985. *Phosphorylated Sugars. Part IX.*[1] *A Simple Synthesis of 2-Deoxy-*D*-ribose 5-Phosphate* [2-*Deoxy*-D-erythro-*pentose 5-(Dihydrogen Phosphate)*].

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A simple synthesis of substantial quantities of chemically and enzymatically pure 2-deoxy-D-ribose (2-deoxy-D-*erythro*-pentose) 5-(dihydrogen phosphate), starting with 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose and passing through 3-deoxy-D-glucose 6-(dihydrogen phosphate), is described.

2-Deoxy-D-ribose 5-phosphate, formed enzymatically from 2-deoxy-D-ribose 1-phosphate through the action of a phosphodeoxyribomutase, was first isolated by Manson and Lampen.[2] The phosphate group was assigned to the 5-position as the compound consumed periodate but did not liberate formaldehyde. A year later, Racker [3] demonstrated that a deoxypentose phosphate (in all probability 2-deoxy-D-ribose 5-phosphate) was formed from D-glyceraldehyde phosphate and acetaldehyde in the presence of extracts of micro-organisms or mouse liver. A third biosynthetic pathway for this sugar phosphate, involving 2-deoxy-D-ribose kinase, was discovered in extracts of *Escherichia coli* [4] and *Lactobacillus plantarum* [5] adapted to growth on 2-deoxy-D-ribose. Although these particular biosynthetic pathways are not responsible for the formation of the 2-deoxy-D-ribose contained in deoxyribonucleic acid,[6] it is firmly established that metabolic pathways involving this sugar exist in micro-organisms and higher animals. The metabolic fate of the deoxyribose and its phosphate, however, is still unknown. This is probably due in part to the fact that deoxyribose 5-phosphate is a difficult substrate to obtain for metabolic studies.

Horecker and his colleagues [7] used the enzymatic condensation of D-glyceraldehyde phosphate with acetaldehyde to prepare this sugar phosphate, and it has also been prepared chemically by two methods. MacDonald and Fletcher [8] described the synthesis of the crystalline cyclohexyl ammonium salt of 2-deoxy-D-ribose dimethyl acetal 5-phosphate, which was converted on acid hydrolysis into a mixture containing essentially the free sugar phosphate;

[1] Part VIII, Lewak and Szabó, *J.*, 1963, 3975.
[2] Manson and Lampen, *J. Biol. Chem.*, 1951, **191**, 94.
[3] Racker, *J. Biol. Chem.*, 1952, **196**, 347.
[4] Jonsen, Laland, and Strand, *Biochim. Biophys. Acta*, 1959, **32**, 117.
[5] Ginsburg, *J. Biol. Chem.*, 1959, **234**, 480.
[6] Hammarsten, Reichard, and Saluste, *J. Biol. Chem.*, 1950, **183**, 105; Reichard, *Biochim. Biophys. Acta*, 1958, **27**, 434; Bagatell, Wright, and Sable, *Fed. Proc.*, 1958, **17**, 184; Bernstein and Sweet, *ibid.*, p. 100; Bertani, Häggmark, and Reichard, *J. Biol. Chem.*, 1963, **238**, 3407.
[7] Domagk and Horecker, *J. Biol. Chem.*, 1958, **233**, 283; Pricer and Horecker, *ibid.*, 1960, **235**, 1292; *Biochem. Prep.*, 1962, **9**, 35.
[8] MacDonald and Fletcher, *J. Amer. Chem. Soc.*, 1959, **81**, 3719.

the compound was not, however, actually isolated. Ukita and Nagasawa[9] reported the synthesis of 2-deoxy-D-ribose 5-phosphate by phosphorylation of a mixture of methyl α- and β-2-deoxyribofuranosides, separation of the 5-phosphate from the 3,5-diphosphate (no 3-phosphate was formed) by chromatography, and hydrolysis of the methyl deoxyriboside 5-phosphate to the phosphate of the free sugar, isolated as the barium salt. Both these syntheses require 2-deoxy-D-ribose as starting material, and thus it still remains difficult to obtain relatively large quantities of the phosphate.

