31. *Carbohydrate Sulphuric Esters. Part III. The Hydrolysis of* iso*Propylidene Glucofuranose Sulphates and Methylglucofuranoside Sulphates.*

By E. G. V. Percival.

The alkaline hydrolysis of the barium salts of 1 : 2-monoacetone glucofuranose-6-sulphate (I), 1 : 2-monoacetone glucofuranose-3-sulphate (II), diacetone glucofuranose-3-sulphate (III), *methylglucofuranoside-6-sulphates* (IV) and *methylglucofuranoside-3-sulphates* (V) has been studied. The sulphate residue of (I) was rapidly eliminated with the production of 1 : 2-monoacetone 3 : 6-anhydroglucofuranose (VI) and 1 : 2-monoacetone glucofuranose (VII), whereas (II) was hydrolysed very slowly to give (VII) only, the rate of hydrolysis being approximately identical with that of (III). The sulphate residues on (IV) and (V) were readily removed and derivatives of 3 : 6-anhydroglucose (VIII) and of glucose (IX) were isolated in both cases.

The conclusion is reached that for sulphates, as distinct from toluene-*p*-sulphonates, ethylene oxide rings are not formed on treatment with alkali, whereas 3 : 6-anhydro-rings are formed [except for (II)]. The significance of these results in connection with the carragheen polysaccharide is discussed.

In our studies on carragheen moss (Buchanan, Percival, and Percival, J., 1943, 51) we assumed that the hydrolysis of sulphuric ester residues would follow a course similar to that of toluene-*p*-sulphonic esters in the sugar group. The sole experimental evidence for this assumption was the isolation of 3 : 6-anhydromethylhexosides from the products of the alkaline hydrolysis of methylglycopyranoside sulphates (Part II, J., 1941, 830). No information was available, however, as to whether the hydrolysis of a carbohydrate sulphate with a suitably placed hydroxyl group would follow the course, familiar for the toluene-*p*-sulphonates, of ethylene oxide ring formation with the accompanying Walden inversion, or not. Owing to the insolubility in non-aqueous solvents of the salts of carbohydrate sulphates, it is not at present feasible to answer this question directly by the isolation of a product containing such a ring system, on treatment with sodium methoxide; but reliance may be placed on an analysis of the products of complete hydrolysis with alkali, since the ethylene oxide ring compounds, if formed, should be decomposed in two ways with the concomitant inversions on both carbon atoms concerned (Peat, *Ann. Reports*, 1939, 258).

From the experiments described below, it appears that ethylene oxide rings are not formed on the alkaline hydrolysis of sulphates, but that in most cases 3 : 6-anhydro-ring formation together with removal of the sulphate group, without inversion, take place.

Ohle and von Vargha (*Ber.*, 1928, **61**, 1203; 1929, **62**, 2435) showed that treatment of 1 : 2-monoacetone glucofuranose-6-toluene-*p*-sulphonate with sodium methoxide gave 1 : 2-monoacetone 5 : 6-anhydroglucose, which on hydrolysis with aqueous alkali yielded 1 : 2-monoacetone glucofuranose (VII) and 1 : 2-monoacetone *l*-idofuranose. As pointed out by Peat (*loc. cit.*), this indicates a preferential formation of the 5 : 6- as against the 3 : 6-anhydride, for 3 : 6-anhydride formation takes place only when the hydroxyl group on C_5 is substituted as in the conversion of 1 : 2-monacetone 5 : 6-ditoluene-*p*-sulphonyl glucose into 1 : 2-monoacetone 3 : 6-anhydro-5-toluene-*p*-sulphonyl glucose by Ohle, von Vargha, and Erlbach (*Ber.*, 1928, **61**, 1211). When, however, barium 1 : 2-monoacetone glucofuranose-6-sulphate (I) was treated with alkali, almost equal amounts of 1 : 2-monoacetone 3 : 6-anhydroglucofuranose (VI) and 1 : 2-monoacetone glucofuranose (VII) were obtained and no *l*-idose derivatives could be detected; here, therefore, we have evidence of the preferential formation of a 3 : 6- as against a 5 : 6-anhydride, contrary to the case of the toluene-*p*-sulphonate.

This observation led to a study of the hydrolysis of barium 1 : 2-monoacetone glucofuranose-3-sulphate (II), but this compound proved to be remarkably resistant to hydrolysis (Table I), in sharp contrast to the

Table I.

