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SYNTHESIS AND CHROMATOGRAPHIC PROPERTIES OF ISOMERIC *O*-β-D-GALACTOPYRANOSYL-D-GALACTOSES, AND OF DIASTEREOISOMERS OF 3,4-*O*- AND 4,6-*O*-(1-CARBOXYETHYLIDENE)-D-GALACTOSE

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ABSTRACT

Partial hydrolysis of β-D-galactopyranans and D-galactose-containing polysaccharides having pyruvic acetal groups gives isomeric *O*-β-D-galactopyranosyl-D-galactoses and *O*-(1-carboxyethylidene)-D-galactoses, respectively. Their chromatographic properties in various systems were investigated as an aid in identification. The best method was a combination of silica gel t.l.c., which separates the 3,4- and 4,6-*O*-(1-carboxyethylidene) derivatives, and paper electrophoresis in borate buffer, which resolves the 2-*O*-, 3-*O*-, 4-*O*-, and 6-*O*-linked galactobioses after removal of the *O*-(1-carboxyethylidene) derivatives. 2-*O*- and 4-*O*-β-D-Galactopyranosyl-D-galactoses were synthesized, as well as the 4 diastereoisomeric 3,4- and 4,6-*O*-(1-carboxyethylidene)-D-galactoses.

INTRODUCTION

Structural studies on β-D-galactopyranans and D-galactose-containing polysaccharides are facilitated by identification of the disaccharides formed on partial hydrolysis. Such investigations are often preceded by chromatographic examination which can give an indication of the chemical structure, and, thus, the chromatographic properties of 2-, 3-, 4-, and 6-*O*-β-D-galactopyranosyl-D-galactose were determined. The O-3 and -6 isomers were readily available, but it was necessary to synthesize 2-*O*-β-D-galactopyranosyl-D-galactose, and also the O-4 isomer, which was not available in our laboratory.

RESULTS AND DISCUSSION

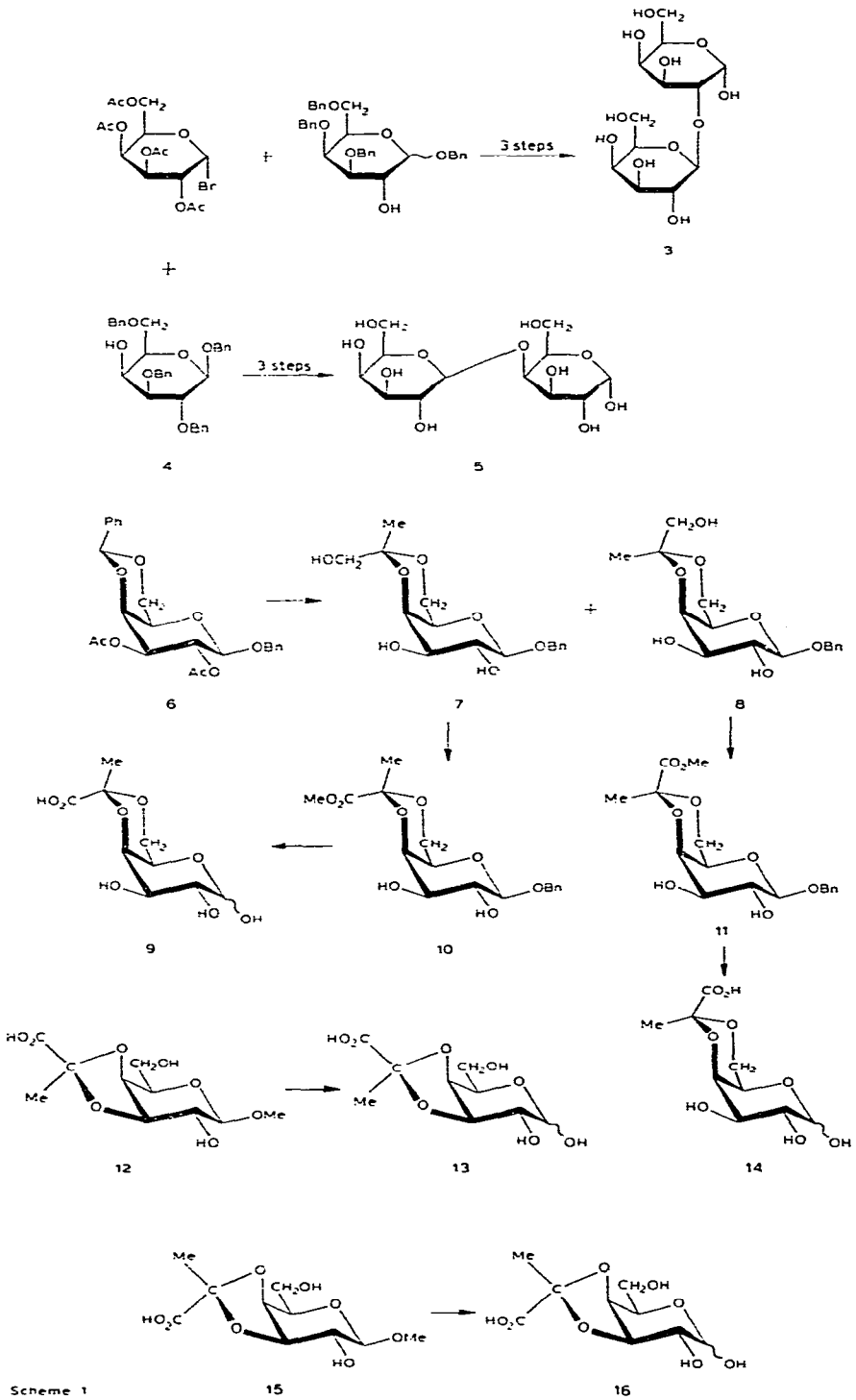
Benzyl 3,4,6-tri-*O*-benzyl-α,β-D-galactopyranoside (**2**) was prepared by treatment of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-galactopyranose[1] with benzyl alcohol containing *p*-toluenesulfonic acid. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) was condensed with benzyl 3,4,6-tri-*O*-benzyl-α,β-D-galactopyranoside (**2**) in dichloromethane solution, in the presence of silver carbonate and a 3 Å molecular sieve. The reaction mixture was successively deacetylated and debenzylated, and the resulting 2-*O*-β-D-galactopyranosyl-D-galactose purified by

