755. *Phosphorylated Sugars. Part VIII.* The Synthesis of Phosphorylated Glucometasaccharinic Acids.*

By Stanislas Lewak and L. Szabó.

The synthesis of 3-*O*-methyl-D-glucose 6-phosphate by two independent methods is described. Alkaline degradation of this compound as well as of D-glucose 3,6-(hydrogen phosphate) yields glucometasaccharinic acid 6-phosphate. In the same conditions D-glucose 3,5-(hydrogen phosphate) yields glucometasaccharinic acid 5-phosphate. The behaviour of these acids toward periodate has been examined and is discussed.

Although neither glucometasaccharinic acid nor its phosphate esters are known to occur in Nature, synthesis of the 6-phosphate has been considered of interest, as this ester is a possible intermediate in the syntheses of 2-deoxy-D-ribose 5-phosphate and 2-keto-3-deoxy D-gluconic acid 6-phosphate, two biochemically important compounds.

As it has been shown by Kenner and Richards [1] that a mixture of α- and β-glucometasaccharinic acids may be obtained in good yield by treatment of 3-*O*-alkylglucoses with oxygen-free lime water, and as it is known that D-fructose 6-phosphate may be degraded by oxidation in a fairly strong alkaline solution to D-arabonic acid 5-phosphate,[2] it was considered that alkaline treatment of 3-*O*-methylglucose 6-phosphate should yield αβ-glucometasaccharinic acid 6-phosphate without concomitant hydrolysis of the phosphate group. Accordingly, the synthesis of 3-*O*-methyl-D-glucose 6-phosphate (V) was attempted.

First, 1,2-*O*-isopropylidene 3-*O*-methyl-D-glucofuranose [3] (I) was treated with diphenyl phosphorochloridate in pyridine solution; in view of the greater reactivity of the primary hydroxyl group formation of the 6-(diphenyl phosphate) (II) was expected. However, when the reaction mixture was worked up in the usual manner, *i.e.*, removal of the pyridine in a vacuum, dissolution of the residue in chloroform, and washing with very dilute, ice-cold acid to remove residual base, the final yield of 3-*O*-methylglucose 6-phosphate (V), obtained on hydrogenolysis of the phenyl groups and mild acid hydrolysis, was only 3—5%. As during the washing of the almost neutral chloroform solution a strong acid was formed and phenol simultaneously liberated, it was concluded that the diphenyl phosphate (II) is unstable in the presence of water and forms, with the neighbouring hydroxyl group, an unstable [4] five-membered cyclic phosphate (III), liberating phenol simultaneously. Brown, Magrath, and Todd have demonstrated [5] that phosphoric triesters are rendered

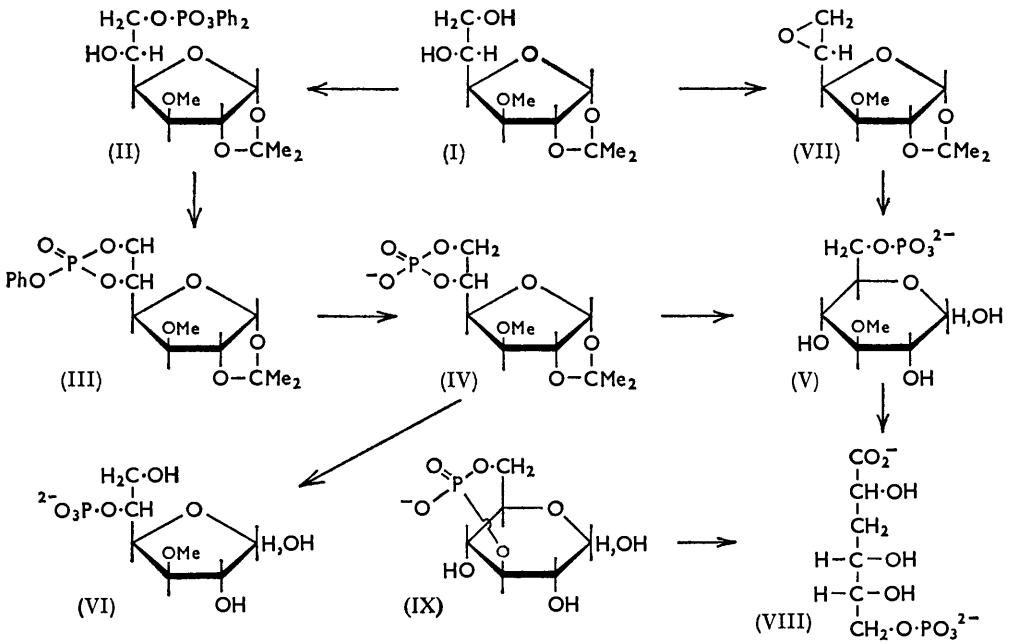
* Part VII, Strobach and Szabó, preceding paper.