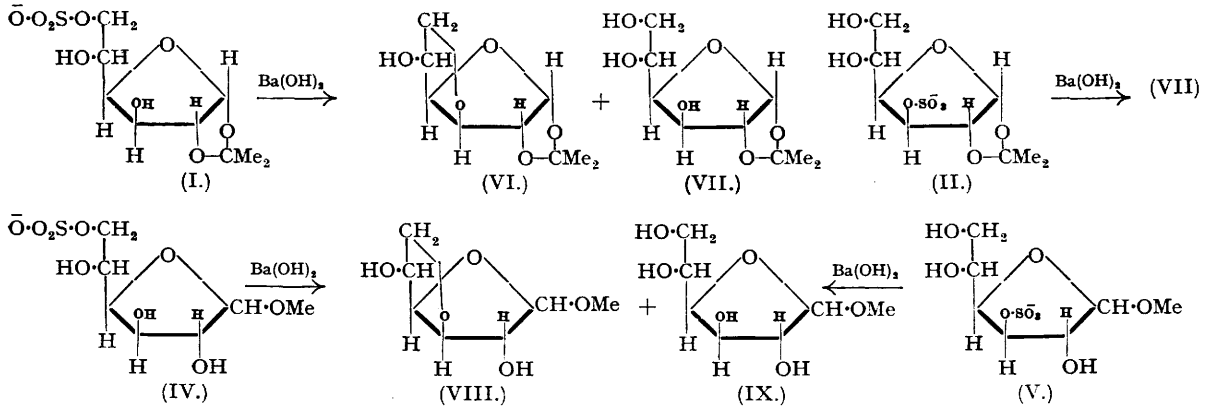
	Time (hours).	% Hydrolysis (mean) in 2·83N-NaOH at 100°.		Time (hours).	% Hydrolysis (mean) in 2·83N-NaOH at 100°.
Barium monoacetone glucose-6-sulphate	0·75	83	Barium methylglucofuranoside-6-sulphates	0·5	55
	1·5	85		1·5	76
	3·5	92		2·5	86
Barium monoacetone glucose-3-sulphate	16	3	Barium methylglucofuranoside-3-sulphates	0·82	55
	47	12		1·5	76
	54	10		3·75	87
Barium diacetone glucose-3-sulphate	15	2			
	47	12·5			

corresponding 6-sulphate, and 1 : 2-monoacetone glucofuranose (VII) was the only product isolated. Apart from the difficulty of hydrolysis this is parallel to the behaviour of the corresponding toluene-*p*-sulphonate, for von Vargha (*Ber.*, 1936, **69**, 2098) obtained 1 : 2-monoacetone glucose quantitatively on hydrolysis of this compound at 100° for 5 hours in aqueous-alcoholic sodium hydroxide (1·25N). Barium diacetone glucofuranose-3-sulphate (III) underwent hydrolysis at the same rate as the monoacetone derivative (cf. Part I, J., 1940, 1475, for the hydrolysis of barium diacetone galactopyranose-6-sulphate), a fact which supports the view that there is no interaction between the sulphate group on C_3 and the hydroxyl group on C_6 when 1 : 2-monoacetone glucofuranose-3-sulphate is treated with alkali.

These results appear to show that 3 : 6-anhydride formation cannot take place when the hydroxyl group on C_3 is esterified when a furanose ring is present. The behaviour of barium 1 : 2-monoacetone glucofuranose-3-sulphate (II) appears to be anomalous, however, since the corresponding derivative of the methylglucofuranosides (V) readily released the sulphate group on treatment with alkali and the products of hydrolysis clearly contained 3 : 6-anhydromethylglucofuranosides (VIII) and methylglucofuranosides (IX), since on suitable treatment 3 : 6-anhydroglucosazone and 3 : 6-anhydrogluconamide, together with the corresponding glucose derivatives, were isolated. Whether the stability of (II) is due to the fact that both the hydroxyl groups on C_2 and C_4 are occupied or is a consequence of the *iso*propylidene bridge between C_1 and C_2 must be left for further investigation.

The alkaline hydrolysis of the barium methylglucofuranoside-6-sulphates (IV) followed a similar course to that of (V), both in speed and in the products formed.

The yield of glucosazone (*ca.* 50%) and 3 : 6-anhydroglucosazone (*ca.* 20%) of the total amount of crude osazone obtained in both cases made necessary a careful search for other hexosazones, to determine whether ethylene oxide ring formation had played a part in the elimination of the sulphate residues. The barium methylglucofuranoside-6-sulphates (IV) would have been expected to yield the corresponding 5 : 6-anhydride, which should have given eventually glucosazone and *l*-sorbosazone (from *l*-idose) in about equal quantities (Ohle and von Vargha, *loc. cit.*) on further hydrolysis and osazone formation, but no sorbosazone was detected.

