344. *Carbohydrate Sulphuric Esters. Part V. The Demonstration of Walden Inversion on Hydrolysis of Barium 1 : 6-Anhydro-β-D-Galactose 2-Sulphate.*

By R. B. Duff.

2 : 3-1 : 6-Dianhydro-β-D-talose (I) has been obtained by the alkaline hydrolysis of *barium 1 : 6-anhydro-β-D-galactose 2-sulphate* (II). An improved method is described for the preparation of carbohydrate sulphates.

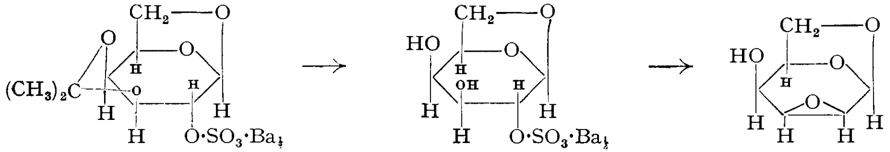
In Parts I (Percival and Soutar, *J.*, 1940, 1475) and II (Duff and Percival, *J.*, 1941, 830) it was shown that barium methylhexoside sulphates (*e.g.*, barium α-methylglucoside sulphate) could be hydrolysed by aqueous alkali to give 3 : 6-anhydromethylhexosides. In Part III (Percival, *J.*, 1945, 119) carbohydrate sulphates (*e.g.*, barium methylglucofuranoside 3-sulphates) in which the sulphate residue was adjacent to a *trans*-hydroxyl group, were studied. It was expected that ethylene oxide anhydrides would be formed on hydrolysis with aqueous alkali and that these would give recognisable products on complete hydrolysis. This was not realised and the conclusion was reached that for sulphates as distinct from toluene-*p*-sulphonates, ethylene oxide rings were not formed on alkaline hydrolysis, but that 3 : 6-anhydro-ring formation, together with the straightforward removal of the sulphate group, occurred.

In Part IV (Duff and Percival, *J.*, 1947, 1675), however, it was established that barium 3-methyl 1 : 2-*iso*propylidene glucofuranose 6-sulphate readily gave 3-methyl 1 : 2-*iso*propylidene 5 : 6-anhydroglucofuranose on treatment with sodium methoxide in methanol. This barium salt was chosen as it was substituted at $C_{(3)}$ and thus the formation of a 3 : 6-anhydride either directly or through the 5 : 6-anhydride (cf. Seebeck, Meyer, and Reichstein, *Helv. Chim. Acta*, 1944, **27**, 1142) was prevented. Further, the material was soluble in organic solvents, and previous difficulties due to decomposition of the ethylene oxide anhydride in aqueous alkali were avoided. As the sulphate residue was attached to $C_{(6)}$, no Walden inversion could be demonstrated with the above material, and attempts to prepare anhydrides, with inversion, from barium 4 : 6-benzylidene α-methylglucoside sulphate and barium 6-methyl β-methylgalactopyranoside 2-sulphate were unsuccessful. In the latter case treatment with sodium methoxide in methanol resulted in extensive decomposition with the production of reducing substances. It was evident that the adjacent sulphate group rendered the glycosidic methoxyl labile to alkali, as in the similar case of the hydrolysis of a 2-hydroxyethanesulphonic acid glycoside recorded by Helferich and Schnorr (*Annalen*, 1941, **547**, 201).

Therefore, in the present work, attention was directed to the synthesis of a carbohydrate sulphate with a grouping on $C_{(1)}$ more stable to alkali than in the previous case. The compound chosen, *barium* 1 : 6-*anhydro*-β-D-*galactose* 2-*sulphate* (II) was obtained from *barium* 3 : 4-iso*propylidene* 1 : 6-*anhydro*-β-D-*galactose* 2-*sulphate* (III), formed on sulphating 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose which was obtained by the method of Hann and Hudson (*J. Amer. Chem. Soc.*, 1942, **64**, 2435). Although (II) was soluble only to a limited extent in methanol, it reacted smoothly with a solution (8%) of sodium in dry methanol at 55° to give 1 : 6-2 : 3-dianhydro-β-D-talose in 36% yield. It was necessary to recrystallise the dianhydride five times to obtain physical and analytical data in agreement with the values given by James *et al.* (*J.*, 1946, 625) and to give no depression in melting point when mixed with an authentic specimen prepared from 1 : 6-anhydro-β-D-galactose 2-toluene-*p*-sulphonate.

