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Note

Synthesis of 6-*O*-methyl-D-galactose 4-sulphate

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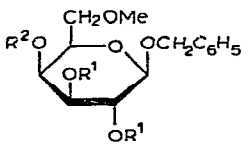
During structural studies of an algal galactan sulphate, a sugar sulphate was isolated which appeared to be a 6-*O*-methylgalactose monosulphate. From its infrared spectrum and other properties, the sulphate group was tentatively assigned to position 4. In order to characterise this as the 4-sulphate, we have now synthesized the D enantiomer by a definitive route.

The most convenient route to derivatives of D-galactose substituted at C-4 is by partial benzoylation of D-galactosides at low temperature[1], which gives the 2,3,6-tri-*O*-benzoyl derivatives preferentially. Benzoylation at low temperature of benzyl 6-*O*-methyl-β-D-galactopyranoside (**1**) gave a mixture of mono-, di-, and tribenzoates. The major product, a crystalline dibenzoate, was isolated by chromatography on silica gel, and was shown to be the expected 2,3-di-*O*-benzoyl-6-*O*-methyl derivative (**2**) as follows. Compound **2** was converted[1] into the tetrahydropyranyl ether **3**, which was then deacylated and methylated to give the tri-*O*-methyl derivative **4**. Removal of the tetrahydropyranyl group by acid hydrolysis, and the glycosidic benzyl group by hydrogenation, gave syrupy 2,3,6-tri-*O*-methyl-D-galactose, characterised as the crystalline 2,3,6-tri-*O*-methyl-D-galactono-1,4-lactone. The free hydroxyl group in compound **2** must, therefore, be on C-4.



1 $R^1 = R^2 = H$
2 $R^1 = Bz$, $R^2 = H$
3 $R^1 = Bz$, R^2 = tetrahydropyranyl
4 $R^1 = Me$, R^2 = tetrahydropyranyl

Sulphation, with pyridine–sulphur trioxide, of derivative **2** gave an amorphous monosulphate, isolated as the barium salt. Deacylation and hydrogenation, to remove the blocking groups, then gave syrupy 6-*O*-methyl-D-galactose 4-(barium sulphate), which crystallised slowly on standing. This sugar was detected on chromatograms by the triphenyltetrazolium hydroxide spray reagent, indicating[2] a free OH group on C-2, and gave a blue colour with the diphenylamine–aniline spray

reagent, indicative[3] of a substituent at C-4. The infrared spectrum showed a C–O–S absorption band at 850 cm^{-1} with a shoulder at 835 cm^{-1}, similar to that given[4] by D-galactose 4-(barium sulphate). Finally, methylation, followed by methanolysis and g.l.c. examination of the derived methyl galactosides, gave a pattern identical with that given by D-galactose 4-(barium sulphate) when subjected to the same treatment.

EXPERIMENTAL

General methods. — The methods of paper chromatography[5] and t.l.c.[6] of sugar sulphates have been described. For other sugar derivatives, t.l.c. was carried out on silica gel with benzene–ethyl acetate (7:3) and benzene–methanol–chloroform (3:3:7). Paper electrophoresis employed 0.1M borate buffer (pH 10) and a potential gradient of 40 volts/cm. Spray reagents for paper chromatography and electrophoresis were *p*-anisidine hydrochloride[7], triphenyltetrazolium hydroxide[2], or diphenylamine–aniline[3]. For t.l.c., the *p*-anisidine–conc. H_2SO_4 reagent[8] was used.

Benzyl 6-O-methyl-β-D-galactopyranoside (**1**). — Dry 6-*O*-methyl-D-galactose[14] (30 g) was acetylated with anhydrous sodium acetate (27 g) and acetic anhydride (150 ml) at 110° for 45 min, in the usual manner, to give a syrupy mixture of tetra-acetates (36 g). The dried syrup (30 g) was converted into the 1-bromo derivative, as described by Haskin *et. al.*[9], and isolated as an oil (26.5 g). Dry benzyl alcohol (200 ml) was stirred with anhydrous calcium sulphate (15 g) and silver oxide (22 g) for 16 h. The 1-bromo derivative (25 g) and iodine (1 g) in dry ether (120 ml) were then added in small portions during 1 h, and the mixture was left at 20° for 96 h. The mixture was filtered, the precipitate was washed with ether, and the combined ethereal solutions were evaporated to a syrup (30 g). The syrup in ethanol (180 ml) was deacetylated by pouring slowly into barium hydroxide (70 g) in water (900 ml), and stirring for 40 h. After being neutralised (CO_2) and filtered, the solution was evaporated to dryness, and the residual solid was continuously extracted with ethyl acetate. Evaporation of the extracts gave a crystalline solid which, after recrystallisation from ethanol–ethyl acetate (1:2), gave pure **1** (9.6 g), m.p. 132–134°, $[\alpha]_D^{20}$ −30.8° (water). The β-D configuration for the product was assumed from the optical rotation and the method of preparation.

Anal. Calc. for $C_{14}H_{20}O_6$: C, 59.2; H, 7.02. Found: C, 58.9; H, 7.05.

