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SYNTHESIS OF THE C-END OF THE TETRAPEPTIDE GASTRIN

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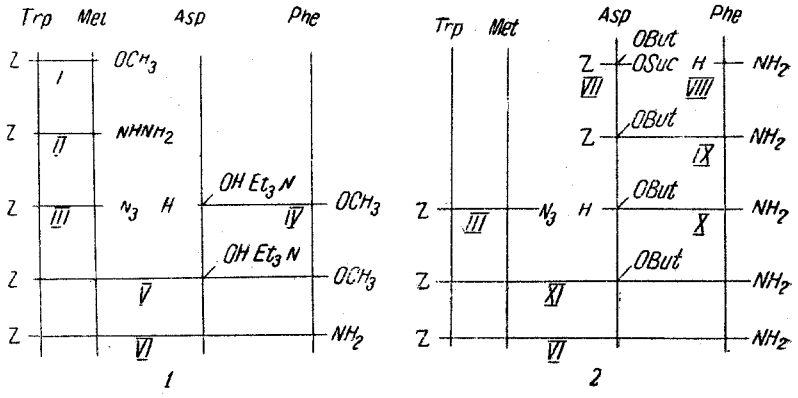
In testing the biological activity of individual fragments of the polypeptide chain of gastrin [1], it was found that the C-end of the tetrapeptide of the latter possesses the complete spectrum of the biological activity of the natural hormone [2, 3].



For biological investigations we synthesized the carbobenzoxy derivatives of the C-terminal tetrapeptide in two ways.* In both cases, we used the 2 + 2 variant for the synthesis and the azide method for linking the fragments. For protection we used carbobenzoxy groups in the case of the amino function and methyl, benzyl, and tert-butyl esters for the carboxyl function.

Carbobenzoxytryptophan and the methyl ester of methionine were condensed in DMF solution by means of dicyclohexylcarbodiimide and also by the mixed anhydride method. The resulting dipeptide—the methyl ester of carbobenzoxytryptophanylmethionine (I), was converted by the action of hydrazine hydrate into the hydrazide (II). The azide of carbobenzoxytryptophanylmethionine (III), which was isolated in the solid state when the hydrazide II was treated with sodium nitrite in an acid medium, was condensed with the triethylammonium salt of the methyl ester of aspartylphenylalanine (IV) in ethyl acetate—DMF. This dipeptide (IV) was also synthesized by the method of Davay et al. [4]. The tetrapeptide—the methyl ester of carbobenzoxytryptophanylmethionylaspartylphenylalanine (V)—after isolation from the reaction medium, was subjected to ammonolysis without further purification. The amide (VI) obtained was purified by recrystallization [4]. The synthesis of the carbobenzoxy derivative of the tetrapeptide VI by the first scheme comprises 12 individual stages.

In the second variant, the dipeptide aspartylphenylalanine was used with a protected β-carboxyl group. The α-succinimidyl β-tert-butyl ester of carbobenzoxyaspartic acid (VII) was synthesized as described by Hofman and Hass [5] and was condensed with the amide of phenylalanine (VIII) in DMF. The carbobenzoxy group was split off from the dipeptide IX by reduction with hydrogen over a palladium catalyst, and the free base—the amide of the β-tert-butyl ester of aspartylphenylalanine (X) was isolated by a known method [4]. After azide coupling, the ester group was split off from the β-tert-butyl ester of carbobenzoxytryptophanylmethionylaspartylphenylalanine (XI) (the compound was not isolated in the analytically pure state) by saponification in anhydrous trifluoroacetic acid. A derivative of the tetrapeptide (VI) was obtained which, in its chromatographic behavior and biological activity was identical with the carbobenzoxytetrapeptide synthesized by the first scheme. The second variant comprises 15 individual stages and has no practical advantages in comparison with the first.