cellulose column-chromatography and crystallized as the α-D anomer (**3**). Another condensation was carried out between **1** and benzyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**), prepared by selective mono-*O*-benzylation of benzyl 2,3-di-*O*-benzyl-β-D-galactopyranoside[2]. This yielded, after an identical series of steps, 4-*O*-β-D-galactopyranosyl-D-galactose (**5**).

Other partial-hydrolysis products have been formed from D-galactose-containing polysaccharides, in addition to D-galactobioses, 3,4-*O*- and 4,6-*O*-(1-carboxyethylidene)-D-galactose being obtained from polysaccharides containing the *O*-carboxyethylidene residue[3-7]. Of the two (*R*) and (*S*) 4,6-*O* isomers **9** and **14**, the diastereo isomer **9** was available as a partial-hydrolysis product of the polysaccharides of *Corynebacterium insidiosum*[7,8] and agar[8]. The other isomer **14**, along with **9**, was synthesized as follows: Benzyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**6**), obtained by acetylation of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside[2], was treated with acetoxyacetone containing a trace of sulfuric acid, to replace the 4,6-*O*-benzylidene with a 4,6-*O*-acetoxyisopropylidene group. On deacetylation of the product, a diastereoisomeric mixture of benzyl 4,6-*O*-hydroxyisopropylidene-β-D-galactopyranosides (**7** and **8**) was formed. This was fractionated on a column of silicic acid to give syrupy **8**, followed by the crystalline isomer **7**. The structures were assigned on the basis of the ^{1}H-chemical shifts of the Me acetal groups[9]. The isomers **7** and **8** were each successively oxidized with oxygen in the presence of platinum and the resulting *O*-(1-carboxyethylidene) derivatives treated with diazomethane to give the *O*-(1-methoxycarbonylethylidene) derivatives **10** and **11**, respectively. Alkaline hydrolysis with cold, aqueous barium hydroxide was followed by conversion of the barium salts into the free acids. These were debenzylated by reduction in the presence of palladium to give the 4,6-*O*-(1-carboxyethylidene)-D-galactoses **9** and **14**.

