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CHEMICAL SYNTHESES OF 4-*O*-α- AND -β-D-GALACTOPYRANOSYL-D-GALACTOSE AND 3-*O*-α- AND -β-D-GALACTOPYRANOSYL-D-GALACTOSE

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ABSTRACT

Reaction of 2,3-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**2**) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of mercuric cyanide and subsequent acetolysis gave 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranose (**4**, 40%) and 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose (**5**, 30%). Similarly, reaction of 2,4-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**3**) gave 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranose (**6**, 46%) and 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose (**7**, 14%). The anomeric configurations of **4**–**7** were assigned by n.m.r. spectroscopy. Deacetylation of **4**–**7** afforded 4-*O*-α-D-galactopyranosyl-D-galactose (**8**), 4-*O*-β-D-galactopyranosyl-D-galactose (**9**), 3-*O*-α-D-galactopyranosyl-D-galactose (**10**), and 3-*O*-β-D-galactopyranosyl-D-galactose (**11**), respectively.

INTRODUCTION

As part of a study[1,2] of the structure of molecules of biological interest, we have synthesised 4-*O*-α-D-galactopyranosyl-D-galactose (**8**), 4-*O*-β-D-galactopyranosyl-D-galactose (**9**), 3-*O*-α-D-galactopyranosyl-D-galactose (**10**), and 3-*O*-β-D-galactopyranosyl-D-galactose (**11**).

4-*O*-α-D-Galactopyranosyl-D-galactose (**8**) is the terminal disaccharide of the ceramide trihexoside isolated from normal human kidney and kidney from a case of Fabry's disease, human erythrocytes, and several other animal sources[3–5]. It has also been obtained by partial hydrolysis of okra mucilage[6], by enzymic reversion of D-galactose with α-D-galactosidase[7,8], and by chemical reduction of esters of 4-*O*-(α-D-galactopyranosyluronic acid)-D-galacturonic acid[9]. 4-*O*-β-D-Galactopyranosyl-D-galactose (**9**) has been isolated from partial hydrolysates of several polysac-

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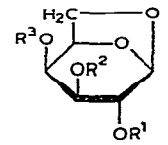

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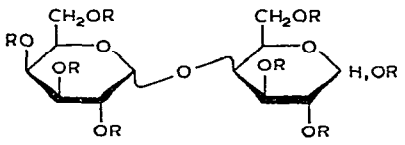
2 $R^1 = R^2 = Ac, R^3 = H$
3 $R^1 = R^3 = Ac, R^2 = H$



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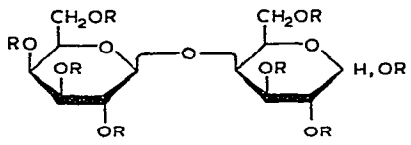
8 R = H



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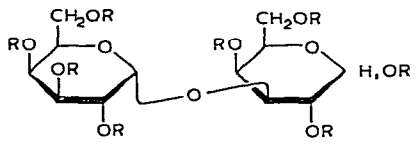

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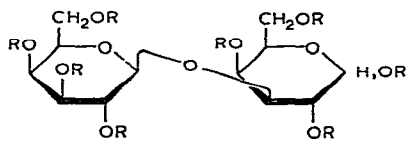

7 R = Ac
11 R = H

charides[10–14]. The physical constants of the synthetic material[15] do not agree, however, with those reported for **9** prepared by other methods. 3-*O*-α-D-Galactopyranosyl-D-galactose (**10**) has been obtained by enzymic polymerization of D-galactose[7,8], and from partial hydrolysates of λ-carrageenin[16] and blood-group B substance[17–19]. 3-*O*-β-D-Galactopyranosyl-D-galactose (**11**) has been isolated from a variety of sources[20–35] and also synthesised[36].