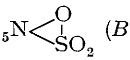
When (II) was heated at 100° for 24 hours with methanolic sodium methoxide, some charring took place and it was necessary to isolate the partly methylated products by continuous extraction. The methoxyl content (11·5—13·4%) of these fractions indicated an impure monomethyl 1 : 6-anhydrohexose (Calc. : 14·35%). That the latter was probably 2-methyl 1 : 6-anhydro-β-D-galactose was indicated by paper chromatography (Partridge, *Nature*, 1946, **158**, 270) in which comparable R_G values were obtained from the above syrups and from authentic 2-methyl 1 : 6-anhydrogalactose after hydrolysis with acid to the corresponding 2-methyl hexose. No evidence was obtained of the presence of 3-methyl 1 : 6-anhydro-β-idose which could also be obtained from the intermediate dianhydrotalose.

An attempt was made to prepare the crystalline 2-methyl 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose from a portion of the syrup. Crystalline material was obtained by cooling with ether and solid carbon dioxide, but attempts to raise the melting point did not succeed owing to difficulty in finding a suitable solvent for recrystallisation and to the small yield. The melting point (30°) of the product was not depressed on mixing with an authentic specimen (m. p. 37°).



(III.) (II.) (I.)

On hydrolysis with aqueous alkali, (II) would be expected to give 2 : 3-1 : 6-dianhydro-β-D-talose (I), followed by 1 : 6-anhydro-β-D-galactose and/or 1 : 6-anhydro-β-D-idose. Paper chromatography was used to investigate this reaction, the material obtained by alkaline hydrolysis of (II) and of an authentic specimen of 2 : 3-1 : 6-dianhydro-β-D-talose being hydrolysed to the corresponding sugars by dilute sulphuric acid. In both cases spots with an R_G value corresponding to that of galactose were observed, but evidence of the formation of idose could not be obtained.

Baumgarten's pyridine sulphuric anhydride,  $C_5H_5N{<}\overset{O}{\underset{SO_2}{|}}$ (*Ber.*, 1926, **59**, 1166, 1978) was used as a sulphating agent in this work. The method used previously involved adding a solution of chlorosulphonic acid in chloroform to a pyridine solution of the sugar at −15°. The mixture was slowly diluted with water, excess sulphuric acid removed by barium carbonate, and chloride ions by silver sulphate.

Pyridine sulphuric anhydride has not previously been used as a sulphating agent for carbohydrates. There is usually little heat evolved on mixing the reagent with a pyridine solution of the carbohydrate derivative, and the temperature can be maintained in the range 15—50°. The mixture is diluted with water, free sulphuric acid removed by barium carbonate, and the filtrate evaporated, to give the pure barium salt of the sugar sulphate. The use of large volumes of silver sulphate solution is thus avoided. When glucose was converted into barium glucose monosulphate by this method, a yield of 96% was obtained, which compares favourably with values previously quoted.

As noted (Percival and Duff, *Nature*, 1946, **158**, 29), realisation of Walden inversion on hydrolysis of carbohydrate sulphates in the laboratory makes it necessary to envisage the interconversion of sugars in nature *via* the ethereal sulphates and ethylene oxide anhydrides.

A mechanism for the conversion of glucose into galactose in animals by way of the 5-phosphate has been indicated by Peat (" Advances in Carbohydrate Chemistry," 1946, II, 54), and the occurrence of 4-sulphates of carbohydrates in nature may have significance for a similar mechanism in plants.

EXPERIMENTAL.

