973. *The Properties of Some Sulphated Derivatives of* D-*Glucose and* D-*Galactose.*

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The properties of some sulphated derivatives of D-glucose and D-galactose have been investigated, including their behaviour on paper chromatography and ionophoresis. The sulphate group can be removed by reduction with lithium aluminium hydride, the parent sugar being regenerated with no trace of deoxy-sugar. The sulphate group in 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose 3-(barium sulphate) resists the action of aqueous sodium or potassium borohydride at temperatures up to 100°. On periodate oxidation of D-glucose 3-(sodium sulphate) and D-galactose 6-(sodium sulphate) the sulphate group does not behave as a simple blocking group.

STRUCTURAL studies on sulphated polysaccharides are complicated by the presence of the sulphate group which makes purification as well as complete acetylation or methylation difficult. Acidic hydrolysis usually leads to simultaneous removal of the sulphate groups and hydrolysis of the glycosidic links, whilst alkali often degrades the molecule severely. In the present work on monosaccharide sulphates we sought information likely to be of value in structural studies on sulphated polysaccharides.

Lithium aluminium hydride has already been used for the reductive fission of carbohydrate nitrates,[1] toluene-*p*-sulphonates,[2] and methanesulphonates,[3] with the regeneration in most cases of the parent alcohol group. The parent alcohol is also regenerated by the action of lithium aluminium hydride on a secondary toluene-*p*-sulphonate, but a deoxy-group is produced by the reduction of a primary toluene-*p*-sulphonate. It has already been reported [4] that the sulphate group is removed from a variety of monosaccharide sulphates by lithium aluminium hydride in refluxing dioxan, and this has now been applied to the barium salts of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose 3-sulphate, αβ-methyl D-glucofuranoside 3-sulphate, 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose 6-sulphate, αβ-methyl D-galactoside 6-sulphate, and β-methyl D-galactoside 2-sulphate: in all cases the parent alcohol was obtained, yields being 43—60%, and no deoxy-sugar could be detected in the product. The retention of isopropylidene and methyl glycosidic groups

[1] Ansell and Honeyman, *J.*, 1952, 2778.
[2] Schmid and Karrer, *Helv. Chim. Acta*, 1949, **32**, 1371.
[3] Smith, *J.*, 1957, 2690.
[4] Grant and Holt, *Chem. and Ind.*, 1959, 1942.

during these reductions is in agreement with the results of other workers. Endres and Oppelt [5] found that α-methyl D-glucoside and salicin were not reduced by lithium aluminium hydride, and Abdel-Akher and Smith [6] reduced 2,3:5,6-di-*O*-isopropylidene-D-mannose to 2,3:5,6-di-*O*-isopropylidene-D-mannitol. The sulphate group in 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose 3-(barium sulphate) was not reduced by the action of aqueous sodium or potassium borohydride at temperatures up to 100°: examination by paper chromatography indicated that there had been some removal of the 5,6-*O*-isopropylidene residue.

The action of periodate on D-glucose 3-(sodium sulphate) and on D-galactose 6-(sodium sulphate) was studied. In both cases the results indicated that the sulphate group does not behave as a simple blocking group, as may be seen by comparison with oxidations of the corresponding *O*-methyl sugars. D-Glucose 3-(sodium sulphate) consumed 1 mol. of 0·1M-sodium periodate at room temperature in the first 3 hr. and a second mol. in 26 hr.; acid was produced, 0·8 equiv. having been formed after 30 hr. These results may be compared with the reaction of 3-*O*-methyl-D-glucose with periodate [7] which, under similar conditions, consumed one mol. of periodate without the liberation of acid. D-Galactose 6-(sodium sulphate) consumed 2·9 mols. of 0·1M-sodium periodate at room temperature within a few minutes and even after 30 hr. this value had not increased; after 20 hr. 2·8 equiv. of acid had been liberated. This may be compared with the reaction of 6-*O*-methyl-D-galactose with periodate [8] which destroyed 4 mols. of periodate. When these periodate oxidations were carried out at 5° the consumption of periodate and the liberation of acid were both slower. When D-glucose 3-(sodium sulphate) was treated with 0·35M-periodate for seven days in the dark no sulphate ions could be detected in the solution, but the reaction of D-galactose 6-(sodium sulphate) with 0·35M-periodate under the same conditions led to the liberation of all the sulphate as sulphate ions. Even with 0·1M-periodate D-galactose 6-(sodium sulphate) liberated 57% of the sulphate in an ionised form in 48 hr.

