Carbohydrate Research, 33 (1974) C5–C7

Preliminary communication

Synthesis of derivatives of the trisaccharide repeating unit of the O-specific polysaccharide from *Salmonella anatum*

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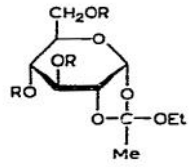
(Received January 22nd, 1974; accepted for publication, February 18th, 1974)

Recently, the structures of several O-specific polysaccharides of Gram-negative bacteria[1–3] have been established. These antigenic polysaccharides are built up of repeating oligosaccharide units composed of from three to six monosaccharide residues. Because of the difficulties, little progress has been made in chemical synthesis in this field and has been limited to disaccharides responsible for particular specific factors of the whole antigen. Synthesis of the complete, biological repeating units of O-specific polysaccharides could be of great value in the search for an understanding of the immunological properties of microbial antigens and their biosynthesis, and would represent a first step towards the chemical synthesis of antigenic polysaccharides. We now describe the synthesis of derivatives of the trisaccharide repeating unit of the O-specific polysaccharide from *Salmonella anatum*, namely[4], β-D-mannopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-α-D-galactopyranose, which appears to be a common component for all *Salmonella* serotypes belonging to group E.

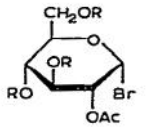
The synthesis involved construction of the trisaccharide starting from the non-reducing end and required the formation of a β-D-mannosyl linkage and the introduction of (1→3)-substitution into a D-galactose residue. The first problem was solved by a glucose–mannose conversion[5] and the second by glycosylation of a galactose derivative[6] having HO-3 and HO-4 unsubstituted.

3,4,6-Tri-*O*-acetyl-α-D-glucopyranose 1,2-(ethyl orthoacetate)[7] (**1**) was saponified with methanolic sodium methoxide to give the ortho ester **2**, which was etherified with benzyl chloride in the presence of methylsulphinyl anion to give syrupy 3,4,6-tri-*O*-benzyl-α-D-glucopyranose 1,2-(ethyl orthoacetate)*(**3**), $[\alpha]_D^{20}$ +33° (*c* 6.6, chloroform); n.m.r. data (CCl_4): δ 7.0–8.0 (15H, 3Ph), 5.6 (*d*, $J_{1,2}$ 5 Hz, H-1), and 1.5 (*s*, 3H, CMe). Treatment of **3** with hydrogen bromide in glacial acetic acid afforded a syrupy glycosyl bromide **4**, $[\alpha]_D^{20}$ +145° (*c* 2.0, chloroform); n.m.r. data (CCl_4): δ 7.0–8.0 (15 H, 3Ph), 6.6 (*d*, $J_{1,2}$

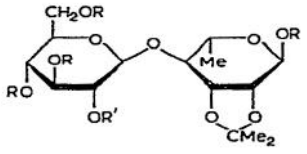
* Satisfactory microanalytical data were obtained for compounds **3**, **5–8**, **11**, and **12**.



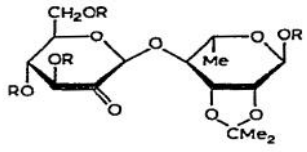
1 R = Ac
2 R = H
3 R = $PhCH_2$



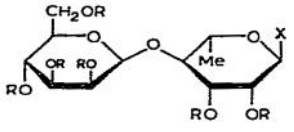
4 R = $PhCH_2$



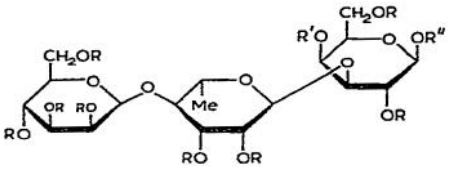
5 R = $PhCH_2$, R′ = Ac
6 R = $PhCH_2$, R′ = H



7 R = $PhCH_2$



8 R = Ac, X = OAc
9 R = Ac, X = Br



10 R = Ac, R′ = H, R″ = $PhCH_2$
11 R = R′ = Ac, R″ = $PhCH_2$
12 R = R′ = R″ = Ac

4.5 Hz, H-1), 1.9 (*s*, 3H, AcO). Glycosylation of benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[8] with **4** in the presence of mercuric cyanide in nitromethane, followed by chromatography on a column of silica gel, gave the syrupy disaccharide derivative **5**, $[\alpha]_D^{20}$ −20° (*c* 3.3, chloroform); n.m.r. data (CCl_4): δ 7.0–8.0 (20H, 4Ph), 1.9 (*s*, 3H, AcO), 1.5 and 1.3 (2*s*, 6H, CMe_2), and 1.32 (*d*, 3H, $J_{5,6}$ 5 Hz, rhamnose CMe). Saponification of **5** with methanolic sodium methoxide gave benzyl 2,3-*O*-isopropylidene-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**6**), m.p. 101–102°, $[\alpha]_D^{20}$ −20° (*c* 2, chloroform). The n.m.r. data for **6** were analogous to those for **5**, except for the absence of the signal for OAc.