Of the well-established general methods for glycoside and disaccharide synthesis, the Koenigs–Knorr reaction[37] and the orthoester method[38] produce 1,2-*trans*-glycosides; the glycal method[39] and the Koenigs–Knorr reaction involving glycosyl halides bearing a non-participating group at C-2 often give 1,2-*cis*-glycosides[40]. In polar solvents in the presence of catalysts carrying strongly complexing anions, the Koenigs–Knorr reaction usually gives a mixture of 1,2-*cis* and *trans*-glycosides[41]. Since the α- and β-linked galactosylgalactoses (**8–11**) were needed for comparison purposes, their syntheses were effected by the last-mentioned procedure, using 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with 2,3-di-*O*-acetyl- (**2**) and 2,4-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**3**). Compounds **2** and **3** are easily prepared by partial acetylation[42] of 1,6-anhydro-β-D-galactopyranose (**1**), and the syntheses described herein provide a straightforward method of obtaining disaccharides **8–11** in reasonable yields.

RESULTS AND DISCUSSION

The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with 2,3-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**2**) in 1:1 nitromethane–benzene in the presence of mercuric cyanide afforded a mixture (90.5%) of α- and β-linked 1,6-anhydro peracetylated disaccharides contaminated by minor by-products. Brief treatment of this mixture with sulphuric acid in acetic anhydride–acetic acid gave 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranose (**4**, 40%) and 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose (**5**, 30%), which were separated by column chromatography. The 100-MHz* spectrum ($CDCl_3$) of **4** showed two doublets at τ 3.65 ($J_{1,2}$ 3.2 Hz) and 4.99 ($J_{1',2'}$ 3.0 Hz), which were assigned to H-1 and H-1′, respectively. The magnitude of the coupling constants are indicative of α configuration. The signals for H-2,2′,3,3′,4 were in the region τ 4.40–4.90 and those for H-4,5,5′ and the protons at C-6 and C-6′ at τ 5.40–6.10. The spectrum of **5** contained signals for H-1 and H-1′ at τ 3.74 and 5.56, respectively. The coupling constants ($J_{1,2}$ 3.0, $J_{1',2'}$ 7.5 Hz) indicated the anomeric configurations to be α at C-1 and β at C-1′. The signals for H-2,2′,3,3′4 appeared at τ 4.60–5.10, and those for H-4,5,5′ and the protons on C-6 and C-6′ at τ 5.60–6.20. The optical rotations (+138° and +54°) of **4** and **5** were in agreement with the proposed configurations. Deacetylation of **4** and **5** gave chromatographically homogeneous **8** and **9**, the melting points and optical rotations of which were identical to those previously reported[6,10] for 4-*O*-α-D-galactopyranosyl-D-galactose (**8**) and 4-*O*-β-D-galactopyranosyl-D-galactose (**9**) prepared by different procedures.

Reaction of tetra-*O*-acetyl-α-D-galactopyranosyl bromide with 2,4-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**3**) under conditions similar to those described above for **2**, followed by acetolysis, gave 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranose (**6**, 46%) and 1,2,4,6-tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose (**7**, 14%). The 100-MHz n.m.r. spectrum ($CDCl_3$) of **7** showed doublets at τ 3.72 and 5.38 assigned to H-1 and H-1′, respectively. The coupling constants ($J_{1,2}$ 3.5, $J_{1',2'}$ 7.2 Hz) were indicative of an α-anomeric configuration at C-1 and β at C-1′. The signals for H-2,2′,4,4′ appeared at τ 4.45–5.20, and those of H-3,5,5′ and the methylenic protons at τ 5.65–6.20. The spectrum of **6** could not be readily analysed. It showed, however, a doublet at τ 3.60 assigned to H-1, with $J_{1,2}$ 3.5 Hz which was indicative of the α configuration. Deacetylation of **7** gave a crystalline disaccharide having physical constants in good agreement with those reported for 3-*O*-β-D-galactopyranosyl-D-galactopyranose (**11**). Deacetylation of **6** gave syrupy 3-*O*-α-D-galactopyranosyl-D-galactose (**10**) which, with acetic anhydride–pyridine, afforded a

*The spectra of the peracetylated disaccharides **4**–**7** have been completely analysed and computed after iterative analysis as independent seven-spin systems. The results of this study will be published elsewhere.

crystalline β-octa-acetate having physical constants identical to those described for the β-octa-acetate prepared from **10** isolated from partial hydrolysates of λ-carrageenin[16].