The application of paper and column chromatography [9,10] and of paper ionophoresis [10] to sugar sulphates has already been described, but only in qualitative terms. We have measured the chromatographic and ionophoretic mobilities of a number of sulphated derivatives of D-glucose and D-galactose, the results being given in Tables 1 and 2. Each R_G value is the average of at least four determinations; the maximum variation was

TABLE 1. R_G *values of sulphated derivatives of* D-*glucose and* D-*galactose* (*acid solvent*).

Derivative	R_G
1,2:5,6-Di-*O*-isopropylidene-D-glucofuranose 3-(barium sulphate)	3·69
1,2-*O*-Isopropylidene-D-glucofuranose 3-(barium sulphate)	1·75
D-Glucose 3-(barium sulphate)	0·37
1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose 6-(barium sulphate)	3·47
D-Galactose 6-(barium sulphate)	0·20
D-Galactose 2-(barium sulphate)	0·18

about 6%. The reducing sugar sulphates gave well-defined circular spots. The spots from the monoisopropylidene derivative were long ellipses, and those from the di-isopropylidene derivatives were long ill-defined streaks.

Ionophoresis was investigated in borate (pH 10), phosphate (pH 10), and acetate (pH 4) buffers. With phosphate and acetate buffers sodium D-glucuronate was used as a reference standard, and the mobilities obtained are referred to as $M_{\text{Glucuronate}}$ values. Each value quoted is the average of at least four determinations, except for two cases marked. The maximum variation was about 2%. The reducing sugars gave well-defined spots in

[5] Endres and Oppelt, *Chem. Ber.*, 1958, **91**, 478.
[6] Abdel-Akher and Smith, *Nature*, 1950, **166**, 1037.
[7] Barker and Smith, *Chem. and Ind.*, 1952, 1035.
[8] Pascu and Trister, *J. Amer. Chem. Soc.*, 1940, **62**, 2301.
[9] Lloyd, *Nature*, 1959, **183**, 109.
[10] Turvey and Clancy, *Nature*, 1959, **183**, 537.

TABLE 2. *Ionophoretic mobilities of sulphated derivatives of* D-*glucose and* D-*galactose.*

Derivative	M_G Borate buffer	$M_{Glucuronate}$ Phosphate buffer	$M_{Glucuronate}$ Acetate buffer
1,2:5,6-Di-*O*-isopropylidene-D-glucofuranose 3-(barium sulphate) ...	0·84	0·88 *	0·75
D-Glucose 3-(barium sulphate)	1·20	0·95	0·82
1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose 6-(barium sulphate)	0·80	0·86 *	0·78
D-Galactose 6-(barium sulphate)	1·29	0·90	0·80
D-Galactose 2-(barium sulphate)	1·32		

* Average of two determinations.

borate and phosphate buffers, but in acetate buffer there was a tendency to streak. The isopropylidene derivatives gave diffused spots or streaks in all three buffers.

EXPERIMENTAL

Paper-partition chromatography was done on Whatman No. 1 filter paper by the descending front technique with upper layers of the following (v/v) solvent systems: (1) butan-1-ol–ethanol–water (4 : 1 : 5), (2) butan-1-ol–glacial acetic acid–water (4 : 1 : 5). Solvent (2) was boiled under reflux for 1 hr. before use.[11] The chromatograms were developed by spraying with (A) benzidine trichloroacetate or (B) aniline hydrogen phthalate. Isopropylidene derivatives were detected by spraying first with trichloroacetic acid in methanol [12] and then with spray (B).

Paper ionophoresis was carried out on Whatman No. 1 filter paper under potential gradients of 25—40 v/cm., with the following buffer solutions: (1) borate buffer (pH 10) [boric acid (7·44 g.) dissolved in 0·1N-sodium hydroxide (1 l.)], (2) phosphate buffer (pH 10) [disodium hydrogen phosphate (8·905 g.) and sodium hydroxide (0·33 g.) dissolved in water (1 l.)], and (3) acetate buffer (pH 4) [0·2N-acetic acid (800 ml.) mixed with 0·2N-sodium acetate (200 ml.)]. The papers were developed in the same manner as for chromatography.

