Phosphorylated Sugars. Part XIII.[1] A New Synthesis of D-Arabinose 5-(Dilithium Phosphate)

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Phosphorylation of 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose with dipotassium hydrogen phosphate yields the corresponding 6-phosphate, which, after acidic hydrolysis and periodate cleavage between C-1 and C-2, gives the stable 2-*O*-benzyl-D-arabinofuranose 5-phosphate. Removal of the benzyl group by hydrogenolysis affords the title compound. In view of the known instability of pentose 5-phosphates D-arabinose 5-phosphate should be stored as its 2-*O*-benzyl derivative.

DICKENS [2] found that D-arabinose 5-phosphate was fermented by yeast; its formation during the metabolism of 6-phosphogluconate by the same organism was subsequently reported.[3] Extracts of *Propionibacterium pentosaceum* can form this sugar phosphate from either L-arabinose or D-ribulose.[4] The first well defined example of a biosynthetic reaction involving D-arabinose 5-phosphate, namely its condensation with phosphoenolpyruvate to give 3-deoxy-D-*manno*-octulosonic acid (KDO) 8-phosphate, mediated by an enzyme from *Pseudomonas aeruginosa*, was discovered by Levin and Racker.[5]

Chemical syntheses have been reported, but the final products were not fully characterised. The two preparations of Levin and Racker [5] involve (*a*) treatment of D-glucose 6-phosphate with 1 mol. equiv. of lead tetraacetate, and (*b*) direct phosphorylation of D-arabinose with polyphosphoric acid; that of Volk [6] involves degradation of D-glucosamine 6-phosphate with ninhydrin. None of these syntheses appears suitable for large-scale preparations.

Levene and Christman [7] phosphorylated 1,2-*O*-isopropylidene-D-arabinose (obtained from D-arabinose in five steps) with phosphoryl chloride; after removal of the isopropylidene group with 0·3N-hydrochloric acid (90°; 2 h), amorphous barium and crystalline brucine salts of D-arabinose 5-phosphate were isolated. The structural assignment was made on the basis of the mode of synthesis, but not further substantiated.

An unequivocal synthesis of D-arabinose 5-phosphate, whereby large quantities of the pure aldose phosphate can be prepared, is now described.

5,6-Anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose [8] (I) was phosphorylated with dipotassium hydrogen phosphate. Whereas 5,6-anhydro-1,2-*O*-isopropylidene-D-glucofuranose [9] and the corresponding 3-*O*-methyl derivative [10] can be easily phosphorylated in position 6 by treatment with this salt in aqueous solution, no, or very little phosphorylated compound was formed when the 3-*O*-benzyl derivative was treated under similar conditions. Nor did reaction in hot aqueous dioxan, methanol, or ethanol give better results. However, good yields of the phosphate (II) were obtained by treating the anhydro-sugar with dipotassium hydrogen phosphate in ethylene glycol containing a little water at 150°. Removal of the isopropylidene group by mild acidic hydrolysis gave 3-*O*-benzyl-D-glucose 6-

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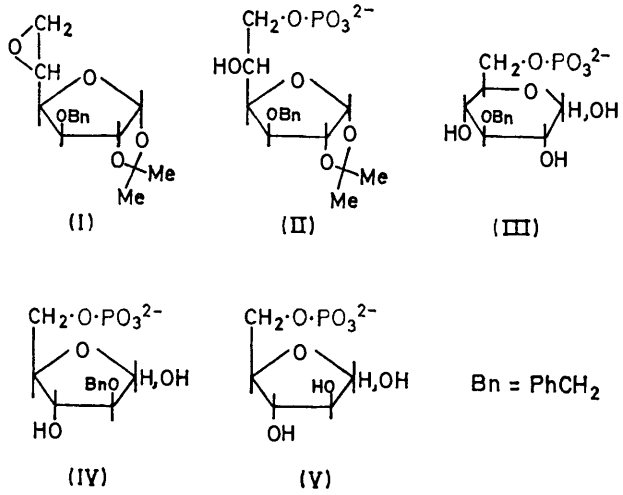
[2] F. Dickens, *Biochem. J.*, 1938, **32**, 1626, 1645; F. Dickens and G. E. Glock, *Nature*, 1950, **166**, 33.
[3] D. B. M. Scott and S. S. Cohen, *J. Biol. Chem.*, 1951, **188**, 509.
[4] W. A. Volk, *J. Biol. Chem.*, 1959, **234**, 1931; 1960, **235**, 1550.
[5] D. H. Levin and E. Racker, *J. Biol. Chem.*, 1959, **234**, 2532.
[6] W. A. Volk, *Biochim. Biophys. Acta*, 1960, **37**, 365.
[7] P. A. Levene and C. C. Christman, *J. Biol. Chem.*, 1938, **123**, 607.
[8] A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, 1946, **29**, 152.
[9] G. P. Lampson and H. A. Lardy, *J. Biol. Chem.*, 1949, **181**, 693.
[10] S. Lewak and L. Szabó, *J. Chem. Soc.*, 1963, 3975.

