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SYNTHESIS OF A HEXAPEPTIDE CONTAINING VALINE, LEUCINE, AND GLUTAMIC ACID

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We have previously reported the synthesis and attractive properties of some optically active peptides containing residues of the amino acids glycine, alanine, leucine, proline, ornithine, lysine, and phenylalanine for blood-sucking mosquitoes [1, 2]. In the present paper we describe the preparation of the methyl ester of the hexapeptide N-benzyloxycarbonyl-(Cbz)-L-phenylalanyl-L-valyl-Oγ-methyl-L-glutamyl-L-leucyl-O-valyl-L-alanine and the intermediate di- and tripeptides with the aim of studying further the dependence of their properties on the amino-acid composition, the sequence and configuration of the amino acids, and the length of the peptide chain.

The hexapeptide (XII) was synthesized from two tripeptides by the carbodiimide method according to the Scheme shown. It must be noted that repeated reprecipitation was necessary to free the substance from dicyclohexylurea.

The dipeptides (VI) and (VIII) and the tripeptides (VII) were obtained by the mixed-anhydride method using isobutyl chloroformate. This method permitted the synthesis of homogeneous substances in good yield (65-98%) and of the glutamyl-containing dipeptide with a free carboxy group, which considerably facilitated the preparation of the desired hexapeptide.

The hydrazide (IX) was readily formed in methanolic solution and was crystallized under ether. The debenzyloxycarbonylation of the tripeptide (X) by hydrogenation over Pd black in methanolic solution in the presence of acetic acid took place without appreciable destruction of the peptide bonds. (See Scheme on next page.)

To confirm the amino-acid composition of the hexapeptide obtained, it was subjected to complete acid hydrolysis with 6 N hydrochloric acid at 105°C for 20 h. The hydrolyzate was investigated chromatographically and electrophoretically. On the chromatogram and on the electrophoretogram five ninhydrin-positive substanes were detected the mobilities of which coincided with those of alanine, valine, leucine, phenylalanine, and glutamic acid.

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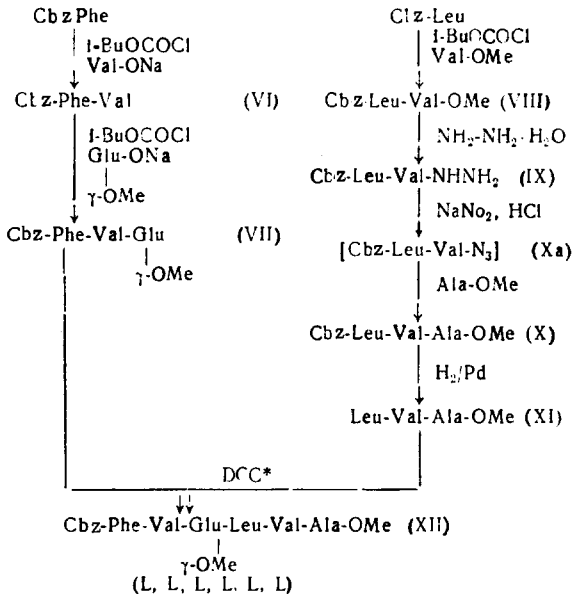

* Dicyclohexylcarbodiimide

EXPERIMENTAL

The chromatographic analysis of the peptides synthesized and of the acid hydrolyzate of the hexapeptide was performed on paper No. 3 (GDR) by the descending method in the following systems: 1) butan-1-ol–water–acetic acid (4 : 5 : 1); 2) butan-1-ol–water–pyridine–acetic acid (30 : 24 : 20 : 6). The electrophoretic investigation of the substances with a free amino group was performed in a "ÉMIB AN USSR" high-voltage horizontal apparatus for electrophoresis (Kiev) on paper in 2 N acetic acid at 800 V. The revealing agents used were solutions of ninhydrin, benzidine, and silver nitrate.

The following were obtained by published methods: N-Cbz-L-phenylalanine (I) [3], N-Cbz-L-leucine (II) [4], the hydrochlorides of the methyl esters of L-valine (III) [5], and of L-alanine (IV) [6], and the hydrochloride of the γ-methyl ester of L-glutamic acid (V) [7].

N-Cbz-L-Phenylalanyl-L-valine (VI). A mixture of 30 g of (I), 30 ml of chloroform and 1.3 ml of triethylamine was cooled to −2°C, and 1.4 ml of isobutyl chloroformate and, after 20 min, a cooled solution of 1.2 g of L-valine in 10 ml of 1 N caustic soda were added. The reaction mixture was stirred vigorously at 0°C for 30 min and at 18°C for 3 h, and the organic layer was separated off, washed with 1 N hydrochloric acid and with water, dried with anhydrous sodium sulfate, and evaporated. Yield 95%, $[\alpha]_D^{20}$ −13.9° (c 2; CH_3OH), R_f, 0.91 (system 1), 0.93 (system 2), $C_{22}H_{26}N_2O_5$.

