SYNTHESIS OF PEPTIDES FROM β-(1-PYRIMIDYL)- AND β-(9-PURINYL)-α-AMINO ACIDS

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The activated ether and dicyclohexylcarbodiimide methods were used to obtain di- and tripeptides of β-(1-thyminyl)-α-alanine and β-(9-adenyl)-α-alanine.

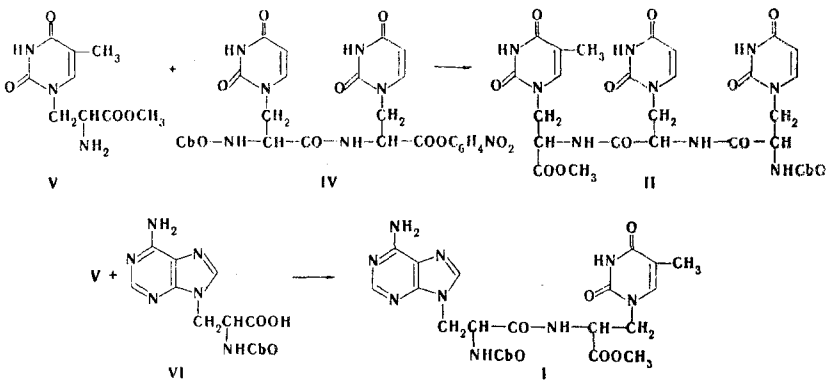
Continuing our research on the synthesis of purinyl peptides [1, 2], we undertook the preparation of heteropeptides from various pyrimidyl- and purinylamino acids.

In the present communication we present the results of the synthesis of a dipeptide – the methyl ester of N-carbobenzoxy-β-(9-adenyl)-α-alanyl-β-(1-thyminyl)-α-alanine (I) – and a tripeptide – the methyl ester of N-carbobenzoxy-DL-willardiylwillardiyl-β-(1-thyminyl)-α-alanine (II). We used the activated ether and dicyclohexylcarbodiimide methods to form the peptide bond. The amino groups were protected in all cases by carbobenzoxylation.

We chose the activated ether method for the synthesis of II. The etherification of N-carbobenzoxy-DL-willardiylwillardiine (III) [1] with p-nitrophenol gave N-carbobenzoxy-DL-willardiylwillardiine p-nitrophenyl ester (IV), which gives II on reaction with β-(1-thyminyl)-α-alanine methyl ester (V).

We used the dicyclohexylcarbodiimide method for the synthesis of dipeptide I. Compound I is formed by the condensation of V with N-carbobenzoxy-β-(9-adenyl)-α-alanine (VI) in the presence of N,N'-dicyclohexylcarbodiimide.

The structures of the compounds obtained were confirmed by the results of hydrolysis, as a result of which the corresponding crystalline amino acids, which were identified by means of partition chromatography and paper electrophoresis, are formed.



EXPERIMENTAL

"Chromatographic C" paper from the Volodarsk Leningrad Factory was used for the chromatography. The following solvent systems were used: butyl alcohol–acetic acid–water (9:1:2) (system A), isopropyl

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alcohol–ammonium hydroxide–water (7:1:2) (system B). The substances were detected on the chromatograms from the UV absorption, while the compounds containing an amino group were developed with ninhydrin. The R_f values presented below pertain to ascending chromatograms.

Electrophoresis was carried out on "Chromatographic M" paper. The following systems were used as electrolytes: 85% formic acid–acetic acid–water (7:5:13, pH 0.7) (system 1), 0.1 N potassium hydroxide (pH 13) (system 2). In working with system 1, a voltage of 350 V was supplied from a rectifier, and the potential gradient on the paper was 9.2 V/cm^2. In work with system 2, the voltage at the output from the rectifier was 600 V, and the potential gradient on the paper was 13.6 V/cm^2. The electrophoresis time varied from 2 to 3 h. The electrophoretic mobilities were calculated from the ratio of the rates of movement of the substances to the voltage of the electrical field.

β-(1-Thyminyl)-α-alanine Methyl Ester (V). An intense stream of dry hydrogen chloride was bubbled without cooling into a suspension of 1.8 g (7 mmole) of β-(1-thyminyl)-α-alanine in 40 ml of absolute methanol. After the amino acid had dissolved, the solution was cooled to 2°, and the introduction of gas was continued until the mixture was saturated, after which it was allowed to stand for 56 h in a refrigerator. The precipitated crystals of the hydrochloride of V were removed by filtration, washed with ether, and dissolved in 20 ml of methanol. The methanol solution was neutralized to pH 7 with triethylamine and allowed to stand at 0° for 24 h. The resulting precipitate was removed by filtration and washed with ether to give 1.2 g (63%) of V with mp 152° and R_f 0.85 (system A) and 0.51 (system B). Found: C 47.47; H 5.46; N 18.78%. $C_8H_{13}N_3O_4$. Calculated: C 47.56; H 5.77; N 18.49%.