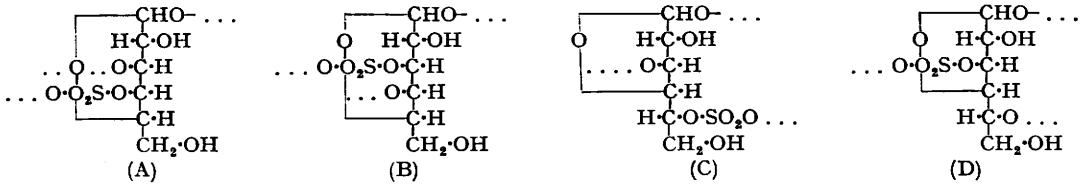


The corresponding 3-sulphate (V) would have given 2 : 3-anhydromethylallofuranosides (Peat and Wiggins, J., 1938, 1088), which should have yielded methylaltro- and methylgluco-furanosides. It is important to note the conclusion of Peat and Wiggins (J., 1938, 1810) that the proportion of methylaltrosides in the products of the alkaline hydrolysis of 2 : 3-anhydromethylallopyranosides is always far higher than that of the accompanying methylglucosides, in fact Robertson and Griffith (J., 1935, 1196) and Robertson and Dunlop (J., 1938, 472) were unable to isolate glucose derivatives in experiments of a similar nature. If ethylene oxide ring formation had taken place to any extent, therefore, on hydrolysis of (V) and suitable treatment allosazone should have been produced in sufficient quantity for recognition, but none was found. There is thus no evidence for the intermediate formation of ethylene oxide rings in the alkaline hydrolysis of (IV) and (V) and it is therefore held that the methylglucofuranosides (IX) are formed by the direct fission of the sulphate residues.

Part of the difficulty in obtaining a high yield of 3 : 6-anhydroglucosazone was found to be due to a *product*, m. p. 108—110°, which was isolated from the hydrolysis products of both the barium methylglucofuranoside-6-sulphate and the corresponding 3-sulphate after removal of the 3 : 6-anhydroglucosazone. This material appeared to be a hexosazone ($C_{18}H_{22}O_4N_4$). When carefully purified 3 : 6-anhydroglucose was subjected to osazone formation, however, of the total crude osazone obtained, *ca.* 20% was found to consist of the osazone usually designated 3 : 6-anhydroglucosazone, m. p. 187—189°, $[\alpha]_D^{17°}$ −151° in methanol, and about the same quantity of the osazone, m. p. 108—110°, which was isolated from (IV) and (V) was isolated from the mother-liquors. This substance must have been produced from 3 : 6-anhydroglucose in the products of hydrolysis of of the methylglucofuranosidesulphates (IV) and (V). Further work is in progress to determine the structure of this substance, which is most probably a hydrate of an anhydroglucosazone, although it is not necessarily directly related to the osazone, m. p. 187—189°. In any case it should be remembered that the structure of " 3 : 6-anhydroglucosazone," which appears to be identical with the anhydroglucosazone of Diels and Meyer

(*Annalen*, 1935, **519**, 157), has not yet been settled (Diels, Meyer, and Onnen, *ibid.*, 1936, **525**, 94; Percival and Percival, J., 1937, 1320).

Returning to the carragheen polysaccharide, four possible formulæ for the repeating unit were postulated (Buchanan, Percival, and Percival, *loc. cit.*) on the evidence of the isolation of 2 : 6-dimethyl galactose from methylated fractions; it may now be added that this polysaccharide does not react with periodic acid, showing the absence of α-glycol groups, which is in agreement with these suggestions and also eliminates certain other theoretical possibilities.



Of these four, (A) was chosen on the ground of the stability to alkali of the sulphate residue, for it was then considered that (B) and (D) would give 3 : 6-anhydrides and (C) a 5 : 6-anhydride with rapid elimination of the sulphate group. The present results show, however, that this particular argument cannot be supported for structure (C). There is no reason, however, why such a structure should not give rise to a 2 : 5-anhydro-ring, and in any event it is unlikely that the carragheen polysaccharide contains galactofuranose units, if the comparison of the rate of hydrolysis with acid with the galactocarolose of Haworth, Raistrick, and Stacey (*Biochem. J.*, 1937, **31**, 640), a polysaccharide containing galactofuranose units, is valid.

Formulation (A) for the major portion of the building units of the carragheen polysaccharide rests therefore on a sounder basis than hitherto, since experimental evidence has now been provided that 3 : 6-anhydride formation can take place when the sulphate residue is located on C_3, at any rate in glucose derivatives.

