SYNTHESIS OF THE HEXAPEPTIDE L-PHENYLALANYL-L-PROLYL-L-GLUTAMYL-DL-PHENYLALANYL-L-VALYL-L-LEUCINE

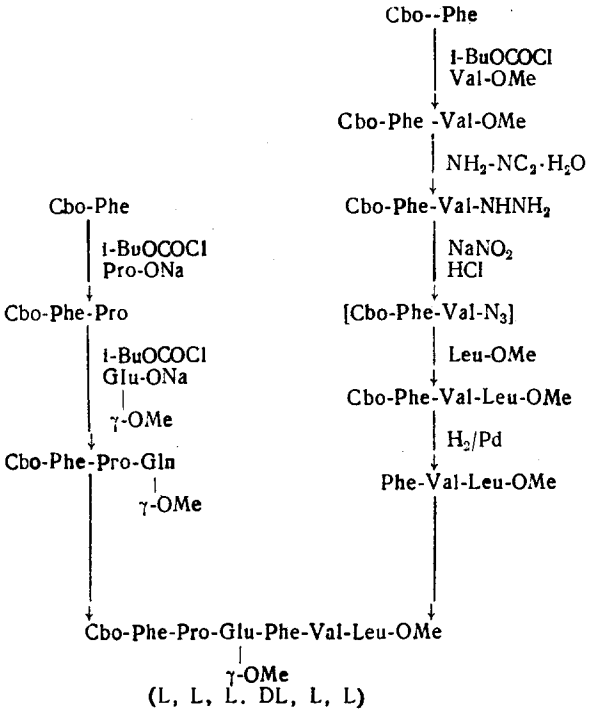
L. V. Fedorova, L. G. Kovalenko,
and N. Ya. Krasnobrizhii

UDC 547.466.1

We have previously reported the synthesis of the optically active peptides lysylleucylornithylphenylalanylprolylornithine with various configurations of the amino acids [1].

The present paper describes the synthesis of the hexapeptide L-phenylalanyl-L-prolyl-L-glutamyl-DL-phenylalanyl-L-valyl-L-leucine in order to study its stability at various pH values of the medium and its capacity for complex-formation with copper, and also its attractive properties for blood-sucking mosquitoes.

The synthesis of the hexapeptide was effected by the mixed-anhydride method from two tripeptides according to the following scheme:



It was impossible to use the milder azide method because hydrazine hydrate reacts simultaneously with the γ-ester group of glutamic acid. This leads to the formation of mixtures of α- and γ-peptides.

The dipeptides were also synthesized by the mixed-anhydride method, which enabled homogeneous substances to be obtained with fairly good yields.

The hydrazides of the di- and tripeptides are readily formed and precipitate from methanolic solution in the form of crystalline compounds.

T. G. Shevchenko Kiev State University. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 44-46, January-February, 1975. Original article submitted June 28, 1973.

The azide of N-Cbo-DL-phenylalanyl-L-valyl-L-leucine is an oily substance, which was treated without isolation in a cooled solution of ethyl acetate in order to avoid decomposition. The process of debenzyloxycarbonylation of the tripeptide by hydrogenation over Pd black took place smoothly without the cleavage of the peptide bonds.

The hexapeptide obtained was hydrolyzed with 6 N hydrochloric acid at 105°C for 20 h, after which it was studied chromatographically to confirm its amino acid composition. On a paper chromatogram, ninhydrin revealed five substances the R_f values of which corresponded to those of glutamic acid, proline, valine, leucine, and phenylalanine.

EXPERIMENTAL

For the chromatographic analysis of the peptides synthesized we chose paper No. 3 manufactured in the GDR and used the descending method in the following systems: 1) butan-1-ol–water–acetic acid (4:5:1), and 2) butan-1-ol–water–pyridine–acetic acid (30:24:20:6). The spots were revealed with benzidine, ninhydrin, and silver nitrate.

We obtained N-benzyloxycarbonyl(Cbo)-L- and -DL-phenylalanines (I) and (II) [2], the hydrochlorides of the methyl esters of L-valine (III) [3] and of L-leucine (IV) [4], and the hydrochloride of the γ-methyl ester of L-glutamic acid (V) by the methods given in the respective literature references.

Methyl Ester of N-Cbo-DL-Phenylalanyl-L-valine (VI). A solution of 3.0 g of (II) in 25 ml of absolute chloroform and 1.4 ml of triethylamine was cooled to –3°C, and 1.5 ml of isobutyl chloroformate was added. After 20 min, a cooled solution of 1.7 g of (III) in 20 ml of chloroform and 1.4 ml of triethylamine was added, and the mixture was left at 0°C for 8 h and at 20°C for 24 h. Then it was washed with 0.5 N hydrochloric acid, with water, with 3% sodium bicarbonate solution, and with water again, and was dried with anhydrous sodium sulfate. The chloroform was distilled off in vacuum, and the oil was reprecipitated with ether from methanol and was crystallized under ether in the cold. The substance is soluble in methanol, ethanol, and ethyl acetate, and insoluble in ether. Yield 97.1%, mp 95-96°C, $[\alpha]_D^{20}$ –10.4° (c 2; CH_3OH), R_f 0.94 (1) and 0.94 (2), $C_{23}H_{28}N_2O_5$.

