SYNTHESIS OF [1-(ETHYL ESTER OF CARBONYLGLYCINE), 2-D-ARGININE, 5-VALINE]ANGIOTENSIN II AND [1-CARBONYLGLYCINAMIDE, 2-D-ARGININE, 5-VALINE]ANGIOTENSIN II

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As the result of an analysis of the structures and functions of angiotensin II (I) and [8-arginine]vasopressin (II) and their analogs, using certain concepts of information theory, we have been able to show that the N- and C-terminal tripeptides of these hormones (I, 1-3 and II, 9-7) contain common structural elements, and also to put forward a hypothesis according to which these fragments fulfill similar functions in the process of hormone-receptor interaction [1]:

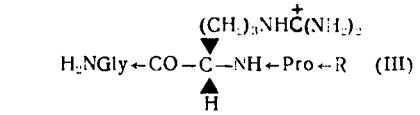
$$\underset{1}{\text{Asp}}\rightarrow\underset{2}{\text{Arg}}\rightarrow\underset{3}{\text{Val}}\rightarrow\text{Tyr}\rightarrow\text{Val}\rightarrow\text{His}\rightarrow\text{Pro}\rightarrow\underset{8}{\text{Phe}} \quad \text{(I)}^*$$

$$\underset{9}{\text{H}_2\text{NGly}}\leftarrow\underset{8}{\text{Arg}}\leftarrow\underset{7}{\text{Pro}}\leftarrow\text{Cys}\leftarrow\text{Asn}\leftarrow\text{Gln}\leftarrow\text{Phe}\leftarrow\text{Tyr}\leftarrow\underset{1}{\text{Cys}} \quad \text{(II)}$$

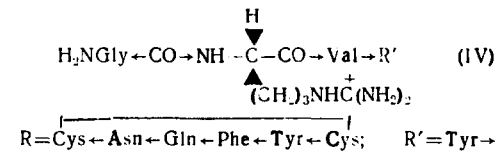
In favor of this hypothesis is the relatively high biological activity of analogs of angiotensin the structure of which in the N-terminal section is similar to the structure of the C-terminal fragment of [8-arginine] vasopressin ([1-carbonylglycinamide, 5-valine]angiotensin II and [1-asparagine, 3-proline, 5-valine]angiotensin II [2, 3]).

For a further investigation of the structural similarity of angiotensin II and [8-arginine]vasopressin, interest was presented by an analog of angiotensin II more fully reflecting the structure of the corresponding C-terminal fragment of [8-arginine]vasopressin — for example, with two modified amino acids at the N-end. As such a compound we selected [1-carbonylglycinamide, 2-D-arginine, 5-valine]angiotensin II(XIII).

In the fully extended peptide chains of the hormones (I), (II), and their analogs the arginine radical has a different spatial arrangement due to the opposite directions of acylation in the skeleton of the chain, for example, in the terminal fragments of [8-arginine]vasopressin (III) and [1-carbonylglycinamide, 5-valine]-angiotensin II (IV):



$$\text{H}_2\text{NGly}\leftarrow\text{CO}-\text{C}(\text{H})(\rightarrow(\text{CH}_2)_3\text{NHC}^{+}(\text{NH}_2)_2)-\text{NH}\leftarrow\text{Pro}\leftarrow\text{R} \quad \text{(III)}$$



$$\text{H}_2\text{NGly}\leftarrow\text{CO}\rightarrow\text{NH}-\text{C}(\text{H})(\rightarrow(\text{CH}_2)_3\text{NHC}^{+}(\text{NH}_2)_2)-\text{CO}\rightarrow\text{Val}\rightarrow\text{R}' \quad \text{(IV)}$$

$$\text{R}=\text{Cys}\leftarrow\text{Asn}\leftarrow\text{Gln}\leftarrow\text{Phe}\leftarrow\text{Tyr}\leftarrow\text{Cys}; \qquad \text{R}'=\text{Tyr}\rightarrow\text{Val}\rightarrow\text{His}\rightarrow\text{Pro}\rightarrow\text{Phe}$$

However, when the arginine side chain rotates around the $C^{\alpha}-C^{\beta}$ bond the guanidine group may acquire very similar spatial values in the two cases (Fig. 1). Consequently, the positive charge of the guanidine group occupies approximately one and the same segment of space, although a difference remains in the spatial directivity of the potential hydrogen bonds on interaction with the "anionic center" of the receptor, for example, with a carboxy group. This noncorrespondence can be eliminated by replacing the 2-arginine residue in the analog (IV) by D-arginine.

