SYNTHESIS OF THE HEXAPEPTIDES GLYCYL-L-VALYL-L-SERYL-L-PROLYL-L-LYSYL-L-LEUCINE

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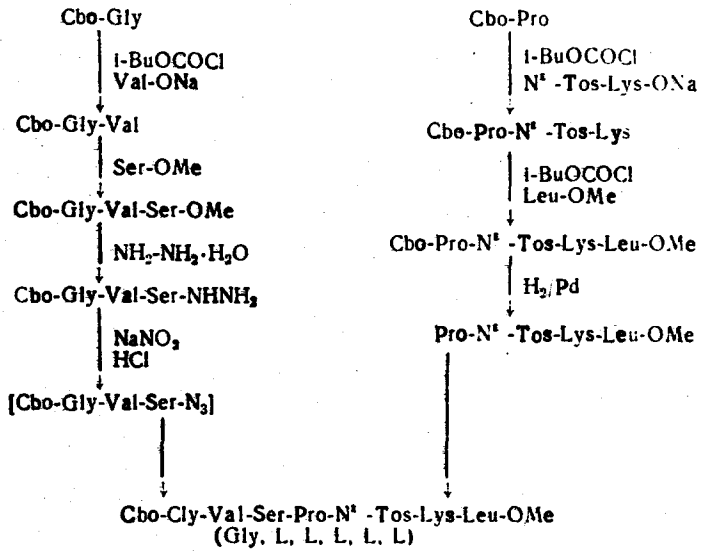
The synthesis of the hexapeptides glycylleucylornithylphenylalanylprolylornithine with various configurations of the amino acids has been performed previously [1].

The present paper describes the synthesis of an optically active linear hexapeptide glycyl-L-valyl-L-seryl-L-prolyl-L-lysyl-L-leucine for the study of its stability and its capacity for complex-formation with copper, and also its attractive properties.

The hexapeptide was synthesized by the azide method in homogeneous solution at a high acidity of the medium and a low temperature in order to exclude side reactions and racemization by the following scheme:



The dipeptides and the tripeptide N-Cbo-L-prolyl-N^{ε}-tos-L-lysyl-L-leucine were obtained by the mixed-anhydride method, which enabled homogeneous substances to be synthesized with high yields.

For the preparation of the methyl ester of N-Cbo-glycyl-L-valyl-L-serine the carbodiimide method proved best in order to exclude side reactions connected with the hydroxy group of the serine.

Repeated reprecipitation was necessary to free the serine tripeptide from dicyclohexylurea.

The hydrazide of the dipeptide precipitated from methanolic solution on the addition of a considerable amount of water.

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The elimination of the N-Cbo group from the methyl ester of N-Cbo-L-prolyl-N^{ε}-tos-L-lysyl-L-leucine by hydrogenation over palladium black took place without the destruction of the peptide bonds.

The substances obtained, with the exception of the tripeptide hydrazide, were oily or highly hygroscopic. To confirm the amino acid composition of the hexapeptide, it was subjected to complete acid hydrolysis with 6 N hydrochloric acid at 105°C for 18 h. The hydrolyzate was investigated chromatographically. On a paper chromatogram, ninhydrin revealed six substances the R_f values of which coincided with those of lysine, serine, glycine, proline, valine, and leucine.

The methyl ester of the hexapeptide synthesized is being tested for its attractive properties for blood-sucking mosquitoes.

EXPERIMENTAL

For the chromatographic analysis of the peptides we used paper No. 3 manufactured in the GDR by 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.

The N-Cbo(benzyloxycarbonyl)glycine (I) [2], N-Cbo-L-proline (II) [3], and the hydrochlorides of the methyl esters of L-serine (III) [4], of L-leucine (IV) [5], and of N^{ε}-tos (tosyl)-L-lysine (V) [6] were obtained as described in the respective references.

The N-Cbo-L-Prolyl-N^{ε}-tos-L-lysine (VI). With stirring, 2.5 g of (II) was dissolved in 30 ml of absolute chloroform and 1.3 ml of triethylamine cooled to −3°C, and 1.4 ml of isobutyl chloroformate was added, followed after 20 min by a cooled solution of 3.4 g of (V) in 20 ml of 1 N solution of caustic soda. The reaction mixture was stirred vigorous for 3 h. The organic layer was separated off, washed with 1 N hydrochloric acid and with water, dried with anhydrous sodium sulfate, and evaporated. The oil was reprecipitated with ether from methanol. The substance was chromatographically homogeneous and was soluble in methanol, ethanol, chloroform, and ethyl acetate. Yield 96.2%, $[\alpha]_D^{20}$ − 27.8° (c 2; CH_3Cl_3), R_f 0.89 (1) and 0.91 (2), $C_{26}H_{33}N_3O_7S$.

N-Cbo-Glycyl-L-valine (VII). Compound (VII) was obtained similarly from 4.18 g of (I), 40 ml of chloroform, 2.5 ml of triethylamine, 2.8 ml of isobutyl chloroformate, and 2.34 g of L-valine in 20 ml of 1 N caustic soda solution. Yield 60.7%, $[\alpha]_D^{20}$ + 5.1 (c 2; CH_3Cl_3), R_f 0.89 (1) and 0.92 (2), $C_{15}H_{20}N_2O_5$.

