dryness, and the residue, which crystallised in the presence of methanol, was partitioned between chloroform and water. After drying ($MgSO_4$), the organic layer was evaporated, and the crystalline product was recrystallised from ethanol–light petroleum to

give monobenzoate **3** (2.1 g, 72%), m.p. 166–168°; $[\alpha]_D$ −91.5° (*c* 1.99, chloroform) (Found: C, 59.3; H, 6.55; N, 4.4. $C_{16}H_{21}NO_6$ calc.: C, 59.6; H, 6.65; N, 4.45%).

Methyl 2-acetamido-2-O-benzoyl-3,6-dideoxy-4-O-methanesulphonyl-α-L-glucopyranoside (**5**). — (*a*) A solution of the 2-benzoate (**3**) (0.39 g) in dry pyridine (1.4 ml) was treated with methanesulphonyl chloride (0.3 ml), and the reaction mixture, which initially became warm, was kept for 1 h at room temperature. Careful decomposition of the reaction mixture by the slow addition of ice–water, with simultaneous scratching of the sides of the reaction flask, afforded crystals. Recrystallisation from acetone–ether–light petroleum afforded compound **5** (0.33 g, 67%), m.p. 164–166°, $[\alpha]_D$ −112° (*c* 1.69, chloroform) (Found: C, 51.0; H, 5.9; N, 3.45; S. 7.8. $C_{18}H_{23}NO_8S$ calc.: C, 50.85; H, 5.75; N, 3.5; S, 8.0%).

(*b*) Methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside (3.76 g) was monobenzoylated as described above. After the reaction mixture had been kept at room temperature for 1 h, methanesulphonyl chloride (4.5 ml) was added, and the reaction mixture was allowed to stand for a further 3 h. Decomposition of the reaction mixture with crushed ice afforded 4.5 g of buff-coloured crystals. Recrystallisation from ethanol yielded methanesulphonate **5** (3.1 g, 44%), m.p. 164–165°, identical with the product described in (*a*).

Methyl 3-acetamido-3,6-dideoxy-α-L-galactopyranoside (**8**). — The 4-methanesulphonate (**5**) (2.33 g) was dissolved in 80% aqueous 2-methoxyethanol (25 ml) containing sodium acetate (2.5 g.), and the solution was heated for 60 h under reflux. The mixture was then evaporated to dryness and extracted twice with boiling acetone (*ca.* 70 ml, 30 ml). Evaporation of the combined extracts gave a crystalline residue that was recrystallised from ethanol–ether–light petroleum to give galactopyranoside **8** (0.76 g, 61%), m.p. 193–196°, $[\alpha]_D$ −220° (*c* 0.92, water). Recrystallisation from ethyl acetate gave the analytical sample, m.p. 199–200°, $[\alpha]_D$ −219° (*c* 0.92, water) (Found: C, 49.25; H, 7.8; N, 6.5. $C_9H_{17}NO_5$ calc.: C, 49.3; H, 7.8; N, 6.4%); lit.[7], m.p. 202–203°, $[\alpha]_D$ −238° (*c* 0.3, water).

3-Acetamido-1,2,4-tri-O-acetyl-3,6-dideoxy-α-L-galactopyranose (**9**). — Methyl 3-acetamido-3,6-dideoxy-α-L-galactopyranoside (0.6 g) was dissolved in a mixture of acetic anhydride (10 ml) and concentrated sulphuric acid (0.3 ml), and kept for 24 h at room temperature. The mixture was then poured onto crushed ice, and the resulting, aqueous solution was quickly neutralised by the addition of solid sodium hydrogen carbonate. Continuous extraction of the solution with chloroform gave, after evaporation of the extract, a white, crystalline solid. The residue was taken up in a little hot chloroform, and some insoluble, inorganic material was filtered off. Addition of light petroleum gave the tri-*O*-acetyl derivative **9** (0.45 g, 50%), m.p. 237–239°, $[\alpha]_D$ −124° (*c* 1.1, chloroform) (Found: C, 50.45; H, 6.6; N, 4.65. $C_{14}H_{21}NO_8$ calc.: C, 50.7; H, 6.55; N, 4.2%)

3-Acetamido-3,6-dideoxy-L-galactose (**10**). — The tri-*O*-acetyl derivative **9** (200 mg) in methanol (6 ml) was treated with N sodium methoxide (0.2 ml), and the change in optical rotation was followed in a 0.1-dm tube [−0.28° (zero, by extrapo-

lation) → −0.26 (40 sec) → −0.252 (1 min) → −0.242 (2 min) → −0.238° (5 min, constant). After 30 min, the solution was de-ionised by the addition of a little Amberlite IR-120 (H^+) resin and then, after removal of the resin, evaporated to dryness. The residue crystallised from ethanol–acetone to give the acetamido sugar (28 mg., 23%), m.p. 173–175°, $[\alpha]_D$ −111° (*c* 0.93, water). A further crop (14 mg, 11%) was obtained by evaporation of the mother liquors to dryness and recrystallisation from ethanol–acetone. The i.r. spectra of both crops were identical with that of a sample of 3-acetamido-3,6-dideoxy-D-galactose isolated from the cell walls of *Xanthomonas campestris* and kindly provided by Dr. G. Ashwell. The physical properties reported[2] for this enantiomorph are m.p. 174–176°, $[\alpha]_D$ +114°.

*Methyl 3-acetamido-3,6-dideoxy-2-*O*-toluene-*p*-sulphonyl-α-*L*-glucopyranoside* (**4**). — Methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside (2.19 g) was suspended in dry pyridine (20 ml) and toluene-*p*-sulphonyl chloride (2.1 g, 1.1 mol.) was added. After standing for 4 h at room temperature, the mixture was diluted with ice–water (*ca.* 200 ml); the precipitated disulphonate (0.28 g, 4%) had m.p. 171–172°, $[\alpha]_D$ −61° (*c* 1.02, chloroform). The mother liquors were then concentrated to dryness to give a crystalline residue that was filtered off with the aid of a little water to give monosulphonate **4** (2.06 g, 55%). Recrystallisation from ethanol–ether–light petroleum gave the hemihydrate (1.72 g, 46%), which melted at 96–98°, resolidified at 98–100°, and then melted again at 145–146°, $[\alpha]_D$ −75° (*c* 1.66, chloroform) [Found (for anhydrous material): C, 51.3; H, 6.3; N, 3.7; S, 8.6; H_2O (for hemihydrate), 2.55. $C_{16}H_{23}NO_7S$ calc.: C, 51.5;H, 6.15; N, 3.75; S, 8.6; H_2O (for hemihydrate), 2.35%].

Benzoylation of the monosulphonate (0.5 g) with benzoyl chloride (0.5 ml) in pyridine (1.5 ml) gave a colourless syrup that slowly crystallised. Careful recrystallisation from ethanol–light petroleum gave methyl 3-acetamido-4-*O*-benzoyl-3,6-dideoxy-2-*O*-toluene-*p*-sulphonyl-α-L-glucopyranoside (240 mg, 38%), m.p. 108–112°, $[\alpha]_D$ −37° (*c* 1.51, chloroform) (Found: C, 57.75; H, 5.95. $C_{23}H_{27}NO_8S$ calc.: C, 57.9; H, 5.65%).

*Methyl 3-acetamido-2,4-di-*O*-acetyl-3,6-dideoxy-α-L-mannopyranoside* (**11**). — A solution of the monotoluene-*p*-sulphonate (**4**) (1 g) and sodium acetate (1 g) in 80% aqueous 2-methoxyethanol was heated for 65 h under reflux. The dark-coloured solution was decolorised with charcoal and evaporated. The dry residue was then treated with acetic anhydride (12 ml) and dry pyridine (10 ml), and the heterogeneous mixture was shaken for 2 h. The mixture was then decomposed by the addition of crushed ice, and neutralised (solid sodium hydrogen carbonate). The resulting solution was continuously extracted with chloroform for 3.5 h, and the extract was concentrated to a brown syrup that was chromatographed on a column of silica gel with chloroform–methanol (20:1). This afforded a syrup that crystallised from ether to give the mannoside (280 mg, 36%) as slightly coloured, sticky crystals. A further recrystallisation from the same solvent gave a white, crystalline product (235 mg, 30%), m.p. 141–143°, $[\alpha]_D$ −31° (*c* 0.94, ethanol) (Found: C, 51.4; H, 6.9; N, 4.8; $C_{13}H_{21}NO_7$ calc.: C, 51.45; H, 7.0; N, 4.6%); lit[7]., m.p. 144.5–146°, $[\alpha]_D$ −29° (ethanol). The i.r. spectrum of the product was identical with that of methyl 3-acetamido-2,4-di-*O*-acetyl-3,6-dideoxy-α-

D-mannopyranoside prepared from mycosamine and kindly provided by Dr. J. D. Dutcher, who reported m.p. 139–141°, $[\alpha]_D$ +31° (ethanol) for this compound.

ACKNOWLEDGMENTS

The author thanks Dr. J. D. Dutcher for samples of mycosamine derivatives, and Dr. L. D. Hall for the 100 Mc.p.s. spectra.

SUMMARY

Selective mono-*O*-benzoylation and mono-*O*-toluene-*p*-sulphonylation of methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside afforded 2-esters in good yields. The methanesulphonate derived from the 2-benzoate underwent solvolysis with sodium acetate in boiling aqueous 2-methoxyethanol to give methyl 3-acetamido-3,6-dideoxy-α-L-galactopyranoside. Acetolysis of this glycoside, followed by de-*O*-acetylation, gave 3-acetamido-3,6-dideoxy-L-galactose, shown to be enantiomorphous with the sugar isolated from *Xanthomonas campestris*. The 2-toluene-*p*-sulphonate underwent solvolysis under similar conditions to give methyl 3-acetamido-3,6-dideoxy-α-L-mannopyranoside.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE SYNTHESIS OF *N*-BENZOYL-D-DAUNOSAMINE*

A. C. RICHARDSON
Department of Chemistry, The University, Reading (Great Britain)
(Received February 3rd, 1967)

INTRODUCTION

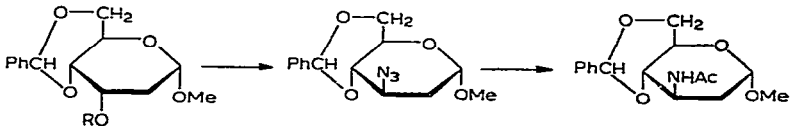
A considerable number of amino sugars are known to occur as components of antibiotics[2] and many have the basic group situated at C-3. Whereas all of the 3-amino-3-deoxyhexoses and 3-amino-3,6-dideoxyhexoses known to occur naturally have been synthesised by straightforward methods[2,3], those containing secondary, in addition to primary, deoxy groups [*e.g.*, desosamine (3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexose)] are less readily synthesised owing to the difficulty of introducing the secondary deoxy group. The synthesis of desosamine has however been achieved from methyl 3-acetamido-3-deoxy-α-D-glucopyranoside by the action of sodium iodide on its 2-*O*-acetyl-4,6-di-*O*-methanesulphonyl derivative. The 4-sulphonate group, normally unreactive towards displacement by iodide, was replaced by virtue of participation of the neighbouring acetamido function. The resulting 4,6-di-iodo derivative was reduced to the appropriate aminotrideoxyglycoside, which was converted into desosamine by standard reactions[4]. Application of this method to the synthesis of methyl 3-acetamido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside[5], which was to be used for the synthesis of rhodosamine (2,3,6-trideoxy-3-dimethylamino-L-*lyxo*-hexose)[6] and daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose)[7], was unsuccessful. Thus, the readily available methyl 3-acetamido-3,6-dideoxy-α-L-galactopyranoside 2-methanesulphonate[8] underwent loss of its methanesulphonate group, when treated with iodide and thiocyanate in various solvents, to give not the covalent 2-iodo and 2-thiocyanato derivatives, but the isomeric oxazolinium salts. As a result of this work, it was decided to investigate the synthesis of rhodosamine and daunosamine by using a precursor which already possessed the 2-deoxy group. This communication describes the use of methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside (**1**) in the synthesis of derivatives of 3-amino-2,3,6-trideoxy-D-*lyxo*-hexose, which are enantiomorphically related to the corresponding derivatives of daunosamine.

RESULTS AND DISCUSSION

Methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside (**1**) was prepared

* For a preliminary communication, see Ref. 1.

from the corresponding *allo*-2,3-epoxide by a modification of the method of Prins[9]. The derived methanesulphonate **2** underwent a facile replacement with azide in boiling *N*,*N*-dimethylformamide to give, with inversion of configuration, the 3-azido-2,3-dideoxy-α-D-*arabino*-hexopyranoside (**3**) in 71% yield. This replacement reaction has already been described by Guthrie and Murphy[10] who used boiling *N*,*N*-dimethylformamide–dioxan (*ca.* 20:1; v/v) for 10 h, but it is more convenient to carry out the reaction in boiling *N*,*N*-dimethylformamide, when it is complete within 10 min, as shown by t.l.c. The azide was hydrogenated catalytically by using Raney nickel, and the resulting crystalline amine, without isolation, was converted into the *N*-acetyl derivative (**4**), isolated in an overall yield of 83%. This compound has been similarly prepared by Guthrie and Murphy[10], and from methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino-α-D-mannopyranoside by catalytic hydrogenation followed by *N*-acetylation[11], although there is some discrepancy between the physical properties of this preparation and those described previously (see Experimental).



(1) R = H
(2) R = Ms

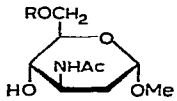
(3)

(4)

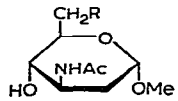
The removal of the benzylidene substituent from **4** presented some difficulty, because boiling, 50%, aqueous acetic acid, conditions under which a benzylidene residue is normally removed selectively from a hexopyranoside[4], caused extensive hydrolysis of the glycosidic group. This difficulty was not unexpected in view of the known lability of 2-deoxypyranosides towards acid[13], but was overcome by the use of methanolic hydrogen chloride which smoothly hydrolysed the benzylidene group, without disruption or anomerisation of the glycosidic group, to give the required product **5** in 85% yield. Reaction of **5** with toluene-*p*-sulphonyl chloride (1.15 moles per mole of **5**) was highly selective, and the 6-sulphonate (**6**) was obtained in 93% yield. Even when a larger excess (*ca.* 2 moles) of the acid chloride was used, the monosulphonate was still isolable in high yield (*ca.* 70%), and t.l.c. indicated that the disulphonate was formed extremely slowly. This result accords with recent work carried out on the selective benzoylation of certain hexopyranosides, which showed the 4-hydroxyl group to be the most hindered to benzoylation due to steric hindrance by the 5-substituent[13,14]. The steric hindrance of the 4-hydroxyl group is increased[3] by the presence of a large substituent at C-3 (acetamido in this case), and it has been found that toluene-*p*-sulphonylation of methyl 6-deoxy-α-L-mannopyranoside 2,3-dibenzoate is possible only when forcing conditions are used[14].

The 6-sulphonate (**6**) underwent displacement by iodide in acetone to give a 75% yield of the 6-iodo derivative (**7**), which, on reductive dehalogenation with Raney nickel–hydrogen, gave methyl 3-acetamido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (**8**) in 86% yield. The next stage of the synthesis was to effect inversion of configuration

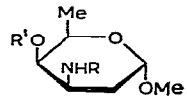
of the 4-hydroxyl group. Since the 4-hydroxyl group was situated *trans* to a neighbouring acetamido group, the inversion was carried out by solvolysis of the derived 4-methanesulphonate in the way described previously[3,8,15]. Some difficulties were



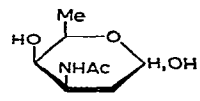
(5) R = H
(6) R = Ts



(7) R = I
(8) R = H



(9) R = Ac, R' = H
(10) R = R' = Ac
(11) R = R' = H
(12) R = Bz, R' = H



(13)

encountered in isolation of the 4-sulphonate because it was water soluble and underwent decomposition in hot solvents. However, it was subsequently found that isolation of the sulphonate was unnecessary and that, after decomposition of the methanesulphonylation reaction mixture with ice–water, the resulting solution could be treated with sodium acetate and boiled to effect the inversion at C-4. After such treatment, the isomeric *lyxo*-hexopyranoside (**9**) was isolated in an overall yield of 42%. The derived 4-acetate (**10**) had an infrared spectrum identical with that of the corresponding derivative prepared from daunosamine[7], but its physical constants (m.p. 183–185°, $[\alpha]_D$ +192°) did not agree with those reported (m.p. 176–178°, $[\alpha]_D$ −130°) for the natural α-L-pyranoside. It would appear from this discrepancy that the glycoside derived from daunosamine was contaminated with the β-L-anomer, and Dr. Arcamone, in a personal communication, has stated that the material used in the original work[7] was found by n.m.r. spectroscopy to contain at least 10% of the β-L-anomer, although the magnitude of the difference in optical rotation would suggest a value nearer 30%. These results illustrate the unreliability of infrared spectroscopy in detecting anomeric impurities in glycosides. Similar results have been obtained with methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranosides, where the i.r. spectrum of the α-anomer is not significantly different from the i.r. spectrum of a 3:1 mixture of the α- and β-anomers[16].

The relationship between the natural sugar and the synthetic product was established by conversion of the synthetic glycoside into the *N*-benzoyl derivative. The *N*-acetylglucoside (**9**) was first de-*N*-acetylated with boiling, aqueous sodium hydroxide, and the resulting amine (**11**), without isolation, was converted into the *N*-benzoyl derivative (**12**) by reaction with benzoic anhydride in ethanol. The *N*-benzoylglycoside (**12**) was remarkable inasmuch as it was hydrolysed to the free sugar by boiling, 25%, aqueous acetic acid in less than 10 minutes. The resulting monosaccharide (**13**) was isolated in 40% yield, and its physical properties and infrared spectrum showed it to be enantiomorphic with *N*-benzoyldaunosamine[7], thus concluding the structural proof of this sugar.

EXPERIMENTAL

For general notes on experimental procedures, see preceding papers.

Methyl 4,6-O-benzylidene-2-deoxy-α-D-ribo-hexopyranoside (**1**). — Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside (25 g) was suspended in dry ether (1000 ml), lithium aluminium hydride (9 g) was added, and the mixture refluxed for 1.5 h. T.l.c. (ether) indicated that only one product had been formed, and that the reaction was probably complete within 30 min. The reaction mixture was decomposed by the addition of water and then filtered. The insoluble material was boiled with chloroform, and these washings were combined with the ethereal filtrate. Evaporation of the combined filtrate and washings, with recrystallisation of the residue from ethanol–light petroleum, gave compound **1** (19.8 g, 79%) m.p. 127–129°, $[\alpha]_D$ +140° (*c* 2.82, chloroform); lit.[9], double m.p. 117–119° and 126–128°, $[\alpha]_D$ +155.6° (chloroform).

Methyl 4,6-O-benzylidene-2-deoxy-3-O-methanesulphonyl-α-D-ribo-hexopyranoside (**2**). — A solution of compound **1** (22.5 g) in dry pyridine (100 ml) was treated with methanesulphonyl chloride (20 ml) and kept at room temperature overnight. Addition of crushed ice to the mixture yielded crystals of the methanesulphonate that were recrystallised from ethanol to give compound **2** (25.2 g, 88%), m.p. 117–119°, $[\alpha]_D$ +138° (*c* 1.2, chloroform); lit.[10], m.p. 118–119°, $[\alpha]_D$ +134°. On standing for several months, some preparations of this compound turned into a black tar.

Methyl 3-azido-4,6-O-benzylidene-2,3-dideoxy-α-D-arabino-hexopyranoside (**3**). — A solution of the 3-methanesulphonate (25 g) in *N*,*N*-dimethylformamide (250 ml) was heated under reflux with sodium azide (25 g) for 15 min, and then poured into water (*ca.* 500 ml) that contained seed crystals of the product obtained from a previous, small-scale experiment. The product initially separated as an oil that quickly solidified, particularly if a little ethanol was added. Recrystallisation from aqueous ethanol afforded the 3-azide (15.3 g, 71%), m.p. 82–83°, $[\alpha]_D$ +111° (*c* 2.75, chloroform); lit.[10], m.p. 82–84°, $[\alpha]_D$ +105°.

Methyl 3-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-arabino-hexopyranoside (**4**). — Compound **3** (13 g) was dissolved in warm methanol (150 ml) and hydrogenated at 63 lb/sq.in in the presence of Raney nickel for 2.5 h. T.l.c. (ether) indicated the reaction was complete. The catalyst was filtered off, and the filtrate was concentrated to a crystalline solid, which was then dissolved in warm ethanol (*ca.* 60 ml). Addition of acetic anhydride (11 ml) caused an immediate precipitation of the 3-acetamido derivative. The mixture was cooled in ice–water, and filtration gave a white, crystalline product (11.4 g, 83%), m.p. 272–274° (subl.), $[\alpha]_D$ +65° (*c* 1.15, chloroform) (Found: C, 62.55; H, 6.95; N, 4.7. $C_{16}H_{21}NO_5$ calc.: C, 62.55; H, 6.85; N, 4.55%); lit.[10,11], m.p. 240–245° (subl.), $[\alpha]_D$ +53°.

Methyl 3-acetamido-2,3-dideoxy-α-D-arabino-hexopyranoside (**5**). — The 3-acetamido-4,6-*O*-benzylidene derivative **4** (6 g) was suspended in a mixture prepared from methanol (100 ml) and acetyl chloride (2 ml) (*i.e.*, effectively, 1% methanolic hydrogen chloride). The mixture was shaken for 40 min at room temperature, and the resulting clear solution was neutralised by the addition of an excess of solid sodium hydrogen carbonate. The neutral solution was decanted from the inorganic material, which was washed thoroughly with methanol, and the combined supernatant liquid and washings were evaporated. The solid residue was extracted with boiling acetone,

and the extract was evaporated to *ca.* 20 ml, when crystallisation commenced. After being kept at −10°, the acetamidoglycoside (3.64 g, 85%) was filtered off; m.p. 137–138°, $[\alpha]_D$ +148° (*c* 1.2, methanol) (Found: C, 49.25; H, 7.65; N, 6.8. $C_9H_{17}NO_5$ calc.: C, 49.3; H, 7.75; N, 6.4%).

Attempted hydrolysis of the 4,6-*O*-benzylidene derivative with 50% aqueous acetic acid appeared to hydrolyse the glycosidic group also, as indicated by t.l.c. (chloroform–methanol, 4:1).

*Methyl 3-acetamido-2,3-dideoxy-6-*O*-toluene-*p*-sulphonyl-α-*D-arabino-*hexopyranoside* (**6**). — Compound **5** (3.02 g) was dissolved in pyridine (7.5 ml) and toluene-*p*-sulphonyl chloride (3 g) was added. The mixture was cooled initially in ice–water and then kept for 2 h at room temperature. Water (*ca.* 250 ml) was then added, and the 6-sulphonate slowly crystallised. After 1 h, the crystals (4.0 g, 80%) were collected, and dried at 65°/1 mm. A further crop of the sulphonate (0.67 g, 13%) separated on storage of the mother liquors. Recrystallisation of the combined crops from ethanol–ether–light petroleum afforded the product as a monohydrate, m.p. 120–123°, $[\alpha]_D$ +125° (*c* 0.8, chloroform) (Found: C, 49.25; H, 6.5; N, 3.55; S, 8.0. $C_{16}H_{23}NOS \cdot H_2O$ calc.: C, 49.1; H, 6.4; N, 3.6; S, 8.2%).

*Methyl 3-acetamido-2,3,6-trideoxy-6-iodo-α-*D-arabino-*hexopyranoside* (**7**). — The 6-toluene-*p*-sulphonate **6** (1.4 g) was dissolved in acetone (30 ml), and the solution was heated under reflux with sodium iodide (3 g) for 16 h. The mixture was evaporated to dryness, and the residue was partitioned between chloroform and water. The aqueous layer was twice re-extracted with chloroform, and the combined chloroform extracts were dried ($MgSO_4$) and evaporated. The crystalline residue was recrystallised from ethanol–light petroleum to give the 6-iodo derivative (0.92 g, 75%). The product was recrystallised from water to give the analytical sample, m.p. 173–174° (decomp.), $[\alpha]_D$ +93° (*c* 1.2, methyl sulphoxide) (Found: C, 33.35; H, 4.8; N, 4.0; I, 40.5. $C_9H_{16}INO_4$ calc.: C, 32.8; H, 4.85; N, 4.25; I, 38.6%).

*Methyl 3-acetamido-2,3,6-trideoxy-α-*D-arabino-*hexopyranoside* (**8**). — The 6-iodo derivative **7** (2.55 g) was dissolved in a mixture of methanol (100 ml) and ethanol (20 ml) containing sodium acetate (2.5 g), and hydrogenated at 60 lb/sq. in with Raney nickel for 16 h. After removal of the catalyst, the solution was evaporated, and a solution of the residue in water was extracted continuously with chloroform for 22 h. Concentration of the extract, followed by recrystallisation of the solid residue from ethyl acetate–light petroleum, gave compound **8** (1.31 g, 86%), m.p. 153–155°, $[\alpha]_D$ +137° (*c* 1.55, methanol). The analytical sample was prepared by recrystallisation from methanol–ether–light petroleum and had m.p. 157.5–158° (Found: C, 53.05; H, 8.4; N, 7.05. $C_9H_{17}NO_4$ calc.: C, 53.2; H, 8.35; N, 6.9%).

*Methyl 3-acetamido-2,3,6-trideoxy-α-*D-lyxo-*hexopyranoside* (**9**). — A solution of the *arabino*-hexopyranoside **8** (0.6 g) in pyridine (2 ml) was treated with methanesulphonyl chloride (0.6 ml), with cooling, and then kept for 3 h at room temperature. It was then decomposed with crushed ice to give a clear solution that was made up to *ca.* 30 ml with water, and sodium acetate (*ca.* 4 g) was added. The resulting solution was boiled for 1 h, cooled, treated with solid sodium hydrogen carbonate until the

evolution of carbon dioxide ceased, and then extracted continuously with chloroform for 18 h. Concentration of the extract yielded a syrup that crystallised upon the addition of a little ethanol. Recrystallisation from acetone yielded compound **9** (0.252 g, 42%), m.p. 176–178°, $[\alpha]_D$ +222° (*c* 1.48, methanol) (Found: C, 53.3; H, 8.45; N, 6.9. $C_9H_{17}NO_4$ calc.: C, 53.2; H, 8.35; N, 6.9%). A further 34 mg (6%) was obtained from the mother liquors by evaporation to dryness, followed by recrystallisation of the residue from ethyl acetate.

Methyl 3-acetamido-4-O-acetyl-2,3,6-trideoxy-α-D-lyxo-hexopyranoside (**10**). — Compound **9** (50 mg) was dissolved in pyridine (0.2 ml), and acetic anhydride (0.15 ml) was added. Some crystalline material separated, but this dissolved during the course of about 1 h when the mixture was shaken. After *ca.* 2 h, the excess of anhydride was decomposed with ice, and the resulting solution was quickly neutralised by the addition of solid sodium hydrogen carbonate and extracted with chloroform (3 × 50 ml). After drying ($MgSO_4$), the combined extracts were concentrated to a crystalline residue, which on two recrystallisations from ethanol–ether–light petroleum gave the 4-acetate (29 mg, 47%), m.p. 183–185° (changing crystalline form from 150° upwards), $[\alpha]_D$ +192° (*c* 0.9, chloroform) (Found: C, 54.1; H, 7.65; N, 5.8. $C_{11}H_{19}NO_5$ calc.: C, 53.9; H, 7.75; N, 5.7%). The i.r. spectrum of this diacetate was identical with that obtained from the methyl pyranoside of daunosamine. The reported values for this derivative [m.p. 176–178°, $[\alpha]_D$ −130°] suggest that it is a mixture of α- and β-L-anomers.

Methyl 3-benzamido-2,3,6-trideoxy-α-D-lyxo-hexopyranoside (**12**). — Compound **9** (100 mg) was dissolved in 2N sodium hydroxide, and the solution was heated for 6 h under reflux and then extracted continuously with chloroform for 16 h. The extract, on evaporation, gave the free base as a crystalline residue that could not be easily recrystallised. It was dissolved in ethanol (1 ml), and benzoic anhydride (150 mg) was added. The mixture was warmed slightly to effect dissolution of the anhydride, and, after 5 min, light petroleum (*ca.* 3 ml) was added to give compound **12** (44 mg, 34%), m.p. 153–155°, $[\alpha]_D$ +168° (*c* 1.02, water) (Found: C, 63.4; H, 7.0. $C_{14}H_{19}NO_4$ calc.: C, 63.4; H, 7.2).

3-Benzamido-2,3,6-trideoxy-D-lyxo-hexose (**13**). — The benzamidoglycoside **12** (40 mg) was dissolved in water (4 ml), and acetic acid (1 ml) was added. The solution was heated on a boiling-water bath for 20 min, although t.l.c. (chloroform–methanol, 4:1) indicated the reaction to be complete within 10 min. Concentration of the solution yielded a syrup that readily crystallised in the presence of a little acetone. The crystals were filtered off with the aid of a little chloroform to give compound **13** (16 mg, 40%), m.p. 150.5–151.5°, $[\alpha]_D$ +110° (*c* 0.86, ethanol) (Found: C, 62.0; H, 6.5. $C_{13}H_{17}NO_4$ calc.: C, 62.15; H, 6.8%). The i.r. spectrum was identical with that of *N*-benzoyldaunosamine for which m.p. 154–156°, $[\alpha]_D$ −107.5° were reported[7].

ACKNOWLEDGMENT

Dr. F. Arcamone is thanked for generously supplying several samples of daunosamine and its derivatives.

SUMMARY

The structure of daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose), the carbohydrate component of the anti-tumour antibiotic daunomycin, has been confirmed by the synthesis of the *N*-benzoyl derivative of the D-enantiomorph from methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside.

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Notes

The conformations of cyclic compounds in solution Part III*. The acetates of dimeric α-hydroxyaldehydes

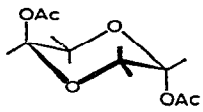
The recent publication by Gardiner[1] concerning the proton magnetic resonance (p.m.r.) spectra of the *O*-trimethylsilyl derivatives of several α-hydroxycarbonyl compounds has prompted us to report our studies of the related acetates of the dimers of glycolaldehyde and of DL-glyceraldehyde. Since Gardiner has already summarized the historical aspects of this problem, it is sufficient to point out that, although Summerbell and Rochen[2] had shown that the acetylated dimer[3] of glycolaldehyde has the gross structure 2,5-diacetoxy-1,4-dioxane (**1**), the stereochemistry of this compound is unknown. A similar situation exists for the acetylated dimer[4] of DL-glyceraldehyde, which is presumed to have the structure 2,5-diacetoxy-3,6-bis(acetoxymethyl)-1,4-dioxane (**2**). We have now assigned configurations and conformations to both of these derivatives.

The simplicity of the p.m.r. spectrum of the acetylated dimer (**1**) of glycolaldehyde (see Experimental) suggests that this derivative is symmetrically substituted, and the magnitudes of the two *vicinal* couplings (1.45 and 0.95 Hz) are consistent[5] with the conformational symmetry depicted in **1a**; the alternative symmetrical conformation **1b** is immediately excluded, as it would require one of the *vicinal* couplings to be *ca.* 8 Hz. In a similar fashion, the possibility that the compound is asymmetrically substituted and undergoing rapid chair–chair inversion between conformations **1c** and **1d** may be excluded; it would require one of the *vicinal* couplings to be *ca.* 5 Hz**. In fact, the very small magnitude of both *vicinal* couplings of conformation **1a** would be anticipated on the basis of the high, total electronegativity[8] of the substituents attached to the C-2—C-3 system (three oxygen substituents), and on the "configurational dependences" arising from these same oxygen substituents[9,10]. The preference for the diaxial conformation **1a** indicates that the anomeric effect[11,12] of the acetoxy group is greater than the steric interactions that are introduced when this substituent is axial. In the absence of more appropriate energy-parameters, it is not worth while pursuing this discussion further[13], beyond pointing out that the results

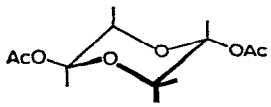
*Part II, *Tetrahedron*, 20 (1964) 1685.

This value was estimated as follows. For 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranose, it is known[6] that $J_{1e,2a} = 3.6$ and $J_{1e,2e} = 1.5$ Hz; for the β-D anomer $J_{1a,2a} = 9.8$ and $J_{1a,2e} = 2.4$ Hz. By using these as limiting values for the couplings of derivative (1**), conformational inversion between the equally populated conformers **1c** and **1d** would result in *vicinal* couplings of *ca.* 5.5 and 3.0 Hz. The estimated[7] couplings of 1,4-dioxane (9.4 and 2.7 Hz) used in this consideration give *vicinal* couplings of 6.0 and 2.7 Hz for the equilibrium **1c** ⇌ **1d**.

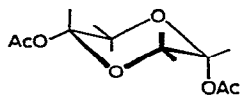
obtained[14] for *trans*-2,5-dichloro-1,4-dioxane indicate that the diaxial conformation is also favored with this derivative.



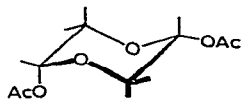
(1a)



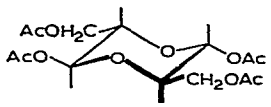
(1b)



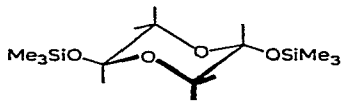
(1c)



(1d)



(2a)



(3)

It is interesting that the corresponding *trans*-di-*O*-trimethylsilyl derivative of glycolaldehyde dimer appears[1] to have a conformational symmetry approximating to that shown in **3**. The difference in conformation between this derivative and the *trans*-diacetate (**1a**) implies that a trimethylsilyloxy substituent has a smaller anomeric effect than an acetoxy substituent.

The spectrum of the acetate of the dimer of DL-glyceraldehyde is quite simple (see Experimental), again reflecting a high degree of conformational symmetry. However, in this case, the *vicinal* coupling between H-2 and H-3 (7.6 Hz) is implicitly consistent with conformation **2a**, which has all four substituents equatorially oriented. The favored conformation of this acetate is the same as that of the per(trimethylsilyl) ether, which shows[1] a *vicinal* coupling of 7.4 Hz.

EXPERIMENTAL

Materials. — Glycolaldehyde and DL-glyceraldehyde were commercial samples (K and K Laboratories, Plainview, N. Y., U.S.A.), which were used as received. The corresponding "dimer acetates" were prepared by treatment of the hydroxyaldehyde with acetic anhydride and pyridine, following the literature procedures; *trans*-2,5-diacetoxy-1,4-dioxane (**1**) had m.p. 158° (lit.[3] m.p. 157–158°); and *trans*-2,5-diacetoxy-3,6-bis(acetoxymethyl)-1,4-dioxane (**2**) had m.p. 155° (lit.[4] m.p. 154°). The latter isomer crystallized readily from the reaction mixture; no attempts were made to isolate other components from the residue.

