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Abstract

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SYNTHESIS OF THE METHYL ESTER OF ADENYLYL-(5 →N)-PHENYLALANINE BY THE CARBODIIMIDE METHOD

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Information on the presence in natural objects of nucleotide peptides with a phosphoamide bond between the nucleotide and peptide fragments is being enriched by ever newer facts ([1,2]). Data obtained by M. A. Prokof' ev and co-workers show that such nucleotide peptides also participate in cellular metabolism ([3]). Recently, reports have also appeared in the literature that elongation of the peptide chain during protein biosynthesis in ribosomes proceeds from the carboxyl end of the peptide ([4]). This fact indicates the possibility that polynucleotide amino acids (peptides), in which the amino end of the amino acid (peptide) is blocked by an oligonucleotide, participate in this process. For a detailed study of the properties of nucleotide amino acids (peptides) with a phosphoamide bond, it is necessary to have a convenient method for synthesizing these compounds.

Such a method has proved to be the "carbodiimide" method, which is widely used in peptide synthesis and also in the synthesis of nucleotide amides. When the methyl ester of phenylalanine is introduced into reaction with adenosine 5 -phosphate (AMP) in the presence of N, N'-dicyclohexylcarbodiimide (DCC), the ester of adenylyl-($5' \to N$)-phenylalanine (I) is formed in a yield of about 50%.

$$\text{AMP} + \text{H}_2\text{NCH}(\text{CH}_2\text{C}_6\text{H}_5)\text{COOCH}_3 \xrightarrow{\text{C}_6\text{H}_{11}\text{N=C=NC}_6\text{H}_{11}} \text{adenylyl-(5 } \to \text{N)-phenylalanine methyl ester (I)}$$

The reaction proceeds for three days at 37°. As a by-product there is formed a phenylalanine derivative of P_1P_2-diadenosine pyrophosphate (II)

$$\text{AD—O—P}(=\text{O})(\text{NHCH}(\text{CH}_2\text{C}_6\text{H}_5)\text{COOCH}_3)\text{—O—P}(=\text{O})(\text{OH})\text{—O—AD} \quad \text{(II), where AD—adenosine}$$

To reduce to a minimum the processes leading to the formation of symmetrical diadenosine pyrophosphate and polynucleotides, which can occur during carbodiimide activation of AMP, a fivefold excess of the amino-acid ester was introduced into the reaction; in addition, triethylamine, which suppresses polynucleotide synthesis, was added to the reaction mixture.

For isolation of the methyl ester of adenylyl-($5' \to N$)-phenylalanine (I), preparative chromatography on paper and chromatography were used

on cellulose powder. The isolated compound is an amorphous, very hygroscopic substance that deliquesces in air and has a low melting point. Compound I is unstable on storage: when kept in a vacuum desiccator at room temperature for a week it decomposes with liberation of phenylalanine methyl ester. In the IR region the substance has a frequency of 844 cm^{-1}, attributed to the stretching vibrations of the P—N bond (^{5}).

The simplicity of the "carbodiimide" method made it possible to obtain in this way a radioactive preparation of methyl adenilyl-($5' \to N$)-phenylalanine ester labeled with (C^{14}) in the carboxyl group of the amino acid. The radioactive compound was isolated by preparative paper chromatography.

Work on the synthesis of other P → N-amino-acid (peptide) derivatives of adenylic acid using the "carbodiimide" method is continuing.

Experimental Part

Systems used in the chromatographic investigation: I —n-butanol saturated with water; II —isoamyl alcohol —5% solution of Na_2HPO_4; III —isopropyl alcohol —conc. NH_3 —water (7 : 1 : 2); IV —n-butanol —water —acetic acid (4 : 5 : 1). Leningrad paper of grade "B" was used for chromatography. R_F values are given for ascending chromatograms. Paper for preparative chromatography was treated with a solution of oxyquinoline and washed with system IV. Electrophoresis on paper was carried out using borate buffer pH 8.29.

Substances were detected on chromatograms and phoregrams by absorption of UV rays, and also with molybdate reagent (^{6}) and the reagent for the cis-glycol grouping (^{7}). Compounds with a free amino group were developed with ninhydrin.

