528. *Phosphorylated Sugars. Part X.*[1] *A Simple Synthesis of 3-Deoxy-*D-erythro-*pentose 5-(Dihydrogen Phosphate)*

By (Mrs.) Patricia Szabó and L. Szabó

A simple synthesis of 3-deoxy-D-*erythro*-pentose 5-(dihydrogen phosphate) from D-glucose *via* 3-deoxy-1,2-*O*-isopropylidene-D-*ribo*-hexofuranose is described. Mild acid hydrolysis of the intermediate 3-deoxy-1,2-*O*-isopropylidene-D-*erythro*-pentofuranose gives 3-deoxy-D-*erythro*-pentose.

In connection with our work on sugar phosphates of biological interest, a simple synthesis of 3-deoxy-D-*erythro*-pentose 5-phosphate and, incidentally, that of the free sugar, has been elaborated. These syntheses seemed of interest in view of the identification [2a] of cordycepin, a metabolite of *Cordyceps militaris*, as 3-deoxyadenosine,[2b] and of the fact that accumulation of its phosphate ester seems to be responsible for its action on nucleic acid biosynthesis.[3]

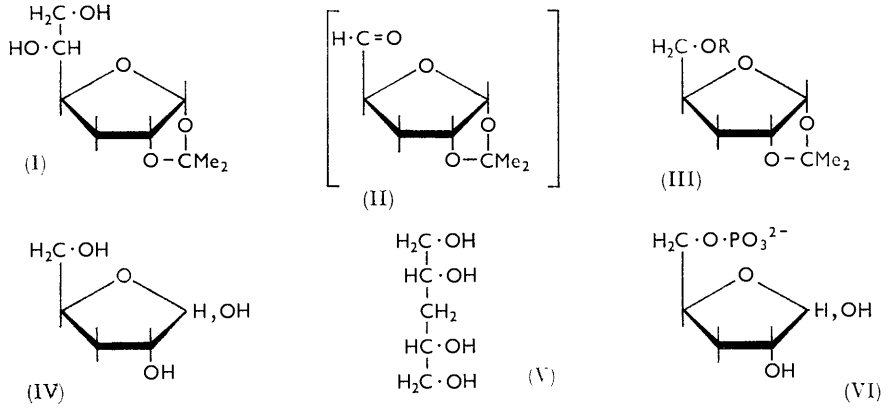
[1] Part IX, P. Szabó and L. Szabó, *J.*, 1964, 5139.

[2] (*a*) E. A. Kaczka, N. R. Trenner, B. Arison, R. W. Walker, and K. Folkers, *Biochem. Biophys. Res. Comm.*, 1964, **14**, 456; (*b*) W. W. Lee, A. Benitez, C. D. Anderson, L. Goodman, and B. R. Baker, *J. Amer. Chem. Soc.*, 1961, **83**, 1906.

[3] H. Klenow, *Biochem. Biophys. Res. Comm.*, 1961, **5**, 156; *Acta Chem. Scand.*, 1963, **17**, 893.

3-Deoxy-D-*erythro*-pentose (then named 3-deoxy-D-xylose) was first prepared by Kent, Stacey, and Wiggins,[4] while the synthesis of the L-sugar has been described by Mukherjee and Todd.[5] Kent *et al.* reported that cleavage of the epoxide ring in methyl 2,3-anhydro β-D-riboside with hydrobromic acid yielded mainly methyl 3-bromo-3-deoxy-β-D-*erythro*-pentoside (3-bromo-xyloside) together with methyl 2-bromo-2-deoxy-β-D-*erythro*-pentoside (2-bromo-arabinoside). Hydrogenation of the 3-bromo-compound followed by mild acid hydrolysis of the 3-deoxy-pentoside thus formed yielded the 3-deoxy-D-*erythro*-pentose as a syrup. Allerton and Overend [6] obtained similar results by cleavage of the epoxide ring in methyl 2,3-anhydro-β-D-riboside with hydrochloric acid and noted that reduction of the anhydro-compound with lithium aluminium hydride also gave predominantly methyl 3-deoxy-β-D-*erythro*-pentoside.