[1] Kenner and Richards, *J.*, 1954, 278; 1957, 3019.
[2] Mandl and Neuberg, *Arch. Biochem. Biophys.*, 1951, **33**, 191; 1952, **37**, 83.
[3] Freudenberg, Dürr, and von Hochstetter, *Ber.*, 1928, **61**, 1735.
[4] Westheimer, "Phosphoric Esters and Related Compounds," *Chem. Soc. Special Publ.* No. 8, 1957, p. 13; Kumamoto, Cox, and Westheimer, *J. Amer. Chem. Soc.*, 1956, **78**, 4558; Cox, Wall, and Westheimer, *Chem. and Ind.*, 1959, 929.
[5] Brown, Magrath, and Todd, *J.*, 1955, 4396.

extremely unstable by the presence of a vicinal hydroxyl group, yielding diesters, five-membered cyclic phosphates, and ultimately a mixture of two isomeric monophosphates. A similar mechanism would yield in our case, first, the cyclic phenyl phosphate (III), then the cyclic hydrogen phosphate (IV), and, after acid hydrolysis, a mixture of the 5- and the 6-(dihydrogen phosphates) (VI and V). The observation that during the washing of the chloroform solution containing the phosphorylated product, the acidity of the aqueous layer increased was taken to indicate that in the presence of water the lability of the neutral ester is enhanced. To minimise the cyclisation, the phosphorylation was therefore carried out in anhydrous benzene, by addition of that amount of pyridine which gave the best yields, as determined experimentally. The use of benzene as solvent also permits elimination of pyridinium hydrochloride formed during the reaction. This is an advantage, as less ion-exchange resin is required during the working-up procedure.

All basic and acidic substances were then removed from the solution in that order, by passing it through columns of ion-exchange resins previously washed with methanol followed by benzene, and the residual, oily, neutral ester obtained after evaporation of the solvent in a vacuum was hydrogenated. In these conditions the yield of 3-*O*-methylglucose 6-phosphate was increased to about 50%. However, as under these circumstances assignment of the phosphate group to the primary hydroxyl group exclusively was doubtful (as any 5,6-cyclic phosphate formed would have hydrolysed during the acid treatment required to remove the isopropylidene group, to yield a mixture of the 5- and 6-phosphates), another route leading to this compound had to be found. This was accomplished as follows.

5,6-Anhydro-1,2-*O*-isopropylidene-3-*O*-methyl-D-glucofuranose [6] (VII) was prepared from 1,2-*O*-isopropylidene-3-*O*-methyl-6-*O*-toluene-*p*-sulphonyl-D-glucofuranose by alkaline treatment, and was treated with dipotassium hydrogen phosphate. The latter



reaction was used by Lampson and Lardy [7] to transform 5,6-anhydro-1,2-*O*-isopropylidene-D-glucofuranose into the 6-phosphate. Isopropylidene-3-*O*-methylglucose 6-phosphate

[6] Kenner and Richards, *J.*, 1954, 3277.
[7] Lampson and Lardy, *J. Biol. Chem.*, 1949, **181**, 693.

was isolated as its barium salt, which was transformed by mild acid hydrolysis into 3-*O*-methylglucose 6-phosphate, identical with the product obtained by phosphorylation of the isopropylidene-3-*O*-methylglucose in benzene (analysis, periodate titration, electrometric titration, and paper chromatography). Both compounds consumed 2 mol. of periodate, the first very fast, the second within 72 hours (no more during the next 50 hours).