The 3,4-*O*-(1-carboxyethylidene)-D-galactoses **13** and **16** were prepared, in quantities sufficient for chromatographic examination, by partial acid-hydrolysis of the methyl 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranosides **12** and **15**, respectively[10,11].

Chromatography of the galactobioses in the presence of borate buffer seemed a promising approach, since Lindberg and Swan[12] separated the 3-*O*, 4-*O*, and 6-*O* isomers of *O*-β-D-galactopyranosyl-D-galactose, which had mobilities relative to glucose (M_{Glc}) of 0.69, 0.50, and 0.83, respectively. As the authors were also able to separate the 2-*O*-, 3-*O*-, 4-*O*-, and 6-*O*-methyl derivatives of D-galactose, it was expected that 2-*O*-β-D-galactopyranosyl-D-galactose would be distinguishable from the other three isomers on paper electrophoretograms. Accordingly, in an aqueous solution of 50mM sodium tetraborate adjusted to pH 10.0, the galactobioses, the 4,6-*O*-(1-carboxyethylidene)-D-galactoses **9** and **14**, and the 3,4-*O*-(1-carboxyethylidene)-D-galactoses **13** and **16** could easily be separated from each other (see Table I). Using pyridine–acetic acid–water, which buffered the solution at pH 5.3, Sömme[13] found that the mobilities of the 3,4- (**13**) and 4,6-isomer (**9**) were almost identical. A better separation of these compounds was obtained by use of 2% aqueous ammo-



Scheme 1

TABLE I

CHROMATOGRAPHIC MOBILITIES OF 2-*O*-, 3-*O*-, 4-*O*-, AND 6-*O*-β-D-GALACTOPYRANOSYL-D-GALACTOSE AND DIASTEREOISOMERS OF 3,4-*O*- AND 4,6-*O*-(1-CARBOXYETHYLIDENE)-D-GALACTOSE, RELATIVE TO THOSE OF D-GALACTOSE

Compound	*Chromatographic method*		
	T.l.c. on silica gel G-60[a]	*Paper chromatography*	*Paper electrophoresis*[b]
O-β-D-Galactopyranosyl-D-galactose			
2-*O*- (**3**)	0.40	0.37	0.54
3-*O*- (**5**)	0.40	0.33	0.73
4-*O*-	0.47	0.41	0.49
6-*O*-	0.30	0.24	0.86
O-(1-Carboxyethylidene)-D-galactose			
(*R*)-3,4-*O*- (**13**)	1.21	1.94	1.21
(*S*)-3,4-*O*- (**16**)	1.36	1.90	1.24
(*R*)-4,6-*O*- (**9**)	1.08	1.52	0.88
(*S*)-4,6-*O*- (**14**)	1.11	1.54	0.84

[a]In 12:3:1:4 (v/v) acetate–acetic acid–formic acid–water. [b]In 50mM sodium tetraborate adjusted to pH 10.0. Under these conditions, *p*-Bromophenol Blue marker had M_{Gal} 0.95 and D-glucose 1.13.

nium carbonate (pH ~9). Relative to *p*-Bromophenol Blue, the 3,4 isomers **13** and **16** had M_{Gal} values of 1.32 and 1.46, respectively. Although the M_{Gal} values of the 4,6 stereoisomers **9** and **14** differed (0.90 and 0.88, respectively), the difference was insufficient to resolve a mixture into two spots. The separation in the presence of ammonium carbonate is superior, however, to that in the borate system in two respects: the silver nitrate and *p*-anisidine–trichloroacetic acid reagents are much more sensitive following removal of the ammonium carbonate buffer by heating the paper, and the *O*-(1-carboxyethylidene) are completely separated from the neutral D-galactose derivatives.

Well-defined separations of the 3,4-*O*- and 4,6-*O*-(1-carboxyethylidene) derivatives of D-galactose were obtained by t.l.c. on silica gel G-60 with three successive chromatographic runs (followed by drying) in ethyl acetate–acetic acid–formic acid–water. The compounds were detected as yellow-brown spots with the *p*-anisidine–trichloroacetic acid reagent* (see Table I). Under the same conditions, the galactobioses were not well separated (see Table I).

