peak, different from that for Ib, was obtained, suggesting that the hydrogenation was more complete than hydrogen uptake indicated. (A less likely explanation is that any unreduced Ib was not extracted from the decomplexed mixture.)

Reduction of 1-Phenyl-2-methyl-3-phospholene (Ic).—The complex formed from 1.71 g (0.0097 mol) of *cis,trans* Ic[8] and 4.0 g (0.16 mol) of nickel chloride in 20 ml of absolute ethanol was reduced over 0.35 g of 10% palladium on charcoal. After 9 days, hydrogen uptake had stopped, with 96% of the theoretical amount of hydrogen consumed. (In another experiment, the measured hydrogen uptake was 71%.) The complex was broken with EDTA as before, and the phosphine was extracted with five portions of ether totaling 450 ml. To 245 ml of the extract was added 5 ml of benzyl bromide. After 3 days, enough of the benzyl bromide salt had formed to permit characterization. It was filtered from the solution; water extraction of the ether solution gave some additional salt. There was obtained 0.281 g, which was recrystallized from ethyl acetate–methanol: mp 202.1–202.5°.

Anal. Calcd for $C_{18}H_{22}BrP$: C, 61.90; H, 6.35; P, 8.87. Found: C, 61.67; H, 6.50; P, 8.96.

The nmr spectrum of the salt (0.067 g in 0.776 g of D_2O, a saturated solution) showed a complicated phenyl region from δ 7.4 to 8.4. Overlapping CCH_3 signals around δ 1.58 indicate a mixture of *cis* and *trans* isomers in the product. The solution was too dilute to gain any other information from the spectrum.

Reduction of 1-Ethyl-4-(2-hydroxyethyl)-1,2,5,6-tetrahydrophosphorin (III).—The nickel chloride complex of phosphine III[9] was formed by adding 10 g (0.058 mol) of III to 7.2 g (0.030 mol) of nickel chloride in 70 ml of absolute ethanol. The complex was hydrogenated over a palladium-on-charcoal catalyst for 7 days at 48 psi, with absorption of 95% of the theoretical amount of hydrogen. The phosphine was then released from the complex as before. The solution was extracted with four 50-ml portions of benzene. The benzene was removed on a rotary evaporator to leave a residue of 6 g (59% crude yield). The residue was fractionated on a spinning-band column to give a 3-g fraction of 1-ethyl-4-(2-hydroxyethyl)phosphorinane at 83–85° (0.15 mm). The infrared spectrum showed no double-bond absorption around 1600 cm^{-1}, and the nmr spectrum showed no vinyl protons.

Anal. Calcd for $C_9H_{19}OP$: C, 62.04; H, 10.99; P, 17.78. Found: C, 61.90; H, 10.98; P, 17.59.

Registry No.—Reduced Ia (benzyl bromide salt), 21473-48-3; reduced Ib (benzyl bromide salt), *cis*, 21473-24-5; reduced Ib (benzyl bromide salt), *trans*, 21492-05-7; reduced Ic (benzyl bromide salt), *cis*, 21473-25-6; reduced Ic (benzyl bromide salt), *trans*, 21473-26-7; 1-ethyl-4-(2-hydroxyethyl)phosphorinane, 21473-27-8.

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(9) L. D. Quin and J. W. Russell, Jr., unpublished work.

Phosphorylation of D-Ribose in Aqueous Solution

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Received February 7, 1969

In the context of prebiological chemistry, the phosphorylation of sugars in aqueous solution is of interest in view of the biological importance of the products. The only relevant work with which we are familiar is that of Calvin,[2] who mentioned that dicyandiamide promotes the formation of ribose 5-phosphate from ribose and orthophosphate. No details were given. We report here our studies on the phosphorylation of ribose using cyanogen and cyanamide as condensing agents.

β-D-Ribofuranose 1-phosphate is the only sugar phosphate (10–20%) formed in the reaction of D-ribose with orthophosphate in the presence of cyanogen or cyanamide. This reaction is an order of magnitude more efficient than the phosphorylation of nucleosides (which lack the reactive sugar hemiacetal function) under comparable conditions.[3]

The reactive phosphorylating species in these reactions are presumably adducts of orthophosphate and cyanogen or cyanamide[3] of the same type as those proposed in related reactions of carbodiimides.[4] We think it unlikely that adducts between ribose and either cyanamide or cyanogen[5] undergo a nucleophilic displacement by orthophosphate.

