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Note

Synthesis of *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose

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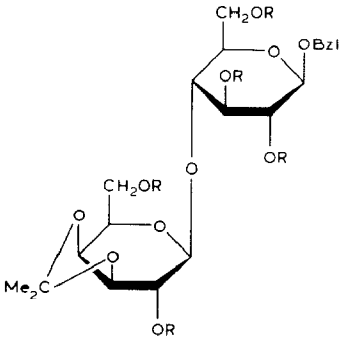
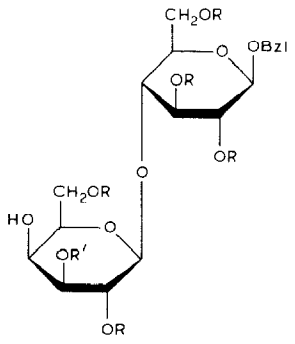
The title trisaccharide is a constituent of the antigenic determinant of the capsular polysaccharide[1] of *Klebsiella* type 73 and we now report its synthesis.

Reaction of benzyl β-D-lactoside (**1**)[2] with 2,2-dimethoxypropane[3] in *N*,*N*-dimethylformamide in the presence of toluene-*p*-sulfonic acid gave two products, and the major was characterised as benzyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucoside[3] (**2**). Compound **2** was benzylated to give benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside[4] (**3**) from which the isopropylidene group was removed, yielding crystalline benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). Condensation of **4** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[5] in the presence of silver carbonate, iodine, and

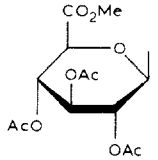


2 R = H
3 R = Bzl



4 R = Bzl, R′ = H

5 R = Bzl, R′ =



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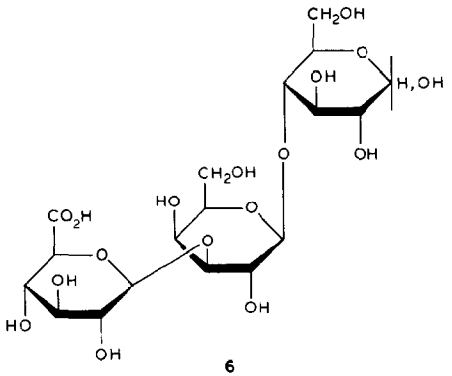

6

Drierite in dichloromethane gave the trisaccharide derivative **5**. Compound **5** was debenzylated and deacetylated in the usual way to give *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**6**).

Hydrolysis[1] of **6** with 0.5M sulfuric acid at 100° gave (p.c.) glucuronic acid, galactose, and glucose. Compound **6** was methylated by the Kuhn method[6], and the product was hydrolysed, reduced, and acetylated. G.l.c. of the resulting alditol acetates revealed derivatives of 2,4,6-tri-*O*-methylgalactose and 2,3,6-tri-*O*-methylglucose. When methylated **6** was reduced with lithium aluminium hydride[1] and the products were converted into the alditol acetates, g.l.c. revealed derivatives of 2,4,6-tri-*O*-methylgalactose, 2,3,6-tri-*O*-methylglucose, and 2,3,4-tri-*O*-methylglucose in equimolar ratios. These data indicated the structure of **6** as shown. Periodate oxidation[1] of **6** also supported the above structure. It is noteworthy that no substitution occurred at position 4′ of the lactose derivative **4**.

EXPERIMENTAL

General. — All reactions were monitored by t.l.c. on Silica Gel G (Merck). Column chromatography was performed on Silica Gel 60 (Merck). P.c. was performed on Whatman No. 1 paper with 9:2:2 ethyl acetate–acetic acid–water and detection with alkaline silver nitrate[7]. All solvents were distilled before use, and all evaporations were conducted at 50° unless stated otherwise. Optical rotations were measured with a Perkin–Elmer Model 241 MC spectropolarimeter. N.m.r. spectra were recorded with a Wilmad FX-100 spectrometer for solutions in $CDCl_3$ (internal Me_4Si). G.l.c. of alditol acetates[8] was performed with a Hewlett–Packard Model 5730A gas chromatograph, having a flame-ionisation detector and glass columns (1.83 m × 6 mm) containing 3% of ECNSS-M on gas Chrom Q (100–120 mesh), at 185° for unmethylated compounds and 170° for methylated compounds. Retention times were measured with respect to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

*Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside* (**3**). — A solution of **2** (2.5 g) in 1,4-dioxane

(20 mL) was stirred with powdered potassium hydroxide (10 g) at 80° whilst benzyl chloride (20 mL) was added drop-wise during 2 h. Heating and stirring were then continued for 3 h. The mixture was cooled (ice-bath), diluted with water, and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with water, dried, filtered, and concentrated. Benzyl alcohol was removed from the syrupy residue by azeotropic distillation with water. Column chromatography (benzene–ether, 2:1) then gave **5** (3.5 g, 72.9%), $[\alpha]_D^{23}$ +9° (*c* 1.2, chloroform); lit.[4] $[\alpha]_D^{25}$ +8.4° (chloroform).

Anal. Calc. for $C_{57}H_{62}O_{11}$: C, 74.20; H, 6.71. Found: C, 74.43; H, 6.82.

*Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**4**). — To a solution of **3** (2 g) in glacial acetic acid (15 mL) at 75° was added drop-wise water (10 mL). Heating was continued for 45 min and the solution was then concentrated. The syrupy residue was crystallised from ethanol to yield **4** (1.2 g, 62.5%), m.p. 115°, $[\alpha]_D^{25}$ +6° (*c* 1.5, chloroform).

Anal. Calc. for $C_{54}H_{58}O_{11}$: C, 72.99; H, 6.67. Found: C, 72.99; H, 6.69.

*Benzyl 2,3,6,2′,6′-penta-*O*-benzyl-3′-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyl)uronate]-β-*D*-lactoside* (**5**). — A mixture of **4** (2 g), active silver carbonate (3 g), Drierite (3 g), and dichloromethane (20 mL) was stirred for 1 h in the dark. Iodine (0.5 g) was then added followed drop-wise by a solution of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (2 g) in dichloromethane (20 mL) during 24 h. The mixture was stirred in the dark at room temperature for a further 20 h, then filtered through Celite, and concentrated. T.l.c. (benzene–ether, 2:1) of the syrupy residue revealed three spots. The major portion of the fastest-moving fraction crystallised from ethanol, and more was obtained by column chromatography (benzene–ether, 1:1) of the material in the mother liquor. The product was recrystallised from ethanol to give **5** (1 g, 37%), m.p. 168°, $[\alpha]_D^{23}$ −10.5° (*c* 1, chloroform); ν_{max} 3600 cm^{-1} (OH). ^{1}H-N.m.r. data: 1.78, 1.98, and 2.00 (3 s, each 3 H, 3 OAc), 3.68 (s, 3 H, COOMe), 7.30 (m, 30 H, 6 Ph).

Anal. Calc. for $C_{67}H_{74}O_{20}$: C, 67.10; H, 6.20. Found: C, 67.24; H, 6.28.

The two slower-moving spots were **4** and partially acetylated methyl glucuronate.

O-(β-D-Glucopyranosyluronic acid)-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**6**). — A solution of **5** (500 mg) in dry methanol (10 mL) was stirred under hydrogen for 24 h at room temperature in the presence of 10% Pd/C (1 g), then filtered through Celite, and concentrated to dryness. To a solution of the residue (150 mg) in dry methanol (10 mL) were added methanolic 2M sodium methoxide (0.5 mL) and, after 3 h, a few drops of water. The mixture was kept at room temperature for 1 h, then neutralised with Dowex 50W-X8 (H^+) resin, filtered, and concentrated to dryness to give **6**, $[\alpha]_D^{23}$ −13° (*c* 1, water). P.c. revealed a single component with $R_{Lactose}$ 0.48.

To a solution of **6** (2 mg) in water (4 mL) was added 0.2M sodium metaperiodate (0.5 mL), and the mixture was kept in the dark for 48 h at 5°. The excess of periodate was decomposed with ethylene glycol and, after 3 h, the mixture was

concentrated to 1 mL, and sodium borohydride (30 mg) was added. The solution was kept at room temperature for 4 h, then cations were removed with Dowex 50W-X8 (H^+) resin, the solution was concentrated to dryness, and boric acid was removed by repeated evaporation of methanol from the residue. G.l.c. of the alditol acetates[8] prepared from the residue revealed galactose and erythritol.

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