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4-*O*-α-D-GALACTOPYRANOSYL-D-GALACTOSE: EFFICIENT SYNTHETIC ROUTES FROM "POLYGALACTURONIC ACID"

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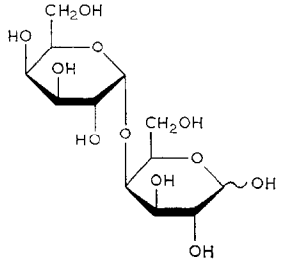
ABSTRACT

Enzymic hydrolysis of "polygalacturonic acid" gave a mixture of oligomers which was fractionated by ion-exchange chromatography. The resulting di- and trisaccharides were treated, respectively, with methanol and ethylene oxide, and the resulting esters were reduced with sodium borohydride. Treatment of the products with acetic anhydride and sulfuric acid, followed by deacetylation, produced the title compound.

INTRODUCTION

Specific attachment by bacterial surface lectins to carbohydrate structures on eukaryotic cell surfaces is now a subject of great interest[1]. Two Swedish groups[2] have suggested that glycolipids (related to the human P blood-group system[3]), located on the surface of uroepithelial cells, act as receptors for the adhesion of uropathogenic *E. coli* bacteria. 4-*O*-α-D-Galactopyranosyl-D-galactose (**1**) is a component of these glycolipids as an α-D-Gal*p*-(1→4)-β-D-Gal group, and simple glycosides of **1** are efficient and specific inhibitors of the agglutination of uropathogenic *E. coli* bacteria with certain red blood cells[4].



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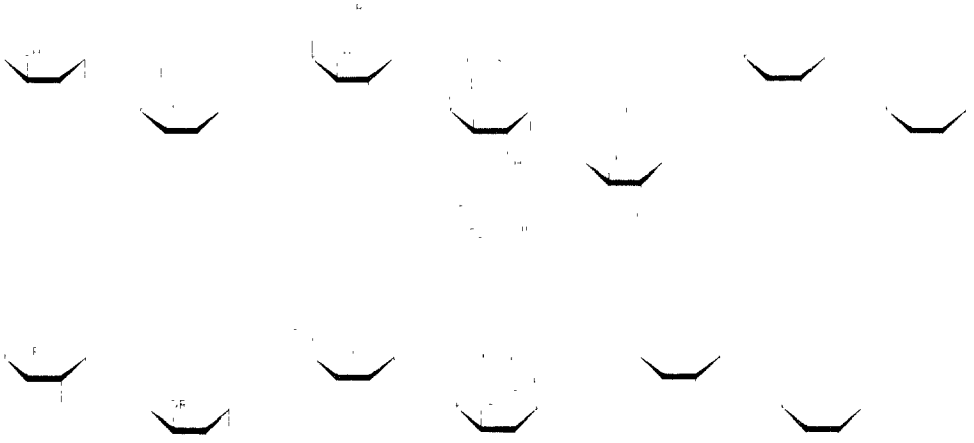
Compound **1** has been prepared from the "di-D-galacturonic acid" **2** by a protection–reduction sequence (diazomethane lithium aluminium hydride)[5] and by multi-step syntheses starting with D-galactose[6]. However, the obnoxious reagents used and/or the low overall yield in these reactions prohibit large-scale synthesis of **1**. We now report a simple and high-yielding route to **1** from pectin, which is an inexpensive starting-material. Enzymic (esterase) hydrolysis of pectin gives poly-α-D-galactopyranuronic acid (commercially available), which in turn can be degraded enzymically to give a mixture mainly of di- and tri-saccharides[7]. The transformation of these compounds (**2** and **3**) into **1** is now reported.

DISCUSSION

Simultaneous esterification and glycosidation (*cf.* ref. 8) of **2** with methanol gave **4**, which was reduced with borohydride to give the methyl α-glycoside **5**. Treatment of **5** with acetic anhydride–pyridine gave the hepta-acetate **6**. With acetic anhydride–sulfuric acid, **5** and **6** were converted into a mixture of the octa-acetates (**7** and **8**) of α- and β-**1**. The α anomer **7** was the preponderant product and was isolated pure by crystallisation. The reaction sequence **2**→**7** is essentially a one-pot reaction, in that that no purification of intermediates is necessary. The overall yield of crystalline **7** was 33%. Chromatography of the material in the mother liquor, followed by crystallisation, raised the yield of **1** to 55%. Deacetylation of **7** or **8** then gave **1**.