Treatment of 3-*O*-methylglucose 6-phosphate (V) with barium hydroxide at room temperature yielded a crystalline barium salt of αβ-glucometasaccharinic acid 6-phosphate (VIII). Barium hydroxide rather than lime water was used, as preliminary experiments have shown that the barium salt of the phosphorylated acid separates easily in crystalline form on concentration of the solution freed from the excess of barium ions by cation-exchange resins. Removal of the excess of barium with carbon dioxide gave a much lower yield, probably owing to adsorption on the precipitated barium carbonate. The same acid was obtained on degradation with barium hydroxide at 100° for a shorter period or with lime water. Although it was not possible to prove the presence of three acid dissociations by potentiometric titration, this could be done by conductometric titration of the salt with standard acid, three different slopes being observed. Attempts to prepare a crystalline lactone failed, but the oily mixture of lactones readily gave a crystalline anilide (presumably a mixture of anilides) isolated as the anilinium salt. Surprisingly, this substance has a very sharp melting point, which can be used to identify the compound and to differentiate it from the glucometasaccharinic acid 5-phosphate (see below). Enzymic dephosphorylation by wheat-germ phosphatase yielded glucometasaccharinic acid, confirming the proposed structure.

Although both methods described above yield pure products, they do not lend themselves to large-scale preparations and thus alternative syntheses of the phosphorylated glucometasaccharinic acid were sought.

In Part III of this series [8] a relatively easy synthesis of D-glucose 3,6-(hydrogen phosphate) was described. As it has been shown [9] that alkaline treatment of D-glucose 3-phosphate yields αβ-glucometasaccharinic acid, *i.e.*, the phosphate group behaves similarly to the methoxyl group and is eliminated by carbon–oxygen fission, it was thought that glucose 3,6-(hydrogen phosphate) (IX) should yield the desired αβ-glucometasaccharinic acid 6-phosphate provided that no intramolecular transesterification intervenes. As preliminary experiments have shown that 1,2-*O*-isopropylidene-D-glucofuranose 3,6-(hydrogen phosphate) is stable for at least 72 hours when treated with barium hydroxide, such transesterification was considered improbable but it could not be rigorously excluded.

Accordingly, when glucose 3,6-(hydrogen phosphate) (IX) was treated with this reagent, authentic αβ-glucometasaccharinic acid 6-phosphate (VIII) was isolated in excellent yield.

Similarly, D-glucose 3,5-(hydrogen phosphate) [8] was degraded to αβ-glucometasaccharinic acid 5-(dihydrogen phosphate). Again, conductometric titration revealed three acid dissociations, and a crystalline anilide was obtained from the oily lactone mixture. The anilinium salt of this anilide also has a very sharp melting point.

The proposed structures were further confirmed by comparison of the behaviour of the two phosphorylated glucometasaccharinic acids towards periodate. The glucometasaccharinic acid 5-phosphate is an α-hydroxy-carboxylic acid not possessing any vicinal hydroxyl groups; it consumes 0·8 mole of sodium periodate in 550 hours, in agreement with previous findings for α-hydroxy-acids.[10] The free 4- and 5-hydroxyl groups of the 6-phosphate constitute a vicinal diol system, and this compound consumes one equivalent of periodate within 2 hours. During the same period of time non-phosphorylated glucometasaccharinic acid reduces 2 mol. of this reagent, thus proving that the structures

[8] Szabó and Szabó, *J.*, 1961, 448.
[9] Brown, Hayes, and Todd, *Chem. Ber.*, 1957, **90**, 936.
[10] Fleury and Boisson, *Compt. rend.*, 1937, **204**, 1264; Huebner, Ames, and Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621; Courtois and Guernet, *Ann. pharm. franç.*, 1958, **16**, 119; Clancy and Whelan, *Chem. and Ind.*, 1959, 673.

assigned to the phosphorylated acids are correct. Periodate reduction by the glucometasaccharinic acid 5-phosphate asymptotically approaches one mol., but with the 6-phosphate and the non-phosphorylated glucometasaccharinic acid, over-oxidation occurs. However, the rate of this is about 50 times slower than the diol cleavage, more than 100 hours being required for reduction of a further mol. of periodate; this and other over-oxidations of sugar phosphates [8] will be discussed later.

