564. *Sulphates of Monosaccharides and Derivatives. Part III.*[1] *Acid Hydrolysis.*

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The hydrolysis of the glycosidic linkages in various glycoside sulphates at 65° in 1·5N-hydrochloric acid has been compared with that in the parent glycosides. The results show that the presence of a sulphate group in a glycoside stabilises the glycosidic linkage to acid, the effect being greater for a 6-sulphate than for a 3-sulphate. The acidic fragmentation of polysaccharide sulphates is discussed in the light of these findings.

Linkage analysis of a polysaccharide by partial acid hydrolysis [2] depends on the production of oligosaccharide fragments, but the constitution of these depends on the relative stabilities to acid hydrolysis of the glycosidic linkages in the polysaccharide. Factors governing this stability include the sugar involved, and the type and configuration of the glycosidic linkage.[3] In polysaccharide sulphates the presence of the sulphate group, which is itself acid-labile, may also influence the rate of hydrolysis of glycosidic linkages, but little is known regarding the magnitude of any such effects. The acid hydrolysis of some methyl glycoside sulphates has now been studied and compared with the hydrolysis of the parent glycosides and of the free sugar sulphates.

Hydrolysis of glycosidic linkages was followed by determination of the reducing power, and the liberation of sulphate by an alkalimetric method. For obvious reasons, it was impracticable to use sulphuric acid for the hydrolyses and hydrochloric acid at 1·5N-concentration was used throughout, corrections being made for loss of sugar due to degradation. Since the hydrolysis of a methyl glycoside sulphate to the free sugar, methanol, and sulphate is the result of two concurrent reaction sequences, a kinetic analysis of the results is difficult. For such an analysis, only the initial rates should be considered (*i.e.*, before the intermediate products have reached significant concentrations) but we are interested in partial hydrolysis studies, which involve a late stage in the overall reaction. The overall reaction is approximately of the first order since the graphical plot of log concentration against time is almost linear. Comparison may therefore be made between the apparent rate coefficients obtained from such a plot and the true rate coefficients for the hydrolysis of a glycoside or of a sugar sulphate. However, the results given in the Table for the methyl glycoside sulphates cannot be regarded as more than a semiquantitative guide to differences in reaction rates.

[1] Part II, *J.*, 1961, 1692.
[2] Peat, Whelan, and Edwards, *J.*, 1955, 358.
[3] Whistler and Smart, " Polysaccharide Chemistry," Academic Press Inc., New York, 1953, p. 54.

A study of the hydrolysis of methyl β-D-galactopyranoside 6-sulphate at various temperatures showed that at 35° no appreciable hydrolysis occurred; at 65° both glycosidic and ester linkages were hydrolysed at a convenient rate for study, and at 100° the rates were considerably accelerated. Between 65° and 100°, the increase in the rate of glycoside hydrolysis was approximately the same as that of ester hydrolysis, indicating that changes in reaction temperature do not appreciably alter the relative rates of hydrolysis of the two

Acid Hydrolysis of Glycose and Glycoside Monosulphates.

Compounds	Reaction temp.	Rate coefficients × 10^5 (sec.$^{-1}$) Ester hydrolysis	Glycoside hydrolysis
Methyl β-D-galactopyranoside 6-sulphate * ...	65°	2·21	1·68
	100	39·1	27·9
Methyl β-D-galactopyranoside	65	—	13·04
D-Galactose 6-sulphate	65	1·86	—
	100	33·2	—
Methyl α-D-glucopyranoside 3-sulphate * ...	65	4·38	0·64
Methyl α-D-glucopyranoside	65	—	1·82
Methyl β-D-glucopyranoside 3-sulphate * ...	65	1·92	1·43
Methyl β-D-glucopyranoside	65	—	4·09
D-Glucose 3-sulphate	65	5·91	—

* Apparent rate coefficients (see text).

linkages (see Table). A more interesting effect is shown by a comparison of the glycoside hydrolysis in a glycoside sulphate with that in the parent glycoside. Thus the rate of hydrolysis of methyl β-D-galactopyranoside is several times faster than that for methyl β-D-galactopyranoside 6-sulphate; for methyl α- and β-D-glucopyranosides the rates are appreciably faster than for their 3-sulphates, but the effect is less pronounced than that shown by the galactoside 6-sulphate. It is apparent, therefore, that a sulphate group stabilises the glycosidic linkage and that a 6-sulphate shows a greater stabilising effect than a 3-sulphate.

The reason for this stabilising effect is not clear but a possible explanation can be given. Recent work [4] has shown that the rate-determining step in the acid-catalysed hydrolysis of glycosides probably involves the formation at position 1 of a carbonium ion from the conjugate acid of the glycoside. This stage probably involves a change in conformation from a chair to a half-chair form [5] and it has been suggested that, during this stage, bulky substituents in equatorial positions increase the non-bonded interactions or repulsions and, as a consequence, increase the stability of these glycosides to acid hydrolysis. Both a 3-sulphate on glucose and a 6-sulphate on galactose occupy equatorial positions in the C1 conformation of these sugars and therefore come within this category.

A further point of interest in this study was the effect of a glycosidic linkage on the rate of hydrolysis of the sulphate group (Table). For methyl α-D-glucopyranoside-3-sulphate compared with glucose 3-sulphate, and for the galactoside 6-sulphate compared with galactose 6-sulphate, the effect was very small. Only in the case of methyl β-D-glucopyranoside 3-sulphate did the presence of a glycosidic linkage decrease appreciably the rate of hydrolysis of the sulphate group. The reason for this is not clear.