Spectral measurements. — The p.m.r. spectra were measured with a Varian HA-100 spectrometer operating in the frequency-sweep mode, and spin-decoupling experiments were also performed in this mode, by using a modified Hewlett–Packard 200 AB audio-oscillator. Tetramethylsilane was used as the internal-lock reference.

In the spectrum of **1**, the approximate quartet at τ 4.06 was assigned to H-2 and H-5, and the signals of the methylene protons at C-3 and C-6 were observed as quartets at τ 5.79 and 6.37. These resonances were analyzed[15] as an ABX system to give the following coupling constants: $J_{3e,3a} = -12.5$; $J_{2e,3a} = +1.45$; $J_{2e,3e} = +0.95$ Hz. This analysis is not strictly valid, as it presupposes that there is no coupling across the ring oxygen atom, which we now know[16] is quite common for carbohydrates and for dioxane derivatives[17]. The acetate group gave a singlet resonance at τ 7.85.

The spectrum of **2** showed the H-2 and H-5 resonance as the low-field doublet at τ 4.25 ($J_{2,3} = 7.6$ Hz). The exocyclic, acetoxymethyl protons (termed 4 and 4′ below) gave resonances as characteristic quartets centered at τ 5.68 and 5.89, and H-3 and H-6 gave resonances at τ 6.09. The latter three resonances were analyzed as an ABM system[15] in which the M-resonance (H-3) was considered to be first-order coupled with an X-resonance (H-2). This analysis gave the following coupling constants: $J_{2a,3a} = +7.6$; $J_{3a,4} = +5.0$; $J_{3a,4'} = +2.8$; $J_{4,4'} = -12.1$ Hz. The acetate methyl resonances were observed at τ 7.87 and 7.93.

The signs of the above couplings were based on determinations reported earlier[18].

ACKNOWLEDGMENT

It is a pleasure to acknowledge financial assistance from the National Research Council of Canada.

Department of Chemistry,
The University of British Columbia,
Vancouver 8, B. C. (Canada)

L. D. HALL

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(Received January 23rd, 1967; in revised form, February 17th, 1967)

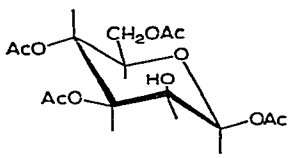
Carbohyd. Res., 4 (1967) 429–432

Methylation of carbohydrates bearing base-labile substituents, with diazomethane–boron trifluoride etherate
II. A new synthesis of 2-*O*-methyl-D-mannose

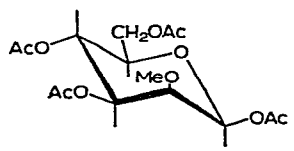
It has been previously shown[1] that monosaccharides having base-labile substituents (*e.g.*, *O*-acetyl groups) could be easily methylated by the use of diazomethane in the presence of a catalytic proportion of boron trifluoride etherate, without migration of the labile substituents.

During the course of other studies, there arose the need for a sample of 2-*O*-methyl-D-mannose; this compound had been prepared by other methods[2], but these are somewhat complicated and therefore, to avoid this difficulty, its preparation was attempted following the sequence used for the synthesis of 2-*O*-methyl-D-glucose and 2-*O*-methyl-D-galactose[1].

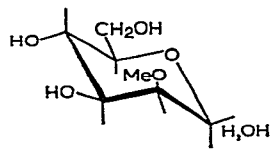
1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose (**1**) was obtained by direct acetylation of D-mannose, following the method described by Helferich and Zirner[3] for the corresponding tetraacetates of D-glucose and D-galactose. For acetylating D-galactose, the last step of the reaction is conducted at a lower temperature and for a shorter time than the similar step for acetylating D-glucose. We have now found that compound **1** is obtained when the reaction is conducted under the conditions used with D-galactose, whereas, when performed as for the acetylation of D-glucose, the only product isolated was 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose. Consequently, the temperature is a very important factor in the reaction and must be controlled with great care. Compound **1** had a melting point different from that of the product prepared by Gakhokidze and Kutidze[4]; possibly, our product is the anomer of the tetraacetate obtained by the Russian workers.



(1)



(2)



(3)

13 B. COXON, *Tetrahedron*, 22 (1966) 2281.
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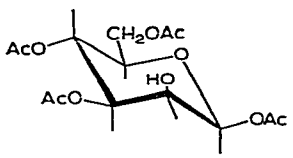
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Methylation of carbohydrates bearing base-labile substituents, with diazomethane–boron trifluoride etherate
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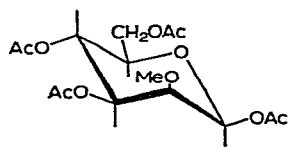
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During the course of other studies, there arose the need for a sample of 2-*O*-methyl-D-mannose; this compound had been prepared by other methods[2], but these are somewhat complicated and therefore, to avoid this difficulty, its preparation was attempted following the sequence used for the synthesis of 2-*O*-methyl-D-glucose and 2-*O*-methyl-D-galactose[1].

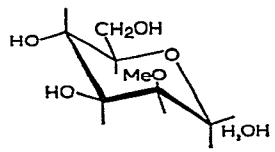
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(1)



(2)



(3)

Methylation of **1** with diazomethane–boron trifluoride etherate in dichloromethane afforded 1,3,4,6-tetra-*O*-acetyl-2-*O*-methyl-β-D-mannopyranose (**2**) in good yield. Compound **2** was deacetylated with sodium methoxide in methanol, yielding 2-*O*-methyl-D-mannose (**3**), which crystallized on nucleation; its physical properties were in good agreement with the values reported in the literature[2].

It is thus possible to prepare, by a simple method, compound **3** — a product hitherto obtained by more laborious and difficult procedures.

EXPERIMENTAL

Melting points are not corrected. Nuclear magnetic resonance spectra were determined in deuteriochloroform or deuterium oxide, with a Varian A-60 n.m.r. spectrometer. Solvents were removed under diminished pressure below 50°.

*1,3,4,6-Tetra-*O*-acetyl-β-*D*-mannopyranose* (**1**). — In a typical experiment, a few mg of D-mannose was added to acetic anhydride (25 ml) followed by 70% perchloric acid (2 drops). To this yellow solution, D-mannose (6.6 g) was added portionwise with continuous stirring during 20 min, the internal temperature being kept at 40–45°. The mixture was kept at room temperature for 60 min, and then cooled to 15°, and phosphorus tribromide (4.3 ml) was added dropwise, the internal temperature being kept at 20–25°. Water (2.3 ml) was now added, and the mixture was kept for 90 min at room temperature. A solution of sodium acetate trihydrate (20 g) in water (25 ml) at 5° was slowly added, the internal temperature being kept at 35–40°, and the resulting yellow solution was kept at this temperature for 25 min. The solution was then poured onto ice, and the mixture was extracted with chloroform (3 × 30 ml); the chloroform extracts were combined, successively washed with cold water, cold sodium hydrogen carbonate solution, and cold water, and dried (magnesium sulfate). The solution was evaporated to dryness, and the residue was crystallized from anhydrous ether. The compound (2.3 g) was recrystallized from the same solvent, yielding pure **1** (1.8 g; 29%), m.p. 164–165°, $[\alpha]_D^{30}$ −23.6°; (*c* 1.4, chloroform); n.m.r. data: τ 4.15 (1-proton doublet, $J_{1,2}$ 1.5 Hz, axial H-1 of β-D form); τ 4.56 (1-proton triplet, J 10 Hz, H-4); τ 4.92 (1-proton quartet, $J_{2,3}$ 3 Hz, $J_{3,4}$ 9 Hz, H-3); τ 6.10 (broad signal, H-5); τ 5.73 (unresolved signal, H-2, H-6).

Anal. Calc. for $C_{14}H_{20}O_{10}$: C, 48.28; H, 5.74. Found: C, 48.64; H, 5.50.

*1,3,4,6-Tetra-*O*-acetyl-2-*O*-methyl-β-*D*-mannopyranose* (**2**). — Compound **1** (2 g) was dissolved in dichloromethane (10 ml) and the solution was cooled to 0°; boron trifluoride etherate (0.05 ml) was added and, while the temperature was kept at 0°, diazomethane in dichloromethane was added until a faint, yellow color persisted in the solution. After 30 min at 0°, a white solid (polymethylene) was filtered off, and the filtrate was evaporated. The residue was dissolved in ethanol, and the product crystallized when the solution was cooled. The compound was recrystallized from ethanol, to give pure **2** (1.15 g); from the mother liquors, a second crop (520 mg) was obtained; total yield, 85%, m.p. 98–99°, $[\alpha]_D^{30}$ −32.3° (*c* 1.1, chloroform). The i.r. spectrum showed no absorption in the hydroxyl region; n.m.r. data: τ 4.16 ($J_{1,2}$

1.5 Hz, 1-proton doublet, H-1); τ 4.57 (1-proton triplet, J 10 Hz, H-4); τ 4.93 (quartet, $J_{2,3}$ 3 Hz, $J_{3,4}$ 9 Hz, H-3); τ 5.76 (H-6), and τ 6.15 (unresolved multiplet, H-2 and H-5); τ 6.39 (OMe).

Anal. Calc. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found: C, 49.77; H, 5.97.

2-O-Methyl-D-mannose (3). — Compound 2 (1 g) was dissolved in methanol (10 ml), and the solution was treated with 0.9M methanolic sodium methoxide (1 ml) and kept for 12 h at room temperature. The solution was neutralized with Dowex-50 W resin, the resin was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in ethanol, and compound 3 crystallized on cooling and nucleating. Pure 3 (376 mg; 70%) had m.p. 138–139°, $[\alpha]_D^{23}$ +6.6 → +4.8° (24 h) (c 1.1, water) in accordance with reported values[2]. The n.m.r. spectrum showed that both anomers were present; τ 4.65 (doublet, $J_{1,2}$ 2 Hz, H-1 of α-D form) and τ 5.03 (doublet, $J_{1,2}$ 4 Hz, H-1 of β-D form); τ 6.53 (OMe).

ACKNOWLEDGMENTS

We thank Dr. G. O. Aspinall (University of Edinburgh) for a sample of crystalline 2-*O*-methyl-D-mannose, Dr. E. Dennler for the measurement of the i.r. and n.m.r. spectra, Dr. B. B. de Deferrari for the microanalyses, and FORGE (New York) for financial support.

Departamento de Química Orgánica,
Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires,
Perú 222, Buenos Aires (Argentina)

J. O. DEFERRARI
E. G. GROS*
I. O. MASTRONARDI

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(Received February 18th, 1967)

Carbohyd. Res., 4 (1967) 432–434

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

Acid hydrolysis of 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine and 1-*N*-acetyl-2-[(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylamine*

In a previous paper[1], the synthesis of 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (**1**) and 1-*N*-acetyl-2-[(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylamine (**2**) has been described. Comparison, by chromatography and electrophoresis, of these compounds with the 2-amino-2-deoxy-D-glucose–L-aspartic acid compound (**3**), isolated from hen-egg albumin[2], showed no significant differences between them. Although it has been concluded by comparison of the physical properties[3–8] that the structure of **3** was identical with that of **1**, the possibility that it was identical with that of **2** was not eliminated. Marks *et al.*[3] supported their conclusion by comparing the rate of hydrolysis of a glycopeptide, which contained approximately one molecule of leucine and one of aspartic acid, with the rates of hydrolysis of the glycine and aspartic acid derivatives of the D-glucose analogs of **1**, and with that of 2-[(β-L-aspartyl)amino]-2-deoxy-D-glucose (**4**)[9]. Recently, Micheel *et al.*[10] identified **4** and the α anomer of **1** in the hydrolyzate of **1**; the hydrolysis was performed with N hydrochloric acid for 40 min at 100° and the identification with an amino acid analyzer using pyridine–acetate–formic acid buffer (pH 3.05) as eluent.

In the present study, the possibility that **3** possesses structure **2** was eliminated by determination of the rates of hydrolysis of **1** and **2**; some observations on the isomerization of **1** during hydrolysis were made. The first-order rate constants of acid hydrolysis of **1** and **2**, under conditions identical to those applied by Micheel *et al.*[10], were calculated from the amounts of ammonia and aspartic acid liberated and determined with an amino acid analyzer using a 0.2N citric acid buffer (pH 3.25) eluent. For compound **1**, the rate constants were 6.4–10.4 $\times 10^{-3}$ min^{-1} and 5.9–10.4 $\times 10^{-3} min^{-1}$, respectively. These values are similar to each other and also to the value that Marks *et al.*[3] obtained in the study of the glycopeptide (Table I). For compound **2**, the rate constants, calculated from the amounts of ammonia and aspartic acid released by the cleavage of the amido linkages at C-1 and C-2 respectively, were 10.0 $\times 10^{-2} min^{-1}$ and 2.1 $\times 10^{-3} min^{-1}$. Therefore, the rates of hydrolysis of *N*-acetyl and *N*-aspartyl groups at C-1 are much higher than those of the corresponding groups at C-2. In addition, the rate of hydrolysis of the *N*-acetyl group at C-1 is much higher than that of the *N*-aspartyl group at the same position. These results show conclusively that, in compound **3**, L-aspartic acid is linked to the 1-amino group of 2-acetamido-2-deoxy-β-D-glucopyranosylamine, in agreement with previous conclusions.

In the chromatogram of the hydrolyzate of **1**, the presence of **1**, **4**, and 2-amino-2-deoxy-D-glucose was observed in addition to that of ammonia and aspartic acid,

*This is paper No. 16 in the series dealing with 2-amino-2-deoxy-D-glucose derivatives. Part XV: see ref. 1.

TABLE I

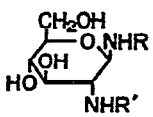
FIRST-ORDER RATE CONSTANTS OF HYDROLYSIS OF AMIDO LINKAGES[a]

Compound	Rate constants[b] (10^{-3} min)		
	Carbohydrate	Ammonia[c]	Aspartic acid[c]
Glycopeptide from egg albumin[3]	—	8.2	—
N-(β-L-Aspartyl)-β-D-glucosylamine[9]	4.9	—	—
N-Glycyl-β-D-glucosylamine[9]	7.8	—	—
4[9]	3.4	—	—
1	—	10.4(30) 5.9(40)[d]	10.4(30) 6.4(40)[d]
2	—	107(30) 100(40)[d]	2.1(40)[e]
5	—	87(20)[d]	—

[a]Each compound was hydrolyzed in N hydrochloric acid at 100°. [b]Each value was calculated from the amounts of liberated substances. [c]The number in parenthesis shows the reaction time. [d]The fragment was liberated from the amido linkages at C-1. [e]The fragment was liberated from the amido linkage at C-2.

but the presence of the α-D anomer of **1**, which was considered to be an intermediate in the transformation[10] of **1** into **4**, was not observed. Compound **4** was identified by comparing it with an authentic sample prepared through hydrolysis and hydrogenolysis of 2-{[α-benzyl *N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-4,6-*O*-benzylidene-2-deoxy-D-glucose[1].

In order to clarify the extent of the *N*→*N* acyl migration observed[10] in compound **1**, 1-*N*-acetyl-2-amino-2-deoxy-β-D-glucopyranosylamine (**5**) was prepared by hydrogenolysis of *N*-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylamine and was hydrolyzed under identical conditions. The rate constant, calculated from



1 R = β-L-aspartyl, R′ = ac
2 R = ac, R′ = β-L-asparyl
5 R = ac, R′ = H

the amount of ammonia, was 8.7×10^{-2} min^{-1} and was similar to that of the *N*-acetyl group of **2**. No N→N acetyl migration was observed, however; *i.e.* 2-acetamido-2-deoxy-D-glucose was not detected, and only 2-amino-2-deoxy-D-glucose hydrochloride could be identified by paper chromatography [ethyl acetate–pyridine–water (12:5:4) and butanol–water (43:7)]. Although it was found that **1** anomerized in 2-(dimethylamino)ethanol solution at room temperature[10], neither acyl migration nor anomerization was observed in the case of **5** under the same conditions, or in pyridine–water (1:1) for 2 days[11]. These observations suggest that the rate of hydrolysis of the 1-*N*-acetyl group of **5** is much too high to permit either acyl migration or the 1-*N*-acyl group to take the α-D configuration in the intermediate step of the migration.

EXPERIMENTAL

Determination of the rate of hydrolysis and analysis of the resulting products. — Each compound (3–4 mmoles) was dissolved in N hydrochloric acid (2 ml) and heated in a sealed tube at 100°. After 20–40 min, the hydrolyzate was diluted with distilled water (10 ml), and aliquots (1 or 2 ml) of this solution were analyzed with an amino acid analyzer Hitachi Model KLA-2, using a column packed with Amberlite CG-120 Type III, 1 × 15 cm for ammonia and 1 × 150 cm for other compounds; the eluent was 0.2N citric acid buffer (pH 3.25) percolating at 30 ml/h at 50°. The amount of each product was determined by comparison with a standard. For example, 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine was hydrolyzed for 40 min. The starting material (54%), 2-[(β-L-aspartyl)amino]-2-deoxy-D-glucose (2.2%), aspartic acid (22%), 2-amino-2-deoxy-D-glucose (9.6%), and ammonia (22%) were eluted with an elution time of 3 h, 4 h, 5 h, 15.5 h, and 3 h, respectively.

2-[(β-L-Aspartyl)amino]-2-deoxy-D-glucose (**4**). — 2-{[α-Benzyl *N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-4,6-*O*-benzylidene-2-deoxy-D-glucose[1] (300 mg) was suspended in 60% acetic acid, and the suspension was heated for 1 h on a boiling water bath. After extraction of the benzaldehyde with petroleum ether, the solution was evaporated to give a crystalline residue (200 mg), which was directly hydrogenated in 50% aq. ethanol in the presence of palladium–charcoal for 24 h at room temperature. After filtration of the catalyst, the filtrate was evaporated, and the residue was crystallized from a small amount of water to give white crystals (89 mg, 60%), m.p. > 275°, $[\alpha]_D^{23}$ +27.0 → 25.5° (after 5 h, *c* 0.5, water); reported values: m.p. > 260°, $[\alpha]_D$ +29 → 22° (*c* 0.28, water)[9]; m.p. 300° (dec.), $[\alpha]_D$ +33.2 → 28.2° (*c* 0.184, water)[10].

Anal. Calc. for $C_{10}H_{18}N_2O_8$: C, 40.81; H, 6.27; N, 9.52. Found: C, 40.75; H, 6.57; N, 9.36).

1-N-Acetyl-2-amino-2-deoxy-β-D-glucopyranosylamine (**5**). — 1-*N*-Acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylamine[1] was hydrogenolyzed in the same manner as described above. Recrystallization from aq. ethanol gave white crystals (yield 90%), m.p. 224–225° (dec.), $[\alpha]_D^{23}$ −4.3° (*c* 0.5, water).

Anal. Calc. for $C_8H_{16}N_2O_5$: C, 43.63; H, 7.32; N, 12.72. Found: C, 43.14; H, 7.10; N, 12.79.

Department of Chemistry,
Tokyo Institute of Technology,
Ookayama, Meguro-ku, Tokyo (Japan)

J. YOSHIMURA
H. HASHIMOTO

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(Received September 15th, 1966; in revised form, January 24th, 1967)

Carbohyd. Res., 4 (1967) 435–438

An improved procedure for the preparation of the anomeric D-xylofuranose tetrabenzoates

The D-xylofuranose tetrabenzoates can be prepared either through the methyl α,β-D-xylofuranosides[1,2] or through 1,2-*O*-isopropylidene-α-D-xylofuranose[2], but the procedure involving the methyl glycosides is superior, in that no intermediates need be isolated and the yields are higher and more consistent[2]. Having repeated this preparation many times, we have effected a number of improvements over the original procedure[1]; and, as these benzoates are useful intermediates for the synthesis of a variety of D-xylofuranose derivatives, the revised preparation is now described.

EXPERIMENTAL

A suspension of pure, finely divided D-xylose (100 g) in anhydrous methanol (2 l) is stirred at room temperature while concentrated sulfuric acid (15 ml) is added. Stirring is continued until the sugar has dissolved (*ca.* 15 min), and the reaction mixture is kept at room temperature. At 15-min intervals, two drops of the solution are added to a mixture of six drops of Fehling solution A ($CuSO_4$) and nine drops of Fehling solution B, and the resulting test solution is heated for 2 min at 100°. Inspection of a consecutive series of such tests (in which the cuprous oxide has settled) clearly reveals the point at which the reducing power of the reaction mixture has virtually disappeared. The time required to reach this point varies, of course, with the temperature of the reaction mixture; it is about 2.5 h at 24°. Prolongation of the reaction time beyond the critical point results in progressively lowered yields, owing to the conversion of the methyl D-xylofuranosides into methyl D-xylopyranosides. At the point where the reducing power has virtually disappeared, part of the acid present is neutralized by shaking the solution for a few minutes with methanol-washed Amberlite IR-45 (OH^-) (50 ml); the solution is then passed through a column (3.7 × 34 cm) of the same ion-exchange resin, and methanol (500 ml) is used to wash the column. The washings are combined with the main solution and concentrated *in vacuo*. The residual syrupy

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mixture of crude methyl D-xylofuranosides is finally kept at 40° (bath)/0.5 mm for 1 h, in order to remove residual methanol; omission of this step results in markedly lowered yields.

The mixture is dissolved in anhydrous pyridine (1 liter) and, after being cooled in an ice bath, the stirred solution is treated gradually with benzoyl chloride (400 ml). After being kept overnight at room temperature, the reaction mixture is cooled, treated with ice–water (50 ml), and kept for 30 min at room temperature; it is then poured onto a stirred mixture of ice and water (2.5 l), and the crude methyl 2,3,5-tri-*O*-benzoyl-D-xylofuranosides are extracted into dichloromethane (4 × 125 ml). The extracts are combined, and washed successively with cold 3N sulfuric acid and saturated aqueous sodium hydrogen carbonate solution, dried (anhydrous sodium or magnesium sulfate), and filtered through a bed of Celite. Concentration of the solution at 35° (bath)/10 mm affords a honey-like syrup.

The syrup is dissolved in a 32% solution of hydrogen bromide in acetic acid (450 ml). After being kept for 1 h at room temperature, the reaction mixture is diluted with dichloromethane (1 liter), and washed successively with water, saturated aqueous sodium hydrogen carbonate solution, and water. While still damp, the solution is added to a mixture of acetone (1800 ml), water (50 ml), and silver carbonate (100 g). The suspension is stirred for 30 min at room temperature, and is then filtered through decolorizing carbon (Darco G-60 or Darco X) overlayered with Celite. The solvents are removed from the filtrate at 40° (bath)/10 mm, giving syrupy 2,3,5-tri-*O*-benzoyl-D-xylofuranose

This syrup is cooled to 0°, and dissolved in a mixture of benzoyl chloride (140 ml) and anhydrous pyridine (500 ml)*. The reaction mixture is kept for 2 h at room temperature, treated with water (10 ml), and kept for 30 min at room temperature. It is then poured onto a mixture of ice and water (2.5 l), and the precipitated syrup is nucleated with crystals** of both anomers of D-xylofuranose tetrabenzoate. As the syrup hardens, the water is decanted, and the solid product is washed with several portions of fresh water. The crystalline mass is then washed with methanol (2 × 300 ml), and finally kept overnight under methanol (500 ml). After being washed with more methanol, followed by drying at room temperature, the mixture of anomeric D-xylofuranose tetrabenzoates weighs, typically, 247 g (65%). For many purposes (such as the preparation of D-xylofuranosides *via* the 2,3,5-tri-*O*-benzoyl-D-xylofuranosyl halides), this mixture is suitable for use without further purification.

The individual anomers may be separated by taking advantage of the relatively low solubility of the α-D anomer in ethyl acetate, and of the acetone solvate of the β-D anomer in acetone. Thus, the mixture is stirred for 5 min at 5° with ethyl acetate (500 ml), and the insoluble portion is removed by filtration at 5°(giving filtrate A);

*If the syrup is dissolved in pyridine prior to the addition of benzoyl chloride, marked darkening results.

**Nucleating crystals of the α-D anomer may be obtained by rubbing a sample of the syrup with methanol; those of the β-D anomer are best obtained by flowing chromatography of a sample of the mixture on neutral alumina.

after being washed with cold ethyl acetate (4 × 25 ml) and dried, this fraction (147 g) melts at 153–156° (corr.) and shows $[\alpha]_D^{20}$ +160° (*c* 0.5, chloroform). It is dissolved in hot ethyl acetate (7 ml/g) and crystallization is allowed to proceed at room temperature and then at 5°. The crystals are removed by filtration (giving filtrate B), washed with three 50-ml portions of cold ethyl acetate (giving filtrate C), and dried, to give pure α-D-xylofuranose tetrabenzoate (123 g, 33%), m.p. 164–165° (corr.), $[\alpha]_D^{20}$ +170° (*c* 1.1, chloroform). Concentration of the mother liquor affords a further crop (8.3 g) of equally pure α-D-xylofuranose tetrabenzoate.

Filtrates A, B, and C are combined, and concentrated at 35° (bath)/10 mm to a heavy syrup which is dissolved in acetone (100 ml); the solution is nucleated* with the acetone solvate of β-D-xylofuranose tetrabenzoate, if necessary. After the solution has been kept for 4 h at 5°, the crystals are removed by filtration, washed with 1:3 (v/v) acetone–methanol (3 × 25 ml) and dried at 55°/1 mm; the acetone-free product (49 g) has m.p. 99–101° (corr.). It is dissolved in boiling methanol (30 ml/g), one third of the methanol is removed by evaporation, and the solution is cooled to room temperature and then to 5°, to give 44 g (12%) of pure β-D-xylofuranose tetrabenzoate, m.p. 108–109° (corr.), $[\alpha]_D^{20}$ +11.2° (*c* 1.0, chloroform).

All mother liquors may readily be processed for further crops of both anomers, the material rich in the α-D anomer being crystallized from ethyl acetate, and the material rich in the β-D anomer being crystallized from acetone.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014 (U. S. A.)

HEWITT G. FLETCHER, JR.
HARRY W. DIEHL

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(Received February 15th, 1967)

Carbohyd. Res., 4 (1967) 438–440

*Nucleating crystals of the acetone solvate may be obtained by crystallizing a sample of chromatographed β-D-xylofuranose tetrabenzoate from acetone.

Gas chromatography of the trimethylsilyl ethers of some less-common disaccharides

Various papers have been published on the identification and determination by gas chromatography of trimethylsilylated (TMS) derivatives of simple sugars[1], their methyl glycosides[2], the more-common disaccharides[1,3], and mixtures of simple sugars[4]. The application of this procedure to hydrolysates of biological materials is proving very valuable[5]. It has considerable advantages over other methods because of the simplicity of the operations involved and the small size of the samples required.

The present note gives the retention times of the TMS ethers of a number of the rarer disaccharides and, where sufficient material was available, of the corresponding alcohols (see Tables I and II). The retention times of the different sugars are determined largely by the conformation of the molecules[6], and, since each disaccharide, when recovered from aqueous solution, exists as an equilibrium mixture of the anomeric, pyranoid forms of the reducing unit and, where not linked at C-4, of small proportions of the furanoid forms, a number of peaks is obtained for each disaccharide. Whilst the multiplicity of these peaks is, in certain instances, advantageous in characterisation, it may cause extensive overlapping and prevent identification of some of the constituents in a complex mixture. In contrast, the disaccharide alcohols, where the reducing unit has been converted into the acyclic form, give single peaks. This results in ready separation of disaccharide alcohols (composed of hexoses, pentoses, or 6-deoxyhexoses), but, on the whole, gives poor separation of isomeric disaccharides.

The less-common disaccharides included in this note were isolated and characterised from the partial hydrolysates of different algal polysaccharides, and the data should be of value to other workers in this field.

EXPERIMENTAL

The trimethylsilyl ethers were prepared by treating the dried disaccharide or alcohol (0.2–10 mg) with chlorotrimethylsilane (0.1 ml) and hexamethyldisilazane (0.2 ml) in dry pyridine (1 ml), and warming the mixture in a closed tube for 2 h at 40°. [In more-recent preparations, it was found to be advantageous to use the ready-mixed reagent, "Sil-Prep", from Applied Science Laboratories Inc., State College, Pennsylvania.] The pyridine was removed on a rotary evaporator under diminished pressure, and the trimethylsilylated disaccharide was dissolved in an appropriate volume of ether depending on the initial weight of disaccharide.

The respective alcohols were formed by treating the disaccharide with a 2% aqueous solution of potassium borohydride for 24 h, followed by neutralisation of the excess borohydride with glacial acetic acid, shaking with Amberlite IR-120 (H^+) resin, evaporation, and several distillations of methanol from the residue to remove borate. The disaccharide alcohol was finally dried with ethanol and benzene before conversion into the TMS derivative.

TABLE I

GAS CHROMATOGRAPHY OF TRIMETHYLSILYL ETHERS OF DISACCHARIDES

Disaccharides	*Apiezon K*[a]	*SE30*[a]
Sucrose	1.0	1.0
Maltose	1.58 vs; 2.31 vw	1.40
Cellobiose	2.32 s; 1.33 m	1.73 s; 1.19 w
Laminaribiose[7]	2.42 s; 2.0 m	1.62
4-*O*-β-D-Mannopyranosyl-D-mannopyranose[8]	1.37 s, w.sh; 2.01 w	1.03 s; 1.4 m
3-*O*-β-D-Galactopyranosyl-D-galactose[9]	1.0	1.0 vs; 1.97 m
6-*O*-β-D-Galactopyranosyl-D-galactose[10]	1.57 s; 2.32 w	2.5
3-*O*-β-D-Galactopyranosyl-L-arabinose[11]	1.03	0.96 s; 0.80 m; 1.19 w
4-*O*-β-D-Mannopyranosyl-L-gulose[12]	1.45	1.31
3-*O*-β-L-Arabinopyranosyl-L-arabinose[9]	0.47 s; 0.37 s	1.1 s; 0.67 m; 0.74 w
4-*O*-β-D-Xylopyranosyl-D-xylose	1.65	0.89 vs; 0.69 w
3-*O*-β-D-Xylopyranosyl-D-xylose[13]	0.95 vs; 1.2 w; 0.76 w	0.56 s; 0.92 w; 0.42 w
2-*O*-α-L-Fucopyranosyl-L-fucose[14]	0.71	0.51
3-*O*-α-L-Fucopyranosyl-L-fucose[14]	0.67	0.46 vs; 0.95 w
4-*O*-α-L-Fucopyranosyl-L-fucose[14]	0.81 s; 0.63 m	0.57 s; 0.51 sh

[a]Retention times at 210°. The relative intensities of peaks are indicated by vs, very strong; s, strong; m, medium; w, weak. Where incomplete resolution of peaks occurs, sh = shoulder.

TABLE II

GAS CHROMATOGRAPHY OF TRIMETHYLSILYL ETHERS OF DISACCHARIDE ALCOHOLS

Disaccharide Alcohol	*Retention times at 210°*	
	Apiezon K	*SE30*
Sucrose	1.0	1.0
Maltitol	1.86	1.90
Cellobiitol	1.50	1.70
Laminaribiitol	2.0	1.90
4-*O*-β-D-Mannopyranosyl-D-mannitol	1.90	2.0
4-*O*-β-D-Mannopyranosyl-L-gulitol	—	2.0
3-*O*-β-L-Arabinopyranosyl-L-arabinitol	0.47	0.37
4-*O*-β-D-Xylopyranosyl-D-xylitol	0.58	0.46
2-*O*-α-L-Fucopyranosyl-L-fucitol	0.89	0.77
3-*O*-α-L-Fucopyranosyl-L-fucitol	0.92	0.75
4-*O*-α-L-Fucopyranosyl-L-fucitol	0.83	0.69
Melibiitol	—	2.7

A Pye Argon Chromatograph, with β-ionisation detector and glass columns (4ft × 4 mm), was used throughout, with argon as the carrier gas at a rate of 70–80 ml/min, and a chart speed of 5 in/h. Several stationary phases were tried. Acceptable results were obtained with (*a*) 15% Apiezon K on Celite, and (*b*) 3% SE30 on Gas Chrom. P, both at 210°. Sucrose was used as a standard, and maltose and cellobiose were included for comparison purposes. The relative retention times (sucrose = unity) are given in the Tables.

The author thanks Mrs. M. Hunt for technical assistance, Dr. R. H. Côté for the gift of the fucobioses, and Professor D. J. Manners for the gift of β-(1→4)-xylobiose. The work was financed by a grant from the Science Research Council, and the gift of a gas chromatograph by the Wellcome Trust.

Chemistry Department,
Royal Holloway College,
Englefield Green, Surrey (Great Britain)

ELIZABETH PERCIVAL

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(Received December 19th, 1966; in revised form, February 23rd, 1967)

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

A FACILE ROUTE TO *trans* CYCLIC CARBONATES OF SUGARS*

W. M. DOANE, B. S. SHASHA, E. I. STOUT, C. R. RUSSELL, and C. E. RIST
*Northern Regional Research Laboratory***, *Peoria, Ill. 61604 (U. S. A.)*
(Received February 27th, 1967)

Carbonic esters of carbohydrates, used as intermediates in syntheses, are often prepared by treatment of the carbohydrate with an alkyl chloroformate in the presence of a base. The type of carbonate formed depends on the structure of the parent compound and on the base selected[1]. Reaction of those pyranosides that possess vicinal *cis*-hydroxyl groups with alkyl chloroformate in the presence of aqueous alkali reportedly[1] gives five-membered, cyclic carbonates. With pyridine or similar organic base at low temperature, only acyclic (alkyl) carbonates result. Regardless of the conditions used, pyranosides that have only vicinal *trans*-hydroxyl groups give the acyclic carbonate[1]. However, we recently published[2] a synthesis of a cyclic carbonate from such hydroxyl groups.

Bokadia and coworkers[3] reported the formation of a cyclic carbonate from a flavan *trans*-3,4-diol upon treatment with ethyl chloroformate and triethylamine. We have examined the reaction of these reagents with pyranoid sugars that have vicinal *trans*-hydroxyl groups, and here report a facile route for formation of cyclic carbonates from such diols. This route appears to have broader applicability than our earlier method[2], which employs a thionocarbonate as the intermediate.