Benzyl 2,3-di-O-benzoyl-6-O-methyl-β-D-galactopyranoside (**2**). — A solution of compound **1** (2.57 g) in anhydrous pyridine (80 ml) was cooled to −40°. Benzoyl chloride (2.3 ml) was added during 1 h with stirring, keeping the temperature below −30°. After a further 3 h, the major component, indicated by t.l.c., appeared to be a dibenzoate. The product was isolated[1] as a syrup (3.6 g), which was fractionated on a column of silica gel (600 g), using benzene–ethyl acetate (7:3) as eluant. In order of elution, the following compounds were isolated: (*a*) benzyl 2,3,4-tri-*O*-benzoyl-6-*O*-methyl-β-D-galactopyranoside (0.41 g), m.p. 140–142°, $[\alpha]_D^{20}$ +126.8° (chloroform).

Anal. Calc. for $C_{35}H_{32}O_9$: C, 70.5; H, 5.37. Found: C, 70.3; H, 5.36.

(*b*) A syrupy dibenzoate (0.12 g).

(*c*) Benzyl 2,3-di-*O*-benzoyl-6-*O*-methyl-β-D-galactopyranoside (**2**, 1.81 g), m.p. 99–100°, $[\alpha]_D^{20}$ +24.8° (chloroform).

Anal. Calc. for $C_{28}H_{28}O_8$: C, 68.3; H, 5.69. Found: C, 68.6; H, 5.79.

(*d*) A monobenzoate (0.74 g), m.p. 104–106°, $[\alpha]_D^{20}$ +6.2° (chloroform).

Anal. Calc. for $C_{21}H_{24}O_7$: C, 65.0; H, 6.19. Found: C, 64.9; H, 6.3.

Proof of structure of compound **2**. — Compound **2** (0.50 g) was converted into the 4-tetrahydropyranyl ether **3**, as described by Williams and Richardson[1]. The ether (0.40 g) had m.p. 149–156°, $[\alpha]_D^{20}$ +55.9° (chloroform).

Anal. Calc. for $C_{33}H_{36}O_9$: C, 68.8; H, 6.25. Found: C, 68.6; H, 6.49.

The ether was debenzoylated with barium methoxide in methanol at 4° for 48 h, and t.l.c. then indicated only one reaction product, which was isolated (0.28 g). Methylation of the product (twice) with methyl iodide (10 ml) and silver oxide (3 g) at 35° for 24 h and 36 h, respectively, gave **4** as a chromatographically pure syrup (0.28 g). A solution of compound **4** in acetone (6 ml) and M hydrochloric acid (5 ml) was left at 20° for 16 h, neutralised with Amberlite IRA-40(HO^-) resin, and concentrated. The syrupy residue (150 mg) was hydrogenated in ethanol–water (20 ml) containing 10% palladium-on-carbon (1 g) at 4 atmos. for 16 h. The catalyst was removed on a filter and the filtrate was concentrated to give a syrup (90 mg), identified chromatographically as 2,3,6-tri-*O*-methyl-D-galactose, and having $[\alpha]_D^{20}$ +81° (water); lit.[10] $[\alpha]_D$ +80°. The syrup (80 mg) in water (20 ml) was oxidised with bromine (0.5 ml) at 37° for 24 h. Excess of bromine was removed by aeration, and the solution was neutralised with silver carbonate and concentrated. The product crystallised from ethanol–light petroleum and was recrystallised from ether to give 2,3,6-tri-*O*-methyl-D-galactono-1,4-lactone, m.p. 98–99°, $[\alpha]_D^{20}$ −40→−29° (water); lit.[10] m.p. 99°, $[\alpha]_D$ −40→−28°).

6-O-Methyl-D-galactose 4-(barium sulphate). — The dibenzoate **2** (3.0 g) was sulphated at 60° with pyridine–sulphur trioxide (10 g) in pyridine (100 ml). T.l.c. revealed that the starting material had all reacted in 0.5 h. The mixture was cooled, and the amorphous monosulphate (3.0 g) was isolated in the usual manner[11] in a chromatographically pure form. The benzoyl groups were removed by treatment with 75mM barium methoxide in methanol (50 ml) at 4° for 48 h, and the benzyl group by hydrogenation (see above). The syrupy product, 6-*O*-methyl-D-galactose 4-(barium sulphate) (363 mg) crystallised slowly on standing. It had $[\alpha]_D^{20}$ +50° (equil., water). The sodium salt, prepared by percolating the barium salt through Zeo-Karb 225(Na^+) resin and evaporating the solution, was used for analysis.

Anal. Calc. for $C_7H_{13}NaO_9S$: S, 10.96. Found: S, 11.0.

The infrared spectrum was recorded for a paraffin paste, and showed a broad absorption band at 1250 cm^{-1} (S=O) and a sharp peak at 850 cm^{-1} with a shoulder at 835 cm^{-1} (C–O–S). On t.l.c.[6], the sugar sulphate had an R_F value of 1, and gave a blue colour with the diphenylamine–aniline spray reagent[3]. On paper, the monosulphate had an R_F value identical with that of D-galactose in three solvent systems, and was detected by the triphenyltetrazolium hydroxide spray[2] and by *p*-anisidine

hydrochloride (orange–red colour). The M_G value in borate buffer was 0.86 (*cf.* 1.05 for D-galactose 4-sulphate).

The sugar sulphate was methylated in *N*,*N*-dimethylformamide, as described by Kuhn *et al.*[12], the product was methanolysed, and the methyl glycosides were analysed[13] by g.l.c. on a 1.5-m column of 15% butane-1,4-diol succinate polyester on Celite at 175°. Peaks with retention times (relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside) of 3.0 (strong), 3.69 (weak), 4.0 (weak), and 4.33 (medium) were obtained. Authentic D-galactose 4-sulphate gave an identical pattern when similarly treated.

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