Chromatography on paper in ethyl acetate–acetic acid–formic acid–water, separated the 3,4- (**13**) from the 4,6-isomer (**9**), the former having a mobility 1.6 times that of the latter[13]. However, with a solvent containing ethyl acetate, acetic acid, and water, the following mobilities, compared with that of D-galactose, were obtained for the 3,4 diastereoisomers **13** (1.94) and **16** (1.90), and for the 4,6 diastereoisomers **9** (1.52) and **14** (1.54). Thus, the 3,4 diastereoisomers were distinguishable from the 4,6 diastereoisomers, but the diastereoisomers themselves were barely resolved. Under the same conditions, the D-galactobiose isomers could be resolved with some

*The 3,4 diastereoisomers **13** and **16** appeared as the only yellow-brown spots, as the only other components of the partial hydrolyzate were the parent methyl glycosides **12** and **15**.

difficulty, having mobilities, compared to that of D-galactose, of 0.37 (**3**, 2-*O*), 0.33 (**5**, 3-*O*), 0.41 (4-*O*), and 0.24 (6-*O*) (Table I).

Gas–liquid chromatography of the acetates of derived galactobiitols, using Dexsil 300 as the liquid phase at 190°, did not appear to be a promising approach as only a partial resolution was obtained. The 2-*O* and 6-*O* derivatives had retention times of 10.0 min and 15.2 min, respectively, and could be separated from the 3-*O* and 4-*O* isomers, which cochromatographed with a retention time of 14.2 min.

In summary, it would appear that the best approach for detection of isomeric *O*-β-D-galactopyranosyl-D-galactoses and 3,4-*O*- and 4,6-*O*-(1-carboxyethylidene)-D-galactoses, in a partial hydrolyzate, is a combination of t.l.c. on silica gel G-60 and paper electrophoresis in borate buffer. Although t.l.c. does not separate the D-galactobioses, the 4 isomeric *O*-(1-carboxyethylidene) derivatives are well resolved. On the other hand, paper electrophoresis resolves the four D-galactobioses as a group, and a mixture of the 4 isomeric *O*-(1-carboxyethylidene) derivatives as a group. Unfortunately, 6-*O*-β-D-galactopyranosyl-D-galactose has the same M_{Gal} as 4,6-*O*-(1-carboxyethylidene)-D-galactose (**9**), and, thus, the method is only satisfactory for detection of D-galactobioses following removal of pyruvic acid acetal derivatives with ion-exchange resin.

EXPERIMENTAL

Thin-layer chromatography. — This separation was carried out on "DC-Alufolien Kieselgel G-60" (E. Merck, Darmstadt, Germany) in 12:3:1:4 (v/v) ethyl acetate–acetic acid–formic acid–water. The solvent was allowed to move a distance of 4.5 cm, and the plate then dried. Following this, the procedure was repeated twice, the solvent being allowed to move successive distances of 9.0 and 18.0 cm. *p*-Anisidine–trichloroacetic acid was used to detect reducing sugars, and 50% aqueous sulfuric acid was used as a general carbohydrate spray.

Paper chromatography. — Whatman No 1 paper was used with 3:1:1 (v/v) ethyl acetate–acetic acid–water as solvent. Spray: *p*-anisidine–trichloroacetic acid.

Paper electrophoresis. — Electrophoretograms were run on Whatman No 1 paper in 50mM sodium tetraborate, adjusted to pH 10.0 with aqueous sodium hydroxide, at 25°, with carbon tetrachloride as coolant. The paper (18 cm wide) was subjected to ~1 kV, the current being 30–35 mA. During the course of the run, the uniformity of development across the paper was checked with a tracking dye, *p*-Bromophenol Blue (R_{Gal} 0.95). Sugars were detected by the silver nitrate–sodium hydroxide dip method, with two successive dips in silver nitrate in acetone [0.1 mL of a saturated solution in dry acetone (25 mL)], and then 3% sodium hydroxide in ethanol. *p*-Anisidine–trichloroacetic acid was also used as spray reagent.