Experimental

The barium salts of the various sugar phosphates very easily take up and lose water of hydration. A barium salt, left in the air until it reaches constant weight, loses the water of hydration if kept over phosphoric oxide in an evacuated desiccator for 24 hr.; in this state the compounds are very hygroscopic. If the water is removed by heating in a vacuum in the presence of a desiccant, some of the phosphates tend to decompose. These changes (except that of decomposition) do not affect the appearance of the product, whose analysis, however, depends on the length of exposure to air. It is, therefore, suggested that samples for analysis should be air-equilibrated for 24 hr. before analysis and that water of hydration should be determined by keeping an air-equilibrated sample in an evacuated desiccator until it reaches constant weight. This practice has been followed in the present work.

3-O-*Methyl*-D-*glucose* 6-(*Dihydrogen Phosphate*).—(*a*) Diphenyl phosphorochloridate (11·5 g.) was added to a solution of 1,2-*O*-isopropylidene-3-*O*-methyl-D-glucofuranose [3] (9·1 g.) in anhydrous benzene (50 ml.). Anhydrous pyridine (10 ml.) was added dropwise to this solution (with stirring). After 2 hr., the pyridine hydrochloride was filtered off and washed with benzene. The combined filtrates and washings were passed through anhydrous Amberlite IR-120 (H^+) (previously washed with ethanol to remove water, then with benzene to remove ethanol), then through anhydrous Amberlite IR-45 (OH^-) (prepared in the same way), and finally through a column identical with the first. The benzene effluent from the third column was concentrated to dryness *in vacuo*, the oily residue dissolved in absolute ethanol (50 ml.), the ethanol removed *in vacuo*, and the residue redissolved in absolute ethanol (300 ml.); it was hydrogenated in the presence of Adams platinum. When the hydrogen uptake ceased, the catalyst was filtered off and the ethanolic solution concentrated *in vacuo*, water being added from time to time so that when all the ethanol had been removed an aqueous solution (250 ml.) remained. This solution was heated for 20 min. on a boiling-water bath, cooled, neutralised (to pH 6·5) with barium hydroxide solution, and concentrated *in vacuo*. The *barium salt* (9·5 g., 55%) which crystallised when acetone was added to the aqueous solution had $[\alpha]_D^{25}$ +23° (*c* 2·6 in H_2O) (Found: C, 19·5; H, 4·0; P, 6·8; loss of wt., 8·1. $C_7H_{13}BaO_9P,2H_2O$ requires C, 18·9; H, 3·8; P, 7·0; H_2O, 8·1%). The *dicyclohexylammonium salt*, prepared by passing an aqueous solution (50 ml.) of the barium salt (2 g.) through Amberlite IR-120 (cyclohexylammonium form), concentration of the effluent, and crystallisation of the residue from ethanol and ether, had m. p. 115° (decomp.), $[\alpha]_D^{25}$ +22° (*c* 2·48 in H_2O) (Found: C, 48·1; H, 8·65; N, 5·9; P, 6·5. $C_{19}H_{41}N_2O_9P$ requires C, 48·3; H, 8·7; N, 5·9; P, 6·6%).

(*b*) 5,6-Anhydro-1,2-*O*-isopropylidene-3-*O*-methyl-D-glucofuranose [6] (34·5 g.) was heated with dipotassium hydrogen phosphate (120 g.) in water (550 ml.), with stirring in a boiling-water bath for 12 hr. and left for 12 hr. to cool. The solution was diluted with its own volume of water and aqueous barium hydroxide was added until all the inorganic phosphate had been precipitated. The precipitate was centrifuged off and washed three times with water. The combined aqueous supernatant layers were neutralised with Amberlite IR-120 (H^+) and concentrated *in vacuo* to a small volume. A small portion of this solution was set aside for the isolation of 1,2-*O*-isopropylidene-3-*O*-methyl-D-glucofuranose 6-(dihydrogen phosphate) (solution A), and the bulk of the solution (solution B) was diluted with water and acidified with Amberlite IR-120 (H^+). The resin was filtered off and thoroughly washed with water, and the combined filtrate and washings were made up to 1300 ml. with water, heated for 30 min. in a boiling-water bath, cooled, and neutralised with aqueous barium hydroxide. The solution was concentrated to a small volume and ethanol was added to precipitate the *barium salt* (31 g., 43·7%). A sample of this salt was reprecipitated from water with ethanol, dried in a desiccator *in vacuo* over phosphoric oxide at room temperature, and then equilibrated in air. It had $[\alpha]_D^{25}$ +23° (*c* 2·6 in H_2O) (Found: C, 19·5; H, 4·0; P, 6·8; loss of wt., 8·1. $C_7H_{13}BaO_9P,2H_2O$ requires C, 18·9; H, 3·8; P, 7·0; H_2O, 8·1%).

