SYNTHESIS OF ANALOGS OF A TETRAPEPSIDE OF GASTRIN WITH A MODIFIED TRYPTOPHAN RESIDUE

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In order to study the interaction of the active fragment of the hormone gastrin (Try–Met–Asp–$PheNH_2$ [1]) with the receptor, we have synthesized some of its analogs containing in place of the tryptophan residue one of the acyl radicals β-(1-naphthyl)acryloyl (A), β-(1-naphthyl)propionyl (B), β-(2-naphthyl) propionyl (C), and α-benzyloxycarbonylamino-β-(1-methylindol-3-yl)propionyl (D). The synthesis of the compounds was effected by the methods of classical peptide chemistry using schemes 1 and (or) 2.

In the first variant of the synthesis (scheme 1), N-hydroxysuccinimide esters of the tryptophan analogs (I, X = A, B, C, D) were condensed with the methyl ester of methionine (II) in dimethylformamide solution. The resulting methionine derivatives (III) were then converted into the hydrazides (IV) and, using the azide method of coupling, were condensed with the β-tert-butyl ester of aspartylphenylalanine (VI) [2]. The tert-butyl ester group in the tetrapeptide derivatives (VII) was eliminated by treatment with trifluoroacetic acid.

According to the second variant of the synthesis (scheme 2), methionine (IX) was added to the methyl ester of aspartylphenylalanine (X) [3] by the stepwise method using hydroxysuccinimide esters, and after the conversion of the tripeptide ester (XI) into the amide (XII) the tert-butoxycarbonyl protection was split off and the resulting compound (XIII) was added to the appropriate N-hydroxysuccinimide ester to give (I, X = A, B, C). The results of biological tests on the compounds obtained have been published in a preceding paper [4].

EXPERIMENTAL

The experiments were performed with amino acids of the L configuration and with anhydrous solvents. All evaporations were performed in a rotary vacuum evaporator at a water-bath temperature of +40 to +50°C. The melting points (uncorrected) were determined in capillaries. The purity of the materials obtained was checked by paper and thin-layer chromatography. For all the compounds, the elementary analyses corresponded to the calculated figures.

N-Hydroxysuccinimide Ester of β-(1-Naphthyl)acrylic Acid (IA). A solution of 0.59 g (2.9 mmoles) of α-naphthylacrylic acid and 0.34 g (2.9 mmoles) of hydroxysuccinimide in 30 ml of dioxane cooled to 0°C was treated with 0.61 g (2.9 mmoles) of dicyclohexylcarbodiimide. The mixture was kept at 0°C for 15 min, and then at room temperature for 20 h. The dicyclohexylurea that deposited was filtered off, and the filtrate was evaporated to dryness. The residue was recrystallized from isopropanol and dried in vacuum over phosphorus pentoxide. The yield of the ester (IA) was 0.49 g (56.2%), mp 159-161°C, composition $C_{17}H_{13}NO_4$.

Compounds IB and IC were synthesized by the same method: the N-hydroxysuccinimide ester of β-(1-naphthyl)propionic acid $C_{17}H_{15}NO_4$ (IB) with a yield of 56.8%, mp 102-105°C (from isopropanol), and the N-hydroxysuccinimide ester of β-(2-naphthyl)propionic acid $C_{17}H_{15}NO_4$ (IC) with a yield of 76.8%, mp 158-160°C (from isopropanol).

Methyl Ester of β-(1-Naphthyl)acryloylmethionine (IIIA). A solution of 0.45 g (1.5 mmole) of the N-hydroxysuccinimide ester of β-(1-naphthyl)acrylic acid and 0.3 g (1.5 mmole) of the hydrochloride of the methyl ester of methionine (II) in 10 ml of DMF cooled to 0°C was treated with 0.21 ml (1.5 mmole) of triethylamine. The mixture was kept at 0°C for 5 min and at room temperature for 1 h. The substance

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was precipitated with water, recrystallized from methanol, and dried at room temperature. The yield of the methyl ester $C_{19}H_{21}NO_3$ (IIIA) was 0.3 g (52%), mp 157-159° C, $[\alpha]_D^{22}$ – 28.3° (c 1; CH_3OH).

