

## Synthesis of 6-Deoxy-D-manno-heptose

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The synthesis of 6-deoxy-D-manno-heptose is reported. The sugar is identical with a 6-deoxyheptose, present in the acid hydrolysate of the lipopolysaccharide from *Yersinia (Pasteurella) pseudotuberculosis* type II A.

During studies of the lipopolysaccharide from *Yersinia (Pasteurella) pseudotuberculosis* type II A<sup>1</sup> the presence of a new monosaccharide component was demonstrated. The new sugar was proved by mass spectrometric evidence to be a 6-deoxyheptose. Its alditol acetate was indistinguishable from 2-(6)-deoxy-D-manno-heptitol acetate on GLC but distinguishable from the corresponding D-galacto-derivative. In the lipopolysaccharide an abequeose (3,6-dideoxy-D-xylo-hexose) residue is  $\alpha$ -linked to the 3-position of the 6-deoxyheptose. In the lipopolysaccharide from *Y. pseudotuberculosis* II B<sup>2</sup> an abequeose residue is  $\alpha$ -linked to the 3-position of a D-mannose residue. The presence of the same O-factor, 5, in both lipopolysaccharides is almost certainly due to the abequeose residue and indicates that it is situated in similar structural environments. For this reason it was assumed that the 6-deoxyheptose had the D-manno-configuration. 6-Deoxyheptoses have not previously been found in Nature but 6-deoxy-D-gluco-heptose has recently been prepared as the 1,2-O-isopropylidene derivative by Rosenthal.<sup>3</sup> In the present communication the synthesis of 6-deoxy-D-manno-heptose is reported.

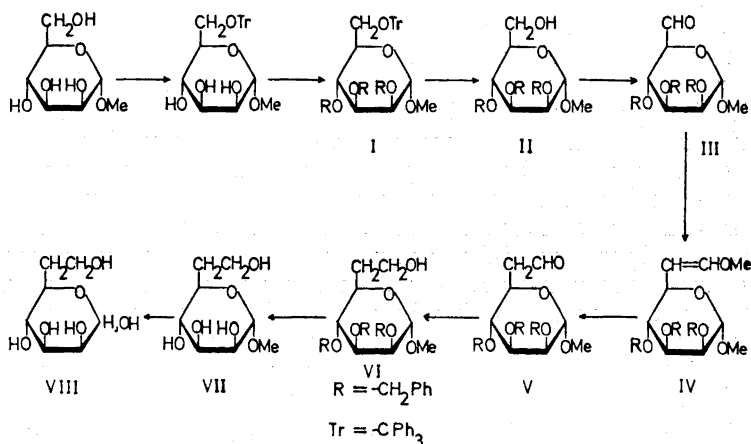
Methyl 2,3,4-tri-O-benzyl- $\alpha$ -D-manno-hexodialdo-1,5-pyranoside (III) was prepared from methyl  $\alpha$ -D-mannopyranoside via tritylation, benzylation, detritylation, and oxidation with methyl sulphoxide-dicyclohexylcarbodiimide.<sup>4</sup> Reaction of III with methoxymethylene triphenylphosphorane produced the vinyl ether (IV) in moderate yield (23 %). Mild acid hydrolysis of IV yielded methyl 2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-manno-heptodialdo-1,5-pyranoside (V). Borohydride reduction of V, followed by catalytic hydrogenation, yielded methyl 6-deoxy- $\alpha$ -D-manno-heptopyranoside (VII), characterized as its crystalline tetraacetate. Acid hydrolysis of VII yielded the amorphous 6-deoxy-D-manno-heptose (VIII). The synthetic product showed  $[\alpha]_D + 25^\circ$  (H<sub>2</sub>O) compared to  $[\alpha]_D + 33^\circ$  (H<sub>2</sub>O) observed for the natural sugar. The latter

value is not very accurate, because of the small amount of material available. The two sugars were indistinguishable on paper chromatography in different systems, in paper electrophoresis in germanate<sup>5</sup> and borate<sup>6</sup> buffers (pH 10.7 and 10.0, respectively) and on GLC of the TMS derivatives, the alditol acetates and the 2,3,4,7-tetra-*O*-methyl-alditol acetates. Mass spectra of the alditol acetates and the 2,3,4,7-tetra-*O*-methyl-alditol acetates were also indistinguishable. It therefore seems safe to conclude that the natural sugar is 6-deoxy-*D*-manno-heptose, in agreement with the assumption based upon immunochemical evidence.

### EXPERIMENTAL

**General methods.** Concentrations were performed at reduced pressure. Melting points are corrected. Optical rotations were determined at room temperature (23–25°) using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded in deuteriochloroform with a Varian A-60 A spectrometer using tetramethylsilane as internal reference. Chemical shifts ( $\delta$ ) are given in ppm downfield from tetramethylsilane. Only pertinent parts of spectra for key compounds are given below. The remainder of the spectra on all the various compounds was invariably in accordance with the presumed structures. MS was performed on a Perkin-Elmer model 270 gas chromatograph-mass spectrometer at a manifold temperature of 200°, an ionization potential of 70 eV, ionization current of 80  $\mu$ A and a temperature at the ion source chamber of 80°. Analytical TLC was performed on "Merck DC-Fertigplatten, Kieselgel F 254" and preparative TLC on 2 mm "Merck PSC-Fertigplatten, Kieselgel F 254". The absorbent for silica gel column chromatography was "Merck, Kieselgel unter 0.08".

**Methyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl- $\alpha$ -*D*-mannopyranoside (I).** Methyl 6-*O*-triphenylmethyl- $\alpha$ -*D*-mannopyranoside<sup>7</sup> (14 g) in benzyl chloride (580 ml) was treated



with sodium hydride (103 g) and the temperature gradually raised until the reaction started. More trityl mannoside (126 g) in benzyl chloride (580 ml) was added dropwise to the reaction mixture at reflux temperature. After 6 h methanol was added. The reaction mixture was partitioned between chloroform and water; the chloroform phase after exhaustive extraction with water was dried over sodium sulphate, filtered and concentrated. A small aliquot of the product was purified by TLC to yield crystals (2 g), m.p. 116–118°,  $[\alpha]_D + 20^\circ$  (*c* 0.51, chloroform). (Found: C 79.7; H 6.35.  $\text{C}_{47}\text{H}_{46}\text{O}_8$  requires: C 79.9; H 6.56.) The remaining product (181 g, 81 %) after concentration in a vacuum to

remove nearly all benzyl methyl ether and dibenzyl ether was sufficiently pure (TLC) for use in the next step.

*Methyl 2,3,4-tri-O-benzyl- $\alpha$ -D-mannopyranoside (II)*. The above product I (70 g) was detritylated by refluxing a solution in acetone (930 ml) and water (234 ml) containing M aqueous hydrochloric acid (70 ml) for 6 h. The reaction mixture was neutralized with aqueous sodium hydrogen carbonate and the product extracted with chloroform. The combined chloroform phases were dried over sodium sulphate, filtered and concentrated. The syrupy product was purified by passage through a silica gel column using toluene-ethyl acetate (4:1) as eluent. The syrupy product (38.3 g, 83 %) had  $[\alpha]_D + 30^\circ$  (c 0.49, chloroform). (Found: C 72.2; H 7.10.  $C_{28}H_{32}O_6$  requires: C 72.4; H 6.94.)

*Methyl 2,3,4-tri-O-benzyl- $\alpha$ -D-manno-hexodialdo-1,5-pyranoside (III)*. Methyl 2,3,4-tri-O-benzyl- $\alpha$ -D-mannopyranoside (II) (3.0 g) was dissolved in dimethyl sulphoxide (5.9 ml). Benzene (1.9 ml), pyridine (0.4 ml), phosphoric acid (0.2 ml), and dicyclohexylcarbodiimide (4.7 g) were added. The mixture was allowed to stand with stirring at room temperature for 5 h. *N,N'*-Dicyclohexylurea was filtered off. Oxalic acid (3.8 g) in methanol (9.4 ml) was added to the filtrate, which then was allowed to stand with stirring at room temperature overnight. *N,N'*-Dicyclohexylurea was filtered off. Excess benzene was added to the filtrate and the solution was extracted with aqueous sodium bicarbonate. The combined aqueous phases were extracted with ethyl acetate and the ethyl acetate extracts combined with the benzene solution. The combined organic phases were dried over calcium sulphate, filtered and concentrated. The syrup obtained was dissolved in the minimum amount of acetone and a further small quantity of *N,N'*-dicyclohexylurea which crystallized out was removed by filtration. The filtrate was concentrated and the crystallization of *N,N'*-dicyclohexylurea from acetone was repeated twice. The final syrup (2.45 g), which was not amenable to chromatographic purification, was used directly in the next step. NMR in deuteriochloroform:  $\delta$  10.0 (1 H) singlet,<sup>4</sup> aldehyde proton;  $\delta$  7.3–7.6 (15 H), multiplets, aromatic protons;  $\delta$  4.95 (1 H), doublet,  $J = 2.5$  Hz, anomeric proton;  $\delta$  4.7–4.9 (6 H), benzyl methylene protons;  $\delta$  3.45 (3 H), singlet, methoxyl protons.

*Methyl 2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-manno-heptopyranoside (VI)*. Methoxymethyl-triphenylphosphonium chloride<sup>8</sup> (8.18 g) in dry diethyl ether (43.5 ml) was enclosed in a serum bottle containing a magnetic stirrer under nitrogen and cooled to  $-10^\circ$ . Butyl lithium (19.2 ml, 30 % in hexane) was added by syringe with stirring and the mixture was allowed to stand with stirring at  $-7^\circ$  to  $-10^\circ$  during 20 min. The 6-aldehyde sugar III (2.9 g) was dissolved in 30 ml diethyl ether and added by syringe. The resulting mixture was allowed to stand with stirring at the above temperature during 1 h and then during 18 h at room temperature. Partitioning between ether and water, followed by preparative TLC (solvent, toluene-ethyl acetate 6:1), yielded the vinyl ether IV (0.73 g). NMR in deuteriochloroform:  $\delta$  7.20–7.45 (15 H), multiplets, aromatic protons;  $\delta$  4.6–4.8 (7 H), benzyl methylene and anomeric proton;  $\delta$  3.56 (3 H), singlet, and  $\delta$  3.32 (3 H), singlet, vinyl ether methyl and methoxyl protons. IR: 1665  $\text{cm}^{-1}$ .