In 1959, a preliminary Note[10] from the present authors' laboratory claimed a chemical synthesis of 2-deoxy-D-ribose 5-phosphate starting with D-glucose. The sequence used was based on the work of Richards[11] who obtained 2-deoxy-D-ribose by applying the Ruff degradation[12] to a mixture of α- and β-glucometasaccharinic acids. The conditions for this reaction being very mild, it was thought likely that a similar degradation of the readily available[1] glucometasaccharinic acid 6-phosphates would yield the desired 2-deoxy-D-ribose 5-phosphate, without concomitant hydrolysis of the phosphate group. Although a seemingly homogeneous phosphate ester of 2-deoxyribose was obtained, subsequent enzymatic analysis * indicated that it was a mixture of isomers containing only about 25% of the 5-phosphate. The isomerisation which occurred during this preparation is now being studied.

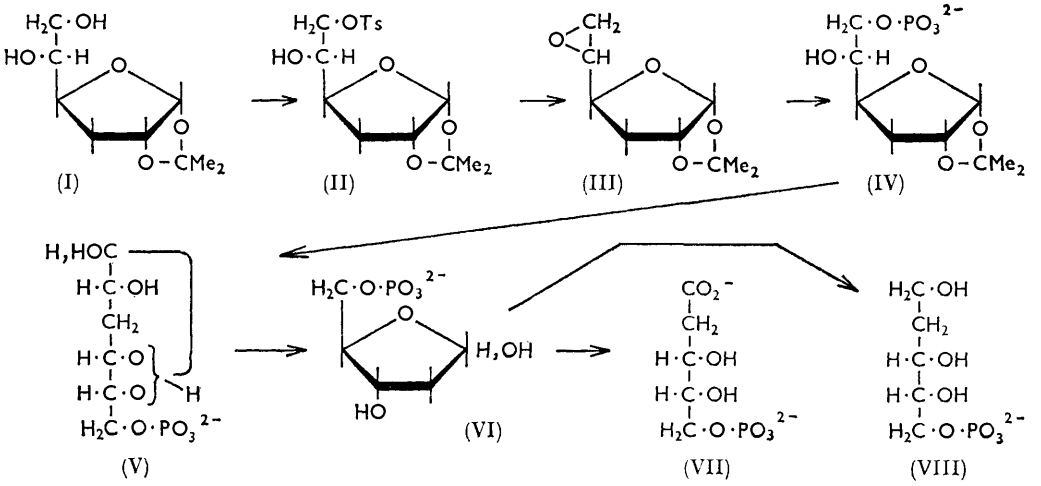
A successful synthesis of 2-deoxy-D-ribose 5-phosphate (VI) was achieved by the following sequence: 3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose, prepared according to Černý *et al.*,[13,14] was partially hydrolysed to 3-deoxy-1,2-*O*-isopropylidene-D-glucofuranose in conditions similar to those used for the preparation of 3-deoxy-1,2-*O*-isopropylidene-D-galactofuranose[15] (Overend and his co-workers,[16] recently published a method of partial hydrolysis of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose which gives the pure 1,2-*O*-isopropylidene derivative in 82% yield). Reaction of this compound with one molar equivalent of toluene-*p*-sulphonyl chloride yielded the oily 6-tosylate (II) from which the 5,6-anhydro-compound (III) was formed by treatment with one molar equivalent of sodium methoxide. The phosphate group was then introduced by treating the anhydride with inorganic phosphate, and 3-deoxy-1,2-*O*-isopropylidene-D-glucofuranose 6-phosphate (IX) was obtained. Mild acid hydrolysis of this compound yielded 3-deoxy-D-glucose 6-phosphate (V) which was isolated as the barium salt. The barium and brucine salts of this ester have been obtained previously by Dahlgard and Kaufmann[17] using another method. Treatment of 3-deoxyglucose 6-phosphate with one mole of sodium metaperiodate (cf. the preparation of 2-deoxy-D-ribose by oxidation of 3-deoxy-D-glucose with sodium periodate[18] and of 3-deoxy-D-mannose with lead tetra-acetate[19]) then gave the required 2-deoxy-D-ribose 5-phosphate (VI). The overall yield from 3-deoxy-1,2-*O*-isopropylidene-D-glucofuranose is 24% and the reaction sequence can easily be run on a scale giving 20—25 grams of the final phosphate ester.

2-Deoxy-D-ribose 5-phosphate has been characterised by elemental analysis of the barium salt. Potentiometric titration revealed two acid dissociations which proves the presence of a mono-esterified phosphoryl radical. Treatment with acid (wheat germ) phosphatase produced only 2-deoxy-D-ribose and inorganic phosphate, as judged by paper

* The authors thank Professor B. L. Horecker for this analysis.