Two observations suggest that the 2-hydroxyl group of ribose is involved in the reaction. Firstly, 2-deoxy-D-ribose is not phosphorylated under the conditions of our reaction; secondly, only the β-phosphate is formed from ribose. It is noteworthy that only the furanose phosphate can be detected, whereas a large number of reactions of ribose are known to produce mixtures of furanose and pyranose isomers.[6]

This reaction may be useful in the synthesis of β-ribofuranose 1-phosphate. Previously reported syntheses involve protected sugars; enzymatic reactions produce exclusively α-ribofuranose 1-phosphate.[4,7]

Experimental Section

Condensation with Cyanogen.—Stirred aqueous solutions of D-ribose and orthophosphate (both initially 0.1–0.2 *M*), at pH's in the 7.0–8.8 range and at 25°, were evacuated through rubber septa. Cyanogen gas was then added by syringe to give final concentrations ranging from 0.02 to 0.44 *M*. After a few minutes, the solutions turned yellowish brown. Samples were withdrawn at intervals for analysis. The phosphate-containing products were studied in various chromatographic systems (see Table I). D-ribose-1-^{14}C was used in some experiments to facilitate the identification of products.

A. Formation of Acid-Labile Phosphate Esters.—The uptake of orthophosphate was measured by the colorimetric method of Lowry and Lopez.[8] A maximal uptake of 18–22% was obtained after 1 day from solutions 0.2 *M* in orthophosphate, cyanogen, and ribose. Chromatography after treatment of the reaction mixture with 0.1 *N* hydrochloric acid for 10 min at 100° showed that all of the sugar phosphate had been hydrolyzed. Only aldose 1-phosphates are so sensitive to acid hydrolysis.[7,9]

B. Test for Alkali-Labile Phosphate Esters.—There was no decomposition of the sugar phosphate formed in the experiment described in A after the reaction mixture had been heated with 0.1 *N* sodium hydroxide for 10 min at 100°. Under such conditions, we found that ribose 5-phosphate was completely destroyed, while both α- and β-ribofuranose 1-phosphates were stable. It had previously been reported that ribose 5-, and 3-, and 2-phosphates readily decompose in aqueous alkali, while aldose 1-phosphates are alkali stable.[9]

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TABLE I
CHROMATOGRAPHIC DATA[a]

Compd	Paper chromatography[b] R_p[A d]	R_p[B]	R_f[C]	R_p[D]	R_f[E]	Paper electrophoresis,[c] borate, $R_{R\text{-}5\text{-}P}$
D-Ribose		1.65	0.70		0.36	
D-Arabinose					0.30	
Ribose 5-phosphate	0.43	1.02	0.43	4.0		1.00
α-Ribofuranose 1-phosphate	0.8	1.21	0.47	2.3		0.92
β-Ribofuranose 1-phosphate	0.6	1.23	0.48	2.5		0.98
Ribopyranose 1-phosphate	0.8	1.07	0.47			0.93
$H_2P_2O_7^{2-}$	0.4	0.79		0.2		
HPO_4^{2-}	1.0	1.0		1.0		

[a] Whatman 3MM paper was used. Sugars and sugar phosphates were detected with an aniline–phthalic acid spray (I. M. Hais and K. Macek, "Paper Chromatography," House of the Czechoslovak Academy of Science, Prague, and Academic Press, New York, N. Y., 1963, p 793). Sugar phosphates and inorganic phosphates were detected by spraying with ammonium molybdate–perchloric acid, followed by uv irradiation to develop the blue color of the phosphomolybdate complex (same reference, p 819). [b] Chromatographic solvents: A, isopropyl alcohol, concentrated ammonia, 0.1 *M* sodium borate (7:1:2); B, *n*-propyl alcohol, concentrated ammonia, water (11:2:7); C, *n*-propyl alcohol, concentrated ammonia, 0.1 *M* sodium borate (11:2:7); D, Methyl Cellosolve, methyl ethyl ketone, 3 *N* ammonia (7:2:3); E, *n*-butyl alcohol, glacial acetic acid, water (4:1:1). [c] Sodium borate buffer (0.05 *M*, pH 9.0); 1000 V during 2 hr. [d] Movement of spots: R_p, relative to orthophosphate; R_f, relative to the solvent front; $R_{R\text{-}5\text{-}P}$, relative to ribose 5-phosphate.

C. Enzymatic Dephosphorylation.—The amount of orthophosphate released from the purified product (see section E) by alkaline phosphatase was within experimental error (about 2%), equal to that released during hydrolysis in hydrochloric acid (0.1 *N*, 10 min, 100°). This confirms that no substantial part of the product is an acid-stable phosphate ester.