N-Cbo-L-Phenylalanyl-L-proline (VII). With stirring, 2.1 g of (I) was dissolved in 20 ml of chloroform and 0.95 ml of triethylamine, the solution was cooled to –2°C, and 1.1 ml of isobutyl chloroformate was added followed after 20 min by a cooled solution of 0.8 g of L-proline in 7 ml of 1 N caustic soda solution. The mixture was stirred vigorously for 3 h, and the organic layer was separated off and was washed with 1 N hydrochloric acid and with water, dried with calcined sodium sulfate, and evaporated, and the residual oil was crystallized under ether. The substance was chromatographically homogeneous and was revealed only by benzidine. Yield 85.2%, mp 110-111°C, $[\alpha]_D^{20}$ +27.7° (c 1; chloroform), R_f 0.87 (1) and 0.91 (2), $C_{22}H_{24}N_2O_5$.

The γ-methyl ester of N-Cbo-L-phenylalanyl-L-prolyl-L-glutamic acid (VIII) was obtained similarly from 1.9 g of (VII), 0.65 ml of triethylamine, 0.9 g of (V), and 0.65 ml of triethylamine in 5 ml of 1 N caustic soda solution, and 0.75 ml of isobutyl chloroformate. Yield 76.3% (oil), $[\alpha]_D^{20}$ –13.8° (c 0.5; CH_3OH), R_f 0.92 (1) and 0.93 (2), $C_{28}H_{33}N_3O_8$.

Hydrazide of N-Cbo-DL-Phenylalanyl-L-valine (IX). A solution of 3.0 g of (VI) in 25 ml of methanol was treated with 1.5 ml of hydrazine hydrate and left at 24°C for 72 h. The hydrazide was filtered off (1.5 g), and from the mother liquor water precipitated another 1.3 g of the substance. It was washed with water, with cooled methanol, and with ether; it was revealed with benzidine and silver nitrate. Yield 93.3%, mp 178-179°C, $[\alpha]_D^{20}$ –19.1° (c 2.5; 50% CH_3COOH), R_f 0.91 (1) and 0.92 (2), $C_{22}H_{28}N_4O_4$.

Methyl Ester of N-Cbo-DL-Phenylalanyl-L-valyl-L-leucine (X). A. Preparation of the Azide (Xa). A solution of 1.2 g of (IX) in 25 ml of a mixture of water and acetic and hydrochloric acids (8:6:1) was cooled to –6°C, a solution of 0.23 g of sodium nitrite in 3 ml of water was added, and the mixture was stirred for 5 min. The azide was extracted with 25 ml of cooled ethyl acetate, and the solution was washed with water, with 3% sodium bicarbonate solution, and with water again, and was dried with calcined sodium sulfate.

B) Preparation of the Methyl Ester of L-Leucine (Xb). A solution of 0.55 g of (IV) and 0.5 ml of triethylamine in 20 ml of chloroform was stirred for 10 min and evaporated, the

residue was dissolved in 20 ml of ethyl acetate, and the solution was filtered and cooled to −3°C.

C) Preparation of the Tripeptide (X). Solutions of (Xa) and (Xb) were mixed, and the mixture was left at 0°C for 10 h and at 20°C for 24 h. Then the product was worked up in a similar manner to (VI). The substance was reprecipitated with ether from methanol and was crystallized under ether in the cold; soluble in methanol, ethanol, chloroform, and ethyl acetate. Yield 91.5%, mp 132-133°C, $[\alpha]_D^{20}$ −15.8° (c 2; CH_3OH), R_f 0.92 (1) and 0.94 (2), $C_{29}H_{39}N_3O_6$.

Hydrochloride of the Methyl Ester of DL-Phenylalanyl-L-valyl-L-leucine (XI). To 0.5 g of (X) in 15 ml of methanol was added 0.05 ml of hydrochloric acid and 0.3 g of Pd black, and then a current of hydrogen was passed until the evolution of carbon dioxide ceased, and the mixture was evaporated. The substance was hygroscopic, and the ninhydrin reaction was positive. Yield 95%, R_f 0.89 (1, electrophoresis), 0.92 (2, electrophoresis).

The γ,α-dimethyl ester of N-Cbo-L-phenylalanyl-L-prolyl-L-glutamyl-DL-phenylalanyl-L-valyl-L-leucine (XII) was obtained in a similar manner to (VI) from 0.3 g of (VIII), 35 ml of chloroform, 0.2 ml of triethylamine, 0.12 ml of isobutyl chloroformate, and 0.3 g of (XI). The oil was reprecipitated with ether from methanol. The substance was chromatographically homogeneous and was soluble in methanol, ethanol, chloroform, acetonitrile, and ethyl acetate. Yield 90% (oil), $[\alpha]_D^{20}$ −20.8° (c 1; CH_3OH), R_f 0.90 (1) and 0.93 (2), $C_{49}H_{64}N_6O_{11}$.

SUMMARY

The synthesis of the γ,α-dimethyl ester of N-Cbo-L-phenylalanyl-L-prolyl-L-glutamyl-DL-phenylalanyl-L-valyl-L-leucine, not previously described in the literature, has been effected.

LITERATURE CITED

1. N. A. Poddubnaya and N. Ya. Krasnobrizhii, Zh. Obshch. Khim., 40, 1137, 1142 (1970); 41, 567 (1971); 42 949 (1972).
2. M. Bergmann. L. Zervas, and J. S. Fruton, J. Biol. Chem., 115, 593 (1936).
3. E. L. Smith, D. H. Spackman, and W. J. Polglase, J. Biol. Chem., 199, 801 (1952).
4. H. F. Schott, J. B. Larkin, L. B. Rockland, and M. S. Dunn, J. Org. Chem., 12, 490 (1947).
5. R. A. Boissonnas, S. Guttman, P. A. Jaquenoud, and J. P. Waller, Helv. Chim. Acta. 38, 1491 (1955).