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Synthesis of Methyl 3-*O*-(3,6-Dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranoside

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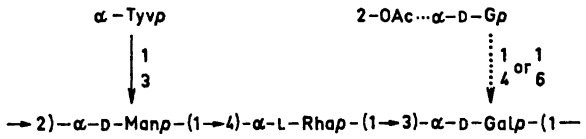
The synthesis of methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranoside, required for immunological studies, is described.

The O-specific side-chains of the *Salmonella* group D_1 lipopolysaccharide may be formulated as follows.[1,2]



The O-factor 9 is thought to be associated with the presence of terminal α-tyvelosyl (3,6-dideoxy-α-D-*arabino*-hexopyranosyl) residues in the side-chain repeating units.[3,4] In order to assess the immunological importance of the nature of the next sugar residue in the repeating unit, the synthesis of methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranoside was undertaken. This disaccharide glycoside incorporates the structural features of the tyvelosylmannose part of the repeating unit, including the suggested anomeric configuration [2] on the mannose moiety. The synthesis of the corresponding β-D-mannoside for comparison of immunological activity will be published at a later date.

Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (I) [5] was partly benzylated with benzyl bromide and silver oxide in dimethylformamide by the method described by Croon and Lindberg.[6] The 2-*O*-benzyl ether Ia was crystallized directly in a 36 % yield from the reaction mixture. The position of attachment of the benzyl group was shown by NMR studies on the derived monoacetate as shown in Fig. 1. The various assignments are given in the figure. The

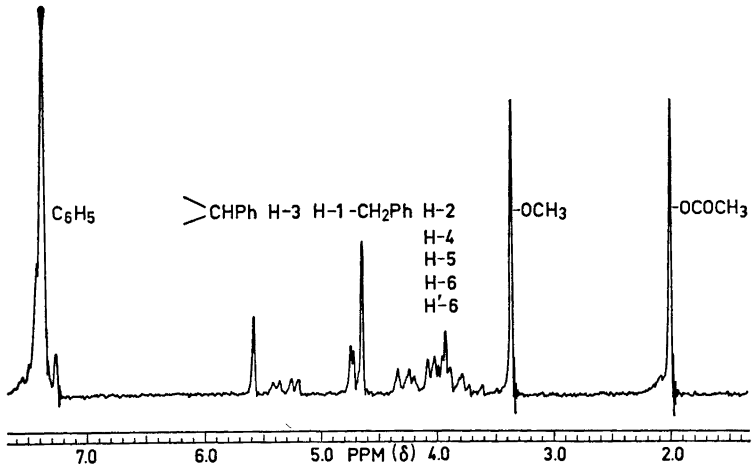


Fig. 1. NMR spectrum of methyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-manno-pyranoside in deuterochloroform.

quartet (1 H) at 5.33 ppm, downfield from the signal given by the anomeric proton, which in turn is observed as a doublet at 4.77 ppm, is assigned to a proton attached to an acetoxylated carbon, by virtue of the well-established deshielding of hydrogens attached to carbinol carbons on *O*-acetylation. It clearly does not couple to the anomeric proton, the coupling constants (first-order analysis) of 4 and 10 Hz, respectively, instead indicate that it is flanked by a *cis*-equatorial and a *trans*-axial proton, and therefore is given by H-3. This was confirmed by decoupling experiments which demonstrated coupling of H-3 to protons in the 4.0 – 4.3 ppm region. The identity of the 2-*O*-benzyl ether Ia also follows from the methylation analysis of Ia as well as of the final product V (see experimental part).

