148. *Carbohydrate Sulphuric Esters. Part II. The Isolation of 3 : 6-Anhydromethylhexosides from Methylhexopyranoside Sulphates.*

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It is shown that alkaline hydrolysis of the barium salts of α- and β-methylglucoside sulphates and α- and β-methylgalactoside sulphates and of α-methylmannopyranoside sulphate yields the corresponding 3 : 6-anhydromethylhexosides.

IN Part I (J., 1940, 1475), the hydrolysis of barium α-methylglucoside sulphate with barium hydroxide was shown to yield an anhydromethylhexoside, m. p. 105—106°, $[\alpha]_D^{18°}$ + 52°. This has now been identified as 3 : 6-anhydro-α-methylglucopyranoside as shown by a comparison of its properties and those of its derivatives (I) with those reported by Haworth, Owen, and Smith (this vol., p. 88) in an extensive study of the properties of this substance (II).

(I.)	(II.)
Anhydro-α-methylhexoside, m. p. 108°, $[\alpha]_D^{15°}$ +52° in water.	3 : 6-Anhydro-α-methylglucopyranoside, m. p. 108°, $[\alpha]_D^{20°}$ +56° in water.
Anhydrohexose, m. p. 121°, $[\alpha]_D$ +53° in water.	3 : 6-Anhydroglucose, m. p. 122°.
Anhydrohexonolactone, m. p. 116—117°.	3 : 6-Anhydrogluconolactone, m. p. 116°.
Dimethyl anhydromethylhexoside, m. p. 64°, $[\alpha]_D^{15°}$ +48° in water.	2 : 4-Dimethyl 3 : 6-anhydro-α-methylglucopyranoside, m. p. 66°, $[\alpha]_D^{20°}$ +50°.
Anhydromethylhexoside obtained after contact with N-H_2SO_4, m. p. 69°, $[\alpha]_D^{15°}$ +163° in water.	3 : 6-Anhydro-α-methylglucofuranoside, m. p. 70°, $[\alpha]_D^{20°}$ +164°.

In attempts to isolate the anhydromethylhexoside without isolating the barium ethereal sulphate, a variable proportion of 3 : 6-anhydro-α-methylglucofuranoside accompanied the product. The formation of this substance is attributed to isomerisation of the pyranoside during the neutralisation of the strongly acid reaction mixture.

Barium β-methylglucopyranoside sulphate yielded 3 : 6-anhydro-β-methylglucopyranoside on alkaline hydrolysis, and the barium salts of α- and *β-methylgalactopyranoside sulphates* yielded the corresponding 3 : 6-anhydromethylgalactosides as shown by direct comparison with authentic specimens. The anhydromethylhexosides reported as derived from α-methylgalactoside in Part I (*loc. cit.*) proved to be impure 3 : 6-anhydro-α-methylglucopyranoside due to the fortuitous presence of glucose in the specimen of galactose used. *Barium α-methylmannopyranoside sulphate* by similar treatment yielded the 3 : 6-anhydro-α-methylmannopyranoside of Valentin (*Coll. Czech. Chem. Comm.*, 1934, **6**, 354), confirmed by the isolation of 3 : 6-anhydroglucosazone from the free sugar.

In the five cases examined, therefore, alkaline hydrolysis of the sulphates obtained by direct interaction of chlorosulphonic acid in pyridine and the methylhexosides yields a 3 : 6-anhydromethylhexoside; this conversion is not complete, however, since in every case some methylhexoside accompanies the product. It is clear, consequently, that either the anhydride formation is not quantitative or the substances examined are mixtures of more than one sulphate, one capable of hydrolysis to the 3 : 6-anhydride and the other to the methylhexoside. The sulphate group responsible for the production of the 3 : 6-anhydride must, however, be attached to C_3 or C_6. The latter is considered to be the more likely, since if, as would be expected, the hydrolysis of a sulphate follows a similar course to that of a *p*-toluenesulphonate, if the residue were on C_3 the 3 : 6-anhydride would be accompanied by other products containing ethylene oxide rings (Peat and Wiggins, J., 1938, 1088), and no evidence for these was found.

Agar contains a proportion of 3 : 6-anhydro-*l*-galactose residues in its molecule, and although it has been pointed out (Hands and Peat, *Chem. and Ind.*, 1938, **57**, 937; Forbes and Percival, J., 1939, 1844) that the sulphate content of the agar used is too low to account for the formation of these residues by hydrolysis during methylation, nevertheless it is well known that a fairly high proportion of sulphur occurs in the soluble fraction of agar (Neuberg and Schwietzer, *Monatsh.*, 1937, **71**, 1, 146). The elimination of sulphate residues with the formation of 3 : 6-anhydro-rings may have taken place at some stage in the development of the alga or in the manufacture of the commercial product.