The product IV (0.70 g) was hydrolyzed by refluxing a solution in 0.125 M aqueous sulphuric acid (10 ml) and acetone (100 ml) for  $4\frac{1}{2}$  h. The hydrolysis mixture containing the 7-aldehyde sugar V was neutralized with barium carbonate, filtered and concentrated. The methyl 2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-manno-heptodialdo-1,5-pyranoside (V) obtained gave the following NMR in deuteriochloroform:  $\delta$  9.75 (1 H), triplet, aldehyde proton;  $\delta$  7.3–7.5 (15 H), multiplets, aromatic protons;  $\delta$  3.34 (3 H), singlet, methoxyl protons. An aliquot of the 6-deoxy-7-aldehyde sugar V was reduced with sodium borodeuteride and then hydrogenated with 5 % palladium on carbon in ethanol. The 7-deuterio analogue of VI thus formed was per-methylated with dimethylsulphinyl sodium and methyl iodide in dimethyl sulphoxide,<sup>9</sup> hydrolyzed, reduced with sodium borohydride and acetylated. The presumed 1,5-di-O-acetyl-6-deoxy-7-deuterio-2,3,4,7-tetra-O-methyl-D-manno-heptitol thus obtained, on MS showed, *inter alia*, the following fragments: *m/e* 220, 205 (both weak), 176, 161, 160, 117, 101, 46, 45 and 43 in accordance with the expected mass spectrum (Fig. 1).<sup>10</sup>

The 7-aldehyde sugar V (40 mg) was reduced with excess sodium borohydride in ethanol-water 19:1 at room temperature overnight, neutralized with Dowex 50(H<sup>+</sup>), filtered, concentrated and then concentrated from methanol several times to remove boric acid. Purification by TLC (solvent, toluene-ethyl acetate 4:1) to yield syrupy VI (0.35 g),  $[\alpha]_D + 39^\circ$  (c 0.60, chloroform). (Found: C 72.9; H 7.30.  $C_{26}H_{34}O_6$  requires: C 72.8; H 7.16.)

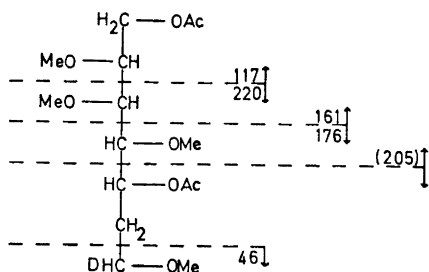


Fig. 1.

NMR in deuteriochloroform:  $\delta$  7.25–7.45 (15 H), multiplets, aromatic protons;  $\delta$  3.32 (3 H), singlet, methoxyl protons.

*Methyl 6-deoxy- $\alpha$ -D-manno-heptopyranoside (VII).* The tri-*O*-benzyl ether VI (100 mg) was hydrogenated in ethanol with 5% palladium on carbon overnight. Filtration and concentration afforded syrupy VII (41 mg),  $[\alpha]_D + 80^\circ$  (c 0.5, water). (Found: C 46.4; H 7.94.  $C_8H_{16}O_6$  requires: C 46.2; H 7.75.) The 6-deoxy glycoside VII (20 mg) was acetylated with acetic anhydride in pyridine (1:1) to yield crystals of methyl 2,3,4,7-tetra-*O*-acetyl-6-deoxy- $\alpha$ -D-manno-heptopyranoside (35 mg), m.p. 77–78°,  $[\alpha]_D + 62^\circ$  (c 0.4, chloroform). (Found: C 51.3; H 6.50.  $C_{16}H_{24}O_{10}$  requires: C 51.1, H 6.43.)

*6-Deoxy-D-manno-heptose (VIII).* The 6-deoxy glycoside VII (20 mg) was hydrolyzed with 0.25 M aqueous sulphuric acid at 100° for 15 h. The hydrolysate was cooled, neutralized with barium carbonate, filtered and concentrated to yield a syrup (13.5 mg),  $[\alpha]_D + 25^\circ$  (c 0.7, water). GLC–MS on the derived 1,2,3,4,5,7-hexa-*O*-acetyl-6-deoxy-D-mannoheptitol gave a retention time (1.25 *versus* hexa-*O*-acetyl-D-glucitol) and mass spectrum indistinguishable from those obtained from the authentic substance, obtained by borohydride reduction and per-acetylation of 2-deoxy-D-manno-heptose.

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