Experimental.

Barium Diacetone Glucofuranose-3-sulphate.—Pure diacetone glucose (8 g.) in dry pyridine (80 c.c.) was cooled to −18° and a solution of chlorosulphonic acid (4 c.c.) in alcohol-free chloroform (25 c.c.) was added during 2 hours so that the temperature never exceeded −15°. After standing overnight at room temperature, the mixture was again cooled to −18°, and a mixture of water (5 c.c.) and pyridine (20 c.c.) added. Dilution with water followed, after which the mixture was made alkaline to phenolphthalein with saturated barium hydroxide solution, the excess of alkali then being removed by carbon dioxide. After evaporation to *ca.* 100 c.c. at 35°/15 mm. in the presence of barium carbonate a slight excess of saturated silver sulphate solution was added, the solution filtered, and silver removed by hydrogen sulphide in the presence of barium carbonate, and the hydrogen sulphide by aeration. After the solution had been made alkaline with barium hydroxide and carbon dioxide passed in, filtration and evaporation in the presence of barium carbonate at 35°/15 mm. was followed by repeated extraction with boiling light petroleum (b. p. 40—60°; 500 c.c.) to remove unchanged diacetone glucose (0·3 g.). The residue was extracted with boiling acetone, filtered, and concentrated to 20 c.c. The addition of ether (200 c.c.), followed by filtration and evaporation, yielded a glass which crystallised in needles on solution in water and evaporation at room temperature. After being washed with alcohol and dried in a vacuum over phosphoric oxide, the product (6·0 g.) had $[\alpha]_D^{16°}$ −10·5° (*c*, 2·4, in water) [Found: Ba, 16·5; S, 7·6; $(CH_3)_2CO$, 26·5. Calc. for $(C_{12}H_{19}O_9S)_2Ba,H_2O$: Ba, 16·5; S, 7·7; $(CH_3)_2CO$, 27·9%].

Barium 1 : 2-*Monoacetone Glucofuranose-3-sulphate.*—Barium diacetone glucose-3-sulphate (3 g.) was kept for 3 hours at 15° with sulphuric acid (60 c.c., 0·5N), made alkaline to thymolphthalein with barium hydroxide, treated with carbon dioxide, filtered, and evaporated at 35°/15 mm. The crystalline product was recrystallised by solution in water (2 c.c.) and alcohol (4 c.c.), followed by the gradual addition, with scratching, to the warm solution, of a mixture of ether and alcohol (2 : 1) until crystallisation commenced. On standing at 0°, filtration, washing with alcohol and drying in a vacuum over phosphoric oxide, the *anhydrous* form of the product previously described by Ohle (*Biochem. Z.*, 1923, **138**, 428) as crystallising with four molecules of alcohol was obtained (2 g.) and had $[\alpha]_D^{16°}$ −15° (*c*, 1·5, in water) [Found: Ba, 18·8; S, 8·8; $(CH_3)_2CO$, 15·7. Calc. for $(C_9H_{15}O_9S)_2Ba$: Ba, 18·6; S, 8·7; $(CH_3)_2CO$, 15·8%].

Barium 1 : 2-*Monoacetone Glucofuranose-6-sulphate.*—1 : 2-Monoacetone glucofuranose was prepared from diacetone glucose (20 g.) by keeping for 210 minutes in sulphuric acid (280 c.c., 0·5N), followed by the addition of barium hydroxide in excess and treatment as previously described. The dry residue was extracted several times with boiling neutral ethyl acetate, after a preliminary extraction with ether to remove any unchanged diacetone glucose. The ethyl acetate extracts on cooling gave pure 1 : 2-monoacetone glucofuranose, m. p. 162°, $[\alpha]_D^{16°}$ −12° (*c*, 2·8, in water).

This material (11 g.) in dry pyridine (60 c.c.) was treated with chlorosulphonic acid (3·7 c.c.) in dry chloroform (35 c.c.) as for the diacetone glucose-3-sulphate except that after removal of hydrogen sulphide the solution was evaporated to dryness in the presence of barium carbonate, and the residue repeatedly extracted with boiling acetone (500 c.c.). These extracts were concentrated to 50 c.c. and the addition of dry ether (100 c.c.) caused the precipitation of a barium salt fraction (I) (8 g.), which was dried over phosphoric oxide in a vacuum and had $[\alpha]_D^{18°}$ −3·8° (*c*, 2·7, in water) [Found: Ba, 18·6; S, 8·4; $(CH_3)_2CO$, 15·0%].