Reaction of AMP with phenylalanine methyl ester

To 490 mg (1.4 mmol) of AMP is added a solution of freshly prepared phenylalanine methyl ester (1.2 g, 7 mmol) in 30 ml of dimethylformamide. The reaction mixture is shaken until dissolved, 0.1 ml of absolute triethylamine and 570 mg (2.8 mmol) of DCC are added; the mixture is left for 3 days in a thermostat at 37°. The precipitated dicyclohexylurea is filtered off. The mixture is chromatographed in system III. With the aid of UV light, molybdate reagent, and the reagent for the cis-glycol grouping, AMP, compounds I and II (R_f in system III respectively 0.10; 0.56; 0.42), and also phenylalanine methyl ester (R_f 0.88) are detected. Compounds I and II are eluted with 50% methanol, the eluates are evaporated in vacuo, and 1 N HCl is added. The solutions are boiled on a water bath for 10 min, cooled, HCl is removed under reduced pressure by adding methanol several times, and the solutions are chromatographed in system IV. In the hydrolysates of compounds I and II the same products are found, namely:

AMP, adenosine, adenine, phenylalanine, and phenylalanine methyl ester (R_f in system IV respectively 0.09; 0.35; 0.5; 0.61; 0.73).

The eluates of compounds I and II are subjected to electrophoresis in borate buffer for 1.5 h (20 V/cm) with cooling of the paper in CCl_4. The rate of movement of compound I toward the anode is approximately 0.5 of the mobility of AMP, and that of compound II is 0.7 of the mobility of AMP. The yields of compounds I and II are respectively 53% and 14% (determined spectrophotometrically ([8]) after chromatography of the reaction mixture in system (III)).

Isolation of methyl adenilyl-($5' \rightarrow N$)-phenylalanine ester

a) Preparative horizontal chromatography on cellulose powder ([9]).

The reaction mixture obtained in dimethylformamide, after removal of the precipitate of dicyclohexylurea, is precipitated with absolute ether in a centrifuge tube. The oil formed is triturated several times with absolute ether, and the precipitate is decanted. 150 mg of the resulting powder is dissolved in

in a small amount of system III; dry paper powder is added to the solution until a thick slurry is obtained, and this is used to fill the trough of the cuvette. Fractionation is carried out in system III at a temperature not exceeding $+5°$, in a closed chamber. After 5 days two zones are detected on the powder (UV). The upper half of the second zone from the start is removed from the cuvette and the cellulose powder is washed with methyl alcohol. The filtrate is evaporated in vacuo at room temperature to dryness. Thirty mg of a yellowish powder is obtained, which is methyl ester of adenylyl-($5' \rightarrow N$)-phenylalanine (I) with a small impurity (2–3%) of compound II. When the reaction mixture is separated at room temperature, no sharp separation of the zones is observed.

b) Preparative chromatography on paper. One hundred fifty mg of the powder precipitated from the reaction mixture with absolute ether is dissolved in 50% methanol and applied as a band to four sheets of chromatographic paper (70 × 60).

Chromatography is carried out in system III. After development under UV, the corresponding zone of the chromatogram is eluted with methanol; the eluate is evaporated in vacuo at room temperature to dryness. On triturating the residue with abs. ether, a yellowish powder is obtained. The substance is dried in a vacuum desiccator over P_2O_5. Yield of I: 77 mg (51%). M.p. 111-112° (with decomp.). R_f 0.56 in system III; 0.69 in system II; 0.14 in system I. Absorption in UV in methyl alcohol: $\lambda_{\max}$258–260 mμ ($\varepsilon = 13\,000$). In the IR region there is a band at 844 cm^{-1}.

Found, %: C 41.60; H 6.12; P 5.87; N 14.01.
$C_{20}H_{25}O_8N_6P \cdot 4H_2O$. Calculated, %: C 41.38; H 5.69; P 5.34; N 14.48.

Compound II. The substance isolated from the paper chromatogram, as described for compound I, was characterized by the ratio adenosine ([10]); phosphorus ([11]): phenylalanine* ([12]), which proved to be, respectively, 2 ∶ 1.7 ∶ 0.8.

Synthesis of methyl ester of adenylyl-($5' \rightarrow N$)-phenylalanine (C^{14})

The reaction between AMP and phenylalanine ester

$$C_6H_5CH_2 - CH(NH_2) - C^{14}OOCH_3$$

was carried out analogously to that described. Into the reaction were introduced 80 mg (0.23 mmole) of AMP; the methyl ester of phenylalanine was obtained from 238.1 mg (1.4 mmole) of phenylalanine (specific activity 21 mCi/g). By the method of preparative chromatography on paper, 30 mg (31%) of radioactive methyl ester of adenylyl-($5' \rightarrow N$)-phenylalanine was isolated. Specific activity of the substance obtained: 14 mCi/g. Total activity: 0.4 mCi. The substance was radiochromatographically homogeneous; the outline peak on the chromatogram coincides with the spot having R_f 0.56 (UV) in system III.

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named after M. V. Lomonosov

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* The amino acid was determined in compound II after hydrolysis for one hour with 0.5 N HCl at 100°.

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