SYNTHESIS OF C-TERMINAL FRAGMENTS OF BOMBESIN AND THEIR ANALOGUES

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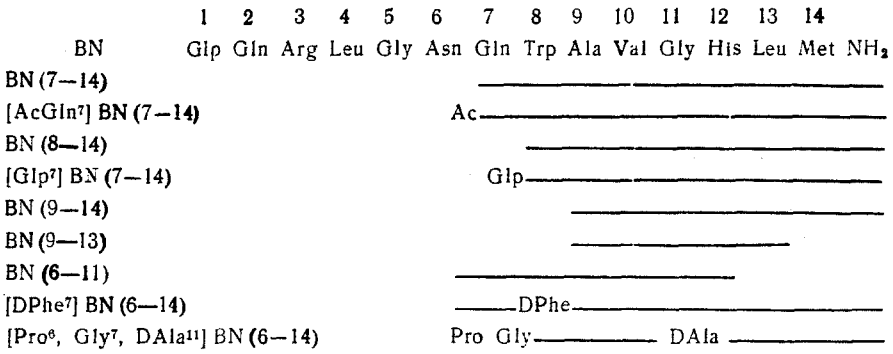
UDC 547.96.07

With the aim of structural-functional studies in the bombesin series, a number of bombesin fragments and analogues have been synthesized. The synthesis was performed by the carbodiimide method and by the activated-ester method. Fragments with the sequences 7-14, 8-14, and 9-14 were obtained by 4 + 4, 3 + 4, and 2 + 4 schemes and a pentapeptide with the sequence 9-13 by a 3 + 2 scheme. Acetylation of the octapeptide BN(7-14) was carried out by the action of acetic anhydride in pyridine. Analogues of the C-terminal nonapeptide of bombesin [DPhe^7]BN(6-14) and [Pro^6, Gly^7, DAla^{11}]BN(6-14) were synthesized by fragment condensation using the 5 + 4 scheme. The individuality of the compounds obtained was confirmed by their chromatographic behavior on plates coated with silica gel, and by the results of amino acid analysis, high-voltage electrophoresis, and high-performance liquid chromatography, and their structures were confirmed by the results of high-resolution ^{1}H NMR spectroscopy (360 MHz). In experiments on rabbits, in a dose of 1 μg with central administration the full hypothermic effect of bombesin was shown by the preparation [AcGln^7]BN(7-14), while the preparation [DPhe^7]BN(6-14) and [Pro^6,Gly^7,DAla^{11}]BN(6-14) possessed only a slight effect (∿1% of the activity of bombesin).

It has been reported in a number of publications that not only the whole molecule of the tetradecapeptide bombesin from amphibia (BN) but also its C-terminal fragments possess activity.

With the aim of a direct search for a reduced structure of amphibian bombesin responsible for the manifestation of biological activity and the subsequent performance of conformational-structural analysis in the series of bombesin-like peptides, we have carried out the synthesis of a number of C-terminal fragments of bombesin and their analogues by classical methods in solution.



The synthesis of shortened fragments with the sequence (7-14) (Scheme 1), 8-14 (Scheme 2), and 9-14 (Scheme 3) was carried out by the condensation of 4 + 4, 3 + 4, and 2 + 4 fragments, respectively. According to Schemes 1-3, the C-terminal methyl component was valine, and it was therefore possible for racemization to have taken place. In order to show the possibility of using the proposed scheme of synthesis, we subjected a hydrolysate of the nonapeptide BN(6-14) obtained previously by an analogous 5 + 4 scheme [4] to stereo-

Leningrad State University. Institute of Organic Synthesis, Latvian SSR Academy of Sciences, Riga. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 554-564, July-August, 1989. Original article submitted June 28, 1988; revision submitted January 6, 1989.

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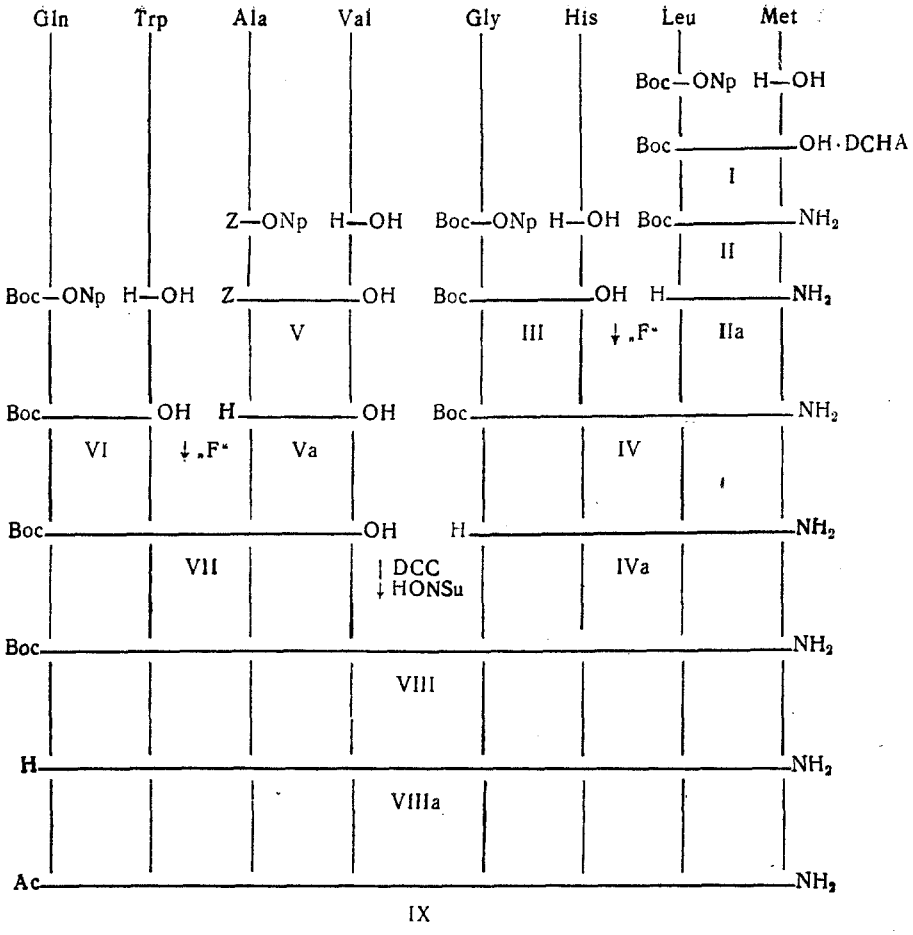
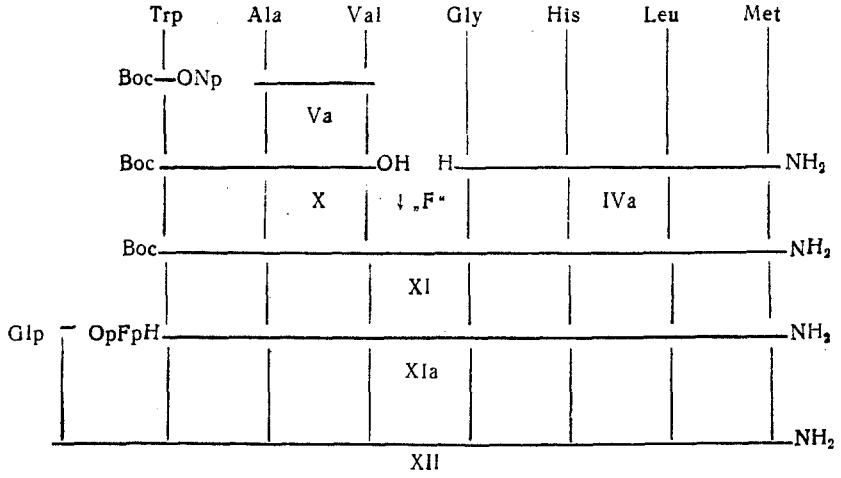


