Carbohydrate Research, 124 (1983) 338–343
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

On the desulphation of carbohydrate sulphates

ALEXANDER B. ROY AND JENNIFER TURNER

Department of Physical Biochemistry, John Curtin School of Medical Research, P.O. Box 334, Canberra City, A.C.T. 2601 (Australia)

(Received June 14th, 1983; accepted for publication, June 29th, 1983)

The desulphation of carbohydrate sulphates by acid or alkali is well known[1], although few systematic studies have been made with well-characterised monosaccharide sulphates. Rees[2] noted that rates of acid hydrolysis could provide a guide to the type of sulphate group, although his data from polysaccharide sulphates could have been complicated by the effects of glycosidic bonds on the reaction[3]. The desulphation of monosaccharide sulphates in alkali, an internal S_N2 reaction, does not appear to have been systematically studied[4]. As we had synthesised and characterised several monosaccharide sulphates[5,6], the opportunity was taken to investigate their rates of desulphation.

Monosaccharides undergo complex reactions in alkali and, to eliminate these effects, the desulphation of the 4-(potassium sulphates) of methyl α-D-glucopyranoside and methyl α-D-galactopyranoside was investigated. The latter compound has been prepared[7] as the barium salt, from methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside. In our hands, it proved difficult[5] to completely remove aromatic compounds after the debenzoylation of similar derivatives, and the syntheses described below, from the 2,3,6-tri-*O*-acetyl-α-D-glycopyranosides, were developed. The glycoside 4-(potassium sulphates) were characterised by ^{13}C-n.m.r. spectroscopy (Table I); the expected, large downfield shifts[5] in the signals from C-4 together with the smaller upfield shifts in those from C-3 and C-5 are clear. The assignments of the resonances were confirmed by their deuterium-induced, differential isotope shifts (d.i.s. shifts)[8], also shown in Table I. The small d.i.s. shifts of the methoxyl resonances are similar to those noted by Pfeffer *et al.*[8] for the parent methyl glycosides.

Harris and Turvey[9] pointed out that, contrary to previous views, the wavelength of the C-O-S group vibration in the i.r. spectra could not be used to distinguish between axial and equatorial sulphate groups. This conclusion is supported by the data in Table II. It is clear that the values for the methyl glucoside and methyl galactoside 4-sulphates are similar, despite the differences in the spectra of the corresponding aldose 4-sulphates.

0008-6215/83/$ 03.00

TABLE I

^{13}C-N M R. DATA FOR METHYL α-D-GALACTOPYRANOSIDE, METHYL α-D-GLUCOPYRANOSIDE, AND THEIR 4-SULPHATES

Compound	*Chemical and d.i.s. shifts (p.p.m.)*[a]						
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*-OCH$_3$*
Methyl α-D-galactopyranoside[b]	100.13	69.17	70.47	70.20	71.59	62.21	55.95
Methyl α-D-galactopyranoside 4-(potassium sulphate)	100.12	69.13	69.35	78.34	70.98	62.04	56.02
	0.03 (0.03)[c]	0.17 (0.17)	0.17 (0.17)	0.03 (0.03)	0.05 (0.03)	0.14 (0.15)	0.04
Methyl α-D-glucopyranoside[b]	100.02	72.20	74.11	70.55	72.46	61.57	55.93
Methyl α-D-glucopyranoside 4-(potassium sulphate)	99.69	71.86	72.55	77.75	70.70	61.28	55.92
	0.00 (0.03)[c]	0.17 (0.17)	0.17 (0.17)	0.04 (0.03)	0.04 (0.03)	0.17 (0.15)	0.04

[a] ^{13}C-Chemical shifts were measured with respect to internal 1,4-dioxane, and converted into the Me_4Si scale using $\delta_{Me_4Si} = \delta_{dioxane} + 67.40$. [b] Data reported by Pfeffer *et al.*[7]. [c] Observed and (in brackets) calculated d.i.s. values for α-D-gluco- and -galacto-pyranoside 4-sulphates.

TABLE II

I R ABSORPTIONS FOR THE C–O–S VIBRATIONS IN SOME ALDOSE AND METHYL GLYCOSIDE 4-(POTASSIUM SULPHATES)

4-(Potassium sulphate)	*C–O–S absorption*[a] *(cm^{-1})*	
D-Galactopyranose	850 (m)	825 (w, sh)
Methyl α-D-galactopyranoside	845 (m)	815 (s)
D-Glucopyranose		817 (m)
Methyl α-D-glucopyranoside	850 (m)	818 (s)

[a]For KBr discs, measured with a Unicam SP1050 spectrophotometer; s, strong; m, medium; sh, shoulder.

