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SYNTHESIS OF 2,5,6- AND 3,5,6-TRI-*O*-METHYL-D-GALACTOSE

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ABSTRACT

Starting from methyl β-D-galactofuranoside, 3,5,6-tri-*O*-methyl-D-galactose (**9**) and 2,5,6-tri-*O*-methyl-D-galactose (**16**) were synthesized. The alditol acetates were prepared from **9** and **16**, and their behavior in g.l.c. was compared. Mass spectra of the alditol acetates from **9** and **16** showed that these compounds gave fragmentations as expected. The alditol acetate from **16** was also prepared by an alternative route.

INTRODUCTION

The presence of galactose in the furanose form has been noted in some complex polysaccharides. For methylation analysis of such a polysaccharide, it was necessary to synthesize 2,5,6-tri-*O*-methyl-D-galactose (**16**) and 3,5,6-tri-*O*-methyl-D-galactose (**9**). Siddiqui and Urbas[1] prepared these compounds by using partial tosylation of methyl 6-*O*-trityl-β-D-galactofuranoside as the crucial step. It was, therefore, of interest to synthesize **9** and **16** without using any partial esterification, because the structures of products from such a reaction may always be ambiguous.

RESULTS AND DISCUSSION

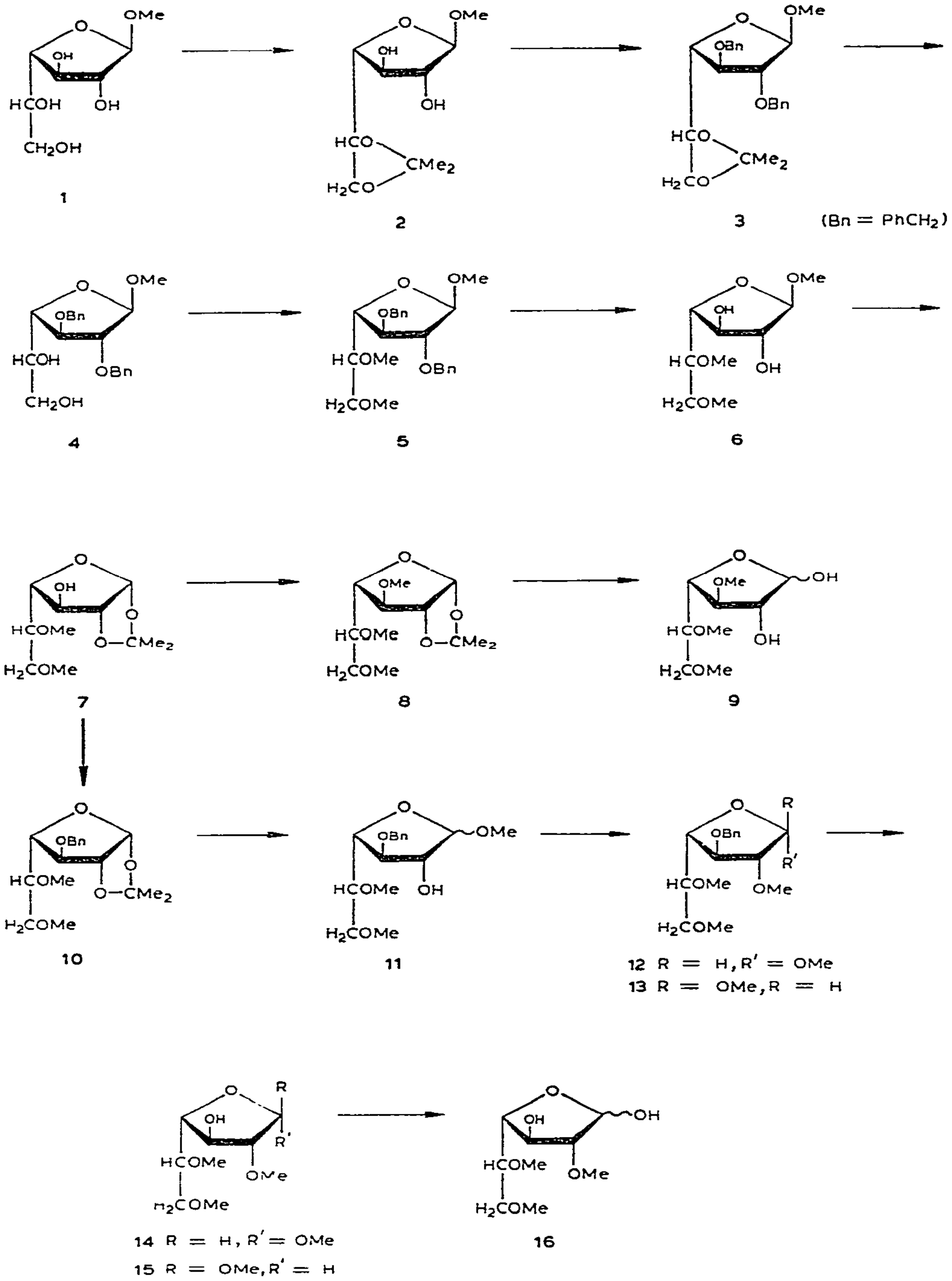
Methyl β-D-galactofuranoside (**1**) was prepared according to the procedure of Augestad and Berner[2]. The per(trimethylsilyl) ether[3] of compound **1** gave a single peak in g.l.c. (column A). Compound **1** was allowed to react with acetone in the presence of *N*,*N*-dimethylformamide[4], anhydrous copper(II) sulfate, and Drierite for 15 days at 27°, with stirring. Methyl 5,6-*O*-isopropylidene-β-D-galactofuranoside (**2**) thus formed had n.m.r. signals at 293 Hz (a one-proton singlet corresponding to H-1), 204 Hz (a three- proton singlet corresponding to the glycosidic methoxyl group), and 84 and 86 Hz (two three-proton singlets for the isopropylidene group). Compound **2** was benzylated to give **3**, from which the isopropylidene group was removed, yielding crystalline methyl 2,3-di-*O*-benzyl-β-D-galactofuranoside (**4**). This compound had m.p. and specific rotation identical to those determined by Saeki and Iwashige[5]. The n.m.r. spectrum of the compound has signals for one methoxyl group and two benzyl groups, apart from the singlet at 293 Hz for H-1.