The reducing-terminal sugar residue of the "tri-galacturonic acid" **3** was used as the glycoside protecting-group in a variation of the above reaction sequence. Esterification of **3** with ethylene oxide in water afforded the tri-ester **9**, which was reduced with borohydride to give the galactitol derivative **10**. Treatment with acetic anhydride



pyridine converted **10** into the dodeca-acetate **11**, whereas treatment with acetic anhydride–sulfuric acid selectively cleaved the galactitol residue and gave a mixture of **7** and **8** together with galactitol hexa-acetate.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded with a Varian XL 200 spectrometer, with Me_4Si or sodium 3-(trimethylsilyl)propionate-d_4 (TSP) as internal standards.

Hydrolysis of "polygalacturonic acid"[7]. — A suspension of "poly-D-galacturonic acid" (50 g; Sigma) in 20mM sodium acetate buffer (1 L, pH 4.5, adjusted with 5M sodium hydroxide) was stirred at 40° and pectinase (1 g, PV 8, Miles Laboratories) was added. The hydrolysis was continued for 24 h at 40° and then terminated by heating at 100° for 5 min. The hydrolysis was monitored by t.l.c. on Silica gel 60 (Merck) with 1-butanol–formic acid–water (4:6:1) and detection by charring with sulfuric acid. The major part of the hydrolysate consisted of di-, tri-, and tetra-mers.

The hydrolysate was passed through a column of Zerolite 225 (H^+) resin (600 mL of resin/L of hydrolysate) and the eluate was lyophilised. A solution of the resulting mixture of oligomers (6 g) in distilled water (20 mL) was applied to a column (60 × 1.6 cm) of Dowex 1 X2 ($HCOO^-$) resin (50–100 mesh) equilibrated with distilled water, washed with 0.2M formic acid (350 mL), and then eluted with a concave gradient of 0.2M formic acid (2 L) to 0.65M (1.2 L), to give fractions containing "di- and tri-galacturonic acid". "Tetra-galacturonic acid" was eluted with M formic acid. Lyophilisation of the appropriate fractions gave **2** and **3** with >90% purity.

	Yield[a] *(g, %)*	*Volume of eluant (mL)*
	0.2 (3)	200
2	1.0 (17)	500–1250
3	2.0 (34)	1650–3750

[a]From 6 g of "poly-D-galacturonic acid".

*1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-* (**7**) *and -β-D-galactopyranose* (**8**). — (*a*) "Di-D-galacturonic acid" (**2**, 20.0 g) was added to a solution of calcium chloride (20 g) in methanol (1.0 L) (the calcium chloride, although not essential, increased the yield of **5**). Duolite (H^+) resin (15 g) was added, and the mixture was boiled under reflux for 18 h, filtered, and concentrated, to give crude **4**, a solution of which in water (200 mL) was treated with a solution of sodium borohydride (6 g) in water (30 mL) during 3 min with occasional stirring. The mixture was kept at ambient temperature for 20 h, acidified [Dowex 50 W-X(2)(H^+) resin], filtered, and co-concentrated with methanol several times. The dried (0.1 Torr)

residue (crude **5**) was stirred with acetic anhydride and pyridine (1:1, 600 mL) at ambient temperature for 24 h. The mixture was concentrated with toluene several times to remove pyridine, and the residue was partitioned between ether (0.5 L) and water (0.2 L). The aqueous phase was extracted with ether (2 × 100 mL) and the combined ether phases were washed with water (50 mL), dried (Na_2SO_4), and co-concentrated with toluene. The pale-yellow residue (31.5 g, mainly **6**) was dried at 0.1 Torr and then stirred with acetic anhydride (320 mL) containing 1% of conc. sulfuric acid at ambient temperature. The reaction was monitored by t.l.c. (ether–toluene, 3:1). After 50 min, the mixture was poured into ether (1 L), washed with ice–water (150 mL), saturated aqueous sodium hydrogencarbonate (150 mL), and ice–water (150 mL), dried (Na_2SO_4), and co-concentrated with toluene. The dried (0.1 Torr) residue (32 g) was crystallised from ethanol, to afford **7** (12.3 g, 33% from **2**), m.p. 151–154°, $[\alpha]_D^{21}$ +135° (*c* 0.5, chloroform); lit.[6b] m.p. 153–154°, $[\alpha]_D^{24}$ +138° (*c* 2, chloroform). Chromatography of the mother liquor, followed by crystallisation, raised the yield to 55%.

(*b*) To a solution of trisaccharide **3** (1.65 g) in water (16.5 mL) was added liquid ethylene oxide (~2 g). The mixture was stirred at room temperature and more ethylene oxide (2–6 g) was added daily. The reaction was monitored by the rise in pH and by t.l.c. (ethyl acetate–acetic acid–water, 2:1:1).

