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SYNTHESIS OF 5-*O*-β-D-GALACTOFURANOSYL-D-GALACTOFURANOSE

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ABSTRACT

Conversion of benzyl αβ-D-galactofuranoside into the 5,6-*O*-[α-(dimethylamino)benzylidene] derivative, followed by acetylation of HO-2 and HO-3, and selective ring opening of the acetal, gave benzyl 2,3-di-*O*-acetyl-6-*O*-benzoyl-αβ-D-galactofuranoside (**4**). The title disaccharide was synthesised from **4** by reaction with 3,4,6-tri-*O*-acetyl-α-D-galactofuranose 1,2-(methyl orthoacetate) followed by removal of protecting groups.

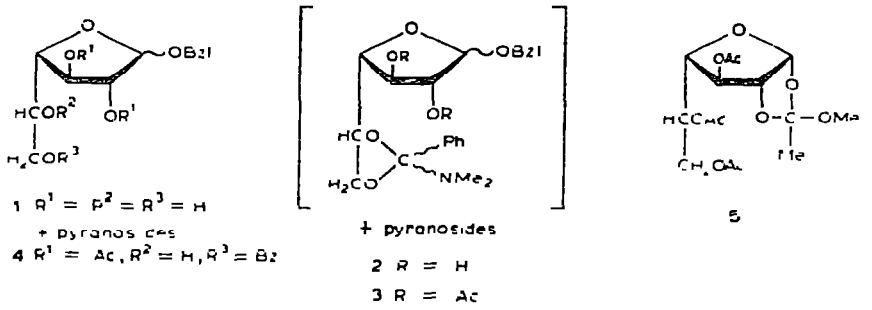
INTRODUCTION

The occurrence of (1→5)-linked D-galactofuranoid units in oligosaccharides has been demonstrated by the isolation of 5-*O*-β-D-glucopyranosyl-D-galactofuranose[1] and 5-*O*-β-D-galactofuranosyl-D-galactofuranose[2] from partial acid hydrolysates of the type-specific substance of Pneumococcus 33B and galactocarolose, respectively. We now report a synthesis of the latter disaccharide.

RESULTS AND DISCUSSION

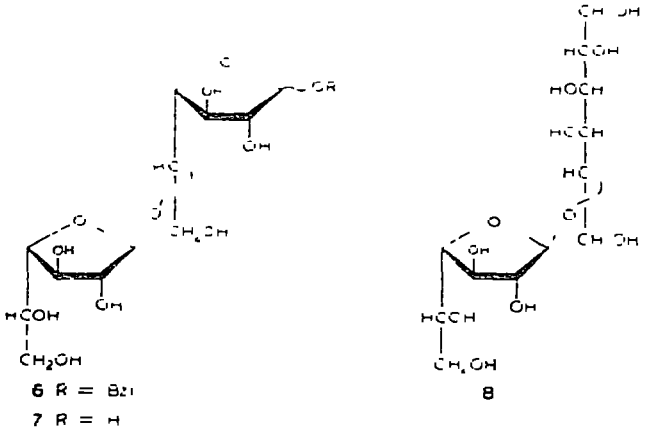
Syrupy benzyl αβ-D-galactofuranoside (**1**) was prepared in 62% yield by treatment of D-galactose diethyl dithioacetal with benzyl alcohol in the presence of mercuric oxide and chloride[3]. Furanosides obtained by this method are frequently syrups[3], although crystalline benzyl β-D-galactofuranoside has been isolated[4] (12%) from **1**. The ^{1}H-n.m.r. spectrum of **1** in methyl sulphoxide-d_6 contained signals for anomeric protons at δ ~4.9 for the furanose forms, made up of an overlapping singlet ($J_{1,2}$ ~0 Hz, H-1β) and a doublet ($J_{1,2}$ 3–4 Hz, H-1α), as observed for 6-aminohexyl D-galactofuranoside[5], and accounting for at least 90% of the mixture. A small doublet at δ 4.73 ($J_{1,2}$ 3.4 Hz) was tentatively assigned to H-1 of the α-pyranoside, but its quantification was hampered by the proximity of resonances for the benzyl methylene group.