Methylation of **4**, and debenzylation of the resulting methyl 2,3-di-*O*-benzyl-

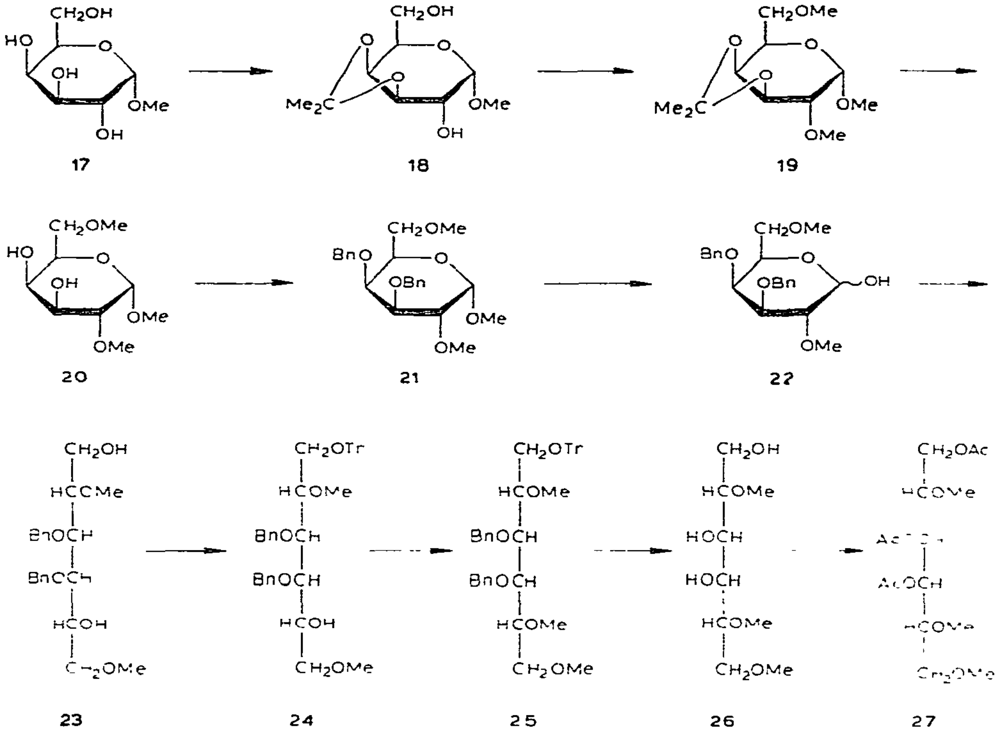
5,6-di-*O*-methyl-β-D-galactofuranoside (**5**) gave methyl 5,6-di-*O*-methyl-β-D-galactofuranoside (**6**). The n.m.r. spectrum of compound **6** has a sharp, one-proton singlet at 294 Hz for H-1. The spectrum also has three three-proton singlets, at 199, 201, and 203 Hz, confirming the presence of three *O*-methyl groups in the compound. On treatment with acetone in the presence of Drierite and a trace of sulfuric acid for 24 h, compound **6** gave 1,2-*O*-isopropylidene-5,6-di-*O*-methyl-α-D-galactofuranose (**7**). The n.m.r. spectrum of compound **7** had a one-proton doublet for H-1 at 348 Hz ($J = 4$ Hz), two three-proton singlets for methoxyl protons at 202 and 210 Hz, and two three-proton singlets for the isopropylidene group at 81 and 92 Hz. Methylation of **7** gave 1,2-*O*-isopropylidene-3,5,6-tri-*O*-methyl-α-D-galactofuranose (**8**), which showed n.m.r. signals at 348 Hz (one-proton doublet, H-1), 205, 207, and 213 Hz (three methoxyl groups), and 82 and 94 Hz (two three-proton singlets for the isopropylidene group). Removal of the isopropylidene group from compound **8** gave 3,5,6-tri-*O*-methyl-D-galactose (**9**).

To prepare 2,5,6-tri-*O*-methyl-D-galactose, compound **7** was benzylated to give 3-*O*-benzyl-1,2-*O*-isopropylidene-5,6-di-*O*-methyl-α-D-galactofuranose (**10**). Compound **10** showed n.m.r. signals at 440 Hz (5 H, Ph), 349 Hz (one-proton doublet, H-1), 198 and 207 Hz (two *O*-methyl singlets), and 82 and 93 Hz (two three-proton singlets, isopropylidene group). Compound **10** was methanolyzed to give methyl 3-*O*-benzyl-5,6-di-*O*-methyl-α,β-D-galactofuranoside (**11**). Compound **11** was methylated to give two compounds, **12** and **13**; these were