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

R. É. Vegner, G. I. Chipens, and Z. P. Auna

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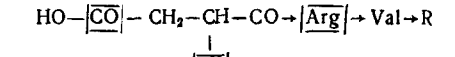
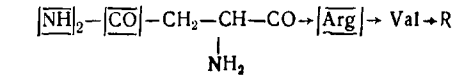
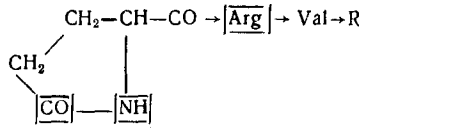
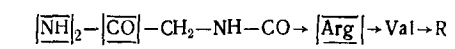
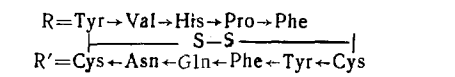
On the basis of an analysis of the interrelationship between the structure and biological activity of analogs of angiotensin II (I) modified in the first position [1-(aspartic acid)], we came to the conclusion [1] that the main structural elements of this position necessary to ensure a high biological activity are CO and NH groups with an adjacent position thanks to a covalent bond – for example, [1-asparagine, 5-valine]angiotensin II (II) [2] and [1-(pyroglutamic acid), 5-valine]angiotensin II (III) [3, 4] – or a definite conformation of the NH_2-terminal residue – for example, [1-(aspartic acid), 5-valine]angiotensin II (I) [2].

I 

II 

III 

IV 

V 

Apparently, the biological activity of the analogs is due not to the stereoelectronic structure of the peptide as a whole but to its signature,* i.e., only the individual sections of the electron cloud of the molecule with the charges characteristic for their shape, density, and distribution [5]. According to the principle of the ambiguity of the cause of an effect [6], compounds with different chemical structures may possess indistinguishable signatures in some situation.

Consequently, by comparing the NH_2- and COOH-terminal tripeptides of angiotensin II (I or II) and of [arginine]vasopressin (IV) it is possible to conclude that their structural elements – the bearers of the signatures – are similar. The proposed structural elements are surrounded by boxes in the formulas. The arrows denote the directions of the peptide linkages (from the NH_2 end to the COOH end). Generally-accepted abbreviations for the amino acids and protective groups are used [7]. The glycine amide in the ninth

*The signature, an idea borrowed from information theory, denotes the part of the characteristics of an object determining its participation in the process considered [6].

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position of the vasopressin molecule (IV) also has CO and NH groups; arginine is "common" to both hormones, and proline and valine may perform similar functions in spatially directing the terminal dipeptides: proline thanks to the rigidly fixed $C_\alpha - N$ bond, and valine through the steric voluminousness of the side chain. The conformations of peptide chains with proline and valine are extremely similar [8].

In order to study the suggested similarity of the bearers of the signatures of angiotensin and vasopressin, it appeared to us to be of interest to synthesize hybrids of these two hormones, i.e., to obtain compounds with interchanged amino-acid residues.

The present paper describes the synthesis of [1-carbonylglycinamide, 5-valine]angiotensin II – an analog of angiotensin in which the NH_2-terminal amino acid (aspartic acid) has been replaced by the COOH-terminal amino acid of vasopressin (glycine amide).

The hybrid (V) was synthesized on the basis of the COOH-terminal heptapeptide of angiotensin II (VI), to which the ethyl ester of N-carbonylglycine was added [9]. After the removal of the protective groups by reduction with hydrogen, the resulting ester of the octapeptide (VIII) was converted into the amide (V) by ammonolysis in methanol.

VI. H–Arg(NO_2)→Val→Tyr→Val→His→Pro→Phe–OBzl(NO_2)
↓ +OC=GlyOEt

VII. EtOGly←CO→Arg(NO_2)→Val→Tyr→Val→His→Pro→Phe–OBzl(NO_2)
↓ H_2/Pd

VIII. EtOGly←CO→Arg→Val→Tyr→Val→His→Pro→Phe–OH
↓ NH_3/MeOH

V. H_2NGly←CO→Arg→Val→Tyr→Val→His→Pro→Phe–OH

The biological activities of the compounds obtained were tested on nephrectomized rats. The values of the pressor activities of the angiotensin analogs synthesized are given below.

Compound	Relative pressor activity in rats %
[1-asparagine, 5-valine]angiotensin II (standard) (II)	100
[1-(ethyl ester of carbonylglycine), 5-valine]angiotensin II (VIII)	18
[1-carbonylglycinamide, 5-valine]angiotensin II (V)	113

In spite of the close similarity of the structures of the amide and ester bonds [10], the activity of the ester (VIII) was considerably lower than the activity of the standard preparation (II). When the ester group was replaced by an amide group, the activity of the resulting hybrid (V) rose more than six-fold. The fact that the activity exceeded that of the standard preparation is apparently due to the retarded inactivation of the hybrid (V) in the organism (action of amino-peptidase blocked because of the absence of a free NH_2-terminal amino group). The high biological activity of the hybrid (V) supports the hypothesis put forward concerning the similarity of the elements of the signatures of angiotensin and vasopressin [11]. A free NH_2-terminal amino group in the first position of angiotensin II is not necessary to ensure high biological activity when a ω-amide group is present.