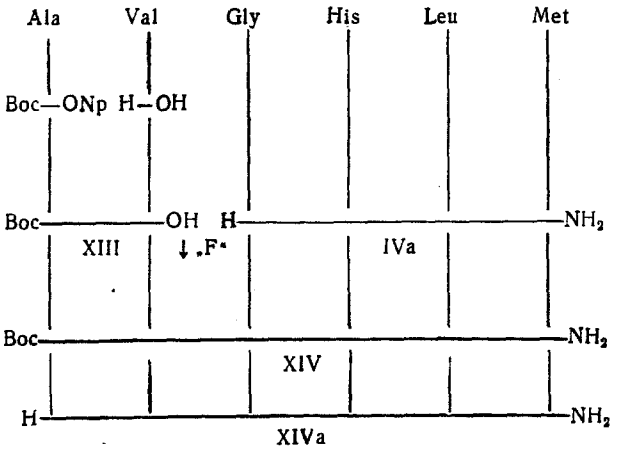
Scheme 1



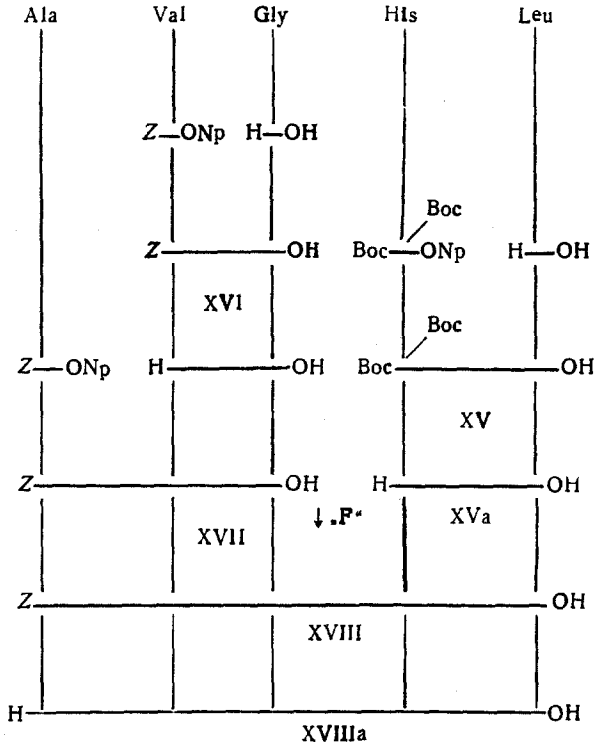
Scheme 2

specific oxidation with the L-amino acid oxidase from the venom of the rattlesnake Crotalis adamanteus [5], which showed the absence of D-valine.

The pentapeptide BN(9-13) was synthesized by a 3 + 2 scheme (Scheme 4). The octapeptide BN(7-14) was acetylated by the action of acetic anhydride in pyridine using Thompson's method [6]. The central fragment of bombesin, the hexapeptide BN(6-11), was obtained by deblocking the corresponding protected hexapeptide. Analogues of the C-terminal nonapeptide of bombesin [DPhe^7]BN(6-14) and [Pro^6,Gly^7,DAla^{11}]BN(6-14) were synthesized by condensing 5 + 4 fragments by a scheme analogous to that for obtaining the BN(6-14) peptide [4].



Scheme 3



Scheme 4

In the process of synthesis, to protect the N-terminal amino groups we used benzyloxycarbonyl (Z) and tert-butoxycarbonyl (Boc) groups, which were eliminated by catalytic hydrogenation and by the action of hydrogen chloride in acetic acid in the presence of 2-mercaptoethanol and dimethyl sulfide [7], respectively. To protect the imidazole group of histidine (Scheme 4) we used a tert-butoxycarbonyl group. The carboxylic functions of the C-terminal amino acids were protected by salt formation.

The protected peptides were purified with the aid of adsorption chromatography, and the deblocked compounds with the aid of chromatography on sulfopropyl-Sephadex C-25 in pyridine-acetate buffer and, in individual cases, with the aid of preparative HPLC.