Following the procedure of Hanessian and Moralioglu[6], **1** was treated with an excess of *N,N*-dimethylbenzamide dimethyl acetal in chloroform to give a mixture (t.l.c.) of two diastereomeric forms of benzyl 5,6-*O*-[α-(dimethylamino)benzylidene]-αβ-D-galactofuranoside (**2**) in virtually quantitative yield. Reaction of **2** with acetic



anhydride in pyridine afforded the 2 3-diacetate **3** which with aqueous acetic acid, gave benzyl 2 3-di-*O*-acetyl-6-*O*-benzoyl-α*β*-D-galactofuranoside (**4**) An overall yield of 69% was obtained for the sequence **1** → **2** → **3** → **4** when the intermediates were not isolated Attempts to purify **2** and **3** resulted in considerable losses due to the lability of the 5,6-acetal groups Compound **4** was purified by column chromatography, and its ^{1}H-n m r. spectrum was consistent with the assigned structure

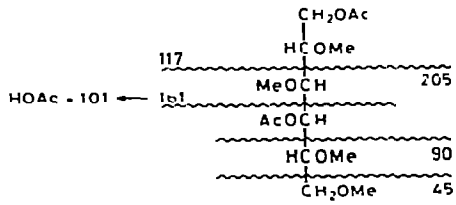
Condensation of 3,5,6-tri-*O*-acetyl-α-D-galactofuranose 1,2-(methyl orthoacetate)[7] (**5**) with **4** in nitromethane in the presence of mercuric bromide, with deacylation of the product, gave a mixture from which benzyl 5-*O*-*β*-D-galactofuranosyl-D-galactofuranoside (**6**) was isolated Although **6** gave one spot in t l c , it contained impurities that were revealed after debenzylation which gave mainly 5-*O*-*β*-D-galactofuranosyl-D-galactofuranose (**7**) The contaminants were reducing disaccharides containing galactopyranose residues (^{1}H-n m r spectroscopy) Purification of **7** was readily achieved by ion-exchange column chromatography[8]



The presence of the (1→5)-linkage in **7** was confirmed by ^{1}H-n m r. spectroscopy, although assignment of the furanoid anomeric protons was only possible on the basis of a 360-MHz spectrum

The physical constants of **7** and those of crystalline 5-*O*-*β*-D-galactofuranosyl-D-galactitol (**8**), obtained upon borohydride reduction of **7** corresponded closely to those reported by Gorin and Spencer[2] The retention times of trimethylsilylated synthetic and authentic **8** in g l c were identical (co-chromatography) (see Experimental)

Reduction of **7** with sodium borodeuteride followed by methylation[9] and then[9 10] hydrolysis, reduction, and acetylation gave 1,4-di-*O*-acetyl-2,3,5,6-tetra-*O*-methyl-D-galactitol (**9**) and 5-*O*-acetyl-1,2,3,4,6-penta-*O*-methyl-D-galactitol-*l*-*d* (**10**), which were identified by g l c –m s and proved the presence of a furanosyl (1→5)-linkage in **7** The ^{1}H-n m r and optical rotation data for **7** and **8** established the intersugar glycosidic linkage to be *β*



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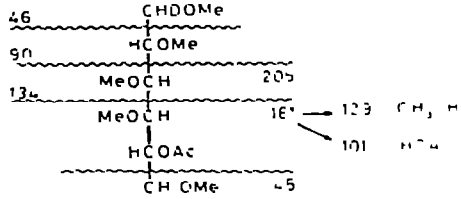

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EXPERIMENTAL

General — 5-*O*-*β*-D-Galactofuranosyl-D-galactitol was a gift from Dr P A J Gorin Pyridine (1 litre) was purified by distillation from chlorosulphonic acid (10 ml) and kept over molecular sieve 5A Nitromethane was purified as described earlier[11] Solutions were concentrated at 40 (bath)/~14 mmHg Specific rotations were determined at ambient temperature with a Perkin–Elmer model 141 polarimeter ^{1}H-N m r. spectra were recorded with Varian EM-390 (90 MHz) and Bruker HX-360 (360 MHz) spectrometers, with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) as the internal standard Directions for the preparation of partially methylated per-*O*-acetylated hexitols and for the interpretation of their g l c –m s data are given in the literature[9 10] Mass spectra (75 eV) were recorded on a Jeol JGC-100/JMS-07 combination [column of 3% of SE-30 on Chromosorb W-AW DMCS (80–100 mesh) at 158 , ion-source temperature 250° accelerating voltage 3 kV, ionizing current 300 *μ*A] G l c. of trimethylsilyl derivatives[12] of sugars was carried out at 227 with a Pye 104 instrument equipped with flame-ionisation detector and glass columns (1 60 m × 4 mm) packed with 3 8% of SE-30 or 3% of OV-17 on Chromosorb W-AW DMCS (80–100 mesh) and a nitrogen flow-rate of 30 ml/min Retention times (T_S) are given relative to that of Me_3Si-sucrose.