EXPERIMENTAL.

Barium Methylhexoside Sulphates.—The method described in Part I (*loc. cit.*) was modified by the use of 1 mol. of chlorosulphonic acid, and in certain cases the method of working up described by Helferich, Löwa, Nippe, and Riedel (*Z. physiol. Chem.*, 1923, **128**, 141) was employed. The products were all extracted thoroughly with boiling alcohol and reprecipitated from aqueous solution with alcohol. The yields were approximately equal to the weight of methylhexoside used.

Barium α-methylglucoside sulphate. It was not found possible to obtain a product with $[\alpha]_D$ + 90° as described in Part I (*loc. cit.*). On several occasions a specimen apparently analytically pure had $[\alpha]_D^{18^\circ}$ + 72° in water, and lower values were obtained when more than 2 mols. of chlorosulphonic acid were used. When 1 mol. of chlorosulphonic acid was used, the barium α-methylglucoside sulphate had $[\alpha]_D^{17^\circ}$ + 81° in water (*c*, 2·0) in good agreement with the value reported by Helferich *et al.* (*loc. cit.*) for the sulphuryl chloride method [Found : Ba, 19·3; OMe, 8·5. Calc. for $(C_7H_{13}O_9S)_2Ba$: Ba, 19·6; OMe, 9·1%].

Barium β-methylglucoside sulphate had $[\alpha]_D^{20^\circ}$ − 12° in water (*c*, 1·0) (Found : Ba, 19·0%).

Barium β-methylgalactoside sulphate had $[\alpha]_D^{17^\circ}$ − 12° in water (*c*, 3·4) (Found : Ba, 18·5%).

Barium α-methylmannoside sulphate had $[\alpha]_D^{18^\circ}$ + 38° in water (*c*, 1·0) (Found : Ba, 18·5%).

Typical Hydrolysis with Barium Hydroxide.—Barium α-methylglucoside sulphate (6·5 g.) was heated for 8 hours at 100° with barium hydroxide (50 g.) in water (300 c.c.). After cooling and filtration the excess of alkali was neutralised with carbon dioxide, and the filtrate evaporated to dryness at 35°/15 mm. Several extractions with alcohol, followed by removal of solvent, gave a syrup (2·35 g.), $[\alpha]_D^{14^\circ}$ + 60° in water (*c*, 1·4). A portion (1·57 g.) of this syrup was extracted thrice with ethyl acetate and α-methylglucoside (m. p. and mixed m. p. 156°) (0·6 g.) was removed. The solution on evaporation gave a syrup (0·9 g.), from which, after treatment with light petroleum, the anhydromethylhexoside (0·43 g.), m. p. 108°, $[\alpha]_D^{15^\circ}$ + 52° in water (*c*, 1·0), crystallised on cooling (Found : C, 48·0; H, 6·9; OMe, 17·4. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·8; OMe, 17·6%). This substance was identified as 3 : 6-anhydro-α-methylglucopyranoside as follows :

Reaction with sulphuric acid. $[\alpha]_D^{13^\circ}$ + 89° (1 min.); + 115° (2 mins.); + 129° (3 mins.); + 140° (8 mins.); + 148° (17 mins.); + 150° (20 mins.); + 145° (35 mins.); + 135° (24 hours) in N-sulphuric acid (*c*, 0·5). When the reaction was arrested after 20 minutes by neutralisation with barium carbonate, a product was obtained which after recrystallisation from ethyl acetate had m. p. 69°, $[\alpha]_D^{16^\circ}$ + 163° in water (*c*, 0·9) (Found : OMe, 16·6%), and was evidently the 3 : 6-anhydro-α-methylglucofuranoside of Haworth, Owen, and Smith (*loc. cit.*).

In another experiment the reaction mixture was heated after standing for 20 minutes until the rotation became constant; the product isolated in good yield in the usual way had m. p. 121°, $[\alpha]_D$ + 53° in water (*c*, 1·0) (Found : C, 44·5; H, 6·2; OMe, nil. Calc. for $C_6H_{10}O_5$: C, 44·7; H, 6·3%). That this was 3 : 6-anhydroglucose was confirmed by conversion into the lactone, m. p. 116—117°, by oxidation with bromine water.