Certain difficulties arose in the isolation of the bombesin(7-14) peptide (VIIIa). According to the results of analytical HPLC, the deblocked octapeptide (VIIIa), after purification with ion-exchange chromatography, contained a small amount of impurity (XII) (Fig. 1). In an attempt to chromatograph the octapeptide (VIIIa) on Woelm silica gel in system 4 this octapeptide was converted almost completely into peptide (XII).

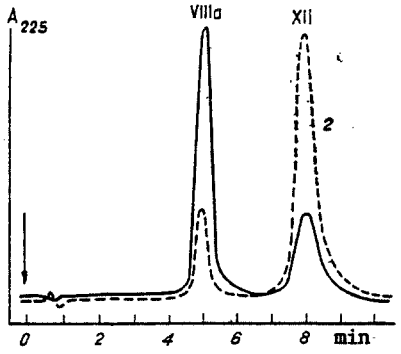


Fig. 1. Profiles of the bombesin-(7-14) peptide (VIIIa) and the [Glp^7]bombesin(7-14) peptide (XII) on an analytical column (HPLC), 0.46 × 15 cm, Zorbax C_8, 22% CH_3CN and 78% 0.2 M NH_4OCOCH_3, rate of flow 2 ml/min, λ 225 nm: 1) after the separation of (VIIIa) on SP-Sephadex C-25 in pyridine-acetate buffer; 2) after the chromatography of (VIIIa) on a column (2 × 50 cm) of Woelm silica gel in the ethyl acetate–pyridine–acetic acid–water (30:20:6:11) system.

TABLE 1. Characteristics of the C-Terminal Fragments of Bombesin and Their Analogues

Compound	Results of amino acid analysis								
	Asp	Glu	Ala	Val	Gly	His	Leu	Met	other amino acids
BN (7—14)	—	1,03	1,02	1,00	1,08	0,97	1,00	0,92	—
[AcGln^7] BN (7—14)	—	1,03	1,00	0,98	1,01	0,98	1,01	0,92	—
BN (8—14)	—	—	1,00	0,95	1,01	0,96	0,99	0,98	—
[Glp^7] BN (7—14)	—	1,00	1,14	1,11	1,07	0,94	0,93	0,98	—
BN (9—14)	—	—	0,99	1,00	1,00	1,00	0,98	0,93	—
BN (9—13)	—	—	1,00	0,83	1,21	1,18	1,22	—	—
BN (6—11)	1,07	1,12	1,06	1,00	1,09	—	—	—	—
[*D*Phe^7] BN (6—14)	1,12	—	1,07	1,02	1,02	1,05	1,00	0,97	Phe 0,94
[Pro^6, Gly^7, *D*Ala^{11}] BN (6—14)	—	—	2,04	1,02	1,00	0,99	0,98	9,97	Pro 1,01

Compound	Yield, %	k'	E_{His}, pH 2,6	$[\alpha]_D^{22}$ (c1,1%; CH_3COOH)	R_f, Merck					
					C	D	B	E	G	A
BN (7—14)	41	4,92	0 81	—36,4	0,65	0,61	0,67	0,33	—	—
[AcGln^7] BN (7—14)	40	—	0,51	—	—	0,36	0,75	0,34	—	—
BN (8—14)	58	3,53	0,83	32,4	0,68	0,67	0,66	—	0,35	—
[Glp^7] BN (7—14)	56	7,84	0,57	36,5	0,73	0,82	0,80	—	—	—
BN (9—14)	54	3,03	0,91	55,0	0,60	0,57	0,62	—	—	—
BN (9—13)	68	1,87	0,84	34,7	0,45	0,44	0,46	—	—	—
BN (6—11)	55	3,17	0,55	—	0,39	—	—	—	—	0,37
[*D*Phe^7] BN (6—14)	49	3,67	0,72	42,2	0,69	—	0,68	0,39	—	—
[Pro^6, Gly^7, *D*Ala^{11}] BN (6—14)	46	5,54	0,80	25,0	0,66	0,69	0,71	—	—	—

According to the literature [8], peptides with N-terminal glutamine readily form five-membered cyclic imides, giving pyroglutamyl peptides. We have performed an independent synthesis of an octapeptide with a pyroglutamic acid residue at the N-end (XII) from the heptapeptide (XIa) and the pentafluorophenyl ester of pyroglutamic acid (Scheme 2). The pyroglutamyl peptide obtained (XII) and the product of the transformation of the octapeptide (VIIIa) isolated by the preparative HPLC proved to be identical according to analytical HPLC and ^{1}H NMR spectroscopy (360 MHz).

The individuality of the compounds obtained was confirmed by their chromatographic behavior on plates coated with Kieselgel 60 F 254 silica gel (Merck, FRG), and by the results of amino acid analysis, high-voltage electrophoresis, and high-performance liquid chromatography, and their structures were confirmed by high-resolution ^{1}H NMR spectroscopy (360 MHz). The yields and physicochemical characteristics of the free peptides are given in Table 1. The influence of the peptides synthesized on thermoregulation has been studied by A. T. Mar'yanovich et al. [9] in experiments on rabbits. The preparations in a dose of 1 μg in 0.2 ml of isotonic NaCl solution were introduced into a lateral ventricle of the brain at a temperature of the medium of +10°C. The full hypothermic effect of bombesin was shown only by the acylated derivatives [AcGln^7]BN(7-14), while the preparations [DPhe^7]BN(6-14) and [Pro^6,Gly^7,DAla^{11}]BN(6-14) possessed only a slight effect (~1% of the activity of bombesin).

