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

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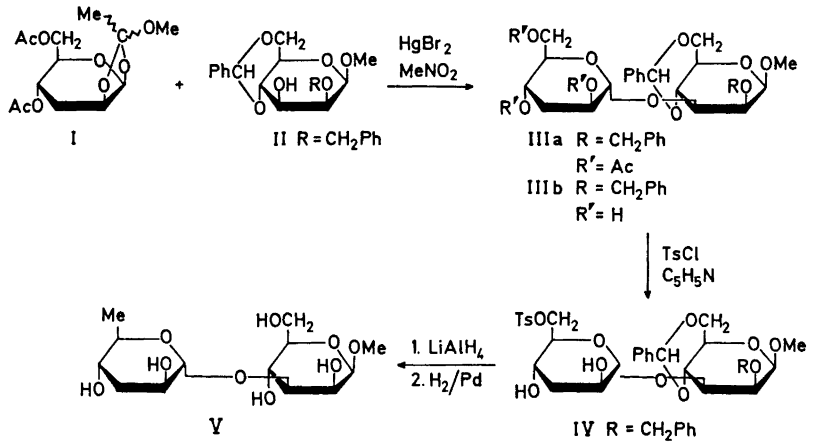
The synthesis of methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-β-D-mannopyranoside, required for immunological studies, is described.

In the *Salmonella* cell-wall lipopolysaccharides, structural variation is associated with the presence of various immunological O-factors.[1] Thus, O-factor 9 in the serogroup D_1 lipopolysaccharides is thought to be associated with an α-tyvelosyl (3,6-dideoxy-α-D-*arabino*-hexopyranosyl) unit being linked to the 3-position of an α-D-mannopyranosyl unit.[1,2] Since it is of immunological interest to definitely ascertain the relation between the chemical structure and the various O-factors, a programme of synthesis of various disaccharide glycosides corresponding to various O-factors has been initiated. In a previous paper the synthesis of methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranoside, corresponding to O-factor 9 of the *Salmonella* serogroup D_1 lipopolysaccharide was described.[3] The D_2 lipopolysaccharide has recently been shown to differ from that of D_1, *inter alia* in the configuration of the mannose units which in the D_2 serogroup is β.[4,5] The present paper describes the synthesis of a 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-β-D-mannopyranoside, required for immunological comparison to the corresponding α-D-mannoside.

4,6-Di-*O*-acetyl-3-deoxy-1,2-*O*-methylorthoacetyl-β-D-*arabino*-hexopyranose (I)[3] was condensed with methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (II)[6] in nitromethane in the presence of mercuric bromide under the general conditions described by Kochetkov and co-workers.[7] Deacetylation of the crude reaction product (III a) yielded IIIb, which was purified. The 3-deoxy-disaccharide derivative III b was converted into the corresponding 3,6-dideoxy-disaccharide by tosylation of the free primary hydroxyl group in III b by means of monotosylation in pyridine at low temperature, purification of IV by chromatography and then reduction of the monotosylate IV with lithium aluminium hydride to yield the corresponding 3,6-dideoxy-disaccharide. The latter, on catalytic hydrogenation afforded V.



The constitution of the disaccharide glycoside V was demonstrated as follows: A methylation analysis, comprising per-methylation,[8] hydrolysis, sodium borohydride reduction, acetylation, and examination of the resulting methylated alditol acetates by GLC–MS [9,10] revealed the presence of 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-mannitol and 1,5-di-*O*-acetyl-3,6-dideoxy-2,4-di-*O*-methyl-D-*arabino*-hexitol in the expected proportions. NMR on per-trimethylsilylated V showed *inter alia* the presence of two anomeric protons [11] with coupling constants less than 3 Hz as expected for V which in both sugar residues has H-1 and H-2 in a gauche relationship (axial-equatorial and equatorial-axial). The immunological evaluation of the product V will be described elsewhere.

EXPERIMENTAL

General methods. Concentrations were performed at reduced pressure. Optical rotations were measured at room temperature (20 – 22°) using a Perkin-Elmer 141 polarimeter. NMR spectra, in $CDCl_3$, were recorded with a Varian A-60 A spectrometer. Tetramethylsilane was used as internal reference and chemical shifts (δ) are given in ppm downfield from this reference. Pertinent parts of the NMR spectra are given in the appropriate sections 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. GLC–MS was run on a Perkin-Elmer 270 combined gas chromatograph-mass spectrometer. The mass spectra were recorded at a manifold temperature of 200°, an ionization potential of 70 eV, ionization current of 80 μA and a temperature at the ion source chamber of 80°.

