SYNTHESIS OF SOME ARGININE- AND LYSINE-CONTAINING FRAGMENTS OF PEPTIDE HORMONES AND INVESTIGATION OF THEIR BIOLOGICAL ACTIVITY

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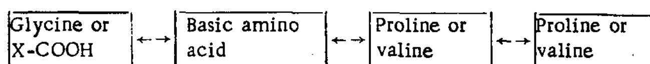
Some peptide hormones and kinins contain fragments of extremely similar structure including from three to five amino acids – so-called "common" fragments. The characteristic feature of the structure of these fragments is the presence of a basic amino acid (arginine, lysine, or ornithine) adjacent to proline or valine [1]:



The similarity of the structures of the fragments permits the assumption that in the formation of a specific response reaction at the level of the receptor they fulfill similar functions. It has been suggested previously [1] that the common fragments participate directly in the formation of a secondary signal [2, 3] and the specificity of their action is ensured by the differing parts of the molecules and that the common fragments of the hormones at the molecular level also correspond to sections of the receptors with similar structures [1]. To check these hypotheses it appeared of interest to synthesize arginine- and lysine-containing fragments of a number of peptide hormones and kinins and to study their biological properties.

The synthesis of the common fragments of the peptides (Table 1) was effected by methods of classical peptide chemistry using dicyclohexylcarbodiimide, mixed anhydrides, and activated esters of acylamino acids for the formation of the peptide bond. After the removal of the protective groups the free peptides were, if this was necessary, purified on a column of carboxymethylcellulose. The nature of the peptides obtained can be judged from Table 2.

The myotropic activity of the substances was investigated as described previously [4], using [1-asparagine, 5-valine]angiotensin II as standard (Table 3 and Fig. 1).

We also studied the influence of the tripeptides of vasopressin (III) and of angiotensin (IV) on the osmotic permeability of the wall of the urinary bladder of the frog Rana temporaria (Table 4).

The results of the biological investigations showed that, in spite of the removal of those parts of the molecule in which the "address" of specific receptors is coded, all the common fragments of the hormones still retained the information necessary for interaction with definite groups of receptors. However, as was to be expected, this interaction for the synthetic peptides did not have the specificity of the natural hormones. This is evidence in favor of the hypothesis that the parts of the receptors corresponding to the common fragments also have a common (universal) structure.

The affinity (pD_2) and internal activity (α) of the fragments tested vary within relatively wide limits. The angles of slope of the cumulative curves also differ, from which it may be concluded that these fragments of peptides affect similar sections of different receptors. Exceptions are angiotensin II and its N-terminal tripeptide.

The results of a comparison of the effect-versus-concentration curves obtained in experiments on the rat colon and uterus show that the receptive system of the latter is less sensitive to the tripeptides con-

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TABLE 1. Common Fragments of Peptide Hormones

Compound	Fragment	Glycine or X-COOH	Basic amino acid	Proline or valine
I	β-MSH [20-22]	Asp←	—Lys←	—Pro
II	Fibrinopeptide B [12-14]	Asp—	→Arg—	→Pro
III	Vasopressin [7-9]	H_2NGly←	—Arg←	—Pro
IV	Angiotensin [1-3]	Asn—	→Arg—	→Val
V	Bradykinin [1-3]		Arg—	→Pro→Pro
VI	ACTH [18-20]		Arg→	Pro→$ValNH_2$

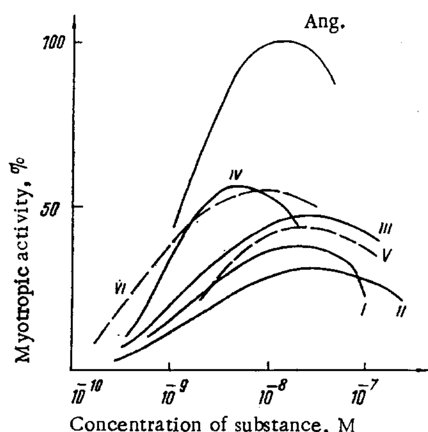

Fig. 1. Cumulative effect-versus-concentration curves for the tripeptides (I-VI) in experiments on the ascending colon of the rat in comparison with [1-asparagine, 5-valine]angiotensin II (ang.).

sidered; in this case, the curves are shifted in the direction of higher concentrations. The highest affinity to the receptor in the rat uterus is possessed by the tripeptide of bradykinin and to that of the rat colon by the tripeptide of angiotensin.