Methylation Experiments.—Two methylations with Purdie's reagents converted the anhydromethylhexoside quantitatively into an oil which crystallised on distillation at 100°/0·1 mm. and on recrystallisation from light petroleum had m. p. 64°, $[\alpha]_D^{14^\circ}$ + 110° in acetone (*c*, 0·3), + 48° in water (*c*, 1·0) (Found : C, 52·7; H, 8·0; OMe, 44·9. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·9; OMe, 45·6%). $[\alpha]_D^{14^\circ}$ + 43·5° (1 min.); 20·5° (6 mins.); 13° (8 mins.); 3° (11 mins.); − 5° (20 mins.); − 22·5° (4 hrs.) constant value, in 0·33N-hydrochloric acid (*c*. 0·4).

This dimethyl methylhexoside was recovered in 90% yield after boiling for 24 hours with sodium methoxide (5%) in methyl alcohol, and oxidation with nitric acid gave no dimethoxysuccinic or -glutaric esters. This agrees with the properties of 2 : 4-dimethyl 3 : 6-anhydro-α-methylglucopyranoside (Haworth, Owen, and Smith, *loc. cit.*).

Shortened Preparation of the Anhydromethylhexosides.—α-Methylglucoside ($[\alpha]_D^{14^\circ}$ + 156·5°) (30 g.) was treated with chlorosulphonic acid (10 c.c.) in pyridine as before. As much pyridine as possible was removed under diminished pressure and the solution was then made alkaline with hot concentrated barium hydroxide solution, pyridine was extracted with ether, and barium hydroxide solution (600 c.c.) was added to the mixture, which was then heated at 100° for 8 hours. Most of the barium hydroxide was removed on cooling, and recrystallisation was carried out four times in an attempt to remove occluded products. The combined mother-liquors were treated with carbon dioxide, and the isolation of the anhydromethylhexosides carried out as usual by evaporation, extraction with alcohol, evaporation and extraction with ethyl acetate. These operations yielded α-methylglucoside, 3 : 6-anhydro-α-methylglucopyranoside (3·5 g.), and a product (2 g.). After removal of the first two substances a crystalline substance (1 g.), m. p. 71° (not depressed on admixture with 3 : 6-anhydro-α-methylglucofuranoside), $[\alpha]_D^{15^\circ}$ + 161° in water (*c*, 0·7), was obtained together with a syrup (1 g.) (Found : C, 47·5; H, 7·15; OMe, 17·8. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·8; OMe, 17·6%). The product did not react with sodium methoxide. Hydrolysis with hot N-sulphuric acid yielded 3 : 6-anhydroglucose, m. p. 120°, $[\alpha]_D^{15^\circ}$ + 53° in water (*c*. 1·0). Methylation yielded a dimethyl anhydromethylhexoside, m. p. 45—46°, $[\alpha]_D^{18^\circ}$ + 204° in water (*c*, 0·7) (Found : C, 52·9; H, 8·0. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·9%), which did not react with sodium methoxide. These results are in agreement with those of Haworth, Owen, and Smith (*loc. cit.*) for 3 : 6-anhydro-α-methylglucofuranoside.

The accompanying syrup, $[\alpha]_D^{18^\circ}$ + 113° in water (*c*, 1·0), was thrice methylated and on distillation an oil (0·7 g.), $n_D^{19^\circ}$ 1·4610, $[\alpha]_D^{17^\circ}$ + 156° (Found : OMe, 40·1%), was obtained which was unchanged after boiling for 48 hours with methyl-alcoholic sodium methoxide (5%); ethylene-oxide ring compounds were therefore absent.

When 3 : 6-anhydro-α-methylglucopyranoside in excess of barium hydroxide solution was added to a solution of chlorosulphonic acid in pyridine in the proportions used in these experiments, the product, after being worked up in the usual way, contained *ca.* 70% of the corresponding furanoside. It is concluded, therefore, that it was at this point that the isomerisation took place and the possibility that the methylglucoside sulphate is present as furanoside is discounted.

