[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, UNITED STATES PUBLIC HEALTH SERVICE]

The Synthesis of Ribose 5-Pyrophosphate and Ribose 5-Triphosphate

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The synthesis and isolation of ribose 5-pyrophosphate and ribose 5-triphosphate is described. Both compounds are converted to the ribulose derivative by phosphoriboisomerase. Ribose 5-pyrophosphate is hydrolyzed to ribose 5-phosphate by inosine diphosphatase.

In the course of an investigation on the release of adenine from adenine nucleotides by enzyme preparations from *Azotobacter vinelandii* it became of interest to study ribose 5-pyrophosphate as a possible intermediate. Since this compound was not available, the carbodiimide procedure of Hall and Khorana[2] for the preparation of uridine diphosphate and triphosphate was employed, with minor modifications, for the preparation of both ribose pyrophosphate and ribose triphosphate.

The barium salt of ribose 5-phosphate was converted to the free acid by treatment with Dowex 50 (H^+ form); phosphoric acid and dicyclohexyl carbodiimide in aqueous pyridine was added. The reaction mixture, after removal of dicyclohexylurea and pyridine, was chromatographed on Dowex 1 (Cl^-). The major products were ribose 5-pyrophosphate which represented 20–25% of the original pentose phosphate, and ribose 5-triphosphate which accounted for about 10% of the starting material. These products were characterized by determining the ratio of total and acid-labile phosphate to pentose. Periodate uptake studies indicated that only the 5-position was esterified.

Ribose 5-pyrophosphate and ribose 5-triphosphate were found to be active in several enzymatic reactions. Both esters were converted by spinach phosphoriboisomerase[3] to the corresponding ribulose derivatives. It was established by determination of inorganic phosphate that this conversion was not preceded by hydrolysis to ribose 5-phosphate.

The diphosphate ester, but not the triphosphate, was hydrolyzed by inosine diphosphatase purified from calf liver acetone powder.[4] This enzyme has been shown to hydrolyze only inosine diphosphate, uridine diphosphate and guanosine diphosphate, of a large number of substrates tested.[4-6]

Ribose triphosphate will serve to esterify glucose in the hexokinase-glucose 6-phosphate dehydrogenase assay system.[7] However, it is inactive with purified hexokinase[8] indicating a transfer from ribose triphosphate to ADP in the test system.

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Experimental

Reaction with Dicyclohexyl Carbodiimide.—1.56 g. of barium ribose 5-phosphate (Schwarz Laboratories) was dissolved in 50 ml. of 0.02 *M* acetic acid and stirred with an excess of Dowex 50 (H^+) (200–400 mesh), until the supernatant solution gave a negative test for barium ion. The resin was removed by filtration, washed, and the combined filtrate and washings, containing 2.65 mmoles of pentose, was lyophilized. To the sirup were added 5 ml. of water, 0.8 ml. of 85% phosphoric acid, 80 ml. of pyridine and 12 g. of dicyclohexyl carbodiimide. The mixture was shaken vigorously for two hours at room temperature, cooled in ice and treated with 50 ml. of water. Precipitated dicyclohexylurea was removed by filtration with suction and washed with cold water.

Chromatography.—The combined filtrate and washings was extracted 6 times with 200-ml. portions of ether. The aqueous phase, 46 ml., was neutralized with 4.5 ml. of 4 *N* KOH and placed on a Dowex 1 (Cl^-) column, 3 cm. in diameter and 16 cm. in length. The adsorbed phosphate esters were eluted by a gradient method,[9] with 4 l. of water in the mixing chamber and a solution containing 0.2 *N* HCl and 0.2 *N* NaCl in the reservoir. The rate of elution was 3.6 ml. per minute and the fraction volume was 25 ml. To reduce hydrolysis of labile esters, chromatography was carried out in the cold room at 2°. Samples (0.025 ml.) were analyzed for pentose.[10]