The residue from the acetone extraction was extracted with water, and evaporation of the aqueous solution after filtration gave a further quantity (5 g.) of material (II), which had $[\alpha]_D^{16°}$ −4·0° (*c*, 3·0, in water) [Found: Ba, 18·9; S, 8·6; $(CH_3)_2CO$, 15·2. Calc. for $(C_9H_{15}O_9S)_2Ba$: Ba, 18·6; S, 8·7; $(CH_3)_2CO$, 15·8%].

Fractions (I) and (II) appear from the analytical results to be the *anhydrous* form of barium 1 : 2-monoacetone glucofuranose-6-sulphate described by Ohle (*loc. cit.*) as containing 1 molecule of alcohol. The purpose of extracting with acetone was to remove any unchanged monoacetone glucose. That any such contaminant was present in (II) is highly unlikely and the similarity of (I) to (II) shows that no appreciable amount was contained in (I).

Barium Methylglucofuranoside-3-sulphates.—Barium monoacetone glucose-3-sulphate (7 g.) in sulphuric acid (200 c.c.; 0·2N) was kept at 38° for 48 hours. An excess of barium carbonate was then added, and the neutral solution filtered and concentrated at 35°/15 mm., to yield a reducing glass, which was extracted twice with boiling alcohol in the presence of barium carbonate. After dissolution in water and precipitation in alcohol, a white powder (5·8 g.) was obtained having $[\alpha]_D^{20°}$ +29° (*c*, 2·2, in water) which was *barium glucose-3-sulphate* [Found: Ba, 20·3; S, 9·6. $(C_6H_{11}O_9S)_2Ba$

requires Ba, 20·95; S, 9·8%]. This substance (4·5 g.) was shaken with dry methanolic hydrogen chloride (80 c.c.; 1·2%) until dissolved, and the reaction stopped by the addition of barium carbonate after 22 hours; the specific rotation was then approximately zero, and the solution was almost non-reducing. After evaporation at 30°/15 mm., in the presence of barium carbonate in a large flask, the residue was extracted with water and treated with silver sulphate solution, followed by hydrogen sulphide and barium carbonate in the usual way. On evaporation a glass, $[\alpha]_D^{20°}$ −7° (*c*, 2·0, in water), was obtained, which was extracted repeatedly with cold ethyl acetate until no residue was obtained on evaporation of the extracts. The solid remaining was purified by dissolution in hot alcohol and precipitation in a large volume of anhydrous ether. The amorphous *product* (2·6 g.) had $[\alpha]_D^{20°}$ −4° (*c*, 2·4, in water) and was an *αβ*-mixture of barium methylglucofuranoside-3-sulphates [Found: Ba, 20·2; S, 9·2; OMe, 9·8. $(C_7H_{13}O_9S)_2Ba$ requires Ba, 20·1; S, 9·4; OMe, 9·1%].

Barium Methylglucofuranoside-6-sulphates.—Barium glucose-6-sulphate was prepared from barium monoacetone glucose-6-sulphate (6 g.) as described for the corresponding 3-sulphate. The product (4·6 g.) had $[\alpha]_D^{17°}$ +30° (*c*, 0·9, in water) (cf. Ohle, *loc. cit.*) [Found: Ba, 20·2; S, 9·5. Calc. for $(C_6H_{11}O_9S)_2Ba$: Ba, 20·95; S, 9·8%].

This substance (4·2 g.) was treated with methanolic hydrogen chloride (90 c.c.; 1·2%) for 26 hours to give, after the treatment described above, an amorphous *product* having $[\alpha]_D^{20°}$ +4° (*c*, 1·5, in water), which was an *αβ*-mixture of barium methylglucofuranoside-6-sulphates [Found: Ba, 19·6; S, 9·3; OMe, 9·6. $(C_7H_{13}O_9S)_2Ba$ requires Ba, 20·1; S, 9·4; OMe, 9·1%].

Hydrolysis Experiments with Sodium Hydroxide (2·83N).—The substance (0·2—0·3 g.) together with barium chloride (0·2 g.) was dissolved in sodium hydroxide solution (5 c.c.: 2·83N) in a tube of Jena glass. The mixture, protected by a condenser and a soda-lime tube, was heated in a boiling water-bath for the time stated; it was then cooled, cautiously acidified with acetic acid, and filtered through a weighed sintered Gooch crucible, to give a direct estimate of the barium sulphate formed (*a*). The filtrate was acidified with concentrated hydrochloric acid and boiled for 4 hours to complete the hydrolysis of the sulphate residues not removed by the alkali, and the barium sulphate (*b*) weighed in the usual way after the addition of barium chloride solution. A parallel experiment (*c*) was carried out on a weighed portion of the material under test, the alkaline hydrolysis being omitted. The results are expressed as the ratio of the weight of barium sulphate to the weight of starting material.