Other electrophoretograms were obtained by use of a buffer of 2% aqueous ammonium carbonate (pH ~9). Prior to development, the papers were heated for 10 min at 100° to remove ammonium carbonate. The *p*-anisidine–trichloroacetic acid reagent was most sensitive when the spots were observed under u.v. light.

Gas–liquid chromatography. — This was performed on D-galactobiitol nona-

acetates, prepared by sodium borohydride reduction of D-galactobioses, followed by treatment with 1:1 (v/v) acetic anhydride–pyridine for 6 h at 100°. The acetates were chromatographed on a 1.2-m column of 3% Dexsil 300 (Analabs Inc., North Haven, CT 06473, U.S.A.) on Chromosorb W (80–100 mesh) at 190°.

Benzyl 3,4,6-tri-O-benzyl-α,β-D-galactopyranoside (**2**). — 3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-galactopyranose, prepared as described previously[1] from 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) (2.5 g), was converted into an anomeric mixture of benzyl glycosides by treatment with benzyl alcohol (30 mL) containing 1% of *p*-toluenesulfonic acid for 1 h at 50°. Following deacetylation and evaporation, the product was chromatographed on a column of silicic acid (eluent: 1:1, v/v, chloroform–Skellysolve B) to give syrupy **2** (1.20 g), $[\alpha]_D^{25}$ +1° (*c* 0.7, ethanol): ^{13}C-n.m.r. ($CDCl_3$) (β-D-anomer): δ 102.2 (C-1) and 82.2 (C-3); (α-D anomer): 98.3 (C-1) and 80.0 (C-3); C-i signal ratio of α to β anomer 1:3.5.

Anal. Calc. for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found C, 75.38; H, 6.47.

2-O-β-D-Galactopyranosyl-α-D-galactose (**3**). — An excess of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) (1.0 g) was added in 5 equal portions over 2 h to a shaken suspension of silver carbonate (2.0 g) and Baker 3 Å molecular sieve (5 g) in dichloromethane (15 mL) containing benzyl 3,4,6-tri-*O*-benzyl-α,β-D-galactopyranoside (**2**) (0.55 g). Filtration followed by deacetylation with methanolic sodium methoxide gave material that was partitioned between ethyl acetate and water. The ethyl acetate layer was evaporated and the residue debenzylated in acetic acid with 5% palladium-on-charcoal in the presence of hydrogen. The product was chromatographed on a cellulose column (eluent: 4:1 v/v, acetone–water) providing 2-*O*-β-D-galactopyranosyl-D-galactose (0.13 g), which crystallized as the α-D anomer (**3**) from methanol–ethanol, m.p. 164–167°, $[\alpha]_D^{25}$ +66→+50° (constant value; *c* 0.6, water); different from lit.[14], m.p. 192–194°, $[\alpha]_D^{25}$ +86→+63°; ^{13}C-n.m.r. (D_2O, 70°): (major α-D anomer) δ 106.08 (C-1'), 93.80 (C-1), and 76.95 (C-2); (β-D anomer): 105.07 (C-1'), 96.84 (C-1), and 79.66 (C-2).

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.00; H, 6.43.

Benzyl 2,3,6-tri-O-benzyl-β-D-galactopyranoside (**4**). — Benzyl 2,3-di-*O*-benzyl-β-D-galactopyranoside[2] (1.45 g) was monobenzylated, in *N*,*N*-dimethylformamide (4 mL) containing silver oxide (1.5 g), by the dropwise addition, over 1 h with shaking, of benzyl bromide (1.3 equiv.). After 18 h, the reaction mixture was diluted with dichloromethane, filtered, and the filtrate evaporated to a syrup. Examination by t.l.c. on silica gel (solvent: 33:1 v/v, chloroform–ethanol) showed 4 spots, the second fastest spot being the 2,3,6 isomer, which was present in the largest proportion. Silicic acid column chromatography (eluent: 1:1 v/v, chloroform–Skellysolve B) provided **4** (0.99 g) as a syrup, $[\alpha]_D^{25}$ −8° (*c* 0.8, ethanol); ^{13}C-n.m.r. ($CDCl_3$): δ 138.7, 138.20, 138.03, 137.6, 128.83, 128.57, 128.45, 128.38, 128.24, 128.05, 127.94, 127.87 127.80, 127.70, 102.7, 80.8, 79.1, 75.3, 73.9, 73.4, 72.6, 71.0, 69.4, and 67.1.