After 6 days, when the pH was 7, the ethylene oxide was removed and the reaction mixture was added dropwise during 5 min to a solution of sodium borohydride (2 g) in water (2 mL) at 20–25°. The mixture was stirred for 12 h at room temperature, acidified (pH 5.5) with acetic acid, filtered, and co-concentrated (40°) several times with methanol. Toluene was evaporated several times from the residue which then was dried in a high vacuum. A solution of the residue (2.3 g) in water (45 mL) was passed through a column (6 × 55 cm) of Sephadex G-15. The fractionation was monitored by t.l.c. (ethyl acetate–acetic acid–water, 2:1:1). Fractions containing **10** (R_t 0.2) were combined and concentrated, to give an amorphous residue (1.5 g) which was stirred with acetic anhydride (50 mL) containing 1% of conc. sulfuric acid at 55°. The reaction was monitored by t.l.c.; after 8 days, the mixture was cooled, diluted with ether, washed with water, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated with several additions of ethanol. The residue was eluted from a column (4 × 60 cm) of silica gel with ethyl acetate–iso-octane (3:1). Appropriate fractions of reasonable homogeneity were combined and concentrated to give **7** (700 mg, 35%) containing a small proportion of **8**. Recrystallisation from ethanol gave pure **7** (425 mg, 21% from **3**).

Characterisation of the intermediate compounds **4**–**6** *and* **11**. — (*a*) *Methyl [methyl 4-O-(methyl α-D-galactopyranosyluronate)-D-galactopyranosid]uronate*[8] (**4**). — Gel filtration (Sephadex G-15, water) of crude **4** [obtained in method (*a*) above] gave a mixture of α-**4** and β-**4** with an αβ-ratio of 7:3 (as determined by n.m.r. spectroscopy). N.m.r. data: ^1H (Me_2SO-d_6 + D_2O, 50°, Me_4Si), δ (*inter alia*) 4.96 (d, *J* 1.5 Hz, H-5), 4.93* (d, *J* 1.5 Hz, H-5), 4.72 (d, *J* 3 Hz, H-1′), 4.69 (d, *J* 4 Hz, H-1), 4.32 (d, *J* 1 Hz, H-5′), 4.29* (d, *J* 1 Hz, H-5′), 4.18 (dd, *J* 3.0 and 1 Hz, H-4′),

4.10* (d, *J* 7.5 Hz, H-1), 3.92 (dd, *J* 3.0 and 1.5 Hz, H-4), 3.69 (s, COOMe), 3.63 (s, COOMe), 3.42* (s, MeO), and 3.29 (s, MeO in α-**4**) (* indicates signals from β-**4**); ^{13}C (D_2O, TSP), δ 174.28, 173.36, 173.0*, 103.38, 103.29*, 102.52, 81.83, 81.04*, 74.17, 72.97*, 72.84, 72.75, 71.46, 71.40*, 71.39*, 70.81, 70.57, 70.45, 60.38*, 58.59, 55.85*, 55.78, and 55.65.

Compound **4** (150 mg) was stirred at room temperature for 2 h with acetic anhydride containing 1% of conc. sulfuric acid and then worked-up as described in (*a*) above for **7**, to give methyl [methyl 2,3-di-*O*-acetyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate)-α-D-galactopyranosid]uronate containing ~15% of the β isomer as a colourless, amorphous solid (200 mg, 88%). ^{13}C-N.m.r. data ($CDCl_3$, Me_4Si): δ 171.10, 170.0, 169.63 (2 C), 169.44, 167.63, 167.16, 97.32 (d, *J* 178 Hz), 97.12 (d, *J* 178 Hz), 76.42 (C-4), 69.17 (2 C), 68.97, 68.09 (2 C), 66.72 (2 C), 56.16 (OMe), 52.57 (COO*Me*), 52.40 (COO*Me*), 20.99, 20.74, 20.58, 20.50, and 20.25.