T l c. was performed on silica gel (Schleicher & Schull TLC Ready Plastic Foil FR-1500) All reactions were monitored by t l c , and mobilities are expressed as R_X.

where X denotes a reference compound or a starting material. The spots were detected by charring with sulphuric acid. Benzyl derivatives were also detected under u.v. light (254 nm). Column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh) with *A*, benzene–propan-2-ol–water (30:14:1), *B*, chloroform–methanol (25:1), *C*, chloroform–methanol (100:1), *D*, acetic acid–ethyl acetate–butan-1-ol–water (6:3:8:1), *E*, water, *F*, ethyl acetate–acetic acid–water (9:2:2), and *G*, propan-2-ol–ethyl acetate–water (5:5:2).

Benzyl D-*galactofuranoside* (**1**). — The crude syrupy product[4] was purified by column chromatography (solvent *A*). The first fraction was concentrated several times from its ethanolic solution to remove residual solvent *A* and yielded **1** as a syrup (62%), $[\alpha]_D$ −23 (*c* 1, methanol). The material gave one spot in t.l.c. (solvents *A* and *G*), and g.l.c. of the Me_3Si derivative gave one peak with a shoulder on the negative slope. ^{1}H-N.m.r. data (methyl sulphoxide-d_6): δ 7.2–7.5 (m, 5 H, aromatic protons), 4.9 (m, 0.9 H, $J_{1,2}$ ~0 and 3–4 Hz, H-1β and H-1α of furanosides), 4.73 [d, $J_{1,2}$ 3.4 Hz, H-1α(?) of pyranoside], and 3.3–4.8 (other protons). [Found (for freeze-dried **1**): C, 57.70, H, 6.67. $C_{13}H_{18}O_6$ calc.: C, 57.77, H, 6.71%].

*Benzyl 2,3-di-O-acetyl-6-O-benzoyl-*D-*galactofuranoside* (**4**). — A mixture of **1** (3.2 g, 11.8 mmol), *N*,*N*-dimethylbenzamide dimethyl acetal[6] (5 g, 25.6 mmol), and chloroform (20 ml) was stirred in the dark at room temperature. After 16 h, t.l.c. (solvent *B*) revealed the absence of **1** and the formation of two closely migrating components at R_1 4 in approximately equal amounts. The solution was cooled, and diluted with chloroform (80 ml) and ice–water (80 ml), and the organic layer was separated, dried (Na_2SO_4), filtered, and concentrated to ~10 ml at 30° (bath). To the solution (containing **2***), pyridine (20 ml) was immediately added followed by acetic anhydride (3.5 ml, 37 mmol). After 24 h, t.l.c. (solvent *B*) revealed the formation of two closely migrating components (**3**, R_2 2.5) in approximately equal amounts and the absence of **3**. The solution was diluted with chloroform (80 ml, 0°), extracted with ice–water (80 ml), dried at 0° (Na_2SO_4), and concentrated, and pyridine was removed from the residue by evaporation of toluene three times therefrom. The product (**3***, 7 g) was immediately dissolved in acetic acid–water (19:1, 40 ml), and the solution was kept at room temperature for 2 h and then poured into 8% aqueous sodium hydrogen carbonate (1 litre). The mixture was extracted with chloroform (3 × 200 ml) and the extract was dried (Na_2SO_4) and concentrated. The residual syrup consisted (t.l.c., solvent *B*) of a major component (**4**) and four minor components having R_4 1.29, 1.23, 0.86, and 0.80. Compound **4** was purified by column chromatography (gradient elution, solvents *C* → *B*) and obtained as a colourless oil (3.75 g, 8.18 mmol, 69% based on **1**), $[\alpha]_D$ −36 (*c* 4, chloroform). ^{1}H-N.m.r. data (chloroform-*d*): δ 7.1–7.6 and 7.9–8.2 (2 m, 5 H, OBz), 7.3 (m, 5 H, Ph), 2.0 (s, 6 H, 2 AcO), 3.23 (d, 1 H, $J_{HO,5}$ 8 Hz, HO-5, disappeared upon deuterium-exchange), and 3.8–5.8 (m, 9 H, sugar ring protons and benzyl-CH_2). (Found: C, 63.31, H, 5.96. $C_{24}H_{26}O_9$ calc.: C, 62.88, H, 5.72%).

*Solutions containing **2** or **3** gave a positive reaction[6] for α-(dimethylamino)benzylidene acetal.