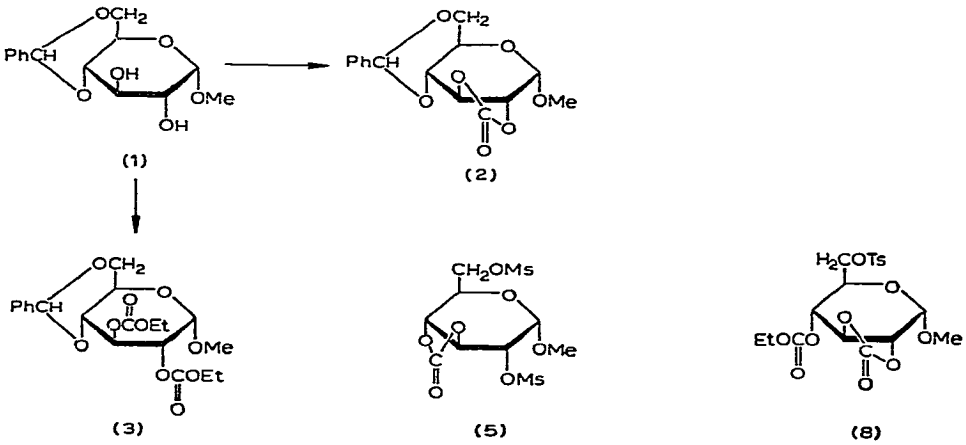
When methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**) was treated with ethyl chloroformate and triethylamine in the molar proportions of 1:5.6:3.6, t.l.c. showed that the reaction product was a multicomponent mixture. The i.r. spectrum of the mixture revealed carbonyl absorption for acyclic and cyclic carbonate groups. When the proportions used were 1:30:10, t.l.c. revealed the presence of a single component having an R_F value identical with that of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate[2] (**2**); this compound was isolated crystalline in 89% yield, and identified as the cyclic carbonate **2**.

Treatment of **1** with ethyl chloroformate and pyridine under similar conditions gave material that showed a single component having an R_F value greater than that of **2**. The analysis of this product was consistent with the structure of methyl 4,6-*O*-benzylidene-2,3-di-*O*-(ethoxycarbonyl)-α-D-glucopyranoside (**3**).

When **1** was treated with 2.3 molar equivalents of ethyl chloroformate and an

*Presented before the Division of Carbohydrate Chemistry, 153rd National Meeting of the American Chemical Society, Miami Beach, Florida, April 1967.
**This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

excess of pyridine, the two monosubstituted isomers, methyl 4,6-*O*-benzylidene-2-*O*-(ethoxycarbonyl)-α-D-glucopyranoside and methyl 4,6-*O*-benzylidene-3-*O*-(ethoxycarbonyl)-α-D-glucopyranoside, were isolated as crystalline derivatives in the ratio of



24:1. When these isomers were treated with triethylamine, neither would cyclize to form the 2,3-carbonate, and they were recovered unchanged. Thus, neither of the mono-*O*-ethoxycarbonyl derivatives of **1** seems to be an intermediate in the formation of **2**.

Treatment of methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside (**4**) with ethyl chloroformate and triethylamine (1:37:13 molar proportions) at 5° gave a major component (t.l.c.). This compound was isolated crystalline in 47% yield, and is formulated as methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside 3,4-carbonate (**5**) by elemental analysis and physical data, and by its quantitative conversion into **4** on mild, alkaline hydrolysis.

Reaction of **4** with ethyl chloroformate and triethylamine in the molar proportions of 1:37:26 at 25° gave two products in about equal amounts, together with unreacted **4**. The product having the higher R_F value gave analyses consistent with the structure of the 3,4-di-*O*-ethoxycarbonyl derivative (**6**) of **4**, whereas the product having the lower R_F value was identified as **5**. The di-*O*-ethoxycarbonyl derivative (**6**) was prepared in quantitative yield by treatment of **4** with ethyl chloroformate and pyridine.

Methyl 6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside (**7**) reacted with ethyl chloroformate and triethylamine (1:37:13 molar proportions) at 5° to give virtually only one product. The i.r. spectrum of this product had absorption bands for cyclic and acyclic carbonate groups, and no absorption for a hydroxyl group. Elemental analysis was consistent with a derivative of **7** that contained one ethoxycarbonyl group and one cyclic carbonate group. As the possibility exists of forming either the 2,3- or 3,4-cyclic carbonate, the reaction product was chromatographed in different solvent systems.

In all systems examined, the product was not resolved and presumably only one isomer was present. On the basis of its i.r. spectrum, to be discussed later, the product is assumed to be methyl 4-*O*-(ethoxycarbonyl)-6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside 2,3-carbonate (**8**).

The reaction of 6-*O*-tritylamylose with ethyl chloroformate and triethylamine also gave a cyclic carbonate derivative. However, the reaction product also contained a considerable proportion of the ethoxycarbonyl derivative, as evidenced by its i.r. spectrum. Although the reaction mixture was kept for several h, there remained a large proportion of unsubstituted hydroxyl groups, as shown by the i.r. spectrum.

We have previously reported that the cyclic carbonate **2** shows two absorption bands for the carbonyl group near 5.45 μ (1835 cm^{-1}) and 5.55 μ (1802 cm^{-1}); we recorded the spectra by using either a film or Nujol mull. As Hough and co-workers[4] have stated that the isomeric methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 2,3-carbonate (which is a *cis*-fused, cyclic carbonate) shows a single absorption band in this region, we assumed that the double peak is characteristic of the *trans*-fused structure. To corroborate this assumption, we prepared the D-mannoside 2,3-carbonate, and compared its i.r. spectrum with those of products **2**, **3**, **5**, **6**, and **8**, measured under similar conditions. The carbonyl stretching-frequencies are recorded in Table I.

TABLE I

CARBONYL STRETCHING-FREQUENCIES OF CARBONATE COMPOUNDS

Compound	$\lambda_{max}^{bromoform}$, cm^{-1}			
Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 2,3-carbonate		1825	1809	
Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate (**2**)	1840	1827	1809	
Methyl 4-*O*-(ethoxycarbonyl)-6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside 2,3-carbonate (**8**)	1841	1822	1810	1750
Methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside 3,4-carbonate (**5**)	1844		1810	
Methyl 4,6-*O*-benzylidene-2,3-di-*O*-(ethoxycarbonyl)-α-D-glucopyranoside (**3**)				1750
Methyl 3,4-di-*O*-(ethoxycarbonyl)-2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside (**6**)				1754

In bromoform solution, the D-mannoside carbonate exhibits two bands, at 1809 and 1825 cm^{-1}. The corresponding *trans*-carbonate (**2**) showed virtually the same bands, but, in addition, a third band, at 1840 cm^{-1}, was seen. This third band was also present in the spectrum of the 6-*O*-*p*-tolylsulfonyl derivative (**8**), which also showed the two bands at 1810 and 1822 cm^{-1} plus a band at 1750 cm^{-1} for the *O*-ethoxycarbonyl group.

The *trans*-fused 3,4-carbonate group of **5** also has the absorption at the highest frequency (1844 cm^{-1}). However, its spectrum was different in that the carbonyl

band near 1825 cm^{-1} was not observed. Compounds **3** and **6** showed carbonyl absorption bands at 1750 and 1754 cm^{-1}, respectively, for the acyclic carbonate groups.

On the basis of the i.r. spectra of the carbonates in bromoform solution, the carbonyl absorption for the *trans*-fused carbonate group in the 1840–1844 cm^{-1} region seems to be characteristic of such a structure.

EXPERIMENTAL

Melting points were taken in a calibrated, Thomas–Kofler* melting-point apparatus. Optical rotations were measured at 5893 Å with a Rudolph* polarimeter. I.r. spectra were recorded with a Perkin–Elmer* Model 621 spectrophotometer with bromoform solutions. T.l.c. was performed on Silica Gel G by using 4:1 (v/v) carbon disulfide–ethyl acetate and detection was with methanol containing 5% of sulfuric acid.

Methyl 4,6-O-benzylidene-α-D-glucopyranoside 2,3-carbonate (**2**). — A solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**, 1.0 g) in *p*-dioxane (5 ml) was treated with ethyl chloroformate (10 ml), and subsequently cooled in an ice–water bath. While the solution was being swirled, a solution of triethylamine (5 ml) in benzene (30 ml) was added dropwise. After being kept for 1 h at 5°, the reaction mixture was successively washed with dilute hydrochloric acid and then water until neutral, dried, and evaporated to a syrup that showed one component by t.l.c. When the syrup was dissolved in chloroform, and hexane was added, the product (**2**) crystallized, to give 970 mg (89% yield), m.p. 115–117° (recrystallized from ether–hexane), $[\alpha]_D^{23}$ +69° (*c* 1.0, chloroform), λ_{max} 1809, 1827, and 1840 cm^{-1} [*trans*-cyclic O(C=O)O].

Anal. Calc. for $C_{15}H_{16}O_7$: C, 58.4; H, 5.2; mol. wt. 308. Found: C, 58.2; H, 5.3; mol. wt. 284 (Rast, in camphor).

The carbonate (**2**) was recovered in 75% yield when a solution of **1** (2.82 g) in *p*-dioxane (25 ml) and benzene (150 ml) was treated with ethyl chloroformate (25 ml) and triethylamine (25 ml) at room temperature.

Methyl 4,6-O-benzylidene-2,3-di-O-(ethoxycarbonyl)-α-D-glucopyranoside (**3**). — To a solution of **1** (5.0 g) in pyridine (20 ml) were added benzene (20 ml) and chloroform (20 ml). The solution was placed in an ice–water bath, and stirred while a solution of ethyl chloroformate (20 ml) and benzene (20 ml) was added dropwise during 2.5 h. The reaction mixture was then diluted with benzene (100 ml) and filtered. The filtrate was evaporated to a syrup which was dissolved in ether, and the solution was successively washed with dilute hydrochloric acid and water, dried (sodium sulfate), and evaporated under diminished pressure at 40°, to give **3** (7.5 g, 99%) as a colorless syrup, $[\alpha]_D^{24}$ +67° (*c* 1.0, chloroform), λ_{max} 1750 cm^{-1} (C=O).

Anal. Calc. for $C_{20}H_{26}O_{10}$: C, 56.3; H, 6.1. Found: C, 56.7; H, 6.5.

Methyl 4,6-O-benzylidene-2-O-(ethoxycarbonyl)-α-D-glucopyranoside and methyl

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

4,6-O-benzylidene-3-O-(ethoxycarbonyl)-α-D-glucopyranoside. — A solution of **1** (10.5 g) in pyridine (25 ml) and benzene (100 ml) was cooled to 5°, and ethyl chloroformate (8 ml) in benzene (100 ml) was added dropwise during 2 h. The reaction mixture was stirred for 4 h at room temperature, and then kept overnight. The clear solution was separated from a syrup that had formed, and was successively washed with N hydrochloric acid and water, dried (sodium sulfate), and evaporated under diminished pressure to a syrup (12.0 g). T.l.c. revealed a minor component that had the same R_F value as **3**, and a major component(s) that streaked behind **3**. The latter was resolved into two components when t.l.c. was performed in 19:1 (v/v) chloroform–acetone. The syrup was dissolved in chloroform (10 ml), hexane (20 ml) was added, and the solution was placed on a column of Adsorbosil (340 g) and eluted with 5:3 (v/v) hexane–chloroform. Compound **3** was eluted first, and gave 3.0 g of syrup. The second component that separated was crystallized from ether–hexane to give 7.2 g, m.p. 100–101°, $[\alpha]_D^{24}$ +96° (*c* 1.0, chloroform). This product was identified as the 2-*O*-ethoxycarbonyl isomer, as it was converted into the (known) methyl 4,6-*O*-benzylidene-3-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside by treatment first with *p*-toluenesulfonyl chloride and then with barium hydroxide. The third fraction separating from the column was crystallized from ether–hexane to give 0.3 g, m.p. 143–144°, $[\alpha]_D^{24}$ +101° (*c* 1.0, chloroform). This product was converted into the (known) methyl 4,6-*O*-benzylidene-2-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside by the procedure described above, and is thus the 3-*O*-ethoxycarbonyl isomer.

Anal. Calc. for $C_{17}H_{22}O_8$: C, 57.5; H, 6.2. Found (for the 2- and 3-isomers, respectively): C, 57.6, 57.1; H, 6.5, 6.3.

Methyl 2,6-di-O-(methylsulfonyl)-α-D-glucopyranoside 3,4-carbonate (**5**). — Methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside[5] (**4**, 0.5 g) was dissolved in *p*-dioxane (5 ml), and ethyl chloroformate (5 ml) was added. The solution was cooled in an ice–water bath, a solution of triethylamine (2.5 ml) in benzene (20 ml) was added dropwise, and the reaction mixture was kept overnight at 5°. The solution was decanted, from the precipitate (*A*), into dilute hydrochloric acid. The organic phase was separated, dried, and evaporated to a syrup. T.l.c. revealed two spots, one for **5** and one having a higher R_F value. When the syrup was dissolved in acetone, compound **5** crystallized readily. More of the product was recovered from precipitate *A* by washing it with water, and dissolving the water-insoluble residue in acetone. A total yield of 250 mg (47%) of crystalline **5** was obtained. Recrystallization from acetone gave m.p. 175–177°, $[\alpha]_D^{23}$ +100° (*c* 1.0, acetone), λ_{max} 1810 and 1844 cm^{-1} [O(C=O)O].

Anal. Calc. for $C_{10}H_{16}O_{11}S_2$: C, 31.9; H, 4.3; S, 17.0; mol. wt. 376. Found: C, 32.1, H, 4.4; S, 16.9; mol. wt. 391 (Rast, in camphor).

The 3,4-carbonate was also formed when **4** (0.5 g), in *p*-dioxane (5 ml) and benzene (30 ml), was treated with ethyl chloroformate (5 ml) and triethylamine (5 ml) at room temperature. After 16 h, the mixture was filtered, and the filtrate was successively washed with N hydrochloric acid and water, dried, and evaporated to a syrup. T.l.c. of the syrup revealed three components having almost equal intensities. The spot of lowest R_F corresponded to the starting compound (**4**), whereas the center spot corres-

ponded to the cyclic carbonate (**5**). The fastest moving component had an R_F value the same as that of methyl 3,4-di-*O*-(ethoxycarbonyl)-2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside (**6**), a product readily obtained by treatment of **4** with ethyl chloroformate and pyridine.

Treatment of **5** *with barium hydroxide.* — To a solution of **5** (75 mg) in acetone (100 ml) was added 10 ml of 1% aqueous barium hydroxide. After 5 min, the mixture was treated with solid carbon dioxide, and filtered. Evaporation of the filtrate gave a syrup (70 mg, 100% yield) that crystallized on addition of a mixture of acetone and chloroform. The i.r. spectrum and m.p. were identical with those of authentic **4**.

*Methyl 3,4-di-*O-(*ethoxycarbonyl*)-*2,6-di-*O-(*methylsulfonyl*)-α-D-*glucopyranoside* (**6**). — A solution of **4** (0.50 g) in pyridine (25 ml) and chloroform (25 ml) was treated with ethyl chloroformate (10 ml) at room temperature. After 1.5 h, the solution was concentrated at 40° under diminished pressure to about 5 ml, and this solution was diluted with chloroform, washed with water, and evaporated to a thin syrup that became viscous after it has been washed with small portions of hexane. When the resulting syrup was dissolved in acetone, and the solvent was removed in an oven at 50°, a crystalline mass remained; it was identified as **6**, yield 710 mg (100%), m.p. 98–103°. Recrystallization from ethanol gave m.p. 118–119°, $[\alpha]_D^{23}$ +98° (*c* 1.0, acetone), λ_{max} 1754 cm^{-1} (C=O).

Anal. Calc. for $C_{15}H_{26}O_{14}S_2$: C, 36.4; H, 5.3; S, 13.0. Found: C, 36.2; H, 5.3; S, 12.7.

*Methyl 4-*O-(*ethoxycarbonyl*)-*6-*O-(p-*tolylsulfonyl*)-α-D-*glucopyranoside 2,3-carbonate* (**8**). — To a solution of methyl 6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside (**7**, 0.5 g) in *p*-dioxane (5 ml) were added benzene (30 ml) and ethyl chloroformate (5 ml). The solution was placed in an ice–water bath, and a solution of triethylamine (2.5 ml) in benzene (20 ml) was added dropwise. After the reaction mixture had been kept for 20 h at 5°, it was successively washed with N hydrochloric acid and water, dried, and evaporated, to give a glassy solid. T.l.c. of this product in several solvents revealed only one spot. The solid weighed 540 mg (84% based on **8**) and gave λ_{max} 1750 cm^{-1} (acyclic carbonate), 1810, 1822, and 1841 cm^{-1} (cyclic carbonate).

Anal. Calc. for $C_{18}H_{22}O_{11}S$: C, 48.4; H, 4.9; S, 7.2; mol. wt. 446. Found: C, 48.4; H, 4.7; S, 7.1; mol. wt. 422 (Rast in camphor).

*Reaction of 6-*O-*tritylamylose with ethyl chloroformate.* — 6-*O*-Tritylamylose (0.5 g), having a degree of substitution of 1.0, was suspended in *p*-dioxane (100 ml) and treated with ethyl chloroformate (10 ml). The mixture was cooled in an ice–water bath, and a solution of triethylamine (4 ml) in *p*-dioxane (30 ml) was added dropwise during 1 h. After the reaction mixture had been kept for several h at 5°, it was added to 500 ml of water. The precipitate that formed was collected, washed several times with water, and dried at 100°. The i.r. spectrum (KBr disc) of this product showed carbonyl absorption for cyclic and acyclic carbonate groups in the ratio of 3:2. A substantial absorption corresponding to hydroxyl groups demonstrated that the substitution was incomplete.

ACKNOWLEDGMENTS

We thank Walter Boyd for recording the i.r. spectra, and Mrs. Clara McGrew and Mrs. Bonita Heaton for the microanalyses.

SUMMARY

Five-membered, cyclic carbonates are formed when vicinal *trans*-hydroxyl groups in D-glucopyranosides are treated with ethyl chloroformate in the presence of triethylamine. With these reagents, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate (**2**), methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside 3,4-carbonate (**5**), and methyl 4-*O*-(ethoxycarbonyl)-6-(*O*-*p*-tolylsulfonyl)-α-D-glucopyranoside 2,3-carbonate (**8**) were prepared. In contrast, when pyridine is the base present, only acyclic carbonates are formed.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

MASSENSPEKTROMETRISCHE UNTERSUCHUNGEN

XVII. MITTEILUNG*. DIE MASSENSPEKTROMETRISCHE BESTIMMUNG DER VERKNÜPFUNGSSTELLEN AMINOZUCKERHALTIGER POLYSACCHARIDE. MASSENSPEKTREN VON DERIVATEN DER 2-AMINO-2-DESOXY-D-GALAKTOSE

K. HEYNS, G. KIESSLING UND D. MÜLLER
Institut für Organische Chemie der Universität Hamburg (Deutschland)

(Eingegangen den 1. Dezember, 1966; modifiziert den 13. Februar, 1967)

Zur Ermittlung der Bindungen zwischen den Bausteinen eines Polysaccharids wird häufig erschöpfend methyliert. Nach der Hydrolyse des Polysaccharids können die einzelnen Zucker papier- oder dünnschichtchromatographisch identifiziert werden. Dazu ist es jedoch notwendig, die entsprechenden Vergleichspräparate herzustellen. Versuche in unserem Institut haben gezeigt, daß man die über Dünnschichtchromatographie aufgetrennten Komponenten eines Gemisches durch die Massenspektren identifizieren kann[2]. Geeignete Derivate für eine massenspektrometrische Untersuchung von Kohlenhydraten sind die Methyläther und die Acetate. Frühere Arbeiten über die Massenspektren peracetylierter Hexosamine[3] und permethylierter Methyl-2-acetamido-2-desoxy-hexopyranoside[4] hatten gezeigt, daß der Einfluß der Zuckerkonfiguration auf die Intensität der einzelnen peaks relativ gering ist. Daher genügt die massenspektrometrische Bestimmung eines Aminozuckers zur Kennzeichnung aller entsprechenden Stereoisomeren. Die Untersuchung permethylierter *N*-Acetyl-aminozucker[5] ergab, daß die Stellung der Amino-Gruppe durch die Massenspektren leicht zu bestimmen ist.

Hydroxy-Gruppen beeinflussen das massenspektrometrische Verhalten permethylierter Kohlenhydrate in ähnlicher Weise. Die bei der Hydrolyse freiwerdenden Hydroxy-Gruppen können daher bei Kenntnis der Fragmentierungsprozesse massenspektrometrisch lokalisiert werden.

Wir haben drei Gruppen von Derivaten der 2-Amino-2-desoxy-D-galaktose (D-Galaktosamin) synthetisiert und deren Massenspektren aufgenommen : (*1*) die partiell methylierten Methyl-2-acetamido-2-desoxy-α-D-galaktopyranoside, (*2*) die Acetate partiell methylierter Methyl-2-acetamido-2-desoxy-α-D-galaktopyranoside und (*3*) die Acetate des partiell methylierten α-D-Galaktosamins.

(*1*) *Die Massenspektren der partiell methylierten Methyl-2-acetamido-2-desoxy-α-D-galaktopyranoside.* Synthetisiert[6] und massenspektrometrisch untersucht wurden : Methyl-2-acetamido-2-desoxy-3,4,6-tri-*O*-methyl-α-D-galaktopyranosid (**1**) und die deuteriummarkierten Verbindungen (**1a**) mit einer NDAc-Gruppe und (**1b**) mit CD_3O-Gruppen an C-4 und C-6, Methyl-2-acetamido-2-desoxy-4,6-di-*O*-methyl-α-D galaktopyranosid (**2**), Methyl-2-acetamido-2-desoxy-3,6-di-*O*-methyl-α-D-galakto-

*XVI. Mitteilung, Ref. 1.

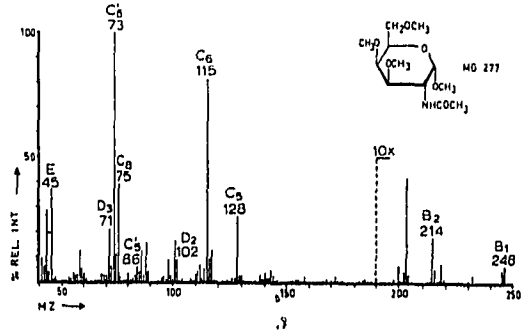


Abb. 1. Methyl-2-acetamido-2-desoxy-3,4,6-tri-*O*-methyl-α-D-galaktopyranosid (**1**).

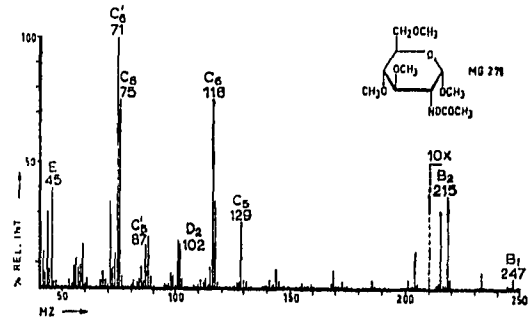


Abb. 1a. Methyl-2-(*N*-deutero-acetamido)-2-desoxy-3,4,6-tri-*O*-methyl-α-D-galaktopyranosid (**1a**).

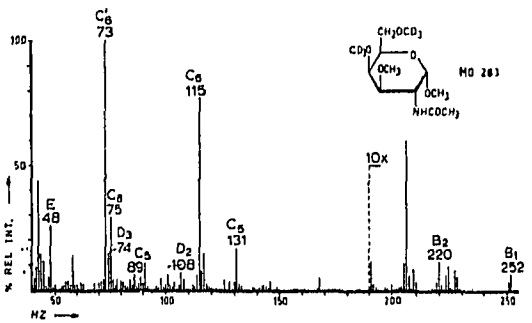


Abb. 1b. Methyl-2-acetamido-2-desoxy-3-*O*-methyl-4,6-di-*O*(trideuteromethyl)-α-D-galaktopyranosid (**1b**).

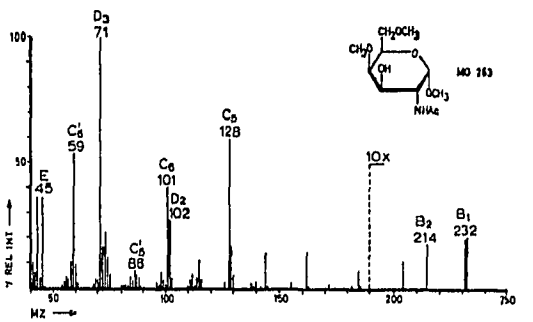


Abb. 2. Methyl-2-acetamido-2-desoxy-4,6-di-*O*-methyl-α-D-galaktopyranosid (**2**).

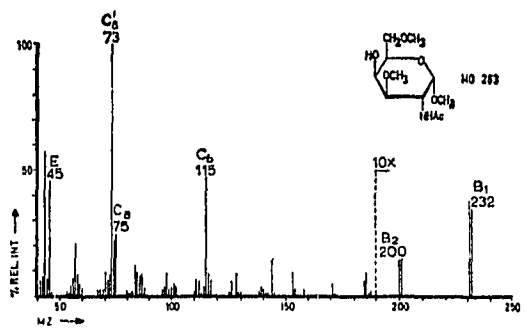


Abb. 3. Methyl-2-acetamido-2-desoxy-3,6-di-*O*-methyl-α-D-galaktopyranosid (**3**).

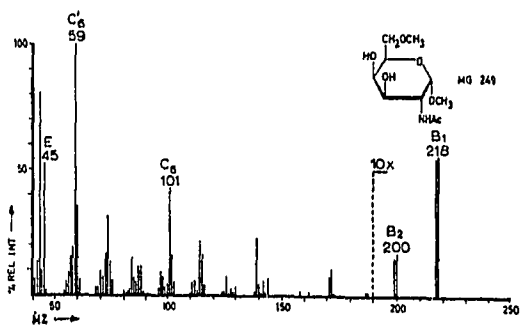


Abb. 4. Methyl-2-acetamido-2-desoxy-6-*O*-methyl-α-D-galaktopyranosid (**4**).

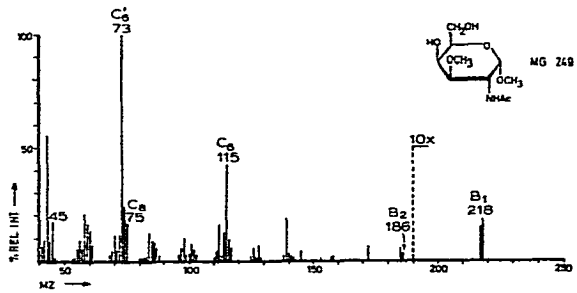


Abb. 5. Methyl-2-acetamido-2-desoxy-3-*O*-methyl-α-D-galaktopyranosid (**5**).

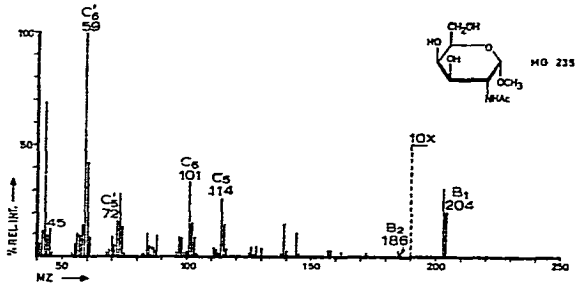


Abb. 6. Methyl-2-acetamido-2-desoxy-α-D-galaktopyranosid (**6**).

pyranosid (**3**), Methyl-2-acetamido-2-desoxy-6-*O*-methyl-α-D-galaktopyranosid (**4**), Methyl-2-acetamido-2-desoxy-3-*O*-methyl-α-D-galaktopyranosid (**5**) und Methyl-2-acetamido-2-desoxy-α-D-galaktopyranosid (**6**).

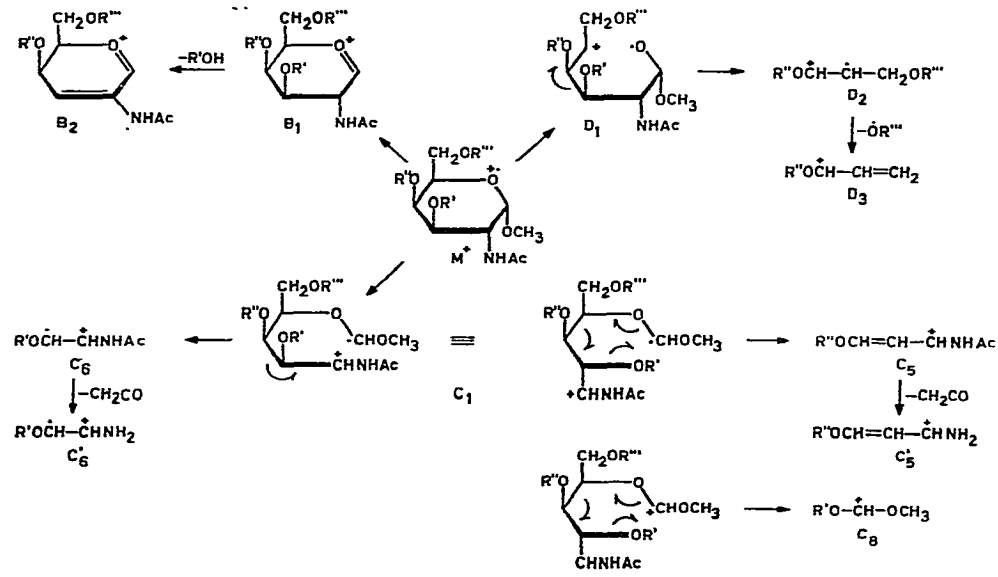


Abb. 7. Überblick über die Fragmentierungsreaktionen der Derivate des Methyl-2-acetamido-2-desoxy-α-D-galaktopyranosids.

Die Massenspektren der Verbindungen **1–6** zeigen die Abbildungen 1–6. Die Fragmentierungsprozesse lassen sich für alle Derivate des Galaktosamins durch die für permethylierte Methyl-pento- und -hexopyranoside entwickelten Vorstellungen[7] erklären. Die Abbildung 7 gibt eine Übersicht über die verschiedenen Primärspaltungen und deren Folgereaktionen.

Der base peak in den Massenspektren permethylierter Methylhexopyranoside entspricht im allgemeinen dem Fragment-Ion C_6. Die Massenzahl (MZ) dieses Radikal-Ions hängt von der Art der Substituenten an den C-Atomen 2 und 3 des Zuckers ab. Variiert wird bei den untersuchten Verbindungen nur R'. Für $R' = CH_3$ hat das Bruchstück C_6 die MZ 115, für R' = H die MZ 101. Durch Eliminierung von Keten aus der Acetamido-Gruppe entstehen aus diesen Fragmenten die Ionen C'_6 der MZ 73 bzw. 59. Das Fragment C'_6 besitzt die höchste Intensität und gibt Auskunft über Substituenten des C-Atoms 3. Eine Ausnahme findet man im Massenspektrum von **2**, bei dem die MZ 71 die höchste Intensität aufweist. Deutlich größer ist auch der peak der MZ 102. Diese Massenzahlen entsprechen den Folgefragmenten D_2 und D_3 der Primärspaltung D. Dieser Prozeß tritt immer dann mit hoher Wahrscheinlichkeit auf, wenn die Bildung des Radikal-Ions C_6 wegen geringer Resonanzstabilisierung der Substituenten an C-2 und C-3 energetisch ungünstig wird. Fehlt die Stabilisierung durch CH_3O-Gruppen auch an den C-Atomen 4 und 6, so wird das Fragment-Ion C'_6 wieder mit der höchsten Intensität gebildet.

Charakteristisch für die Massenspektren permethylierter Monosaccharide ist eine Umlagerungsreaktion, bei der der Substituent des C-Atoms 3 über einen sechsgliedrigen Übergangszustand nach dem C-Atom 1 des Pyranringes wandert. Je nach Verbleib der Ladung entstehen das Allyl-Ion C_5, das Keten eliminieren kann, oder das ebenfalls resonanzstabilisierte Fragment-Ion C_8. Das Ion C_5 findet man mit höherer Intensität nur bei $R'' = CH_3$; eine Ausnahme bildet **6**. Das Fragment C_8 tritt nur bei $R' = CH_3$ auf.

Die Abspaltung der Methoxy-Gruppe an C-1 ergibt das resonanzstabilisierte Fragment B_1. Eliminierung von Methanol und Wasser sind weitere Folgereaktionen dieser Primärspaltung. Da ein Molekül-Ion bei allen Verbindungen praktisch nicht auftritt, hat das Ion B_1 in den Massenspektren die höchste Massenzahl. Diese Massenzahl gibt Auskunft über die Anzahl der freien Hydroxy-Gruppen.

Neben der Glykosid-Gruppe kann auch die Seitenkette des C-Atoms 5 abgespalten werden. Bei diesem Fragmentierungsprozeß bleibt die Ladung überwiegend bei der Seitenkette. Für $R''' = CH_3$ findet man in den Massenspektren das Bruchstück E $CH_2{=}\overset{+}{O}CH_3$ der MZ 45 mit höherer Intensität.

Die betrachteten Fragmentierungsreaktionen lassen eine eindeutige Bestimmung der Anzahl und der Stellung von freien Hydroxy-Gruppen zu. Bei unseren Untersuchungen fehlten das Methyl-2-acetamido-2-desoxy-3,4-di-*O*-methyl-α-D-galaktopyranosid und das Methyl-2-acetamido-2-desoxy-4-*O*-methyl-α-D-galaktopyranosid. Da die Zerfallsreaktionen dieser Verbindungsklasse weitgehend bekannt sind, kann man die Massenzahl und Intensität der hier betrachteten Fragment-Ionen abschätzen. Die Tabelle I gibt einen Überblick über die Massenzahlen und Intensitäten der

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TABELLE I

CHARAKTERISTISCHE MASSENZAHLEN UND REL. INTENSITÄTEN DER PARTIELL METHYLIERTEN METHYL-2-ACETAMIDO-2-DESOXY-α-D-GALAKTOPYRANOSIDE

	1		**2** *3-OH*		**3** *4-OH*		*6-OH*		**4** *3,4-OH*		*3,6-OH*		**5** *4,6-OH*		**6** *3,4,6-OH*	
Ion	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*
B_1	246	0.4	232	2.1	232	3.5	232	~ 2	218	5.6	218	~ 2	218	1.8	204	2.2
C_5	128	26	128	59			128	~20			128	~20			114	26
C'_6	73	100	59	54	73	100	73	100	59	100	59	100	73	100	59	100
C_8	75	39			75	24	75	~35					75	17		
D_3	71	21	71	100												
E	45	37	45	36	45	46			45	52						

Fragment-Ionen B_1, C_5, C'_6, C_8, D_3 und E. Dabei wurden mit Ausnahme des Bruchstücks B_1 nur Ionen betrachtet, die mit mehr als 15% relativer Intensität auftreten.

