Communication to the Editor

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## Synthesis of 2-Deoxy-D-ribose 5-Phosphate

2-Deoxy-D-ribose 5-phosphate was identified as a reaction product of enzymatic phosphorolysis and subsequent mutasis of deoxyribonucleosides in the presence of inorganic phosphate catalyzed by crude extracts of *Escherichia coli*.[1,2] Further, the same phosphate was reported as an enzymatic aldol-condensation product of D-glyceraldehyde 3-phosphate and acetaldehyde by purified deoxyribose-phosphate-aldolase obtained from *E. coli*.[3]

Thus, this phosphate is an interesting one in several important pathways in intermediary metabolism, but no chemical synthesis of this phosphate has hitherto been encountered in the literature. In 1952, Allerton, *et al.*[4] reported chemical synthesis of its isomer, 2-deoxy-L-ribose 5-phosphate, and several related phosphates, but no detail of experiment was recorded.

This communication deals with chemical synthesis of 2-deoxy-D-ribose 5-phosphate.

$\alpha\beta$-Methyl 2-deoxy-D-ribofuranoside (I)[5] was reacted with equimolar amount of diphenyl phosphorochloridate in dehyd. pyridine. After removal of the solvent, the reaction product was dissolved in $CHCl_3$ and extracted with ice water to separate both unreacted (I) and diphenyl phosphate. The syrupy residue obtained after removal of $CHCl_3$ was tested by paper chromatography and paper electrophoresis,*1 and each gave a single spot which showed positive phosphorus and 2-deoxysugar reaction. This single spot was established as that of a mixture of $\alpha\beta$-methyl 2-deoxy-D-ribofuranoside 5-(diphenyl-phosphate) (IIa) and 3,5-bis(diphenyl-phosphate) (IIb).

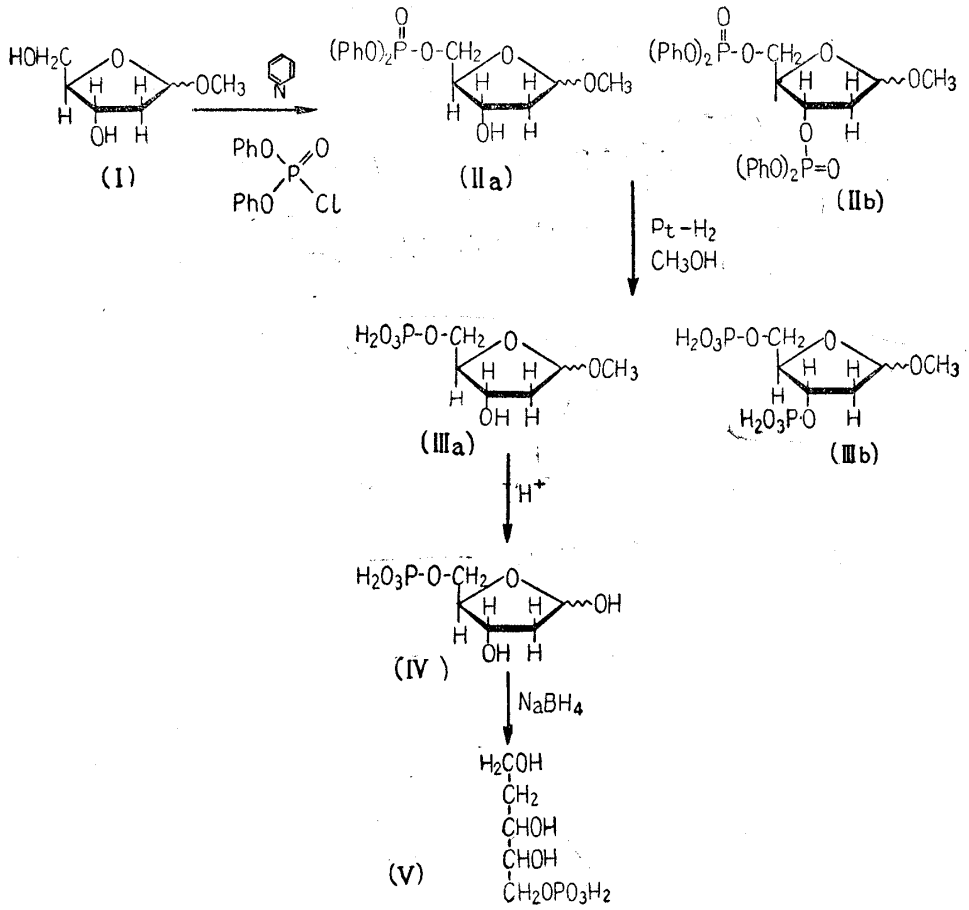
On catalytic hydrogenation of this syrup in dehyd. MeOH with Adams' platinum oxide, two products (IIIa and IIIb) giving respective Rf values of 0.76 and 0.28 were formed in a ratio of 4 to 1. These products were separated by EtOH precipitation from aqueous solution of their mixed Ba salts and the final purification of each Ba salt was performed by cellulose column chromatography.*2