4,6-Di-*O*-acetyl-3-deoxy-1,2-*O*-methylorthoacetyl-β-D-*arabino*-hexopyranose (II) was obtained from 3-deoxy-D-*arabino*-hexose by the general method described by Kochetkov and co-workers,[8] but using the glycosyl halide preparation involving the action of titanium tetrachloride upon the fully acetylated sugar, as described by Zinner and Wulf.[9] Condensation of the orthoester II with the mannoside Ia using the two-stage glycosylation method described by Kochetkov and co-workers [8] yielded the substituted disaccharide IIIa. The crude reaction product was deacetylated to yield IIIb, which was obtained in pure form by two-phase solvent partitioning followed by chromatography on silica gel. The transformation of the hydroxymethyl group of the 3-deoxy-hexose moiety in IIIb into a methyl group was performed as follows: Mono-tosylation of the primary hydroxyl in IIIb with *p*-toluenesulphonyl chloride in pyridine at low temperature and chromatographic purification of the product afforded IV which can be obtained in good yield by recycling unreacted starting material. Treatment of the tosylate IV with lithium aluminium hydride afforded a product which without purification was converted into the di-

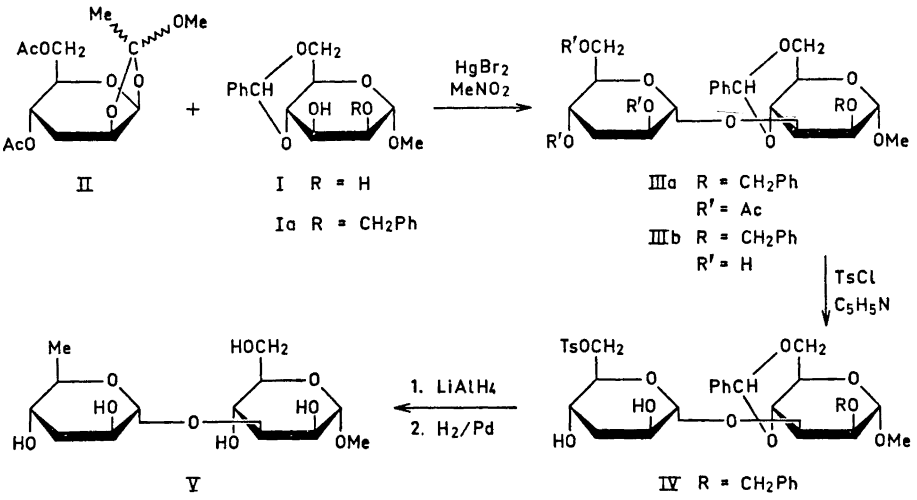


Fig. 2.

saccharide glycoside V by catalytic hydrogenation. The identity of V follows from its method of synthesis, from methylation analysis of V, from the NMR spectrum of V and the various intermediates (see experimental part) and from elemental analysis.

The immunochemical evaluation of the product V will be described elsewhere.

EXPERIMENTAL

Concentrations were performed at reduced pressure. Melting points are corrected. Optical rotations were determined at room temperature (20 – 22°) using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded with a Varian A-60 A spectrometer with a V-6058 A unit for decoupling experiments. Tetramethylsilane was used as internal reference and chemical shifts (δ) are given in ppm downfield from this reference. Pertinent parts of the various NMR spectra are given in the appropriate section below, the remainder of the spectra were invariably in accordance with the presumed structures. TLC was performed on silica gel F_{254} (Merck). Sulphuric acid was used as spray reagent.

Methyl 2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (Ia). Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside I (140 g) in dry dimethylformamide (1400 ml) was benzylated with benzyl bromide (72 ml) and silver oxide (280 g). After stirring at room temperature for 48 h, chloroform (1000 ml) was added and the mixture filtered through kieselguhr. Concentration of the filtrate yielded a thick syrup which upon addition of ethanol deposited crystals. Recrystallization from ethanol gave 66 g crystals, m.p. 42 – 44°, $[\alpha]_D + 2°$ (*c*, 1.0 in chloroform). (Found: C 67.8; H 6.70; O 25.6. $C_{21}H_{24}O_6$ requires: C 67.7; H 6.50; O 25.8.) A small part of the material was methylated in methyl sulphoxide under nitrogen with methyl sulphinyl sodium and methyl iodide as described by Hakomori.[10] The product was hydrogenated in ethanol with palladium on carbon and then hydrolyzed overnight with 0.25 M aqueous sulphuric acid at 100°, the hydrolysate neutralized with Dowex 3 (free base), filtered and concentrated. The presumed 3-*O*-methyl-D-mannose was converted into the corresponding alditol acetate which was shown by GLC – MS [11,12] to be indistinguishable from authentic 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methylmannitol.

