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Note

Synthesis of tri-*O*-methyl derivatives of D-*glycero*-L-*manno*-heptose

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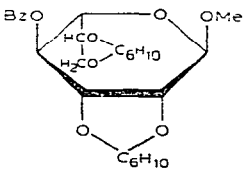
In connection with work on the structure of heptose-containing bacterial lipopolysaccharides, partially methylated heptitol acetates were required as standards. The synthesis of two tri-*O*-methyl derivatives of D-*glycero*-L-*manno*-heptose is now reported.

Partial acidic hydrolysis of methyl 4-*O*-benzoyl-2,3:6,7-di-*O*-cyclohexylidene-β-D-*glycero*-L-*manno*-heptoside[1] (**1**) yielded a monocyclohexylidene derivative (**2**) which reduced 1 mol. of periodate to yield 1 mol. of formaldehyde. This result shows that the 6,7-*O*-cyclohexylidene group had been hydrolysed, as expected by analogy with the partial acidic hydrolysis of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose[2]. Saponification of the benzoyl group in **2** followed by methylation and acidic hydrolysis afforded the 4,6,7-tri-*O*-methylheptose **3**, which was reduced and acetylated; the product was characterised by g.l.c.–m.s.



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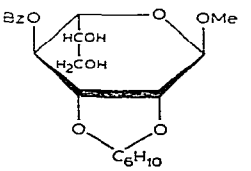

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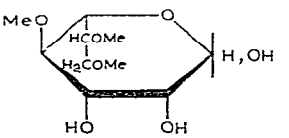

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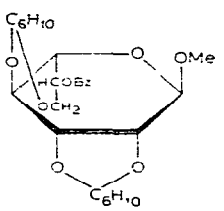

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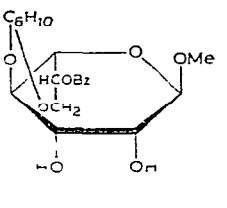

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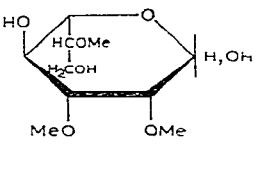

6

The selective removal of one cyclohexylidene group from methyl 6-*O*-benzoyl-2,3:4,7-di-*O*-cyclohexylidene-β-D-*glycero*-L-*manno*-heptoside[1] (**4**) proved to be more difficult. From the product obtained by partial acidic hydrolysis, the methyl 6-*O*-benzoyl-4,7-*O*-cyclohexylideneheptoside **5** could be crystallized. The structure of **5** was proved by periodate oxidation (1 mol. of oxidant consumed, but no formaldehyde liberated) followed by borohydride reduction, saponification, acidic hydrolysis, and acetylation. The product thus obtained was identical by g.l.c.–m.s. with authentic lyxitol penta-acetate.

Saponification of the benzoyl group of **4**, followed by neutralization with an acidic resin, gave two products which were detectable by t.l.c. but not separable. That the acidic treatment provoked a partial ring contraction of the cyclohexylidene group from 4,7 to 6,7 was shown in two ways. Firstly, the mixture was treated with periodate, reduced with borohydride, hydrolysed with acid, and acetylated. G.l.c.–m.s. then showed two peaks, corresponding to threitol tetra-acetate and lyxitol penta-acetate. Secondly, the debenzoylated mixture was methylated, and the product was hydrolysed with acid, reduced with borohydride, and acetylated. The two tri-*O*-methylheptitol tetra-acetates thus formed were separated by g.l.c. and shown by m.s. to be tetra-acetates of a 2,3,6-tri-*O*-methylheptitol (expected product from **4**, and major component of the mixture) and 2,3,4-tri-*O*-methylheptitol (arising from the product of ring contraction).

When debenzoylation of **4** was not followed by neutralisation, a crystalline product was obtained which gave only peracetylated lyxitol in the first series of reactions described above, and only peracetylated 2,3,6-tri-*O*-methylheptitol in the second series.