[9] Ukita and Nagasawa, *Chem. and Pharm. Bull.* (*Japan*), 1959, **7**, 655.
[10] Lewak, Derache, and Szabó, *Compt. rend.*, 1959, **248**, 1837.
[11] Richards, *J.*, 1954, 3638.
[12] Ruff, *Ber.*, 1896, **31**, 1573.
[13] Černý and Pacák, *Coll. Czech. Chem. Comm.*, 1956, **21**, 1003.
[14] Černý, Pacák, and Jina, *Monatsh.*, 1963, **94**, 632.
[15] Antonakis, Dowgiałło, and Szabó, *Bull. Soc. chim. France*, 1962, 1355.
[16] Hedgley, Overend, and Rennie, *J.*, 1963, 4701.
[17] Dahlgard and Kaufmann, *J. Org. Chem.*, 1960, **25**, 781.
[18] Gorin and Jones, *Nature*, 1953, **172**, 1051.
[19] Rembarz, *Chem. Ber.*, 1962, **95**, 1565.

chromatography. The ratio of 2-deoxyribose (as measured by the diphenylamine method of Dische [20]) to phosphorus (determined according to Macheboeuf and Delsal [21]) was 1. Enzymatic analysis * showed that the compound was over 99% pure. Oxidation of 2-deoxy-D-ribose 5-phosphate by bromine in the presence of barium carbonate yielded the corresponding phosphorylated acid (VII), and its reduction with sodium borohydride gave



Ts = toluene-*p*-sulphonyl.

2-deoxyribitol 5-phosphate (VIII). Under the conditions described in the Experimental section, the 2-deoxy-D-ribose 5-phosphate consumed four moles of periodate in 50 hours, the oxidation then continuing very slowly. Two moles of titratable acid were liberated Under the same conditions, 2-deoxy-D-ribose reduced five moles of periodate, liberating three moles of acid.

Experimental

Chloroform and ether solutions were dried over anhydrous sodium sulphate before removal of the solvent. Unless otherwise stated, evaporations were conducted under reduced pressure and specific rotations were determined for aqueous solutions.

3-Deoxy-1,2-O-isopropylidene-D-glucofuranose.—To a solution of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose [13,14] (107 g.), in acetone (1 l.) heated under reflux on a water-bath, was added rapidly 0·1N-hydrochloric acid (110 ml.) at 58°. The mixture was left exactly 7 min. under reflux then poured on to stirred IR-45 resin (OH^- form) (400 g.) wetted with a little water. The resin was filtered from the neutral solution and washed with ethanol. The combined filtrate and washings were evaporated to dryness and the residue was taken up in anhydrous ether (400 ml.) (if there was any insoluble residue, the ethereal solution was decanted) and dried. The sodium sulphate was filtered off, washed well with anhydrous ether, and the combined filtrate and washings (1700 ml.) were put on a column (ϕ 43 mm.) of alumina (700 g.) in anhydrous ether. The column was washed with anhydrous ether (4 l.) and the eluate evaporated to dryness. The residue (starting material) (77·5 g.) was rehydrolysed as above and put on the same alumina column which was then washed again with anhydrous ether (3 l.). The starting material recovered (52·5 g.) from the eluate was rehydrolysed as above and put on the same column which was washed with ether (3 l.) to give starting material (34 g.). The column was then washed with methanol (4 l.), the eluate was evaporated to dryness, and the residue (44 g.,

* We thank Professor E. Racker for these analyses.

[20] Dische, *Mikrochem.*, 1930, **2**, 4.
[21] Macheboeuf and Delsal, *Bull. Soc. Chim. biol.*, 1943, **25**, 116.

yield 73%) crystallised spontaneously. This crude product can be used for the following reaction. A sample recrystallised from acetone–hexane had m. p. 80—81° (lit.,[13,16] 84°).

3-*Deoxy*-1,2-O-*isopropylidene*-6-*toluene*-p-*sulphonyl*-D-*glucofuranose*.—Toluene-*p*-sulphonyl chloride (13·5 g.) was dissolved in a solution of the above acetal (12·8 g.) in chloroform (165 ml.) freshly distilled from phosphoric oxide. Anhydrous pyridine (13·7 ml.) was added dropwise to this solution, which had been cooled to −50°. The mixture was put back into the cooling bath and the whole was left to come to room temperature and left overnight. A few drops of water were added and the solvent was removed below 30°. The residual oil was dissolved in chloroform to give a cloudy solution which was washed with water, ice-cold sulphuric acid (1%) (the chloroform layer then became clear) until the aqueous layer remained acid, sodium hydrogen carbonate solution, and twice with water, dried, and evaporated to give an oil (20·25 g., 90%).

