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REACTION OF METHYL β-D-*glycero*-L-*manno*-HEPTOPYRANOSIDE WITH CYCLOHEXANONE: SYNTHESIS OF THE 4- AND 6-METHYL ETHERS OF D-*glycero*-L-*manno*-HEPTITOL*

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ABSTRACT

Acid-catalysed condensation of methyl β-D-*glycero*-L-*manno*-heptopyranoside with cyclohexanone yielded an approximately 3:1 mixture of the 2,3:6,7- and 2,3:4,7-di-*O*-cyclohexylideneheptosides (**1** and **2**), which could be separated either as their benzoates (**3** and **4**) or as their methyl ethers (**5** and **6**). The latter compounds afforded the 4- and 6-methyl ethers (**7** and **8**) of D-*glycero*-L-*manno*-heptitol.

INTRODUCTION

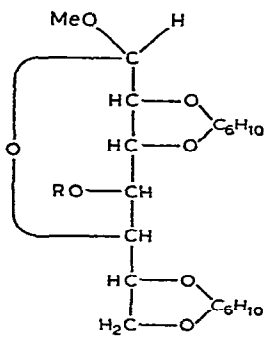
In connection with work on the structure of heptose-containing bacterial lipopolysaccharides, partially methylated methyl heptosides and heptitol acetates were required as standards. Although L-*glycero*-D-*manno*-heptose is the most widely occurring heptose in the lipopolysaccharides of Gram-negative bacteria, because of the difficulties inherent in the synthesis of this sugar[1], and also in view of the fact that the gas–liquid chromatographic and mass-spectral characteristics of its derivatives will be identical to those of the derivatives of D-*glycero*-L-*manno*-heptose, it was considered expedient to use the latter sugar in the present work. This paper describes the characterisation of two di-*O*-cyclohexylidene derivatives of methyl β-D-*glycero*-L-*manno*-heptopyranoside[2], and the synthesis from these of the 4- and 6-methyl ethers of the heptoside and of the derived heptitol.

RESULTS AND DISCUSSION

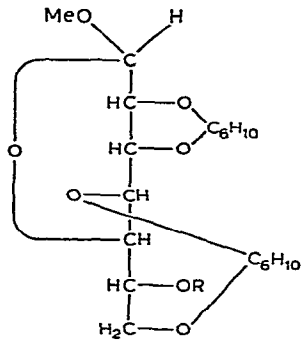
By analogy with the behaviour of benzyl β-D-*glycero*-D-*gulo*-heptopyranoside[3], it was expected that the acid-catalysed condensation of methyl β-D-*glycero*-L-*manno*-heptopyranoside with cyclohexanone would afford the 2,3:6,7-di-*O*-cyclohexylidene derivative and hence the 4-methyl ether. However, it was found that the condensation gave rise to two di-*O*-cyclohexylidene derivatives in the ratio ~3:1. These could be

*Dedicated to the memory of Dr. Hewitt G. Fletcher, Jr.

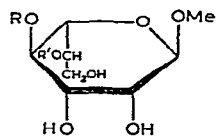
separated either as their benzoates (**3** and **4**) or as their methyl ethers (**5** and **6**). Debenzoylation of **3** and **4** followed by methylation gave **5** and **6**, respectively. These methyl ethers were used to determine the positions of the cyclohexylidene groups.



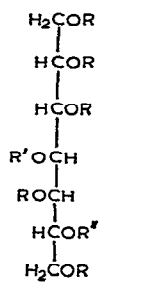
1 R = H
3 R = Bz
5 R = Me



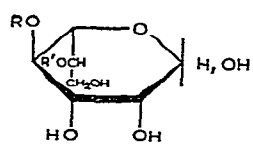
2 R = H
4 R = Bz
6 R = Me



7 R = Me; R' = H
10 R = H ; R' = Me



9 R' = Me; R = R'' = H
12 R'' = Me; R = R' = H



8 R = Me; R' = H
11 R = H ; R' = Me

The structure of the major product was determined in the following way. Removal of the cyclohexylidene groups from **5** by mild hydrolysis with acid yielded a mono-*O*-methyl methyl heptoside which reduced exactly two molar equivalents of periodate with the concomitant formation of one molar equivalent of formaldehyde, thus showing the methyl group to be located at O-4; a 2-methyl ether would be overoxidised under the conditions used. In addition, the mono-*O*-methylheptitol **9**, obtained by total acidic hydrolysis of **5** followed by reduction of the free sugar **8** with sodium borohydride, reduced four molar equivalents of periodate (and was then slowly overoxidised) and yielded two molar equivalents of formaldehyde, in agreement with the assignment of the methyl group to position 4. Finally, the mass spectrum of peracetylated **9** was characteristic of 4-*O*-methyl substitution[4] [primary fragment

m/e 261 (100%), corresponding to fission between C-3–C-4 or C-4–C-5]. The major product of the condensation was thus the 2,3:6,7-dicyclohexylidene derivative **1**.

