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SULPHATES OF MONOSACCHARIDES AND DERIVATIVES

PART IX[1]. THE CONFORMATIONS OF GLYCOSIDE SULPHATES IN SOLUTION DETERMINED BY N.M.R. SPECTROSCOPY

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ABSTRACT

The chemical shifts and coupling constants for the protons in a number of α-D-glucopyranoside and α- and β-D-galactopyranoside sulphates have been measured in aqueous solution. The values recorded are in agreement with a *C1*(D) conformation for both series of glycoside sulphates. The deshielding effect of the sulphate group on geminal and vicinal protons has been determined. A geminal proton is deshielded by about 0.6 p.p.m., but some variation with orientation is apparent. The effect of a sulphate group on a vicinal proton is dependent on the relative orientations of the two groups, and is largest when both groups are equatorial in the pyranoside ring. The solvent shift produced by adding pyridine to the aqueous solution of the glycoside sulphates is a useful indication of the position occupied by the sulphate group, since protons geminal to sulphate groups move rapidly downfield relative to the other sugar protons, but not as rapidly as the HOD line.

INTRODUCTION

In the preceding paper[1], we commented on the infrared spectra of some glycoside sulphates, and adduced evidence from their optical rotations that, in aqueous solution, the glycoside sulphates of D-glucose and D-galactose appear to retain the *C1*(D) conformation. Although n.m.r. is now used routinely for examining the conformations of various sugar derivatives in solution, no previous study has been made of the sulphate derivatives. We now report the results of such a study, with particular reference to the conformations adopted by glycoside sulphates in aqueous solution and to the effect of the sulphate groups on the proton resonances. A study has also been made of the effect of adding pyridine to the aqueous solution of glycoside sulphates and the application of this method as an aid in assigning the spectral lines.

EXPERIMENTAL

The glycoside sulphates used in this study have been described in previous communications[2,3]. All spectra were recorded initially at 60 MHz on a Perkin–Elmer

R10 spectrometer with spin decoupler, and at the normal probe temperature (33.5°). Chemical shifts were measured with an accuracy of 0.02 p.p.m. and coupling constants to 0.5 Hz. For better resolution, a few spectra were also recorded at 100 MHz on a Varian HA100 spectrometer at the University of East Anglia. Samples were prepared by equilibration of the glycoside sulphates in D_2O, followed by evaporation to dryness and subsequent dissolution in D_2O to give almost saturated solutions. Where viscous solutions resulted, dilution with D_2O was necessary to give better spectral resolution. Where possible, barium salts were used but, in the cases of sparing solubility, conversion into the sodium salt gave greater solubility. Such changes in the cation did not alter the spectra.

Initially, sodium 4,4-dimethyl-4-silapentane-1-sulphonate was used as internal standard but, for comparative purposes in mixed solvents, acetonitrile was used as internal standard. The τ-value of the protons of acetonitrile in D_2O was taken to be 8.00. The effect of pyridine addition to aqueous solution of the sulphates was followed by using acetonitrile as internal standard. The τ-values of acetonitrile in various concentrations of aqueous pyridine were measured relative to an external standard of tetramethylsilane in a capillary; corrections for changes in the bulk susceptibility due to addition of pyridine to aqueous solution were made by using Wiedmann's additivity law.

RESULTS AND DISCUSSION

The conformations of sugars and their derivatives in solution have been effectively studied by n.m.r. since 1958, when it was reported[4] that equatorial protons resonate at lower field than axial, and that *trans*-diaxial protons have coupling constants much larger than for other orientations of vicinal protons. That the magnitude of the coupling constant is a function of the dihedral angle between two vicinal protons was first formalised by the equation of Karplus[5], but this equation has since been modified to take account of effects other than the geometry of the protons[6,7]. Although the τ-values and coupling constants of ring protons can give a good indication of the overall conformation of the ring, small changes in the values of either cannot be related closely to small changes of conformation[8]. For this reason, τ-values and coupling constants are interpreted only in terms of gross conformation, and are reported only for those protons to which a first-order treatment of the spectra could reasonably be applied.

In assigning resonance lines to the protons of glycoside sulphates, the first consideration was to locate the protons geminal to the sulphate group. In some cases, notably that of benzyl β-D-galactopyranoside 4-sulphate, this simply meant comparing the spectrum of the sulphated derivative with that of the parent compound, some of whose lines have already been assigned. Benzyl β-D-galactopyranoside gave signals at τ 5.18 (quartet, benzyl CH_2), 5.54 (H-1), and 6.0–6.4(6-proton multiplet, H-2–H-6). For the 4-sulphate, a quartet at τ 5.11 and a broad signal at τ 5.48 of the correct intensities are assigned to the benzyl CH_2 protons and H-1, respectively. The signals