EXPERIMENTAL

L-Amino acids (Reanal) were used for the synthesis. The necessary derivatives were prepared by known methods [10, 11]. Melting points were determined on a Kofler instrument and are given without correction. The individuality of the compounds obtained was checked with the aid of TLC on plates with a fixed layer of Kieselgel 60 F 254 silica gel (Merck). Chromatographic mobilities in the following systems are given: 1) butan-1-ol–acetic acid–water (3:1:1); 2) butan-1-ol–acetic acid–pyridine–water (30:6:20:24); 3) butan-1-ol–acetic acid–pyridine–water (10.5:1:6:7.5); 4) ethyl acetate–pyridine–acetic acid–water (30:20:6:11); 5) butan-1-ol–acetic acid–water (4:1:5) (upper phase); 6) ethyl acetate; and 7) benzene–acetone–acetic acid (50:25:1). Electrophoretic mobilities were determined with respect to histidine on FN-12 paper (Filtrak) at a potential gradient of 30 V·cm^{-1} in 2% acetic acid (pH 2.6). The amino acid analysis of peptides that had been hydrolyzed in sealed tubes with 6 N HCl at 110°C for 20 h was performed on an LKB 4102 analyzer (Sweden). Elementary analyses were conducted on a Hewlett-Packard 185 automatic C,H,N-analyzer. Specific rotations were determined on a Perkin-Elmer 141 polarimeter. HPLC was conducted on a Du Pont 830 liquid chromatograph (USA): column 4.6 mm × 15 cm, Zorbax C_8, 22% CH_3CN and 78% 0.2 M CH_3COONH_4, rate of flow 2 ml/min, λ 225 nm (analytical variant); column 21.2 mm × 25 cm, Zorbax C_8, rate of flow 20 ml/min, λ 230 nm (preparative variant). Ion-exchange chromatography was performed on SP-Sephadex C-25 in pyridine-acetate buffer in the gradient regime with a range of molarity of the buffer of from 0.05 to 1.0 M, pH 5.2 (column 1.3 × 23 cm, rate of elution 30 ml/h, detection at λ 280 nm). The detecting instrument used was a Uvicord II (LKB, Sweden). Woelm (FRG) or Silpearl (Czechoslovakia) silica gel was used for column chromatography with a 2.5 × 50 cm column at a rate of elution of 30 ml/h. The results of the elementary analyses of all the protected peptides corresponded to the calculated figures.

^{1}H NMR spectra were obtained on a WM-360 spectrometer (Bruker) with a working frequency of 360 MHz. Chemical shifts were determined relative to the internal standard TMS with an accuracy of ±0.01 ppm.

1. BocLeuMetOH·DCHA (I). A solution of 2.0 g (13.40 mmole) of methionine in 13.4 ml of 1 N NaOH was treated with 5.2 g (14.8 mmole) of BocLeuONp in 20 ml of dioxane. The reaction mixture was kept for 48 h and was then evaporated to small volume, diluted with water, and extracted with ethyl acetate. The aqueous layer was acidified with 1 N H_2SO_4, the oil that separated out was extracted with ethyl acetate (3 × 40 ml), and the combined extract was washed with water, dried over Na_2SO_4, and evaporated. The oily residue was dissolved in 25 ml of ether, and 2.7 ml (13.4 mmole) of dicyclohexylamine was added. The precipitate was filtered off and was dried over P_2O_5. Recrystallization from ethyl acetate yielded 5.8 g (80%) of a product with mp 152-154°C, Rf 0.82 (system 2). $C_{28}H_{53}N_3O_5S \cdot 1/2H_2O$.

2. BocLeuMetNH$_2$ (II). A suspension of 5.0 g (9.2 mmole) of substance (I) in 50 ml of ethyl acetate was washed with a 2% solution of H_2SO_4 and with water, and was dried over Na_2SO_4 and evaporated. The residue was dissolved in 2 ml of methanol, and an ethereal solution of diazomethane (9.4 mmole, calculated on the nitrosomethylurea used) was added. The solvent

was evaporated off, the residue was dissolved in 3 ml of methanol, and 100 ml of a solution of ammonia in methanol was added. The reaction mixture was left for three days and was evaporated, and the residue was crystallized under hexane and was recrystallized from ethyl acetate. This gave 3.2 g (97%) of product with mp 156-158°C (lit.: 158-159°C [12]), Rf 0.55 (system 6). $C_{16}H_{31}O_4N_3S \cdot 1/2H_2O$.

3. BocGlyHisOH (III) was obtained from 12.9 mmole of histidine and 14.2 mmole of BocGlyONp by method 1. After reprecipitation from propan-2-ol with ether, 3.9 g (97%) of product was obtained with mp 128-130°C (decomp.), Rf 0.30 (system 1). $C_{13}H_{20}N_4O_5 \cdot H_2O$.

4. BocGlyHisLeuMetNH$_2$ (IV). At −5°C, with stirring, 2.7 g (3.6 mmole) of "complex F" [13] was added to a solution of 1.1 g (3.6 mmole) of the dipeptide (III) in a mixture of 24 ml of dioxane and 8 ml of DMF. The reaction mixture was stirred at −5°C for 1 h and at room temperature for 30 min. A solution of 1.3 g (3.6 mmole) of dipeptide (II) in 1 ml of glacial acetic acid was treated with 0.1 ml of 2-mercaptoethanol, 0.1 ml of dimethyl sulfide, and 5.7 ml (12.6 mmole) of a 2.2 N solution of HCl in glacial acetic acid. The mixture was left for 20 min, after which the solvent was evaporated off, and the residue was treated with ether, filtered off, dried, and treated with propan-2-ol. This gave 1.1 g (99%) of product (IIa), Rf 0.38 (system 1), E_{His} 0.86. A solution of 1.1 g (3.6 mmole) of the hydrochloride of (IIa) in 3 ml of DMF was treated at 0°C with 0.45 ml (3.6 mmole) of N-ethylmorpholine and a solution of the pentafluorophenyl ester of the dipeptide (III) filtered free from DCHU. The reaction mixture was left for 24 h, evaporated to small volume, and dissolved in 20 ml of water-saturated n-butanol, and this solution was washed with 2% acetic acid, water, 5% $NaHCO_3$ solution, and water again. After two treatments with hot ethyl acetate, 1.2 g (58%) of product (IV) was obtained with mp 164-167°C, Rf 0.34 (system 1). $C_{24}H_{41}N_7O_7S \cdot 1/2H_2O$. Amino acid analysis: Gly 1.09 (1), His 0.96 (1), Leu 1.00 (1), Met 0.93 (1).

