## 232. *Carbohydrate Phosphoric Esters. Part I. The Alkaline Hydrolysis of α-Methylglucopyranoside*-6-*phosphate, Methylglucofuranoside*-3-*phosphates and* iso*Propylidene Glucofuranose*-3- *and* -6-*phosphates.*

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The alkaline hydrolysis of barium α-methylglucopyranoside-6-phosphate, barium methylglucofuranoside-3-phosphates and barium *iso*propylidene glucose-3- and -6-phosphates has been studied and in no case was any evidence found of Walden inversion or anhydride formation.

Although Robinson (*Nature*, 1927, **120**, 44, 656) made the attractive suggestion that the hydrolysis of phosphoric esters with accompanying Walden inversion might account for the production in nature of *d*-galactose and *d*-ribose from glucose-4-phosphate and xylose-3-phosphate respectively, no evidence for Walden inversions in the hydrolysis of sugar phosphates has been recorded (Gardner, *J. Org. Chem.*, 1943, **8**, 111; Raymond, " Organic Chemistry," Gilman, New York, 1938, 1480).

In all cases where Walden inversions occur with sugar derivatives with the exception of compounds substituted on the potential reducing groups, the intermediate formation of a ring system such as the ethylene oxide ring is necessary, as in the hydrolysis of appropriately substituted toluene-*p*-sulphonates (Peat, *Annual Rep.*, 1939, 258). From experiments on the alkaline hydrolysis of carbohydrate sulphates it would appear that Walden inversions do not take place and ethylene oxide rings are not formed, although 3 : 6-anhydrides were isolated by the alkaline hydrolysis of the methylglycopyranosides of barium glucose-, galactose-, and mannose-6-sulphates, methylglucofuranoside-3- and -6-sulphates and 1 : 2-monoacetone glucofuranose-6-sulphate (Duff and Percival, *J.*, 1941, 830; Percival, this vol., p. 119). In the experiments here described, however, no evidence for the formation of anhydro rings of any kind or of Walden inversions could be found when phosphoric esters of certain glucose derivatives were hydrolysed with alkali. Furthermore the phosphate residues were removed with greater difficulty than for the corresponding sulphates. The results may be summarised thus : (1) barium α-methylglucopyranoside-6-phosphate gave α-methylglucopyranoside (47%) after hydrolysis for 72 hours with N-barium hydroxide solution at 100°; (2) barium methylglucofuranoside-3-phosphates (αβ-mixture) gave methylglucofuranosides (52%); (3) barium 1 : 2-monoacetone glucofuranose-3-phosphate gave 1 : 2-monoacetone glucofuranose (11%); (4) barium 1 : 2-monoacetone glucofuranose-6-phosphate the same product (22%). Substantial amounts of the unchanged starting materials were recovered from experiments (3) and (4). The product obtained from (2), when hydrolysed with dilute acid, gave crystalline α-*d*-glucose together with a syrup, but from this latter on osazone formation glucosazone was the only substance isolated. A syrup also accompanied the monoacetone glucofuranose obtained in (4); in this case also, glucosazone was the only product identified after hydrolysis and osazone formation. No 3 : 6-anhydroglucosazone was found although the detection of this substance has been simplified by the discovery that 3 : 6-anhydroglucosazone acetate is more readily crystallisable than the parent osazone.

It should be pointed out that Seebeck, Meyer, and Reichstein (*Helv. Chim. Acta*, 1944, **27**, 1142) have thrown doubt on the conclusions of Ohle and von Vargha (*Ber.*, 1929, **62**, 2435) that *l*-idose can be obtained from 1 : 2-*iso*propylidene-5 : 6-anhydroglucose, since this latter substance is readily converted into the corresponding 3 : 6-anhydride. If this is the case, the possibility that the alkaline hydrolysis of the methylglucofuranoside-6-sulphates and the 1 : 2-*iso*propylidene glucofuranose-6-sulphate (Percival, *loc. cit.*) might take place *via* the 5 : 6-anhydride cannot be ignored although the failure to isolate *l*-sorbosazone was held to discount this possibility. Owing to the fact that no 3 : 6-anhydroglucosazone was isolated in the present investigation this difficulty does not arise here.