The structure of the minor product was established similarly. The fact that the mono-*O*-methyl methyl heptoside **10**, obtained by mild hydrolysis of the methyl ether **6** with acid, reduced two molar equivalents of periodate, but yielded no formaldehyde, showed the methyl group to be located at position 6 or 7. The mass spectrum of peracetylated **10** indicated the methyl group to be at position 6 [primary fragment *m/e* 117 (100)%, arising by cleavage between C-5 and C-6]. The 6-position of the methyl group was definitely established in two ways. Firstly, the mono-*O*-methylheptitol **12**, obtained by total hydrolysis of **6** with acid followed by reduction of the resulting free sugar **11** with sodium borohydride, reduced four molar equivalents of periodate, one molar equivalent of formaldehyde being formed concomitantly. Secondly, the mass spectrum of peracetylated **12** was characteristic for a 6-*O*-(2-*O*-)methylheptitol [primary fragment *m/e* 117 (100%), arising by cleavage between C-5 and C-6]. The minor product was thus the 2,3:4,7-di-*O*-cyclohexylidene derivative **2**.

To our knowledge, this is the first example in the sugar series of a 7-membered cyclic acetal involving a ketone. Molecular space-filling models show the molecule to have a compact, strainless structure with all its bulky groups in equatorial positions. The same models show that a six-membered 4,6-acetal would be sterically unfavorable: the bulky substituent on C-6 occupies an axial position, and the position of the cyclohexylidene group is such that a very crowded structure results. It remains to be seen if the formation of the 7-membered cyclic acetal is under kinetic or thermodynamic control.

EXPERIMENTAL

General. — Melting points were determined on a Kofler hot-plate and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. G.l.c.–m.s. was performed with a Varian Aerograph 2700 equipped with a flame detector and coupled to a Dupont 21-492B spectrometer. The carrier gas was helium, at a flow rate of 26 ml/min. The stainless-steel columns used were packed with (*1*) 5% of XE60 on Varaport 30 (100–120 mesh), 10 ft × 1/8 in., and (*2*) 3% of SE 30 on the same support, 5 ft × 1/8 in. Retention times are given with respect to hexa-*O*-acetyl-D-glucitol. The mass spectra of all compounds, except **3** and **4** which were introduced directly, were recorded after passage through column *2*. The peak intensities are given as percentages of the major peak of the spectrum. T.l.c. was performed on plastic sheets coated with silica gel (F 1500 LS 254; Schleicher and Schüll), and p.l.c. on glass plates coated with a 1.5-mm layer of silica gel (Merck 60 PF_{254}), using the following solvent systems: ethyl acetate–hexane (1:3, *A*; 1:2, *B*; 1:5, *C*; 1:2.5, *D*), chloroform–methanol (6:1, *E*). Compounds eluted with methanol were freed from contaminating inorganic material by filtration with charcoal. Ascending chromatograms were run in butan-1-ol–ethanol–water (4:5:1, organic phase; solvent *F*) on Whatman No. 1 paper, without time or temperature control. Free sugars were

detected with aniline hydrogen phthalate[5], and heptitols with silver nitrate[6] after the papers had been sprayed with a solution of potassium periodate. Evaporations were carried out *in vacuo* below 40°. All compounds were dried *in vacuo* (P_2O_5) before analysis. Periodate oxidations were performed by the method of Avigad[7], using solutions that were 2mM with respect to substrates reducing two molar equivalents of periodate, and mM for those reducing more, and 10mM with respect to periodate. Formaldehyde was determined with chromotropic acid[8]. Acetylations were carried out in stoppered tubes at 110° for 2 h using acetic anhydride–sodium acetate. Acetic anhydride was removed by distillation with toluene, the residual acetates were dissolved in ethyl acetate, sodium acetate was centrifuged off, and the supernatant solutions were used for g.l.c. and m.s. This method of acetylation gave pure products.

*Methyl 2,3:6,7- and 2,3:4,7-di-*O*-cyclohexylidene-β-*D*-glycero-*L*-manno-heptopyranosides* (**1** *and* **2**). — A suspension of methyl *β-*D*-glycero-*L*-manno*-heptopyranoside[9] (2 g) in cyclohexanone (3.6 ml) containing conc. sulphuric acid (0.18 ml) was vigorously shaken overnight. Heptane (10 ml) was added and the solution was decanted from a small amount of oil which was then washed with heptane. The heptane solution was washed with aqueous sodium hydrogen carbonate and water, and dried (Na_2SO_4). The residue (3 g) obtained after removal of the solvent was chromatographed on a column of silicic acid (Mallinckrodt 100 mesh, 50 × 4 cm) with solvent *A* (30 ml/h). The fractions containing the di-*O*-cyclohexylideneheptosides (2 g, 58%) were concentrated, and the residue was dried (Found: C, 62.33; H, 8.28. Calc. for $C_{20}H_{32}O_7$: C, 62.50; H, 8.33%).

