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Note

A novel preparation of D-fructopyranose 5-sulphate

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We have described[1] the synthesis and characterisation of a series of galactopyranose and glucopyranose sulphates, and their use in an investigation[2] of the specificity of sulphatase A. For the completion of this work, D-glucofuranose 5-sulphate was sought. The preparation[3] of D-glucofuranose 5-phosphate from 3-*O*-acetyl-1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-α-D-glucofuranose suggested a route to D-glucofuranose 5-sulphate.

The reaction of 3,6-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose with pyridine–sulphur trioxide followed by deacetylation gave the expected 1,2-*O*-isopropylidene-α-D-glucofuranose 5-(barium sulphate) (**1**), which was characterised by ^{13}C-n.m.r. spectroscopy (Table I). As with other monosaccharide sulphates[1], there was a large downfield shift (7.33 p.p.m.) in the signal for C-5, which carries the sulphate group, and smaller upfield shifts (1.61 and 2.57 p.p.m., respectively) in the signals for C-4 and C-6.

Autohydrolysis of the free-acid form of **1** removed the isopropylidene group and gave a sulphate ester of a reducing sugar, but the p.m.r. spectrum showed that it was not D-glucofuranose 5-sulphate because there was no signal for an anomeric proton. Also, the ^{13}C-n.m.r. spectrum was not consistent with a glucofuranose derivative. Proton-coupled, ^{13}C-n.m.r. spectroscopy showed that the compound contained one quaternary, three secondary, and two primary carbon atoms, and the spectrum was consistent with D-fructopyranose 5-(potassium sulphate). There was a large downfield shift (8.32 p.p.m.) in the signal for C-5, and smaller upfield shifts (1.46 and 2.47 p.p.m., respectively) in the signals for C-4 and C-6. As shown in Table I, the deuterium-induced, differential isotope shifts (d.i.s. shifts)[4] confirmed these assignments. Only for C-2 was there a significant discrepancy between the observed and calculated d.i.s. values, and a similar low value (0.12) has been observed[4] for C-2 in β-D-fructopyranose.

D-Fructose 5-sulphate was a substrate for sulphatase A: with K_m and V values of 48 mM and 13 μmol.mg^{-1}.min^{-1}, respectively: these values are comparable to

TABLE I

^{13}C-N.M.R. DATA FOR 1,2-*O*-ISOPROPYLIDENE-D-GLUCOSE, D-FRUCTOSE, AND THEIR 5-SULPHATES

	Chemical and d.i.s. shifts (p.p.m.)[a]								
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C*	*CH*$_3$	
1,2-*O*-Isopropylidene-D-glucofuranose	105.43	85.14	74.33	80.51	69.16	64.23	113.42	26.31	25.88
1,2-*O*-Isopropylidene-D-glucofuranose 5-(barium sulphate)	105.42	84.93	74.27	78.90	76.69	61.66	113.57	26.36	25.92
β-D-Fructopyranose	64.91	98.89	68.57	70.68	70.16	64.24	—	—	—
β-D-Fructopyranose 5-(potassium sulphate)	64.64	98.90	68.50	69.22	78.48	61.77	—	—	—
	0.19 (0.18)[b]	0.11 (0.17)	0.22 (0.20)	0.16 (0.17)	0.00 (0.03)	0.00 (0.00)			

[a] ^{13}C-Chemical shifts were measured with respect to internal 1,4-dioxane and converted to the Me_4Si scale by using the relationship $\delta_{Me_4Si} = \delta_{dioxane} + 67.40$. [b] Values are for the observed and (in brackets) calculated d.i.s. values for D-fructose 5-sulphate.

those for D-glucose 3-sulphate[2]. Moreover, the liberated sugar had the chromatographic properties of fructose (see Experimental).

Unlike gluco- and galacto-pyranose sulphates[1], the fructopyranose 5-sulphate was precipitated by ethanol as a single anomer, as shown by ^{13}C-n.m.r. spectroscopy. D-Fructopyranose exists[4] in solution mainly as the β anomer, and this is likely also for the 5-sulphate which had an $[\alpha]_D$ value (water) of $-86°$.

