SYNTHESIS OF [1-ASPARAGINE, 3-GLYCINE, 5-VALINE]ANGIOTENSIN II

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In accordance with the program for the production of glycine-containing analogs of angiotensin II that has been reported [1, 2], we have synthesized [1-asparagine, 3-glycine, 5-valine]angiotensin II (compound II, Table 1). The results of an investigation of the biological activity of this analog enable us to judge the functions and values of the side chains of different amino acids in position 3 of the angiotensin II molecule [1]. This question is of fundamental importance in connection with the structural similarity of a number of physiologically active peptides that has been found [3]. If the "common" fragments of the type of proline/valine-arginine/lysine-aspartic acid/glycine fulfill similar functions, as is suggested [4], similar changes in their structures (for example, the replacement of a proline or valine residue by glycine) should lead to similar changes in their biological activities.

It is known that a modification of the amino-acid residue in position 3 of the angiotensin molecule (IV-VII), with a rare exception (VIII), has little effect on its biological activity. This contradicts the results obtained on modifying the proline residue (an assumed structural and functional equivalent of a valine residue) in the vasopressin molecule (IX, X) [10, 11].

Compound (II) was synthesized by the 2 +2 +4 scheme. The hydrazide of tert-butoxycarbonyl-glycyl-tyrosine [13] was added by the azide method to the tetrapeptide consisting of the nitrobenzyl ester of valyl-histidylprolylphenylalanine [1, 12]. The protected hexapeptide – the p-nitrobenzyl ester of tert-butoxy-carbonyl-glycyltyrosylvalylhistidylprolylphenylalanine – was obtained. After the splitting off the tert-butoxycarbonyl group under the action of hydrogen chloride in acetic acid, benzyloxycarbonyl-asparaginyl-nitroarginine [14] was added by the dicyclohexylcarbodiimide method using N-hydroxysuccinimide [15], which avoids racemization. The protected octapeptide was hydrogenated to eliminate the protective groups. After purification on carboxymethylcellulose in a concentration gradient of ammonium acetate, the free octapeptide (II) was obtained. The hydrogenation of the p-nitrobenzyl ester of the hexapeptide likewise gave the free hexapeptide (III). The pressor activity of [1-asparagine, 3-glycine, 5-valine]angiotensin II on nephrectomized rats proved to be only 0.5% and that of the corresponding COOH-terminal hexapeptide only 0.01% of the activity of [1-asparagine, 5-valine]angiotensin II.

The sharp fall in the pressor activity of angiotensin with the replacement of the valine in position 3 by glycine shows the fundamental importance of the side chain of this amino acid in ensuring a biological response reaction. It must be observed that the activity of the analog II is of the same order of magnitude as the activity of the COOH-terminal hexapeptide of angiotensin II [12]. Thus, the replacement of valine by glycine does not provide the possibility of transmitting the part of the information coded in the NH_2-terminal dipeptide Asp–Arg–. In the interaction of the hormone with the receptor, the side chain of the valine may make its own contribution both directly and by determining the spatial arrangement of the adjacent arginine residue, the guanidine group of which is of fundamental importance in the determination of the pressor reaction [2].

The results of a comparison of the biological activities of the compounds listed in Table 1 with measurements performed on molecular models permit the assumption that the side chain of the amino acid in

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TABLE 1. Results of a Comparison of the Pressor Activities of Some Analogs of Angiotensin and Vasopressin Modified in Positions 3 and 7, Respectively

Compound No.	Primary structure	Pressor activity*	Literature data
I	Asp—Arg—Val—Tyr—Val—His—Pro—Phe	100	[5]
II	Asn—Arg—Gly—Tyr—Val—His—Pro—Phe	0,5	—
III	Gly—Tyr—Val—His—Pro—Phe	0,01	—
IV	Asn—Arg—Pro—Tyr—Val—His—Pro—Phe	30	[12]
V	Asp—Arg—Pro—Tyr—Ile—His—Pro—Phe	40	[6]
VI	Asp—Arg—Ala—Tyr—Ile—His—Pro—Phe	68	[7]
VII	Asn—Arg—Leu—Tyr—Val—His—Pro—Phe	100	[8]
VIII	Asp—Arg—NHNHCO—Tyr—Val—His—Pro—Phe	0,5	[9]
IX	Cys—Tyr—Phe—Gln—Asn—Cys—Gly—Lys—GlyNH$_2$	0,15	[10]
X	Cys—Tyr—Phe—Gln—Asn—Cys—Val—Lys—GlyNH$_2$	0,7	[11]