separated by column chromatography. On methanolysis, **12** or **13** gave a mixture of **12** and **13**. Compound **13**, which was the major component, had a negative rotation and was, therefore, methyl 3-*O*-benzyl-2,5,6-tri-*O*-methyl-β-D-galactofuranoside. The n.m.r. spectrum of compound **13** confirmed the presence of four methoxyl groups and one benzyl group, and it showed a sharp singlet at 294 Hz for H-1. Debenzylation of **12** and **13** afforded two methyl glycosides (**14** and **15**) which were interconvertible on methanolysis, as confirmed by g.l.c. (column C). The methyl glycoside **15** (from **13**) was hydrolyzed to give 2,5,6-tri-*O*-methyl-D-galactose (**16**).

In order to confirm the structures of **7** and **16**, the alditol acetate of **16** was synthesized by a different route. Starting from methyl α-D-galactopyranoside[6] (**17**), methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside (**18**) was prepared by treatment with acetone in the presence of anhydrous copper(II) sulfate, *N*,*N*-dimethylformamide[4], and Drierite. The n.m.r. spectrum of **18** has a one-proton doublet at 286 Hz (H-1), one three-proton singlet at 208 Hz (*O*-methyl), and two three-proton singlets at 81 and 91 Hz (isopropylidene methyl groups). Methylation of compound **18**, to give **19**, removal of the isopropylidene group to give **20**, and benzylation of **20** gave methyl 2,6-di-*O*-methyl-3,4-di-*O*-benzyl-α-D-galactopyranoside (**21**). The glycosidic methyl group was removed from **21** by hydrolysis, and the product (**22**) was reduced with sodium borohydride to give **23** in crystalline form. The n.m.r. spectrum of **23** revealed the presence of two methoxyl groups (at 199 and 208 Hz), and two benzyl groups (10 H, at 441 Hz, 2 Ph; and 4 methylene protons of 2 benzyl groups, at 278 Hz).