The comparative results of a study of the water permeability of the urinary bladder of the frog (see Table 4) appear extremely interesting.* As the experiments showed, the tripeptide of vasopressin (III) considerably increases the osmotic permeability. Its activity even exceeds that of the amide of the cyclic hexapeptide vasopressin (see Table 4). Conversely, the tripeptide of angiotensin (IV) is only feebly active.

In these experiments, confirmation was obtained of the hypothesis put forward previously according to which a number of biologically active peptides have two centers – one in which the bulk of the information required for the specific "recognition" and binding of the peptide by the receptor is coded and the other common one making the fundamental contribution to the formation of the secondary signal [2].

EXPERIMENTAL

Methods. The peptides were synthesized from amino acids of the L series. The melting points were determined in open capillaries (without correction) and the angles of optical rotation on a Perkin-Elmer 141 polarimeter. The purity of the protected peptides was checked by thin-layer chromatography on Silufol plates in the following solvent systems: 1) chloroform–methanol (8:2); 2) chloroform–acetone (8:2); and 3) hexane–ethyl acetate (1:5).

The purity of the peptides with free amino groups was checked by paper chromatography in systems 4) butan-1-ol–acetic acid–water (5:1:2) and 5) sec-butanol–3% ammonia solution (8:3), and also by paper electrophoresis at 20 V/cm in 1 N acetic acid for 30 min. The spots were revealed with ninhydrin (free amino group), the Sakaguchi reagent (free guanidine group), and the chlorine–benzidine reagent (peptide bonds); peptides containing the heat-labile tert-butoxycarbonyl group were revealed with ninhydrin after heating to 130°C.

To isolate the protected peptides, their solutions in organic solvents were washed with 0.5 N hydrochloric or citric acid (the tert-butoxycarbonylpeptides at 0°C), with water, with 5% sodium hydrogen carbonate solution, and with water again. The free peptides were purified on a column of carboxymethylcellulose in a linearly increasing concentration gradient of ammonium acetate (400 ml of a 0.2 M solution of ammonium acetate in the reservoir and 400 ml of water in the mixer, pH 6.5). A column with dimensions of 3×40 cm was used for 1 g of crude tripeptide. If the peptide contained arginine at the N- end, the concentration of the ammonium acetate solution was increased to 0.25 M. Fractions of 10 ml each were collected every 8 min. The contents of the tubes with the required fraction (determined by paper electrophore-

* The investigations were performed by E. Shakhmatova under the direction of Yu. V. Natochin in the I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry of the Academy of Sciences of the USSR in Leningrad by the method of Natochin et al. [5].

TABLE 2. Chemical Characteristics of the Peptide Fragments Synthesized and Used for Biological Tests

Compound	Amino acid sequence	mp (dec.), °C	$[\alpha]_D^{20}$ (solvent water), deg	R_f in system 4	R_f in system 5	E_{Arg} (1 N AcOH)	Found, % C	Found, % H	Found, % N	Empirical formula	Calc., % C	Calc., % H	Calc., % N
I	Pro-Lys-Asp	216–218	–39,4 (c 0,9)	0,17	0,10	0,59	48,20	7,23	14,72	$C_{15}H_{26}N_4O_6 \cdot H_2O$	47,86	7,50	14,88
II	Asp-Arg-Pro	124	–36,4 (c 1,1)	0,10	0,05	0,74	38,14	7,61	17,36	$C_{15}H_{26}N_6O_6 \cdot 5H_2O$	37,80	7,62	17,64
III	Pro-Arg-GlyNH₂	Oil*	—	0,18	0,15	1,15	—	—	—	—	—	—	—
IV	Asn-Arg-Val	110	–18,1 (c 1,0)	0,22	0,11	0,80	40,38	8,13	19,48	$C_{15}H_{29}N_7O_5 \cdot CH_3COOH \times 3H_2O$ †	40,71	7,84	19,54
V	Arg-Pro-Pro	130–135	–96,5 (c 0,6)	0,21	0,14	1,00	46,30	7,88	18,29	$C_{16}H_{28}N_6O_4 \cdot CH_3COOH \times 2H_2O$ ‡	46,54	7,82	18,09
VI	Arg-Pro-ValNH₂	70	–3,4 (c 1,0)	0,16	0,09	0,96	40,09	8,51	16,80	$C_{16}H_{31}N_7O_3 \cdot 2CH_3COOH \times 6H_2O$	40,19	8,60	16,41