*The arrows denote the direction of acylation.

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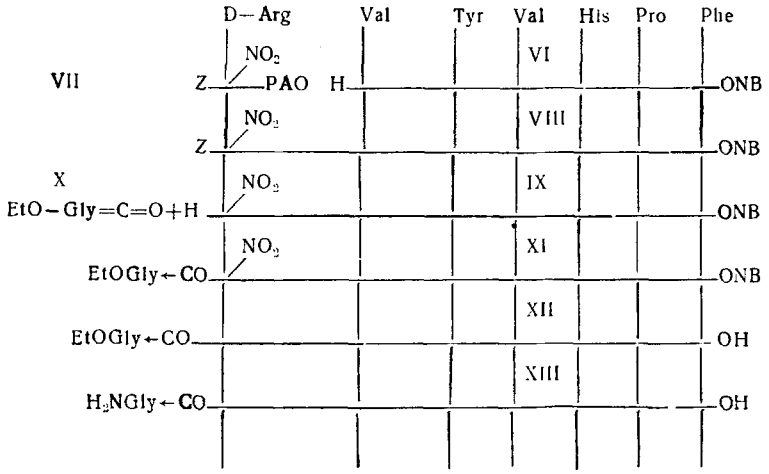
TABLE 1

Compound No.	Compound	Pressor activity, % *	Literature
XII	[1-(Ethyl ester of carbonylglycine), 2-D-arginine, 5-valine]angiotensin II	1,5	—
XIII	[1-Carbonylglycinamide, 2-D-arginine, 5-valine]angiotensin II	3,4	—
XIV	[1-Asparagine, 2-D-arginine, 5-isoleucine]angiotensin II	3	[4]
XV	[1-Glycine, 2-D-arginine, 5-isoleucine]angiotensin II	14	[5]

* Relative to [1-asparagine, 5-valine]angiotensin II on intact rats.

The scheme of the synthesis of [1-carbonylglycinamide, 2-D-arginine]angiotensin (XIII), beginning with the heptapeptide (IX) did not differ from that for the L-arginine analog [2]. The heptapeptide (IX) was obtained by acylating the hexapeptide (VI) with the pyridine-4-carbaldehyde oxime ester of nitroarginine (VII) with a subsequent splitting out of the benzyloxycarbonyl group with hydrogen bromide in glacial acetic acid.



After reduction and purification on carboxymethylcellulose, the compounds obtained — [1-(ethyl ester of carbonylglycine), 2-D-arginine]angiotensin II (XII) and its amide (XIII) — were tested for pressor activity (Table 1).

The activity of the amide (XIII) was practically identical with that of [1-asparagine, 2-D-arginine, 5-isoleucine]angiotensin II (XIV) but less than the activity of [1-glycine, 2-D-arginine]angiotensin II (XV). As in the case of the [2-L-arginine] analog, passage from the ester (XII) to the amide (XIII) was accompanied by an increase in biological effect.

The low activities of compounds (XII) and (XIII) are apparently due to a lack of steric correspondence of the N-terminal dipeptides of the analogs and the corresponding section of the receptor system. If this is the case, it could be concluded that the N- and C-terminal fragments of angiotensin and vasopressin have only individual structural elements in common and not the whole spatial electronic structure ensuring their interaction with receptors. However, if the modification of the terminal fragment also has the conformation of the whole molecule and as a result of this the conditions of "recognition" and complex formation with the receptor change, this conclusion may prove to be erroneous. In this respect, it is interesting to observe that the R_f constants of (IX) (XII), and (XIII) and the corresponding analogs of angiotensin II with L-arginine [2] are different (see Experimental section). For the further investigation of this question, interesting information can be obtained by determining and comparing the myotropic activities of the terminal fragments of angiotensin, vasopressin, and their analogs with D-arginine, and also by studying the optical rotatory dispersion curves of the compounds synthesized.