The present synthesis of the 3-deoxy-pentose was achieved by treating the now readily accessible 1,2-*O*-isopropylidene-3-deoxy-D-*ribo*-hexose (I) [7,8] with one mol. of sodium metaperiodate and reducing the dialdehyde (II) thus obtained with Raney nickel to give the crystalline 1,2-*O*-isopropylidene-3-deoxy-D-*erythro*-pentofuranose (III; R = H). This sugar was further characterised as its crystalline 5-toluene-*p*-sulphonyl ester (III; R = $-SO_2{\cdot}C_7H_7$). Mild acid hydrolysis readily removed the isopropylidene group from the ketal (III; R = H) yielding the free sugar (IV) as a syrup, which on treatment with toluene-*p*-sulphonylhydrazine gave the crystalline hydrazone. The sugar was also reduced to give the crystalline 3-deoxy-D-*erythro*-pentitol (V), identical with that of Kent *et al.*[4] A mixture of 2-deoxy-D-*erythro*-pentose (2-deoxy-D-ribose) and the above 3-deoxy-pentose can be easily separated by low-voltage paper electrophoresis in borate buffer at pH 10.



When a sample of natural cordycepin, kindly donated by Dr. G. E. Boxer of Merck, Sharp, and Dohme, was hydrolysed with N-acid and the hydrolysate submitted to electrophoresis, it was found that the reducing sugar which was liberated from the nucleoside had the same mobility as 3-deoxy-D-*erythro*-pentose, thus further confirming the structure suggested [2a] for cordycepin.

Treatment of 1,2-*O*-isopropylidene-3-deoxy-D-*erythro*-pentofuranose with diphenyl phosphorochloridate gave the crystalline diphenyl phosphate [III; R = $-PO(OC_6H_5)_2$] which, after hydrogenation, yielded the 5-phosphate (III; R = $-PO_3^{2-}$) isolated as its crystalline barium salt. 3-Deoxy-D-*erythro*-pentose 5-phosphate (VI), also isolated as the barium salt, was obtained from this compound after removal of the isopropylidene group by mild acid hydrolysis.

[4] P. W. Kent, M. Stacey, and L. F. Wiggins, *J.*, 1949, 1232.
[5] S. Mukherjee and A. R. Todd, *J.*, 1947, 969.
[6] R. Allerton and W. G. Overend, *J.*, 1951, 1480.
[7] M. Černý and J. Pacák, *Coll. Czech. Chem. Comm.*, 1956, **21**, 1003; M. Černy, J. Pacák, and V. Jína, *Monatsh.*, 1963, **94**, 632.
[8] E. J. Hedgley, W. G. Overend, and R. A. C. Rennie, *J.*, 1963, 4701.

With 2-naphthol–concentrated sulphuric acid, the free sugar gave a non-specific brown colour instead of the blue colour normally observed with pentoses.[9] The Webb and Levy test [10] as modified by Fromme *et al.* for 3-deoxy-sugars [11] was negative. This confirms the observation [11,12] that 2-deoxy-tetroses give a negative Webb and Levy test. Both the free sugar and its 5-phosphate gave a weak colour in Dische's diphenylamine test [13] for 2-deoxy-sugars, the phosphate giving a coloration that was about one tenth of the intensity of that given by 2-deoxy-D-ribose. Allerton *et al.*[14] found that 3-deoxy-L-*erythro*-pentose gave a coloration having about one thirtieth the intensity of that given by 2-deoxy-D-ribose in the Dische test.

When treated with periodate in the usual conditions,[15] the phosphate ester (VI) reduced one molar equivalent of periodate in a few minutes, no more periodate being reduced for the next 48 hr.; one molar equivalent of acid was formed. Paper chromatography of the reaction mixture revealed as the only phosphate ester present a substance having the mobility which would be expected for 2-deoxy-D-*glycero*-tetrose 4-phosphate (2-deoxy-D-erythrose 4-phosphate). The isolation and characterisation of this compound .whose behaviour as an acceptor aldehyde in the transketolase reaction should be of interest, will be reported elswhere.

Experimental

Chloroform solutions were dried (Na_2SO_4) before removal of the solvent. Unless otherwise stated, all evaporations were conducted under reduced pressure.