1
2
3
(Bn = PhCH₂)
4
5
6
7
8
9
10
11
12 R = H, R′ = OMe
13 R = OMe, R = H
14 R = H, R′ = OMe
15 R = OMe, R′ = H
16

Tritylation of **23** gave 3,4-di-*O*-benzyl-5,6-di-*O*-methyl-1-*O*-trityl-D-galactitol (**24**). The n.m.r. spectrum of compound **24** confirmed the presence of two benzyl groups and one trityl group (broad, 25-proton signal at ~440 Hz), four methylene protons of benzyl groups (~276 Hz), and two *O*-methyl groups (196 and 204 Hz). Compound **24** was methylated, and the product (**25**) treated with hydrogen in the presence of 10% palladium-on-charcoal, giving 2,5,6-tri-*O*-methyl-D-galactitol (**26**), which was acetylated to 1,3,4-tri-*O*-acetyl-2,5,6-tri-*O*-methyl-D-galactitol (**27**).



Methyl 3,5,6-tri-*O*-methyl-α,β-D-galactofuranoside, prepared from **9** by refluxing with 4% hydrogen chloride in methanol, gave in g.l.c. (column C) two peaks having retention times (R_t) 2.7 and 5.2, compared to that of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside as unity. Methyl 2,5,6-tri-*O*-methyl-β-D-galactofuranoside (**15**), obtained from **13** by debenzylation, gave a single peak having R_t 4.25, whereas the corresponding α-glycoside (**14**), obtained from **12** by debenzylation, gave a single peak at R_t 7.4. The alditol acetates from **9** and **16** were prepared by standard methods[7], and they were compared by g.l.c. (column B). The alditol acetate from **9** had R_t 2.21, compared to that of the alditol acetate from 2,3,4,6-tetra-*O*-methyl-D-glucose as unity. The alditol acetate from **16** and the alditol acetate **27** were identical in g.l.c. (column B), both having R_t 2.27.

The mass spectra of the alditol acetates from **9** and **16**, and of **27**, were recorded. The primary, fragmentation peaks are summarized in Table I.

TABLE I

PRIMARY FRAGMENTS IN THE MASS SPECTRA OF ALDITOL ACETATES

Compound	m/e						
	45	*89*	*117*	*189*	*205*	*277*	*305*
9 (as alditol acetate)	+	+		+	+		+
16 (as alditol acetate)	+	+	+			+	+
27	+	+	+			+	+

Fragmentation in the alditol acetates of compounds **9** and **16**, and in the alditol acetate **27** occurred as expected[9]. One notable feature of all of the spectra was the presence of a strong peak at *m/e* 305, formed by cleavage between C-5 and C-6. The weak peak at *m/e* 277 for **16** (as alditol acetate) and **27**, formed by breakage of the C-1–C-2 bond, is also noteworthy.

EXPERIMENTAL

General. — All of the reactions were monitored by thin-layer chromatography. Thin layers were prepared on microscope slides (7.5 × 2.5 cm) with Silica gel G (E. Merck, Germany). Column chromatography was performed on Silica gel 60 (E. Merck, Germany). Solvents were always mixed in volume per volume (v/v).

N.m.r. spectra were recorded with a Varian A-60A spectrometer in chloroform-*d*, with tetramethylsilane as the internal standard. Optical rotations were determined with a Perkin–Elmer 241 spectropolarimeter. Mass spectra were recorded with a Hitachi Mass Spectrometer Model RMU-6L.