The analyses were performed by R. F. Platnietse.

EXPERIMENTAL

The experiments were performed with amino acids of the L series, except for the glycine. The melting points were determined in open capillaries without correction; the angles of optical rotation were read on a Jasco ORD/UV-5 spectropolarimeter at 22°C. The purities of the products were checked by chromatography on "slow" paper of the Leningrad No. 2 paper mill in the solvent systems 1) butan-1-ol–acetic acid–water (5 : 1 : 2) [12] and 2) sec-butanol–3% ammonia solution (3 : 1) [13], and also by paper electrophoresis at 15 V/cm for 1.5 h. The electrophoretic mobilities are expressed as the ratios of the distances migrated by the substances under investigation and by histidine (E_{His}). The C, H, and N analytical figures corresponded to the calculated values.

The amino-acid composition after the hydrolysis of the peptide (with 6 N hydrochloric acid at 110°C for 24 h) was analyzed on a Biocal BC-200 automatic analyzer.

p-Nitrobenzyl Ester of [1-(Ethyl ester of carbonylglycine), 2-nitroarginine, 5-valine]angiotensin II (VII). A solution of 1.11 g (1.0 mmole) of the heptapeptide consisting of the p-nitrobenzyl ester of nitroarginylvalyltyrosylvalylhistidylprolyphenylalanine (VI) in 2 ml of dimethylformamide was treated with 0.14 ml (1.1 mmole) of the ethyl ester of carbonylglycine, and the mixture was left at room temperature for 24 h. Then 50 ml of water was added, and the precipitate that deposited was filtered off with suction, washed with water, and dried in vacuum over phosphorus pentoxide. Yield 1.10 g (90%), mp 180-182°C. After two crystallizations from ethanol, mp 190-191°C, $[\alpha]_D$ −62.8° (c 1.05; acetic acid), R_f 0.91 (1) and 0.94 (2); composition $C_{57}H_{75}N_{15}O_{16} \cdot H_2O$.

[1-(Ethyl ester of carbonylglycine),5-valine]angiotensin II (VIII). A solution of 0.25 g (0.2 mmole) of the p-nitrobenzyl ester of [1-(ethyl ester of carbonylglycine), 2-nitroarginine, 5-valine]angiotensin II (VII) in 10 ml of methanol–acetic acid–water (10 : 1 : 1) was hydrogenated in the presence of palladium black for 20 h. After the catalyst had been removed, the filtrate was evaporated to dryness and the residue was dried in vacuum over potassium hydroxide. Then it was dissolved in 10 ml of a mixture of tert-butanol and a 0.01 M aqueous solution of ammonium acetate (1 : 1), and the resulting solution was passed through a column (2×20 cm) containing carboxymethylcellulose in the H^+ form. The column was washed with the same solvent (90 ml) and then with an aqueous solution of ammonium acetate containing a linearly increasing concentration gradient (300 ml of 0.001 M + 300 ml of 0.1 M, pH 6.5), 15-ml fractions being collected. The contents of test-tubes 8-15 (selected by means of their absorption at 272 nm) were combined, partly evaporated in vacuum, and dried by sublimation at room temperature. After repeated sublimation drying at 40°C, the yield of chromatographically and electrophoretically pure product (VII) was 84 mg (40%). It decomposed above 210°C, $[\alpha]_D$ −44.0° (c 0.5; 50% acetic acid), R_f 0.76 (1), 0.81 (2), E_{His} 0.40 (1 N acetic acid). The Pauli and Sakaguchi reactions were positive and the ninhydrin reaction was negative.

In the wavelength range from 240 to 290 nm, λ_{max} 272 nm, λ_{min} 252 nm; $C_{50}H_{71}N_{13}O_{12} \cdot 5H_2O \cdot CH_3 \cdot COOH$.

[1-Carbonylglycinamide, 5-valine]angiotensin II (V). A solution of 50 mg of the ethyl ester of the octapeptide (VIII) in 2 ml of methanol was saturated with ammonia at 0°C. Then it was left at room temperature for 48 h, and the precipitate that had deposited was filtered off, washed with cold methanol, and kept in vacuum over phosphorus pentoxide. After sublimation-drying from acetic acid, the yield of substance (V) was 25 mg. It decomposed above 235°C, $[\alpha]_D$ −52.9° (c 0.34; 50% AcOH), R_f 0.53 (1), 0.18 (2), E_{His} 0.41 (1 N acetic acid). It showed the Pauli and Sakaguchi reactions. Amino-acid analysis: arginine 1.10; valine 1.93; histidine 1.00; glycine 0.90; proline 1.17; tyrosine 0.90; and phenylalanine 1.10.

SUMMARY

[1-Carbonylglycinamide, 5-valine]angiotensin II – a biologically highly active hybrid of angiotensin and vasopressin in which the NH_2-terminal amino acid of angiotensin has been replaced by the COOH-terminal amino acid of vasopressin – has been synthesized.

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