EXPERIMENTAL

General. — Melting points were measured in capillary tubes on a Büchi apparatus and are uncorrected. T.l.c. was performed on silica gel G (E. Merck, Darmstadt) with detection by sulphuric acid. Column chromatography was performed on silica gel Merck (60–230 mesh). P.c. was conducted on Whatman No. 1 paper with detection by silver nitrate[43]. The i.r. spectra were recorded for KBr discs, using a Perkin–Elmer 457 spectrometer. N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si), using a Varian XL-100 spectrometer. Optical rotations were determined with a Perkin–Elmer 141 polarimeter.

1,2,3,6-Tetra-O-*acetyl-4*-O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*galactopyranosyl*)-α-D-*galactopyranose* (**4**) *and 1,2,3,6-tetra*-O-*acetyl-4*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-α-D-*galactopyranose* (**5**). — A solution of 2,3-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose[42] (**2**; 0.9 g, 3.7 mmoles) in 1:1 nitromethane–benzene (95 ml) was concentrated until 25 ml of the solvent mixture had distilled. After cooling to 40°, mercuric cyanide (0.932 g, 3.7 mmoles) and tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.487 g, 3.7 mmoles) were added and the mixture was stirred at 40° for 50 h. Further additions of mercuric cyanide (0.471 g, 1.8 mmoles) and glycosyl bromide (0.743 g, 1.8 mmoles) were made after 20, 28, and 40 h. After 50 h, the mixture was cooled to room temperature, diluted with benzene, filtered, washed with 5% aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue showed two main spots of similar mobility on t.l.c. (5:3 ethyl acetate–benzene). Column chromatography using the same solvent system gave, first, fast-moving impurities and then a syrupy mixture (1.9 g, 90.5%) of the main products. A solution of the syrup (1.9 g) in cold 7:3 acetic anhydride–acetic acid (78 ml) containing conc. sulphuric acid (1.6 ml) was kept for 15 min at room temperature, then neutralized with 5% aqueous sodium hydrogen carbonate at 0°, and extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue (2.1 g), which showed two main spots on t.l.c. (3:1 ether-benzene), was subjected to column chromatography. Elution with 3:1 ether–benzene first gave **4** (0.875 g, 40%), m.p. 153–154° (from ethanol), $[\alpha]_D^{24}$ +138° (*c* 2, chloroform).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.64. Found: C, 49.73; H, 5.75.

Subsequently, **5** (0.655 g, 30%) was eluted having m.p. 189–190° (from ethanol), $[\alpha]_D^{25}$ +54° (*c* 1.8, chloroform).

Anal. Found: C, 49.49; H, 5.76.

1,2,4,6-Tetra-O-*acetyl-3*-O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*galactopyranosyl*)-α-D-*galactopyranose* (**6**) *and 1,2,4,6-tetra*-O-*acetyl-3*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-α-D-*galactopyranose* (**7**). — A solution of 2,4-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose[42] (**3**; 1.80 g, 7.3 mmoles) in 1:1 nitromethane–benzene (185 ml) was concentrated until 50 ml of the solvent mixture had distilled. The solution was