Compounds IIIB and IIIC were synthesized by the same method: the methyl ester of β-(1-naphthyl) propionylmethionine $C_{19}H_{23}NO_3S$ (IIIB) with a yield of 80.5%, mp 91-93° C (from methanol–water), $[\alpha]_D^{22}$ – 51.4° (c 2; CH_3OH), and the methyl ester of β-(2-naphthyl)propionylmethionine $C_{19}H_{23}NO_3S$ (IIIC) with a yield of 77%, mp 76-78° C (from methanol–water), $[\alpha]_D^{22}$ – 28.8° (c 2; CH_3OH).

Methyl Ester of α-Benzyloxycarbonylamino-β-(1-methylindol-3-yl)propionylmethionine (IIID). To a solution of 0.40 g (0.2 mmole) of the hydrochloride of the methyl ester of methionine in 10 ml of anhydrous chloroform cooled to 0° C were added 0.28 ml (0.2 mmole) of triethylamine and a solution of 0.73 g (0.2 mmole) of α-benzyloxy-carbonylamino-β-(1-methylindol-3-yl)propionic acid in 10 ml of chloroform. With stirring, 0.42 g (0.2 mmole) of dicyclohexylcarbodiimide was added to the resulting solution, and the mixture was left overnight at +5° C. The yield of the dipeptide $C_{26}H_{31}N_3O_5S$ (IIID) was 0.73 g. After recrystallization from ethanol–petroleum ether, 0.53 g (52.5%) of a product with mp 117-120° C was obtained.

Hydrazide of β-(1-Naphthyl)acryloylmethionine (IVA). A solution of 0.32 g (0.87 mmole) of the methyl ester of β-(1-naphthyl)acryloylmethionine (III) in 7 ml of methanol was treated with 0.5 ml of hydrazine hydrate, and the mixture was boiled for 30 min. Then it was left at room temperature for 20 h. The crystals that had deposited were filtered off and dried in vacuum over phosphorus pentoxide. The yield of the hydrazide of β-(1-naphthyl)acryloylmethionine $C_{18}H_{21}N_3O_2S$ (IVA) was 0.25 g (83.3%), mp 246-248° C (from dioxane methanol), $[\alpha]_D^{25}$ + 13.0° (c 1; CH_3COOH).

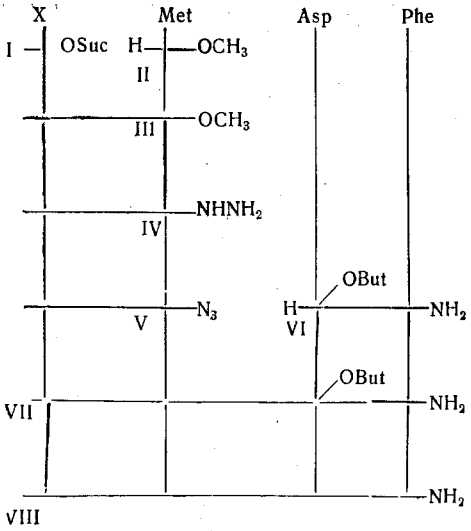
By the same method we synthesized the hydrazide of β-(1-naphthyl)propionylmethionine $C_{18}H_{23}N_3O_2S$ (IVB) with a yield of 83.3%, mp 196-197.5° C (from methanol), $[\alpha]_D^{25}$ – 126° (c 1; CH_3COOH), the hydrazide of β-(2-naphthyl)propionylmethionine $C_{18}H_{23}N_3O_2S$ (IVC) with a yield of 63.3%, mp 186-187° C (from ethanol), $[\alpha]_D^{25}$ – 12.5° (c 1; CH_3COOH), and the hydrazide of α-benzyloxycarbonylamino-β-(1-methylindol-3-yl)-propionylmethionine $C_{25}H_{31}N_5O_4S$ (IVD) with a yield of 75.6%, mp 211-214° C.