(2) *Die Massenspektren der Acetate der partiell methylierten Methyl-2-acetamido-2-desoxy-α-D-galaktopyranoside.* Die Acetylierung der Verbindugen **2–6** ergab die folgenden Derivate : Methyl-2-acetamido-3-*O*-acetyl-2-desoxy-4,6-di-*O*-methyl-α-D-galaktopyranosid (**7**), Methyl-2-acetamido-4-*O*-acetyl-2-desoxy-3,6-di-*O*-methyl-α-D-galaktopyranosid (**8**), Methyl-2-acetamido-3,4-di-*O*-acetyl-2-desoxy-6-*O*-methyl-α-D-galaktopyranosid (**9**), Methyl-2-acetamido-4,6-di-*O*-acetyl-2-desoxy-3-*O*-methyl-α-D-galaktopyranosid (**10**), Methyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-desoxy-α-D-galaktopyranosid (**11**) und Methyl-2-acetamido-2-desoxy-3,4,6-tri-*O*-(trideuteroacetyl)-α-D-glucopyranosid (**11a**).

Obwohl durch die Einführung der Acetyl-Gruppen das Molekulargewicht ansteigt, haben wir die Acetate gewählt, da diese thermisch stabil sind und wegen ihrer Flüchtigkeit eine gaschromatographische Vortrennung ermöglichen. Die Bestimmung der Stellung der freien Hydroxy-Gruppen kann auch durch Nachmethylierung mit Trideuteromethyljodid erfolgen[8], doch entfällt bei dieser Methode die Trennmöglichkeit durch Dünnschicht- oder Gaschromatographie.

Die Massenspektren der Acetate **7–11a** (Abb. 8–12a) lassen sich ebenfalls durch die in Abbildung 7 gezeigten Fragmentierungsmechanismen erklären. Die Fragmente mit einer oder zwei Acetyl-Gruppen sind nur um 42 bzw. 84 Masseneinheiten (ME) zu höheren Massenzahlen verschoben. Wegen der leichten Keteneliminierung werden sie mit vergleichbarer Intensität von Folge-Ionen bei einer um 42 ME kleineren Massenzahl begleitet.

Der base peak entspricht bei allen untersuchten Derivaten dem Fragment C_6 mit den C-Atomen 2 und 3 des Pyranringes. Für $R' = CH_3$ findet man das Haupt-Ion bei der MZ 115 und mit fast gleicher Intensität das unter Verlust von Keten entstehende Bruchstück C'_6 der MZ 73. Eine Acetyl-Gruppe an C-3 verschiebt die MZ des Ions C_6 nach 143, durch Keteneliminierung werden die Tochterfragmente der MZ 101 und 59 gebildet. Dabei wird im ersten Schritt bevorzugt Keten aus der Acetoxy-Gruppe abgespalten. Der größte peak ist in diesem Fall die MZ 101. Ebenfalls charakteristisch für eine Methoxy-Gruppe an C-3 ist die Umlagerungsreaktion des Primär-Ions C_1, bei der die CH_3O-Gruppe von C-3 nach C-1 wandert unter Bildung des resonanzstabilisierten Ions C_8 der MZ 75 (Abb. 7).

In der gleichen Umlagerungsreaktion kann auch das Allyl-Ion C_5 gebildet werden, das eine Bestimmung des Substituenten an C-4 ermöglicht. Wegen der geringeren Resonanzeffekte der Amid-Gruppe an C-2 bleibt bei diesem Prozeß die Ladung überwiegend an C-1. Man beobachtet ein Fragment-Ion C_5 mit mittlerer Intensität nur bei $R'' = CH_3$. Fehlt die induktive Stabilisierung durch die Methyl-Gruppe (d.h. $R'' = Ac$), so wird das Allyl-Ion C_5 praktisch nicht gebildet (Ausnahme 11).

Kennzeichnend für eine Methoxy-Gruppe an C-6 ist wieder die Primärspaltung E. Die Ladung bleibt bevorzugt bei der Seitenkette der MZ 45. Dieses Fragment wird mit 30–40% relativer Intensität gefunden.

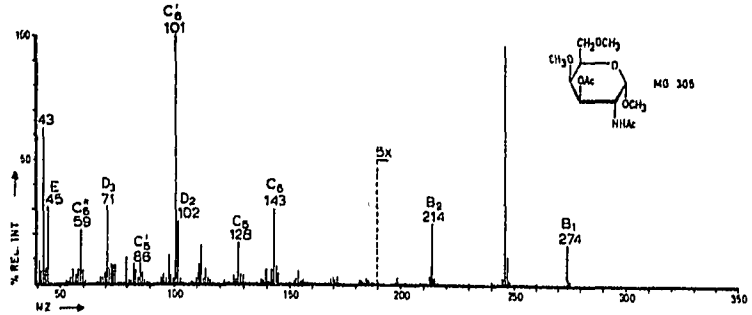


Abb. 8. Methyl-2-acetamido-3-*O*-acetyl-2-desoxy-4,6-di-*O*-methyl-α-D-galaktopyranosid (7).

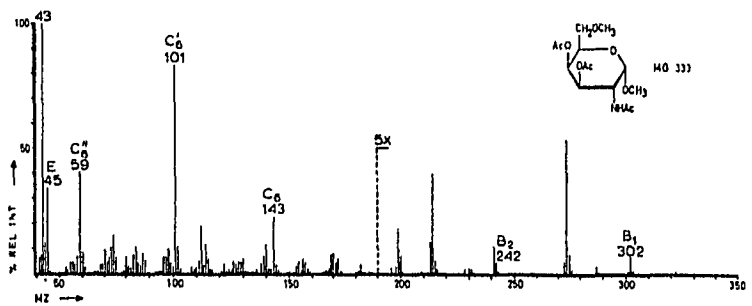


Abb. 10. Methyl-2-acetamido-3,4-di-*O*-acetyl-2-desoxy-6-*O*-methyl-α-D-galaktopyranosid (9).

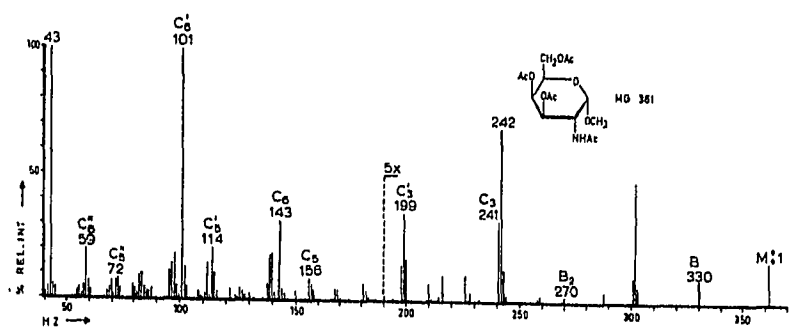


Abb. 12. Methyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-desoxy-α-D-galaktopyranosid (**11**).

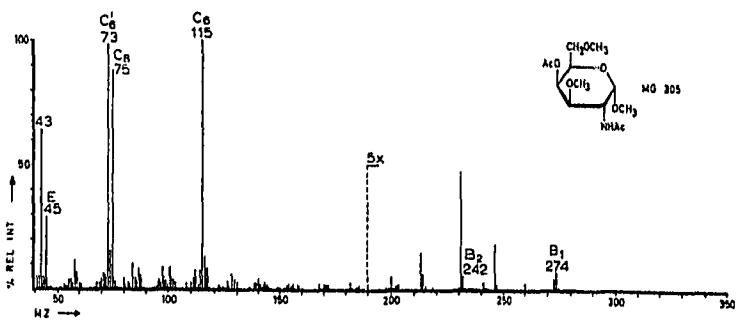


Abb. 9. Methyl-2-acetamido-4-*O*-acetyl-2-desoxy-3,6-di-*O*-methyl-α-D-galaktopyranosid (8).

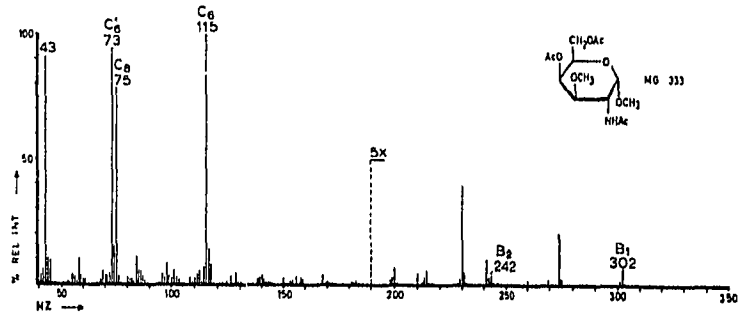


Abb. 11. Methyl-2-acetamido-4,6-di-*O*-acetyl-2-desoxy-3-*O*-methyl-α-D-galaktopyranosid (**10**).

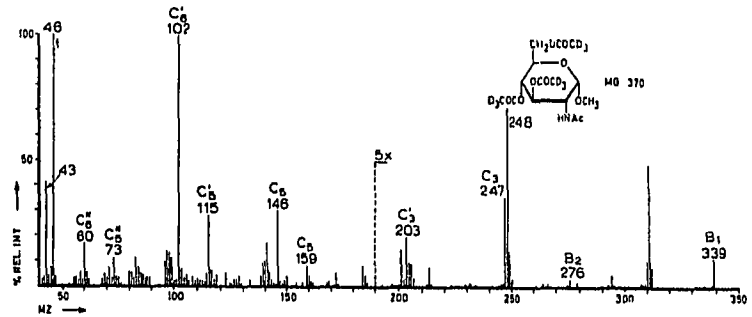


Abb. 12a. Methyl-2-acetamido-2-desoxy-3,4,6-tri-*O*-(trideuteroacetyl)-α-D-glucopyranosid (**11a**).

Die Primärspaltung D hat nur für das Derivat 7 Bedeutung. Die Bildungswahrscheinlichkeit der Fragmente D_2 MZ 102 und D_3 MZ 71 nimmt zu, da die Entstehung des Ions C_6 durch die geringere Stabilisierung der Acetyl-Gruppe an C-3 energetisch benachteiligt wird.

Die Anzahl der Acetyl-Gruppen läßt sich aus der MZ des Primär-Ions B_1 bestimmen. Nur dieses Ion weist am oberen Ende des Spektrums eine höhere Intensität auf. Die MZ für B_1 ist entsprechend für eine Acetoxy-Gruppe 274, für zwei 302 und für drei 330. In der Tabelle II sind die wichtigsten Massenzahlen und Intensitäten zusammengefaßt, die eine eindeutige Zuordnung ermöglichen. Auch hier haben wir die Daten für die Galaktosamin-Derivate mit Acetoxy-Gruppen an C-6 und an C-3 und C-6 auf Grund der bekannten Fragmentierungsreaktionen geschätzt.

TABELLE II

CHARAKTERISTISCHE MASSENZAHLEN UND REL. INTENSITÄTEN DER ACETATE PARTIELL METHYLIERTER METHYL-2-ACETAMIDO-2-DESOXY-α-D-GALACTOPYRANOSIDE

	7		**8**				**9**				**10**		**11**	
	3-OAc		*4-OAc*		*6-OAc*		*3,4-OAc*		*3,6-OAc*		*4,6-OAc*		*3,4,6-OAc*	
Ion	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*
B_1	274	3.3	274	1.4	274	~ 2	302	1.7	302	~1.5	302	1.3	330	1.9
C_5	128	17			128	~15			128	~15				
C'_5													114	21
C_6	143	30	115	100	115	100	143	27	143	~30	115	100	143	32
C'_6	101	100	73	98	73	~90	101	100	101	100	73	95	101	100
C_8			75	87	75	~90					75	88		
D_3	71	32												
E	45	31	45	29			45	41						

(*3*) *Massenspektren der Acetate des partiell methylierten* D-*Galaktosamins.* Die Acetylierung der Hydrolyseprodukte ist der einfachste Weg zu Derivaten, die für eine Trennung und massenspektrometrische Analyse geeignet sind. Wir haben daher einige dieser Verbindungen hergestellt und die Massenspektren aufgenommen: 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-desoxy-α-D-galaktopyranose (**12**), 2-Acetamido-2-desoxy-1,3,4,6-tetra-*O*-(trideuteroacetyl)-α-D-galaktopyranose (**12a**), 2-Acetamido-1,4,6-tri-*O*-acetyl-2-desoxy-3-*O*-methyl-α-D-galaktopyranose (**13**), 2-Acetamido-1,3,6-tri-*O*-acetyl-2-desoxy-4-*O*-methyl-α-D-galaktopyranose (**14**), 2-Acetamido-1,3,4-tri-*O*-acetyl-2-desoxy-6-*O*-methyl-α-D-galaktopyranose (**15**), 2-Acetamido-1,4-di-*O*-acetyl-2-desoxy-3,6-di-*O*-methyl-α-D-galaktopyranose (**16**) und 2-Acetamido-1,6-di-*O*-acetyl-2-desoxy-3,4-di-*O*-methyl-α-D-galaktopyranose (**17**).

Da das Massenspektrum des Galaktosamin-pentaacetats dem der Penta-*O*-acetylglucose sehr ähnlich ist, abgesehen von Massenverschiebungen auf Grund von O = 16 und NH = 15, versuchten wir, die von Biemann und Mitarbeitern formulierten Fragmentierungsmechanismen des Glucosepentaacetats[9] auf das peracetylierte Galaktosamin zu übertragen. Dabei ergaben sich gewisse Widersprüche mit unseren

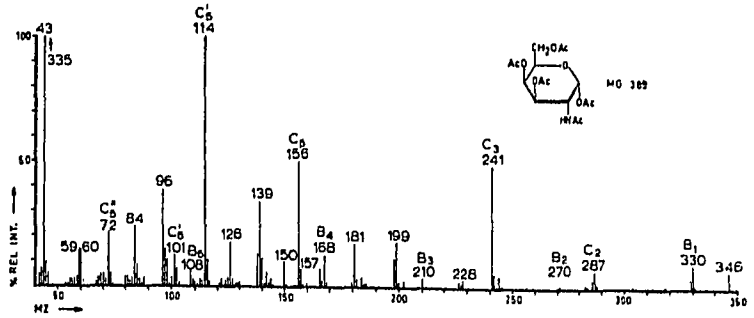


Abb. 13. 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-desoxy-α-D-galaktopyranose (**12**).

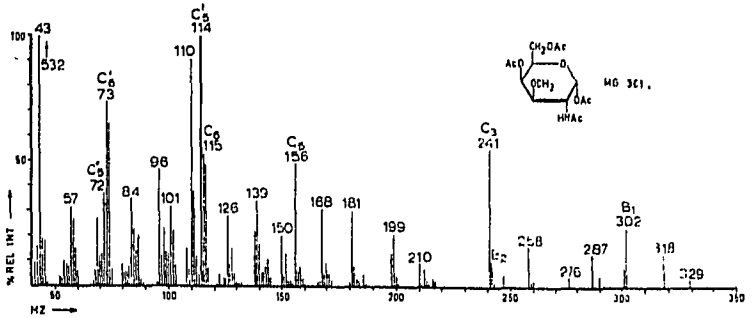


Abb. 14. 2-Acetamido-1,4,6-tri-*O*-acetyl-2-desoxy-3-*O*-methyl-α-D-galaktopyranose (**13**).

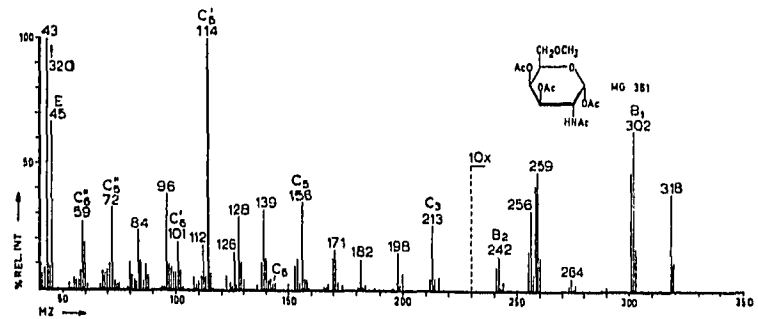


Abb. 16. 2-Acetamido-1,3,4-tri-*O*-acetyl-2-desoxy-6-*O*-methyl-α-D-galaktopyranose (**15**).

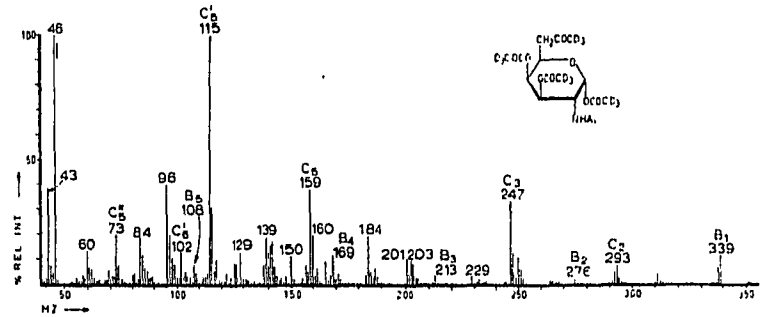


Abb. 13a. 2-Acetamido-2-desoxy-1,3,4,6-tetra-*O*-(trideuteroacetyl)-α-D-galaktopyranose (**12a**).

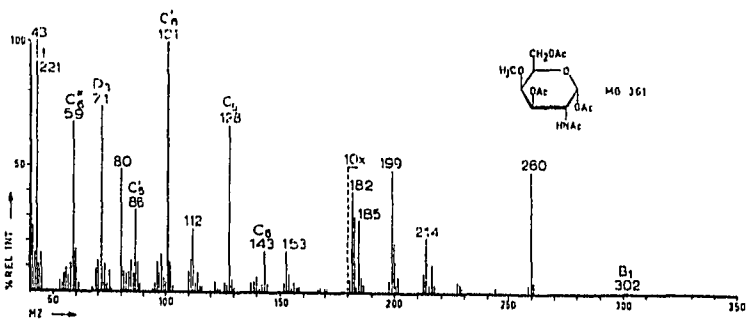


Abb. 15. 2-Acetamido-1,3,6-tri-*O*-acetyl-2-desoxy-4-*O*-methyl-α-D-galaktopyranose (**14**).

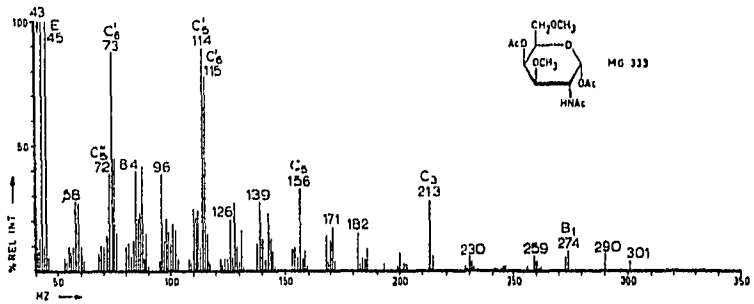


Abb. 17. 2-Acetamido-1,4-di-*O*-acetyl-2-desoxy-3,6-di-*O*-methyl-α-D-galaktopyranose (**16**).

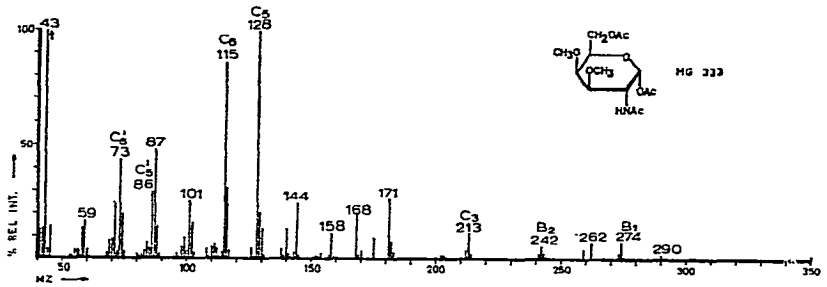


Abb. 18. 2-Acetamido-1,6-di-*O*-acetyl-2-desoxy-3,4-di-*O*-methyl-α-D-galaktopyranose (**17**).

eigenen Ergebnissen, so daß wir in einer neuen systematischen Untersuchung der peracetylierten Pento- und Hexopyranosen[10] die Fragmentierungsprozesse überprüften und neu formulierten. Das Massenspektrum des peracetylierten D-Galaktosamins (Abb. 13) läßt sich durch die gleichen Zerfallsreaktionen deuten. Wie bei Polyacetaten zu erwarten ist, hat das Acetyl-Ion der MZ 43 die höchste Intensität. Da diese Massenzahl wenig charakteristisch ist, wurden die Massenspektren auf den zweitgrößten peak bezogen. Die Massenspektren der Verbindungen **12a–17** zeigen die Abbildungen 13a–18.

Haupt-Ionen sind wieder die Bruchstücke C_5 und C_6 mit ihren Tochterfragmenten C'_5 und C'_6. Dabei zeigen im allgemeinen die Allyl-Ionen C_5 oder C'_5 den größten peak, bei **14** mit einer Methoxy-Gruppe an dem C-Atom 4 ist das Radikal-Ion C'_6 $HO\dot{C}H\text{-}CH{=}N^+HAc$ mit der größten Intensität vertreten.

Neu in den Massenspektren der Acetate[10] ist eine Fragmentreihe, die einen anderen Zerfallsweg des Primär-Ions C_1 darstellt. Eine ähnliche Reaktion findet man auch bei den permethylierten Methyl-hexopyranosiden, allerdings mit geringer Intensität. Die Abbildung 19 gibt einen Überblick über diesen Fragmentierungsmechanismus des peracetylierten Galaktosamins.

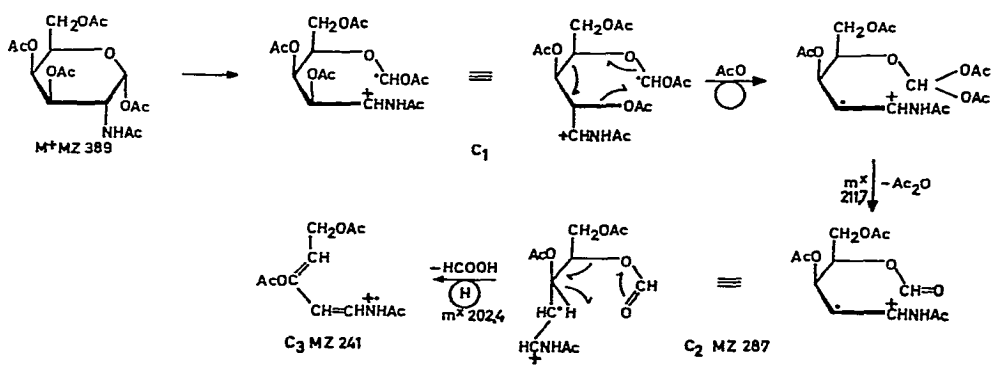


Abb. 19. Bildungsmechanismus des Fragments C_3.

Bei Elektronenstoß tritt eine Spaltung der C-1/C-2 Bindung ein. Das Primär-Ion C_1 lagert sich unter Wanderung der Acetoxy-Gruppe an C-3 nach C-1 in ein offenkettiges Ion mit geminalen Acetoxy-Gruppen um. Während im Fall der permethylierten Derivate diese Gruppierung stabil ist, erfolgt hier leicht die Eliminierung von Acetanhydrid zum Ion C_2. Die Spaltung der C-4/C-5 Bindung führt in üblicher Weise zu dem Allyl-Ion C_5. Ein dem Ion C_8 (siehe Abb. 7) entsprechendes Fragment fehlt. Andererseits kann in dem Ion C_2 in einer McLafferty-Umlagerung der Wasserstoff des C-Atoms 4 an die Carbonyl-Gruppe des C-1 angelagert werden, das dann als Ameisensäure eliminiert wird. Das gebildete Radikal-Ion C_3 ist resonanzstabilisiert mit Verteilung der Ladung über die Kohlenstoffkette.

Während der weitere Zerfall des Radikal-Ions C_3 bei den peracetylierten Hexopyranosen bei der Massenzahl 98 endet, findet man im Massenspektrum des Galaktosamin-pentaacetats neben der MZ 97 den größten peak bei der MZ 96. Die Abbildung 20 zeigt ein mögliches Reaktionsschema. Das Fragment der MZ 97 entspricht der Reaktion der Hexopyranosen. Neben der hier gezeigten Zweistufeneliminierung von Keten und Essigsäure bzw. vice versa muß man als Hauptreaktion die Eliminierung von Acetanhydrid (oder Essigsäure + Keten) in einem Schritt annehmen[10]. Die zweite Zerfallsreaktion beginnt mit der Abspaltung einer Acetyl-Gruppe, bevorzugt von dem C-Atom 2. Es folgt die Eliminierung von Essigsäure und Keten, auch hier überwiegend in einem Schritt als Acetanhydrid (oder Essigsäure + Keten). Die genaue Massenbestimmung der MZ 96 und 97 in den Massenspektren von **12** und **12a** ergab in beiden Fällen für die MZ 96 ein Singulett der Zusammensetzung C_5H_6NO und für die MZ 97 ein Duplett mit den Bruttoformeln C_5H_6NO und $C_5H_5O_2$ im Verhältnis 2:1. Das stickstoff-freie Fragment kann auf dem gleichen Weg wie das Ion der MZ 96, jedoch unter Abspaltung von Acetamid, gebildet werden.

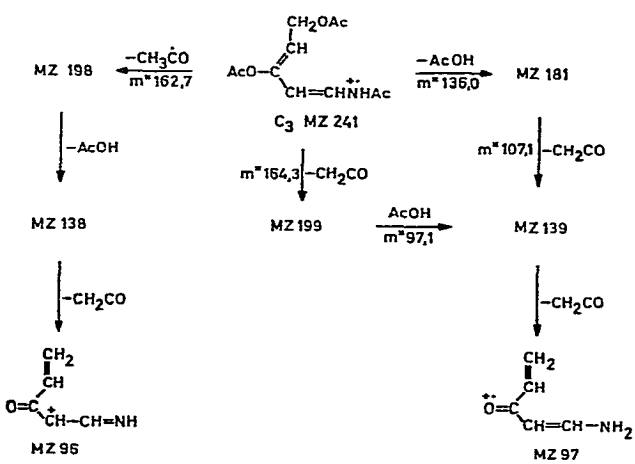


Abb. 20. Zerfallsreaktionen des Radikal-Ions C_3.

Ein Fragment-Ion C_3 der MZ 241 beobachtet man ebenfalls in dem Massenspektrum der Verbindung **13**. Bei einer Methoxy-Gruppe an dem C-Atom 4 oder 6

findet man dieses Ion bei der MZ 213. Dabei ist die Intensität dieses Radikal-Ions bei einer Methoxy-Gruppe an C-4 deutlich geringer. Gleichzeitig treten die Radikal-Ionen C_6, C'_6 und D_3 mit höherer Intensität auf.

Die Ionen der Primärspaltung B sind mit Ausnahme von **14** stärker als bei den Methyl-glycosiden vertreten. Da bei den partiell methylierten Acetaten der erste Schritt der Reihe B die Abspaltung der glycosidischen Acetoxy-Gruppe (59 ME) zum Fragment B_1 ist, aber aus dem Molekül-Ion auch Methanol oder die Acetyl-Gruppe eliminiert werden kann, ist das Ion B_1 nicht mehr das mit der höchsten Massenzahl im Spektrum. Die Primärspaltung E ist wieder an eine Methoxy-Gruppe an C-6 gebunden.

Die Tabelle III zeigt die wichtigsten Ionen-Typen mit ihren Massenzahlen und relativen Intensitäten. Aufgenommen wurden wieder nur Ionen mit mehr als 10%

TABELLE III

CHARAKTERISTISCHE MASSENZAHLEN UND REL. INTENSITÄTEN DER ACETATE PARTIELL METHYLIERTER D-GALAKTOSAMINE

	12		**13**		**14**		**15**		**16**		**17**			
			3-OCH₃		*4-OCH₃*		*6-OCH₃*		*3,4-OCH₃*		*3,6-OCH₃*		*4,6-OCH₃*	
Ion	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*	*MZ*	*Int.*
C_3	241	49	241	56	213	0,8	213	26	213	12	213	28	185	
C_5	156	50	156	49	128	67	156	35	128	100	156	33	128	
C'_5	114	100	114	100	86	33	114	100	86	30	114	88	86	
C_6			115	53	143	16			115	86	115	78	143	
C'_6	101	12	73	74	101	100	101	19	73	44	73	88	101	
D_3					71	74			71	25			71	
E							45	67			45	100	45	

relativer Intensität. Da der Einfluß der Methoxy-Gruppen auf das Fragmentierungsverhalten der Acetate sehr groß ist, können wir bei der noch fehlenden Verbindung mit Methoxy-Gruppen an C-4/C-6 wohl Angaben über die Massenzahlen der Haupt-Ionen, aber nicht über ihre Intensitäten machen.

DISKUSSION

Die Ergebnisse der hier vorliegenden Untersuchung zeigen, daß die Massenspektrometrie eine einfache Unterscheidung der verschiedenen Derivate des Galaktosamins zuläßt. Innerhalb einer Substanzgruppe lassen sich die Anzahl und die Stellung der freien bzw. acetylierten Hydroxy-Gruppen durch die Massenzahlen und relativen Intensitäten einiger weniger Ionen eindeutig bestimmen. Die Methode ist sehr empfindlich. Zur Aufnahme eines Massenspektrums genügen Bruchteile eines Mikrogramms. Die gezeigten Fragmentierungsmechanismen gelten für alle Stereoisomeren. Die verschiedenen Methyläther können über Dünnschicht-, Säulen- oder Gaschromatographie getrennt werden. Es genügt die massenspektrometrische Bestimmung des jeweiligen Derivates eines beliebigen Hexosamins, um alle übrigen 2-Amino-

zucker zu bestimmen. Die Zuordnung der Konfiguration des Aminozuckers muß auf andere Weise erfolgen.

EXPERIMENTELLER TEIL

Die Verbindungen **1–17** wurden nach den in der Literatur[6] beschriebenen Verfahren hergestellt. Die Massenspektren wurden bei einer Elektronenenergie von 70 eV mit einem Massenspektrometer Typ CH-4 der ATLAS Mess- und Analysentechnik GmbH Bremen aufgenommen. Die Proben wurden direkt in die Ionenquelle TO-4 eingebracht, die Temperatur betrug 90–150°. Genaue Massenbestimmungen einiger Fragmente wurden mit einem doppelfokussierenden Gerät SM-1 der gleichen Firma vorgenommen.

DANK

Dem Bundesministerium für wissenschaftliche Forschung danken wir für die finanzielle Unterstützung dieser Arbeit.

ZUSAMMENFASSUNG

Die Massenspektren geeigneter Derivate ermöglichen die Bestimmung der Verknüpfungsstellen von aminozuckerhaltigen Polysacchariden. Untersucht wurden die partiell methylierten Methyl-2-acetamido-2-desoxy-D-galaktopyranoside, die entsprechenden acetylierten Verbindungen und die Acetate der partiell methylierten 2-Acetamido-2-desoxy-D-galaktose.

SUMMARY

Mass spectrometry is a useful technique for the determination of the linkage in polysaccharides containing amino sugars. Suitable derivatives are the methyl ethers and the acetates. The mass spectra and fragmentation patterns of partially methylated methyl-2-acetamido-2-deoxy-D-galactopyranosides, the corresponding acetylated compounds, and the acetates of partially methylated derivatives of 2-acetamido-2-deoxy-D-galactose are discussed.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

NITROGEN-CONTAINING CARBOHYDRATE DERIVATIVES

PART XV*. THE REACTION OF HYDRAZINE WITH AZIDO SUGARS

R. D. GUTHRIE,
The Chemical Laboratory, University of Sussex, Brighton (Great Britain)
AND D. MURPHY,
Chemistry Department, Bedford College, London N.W. 1 (Great Britain)

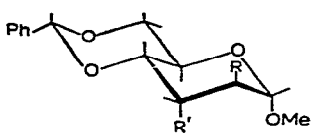
(Received February 13th, 1967)

INTRODUCTION

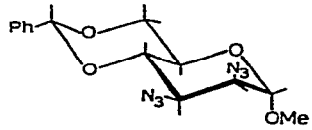
In part IX[1] of this series, we described an attempt to prepare methyl 2,3-diazido-4-6-*O*-benzylidene-2,3-dideoxy-α-D-alloside by the nucleophilic displacement with azide ion of the sulphonate group in methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-altroside 2-toluene-*p*-sulphonate (**1**). In a similar reaction, the corresponding 2-azido-2-deoxy-3-*O*-toluene-*p*-sulphonyl derivative (**2**) gave the 2,3-diazido-mannoside **5** in 49% yield. The lack of reaction of compound **1** was ascribed to the steric hindrance of the groups on C-1 and C-3 in preventing approach to the rear side of C-2.

A similar effect has been discussed elsewhere[2]. It has been shown that sulphonic esters that are unreactive towards azide ion can be displaced by hydrazine. Compound **1** was therefore refluxed with the latter reagent and, surprisingly, gave[1] a good yield (72%) of methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (**6**).

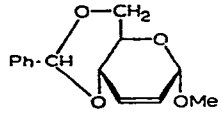
We have now studied this reaction further and find that it has many interesting features, for which we are not yet able to find an explanation.



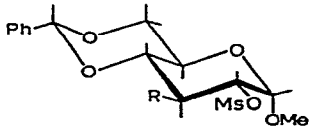
	R	R'
(1)	OTs	N_3
(2)	N_3	OTs
(3)	OH	N_3
(4)	N_3	OH



(5)



(6)



(7) R = N_3

(8) R = H

*Part XIV, C. B. Barlow and R. D. Guthrie, *J. Chem. Soc.*, in press.

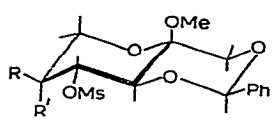
DISCUSSION

Further experiments on compound **1** showed that a reaction time of 10 min was sufficient. The yield of olefinic sugar **6** was reproducible, and a similar yield was obtained from the corresponding methanesulphonate. A similar reaction on the 2-azido-2-deoxy-3-*O*-toluene-*p*-sulphonyl derivative (**2**) also gave the olefinic sugar **6** (70–75% yield.)

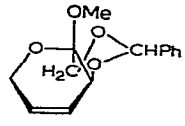
In order to obtain more information about this reaction, other azido-sugar derivatives available in our laboratories were treated with hydrazine. Methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-altroside (**3**) and the corresponding 2-azido compound (**4**) gave only the corresponding methyl amino-4,6-*O*-benzylidene-deoxy-α-D-altrosides (70–80%). No other products could be detected.