On recrystallization of cyclohexylammonium salt of (IIIa) from dehyd. EtOH-$Et_2O$, the dicyclohexylammonium $\alpha\beta$-methyl 2-deoxy-D-ribofuranoside 5-phosphate was obtained as fine needles of m.p. 173°(decomp.), Rf 0.76 (*Anal.* Calcd. for $C_{18}H_{39}O_7N_2P$ : C, 50.70; H, 9.22; N, 6.57; P, 7.27. Found : C, 50.80; H, 9.16; N, 6.59; P, 7.51. $[\alpha]_D^{17}$ +32.88°(c= 1.57, $H_2O$)). The cyclohexylammonium salt of (IIIb) was recrystallized from a mixture of MeOH and *iso*-PrOH to give tricyclohexylammonium $\alpha\beta$-methyl 2-deoxy-D-ribofuranoside 3,5-diphosphate as needles with m.p. 215~216°(decomp.), Rf 0.28 (*Anal.* Calcd. for

*1 In paper chromatography, samples were applied on Toyo Roshi No. 53 filter paper and run ascendingly with the solvent system of *n*-PrOH:conc. $NH_4OH$:$H_2O$=6:3:1. Paper electrophoresis was performed on the same filter paper using a buffer solution of pH 6.0, consisting of pyridine (10 cc.):AcOH(2 cc.):*n*-BuOH(20 cc.):$H_2O$(500 cc.), and subjected to a potential of 20~40 V/cm. for 30~60 min. Spots on each chromatogram were detected by phosphorus and 2-deoxysugar reactions.

*2 Toyo Roshi cellulose powder (100~200 mesh) column was prepared in a mixed solvent (*iso*-PrOH: conc. $NH_4OH$:$H_2O$=7:1:2) and eluted with the same solvent. Phosphorus-containing fractions were checked by both paper chromatography and electrophoresis.

1) M. Friedkin, H.M. Kalckar : J. Biol. Chem., **184**, 437(1950).
2) L.A. Manson, J.O. Lampen : *Ibid.*, **191**, 95(1951).
3) E. Racker : *Ibid.*, **196**, 347(1952).
4) R. Allerton, W.G. Overend, M. Stacey : Chem. & Ind.(London), **1952**, 952.
5) W.G. Overend, M. Stacey, L.F. Wiggins : J. Chem. Soc., **1949**, 2836.




$C_{24}H_{53}O_{10}N_3P_2 \cdot H_2O$ : C, 46.19; H, 8.89; N, 6.73; P, 9.94. Found : C, 45.84; H, 8.73; N, 6.48; P, 9.88. $[\alpha]_D^{21}$ +18.17°(c=1.15, $H_2O$)).

The aqueous acid solution (pH 1.25) obtained by decationization of the cyclohexylammonium salt of (IIIa) was kept for 1 hour at 37°. By this treatment, (IIIa) was completely hydrolyzed to 2-deoxy-D-ribose 5-phosphate (IV) which showed Rf value of 0.47. (IV) was converted to its Ba salt and purified by repeated precipitation from its aqueous solution with EtOH (*Anal.* Calcd. for $C_5H_9O_7BaP$ : C, 17.18; H, 2.87; P, 8.87. Found: C, 17.20; H, 3.30; P, 8.42. $[\alpha]_D^{28}$ +16.51°(c=2.12, $H_2O$)).

The Ba salt of (IV) thus obtained was hydrolysed with *N* HCl at 95° for 40 min. to inorganic phosphate and 2-deoxy-D-ribose, and its hydrolysis curve was almost similar to that of 2-deoxy-D-ribose 5-phosphate[3] isolated enzymatically.

For further confirmation of the structure of (IV), the Na salt of (IV) was reduced with $NaBH_4$ to obtain the phosphate of 2-deoxy-D-ribitol. The cyclohexylammonium salt of (V) was recrystallized from dehyd. EtOH-$Et_2O$ to fine needles, m.p. 165°, Rf 0.63 (*Anal.* Calcd. for $C_{17}H_{39}O_7N_2P$ : C, 49,23; H, 9.48; N, 6.76; P, 7.48. Found : C, 48.76; H, 9.34; N, 6.31; P, 7.81. $[\alpha]_D^{17}$ −10.0°(c=1.20, $H_2O$)). On periodate oxidation, cyclohexylammonium salt of (V) consumed 0.98 mole of the reagent giving, not formaldehyde,[6] but glycolaldehyde phosphate[7] as the reaction product.

From above-described experiments on phosphorylation of $\alpha\beta$-methyl 2-deoxy-D-ribofuranoside (I) with diphenyl phosphorochloridate, it was established that the primary hydroxyl group of (I) was preferentially phosphorylated to (IIa), further phosphorylation

6) W. R. Frisell, L. A. Meech, C. G. Mackenzie : J. Biol. Chem., **207**, 709(1954).
7) J. Baddiley, J. G. Buchanan, B. Carss, A. P. Mathias, A. R. Sanderson : Biochem. J., **64**, 599 (1956).

occurred partially at C-3 hydroxyl group of the latter to give (IIb), and that no selective phosphorylation of (I) at its C-3 secondary hydroxyl group was observed.

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