*The pressor activities of the angiotensin analogs are expressed in percentages with respect to the corresponding [5-isoleucine]- or [5-valine]angiotensin II or its amide. The activities of the vasopressin analogs (IX, X) are given in IU/mg.

position 3 serves mainly for the spatial direction of the terminal amino-acid residues by limiting their mobility. This can be observed both in solution (intramolecular interaction) and also – possibly to a still greater extent – during the interaction of the residue with the corresponding point of the receptor (intermolecular, probably hydrophobic, interactions). Although proline and valine impart approximately the same directions to the terminal dipeptides of angiotensin and vasopressin, the replacement of proline by valine in the latter case (X) leads to a marked inactivation of the hormone. Here the steric correspondence of the vasopressin molecule and the receptor is more strongly expressed [10, 11].

The pharmacological investigation of the compounds obtained was performed by Z. P. Auna and the amino-acid analyses were performed by R. F. Platnietse.

EXPERIMENTAL

The melting points were determined in open capillaries without correction, and the angles of optical rotation were determined on a Perkin-Elmer 141 polarimeter at 21°C. The solvent systems for descending paper chromatography were as follows: 1) butan-1-ol–acetic acid–water (5 : 1 : 2), and 2) sec-butanol–3% aqueous ammonia solution (3 : 1). "Slow" paper of the Volodarskii Leningrad mill was used. Electrophoresis was performed for 45 min at a potential difference of 20 V/cm on FN-16 paper; the electrophoretic mobilities were expressed as the ratios of the distances travelled by the compound under investigation and by histidine (E_{his}). The amino-acid compositions of the three peptides after their hydrolysis (6 N hydrochloric acid, 24 h at 105°C) were determined on a "Biocal BC-200" automatic amino-acid analyzer.

The analyses of all the compounds corresponded to the calculated figures.

p-Nitrobenzyl Ester of tert-Butoxycarbonyl-glycyltyrosylvalylhistidylprolylphenylalanine (XI). At –20°C, 6 ml of a 3.3 N solution of hydrogen chloride in tetrahydrofuran and 0.7 ml (5 mmoles) of n-butyl nitrite were added to a solution of 1.8 g (5 mmoles) of the hydrazide of tert-butoxycarbonyl-glycyltyrosine in 4.5 ml of dimethylformamide. The mixture was stirred at the same temperature for 6 min, and then 20 ml of cooled ethyl acetate was added and the acid solution of azide so obtained was neutralized with triethylamine. Then a cooled solution of 3.1 g (5 mmoles) of the p-nitrobenzyl ester of valylhistidylprolylphenylalanine in 25 ml of ethyl acetate was added. After being kept at +4°C for 48 h, the mixture was washed successively with 1 N hydrochloric acid, water, 1 N sodium hydrogen carbonate solution, water again, and a saturated solution of sodium chloride, and then it was dried with sodium sulfate and evaporated to dryness. After titration with ether and drying over phosphorus pentoxide, the yield of protected hexapeptide $C_{48}H_{59}N_9O_{12}$ (XI) was 3.8 g (80%). For analysis, the substance was reprecipitated from ethanol with ether. Mp 176-180°C (decomp.), softening at 147°C; $[\alpha]_D$ –52.2° (c 0.5; methanol); R_f 0.94 (1), 0.96 (2); E_{His} 0.28 (5 N acetic acid); spots revealed with Pauli's reagent.

Dihydrochloride of the p-Nitrobenzyl Ester of Glycyltyrosylvalylhistidylprolylphenylalanine (XII). A solution of 3.0 g (3.15 mmoles) of the hexapeptide (XI) in 15 ml of a 1 N solution of hydrogen chloride in glacial acetic acid was kept at room temperature for 1 h. After the addition of 200 ml of ether, the precipitate that had deposited was kept at 0°C for 1 h, and it was then filtered off and reprecipitated from ethanol with acetone. The yield of the dihydrochloride $C_{43}H_{53}N_9O_{10}Cl_2$ (XII) was 2.0 g (70%), mp 168-170°C (decomp.), R_f 0.63 (1) (yellow coloration with ninhydrin).

p-Nitrobenzyl Ester of Benzyloxycarbonyl-asparaginylnitroarginylglycyltyrosylvalylhistidylprolyphenylalanine (XIII). To a solution of 0.5 g (0.6 mmole) of the dihydrochloride of the hexapeptide ester (XII) in 2 ml of dimethylformamide were added 0.17 ml (1.2 mmole) of triethylamine, 0.14 g (1.2 mmole) of N-hydroxysuccinimide [16], 0.38 g (0.8 mmole) of benzyloxycarbonyl-asparaginylnitroarginine, and at −5°C, 0.13 g (0.5 mmole) of dicyclohexylcarbodiimide dissolved in 2 ml of tetrahydrofuran. The reaction mixture was kept at 0°C for 48 h, after which the dicyclohexylurea that had precipitated was filtered off, and the precipitate was washed with 2 ml of dimethylformamide. The filtrate was treated with 50 ml of saturated sodium chloride solution and 10 ml of a 1 N solution of sodium hydrogen carbonate. The precipitate that formed was washed with water, 0.25 N hydrochloric acid solution, and water again, and was dried over phosphorus pentoxide. The yield of $C_{61}H_{74}N_{16}O_{17}$ (XIII) was 0.6 g (76%).