The rates of acid hydrolysis of monosaccharide and glycoside sulphates, in 0.25M HCl at 100°, are given in Table III. The values for the latter are only apparent because of the simultaneous occurrence, at comparable rates, of both sulphate-ester and glycoside hydrolysis[3]. The general pattern of reactivity is as expected: glycosyl sulphate ≫ secondary equatorial sulphate > axial secondary sulphate > primary sulphate, assuming that the principal conformation[10] of the aldohexose sulphates in solution is $^4C_1$(D) and that of fructopyranose 5-sulphate is, like fructose[11], $^2C_5$(D). The differences between the rates of hydrolysis of the different types of sulphate ester are not sharp, however, and their value as diagnostic tools is limited. For example, the rates of acid hydrolysis of galactose 3-sulphate (equatorial) and galactose 4-sulphate (axial) are similar.

There are much greater differences in the rates of desulphation of the monosaccharide sulphates in 0.25M NaOH at 60° (Table III). Several reactions

TABLE III

RATES OF DESULPHATION OF SUGAR SULPHATES

Compound	*In 0.25M HCl at 100°*		*In 0.25M NaOH at 60°*
	$k \times 10^4$ *(s^{-1})*	$t_{1/2}$ *(min)*	$t_{1/2}$ *(min)*
D-Fructopyranose 5-sulphate	1.3	92	23
D-Galactose 2-sulphate	3.8	30	220
D-Galactose 3-sulphate	2.3	51	3
D-Galactose 4-sulphate	1.7	69	11
Methyl α-D-galactopyranoside 4-sulphate	—	80	[a]
D-Galactose 6-sulphate	0.93	120	19
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl sulphate	—	1.5	[b]
D-Glucose 2-sulphate	3.7	31	200
D-Glucose 3-sulphate	5.0	23	1
D-Glucose 4-sulphate	5.4	21	13
Methyl α-D-glucopyranoside 4-sulphate	—	25	900
D-Glucose 6-sulphate	0.99	120	22
D-Mannose 2-sulphate	1.3	92	160

[a]Stable. [b]Complete within 30 s.

must occur simultaneously, and t.l.c. of the reaction mixtures showed four or more products with R_F values ranging from 0.2 to 0.7 (*cf.* glucose, R_F 0.2). Both reducing and non-reducing substances were present. The generalisation[1,4] that desulphation of a carbohydrate sulphate in alkali will occur only when an adjacent *trans*-hydroxyl group is present does not apply to the monosaccharide sulphates, presumably because of alkali-induced changes originating at C-1. When these changes are prevented, the generalisation is valid. Thus, methyl α-D-galactopyranoside 4-sulphate, unlike galactose 4-sulphate, is stable in 2.5M NaOH at 60°, whereas methyl α-D-glucopyranoside 4-sulphate, in which HO-3 is *trans* to the sulphate, is desulphated with a $t_{1/2}$ of ~50 min. In 0.25M NaOH at 60°, the $t_{1/2}$ of the latter reaction is ~15 h, which is much greater than that for any of the monosaccharide sulphates.

The aldohexose 2-sulphates are more stable to alkali than the other isomers (Table III), which may be due to retardation of such alkali-induced reactions as the Lobry de Bruyn–Alberda van Ekenstein transformation or the formation of saccharinic acids. Several products were formed but not identified, although ^{13}C-n.m.r. spectroscopy showed the presence of carboxyl-containing compounds.

EXPERIMENTAL

General methods. — The techniques used in i.r., p.m.r., and ^{13}C-n.m.r. spectroscopy have been described previously[5,6], as has the t.l.c. of sugars[6] on phosphate-impregnated silica gel[12]. Reducing sugars were detected with 1-naphthylamine, and 0.5% $KMnO_4$ in M NaOH[13] was used generally for hydroxyl-containing compounds.

Monosaccharide sulphates. — D-Mannopyranose 2-(barium sulphate), a gift from Dr. J. R. Turvey (University College, Bangor), was converted into the potassium salt by passage through Dowex 50 (H^+) resin and neutralisation of the eluate with $KHCO_3$. The other aldo- and keto-hexose sulphates were those synthesised and characterised previously[5,6].

Methyl α-D-glucopyranoside 4-(potassium sulphate). — Methyl 2,3,6-tri-*O*-acetyl-α-D-glucopyranoside was prepared from the glucoside (31 g), *via* the 4,6-*O*-benzylidene derivative, as described for the parent hexose[5]. Only the tetra-acetate could be crystallised from the resulting syrupy mixture of tri- and tetra-acetates, and the remaining crude triacetate (5.8 g) was treated[5] with 1.5 mol of pyridine–sulphur trioxide in pyridine for 1.5 h at 60°. After standing overnight at room temperature, methyl 2,3,6-tri-*O*-acetyl-α-D-glucopyranoside 4-(barium sulphate) (4.3 g) was isolated as usual[5], and deacetylated with methanolic barium methoxide to give methyl α-D-glucopyranoside 4-(barium sulphate) (2.9 g). Part (2.8 g) of this compound was converted into the title compound (1 g) by passage through a column of Dowex 50 (K^+) resin followed by precipitation with ethanol. The product had $[\alpha]_D$ +109° (*c* 1, water). P.m.r. data (D_2O): δ 4.82 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1β) and 3.42 (s, 3 H, OMe).