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 instrument equipped with a flame-ionisation detector and coupled to a DuPont 21-492B spectrometer. The spectra were obtained by electron impact at 70 eV with a source temperature of 270°. The peak intensities are given as percentages of the major peak of the spectrum. G.l.c. was performed on a stainless-steel column (1/8 in. × 5 ft) packed with 3% of methyl silicone on Varaport 30 (100–120 mesh). T.l.c. was performed on plastic sheets coated with silica gel (F1500LS254; Schleicher and Schüll), and p.l.c. on glass plates coated with a 1.5-mm layer of silica gel (Merck 60 PF_{254}), with *A* chloroform–methanol (6:1) or *B* ethyl acetate–hexane (1:2.5). All concentrations were carried out *in vacuo* at 40°. Periodate oxidations were performed by the method of Avigad[3], and formaldehyde was determined with chromotropic acid[4]. Acetylations were carried out with acetic anhydride–sodium acetate at 110° for 1 h. Acetic anhydride was removed by co-distillation with toluene, the residual acetates were dissolved in ethyl acetate, sodium acetate was removed by centrifugation, and the supernatant solutions were used for g.l.c.–m.s.

*Methyl 4-O-benzoyl-2,3-O-cyclohexylidene-β-*D-glycero-L-manno-*heptopyranoside* (**2**). — To a cold solution of **1**[1] (1.15 g) in methanol (30 ml), 0.02M methanolic hydrogen chloride (30 ml) was added. The solution was stored for 2 h at 21°, and then neutralised with Amberlite IR-45 (HO^-) resin, filtered, and concentrated. The residue was dissolved in chloroform (2 ml) and purified by p.l.c. (solvent *A*) to give **1** (234 mg) and syrupy **2** (444 mg, 58% based on starting material used), $[\alpha]_D^{21}$ −25° (*c* 1, methanol) (Found: C, 60.6; H, 6.9. $C_{21}H_{28}O_8$ calc.: C, 61.8; H, 6.9%).

Compound **2** reduced 1 mol. of periodate and formed 1 mol. of formaldehyde. The periodate-oxidised material was reduced with sodium borohydride, and the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness. Methanol was evaporated several times from the residue to remove borate. The residue was debenzoylated with methanolic sodium methoxide, and the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness, and the residue was heated at 100° for 0.5 h with 0.1M HCl. The solution was concentrated to dryness and the residue was reduced and acetylated. On g.l.c., it gave a single peak having a retention time identical to that of mannitol hexa-acetate.

*4,6,7-Tri-*O*-methyl-*D-glycero-L-manno-*heptose* (**3**). — To a solution of **2** (0.38 g) in anhydrous methanol (10 ml) was added freshly prepared 0.25M methanolic sodium methoxide (2 ml), and the mixture was boiled under reflux for 45 min. The cooled solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness.

The dried residue (0.3 g) was dissolved in methyl iodide (25 ml), and silver oxide (2 g) was added in portions to the refluxing solution during 6 h. The mixture was diluted with chloroform and filtered, and the precipitate was washed with hot chloroform. The combined filtrate and washings were concentrated, and the residue was dissolved in chloroform and purified by p.l.c. (solvent *B*). The methyl tri-*O*-methyl-*O*-cyclohexylideneheptoside (0.2 g) thus obtained was dissolved in methanol (8 ml), M HCl (8 ml) was added, and the mixture was heated in an open vessel with stirring in an oil bath at 100° until all of the methanol had distilled off (20 min), and then for 2.75 h in a sealed vessel. The solution was cooled, neutralised with Amberlite IR-45 (HO^-) resin, filtered, and concentrated to dryness. The residue was dissolved in methanol and purified by p.l.c. (solvent *A*) to give syrupy **3**, $[\alpha]_D^{22}$ −30° (*c* 1, methanol) (Found: C, 47.7; H, 7.85. $C_{10}H_{20}O_7$ calc.: C, 47.6; H, 7.9%).