D. Chromatographic Analyses.—Samples from reaction mixtures containing D-ribose-1-^{14}C were analyzed by paper chromatography in solvent B. The resulting paper strips were analyzed on a radiochromatogram scanner and the ratio of ^{14}C activity in the ribose to that in the ribose phosphate regions was determined. In a run initially 0.1 *M* in ribose and phosphate and 0.05 *M* in cyanogen (pH 7.0 or 8.0; 3 hr at 24°) the yield of ribose phosphate was 9%. With 0.2 *M* cyanogen under the same conditions the yield was 20%.

Cochromatography of the reaction mixtures with the different ribose phosphates in system A for 6 days gave coincidence of the ^{14}C activity only with a spot corresponding to β-ribofuranose 1-phosphate.

E. Isolation of the Ribose Phosphate.—In one run, the reaction mixture (containing initially 0.2 *M* each of sodium phosphate, D-ribose, and cyanogen at pH 8.0), after standing overnight at 25°, was treated with barium acetate to remove the unreacted orthophosphate. Crude barium ribose phosphate was then precipitated by addition of ethanol as a brownish sticky powder (yield 27%, after overnight vacuum drying). In a similar experiment, the crude reaction mixture was passed through a column of Dowex 2-X8 anion-exchange resin in the formate form and was eluted with a 0.1 *M* formate buffer (pH 5.0).[10] The effluent was treated with barium acetate and then ethanol, as above, yielding a small amount of purified product. After twice dissolving the product in water, precipitating with ethanol, and drying overnight under vacuum, shiny white crystals were obtained. *Anal.* Calcd for $C_5H_9O_8PBa \cdot 1H_2O$:[7] P, 8.1. Found: P, 8.0. The product gave only one spot, identical with that given by β-ribofuranose 1-phosphate, on descending paper chromatography in solvent A.

After hydrolysis overnight in 0.01 *N* hydrochloric acid at room temperature, chromatography of the hydrolysate (in solvent E) gave only one spot, that of D-ribose. No D-arabinose could be detected.

F. Rate of Acid Hydrolysis.—Rates of hydrolysis were measured by dissolving weighed portions of each sugar phosphate in 0.010 *N* hydrochloric acid and maintaining the solutions at 25.0°. Aliquots were analyzed for orthophosphate by measuring the absorption at 700 mμ of the phospho–molybdate complex.[8]

The half-life of our purified product at 25° in 0.010 *N* hydrochloric acid was 3 ± 1 hr compared with 2.0 ± 0.4 hr for α-ribofuranose 1-phosphate, 2.4 ± 0.4 hr for β-ribofuranose 1-phosphate and 70 ± 20 hr for ribopyranose 1-phosphate. The rates for the α- and β-ribofuranose 1-phosphates are similar, and within experimental error equal to that of our product. They are much larger than the rate for acid hydrolysis of ribopyranose 1-phosphate, in agreement with a previous report.[7] Consequently ribopyranose 1-phosphate is not a substantial component of our product.

G. Attempt to Phosphorylate 2-Deoxy-D-ribose.—2-Deoxy-D-ribose and orthophosphate (both 0.1 *M*, at pH 7.0 or 8.8 and at 25 or 65°) did not undergo phosphorylation in the presence of cyanogen (0.22 *M*).

Condensation with Cyanamide.—Dilute aqueous solutions of D-ribose, orthophosphate, and cyanamide at pH 7.0 were placed in a bath at 65°. When the three components were each initially 0.02 *M* in concentration, formation of a ribose phosphate could be detected by paper chromatography (solvent B) after 7 days of reaction. The product was identified as an aldose 1-phosphate by its acid lability and alkali stability. With 0.25 *M* cyanamide, 0.1 *M* D-ribose (with added D-ribose-1-^{14}C), and 0.1 *M* orthophosphate, the yields of ribose 1-phosphate after 21 and 72 hr were 6 and 8%, respectively. They did not increase after that. The product was identified as β-ribofuranose 1-phosphate by descending development in solvent A for 6 days.

Registry No.—D-Ribose, 58-91-3; β-ribofuranose 1-phosphate, 21317-51-1.

Acknowledgment.—We are grateful to Professor H. G. Khorana for a sample of ribopyranose 1-phosphate and to Dr. Ch. Degani for carrying out some of the chromatographic separations. This work was supported in part by Grant No. GB 5303 from the National Science Foundation.

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Chlorinolysis of Cysteine Ethyl Ester Hydrochloride. An Efficient Route to Certain Chloramino Acid Derivatives[1]

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Received April 17, 1969

Chlorinolysis of crystine diester derivatives with molecular chlorine to give the corresponding 2-amino-3-chloropropionates (**4**) in high yield was first reported

(1) Presented in part at the 4th Middle Atlantic Regional Meeting of the American Chemical Society, Washington, D. C., Feb. 15, 1969.