phosphate (III), which was cleaved with 1 mol. equiv. of periodate to yield 2-*O*-benzyl-D-arabinose 5-phosphate (IV) in almost quantitative yield. Removal of the benzyl group by hydrogenolysis led to the free sugar phosphate (V), which, when freshly isolated as the



neutral lithium salt, reduced 3 mol. equiv. of periodate, no formaldehyde being liberated. This lithium salt had $[\alpha]_D$ +13° (in 0·1N-HCl); Levene and Christman [7] found $[\alpha]_D$ −18·8° for the barium salt. In one case when the hydrogenolysis was abnormally long (the sugar phosphate was in contact with the catalyst in acid medium for 48 h, whereas a normal hydrogenolysis requires 2—3 h) a sugar phosphate giving the analytical figures expected for a pentose phosphate was isolated, but it reduced only 2·4 mol. equiv. of periodate and gave rise to 0·2 mol. equiv. of formaldehyde. The lithium salt of this phosphate had $[\alpha]_D$ +6·6° (in 0·1N-HCl). Both the discrepancy in the $[\alpha]_D$ values and the release of formaldehyde are probably due to partial phosphate migration and/or to ketose formation.

Since transformation of the neutral barium salts of D-ribose 5-phosphate [11] and of D-xylose 5-phosphate [12] into the corresponding ketose phosphates at room temperature has been observed previously, we recommend that D-arabinose 5-phosphate should be freshly prepared as needed from the stable 2-*O*-benzyl derivative.

EXPERIMENTAL

All evaporations were carried out under reduced pressure. The purity of the phosphate esters was verified by chromatography on Whatman No. 1 paper with propan-2-ol–ammonia (*d* 0·880)–water (7 : 1 : 2) as solvent and by electrophoresis on Whatman 3MM paper in 0·2M-pyridine–acetic acid buffer (pH 5). The phosphates were revealed by the Hanes–Isherwood reagent [13] and also, in the case of reducing sugar phosphates, by use of aniline hydrogen phthalate.[14] The lithium salts of the phosphates were dried *in vacuo* (P_2O_5) at room temperature and then equilibrated in air. Periodate was estimated by the method of Avigad [15] and formaldehyde with chromotropic acid.[16] Optical rotations were determined for aqueous solutions with a Perkin-Elmer 141 polarimeter, unless otherwise stated.

3-O-*Benzyl*-1,2-O-*isopropylidene*-D-*glucofuranose* 6-(*Dihydrogen Phosphate*) (II).—To a solution containing dipotassium hydrogen phosphate (50 g) in water (60 ml) and ethylene glycol (180 ml), stirred and heated at 150°, 5,6-anhydro-3,*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose [8] (25 g) was added dropwise during 6 h 15 min. The resulting solution was then stirred for a further 16 h at 110°. A saturated solution of barium hydroxide was added dropwise to the cooled, vigorously stirred solution until no further precipitation of inorganic phosphate occurred. The precipitate was filtered off, suspended in hot water (1 l), and again filtered off. This process was repeated once. The combined filtrates and washings were passed through a column of Amberlite IR 120 ion-exchange resin (NH_4^+) to remove all barium and potassium ions. The effluent and washings from the column were concentrated to dryness and the remaining ethylene glycol was removed at 0·1—0·2 mmHg. The residue was dissolved in water (100 ml), a solution of barium bromide dihydrate (30 g) in water (50 ml) was added, and the pH of the stirred solution was brought to 7—7·5 with aqueous barium hydroxide. The precipitate was filtered off and washed with a little water, then with ethanol. The crude barium salt was dissolved in water with the aid of IR 120 (H^+) resin and the filtered solution was neutralised with aqueous barium hydroxide. The precipitate (36 g) when filtered off and washed with water and ethanol showed one spot for phosphate; it still contained barium bromide. (Some preparations were contaminated with ethylene glycol phosphate; this could be removed either at this stage by repeated precipitations of the barium salt, or in the following step by reprecipitation of the lithium salt.) A sample was converted into the *biscyclohexylammonium salt* with IR 120 resin (cyclohexylammonium form). This salt, crystallised from ethanol, had $[\alpha]_D^{23}$ −8·9° (*c* 1) (Found: C, 55·5; H, 8·6; N, 4·7; P, 5·1. $C_{28}H_{49}N_2O_9P,H_2O$ requires C, 55·4; H, 8·4; N, 4·6; P, 5·1%).