Precipitation of the Barium Salts.—Four well separated peaks were obtained (Fig. 1). In peaks I and III the ratio of organic phosphorus[11] to pentose was approximately 1.0;

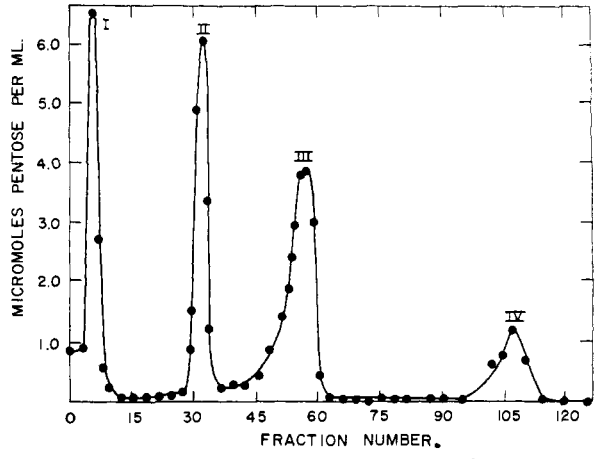

Fig. 1.—Separation of the reaction products by ion-exchange chromatography.

these were discarded. The fractions from peaks II and III, which contained organic phosphorus and pentose in ratios of approximately 2 and 3, respectively, were pooled and precipitated as follows: fractions 52–60, containing approximately 600 μmoles of pentose in 244 ml. were adjusted to *p*H 6.5 with 0.64 ml. of saturated KOH solution. The barium salts were precipitated by the addition of 2 ml. of *M* barium acetate and 750 ml. of ethanol. The precipitate

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was collected by centrifugation, washed with 100 ml. of 80% ethanol and dried *in vacuo* over KOH. The yield was 258 mg. Fractions 102–112, containing about 225 μmoles of pentose in 259 ml. were adjusted to *p*H 6.5 with 1.5 ml. of saturated KOH solution and the barium salts precipitated with 1 ml. of *M* barium acetate and 300 ml. of ethanol. The precipitate, washed and dried as described for peak II, weighed 142 mg.

Analytical Data.—Aliquots of the dried barium salts were dissolved in dilute acid and analyzed for pentose, total organic phosphorus and acid labile phosphorus. On the basis of pentose content the products were approximately 80% pure: peak III, ribose 5-pyrophosphate: $C_5H_9O_{11}Ba_{1.5}P_2$; pentose calcd. 29.3%, found 24.2%; peak IV, ribose 5-triphosphate; $C_5H_9O_{14}Ba_2P_3$; pentose calcd. 22.7%, found 18.2%.

The results of phosphate analyses are shown in Table I. Very little inorganic phosphorus was present in either fraction and the ratios of organic phosphorus and acid labile phosphorus were consistent with the presence of pyrophosphate and triphosphate esters. The esters consumed 3.5 and 3.0 μmoles of periodate, respectively, per μmole of pentose which is consistent with esterification in the 5-position only. Paper chromatography in the solvent of Paladini and Leloir[12] separates ribose 5-phosphate (R_f = 0.52) from the pyrophosphate ester (R_f = 0.42) and triphosphate ester (R_f = 0.33). The compounds were in the expected positions relative to one another, when compared with the corresponding uridine and adenosine derivatives.

TABLE I

RATIO OF PHOSPHORUS TO PENTOSE IN THE BARIUM SALTS

Analysis[a]	Ribose 5-pyrophosphate μmoles per ml.	Ribose 5-pyrophosphate Molar ratio	Ribose 5-triphosphate μmoles per ml.	Ribose 5-triphosphate Molar ratio
Pentose	19.0	1.0	15.3	1.0
Inorganic phosphorus	0.9	..	1.3	
Acid labile phosphorus[b]	18.2	0.96	29.3	1.92
Total organic phosphorus	40.0	2.10	48.3	3.15

[a] These analyses were carried out on solutions of the potassium salt obtained by dissolving the barium salt in dilute acetic acid and adding a slight excess of K_2SO_4. [b] Hydrolyzed by heating for 10 minutes at 100° in *N* H_2SO_4.