5. ZAlaValOH (V) was obtained from 2.0 g (17.2 mmole) of valine and 6.5 g (18.8 mmole) of ZAlaONp by method 1. This gave 5.1 g (94%) of product (V), mp 142-144°C, Rf 0.66 (system 5). $C_{16}H_{22}N_2O_5$.

6. BocGlnTrpOH (VI) was obtained from 2.0 g (9.8 mmole) of tryptophan and 3.9 g (10.8 mmole) BocGlnONp in 20 ml of dioxane in a similar way to method 1, with a yield of 3.6 g (86%) after recrystallization from propan-2-ol–hexane. mp 173-175°C (decomp.), Rf 0.66 (system 6). $C_{21}H_{28}N_4O_6$.

7. BocGlnTrpAlaValOH (VII). A solution of 2.6 g (6.0 mmole) of the dipeptide (VI) in a mixture of 45 ml of dioxane and 15 ml of DMF was cooled to 0°C, and, with vigorous stirring, 4.6 g (6.0 mmole) of "complex F" was added. The mixture was stirred at 0°C for 30 min and then at room temperature for 1 h.

The dipeptide (V) (1.9 g, 6.0 mmole) was dissolved in 100 ml of methanol and was hydrogenated over a Pd/C catalyst. The free peptide that had precipitated was dissolved by the addition of 6 ml (6.0 mmole) of 1 N NaOH, the catalyst was filtered off, the solvent was evaporated, and the resulting residue was treated with a solution of the pentachlorophenyl ester of the dipeptide (VI) in dioxane. The reaction mixture was left for 24 h, after which, under the conditions of separation of peptide (V) and two treatments with hot ethyl acetate, 2.7 g (75%) of product (VII) was obtained: $C_{29}H_{42}N_6O_8 \cdot H_2O$, mp 164-166°C, Rf 0.85 (system 1).

8. BocGlnTrpAlaValGlyHisLeuMetNH$_2$ (VIII). The tetrapeptide (IV) (0.60 g; 1.1 mmole) was deblocked by the action of 2.7 ml (3.8 mmole) of a 2 N solution of HCl in glacial acetic acid for 40 min, after which the solution was evaporated and the substance was recrystallized from propan-2-ol. This gave 0.55 g (98%) of substance (IVa), E_{His} 1.02. At −5°C, with stirring, 0.14 ml (1.14 mmole) of N-ethylmorpholine, 0.30 g (0.57 mmole) of the tetrapeptide (VII), 0.72 g (0.63 mmole) of N-hydroxysuccinimide, and 0.12 g (0.57 mmole) of DCC in 3 ml of DMF were added successively to 0.34 g (0.57 mmole) of the tetrapeptide (IVa) in 3 ml of DMF. The reaction mixture was stirred at −5°C for 2 h and at room temperature for 48 h. Then the DCU was filtered off, the reaction mixture was evaporated, the reaction product was extracted with n-butanol (3 × 30 ml), the extract was washed with 2% acetic acid and with water, and was evaporated, and the residue was recrystallized from methanol–ethyl acetate. Of the 0.21 g of substance so obtained 0.12 g was chromatographed on Woelm silica gel in system 1. This gave 0.067 g (57%) of substance (VIII), Rf 0.58 (system 1), 0.71 (system 5), 0.57 (system 4), 0.70 (system 3), E_{His} 0.52. Amino acid analysis: Glu 1.09 (1), Gly 1.01 (1), Ala 1.00 (1), Val 0.91 (1), Met 0.89 (1), Leu 0.95 (1), His 0.96 (1).

9. GlnTrpAlaValGlyHisLeuMetNH$_2$ (VIIIa). Compound (VIII) (0.21 g) was deblocked by the action of 0.35 ml of a 2 N solution of hydrogen chloride in acetic acid, and 0.182 of the product was purified by ion-exchange chromatography, giving 0.106 g of substance. After the purification of 0.046 g of the product by preparative HPLC, 0.037 g of substance (VIIIa) was obtained (see Table 1).

10. AcGlnTrypAlaValGlyHisLeuMetNH$_2$ (IX). To 0.050 g (0.049 mmole) of substance (VIIIa) in 5 ml of redistilled pyridine was added 0.5 ml of acetic anhydride. After 1 h the reaction mixture was evaporated, and the residue was dissolved in water and treated with Dowex 50 × 8 resin (H^+). The substance so obtained (0.031 g) was treated with 2-mercaptoethanol at 40°C for 4 h and was purified on a 1.0 × 30 cm column of Silpearl silica gel in solvent system 2. This gave 0.011 g of substance (see Table 1).

11. BocTrpAlaValOH·DCHA was obtained from 2.0 g (6.2 mmole) of the dipeptide (Va) and 2.9 g (6.8 mmole) of BocTrpONp by method 1. After recrystallization from ethyl acetate–hexane, 2.5 g (61%) of the DCHA salt of substance (X) was obtained with mp 121-123°C, R_f 0.67 (system 6). $C_{36}H_{57}N_5O_6 \cdot H_2O$.

12. BocTrpAlaValGlyHisLeuMetNH$_2$ (XI). A solution of 0.30 g (0.46 mmole) of the DCHA salt of substance (X) in ethyl acetate was washed with 10% $KHSO_4$ and with water, and was dried and evaporated. The residue was dissolved in 15 ml of dioxane, the solution was cooled to 0°C, and, with stirring, 0.35 g (0.46 mmole) of "complex F" was added. The reaction mixture was stirred at 0°C for 30 min and at room temperature for 1 h. Then it was filtered from the DCU and was added to a solution of 0.24 g (0.46 mmole) of the tetrapeptide (IVa) and 0.12 ml (0.92 mmole) of N-ethylmorpholine in 5 ml of DMF. The new reaction mixture was left for 24 h and was worked up under the conditions of experiment 8. After recrystallization from methanol–ethyl acetate, 0.23 g of substance (XI) was obtained, with R_f (main spot) 0.53 (system 1).