TABLE II.

	Time (hrs.).	*a*.	*b*.	*c*.	% Hydrolysis, 100*a*/*c*.	% Hydrolysis, 100 (1 − *b*/*c*).
Barium monoacetone glucose-6-sulphate	0·75	0·51	0·095	0·63	81	85
	1·5	0·53	0·085	,,	84	86·5
	3·5	0·57	0·04	,,	90·5	93·5
Barium monoacetone glucose-3-sulphate	16	0·02	0·62	0·64	3	3
	47	0·07	0·56	,,	11	12·5
Barium diacetone glucose-3-sulphate	15	0·01	0·545	0·56	2	3
	47	0·07	0·49	,,	12·5	12·5
Barium methylglucofuranoside-3-sulphates	0·82	0·40	0·28	0·67	60	58
	1·5	0·47	0·185	,,	70	73
	3·75	0·575	0·08	,,	86	88
Barium methylglucofuranoside-6-sulphates	0·5	0·375	0·31	0·68	55	54
	1·5	0·51	0·155	,,	75	77
	2·5	0·58	0·09	,,	85	87

Hydrolysis Experiments with Barium Hydroxide. Barium 1 : 2-*Monoacetone Glucofuranose-6-sulphate.*—Fraction II (4·0 g.) in water (150 c.c.) was heated for 15 hours at 100° with crystalline barium hydroxide (32 g.). Alcohol (150 c.c.) was then added, and the mixture cooled and filtered, the precipitate being washed several times with hot alcohol. Carbon dioxide was passed through the filtrate, which was then evaporated to dryness at 35°/15 mm. The residue was extracted with acetone, and removal of solvent gave a syrup (1·94 g.) which crystallised rapidly. After standing for some days and extraction with ether the crystalline material remaining (0·95 g.) consisted of 1 : 2-monoacetone glucofuranose, m. p. 158°, raised to 162° on recrystallisation from acetone–ether, not depressed by an authentic specimen, $[\alpha]_D^{17°}$ −11·8° (*c*, 3·0, in water). The ethereal extracts on evaporation gave a syrup (0·91 g.). A portion of the syrup (0·1 g.) was hydrolysed at 90° with 0·1N-sulphuric acid and treated with phenylhydrazine hydrochloride and sodium acetate. Needles of an osazone, m. p. 186°, were obtained, not depressed by 3 : 6-anhydroglucosazone.

The syrup crystallised completely after 2 weeks and was proved to be 1 : 2-monacetone 3 : 6-anhydroglucofuranose. Recrystallisation from ether–light petroleum gave needles, $[\alpha]_D^{16°}$ +29° (*c*, 1·3, in water), m. p. 55°, not depressed by an authentic specimen prepared from 1 : 2-monoacetone 5 : 6-ditosyl glucose (Ohle, v. Vargha, and Erlbach, *loc. cit.*).

The substance (0·7 g.) on hydrolysis with sulphuric acid (10 c.c., 0·1N) for 47 hours at 37° gave a crystalline product (0·45 g.), m. p. 121—122°, not depressed by an authentic specimen of 3 : 6-anhydroglucose, $[\alpha]_D^{17°}$ +53° (*c*, 1·5, in water).

In another experiment fraction (I) (4·5 g.) was hydrolysed for 19 hours as above to give after recrystallisation 1 : 2-monoacetone glucofuranose (0·88 g.) and 1 : 2-monoacetone 3 : 6-anhydroglucofuranose (0·82 g.).

Search for Idose Derivatives.—The mother-liquors from all the recrystallisations of the monoacetone glucofuranose (A) and the anhydro-compound (B) were hydrolysed separately and treated with phenylhydrazine acetate. Several crops of osazones were obtained from each and these were examined to see if *l*-sorbosazone was present.

Three crops of osazone (0·3 g.) from (A) had m. p. 192—195°, rising to 204° on recrystallisation, not depressed on admixture with glucosazone. An impure product, m. p. 175—180°, raised to 192° on recrystallisation, was obtained from the mother-liquor, but this was clearly not *l*-sorbosazone, m. p. 164°.

Two crops of osazone (0·2 g.) were obtained from (B), m. p. 160—178°, depressed to 140° on admixture with *l*-sorbosazone. Recrystallisation gave slightly impure 3 : 6-anhydroglucosazone, m. p. 182°, as the only identifiable product.