N-Carbobenzoxy-DL-willardiylwillardiine p-Nitrophenyl Ester (IV). A 0.06 g (0.5 mmole) sample of p-nitrophenol and 0.1 g (0.5 mmole) of dicyclohexylcarbodiimide were added with vigorous stirring at 12° to 0.24 g (0.5 mmole) of III in 25 ml of dry dioxane, and the mixture was stirred at 12° for 2 h and allowed to stand at room temperature for 24 h. The precipitated dicyclohexylurea was removed by filtration, and the filtrate was vacuum evaporated to give an oil, which crystallized on trituration with dry ethanol to give 0.21 g (72.4%) of IV with mp 217° (nitromethane) and R_f 0.03 (system A) and 0.12 (system B).

N-Carbobenzoxy-DL-willardiylwillardiyl-β-(1-thyminyl)-α-alanine Methyl Ester (II). A solution of 0.1 g (0.2 mmole) of IV and 0.04 g (0.2 mmole) of V in 10 ml of dry dimethylformamide was held at room temperature for 5 days. The solvent was evaporated in vacuo to give a yellow oil, which crystallized on trituration with dry ethanol. The crystals were removed by filtration and washed with acetone and absolute ethanol to give 0.085 g (77%) of II with mp 259° and R_f 0.33 (system A) and 0.44 (system B). The electrophoretic mobilities* were $-6.03 \cdot 10^{-4}$ cm$^2 \cdot$ V$^{-1} \cdot$ sec^{-1} (system 1) and $+1.93 \cdot 10^{-3}$ cm$^2 \cdot$ V$^{-1} \cdot$ sec^{-1} (system 2).

N-Carbobenzoxy-β-(9-adenyl)-α-alanine (VI). An 0.84 ml (4.9 mmole) sample of carbobenzoxy chloride was added with stirring to a cooled (to 0°) solution of 2.3 g (9 mmole) of β-(9-adenyl)-α-alanine in 24 ml of water and 7.3 ml of 2.0 M sodium hydroxide. After 10 min, another 2.7 ml of 2.0 M sodium hydroxide and 0.84 ml (4.9 mmole) of carbobenzoxy chloride were added, and the suspension was stirred at 0° for 3 h, allowed to stand at room temperature for 2 h, and extracted with ether. The aqueous layer was cooled and acidified with concentrated HCl to pH 2-3, and the liberated oil began to crystallize at 5° to give 2.4 g (69.7%) of a product with mp 239-240° (absolute ethanol) and R_f 0.33 (system A) and 0.23 (system B). Found: C 54.55; H 4.67; N 23.26%. $C_{16}H_{16}N_6O_4$. Calculated: C 53.92; H 4.53; N 23.58%. The electrophoretic mobilities were $-1.126 \cdot 10^{-3}$ cm$^2 \cdot$ V$^{-1} \cdot$ sec^{-1} (system 1) and $+1.049 \cdot 10^{-3}$ cm$^2 \cdot$ V$^{-1} \cdot$ sec^{-1} (system 2).

N-Carbobenzoxy-β-(9-adenyl)-α-alanyl-β-(1-thyminyl)-α-alanine Methyl Ester (I). A 0.19 g (0.9 mmole) sample of V was dissolved in 10 ml of dimethylformamide, and 0.18 g (0.9 mmole) of dicyclohexylcarbodiimide was added in portions with stirring. The mixture was stirred for 30 min, a solution of 0.3 g (0.9 mmole) of VI in methylene chloride was added, and the new mixture was allowed to stand for 24 h. Glacial acetic acid (0.1 ml) was added, and the precipitated dicyclohexylurea was removed by filtration. The filtrate was vacuum evaporated to give 0.34 g (68.6%) of a product with mp 185-186° (absolute ethanol) and R_f 0.29 (system A) and 0.32 (system B). Found: C 53.07; H 4.73. N 22.55%. $C_{25}H_{27}N_9O_7$. Calculated: C 53.08; H 4.82; N 22.29%. The electrophoretic mobilities were $-1.061 \cdot 10^{-3}$ cm$^2 \cdot$ V$^{-1} \cdot$ sec^{-1} (system 1) and $+1.641 \cdot 10^{-3}$ cm$^2 \cdot$ V$^{-1} \cdot$ sec^{-1} (system 2).

Hydrolysis of I and II. A 10 mg sample of the compound was heated for 48 h on a boiling-water bath with 1 ml of 6 N hydrochloric acid, and the presence of the corresponding amino acids of the peptide in the resulting solution was proved by paper chromatography and paper electrophoresis.

*The plus and minus signs indicate migration of the substance to the cathode or anode, respectively.

LITERATURE CITED

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