then cooled at 40°, mercuric cyanide (1.86 g, 7.3 mmoles) and tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2.97 g, 7.3 mmoles) were added, and the stirred mixture was kept at 40° for 52 h. Further additions of mercuric cyanide (0.9 g) and glycosyl bromide (1.45 g) were made after 23, 30, and 46 h. After 52 h, the reaction mixture was cooled to room temperature, diluted with benzene, filtered, washed with 5% aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue (7.5 g), which showed on t.l.c. (5:3 ethyl acetate–benzene) two main spots and small amounts of fast-moving impurities, was subjected to column chromatography. Elution with 5:3 ethyl acetate–benzene removed first the impurities and then gave a syrupy mixture (3.2 g, 76%) of 1,6-anhydro peracetylated disaccharides. A solution of this syrup (2.9 g) in 7:3 acetic anhydride–acetic acid (110 ml) containing conc. sulphuric acid (2.2 ml) was kept at room temperature for 90 min and then at 50° for 30 min. The solution was cooled, neutralized with 5% aqueous sodium hydrogen carbonate at 0°, and extracted with chloroform. The chloroform layer was washed with water, dried ($MgSO_4$), and concentrated *in vacuo* to give a syrup (3.4 g) which showed two main spots on t.l.c. (5:1 ether–benzene). The syrup was subjected to column chromatography. Elution with 5:1 ether–benzene first afforded **6** (1.55 g, 46%), m.p. 97–100° (from ethanol), $[\alpha]_D^{23}$ +137° (*c* 2, chloroform).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.64. Found: C, 49.28; H, 5.89.

Subsequently, **7** (0.46 g, 14%) was eluted having m.p. 185° (from ethanol), $[\alpha]_D^{25}$ +54° (*c* 1.5 chloroform).

Anal. Found: C, 49.58; H, 5.36.

Deacetylations. — To a solution of **4** (0.22 g, 0.32 mmole) in methanol (2.2 ml), 0.2M methanolic sodium methoxide (0.65 ml) was added, and the solution was stirred for 30 min at room temperature. The solution was neutralized with Amberlite IR-120(H^+) resin and concentrated *in vacuo* to give 4-*O*-α-D-galactopyranosyl-D-galactose (**8**; 0.08 g, 85%), m.p. 212–213° (from methanol–1-butanol–water), $[\alpha]_D^{26}$ +167→170° (*c* 1, water), R_{GAL} 0.70 (p.c., 10:4:3 ethyl acetate–pyridine–water); lit.[6] m.p. 210–211°, $[\alpha]_D^{26}$ +177°.

Likewise, the following compounds were obtained.

4-*O*-β-D-Galactopyranosyl-D-galactose (**9** from **5**), m.p. 204–206° (from 0.5:10 water–acetone), $[\alpha]_D^{26}$ +76→+69° (*c* 1.1, water), R_{GAL} 0.78 (p.c., 10:4:3 ethyl acetate–pyridine–water); lit.[10] m.p. 204°, $[\alpha]_D$ +68° (*c* 1, water).

3-*O*-α-D-Galactopyranosyl-D-galactose (**10** from **6**, 74%), $[\alpha]_D^{26}$ +149° (*c* 1.6, water), R_{GAL} 0.77 (p.c., 10:4:3 ethyl acetate–pyridine–water); lit.[16] $[\alpha]_D^{25}$ +155° (*c* 0.3, water).

3-*O*-β-D-Galactopyranosyl-D-galactose (**11** from **7**), m.p. 165–168° (from 0.5:10 water–acetone), $[\alpha]_D^{26}$ +71→+62° (*c* 1, water), R_{GAL} 0.72 (p.c., 10:4:3 ethyl acetate–pyridine–water); lit.[23] m.p. 165°, $[\alpha]_D^{24}$ +64° (*c* 0.8, water).

1,2,4,6-Tetra-O-*acetyl-3*-O-(*2,3,4,6-tetra*-O-*acetyl-α-D-galactopyranosyl*)-*β*-D-*galactopyranose.* — To a solution of **10** (0.130 g) in pyridine (2 ml), acetic anhydride (1.2 ml) was added and the solution was kept overnight at room temperature. After the usual work-up, the acetate (0.118 g) was purified by p.l.c. (1:3 benzene–ether) and

recrystallized from ethanol to give the title compound, m.p. 156–157°, $[\alpha]_D^{26}$ +114° (*c* 0.95, chloroform); lit.[16] m.p. 157.5–158.5°, $[\alpha]_D^{25}$ +110.2° (*c* 0.5, chloroform). N.m.r. data: τ 4.39 (*d*, $J_{1,2}$ 8.2 Hz).

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