Amide of β-(1-Naphthyl)acryloylmethionyl–β-tert-butyl-aspartylphenylalanine (VIIA). A solution of 171 mg (0.5 mmole) of the hydrazide of β-(1-naphthyl)acryloylmethionine (IVa) in a mixture of 10 ml of glacial acetic acid, 4 ml of 1 N hydrochloric acid, and 4 ml of water was cooled to –10° C, and, with stirring, a cold solution of 38 mg of sodium nitrite in 0.5 ml of water was added in one portion, and stirring was continued at –5° C for 10 min. The azide (VA) precipitated in the form of white crystals, which were extracted with 30 ml of ethyl acetate. The organic layer was washed successively, with cooling, with 20 ml of water, 20 ml of 1 N sodium bicarbonate solution, and 20 ml of water. Then it was dried over sodium sulfate for 5 min. To the cold solution was added 167 mg (0.5 mmole) of the amide of β-tert-butyl-aspartylphenylalanine (VI) [2] in 5 ml of DMF. The solution was kept at +2° C for 48 h and was then washed with 20 ml of 5 N hydrochloric acid, 10 ml of water, 25 ml of 3% sodium bicarbonate solution, and 20 ml of water. The ethyl acetate was evaporated off and the residue was crystallized under water. The amide (VIIA) was dried in vacuum over phosphorus pentoxide. Yield 220 mg (68.3%), mp 211-214° C (from methanol).

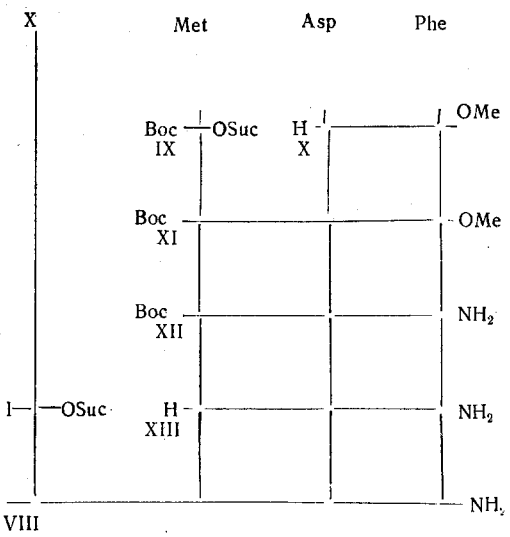
The amide of β-(1-naphthyl)propionylmethionyl–β-tert-butyl-aspartylphenylalanine (VIIB) was synthesized similarly with a yield of 64.2%, mp 218-220° C, and so were the amide of β-(2-naphthyl)propionyl-methionyl–β-tert-butylaspartylphenylalanine (VIIC) with a yield of 39%, mp 191-195° C, and the amide of α-benzyloxycarbonylamino-β-(1-methyl-indol-3-yl)propionylmethionylaspartylphenylalanine (VIIID) with a yield of 40%, mp 195-205° C.

Amide of β-(1-Naphthyl)acryloylmethionylaspartylphenylalanine (VIIIA). A solution of 50 mg of the amide of β-(1-naphthyl)acryloylmethionyl–β-tert-butylaspartylphenylalanine in 0.6 ml of anhydrous trifluoroacetic acid was kept at room temperature for 15 min. The amide (VIIIA) was precipitated with ether, filtered off, carefully washed with ether, and dried in vacuum over caustic potash. The yield of the amide of β-(1-naphthyl)acryloylmethionylaspartylphenylalanine, $C_{31}H_{36}O_6N_4S$ (VIIIA) was 30 mg (65.5%), mp 222-224°C.