Hydrolysis of Barium β-*Methylglucopyranoside Sulphate.*—When the hydrolysis was carried out on this sulphate (10 g.) as described above, a syrup was obtained, from which, after solution in ethyl acetate, β-methylglucoside crystallised (0·2 g.). On distillation at 170—175°/0·01 mm. a syrupy product (2·5 g.), $n_D^{17^\circ}$ 1·4920, was obtained, $[\alpha]_D^{16^\circ}$ − 136° in water (*c*, 1·3) (Found: OMe, 17·4%), which appeared to be identical with the 3 : 6-anhydro-β-methylglucopyranoside of Haworth, Owen, and Smith (*loc. cit.*). $[\alpha]_D^{17^\circ}$ − 110° (1 hr.); − 78° (4 hrs.); − 55° (8·6 hrs.), − 16° (22 hrs.), in 0·1N-sulphuric acid (*c*, 1·0). After neutralisation with barium carbonate, followed by filtration and evaporation under reduced pressure, 3 : 6-anhydroglucose, m. p. 122°, unchanged on admixture with an authentic specimen, $[\alpha]_D^{17^\circ}$ + 52° in water (*c*, 1·0), was obtained.

Hydrolysis of Barium β-*Methylgalactopyranoside Sulphate.*—The sulphate (13 g.) was hydrolysed at 100° with barium hydroxide solution as before. The alcohol-soluble portion of the hydrolysate was repeatedly extracted with ethyl acetate; the extracts deposited β-methylgalactoside (2 g.), m. p. 173°, followed by 3 : 6-anhydro-β-methylgalactoside (4 g.), m. p. 114—116° alone or mixed with an authentic specimen. Concentration of the mother-liquors and treatment with light petroleum yielded further small quantities of this substance and no other product could be detected.

In another experiment using the shortened process, β-methylgalactoside (20 g.) gave 3 : 6-anhydro-β-methylgalactoside (5 g.), m. p. 118°, $[\alpha]_D^{19^\circ}$ − 113·5° in water (*c*, 1·0). No other products could be isolated. Conversion into the free sugar, followed by osazone formation, readily gave 3 : 6-anhydrogalactosazone in good yield, m. p. 216°, unchanged on admixture with an authentic specimen.

Hydrolysis of Barium α-*Methylgalactopyranoside Sulphate.*—A similar result was obtained in this case, both the direct and the shortened method being used, although owing to its greater solubility the yields of crystalline 3 : 6-anhydro-α-methylgalactopyranoside were smaller. This had m. p. 140°, unchanged on admixture with an authentic specimen. $[\alpha]_D$ + 78° in water (*c*, 1·0); + 73° (12 mins.); + 61° (17 mins.); + 57° (77 mins.); + 43° (115 mins.); + 25° (7 hrs.) (constant value), in N/10-sulphuric acid (*c*, 1·0) (cf. Haworth, Jackson, and Smith, J., 1940, 620). The free sugar was converted into the osazone and 3 : 6-anhydrogalactosazone, m. p. 216—217°, was readily obtained.

The filtrates after the removal of 3 : 6-anhydro-α-methylgalactoside deposited small quantities of the product, m. p. 107°, $[\alpha]_D^{15^\circ}$ + 50° in water, described in Part I (*loc. cit.*). In dilute acid the rotation changed rapidly in the same way as for 3 : 6-anhydro-α-methylglucopyranoside and this was confirmed by the isolation of 3 : 6-anhydro-α-methylglucofuranoside, m. p. 69°, unchanged on admixture with the product obtained from α-methylglucoside. The depression of m. p. with the anhydromethylhexoside isolated from methylglucoside sulphate was not confirmed.

Hydrolysis of Barium α-*Methylmannoside Sulphate.*—The sulphate (1·2 g.) was hydrolysed with excess of barium hydroxide solution as before. The alcohol-soluble fraction on extraction with ethyl acetate yielded α-methylmannopyranoside, m. p. 188°, and the filtrate deposited crystals (0·5 g.) of a product, m. p. 130°, $[\alpha]_D^{19°}$ + 95° in water (*c*, 3·0) (Found : C, 47·5; H, 7·0; OMe, 17·8. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·8; OMe, 17·6%). Valentin (*loc. cit.*) reports m. p. 130—132°, $[\alpha]_D$ + 97° for 3 : 6-anhydro-α-methylmannopyranoside.

Hydrolysis, followed by osazone formation, gave an osazone, m. p. 182—183°, identical with that prepared from 3 : 6-anhydromethylglucoside (Found : N, 16·0. Calc. for $C_{18}H_{20}O_3N_4$: N, 16·5%).

It was shown that a large excess of barium hydroxide solution had no effect on α-methylglucoside when heated for long periods at 100° and 3 : 6-anhydro-α-methylglucopyranoside was recovered unchanged in good yield after being heated for 30 hours at 100° with this reagent.

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