In later experiments, the separation was performed by p.l.c. (solvent *B*, two irrigations; elution of dicyclohexylidene derivatives with chloroform), which was time-saving and gave the same yield.

*Methyl 4-*O*-benzoyl-2,3:6,7- and 6-*O*-benzoyl-2,3:4,7-di-*O*-cyclohexylidene-β-*D*-glycero-*L*-manno-heptopyranosides* (**3** *and* **4**). — The above mixture of **1** and **2** (2 g) in pyridine (60 ml) was treated with benzoyl chloride (1.5 ml). The reaction mixture was worked-up in the usual way[2], and the precipitate was dried. The mixture of benzoates (2.4 g, 95%) was fractionated by p.l.c. (solvent *C*, bands eluted with chloroform).

The slower-moving compound **3** (1.7 g) crystallised from ethanol and had m.p. 96°, $]\alpha[_D^{22}$ −11.7° (*c* 0.87, chloroform); m.s.: 488 (24) (M^+), 445 (28), 347 (7), 201 (10), 155 (12), 153 (9), 141 (18), 140 (9), 105 (100), 99 (8), 81 (8), 77 (9), and 55 (17) (Found: C, 66.22; H, 7.37. Calc. for $C_{27}H_{36}O_8$: C, 66.39; H, 7.37%).

Compound **4**, crystallised from ethanol–ethyl acetate (95:5), had m.p. 187–188°, $[\alpha]_D^{22}$ −61° (*c* 0.86, chloroform); m.s.: 488 (33) (M^+), 445 (20), 347 (27), 177 (18), 155 (8), 153 (13), 140 (13), 105 (100), 99 (10), 81 (13), 77 (13), and 55 (20) (Found: C, 66.68; H, 7.40%).

*Methyl 4-*O*-methyl-2,3:6,7- and 6-*O*-methyl-2,3:4,7-di-*O*-cyclohexylidene-β-*D*-glycero-*L*-manno-heptopyranosides* (**5** *and* **6**). — A solution of **1** and **2** (1.4 g) in methyl iodide (25 ml) was boiled under reflux, and silver oxide (3.5 g) was added in portions during 8 h; t.l.c. (solvent *C*) then showed methylation to be virtually com-

plete. The mixture was diluted with hot chloroform (100 ml) and filtered. The solvents were removed and the methyl ethers were separated by p.l.c. (solvent *C*, thorough elution of bands with chloroform).

The slower-moving, syrupy **5** (0.8 g) had $[\alpha]_D^{22}$ −25.5° (*c* 0.98, chloroform); m.s.: 398 (100) (M^{+}), 368 (18), 354 (98), 257 (22), 251 (9), 185 (17), 183 (10), 171 (8), 155 (36), 153 (13), 141 (20), 140 (20), 125 (12), 113 (13), 111 (10), 99 (17), 97 (16), 87 (8), 81 (18), 71 (14), 55 (45), and 45 (20) (Found: C, 63.30; H, 8.53. Calc. for $C_{21}H_{34}O_7$: C, 63.30; H, 8.54%).

Compound **6**, crystallised from methanol, had m.p. 90–92°, $[\alpha]_D^{22}$ −2° (*c* 0.63, chloroform); m.s.: 398 (100) (M^{+}), 368 (4), 354 (45), 257 (50), 227 (6), 225 (5), 213 (15), 185 (11), 183 (5), 155 (16), 153 (10), 143 (13), 140 (12), 129 (7), 127 (6), 125 (6), 113 (12), 99 (14), 87 (23), 81 (12), 71 (14), 59 (15), 58 (18), 55 (26), and 45 (11) (Found: C, 63.34; H, 8.47%).

*Methyl 4-O-methyl-β-*D-glycero-L-manno-*heptopyranoside* (**7**). — In a 25-ml open-flask, 0.5M sulphuric acid (10 ml) was added to a solution of **5** (0.29 g) in methanol (10 ml), and the mixture was heated with stirring for 25 min at 90°, then cooled, and neutralised with Amberlite IR-45 (HO^{-}) resin. The resin was filtered off and the filtrate evaporated to dryness. The residue was dissolved in methanol and purified by p.l.c. (solvent *E*, elution with methanol). The amorphous, hygroscopic methyl ether had $[\alpha]_D^{20}$ −91° (*c* 2, methanol) (Found: C, 43.85; H, 7.61. Calc. for $C_9H_{18}O_7 \cdot 0.5H_2O$: C, 43.72; H, 7.69%).