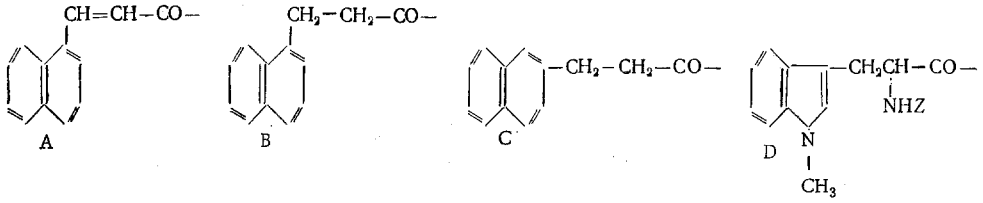
Compounds (VIIIB, C and D) were obtained by the same method: the amide of β-(1-naphthtl)propionyl-methionylaspartylphenylalanine $C_{31}H_{36}O_6N_4S$ (VIIIB) with a yield of 63.5%, mp 229-232°C, $[\alpha]_D^{22}$ –51° (c 1.3; DMF), the amide of β-(2-naphthyl)propionylmethionylaspartylphenylalanine, $C_{31}H_{36}O_6N_4S$ (VIIIC) with a yield of 63%, mp 214-215°C, $[\alpha]_D^{22}$ –45° (c 1; DMF), and the amide of α-benzyloxycarbonylamino-β-(1-methylindol-3-yl)-propionylmethionylaspartylphenylalanine $C_{30}H_{44}N_8O_6S$ (VIIID) with a yield of 60.5%, mp 226-229°C.



Scheme 1 (X=A, B, C or D)



Scheme 2 (X=A, B or C)



Amide of tert-Butoxycarbonylmethionylaspartylphenylalanine (XII). A solution of 2.64 g (9 mmoles) of the methyl ester of aspartylphenylalanine [3] and 3.15 g (9 mmoles) of the N-hydroxysuccinimide ester of tert-butoxycarbonylmethionine in 50 ml of DMF and 6 ml of water was treated with 1.26 ml of triethylamine. The mixture was kept at room temperature for 2 h and was then cooled to 0° C and acidified with a 10% solution of citric acid to pH 3. The oil that deposited was extracted with ethyl acetate (3 × 40 ml), washed with ice water, and dried over sodium sulfate. The ethyl acetate was evaporated off, the residue was dissolved in 50 ml of methanol, and the solution was saturated with ammonia at 0° C and kept at the same temperature for 18 h. The organic solvent was evaporated off and the residue was again dissolved in 50 ml of methanol. The solution was cooled and acidified with 10% citric acid to pH 3, and then 40 ml of ice water was added. The white crystals that deposited were filtered off, washed with ice water, and dried at room temperature. The yield of the amide (XII) was 2.4 g (50%), mp 200-202° C (decomp.) $[\alpha]_D^{22} - 39.0$ (c 1.0; DMF). Literature data: mp 209-210° C (decomp.), $[\alpha]_D^{22} - 39.3°$ [3]. The tert-butoxycarbonyl group was eliminated from the compound obtained by means of a solution of hydrogen chloride in glacial acetic acid [3].

Amide of β-(1-Naphthyl)acryloylmethionylaspartylphenylalanine (VIIIA). A solution of 114 mg (0.25 mmole) of the hemihydrate of the hydrochloride of the amide of methionylaspartylphenylalanine (XIII) and 74 mg (0.25 mmole) of the N-hydroxysuccinimide ester of naphthylacrylic acid in 2 ml of DMF and 0.2 ml of water was treated with 0.07 ml of triethylamine and kept at room temperature for 3 h. Then 20 ml of water was added and the mixture was acidified with 1 N hydrochloric acid to pH 3. The white crystals that deposited were filtered off, washed with water, and dried at room temperature. Yield 70 mg (47.5%), mp 222-224° C.

In the same way we synthesized the amide of β-(1-naphthyl)-propionylmethionylaspartylphenylalanine (VIIIB) with a yield of 81%, mp 229-232° C, and the amide of β-(2-naphthyl)propionylmethionylaspartylphenylalanine (VIIIC) with a yield of 82%, mp 214-215° C. In their chromatographic behavior, these compounds were identical with authentic compounds obtained by the first scheme.

SUMMARY

New derivatives of the COOH-terminal tetrapeptide of gastrin containing in place of the tryptophan residue the acyl radicals β-(1-naphthyl)acryloyl, β-(1-naphthyl)propionyl, β-(2-naphthyl)propionyl, and α-benzyloxycarbonylamino-β-(1-methylindol-3-yl)propionyl have been synthesized.

LITERATURE CITED

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