3-*Deoxy*-1,2-O-*isopropylidene*-D-erythro-*pentose*.—3-Deoxy-1,2-*O*-isopropylidene-D-*ribo*-hexose [8] (4·5 g.) was dissolved in water (10 ml.) and the solution was cooled in an ice-bath. A solution of sodium metaperiodate (5·66 g.) in water (60 ml.) was added slowly over a period of 1·5 hr., the pH being kept near the Methyl-red end-point by addition of dilute sodium carbonate solution. The aqueous solution was then extracted with chloroform (10 × 70 ml.), the chloroform solution was dried and evaporated to dryness below 40°. The residue (3·7 g.) was dissolved in 95% ethanol and reduced over Raney nickel. When the uptake of hydrogen had ceased, the catalyst was filtered off and the ethanolic solution evaporated to dryness. The crystalline residue was dissolved in hexane and filtered hot to remove a slight insoluble residue (0·15 g.). The *isopropylidene-deoxy-pentose* (2·5 g.) crystallised when the solution was cooled. A further crop (220 mg.) of material was obtained from the mother-liquors (yield 2·7 g., 71%). The compound had m. p. 74—76°, $[\alpha]_D$ −3·0° (*c* 0·106 in $CHCl_3$) (Found: C, 55·0; H, 8·2; O, 36·7. $C_8H_{14}O_4$ requires C, 55·15; H, 8·1; O, 36·7%).

3-*Deoxy*-1,2-O-*isopropylidene*-5-*toluene*-p-*sulphonyl*-D-erythro-*pentose*.—Toluene-*p*-sulphonyl chloride (60 mg.) was added to a cold solution of the above compound (50 mg.) in anhydrous pyridine (2 ml.). The mixture was left for 3 days at room temperature, a few drops of water were added and after 2 hr. the pyridine was removed. The residue was taken up in chloroform (5 ml.) and the chloroform solution was washed successively with iced water, ice-cold sulphuric acid (1% v/v), water, a saturated solution of sodium bicarbonate and finally with water, and dried. The chloroform was removed and the residue dissolved in ether. On addition of hexane to the ethereal solution, the *toluene*-p-*sulphonate* crystallised. After having been recrystallised once from the same mixture of solvents, it had m. p. 61—62°, $[\alpha]_D^{25}$ −5·0° (*c* 0·0425 in $CHCl_3$) (Found: C, 55·0; H, 6·1; S, 9·65. $C_{15}H_{20}O_6S$ requires C, 54·9; H, 6·1; S, 9·75%).

3-*Deoxy*-D-erythro-*pentose*.—3-Deoxy-1,2-*O*-isopropylidene-D-*erythro*-pentose (300 mg.) was heated for 2 hr. in a boiling-water bath with 0·05N-HCl (10 ml.). (During this time $[\alpha]_D$ changed from −19·5° to −2·0°.) The solution was cooled, neutralised with silver carbonate, filtered (charcoal), and evaporated to dryness below 40°. The product was dried several days *in vacuo* over phosphoric oxide. It had n_D^{28} 1·4933, $[\alpha]_D^{25}$ −11·2 (*c* 1·94 in H_2O) (Kent *et al.*[4] give n_D^{16}

[9] P. Thomas, *Bull. Soc. Chim. biol.*, 1925, **7**, 102.
[10] J. M. Webb and H. B. Levy, *J. Biol. Chem.*, 1955, **213**, 107.
[11] I. Fromme, O. Lüderitz, H. Stierlin, and O. Westphal, *Biochem. Z.*, 1958, **330**, 53.
[12] K. Himmelspach and O. Westphal, *Annalen*, 1963, **668**, 165.
[13] Z. Dische, *Mikrochem.*, 1930, **8**, 4.
[14] R. Allerton, W. G. Overend, and M. Stacey, *J.*, 1952, 255.
[15] D. R. Strobach and L. Szabó, *J.*, 1963, 3970.

1·4610 and $[\alpha]_D^{24}$ −6·3°; Mukherjee and Todd [5] describe the enantiomorph as having $[\alpha]_D$ +8·7°).

3-*Deoxy*-D-erythro-*pentose Toluene*-p-*sulphonylhydrazone*.—The above sugar (70 mg.), dissolved in methanol (3 ml.), was refluxed for 45 min. with toluene-*p*-sulphonylhydrazine (65 mg.). The mixture was then cooled and concentrated to small volume (*ca.* 1 ml.). The crystals which formed on cooling were redissolved and the hot solution was filtered with charcoal. After another crystallisation from methanol, the *toluene*-p-*sulphonylhydrazone* had m. p. 143—144°, $[\alpha]_D^{25}$ −5·0° (*c* 0·1 in EtOH) (Found: C, 47·7; H, 5·85; N, 9·2; S, 10·4. $C_{12}H_{18}N_2O_5S$ requires C, 47·7; H, 6·0; N, 9·3; S, 10·6%).