Enzymatic Tests.—The activity of ribose 5-pyrophosphate and ribose 5-triphosphate with spinach phosphoriboisomerase is shown in Table II. On the basis of the cysteine carbazole reaction[13,14] these esters appear to be converted to ribulose 5-pyrophosphate and ribulose 5-triphosphate since no inorganic phosphate was formed. However, the reaction products have not been isolated. The relative rates of reaction compared with ribose 5-phosphate were 1.0 and 0.3%, respectively. In these experiments on prolonged incubation, approximately one-third of the ribose pyrophosphate and ribose triphosphate added was converted to ketopentose as measured by the cysteine–carbazole reaction.

TABLE II

RATE OF REACTION WITH PHOSPHORIBOISOMERASE

Substrate	Ketopentose formed[a]
Ribose 5-phosphate	10,000
Ribose 5-pyrophosphate	1,000
Ribose 5-triphosphate	395

[a] The incubation mixtures (0.7 ml.) contained 2.2 μmoles of substrate, 2 μmoles of cysteine, 70 μmoles of tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.0 and phosphoriboisomerase. After 10 min. at 38° ketopentose was estimated by the cysteine–carbazole test[13] as modified by Axelrod and Jang.[14] In the case of ribose 5-pyrophosphate and ribose 5-triphosphate, 10 μg. of protein was used; with ribose 5-phosphate the quantity of enzyme was 0.1 μg. The enzyme solution contained 12 mg. of protein per ml. The quantity of ketopentose formed is calculated as μmoles formed per ml. of enzyme in 10 minutes at 38°.

TABLE III

HYDROLYSIS OF RIBOSE PYROPHOSPHATE BY INOSINE DIPHOSPHATASE

Time, min.	Substrate	Inorganic phosphate formed,[a] μmoles
30	Ribose pyrophosphate	0.25
85	Ribose pyrophosphate	.45
150	Ribose pyrophosphate	.50
346	Ribose pyrophosphate	.50
30	Inosine diphosphate	.40

[a] The incubation mixture (0.09 ml.) contained 0.035 ml. of 0.035 *M* veronal–acetate, *p*H 7, containing 0.004 *M* $MgCl_2$, 0.02 ml. of 0.15% albumin, 0.005 ml. of 0.33 *M* glutathione containing 0.002 *M* sodium ethylenediamine tetraacetate, 3.6 μgrams of purified inosine diphosphatase,[4] and 0.6 μmole of ribose pyrophosphate or inosine diphosphate. Incubation was at 37.5°. Analysis for inorganic phosphate was by the method of Fiske and Subbarow.[11] In the absence of enzyme, less than 0.01 μmole of inorganic phosphate was formed in 100 minutes with either substrate.

As shown in Table III, ribose 5-pyrophosphate is cleaved by inosine diphosphatase.

The following enzyme systems were found to be inactive with either or both ribose 5-pyrophosphate and ribose 5-triphosphate: yeast hexokinase,[8] muscle myokinase[15] and phosphoribulokinase.[3] In the latter case, the determination was carried out in the presence of phosphoriboisomerase.

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The Preparation and Metal Complexing of 2-(2′-Pyridyl)-10-hydroxybenzo[h]quinoline-4-carboanilide

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A new type of chelating agent 2-(2′-pyridyl)-10-hydroxybenzo[h]quinoline-4-carboanilide (I) has been prepared and its solution complexes examined. In methanol, I formed 1:1 complexes which exhibited a relative order of stability: $Cu^{II} > Ni^{II} > Co^{II} > Zn^{II} > Mn^{II} > Mg^{II}$.

A chelating agent (I), combining the essential features of the extensively studied 8-hydroxyquinoline[2] and 2,2′-bipyridyl[3] systems, has been synthesized and examined as to its complexing with

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