The conversion of a 5-substituted 1,2-*O*-isopropylidene-α-D-glucofuranose into a 5-substituted β-D-fructopyranose apparently has not been described hitherto and presumably involves a Lobry de Bruyn–Alberda van Ekenstein transformation[5] of D-glucofuranose 5-sulphate. The slow transformation of glucose into fructose in acid solution is long known[6], but the conversion noted here, as judged by the disappearance of the n.m.r. signal for the anomeric proton, was essentially complete in 30 min at 70°. The reaction is also unusual in that the ketose is the main product, whereas, in most such transformations, a complex mixture of sugars is obtained[5]. If the transformation is a general one, it could be useful for the synthesis of 5-substituted fructopyranoses, although other routes[7] are available.

The above results cast doubt on the structure of the presumed D-glucofuranose 5-phosphate[3], but it should be noted that Fitzgerald[8] suggested a specific role for the sulphate group in the Tris-catalysed isomerisation of glucose 6-sulphate to fructose 6-sulphate.

EXPERIMENTAL

General methods. — The general chemical and spectroscopic methods were described in the previous paper[1].

1,2-O-Isopropylidene-α-D-glucofuranose 5-(barium sulphate). — 3,6-Di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose[9] (7.3 g, 24 mmol) was treated overnight with pyridine–sulphur trioxide (5.8 g, 36 mmol) in pyridine (100 mL) at room temperature and the product (13 g) was isolated by the usual methods[1]. This was deacetylated with methanolic barium methoxide[1] to give the crude, title compound (10 g). P.m.r. data (D_2O): δ 5.83 (d, $J_{1,2}$ 3.66 Hz, H-1).

β-D-Fructopyranose 5-(potassium sulphate). — The foregoing compound (two lots of 4.0 and 6.0 g) was converted into the free acid by passage through a column of Dowex-50(H^+) resin and the acidic eluate (~100 mL for each lot) was kept for 40 min at 80°. Removal of the isopropylidene group was confirmed by p.m.r. spectroscopy, and the barium salt of the sulphate ester was obtained in the usual manner[1].

This crude barium salt (10 g) was converted into the free acid, as described above, and thence[10] into the brucine salt. Three recrystallisations from aqueous acetone[10] gave a product (3.6 g), m.p. 176–178° (dec.), $[\alpha]_D$ −65° (water).

Anal. Calc. for $C_6H_{11}O_9S \cdot C_{23}H_{27}N_2O_4$: C, 53.2; H, 5.85; N, 4.28; S, 4.90. Found: C, 52.9; H, 4.57; N, 4.17; S, 4.57.

The pure brucine salt (2.3 g) was converted[10] into the potassium salt, which was precipitated from aqueous solution with ethanol[1] to give the title compound

(0.9 g), $[\alpha]_D$ −86° (water). The tenaciously retained ethanol of solvation was detectable by ^{13}C-n.m.r. spectroscopy, and there was no n.m.r. signal (D_2O) for an anomeric proton.

Anal. Calc. for $C_6H_{11}KO_9S$: C, 24.2; H, 3.72; S, 10.8. Found: C, 23.9; H, 4.02; S, 9.28.

Enzymic hydrolysis of D-*fructopyranose 5-sulphate.* — A solution of D-fructose 5-(potassium sulphate) (25 μmol) in 0.1M pyridine–acetic acid buffer (pH 5.6, 0.25 mL) was treated with 250 μg of sulphatase A for 4 days at room temperature, when analysis[2] revealed ~50% hydrolysis. Dilution (10-fold) of a sample of the hydrolysate with water followed by t.l.c.[11] [phosphate-impregnated Silica gel 60 (Merck), acetone–2-propanol–0.1M lactic acid (2:2:1), detection with orcinol[12] and 1-naphthol–phosphoric acid[13]] revealed fructose, R_f 0.20 (*cf.* 0.26 for glucose)

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