* Literature data [10] for the deflavianate $C_{13}H_{25}N_7O_3 \cdot 2C_{10}H_6N_2O_8S \cdot 1^1/_2H_2O$ – mp 180°C, $[\alpha]_D^{24}$ –18.2° [c 1; acetone–water (1 : 1)]; the synthesis was performed by O. S. Papsuevich by the *method* of Studer and du Vigneaud [11].

† Literature data [12] for $C_{15}H_{29}N_7O_5 \cdot CH_3COOH \cdot 2H_2O$ – mp 114°C (decomp.), $[\alpha]_D^{20}$ –13° (c 1.0; water).

‡ Literature data [13] for $C_{16}H_{28}N_6O_4 \cdot CH_3COOH \cdot 1^1/_2H_2O$ – mp 125°C, $[\alpha]_D^{24}$ – 81.8° (c 0.38; water).

** Literature data [14] for $C_{16}H_{31}N_7O_3 \cdot 2CH_3COOH \cdot 2H_2O$-$[\alpha]_D^{30}$ –49.0° (c 1.02; 10% AcOH).

sis) were combined and evaporated in vacuum. The ammonium acetate was eliminated at 50°C (2 mm) and the product was lyophilized from water or acetic acid.

Pharmacological Tests. All the results are the means of 5-7 experiments. The cumulative-effect-versus-concentration curves were obtained by Van Rossum's method [4]. In the investigation of myotropic activity on the isolated ascending rat colon, the peptides were dissolved in Krebs solution [8] and in the investigation on the rat uterus in Jalon's solution [9].

Dibenzyl α-tert-Butoxycarbonyl-ε-N-benzyloxycarbonyllysylaspartate (VII). To a solution of 34.0 g (70 mmoles) of the p-toluenesulfonate of dibenzyl aspartate in 100 ml of dimethylformamide were added 9.7 ml (70 mmole) of triethylamine, a solution of 26.6 g (70 mmoles) of α-tert-butoxycarbonyl-ε-N-benzyloxycarbonyllysine [16] in 60 ml of dimethylformamide, and, after cooling to –5°C, a solution of 14.4 g (70 mmoles) of dicyclohexylcarbodiimide in 15 ml of dimethylformamide. The reaction mixture was left at 0°C for 24 h, the precipitate that had deposited was filtered off, and the filtrate was evaporated in vacuum (50°C). The residue was dissolved in ethyl acetate and the solution was washed, dried with sodium sulfate, evaporated to small volume, and crystallized by the addition of heavy petroleum ether. The yield of the dipeptide (VII) was 33.1 g (70%), mp 96-98°C, $[\alpha]_D^{20}$ –12.6° (c 1.0; dimethylformamide), R_f 0.5 (2), 0.88 (3). Composition (%): C 65.43; H 6.76; N 6.23; $C_{37}H_{45}N_3O_9$.

Dibenzyl Benzyloxycarbonylprolyl-ε-N-benzyloxycarbonyllysylaspartate (VIII). A solution of 16.9 g (25 mmoles) of the tert-butoxycarbonyl derivative (VII) in 30 ml of acetic acid was treated with 100 ml of a 1 N solution of hydrogen chloride in glacial acetic acid. The mixture was kept at room temperature for 1 h and was then evaporated in vacuum to one half its initial volume, and 500 ml of ether was added. The hydrochloride of the dibenzyl ester of the dipeptide, which precipitated in the form of an oil, was triturated several times with ether and was kept in vacuum over potassium hydroxide. Of the resulting oil,* 10.0 g (16 mmoles) was dissolved in 20 ml of dimethylformamide, and to this solution were added triethylamine to give a weakly alkaline reaction, a solution of 5.0 g (20 mmoles) of benzyloxycarbonylproline, and, after cooling to –5°C, 4.1 g (20 mmoles) of dicyclohexylcarbodiimide. The mixture was kept at 0°C for 24 h, the

* The hydrochloride crystallized after one month. By treating an aqueous solution of the hydrochloride with potassium carbonate it is possible to isolate the free peptide in the solid state, but it rapidly becomes electrophoretically inhomogeneous (spots revealed by the chlorine–benzidine reagent), probably because of cyclization to form a piperazinedione.