1,2-O-*Isopropylidene*-3-O-*methyl*-D-*glucofuranose* 6-(*Dihydrogen Phosphate*).—Solution A from above was diluted with a little water and passed through Amberlite IR-120 (NH_4^+). Aqueous barium hydroxide was added to the effluent to give pH ~10 and nitrogen was bubbled through the solution for 24 hr. The solution was then concentrated to a small volume and filtered. On addition of ethanol, the *barium salt* crystallised. It was dried *in vacuo* over P_2O_5 and then equilibrated in air. The dihydrate had $[\alpha]_D^{20}$ −21·6° (*c* 0·24 in H_2O) (Found: C, 25·0; H, 4·5; P, 6·4; loss of wt., 7·6. $C_{10}H_{17}BaO_9P,2H_2O$ requires C, 24·7; H, 4·3; P, 6·4; H_2O, 7·4%).

αβ-*Glucometasaccharinic Acid* 6-(*Dihydrogen Phosphate*).—(*a*) A solution of the barium salt (10 g.) of 3-*O*-methyl-D-glucose 6-(dihydrogen phosphate) in water (40 ml.) was poured into 0·3N-barium hydroxide (400 ml.) and left for 2 days at room temperature. The solution, at first green, became colourless and slightly turbid after a few hours. It was neutralised with Amberlite IR-120 (H^+), the resin was filtered off and washed with water, and the combined filtrate and washings were adjusted to pH 8 with aqueous barium hydroxide and concentrated *in vacuo* to a small volume. The barium salt which crystallised spontaneously was filtered off and redissolved in the minimum amount of water. The aqueous solution was decolorised with charcoal and concentrated. The barium salt was filtered off and, after concentration of the mother-liquors and addition of ethanol, an additional crop of crystals was obtained. The mixed *barium salts* (9·4 g., 93%) of αβ-glucometasaccharinic acid 6-phosphate thus obtained had $[\alpha]_D^{22}$ −5·5° (*c* 5 in H_2O) (Found: C, 14·6; H, 2·8; P, 6·1; loss of wt. *in vacuo* over P_2O_5 at 60°, 7·2. $C_6H_{10}Ba_{1.5}O_9P,2H_2O$ requires C, 14·4; H, 2·8; P, 6·2; H_2O, 7·2%).

(*b*) The barium salt (10 g.) of D-glucose 3,6-(hydrogen phosphate) [8] was degraded as in (*a*), and the mixture of *barium salts* (9 g.) isolated in the same way (Found: C, 14·4; H, 2·8; P, 6·3%).

(*c*) Degradation of the barium salt (100 mg.) of D-glucose 3,6-(hydrogen phosphate) in water (4 ml.) with 0·3N-barium hydroxide (40 ml.) at 100° for 1 hr., followed by the usual working-up, also gave αβ-glucometasaccharinic acid 6-phosphate (85 mg.).

(*d*) The barium salt (500 mg.) of the 3,6-cyclic phosphate was dissolved in water (3 ml.), and barium ions were removed with Amberlite IR-120 (H^+). The resin was filtered off, and the aqueous solution was added to 0·045N-calcium hydroxide (120 ml.) and left at room temperature for 2 days. Calcium ions were removed with IR-120 (H^+), and the filtered solution was neutralised with aqueous barium hydroxide and worked up as before to give the same mixture of barium salts (470 mg.).

