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## Note

# A new synthesis of 3-*O*-β-D-galactopyranosyl-D-galactose

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3-*O*-β-D-Galactopyranosyl-D-galactose (**5**) is a component of the specific carbohydrate–protein linkage region present in several connective tissue proteoglycans[1]. This disaccharide has also been isolated from a variety of other sources[2], and a particularly suitable starting material for large-scale preparation is larch D-arabino-D-galactan, from which **5** may be obtained by graded acid hydrolysis and chromatography on charcoal[3,4]. Since this procedure requires a search for the proper species of larch tree (*e.g., Larix occidentalis*) and subsequent isolation of the polysaccharide, the time and effort involved may be considerable, and this has prompted us to develop a more rapid chemical synthesis. After a simple method for the preparation of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**2**) became available[5], a convenient procedure based on the condensation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in a Koenigs–Knorr reaction could be designed. Although the chemical synthesis of **5** has been previously described by Ball and Jones[6] and, more recently, by Chacon-Fuertes and Martin-Lomas[7], the procedures used by these authors are more time-consuming and give lower yields than the method reported here. Di-*O*-isopropylidene derivatives have been employed earlier in similar syntheses. Bächli and Percival[8] obtained laminarabiose in 9% yield from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and, recently, Lemieux *et al.*[9] used **2** in a halide-ion-catalyzed, glycosidation reaction.

1,2:5,6-Di-*O*-isopropylidene-α-D-galactofuranose (**2**) was synthesized as described[5] with the slight modification that the final crystallizations were performed in different solvents. The physical properties and $^1$H-n.m.r. spectrum were identical to those reported[10,11]. Compound **2** was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) under conditions similar to those reported for the D-glucose series[8], with the exception that the reaction was carried out at a higher temperature (40–45°), which decreased the reaction time and gave comparable yields of 1,2:5,6-di-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-

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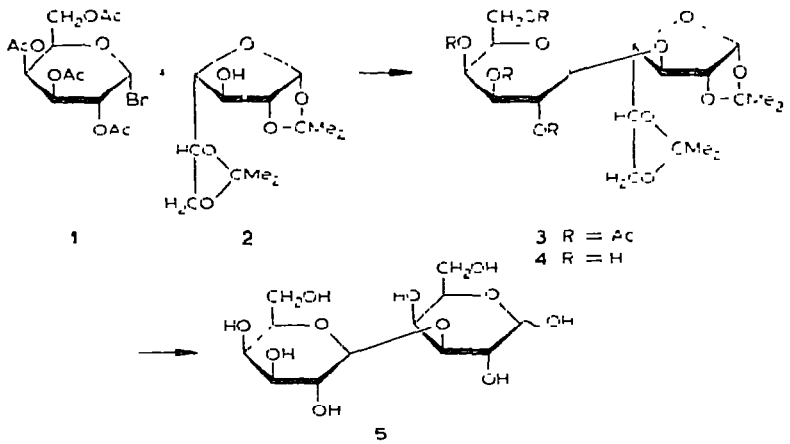
pyranosyl)-α-D-galactofuranose (**3**). The course of the reaction was followed by t.l.c. which showed that **3** ($R_1$ 0.84) was present after only a few minutes. In addition, there was simultaneous formation of two by-products. Compound **3** was not purified on a preparative scale at this stage, since an initial attempt at separation by column chromatography failed to resolve the blocked disaccharide from the faster-moving by-product. The entire mixture was therefore subjected to *O*-deacetylation with sodium methoxide which yielded 3-*O*-β-D-galactopyranosyl-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**4**) as the major product. Preferential extraction with acetone, followed by treatment with ether, gave pure **4** having physical properties, composition, and $^{13}$C-n.m.r. spectrum consistent with the presumed structure.