TABLE 3. Indices of the Affinity (pD_2) for the Receptor and the Internal Activity (α) of the Tripeptides (I-VI) on the Isolated Ascending Colon and Uterus of the Rat as Compared with [1-Asparagine, 5-valine]angiotensin II

Compound	Acid sequence	Rat colon		Rat uterus	
		pD_2	α	pD_2	α
I	Pro-Lys-Asp	8,99	0,39	5,73	0,48
II	Asp-Arg-Pro	8,78	0,31	6,93	0,33
III	Pro-Arg-GlyNH_2	8,80	0,46	7,50	0,49
IV	Asn-Arg-Val	9,27	0,55	6,36	0,89
V	Arg-Pro-Pro	8,78	0,44	9,62	0,58
VI	Arg-Pro-ValNH_2	9,24	0,54	5,73	0,25
	Angiotensin II	9,70	1,00	8,27	1,00

TABLE 4. Permeability of the Wall of the Urinary Bladder of the Frog under the Action of the Tripeptides (III) and (IV) and the Amide of the Cyclic Hexapeptide Vasopressin in Comparison with Pituitrin

Compound	Nature	Dose, mg/ml	No. of expts.	Permeability, mg of H_2O/cm^2 · min at gradient of 1:10 [5]
III	Tripeptide of vasopressin	0,01	6	0,19 ±0,09
		0,1	6	0,14 ±0,07
		1,0	6	0,16 ±0,07
	Control		6	0,028±0,003
IV	Tripeptide of angiotensin	0,01	5	0,049±0,017
		0,1	5	0,082±0,021
		1,0	5	0,093±0,012
	Amide of the cyclic hexapeptide of vasopressin [7]	0,3		от 0,064±0,013 до 0,094±0,029
	Pituitrin	1 MU/ml	5	1,89±0,20

precipitate that had deposited was filtered off, and the filtrate was evaporated in vacuum. The residue was dissolved in ethyl acetate and the solution was washed, dried with sodium sulfate, and evaporated to small volume, and crystallization was induced by the addition of ether. The yield of the tripeptide (VIII) was 3.5 g (27%), mp 81-83°C, $[\alpha]_D^{20}$ −27.9° (c 0.7; dimethylformamide), R_f 0.43 (1). Composition $C_{45}H_{50}N_4O_{10}$.

Prolyllysylaspartic Acid Monohydrate (I). A solution of 1.5 g (1.85 mmole) of the tripeptide dibenzyl ester (VIII) with 45 ml of methanol−acetic−water (4 : 4 : 1) was hydrogenated in the presence of palladium black for 14 h. The catalyst was filtered off, the filtrate was evaporated in vacuum, and the residue was dried over potassium hydroxide. The dry residue was purified on a column of carboxymethylcellulose. After lyophilization from water, the yield of free tripeptide (I) was 0.4 g (60%).

β-Benzyl Ester of Benzyloxycarbonylaspartylnitroarginylproline (IX). To a solution of 7.1 g (20 mmoles) of β-benzyl benzyloxycarbonylaspartate [17] in 20 ml of dimethylformamide were added 2.8 ml (20 mmoles) of triethylamine, and, at −15°C, 2.5 ml (20 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min, and then a suspension of 5.1 g (16 mmoles) of nitroarginylproline [18] in dimethylformamide−water (1 : 1) containing 2.2 ml (16 mmoles) of triethylamine was added. Stirring at −5°C was continued for another 4 h and then the mixture was left at 0°C for 24 h. The solvent was evaporated off in vacuum and the residue was distributed between 50 ml of ethyl acetate and 50 ml of 1 N HCl. The ethyl acetate layer was washed with water three times, dried with sodium sulfate, evaporated in vacuum, and triturated with ether. The resulting precipitae was twice reprecipitated from ethyl acetate with ether. The yield of tripeptide (IX) was 3.2 g (31%), decomp. p. above 100°C, $[\alpha]_D^{20}$ −39.4° (c 0.7; ethanol). Composition (%): C 54.59; H 5.72; N 14.60. $C_{30}H_{37}N_7O_{10}$.