In view of the reported racemisation of optically active s-butyl hydrogen sulphate when treated with acid,[6] both of the methyl glucoside sulphates and D-glucose 3-sulphate were completely hydrolysed and the products examined. The only sugar product detected in each case was glucose. From galactose 6-sulphate and the galactoside 6-sulphate only galactose is expected and this was verified.

From these results it is concluded that it should be possible to isolate mono- and oligosaccharide sulphates from partial acid-hydrolysates of sulphated polysaccharides. The

[4] Banks, Meinwald, Rhind-Tutt, Shaft, and Vernon, *J.*, in the press.
[5] Foster and Overend, *Chem. and Ind.*, 1955, 566; Edward *ibid.*, 1955, 1102.
[6] Burwell, *J. Amer. Chem. Soc.*, 1945, **67**, 220.

recent isolation of galactose 6-sulphate from one seaweed mucilage [7] and the production of oligosaccharide sulphates from another [8] support this view. It is further suggested that, during the early stages of hydrolysis of a sulphated polysaccharide, there would be a tendency for oligosaccharides with sulphate on sugar units other than the reducing-end unit to accumulate.

Experimental

Preparative and Analytical Techniques.—These were as previously described.[1]

Methyl α-D-glucopyranoside 3-(ammonium sulphate), having $[\alpha]_D^{18}$ +78·6° (*c* 2, in H_2O) and m. p. 165—166° (decomp.) (Found: S, 11·7. $C_7H_{17}NO_9S$ requires S, 11·1%), was isolated from the products obtained by sulphation of methyl α-D-glucoside. When it (0·15 g.) was oxidised in 4—5 molar excess of 46mM-periodate at room temperature the following results were obtained:

Time (hr.)	0·33	0·66	1·00	19
Periodate consumed (mol.)	0·00	0·08	0·17	0·22
Formic acid liberated (mol.)	0·05	0·07	0·08	0·13

These results are consistent with a methyl glucopyranoside 3-sulphate.

Methyl β-D-glucopyranoside 3-(ammonium sulphate), similarly isolated, had $[\alpha]_D^{16}$ −13·3° (*c* 1 in H_2O) and m. p. 155° (decomp.) (Found: S, 11·3%). Periodate oxidation, carried out as above, gave the following results:

Time (hr.)	1·0	5·0	24	48
Periodate consumed (mol.)	0·0	0·0	0·0	0·0
Formic acid liberated (mol.)	0·0	0·0	—	—

These results are in excellent agreement with expectation for methyl β-D-glucopyranoside 3-(ammonium sulphate).

Acid Hydrolysis.—Samples (1 mmole) of various glycoses, glycose monosulphates, glycosides, and glycoside monosulphates were dissolved in 1·5N-hydrochloric acid (100 ml.), previously heated to the temperature of the experiment, and kept at the selected temperature. Serial samples (*ca.* 5 ml.) were withdrawn, quickly cooled in ice-water to stop hydrolysis, and then allowed to equilibrate to room temperature.

The free sulphate was determined by mixing a portion (1 ml.) of the cooled reaction mixture in a Pyrex centrifuge tube (10 ml. capacity) with benzidine reagent [9] (5 ml.). After being kept for 5 min. at room temperature, the tube was immersed in ice-water for 10 min. and then centrifuged at 3000 r.p.m. for 5 min. The supernatant liquid was carefully decanted and the precipitate washed twice with 95% (v/v) aqueous ethanol (5 ml.) and centrifuged as above. To the precipitate was then added hot carbonate-free distilled water (2—2·5 ml.) and Phenol Red solution (0·01% in water; 0·1 ml.), and the tube was placed in a boiling-water bath. After a few minutes most of the precipitate usually dissolved. The tube was then removed from the bath, and a fine Pyrex jet was introduced into the tube for the delivery of carbon dioxide-free air to stir the mixture, which was titrated against 2mM-sodium hydroxide until the yellow colour of the indicator underwent the first change of colour. The tube was then placed in the boiling-water bath for 0·5—1 min. to ensure complete dissolution of the benzidine sulphate. The titration was continued if the yellow colour returned. For each determination duplicate readings were taken and each reading was corrected by subtracting a blank value obtained by titrating indicator and carbonate-free distilled water in the same way. The inorganic sulphate in terms of moles of sulphate per 100 ml. was derived by multiplying the titre figure by 10^{-4}. Control experiments had shown that the recovery of sulphate by this procedure varied from 98 to 103%.

The reducing power was determined on a portion (1—2 ml.) of the cooled reaction mixture by the Somogyi titrimetric method,[10] a correction being made for the loss of reducing power through destruction of sugar by hydrochloric acid.

[7] Turvey and Rees, *Nature*, 1961, **189**, 831.
[8] Painter, *Chem. and Ind.*, 1959, 1488.
[9] "The B.D.H. Book of Organic Reagents for Analytical Use," The British Drug Houses Ltd., London, 8th edn., p. 16.
[10] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.

Complete Acid Hydrolysis of Glycose and Glycoside Monosulphates.—Quantities of the sugar sulphates were separately hydrolysed in 1·5N-hydrochloric acid for 5 hr. The hydrolysates were then cooled, neutralised with silver carbonate, and filtered and the soluble silver was removed with hydrogen sulphide. The solutions were again filtered and the clear filtrates separately concentrated under reduced pressure. Samples of the residues were examined by partition chromatography on paper and by ionophoresis. The only sugar detected in each case was the parent sugar.

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