The biological activity of the substances isolated was tested in experiments on cats. After the subcutaneous injection of a solution of the peptide into nonnarcotized cats, the gastric juice was directed through special fistulas and the content of free and bound HCl in it was determined by the acidimetric method, and the MR_{50} value, i.e., the dose of peptide the introduction of which caused a secretion of 50% (of the maximum of secretion) of HCl, was also determined. For the preparations that we had obtained, the MR_{50} value was 11.5 μg/kg bodyweight (mean result from experi-

*The following symbols are used in the paper: Z) carbobenzoxy group; OBut) tert-butyl ester; OSuc) succinimidyl ester; Et_3N) triethylamine; and DMF) dimethylformamide.

ments on three cats). For comparison, the same experiments were carried out with histamine; the MR_{50} value for histamine was 109 μg/kg. The ratio of MR_{50} for histamine to the MR_{50} for gastrin was 9.4. Thus, the tetrapeptide gastrin is 9.4 times more active than histamine. These results are approximately comparable with those given in the literature. Thus, Konturek, in experiments on dogs obtained the following MR_{50} values: for the tetrapeptide 15 μg/kg; for histamine 100 μg/kg. The ratio of the MR_{50} for histamine to the MR_{50} for gastrin is 6.7. According to the same results, the MR_{50} value for a heptadecapeptide of gastrin is 3 μg/kg [6].

Experimental

The work was carried out with amino acids of the L configuration and with anhydrous solvents. The chromatographic analyses were performed on "Leningrad medium" paper by the descending method in the butan-1-ol—acetic acid—water (4 : 1 : 5) system; the spots were revealed with benzidine and Ehrlich's reagent (only the tryptophan-containing peptides).

Methyl ester of carbobenzoxytryptophanylmethionine (I). A) A suspension of 0.34 g (1 mM) of carbobenzoxytryptophan [7] in 5 ml of chloroform cooled to 0° C was treated with 0.14 ml (1 mM) of triethylamine. The mixture was cooled to −10° C, 0.1 ml (1 mM) of ethyl chlorocarbonate was added, and stirring was performed at −5° C for 20 min (solution A). A suspension of 0.2 g (1 mM) of the hydrochloride of the methyl ester of methionine [8] in 2 ml of chloroform cooled to 0° C was treated with 0.14 ml (1 mM) of triethylamine and the resulting mixture was kept for 10 min and was then added to solution A. The solution so obtained was stirred at 0° C for 1 hr and was then left at room temperature for 16 hr. The reaction mixture was washed with water (5 ml), 1 N HCl (5 ml), and 3% sodium bicarbonate solution (2 × 5 ml). The organic layer was dried over sodium sulfate and the chloroform was evaporated off. The oily residue crystallized over hexane. The yield of the methyl ester of carbobenzoxytryptophanylmethionine (I) was 0.3 g (60%), mp 132–134° C.

After recrystallization from ethyl acetate—petroleum ether, mp 134–135° C; R_f 0.89, $[\alpha]_D^{22}$ −22.15° (c 2; methanol).

Found, %: C 62.16; H 5.46; N 8.73; S 6.52. Calculated for $C_{25}H_{29}N_3O_5S$, %: C 62.10; H 6.04; N 8.68; S 6.60.

B) A solution of 1.69 g (5 mM) of carbobenzoxytryptophan [7] and 0.99 g (5 mM) of the hydrochloride of the methyl ester of methionine [8] in 35 ml of DMF cooled to −5° C was treated with 0.69 ml (5 mM) of triethylamine. The mixture was kept at 0° C for 5 min, cooled to −10° C, and treated with a solution of 1.03 g (5 mM) of dicyclohexylcarbodiimide in 5 ml of DMF. The mixture was left at −5° C for 20 min and then at 0° C for 24 hr and at room temperature for 3 hr. The dicyclohexylcarbonylurea that had deposited was filtered off and the filtrate was evaporated to dryness. The residue obtained was dissolved in 40 ml of ethyl acetate and the solution was washed with 20 ml of water, 20 ml of 1 N HCl, two 15-ml portions of 1 M sodium bicarbonate solution, and 20 ml of water. The solution was dried over magnesium sulfate and evaporated. The oily residue crystallized over hexane. The yield of the methyl ester of the dipeptide I was 2.2 g (91%), mp 129–134° C. After recrystallization from ethyl acetate—petroleum ether, mp 132–134° C.