G.l.c. was performed with a Hewlett–Packard Model 5731 Gas Chromatograph. Column A (1.83 m × 3.2 mm) was stainless steel packed with 3% of SE 52 on Chromosorb W at 160°, column B (1.83 m × 6.4 mm) was glass packed with 3% of ECNSS-M on Gas Chrom Q at 165°, and column C (1.83 m × 3.2 mm) was stainless steel packed with 15% of diethyleneglycol succinate on Chromosorb W at 190°.

Methyl 5,6-O-isopropylidene-β-D-galactofuranoside (**2**). — To a solution of methyl β-D-galactofuranoside (**1**; 4 g) in dry *N,N*-dimethylformamide (50 ml) were added dry acetone (300 ml), anhydrous copper(II) sulfate (60 g), and Drierite (30 g). The mixture was stirred for 15 days, filtered through Celite, and the filtrate evaporated under diminished pressure to a syrup. T.l.c. in ethyl acetate showed two spots—one for the product, and one for unreacted **1**. Column chromatography with the same solvent gave **2** as a thick syrup; yield 1.7 g (35.2%), $[\alpha]_D^{27}$ −80° (*c* 1, chloroform); lit.[5] $[\alpha]_D^{20}$ −82°.

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.74. Found: C, 51.19; H, 7.90.

Methyl 2,3-di-O-benzyl-5,6-O-isopropylidene-β-D-galactofuranoside (**3**). — A solution of the isopropylidene acetal **2** (900 mg) in 1,4-dioxane (8 ml) was mixed with powdered potassium hydroxide (4.5 g) in a three-necked flask. Benzyl chloride (5 ml) was added dropwise during 2 h while the mixture was stirred and heated at 80°; after the addition, heating and stirring were continued for 3 h. The mixture was cooled in an ice bath, diluted with water, and extracted with three 50-ml portions of dichloromethane. The extracts were combined, washed with water, dried (anhydrous sodium sulfate), and filtered. The filtrate was evaporated to a syrup, from which benzyl alcohol was removed by azeotropic distillation with water. Column chromatography using 9:1 benzene–ether gave pure **3** (1.3 g, 81.6%) having $[\alpha]_D^{27}$ $-61°$ (*c* 3, chloroform); lit.[5] $[\alpha]_D^{20}$ $-62°$.

Anal. Calc. for $C_{24}H_{30}O_6$: C, 69.55; H, 7.23. Found: C, 69.27; H, 7.40.

Methyl 2,3-di-O-benzyl-β-D-galactofuranoside (**4**). — To a solution of **3** (1.2 g) in glacial acetic acid (15 ml) was added water (10 ml) dropwise while the flask was kept in a water bath at 75°. Heating was continued for 45 min, and the solution was evaporated under diminished pressure. The resulting syrup crystallized from cyclohexane, yielding 930 mg (86%) of **4**; m.p. 86–88°, $[\alpha]_D^{27}$ $-78.5°$ (*c* 2, chloroform); lit.[5] m.p. 90°, $[\alpha]_D$ $-79.4°$.

Anal. Calc. for $C_{21}H_{26}O_{61}$ C, 67.40; H, 6.99. Found: C, 67.30; H, 7.02.

Methyl 2,3-di-O-benzyl-5,6-di-O-methyl-β-D-galactofuranoside (**5**). — To a solution of compound **4** (880 mg) in *N*,*N*-dimethylformamide (50 ml) were added silver oxide (7 g) and Drierite (6 g). The mixture was stirred for 30 min; methyl iodide (5 ml) was then added, and the mixture was stirred for 24 h. Chloroform (50 ml) was added while the mixture was vigorously stirred. The solid was filtered off through a Celite bed, and the filtrate was evaporated to a thin syrup. An excess of chloroform (100 ml) was added, the suspension was filtered, and the filtrate was washed with water, dried (anhydrous sodium sulfate), and evaporated to a thick syrup. The product was purified by column chromatography, using 9:1 benzene–ether as the solvent, yielding pure **5** (850 mg, 90.3%), $[\alpha]_D^{27}$ $-63.5°$ (*c* 1, chloroform).

Anal. Calc. for $C_{23}H_{30}O_6$: C, 68.72; H, 7.51. Found: C, 68.44; H, 7.48.