5,6-*Anhydro*-3-*deoxy*-1,2-O-*isopropylidene*-D-*glucofuranose*.—To the above oil (20·25 g.) dissolved in chloroform (freshly distilled from phosphoric oxide) (85 ml.) and cooled in an acetone–dry-ice bath was added a solution of sodium (1·3 g.) in methanol (28 ml.) also cooled. The mixture was then taken out of the bath and shaken vigorously. As the temperature rose, the mixture became solid and gelatinous and after 15 min. reliquefied. At this stage, it was diluted with chloroform and the chloroform solution was washed with water, a fresh solution of sodium hydrogen carbonate, and water. The combined aqueous layers were re-extracted 3 times with chloroform and the combined chloroform solutions were dried and evaporated to give an oil which was purified by distillation. The 5,6-*anhydro-sugar* (7·25 g., 62%) had b. p. 53°/0·01 mm., $[\alpha]_D^{24}$ −21·4° (*c* 2·05 in EtOH), $n_D^{22\cdot5}$ 1·4570 (Found: C, 57·9; H, 7·45; O, 34·3. $C_9H_{14}O_4$ requires C, 58·1; H, 7·5; O, 34·4%).

3-*Deoxy*-1,2-O-*isopropylidene*-D-*glucofuranose* 6-(*Dihydrogen Phosphate*).—A solution of dipotassium hydrogen phosphate (25·2 g.) in water (100 ml.) was added to the foregoing oil (7·2 g.). The heterogeneous mixture became homogeneous after being heated at 50—60° with stirring for 16 hr. The solution was cooled, washed with a little ether, and diluted to 200 ml. with water, and the inorganic phosphate was precipitated by addition of barium hydroxide solution. Barium phosphate was filtered off and washed thoroughly with water. The pH of the combined filtrate and washings was adjusted to 7·2 with IR-120 resin (H^+), the solution was passed through a column of IR-120 resin (NH_4^+), and the column washed with water. Barium hydroxide solution (10 ml.) was added to the combined eluates which were evaporated under nitrogen, barium hydroxide solution being added in 10 ml. portions each time the pH fell until the solution remained alkaline and no more ammonia could be detected in the distillate. At a volume of 100 ml., the mixture was filtered (charcoal), the pH was adjusted to 7·0 with a trace of Amberlite-IR-120 (H^+), and the solution was further concentrated (40 ml.) and heated on a water-bath. The crystalline *barium salt* (8·8 g.) which formed was filtered off from the hot solution. A further crop (1·4 g.) was obtained by addition of ethanol to the concentrated mother-liquors (total yield 10·2 g., 63%). The compound had $[\alpha]_D^{20}$ −6·8° (*c* 0·47) (Found: C, 25·75; H, 3·65; P, 7·5. $C_9H_{15}BaO_8P$ requires C, 25·8; H, 3·6; P, 7·4%).

3-*Deoxy*-D-*glucose* 6-(*Dihydrogen Phosphate*).—The preceding compound (5 g.) was suspended in water (70 ml.) and acidified with Amberlite IR-120 (H^+). The resin was filtered off and washed with water. The combined filtrate and washings (150 ml.) were heated for 13 min. on a water-bath, cooled, neutralised (pH 6·9) with barium hydroxide solution, filtered (charcoal), and concentrated to a small volume.. The barium salt (4·3 g., 95%) was precipitated from the aqueous solution by addition of ethanol, filtered off, washed with ethanol, and dried over phosphoric oxide *in vacuo*. When equilibrated in air, the *dihydrate* had $[\alpha]_D^{20}$ +6·6 (*c* 0·4) (Found: C, 17·6; H, 3·8; P, 7·6; H_2O, 8·5. $C_6H_{11}BaO_8P,2H_2O$ requires C, 17·35; H, 3·6; P, 7·5; H_2O, 8·7%).