3-*O*-β-D-Galactopyranosyl-D-galactose (**5**) was obtained by hydrolysis of the isopropylidene groups. Since partial rupture of the glycosidic linkage has been reported[12], two different hydrolytic procedures were tested. When **4** was treated with 0.5M sulfuric acid, a considerable amount of D-galactose was formed. Hydrolysis with a cation-exchange resin ($H^+$) at 45° gave the best results, yielding 3-*O*-β-D-galactopyranosyl-D-galactose (**5**) as the major product, which was subsequently purified by crystallization from anhydrous methanol. The properties of **5** were identical with those of an authentic sample isolated from larch arabinogalactan[13]. In addition, the chemical shift at δ 104.7 of the anomeric C-1 atom in the $^{13}$C-n.m.r. spectrum corroborated the β-D configuration, since chemical shifts for the non-reducing anomeric carbons of 3-*O*-(α-D-galactopyranosyl)-α-D-galactopyranose and 3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranose, as reported by Lemieux *et al.*[14], are 95.5 and 96.7 p.p.m., respectively.

In an attempt to improve the yield of the partially blocked disaccharide **4**, **1** and **2** were condensed in nitromethane in the presence of mercuric cyanide, but the reaction pattern was complex and, although the yield after deacetylation *a priori* seemed higher, the isolated, chromatographically-pure product was in fact con-

taminated with a compound having a different structure; the possibility of formation of the α-D-linked disaccharide was not investigated, since the aim of the synthesis was to prepare 3-*O*-β-D-galactopyranosyl-D-galactose. Hydrolysis gave, in addition to the expected disaccharide **5**, at least three additional compounds, and it was necessary to resort to paper chromatography in order to isolate pure 3-*O*-β-D-galactopyranosyl-D-galactose in 16% yield.

The $^{13}$C-n.m.r. signals of **2** were assigned as follows: The two low-field signals of relatively small intensity at δ 113.3 and 109.8 were readily assigned to the carbon atoms of the isopropylidene groups bearing no hydrogen atoms; the signal at δ 106.0 was attributed to the anomeric carbon atom C-1′; and the high-field signal at δ 66.4 was assigned to C-6′, the only carbon atom bearing two hydrogen atoms. The remaining signals at δ 88.7 and 87.7 were attributed to C-2′ and C-4′, respectively, and the signal at δ 76.8 to C-3′ and C-5′ by comparison with compounds having similar structures[15,16].

The assignments of the $^{13}$C-n.m.r. signals of **4** were deduced from the signals of the corresponding monosaccharide **2** and of D-galactose[17]. At low field, the low-intensity signals at δ 113.7 and 109.9 were readily assigned to the carbon atoms of the isopropylidene groups bearing no hydrogen atoms. The signals at δ 106.0 and 104.1 were attributed to C-1′ and C-1, respectively. Substitution at C-3′ of the furanose ring had no effect on the chemical shift of C-1′, whereas substitution at C-1 of the pyranose ring causes a downfield shift of 6.6 p.p.m. relative to the corresponding signal of D-galactose[17]. The chemical shift of the anomeric carbon atom C-1 is also consistent with the expected β-D configuration of the glycosidic linkage, since in the α-D series, C-1 would resonate at much higher field[15]. The signals at δ 86.8 and 85.9 were assigned to C-2′ and C-4′, respectively, of the furanose ring. Both signals were shifted upfield by 1.9 and 1.8 p.p.m., respectively, after substitution at C-3′. The carbon atom C-3′ involved in the glycosidic linkage showed a signal at δ 83.3, a downfield shift of 6.5 p.p.m. from the signal of the corresponding carbon atom in the monosaccharide **2**. The two signals at δ 76.9 and 66.4 were assigned to C-5′ and C-6′, respectively, of the furanose ring, and by comparison with the signals of D-galactose[17]. The other signals at δ 76.4, 74.6, 72.1, 69.8, and 62.3 were assigned to C-5, C-3, C-2, C-4, and C-6, respectively, of the pyranose ring.