*Methyl 3-O-(3-deoxy-α-D-*arabino*-hexopyranosyl)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (III b).* 4,6-Di-*O*-acetyl-3-deoxy-1,2-*O*-methylorthoacetyl-β-D-*arabino*-hexopyranose (I) [3] (6.1 g) was dissolved in nitromethane (155 ml). Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (II) [6] (7.5 g) was added. Solvent was distilled off at constant volume by the continuous addition of nitromethane for 1 h. Mercuric bromide (516 mg) was added and the mixture was refluxed overnight. After filtration, the product was concentrated to a syrup (14.7 g) and deacetylated with ammonia in methanol (275 ml methanol containing 100 ml ammonia-saturated methanol), for 72 h. The solution was concentrated to dryness. The residue, in toluene–hexane 1:1 was extracted with water, leaving most of the aglycone in the organic phase. The product III b was extracted from the aqueous phase with chloroform. The chloroform extracts

contained III b together with some aglycone II. Syrupy III b was obtained in a chromatographically pure state after separation on silica gel (solvent, ethyl acetate – methanol – water 85:10:5), 1.86 g, $[\alpha]_D - 30°$ (*c*, 0.5 in chloroform). (Found: C 62.4; H 6.79; O 30.7. $C_{47}H_{84}O_{10}$ requires C 62.5; H 6.61; O 30.9.)

Methyl 3-O-(3,6-dideoxy-α-D-arabino-hexopyranosyl)-β-D-mannopyranoside (V). The above product III b (600 mg) in pyridine (10 ml) was tosylated with *p*-toluenesulphonyl chloride (200 mg) in pyridine (7 ml). The two solutions were combined at $-25°$ and allowed to stand at this temperature for 24 h after which more *p*-toluenesulphonyl chloride (100 mg) in pyridine (3.5 ml) at $-25°$ was added. The reaction was followed by TLC (ethyl acetate – methanol – water 85:10:5 and chloroform – ether 1:9). After a further 24 h at $-25°$, water was added to turbidity and then, dropwise, pyridine to just solution. After standing at room temperature for 30 min the solution was poured onto ice-water. The product was extracted with chloroform. The combined chloroform extracts were dried over magnesium sulphate with solid barium carbonate added in order to neutralize any acid present, filtered and concentrated. The product IV was purified by TLC (solvent, chloroform – ether 1:9 and ethyl acetate – methanol – water 85:10:5) to yield 410 mg monotosylate IV. NMR; the presence of one tosyl group only is shown by the following parameters: δ 7.2 – 7.9, (14H), multiplets, aromatic protons, δ 5.50, (1H), singlet, benzylidene methine proton, δ 3.56, (3H), singlet, methoxyl protons, δ 2.40, (3H), singlet, toluene methyl protons. The tosylate IV in tetrahydrofurane (40 ml) was reduced with lithium aluminium hydride (175 mg) at reflux temperature for 2 h. After destroying excess hydride by the sequential addition of ethyl acetate, ethanol, and water, the mixture was neutralized with aqueous phosphoric acid. The neutral mixture was filtered. Organic solvents were removed by concentration. The resulting aqueous phase was thoroughly extracted with chloroform, the combined chloroform phases dried over magnesium sulphate, filtered, concentrated and purified by TLC (solvent, ethyl acetate – methanol – water 85:10:5) to yield 270 mg of a chromatographically homogeneous syrup. NMR: δ 7.2 – 7.6. (10H), multiplets, aromatic protons, δ 5.52, (1H) singlet, benzylidene methine proton, δ 3.55, (3H) singlet, methoxyl protons. The syrup (270 mg) in ethanol (35 ml) was hydrogenated with 10 % palladium on carbon to yield the title compound V as a chromatographically pure syrup (175 mg) $[\alpha]_D + 23°$ (*c*, 0.5 in water). The disaccharide V was too hygroscopic for a satisfactory analysis to be obtained. The NMR on the penta(trimethylsilyl) derivative of V showed the presence of two anomeric protons only in a ratio of 1:1 at δ 4.12 and 4.53, respectively. Methylation analysis [8–10] as previously described [8] gave two products, 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 in accordance with the structure V.

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