Treatment of methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-glucoside 2-methanesulphonate (**7**) under the same conditions gave a product (70%), which, from its n.m.r. spectrum, contained a C-CH_2-C group and had retained the mesyl group. Spin-decoupling experiments showed that the CH_2 group was at C-3, and that the mesyl group was probably equatorial. The structure of the product was confirmed as methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside 2-methanesulphonate (**8**) by comparison with a sample prepared by mesylation of authentic[4] methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside.

Similar hydrazinolysis of the *cis*-diazido compound **5** gave the olefinic sugar **6** (64%). Careful evaporation of the aqueous hydrazine filtrate gave a small amount of a waxy solid whose appearance and physical properties were consistent with its being hydrazinium azide[5]. Acetylation of the remaining residue gave crystalline methyl 2,3-diacetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D mannoside (10%). In this *cis*-elimination, one azide group must act as the leaving group.



	R	R'
(9)	N_3	H
(10)	H	N_3
(11)	H	H



(12)

Recent work[6] on azido-ketosides has given two other products that were useful to test under hydrazinolysis. Methyl 5-azido-1,3-*O*-benzylidene-5-deoxy-α-L-sorbo-pyranoside 4-methanesulphonate (**9**) (*eq-eq* N_3-OMs) gave only one product, the deoxy-sulphonyloxy compound **11***, indicating that the same type of reaction had occurred as with the *eq-eq*-glucoside (**7**). The structure of compound **11** was established by isolation of the same product from Raney-nickel desulphurisation of methyl 1,3-*O*-benzylidene-5-deoxy-5-thiocyano-β-D-fructopyranoside 4-methanesulphonate[6].

*Since this product has a CH_2 group at C-5, it may be considered as a derivative of either methyl α-L-sorbopyranoside or methyl β-D-fructopyranoside.

Hydrazinolysis of methyl 5-azido-1,3-*O*-benzylidene-5-deoxy-β-D-fructopyranoside 4-methanesulphonate (**10**) (*cis* system) gave the olefinic sugar **12**, identical with an authentic sample prepared by the action of sodium iodide and zinc dust in *N*,*N*-dimethylformamide[7] on methyl 1,3-*O*-benzylidene-α-L-sorboside 4,5-bis(toluene-*p*-sulphonate).

The reactions described above fall distinctly into three groups. Firstly, the *trans*-diaxial (**1** and **2**) and *cis*-axial, equatorial systems (**5** and **10**) (all having an axial azide group) undergo elimination, with formation of an olefinic product. Secondly, the *trans*-diequatorial systems **7** and **9** both undergo reduction of the C-N_3 group to C-H, and, finally, with the azido alcohols **3** and **4**, there is straightforward reduction of the azido group to an amino group. Since elimination occurs from both *cis* and *trans* systems, it appears that the azido centre is first converted into an sp^2-hybridised form, so that both types become stereochemically similar. However, the fact that sorboside **9** and fructoside **10** gave different products rules out any mechanism of the type of the Wolff-Kishner reduction–elimination[8], which goes *via* a hydrazone intermediate; derivatives **9** and **10**, which differ only in the configuration about the azido group on C-5, would give the same hydrazone and hence the same products.

Presumably, in the hydrazinolysis of all of the azides described above, the azido group must undergo the same initial change; the subsequent steps then depend on the stereochemistry and on the neighbouring functional groups. Two reaction paths seem possible : (*i*) formation of a nitrene and its reaction with hydrazine, or (*ii*) attack of hydrazine on the azido group and subsequent breakdown of the pentazole intermediate. At present, we are unable to devise a scheme to embrace the differences in reaction between the variously orientated azides.

EXPERIMENTAL

All solutions were concentrated at diminished pressure. Where possible, compounds were identified by mixed m.p. and by infrared spectroscopy; new compounds had i.r. and p.m.r. spectra consistent with the assigned structures.

Preparation of methanesulphonates. — (*a*) *Methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-glucoside 2-methanesulphonate* (**7**). Methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-glucoside[9] was treated in the usual way with excess of methanesulphonyl chloride in pyridine. The white, solid product, after two recrystallisations from propan-2-ol, gave compound **7** (80%), m.p. 129–130°, $[\alpha]_D^{20}$ +80.4° (*c* 1.54, chloroform) (Found: C, 47.0; H, 5.1. $C_{15}H_{19}N_3O_7S$ calc.: C, 46.8; H, 5.0%).

(*b*) *Methyl 4,6-O-benzylidene-3-deoxy-α-D-ribo-hexopyranoside 2-methanesulphonate* (**8**). — Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside[4] was esterified in the usual way to give, after recrystallisation from ethanol and then propan-2-ol, compound **8** (47%), m.p. 154.5–155°, $[\alpha]_D^{26}$ +69.7° (*c* 0.54, chloroform); molecular ion, m/e 344 (Found: C, 52.5; H, 5.4. $C_{15}H_{20}O_7S$ calc.: C, 52.3; H, 5.8%; mol. wt, 344). N.m.r. data: τ 2.48–2.75 (5-proton multiplet, phenyl), 4.52 (1-proton singlet, benzylic H), 5.16 (1-proton doublet, $J_{1,2}$ ~2.5 c.p.s., H-1), 5.30 (1-proton multiplet,

H-2), 5.70–6.50 (4-proton multiplet, H-4,5,6,6'), 6.57 (3-proton singlet, OCH_3), 7.00 (3-proton singlet, mesyl CH_3), 7.53–7.90 (2-proton multiplet-H-3,3').

(*c*) *Methyl 1,3-O-benzylidene-5-deoxy-β-*D-threo-*hexulopyranoside 4-methanesulphonate* (**11**). Methyl 1,3-*O*-benzylidene-5-deoxy-5-thiocyano-β-D-fructopyranoside 4-methanesulphonate[6] (2 g) in ethanol (50 ml) was treated with Raney nickel (20 g), and the mixture was boiled for 4 h under reflux. Filtration and evaporation gave a white solid, which, on three recrystallisations from propan-2-ol, gave compound **11** (51%), m.p. 102–103°, $[\alpha]_D^{20}$ −58.5° (*c* 0.85, chloroform) (Found: C, 52.5; H, 5.4. $C_{15}H_{20}O_7S$ calc.: C, 52.3; H, 5.8%).

*Methyl 1,3-O-benzylidene-4,5-dideoxy-α-*L-glycero-*hex-4-enulopyranoside.* — Methyl 1,3-*O*-benzylidene-α-L-sorbopyranoside 4,5-bis(toluene-*p*-sulphonate)[6] (2 g), sodium iodide (20 g), and zinc dust (10 g) were boiled in *N*,*N*-dimethylformamide (40 ml) for 2 h with stirring. Insoluble material was removed by filtration, and the filtrate was poured into ice–water (400 ml). Ether extraction, washing, drying, and evaporation of the extract gave a white solid, which, on two recrystallisations from light petroleum, gave the product (39%), m.p. 103–104°, $[\alpha]_D^{20}$ +146° (*c* 1.0, chloroform) (Found: C, 67.6; H, 6.5. $C_{14}H_{16}O_4$ calc.: C, 67.7; H, 6.5%). N.m.r. data: τ 2.35–2.8 (5-proton multiplet, phenyl), 4.2 (2-proton singlet, H-4,5), τ 4.32 (1-proton singlet, benzylic H), 5.50–6.42 (5-proton multiplet, H-1,1',3,6,6'), 6.56 (3-proton singlet, OCH_3).

Reactions with hydrazine. — In each case, the azido compound (2 g) in 95% hydrazine (20 ml) was heated from room temperature to boiling point. A brisk effervescence occurred during the heating. The solution was then refluxed for 5–10 min and poured into water (200 ml). Only traces of starting material were found in any of the reactions if the mixture was poured into water as soon as the effervescence had ceased; this usually occurred before the boiling point was reached. The reactions were carried out in 250-ml flasks in an effort to control the effervescence. In an initial experiment (50-ml flask), hydrazine was driven out of the reaction system and came into contact with metal supports; a violent explosion resulted, completely shattering the apparatus, but no personal injury resulted, as suitable protective clothing was being worn.

(*a*) *Methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-*D-*altroside 2-toluene-*p-*sulphonate* (**1**) gave a white, solid product, m.p. 109–111°, which, on recrystallisation from ethanol, gave methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (**6**) (72%), m.p. 116–117°, mixed m.p. 115–116° with an authentic sample[10].

(*b*) *Methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-*D-*altroside 3-toluene-*p-*sulphonate* (**2**) gave olefin **6** (72%), m.p. 115–116°.

(*c*) The corresponding methyl 2-(and 3-)azido-altroside 3-(and 2-)methanesulphonates each gave olefin **6** (70–75%), m.p. 115–116°.

(*d*) *Methyl 2,3-diazido-4,6-O-benzylidene-2,3-dideoxy-α-*D-*mannoside* (**5**)[1] gave a white, solid precipitate, which, on recrystallisation from ethanol, gave olefin **6** (64%), m.p. 115–116°. Evaporation of the aqueous hydrazine mother-liquor gave a waxy residue that was recrystallised from dry propan-2-ol to give white crystals of

hydrazine hydrogen azide[5] (10% based on assigned structure), m.p. 50° (decomp.). Acetylation of the residue obtained by evaporation of the propan-2-ol mother-liquor gave a white solid, which, on recrystallisation from propan-2-ol–light petroleum, gave methyl 2,3-diacetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-mannoside[1] (9%), m.p. 300° (decomp.).

(*e*) *Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-*D*-altroside* (**4**) gave, after evaporation of the aqueous hydrazine, a white solid, which, on recrystallisation from ethanol, gave methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-altroside[9,11] (73%), m.p. 164–166°.

(*f*) *Methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-*D*-altroside* (**3**) similarly gave methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-altroside[9,12] (75%), m.p. 185–187°.

(*g*) *Methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-*D*-glucoside 2-methanesulphonate* (**7**) gave a white, crystalline product, which, on recrystallisation from propan-2-ol, gave methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside 2-methanesulphonate (**8**) (78%), m.p. 156–157°, $[\alpha]_D^{26}$ +68.3° (*c* 0.46, chloroform), identical with the authentic sample described above.

(*h*) *Methyl 5-azido-1,3-*O*-benzylidene-5-deoxy-α-*L*-sorbopyranoside 4-methanesulphonate*[6] (**9**) gave, on recrystallisation of the solid product from propan-2-ol, methyl 1,3-*O*-benzylidene-5-deoxy-β-D-*threo*-hexulopyranoside 4-methanesulphonate (**11**) (69%), m.p. 102–103°, identical with the authentic compound described above.

(*i*) *Methyl 5-azido-1,3-*O*-benzylidene-5-deoxy-β-*D*-fructopyranoside 4-methanesulphonate* (**10**) gave a white, crystalline product, which, on recrystallisation from propan-2-ol, gave methyl 1,3-*O*-benzylidene-4,5-dideoxy-α-L-*glycero*-hex-4-enulopyranoside (**12**) (75%), m.p. 104.5–105.5°, $[\alpha]_D^{20}$ +146° (*c* 0.27, chloroform). The mixed m.p. with the authentic sample described above was 102–103°.

ACKNOWLEDGMENTS

Part of this work was carried out in the Chemistry Department, University of Leicester, and was supported by the U. S. Department of the Army through its European Office. We thank Mrs. A. G. Pike for preparing the authentic sample of methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside 2-methanesulphonate. The n.m.r. spectra (Varian HA-100) were kindly determined by Mr. R. J. Suffolk, and the mass spectrum (AEI-MS9) by Mr. F. G. Bloss.

SUMMARY

The reaction of a number of azido sugars with hydrazine has been studied. Vicinal azido-hydroxyl compounds gave the appropriate amino-alcohol, *trans* (*ax-ax*) and *cis* (*ax-eq*) azido-sulphonates both gave the corresponding olefin; *trans* (*eq-eq*) azido-sulphonates gave deoxy-sulphonates in which the HCN_3 group had been reduced to CH_2. Possible mechanisms are discussed.

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Carbohydrate Research.
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PROTEIN–CARBOHYDRATE INTERACTION

VIII. A TURBIDIMETRIC METHOD FOR THE ANALYSIS OF D-MANNOSE

R. D. PORETZ AND I. J. GOLDSTEIN*

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Mich. (U. S. A.)

(Received February 27th, 1967)

The quantitative analysis of a specific sugar in the presence of other carbohydrates has always been an involved and time-consuming process. Only with the advent of the glucose oxidase–peroxidase colorimetric method[1–3] has it been possible to analyze routinely for D-glucose in the presence of other sugars. Similarly, D-galactose can be estimated by using galactose oxidase[4]. Both of these oxidases show a high degree of specificity for their respective substrates and have proved to be extremely efficient analytical tools. Enzymic procedures are also available for several other carbohydrates (*cf.* ref. 5).

The determination of D-mannose, especially in a mixture of sugars, remains a tedious process. It usually involves paper-chromatographic or -electrophoretic separation of the components of the mixture, followed by elution of developed chromophores obtained by a spray reagent, or alternatively, by elution of the appropriate sugar followed by colorimetric or reductometric analysis (*cf.* ref. 6). Gas–liquid chromatography has also found wide application in the analysis of sugar mixtures, although this usually requires further analysis of the various anomeric forms of individual sugars (*cf.* ref. 7).

Morgan and Watkins[8] have shown that certain mono- and oligo-saccharides specifically inhibit the interaction of the phytohemagglutinins from various plant-seeds with red blood-cells of types A and B. Similarly, it has been shown in this laboratory[9] that D-mannose and D-glucose, and some of their derivatives, inhibit the interaction of concanavalin A (a phytohemagglutinin from jack bean) with certain branched α-D-glucans and α-D-mannans.

This communication reports, for the analysis of D-mannose, a procedure that depends upon estimation of the degree to which the turbidity that is formed on interaction of concanavalin A with glycogen is inhibited by D-mannose. The application of this procedure to the determination of D-mannose in a variety of polysaccharides is described.

MATERIALS AND METHODS

Concanavalin A was extracted from jack-bean meal, and purified by the method of Agrawal and Goldstein[10]. It was stored as a stock solution (2.8 mg/ml) in

*Established Investigator of the American Heart Association.

saturated sodium chloride. The protein was stable for long periods of time when stored at 4°. A diluted solution that contained 60 μg/ml of protein in 0.1N sodium phosphate buffer, pH 7.0, and that was approximately 0.1N with respect to sodium chloride, was used for the analysis.

Carbohydrates. — Oyster glycogen was purchased from Distillation Products Industries (Rochester, New York). Chromatographically "pure" D-mannose and D-glucose were purchased from Mann Research Laboratories, Inc. (New York, New York). The D-mannose, which contained impurities migrating as oligosaccharides on paper chromatograms, was further purified by repeated recrystallization from acetic acid followed by cellulose-powder, column chromatography [elution solvent: 10:4:3 (v/v) ethyl acetate–pyridine–water]. The remaining sugars were obtained commercially, and were used without further purification.

The following polysaccharides were gifts from the sources noted: yeast mannan from *Saccharomyces cerevisiae* (Prof. S. Peat); galactomannan from *Leucaena glauca* (Dr. A. M. Unrau); glucomannan from Aspen wood and galactoglucomannan from Eastern Hemlock wood (Prof. T. E. Timell); galactoglucomannan from wood of *Picea abies* Karst (Prof. B. Lindberg); and guar gum (Meer Corporation, New York).

Reagents. — Phenol (analytical reagent) and Amberlite IR-45 anion-exchange resin were purchased from Mallinkrodt Chemical Works (St. Louis, Missouri). The phenol was redistilled, and prepared a an aqueous solution (80% w/w). Concentrated sulfuric acid (reagent grade) was a product of E. I. DuPont de Nemours and Co. (Wilmington, Delaware); Glucostat and Galactostat were products of the Worthington Biochemical Corp. (Freehold, New York).

PROCEDURE

Turbidimetric analysis. — To a Bausch and Lomb (Rochester, New York) Spectronic 20 tube (0.5 inch diameter) were added 0.95 ml of an aqueous solution of D-mannose (320–740 μg/ml) and 0.05 ml of glycogen solution (8.60 mg/ml). The same amount of glycogen, diluted to 1.0 ml with water and containing no inhibitor, was used as a control (hereafter called the uninhibited reference). The reaction was initiated by the rapid addition of 2.0 ml of concanavalin A solution (60 μg/ml). The solution was stirred immediately with a polyethylene rod, and incubated at 25°. After exactly 20 min, the resulting turbidity was measured at 420 nm in a Bausch and Lomb Spectronic 20 spectrophotometer, and recorded as optical density units (O.D.). Blank readings were obtained by clarifying the reaction mixture by the addition of crystalline methyl α-D-mannopyranoside (*ca.* 0.5 mg), an excellent inhibitor of the turbidimetric reaction[9]. The optical density of the clear solutions was determined after 5 min, and this value was subtracted from the optical density of the solutions prior to the addition of methyl α-D-mannopyranoside, to give a value referred to as corrected O.D.

Each analysis of a single, unknown solution was performed at three different dilutions, each in duplicate. A similar protocol was followed for standard solutions of D-mannose and D-glucose. The percent inhibition was calculated as follows:

$$\text{Percent inhibition} = \frac{\text{Corrected O.D. (uninhibited reference)} - \text{Corrected O.D. (inhibited)}}{\text{Corrected O.D. (uninhibited reference)}} \times 100.$$

Hydrolysis of polysaccharides. — Hydrolysis of polysaccharides (5–8 mg/ml) was conducted in sealed tubes in 1.5N sulfuric acid for 5–6 h in a boiling water-bath. The hydrolyzates were neutralized with Amberlite IR-45 (OH^-) resin (*ca.* 1 g/ml of hydrolyzate), the resin was washed thoroughly, and the solutions were evaporated to dryness *in vacuo* in a rotary evaporator. The residue was dissolved in sufficient water to produce a concentration of 4–6 mg of total sugar/ml, based upon the weight of the starting material.

During the course of these studies, it was noted that flocculation occurred during the 20-min incubation period of the analysis, when barium carbonate was used for neutralizing the sulfuric acid in the hydrolyzates. Therefore, the hydrolyzates were deionized with Amberlite IR-45 (OH^-) resin prior to analysis for carbohydrates. It was also noted that sodium chloride in concentrations up to 2N had no effect on the estimation of D-mannose. Additional information concerning the effect of various factors on the concanavalin A–glycogen interaction will be reported elsewhere[11].

Quantitative paper-chromatography. — Whatman No. 1 chromatographic filter paper was used for the quantitative, chromatographic separation of sugar mixtures, and was irrigated with either 10:4:3 (v/v) ethyl acetate–pyridine–water or butanone–water azeotrope. The D-mannose was eluted with water, and analyzed by the phenol–sulfuric acid method[12]. D-Glucose was analyzed by the Glucostat procedure, performed according to the micro method of Worthington Biochemical Corp. (manufacturer's directions) and D-galactose was analyzed by the Galactostat method (same manufacturer's directions).

RESULTS

Standard curves of the log of the weight (in mg) of D-mannose and D-glucose *versus* percent inhibition are shown in Fig. 1. Standard curves were prepared within the

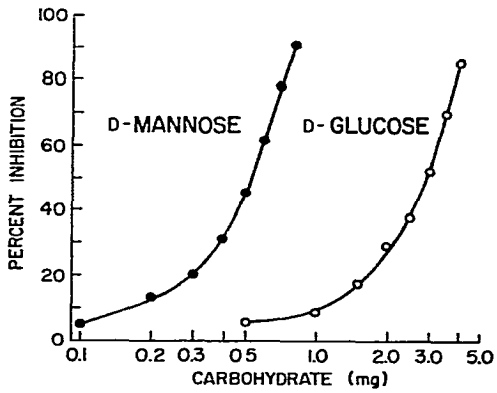


Fig. 1. Inhibition of concanavalin A–glycogen interaction by D-mannose and D-glucose. Concanavalin A, 120 μg; glycogen, 430 μg; in a total volume of 3.0 ml.

range of 10–90% inhibition by using eight different concentrations of D-mannose and D-glucose. It may be seen that, as an inhibitor, D-mannose is 5.4 times as effective as D-glucose. At least three concentrations of D-mannose (chosen to produce 25–75% inhibition), each in duplicate, were used as standards and were included with each analysis of unknowns.

When D-mannose was the sole sugar present, inhibition analysis, followed by reference to the standard curve for D-mannose, gave directly the quantity of this sugar present. The presence of noninhibitory sugars did not affect the determination of D-mannose by this method (*cf.* Table I).

TABLE I

TURBIDIMETRIC DETERMINATION OF D-MANNOSE IN THE PRESENCE OF D-GLUCOSE OR D-GALACTOSE

Synthetic mixtures, mg			*Total* D-*mannose-inhibiting equivalents*[a], *mg*	D-*Glucose, in* D-*mannose-inhibiting equivalents, mg*	D-*Mannose found, mg*	*Recovery, %*
D-Mannose	D-Glucose	D-Galactose				
0.200	0.500	—	0.290	0.093	0.197	98.5
0.300	0.500	—	0.401	0.093	0.308	102
0.400	0.500	—	0.489	0.093	0.396	99
0.300	—	1.95	0.312	—	0.312	104
0.400	—	1.65	0.408	—	0.408	102
0.500	—	1.35	0.500	—	0.500	100

[a]Converted from the percent inhibition, directly from the standard graph (Fig. 1).

It was observed that the inhibiting power of each sugar in a mixture of D-mannose and D-glucose was unaffected by the presence of the second sugar in the mixture. Thus, the inhibiting power of a mixture of the two sugars was additive. Since, as an inhibitor, D-mannose was 5.4 times as effective as D-glucose (as observed from the standard curves at the 50%-inhibition level), it was possible to calculate the theoretical inhibition of a mixture of these two sugars. Thus, a mixture of 0.30 mg of D-mannose and 1.62 mg of D-glucose would yield an inhibiting power equivalent to 0.60 mg of D-mannose alone: [0.30 mg of D-mannose + (1.62 mg of D-glucose)/5.4 = 0.60 mg of D-mannose].

Synthetic mixtures of D-glucose and D-mannose were readily analyzed in the following manner. A Glucostat determination gave the quantity of D-glucose present. (D-Mannose did not interfere in this determination.) The amount of D-glucose present was then converted into "D-mannose-inhibiting equivalents" (M.I.E.), expressed as mg of D-mannose, by employing the following relationship:

M.I.E. (mg of D-mannose) =

$$\text{mg of D-glucose} \times \frac{\text{(mg of D-mannose required for 50\% inhibition)}}{\text{(mg of D-glucose required for 50\% inhibition)}}.$$

Values for the 50%-inhibition levels were taken from the respective standard curves.

An inhibition determination was then performed on the D-glucose–D-mannose mixture by employing the standard curve for D-mannose. The amount of D-mannose present in the mixture is calculated by subtracting the contribution of D-glucose, in terms of M.I.E., from the total amount of D-mannose.

Table I shows the results obtained for the analysis of D-mannose in synthetic mixtures of D-mannose and D-glucose. It is evident that, after correcting for the known amount of D-glucose, the values obtained for D-mannnose are in excellent agreement with the amounts present. Table I also demonstrates that D-galactose, a common constituent of many heteromannans, has no effect on the estimation of D-mannose.

A comparison of the analysis of D-mannose in a variety of mannans, by the combination of quantitative, paper-chromatographic separation and the phenol–sulfuric acid method[12], and by the concanavalin A, turbidimetric assay, is shown in Table II. There is excellent agreement between these two empirical procedures.

TABLE II

COMPARISON OF THE TURBIDIMETRIC AND PHENOL–SULFURIC ACID METHODS FOR THE DETERMINATION OF D-MANNOSE IN VARIOUS MANNANS

Mannan	*Constituents*	*Percent of* D-*mannose in hydrolyzates*	
		Concanavalin A method	*Phenol–H_2SO_4[a] method*
Saccharomyces cerevisiae	D-mannose	94.5	100.0[b]
Leucaena glauca	D-mannose, D-galactose[c]	59.3	58.3
Guar gum	D-mannose, D-galactose[c]	57.4	59.9
Aspen wood	D-mannose, D-glucose[d]	62.0	64.8
Eastern Hemlock wood	D-mannose, D-galactose[c], D-glucose[d]	68.7	69.5
Picea abies Karst[e]	D-mannose, D-galactose[c], D-glucose[d]	70.6	71.4

[a]Paper-chromatographic separation, followed by elution of the D-mannose and analysis by the phenol–H_2SO_4 method (see ref. 11). [b]Normalized to 100.0. [c]Determined by the Galactostat method. [d]Determined by the Glucostat method. [e]This mannan was initially dissolved in 0.3N NaOH, and H_2SO_4 was added to yield a final acid concentration of 1.5N.

The effect, on the analysis of D-mannose, of some common carbohydrate constituents found in polysaccharides and glycoproteins is shown in Table III. Only slight interference occurs at high concentrations of D-arabinose and 2-amino-2-deoxy-D-glucose.

DISCUSSION

The concanavalin A turbidimetric procedure for the analysis of D-mannose is a simple, rapid, and precise method that makes use of the Landsteiner inhibition technique in which a species of low molecular weight (hapten) competes with an antigen for the binding sites on a specific antibody. The resemblance of concanavalin A–polysaccharide interaction to the antibody–antigen system has been discussed previously[9,13–15]. Here, the extent to which a specific inhibitor of the system

(D-mannose) inhibits the turbidimetric reaction between concanavalin A and glycogen is exploited for the quantitative determination of D-mannose.

TABLE III

EFFECT OF SOME ALDOSES ON THE TURBIDIMETRIC DETERMINATION OF D-MANNOSE

Sugar, mg	D-*Mannose, mg*		
	Present	*Found*	*Recovery* %
D-Arabinose (1.00)	0.500	0.522	104
D-Arabinose (1.50)	0.500	0.536	107
D-Xylose (2.00)	0.500	0.511	102
D-Xylose (3.00)	0.500	0.513	103
L-Fucose (5.00)	0.500	0.505	101
2-Amino-2-deoxy-D-glucose (1.00)	0.500	0.522	104
2-Amino-2-deoxy-D-glucose (1.50)	0.500	0.536	107
2-Amino-2-deoxy-D-galactose (3.00)	0.500	0.510	102
L-Rhamnose (5.00)	0.500	0.505	101

By this procedure it is possible to estimate D-mannose in the range of 300–750 μg. Modification of the assay by proportionally decreasing the components and the volume of the system could result in its use as a microanalytical tool for the determination of D-mannose.

A typical analysis required approximately one h. The length of time used for the incubation period may be decreased, but this causes a decrease in the resultant turbidity. Concurrent studies on the turbidimetric technique for studying polysaccharide–concanavalin A interaction has revealed the time-dependency of the reaction. Thus, with a longer incubation period, a parallel decrease in the percentage inhibition was observed[11]; this necessitated the use of larger aliquots of sample to produce a given percentage inhibition.

Because the amount of sugar needed to cause a given degree of inhibition is a function of the ratio of concanavalin A to glycogen, it was necessary to establish the conditions for maximum sensitivity to inhibition by D-mannose. This was accomplished by studying the initial rate of development of turbidity when various amounts of glycogen were allowed to react with a given amount of concanavalin A (120 μg). The ratio that yielded the highest initial rate of formation of turbidity (430 μg of glycogen: 120 μg of concanavalin A, commonly called the ratio of optimum proportions) was chosen for this study (*cf.* ref. 11).

It has been suggested[9,13,14] that the 2-deoxy-D-*arabino*-hexopyranose or D-arabinofuranose moiety is the minimal structural requirement for the binding of a monosaccharide to concanavalin A. Of the sugars in Table III, only 2-amino-2-deoxy-D-glucose and D-arabinofuranose possess these configurational features. Indeed, of the sugars tested, only 2-amino-2-deoxy-D-glucose and D-arabinose caused somewhat higher results than expected, when in concentrations equivalent to approximately three times that of D-mannose. Compensation for the presence of large proportions of such interfering substances may be made in a manner similar to that

used for the D-glucose correction. Thus, if the amount of an interfering substance is known, the D-mannose-inhibition equivalent, namely,

$$\text{mg of interfering substance} \times \frac{\text{mg of D-mannose needed for 50\% inhibition}}{\text{mg of substance needed for 50\% inhibition}},$$

of this substance may be subtracted from the total amount of sugar obtained directly from the standard graph. The consistent agreement that is obtained for the analysis of D-mannose by the concanavalin A method (with the above correction) and the method employing paper-chromatographic separation and phenol–sulfuric acid assay, in heteromannans containing D-glucose, is illustrated in Table II.

To the best of the authors' knowledge, this is the first report of the use of plant lectins for the specific, quantitative analysis of a sugar. It suggests the possibility that other carbohydrates may be analyzed in a similar manner by the use of other lectins, or of possible antihapten antibodies specific for a particular sugar or other haptens.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institutes of Health (AM-10171) and the Corn Industries Research Foundation, Inc.

SUMMARY

A unique, spectrophotometric method for the quantitative estimation of D-mannose in the presence of other sugars is described. The procedure depends on the degree to which the turbidity formed on interaction of concanavalin A with glycogen is specifically inhibited by D-mannose. The analysis, whose useful range is 300 to 750 μg/ml, gives essentially the same results as other methods commonly used. A correction for the presence of D-glucose, which also inhibits glycogen–concanavalin A interaction, is readily achieved by use of the Glucostat reagent.

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Printed in Belgium

SYNTHESIS OF THE METHYL D-ALLOPYRANOSIDES AND OF D-ALLOSE FROM 1,2:5,6-DI-*O*-ISOPROPYLIDENE-3-*O*-*p*-TOLYLSULFONYL-α-D-GLUCOFURANOSE

RAJENDRA AHLUWAHLIA, S. J. ANGYAL, AND M. H. RANDALL
School of Chemistry, The University of New South Wales, Kensington, N.S.W. (Australia)
(Received February 13th, 1967)

INTRODUCTION

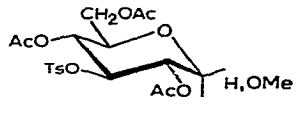
The reaction of sulfonic esters of sugars with sodium benzoate in *N,N*-dimethylformamide—introduced by B. R. Baker and co-workers[1,2]—has been used successfully for the interconversion of sugars[3]. The reaction may take a variety of courses[4], but, when the neighboring hydroxyl groups are esterified, the usual outcome is a displacement of the sulfonyloxy group, with inversion, by a hydroxyl or acyloxy group. In some cases, however, the reaction fails unpredictably; the factors influencing the reaction of secondary sulfonyloxy groups in carbocyclic or heterocyclic rings are not yet clearly understood. Thus, methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(methylsulfonyl)-α-D-mannopyranoside was reported to be resistant to displacement by sodium benzoate in *N,N*-dimethylformamide[5], whereas the corresponding derivatives of D-galactose[2,5] and D-glucose[6] undergo displacement. It has been reported[7] that only the 4-sulfonyloxy group is replaced in methyl 2-*O*-benzoyl-3,4-di-*O*-*p*-tolylsulfonyl-β-D-arabinoside.

Hughes and Speakman have recently reported[8] that 1,2,4,6-tetra-*O*-benzoyl-3-*O*-*p*-tolylsulfonyl-β-D-glucopyranose undergoes a displacement reaction with benzoate ion in *N,N*-dimethylformamide to give penta-*O*-benzoyl-β-D-allopyranose, but that the corresponding α-D anomer fails to react. At that time, we were engaged in similar work. Initially, we attempted the displacement on the tetra-*O*-acetyl derivatives of 3-*O*-*p*-tolylsulfonyl-D-glucose, but found that extensive decomposition occurs. For this reason, we decided to use the methyl glycopyranosides of 3-*O*-*p*-tolylsulfonyl-D-glucopyranose, in the form of their triacetates, because they are more stable, and found—in contrast to the results of Hughes and Speakman[8] — that both anomers react, to give methyl α- and β-D-allopyranoside, respectively.

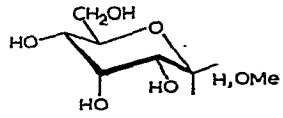
RESULTS AND DISCUSSION

The anomeric pair of compounds, methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-α- and β-D-glucopyranoside (**1a** and **1b**) was prepared by a modification of the method of Peat and Wiggins[9] involving methanolysis of 1,2:5,6-di-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-α-D-glucofuranose followed by acetylation. The reaction mixture was poured into water, from which the two anomers were precipitated in 72% yield; they

were separated from each other by fractional recrystallization from methanol. Extraction of the aqueous mother liquors with chloroform gave an oil which, on investigation by n.m.r. spectroscopy, showed the presence of methoxyl, acetyl, and isopropylidene groups, but not of *p*-tolylsulfonyl groups. The side reaction involved in the formation of this product is being studied further*.



1a α-D anomer
1b β-D anomer



2a α-D anomer
2b β-D anomer

The reaction of methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (**1b**) with sodium benzoate in boiling *N*,*N*-dimethylformamide was complete in 15 h, and was accompanied by very little discoloration or decomposition. After evaporation of the solvent, and dissolution of the residue in dilute sodium hydrogen carbonate, the products were extracted into chloroform. Examination of this extract by t.l.c. revealed the presence of two products, which were separated by column chromatography on silica gel. Both products gave methyl 2,3,4,6-tetra-*O*-acetyl-β-D-allopyranoside (as revealed by g.l.c.) on saponification and subsequent acetylation; the faster-moving component contained a benzoate group, whereas, in the slower-moving one, the presence of hydroxyl groups could be shown. These two products accounted, however, for only *ca.* 70% of the theoretical yield. A further amount of methyl β-D-allopyranoside was recovered from the aqueous layer by evaporation and saponification. This material was originally in a form more soluble in water than in chloroform, and therefore probably contained more than one hydroxyl group. Methyl β-D-allopyranoside (**2b**) was obtained in almost theoretical yield from these different fractions, but obviously, it was produced during the reaction in several forms, differing in the extent of acylation. In subsequent experiments, the reaction mixture was subjected to acetylation, to give products that were soluble in chloroform.

Methyl 2,3,4-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside (**1a**) required 66 h for complete reaction with sodium benzoate in boiling *N*,*N*-dimethylformamide, but, even during this long time, the mixture colored only slightly. The total yield of methyl α-D-allopyranoside (**2a**), isolated as its tetraacetate, was 92%, but, again, some of the reaction products were not soluble in chloroform, and were therefore assumed to contain free hydroxyl groups. Gas–liquid chromatography showed the absence of any other methyl glycoside.