Removal of the isopropylidene group from **6** with trifluoroacetic acid in chloroform, followed by hydrogenolysis over a palladium catalyst, and treatment with acetic anhydride in pyridine afforded 4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranose hepta-acetate[9], m.p. 98–100°.

Oxidation of **6** with the Pfitzner–Moffatt reagent[10] (100 h, ~20°) gave benzyl 2,3-*O*-isopropylidene-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-*arabino*-hexopyranosylulose)-α-L-rhamnopyranoside (**7**), m.p. 102–104°, $[\alpha]_D^{20}$ −40° (*c* 1.7, chloroform); n.m.r. data ($CDCl_3$): 7.0–8.0 (20H, 4Ph), 1.3 and 1.5 (2*s*, 6H, CMe_2), and 1.32 (*d*, 3H, $J_{5,6}$ 5 Hz, rhamnose CMe). Hydrogenation of **7**, first over a platinum catalyst until the i.r. absorption for C=O had disappeared, and then over a palladium catalyst (debenzylation), followed by esterification with acetic anhydride in pyridine, removal of the isopropylidene group by trifluoroacetic acid in chloroform, and acetylation gave syrupy 4-*O*-β-D-mannopyranosyl-α-L-rhamnose hepta-acetate (**8**), $[\alpha]_D^{20}$ −57° (*c* 2, chloroform); n.m.r. data ($CDCl_3$): δ 5.9 (*d*, $J_{1,2}$ 2 Hz, H-1 of rhamnose), 7.9–8.1 (21H, 7AcO), and 1.3 (*d*, 3H, $J_{5,6}$ 5 Hz, rhamnose CMe); mass spectrum: *m/e* 560 (M − AcOH), 331 (mannose moiety), and 273 (rhamnose moiety). Deacetylation of **8** with methanolic sodium methoxide, followed by hydrolysis (100°, 2 h)

with M sulphuric acid, afforded only mannose and rhamnose (identified by g.l.c.). The structure of **8** was supported by the results of methylation analysis which gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol, which were identified by g.l.c. and combined g.l.c.–m.s.

Treatment of **8** with hydrogen bromide in glacial acetic acid gave the glycosyl bromide **9**, which was condensed without further purification with benzyl 2,6-di-*O*-acetyl-β-D-galactopyranoside[6], using (*1*) boiling benzene and silver carbonate (Koenigs–Knorr) and (*2*) nitromethane and mercuric cyanide at room temperature (Helferich modification), to afford the trisaccharide derivative **10**, which was characterised, after acetylation, as benzyl 2,4,6-tri-*O*-acetyl-3-*O*-[2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranosyl]-β-D-galactopyranoside (**11**), m.p. 210–212°, $[\alpha]_D^{20}$ −40.5° (*c* 1, chloroform); mass spectrum: *m/e* 331, 379, 561, 609, and 854 (M–102). Methylation analysis of **11** gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol, and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol in the ratios 1:1:1, which were identified by comparison (g.l.c.) with authentic samples and by combined g.l.c.–m.s. Hydrogenation of **11** over a palladium catalyst and esterification of the product with acetic anhydride in pyridine gave an amorphous deca-acetate (**12**), $[\alpha]_D^{20}$ −11° (*c* 1, chloroform), which was probably a mixture of anomers; mass spectrum: *m/e* 331, 561, and 848 (M − AcOH).

In addition to the (1→4), (1→3)-linked trisaccharide **10**, a trisaccharide containing two (1→4)-linkages was isolated from the reaction mixture and converted into an amorphous acetate, $[\alpha]_D^{20}$ −33° (*c* 2, chloroform). Hydrogenolysis of this acetate over a palladium catalyst and acetylation of the product gave a deca-acetate, m.p. 87–90°, $[\alpha]_D^{20}$ +5° (*c* 1.85, chloroform). The structures of derivatives of the isomeric (1→4), (1→4)-linked trisaccharide were proved by mass spectrometry and methylation analysis, as for the trisaccharide **10**.

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