In conclusion, reference may be made to the isolation by Levene and Raymond (*J. Biol. Chem.*, 1929, **83**, 619) of an anhydrohexosazone, in unspecified yield, from barium glucose-3-phosphate prepared by the acid hydrolysis of diacetone glucose-3-phosphate; despite several attempts we have been unable to repeat this work. The reported properties of the anhydro-osazone in question, however, are not inconsistent with the fact that it might have been the anhydroglucosazone isolated by Diels and Meyer (*Annalen*, 1935, **519**, 157) on heating glucosazone in alcohol with a trace of sulphuric acid, and it may be noted that before treatment with phenylhydrazine and acetic acid, Levene and Raymond removed barium from their salt with this reagent. Although Diels and Meyer (*loc. cit.*) claimed their anhydroglucosazone to be identical with 3 : 6-anhydroglucosazone it is now known (Percival, this vol., p. 783) that this is certainly not the case; it appears to us that the isolation of an anhydrohexosazone by Levene and Raymond (*loc. cit.*) following the acid hydrolysis of a phosphoric ester may not in fact have been directly connected with the hydrolysis of such an ester but with some abnormal condition prevailing during osazone formation.

## EXPERIMENTAL.

*Preparation of Barium α-Methylglucopyranoside-6-phosphate.*—α-Methylglucopyranoside (m. p. 166°) (10 g.) was converted into barium α-methylglucoside-6-phosphate according to the method of Fischer (*Ber.*, 1914, **47**, 3193). After continuous extraction with alcohol under reflux for 24 hours the product, dissolved in the minimum quantity of water, was filtered into alcohol to give a white precipitate which was separated and dried (11 g.). It had $[\alpha]_D^{16°}$ +95° (*c*, 1·4, in water) (Found : OMe, 7·2; P, 7·6. Calc. for $C_7H_{13}O_9PBa$ : OMe, 7·6; P, 7·6%).

*Alkaline Hydrolysis.*—Barium α-methylglucoside-6-phosphate (4·6 g.) was hydrolysed in water (150 c.c.) at 100° with barium hydroxide (24 g.) for 72 hours. The alcohol soluble portion of the hydrolysate gave a colourless crystalline solid (1·45 g., 47%); this was recrystallised from hot alcohol and the crystals (m. p. 166°) obtained were identified as α-methylglucoside (mixed m. p.) and had $[\alpha]$ +153° (*c*, 1·5, in water). The hydrolysis was repeated a number of times but all attempts to isolate any other product were unsuccessful.

*Preparation of Barium* 1 : 2-*Monoacetone Glucofuranose-6-phosphate and its Hydrolysis with Alkali.*—Monoacetone glucose (5 g.) in dry pyridine (18 c.c.) was converted into monoacetone glucose-6-phosphate according to the method used for the preparation of diacetone glucose-3-phosphate. The product, after treatment with silver sulphate, was extracted with hot ethyl acetate, dissolved in water, filtered and the water removed by distillation at 40°/15 mm. to yield a white solid (4·8 g.), $[\alpha]_D^{15°}$ +12° (*c*, 1·4 in water) (Found : Ba, 29·6; $Me_2CO$, 13·8. Calc. for $C_9H_{15}O_9PBa$ : Ba, 31·5; $Me_2CO$, 13·3%).

The above product (3·5 g.) was hydrolysed with barium hydroxide at 100° as before. The product was extracted thoroughly with ethyl acetate and left a residue which was dissolved in water, filtered and evaporated to give a white solid (1·36 g.), $[\alpha]_D^{17}$ +10° (*c*, 1·2 in water) [Found : Ba, 27·9; $Me_2CO$, 12·5%]. This was largely unchanged material. Evaporation of the ethyl acetate extracts gave a residue (0·55 g.) which, after extraction several times with cold dry ether, left a crystalline residue (0·35 g.), m. p. 162°, identified as 1 : 2-monoacetone glucofuranose; $[\alpha]_D^{18°}$ −11° (*c*, 1·5 in water).

When the ether was removed from the ethereal extracts a colourless syrup (0·17 g.) remained. This, after standing for several weeks, partly crystallised. The crystals (0·04 g.) were separated on porous porcelain and had m. p. 159°, not depressed by monoacetone glucoses and the total yield was 0·39 g., 22% of the theoretical.