(*b*) *Methyl 4*-O-α-D-*galactopyranosyl*-α-D-*galactopyranoside* (**5**). A solution of **6** (2.5 g) [obtained as in method (*a*) above] in tetrahydrofuran (10 mL) and methanol (20 mL) was stirred with 0.1M methanolic sodium methoxide (3 mL) for 24 h. The product (needles) was collected, washed with methanol, and dried (0.1 Torr), to give **5** (1.08 g, 79%), m.p. 210–211°, $[\alpha]_D^{22}$ +228° (*c* 0.5, water). N.m.r. data: ^{1}H (Me_2SO-d_6 + D_2O, 50°, Me_4Si), δ (*inter alia*) 4.82 (d, 1 H, *J* 3.5 Hz, H-1′), 4.59 (d, 1 H, *J* 2.5 Hz, H-1), 3.27 (s, 3 H, MeO); ^{13}C (D_2O, TSP), δ 103.33 (d, *J* 170 Hz, C-1), 102.33 (d, *J* 170 Hz, C-1′), 81.73, 73.94, 73.85, 72.07, 72.00, 71.85, 71.47, 71.26, 63.47 (t, 2 C, *J* 144 Hz, C-6,6′), and 58.05 (q, *J* 144 Hz, MeO).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79. Found: C, 43.72; H, 6.78.

(*c*) *Methyl 2,3,6-tri*-O-*acetyl-4*-O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*galactopyranosyl*)-α-D-*galactopyranoside* (**6**). Crystallisation of crude **6** [obtained in method (*a*) above] from methanol gave needles, m.p. 176–178.5°, $[\alpha]_D^{22}$ +160° (*c* 0.9, chloroform). N.m.r. data: ^{1}H ($CDCl_3$, Me_4Si), δ 5.56 (bd, 1 H, *J* 3 Hz, H-4′), 5.39 (dd, 1 H, *J* 11 and 3 Hz, H-3′), 5.30–5.19 (3 H, H-2,2′,3), 5.00 (bs, 2 H, H-1,1′), 4.54 (bt, 1 H, *J* 7 Hz), 4.36 (dd, 1 H, *J* 13 and 9 Hz), 4.24–4.04 (5 H), 3.42 (s, 3 H, MeO), 2.14 (s, 6 H), 2.10, 2.08, 2.07, 2.04, and 2.00 (5 s, each 3 H); ^{13}C ($CDCl_3$, Me_4Si), δ 170.54, 170.50, 170.42, 170.30, 170.17, 169.99, 169.40, 99.03 (d, *J* 172 Hz, C-1′), 97.19 (d, *J* 175 Hz, C-1), 77.69, 69.44, 68.28, 67.80 (3 C), 67.40, 66.97, 62.37 (CH_2), 60.64 (CH_2), 55.39 (MeO), 20.96, 20.80, 20.78, 20.77, 20.73, and 20.66 (2 C).

Anal. Calc. for $C_{27}H_{38}O_{18}$: C, 49.85; H, 5.89. Found: C, 49.83; H, 5.86.

(*d*) α-D-*Gal*p-(1→4)-α-D-Gal*p*-(1→3)-*galactitol dodeca-acetate* (**11**). Compound **10**, obtained in method (*b*) above, was treated with acetic anhydride containing 1% of conc. sulfuric acid for 3 h. Work-up as above, followed by chromatography on silica gel with ethyl acetate–iso-octane (3:1), gave **11** as a colourless, amorphous solid, $[\alpha]_D^{22}$ +93° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$, Me_4Si): ^{1}H, δ 5.58 (dd, 1 H, *J* 3.0 and 1 Hz, H-4″), 5.54–5.10 (8 H), 5.01 (d, 1 H, *J* 3.5 Hz, H-1″), 4.60–4.00 (12 H), 2.14, 2.12, 2.11, 2.105, 2.09, 2.09, 2.085, 2.08, 2.07, 2.06, 2.03, and 1.99 (12 s, each 3 H, 12 AcO); ^{13}C, δ 170.54, 170.43 (3 C), 170.30 (2 C), 170.18, 170.08, 169.97,

169.93, 169.85, 169.73, 99.23 (d, *J* 172 Hz), 97.79 (d, *J* 174 Hz), 77.56, 75.92, 70.59, 70.44, 69.06, 69.01, 68.94, 68.26, 67.78, 67.43, 67.37, 67.27, 67.01, 62.10, 61.79, 60.51, 20.99, 20.83, 20.72 (2 C), 20.709 (2 C), 20.706 (2 C), 20.67 (3 C), and 20.56.

4-O-α-D-Galactopyranosyl-D-galactose (**1**). — A solution of **7** (5 g) in methanol (100 mL) was treated with 0.1M methanolic sodium methoxide for 4 days and then diluted with water, and the methanol was evaporated. The aqueous solution was neutralised with Dowex 50W-X2 (H^+) resin, treated with charcoal, filtered, and concentrated. Crystallisation of the residue from methanol–water gave **1** (2 g, 78%), m.p. 211–213°, $[\alpha]_D^{26}$ +171° (*c* 1, water); lit.[6a] m.p. 210–211°, $[\alpha]_D^{26}$ +177°.

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