M.s. of peracetylated **7**: 375 (4), 287 (5), 261 (5), 213 (6), 189 (6), 169 (6), 159 (4), 153 (7), 142 (14), 129 (100), 116 (20), 111 (8), 103 (6), 100 (16), 99 (14), 87 (39), 74 (42), and 43 (61).

*4-O-Methyl-*D-glycero-L-manno-*heptose* (**8**). — M Hydrochloric acid (12 ml) was added to a hot solution of **5** (0.33 g) in methanol (8 ml), and the stirred solution was heated in an open flask at 100° for 40 min. The flask was then stoppered and heated for a further 2 h. The solution was cooled and passed through a column of IR-45 (HO^{-}) resin (20 ml), and the column was thoroughly washed with water. The effluents and washings were concentrated to dryness. The residue was purified by p.l.c. (solvent *E*, three developments; band eluted with methanol) to give **8** (98 mg) as a syrup having $[\alpha]_D^{22}$ −38° (*c* 0.9, methanol), R_F (solvent *F*) 0.28 (Found: C, 43.02; H, 7.20. Calc. for $C_8H_{16}O_7$: C, 42.85; H, 7.15%).

*4-O-Methyl-*D-glycero-L-manno-*heptitol* (**9**). — Sodium borohydride (43 mg) in water (1 ml) was added dropwise to a stirred solution of **8** (43 mg) in water (2 ml), and the stirred solution was left overnight, decationised with Amberlite IR-120 (H^{+}) resin, filtered, and evaporated to dryness. Borate was removed by numerous evaporations with methanol, and the residue (40 mg) was crystallised from methanol to give **9**, m.p. 115–117°, $[\alpha]_D^{22}$ −16.25° (*c* 1.84, water), R_F (solvent *F*) 0.17 (Found: C, 41.99; H, 7.85. Calc. for $C_8H_{18}O_7$: C, 42.48; H, 7.96%).

M.s. of peracetylated **9**: 261 (68), 201 (20), 187 (14), 159 (20), 141 (11), 127 (63), 117 (7), 115 (8), 99 (42), 87 (16), 85 (37), and 43 (100).

*Methyl 6-O-methyl-β-*D-glycero-L-manno-*heptopyranoside* (**10**). — 0.5M

Sulphuric acid (2 ml) was added to a hot, stirred solution of **6** (54 mg) in methanol (4 ml) in an open tube, and the mixture was heated for 25 min at 90°, cooled, and neutralised with IR-45 (HO^-) resin. The filtered solution was concentrated to dryness, and the residue was dissolved in methanol and purified by p.l.c. (solvent *E*, elution with methanol). The syrupy residue had $[\alpha]_D^{20}$ −69° (*c* 1, methanol) (Found: C, 43.49; H, 7.70. Calc. for $C_9H_{18}O_7 \cdot 0.5H_2O$: C, 43.72; H, 7.69%).

M.s. of peracetylated **10**: 375 (4), 333 (5), 289 (16), 213 (9), 199 (19), 171 (9), 169 (14), 157 (14), 153 (9), 139 (19), 129 (15), 127 (34), 117 (100), 115 (12), 103 (7), 99 (10), 87 (17), 85 (10), and 43 (70).

6-O-Methyl-D-glycero-L-manno-*heptose* (**11**). — M Hydrochloric acid (10 ml) was added dropwise to a hot solution of **6** (0.31 g) in methanol (10 ml), and the stirred solution was heated in an open flask at 100° for 40 min. The flask was then stoppered and heated for a further 3 h at 100°. The solution was cooled and passed through a column of IR-45 (HO^-) resin (20 ml), and the effluent and washings were concentrated to dryness. The residue was dissolved in methanol and purified by p.l.c. (solvent *E*, three developments; band eluted with methanol). Syrupy **11** (96 mg) had $[\alpha]_D^{22}$ −22.5° (*c* 1, methanol), R_F (solvent *F*) 0.32 (Found: C, 42.99; H, 7.28. Calc. for $C_8H_{16}O_7$: C, 42.85; H, 7.15%).

6-O-Methyl-D-glycero-L-manno-*heptitol* (**12**). — Compound **11** (70 mg) was reduced with sodium borohydride (70 mg), as described for **9**, to give **12** (65 mg), m.p. 129–130° (from methanol), $[\alpha]_D^{22}$ −1.4° (*c* 1.54, water), R_F (solvent *F*) 0.22 (Found: C, 42.44; H, 7.93. Calc. for $C_8H_{18}O_7$: C, 42.48; H, 7.96%).

M.s. of peracetylated **12**: 419 (2), 405 (5), 201 (5), 169 (13), 159 (5), 117 (100), and 43 (54).

Compounds **9** and **12** gave acetylated products that had retention times of 2.04 and 1.40, respectively, on column **1** heated isothermally at 220°.

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