2-*Deoxy*-D-*ribose* 2-(*Deoxy*-D-erythro-*pentose*) 5-(*Dihydrogen Phosphate*).—The barium salt of 3-deoxy-D-glucose 6-phosphate (2·2 g.) was dissolved in water and stirred with Amberlite IR-120 (pyridinium form) until the supernatant liquor gave a negative test for barium ions. The resin was filtered off and washed with water. A solution of sodium metaperiodate (1·186 g.) in water (30 ml.) was added to the ice-cooled combined filtrate and washings (220 ml.), and left in a cold-room overnight. Barium hydroxide solution was added slowly to the cold, stirred solution to bring the pH to 6·9, barium iodate was filtered off, the solution was concentrated to remove pyridine, and the pH was again adjusted to 6·9 with barium hydroxide solution. The solution was concentrated (15 ml.) and the barium salt (1·4 g.) of 2-deoxy-D-ribose 5-phosphate was precipitated with methanol, centrifuged off, washed with methanol, and dried (P_2O_5). The

compound was reprecipitated from water with ethanol, centrifuged off, washed twice with ethanol, dried *in vacuo* at 40° over phosphoric oxide, and equilibrated in air. It had $[\alpha]_D^{20}$ +10·8° (*c* 0·52) (Found: C, 16·2; H, 3·25; P, 8·1; H_2O, 7·05. Calc. for $C_5H_9BaO_7,1{\cdot}5H_2O$: C, 16·2; H, 3·2; P, 8·2; H_2O, 7·2%).

The content of 2-deoxy-D-ribose, determined by the Dische diphenylamine test [20] using 2-deoxy-D-ribose as standard, was 99% of theory. A solution containing 114 μmoles/ml. of the barium-free sugar phosphate according to the diphenylamine test, contained 104 and 116 μmoles/ml. in two enzyme assays.

In a solution which was 0·004M in sugar phosphate and 0·022M in sodium metaperiodate, the reduction of four moles of periodate (estimated by the method of Fleury and Lange [22]) was practically complete after 24 hr. (3·75 moles) and was complete after 50 hr. (4·0 moles), the uptake thereafter being very slow (4·2 moles after 146 hr.). Two moles of titratable acid were formed.

2-Deoxy-D-ribonic Acid (*2-Deoxy-D-*erythro-*pentonic Acid*), 5-(*Dihydrogen Phosphate*).—2-Deoxy-D-ribose 5-phosphate (94 mg.) in water (2 ml.) was stirred with barium carbonate (118 mg.) and diluted bromine water was added dropwise until the bromine colour persisted. The mixture was left in a cold-room overnight, the solids were filtered off, and barium hydroxide solution was added to give a pH of 10. The solution was heated on a water-bath, filtered, cooled, and the pH adjusted to 8 with dilute hydrobromic acid. The solution was concentrated slightly, filtered (charcoal), then concentrated to a small volume. The *barium salt* (88 mg., 75%), precipitated with methanol, centrifuged off, washed twice with methanol, dried *in vacuo* over phosphoric oxide, and equilibrated in air, had $[\alpha]_D^{20}$ +1·95° (*c* 0·2) (Found: C, 13·0; H, 2·7; P, 6·5; H_2O, 7·8. $C_5H_8Ba_{1.5}O_8P,2H_2O$ requires C, 12·8; H, 2·6; P, 6·6; H_2O, 7·7%).

2-Deoxy-D-ribitol (*2-Deoxy-D-*erythro-*pentitol*) 5-(*Dihydrogen Phosphate*).—A solution of the barium salt of 2-deoxy-D-ribose 5-phosphate (206 mg.) in water (8 ml.) was added dropwise to a stirred solution of potassium borohydride (210 mg.) in water (2 ml.). The turbid mixture was left at room temperature overnight and acidified with Amberlite IR-120 (H^+). The resin was filtered off and washed with water, and the combined filtrate and washings were concentrated to dryness. The residue was triturated with water (10 ml.) and an insoluble precipitate was filtered off (this precipitate contained very little phosphorus and was not further examined). The filtrate was concentrated to a small volume. The *barium salt* was precipitated with methanol, centrifuged off, washed with methanol, reprecipitated from water with methanol, dried *in vacuo* over phosphoric oxide, and equilibrated in air, $[\alpha]_D^{20}$ −16·8° (*c* 0·2) (Found: C, 15·5; H, 3·7; P, 8·0; H_2O, 9·0. $C_5H_{11}BaO_7,2H_2O$ requires C, 15·5; H, 3·9; P, 8·0; H_2O, 9·3%).

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[22] Fleury and Lange, *J. Pharm. Chim.*, 1933, [8], **17**, 107.