Displacement of the *p*-tolylsulfonyloxy group under the conditions of the reaction can occur by two possible mechanisms: (i) direct displacement by a benzoate ion to give a benzoylated compound, or (ii) internal displacement by a neighbouring, *trans*, acyl group[10] to give first a cyclic acyloxonium ion, and ultimately, a compound having a

**Note added June 5th, 1967:* The product has been shown to be 3,6-anhydro-4,5-*O*-isopropylidene-D-allose dimethyl acetal.

free hydroxyl group. Hughes and Speakman[8] observed only the first mechanism in the reaction of 1,2,4,6-tetra-*O*-benzoyl-3-*O*-*p*-tolylsulfonyl-β-D-glucopyranose; their product was the pentabenzoate of β-D-allopyranose, and they did not isolate any compound containing a free hydroxyl group. In other cases of benzoate exchange, however, participation of neighboring acyl groups has been well established. For example, the reaction of methyl 2-*O*-benzoyl-5-deoxy-3-*O*-(methylsulfonyl)-α,β-D-xylofuranoside with sodium benzoate in *N*,*N*-dimethylformamide has been shown[11] to occur mainly by acyl participation. The reactions of acetylated derivatives of *p*-tolylsulfonyl-(+)- and (−)-inositols also proceed by neighboring-group participation[12]. If fluoride ion, a poor nucleophile, is used instead of benzoate ion, displacement occurs by participation of a neighboring acyloxy group[13].

Participation of a neighboring group may explain the formation of compounds containing one hydroxyl group. Those containing more than one must have been formed in some other way, and it was postulated that, during the prolonged heating with benzoate ion (which is probably a strong base in *N*,*N*-dimethylformamide), hydrolysis of acyl groups occurs. It was, indeed, found that there is a loss of acetyl groups when methyl tetra-*O*-acetyl-α-D-allopyranoside is heated with sodium benzoate in *N*,*N*-dimethylformamide. This deacylation explains why esters of sugars, in contrast to their methyl glycopyranosides, decompose under the conditions of benzoate exchange; the use of glycopyranosides is recommended in the sodium benzoate–*N*,*N*-dimethylformamide reaction.

Our reaction occurs to a large extent through direct displacement by benzoate ion; the presence of benzoate groups in the product can be shown by saponification, and isolation of benzoic acid. Whether the products containing hydroxyl groups were all formed by hydrolysis, or were partly formed by a reaction proceeding through a cyclic ion, has not yet been determined. Participation of an adjacent acyl group in the displacement requires an antiparallel orientation of the *p*-tolylsulfonyloxy and the acyloxy groups that is not readily achieved with the *gluco* configuration; it requires much less energy for cyclohexanediols, the optically active inositols, or furanoses; that is, for the compounds for which that mechanism has been observed.

Very little has been reported about the methyl D-allopyranosides. The β-D anomer has been prepared once in small amounts[14], but the α-D anomer has not been described. We have found that methyl β-D-allopyranoside (**2b**) crystallizes readily, but the α-D anomer (**2a**) has thus far remained a syrup. On the other hand, the tetraacetate of methyl α-D-allopyranoside is crystalline, but that of the β-D anomer has not yet been crystallized. A mixture of the anomers is, therefore, readily separated by crystallizing first the β-D-glycoside and then, after acetylation, the tetraacetate of the α-D-glycoside.

The molecular rotation of methyl α-D-allopyranoside (**2a**) in aqueous solution is +260.5°, in reasonable agreement with the value (+309°) calculated by Whiffen's method[15] for the *C1*(D) conformation. It is worth mentioning that the molecular rotation[16] reported for methyl 6-deoxy-α-D-allopyranoside is only +96°, far from the calculated value of +279°; however, this product was obtained as an oil from mother

liquors after crystallization of the β-D anomer, and probably still contained some methyl 6-deoxy-β-D-allopyranoside.

The n.m.r. spectra of methyl α- and β-D-allopyranoside clearly show the signals of H-1, H-2, and H-3, from which $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ can be derived. The coupling constants fully define the molecular shapes as the *CI*(D) conformations. These two glycosides represent instances in which the full chair structure (not just the dihedral angle at the anomeric carbon atom) is indicated by the n.m.r. spectrum. The α-D anomer (**2a**) shows long-range coupling between H-1 and H-3, typical of the 1*e*–3*e* arrangement[17].

Hydrolysis of the methyl D-allopyranosides gives D-allose. The sequence of reactions here described represents a convenient synthesis of D-allose, although nowadays, modern methods[18,19] involving the oxidation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose may be preferable. For the synthesis of D-allose, it is not necessary to separate the anomers of methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-D-glucopyranoside; the crude mixture of the anomers may be treated with sodium benzoate in *N*,*N*-dimethylformamide and the resulting mixture of methyl D-allopyranosides hydrolyzed to D-allose.

In order to explain why the α-D anomer of 1,2,4,6-tetra-*O*-benzoyl-3-*O*-*p*-tolylsulfonyl-D-glucopyranose does not yield a D-allose derivative, Hughes and Speakman[8] assumed that the axial benzoyloxy group on C-1 hinders the rearside approach to C-3. Our results seem to support their assumption; although, in our example, the axial group on C-1, a methoxyl group, is smaller, the α-D anomer reacts considerably more slowly than the β-D anomer. A direct comparison indicates that the ratio of reaction rates is about 8:1. The unreactivity of the compounds mentioned in the introduction, namely, methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(methylsulfonyl)-α-D-mannopyranoside[5] and methyl 2-*O*-benzoyl-3,4-di-*O*-*p*-tolylsulfonyl-β-D-arabinoside[7], is also explained by assuming hindrance by an axial group on that side of the molecule from which the nucleophile has to attack. Another compound, methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-*p*-tolylsulfonyl-α-L-mannopyranoside, in which approach of a nucleophile is similarly hindered, reacts with sodium acetate in *N*,*N*-dimethylformamide by ring contraction, instead of by displacement[20]. A similar explanation for the unreactivity of some methylsulfonyl derivatives towards sodium azide has been proposed by Dick and Jones[21].

EXPERIMENTAL

Melting points were determined on a Kofler microstage apparatus and are uncorrected. Evaporations of solvents were conducted under diminished pressure. *N*,*N*-Dimethylformamide was dried by storing it over barium oxide for 24 h, filtering, and distilling; the fraction boiling at 150–152° was used for the exchange reactions.

Analytical t.l.c. was performed with silica gel G on microscope slides with 1:1 benzene–ether. The components were detected by exposure to iodine vapor or, if that failed, by 1:9 chlorosulfonic acid–acetic acid for *ca.* 10 min at 110°.

For g.l.c., a 4-ft polyester (1.5% LAC-1-R 296 on Celite) column was used in a

custom-built instrument. The carrier gas was nitrogen, at a flow rate of 30–40 ml/min. The best operating temperature was 215°, with the injection block at 200°. The retention times of methyl 2,3,4,6-tetra-*O*-acetyl-α- and β-D-allopyranoside relative to methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside as unity, were 0.88 and 1.1, respectively.

*Anomeric methyl 2,4,6-tri-*O*-acetyl-3-*O*-p-tolylsulfonyl-D-glucopyranosides.* — 1,2:5,6-Di-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-α-D-glucofuranose[22] (30 g) and 2% methanolic hydrogen chloride (800 ml) were refluxed for 27 h. After neutralization with sodium hydrogen carbonate, the inorganic salts were filtered off, and the filtrate was concentrated to an oil, which was acetylated for 3 h at 100° with acetic anhydride (100 ml) and anhydrous sodium acetate (10 g).

On pouring the solution into iced sodium hydrogen carbonate solution and storing the mixture overnight at 0°, a mixture (25 g, 72%) of the anomers separated. Recrystallization from methanol (100 ml) gave methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (**1b**, 5.9 g), m.p. 135°, raised by recrystallization to 138° (lit.[9] 138°). On storage, the mother liquors gave an anomeric mixture, m.p. 90–135°, which, on recrystallization from ethanol, gave methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside (**1a**, 1.6 g), m.p. 93–94°, $[\alpha]_D^{24}$ +83° (chloroform) [lit.[9] m.p. 97°, $[\alpha]_D^{16}$ +87.1° (chloroform)].

*Reaction of methyl 2,4,6-tri-*O*-acetyl-3-*O*-p-tolylsulfonyl-β-D-glucopyranoside with sodium benzoate.* — (a) The title compound (1.5 g), *N*,*N*-dimethylformamide (60 ml), and sodium benzoate (1.5 g) were boiled for 15 h. After the solvent had been removed and the remaining solid had been dissolved in dilute sodium hydrogen carbonate, the solution was extracted with chloroform (5 × 20 ml) and the aqueous layer was kept. The extract was washed with water (2 × 15 ml) (and the washings were kept), dried (magnesium sulfate), and concentrated to an oil (1.2 g). Deacetylation with methanolic sodium methoxide, followed by de-ionization with IR-120 (H^+) resin, and concentration, yielded methyl β-D-allopyranoside (**2b**). After recrystallisation from ethanol, the allopyranoside (0.43 g, 71.7%) had m.p. 154–155°, $[\alpha]_D^{24}$ −50.8° (*c* 5.7, water) [lit.[14] m.p. 150–151°, $[\alpha]_D$ −53° (*c* 2, water)].

The original, aqueous solution and the aqueous washings of the chloroform extract were combined and evaporated to dryness, and the product was treated with methanolic sodium methoxide. After deionization with a mixed-bed, ion-exchange resin, the solution was concentrated to yield a further amount (0.16 g, 26%) of methyl β-D-allopyranoside, m.p. 156°. The overall yield from the sulfonate was, therefore, 97%. N.m.r. data were: δ 4.57 (doublet, $J_{1,2}$ 8.1 Hz, H-1), δ 4.13 (triplet with broad center line, $J_{2,3}$ 3.1, $J_{3,4}$ 2.4 Hz, H-3), δ 3.83–3.51 (multiplet, H-4,5,6), δ 3.60 (singlet, OMe), δ 3.40 (pair of doublets, $J_{1,2}$ 8.1, $J_{2,3}$ 3.1 Hz., H-2).

(b) In a similar experiment, methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (0.52 g), sodium benzoate (0.5 g), and *N*,*N*-dimethylformamide (20 ml) were boiled together for 15 h. After the solvent had been evaporated, the residual solid was dissolved in sodium hydrogen carbonate solution, and extracted with chloroform (4 × 75 ml). The extract was washed with water (2 × 20 ml), dried

(magnesium sulfate), and evaporated to an oil (0.39 g) which, on investigation by t.l.c., showed two components, having R_F *ca.* 0.5 and *ca.* 0.25.

The components were separated by preparative t.l.c. The component having R_F *ca.* 0.5 showed no hydroxyl absorption by infrared spectroscopy, but the component having R_F *ca.* 0.25 showed hydroxyl absorption.

Saponification with methanolic sodium methoxide, followed by reacetylation with 1:1 pyridine–acetic anhydride gave, in each case, one product which was indistinguishable by g.l.c. from authentic methyl 2,3,4,6-tetra-*O*-acetyl-β-D-allopyranoside.

*Reaction of methyl 2,4,6-tri-*O*-acetyl-3-*O*-p-tolylsulfonyl-α-D-glucopyranoside with sodium benzoate.* — The title compound (2.7 g), *N,N*-dimethylformamide (108 ml), and sodium benzoate (2.7 g) were boiled together for 66 h. After removal of the solvent, the residual solid was acetylated with 1:1 pyridine–acetic anhydride (50 ml). Removal of the acetylating reagent gave a solid that was dissolved in dilute sodium hydrogen carbonate solution. The chloroform extract (3 × 30 ml) of the resulting solution was washed with water (2 × 20 ml), dried (magnesium sulfate), and evaporated to a solid (2.26 g), which was saponified with methanolic sodium methoxide and the solution deionized with IR-120 (H^+) resin. Evaporation of the resulting solution gave syrupy methyl α-D-allopyranoside (1.02 g, 92%).

Acetylation with pyridine–acetic anhydride gave methyl 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranoside, m.p. 115–116°, $[\alpha]_D^{25}$ +97.7° (*c.* 1.3, chloroform).

Anal. Calc. for $C_{15}H_{22}O_{10}$: C, 49.7; H, 6.1. Found: C, 49.8; H, 6.1.

Methyl α-D-allopyranoside. — Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranoside was saponified with methanolic sodium methoxide. The resulting solution was deionized with IR-120 (H^+) resin, and evaporated to yield syrupy methyl α-D-allopyranoside, $[\alpha]_D^{25}$ +134° (*c* 0.5, water). N.m.r. data: δ 4.78 (pair of doublets, $J_{1,2}$ 4.0, $J_{1,3}$ 0.9 Hz, H-1), δ 4.08 (broadened triplet, $J_{2,3} = J_{3,4}$ *ca.* 3.0 Hz, H-3), δ 3.85–3.55 (multiplet, H-2,4,5,6), δ 3.41 (singlet, OMe).

Anal. Calc. for $C_7H_{14}O_6$: C, 43.3; H, 7.3. Found: C, 43.1; H, 6.9.

Comparison of the rate of reaction of the α-D and β-D anomers with sodium benzoate. — In a mixture of the anomeric *p*-toluenesulfonic esters (**1a** and **1b**) the α:β ratio was determined as being 2:1, by comparison of the *O*-methyl signals in the n.m.r. spectrum (δ 3.41 for the α-D and 3.48 for the β-D anomer). The mixture (0.13 g) was boiled with *N,N*-dimethylformamide (10 ml) and sodium benzoate (0.13 g); at intervals, samples of the mixture were removed and evaporated to dryness. After treatment with methanolic sodium methoxide for 0.75 h at 90°, the samples were evaporated to dryness and acetylated with 1:1 pyridine–acetic anhydride at 90°. Each sample was then concentrated, dissolved in dilute sodium hydrogen carbonate solution, and extracted into chloroform. The ratio of methyl 2,3,4,6-tetra-*O*-acetyl-α- to β-D-alloside was determined by g.l.c., and found to be: 5.5 h, 1:2.0; 23 h, 1:1.1; 29 h, 1:0.7; 35 h, 1:0.55; 49 h, 1:0.45. In the early stages of the reaction, a number of peaks of shorter retention time appeared, but they were not present after 49 h; they probably represent epoxides or methyl ethers formed from the *p*-tolylsulfonyl compounds by the action of sodium methoxide.

D-*Allose*. — A mixture of methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-α- and β-D-glucopyranosides (3.1 g, m.p. 85–135°) was treated with sodium benzoate and *N*,*N*-dimethylformamide by the method described for the α-D anomer. The resulting oil (1.2 g), which was a mixture of methyl α- and β-D-allopyranoside, was dissolved in 3N sulfuric acid (30 ml), and the solution heated for 2.5 h at 100°, cooled, neutralized with barium carbonate, and filtered; evaporation of the filtrate yielded an oil (1.1 g), which has not yet crystallized.

Column chromatography, with cellulose powder (120 g) as absorbent and 8:2 acetone–water as irrigant, gave a fraction (0.5 g) that crystallized from ethanol–water to give β-D-allose, m.p. 131–132°, mixed m.p. 130–131° (lit.[23] m.p. 128–128.5°). The early fractions contained 1,6-anhydro-β-D-allopyranose (about 14%), which could be hydrolyzed by acid to yield more D-allose.

*Reaction of sodium benzoate with methyl 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranoside*. — The title compound (0.04 g), *N*,*N*-dimethylformamide (2 ml), and sodium benzoate (0.04 g) were boiled together for 18 h. After removal of the solvent, and dissolution of the resulting solid in dilute sodium hydrogen carbonate solution, the solution was extracted with chloroform (3 × 10 ml). The extract was washed with water (2 × 5 ml), dried (magnesium sulfate), and evaporated to an oil (0.03 g), having ν_{max} *ca.* 3500 cm^{-1} (OH) and *ca.* 1725 cm^{-1} (C=O). Investigation of the oil by t.l.c. showed several components, the main component corresponding to methyl 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranoside. The other components had R_F values lower than that of the main component.

Reacetylation of the oil with 1:1 pyridine–acetic anhydride (2 ml) gave an oil (0.032 g) that was homogeneous by t.l.c., and indistinguishable from the title compound. Crystallization of the oil from ethanol gave methyl 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranoside (0.015 g), m.p. 115–116°, m.p. in admixture with authentic compound, 116°.

ACKNOWLEDGMENTS

This investigation was supported by a U. S. Public Health Service Grant from the National Heart Institute (to R. A.) and by a grant from the Australian Research Grants Committee (to M. H. R.). We thank Mrs. R. Milfull and Mr. G. C. Irving for assistance with many of the experiments.

SUMMARY

The two anomers, methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-α- and β-D-glucopyranoside, readily prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, react with sodium benzoate in *N*,*N*-dimethylformamide to give methyl α- and β-D-allopyranoside, respectively. The α-D anomer reacts much more slowly, presumably because of steric hindrance. This reaction offers a convenient method for the synthesis of D-allose.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS AND PROPERTIES OF ANOMERICALLY UNSUBSTITUTED HEPTA-*O*-ACETYL DISACCHARIDES*

R. M. ROWELL AND M. S. FEATHER

*Division of Wood Chemistry Research, Forest Products Laboratory***,
*Forest Service, U. S. Department of Agriculture, Madison,
Wisconsin 53705 (U. S. A.)*

(Received January 30th, 1967; in revised form, March 31st, 1967)

INTRODUCTION

The reaction[1] between 1,2,3,4,6-penta-*O*-acetyl-D-glucose and piperidine to give the partially deacetylated, condensation product, *N*-(3,4,6-tri-*O*-acetyl-D-glucosyl)-piperidine, is of considerable utility, as it provides the starting material for the preparation of some 2-*O*-substituted derivatives of D-glucose[1,2]. This reaction, when recently extended to cellobiose octaacetate, was found to produce an analogous end-product, and it permitted the preparation of 4-*O*-β-D-glucopyranosyl-2-*O*-methyl-D-glucose. Despite the obvious possibilities that this reaction possesses for the production of substituted carbohydrate derivatives and partially deacetylated sugars, it has received little attention from carbohydrate chemists.

This paper describes some additional experiments concerning this reaction, with β-cellobiose octaacetate and the octaacetates of some other disaccharides. If the reaction of β-cellobiose octaacetate with piperidine is halted after a short time, there is produced, in good yield, a nonnitrogenous, crystalline material having the properties and analysis of a heptaacetate. In view of the structure of the end product of the reaction, it is probable that this material was produced by *O*-deacetylation either at C-1 or C-2 of the reducing D-glucose residue. The chemical and physical properties of this material, including n.m.r. spectra, indicate that the product is the anomerically unsubstituted heptaacetate having the α-D configuration (A).

Previous workers have reported the isolation of heptaacetates of such disaccharides as cellobiose[4-7], celtrobiose[8], gentiobiose[9], 4-*O*-β-D-glucosyl-D-mannose[10], lactose[4,11], maltose[4,11-14], melibiose[15], neolactose[16], and turanose[17]. The general method of preparation has involved hydrolysis of the corresponding acetylated glycosyl halide derivatives. That these reaction conditions may not always result in the isolation of the anomerically unsubstituted acetate is evident from the studies of Corbett and co-workers[7], who recently isolated three different hepta-*O*-acetylcellobioses from a hydrolyzate of the acetylated glycosyl bromide. The reaction between piperidine and cellobiose octaacetate appears to produce only one heptaacetate

*Presented at the 153rd National Meeting of the American Chemical Society, Miami Beach, Florida, April 1967.

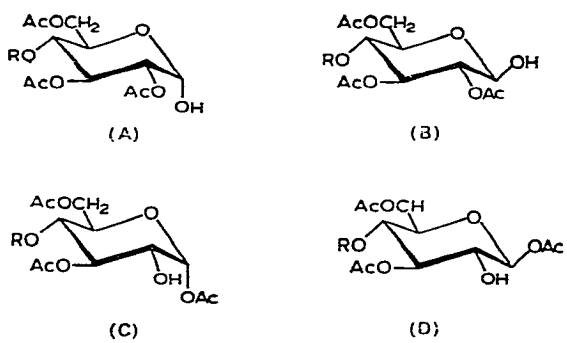
**In cooperation with the University of Wisconsin, Madison, Wisconsin.

(chromatographic evidence). The reaction proceeds equally well when applied to the octaacetates of maltose, melibiose, and lactose.

RESULTS AND DISCUSSION

On the basis of the end products of the reaction, the heptaacetate is probably one member of the two anomeric pairs shown below.

Lemieux and Morgan[18] examined the n.m.r. spectra of some acetates of D-glucose in chloroform-*d*, and concluded that a signal at τ 7.82 is indicative of an axial acetoxy group. A signal at τ 3.77 was assigned to an anomeric proton in the equatorial orientation. These assignments were tested in the cellobiose series with the octaacetates of α- and β-cellobiose, and found to be valid. The n.m.r. spectrum of the heptaacetate in chloroform did not have signals near τ 3.77 or τ 7.82, and structure C was, therefore, eliminated as a possibility. A signal for H-1 near τ 3.77 would appear if the acetoxy group were present at C-1; the absence of such a signal thus provides additional evidence that the C-1 hydroxyl group of the heptaacetate is unsubstituted. The heptaacetate mutarotates in anhydrous pyridine from $+33.4 \rightarrow +23°$ (in 24 h), indicating that the reducing moiety has the α-D configuration. That



R = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-

this change in optical rotation was a true mutarotation, and not an acetyl migration, is indicated by the fact that the starting material could be recovered in good yield from the mutarotated product, and the n.m.r. signals of the acetoxy protons were identical before and after mutarotation. In addition, acetylation of the heptaacetate with pyridine–acetic anhydride gave high yields of α-cellobiose octaacetate. This information suggests that structures B and D may be excluded, and structure A (**1**) is the only one consistent with the experimental results.

It is noteworthy that further treatment of **1** with piperidine readily gave the known *N*-[3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]piperidine.

Methylation (or ethylation) of **1** with the appropriate alkyl iodide in the

presence of silver oxide and *N,N*-dimethylformamide afforded crystalline material that, on hydrolysis, gave no alkyl ethers. Optical rotatory measurements indicated that, in each case, both anomeric glycosides were formed, in the approximate ratio of 1:1. Benzoylation also gave a crystalline product that was, presumably, 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-*O*-benzoyl-α-cellobiose.

The physical properties and chemical reactions undergone by **1** are similar to, but not identical with, those of the product that Corbett *et al.*[7] reported as having the same structure. Although the melting points of the two products are almost the same, the compound described in this report showed no mutarotation in chloroform, whereas Corbett *et al.*[7] reported $[\alpha]_D$ +33.3 → +25.0° (24 h). With anhydrous pyridine as the solvent, however, the mutarotation values now found for **1** are almost identical with theirs[7].

β-Maltose octaacetate reacted with piperidine in a similar manner, to give a crystalline heptaacetate whose mutarotation in chloroform, and also in pyridine, (+84 → 114° in 24 h) indicated the β-D configuration of the reducing moiety. Acetylation of this compound with acetic anhydride in pyridine gave only β-maltose octaacetate. The n.m.r. spectra in the acetoxy-proton region were identical before and after mutarotation, and did not show a signal at τ 7.82 or at τ 3.77. On the basis of this evidence, the heptaacetate of maltose may be assigned the structure 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucose (**2**), which is analogous to that found for the heptaacetate of α-cellobiose except that it has the opposite anomeric configuration. Methylation of **2** afforded an amorphous material that, on deacetylation, gave a syrup. Measurements of optical rotation suggested that this syrup was an approximately 1:1 mixture of methyl maltosides.

β-Lactose octaacetate likewise reacted with piperidine to give an amorphous product; its R_F value was similar to those of the disaccharide heptaacetates **1** and **2**. It has not yet been examined further.

Under the same conditions, β-melibiose octaacetate gave a crystalline product whose analysis corresponded to that for a heptaacetate and whose mutarotation values suggested that it is a β-D anomer. The preparation of this heptaacetate shows that the reaction is not limited to (1→4)-linked disaccharides. The n.m.r. spectra in the latter two cases did not give meaningful information about substitution at C-1, because the molecule contains a D-galactose moiety having an axial acetoxy group at C-4. In view of the chemical and physical properties of the latter two heptaacetates, and the preparative methods employed for their synthesis, it seems probable that they are also the anomerically unsubstituted derivatives.

EXPERIMENTAL

The disaccharide octaacetates were prepared by acetylation of the parent sugar with acetic anhydride and sodium acetate[19] and, hence, were the β-D anomers. N.m.r. spectra were determined at 60 MHz with a Varian A-60 spectrometer. Samples, at a concentration of 200 g/l in either chloroform or pyridine, were measured at 40°

with tetramethylsilane (τ = 10.00) as the internal standard. The purity of crystalline products was determined by t.l.c. on microscope slides coated with silica gel H and irrigated with 1:1 (v/v) hexane–ethyl acetate. Components were located by spraying with 10% sulfuric acid in ethanol and then charring until permanent spots became visible. Acetyl groups were determined qualitatively by the ferric hydroxamate spray-reagent[20]. Paper chromatography was performed on Whatman No. 1 paper, with a solvent system of 18:3:1:4 (v/v) ethyl acetate–acetic acid–formic acid–water, and the papers were sprayed with silver nitrate solution.

4-O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2,3,6-tri-O-acetyl-α-D-glucopyranose (**1**). — To 25 g of octa-*O*-acetyl-β-cellobiose (m.p. 202°) in 100 ml of tetrahydrofuran was added 25 ml of piperidine with stirring. After 1 h at 25°, dissolution was complete. After the solution had been stirred for an additional h, it was poured into 500 ml of ice–water. The aqueous solution was extracted with three 100-ml portions of chloroform, and the extracts were combined, washed successively with ice-cold, dilute, hydrochloric acid solution, saturated aqueous sodium hydrogen carbonate solution, and water, dried (sodium sulfate), and evaporated under diminished pressure to a thick syrup that crystallized on standing. Recrystallization from ethanol gave 19 g (81%) of **1**, m.p. 209°, $[\alpha]_D^{22}$ +33.4° (*c* 2.22, pyridine).

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.1; H, 5.69; acetyl, 47.4. Found: C, 49.3; H, 5.78; acetyl, 47.5.

Reactions of compound **1** — *A. Anomerization.* Compound **1** (2.2 g) was dissolved in 50 ml of pyridine, and the solution was kept for 24 h. During this time, a separate sample showed change in rotation: $[\alpha]_D^{22}$ +33.4 → +23.0° (*c* 2.22). At the end of this time, the pyridine solution was evaporated to a thick syrup that crystallized. Recrystallization from ethanol gave 1.7 g of **1**, m.p. 207°, $[\alpha]_D^{22}$ +29.4°. The n.m.r. signals from the acetoxy protons before and after mutarotation were identical. Compound **1** showed no mutarotation in chloroform during 48 h.

B. Acetylation. Compound **1** (2 g) was dissolved in 20 ml of pyridine, the solution was cooled to 0°, acetic anhydride (15 ml) was added, and the mixture was kept for 16 h at 25°. On dilution of the solution with 250 ml of ice-water, 1.6 g (75%) of crystals was obtained. After recrystallization from ethanol, this material had m.p. and mixed m.p. with an authetic sample of α-cellobiose octaacetate of 226°, and $[\alpha]_D^{22}$ +40.6° (*c* 1.6, chloroform).

C. Benzoylation. Compound **1** (2 g) was benzoylated in the usual way with pyridine and benzoyl chloride to give 1.7 g (73%) of a monobenzoate of **1**, m.p. 218°, $[\alpha]_D^{22}$ +59.8° (*c* 0.84, chloroform).

Anal. Calc. for $C_{33}H_{40}O_{19}$: C, 53.5; H, 5.41. Found: C, 53.6; H, 5.42.

D. Methylation. Compound **1** (1 g), methyl iodide (5 ml), silver oxide (2 g), and *N,N*-dimethylformamide (10 ml) were stirred together for 16 h at 25°. The mixture was filtered through Celite, diluted with benzene, and evaporated to a thick syrup, which crystallized from ethanol; m.p. 170°, $[\alpha]_D^{22}$ +16.1° (*c* 0.87, chloroform).

Deacetylation of this material, followed by hydrolysis for 2 h at 100° in N sulfuric acid, gave a hydrolyzate for which paper chromatography indicated a glucose as the

only product. Known methyl hepta-*O*-acetyl-α-cellobioside[21] has m.p. 185°, $[\alpha]_D^{25}$ +55.7°, and methyl hepta-*O*-acetyl-β-cellobioside[22] has m.p. 187°, $[\alpha]_D^{25}$ −25.7°.

E. Ethylation. Compound **1** (1 g) was ethylated with ethyl iodide, silver oxide, and *N,N*-dimethylformamide, as described for the methylation of **1**. Crystallization of the syrupy product from ethanol gave material having m.p. 152°, $[\alpha]_D^{22}$ +8.3° (*c* 0.8, chloroform). Known ethyl hepta-*O*-acetyl-α-cellobioside[23] has m.p. 175°, $[\alpha]_D^{25}$ +57.2°, and ethyl hepta-*O*-acetyl-β-cellobioside[7] has m.p. 190°, $[\alpha]_D^{25}$ −23.5°. Hydrolysis of this material, as described for the methylation product, gave only a glucose.

F. Preparation of N-(*3,6,2',3',4',6'-hexa*-O-*acetyl-β-cellobiosyl*)*piperidine.* Compound **1** (5 g) was mixed with 25 ml of tetrahydrofuran, and to the slurry was added 5 ml of piperidine; the mixture was stirred for 36 h at 25°, and then poured into 100 ml of ice-water. The aqueous mixture was extracted with three 50-ml portions of chloroform, the extracts were combined, and the piperidine was neutralized with cold, 30% aqueous acetic acid. The chloroform solution was washed with water, dried (sodium sulfate), and evaporated to a syrup, which crystallized. Recrystallization from methanol gave 0.4 g, m.p. 185° (lit.[3] m.p. 189°). By t.l.c., the product was identical with an authentic sample.

*4-*O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*glucopyranosyl-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranose* (**2**). — β-Maltose octaacetate (25 g, m.p. 160°) was treated with piperidine as described for the preparation of **1**. Crystallization of the final syrup from ethyl acetate–hexane, followed by two recrystallizations from ethanol, gave 17.8 g (76%) of **2**, m.p. 188°, $[\alpha]_D^{22}$ +84 → 114° (*c* 0.928, pyridine, 24 h)*.

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.1; H, 5.69; acetyl, 47.4. Found: C, 49.0; H, 5.81; acetyl, 47.4.

Reactions of compound **2**. — *A. Acetylation.* Compound **2** (2 g) was acetylated with acetic anhydride in pyridine as described for **1**. The yield was 1.4 g (66%); m.p. and mixed m.p. with authentic β-maltose octaacetate, 159–160°, $[\alpha]_D^{22}$ +60.1° (*c* 1.4, chloroform).

B. Methylation. Compound **2** (1 g) was methylated as described for **1**. The resulting syrup, which did not crystallize, was deacetylated with sodium methoxide in methanol, to give syrup having $[\alpha]_D^{22}$ +125° (*c* 0.97, water). Known methyl α-maltoside[24] has $[\alpha]_D^{22}$ +184°, and methyl β-maltoside[25] has $[\alpha]_D^{25}$ +84°.

Reaction of the octaacetates of melibiose and lactose with piperidine. — β-Melibiose octaacetate (25 g, m.p. 177°) was treated with piperidine as described for **1**. The final syrup crystallized on standing, and after two recrystallizations from ethanol, gave 15.5 g (66.5%) of product, m.p. 199–200°, $[\alpha]_D^{22}$ +151 → 163° (*c* 2.38, pyridine, 24 h).

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.1: H, 5.69; acetyl, 47.4. Found: C, 49.3; H, 5.95; acetyl, 47.5.

*J. E. Hodge and B. E. Fisher also prepared this compound by this method, and observed m.p. 190–191°, $[\alpha]_D^{22}$ +73 → +109.7° (chloroform) (personal communication, November 3rd, 1966).

In a similar way, 25 g of β-lactose octaacetate (m.p. 90°) reacted, to give 19 g of a syrup. As with the other heptaacetates, this compound had a thin-layer chromatographic mobility approximately half that of the starting material.

SUMMARY

The octaacetates of β-cellobiose and β-maltose react with piperidine in tetrahydrofuran to give crystalline, anomerically unsubstituted heptaacetates, having, respectively, the α- and β-D configurations for the reducing moiety. The structural assignments were made on the basis of chemical reactions and n.m.r. spectroscopy. These heptaacetates underwent acetylation with retention of configuration and, on methylation, gave methyl α- and β-D-glycosides in the approximate ratio of 1:1.

The octaacetates of β-melibiose and β-lactose likewise react readily with piperidine in tetrahydrofuran to give heptaacetates, which were not fully characterized. Available data indicate that these compounds are also anomerically unsubstituted at the reducing moiety.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

A NEW METHOD OF SYNTHESIS OF INDOLYL GLYCOSES

YU. A. ZHDANOV, V. I. KORNILOV, AND G. V. BOGDANOVA
The Rostov-on-Don State University, Rostov-on-Don (U.S.S.R.)

(Received November 7th, 1966; in revised form, March 6th, 1967)

INTRODUCTION

α-Diazocarbonyl compounds, because of their tendency to form reactive α-oxo carbenes[1], possess great synthetic possibilities for the formation of new carbon–carbon bonds. Thus, substituted indole derivatives have been obtained from the reaction of indole with diazoacetic ester[2], some diazoketones, and esters of ω-diazo-oxo acids[3,4].

In a preliminary communication[5], it was shown that acetylated 1-deoxy-1-diazoketoses afford α-oxo carbenes that may be used for the synthesis of *C*-substituted sugars. In the case of indole, the sugar residue is linked to the β-position of the indole ring to form the corresponding 1-*C*-substituted 1-deoxy derivatives of higher ketoses.

EXPERIMENTAL

Acetylated aldonic acid chlorides were prepared by heating the aldonic acid acetates in dry ether with phosphorus pentachloride[6]. 1-Deoxy-1-diazoketoses were synthesized by the action of ethereal diazomethane on the acetylated aldonic acid chlorides[7,8]. Infrared spectra (mineral oil) were obtained with a IKS-14 spectrophotometer; ultraviolet spectra were recorded for methanolic solutions (10^{-5}M) with a SF-4 spectrophotometer.

Penta-O-acetyl-D-gluconic acid. — To acetic anhydride (150 ml), cooled with ice, 70% perchloric acid (15.5 g) was added whilst the temperature of the mixture was kept below 20°. Calcium D-gluconate (15 g) was then added in small portions so that the temperature did not rise above 40°. The mixture was kept for 20 min at 40°, cooled, poured into ice–water (500 ml), and stirred for 1 h. The product was extracted with chloroform (6 × 50 ml), and the combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The syrupy residue was dissolved in hot toluene (100–150 ml), and the solution was kept in a refrigerator overnight. The product was washed with light petroleum and air-dried; yield, 15 g (78.5%); m.p. 110–112°; lit.[12], 110–111°.