Methyl 5,6-di-O-methyl-β-D-galactofuranoside (**6**). — To a solution of **5** (800 mg) in ethanol (18 ml) was added 10% palladium-on-charcoal (200 mg), and the mixture was stirred under hydrogen for 16 h at room temperature. The mixture was filtered through Celite, and the filtrate evaporated to dryness, giving 430 mg (97%) of **6** having $[\alpha]_D^{27}$ $-103.9°$ (*c* 0.5, chloroform).

Anal. Calc. for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 48.75; H, 8.32.

1,2-O-Isopropylidene-5,6-di-O-methyl-α-D-galactofuranose (**7**). — To a solution of **6** (400 mg) in dry acetone (80 ml) was added Drierite (7 g), and the mixture was stirred for 1 h. Sulfuric acid (0.6 ml) was added dropwise, while the mixture was stirred and cooled in ice. Stirring was continued for 24 h, the acid was neutralized with calcium oxide, and the suspension was filtered through a Celite bed. The filtrate was evaporated to dryness, and the product was purified by column chromatography using 1:1 benzene–ether as the solvent, giving **7** (250 mg, 56%), $[\alpha]_D^{27}$ −4.6° (*c* 2, chloroform).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.11. Found: C, 53.15: H, 8.20.

*3,5,6-Tri-*O*-methyl-*D*-galactofuranose* (**9**). — The isopropylidene acetal **7** (150 mg) was methylated as described in the preparation of **5**. On column chromatography using 9:1 benzene–ether as solvent, the crude product gave pure **8** (140 mg, 88%) as a syrup having $[\alpha]_D^{27}$ −28.6° (*c* 1, chloroform).

The isopropylidene group in compound **8** was removed as described in the preparation of **4**. The product was purified by column chromatography, using ethyl acetate as the solvent, giving pure **9** (120 mg, 94%) as a thick glass, $[\alpha]_D^{27}$ −41° (*c* 1, water); lit.[1] $[\alpha]_D^{27}$ −40.5° (water).

Anal. Calc. for $C_9H_{18}O_6$: C, 48.60; H, 8.11. Found: C, 48.37; H, 8.20.

*Methyl 3-*O*-benzyl-2,5,6-tri-*O*-methyl-β-*D*-galactofuranoside* (**13**). — The isopropylidene acetal **7** (100 mg) was benzylated as already described, and the product was purified by column chromatography with 9:1 benzene–ether, to give pure **10** (125 mg) as a syrup having $[\alpha]_D^{27}$ −36° (*c* 1, chloroform). A solution of compound **10** in 4% hydrogen chloride in methanol was boiled under reflux for 8 h. The solution was made neutral (silver carbonate), the suspension filtered, and the filtrate (showing one spot in t.l.c. with 1:1 benzene–ether) was evaporated to a syrup. This was methylated as already described. The product gave two spots in t.l.c. with 4:1 benzene–ether, and the components were separated by column chromatography. On methanolysis, both compounds gave a mixture of the same two compounds. The major component (**13**) (yield 84 mg, 70%) had $[\alpha]_D^{27}$ −107° (*c* 0.6, chloroform) and the higher mobility. The minor component **12** (yield 14 mg, 12%) had $[\alpha]_D^{27}$ +36° (*c* 0.2, chloroform), and the lower mobility.

Anal. Calc. for $C_{17}H_{20}O_6$: C, 62.55; H, 8.03. Found: C, 62.40: H, 8.20.

*2,5,6-Tri-*O*-methyl-*D*-galactofuranose* (**16**). — Compound **13** (70 mg) was debenzylated as already described, giving **15** (pure by t.l.c. in 1:1 benzene–ether) as a syrup (45 mg; 92%), $[\alpha]_D^{27}$ −76.9° (*c* 0.2, chloroform). To a solution of compound **15** (45 mg) in 1,4-dioxane (1 ml) was added 0.5M sulfuric acid (4 ml), and the solution was boiled under reflux for 5 h, made neutral with barium carbonate, and the suspension filtered. The filtrate was evaporated to a syrup which was pure by t.l.c. in ethyl acetate; yield of **16**, 30 mg (91%); $[\alpha]_D^{27}$ −15° (*c* 0.5, water); lit.[1] $[\alpha]_D^{27}$ −15.9° (water).