Anal. Calc. for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.71; H, 6.39.

4-O-β-D-Galactopyranosyl-α-D-galactose (**5**). — This disaccharide was obtained by following a procedure similar to that described above for the preparation of **3**, except that **4** was used as acceptor. The final purification was carried out by

cellulose column-chromatography (eluent: 4:1 v/v, acetone–water), and the resulting 4-*O*-β-D-galactopyranosyl-D-galactose (82 mg) crystallized as the α-D anomer **5** from methanol, m.p. 213–216°, $[\alpha]_D^{25}$ +77→+60° (constant value; *c* 0.3, water); lit.[15] m.p. 210–212°, $[\alpha]_D^{25}$ +67°.

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found C, 42.01; H, 6.33.

Benzyl 2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranoside (**6**). — Benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside[2] (5.0 g) was acetylated by heating for 1 h at 100° a solution in acetic anhydride (10 mL) and pyridine (10 mL). The solution was evaporated and the residue crystallized twice from 2:1 (v/v, ethanol–hexane) (yield, 5.8 g), m.p. 188–189°, $[\alpha]_D^{25}$ +57° (*c* 1.9, pyridine).

Anal. Calc. for $C_{24}H_{26}O_8$: C, 65.17; H, 5.92. Found: C, 65.27; H, 5.92.

Benzyl 4,6-O-[(R)- and (S)-hydroxyisopropylidene]-β-D-galactopyranoside (**7** *and* **8**). — Benzyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside (2.5 g) was shaken in acetoxyacetone (20 mL) containing sulfuric acid (0.01 mL) until dissolution was complete. After being kept for a further 24 h, the solution was added to ice-cold, aqueous sodium hydrogencarbonate, and the mixture was extracted with ethyl acetate. Evaporation gave a syrup that was deacetylated with 0.1M sodium methoxide in methanol (40 mL) for 1 h. The solution was evaporated and the residue de-ionized with mixed ion-exchange resins. Examination of the product by t.l.c. on silica gel (solvent: 23:2 v/v, chloroform–ethanol) gave two principal spots having R_F 0.18 and 0.20 with a minor spot at R_F 0.10 corresponding to benzyl β-D-galactopyranoside. The mixture was chromatographed on a column of silicic acid (eluent: 25:1 v/v, chloroform–methanol) providing a syrupy material having R_F 0.20 (283 mg), corresponding to stereoisomer **8**, $[\alpha]_D^{25}$ −54° (*c* 0.5, water); ^{13}C-n.m.r. (D_2O): δ 137.9, 129.89, 129.61, 102.4, 100.6, 72.8, 72.5, 71.5, 69.4, 68.9, 67.6, 63.3, and 15.2.

Anal. Calc. for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 58.54; H, 6.93.

The stereoisomer having R_F 0.18 (256 mg) crystallized from ethyl acetate (yield 161 mg), m.p. 172°, $[\alpha]_D^{25}$ −44° (*c* 0.5, ethanol); ^{13}C-n.m.r. (D_2O); δ 137.9, 129.89, 129.60, 102.4, 100.8, 72.8, 72.4, 71.4, 69.7, 67.2, 63.6, 59.0, and 25.0.

Anal. Calc. for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 58.93; H, 6.83.

Benzyl 4,6-O-[(R)- and (S)-(1-methoxycarbonylethylidene)]-β-D-galactopyranoside (**10** *and* **11**). — These compounds were prepared from **7** and **8**, respectively, by successive oxidation with Adams' platinic oxide catalyst providing the *O*-(1-carboxyethylidene) derivatives. Treatment with diazomethane gave the methyl esters **10** and **11**, respectively, which were purified by silicic acid column chromatography. This procedure has been previously described for the conversion of methyl 3,4-*O*-hydroxyisopropylidene-β-D-fucopyranosides into *O*-(1-methoxycarbonylethylidene) derivatives[10,11].