Barium 3 : 4-iso*Propylidene* 1 : 6-*Anhydro-β*-D-*galactose* 2-*Sulphate*.—3 : 4-*iso*Propylidene 1 : 6-anhydro-β-D-galactose (7·73 g.) was dissolved in dry redistilled pyridine (150 c.c.), and pyridine sulphuric anhydride (20 g., 3·3 mols.) added with shaking. The mixture was stirred with a vigorous current of nitrogen and heated at 50° for 2½ hours, and then poured, with stirring, into excess of a suspension of barium carbonate in water. After removal of barium salts by filtration, the solution was evaporated to dryness at 30°/15 mm. in the presence of a little barium carbonate, and the residue dried with alcohol and benzene. Unchanged material was removed by four extractions with hot ethyl acetate. Dissolution in water, followed by filtration and evaporation to dryness, gave the pure *barium* salt (11·9 g., 89%), $[\alpha]_{\rm D}^{15^\circ}$ −35·0° (*c*, 1·9 in water) (Found : Ba, 20·2 ; SO_4, 28·4 ; acetone, 14·2. $C_9H_{13}O_8SBa_{\frac{1}{2}}$ requires Ba, 20·8 ; SO_4, 29·1 ; acetone, 16·6%).

Barium 1 : 6-*Anhydro-β*-D-*galactose* 2-*Sulphate*.—Barium 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose 2-sulphate (11·6 g.) in water (70 c.c.) was treated with 0·2N-sulphuric acid (300 c.c.), and the mixture was kept at 37° for 48 hours. Sulphuric acid was removed as usual and the *product* isolated by evaporation at 30°/15 mm. (8·9 g.) ; $[\alpha]_{\rm D}^{15^\circ}$ −10·6° (*c*, 1·2 in water) (Found : Ba, 22·3 ; SO_4, 30·7 ; acetone, 0·2. $C_6H_9O_8SBa_{\frac{1}{2}}$ requires Ba, 22·2 ; SO_4, 31·0%).

Hydrolysis of Barium 1 : 6-*Anhydro-β*-D-*galactose* 2-*Sulphate with Sodium Methoxide*.—The preceding barium salt (3·6 g.) was heated under reflux for 30 minutes with anhydrous methanol (100 c.c.) but did not dissolve completely. Sodium (5·0 g.), dissolved in methanol (60 c.c.), was added to the cooled suspension, and the mixture heated at 55° for 2 hours while being stirred with a current of nitrogen. Insoluble salts were removed by centrifugation, and the supernatant liquor neutralised (phenolphthalein) with carbon dioxide, water being added from time to time to dissolve precipitated sodium carbonate. The solution was extracted with chloroform (ten times), the combined extracts (1 l.) were dried (Na_2SO_4), and the filtered solution was evaporated at 35°/15 mm. The dry residue was extracted three times with ethyl acetate, and the combined extracts were evaporated to small bulk. Light petroleum (b. p. 40—60°) was added, and the solution decanted from precipitated gummy material. Further addition of light petroleum gave a cloudy liquid which deposited needles (0·6 g., 36%), m. p. 122—124°, when kept at 0°. Purified by recrystallisation five times from ethyl acetate and light petroleum (b. p. 40—60°), the product had m. p. 131—132°, not altered on admixture with authentic 1 : 6-2 : 3-dianhydro-β-D-talose prepared from 1 : 6-anhydro-β-D-galactose 2-toluene-*p*-sulphonate (Found : C, 50·2 ; H, 5·8. Calc. for $C_6H_8O_4$: C, 50·0 ; H, 5·6%), $[\alpha]_{\rm D}^{18^\circ}$ −88·0° (*c*, 1·4 in water). James *et al.* (*loc. cit.*) give m. p. 132° and $[\alpha]_{\rm D}^{20^\circ}$ −88° (*c*, 0·76 in water).