for a group of five protons at τ 6.0–6.4 must be common to both compounds, but in the sulphate a new absorption at τ 5.22, due to one proton, can reasonably be attributed to H-4. Addition of pyridine to the D_2O solution of the 4-sulphate resulted in some movement of all the signals[9], but the H-4 signal, originally at τ 5.22, moved markedly downfield, compared to the other protons, until, in 63% (v/v) aqueous pyridine, it was clearly observed as a broad, unresolved singlet, 0.4 p.p.m. to lower field than the benzyl CH_2 protons. These results suggest that replacement of OH by OSO_3^-, in the pyranose ring, resulted in deshielding of the geminal proton by ~0.8 p.p.m. Similarly, in Table I, the τ-values of the various protons in methyl 4,6-*O*-benzylidene-α-D-galactopyranoside are compared with those of the derived 2,3-disulphate and 2,3-dinitrate. Despite the difference in solvents used, there is a close similarity between the effects of the nitrate and sulphate groups, except that the deshielding effect of nitrate groups on geminal protons is larger than that of sulphates, as expected from relative inductive effects. Again, the sulphate groups deshield geminal protons by ~ 0.8 p.p.m.

TABLE I

τ-VALUES FOR PROTONS IN METHYL 4,6-*O*-BENZYLIDENE-α-D-GALACTOPYRANOSIDE AND ITS 2,3-DISULPHATE AND 2,3-DINITRATE

Compound (solvent)	*τ-Value*							
	BzH	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*OCH_3*
Parent glycoside ($CDCl_3$)	4.36	5.01	6.02	6.02	5.7	6.29	5.71, 5.85	6.50
2,3-Disulphate (D_2O)	4.19	4.67	5.2	5.2	5.2	6.05	5.76	6.52
2,3-Dinitrate ($CDCl_3$)	4.20	4.66	4.2	4.2	5.26	6.10	5.60, 5.72	6.41

In those cases where considerable overlapping of peaks occurred, addition of pyridine in increasing concentration to the D_2O solution of the sulphate served to move the HOD signal downfield and to distinguish protons geminal to sulphate groups since, relative to other protons and to the internal standard (acetonitrile), the signals for these protons moved most markedly downfield. By selecting suitable concentrations of pyridine in D_2O as solvent, it was frequently possible to observe coupling constants which were obscured either by signal overlap or by virtual coupling in D_2O alone. In no case was addition of pyridine observed to alter the coupling constant of any proton, thus implying that the conformation of the glycoside sulphate in aqueous pyridine was essentially the same as that in water.

Rationalisation of the effect of pyridine in apparently deshielding protons at sulphated positions only becomes possible if the absolute effect of pyridine on the internal standard (acetonitrile) is taken into account. After being corrected for changes in bulk susceptibility, the τ-values relative to an external TMS standard, of acetonitrile in water, 50% and 70%(v/v) aqueous pyridine were 7.4, 7.9, and 8.1, respectively. The shielding of the protons in acetonitrile by pyridine is in accord with the known effect of aromatic solvents on polar solutes. Formation of a collision complex, such

TABLE II

τ-VALUES AND COUPLING CONSTANTS FOR SOME GLYCOSIDE SULPHATES

Compound (barium salt)	*τ-Value (coupling constants in Hz)**							
	OCH_3	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*Others*
Methyl α-D-glucopyranoside 2,3-disulphate	6.59	4.85 (3.5)	5.66 (3.5, 10.0)	5.5(m)[a]	———	6.0–6.5	———	
Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-disulphate[b]	6.52	4.66 (3.5)	5.42 (3.5, 10.0)	5.2(m)[a]	———	5.6–6.4	———	4.38[c]
Methyl α-D-galactopyranoside 2,3-disulphate	6.60	4.83[a]	5.45(m)	5.45(m)	5.62[a]	———	6.0–6.4 ———	
Methyl β-D-galactopyranoside 2,3-disulphate	6.49	———	———	5.4–5.7 ———	———	6.26[a]	6.26[a]	
Methyl 4,6-*O*-benzylidene-α-D-galactopyranoside 2,3-disulphate	6.52	4.67[a]	5.2(m)	5.2(m)	5.2(m)	6.05(s)	5.76(s)	4.19[c]
Methyl 4,6-*O*-benzylidene-β-D-galactopyranoside 2,3-disulphate	6.41	———	———	5.3(m)	———	6.20(m)[a]	5.74(d)[a]	4.22[e]
Methyl α-D-galactopyranoside 2,6-disulphate	6.62	4.86 (3.5)	5.55 (3.5, 10.0)	~6.15	———	5.8–6.0	———	
Benzyl β-D-galactopyranoside 2,6-disulphate[c]		5.40 (7.75)	5.60 (7.75, 9.25)	6.16 (9.25, 3.0)	5.93 (3.0, 0.5)	6.07 (0.5, 6.25)	5.76 (6.25)	5.13 (12.25)[f]
Methyl α-D-galactopyranoside 4-sulphate	6.61	5.12	———	5.8–6.4 ———	5.27	———	5.8–6.4 ———	
Methyl β-D-galactopyranoside 4-sulphate	6.46	5.63 (7.0)						
Benzyl β-D-galactopyranoside 4-sulphate		5.48 (7.0)[a]	———	6.0–6.4 ———	5.22[a]	———	6.0–6.4 ———	5.11 (12.0)[f]
Methyl α-D-glucopyranoside 4,6-disulphate[c]	6.62	5.17 (3.25)	6.30 (3.25, 9.25)	———	———	5.4–6.2	———	
Methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside 4,6-disulphate	6.54		6.38 (3.5, 10.0)		(11.0)[d]			