## EXPERIMENTAL

*General methods.* — P.m.r. spectra were recorded with a Brucker HX-90 spectrometer operating in the frequency-sweep mode at 28°, with $Me_4Si$ as internal standard. $^{13}$C-n.m.r. spectra were measured at 35° on the same spectrometer operating at 22.6 MHz in the pulsed, Fourier-transform mode with proton-noise decoupling (Nicolet 1085 computer; $^2$H lock). Typical spectrometer parameters were: 20° flip angle, 4000-Hz spectral width, 4 K data points, and 1-sec recovery time. Chemical shifts are reported in p.p.m. downfield from $Me_4Si$. For spectra recorded in $D_2O$, internal acetone was used as a secondary, chemical-shift standard (chemical shift,

$\delta$ 30.6). The carbohydrates (80 mg) were dissolved in $D_2O$ (1 ml) in a 10-mm n.m.r tube fitted with a Teflon vortex plug. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter at 23°. Silica gel microslides (Quanta/Gram MQ1F) and preparative t.l.c. plates (PQ4F) (Quantum Industries, Fairfield, New Jersey) were irrigated with (*A*) 9:1 benzene–methanol, (*B*) 19:1 benzene–methanol, (*C*) 4:5:1 butanol–acetone–water, and (*D*) 4:5:3 butanol–acetone–water; the products were detected by spraying with 50% $H_2SO_4$, followed by heating at 130°. The solvent mixtures used for paper chromatography were: (*E*) 3:1:1 ethyl acetate–acetic acid–water, and (*F*) 10:4:3 ethyl acetate–pyridine–water; the products were detected with the silver nitrate–sodium hydroxide reagent[18]. All solvent concentrations are v/v. Concentration of solvents was performed *in vacuo* at 38°, unless otherwise stated. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee, 37921.

*1,2:5,6-Di-O-isopropylidene-α-D-galactofuranose* (**2**) — 1,2:5,6-Di-*O*-isopropylidene-α-D-galactofuranose was synthesized according to Morgenlie[5], except that the final sirupy residue was extracted four times with petroleum ether (30–60°) and the yellowish material crystallizing in the extract was recrystallized from cyclohexane to give pure **2**. The insoluble residue was dissolved in cyclohexane, whereupon the remainder of **2** crystallized (combined yield, 4.06 g, 20%), m.p. 96–97°, $[\alpha]_D^{23}$ −33° (*c* 1.0 methanol); p.m.r. (acetone-$d_6$): $\tau$ 4.16 (d, $J_{1,2}$ 4 Hz, H-1), 5.48 (d of d, $J_{2,3}$ 1.3 Hz, H-2), 5.69 (q, H-5), 5.93 (m, H-6 and H-3), 6.22 (m, H-4 and H-6'), 8.50 and 8.62 (2 s, 6 H, $CMe_2$), and 8.68 (1 s, 6 H, $CMe_2$); $^{13}$C-n.m.r. [$(CD_3)_2CO$]: see Discussion.

*Anal.* Calc. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.50; H, 7.90.

*3-O-β-D-Galactopyranosyl-1,2:5,6-di-O-isopropylidene-α-D-galactofuranose* (**4**). — A solution of **2** (1.0 g) in benzene (20 ml) containing Drierite (8 g) was stirred at 40–45°. After 4 h, $Ag_2CO_3$ (4 g) and $I_2$ (0.7 g) were added, followed over a period of 1 h by the addition of a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) (3 g) in benzene (15 ml). The progress of the reaction was followed by t.l.c. in solvent *B*. In addition to the desired product ($R_1$ 0.84), two by-products were formed ($R_1$ 0.92 and 0.42). After complete reaction of **1**, additional **1** (3 g) in benzene (12 ml) and $Ag_2CO_3$ (4 g) were added, and the reaction was continued until complete disappearance of **1**. The reaction mixture was filtered over Celite, and the insoluble residue was washed with benzene. The combined filtrate and washings (60 ml) were extracted successively twice with water (50 ml), once with a solution of $Na_2S_2O_3$ (50 ml), and once with water (50 ml). Following evaporation, the glassy residue (2.83 g) was dissolved in 0.1M methanolic sodium methoxide (18 ml) and, after 0.5 h, the solution was diluted with an equal volume of methanol and then neutralized with AG 50W ion-exchange resin. Evaporation gave a glassy material (1.82 g), composed of one major product, **4** ($R_F$ 0.70, solvent *C*), two slow-moving compounds (one having the $R_F$ value of D-galactose), and one very fast-moving compound. This mixture was treated with dry acetone (18 ml), the solid product was filtered off, and the filtrate was evaporated *in vacuo*. The fast-moving compound was removed from

the sirupy residue (1.2 g) by trituration with cold ether (18 ml) to give chromatographically pure **4** (0.90 g, 30%), $[\alpha]_D^{23} - 10°$ (*c* 1.0 methanol); $^{13}$C-n.m.r. [$(CD_3)_2CO$]: see Discussion.