13. TrpAlaValGlyHisLeuMetNH$_2$ (XIa). The deblockage of 0.23 g of substance (XI) was achieved by the action of 0.9 ml of a 2 N solution of hydrogen chloride in acetic acid. The dihydrochloride was worked up as in experiment 4, and 0.221 g of the product was purified by ion-exchange chromatography. This gave 0.160 of product the additional purification of 0.100 g of which by preparative HPLC yielded 0.081 g of substance (XIa) (see Table 1).

14. GlpTrpAlaValGlyHisLeuMetNH$_2$ (XII) was obtained from 0.340 g (0.381 mmole) of the dihydrochloride of (XIa), 0.100 ml (0.762 mmole) of N-ethylmorpholine, and 0.220 g (0.762 mmole) of Glp-OPFP in 10 ml of DMF. The product was worked up as in experiment 8. Then 0.176 g of the substance was purified by ion-exchange chromatography. This gave 0.099 g of substance (XII) (see Table 1).

15. BocAlaValOH (XIII) was obtained under the conditions of experiment 5 from 2.0 g (17.1 mmole) of valine, 17.1 ml of 1 N NaOH, and 5.3 g (18.8 mmole) of BocAlaONp. After recrystallization from ethyl acetate–hexane, 4.2 g (86%) of substance (XIII) was obtained, with mp 154-155°C, R_f 0.37 (system 7).

16. BocAlaValGlyHisLeuMetNH$_2$ (XIV) was obtained under the conditions of experiment 12 from 0.24 g (0.85 mmole) of the dipeptide (XIII), 0.65 g (0.85 mmole) of "complex F," 0.30 g (0.57 mmole) of the tetrapeptide (IVa), and 0.14 ml (1.14 mmole) of N-ethylmorpholine. After recrystallization from methanol–ethyl acetate, 0.35 g of substance (XIV) was obtained, with R_f (main spot) 0.43 (system 1).

17. AlaValGlyHisLeuMetNH$_2$ (XIVa). The deblockage of 0.196 g of substance (XIV) was achieved by the action of 0.47 ml of 2 N hydrogen chloride in acetic acid. The dihydrochloride was worked up as in experiment 4, and 0.154 g of the product was purified by partition chromatography on Sephadex G-25 Fine in the two-phase system butan-1-ol–1% acetic acid–pyridine (5:11:3), 3.0 × 80 cm column. Fractions with a volume of 2.5 ml were collected every 15 min. Fractions 41-58 yielded 0.083 g of the hexapeptide (XIVa) (see Table 1).

18. Boc$_2$HisLeuOH (XV) was obtained from 0.6 g (4.8 mmole) of leucine and 2.5 g (5.3 mmole) of Boc$_2$HisONp by method 1. After recrystallization from ethyl acetate–hexane, 1.2 g (55%) of product (XV) was obtained, with mp 113-115°C, R_f 0.63 (system 7). $C_{22}H_{36}N_4O_7$.

19. ZValGlyOH (XVI). This was obtained from 1.0 g (13.3 mmole) of glycine, 13.3 ml of 1 N NaOH, and 5.5 g (14.6 mmole) of ZValONp in a similar way to substance (V). The yield after recrystallization from propan-2-ol–hexane was 3.6 g (88%), mp 145-146°C, R_f 0.29 (system 1), 0.65 (system 2). $C_{15}H_{20}N_2O_5$.

20. ZAlaValGlyOH (XVII). The dipeptide (XVI) (3.0 g; 9.7 mmole) was hydrogenated in methanol over a Pd/C catalyst, and the residue was dissolved in 9.7 ml of 1 N NaOH solution

and was treated with a solution of 3.7 g (10.7 mmole) of ZAlaONp in 20 ml of dioxane. The mixture was worked up as in experiment 5. After recrystallization from propan-2-ol–hexane, 2.8 g (76%) of a substance with mp 210-213°C, Rf 0.90 (system 1), was obtained. $C_{18}H_{25}N_3O_6 \cdot 1/2H_2O$

21. ZAlaValGlyHisLeuOH (XVII). a) With stirring, 1.6 g (2.1 mmole) of "complex F" was added to 0.78 (2.1 mmole) of ZAlaValGlyOH in a mixture of 15 ml of dioxane and 5 ml of DMF.

b) Substance (XV) (1.1 g; 2.4 mmole) was deblocked under the action of 8.4 ml (16.8 mmole) of a 2 N solution of HCl in acetic acid. This gave 0.67 g (81%) of substance (XVa), with E_{His} 0.96.

c) With cooling to 0°C and stirring, 0.73 ml (5.88 mmole) of N-ethylmorpholine and the solution of ZAlaValGlyOPFP freed from DCU by filtration were added successively to a solution of 0.67 g (1.96 mmole) of substance (XVa) in 5 ml of DMF. The product was worked up as in experiment 8. This gave 0.59 g of substance (XVIII), Rf (main spot) 0.62 (system 3), which was purified by adsorption chromatography (column) on Silpearl silica gel in solvent system 1 (with the collection of 10-ml fractions). The combined fractions 26-32 were evaporated, and the substance was treated with ether and dried. This gave 0.39 g (32%) of substance (XVIII), mp 213-216°C, Rf 0.62 (system 3). $C_{30}H_{43}N_7O_8 \cdot 3H_2O$.

22. AlaValGlyHisLeuOH (XVIIIa). Substance (XVIII) (0.250 g) was hydrogenated in 70% aqueous methanol over Pd/C catalyst. This gave 0.176 g of the deblocked peptide with Rf (main spot) 0.46 (system 4), 0.130 g of which was purified by preparative HPLC (4% of CH_3CN and 96% of 0.2 M NH_4OCOCH_3). This gave 0.099 g of substance (XVIIIa) (see Table 1).