Methyl Ester of N-Cbo-L-Prolyl-N^{ε}-tos-L-lysyl-L-leucine (VIII). A solution of 1.9 g of (VI) in 30 ml of chloroform and 0.5 ml of triethylamine was cooled to −5°C, and then 0.53 ml of isobutyl chloroformate was added. After 20 min, a cooled solution of 0.62 g of (IV) in 10 ml of chloroform and 0.5 ml of triethylamine was added. The solution was left at 0°C for 12 h and at 20°C for 20 h, and was then washed with 0.5 N hydrochloric acid, with water, with 3% sodium bicarbonate solution, and again with water, dried with calcined sodium sulfate, and evaporated. Yield 95.6%, $[\alpha]_D^{20}$ −43.3° (c 2; CH_3OH), R_f 0.90 (1) and 0.94 (2), $C_{33}H_{46}N_4O_8S$.

Methyl Ester of N-Cbo-Glycyl-L-valyl-L-serine (IX). A solution of 0.8 g of (VII) in 30 ml of chloroform and 0.35 ml of triethylamine was cooled to 0°C, and then 0.4 g of (III) and 0.7 g of dicyclohexylcarbodiimide were added. The reaction mixture was left at 0°C for 5 h and at 20°C for 12 h. A considerable amount of dicyclohexylurea was separated off, the solvent was distilled off in vacuum, the residue was dissolved in 40 ml of ethyl acetate, and the solution was washed in the same way as for (VIII). The oil was reprecipitated with ether from methanol. Yield 90.9%, $[\alpha]_D^{20}$ −6.6 (c 2; CH_3OH), R_f 0.90 (1) and 0.91 (2), $C_{18}H_{27}N_3O_7$.

Hydrazide of N-Cbo-Glycyl-L-valyl-L-serine (X). A solution of 0.5 g of (IX) in 15 ml of methanol was treated with 0.7 ml of hydrazine hydrate and the mixture was left at 20°C for 48 h. On the addition of water, a considerable amount of a crystalline substance deposited, which was revealed with benzidine and silver nitrate. Yield 98.0%, $[\alpha]_D^{20}$ +6.1 (c 2; CH_3COOH), R_f 0.90 (1) and 0.91 (2), $C_{18}H_{27}N_5O_6$.

Hydrochloride of the Methyl Ester of L-Prolyl-N^{ε}-tos-L-lysyl-L-leucine (XI). A solution of 0.5 g of (VIII) in 15 ml of methanol was treated with 0.05 ml of hydrochloric acid and 0.3 g of Pd black, a current of hydrogen was passed until the evolution of carbon dioxide ceased, and then the reaction mixture was evaporated and the residue was reprecipitated with ether from methanol. The substance was hygroscopic and chromatographically homogeneous and gave a positive ninhydrin reaction. Yield 90.3%, R_f 0.70 (1) and 0.86 (2).

Methyl Ester of N-Cbo-Glycyl-L-valyl-L-seryl-L-prolyl-N^{ε}-tos-L-lysyl-L-leucine (XII). A) Preparation of the Azide (XIIa). A solution of 0.3 g of (X) in 30 ml of a mixture of water, acetic acid, and hydrochloric acid (8:6:1) was cooled to −4°C, a solution of 0.1 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 extract was washed with water, with 3% sodium bicarbonate solution, and with water again, and was dried with calcined sodium sulfate. Yield 75.7%, $[\alpha]_D^{20}$ −34.6 (c 0.5; CH_3OH), R_f 0.93 (1) and 0.94 (2), $C_{43}H_{63}N_7O_{12}S \cdot 2H_2O$.

B) Preparation of the Methyl Ester of L-Prolyl-N^{ε}-tos-L-lysyl-L-leucine (XIIb). A solution of 0.4 g of (XI) in 15 ml of chloroform and 0.12 ml of triethylamine was stirred for 15 min and was then evaporated. The residue was dissolved in 20 ml of ethyl acetate, and the solution was cooled to −3°C.

C) Preparation of the Hexapeptide (XII). Solutions of (XIIa) and (XIIb) were mixed, and the mixture was kept at −1°C for 15 h and at 20°C for 24 h. Then it was worked up in a similar manner to (XIIa). The substance was reprecipitated with ether from methanol and was crystallized under ether in the cold; chromatographically homogeneous, very hygroscopic, soluble in methanol, ethanol, chloroform, and ethyl acetate.

SUMMARY

The synthesis of the methyl ester of the hexapeptide N-Cbo-glycyl-L-valyl-L-seryl-L-prolyl-N^{ε}-tos-L-lysyl-L-leucine, not described in the literature, has been affected.

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 and L. Zervas, Ber., 65, 1192 (1932).
3. B. F. Erlanger and H. Sachs, J. Amer. Chem. Soc., 76, 1806 (1954).
4. J. I. Harris and J. S. Fruton, J. Biol. Chem., 191, 143 (1951).
5. K. Weil and W. Kuhn, Helv. Chim. Acta, 29, 784 (1946).
6. R. Roeske, F. Steward, R. Stedman, and V. Vigneaud, J. Amer. Chem. Soc., 78, 5883 (1956).