Barium 1 : 2-*Monoacetone Glucofuranose-3-sulphate.*—This substance (1·5 g.) in water (60 c.c.) was heated at 100° for 19 hours with barium hydroxide (10 g.). After removal of the excess of barium hydroxide, which partly crystallised on cooling, from solution by carbon dioxide, filtration and evaporation at 40°/15 mm. gave a product which contained no material extractable with boiling ether. The residue crystallised on treatment with water and on recrystallisation the starting material (1·0 g.), $[\alpha]_D^{16°}$ −14° (*c*, 2·0, in water), was obtained.

In a second experiment the crystalline barium salt (3 g.) in water (180 c.c.) was heated for 72 hours with barium hydroxide (33 g.) and treated as before. No ether-soluble products were obtained and recrystallisation of the residue from alcohol–water–ether gave the starting material (2·1 g.), $[\alpha]_D^{15°}$ −15° (*c*, 2·5, in water) (Found: Ba, 18·4%).

The mother-liquors were evaporated to give a solid which, when extracted with chloroform, gave a syrup (0·1 g.); this crystallised overnight and had m. p. 152°, raised to 162° on one recrystallisation, not depressed by monoacetone glucose.

Barium Diacetone Glucofuranose-3-sulphate.—Hydrolysis of this material (2·5 g.) as described above for 72 hours was followed by removal of barium hydroxide and evaporation at 40°/15 mm. The residue was extracted three times with boiling ether (200 c.c.); evaporation of the solution gave diacetone glucose (0·05 g.), m. p. 110°, $[\alpha]_D^{16°}$ −18·5° (*c*, 0·7, in water).

Barium Methylglucofuranoside-3-sulphates.—The substance (2·25 g.) in water (70 c.c.) was heated for 6 hours at 100° with crystalline barium hydroxide (20 g.) in an atmosphere of nitrogen, the subsequent working up being as described for the monoacetone-6-sulphate except that the residue, after the treatment with carbon dioxide, etc., was extracted with alcohol instead of acetone. By this means a syrup (0·81 g.; 67% of the theo.) was obtained (Found: OMe, 16·5%), $[\alpha]_D^{16°}$ +1° (*c*, 1·9, in 0·2N-sulphuric acid), rising to +45° after 7 hours at 100°. Neutralisation with barium carbonate, followed by evaporation and extraction with alcohol, etc., gave a reducing syrup (0·65 g.) which restored the colour to Schiff's reagent after 10 minutes.

Oxidation and Isolation of 3 : 6-Anhydrogluconamide.—The sugars (0·3 g.) in water (10 c.c.) were kept for 6 days with bromine (0·5 c.c.), which was then removed by aeration and the organic acids isolated in the usual way by neutralisation with silver carbonate, followed by treatment with hydrogen sulphide. The mixed acids in methanol were then esterified by excess of diazomethane dissolved in ether, and after removal of solvents the mixed esters were dissolved in saturated methanolic ammonia. After 2 days gluconamide (0·1 g.), m. p. 135°, raised to 144° on recrystallisation, was obtained. The mother-liquors after the removal of this amide crystallised to give an amide (0·05 g.), m. p. 148°, raised to 160° on recrystallisation, not depressed by 3 : 6-anhydrogluconamide. It showed $[\alpha]_D^{17°}$ +106° (*c*, 2·0, in water) (Found: N, 7·9. Calc. for $C_6H_{11}O_5N$: N, 7·9%).

Osazone Formation.—Another portion of the reducing sugars (0·3 g.) was treated with phenylhydrazine hydrochloride (0·6 g.), sodium acetate (1·0 g.), and sodium bisulphite (0·1 g.) in the usual way to give 3 crops of a crude osazone (0·25 g.), m. p. 130—195°. Glucosazone (0·12 g.), m. p. 209°, not depressed by an authentic specimen, separated on treatment with cold alcohol. The solution was allowed to evaporate, and the residue treated with warm acetonitrile. Crystals (0·02 g.), m. p. 185°, not depressed by 3 : 6-anhydroglucosazone, separated overnight (Found: N, 16·3. Calc. for $C_{18}H_{20}O_3N_4$: N, 16·5%).