The syrup (0·12 g.) obtained from the porous porcelain was hydrolysed with 0·1N sulphuric acid (10 c.c.) at 45° for 47 hours. The product (0·1 g.), isolated in the usual way, had $[\alpha]_D^{15^\circ}$ +44° (*c*, 1·0 in water) and was subjected to osazone formation to give four crops of osazone (0·043 g.) having m. p. 165—170°, depressed to 140° on admixture with *l*-sorbosazone (m. p. 165°). After several crystallisations from alcohol a small amount of crystalline product having m. p. 185—186° was obtained; m. p. depressed to 170° by admixture with 3 : 6-anhydroglucosazone (m. p. 185°). Acetylation with acetic anhydride and pyridine at room temperature failed to yield any crystallisable acetate, and, in particular, the easily crystallisable 3 : 6-anhydroglucosazone acetate. This work was repeated three times, but it was impossible to obtain identifiable products other than glucosazone and 1 : 2-monoacetone glucose.

*Barium Diacetone Glucofuranose-3-phosphate.*—Pure diacetone glucose (10 g.) in dry pyridine (37·5 c.c.) was treated with phosphorus oxychloride (5 c.c.) in pyridine (25 c.c.) according to the method of Josephson and Proffe (*Annalen*, 1930, **481**, 91; cf. Raymond and Levene, *J. Biol. Chem.*, 1929, **83**, 619). After the addition of saturated barium hydroxide solution until alkaline to phenolphthalein and removal of the excess pyridine by distillation at 35°/15 mm. the solid product was dissolved in water (100 c.c.) and a saturated solution of barium hydroxide added until faintly alkaline to thymolphthalein. The solution was decolorised with charcoal, filtered and the filtrate evaporated to give a white solid. Unchanged diacetone glucose was removed by repeated extraction with ligroin (6 litres) under reflux for 3 days. The residue, dissolved in water (75 c.c.), was treated with an excess of silver sulphate, the solution filtered, the excess of silver ion removed by hydrogen sulphide in the presence of barium carbonate, followed by aeration, treatment with barium hydroxide and carbon dioxide. The resulting solution, after filtration, was evaporated to dryness at 40°/15 mm. to yield a non-reducing white solid. This was purified by solution in acetone and precipitation with ligroin (b. p. 40—60°), followed by several extractions with the same solvent. The product (12·5 g.) had $[\alpha]_D^{18^\circ}$ −3·6° (*c*, 1·0 in water) (Found : Ba, 26·0; P, 6·9; $Me_2CO$, 25·0. Calc. for $C_{12}H_{19}O_9PBa$ : Ba, 28·9; P, 6·5; $Me_2CO$, 24·4%). The product contained therefore a small amount of the acid phosphate (cf. Josephson and Proffe, *loc. cit.*).

*Barium* 1 : 2-*Monoacetone Glucofuranose*-3-*phosphate and its Hydrolysis with Alkali.*—The above salt (12 g.) was dissolved in water (50 c.c.) and treated with 0·5N sulphuric acid (70 c.c.) for 165 minutes at room temperature. The solution, made alkaline to thymolphthalein with saturated barium hydroxide (30 c.c.), was treated with carbon dioxide, filtered, and the filtrate and washings on evaporation gave a yellow solid which was extracted with ethyl acetate under reflux for 14 hours to leave a pale yellow residue (9·8 g.), $[\alpha]_D^{15^\circ}$ +8° (*c*, 1·7, in water) (Found : Ba, 30·8; $Me_2CO$, 13·1. Calc. for $C_9H_{15}O_9PBa$ : Ba, 31·5; $Me_2CO$, 13·3%).

Barium 1 : 2-monoacetone glucose-3-phosphate (3 g.) was hydrolysed with barium hydroxide as before. After cooling and filtration, the excess of alkali was neutralised with carbon dioxide and the filtrate evaporated to dryness at 35°/15 mm. to give a white solid : this was extracted several times with ethyl acetate under reflux. The remaining white solid (1·94 g.) had $[\alpha]_D^{18^\circ}$ +6° (*c*, 1·6, in water) (and was for the most part unchanged material) [Found : Ba, 29·6; $Me_2CO$, 13·2%]. The ethyl acetate extracts gave a white solid (0·164 g.) which was recrystallised from ether. It had m. p. 161°, and was identified as 1 : 2-monoacetone glucofuranose, $[\alpha]_D^{17^\circ}$ −12° (*c*, 0·9, in water) (Found : $Me_2CO$, 25·5. Calc. for $C_9H_{16}O_6$ : $Me_2CO$, 26·5%). The unchanged material was submitted to further hydrolyses with barium hydroxide but monoacetone glucose was the only compound isolated in each case.