N-Cbz-L-Phenylalanyl-L-valyl-Oγ-methyl-L-glutamic acid (VII) was obtained similarly from 1.27 g of (VII) in 20 ml of chloroform, 0.4 ml of triethylamine, 0.5 ml of isobutyl chloroformate, and 0.8 g of (V) in 4.0 ml of 1 N caustic soda solution and 0.6 ml of triethylamine. The oil was reprecipitated with ether from methanol. Yield 63.9%, $[\alpha]_D^{20}$ − 17.3° (c 2; $CHCl_3$); R_f 0.90 (system 1), 0.93 (system 2), $C_{28}H_{35}N_3O_8$.

Methyl Ester of N-Cbz-L-Leucyl-L-valine (VIII). A mixture of 2.2 g of (II), 30 ml of chloroform, and 1.2 ml of triethylamine was cooled to −5°C and 1.3 ml of isobutyl chloroformate and, after 20 min, a cooled solution of 1.4 g of (III) in 20 ml of chloroform and 1.2 ml of chloroform were added. The reaction mixture was left at 0°C for 19 h and at 20°C for 24 h and was then washed with 0.5 N hydrochloric acid, water, 3% sodium bicarbonate solution, and water again, and was dried with calcined sodium sulfate and evaporated. Yield 98.7%, $[\alpha]_D^{20}$ −20.8° (c 2; CH_3OH); R_f 0.93 (system 1), 0.94 (system 2), $C_{20}H_{30}N_2O_5$.

Hydrazide of N-Cbz-L-Leucyl-L-valine (IX). To 2.0 g of (VIII) in 20 ml of methanol was added 3 ml of hydrazine hydrate, and the mixture was left at 20°C for 36 h and was evaporated. The oil was repeatedly dissolved in methanol and evaporated. Yield 96.5%. The substance was chromatographically homogeneous, the spots being revealed with silver nitrate; mp 148–149°C, $[\alpha]_D^{20}$ −31.2° (c 2; 50% CH_3OH), R_f 0.88 (system 1), 0.90 (system 2), $C_{13}H_{30}N_4O_4$.

Methyl Ester of N-Cbz-L-Leucyl-L-valyl-L-alanine (X). Preparation of the Azide (Xa). A solution of 1.5 g of (IX) in 30 ml of water–acetic acid–hydrochloric acid (8 : 6 : 1) was cooled to −4°C,

0.3 g of sodium nitrite in 3 ml of water was added, and the mixture was stirred for 5 min. The azide, produced in the form of an oil, was extracted with 30 ml of ethyl acetate, and the extract was washed with water, 3% sodium sulfate. Yield 74.3%, mp 165-166°C, $[\alpha]_D^{20}$ −29.5° (c 2; CH_3OH), R_f 0.91 (system 1), 0.94 (system 2), $C_{23}H_{35}N_3O_6$.

Preparation of the Methyl Ester of L-Alanine (Xb). A mixture of 0.6 g of (IV), 10 ml of chloroform, and 0.6 ml of triethylamine was stirred for 15 min and evaporated, the residue was extracted with 20 ml of ethyl acetate, and the extract was cooled to −3°C.

Preparation of the Tripeptide (X). Cooled solutions of (Xa) and (Xb) were mixed, and the mixture was left at 0°C for 17 h and at 20°C for 6 h and was then worked up as for the preparation of (VIII). The substance is soluble in methanol, ethanol, chloroform, ethyl acetate, and acetonitrile.

Acetate of the Methyl Ester of L-Leucyl-L-valyl-L-alanine (XI). A solution of 0.8 g of compound (X) in 20 ml of ethanol was treated with 0.1 ml of acetic acid and 0.5 g of Pd black, and a current of hydrogen was passed until the evolution of carbon dioxide ceased; then the reaction mixture was evaporated. The oil was reprecipitated with ether from methanol. The substance was chromatographically homogeneous and gave a positive ninhydrin reaction. Yield 74.5%; electrophoresis in 2 N CH_3COOH at 20 B/cm l = −6.8 cm, 2 h; R_f 0.77 (system 1), 0.88 (system 2).

Methyl Ester of N-Cbz-L-Phenylalanyl-L-valyl-Oγ-methyl-L-glutamyl-L-leucyl-L-valyl-L-alanine (XII). To a solution of 0.25 g of (XI) in 20 ml of chloroform and 0.1 ml of triethylamine cooled to 0°C were added 0.3 g of (VII) and 0.2 g of dicyclohexylcarbodiimide. The reaction mixture was left at 0°C for 12 h and at 20°C for 49 h, and then 0.1 ml of acetic acid was added, the dicyclohexylurea was filtered off, the solvent was evaporated, the residue was extracted with 40 ml of ethyl acetate, and the extract was washed with 0.5 N hydrochloric acid, 3% sodium bicarbonate solution, and water, dried with anhydrous sodium sulfate, and evaporated. The oil was reprecipitated with ether from methanol. The substance was chromatographically homogeneous and soluble in methanol, ethanol, ethyl acetate, chloroform, and methylene chloride. Yield 85.1%, $[\alpha]_D^{20}$ −20.8° (c 1; CH_3OH), R_f 0.92 (system 1), 0.94 (system 2), $C_{43}H_{62}N_6O_{12}$.

SUMMARY

The previously unreported methyl ester of N-benzyloxycarbonyl-L-phenylalanyl-L-valyl-Oγ-methyl-L-glutamyl-L-leucyl-L-valyl-L-alanine has been synthesized.

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