SYNTHESIS OF SOME DERIVATIVES OF THE C-TERMINAL TETRAPEPTIDE OF THE HORMONE GASTRIN

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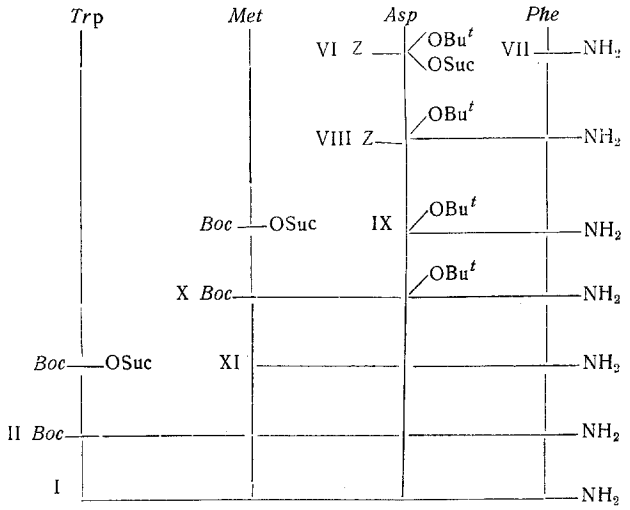
Continuing investigations in the field of the physiology and chemistry of the hormone gastrin [1, 2], we have synthesized an active fragment of gastrin [3] – the C-terminal tetrapeptide (I) and some of its derivatives (II-V):

Trp·Met·Asp·PheNH$_2$·HCl,	I
Boc-Trp·Met·Asp·PheNH$_2$,	II
C$_2$H$_5$OCO-*Trp·Met·Asp·PheNH$_2$*,	III
C$_2$H$_5$OCOCH$_2$NHCO-*Trp·Met·Asp·PheNH$_2$*,	IV
Boc-β-Ala·Trp·Met·Asp·PheNH$_2$	V

The synthesis of the tetrapeptide was effected by the succinimidyl ester method in accordance with the following scheme*:



The amide of phenylalanine (VII) was obtained by the ammonolysis of the ethyl ester of phenylalanine [6]. The method described previously [5] was used for the synthesis of α-succinimidyl β-tert-butyl carbobenzoxyaspartate (VI). A 5- to 10-fold increase in the amounts and the use of unrecrystallized intermediates made it possible to raise the yield of VI by a factor of 1.5 in comparison with that obtained previously [5]. The condensation of (VI) and (VII) in DMF solution gave the β-tert-butyl ester of the amide of benzyloxycarbonylaspartylphenylalanine (VIII), the catalytic hydrogenation of which in 90% acetic acid over palladium black led to the formation of the β-tert-butyl ester of the amide of aspartylphenylalanine (IX). The

*The generally adopted symbols [4] are used in this paper.

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latter, on reaction with the succinimidyl ester of tert-butyloxycarbonylmethionine [8] gave the amide of butyloxycarbonylmethionyl(β-tert-butyl)aspartylphenylalanine (X). The simultaneous removal of the butyloxycarbonyl and the β-tert-butyl groups of the tripeptide (X) was performed by treating it with 2 N HCl in acetic acid. The condensation of the hydrochloride of the amide of methionylaspartylphenylalanine (XI) with the succinimidyl ester of tert-butyloxycarbonyltryptophan [8] in DMF solution gave the amide of tert-butyloxycarbonyltryptophanylmethionylaspartylphenylalanine (II). The elimination of the tert-butyloxycarbonyl protective group of the tetrapeptide (II) led to the hydrochloride of the C-terminal tetrapeptide of gastrin (I).

The condensation of the tetrapeptide (I) with the ethyl ester of carbonylglycine [9] and with the succinimidyl ester of tert-butyloxycarbonyl-β-alanine gave, respectively, the ethyl ester of the amide of N-carbonylglycyl-N'-tryptophanylmethionylaspartylphenylalanine (IV) and the amide of tert-butyloxycarbonyl-β-alanyltryptophanylmethionylaspartylphenylalanine (V). The ethoxycarbonyltetrapeptide (III) was synthesized from (XI) and the succinimidyl ester of ethoxycarbonyltryptophan (XIV).

An investigation of the biological activity of the derivatives of the C-terminal tetrapeptide and pentapeptide of gastrin that had been synthesized was carried out in the pharmacology laboratory of the Institute of Organic Synthesis* in experiments on dogs by determining the intraventricular pH. All the derivatives cause an increase in the secretion of acid, (IV) showing an activity beginning at a dose of 7 μg/kg, and the others at a dose of 1 μg/kg. The mean indices [latent period, time of appearance of the maximum pH, and the duration of the action for compounds (I) and (V) with the administrations of doses of 1, 2, 3, 7, and 15 μg/kg] differed only slightly.