Hydrazide of carbobenzoxytryptophanylmethionine (II). A solution of 1.5 g (3 mM) of the methyl ester of carbobenzoxytryptophanylmethionine (I) and 0.6 ml (12 mM) of hydrazine hydrate in 6 ml of ethanol was boiled for 1.5 hr. Then it was left at room temperature for 20 hr. The crystals that had deposited were filtered off and dried in vacuum over phosphorus pentoxide. The yield of the hydrazide of carbobenzoxytryptophanylmethionine (II) was 1.23 g (82%), mp 185–188° C. After recrystallization from dioxane—ethanol, mp 189–190.5° C, R_f 0.97.

Found, %: C 59.38; H 6.59; S 6.55; N 14.04. Calculated for $C_{24}H_{29}N_5O_4S$, %: C 59.60; H 6.04; S 6.62; N 14.48.

Triethylammonium salt of the methyl ester of carbobenzoxytryptophanylmethionylaspartylphenylalanine. (V). With stirring, a cold solution of 90 mg of sodium nitrite in 1 ml of water was added in one portion to a solution of 0.48 g (1 mM) of the hydrazide of carbobenzoxytryptophanylmethionine (II) in a mixture of 12 ml of glacial acetic acid, 8 ml of 1 N HCl, and 8 ml of water cooled to −5° C. The mixture was kept at −5° for 10 min. The azide III deposited in the form of white crystals, which were extracted with ethyl acetate (2 × 30 ml). With cooling, the ethyl acetate layer was washed with water (30 ml), 3% sodium bicarbonate solution (3 × 15 ml), and water (30 ml). Then it was dried over sodium sulfate for 5 min. To the cold solution was added 0.29 g (1 mM) of the methyl ester of aspartylphenylalanine (IV) in 15 ml of DMF and 0.14 ml (1 mM) of triethylamine. The solution was kept at +5° C for 86 hr. After evaporation of the solvent, the crystalline residue was washed with petroleum ether and dried in vacuum over caustic potash. The yield of the triethylammonium salt of the methyl ester of the tetrapeptide (V) was 0.5 g (67.5%), mp 58–68° C. The substance was used for the preparation of the amide of the tetrapeptide (VI) without additional purification.

Amide of the β-tert-butyl ester of carbobenzoxyaspartylphenylalanine (IX). A solution of 0.2 g (1 mM) of phenylalanine hydrochloride [10] in 2 ml of DMF was treated with 0.14 ml (1 mM) of triethylamine. The triethylamine salt that deposited was filtered off and the resulting solution of the amide of phenylalanine (VIII) was treated with 0.42 g (1 mM) of the α-succinimidyl β-tert-butyl ester of carbobenzoxyaspartic acid (VII) and was kept at room temperature for 1 hr. Then, with stirring, the solution was poured into 15 ml of water. The dipeptide (IX) that separated out was filtered off and dried in the air. Yield 0.42 g (89%), mp 147–158° C. After recrystallization from ethyl acetate, mp 156–157° C (158.5–159.5° C [4]).

Found, %: C 63.28; H 6.95. Calculated for $C_{25}H_{31}N_3O_6$, %: C 63.90; H 6.70.