Following the same technique, penta-*O*-acetyl-D-galactonic acid (83%), m.p. 128–130° (lit.[12], 129–131°), and tetra-*O*-acetyl-L-arabinonic acid (50%), m.p. 132–135°, (lit.[12], 135–136°), were prepared from calcium D-galactonate and calcium L-arabinonate, respectively.

Penta-O-acetyl-1-deoxy-1-(indol-3-yl)-keto-D-gluco-*heptulose* (**2a**). — To a mixture of indole (3.5 g; 0.08 mole), dry toluene (5 ml), and a catalytic quantity of copper powder at 95–100°, was added dropwise a solution of penta-*O*-acetyl-1-deoxy-1-diazo-*keto*-D-*gluco*-heptulose (4.3 g; 0.01 mole) in toluene (50 ml). Liberation of nitrogen began at once, and when this was complete, the catalyst was filtered off. The toluene was removed by distillation, and the syrupy residue was kept *in vacuo* at 95–100° for *ca.* 3 h in order to sublime the major part of the unchanged indole.

The syrup was treated 5–6 times with hot, light petroleum, and was then dissolved in benzene (50 ml) and gradually poured into light petroleum (500 ml). The product separated as a dark syrup that was dissolved in chloroform–methanol (19:1) and shaken with a small quantity of alumina for several hours. The alumina was filtered off, and the filtrate was evaporated *in vacuo*. The residue was dissolved in benzene (50 ml) and poured into light petroleum (0.5 l). The crude product was precipitated as a yellow, amorphous powder, and was purified by repeated re-precipitation from light petroleum, the material precipitated initially being discarded. The product (1.8 g, 35%) finally obtained was a yellow, amorphous powder that was soluble in most organic solvents, but insoluble in water and light petroleum; ν_{max}^{NaCl} 1758 (OAc), 1718 (C=O), 1640 and 1517 cm^{-1} (C=C); ν_{max}^{LiF} 3340–3320 cm^{-1} (N–H); λ_{max} 222 (ε 26,500) and 280 nm (7500) (Found: C, 57.94; H, 5.31; N, 2.53. $C_{25}H_{29}NO_{11}$ calc.: C, 57.80; H, 5.58; N, 2.69%).

Oxidation of penta-O-acetyl-1-deoxy-1-(indol-3-yl)-keto-D-gluco-*heptulose*[4]. — Compound **2a** (1.55 g) was suspended in 1% aqueous sodium hydroxide (75 ml), and oxygen was passed through the suspension for 30–40 h at room temperature. The resulting, resinified solution was extracted with ether, acidified (pH 5), and again extracted with ether. The combined ethereal extracts were treated with charcoal and dried with sodium sulphate. Paper chromatography with chloroform–acetic acid (50:1) or butyl alcohol–ammonia revealed the presence of indole-3-carboxylic acid.

Penta-O-acetyl-1-deoxy-1-(indol-3-yl)-keto-D-galacto-*heptulose* (**2b**). — Following the procedure described for the preparation of **2a**, compound **2b** (1.5 g, 35%) was obtained as a bright-yellow powder from penta-*O*-acetyl-1-deoxy-1-diazo-*keto*-D-*galacto*-heptulose (4.3 g, 0.01 mole) and indole (3.5 g, 0.03 mole). The product had ν_{max}^{NaCl} 1755 (OAc), 1714 (C=O), 1648 and 1517 cm^{-1} (C=C); λ_{max} 222 (ε 26,800) and 280 nm (7500) (Found: C, 58.20; H, 5.49; N, 2.50. $C_{25}H_{29}NO_{11}$ calc.: C, 57.80; H, 5.58; N, 2.69%).

Tetra-O-acetyl-1-deoxy-1-(indol-3-yl)-keto-L-*fructose* (**2c**). — According to the directions given for obtaining **2a**, tetra-*O*-acetyl-1-deoxy-1-diazo-*keto*-L-fructose (3.5 g, 0.01 mole) and indole (3.5 g, 0.03 mole) gave compound **2c** (1.33 g, 31%) as a yellow, amorphous powder that was soluble in most organic solvents, and insoluble in water and light petroleum; ν_{max}^{NaCl} 1758 (OAc), 1716 (C=O), 1648 and 1510 cm^{-1} (C=C); λ_{max} 222 (ε 26,800) and 280 nm (7500) (Found: C, 59.40; H, 5.80; N, 2.79. $C_{22}H_{25}NO_9$ calc.: C, 59.06; H, 5.59; N, 3.23%).

1-Deoxy-1-(indol-3-yl)-D-gluco-*heptulose* (**4a**). — Compound **2a** (1.38 g; 0.0026 mole) was added to a solution prepared from sodium (0.14 g) and methanol

(30 ml). The mixture was kept for 2 h at room temperature, a small excess of a cation exchanger (KY-2, H^+) was added, and the mixture was kept overnight. The cation exchanger was filtered off, the solution was concentrated to 20 ml, and the product was precipitated with a large excess of ether. The brown, amorphous powder was filtered off, redissolved in methanol and boiled with charcoal for 2 h, and the product was precipitated with ether.

Compound **4a**, which was soluble in methanol and ethanol, less soluble in water, and insoluble in non-polar, organic solvents, had ν_{max}^{LiF} 3396 (N–H) and 3324–3260 cm^{-1} (OH); ν_{max}^{NaCl} 1710 cm^{-1} (weak, C=O); λ_{max} 222 (ε 27,500) and 278 nm (10,700) (Found: C, 58.40; H, 5.87; N, 4.37. $C_{15}H_{19}NO_6$ calc.: C, 58.25; H, 6.14; N, 4.53%).

To a portion of **4a** (0.0408 g) were added water (2 ml), saturated, aqueous sodium hydrogen carbonate (2 ml), and aqueous sodium periodate (5 ml, saturated at room temperature)[10]. The mixture was kept overnight in the dark, and then diluted to 25 ml. Samples (2 ml) were withdrawn and added to saturated, aqueous sodium hydrogen carbonate (10 ml) and 20% aqueous potassium iodide (2 ml). The mixtures were kept for 15 min in the dark. The liberated iodine was titrated with 0.2N sodium thiosulphate in the presence of starch. Compound **4a** consumed 4.13 mol. of sodium periodate.

1-Deoxy-1-(indol-3-yl)-D-galacto-*heptulose* (**4b**). — According to the procedure described for the preparation of **4a**, penta-*O*-acetyl-1-deoxy-1-(indol-3-yl)-*keto*-D-*galacto*-heptulose (**2b**, 1.38 g) was converted into compound **4b** (0.3 g, 37%), obtained as a brown, amorphous powder, ν_{max}^{LiF} 3390 (NH) and 3320–3260 cm^{-1} (OH); ν_{max}^{NaCl} 1718 cm^{-1} (weak, C=O); λ_{max} 222 (27,800) and 278 nm (10,650) (Found: C, 58.02; H, 5.89; N, 4.56. $C_{15}H_{19}NO_6$ calc.: C, 58.25; H, 6.14; N, 4.53%).

1-Deoxy-1-(indol-3-yl)-L-*fructose* (**4c**). — In a manner similar to that described for **4a**, tetra-*O*-acetyl-1-deoxy-1-(indol-3-yl)-*keto*-L-fructose (1.5 g) was converted into compound **4c** (0.36 g, 40%) obtained as a brown, amorphous powder that was readily soluble in methanol, less soluble in water, and insoluble in non-polar solvents; ν_{max}^{NaCl} 1710 (weak, C=O), 1608 cm^{-1} (C=C); λ_{max} 222 (ε 27,600) and 278 nm (10,735) (Found: C, 59.81; H, 6.32; N, 5.35. $C_{14}H_{17}NO_5$ calc.: C, 60.21; H, 6.09; N, 5.01%).

1-Deoxy-1-(indol-3-yl)-D-galacto-*heptulopyranosylamine* (**6b**). — Compound **2b** (2 g) was dissolved in dry methanol (50 ml), and dry ammonia was passed through the mixture (cooled with ice) for 3 h. The reaction mixture was then kept overnight at room temperature, the methanol was evaporated, and the residual syrup was extracted several times with warm ether. The syrup was re-dissolved in methanol, and the solution was shaken with charcoal for 2 h and diluted with ether. The resulting product was repeatedly washed with dry ether to yield compound **6b** (0.98 g, 80%) as a light-brown, amorphous powder that was purified by several re-precipitations from methanol with ether. The product was readily soluble in methanol and ethanol, but less soluble in water, and had ν_{max}^{NaCl} 1664 (NH_2), 1610 and 1554 cm^{-1} (C=C); ν_{max}^{LiF} 3290–3220 cm^{-1} (OH); λ_{max} 222 (ε 19,112) and 278 nm (7062) (Found: C, 58.30; H, 6.10; N, 9.30. $C_{15}H_{20}N_2O_5$ calc.: C, 58.63; H, 6.49; N, 9.12%).

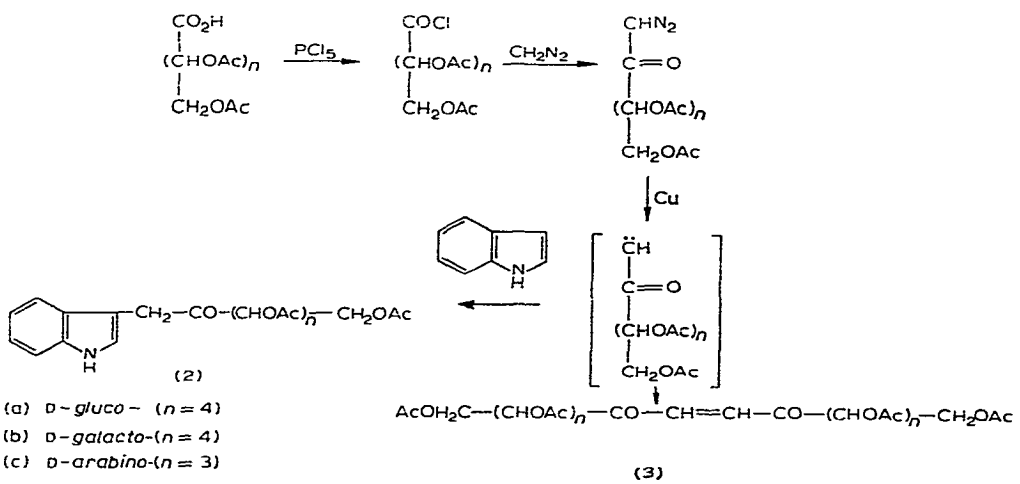
1-Deoxy-1-(indol-3-yl)-D-gluco-*heptulopyranosylamine* (**6a**). — By a procedure

similar to that used for the preparation of **6b**, compound **2a** (2 g) was converted into compound **6a** (0.8 g, 60%), obtained as a light-brown, amorphous powder that was soluble in alcohol and moderately soluble in water. Compound **6a** had ν_{max}^{NaCl} 1662 (NH_2), 1608 and 1560 cm^{-1} (C=C); ν_{max}^{LiF} 3295–3216 cm^{-1} (OH); λ_{max} 222 (ε 19,000) and 278 nm (7058) (Found: C, 58.59; H, 6.30; N, 9.35. $C_{15}H_{20}N_2O_5$ calc.: C, 58.63; H, 6.49; N, 9.12%). The product consumed 3.2 mol. of sodium periodate under standard conditions[10].

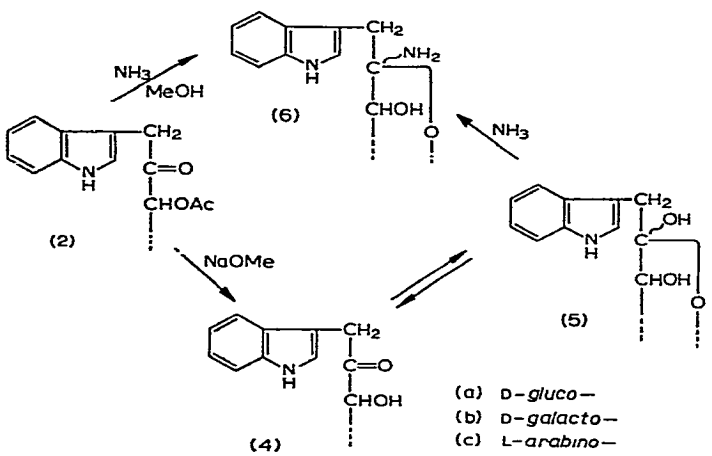
*1-Deoxy-1-(indol-3-yl)-*L*-fructopyranosylamine.* — By the method described for **6b**, compound **2c** (2 g) was converted into compound **6c** (0.6 g, 50%) obtained as a light-brown, amorphous powder, ν_{max}^{NaCl} 1664 (NH_2), 1608 and 1560 cm^{-1} (C=C); λ_{max} 222 (ε 19,150) and 278 nm (7060) (Found: C, 60.22; H, 6.10; N, 9.87. $C_{14}H_{18}N_2O_4$ calc.: C, 60.43; H, 6.47; N, 10.07%).

DISCUSSION

The decomposition of diazoketoses in the presence of indole proceeds with the formation of β-substituted derivatives. However, other reactions, may occur, for example, dimerisation[5,11] of the initial oxo-carbene into the corresponding diacylethylene (3) (Scheme 1). This difficulty may be overcome by adding the diazoketose to a considerable excess (2.5–3-fold) of indole. The purity of the compounds synthesized was checked by thin-layer chromatography on alumina by using chloroform–methanol (30:1) and detection with concentrated sulphuric acid. Solutions of 1-*C*-indolyl derivatives became dark in colour on standing, whereas the isolated products are stable, yellow, amorphous powders. The dark colour of the solutions makes polarimetry very difficult. The constitutions of the amorphous indole derivatives were indicated by elemental analysis, infrared and ultraviolet spectroscopy, alkaline oxidation with oxygen to give indole-3-carboxylic acid, and periodate oxidation.



In the infrared spectra of the acetylated indole derivatives, the strong bands at 1758 and 1220 cm^{-1} are characteristic of the acetyl group. The band at *ca.* 1718 cm^{-1} corresponds to the carbonyl absorption of the ketone, and the weak absorptions at 1640 and 1517 cm^{-1}, together with the band at *ca.* 3025 cm^{-1}, may be assigned to the aromatic residue. The strong band at 3340–3320 cm^{-1} is assigned to the N–H bond of the indole ring. The ultraviolet spectra of the acetylated samples had maxima at 222 (ε 26,800) and 280 nm (7500) consistent with the spectrum of indole. Deacetylation (sodium methoxide) gave material having absorptions in its infrared spectrum at 3396 (indole N–H), and 3324–3260 cm^{-1} (hydroxyl). The presence in the deacetylated compounds of a weak carbonyl absorption at 1710 cm^{-1}, and the data of periodate oxidation, indicate the existence of a tautomeric equilibrium between an open-chain structure and a pyranoid ring, characteristic of unprotected ketoses. This view is supported by the following facts. (*a*) Under the conditions of deacetylation by ammonia, the nitrogen content of the reaction products corresponds to the substitution of a glycoside hydroxyl in compound **5** by an amino group (structure **6**). (*b*) Amino compounds having structure **6** may be prepared from samples deacetylated by sodium methoxide. This is possible only if a hemiacetal hydroxyl group is available, that is, the deacetylated compounds must have structure **5**, so that a reaction may occur that is analogous to the formation of glycosylamines from unprotected sugars (Scheme 2).



SUMMARY

The copper-catalysed decomposition of acetylated 1-deoxy-1-diazoketoses in the presence of indole affords acetates of higher 1-deoxy-1-(indol-3-yl)ketoses. The constitution of the products was indicated by elemental analysis, infrared and ultraviolet spectroscopy, destructive oxidation, and periodate oxidation.

Deacetylation of the acetates with sodium methoxide gave the 1-deoxy-1-(indol-3-yl)ketoses, whereas, on deacetylation by ammonia, 1-deoxy-1-(indol-3-yl)ketosyl-

amines were formed. Periodate-oxidation data indicated that the ketosylamines contain a stable, pyranoid ring.

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Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE PREPARATION OF AMINO SUGARS FROM METHYL GLYCOPYRANOSIDULOSES: METHYL 4-ACETAMIDO-4,6-DIDEOXY-α-L-TALOPYRANOSIDE

S. W. GUNNER, W. G. OVEREND, AND N. R. WILLIAMS

Department of Chemistry, Birkbeck College (University of London), Malet Street, London, W. C. 1 (Great Britain)

(Received February 7th, 1967; in revised form, March 23rd, 1967)

INTRODUCTION

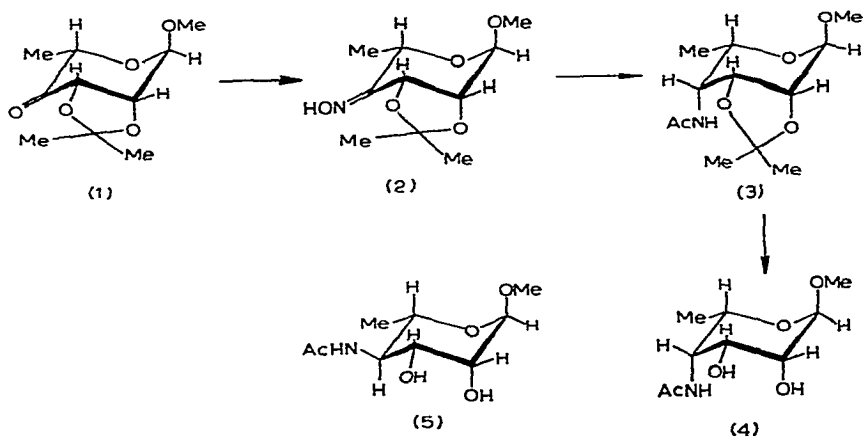
In a recent report[1] from this laboratory, a route to amino sugars from partially protected glycopyranosides by sequential oxidation to the glycopyranosidulose, oximation, reduction of the oxime, and removal of protecting groups was evaluated. Several 2-amino-2-deoxy sugars have been prepared in this way, and the procedure has now been extended to include the synthesis of derivatives of 4-amino-4-deoxy sugars. Members of this class are of interest primarily because of their occurrence in antibiotics[2–4] and the possibility of forming furanoid derivatives containing nitrogen in the ring[5]. Studies of this type of ring system are important in relation to the destruction of 4-amino-4-deoxy sugars and nonulosaminic acids under acidic conditions of hydrolysis, a factor which complicates studies by fragmentation methods of materials containing these sugars.

DISCUSSION

The glycopyranosidulose used as starting material was methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose (**1**). This compound was prepared first in our laboratory by Dr. B. M. Gough[6] by oxidation of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside) with the chromium trioxide–pyridine complex employing essentially the conditions described by Burton *et al.*[7]. The oxidation has been carried out also at a higher temperature without loss of yield, which is about 50% (obtained after recycling unreacted material several times). Subsequently, oxidation with ruthenium tetroxide was shown to be a much better method giving yields of 80–90% of purer product. The oxoglycoside was characterised as its crystalline 2,4-dinitrophenylhydrazone. It also afforded a crystalline oxime (**2**), and this was reduced with lithium aluminium hydride in ether to give preponderantly one compound which, after *N*-acetylation, was isolated pure and in crystalline form. The *N*-acetyl derivative (**3**) could be deacetonated to give a solid methyl 4-acetamido-4,6-dideoxy-α-L-hexoside (**4**) which yielded a crystalline 2,3-diacetate. Attempts to obtain the free 4-amino-4,6-dideoxyhexose, by hydrolysis under various conditions of either the methyl 4-acetamido-4,6-dideoxy-α-L-hexoside or its 2,3-diacetate, were unsuccessful.

There are several reasons for believing that the amino-sugar derivatives now described have the L-*talo* configuration. (*i*) The steric course of reduction of oximes is less well-documented than is the case for ketones, but indications are that they are similar[1]. Previously, it had been shown[8] that stereospecific reduction of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose with lithium aluminium hydride gives a hexoside having the L-*talo* configuration, and so reduction with lithium aluminium hydride of the oxime of this glycopyranosidulose might be expected to lead to an L-*talo*-aminoglycoside having the amino group axially located in the preferred conformation.

(*ii*) The n.m.r. spectra of the methyl 4-acetamido-4,6-dideoxy-α-L-hexoside (**4**) and its 2,3-*O*-isopropylidene and 2,3-di-*O*-acetyl derivatives show coupling constants for the anomeric proton $J_{1,2}$ of ~1.0 c.p.s., suggesting the *1C* conformation with an axial glycosidic methoxyl group, and a chemical shift for the CH_3 protons in the acetamido group of τ 7.94–7.96, compatible with an axial rather than an equatorial acetamido group[9] which would be present in the *manno* isomer (**5**). A further analysis



using a 100 Mc.p.s. spectrometer showed H-5 as an octet centred at τ ~5.9, which, on irradiation of the C-5-methyl doublet at τ 8.86, collapsed to a doublet at τ 5.95, $J_{4,5}$ ~2 c.p.s. The low value for $J_{4,5}$ indicates that these protons cannot be diaxial, so that the acetamido group must be axial with H-4 equatorial and H-5 axial. (*iii*) In general, the replacement of a hydroxyl group by an amino group in pyranosides does not affect substantially their molecular rotations[10–13]. A comparison of $[M]_D$ of the product from reduction of the oxime with those of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*talo*- and -*manno*-pyranoside, respectively, indicates the *talo* configuration for the major component of the reaction product. This conclusion is reinforced by examining the $[M]_D$ values of methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-L-mannoside and -taloside and the triacetate obtained from the reduction product by sequential *N*-acetylation, deacetonation, and *O*-acetylation. The values are given in Table I. (*iv*) Recently, and well after our work was first recorded[14], Stevens *et al.*[15] outlined a similar sequence to

ours working with the D-isomers. They described the preparation of methyl 4-acetamido-4,6-dideoxy-α-D-taloside from methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside. The configurational assignment was based on mass spectral and n.m.r. measurements.

TABLE I

COMPARISON OF THE MOLECULAR ROTATION OF DERIVATIVES OF 4-AMINO-4,6-DIDEOXY-α-L-TALOSE WITH RELATED COMPOUNDS

Compound	$[M]_D$	*Solvent*	*Reference*
Methyl 4-amino-4,6-dideoxy-2,3-*O*-isopropylidene-α-L-taloside	−11,000[a]	MeOH	This work
Methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside	−11,350	EtOH	22
Methyl 6-deoxy-2,3-*O*-isopropylidene α-L-*mannopyranoside*	− 2,600	MeOH	23
Methyl 4-acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-L-talopyranoside	−24,000	$CHCl_3$	This work
Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-L-taloside	−23,000	MeOH	8
Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-L-mannoside	−16,300	$C_2H_2Cl_4$	24

[a]Based on reduction product from the oxime (2) being 90% L-*talo* isomer.

Compared to other secondary ring sulphonates, nucleophilic displacement reactions of methyl pyranoside 4-sulphonates are considered to occur with relative ease[16], and the unassisted displacement of a 4-*O*-sulphonyl derivative by an azide ion theoretically should afford the epimeric 4-azido derivative by an S_N2 process, and subsequently by reduction a 4-amino sugar[2,17]. Consequently, an attempt was made to devise an alternative synthesis of methyl 4-acetamido-4,6-dideoxy-2,3-*O*-isopropylidene-α-L-taloside by treating methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-mesyl-α-L-mannoside (*A*) with an excess of sodium azide in *N*,*N*-dimethylformamide. However, the reaction led to a product which was not fully characterised, and this approach was not pursued. Hanessian[18] has shown recently that an unusual rearrangement occurs during the displacement and the product is methyl 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-α-L-talofuranoside. An identical rearrangement was observed by Stevens and co-workers[19] when the D-enantiomer of compound *A* was treated with lithium azide in *N*,*N*-dimethylformamide.

EXPERIMENTAL

General. — Infrared absorption spectra were measured with a Perkin–Elmer "Infracord" spectrometer. N.m.r. spectra were measured with a Varian A-60 spectrometer operating at 60 Mc.p.s. on 10% solutions in deuteriochloroform with tetramethylsilane as internal standard. Paper chromatography was effected by the descending method with Whatman No. 1 paper in butyl alcohol–ethanol–water (4:1:5) as eluant and detection by silver nitrate–acetone dip followed by sodium hydroxide–ethanol spray[20]. Thin-layer chromatography (t.l.c.) was performed with Desaga equip-

ment by using Silica gel G (Merck A. G., Darmstadt, Germany) with either benzene–methanol in the following proportions (*i*) 99:1, (*ii*) 19:1, (*iii*) 1:1, or (*iv*) chloroform–light petroleum (b.p. 40–60°) (3:1) as solvent system. Indication was either by (*a*) the anisaldehyde spray reagent[21] or (*b*) the ammoniacal silver nitrate spray.

Methyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose. — Chromium trioxide (70 g) was added slowly with stirring to dry pyridine (1.9 l) at 0–5°. After the yellow complex had formed, methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (40.5 g) in pyridine (100 ml) was added. The mixture was stirred for 2 h at room temperature, and then was brought to reflux in the course of 1 h, boiled with stirring for 1 h, and cooled. The product was isolated by removal of pyridine and extraction of the powdered residue with ether (5 × 750 ml). The oxo sugar was obtained from the syrupy product by repeated fractional distillation as a clear, colourless, mobile liquid (13.6 g, 34%; increased yields could be obtained by re-oxidising recovered starting material), b.p. 78–80°/0.45 mm, 48–49°/0.03 mm, $[\alpha]_D^{20}$ −101° (*c* 3.5, methanol), υ_{max} 1750 cm^{-1} (C=O). Gough[6] reported b.p. 68–70°/0.2 mm, $[\alpha]_D^{22}$ −103°.

A mixture of the oxoglycoside (300 mg) and 2,4-dinitrophenylhydrazine (300 mg) in methanol (3 ml) was treated with two drops of N sodium hydroxide and heated under reflux for 2.5 h. After cooling, filtration, and evaporation, the syrupy residue in chloroform was passed through a column of neutral alumina (10 × 2 cm). From the eluate, crystals were obtained, and, on recrystallisation from aqueous ethanol, the 2,4-dinitrophenylhydrazone (300 mg, 55%) was obtained as yellow needles, m.p. 151.5–152°, $[\alpha]_D^{20}$ +303° (*c* 0.36, chloroform), υ_{max} 3350, 1650 cm^{-1}.

Anal. Calc. for $C_{16}H_{20}N_4O_8$: C, 48.5; H, 5.1; N, 14.1. Found: C, 48.1; H, 5.2; N, 14.0.

Methyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose oxime. — A solution of the glycopyranosidulose (4.9 g) in water (75 ml) and methanol (10 ml) was treated with hydroxylamine hydrochloride (1.88 g) in N sodium hydroxide (15 ml). After its pH had been adjusted to 8, the solution was stirred for 7 h at 65°, then for 15 h at room temperature, and finally for 1 h at 80°. The clear, colourless solution was cooled in ice, and adjusted to pH 6–7 by addition of *N* hydrochloric acid. After brief storage at 0°, colourless crystals separated. After several hours, these were harvested. Processing of the evaporated mother liquors gave more solid material. The combined solid was recrystallised twice from aqueous acetone to yield the oxime (3.65 g, 70%) as colourless needles, m.p. 128–128.5°, $[\alpha]_D^{20}$ −153° (*c* 1.3, methanol), ν_{max} 1660–1620 cm^{-1} (C=N), R_F 0.75 [t.l.c., solvent (*ii*) and spray (*a*)].

Anal. Calc. for $C_{10}H_{17}NO_5$: C, 51.9; H, 7.4; N, 6.1. Found: C, 52.2; H, 7.3; N, 5.95.

Reduction of methyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose oxime. — The oxime (2.31 g) in dry ether (10 ml) was added dropwise to a stirred mixture of lithium aluminium hydride (3 g) in dry ether (150 ml) at room temperature. After being heated under reflux with stirring for 2 h, the mixture was cooled in an ice bath, and excess of hydride was destroyed by slow addition of water. The ethereal layer

was decanted, and the residue washed with ether (3 × 100 ml). The combined ethereal solutions were dried and evaporated to a clear, yellow syrup which was shown by t.l.c. to contain components having the following R_F values: 0.86 (*ca.* 90%), 0.80 (5–10%), 0.10 (trace) [all in solvent (*i*), and detection by reagents (*a*) or (*b*)]. Both faster-moving components gave a positive result in the spray test with ninhydrin.

The crude reduction product (2 g) in warm ethanol (20 ml) was acetylated [acetic anhydride (2.5 ml), 60°, 3 min] to give a viscous, yellow syrup which crystallised on trituration with solvent. Sublimation (90–100°/0.02 mm) gave white crystals (2 g); t.l.c. in several solvent systems showed one major component, together with a small proportion (*ca.* 5–10% by visual estimation) of another. (As prolonged heating during sublimation led to pyrolytic breakdown of the crystals, purification by this method was subsequently undertaken batchwise on small amounts of material.) Two recrystallisations from acetone gave the acetamido derivative as small, colourless prisms (1.6 g, 70%), m.p. 152.5–153°, $[\alpha]_D^{20}$ −31° (*c* 1.22, methanol) ν_{max} 3300, 1560 (NH), 1650 (NAc) cm^{-1}, R_F 0.75 [t.l.c., solvent (*i*), reagent (*a*)], 0.44 [solvent (*ii*), reagent (*a*)].

Anal. Calc. for $C_{12}H_{21}NO_5$: C, 55.6; H, 8.2; N, 5.4. Found: C, 56.0; H, 8.2; N, 5.4.

The second component, assumed to be the epimeric *N*-acetyl derivative, was not isolated.

Methyl 4-acetamido-4,6-dideoxy-α-L-talopyranoside. — The above acetonated *N*-acetyl derivative (1.5 g) in methanol (25 ml) was shaken at room temperature with ion-exchange resin [7.5 g of Amberlite IR-120 (H^+) which had been washed with methanol]. Deacetonation was followed by t.l.c. and was complete in 20 h; the solution was then decanted, and the resin washed with more methanol (3 × 20 ml). The combined methanolic solutions were evaporated to a solid residue (0.9 g) which was recrystallized several times from ethyl acetate as colourless prisms (0.75 g, 59%), m.p. 184.5–185°, $[\alpha]_D^{20}$ −148° (*c* 1.22, methanol), ν_{max} 1660 (NAc), 3400, 1550 (NH) cm^{-1}, R_F 0.73 [t.l.c., solvent (*iii*), reagent (*a*)], 0.68 (paper chromatography).

Anal. Calc. for $C_9H_{17}NO_5$: C, 49.3; H, 7.8; N, 6.4. Found: C, 49.5; H, 8.05, N, 6.5.

A solution of the crystals (0.05 g) in either N or 0.1N hydrochloric acid (5 ml) at 100° became dark after 10 min and afforded a tar which was shown by paper-chromatographic analysis to contain several components. Use of 2N hydrochloric acid or 50% acetic acid for 10 days at room temperature did not lead to reducing sugar as revealed with Fehling's solution.

Acetylation of the 4-acetamido derivative (202 mg) in pyridine (1.5 ml) with acetic anhydride (1.5 ml) for 24 h at room temperature gave a viscous syrup which crystallised on storage. Recrystallisation from acetone–ether–light petroleum (b.p. 60–80°) gave the 2,3-di-*O*-acetyl derivative (230 mg, 82%) as fine, colourless needles, m.p. 137–137.5°, $[\alpha]_D^{20}$ −79° (*c* 1.1, chloroform), ν_{max} 3400, 1550 (NH), 1660 (NAc), 1760 (C=O) cm^{-1}.

Anal. Calc. for $C_{13}H_{21}NO_7$: C, 51.5; H, 7.0; N, 4.6. Found: C, 51.9; H, 7.0; N, 4.65.

Methyl 6-deoxy-2,3-O-isopropylidene-4-O-methanesulphonyl-α-L-mannopyranoside. — Mesyl chloride (3 ml) was added to an ice-cold solution of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (7 g) in dry pyridine (20 ml). After storage for 48 h at 0°, the mixture was poured into stirred, crushed ice (100 ml). The precipitate was collected by filtration and recrystallised from aqueous ethanol; yield 8.8 g (93%), m.p. 130–131°, $[\alpha]_D^{20}$ −4.6° (*c* 1.6, chloroform), R_F 0.50 [t.l.c., solvent (*iv*),spray (*a*)]. Hanessian[18] reported m.p. 128–129° for this compound, and Stevens *et al.*[19]gave m.p. 128–129° for the D-isomer.

Anal. Calc. for $C_{11}H_{20}O_7S$: C, 44.6; H, 6.8; S, 10.8. Found: C, 44.6; H, 6.9; S, 11.7.

A mixture of the 4-*O*-mesyl derivative (7.2 g) and sodium azide (14.7 g) in dry *N*,*N*-dimethylformamide (150 ml) was heated under reflux for 48 h. After processing, a pale-yellow syrup (5.6 g) was obtained from which a small amount of unreacted material (0.8 g) was deposited on standing. T.l.c. showed the syrup to contain four components, (*i*) starting material, (*ii*) an azido-derivative, (*iii*) and (*iv*) unidentified substances in small proportion. After column chromatography on neutral alumina with chloroform–light petroleum (b.p. 40–60°) (3:1), the azido derivative was obtained as a colourless, mobile syrup (3.6 g, 69%), $[\alpha]_D^{20}$ −17° (*c* 2.4, methanol), ν_{max} 2100 cm^{-1}, R_F 0.66 [t.l.c., solvent (*iii*), spray reagent (*a*)]. Hanessian[18] gave $[\alpha]_D^{25}$ −21° in methanol for methyl 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-α-L-talofuranoside.

ACKNOWLEDGMENTS

The authors thank Drs. P. M. Collins and R. J. Ferrier for helpful discussions, and Mr. J. Hoyle of the Chemistry Department, University of Alberta, Edmonton for the 100 Mc. n.m.r. spectra. The work was made possible by the generous financial support provided by the Medical Research Council and the Governors of Birkbeck College.

SUMMARY

A route to derivatives of amino sugars from partially protected glycopyranosides by sequential oxidation to the corresponding glycopyranosidulose, oximation, and reduction of the oxime has been extended by the conversion of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside into derivatives of a 4-amino-4,6-dideoxy-hexose. Some evidence regarding the configuration of the amino-sugar derivatives is included.