Benzyl 5-O-β-D-galactofuranosyl-D-galactofuranoside (**6**) — 3,5,6-Tri-*O*-acetyl-α-D-galactofuranose 1,2-(methyl orthoacetate)[7] (**5**, 2.28 g, 6.29 mmol) and **4** (2.3 g, 5.02 mmol) were dissolved in nitromethane (25 ml). The solvent was distilled at atmospheric pressure with simultaneous addition of nitromethane to keep the volume constant. Mercuric bromide (84 mg) was added after a few ml of solvent had distilled, and distillation was then continued for 4 h. The mixture was cooled, pyridine (1 ml) was added, the precipitate was removed, and the filtrate was concentrated. The resulting syrup (6 g) was deacylated in 0.02M methanolic sodium methoxide (100 ml). After completion of the reaction (t.l.c., solvents *A* and *B*), the solution was neutralized with Amberlite IR-120 (H^+) resin, and concentrated to dryness. The residue was eluted from a column[8] (1.5 m × 2 cm) of Dowex 50 X4 (K^+) resin with solvent *E* at 0.4 ml/min. D-Galactose, methyl β-D-galactofuranoside, **6**, and **1** were separately eluted. The fraction containing **6** was freeze-dried to yield a syrup (262 mg, 12%) $[\alpha]_D$ −113 (*c* 8, methanol) which was homogeneous in t.l.c. (solvent *A*, R_{GAL} 2). ^{1}H-N.m.r. data (deuterium oxide): δ 7.5 (m, 5 H, Ph), 5.0–5.3 (m, 2 H, anomeric protons), 3.6–4.3 (m, sugar ring protons), and 4.7–4.9 (m, benzyl-CH_2) partially masked by an HOD signal. (Found: C, 50.10; H, 6.78. Calc. for $C_{19}H_{28}O_{11}$, 1.3H_2O: C, 50.06; H, 6.77%.)

5-O-β-D-Galactofuranosyl-D-galactofuranose (**7**) — A vigorously stirred solution of **6** (250 mg, 0.58 mmol) in 90% aqueous ethanol (100 ml) was hydrogenated at atmospheric pressure over 10% palladium-on-charcoal (200 mg) for 2.5 h. Removal of the catalyst and concentration left a residue which consisted mainly of **7** (t.l.c., solvent *D*; R_{GAL} 0.85) with minor components having R_{GAL} 0.60, 0.40 and 0.30. These impurities proved to be, at least partly, disaccharides containing galactopyranoses, as shown by ^{1}H-n.m.r. spectroscopy (deuterium oxide): δ 4.63 (d, 0.2 H, $J_{1,2}$ 7.5 Hz, H-1β pyranose), 5.0–5.3 (m, 1.8 H, overlapping signals for H-1 of α- and β-furanose and α-pyranose) and 3.4–4.3 (m, 12 H, sugar ring protons). Ion-exchange chromatography[8] (solvent *E*), as described for **6**, yielded **7** (120 mg, 61%) as a syrup, $[\alpha]_D$ −64 (*c* 2, water). lit.[2] $[\alpha]_D$ −65° (*c* 3.8, water). The freeze-dried sugar had $[\alpha]_D$ −74 (*c* 2, water) and R_{GAL} 0.85 (descending p.c., solvent *F*), lit.[2] R_{GAL} 0.9. The 360-MHz ^{1}H-n.m.r. spectrum of **7** in deuterium oxide is consistent with a 2.3 α:β-mixture: δ 5.197 ($J_{1,2}$ 3.4 Hz, H-1α), 5.231 ($J_{1,2}$ ~0 Hz, H-1′β), and 5.244 ($J_{1,2}$ ~0 Hz, H-1β), 5.206 ($J_{1,2}$ ~0 Hz, H-1′β). [Found (for freeze-dried **7**): C, 41.62; H, 6.37. $C_{12}H_{22}O_{11}$ calc.: C, 42.11; H, 6.48%.]

5-O-β-D-Galactofuranosyl-D-galactitol (**8**) — Reduction[2] of **7** (40 mg, 0.12 mmol) with sodium borohydride (40 mg) gave a crude alditol (38 mg, 94%) which was homogeneous in t.l.c. (solvent *G*, R_{GAL} 1) and by ^{1}H-n.m.r. spectroscopy (deuterium oxide): δ 5.27 (s, 1 H, $J_{1,2}$ ~0 Hz, H-1′) and 3.4–4.3 (m, 14 H, other protons). When recrystallized from methanol–ethanol, **8** had m.p. 148–150°, $[\alpha]_D$ −63 (*c* 2, water), lit.[2] m.p. 149–151°, $[\alpha]_D$ −65° (water).

On g.l.c. of the Me_3Si derivatives on SE-30 and OV-17, the following T_S values were observed: **7** 0.90, 1.13 and 0.90, 1.15, **8** 1.37 and 1.38.

Methanolysis of **8** (1 mg) followed by g.l.c., as devised by Clamp *et al.*[13,14],

showed the presence of galactose and galactitol in a 1 1 ratio Alditol acetate analysis[9, 10] of 8-d_1 was in accordance with the assigned structure

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