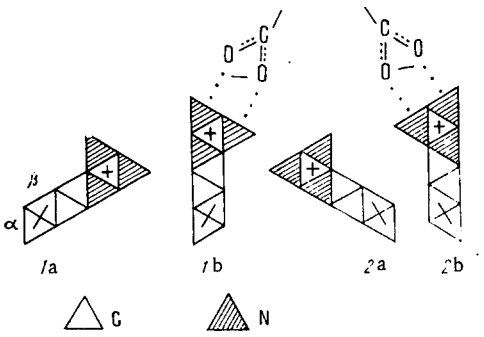


Fig. 1. Spatial direction of the arginine side chain in the completely extended peptide chain before (a) and after (b) rotation around the $C^{\alpha}-C^{\beta}$ bond in angiotensin (1), [8-arginine]vasopressin (2), and [2-D-arginine]angiotensin (2).

The pharmacological investigation of the compounds isolated was performed by Z. P. Auna.

EXPERIMENTAL

The experiments were performed with amino acids of the L series, with the exception of glycine and D-arginine. The melting points were determined in open capillaries without correction, and the angles of optical rotation were determined on a Perkin-Elmer 141 spectropolarimeter at 20°C. The peptides obtained were characterized by ascending chromatography (Filtrak FN-15) in systems 1) butan-1-ol–acetic acid–water (5:1:2) and 2) sec-butanol–3% aqueous ammonia (8:3), and also by paper electrophoresis (Filtrak FN-16, 20 V/cm, 30 min, 1 N acetic acid). In order to compare the R_f values of compounds (IX), (XII), and (XIII) and the corresponding 2-L-arginine analogs, chromatography was performed under the same conditions with the deposition of 10 μg of substance at each point.

The C, H, and N analyses corresponded to the calculated figures.

p-Nitrobenzyl Ester of Benzyloxycarbonylnitro-D-arginylvalyltyrosylvalylhistidylprolylphenylalanine (VIII). To a solution of 1.36 g (3.84 mmoles) of benzyloxycarbonylnitro-D-arginine [6] in 10 ml of tetrahydrofuran were added 0.52 g (4.22 mmoles) of pyridine-4-carbaldehyde oxime and, at −5°C, a solution of 0.79 g (3.84 mmoles) of dicyclohexylcarbodiimide in 5 ml of tetrahydrofuran [7]. The solution was left at −5°C for 6 h, the dicyclohexylurea that had deposited was filtered off, and the activated ester (VII) was precipitated with petroleum ether. The product obtained, after brief drying in air, was dissolved in 3 ml of tetrahydrofuran and this solutin was treated with a solution of 2.3 g (2.56 mmoles) of the p-nitrobenzyl ester of valyltyrosylvalylhistidylprolylphenylalanine [8, 9] in 5 ml of dimethylformamide and with 0.15 ml of acetic acid [10] and was left at room temperature for 48 h. Then ether was added and the resulting precipitate was filtered off and was triturated with a 5% solution of sodium bicarbonate, with water, with a 0.5 N solution of hydrochloric acid, with water, again with 5% sodium bicarbonate solution, and with water and was dried in vacuum over phosphorus pentoxide. The yield of the heptapeptide (VIII) was 2.3 g (74%), mp 165-170°C, $[\alpha]_D^{20}-27.4°$ (c 0.4; dimethylformamide), R_f 0.96 (2). Composition (%): C 58.89; H 6.17; N 15.85. $C_{60}H_{74}N_{14}O_{15}$.

p-Nitrobenzyl Ester of Nitro-D-arginylvalyltyrosylvalylhistidylprolylphenylalanine (IX). A solution of 1.7 g (1.40 mmoles) of the heptapeptide (VIII) in 5 ml of acetic acid was treated with 20 ml of a 20% solution of hydrogen bromide in glacial acetic acid and the mixture was kept at room temperature for 2 h. Then 0.5 liter of ether was added and the oil that separated out was dissolved in a small amount of water, rapidly separated from the ethereal layer, and washed several times with ether, and at 0°C the pH was brought to 9.0 with a saturated solution of potassium carbonate. The precipitate that deposited was filtered off, washed with water, and dried in vacuum over phosphorus pentoxide. The yield of the heptapeptide ester (IX) was 1.25 g (80%), decomp. p. above 190°C, $[\alpha]_D^{20}-40.0°$ (c 0.6; dimethylformamide), R_f 0.69 (1)* (ninhydrin, Pauly, positive spot). Composition (%): C 56.58; H 6.34; N 17.55. $C_{52}H_{68}N_{14}O_{13}$.