3-O-*Benzyl*-D-*glucose* 6-(*Dihydrogen Phosphate*) (III).—A suspension of the barium salt (12 g) of the foregoing phosphate in water was decationised with IR 120 (H^+) resin; the resin was filtered off and washed with water, and the combined filtrate and washings (*ca.* 100 ml) were heated on a boiling water bath for 25 min, cooled, neutralised (pH 6·9) with aqueous barium hydroxide, treated with charcoal, and concentrated to a small volume. The barium salt (9·4 g) was precipitated with acetone. This salt was suspended in water and decationised with IR 120 (H^+) resin. The solution was neutralised (pH 6·98) with lithium hydroxide and concentrated (to *ca.* 15 ml). The *lithium salt* (5 g), precipitated with ethanol, had $[\alpha]_D^{23}$ +25° (*c* 1) (Found: C, 40·6; H, 5·0; P, 8·3. $C_{13}H_{17}Li_2O_9P,H_2O$ requires C, 41·0; H, 5·0; P, 8·2%).

2-O-*Benzyl*-D-*arabinose* 5-(*Dilithium Phosphate*) (IV).—To an aqueous solution (110 ml) of the preceding lithium salt (1·52 g), decationised with IR 120 (H^+) and neutralised with

[11] B. Axelrod and R. Jand, *J. Biol. Chem.*, 1954, **209**, 847.
[12] J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, 1957, **79**, 1194.
[13] C. S. Hanes and F. A. Isherwood, *Nature*, 1949, **164**, 1107.
[14] S. M. Partidge, *Nature*, 1949, **164**, 443.
[15] G. Avigad, *Carbohydrate Res.*, 1969, **11**, 119.
[16] D. A. MacFadyen, *J. Biol. Chem.*, 1945, **158**, 107.

pyridine, was added a solution of sodium periodate (0·934 g) in water (20 ml). The mixture was kept for 40 h at 4°. Half an hour after addition of a few drops of ethylene glycol, aqueous barium hydroxide was added with stirring until no further precipitation of barium iodate occurred. The precipitate (0·94 g) was filtered off and a solution (1·4 ml) of hydriodic acid (57% w/v) was added to the filtrate. The iodine formed was extracted with benzene. The aqueous solution was completely decationised [IR 120 (H^+)], neutralised (pH 7) with lithium hydroxide, and concentrated to a small volume. The *lithium salt* (1·2 g), precipitated with ethanol, centrifuged off, and thoroughly washed with ethanol, had $[\alpha]_D^{23}$ −3° (*c* 1) (Found: C, 39·8; H, 4·7; P, 8·5. $C_{12}H_{15}Li_2O_8P,1{\cdot}5H_2O$ requires C, 40·1; H, 5·0; P, 8·6%).

D-*Arabinose* 5-(*Dilithium Phosphate*) (V).—The lithium salt of the preceding compound (300 mg) was dissolved in water, acidified by addition of IR 120 (H^+) resin, and hydrogenated over palladium. The catalyst was filtered off and the filtrate neutralised (pH 7·1) with lithium hydroxide solution and freeze-dried. The residue was dissolved in a little water and the lithium salt of the phosphate precipitated with ethanol. The *precipitate*, when centrifuged off, washed twice with ethanol, and then with ether, had $[\alpha]_D^{22}$ +10° (*c* 2), $[\alpha]_D^{22}$ +13° (*c* 2 in 0·1N-HCl) (Found: C, 22·4; H, 4·2; P, 11·2. $C_5H_9Li_2O_8P,1{\cdot}5H_2O$ requires C, 22·3; H, 4·4; P, 11·5%). This compound reduced 2·9 mol. equiv. of periodate; no formaldehyde was formed.

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