Action of Lithium Aluminium Hydride on 1,2:5,6-*Di*-O-*isopropylidene*-D-*glucose* 3-(*Barium Sulphate*).—To 1,2:5,6-di-*O*-isopropylidene-D-glucose 3-(barium sulphate) [13] (1·87 g.) in dry dioxan (90 ml.) was added lithium aluminium hydride (1·87 g.), and the solution was boiled under reflux for 25 hr. More lithium aluminium hydride (0·50 g.) was added and the solution was boiled under reflux for a further 23 hr. Excess of lithium aluminium hydride was decomposed by water, and the solution was acidified with sulphuric acid and then neutralised with barium carbonate. The filtered solution was extracted three times with ether. Evaporation of the combined and dried extracts gave a syrup (0·65 g., 55%), which solidified in two days at room temperature. Paper chromatography revealed only one component, with a mobility corresponding to that of 1,2:5,6-di-*O*-isopropylidene-D-glucose. Recrystallisation from light petroleum (b. p. 60—80°) gave material (0·48 g.) of m. p. 104°. The mother-liquor from this recrystallisation was evaporated to dryness to give a gum, which was hydrolysed with 2N-sulphuric acid. Examination of this hydrolysate by paper chromatography indicated the presence of D-glucose only. It appears therefore that no deoxy-sugar had been formed. The crystalline product was recrystallised once more to give material with m. p. 107°, mixed m. p. 108° with 1,2:5,6-di-*O*-isopropylidene-D-glucose (Found: C, 55·75; H, 7·5. Calc. for $C_{12}H_{20}O_6$: C, 55·4; H, 7·7%).

Action of Lithium Aluminium Hydride on 1,2:3,4-*Di*-O-*isopropylidene*-D-*galactose* 6-(*Barium Sulphate*).—This sulphate [14] (1·59 g.) in dry dioxan (80 ml.) was treated with two portions of lithium aluminium hydride (1·59 g. + 0·74 g.) as before. The product, extracted from the aqueous solution with chloroform, was obtained as a gum (0·58 g.) that contained no sulphate. A small portion of it was hydrolysed and the hydrolysate examined by paper chromatography. Only one compound, with a mobility corresponding to that of galactose, was detected. The

[11] Edington and E. E. Percival, *J.*, 1955, 3554.
[12] Foster and Hancock, *J.*, 1957, 968.
[13] E. G. V. Percival, *J.*, 1945, 119.
[14] E. G. V. Percival and Soutar, *J.*, 1940, 1477.

gum was characterised as 1,2:3,4-di-*O*-isopropylidene-D-galactose by conversion into 1,2:3,4-di-*O*-isopropylidene-D-galactose 6-acetate,[15] m. p. 106—108° (m. p. given [13] 109—110°) (Found: C, 55·5; H, 7·3. Calc. for $C_{14}H_{22}O_7$: C, 55·6; H, 7·3%).

Action of Lithium Aluminium Hydride on αβ-*Methyl* D-*Glucofuranoside* 3-(*Barium Sulphate*).—This sulphate [13] (0·59 g.) in dry dioxan (80 ml.) was treated with two portions of lithium aluminium hydride (0·57 g. + 0·28 g.) as before. After destruction of the excess of hydride the solution was de-ionised with ion-exchange resins. The solvent was evaporated under reduced pressure to leave a gum (0·15 g.), which did not solidify. A small portion of it was hydrolysed and the hydrolysate examined by paper chromatography. Only one compound, with a mobility corresponding to that of glucose, was detected, and no sulphate was detected in the hydrolysate. The gum was characterised as α-methyl D-glucofuranoside by the preparation from it of the crystalline tetracarbanilate, m. p. 218—221° (Found: C, 63·1; H, 5·0; N, 8·4. Calc. for $C_{35}H_{34}N_4O_{10}$: C, 62·6; H, 5·2; N, 8·35%). A tetracarbanilate prepared from α-methyl D-glucofuranoside had the same m. p. and mixed m. p. as this product.