p-Nitrobenzyl Ester of [1-(Ethyl Ester of Carbonylglycine), 2-Nitro-D-arginine, 5-Valine]angiotensin II (XI). A solution of 1.1 g (1.0 mmole) of the heptapeptide ester (IX) in 3 ml of dimethylformamide was treated with 0.14 ml (1.1 mmole) of the ethyl ester of carbonylglycine (X) [11], and the mixture was left at room temperature for 24 h. On the following day, it was poured into 50 ml of a 10% solution of sodium chloride acidified with hydrochloric acid to pH 3.0. The oil that separated out was triturated with water until it formed a solid, which was filtered off, washed with water, and dried over phosphorus pentoxide. The yield of the octapeptide (XI) was 0.8 g (65%), mp 191-193°C (decomp.), $[\alpha]_D^{20}-32.6°$ (C 0.5; acetic acid), R_f 0.94 (1), 0.97 (2). Composition (%): C 55.57; H 6.16; N 16.78. $C_{57}H_{75}N_{15}O_{16}$.

[1-(Ethyl Ester of Carbonylglycine), 2-D-arginine, 5-valine]angiotensin II (XII). A solution of 0.5 g (0.41 mmole) of the octapeptide (XI) was hydrogenated in 30 ml of methanol–acetic acid–water (10:1:1) in the presence of palladium black for 30 h. After the catalyst had been filtered off, the filtrate was evap-

* For the p-nitrobenzyl ester of the [2-nitro-L-arginine]heptapeptide, R_f 0.76 (1) [2].

orated to dryness and was kept in vacuum over potassium hydroxide. The residue was dissolved in 10 ml of dimethylformamide–water (1:1) and deposited on a column of carboxymethylcellulose (3×40 cm) in the H^+ form. Elution was performed with 50 ml of the same solution and then with aqueous ammonium acetate with a linearly increasing concentration gradient from 0 to 0.25 M (500 ml of water + 500 ml of 0.25 M solution, pH 6.5). The fraction eluted by an approximately 0.17 M solution of ammonium acetate was evaporated to dryness, and the ammonium acetate was driven off in vacuum at 40°C/2 mm. The yield of the ester (XII) in the form of the tetrahydrate of the acetate was 0.2 g (41%), decomp. p. above 200°C, $[\alpha]_D^{20}$ −25.0° (c 0.4; 50% acetic acid), R_f 0.63 (1), 0.42 (2),* E_{Arg} 0.61 (1 N acetic acid, Pauly, Sakaguchi, positive spot). Composition (%): C 53.33; H 6.84; N 15.25. $C_{52}H_{75}N_{13}O_{14} \cdot 4H_2O$.

[1-Carbonylglycinamide, 2-D-arginine, 5-valine]angiotensin II (XIII). A solution of 100 mg (0.085 mmole) of the ester (XII) in 10 ml of methanol saturated with ammonia at 0°C was left at room temperature for 72 h and was then evaporated to dryness and the residue was triturated with ether. The yellow-brown powder was dissolved in 2 ml of water and purified on a column of carboxymethylcellulose in a similar manner to the ester (XII). The fraction issuing at a concentration of ammonium acetate of about 0.22 M was evaporated, and the ammonium acetate was driven off at 40°C/2 mm. The yield of the amide (XIII) in the form of the pentahydrate of the acetate was 70 mg (71%), decomp. p. above 240°C, $[\alpha]_D^{20}$ −36.6° (c 0.4; 50% acetic acid), R_f 0.21 (1); 0.19 (2),† E_{Arg} 0.62 (Pauly, Sakaguchi, positive spot). Composition (%): C 51.54; H 6.66; N 16.59. $C_{50}H_{72}N_{14}O_{13} \cdot 5H_2O$.

SUMMARY

[1-(Ethyl ester of carbonylglycine), 2-D-arginine, 5-valine]angiotensin II and [1-carbonylglycinamide, 2-D-arginine, 5-valine]angiotensin II have been synthesized and their pressor activities have been determined.

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* For the [2-L-arginine] ester R_f 0.65 (1), 0.48 (2) [2].
† For the [2-L-arginine] amide, R_f 0.20 (1), 0.17 (2) [2].