*4,6-Di-*O*-acetyl-3-deoxy-1,2-*O*-methylorthoacetyl-β-*D*-*arabino*-hexopyranose (II).* 3-Deoxy-D-*arabino*-hexose (37 g) was acetylated at room temperature with pyridine (450 ml) and acetic anhydride (450 ml). The solution was poured into ice-water (2500 ml) and the mixture extracted with chloroform. The combined chloroform extracts were washed with, in turn, cold 0.25 M aqueous sulphuric acid, saturated aqueous sodium bicarbonate and finally water. The chloroform layer was dried over magnesium sulphate, filtered and concentrated to yield 49 g of a syrup. This was dissolved in ethanol-free chloroform (400 ml) and the resulting solution cooled to −20°. Titanium tetrachloride (35 ml) in ethanol-free chloroform (150 ml) was added with stirring at this temperature, during 1.5 h. The reaction mixture was then kept at 60° for 3 h, cooled to room temperature, filtered and concentrated. The product was dissolved in ethanol-free chloroform (400 ml) and the chloroform solution was washed with ice-water. The chloroform layer was dried over magnesium sulphate, filtered and concentrated to dryness. The resulting syrup was dissolved in ethyl ether and decolourized with activated charcoal, filtered and concentrated to a brown oil (39 g). The crude tri-*O*-acetyl-3-deoxy-D-*arabino*-hexosyl chloride was used directly in the next reaction step. The acetochloro sugar (39 g) in nitromethane (120 ml) was treated with methanol (75 ml) and 2,6-lutidine (35 ml) during 48 h at 37°. Aqueous silver nitrate (300 ml, 2 M), water (400 ml) and acetone (500 ml) were added and the mixture filtered. Ethanol-free chloroform (1000 ml) and hexane (2500 ml) were added to the filtrate. After shaking, the organic layer was washed twice with water, dried over magnesium sulphate, filtered and concentrated to a light-brown oil. This was purified on silica gel (solvent toluene : ethyl acetate 1 : 1) to yield a syrup (14 g), $[\alpha]_D + 10°$ (*c*, 0.9 in chloroform). (Found: C 51.4; H 6.50; O 42.2. $C_{13}H_{20}O_8$ requires: C 51.3; H 6.63; O 42.2.) NMR in deuterochloroform: orthoester methoxyl (3 H) at 3.45 and 3.30 ppm (ratio 1 : 3), *O*-acetyl (6 H) 2.08 ppm, orthoester methyl (3 H) at 1.7 and 1.52 ppm (ratio 3 : 1).

*Methyl 3-*O*-(3-deoxy-α-*D*-arabino-hexopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside (IIIb).* The above orthoester II (13 g) was dissolved in nitromethane (350 ml). The partially protected mannoside Ia (16 g) was added. The solution was heated to boiling and methanol removed by co-distillation with nitromethane while keeping the volume constant by continuous addition of nitromethane. The removal from the reaction solution of methanol was followed by GLC. After 1.5 h no further methanol in the distillate was observed. After a further 30 min the distillation was interrupted. NMR on a small sample in nitromethane: The signals from the orthoester methoxyls in II had vanished, the only methoxyl signal observed was that due to the substituted methyl α-D-mannoside moiety. Pertinent NMR parameters, shifts being measured from nitromethane with a presumed chemical shift at 4.33 ppm: aromatic protons (10 H) at 7.4−7.5 ppm, methoxyl (3 H) at 3.38 ppm, acetoxyl (6 H) at 2.06 and 2.02 ppm, orthoester methyl (3 H) 1.53 and 1.44 ppm. Mercuric bromide (1.1 g) was added and the mixture was refluxed for 22 h, filtered and concentrated. The crude reaction product containing IIIa was immediately deacetylated with 1.67 % ammoniacal methanol (300 ml) for 72 h. The solution was concentrated to dryness. The residue in toluene (200 ml) and hexane (200 ml) was shaken with water which extracted IIIb leaving almost all the aglycone Ia in the organic phase. Extraction of the combined aqueous layers with chloroform extracted IIIb. The extractions were monitored by TLC (solvent ethyl acetate-methanol-water 85 : 10 : 5). The combined chloroform extracts were dried over magnesium sulphate, filtered and concentrated. The product was purified on silica gel. A chromatographically homogeneous syrup was obtained (2.98 g), $[\alpha]_D + 44°$ (*c*, 1.0 in chloroform). (Found: C 62.3; H 6.54; O 30.7. $C_{27}H_{34}O_{10}$ requires C 62.5; H 6.61; O 30.9.)