The 4,6-*O*-[(*R*)-hydroxyisopropylidene] derivative **7** (151 mg) was converted into syrupy benzyl 4,6-*O*-[(*R*)-(1-methoxycarbonylethylidene)]-β-D-galactopyranoside (**10**) (48 mg), $[\alpha]_D^{25}$ −25° (*c* 0.8, ethanol); ^{13}C-n.m.r. ($CDCl_3$): δ 137.1, 128.53, 128.17, 128.02, 101.5, 101.4, 72.7, 71.5, 70.9, 68.6, 67.2, 64.2, 52.7, and 20.9.

Anal. Calc. for $C_{17}H_{22}O_8$: C, 57.62; H, 6.22; Found: C, 57.91; H, 6.33.

The 4,6-*O*-[(*S*)-hydroxyisopropylidene] derivative **8** (134 mg) gave syrupy benzyl 4,6-*O*-[(*R*)-(1-methoxycarbonylethylidene)]-β-D-galactopyranoside (**11**) (35 mg), $[\alpha]_D^{25}$ −25° (*c* 0.7, ethanol); ^{13}C-n.m.r. ($CDCl_3$): δ 137.1, 128.55, 128.15, 128.02, 101.7, 74.5, 70.9, 66.4, 65.5, 65.0, 52.7, and 15.8.

Anal. Calc. for $C_{17}H_{22}O_8$: C, 57.62; H, 6.22. Found: C, 57.34; H, 6.51.

4,6-O-[(R)- and (S)-(1-Carboxyethylidene)]-α,β-D-galactose (**9** *and* **14**). — Benzyl 4,6-*O*-[(*R*)-(1-methoxycarbonylethylidene)]-β-D-galactopyranoside (**10**) was treated overnight with aqueous barium hydroxide. Excess alkali was neutralized with carbon dioxide, the suspension filtered, and the filtrate treated with Amberlite IR-120 (H^+) cation-exchange resin. The resulting solution, containing benzyl 4,6-*O*-[(*R*)-(1-carboxyethylidene)]-β-D-galactopyranoside, was evaporated and the residue debenzylated in acetic acid with 5% palladium-on-charcoal in the presence of hydrogen. The product was chromatographed on a cellulose column (eluent: 3:1:1 v/v ethyl acetate–acetic acid–water), to provide **9**, $[\alpha]_D^{25}$ +42° (*c* 0.2, water); ^{13}C-n.m.r. (D_2O): δ 100.2, 97.2, 93.8, 73.0, 72.6, 72.4, 69.1, 68.7, 66.8, 66.5, 66.4, 63.7, 62.9, and 26.1; ^{1}H-n.m.r. (D_2O): δ 1.98.

Anal. Calc. for $C_9H_{14}O_8$: C, 43.20; H, 5.64. Found: C, 42.82; H, 5.33.

Benzyl 4,6-*O*-[(*S*)-(1-methoxycarbonylethylidene)]-β-D-galactopyranoside (**11**) was converted, in a similar way, into **14**, $[\alpha]_D^{25}$ +53° (*c* 0.2, water); ^{13}C-n.m.r. (D_2O): δ 99.3, 97.1, 93.9, 72.6, 72.4, 70.8, 70.3, 69.1, 68.6, 67.2, 66.8, 64.2, 63.3, and 17.6; ^{1}H-n.m.r. (D_2O): δ 2.12.

Anal. Calc. for $C_9H_{14}O_8$: C, 43.20; H, 5.64. Found: C, 42.91; H, 5.94.

3,4-O-[(R)- and (S)-(1-Carboxyethylidene)]-α,β-D-galactose (**13** *and* **16**). — Methyl 3,4-*O*-[(*R*)-(1-carboxyethylidene)]-β-D-galactopyranoside[10,11] (**12**) (10 mg) was partially hydrolyzed with 0.5M sulfuric acid (0.5 mL) for 1 h at 70°. The solution was neutralized ($BaCO_3$), the suspension filtered, and the supernatant solution treated with Amberlite IR-120 (H^+) cation-exchange resin, and the solution lyophilized. The other stereoisomer **15** was partially hydrolyzed under similar conditions to give a product containing the stereoisomer **16**.

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