EXPERIMENTAL

The work was carried out with amino acids having the L configuration. The purity of the products was checked by chromatography on paper (1) and in thin layers of silica gel (2), silica gel G (3), alumina (4), and Silutol (5) in the systems: 1) butanol–acetic acid–water (4:1:5); 2) butanol–acetic acid–water–pyridine (15:3:12:10); and 3) isopropanol–ammonia–water (5:1:1). The analyses of all the compounds corresponded to the calculated figures.

Z–Asp(Bu^t) · PheNH$_2$ (VIII). A suspension of 18.6 g (0.093 mole) of (VII) [6] in 150 ml of DMF was cooled to −5 to 0°C, and 12.9 ml (0.093 mole) of triethylamine and 37.6 g (0.093 mole) of (VI) [5] were added. The mixture was stirred at −5 to +5°C for 4 h, left at room temperature overnight, and poured into 450 ml of ice water. The precipitate was filtered off with suction and washed with ice water. Then it was dried in vacuum over phosphorus pentoxide. Yield 30.0 g (69%), mp 157-158°C (literature data: 157-158.5°C [7]). R_f (A-1) 0.56, (A-2), 0.74, (A-5) 0.42.

Boc–Met · Asp(Bu^t) · PheNH$_2$ (X). A solution of 2.5 g (7.5 mmoles) of IX in 20 ml of DMF was cooled to −5 to 0°C and, with stirring, 2.6 g (7.5 mmoles) of Boc–Met–OSuc [8] was added. The solution was stirred at 0-5°C for 4-5 h and at room temperature for 4-4.5 h and was then poured into 200 ml of cold water. The precipitate was filtered off with suction, washed with cold water, and recrystallized from the minimum amount of isopropanol–water. Yield 3.65 g (85%), composition $C_{27}H_{41}N_4O_7S$, mp 158.5-159°C: $[\alpha]_D^{20}$ −30 (c 1; DMF); R_f (A-2) 0.95.

Boc–Trp · Met · Asp · PheNH$_2$ (II). A solution of 0.83 g (2 mmoles) of XI [7] in 10 ml of DMF was cooled to −5 to 0°C, 0.56 ml (4 mmoles) of triethylamine and 0.81 g (2 mmoles) of Boc–Trp–OSuc [8] were added, and the mixture was stirred at 0-5°C for 4 h and at room temperature for 5 h. Then it was poured into 40 ml of ice water containing 0.4 ml of acetic acid, left in a cold place for 2-3 h, and filtered with suction, and the residue was washed with water and dried in vacuum at 35-40°C over phosphorus pentoxide. Yield 1.22 g (87%), mp 200-200.5°C. After recrystallization from methanol and water the yield was 1.15 g (83%), mp 206-208°C (literature data: 209-210°C [7]), $[\alpha]_D^{20}$ −37.2° (c 1, DMF) (literature data: $[\alpha]_D^{22}$ −35.7° (c 1; DMF [7])). R_f (A-5) 0.55, (A-4) 0.7.

Trp · Met · Asp · PheNH$_2$ · HCl (I). The removal of the tert-butoxycarbonyl protective group from (II) was performed by the method of Davey et al. [7]. The product had mp 221-221.5°C (literature data: 215-216°C [7]), $[\alpha]_D^{20}$ −30° (c 1; DMF) (literature data: $[\alpha]_D^{22}$ −31.7°, c 1; DMF), R_f (A-3) 0.64.

*The results of the physiological investigation of the gastrin derivatives were given to us by Z. A. Atare.

$C_2H_5OCOCH_2NHCO-Trp \cdot Met \cdot Asp \cdot PheNH_2$ (IV). A suspension of 0.1 g (0.158 mmole) of (I) in 5 ml of dioxane was treated with 0.045 ml (0.32 mmole) of triethylamine and 0.02 ml (0.152 mmole) of the ethyl ester of carbonylglycine [9]. The mixture was shaken for 4 h and was left overnight at room temperature. Then 0.02 ml (0.35 mmole) of acetic acid was added, and the mixture was stirred and treated with 30 ml of ether. Yield 0.094 g, mp 189-195°C. The product was treated several times with the minimum amount of hot water, cooled, and filtered, and the residue was dissolved in 100 ml of methanol. This solution was filtered and evaporated in a rotary evaporator, and the residue was dried in vacuum over phosphorus pentoxide at 50-60°C. Yield 0.03 g (25%), composition $C_{34}H_{43}N_7O_9S$, mp 205-206°C, $[\alpha]_D^{20}$ −38.0° (c 0.4; DMF); R_f (A-5) 0.47.