Aspartylarginylproline Pentahydrate (II). In the presence of palladium black in 20 ml of ethanol−acetic acid−water (6 : 1 : 1), 1.0 g (1.5 mmole) of the benzyloxycarbonyltripeptide (IX) was hydrogenated for 30 h. The catalyst was filtered off, the filtrate was evaporated in vacuum, and the residue was kept over potassium hydroxide and was then separated in a column of carboxymethylcellulose. The required tripeptide was eluted by approximately 0.15 M ammonium acetate. After lyophilization from water, the yield of the tripeptide (II) in the form of the pentahydrate was 0.5 g (70%).

Benzyl Ester of tert-Butoxycarbonylnitroarginylvaline (X). To a solution of 19.0 g (60 mmoles) of tert-butoxycarbonylnitroarginine [19] in 60 ml of dimethylformamide were added 8.3 ml (60 mmoles) of triethylamine and, at −15°C, 7.7 ml (60 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min, and a solution of 12.5 g (60 mmoles) of the benzyl ester of valine* in 30 ml of ethyl acetate was added. Then stirring was continued at −5°C for 4 h. The triethylammonium chloride that had deposited was filtered off, and the filtrate was evaporated in vacuum. The residue was dissolved in ethyl acetate and the solution was washed, dried with sodium sulfate, and evaporated again in vacuum. The resulting oil was reprecipitated twice from ethyl acetate with petroleum ether. After keeping in vacuum (2 mm), the yield of the dipeptide (X) in the form of an amorphous powder was 23 g (75%), mp 85-91°C (decomp.), $[\alpha]_D^{20}$ −28.3° (c 1.2; ethanol), R_f 0.75 (1), 0.34 (3). Composition (%): C 54.04; H 7.08; N 16.21. $C_{23}H_{36}N_6O_7$.

Benzyl Ester of Benzyloxycarbonylasparaginylnitroarginylvaline (XI). A solution of 10 g (20 mmoles) of the tert-butoxycarbonyldipeptide (X) in 15 ml of acetic acid was treated with 80 ml of a 1 N solution of hydrogen chloride in glacial acetic acid. The mixture was kept at room temperature for 1 h and was then evaporated in vacuum to half its initial volume and was treated with ether. The oil that separated out was triturated several times with ether, and it was dissolved in 20 ml of water and the pH of the solution was brought to 9.0 with saturated potassium carbonate solution. The oil that separated out was extracted with ethyl acetate, and the ethyl acetate layer was washed with water and dried with sodium sulfate, and the solvent was evaporated off in vacuum. The benzyl ester of nitroarginylvaline remaining in the form of an oil (6.1 g or 15 mmoles) [R_f 0.57 (4), E_{Arg} 0.66] was dissolved in 40 ml of dimethylformamide containing 7.0 g (18 mmoles) of the p-nitrophenyl ester of benzyloxycarbonylasparagine [20]. Then 1.0 ml of acetic acid was added and the mixture was left at room temperature for 48 h. After this, the solution was poured into a mixture consisting of 300 ml of a 10% solution of sodium chloride and 100 ml of ethyl acetate. The small amount of precipitate was filtered off (and discarded), and the ethyl acetate layer was washed five times with 1 N ammonium hydroxide solution, with saturated sodium hydrogen carbonate solution, and with water. The first portion of the tripeptide (XI), which deposited during extraction, (2.6 g, 32%, mp 135-138°C) was filtered off and was washed with ethyl acetate and ether. Evaporation of the combined ethyl acetate layer and the addition of ether gave a second fraction of the tripeptide (XI) (3.9 g, mp 120-124°C). The total yield was 80%. After recrystallization from ethanol−ethyl acetate, mp 143-145°C $[\alpha]_D^{20}$ −141° (c 1.0; dimethylformamide), R_f 0.44 (1). Composition (%): C 57.18, H 6.22; N 15.87. $C_{26}H_{34}N_6O_7$.