Amide of the β-tert-butyl ester of aspartylphenylalanine (X). Substance IX was dissolved in 25 ml of 90% acetic acid and was hydrogenated in the presence of palladium black. After the cessation of hydrogenation, the catalyst was filtered off and the filtrate was evaporated. The residue was evaporated several times with small portions of benzene. The resulting dry acetate of the amide of the β-tert-butyl ester of aspartylphenylalanine was used for the isolation of the base as described by Davay et al. [4]. The yield of the dipeptide **X** was 1.42 g (84%), mp 122–124° C (123–124° C [4]).

Amide of the β-tert-butyl ester of carbobenzoxytryptophanylmethionylaspartylphenylalanine (XI). With stirring, a cold solution of 38 mg of sodium nitrite in 0.5 ml of water was added in one portion to a solution of 242 mg (0.5 mM) of the hydrazide of carbobenzoxytryptophanylmethionine (II) in a mixture of 6 ml of glacial acetic acid, 4 ml of 1 N HCl, and 4 ml of water cooled to −5° C. The mixture was stirred at −5° C for 10 min. The azide (III) deposited in the form of white crystals, which were extracted with 30 ml of ethyl acetate. With cooling, the organic layer was washed successively with water (10 ml), 3% sodium bicarbonate solution (4 × 15 ml), and water (10 ml). After being dried over sodium sulfate for 5 min, the cold solution was treated with 167 mg (0.5 mM) of the amide of the β-tert-butyl ester of aspartylphenylalanine (**X**) in 2 ml of DMF. The solution was kept at 0° C for 48 hr and was then washed with 0.2 N HCl (20 ml), 3% sodium bicarbonate solution (20 ml), and water (10 ml). The substance was precipitated from ethyl acetate with benzene. The crystals that deposited were dried in vacuum over phosphorus pentoxide. The yield of the tetrapeptide **XI** was 0.24 g (61%), mp 174–179° C. After recrystallization from ethanol, mp 191–192° C.

Amide of carbobenzoxytryptophanylmethionylaspartylphenylalanine (VI). A) The unpurified triethylammonium salt of the methyl ester of carbobenzoxytryptophanylmethionylaspartylphenylalanine (**V**) (0.5 g) was dissolved in 10 ml of methanol saturated with dry ammonia at 0° C. The solution was kept at +5° C for 3 days and was then evaporated.

The oily residue was dissolved in 30 ml of methanol and, with cooling, the solution was acidified with 0.2 N HCl to pH 3. The amide of the tetrapeptide (**VI**) precipitated in the form of white crystals. The substance was filtered off, washed with water to neutrality, and dried in vacuum over phosphorus pentoxide. The yield of the amide of the tetrapeptide (**VI**) was 0.39 g (76.5%), mp 204–212° C

After recrystallization from 2-ethoxyethanol and water, mp 215° C (decomp.), (237–238° C decomp. [4], $[\alpha]_D^{22}$ −36.4° (c 1; DMF), $[\alpha]_D^{22}$ −41° (c 1; DMF [4]), R_f 0.94.

Found, %: C 59.99; H 5.95; S 4.67; N 11.91. Calculated for $C_{37}H_{42}N_8O_6S$, %: C 60.80; H 5.79; S 4.38; N 11.50.

B) A solution of 0.11 g of the amide of the β-tert-butyl ester of the carbobenzoxytetrapeptide (**XI**) in 2 ml of anhydrous trifluoroacetic acid was kept at room temperature for 15 min. The substance was precipitated with ether, filtered off, carefully washed with ether, and dried in vacuum over caustic potash. The yield of the amide of the carbobenzoxytetrapeptide (**VI**) was 80 mg (79.5%), mp 208–210° C (decomp.), (237–238° C, decomp. [4]), R_f 0.94.

Conclusions

1. The carbobenzoxy derivative of the C-terminal tetrapeptide of gastrin—the amide of carbobenzoxytryptophanylmethionylaspartylphenylalanine—has been synthesized by two different routes.

2. In nonnarcotized cats the tetrapeptide obtained causes the secretion of gastric juice with a high content of hydrochloric acid (MR_{50} = 11.5 μg/kg).

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