Anal. Calc. for $C_7H_{13}KO_9S \cdot H_2O$: C, 25.45; H, 4.58; S, 9.71. Found: C, 25.53; H, 4.52; S, 9.01.

Methyl α-D-galactopyranoside 4-(potassium sulphate). — This compound (1 g), prepared as described above from a syrupy mixture (3.9 g) of methyl 2,3,6-tri-*O*-acetyl-α-D-galactopyranoside and the corresponding tetra-acetate, had $[\alpha]_D$ +129° (*c* 1, water); lit.[7] $[\alpha]_D$ +110° (Ba salt) ($[M]_D$ 42,600 and 37,300[9], respectively). P.m.r. data (D_2O): δ 4.86 (d, 1 H, $J_{1,2}$ 2.9 Hz, H-1β) and 3.42 (s, 3 H, OMe).

Anal. Calc. for $C_7H_{13}KO_9S \cdot H_2O$: C, 25.45; H, 4.58; S, 9.71. Found: C, 25.12; H, 4.51; S, 9.53.

Acid hydrolysis. — Portions (0.7 mL) of a solution of each sulphate ester (~15 mg) in cold 0.25M HCl (10 mL) were sealed in ampoules which were then immersed in boiling water for the appropriate time. Duplicate samples (0.25 mL) of each cooled hydrolysate were diluted with aqueous 4% trichloroacetic acid (4 mL), and the released sulphate was determined by the turbidimetric method[14].

In general, the data fitted the first-order rate equation up to ~90% hydrolysis. With 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl sulphate, where the rate of hydrolysis was too rapid to allow accurate data to be obtained by the above method, and with the glycoside sulphates, where glycoside hydrolysis and sulphate hydrolysis were proceeding simultaneously[3], $t_{1/2}$ values were estimated directly from the progress curves of the reactions.

Desulphation in alkali. — A solution of each sulphate ester (15 mg) in 0.25M NaOH (10 mL) was kept at 60°. At intervals, samples (0.25 mL) were removed, and diluted with aqueous 4% trichloroacetic acid (4 mL), and the released sulphate was determined turbidimetrically[14]. Most of the reactions were rapid and not first-order, so that $t_{1/2}$ values were estimated from the progress curves.

The glycoside sulphates were treated with 2.5M NaOH at 60° and the reactions were stopped with aqueous 6.4% trichloroacetic acid (4 mL). Methyl α-D-galactopyranoside 4-sulphate was stable, but the *gluco* isomer was desulphated by a first-order reaction.

ACKNOWLEDGMENTS

We thank Dr. M. D. Fenn for help with the n.m.r. measurements, and the staff of the ANU Analytical Chemistry Service for the elemental analyses.

REFERENCES

1 E. G. V. PERCIVAL, *Q. Rev. Chem. Soc.*, 4 (1949) 369–384.
2 D. A. REES, *Biochem J.*, 88 (1963) 343–345
3 M. J. CLANCY AND J. R. TURVEY, *J Chem. Soc.*, (1961) 2935–2938
4 J. R. TURVEY *Adv. Carbohydr. Chem.*, 20 (1965) 183–218.
5 P. J. ARCHIBALD, M. D. FENN, AND A. B. ROY, *Carbohydr Res.*, 93 (1981) 177–190
6 A. MARKER, A. B. ROY, AND J. TURNER, *Carbohydr. Res.*, 114 (1983) 331–334.
7 M. J. HARRIS AND J. R. TURVEY, *Carbohydr. Res*, 9 (1969) 397–405.

8 P. E. PFEFFER, K. M. VALENTINE, AND F. W. PARRISH, *J. Am. Chem. Soc.*, 101 (1979) 1265–1274.
9 M. J. HARRIS AND J. R. TURVEY, *Carbohydr. Res.*, 15 (1970) 51–56.
10 M. J. HARRIS AND J. R. TURVEY, *Carbohydr. Res.*, 15 (1970) 57–63.
11 P. L. DURETTE AND D. HORTON, *Adv. Carbohydr. Chem. Biochem.*, 26 (1971) 49–125.
12 S. A. HANSEN, *J. Chromatogr.*, 107 (1975) 224–226.
13 G. W. HAY, B. A. LEWIS, AND F. SMITH, *J. Chromatogr.*, 11 (1963) 479.
14 K. S. DODGSON, *Biochem. J.*, 78 (1961) 312–319.