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Abstract

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SYNTHESIS OF NUCLEOTIDE COENZYMES AND RELATED COMPOUNDS

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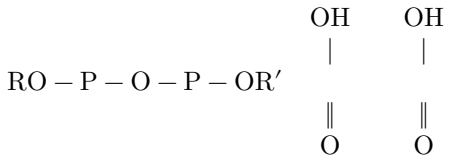
In a preceding paper ([1]) we demonstrated the possibility of using, in the synthesis of nucleotide coenzymes of the ATP type, P-amino-acid derivatives of nucleoside 5′-phosphate. The study of this reaction is of interest in connection with the possibility that such a process may occur in the cell, where activation of the phosphate residue of a nucleotide may be effected not simply by ammonia, but by amino acids or even proteins (enzyme systems). In seeking to synthesize unsymmetrical nucleotide coenzymes of the type



(where R is a nucleoside, R′ is a sugar residue), in the present work we studied the reaction between the methyl ester of N-(2′:3′-isopropylideneadenosine-5′-phospho)-phenylalanine (II) and ribose 5-phosphate or glucose 6-phosphate. The starting nucleoside 5′-phosphoamino acid (II) was prepared by hydrogenolysis of the methyl ester of N-(2′:3′-isopropylideneadenosine-5′-benzylphospho)-phenylalanine ([1]) in the presence of triethylamine.

[structural formula] (I) $\xrightarrow[(C_2H_5)_3N]{H_2/Pd}$ [structural formula] (II)

It should be noted that hydrogenolysis of I in the presence of triethylamine proceeds smoothly, and the triethylammonium salt II, obtained in high yield, is stable*. Preparation of the free compound II from the salt (by addition

* In contrast to the extremely unstable free compound II ([1]).

equimolar amount of hydrogen chloride in dioxane) was carried out immediately before introducing it into the reaction with the pyridinium or triethylammonium salt of the sugar phosphate. The reaction was conducted in dry pyridine at room temperature for 3 days.

2',3'-O-isopropylideneadenosine-5'-O-P(= O)(OH)NHCH($CH_2C_6H_5$)$COOCH_3$ + HO−P(= O)(OH)−O−sugar ⟶

2',3'-O-isopropylideneadenosine-5'-O-P(= O)(OH)−O−P(= O)(OH)−O−sugar (II, III)

where the sugar is ribose or glucose.

The formation of 2′:3′-isopropylideneadenosine-5′-diphosphosugar (iPr-ADP-sugar) (III) was demonstrated chromatographically. The yield of III, determined from absorption in the ultraviolet region ([2]), is 25-40%. The structure of III was established by hydrolysis with 0.01 *N* HCl (100°, 10 min), as a result of which adenosine, adenosine 5′-phosphate, adenosine 5′-diphosphate, and the sugar (ribose or glucose) were detected.

2',3'-O-isopropylideneadenosine-5'-O-P(= O)(OH)−O−P(= O)(OH)−O−sugar

When not the free compound II, but its triethylammonium salt, is introduced into the reaction, the nucleotide-coenzyme synthesis proceeds much more slowly. In some cases dioxane may be used as the solvent instead of pyridine; however, the yield of iPr-ADP-glucose when the reaction is carried out in this solvent is considerably lower than in pyridine.

Thus, it was established that the P-amino acid derivatives of adenosine 5′-phosphate are sufficiently reactive and can take part in nucleotide-coenzyme synthesis.

Experimental Part

The systems used in the chromatographic study were: I–*n*-butanol, saturated with water; II–isopropyl alcohol–1% solution of $(NH_4)_2SO_4$ (2 : 1) ([3]); III–96% ethanol–0.5 *M* ammonium acetate (pH 3.5) (5 : 2) ([4]); IV–*n*-butanol–water–acetic acid (4 : 5 : 1).

Paper electrophoresis was carried out using buffers: I–0.075 M ammonium acetate (pH 3.5); II–borate (pH 8.28).

Reagents for detection of sugars: reagent I–a mixture of equal volumes of 0.1 N $AgNO_3$ and 5 N NH_4OH ([5]); reagent II–a mixture of 1 volume of a 1 N solution of aniline in butanol with 2 volumes of 2 N H_3PO_4 in butanol ([6]). After spraying with these reagents, the chromatograms were heated for 10 min at 105°.

The yields of nucleotide coenzymes were determined by ultraviolet spectrophotometry ([2]).

1. **Triethylammonium salt II.** To a solution of 100 mg of I ([1]) in 20 ml of ethanol, 0.08 ml of dry triethylamine is added. The mixture is subjected to hydrogenolysis ([7]) over 10 mg of palladium black for 40–50 min (room temperature, atmospheric pressure). The catalyst is filtered off, the filtrate is evaporated in vacuum at 30°, and the residue is dissolved in chloroform. The chloroform solution is poured into dry petroleum ether. The triethylammonium salt II is isolated and reprecipitated another 2–3 times. Yield 75 mg (70%), m.p. 92–94° (decomp.); $R_f = 0.47$ in system I. The substance was dried for 24 h over P_2O_5 in vacuum.