Trihydrate of the Acetate of Asparaginylarginylvaline (IV). The benzyloxycarbonyltripeptide (XI) (2.2 g; 4 mmoles) was hydrogenated in 30 ml of methanol−acetic acid−water (6 : 1 : 1) in the presence of palladium black for 30 h. The catalyst was filtered off, the filtrate was evaporated, and the residue was kept in vacuum over potassium hydroxide. The crude product was purified on a column of carboxymethylcellulose. The required fraction, which issued at a concentration of ammonium acetate of about 0.15 M, was lyophilized from water. The yield of the tripeptide (IV) was 1.2 g (60%).

Hydrochloride of the Benzyl Ester of Prolylproline (XII). A solution of 10 g (25 mmoles) of the benzyl ester of tert-butoxycarbonylprolylproline (an oil) [21] in 20 ml of acetic acid was treated with 120 ml of a 1 N solution of hydrogen chloride in glacial acetic acid. The mixture was kept at room temperature for 1 h, the solvent was evaporated off in vacuum, and the residue was triturated with ether several times. After recrystallization from methanol with the addition of ether, the yield of the hydrochloride (XII) was 7.0 g (83%), mp 181-183°C; $[\alpha]_D^{20}$ −140° (c 0.7; ethanol), R_f 0.47 (4), 0.89 (5), E_{Arg} 0.80. Composition (%): C 60.54; H 7.00; N 8.37. $C_{17}H_{22}N_2O_3 \cdot HCl$.

Benzyl Ester of tert-Butoxycarbonylnitroarginylprolylproline (XIII). To a solution of 2.5 g (7.8 mmoles) of tert-butoxycarbonylnitroarginine in 15 ml of dimethylformamide were added 1.1 ml (7.8 mmoles) of triethylamine and, at −15°C, 1.0 ml (7.8 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min and a suspension of 2.5 g (7.4 mmoles) of the hydrochloride of the dipeptide (XII) and 1.0 ml (7.4 mmoles) of triethylamine in 10 ml of dimethylformamide were added. The mixture was then stirred at −5°C for another 4 h, the precipitate of triethylammonium chloride was filtered off, and the solvent was evaporated in vacuum (50°C). The residue was dissolved in ethyl acetate and the solution was washed, dried with sodium sulfate, evaporated to small volume, and treated with ether to induce crystallization. After recrystallization from ethyl acetate, the yield of the tripeptide (XIII) was 2.9 g (65%), mp 109-113°C; after recrystallization from chloroform−carbon tetrachloride, mp 115-117°C, $[\alpha]_D^{20}$ −143° (c 0.5; ethanol), R_f 0.62 (1). Composition (%) C 55.49; H 6.88; 16.22. $C_{28}H_{41}N_7O_8$.

* Obtained in the form of an oil from the toluenesulfonate [15] by treating it with a mixture of ethyl acetate and a solution of potassium carbonate (pH 9.0) and evaporating the ethyl acetate layer.

Dihydrate of the Acetate of Arginylprolylproline (V). A solution of 2.0 g (3.3 mmoles) of the tert-butoxycarbonyltripeptide (XIII) in 5 ml of acetic acid was treated with 20 ml of a 1 N solution of hydrogen chloride in glacial acetic acid. The mixture was kept at room temperature for 1 h and evaporated in vacuum, and the residue was triturated with ether. It was then kept for several days over potassium hydroxide and was dissolved in a mixture of methanol, acetic acid, and water and hydrogenated in the presence of palladium black for 30 h. The catalyst was filtered off, the filtrate was evaporated in vacuum and the residue was lyophilized from water. The yield of the chromatographically pure tripeptide (V) in the form of the dihydrate of the acetate was 1.2 g (79%).