In another experiment the barium salt (1·97 g.) was heated under reflux overnight with a solution of sodium (1·5 g.) in methanol (40 c.c.). The solution was neutralised with carbon dioxide, diluted with water, and transferred to a continuous-extraction apparatus. Extraction with light petroleum (b. p. 40—60°) for 4 days, followed by removal of the solvent, gave a syrup (0·026 g.) (Found : OMe, 11·5%). A similar operation with the use of benzene gave a pale syrup (0·109 g.) (Found : OMe, 13·4%). Finally the solution was extracted continuously with chloroform for 4 days, to give a clear syrup (0·195 g.) (Found : OMe, 12·7%). The material extracted as above was thought to be an impure methyl anhydrohexose (Calc. : OMe, 14·35%), and the fractions were further investigated on the paper chromatogram (Partridge, *loc. cit.*) with tetramethyl glucose as standard. In a typical experiment the syrup (from the benzene extract) gave spots of R_G 0·094 and 0·214. The syrup (from the chloroform extract) gave spots of R_G 0·094 and 0·212. 2-Methyl galactose containing a little galactose (from 2-methyl 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose) gave spots of R_G 0·093 and 0·215.

In the above experiments the 1 : 6-anhydrides were hydrolysed to the sugars by treatment for 18 hours at 100° in a sealed tube with 2N-sulphuric acid. Acid was then removed with barium carbonate, the mixture centrifuged, and the supernatant liquor spotted on the chromatogram. The R_G values found agree reasonably well with those given by Brown *et al.* (*Nature*, 1948, **161**, 720) for galactose (R_G 0·075) and 2-methyl galactose (R_G 0·205). In a further attempt to characterise the syrup the material (0·090 g.) from the chloroform extract was converted into the *iso*propylidene compound by the method of Ohle and Thiel (*Ber.*, 1933, **66**, 525) as modified by McPhillamy and Elderfield (*J. Org. Chem.*, 1939, **4**, 150). The product was distilled at 110—120° (bath-temperature)/0·01 mm. ; it crystallised on freezing with solid carbon dioxide–ether ; yield, 0·030 g. ; m. p. 30°, unchanged on mixing with authentic 2-methyl 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose, m. p. 37°.

Hydrolysis of Barium 1 : 6-*Anhydro-β*-D-*galactose* 2-*Sulphate with Aqueous Alkali*.—The barium salt (0·06 g.) was heated for 18 hours at 100° in a sealed tube with barium hydroxide (1·04 g.) and water (2 c.c.). The solution was neutralised (phenolphthalein) with carbon dioxide, the filtrate taken to dryness at 30°/15 mm., and the syrupy residue heated for 18 hours at 100° with 2N-sulphuric acid (5 c.c.). After removal of sulphuric acid with barium carbonate and evaporation of the filtrate, the residue was dissolved in a few drops of water and examined by paper chromatogram.

1 : 6-2 : 3-Dianhydro-β-D-talose (0·043 g.) was treated similarly to provide a standard. In both cases spots with R_G 0·075, corresponding to galactose, were obtained, and idose, if formed, was not separated.

Barium Glucose Monosulphate.—Glucose (10 g.) was mixed at room temperature with dry pyridine (150 c.c.) and pyridine sulphuric anhydride (13·7 g., 1·5 mol.) (prep. : "Inorganic Syntheses," 1946, **11**, 173, McGraw-Hill, New York). The mixture was shaken at intervals for 4 hours and kept overnight in a stoppered flask ; sulphuric acid was then removed by adding water (200 c.c.) and barium carbonate (50 g.) with agitation. The solution was heated at 50° for 2 hours to complete neutralisation, and excess of barium salts removed by filtration. The filtrate was evaporated to small bulk at 35°/15 mm. and poured into absolute alcohol (1 l.) with mechanical stirring, and the precipitate was dried *in vacuo* over phosphoric oxide ; yield, 17·5 g. (96%), $[\alpha]_{\rm D}^{24^\circ}$ +32° (*c*, 2·9 in water at equilibrium) (Found : Ba, 20·5 ; SO_4, 29·8. Calc. for $C_6H_{11}O_9SBa_{\frac{1}{2}}$: Ba, 21·0 ; SO_4, 29·3%).

2-*Methyl* 3 : 4-iso*Propylidene* 1 : 6-*Anhydro-β-*D*-galactose.*—3 : 4-*iso*Propylidene 1 : 6-anhydro-β-D-galactose was methylated three times with Purdie's reagents. The product had m. p. 37°.

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