Preparation of β-*Methyl* D-*Galactopyranoside* 2-(*Barium Sulphate*).—Crude β-methyl 3,4-*O*-isopropylidene-6-trityl-D-galactopyranoside (1·75 g.), m. p. 135—142°, $[\alpha]_D$ −16·2° (*c* 3·4 in $CHCl_3$), was dissolved in dry pyridine (15 ml.), and pyridine–sulphur trioxide complex (2·5 g.) added. The mixture was kept at room temperature for 2 days, heated to 50—55° for 3½ hr., and poured into water; a white precipitate was formed. The suspension was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure to give a white solid (0·25 g.). This product gave a white precipitate with dilute sulphuric acid and also with hot dilute hydrochloric acid, showing the presence of both barium and sulphate. It was hydrolysed to β-methyl D-galactopyranoside 2-(barium sulphate) by leaving it in water (25 ml.) and 2N-sulphuric acid (5 ml.) at room temperature for 48 hr.; the solution was then neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure to give a white solid (0·18 g.).

Action of Lithium Aluminium Hydride on β-*Methyl* D-*Galactopyranoside* 2-(*Barium Sulphate*).—This sulphate (60 mg.) in dry dioxan (25 ml.) was treated with two portions of lithium aluminium hydride (50 mg. + 50 mg.) as before. After destruction of the excess of hydride, the solution was de-ionised and evaporated to dryness under reduced pressure to give a solid (15 mg.). After recrystallisation from ethanol this had m. p. 170—172° (Found: C, 43·1; H, 7·1. Calc. for $C_7H_{14}O_6$: C, 43·4; H, 7·2%), showing it to be β-methyl D-galactoside. The mother-liquor from the recrystallisation was evaporated to dryness, the residue hydrolysed with dilute mineral acid, and the hydrolysate examined by paper chromatography. Only one compound, with a mobility corresponding to that of galactose, could be detected.

Action of Lithium Aluminium Hydride on αβ-*Methyl* D-*Galactoside* 6-(*Barium Sulphate*).—This sulphate (0·39 g.) in dry dioxan (70 ml.) was treated with two portions of lithium aluminium hydride (0·40 g. + 0·19 g.) as before. After destruction of the excess of hydride the solution was de-ionised and evaporated to dryness under reduced pressure to give a gum (0·10 g.). This contained no barium and gave no sulphate on hydrolysis. The hydrolysate was examined by paper chromatography and the presence of one compound only, with a mobility corresponding to that of galactose, was indicated. Attempts to prepare the acetate were unsuccessful.

Action of Potassium and Sodium Borohydride on 1,2:5,6-*Di*-O-*isopropylidene*-D-*glucose* 3-(*Barium Sulphate*).—A solution of 1,2:5,6-di-*O*-isopropylidene-D-glucose (0·5 g.) in water (3 ml.) was added dropwise to an 8% aqueous solution (3 ml.) of potassium borohydride and the mixture was kept at room temperature for 24 hr. The excess of hydride was decomposed with 2N-sulphuric acid, and the resulting solution neutralised with barium carbonate and evaporated to dryness under reduced pressure. The residue was extracted with acetone, and the extract was filtered and evaporated to dryness. The product was examined by paper chromatography (acid solvent), two well-defined spots being obtained, whose mobilities corresponded one to that of the starting material and the other to that of 1,2-*O*-isopropylidene-D-glucose 3-(barium sulphate). This product gave sulphate ions on hydrolysis. The use of larger amounts of potassium borohydride at temperatures up to 100° gave similar results. When sodium borohydride was used at 100° paper chromatography of the product indicated the presence of 1,2-*O*-isopropylidene-D-glucose 3-(barium sulphate) but no starting material.

General Conditions for Periodate Oxidations.—All periodate oxidations were carried out in

[15] Hockett, Fletcher, and Ames, *J. Amer. Chem. Soc.*, 1941, **63**, 2516.

the dark. Samples (5 ml.) were removed at intervals for analysis. The periodate remaining in the solution was destroyed with sodium arsenite and the excess of arsenite back-titrated with iodine. The acid liberated was titrated with 0·01N-sodium hydroxide, to Methyl Red as indicator.