*Multiplicity of signals; (s) = singlet, (d) = doublet, (m) = multiplet. [a]Broad signal due to virtual coupling. [b]Sodium salt. [c]At 100 MHz. [d]Coupling constant obtained after addition of pyridine. [e]Benzylidene CH. [f]Benzyl CH_2.

that the methyl group lies within the shielding cone of the magnetically anisotropic, aromatic ring, is considered to be the chief factor in this solvent shift[9–11]. When allowance is made for the solvent shift of the internal standard, it is seen that, in absolute terms, ring protons in the sugar sulphate are being shielded by added pyridine, but to a lesser extent than the standard, and protons geminal to the sulphate group are shielded least of all. This implies that the pyridine is being repelled by the sulphate groups, or is forced to assume a geometry such that the geminal proton no longer lies within the shielding cone of the aromatic ring. Other ring protons are, however, being shielded to a certain extent. For practical purposes, it is more convenient to measure this solvent shift relative to that of the internal standard, and the shift appears, therefore, as a move of the resonances downfield, particularly of the protons at the sulphated position. Fig. 1 illustrates the relative downfield-shift of selected protons in methyl α-D-galactopyranoside 4-sulphate when pyridine is added.

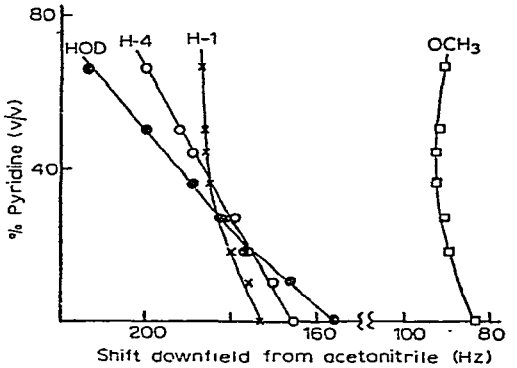

Fig. 1. Effect on proton chemical-shifts of adding pyridine to aqueous solutions of methy α-D-galactopyranoside 4-sulphate.

Table II lists the τ-values and coupling constants, where these could be measured accurately, for most of the glycoside sulphates examined. Virtual coupling occurred frequently, as indicated in Table II, and this had the effect of masking small coupling constants (0–3Hz), although larger coupling constants could still be observed. In terms of ring conformations, the τ-values listed are in good agreement with *CI*(D) conformations for all of the glucoside and galactoside sulphates, allowances being made for the observed deshielding effect of the sulphate group (see later). Where comparison could be made between α and β anomers, the α-D form gave the resonance for the axial methoxyl group at higher field than for the β-D form, as expected[4], and the equatorial H-1 of the α-D form resonated at lower field than the axial H-1 of the β-D form (Table III). Similarly, the coupling constants $J_{1,2}$ and $J_{2,3}$ were usually in agreement with those expected from the *CI*(D) conformation, with only small departures from previously reported values[4,13]. This general conclusion that sulphation of these glycosides has little effect on the conformation adopted in aqueous solution agrees with the previous conclusion obtained from a consideration of optical rotations[1].

TABLE III

COMPARISON OF METHYL AND H-1 PROTON RESONANCES IN GLYCOSIDES AND GLYCOSIDE SULPHATES

Compound	*τ-Value*	
	OCH_3	*H-1*
Methyl α-D-glucopyranoside	6.61	5.17
2,3-disulphate	6.59	4.85
4,6-disulphate	6.62	5.17
Methyl α-D-galactopyranoside	6.61	5.12
4-sulphate	6.61	5.12
2,3-disulphate	6.60	4.83
3,4-*O*-isopropylidene 2,6-disulphate	6.62	
Methyl β-D-galactopyranoside	6.47	5.72
4-sulphate	6.46	5.63
2,3-disulphate	6.49	5.5
2,6-disulphate	6.48	

The deshielding effect of a sulphate group on geminal and vicinal protons can readily be obtained from the τ-values listed in Tables I and II, together with those obtained for the unsulphated glycosides. The deshielding effect on a geminal, axial proton is about 0.60 p.p.m. Where two sulphate groups occur geminal and vicinal equatorial to an axial proton, the deshielding increases to *ca.* 0.75 p.p.m. For a proton geminal to an axial sulphate (*e.g.* galactopyranoside 4-sulphates), the value is rather more variable (0.58–0.71 p.p.m.). The equatorial H-1 of both series of α-D-glycosides is deshielded (*a*) 0.27 p.p.m. by a *cis*, equatorial 2-sulphate, (*b*) 0.30 p.p.m. by a diequatorial 2,3-disulphate and (*c*) 0.06 p.p.m. by an equatorial 3-sulphate. An axial H-1 is deshielded by *ca.* 0.18 p.p.m. by an equatorial 2-sulphate. This suggests that an equatorial 2-sulphate has rotamer states in which, on average, it is closer to an equatorial H-1 than to an axial H-1, thus deshielding the former to the greater extent.

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