Anilide of αβ-*Glucometasaccharinic Acid* 6-(*Dihydrogen Phosphate*).—The barium salt (1 g.) of the mixed αβ-glucometasaccharinic acid 6-phosphate was dissolved in water and, after conversion of the salt into the free acid by addition of Amberlite IR-120 (H^+) and removal of the resin, the aqueous solution was concentrated to dryness *in vacuo*. The syrupy residue was dried over phosphoric oxide, and aniline (3 ml.) and ethanol (in quantity sufficient to give a homogeneous solution, less than 2 ml.) were added. The solution was heated for 1 hr. on a boiling-water bath. Ethanol, the water formed in the reaction, and aniline were then removed *in vacuo* in an atmosphere of nitrogen. The oily residue was dissolved in ethanol and ethyl acetate was added to the hot solution until crystallisation started. After recrystallisation, first from ethanol and then from methanol, the acid *anilinium salt* of the anilide (100 mg.) had m. p. 155—156° (Found: C, 50·3; H, 5·9; N, 6·4; P, 7·4. $C_{18}H_{25}N_2O_8P$ requires C, 50·5; H, 5·85; N, 6·6; P, 7·1%).

Enzymic Dephosphorylation of αβ-*Glucometasaccharinic Acid* 6-(*Dihydrogen Phosphate*).—To an aqueous solution (1·5 ml.) of the free acid, obtained from the barium salt (50 mg.) of glucometasaccharinic acid 6-phosphate, were added 0·1N-sodium acetate–acetic acid buffer of pH 5·5 (10 ml.) and a solution of wheat-germ acid phosphatase (8 mg.) in water (1 ml.). The mixture was left for 1 hr. at room temperature, then concentrated *in vacuo*. Ethanol was added, the precipitated proteins were filtered off, and the ethanol was removed *in vacuo*. The residue was dissolved in water, acidified with an excess of Amberlite IR-120 (H^+) and, after removal of the resin, the aqueous solution was concentrated *in vacuo*. The residue was identical on paper chromatograms in pyridine–propan-1-ol–water (1 : 1 : 1) and propan-1-ol–aqueous ammonia (*d* 0·88)–water (7 : 1 : 2) with authentic αβ-glucometasaccharinic acid (R_F, in the first solvent $0·48_{acid}$ and $0·72_{lactone}$, and in the second 0·51). The residue, after being dried, dissolved in ethanol (1 ml.), treated with aniline (1 ml.) at 100° for 1 hr., and freed from aniline and ethanol, gave an anilide chromatographically identical with that of authentic

αβ-glucometasaccharinic acid [R_F in acetone–benzene–water (9 : 2 : 1) containing 40 mg. of rhodamine B per litre,[11] 0·68].

αβ-*Glucometasaccharinic Acid* 5-(*Dihydrogen Phosphate*).—Degradation of D-glucose 3,5-(hydrogen phosphate) [4] (5 g.) with barium hydroxide, carried out as for 3-*O*-methylglucose 6-phosphate and glucose 3,6-phosphate, gave the barium salts (4·8 g.) of αβ-glucometasaccharinic acid 5-phosphate, which were less soluble in water than the corresponding 6-phosphate and, after crystallisation from this solvent, had $[\alpha]_D^{22}$ −6·7° (*c* 5 in H_2O) (Found: C, 14·85; H, 2·8; P, 6·2. $C_6H_{10}Ba_{1.5}O_9P,2H_2O$ requires C, 14·4; H, 2·8; P, 6·2%).

Anilide of αβ-*Glucometasaccharinic Acid* 5-(*Dihydrogen Phosphate*).—The barium salt (1 g.) of αβ-glucometasaccharinic acid 5-phosphate was transformed into the lactone in the same way as the 6-phosphate, for preparation of the anilide. The oil thus obtained was dissolved in ethanol (2 ml.), aniline (3 ml.) was added, and the mixture was heated on a boiling-water bath for 1 hr. The ethanol and aniline were then removed *in vacuo* and the solid residue was crystallised from 80% ethanol. After recrystallisation from methanol, the *anilinium salt* of the anilide (380 mg.) had m. p. 149—151° (Found: C, 50·5; H, 6·0; N, 6·6; P, 7·5%).

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CENTRE DE RECHERCHES SUR LA CELLULE NORMALE ET CANCÉREUSE,
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE,
VILLEJUIF, SEINE, FRANCE. [*Received, January 5th,* 1963.]

[11] Green, *J. Amer. Chem. Soc.*, 1954, **76**, 5791; 1956, **78**, 1894.