This work was repeated three times with identical results.

*Barium Glucose*-3-*phosphate.*—Barium 1 : 2-monoacetone glucose-3-phosphate (4·55 g.) was dissolved in water (100 c.c.) and treated with N-sulphuric acid (25 c.c.) at 40° for 43 hours. The product was neutralised, filtered and evaporated to dryness at 35°/15 mm. to yield a white solid (3 g.), $[\alpha]_D^{15^\circ}$ +29° (*c*, 1·0, in water) (cf. Josephson and Proffe, *loc. cit.*) (Found : Ba, 32·5. Calc. for $C_9H_{11}O_9PBa$ : Ba, 34·7%).

*Barium Methylglucofuranoside*-3-*phosphates and their Hydrolysis with Alkali.*—Barium glucose-3-phosphate (3·5 g.) was shaken at 15° with methanolic hydrogen chloride (60 c.c., 1·3%) until the specific rotation had fallen to a constant value (120 hours). After neutralisation with barium carbonate and removal of the solvent the mixture of salts was dissolved in water and treated with excess silver sulphate and the product worked up in the usual way to yield a glass. This was extracted thoroughly with ethyl acetate under reflux to remove methylglucofuranosides and the *product* (2·5 g.) had $[\alpha]_D^{17^\circ}$ +4° (*c*, 1·0, in water) (Found : Ba, 32·0; P, 7·0; OMe, 6·8. $C_7H_{13}O_9PBa$ requires Ba, 33·6; P, 7·6; OMe, 7·6%).

The above product (2·45 g.) was hydrolysed at 100° with barium hydroxide solution for 72 hours as before and the residue obtained, after neutralisation and evaporation, was extracted repeatedly with alcohol to give a syrupy glass (0·61 g.) (Found : OMe, 13·9. Calc. for $C_7H_{14}O_6$ : OMe, 16·0%). The product (0·57 g.) was hydrolysed with 0·2N sulphuric acid (20 c.c.) at 100° for 7 hours. After neutralisation with barium carbonate, the solution was evaporated and extraction with alcohol yielded a reducing syrup (0·4 g.) which was purified by dissolution in water, filtration and evaporation, followed by drying with alcohol and benzene; the resulting product (0·36 g.) had $[\alpha]_D^{17^\circ}$ +59·7° (*c*, 1·8 in water). On standing for some months this syrup partly crystallised. The crystals (0·1 g.), separated from the syrup by treatment with ethyl acetate, had m. p. 150°, not depressed by *d*-glucose (m. p. 150°). The remainder was subjected to osazone formation to give two crops of osazone (0·16 g.), m. p. 207° not depressed by an authentic specimen of glucosazone (m. p. 208°). Recrystallisation from alcohol and evaporation of the mother liquors gave a small quantity (0·03 g.) of a syrup which could not be crystallised. This syrup was acetylated with acetic anhydride and pyridine but the acetate so obtained could not be crystallised from aqueous alcohol in contrast with the easily crystallisable 3 : 6-anhydroglucosazone acetate.

*Attempted Isolation of an Anhydrohexosazone from Barium Glucose*-3-*phosphate.*—Barium glucose-3-phosphate (1·9 g.) was treated with the calculated quantity of sulphuric acid, filtered, and heated with sodium acetate (2 g.), phenylhydrazine (2 c.c.) and acetic acid (1·5 c.c.) at 100° for 45 minutes. A crystalline product (0·6 g.) was obtained, m. p. 118—120°, $[\alpha]_D^{17^\circ}$ −62° (*c*, 0·2, in pyridine–alcohol) (Found : N, 16·3; P, 3·95%). The product could not be recrystallised and the analyical figures of three such preparations were too variable to permit the assignment of a formula. It appears highly probable, however, to be a phenylhydrazine salt of glucosephenylosazone-3-phosphate; cf. the corresponding derivative obtained from glucose-6-phosphate, m. p. 154—154·5° (Robison and King, *Biochem. J.*, 1931, 323). Attempts to isolate the anhydrohexosazone reported by Levene and Raymond (*loc. cit.*) were abortive.

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