Anal. Calc. for $C_9H_{18}O_6$: C, 48.60; H, 8.11. Found: C, 48.42; H, 8.40.

*Methyl 3,4-*O*-isopropylidene-α-*D*-galactopyranoside* (**18**). — Methyl α-D-galactopyranoside[6] (**17**, 8 g) in *N*,*N*-dimethylformamide (30 ml) was stirred with acetone (120 ml), anhydrous copper(II) sulfate (25 g), and Drierite (20 g) for 15 days at room temperature. T.l.c. in ethyl acetate then showed two spots (in addition to one for a trace of unreacted material). Separation of the two products was effected by column chromatography. The faster-moving component **18** crystallized from ethyl acetate; 4 g (41.4%), m.p. 104–105°, $[\alpha]_D^{27}$ +167° (*c* 1, water); lit.[8] $[\alpha]_D^{27}$ +168° (water).

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.27; H, 7.75. Found: C, 51.22; H, 7.80.

The slower-moving component (30%) is probably the 4,6-isopropylidene derivative of **17**, as revealed by its n.m.r. spectrum and its behavior with trityl chloride. It had $[\alpha]_D^{27}$ +139° (water), and it could not be crystallized.

*3,4-Di-O-benzyl-2,6-di-O-methyl-*D*-galactitol* (**23**). — Compound **18** (3.5 g) was methylated as described in the preparation of **5**. The product was purified by column chromatography with 1:1 benzene–ether, yielding 3.1 g (80%) of **19** in the form of a thick syrup, $[\alpha]_D^{27}$ +144° (*c* 2, chloroform). The isopropylidene group of **19** was removed as already described, giving compound **20**. This was chromatographed (ethyl acetate), and the pure **20** was benzylated as already described, to give **21**, which was purified by column chromatography with 10:1 benzene–ether. The dibenzyl ether **21** (2.9 g) was boiled under reflux for 5 h with 0.5M sulfuric acid in 1:3 water–1,4-dioxane (40 ml). The solution was made neutral with barium carbonate, the suspension filtered, and the filtrate evaporated to a syrup (**22**). T.l.c. showed a single spot, R_F <0.5 that for **21**. The syrupy **22** was treated with 4% sodium borohydride in 95% ethanol (50 ml) for 16 h. Sodium ions were then removed by adding Dowex 50 resin, and boric acid was removed by repeated evaporation with methanol. The product was finally purified by column chromatography with 1:1 benzene–ether, yielding 2.5 g (90%) of **23** which crystallized from benzene; m.p. 75–77°; $[\alpha]_D^{27}$ −7° (*c* 1, chloroform).

Anal. Calc. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.7. Found: C, 67.45; H, 7.8.

*1,3,4-Tri-O-acetyl-2,5,6-tri-O-methyl-*D*-galactitol* (**27**). — Compound **23** (2.4 g) was tritylated with 1.5 molar equivalents of trityl chloride in pyridine for 4 days. Treatment of the product by column chromatography gave **24** (2.9 g, 75%) as a thick glass having $[\alpha]_D^{27}$ −5° (*c* 1, chloroform).

Methylation of **24** (2 g) was achieved as already described, and column chromatography of the product with 9:1 benzene–ether gave pure **25** (1.2 g, 60%), $[\alpha]_D^{27}$ −2° (*c* 1, chloroform).

Anal. Calc. for $C_{42}H_{46}O_6$: C, 78.00; H, 7.17. Found: C, 78.10; H, 7.27.

Detritylation and debenzylation of compound **25** (1 g) were achieved in a single step by hydrogenolysis over Pd/C as already described. Purification of the product by column chromatography (ethyl acetate) yielded **26** (280 mg, 80%), $[\alpha]_D^{27}$ −2° (*c* 2, chloroform). Acetylation of this material gave **27**, $[\alpha]_D^{27}$ −6.5° (*c* 0.2, chloroform).

Anal. Calc. for $C_9H_{20}O_6$: C, 49.20; H, 8.99. Found: C, 49.30; H, 9.05.

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