3-*Deoxy*-D-erythro-*pentitol*.—3-Deoxy-1,2-*O*-isopropylidene-D-*erythro*-pentose (200 mg.) in water (2·5 ml.) was heated in a boiling-water bath with finely ground IR-120 resin (H^+ form) for 30 min. The resin was filtered off and washed with water (2·5 ml.) and the combined filtrate and washings were added dropwise to a solution of potassium borohydride (355 mg.) in water (2 ml.). The reaction mixture was left overnight at room temperature, the excess of borohydride was destroyed with IR-120 resin (H^+ form), the resin was filtered off and washed with water, and the combined filtrate and washings were lyophilised. Methanol was added to the residue and then distilled off at atmospheric pressure and this process was repeated until the distillate contained no more methyl borate. The residue was crystallised from ether–hexane and had m. p. 66—67° (lit.,[4] 68—69°).

3-*Deoxy*-1,2-O-*isopropylidene*-D-erythro-*pentose* 5-*Diphenyl Phosphate*.—Diphenyl phosphorochloridate (926 mg.) in anhydrous pyridine (2 ml.) was added to a solution of 3-deoxy-D-*erythro*-pentose (500 mg.) in anhydrous pyridine (4 ml.) and the mixture was left overnight at room temperature. A few drops of water were added and the pyridine was removed. The crystalline residue (1·15 g.) was triturated with cold water and then recrystallised from hot aqueous (50%) ethanol. The *diphenyl phosphate* had m. p. 74°, $[\alpha]_D^{25}$ −13·6° (*c* 0·14 in EtOH) (Found: C, 58·9; H, 5·7; P, 7·7. $C_{20}H_{23}O_7P$ requires C, 59·1; H, 5·7; P, 7·6%).

3-*Deoxy*-1,2-O-*isopropylidene*-D-erythro-*pentose* 5-(*Dihydrogen Phosphate*).—The above compound (0·9 g.) dissolved in ethanol was hydrogenated in the presence of Adams platinum catalyst. When the theoretical quantity of hydrogen had been absorbed, the catalyst was filtered off and the filtrate was neutralised with barium hydroxide solution. The solvents were removed and the *barium salt of the phosphate* was crystallised in hot water. An additional crop of crystals was obtained by addition of ethanol to the concentrated mother-liquors (yield 0·73 g., 95%). The salt had $[\alpha]_D^{25}$ −24·8° (*c* 0·05 in H_2O) (Found: C, 23·4; H, 4·1; P, 7·3. $C_8H_{13}BaO_7P,H_2O$ requires C, 23·6; H, 3·7; P, 7·6%).

3-*Deoxy*-D-erythro-*pentose* 5-(*Dihydrogen Phosphate*).—The above barium salt (0·3 g.) was suspended in water (3 ml.) and barium ions were removed with Amberlite IR-120 resin (H^+ form). The resin was filtered off and washed with water and the combined filtrate and washings (total volume 5·5 ml.) were heated for 10 min. in a boiling-water bath, cooled and neutralised (pH 6·9) with barium hydroxide solution. The solution was filtered with charcoal and concentrated to small volume (*ca.* 1 ml.). Ethanol was added to precipitate the *barium salt of the phosphate* which was dried *in vacuo* (P_2O_5) and then equilibrated in air. It had $[\alpha]_D^{25}$ −10·65° (*c* 0·122 in H_2O) (Found: C, 16·5; H, 3·4; P, 8·6; H_2O, 4·7. $C_5H_9BaO_7P,H_2O$ requires C, 16·3; H, 3·0; P, 8·4; H_2O, 4·9%).

Hydrolysis and Electrophoresis of Cordycepin.—Cordycepin (7·48 mg.) was heated with 1N-hydrochloric acid (50 ml.) for 10 min. at 100° in a sealed tube. The warm solution was used for electrophoresis experiments. In a typical electrophoresis run in borate buffer at pH 10 at approximately 400v for 3 hr., 2-deoxy-D-ribose moved 6·5 cm. from the origin and cordycepin and 3-deoxy-D-*erythro*-pentose moved 7·5 cm.

INSTITUT DE BIOCHIMIE, FACULTÉ DES SCIENCES,
ORSAY, SEINE ET OISE, FRANCE.

[*Received, October 13th*, 1964.]