Amide of tert-Butoxycarbonylprolylvaline (XIV). To a solution of 16 g (75 mmoles) of tert-butoxycarbonylproline [22] in 60 ml of dimethylformamide was added 10.3 ml (75 mmoles) of triethylamine and, at −15°C, 9.6 ml (75 mmoles) of n-butyl chloroformate. After the mixture had been stirred at −5°C for 20 min, a solution of 11.5 g (75 mmoles) of the hydrochloride of valinamide [23] and 10.3 ml (75 mmoles) of triethylamine in 40 ml of dimethylformamide were added to the mixed anhydride so formed. The reaction mixture was kept at −5°C for 4 h, the precipitate of triethylammonium chloride was filtered off, the filtrate was evaporated to small volume, diluted with ethyl acetate, washed, and dried with sodium sulfate, and the solvent was evaporated off in vacuum. The yield of the amide of the dipeptide (XIV) was 17 g (73%). For analysis, the substance was recrystallized from ethyl acetate. mp 96-98°C, $[\alpha]_D^{20}$ −77.5° (c 1.0; ethanol), R_f 0.18 (1). Composition (%): C 57.88; H 8.80. N 13.03. $C_{15}H_{27}N_3O_4$.

Hydrochloride of the Amide of Prolylvaline (XV). A solution of 10 g (32 mmoles) of the tert-butoxycarbonyl derivative (XIV) in 100 ml of a 1 N solution of hydrogen chloride in glacial acetic acid was kept at room temperature for 1 h. Then the solution was evaporated to small volume and the residue was triturated with ether. After recrystallization from methanol with the addition of ether, the yield of the hydrochloride of the amide of the dipeptide (XV) was 6.9 g (86%), mp 260°C, $[\alpha]_D^{20}$ −19.5° (c 0.4; dimethylformamide), R_f 0.40 (4), 0.79 (5), E_{Arg} 0.91. Composition (%): C 48.24; H 8.28; N 16.64. $C_{10}H_{19}N_3O_2 \cdot HCl$.

t-Butoxycarbonylnitroarginylprolylvalinamide (XVI). To a solution of 7.0 g (22 mmoles) of tert-butoxycarbonylnitroarginine in 40 ml of dimethylformamide were added 3.0 ml (22 moles) of triethylamine and, at −15°C, 2.8 ml (22 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min, and then 5 g (20 mmoles) of the hydrochloride (XV) was added. The mixture was stirred at the same temperature for a further 4 h, and the precipitate of triethylammonium chloride that had deposited was filtered off. The filtrate was evaporated in vacuum and the residue was twice reprecipitated from ethanol with ethyl acetate. The yield of tripeptide (XVI) was 4.8 g (47%), decomp. p. above 140°C, $[\alpha]_D^{20}$ −22.4° (c 0.4; dimethylformamide), R_f 0.59 (1). Composition (%): C 48.89; H 7.32; N 21.56. $C_{21}H_{38}N_8O_7$.

Diacetate of the Hexahydrate of Arginylprolylvalinamide (VI). A solution of 1.5 g (2.9 mmoles) of the tripeptide (XVI) in 15 ml of a 1 N solution of hydrogen chloride in glacial acetic acid was kept at room temperature for 1 h, and then the solvent was evaporated off in vacuum and the residue was triturated with ether. The hydrochloride of nitroarginylprolylvalinamide so obtained was hydrogenated in 20 ml of methanol−acetic acid−water (6 : 1 : 1) in the presence of palladium black for 30 h. The catalyst was filtered off, the filtrate was evaporated in vacuum, and the residue was kept over potassium hydroxide and then was separated on a column of carboxymethylcellulose. The tripeptide was eluted at a concentration of ammonium acetate solution of 0.2 M. After lyophilization from acetic acid and reprecipitation from methanol with ether, the yield of the hygroscopic tripeptide in the form of the hexahydrate of the diacetate (VI) was 1.1 g (62%).

SUMMARY

1. The tripeptides of the ACTH sequence [18-20], the angiotensin sequence [1-3], the vasopressin sequence [7-9], the bradykinin sequence [1-3], the β-MSH sequence [20-22], and the fibrinopeptide B sequence [12-14] have been synthesized.

2. It has been shown that these compounds possess considerable myotropic activity in vitro experiments on isolated organs of the smooth musculature, but lack the specificity of the action of the natural hormones.

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