Boc−β-Ala (XII). A suspension of 7.8 g (0.8 mole) of β-alanine in a mixture of 20 ml of dioxane and 20 ml of water was treated with a 4 N solution of caustic soda to pH 8.9 and with 13 ml of tert-butyl azidoformate. The mixture was heated to 50-55°C, the pH being maintained at 8.9 by means of an automatic block titrator. The reaction was complete after 2.5-3 h. The excess of azide and part of the dioxane were evaporated off in a rotary evaporator in vacuum at 50°C, and the residue was cooled to 0-5°C and acidified to pH 2 with 20% citric acid. Then it was extracted several times with ethyl acetate, and the ethyl acetate extract was dried with magnesium sulfate, filtered, and evaporated in a rotary evaporator in vacuum. The residual oil crystallized under petroleum ether. Yield 13.0 g (85.5%), mp 75-77°C (decomp.). The product recrystallized from ether−petroleum ether, with the composition $C_8H_{15}NO_4$, had mp 82-83°C (literature data: 73-74°C [12]), R_f (A-4) 0.67.

Boc−β-Ala−OSuc (XIII). A solution of 1.8 g (9.5 mmoles) of (XII) and 1.09 g (9.5 mmoles) of N-hydroxysuccinimide [8] in 5 ml of dioxane and 5 ml of methylene chloride was cooled to 0°C and, with stirring, 1.9 g (9.5 mmoles) of dicyclohexylcarbodiimide was added. The mixture was stirred at 0-5°C for 5 h and was left at room temperature overnight. The dicyclohexylurea that had deposited was filtered off, and the solvent was evaporated off. Yield 2.33 g (86%), mp 110-112°C. It was recrystallized from isopropanol−hexane. Yield 1.55 g (57%). The product additionally recrystallized from ethyl acetate−petroleum ether had the composition $C_{12}H_{18}N_2O_6$ with mp 112-113°C, R_f (A-3) 0.78, (B-3) 0.74.

Boc−β-Ala · Trp · Met · Asp · $PheNH_2$ (V). A mixture of 0.16 g (0.25 mmole) of (I) and 3 ml of DMF cooled to 0-5°C was treated with 0.068 g (0.24 mmole) of (XIII) and 0.07 ml of triethylamine. The solution was stirred at 0-5°C for 4-5 h and was left at room temperature overnight. Then it was poured with stirring into 10 ml of ice water containing 0.05 ml of acetic acid and the resulting precipitate was filtered off, mp 212-213°C (decomp.). It was recrystallized from acetic acid and water. Yield 0.14 g (79%), composition $C_{37}H_{49}N_7O_7S$, mp 213°C (literature data: 229-230°C [7]); $[\alpha]_D^{20}$ −30° (c 1; DMF) (literature data: $[\alpha]_D^{22}$ −28.8°, c 1; DMF [7]), R_f (A-5) 0.78, (A-4) 0.66.

$C_2H_5OCO-Trp-OSuc$ (XIV). At 0-5°C, 1.3 g (6.3 mmoles) of dicyclohexylcarbodiimide was added to a solution of 1.66 g (6.0 mmoles) of ethoxycarbonyl-L-tryptophan [13] and 0.73 g (6.3 mmoles) of N-hydroxysuccinimide [8] in 12 ml of a mixture of dioxane and methylene chloride (1:1), and the mixture was stirred at 0-5°C for 3 h and was left at room temperature overnight. The dicyclohexylurea was filtered off. The solution was evaporated in vacuum at 35-40°C. The residual oil was crystallized under petroleum ether. The yield of crude product with mp 135-140°C was quantitative. After recrystallization from isopropanol it had the composition $C_{18}H_{19}N_3O_6$, mp 145-146°C, R_f (A-1) 0.9, (B-2) 0.94.

SUMMARY

The synthesis has been effected of the C-terminal tetrapeptide of gastrin Trp · Met · Asp · $PheNH_2$ · HCl (I) and its derivatives Boc−Trp · Met · Asp · $PheNH_2$ (II), $C_2H_5OCO-Trp \cdot Met \cdot Asp \cdot PheNH_2$ (III), $C_2H_5OCOCHNH_2CO-Trp \cdot Met \cdot Asp \cdot PheNH_2$ (IV), and Boc−β-Ala · Trp · Met · Asp · $PheNH_2$ (V).

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