23. BocGlnTrpAlaValGlyOH (XIX). The tripeptide (XVII) (2.2 g; 5.8 mmole) was hydrogenated in methanol over Pd/C catalyst. A solution of 2.7 g (6.4 mmole) of substance (VI) in a mixture of 30 ml of dioxane and 10 ml of DMF was cooled to 0°C, and 4.8 g (6.4 mmole) of "complex F" was added. The resulting pentafluorophenyl ester was added to the sodium salt of substance (XVIIa), and the reaction mixture was left for 12 h. Then it was worked up as in experiment 7. After two treatments with hot methanol 2.4 g (62%) of a product with mp 248-250°C (decomp.), Rf 0.63 (system 1) was obtained. $C_{31}H_{45}N_7O_9$.

24. BocAsnGlnTrpAlaValGlyOH (XX). The hydrochloride of (XIXa) obtained by the deblocking of 1.5 g (2.2 mmole) of (XIX) as in experiment 4 was dissolved in 3 ml of DMF, and 0.56 ml (4.4 mmole) of N-ethylmorpholine and 1.0 g (2.6 mmole) of BocAsnOPFP were added. The reaction mixture was left for 24 h and was worked up as in experiment 7. After recrystallization from DMF–ethyl acetate, 0.81 g (47%) was obtained of a substance with mp 215-218°C (decomp.), Rf 0.50 (system 1). $C_{35}H_{51}N_9O_{11}$.

25. AsnGlnTrpAlaValGlyOH (XXa). The deblockage of 0.222 g (0.29 mmole) of substance (XX) by the action of hydrogen chloride in glacial acetic acid gave 0.177 g of a substance, which was purified on a column of SP-Sephadex C-25 in 0.005 M pyridine-acetate buffer and then by preparative HPLC. This yielded 0.106 g of the pentapeptide (XXa) (see Table 1).

26. ZDPheTrpOH (XXI) was obtained from 1.7 g (8.4 mmole) of tryptophan and 3.9 g (9.3 mmole) of ZDPheONp by method 1. Recrystallization from ethyl acetate–hexane gave 3.1 g (74%) of a product with mp 146-147°C, Rf 0.54 (system 1).

27. BocAsnDPheTrpOH (XXII). Substance (XXI) (2.0 g; 4.1 mmole) was hydrogenated in methanol over a Pd/C catalyst, and 0.52 ml (4.1 mmole) of NEM and 1.8 g (4.5 mmole) of BocAsnOPFP were added. The subsequent operations were as in experiment 1. After two recrystallizations from ethyl acetate, 0.9 g (39%) of a product with mp 145-150°C, Rf 0.67 (system 5) was obtained.

28. BocAsnDPheTrpAlaValOH (XXIII). Substance (V) (0.51 g; 1.6 mmole) was hydrogenated in methanol over a Pd/C catalyst. The deblocked peptide was dissolved in 1 ml of DMF, and 0.20 ml (1.6 mmole) of NEM and BocAsnDPheTrpOPFP, obtained from 0.90 g (1.6 mmole) of the tripeptide (XXII) and 1.21 g (1.6 mmole) of complex F in a mixture of 15 ml of dioxane and 5 ml of DMF, were added. Then the mixture was worked up as in experiment 8. This gave 0.65 g (45%) of a product with mp 211-214°C (decomp.), Rf 0.82 (system 1). $C_{37}H_{49}N_7O_9$.

29. BocAsnDPheTrpAlaValGlyHisLeuMetNH$_2$ (XXIV). A solution of 0.300 g (0.57 mmole) of the dihydrochloride of (IVa) in 5 ml of DMF was cooled to −5°C, and, with stirring, 0.143 g (1.14 mmole) of NEM, 0.418 g (0.57 mmole) of the pentapeptide (XXIII), 0.072 g (0.62 mmole) of HONSu, and a solution of 0.120 g (0.57 mmole) of DCC in 2 ml of DMF were added successively. The reaction mixture was stirred at −5°C for 1 h and was then left at room temperature

for 48 h. It was worked up as in experiment 8. This gave 0.397 g of substance with Rf (main spot) 0.51 (system 1).

30. AsnDPheTrpAlaValGlyHisLeuMetNH$_2$ (XXIVa). The action of 0.38 ml of 2 N HCl in acetic acid led to the deblocking of 0.255 g of the product (XXIV) that had been obtained, and 0.221 g of the deblocked substance was purified on SP-Sephadex C-25 in pyridine-acetate buffer. This gave 0.121 g of substance (see Table 1.).

31. BocDAlaHisOH (XXV) was obtained from 2.0 g (12.9 mmole) of histidine and 4.1 g (14.3 mmole) of BocDAlaOSu by method 3. After reprecipitation from propan-2-ol with ether, 3.3 g (80%) of substance was obtained with mp 122-124°C, Rf 0.38 (system 1), $[\alpha]_D^{20}$ 16.8° (*c* 1; DMF). $C_{14}H_{22}N_4O_5 \cdot 1/2\,H_2O$.

32. BocDAlaHisLeuMetNH$_2$ (XXVI) was obtained under the conditions of experiment 4 from 1.8 g (5.5 mmole) of (XXV), 4.2 g (5.5 mmole) of "complex F," 1.6 g (5.4 mmole) of (IIa), and 0.7 ml (5.4 mmole) of NEM in an amount of 1.8 g (58%) with mp 173-175°C, Rf 0.57 (system 1). $[\alpha]_D^{20}$ —8.6° (*c* 1; DMF). $C_{25}H_{43}N_7O_6S \cdot 1/2\,H_2O$. Amino acid analysis: His 0.96 (1), Ala 1.09 (1), Leu 1.00 (1), Met 0.93 (1).

33. ZTrpAlaValOH (XVII). The dipeptide (V) (2.0 g; 6.2 mmole) was hydrogenated in methanol over Pd/C catalyst, and to the deblocked peptide was added 3.4 g (6.7 mmole) of ZTrpOPFP, and the mixture was worked up as in experiment 1. This gave 2.9 g (91%) of product with mp 174-177°C, Rf 0.93 (system 1), $[\alpha]_D^{20}$ 13.0° (*c* 1; DMF). $C_{27}H_{32}N_4O_6 \cdot 1/2\,H_2O$.