*Anal.* Calc. for $C_{18}H_{30}O_{11} \cdot H_2O$: C, 49.08; H, 7.32. Found: C, 49.39; H, 7.29.

*3-O-β-D-Galactopyranosyl-D-galactose* (**5**). — (*a*). *From* **4**. To a solution of **4** (0.20 g) in water (12 ml), was added AG 50W ($H^+$, 3 ml) cation-exchange resin. The reaction mixture was maintained at 45° without stirring for 18 h. The resin was removed by filtration and washed with water. The combined filtrate and washings were evaporated *in vacuo* at 20° to a volume of about 2 ml. The solution was diluted with methanol (10 ml) and evaporated again *in vacuo* to about 2 ml. This procedure was repeated twice before a final evaporation to dryness. The sirypy residue showing on t.l.c. one major product ($R_{Gal}$ 0.7, solvent *D*), and two minor products ($R_{Gal}$ 0.4 and 1.0) was dried *in vacuo* over $P_2O_5$ and dissolved in anhydrous methanol (1 ml). A crystalline material slowly separated which, after several hours, was filtered off, washed with small amounts of methanol, and dried to yield 60 mg of chromatographically pure **5**. A second quantity of **5** (42 mg) was obtained after evaporation of the combined mother liquor and washings and subsequent addition of a small amount of anhydrous methanol (total yield 0.102 g, 66%), m.p. 201–204°, $[\alpha]_D^{23}$ +64° (*c* 0.73 water); $^{13}$C-n.m.r. ($D_2O$): δ 104.7 (C-1), 96.7 (C-1'e), 92.6 (C-1'a); indistinguishable from an authentic sample on t.l.c. in solvent *D* and chromatography on Whatman No. 1 paper in solvents *E* and *F*; lit.[13]: m.p. 200–203°, $[\alpha]_D$ +60° to +65°.

*Anal.* Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.47. Found: C, 41.86; H, 6.56.

(*b*). *From* **2**. — A stirred solution of **2** (1.04 g) in 1:1 nitromethane–benzene (80 ml) was evaporated until approximately 40 ml of the solvent mixture had distilled, and the reaction mixture was cooled to room temperature. $Hg(CN)_2$ (1.02 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) (1.65 g) were added to the stirred mixture. Further additions of **2** (0.85 g) and $Hg(CN)_2$ (0.50 g) were made after 18 h, and of **1** (0.50 g) 6 h later, and stirring was continued for another 18 h. The solution was diluted with benzene, washed successively, once with a saturated solution of $NaHCO_3$ (70 ml) and twice with water (70 ml), dried ($Na_2SO_4$), and evaporated *in vacuo*. The glassy material (3.48 g) was dissolved in 0.1M methanolic sodium methoxide (35 ml) and, after complete deacetylation, the solution was diluted with an equal volume of methanol and neutralized with AG 50W ($H^+$) cation-exchange resin. The resin was removed by filtration, washed with methanol, and the filtrate and washings were evaporated *in vacuo*. As shown by t.l.c. in solvent *C*, the glassy residue (2.02 g) was composed of one major compound ($R_F$ 0.70) and four by-products, one having the same chromatographic mobility as D-galactose. The residue was triturated with dry acetone (40 ml), the solid material was filtered off, and the solution was evaporated *in vacuo*. The yellowish, sirupy residue (1.5 g) was triturated with cold ether, and the amorphous material filtered off and dried (0.86 g). The product ($R_F$ 0.70, solvent *C*) was slightly contaminated with a compound having a lower mobility. Part of the crude amorphous compound (0.14 g) was hydrolyzed with a resin, as described earlier, to yield a sirupy material (0.10 g) containing **5** in

addition to at least three other products. Preparative paper chromatography of this material on Whatman No. 3MM paper in solvent *E*, followed by elution with water, evaporation to dryness, and subsequent addition of anhydrous methanol, gave **5** (18 mg, 16%).

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