*Methyl 3-*O*-(3,6-dideoxy-α-*D*-arabino-hexopyranosyl)-α-*D*-mannopyranoside (V).* The above disaccharide glycoside IIIb (510 mg) was dissolved in pyridine (20 ml) and the solution cooled to −30°. *p*-Toluenesulphonyl chloride (190 mg) in pyridine (10 ml) at the same temperature was slowly added with stirring. The reaction was monitored by TLC (solvent ethyl acetate-methanol-water 85 : 10 : 5). After 48 h at −30° a further 190 mg *p*-toluenesulphonyl chloride in pyridine (10 ml) was added and the reaction mixture allowed to stand at −30° for a further 24 h. Water was added to turbidity and then pyridine, dropwise, until a clear solution was obtained. After 30 min at room temperature the solution was concentrated. TLC (solvent ethyl acetate-methanol-water 85 : 10 : 5) indicated the presence of starting material IIIb in addition to two faster-moving com-

ponents, the slower of which predominated strongly. The mixture was separated on silica gel in the above solvent. A monotosylate (presumed IV) was obtained (266 mg). The following NMR parameters (in deuterochloroform) showed the presence of one tosyl group: Aromatic protons (14 H) at 8.0 – 7.3 ppm, benzylidene methine (1 H) at 5.62 ppm, benzyl methylene (2 H) at 4.80 ppm. The monotosylate IV (266 mg) in tetrahydrofurane (30 ml) containing lithium aluminium hydride (100 mg) was refluxed for 1.5 h. Excess hydride was destroyed by the sequential addition of ethyl acetate, ethanol and water. The mixture was neutralized with orthophosphoric acid, filtered and concentrated to a chromatographically homogeneous syrup (174 mg). NMR in deuterochloroform: Aromatic protons (10 H) at 7.4 ppm, benzylidene methine (1 H) at 5.64 ppm, benzyl methylene (2 H) at 4.82 ppm, anomeric protons (1 H each, $J = 1$ Hz for both) at 5.00 and 4.78 ppm, respectively. The syrup (174 mg) was hydrogenated with 5 % palladium on charcoal in ethanol to yield V as a chromatographically pure syrup (115 mg), $[\alpha]_D + 84°$ (*c*, 0.5 in water). (Found for the pentaacetate of V, prepared by acetylation with acetic anhydride in pyridine: C 51.7; H 6.44; O 41.7. $C_{23}H_{34}O_{14}$ requires: C 51.7, H 6.41, O 41.9.) The disaccharide glycoside V itself was too hygroscopic for a satisfactory analysis to be obtained. A small sample of V (3 mg) was subjected to methylation analysis using the Hakomori methylation procedure [10] and the GLC-MS procedure described by Björndal and co-workers,[11,12] as described above for II, but using a milder hydrolysis procedure for the methylated material, 0.125 M aqueous sulphuric acid for 8 h at 100°, to minimize acidic degradation of the tyvelose derivative. The alditol acetates finally obtained were indistinguishable by GLC-MS from 1,5-di-*O*-acetyl-3,6-dideoxy-2,4-di-*O*-methyl-D-*arabino*-hexitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-mannitol, respectively.

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