34. BocProGlyOH (XXVIII) was obtained under the conditions of experiment 5 from 1.0 g (17.5 mmole) of glycine, 17.5 ml (17.5 mmole) of 1 N NaOH, and 6.1 g (19.2 mmole) of BocProOSu. Recrystallization from ethyl acetate—hexane led to the isolation of 3.1 g (64%) of a substance with mp 161-163°C (decomp.), Rf 0.73 (system 1), $[\alpha]_D^{20}$ —29.0° (*c* 1; DMF). $C_{12}H_{20}N_5O_5$.

35. BocProGlyTrpAlaValOH (XXIX). The hydrogenation of 2.1 g (4.2 mmole) of (XXVII) was carried out in methanol—acetic acid over Pd/C catalyst. The deblocked peptide was dissolved in 10 ml of DMF, and to this solution were added 0.53 ml (4.2 mmole) of NEM and the BocProGlyOPFP obtained from 1.2 g (4.2 mmole) of (XXVIII) and 3.2 g (4.2 mmole) of "complex F." The mixture was worked up as in experiment 8. After two treatments with hot ethyl acetate, 1.6 g (61%) was obtained of a product with mp 200-204°C, Rf 0.58 (system 4). $C_{31}H_{44}N_6O_8$.

36. BocProGlyTrpAlaValDAlaHisLeuMetNH$_2$ (XXX) was obtained in a similar way to substance (VIII) from 0.60 g (0.95 mmole) of (XXIX), 0.20 g (0.97 mmole) of DCC, 0.11 g (1.1 mmole) of HONSu, 0.24 ml (1.9 mmole) of NEM, and 0.54 g (0.95 mmole) of (XXVIa) in an amount of 0.44 g after recrystallization from isopropyl alcohol—ethyl acetate, Rf (main spot) 0.51 (system 1), and 0.220 g of the product was purified by adsorption chromatography on Silpearl silica gel (column) in solvent system 1. Fractions 68-85 (rate of elution 7 ml/h, fraction volume 5 ml) yielded 0.123 g (55%) of a substance with Rf 0.51 (system 1), 0.89 (system 2), 0.82 (system 3), E_{His} 0.52, $[\alpha]_D^{20}$ —23.0° (*c* 1; DMF). Amino acid analysis: Pro 1.06 (1), Gly 1.00 (1), Ala 1.88 (2), Val 1.04 (1), Leu 1.08 (1), Met 1.05 (1), His 0.98 (1).

37. ProGlyTrpAlaValDAlaHisLeuMetNH$_2$ (XXXa). After the deblockage of 0.220 g of substance (XXX) by the action of 0.33 ml of 2 N HCl in glacial acetic acid and purification on a column of SP-Sephadex C-25 in pyridine-acetate buffer, 0.146 g (68%) of product was obtained. Then 0.037 g of the crude substance (XXXa) was purified by preparative HPLC (25% of CH_3CN and 75% of 0.2 M NH_4OCOCH_3). This gave 0.025 g of substance (XXXa) (see Table 1).

SUMMARY

1. The synthesis has been effected of a number of C-terminal fragments of the tetradecapeptide bombesin from amphibia and their analogues: BN(7—14); [AcGln^7]BN(7—14); BN(8—14); BN(7—14); BN(9—14); BN(9—13); BN(6—11); [*D*Phe^7]BN(6—14); and [Pro^6, Gly^7, *D*Ala^{11}] BN(6—14).

2. The influence of the peptides synthesized on thermoregulation has been studied in experiments on rabbits. In a dose of 1 μg with central administration the full hypothermic effect of bombesin was shown by the preparation [AcGln^7]Bn(7-14), while [DPhe^7]BN(6-14) and [Pro^6,Gly^7,DAla^{11}]BN(6-14) possessed a very slight effect (~ 1% of the activity of bombesin).

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VASOACTIVE PEPTIDES FROM VENOM OF THE WASP Polistes gallicus. ISOLATION AND PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES

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UDC 547.993

Six vasoactive peptides have been isolated in the homogeneous form from the venom of the wasp Polistes gallicus. Their physicochemical characteristics have been investigated. The wasp kinins exhibit myotropic and hypotensive effects. One of the kinins shows a prolonged hypotensive action.

The biological properties of vasoactive peptides and, in particular, kinins are shown in very low concentrations [1] and are mediated by their specific interaction with receptor structures concentrated in the smooth tissue of the blood vessels and capillaries [2, 3]. The structural-functional laws of the differential activity of the kinins have scarcely been studied, but numerous attempts have been made at the chemical modification of the molecules of vasoactive peptides with the aim of obtaining analogs with a prolonged action [4]. It is obvious that a structural-functional investigation of natural vasoactive peptides is necessary for the synthesis of new peptide bioregulators.

In the present paper we give information on the isolation and the physicochemical and functional characteristics of vasoactive peptides from the venom of the wasp Polistes gallicus.

The vasoactive peptides forming components of the venom were identified by a biotest [5] based on measuring the myotropic activity of the venom on the neck of the rat uterus or on guinea-pig ileum. To distinguish between the contractions caused by peptides and by biogenic amines, we used treatment with proteolytic enzymes or spectific antagonists of histamine and of serotonin (atropine sulfate, cyproheptadine). It is interesting to note that when antibodies to bradykinin (BK) were present in the incubation medium a partial elimination of the myotropic activity of the venom was observed. In other words, the venom contained peptides (BK analogues), exhibiting a substantial myotropic activity.

As a result of the fractionation of the wasp venom on a TSK Hw-40 column (separation of the peptides according to molecular mass) and under conditions of low ionic strength (on the

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Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 564-568, July-August, 1989. Original article submitted August 31, 1988; revision submitted January 2, 1989.

 0009-3130/89/2504-0484 $12.50