The residue was recrystallised from aqueous alcohol several times to give an osazone (0·05 g.), m. p. 180—182°, m. p. 179° on admixture with 3 : 6-anhydroglucosazone (m. p. 187°), $[\alpha]_D^{18°}$ +140° (*c*, 0·3, in methanol), and was evidently mainly 3 : 6-anhydroglucosazone. The mother-liquors from which this material had been deposited, on warming and dilution with water deposited an *osazone* (0·01 g.), m. p. 107—109° (Found: N, 15·6. $C_{18}H_{22}O_4N_4$ requires N, 15·6%).

A second experiment on another portion (0·52 g.) of the reducing sugars obtained from barium methylglucofuranoside-3-sulphate gave a crude osazone (0·4 g.), from which glucosazone (0·20 g.), 3 : 6-anhydroglucosazone (0·07 g.), m. p. 186—187°, and the hexosazone (0·04 g.), m. p. 109°, but no other identifiable products were isolated. Special attention was paid to the fraction insoluble in cold alcohol, but this proved to be entirely glucosazone and no product obtained on fractional crystallisation resembling allosazone (m. p. 178°) could be detected, since no fraction had a m. p. lower than 205°.

Barium Methylglucofuranoside-6-sulphates.—The substance (3·5 g.) was hydrolysed with barium hydroxide as described above to give a syrupy mixture of methylglycosides (1·3 g.; OMe, 16·3%), $[\alpha]_D^{18°}$ +10° (*c*, 2·0, in 0·2N-sulphuric acid), raised to +50° on heating for 8 hours at 100°. The free sugars (1·0 g.) so obtained rapidly restored the colour to Schiff's reagent.

Isolation of 3 : 6-Anhydrogluconamide.—The bromine oxidation of 0·3 g. gave, on the treatment previously described, gluconamide (0·1 g.) and 3 : 6-anhydrogluconamide (0·06 g.), m. p. 159°, not depressed by an authentic specimen, $[\alpha]_D^{17°}$ +108° (*c*, 1·0, in water).

Osazone Formation.—The reducing sugars (0·6 g.) gave a crude osazone (0·47 g.), m. p. 160—200°, from which pure glucosazone (0·24 g.) was isolated on treatment with cold alcohol, 3 : 6-anhydroglucosazone (on treatment with acetonitrile as before), (0·03 g.), m. p. 187°, not depressed by an authentic specimen, and the hexosazone (0·05 g.), m. p. 108—110°, not depressed by the specimen prepared from the 3-sulphate.

The remaining osazone (0·1 g.) at one stage of its purification had m. p. 162—165°, but that it was not *l*-sorbosazone was shown by the fact that the m. p. fell to 135° on admixture with a specimen of that substance. On further recrystallisation (0·06 g.) the m. p. was raised to 176°, m. p. 174° on admixture with 3 : 6-anhydroglucosazone, $[\alpha]_D^{17°}$ −140° (*c*, 0·25, in methanol) (Found: N, 16·4%). This fraction was therefore mainly 3 : 6-anhydroglucosazone. From the mother-liquors the osazone (0·01 g.), m. p. 110°, was isolated.

Osazone Formation with Pure 3 : 6-Anhydroglucose.—3 : 6-Anhydroglucose (0·5 g.), m. p. 120—121°, thrice recrystallised, in water (20 c.c.) was heated for 4 hours with phenylhydrazine hydrochloride, sodium acetate and sodium bisulphite in the usual way. The crude osazone (0·56 g.) had m. p. 140—180°. Treatment with cold alcohol gave prisms (0·11 g.), m. p. 185—186°, raised to 187—188° on recrystallisation, $[\alpha]_D^{17°}$ −134° (*c*, 0·4, in ethanol), −150° (*c*, 0·4 in methanol) (Found: C, 63·2; H, 6·0; N, 16·4. Calc. for $C_{18}H_{20}O_3N_4$: C, 63·5; H, 5·9; N, 16·5%).

The alcoholic solution from which these crystals separated was warmed to 70° and diluted with water to turbidity; cooling gave an osazone, m. p. 177°, raised to 180—182° on recrystallisation. This product (0·2 g.) had not the crystalline form of the first product but appeared to consist of aggregates. Nevertheless the specific rotation was $[\alpha]_D^{18°}$ −147° (*c*, 0·4, in methanol) and the m. p. was not depressed on admixture with the first specimen. The filtrates from the recrystallisations, on warming and dilution with water, gave a pale yellow osazone (0·11 g.), flaky when dry, m. p. 106°, raised to 108—110° on recrystallisation, not depressed by the specimens prepared above from the methylglucofuranoside-3- and -6-sulphates (Found: C, 60·2; H. 5·9; N, 15·5. $C_{18}H_{22}O_4N_4$ requires C, 60·2; H, 6·2; N, 15·6%).

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