Table 1

Substance	R_f values in system II	R_f values in system III	R_f values in system IV
Adenosine	0.74	0.74	0.37
Adenosine 5′-phosphate	0.41	0.54	
Adenosine 5′-diphosphate	0.22	0.32	
Ribose	0.62	0.71	0.24
Glucose	0.54	0.64	0.14

$$C_{23}H_{29}O_8N_6P \cdot (C_2H_5)_3N \cdot H_2O.$$

Found, %: C 51.85; H 6.52; P 4.8; N 14.4
Calculated, %: C 52.00; H 6.90; P 4.65; N 14.7

2. **Tributylammonium salt of ribose 5-phosphate.** 0.5 g ($1.3 \cdot 10^{-3}$ mole) of the barium salt of ribose 5-phosphate is suspended in 10 ml of water, 5 g of KU-2 (H^+) is added, and the mixture is stirred for 5 min. The resin is filtered off and washed with water; to the combined filtrates 0.66 ml ($2.8 \cdot 10^{-3}$ mole) of tributylamine is added, the mixture is subjected to lyophilic drying, and the residue is dissolved in abs. pyridine. The tributylammonium salt of glucose 6-phosphate is prepared analogously.

3. **Tributylammonium salts of phosphoric and pyrophosphoric acids.** To 0.44 g of a mixture of phosphoric and pyrophosphoric acids ([8]), 1 ml of dry tributylamine is added; the mixture is evaporated in vacuum (60–70°). Abs. dioxane is added to the residue.

4. **2′:3′-Isopropylideneadenosine 5′-diphosphoribose (ip-ADP-ribose).** 20 mg ($3.1 \cdot 10^{-5}$ mole) of the triethylammonium salt II

is dissolved in 1 ml of abs. dioxane, and 0.03 ml of a 1.03 N solution of hydrogen chloride in abs. dioxane is added ($3.1 \cdot 10^{-5}$ mole HCl). The precipitate is filtered off; to the filtrate $1.2 \cdot 10^{-4}$ mole of the tributylammonium salt of ribose 5-phosphate in dry pyridine is added. The mixture is left for 3 days at 20°, protected from atmospheric moisture, and chromatographed in system III. II ($R_f = 0.37$), 2′:3′-isopropylideneadenosine 5′-phosphate ($R_f = 0.69$), and a spot of unknown composition ($R_f = 0.50$) are detected. The lower spot is eluted, the eluate is evaporated in vacuum, and 0.01 N HCl is added. The solution is boiled for 10 min, cooled, and chromatographed in systems II, III, and IV. On the chromatograms, after inspection in ultraviolet light and spraying with sugar reagents, AMP, ADP, adenosine, and ribose are detected (for R_f values in systems II, III, and IV see Table 1). The eluate of the ADP spot is subjected to electrophoresis in buffer II for 2 h (7 V/cm, with cooling of the paper in carbon tetrachloride). On the electropherogram, one spot is detected, moving at the same rate as the control sample of ADP. Yield of ip-ADP-ribose 25% (determined spectrophotometrically after chromatography in system III).

5. **2′:3′-Isopropylideneadenosine-5′-diphosphoglucose** (ip-ADP-glucose). To $3.1 \cdot 10^{-5}$ mole of II, prepared as described above, $1.2 \cdot 10^{-2}$ mole of the tributylammonium salt of glucose 6-phosphate in dry pyridine is added. The mixture is left for 3 days at 20°, protected from atmospheric moisture, and chromatographed in system III. On the chromatogram, in addition to II ($R_f = 0.87$) and ip-AMP ($R_f = 0.69$), a spot with $R_f = 0.47$ is detected. This spot is eluted; the eluate is evaporated, hydrolyzed as described above, and chromatographed in systems II, III, and IV. Adenosine, AMP, ADP, and glucose are detected (for R_f values, see Table 1). The yield of ip-ADP-glucose is 37%.

6. **ip-ADP and ip-ATP.** These were obtained analogously from II and the tributylammonium salts of phosphoric and pyrophosphoric acids in absolute dioxane. The reaction mixture is subjected to electrophoresis in buffer I for 2 h (15 V/cm). In addition to II and ip-AMP, the electropherogram shows ip-ADP and ip-ATP, migrating at the same rate as control samples of ADP and ATP. The yields of ip-ADP and ip-ATP are, respectively, 39 and 27%.

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Note: Figure translations are in progress. See original paper for figures.