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Notes

Muramic acid having a reduced side-chain: 2-amino-2-deoxy-3-*O*-[(*S*)-1-(hydroxymethyl)ethyl]-D-glucose*

INTRODUCTION

In the structure proposed for the peptidoglycan backbone of bacterial cell-walls[1], some of the *N*-acetylmuramic acid [2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose] residues are linked to a peptide component through the carboxyl group of the lactyl side-chain, whereas other residues have a free carboxyl group. In order to determine the location and the proportion of these free groups in the

*Amino sugars LIII. This is publication No. 427 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. This investigation was supported by research grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service (Grant AI-06692) and the National Science Foundation (Grant GB-5031). Preliminary communication: *Abstracts Papers Am. Chem. Soc. Meeting*, 145 (1963) 18D.

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Muramic acid having a reduced side-chain: 2-amino-2-deoxy-3-*O*-[(*S*)-1-(hydroxymethyl)ethyl]-D-glucose*

INTRODUCTION

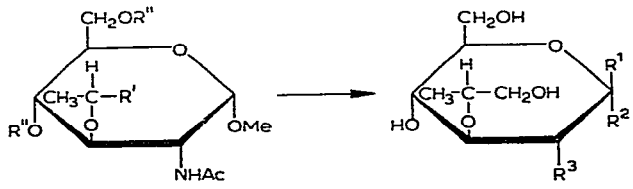
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peptidoglycan backbone, Salton[2] proposed to reduce the carboxylic group (after esterification), hydrolyze the peptidoglycan, and isolate the reduced *N*-acetylmuramic acid[2]. For the purpose of comparison, the synthesis of this reduced compound, namely 2-amino-2-deoxy-3-*O*-[(*S*)-1-(hydroxymethyl)ethyl]-α-D-glucose (**4**), was undertaken and is described in the present paper.

RESULTS AND DISCUSSION

Reduction of methyl 2-acetamido-3-*O*-[D-1-(methyl carboxylate)ethyl]-2-deoxy-α-D-glucopyranoside[3] (**1**) with lithium borohydride afforded, in 91% yield, crystalline



(1) R'= CO_2Me , R''= H
(2) R'= CH_2OH , R''= H
(3) R'= CH_2OAc , R''= Ac

(4) R^1= H , R^2= OH , R^3= NH_2
(5) R^1= H , R^2= OH , R^3= $NH_3^+Cl^-$
(6) R^1= OH, R^2= H , R^3= N=CH–$C_{10}H_6OH$

methyl 2-acetamido-2-deoxy-3-*O*-[(*S*)-1-(hydroxymethyl)ethyl]-α-D-glucopyranoside (**2**), which was further characterized by a crystalline tri-*O*-acetyl derivative (**3**). Hydrolysis of **1** gave, in good yield, the crystalline 2-amino-2-deoxy-3-*O*-[(*S*)-(hydroxymethyl)ethyl]-α-D-glucopyranose (**4**), isolated as the crystalline hydrochloride. Compound **4** was further characterized by a crystalline Schiff base (**6**) with 2-hydroxy-1-naphthaldehyde, but attempts to obtain a crystalline *N*-acetyl derivative failed.

EXPERIMENTAL*

*Methyl 2-acetamido-2-deoxy-3-*O*-[(S)-1-(hydroxymethyl)ethyl]-α-D-glucopyranoside* (**2**). — Compound **1** (200 mg, see ref. 3) was added to a 0.5M solution of lithium borohydride in dry tetrahydrofuran (3 ml). The solution was kept for 24 h at room temperature, and then the excess of lithium borohydride was decomposed by addition, with cooling, of 5% acetic acid (20 ml). The solution was passed through Dowex-50 (X-8, 50–200 mesh, H^+) resin, and the eluate and washings were evaporated to dryness. Abs. ethanol and then methanol were successively added and evaporated to remove boric acid. The colorless syrup was crystallized and recrystallized from acetone–ether to give 165 mg (91%) of rhombic prisms, m.p. 185–7°, $[\alpha]_D^{25}$ +108° (*c* 0.42, methanol).

Anal. Calc. for $C_{12}H_{23}NO_7$: C, 49.14; H, 7.90. Found: C, 49.01; H, 7.84.

Acetylation of **2** (50 mg) was performed with dry pyridine (3 ml) and acetic anhydride (2.5 ml) for 12 h at room temperature, and then for 0.5 h at 60°. An excess of methanol was added to the ice-cold solution. The solution was evaporated, the last traces of pyridine and acetic acid being removed by adding and distilling off toluene.

*For conditions, see previous publication[4].

The crystalline residue, methyl 2-acetamido-3-*O*-[(*S*)-1-(acetoxymethyl)ethyl]-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**3**) was recrystallized twice from acetone–pentane to give 44 mg (78%) of long needles, m.p. 137–8°, $[\alpha]_D^{27}$ +84° (*c* 0.46, chloroform).

Anal. Calc. for $C_{18}H_{29}NO_{10}$: C, 51.54; H, 6.97. Found: C, 51.45; H, 7.00.

*2-Amino-2-deoxy-3-*O*-[(S)-1-(hydroxymethyl)ethyl]-α-D-glucopyranose hydrochloride* (**5**). — A solution of **2** (30 mg) in 3N hydrochloric acid (1.5 ml) was boiled overnight under reflux. After being cooled, the solution was evaporated, and the residue was dried by successive additions of abs. ethanol and toluene, and evaporation, and kept in a desiccator over soda-lime. The residue was crystallized twice from methanol–acetone to give 20 mg (72%) of monoclinic prisms, m.p. 176–8° (dec.), $[\alpha]_D^{24}$ +68 (4 min) → +48° (10 h) (*c* 0.99, water).

Anal. Calc. for $C_9H_{20}ClNO_6$: C, 39.49; H, 7.37; Cl, 12.95. Found: C, 39.57; H, 7.48; Cl, 13.00.

*2-Deoxy-3-*O*-[(S)-1-(hydroxymethyl)ethyl]-2-[(2-hydroxy-1-naphthylmethylene)amino]-β-D-glucopyranose* (**6**). — To a solution of **5** (20 mg) in water (0.5 ml) was added a solution of 2-hydroxy-1-naphthaldehyde (50 mg) and sodium acetate trihydrate (35 mg) in methanol (4 ml). After being mixed, the solution was kept in the dark for 3 h at room temperature, and then evaporated (bath temperature, 20° max). The residue was dissolved in benzene, and the solution was chromatographed on 5 g of silica gel. Benzene–ether (4:1) eluted the excess of 2-hydroxy-1-naphthaldehyde. Ethyl acetate–acetone (2:1) eluted crystalline fractions, which were recrystallized from acetone–methanol to give yellow prisms (24 mg, 84%), m.p. 178–180° (dec.), $[\alpha]_D^{20}$ +118 (7 min) → +294° (48 h), $[\alpha]_{5461}^{20}$ +122 (7 min) → +299° (48 h) (*c* 0.36, methanol).

Anal. Calc. for $C_{20}H_{25}NO_7$: C, 61.37; H, 6.44. Found: C, 61.26; H, 6.56.

ACKNOWLEDGMENT

The authors thank Charles Pfizer and Co. for a gift of 2-acetamido-2-deoxy-D-glucose.

Laboratory for Carbohydrate Research,
Departments of Biological Chemistry
and Medicine,
Harvard Medical School and
Massachusetts General Hospital,
Boston, Massachusetts 02114 (U. S. A.)

ROGER W. JEANLOZ
EVELYNE WALKER

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(Received February 27th, 1967)

Carbohyd. Res., 4 (1967) 504–506

Preparation of an oligoguluronide from sodium alginate

Alginic acid is a polymer of D-mannuronic acid and L-guluronic acid[1], which can be separated, by several precipitation techniques[2-5], into fractions that are rich in D-mannurono- and L-gulurono-lactones. The two uronic acids occur in the same uronide chain[6], and acid-resistant sections containing 80–90% of L-guluronic acid or 80–90% of D-mannuronic acid have been demonstrated[7]. Special interest in these compounds has been stimulated recently by the demonstration that dietary supplementation with sodium alginate produces inhibition of strontium absorption in rats[8,9], without alteration of calcium absorption. In humans[10,11], a similar effect has been reported for strontium.

The highest content of L-guluronic acid found in naturally occurring alginate extracted from readily available seaweed is[12] 73%. In addition, oligouronides containing 90% of L-guluronic acid have been prepared by partial hydrolysis with acid[7]. By combining selective precipitation and partial, acid hydrolysis, an oligouronide having a degree of polymerization of about 13 (12.5 from determination of reducing power, and 12.9 from determination of intrinsic viscosity), and containing 97% of L-guluronic acid, has now been prepared from a commercially available alginate. The effect of this material on the absorption of strontium will be reported elsewhere.

EXPERIMENTAL AND RESULTS

A commercial sodium alginate (Manucol SS/LD/2 — Alginate Industries Ltd.), used as a starting material, contained 71% of L-guluronic acid.

Preparation of an oligouronide having a high content of L-*guluronic acid.* — (*a*) *Selective precipitation.* A 1% solution of sodium alginate containing 0.35N magnesium chloride was precipitated by the addition of 2% (v/v) Industrial Methylated Spirits (IMS). The precipitated magnesium alginate was separated by centrifugation and suspended in water, and the pH was adjusted to 1.5 with hydrochloric acid. The precipitated alginic acid was centrifuged, washed twice with water, resuspended in 25% of the original volume of water, and then neutralized with sodium hydroxide solution. A one-tenth volume of 25% sodium chloride solution was then added, followed by a half volume of IMS. The precipitated sodium alginate was separated by centrifugation and washed twice with 50% IMS, twice with 75% IMS, and twice with 100% IMS, and dried *in vacuo* over anhydrous calcium chloride. Yield, 20–25%. The resulting sodium alginate contained 85–90% of L-guluronic acid.

(*b*) *Partial hydrolysis with acid.* One part of the air-equilibrated product was dissolved in 75 parts of water, 20 parts of 5N hydrochloric acid were added, and the total volume was made up to 100 parts. The mixture of precipitated alginic acid and N hydrochloric acid was then heated for 6 h at 100° in a sealed vessel, cooled, and centrifuged, and the supernatant was discarded. The precipitated oligouronic acid was washed twice with water and resuspended in 40 parts of water before being neutralized

with sodium hydroxide solution. The sodium oligouronide was precipitated by the addition of 4 parts of 25% sodium chloride solution and a half volume of IMS, washed, and dried as before. The overall yield was 12–15% of a white powder that was readily soluble in water and had $[\alpha]_D^{25}$ −128° (*c* 1.0, water).

Analysis of alginate and oligouronide for uronic acids[12]. — The uronide was completely hydrolysed with sulphuric acid, and the uronic acids were separated by anion-exchange chromatography, and determined colorimetrically.

Sulphuric acid (80%, 0.5 ml) was added to 50 mg of the air-equilibrated material contained in a 15-ml Bijou bottle cooled in an ice bath. The mixture was maintained for 18 h at 20°, water (6.5 ml) was then added, and the sealed mixture was heated for 5 h at 100°. Calcium carbonate (1 g) was added, and, after centrifugation, the supernatant was drawn off. The precipitate was washed twice with water, and the supernatant and washings were combined and diluted to 25 ml. A 5-ml aliquot of this solution was brought to pH 8.0 by the addition of 0.1N sodium hydroxide, and the resulting solution was applied to a column (12 × 120 mm) of Bio-Rad AG1 × 8 anion-exchange resin (200–400 mesh, AcO^-) (Bio-Rad Laboratories, Richmond, California). The column was eluted with 300 ml of 1–2.4N acetic acid in a linear gradient at 13.6 ml/h; the column temperature was kept at 30°. Fractions (5 ml) were collected, and analysed for uronic acid by the orcinol reaction[13]. The peaks for guluronic and mannuronic acid appeared between tubes 20 and 45. A correction was made for the different rates of breakdown of guluronic and mannuronic acids during hydrolysis[12]. The guluronic acid content was calculated on the assumption that the alginate and oligouronide are composed entirely of residues of L-guluronic and D-mannuronic acid.

Initially, the guluronic and mannuronic acids were identified by chromatography on Whatman No. 1 paper with pyridine–ethyl acetate–acetic acid–water (5:5:1:3)[1].

Determination of degree of polymerization. — (*a*) *From reducing power.* A modified Hagedorn–Jensen method was used[13,14] with the substitution of 0.005N sodium arsenite for 0.005N sodium thiosulphate; the final conditions of titration were altered to take this into account.

(*b*) *From viscosity.* Intrinsic viscosity $[\eta]$ was determined by extrapolating to zero concentration the specific viscosities of several concentrations of oligouronide in 0.1N sodium chloride. The degree of polymerization was calculated[15] from the expression D. P. = 58 × $[\eta]$.

DISCUSSION

The preparation of a uronide consisting mainly of residues of L-guluronic acid was stimulated by the earlier observation[9] that, in rats, strontium absorption decreased as the L-guluronic acid content of sodium alginate, given as a dietary additive, increased. Further experiments have demonstrated that sodium oligouronide prepared by the present method, when fed to rats, is by far the most effective means of selectively decreasing strontium absorption. The effect of chain length is currently being investigated.

I thank Dr. G. E. Harrison and Mr. T. E. F. Carr of this Unit, and Dr. Elizabeth Percival of Royal Holloway College, London, for advice and helpful criticisms in the preparation of the manuscript, and Jill Pollard for technical assistance.

Medical Research Council,
Radiobiological Research Unit,
Harwell,
Didcot, Berkshire (Great Britain)

E. R. HUMPHREYS

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(Received February 16th, 1967)

An unusual distribution of substituents on etherification of the anomeric methyl 4,6-*O*-benzylidene-D-glucosides

We report here an unexpected distribution of *O*-(2-diethylaminoethyl) substituents at the C-2 and C-3 hydroxyl groups of the α (**1a**) and β (**1b**) anomers of methyl 4,6-*O*-benzylidene-D-glucoside. Compound **1a** has been reported to react predominantly at the C-2 hydroxyl group with (benzylthio)carbonyl chloride, benzoyl chloride, or *p*-toluenesulfonyl chloride in dry pyridine[1,2]. Under the same conditions, compound **1b** reacts with (benzylthio)carbonyl chloride to yield 40% of a disubstituted derivative and only a small proportion of monosubstituted derivative; recovery of **1b**[2], 40%. The latter results seem to indicate that the hydroxyl groups at C-2 and C-3 exhibit (equally?) low reactivities and that the hydroxyl group adjacent to a newly substituted position possesses enhanced reactivity. Essentially equal reactivities for the hydroxyl groups of **1b** were observed on methylation with methyl sulfate in aqueous solution[3].

We have found that both **1a** and **1b** react preferentially at the C-3 hydroxyl

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We have found that both **1a** and **1b** react preferentially at the C-3 hydroxyl

group with 2-diethylaminoethyl chloride. The product from the reaction of **1a** in 20% sodium hydroxide solution with 1.1 molar proportions of 2-diethylaminoethyl chloride (16 h, room temperature) contained 2.35% of nitrogen. On being processed; it yielded 6.2% of the 2-ether, 12.3% of the 3-ether, 19.5% of a di-ether, and 59.5% of unchanged **1a**. Under the same conditions, the reaction product from **1b** contained 3.62% of nitrogen; it yielded 7.4% of the 2-ether, 12.1% of the 3-ether, 35.8% of a di-ether, and 46.8% of unchanged **1b**.

The products having a single diethylaminoethyl substituent, on O-2 or O-3, were identified in the mixture obtained after hydrolytic removal of the benzylidene and methyl groups from the mixture of products from the original reaction. Chromatographic comparisons were made with authentic samples of 2-*O*- and 3-*O*-(2-diethylaminoethyl)-D-glucopyranose prepared by unambiguous methods. The details of these syntheses and of this type of chromatographic comparison are described in an earlier publication[4]. The chromatographic analyses were made with an F & M model 1609 instrument by use of a 1/4-in × 2-ft column carrying an XE-60 liquid phase of 8.5% on Chromsorb W. These analyses were confirmed on a 1/4-in × 6-ft column carrying an SE-52 liquid phase of 19%.

Paper electrophoresis of the reaction products in 0.75M formic acid (visualized with aniline dihydrogen phosphate) showed the presence of three components: a glucose, a mono-*O*-(2-diethylaminoethyl)glucose (the 2- and 3-ethers are not separable from each other under these conditions), and a 2,3-di-*O*-(2-diethylaminoethyl)-glucose (the only other product possible from these reactions).

For quantitative determinations, the D-glucose, the fraction containing mono-*O*-(2-diethylaminoethyl)-D-glucoses, and the 2,3-di-*O*-(2-diethylaminoethyl)-D-glucose were separated by electrophoresis in 0.75M formic acid at 75 volts/cm on Whatman 3MM paper, and each component was located by means of guide strips cut from the edges of the paper. Each section of the paper containing a component was cut out, and eluted with water. Glucose was determined colorimetrically by the phenol–sulfuric acid reagent of Dubois *et al.*[5]. The total monosubstituted glucoses were measured by the hypoiodite method of Hirst, Hough, and Jones[6]. The disubstituted glucose could not be determined by this method, because of the presence of an interfering substance in the eluate. However, this fraction could be calculated from that part of the nitrogen content that was not accounted for by the monosubstituted glucose. The ratio of 2-*O*-(2-diethylaminoethyl)-D-glucose to the 3-ether was determined by g.l.c. of the trimethylsilyl derivatives on an XE-60 column[4], and the quantities of the individual isomers were calculated from this ratio. Independent determinations of the derivatives in this manner accounted for all but 2.5% of the total weight of the sample taken.

Although this is the first instance to be reported of a reaction that occurs predominantly at the C-3 hydroxyl group of a D-glucose residue, it appears that 2-diethylaminoethyl chloride is not unique in this respect. Bullock[7] has observed that addition predominates at the C-3 hydroxyl group in the reaction of **1a** with methyl vinyl sulfone.

It is significant that the ratio of 2-*O*- to 3-*O*-substitution by 2-diethylaminoethyl groups is slightly higher for **1b**, and that the proportion of disubstituted product from **1b** is substantially higher than from **1a**. These differences in the reactivity of the anomers are interpreted to be the consequence of a lower degree of steric interference between the anomeric methoxyl group of the β-D anomer and the alkylating agent approaching O-2 of the D-glucoside.

We believe these results to be pertinent to development of an understanding of the factors that influence substituent distribution in synthetic derivatives of cellulose and starch. All of the evidence to date[4] for reactions involving the β-D-(1→4)-linked D-glucopyranosyl residues of cellulose shows that reaction predominates at the C-2 or the C-6 hydroxyl group, depending on the type and specific nature of the reagent. The reaction of 2-diethylaminoethyl chloride with cotton cellulose (under the conditions employed here with **1a** and **1b**) introduces substituents at the C-2, C-3, and C-6 hydroxyl groups[4] in the proportions of 1.27:0.35:1.00, *i.e.*, 3.6 times as much reaction occurs at the C-2 hydroxyl group as at the C-3 hydroxyl group.

Southern Regional Research Laboratory,
Southern Utilization Research and Development Division,
Agricultural Research Service,
U. S. Department of Agriculture,
New Orleans, Louisiana 70119 (U. S. A.)

E. J. ROBERTS
S. P. ROWLAND

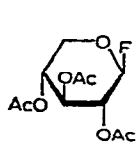
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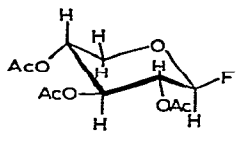
(Received January 16th, 1967)

The favored conformation of 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl fluoride and other pentopyranosyl fluorides: the anomeric effect of a fluorine substituent*

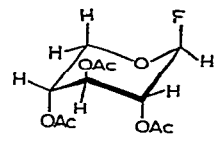
In a previous communication, we reported[1] that measurement of the fluorine chemical shifts and *vicinal* ^{19}F–^{1}H coupling constants of aldopyranosyl fluorides provides a direct method for identifying the anomeric configurations of such derivatives. We have since investigated in detail the 100-MHz, and in several instances the 220-MHz, proton spectra of these derivatives, and now report an interesting conformational property of the α- and β-(D or L)-pentopyranosyl fluorides. These derivatives all show a most marked preference for that conformation in which the fluorine substituent is axial.



(1)



(1a)



(1b)

The limiting case is 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl fluoride** (**1**). Analysis of the 100-MHz p.m.r. spectrum of this derivative gave the following *vicinal* coupling constants***:

$J_{1,F}$ +52.1, $J_{2,F}$ +6.9, $J_{1,2}$ 3.5, $J_{2,3}$ 5.7, $J_{3,4}$ 5.7, $J_{4,5e}$ 3.1, $J_{4,5a}$ 4.3, and $J_{5e,5a}$ 12.5 Hz. These values are clearly inconsistent with the *C1* (D) conformation (**1a**), and indicate that this derivative has a significant tendency to assume the *1C* (D) conformation (**1b**), despite the fact that this requires that all of the "bulky" substituents be axial. This conclusion is supported by the observation that $J_{5e,5a}$ is 12.5 Hz (that of the corresponding α-D anomer is 10.4 Hz), which indicates that the acetoxy substituent at C-4 is axial[3–5]. Furthermore, all of the ring protons exhibit a number of long-range couplings (as distinct from "virtual" couplings[6]), which are known to be favored[7], although by no means exclusively[8], and to occur between protons having a 1,3-diequatorial disposition. A similar consideration of the 100- and 220-MHz* spectra of 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl fluoride indicates that this derivative also favors the *1C* (D) conformation.

*Financial assistance to L. D. Hall from the National Research Council of Canada and from the National Cancer Institute of Canada is gratefully acknowledged. Presented in part at the 3rd International Fluorine Symposium, August 30–September 2, 1965, Munich, Germany.

**Prepared from 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide by treatment with silver fluoride in acetonitrile (*cf.* ref. 2).

***Data for a solution in acetone-d_6; no significant difference was observed for solutions in benzene-d_6, chloroform-*d*, or acetonitrile.

In view of the above results, it is hardly surprising that the other pentopyranosyl fluorides that we have studied also favor the conformation having a fluorine substituent axial. It seems reasonable to assign assumption of this structure to the anomeric effect[9,10] of the fluorine substituent.

Although the above discussion has been couched in terms of *Cl* and *1C* conformations, we have some evidence that this may be a slight oversimplification. For example, the *vicinal* ^1H–^1H couplings of (**1**) do not seeem to be consistent with an exclusive population of the *1C* (D) conformation (**1b**). Furthermore, the ^{19}F chemical shift changes as the temperature is varied between room temperature and −70°. These observations may reflect a rapid conformational equilibrium between the two possible chair conformations of (**1**). However, the existence of this derivative in a non-chair conformation cannot yet be excluded. We plan ultra-low temperature (−150°) measurements for investigation of this point.

Results from other laboratories indicate that the anomeric effect of other halogen substituents is also significant[4,11]. Horton and co-workers[12] have observed that the 1-chloro counterpart of (**1**) also favors the *1C* (D) conformation. Our findings have important implications regarding the steric course of electrophilic additions to glycals of pentoses, which will be described elsewhere.

Department of Chemistry,
The University of British Columbia,
Vancouver 8, B. C. (Canada)

L. D. HALL
J. F. MANVILLE

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(Received February 8th 1967; in revised form, March 29th, 1967)

*Kindly measured by Dr. N. S. Bhacca, Varian Associates, Palo Alto, California; this aspect will be the subject of a joint communication elsewhere.

The stereospecificity of long-range coupling constants of saturated carbohydrate derivatives*

It is now generally realized that the favored stereochemistry for proton–proton coupling across four bonds (^4J) in saturated carbohydrate derivatives[1], and other saturated cyclic systems[2], requires that the protons involved have the "planar M" relationship[2]. On this basis, there has been a tendency to assume that the observation of any long-range coupling for a pyranoid carbohydrate implies unequivocally that the protons involved have a 1,3-diequatorial orientation ($^4J_{e,e}$). We now report typical examples which demonstrate that long-range couplings can occur between protons having 1,3-axial:equatorial ($^4J_{a,e}$) and, possibly, 1,3-diaxial ($^4J_{a,a}$) orientations. The signs of ^4J couplings appear to exhibit a regular stereospecificity[3,4]. All spectra were measured with a Varian HA-100 spectrometer, and the source and magnitude of all couplings were confirmed by frequency-swept, double- and triple-resonance experiments. In all cases, the "virtual-coupling" limits of Musher and Corey[5] were exceeded.

$^4J_{e,e}$ Coupling: Such couplings occur extensively, and vary in magnitude from 1.2 to 1.6 Hz. For example, 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**1**) shows $|J_{1,3}| = 1.2$ Hz, $|J_{2,4}| = 1.25$ Hz, and $|J_{3,5}| = 1.5$ Hz. For 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-mannopyranose (**2**), $J_{1,3} = +1.6$ Hz, and $J_{3,5} = +1.5$ Hz, and for 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-idopyranose $|J_{4,6}|_{exo} = +1.2$ Hz. Thus far, all of the signs determined in this series are *positive*.

$^4J_{e,a}$ Coupling: Most of these give rise to splittings of 0.5 ±0.2 Hz, and all signs so far determined have been found to be *negative*. For example, compound **2** shows $|J_{2,4}| = 0.45$ Hz, 3,4,6-tri-*O*-acetyl-1-*O*-benzoyl-2-deoxy-2-iodo-α-D-mannopyranose (**3**) shows $J_{1,3} = -0.65$ Hz, and 3,4,6-tri-*O*-acetyl-2-deoxy-2-iodo-α-D-glucopyranosyl fluoride shows $J_{1,3} = -0.4$ Hz.

Although the above *numerical* distinction between $^4J_{e,e}$ and $^4J_{a,e}$ couplings appears to be quite unequivocal, it is clearly desirable that further signs be determined before the generality of the above inversion in sign is assumed. It is interesting that Barfield has calculated[3] that $^4J_{e,e}$ couplings should be *positive*, whereas the Dirac–Van Vleck vector model predicts[6] a *negative* sign for *all* ^4J couplings**.

Intermediate values of ^4J couplings may be found for derivatives that are undergoing conformational inversion at room temperature. Thus 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose (**4**), for which there is such evidence[7], shows $J_{2,4} = +0.8$ Hz.

We have also found two further classes of long-range coupling that have not previously been reported for saturated carbohydrate derivatives. First, ^4J couplings

*Presented, in part, at the 152nd meeting of the American Chemical Society, New York, N. Y., September 11–16, 1966.

**So far, we have only observed one *possible* $^4J_{a,a}$ coupling. Methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucuronate shows an apparent $J_{2,4}$ of +0.75 Hz; although this spectrum does not fall within the "virtual-coupling" limits[5], the above splitting is slightly solvent-dependent and hence its numerical magnitude cannot be relied upon.

can occur across the ring oxygen atom ($J_{1,5}$). For example, **3** shows a $|^4J_{e,a}|$ coupling of 0.65 Hz. $^4J_{e,e}$ Couplings, through the ring oxygen atom, appear to be smaller in magnitude, although not necessarily smaller in absolute value, than their $^4J_{e,a}$ counterparts; thus **1** and **2** show couplings of *ca.* |0.1| Hz. Second, we have found that ^5J coupling occurs quite widely; thus, both **1** and **4** show $J_{1,4}$ *ca.* 0.1 Hz, and **1** shows $J_{2,5}$ 0.5 Hz.

It is interesting that *furanoid* derivatives also exhibit an extensive array of long-range coupling-constants, including those across the ring oxygen atom[8,9]. This widespread occurrence of both ^4J and ^5J couplings in the spectra of carbohydrate derivatives is undoubtedly responsible for the difficulties that are often experienced in obtaining spectra having "natural" line-widths. For example, the H-1 resonance of the D-*gluco* derivative (**1**) is spin-coupled to every one of the other ring protons. Dr. J. D. Stevens (University of New South Wales, Australia) has also observed long-range coupling for furanoid derivatives[9].

Recent studies of dioxane derivatives[10] appear to be in accord with our results (see also ref. 11 for a brief summary). A ^5J coupling has recently been reported for *endo*-tricyclo[3,2,1,$0^{2,4}$]oct-6-ene[12].

We are investigating the generality of these findings, in particular the sign change found for ^4J couplings.

ACKNOWLEDGMENTS

Financial support from the National Research Council of Canada (to L. D. H.) is gratefully acknowledged.

Department of Chemistry, L. D. HALL
The University of British Columbia, J. F. MANVILLE
Vancouver 8, B. C. (Canada) A. TRACEY

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(Received February 8th, 1967; in revised form, March 29th, 1967)

The synthesis of *proto*-quercitol*

A colorless, crystalline compound, $C_6H_{12}O_5$, isolated by Braconnot[2] from the acorns of an oak tree (genus *Quercus*) and later named *quercitol*, was recognized as a new compound by Dessaignes[3]. Its structure (**1**) was established 82 years ago by Kanonnikoff[4], and its configuration (**2**) was determined 35 years ago by Posternak[5], but no synthesis has been reported.

In 1965, Angyal and co-workers[6] heated natural (−)-*vibo*-quercitol with 95% acetic acid containing a little sulfuric acid. At equilibrium, the product was shown by g.l.c. to contain acetylated *proto*-quercitol, but none was actually isolated[7].

As some authors have employed "quercitol" as a generic name (without official sanction) for the ten diastereoisomeric cyclohexanepentols, the diastereoisomer **2** (or **3**) is more explicitly termed *proto*-quercitol[8]. The *proto*-quercitol first discovered was dextrorotatory; more recently, Plouvier[9] has found the levorotatory form (**3**) in leaves of the tree *Eucalyptus populnea*. From Posternak's chemical correlations[5], it is now possible to assign the *absolute* configuration (**2**) to the dextrorotatory enantiomer. We use the systematic fractional prefix "L-(134/25)" to specify this configuration (**2**) of cyclohexanepentol.

The ten quercitols (cyclohexanepentols) constitute possibly the largest *all-known* family of diastereoisomers in organic chemistry. Although the *proto* diastereoisomer was the first to be discovered, it was the last to be synthesized.

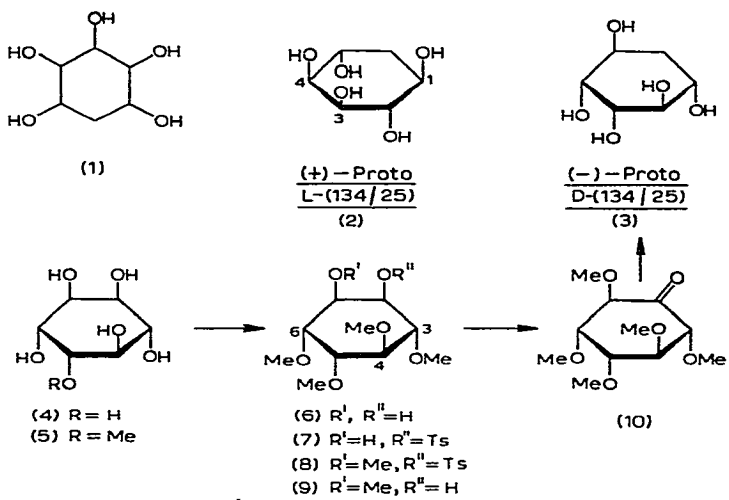
For convenience, our present synthesis started with (−)-inositol (**4**) (or quebrachitol, **5**), and thus led to (−)-*proto*-quercitol (**3**); if desired, the *identical* procedures could be applied to the well known (+)-inositol, to give (+)-*proto*-quercitol (**2**). Application of essentially identical procedures to DL-inositol would constitute a total synthesis of DL-*proto*-quercitol, because the total synthesis of DL-inositol has been reported[10]. (It is possible that some of the racemic intermediates would have different solubilities, and thus require different volumes of crystallizing solvents for best results.) DL-*proto*-Quercitol is not known to occur in Nature. Our synthesis of active *proto*-quercitol will not become "total" until DL-inositol has been resolved; Tanret[11] described the basis for a possible microbiological resolution.

First, we prepared the isopropylidene acetal of the hexol tetramethyl ether **6**, by methylating the isopropylidene acetal[8] of quebrachitol (**5**) or, somewhat less conveniently, by methylating the monoisopropylidene acetal[8] of (−)-inositol (**4**).

*Part XXXI in the series Alicyclic Carbohydrates. For part XXX, see Ref. 1.

The resulting syrup was deacetonated in the usual manner, to give the hexol tetramethyl ether* **6**, m.p. 90–92°. Selective *p*-toluenesulfonation of the equatorial hydroxyl group at C-2 gave **7**, m.p. 115–116°, which gave, on further methylation, the 2-*O*-*p*-tolylsulfonyl pentamethyl ether (**8**) as a syrup.

Treatment of **8** with sodium methoxide–ethanol gave the hexol pentamethyl ether **9** as a syrup, characterized as its monobenzoate, m.p. 88–90°. Oxidation of **9** with ruthenium dioxide–sodium periodate[12] gave (−)-*proto*-inosose pentamethyl ether (**10**), characterized as its (2,4-dinitrophenyl)hydrazone, m.p. 202–203°.



Treatment of **10** with 1,2-ethanedithiol, and reduction of the crude product with Raney nickel in boiling absolute ethanol, gave a pentol pentamethyl ether. On cleavage, this ether gave the desired (−)-*proto*-quercitol (**3**) as colorless needles, m.p. 238–239°, $[\alpha]_D^{25}$ −25.1° (*c* 1, water), (reported[9] m.p. 237°, $[\alpha]_D$ −26°). The pentabenzoate had m.p. 154–155°, $[\alpha]_D^{25}$ −62.8° (*c* 1, ethyl acetate) (reported[13] for the dextro form, m.p. 155°, $[\alpha]_D^{25}$ +61°). The i.r. spectra of the pentol and pentabenzoate were identical with those of authentic samples of the dextro form.

By recrystallizing a mixture of equal weights of the two enantiomers, we obtained DL-*proto*-quercitol, m.p. 238–239°, $[\alpha]_D$ 0° (within experimental error) (reported[9] m.p. 237°).

The diastereoisomeric configuration of (+)-*proto*-quercitol, which was first established[5] by laborious chemical correlations, has recently been confirmed[14] by p.m.r. spectroscopy at 220 MHz with a superconducting solenoid[15].

*Structures of the principal intermediates were confirmed by microanalysis, and by i.r. and p.m.r. spectroscopy; details will be reported elsewhere.

ACKNOWLEDGMENT

This work was supported by a grant to the Institute of Chemical Biology, University of San Francisco, from the National Institutes of Health, U. S. Public Health Service (Grant No. CA-07250).

G. E. MCCASLAND*
M. O. NAUMANN**

Department of Chemistry,
University of San Francisco,
San Francisco, California 94117 (U. S. A.)

LOIS J. DURHAM

Department of Chemistry,
Stanford University,
Stanford, California 94305 (U. S. A.)

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(Received February 17th, 1967)

Carbohyd. Res., 4 (1967) 516–518

*To whom any correspondence should be addressed (University of San Francisco).
**Postdoctoral Fellow of the U. S. Public Health Service, at the University of San Francisco, 1965–1967.