Sugar sulphates are most conveniently prepared and isolated as barium salts but, since barium periodate is insoluble, it is necessary to remove barium ions before carrying out periodate oxidations. Two methods of doing this were tried. The first was to add a slight excess of sodium sulphate to the carbohydrate barium sulphate and carry out the oxidation in the presence of the barium sulphate precipitated. The second was to obtain the sodium salt of the sugar sulphate by treating the barium salt with a cation-exchange resin and exactly neutralising the resulting acid solution with sodium hydroxide. Both methods gave satisfactorily reproducible results. Working with the sodium salts is complicated by the fact that they are very deliquescent.

Periodate Oxidations at Room Temperature.—Oxidation of D-*glucose* 3-(*sodum sulphate*). (*a*) To a solution of D-glucose 3-(barium sulphate (0·19 g.) in water (5 ml.) was added a slight excess of sodium sulphate followed by 0·1M-sodium metaperiodate (45 ml.).

Time (hr.)	0·5	1	2·5	4	6	24	30
Periodate consumed (mol.)	0·68	0·80	0·95	1·07	1·25	1·94	2·02

(*b*) To a solution of D-glucose 3-(barium sulphate) (0·06 g.) in water (2 ml.) was added a slight excess of sodium sulphate followed by 0·1M-sodium metaperiodate (28 ml.).

Time (hr.)	0·5	1·5	3	5	24
Acid liberated (equiv.)	0·09	0·24	0·39	0·43	0·74

Oxidation of D-*galactose* 6-(*sodium sulphate*). (*a*) To a solution of the sulphate (0·14 g.) in water (5 ml.) was added a slight excess of sodium sulphate followed by 0·1M-sodium metaperiodate (45 ml.).

Time (hr.)	0·5	1	2	4	6	24	30
Periodate consumed (mol.)	2·83	2·84	2·86	2·87	2·87	2·95	3·02

(*b*) To a solution of the sulphate (0·04 g.) in water (2 ml.) was added a slight excess of sodium sulphate followed by 0·1M-sodium metaperiodate (23 ml.).

Time (hr.)	0·5	2	5	24
Acid liberated (equiv.)	1·74	2·34	2·59	2·79

Periodate Oxidations at 5°.—Oxidation of D-*glucose* 3-(*sodium sulphate*). This sulphate (0·19 g.) was dissolved in 0·1M-sodium metaperiodate solution (80 ml.):

Time (hr.)	0·5	1·5	2·5	4	6	24	30	48
Periodate consumed (mol.)	0·64	0·76	0·81	0·95	0·99	1·36	1·57	1·95
Acid liberated (equiv.)	0·06	0·13	0·17	0·24	0·29	0·55	0·65	0·78

Oxidation of D-*galactose* 6-(*sodium sulphate*). This sulphate (0·16 g.) was dissolved in 0·1M-sodium metaperiodate solution (85 ml.):

Time (hr.)	0·5	1	2	4	6	24	30	48
Periodate consumed (mol.)	2·17	2·46	2·55	2·65	2·80	2·88	2·91	3·04
Acid liberated (equiv.)	1·25	1·58	1·85	2·14	2·24	2·47	2·55	

Estimation of sulphate liberated during oxidation of D-*glucose* 3-(*sodium sulphate*) *and* D-*galactose* 6-(*sodium sulphate*). Sulphate ions produced during the oxidation were estimated by precipitation as barium sulphate. Iodate and periodate ions were first removed by addition of dilute nitric acid followed by a slight excess of potassium iodide. The iodine produced was removed by extraction with chloroform.

D-Glucose 3-(sodium sulphate (0·15 g.) was dissolved in 0·35M-sodium metaperiodate solution (45 ml.). After 7 days no sulphate ions were detected in the solution.

D-Galactose 6-(sodium sulphate) (0·15 g.) was dissolved in 0·35M-sodium metaperiodate (45 ml.). The whole of the sulphate had been liberated as sulphate ions after 48 hr.

D-Galactose 6-(sodium sulphate) (0·06 g.) was dissolved in 0·1M-sodium metaperiodate (30 ml.). After 48 hr. 57% of the sulphate had been liberated as sulphate.

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