For analysis, the compound was reprecipitated twice from hot ethanol. Mp 187-190°C (decomp.), softening at 150°C, $[\alpha]_D$ −44.1° (c 2; methanol), R_f 0.94 (1), 0.96 (2), E_{His} 0.25 (5 N acetic acid), the spots being revealed with Pauli's reagent and the benzidine reagent [17].

[1-Asparagine, 3-glycine, 5-valine]angiotensin II (II). The protected octapeptide (XIII) (0.42 g) was dissolved in 25 ml of methanol–acetic acid–water (10 : 1 : 1) and was hydrogenated for 24 h in the presence of palladium black. The end of the hydrogenation was determined by means of an electrophoregram, after which the catalyst was filtered off, the filtrate was evaporated in vacuum, and the residue was triturated with ethyl acetate. The white powder obtained was dissolved in 5 ml of a 0.001 M solution of ammonium acetate and passed through a column (2×30 cm) of carboxymethylcellulose in the H^+ form in a concentration gradient of ammonium acetate of from 0.001 M in the mixing chamber (300 ml) to 0.1 M in the reservoir (300 ml, pH 6.5). The rate of elution was 120 ml/h. The fraction that issued at a concentration of the buffer solution of about 0.05 M was collected on the basis of λ_{max} 280 nm, and it was evaporated at 40°C to small volume and lyophilized. After relyophilization from acetic acid, the yield of octapeptide with the composition $C_{46}H_{64}N_{14}O_{11} \cdot CH_3COOH \cdot 5H_2O$ (II) was 240 mg. Mp 175°C (decomp.), softening at 160°C, $[\alpha]_D$ −40.0° (c 0.5; water), R_f 0.05 (1) and 0.76 (2), E_{His} 0.83 (1 N acetic acid), the spot being revealed with ninhydrin and the Pauli and Sakaguchi reagents.

Amino-acid analysis: arginine 1.02; aspartic acid 0.90; valine 1.10; histidine 1.02; glycine 1.00; proline 1.00; tyrosine 0.99; phenylalanine 1.06.

Glycyltyrosylvalylhistidylprolylphenylalanine (III). The hexapeptide ether dihydrochloride (XII) (0.22 g) was dissolved in 20 ml of methanol–acetic acid–water (10 : 1 : 1) and was hydrogenated for 12 h in the presence of palladium black. The catalyst was filtered off, the filtrate was evaporated to dryness in vacuum, and the residue was triturated with ethyl acetate. The resulting powder was dissolved in 4 ml of a 0.001 M solution of ammonium acetate and was chromatographed through a column (2×20 cm) of carboxymethylcellulose in the H^+ form in a concentration gradient of ammonium acetate of from 0.001 M in the mixing chamber (200 ml) to 0.1 M in the reservoir (200 ml, pH 6.5). The rate of elution was 120 ml/h. The fraction issuing at a concentration of the buffer solution of about 0.02 M, was collected on the basis of λ_{max} 280 nm, and it was evaporated at 40°C to small volume and lyophilized. After relyophilization from acetic acid, the yield of the hexapeptide $C_{36}H_{46}N_8O_8 \cdot 4H_2O$ (III) was 90 mg. Mp 160°C (decomp.), $[\alpha]_D$ −42.1° (c 0.5; 50% acetic acid), R_f 0.27 (1) and 0.93 (2), E_{His} 0.69 (1 N acetic acid).

Amino-acid analysis: valine 0.96; histidine 1.00; glycine 1.03; proline 0.99; tyrosine 0.98; phenylalanine 0.90.

SUMMARY

1. [1-Asparagine, 3-glycine, 5-valine]angiotensin II has been synthesized by the classical methods of peptide chemistry and its pressor activity has been determined.

The main function of the amino acid in position 3 of the angiotensin molecule is to ensure the spatial direction of the arrangement of the NH_2-terminal dipeptide by steric (intramolecular) and hydrophobic (intermolecular) interactions.

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