Reduction of **3** with sodium borohydride and acetylation of the product gave the peracetylated tri-*O*-methylheptitol which had retention times of 0.85 and 0.70 with respect to those of D-glucitol hexa-acetate and D-*glycero*-L-*manno*-heptitol hepta-acetate, respectively. Mass spectrum: *m/e* 363 (M−59, 58%), 333 (19), 275 (93), 217 (38), 205 (34), 183 (9), 173 (13), 169 (10), 159 (10), 157 (14), 155 (13), 145 (9), 141 (25), 129 (11), 117 (14), 115 (9), 113 (19), 101 (100), 99 (18), 89 (50), 45 (13), and 43 (50).

*Methyl 6-*O*-benzoyl-4,7-*O*-cyclohexylidene-β-*D-glycero-L-manno-*heptopyranoside* (**5**). — A solution of **4**[1] (1.08 g) in chloroform (30 ml) and 0.06M methanolic

hydrogen chloride (30 ml) was kept for 2 h at room temperature, and then neutralised with Amberlite IR-45 (HO^-) resin, filtered, and concentrated to dryness. The residue was heated in methanol (5 ml), and insoluble **4** was collected and rehydrolysed as described above. After four such hydrolyses, the methanolic supernatants were combined and concentrated to a small volume, and the residue was purified by p.l.c. (solvent *A*) to give **5** (25% yield based on starting material recovered), m.p. 150–151° (from ethyl acetate–hexane), $[\alpha]_D^{21}$ −89° (*c* 0.5, methanol) (Found: C, 61.6; H, 7.0. $C_{21}H_{28}O_8$ calc.: C, 61.8; H, 6.9%).

Compound **5** reduced 1 mol. of periodate: no formaldehyde was formed. Treatment of the oxidation mixture, as described above for **2**, gave a product showing a single peak on g.l.c. which was indistinguishable from that of lyxitol penta-acetate.

2,3,6-Tri-O-methyl-D-glycero-L-manno-*heptose* (**6**). — A solution of **5** (0.1 g) in methanol (3 ml) was boiled under reflux for 1 h with 2.5M methanolic sodium methoxide (1 ml), then cooled, and concentrated to dryness. The residue was dissolved in methyl iodide (25 ml), and silver oxide (2 g) was added to the refluxing solution during 6 h. The mixture was cooled, and the solids were collected, and washed with hot chloroform. The combined filtrate and washings were concentrated to dryness to give syrupy **6** which was homogeneous on t.l.c. (solvent *A*) and had $[\alpha]_D^{21}$ −7° (*c* 1.76, ethanol) (Found: C, 47.4; H, 7.8. $C_{10}H_{20}O_7$ calc.: C, 47.6; H, 7.9%).

The tri-*O*-methylheptitol tetra-acetate derived from **6** had retention times of 1.03 and 0.85 with respect to those of D-glucitol hexa-acetate and D-*glycero*-L-*manno*-heptitol hepta-acetate, respectively. Mass spectrum: *m/e* 363 (M − 59, 2%), 349 (3), 305 (34), 161 (15), 143 (23), 129 (20), 117 (100), 101 (38), 97 (20), 87 (16), and 43 (65).

When the mixture obtained on debenzoylation of **5** was neutralized with Amberlite IR-120 (H^+) resin, two products were detectable (but not separable) on t.l.c. (solvent *A*). The mixture was methylated as described above, and the products were converted into the alditol acetates which were separable on g.l.c. and identified as the tetra-acetates of 2,3,4- and 2,3,6-tri-*O*-methyl-D-*glycero*-L-*manno*-heptitol. The 2,3,4-tri-*O*-methyl derivative had retention times of 0.99 and 0.81 with respect to those of D-glucitol hexa-acetate and D-*glycero*-L-*manno*-heptitol hepta-acetate, respectively. Mass spectrum: *m/e* 161 (28%), 143 (34), 117 (48), 101 (57), and 43 (100).

ACKNOWLEDGMENT

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