Angiotensin II Analogs. II.[1] *cyclo*-(-Val-Tyr-Ile-His-Pro-Phe-)

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The hexapeptide, Val-Tyr-Ile(or Val)-His-Pro-Phe, representing a carboxyl terminal portion of the tissue hormone, angiotensin II, has been reported variously as <0.1–1% as potent in pressor assays as the parent octapeptide.[2-4] This low activity of the hexapeptide has been significantly enhanced by extension of the peptide at the amino end by a variety of amino acids or their analogs, among the most effective being the Asp-Arg- dipeptide of natural angiotensin II.[5] Significant alteration of the carboxyl end resulted in virtual loss of activity.

One possible role of peptide backbone extension is the stabilization of a preferred conformation for the essential functional groups of the hexapeptide sequence, such as the strained α helix proposed by Smeby.[6a] A variety of physical measurements on the hormone and its analogs have been interpreted in support of,[6a] and in opposition to,[7] such an ordered structure for angiotensin II. Thin-film dialysis studies by Craig[6b,c] have indicated a preferred time-average structure with a rather spherical conformation of near-minimal size at neutral or acidic pH. Since the cyclic hexapeptide represents a more rigid and compact structure, its synthesis and biological comparison with the linear hexapeptide and with some related cyclic peptides was undertaken. The importance of the tyrosine and histidine residues for the biological effects of angiotensin II[5] made it of interest to examine for angiotensin-like activities the cyclic peptides of Kopple[8] containing these amino acids spaced at various intervals, primarily by glycine.

Myotropic activity was tested in the guinea pig ileum.[9] Analogs showing a significant response were tested for pressor activity in nephrectomized pentolinium-treated male rats anesthetized with pentobarbital.[10] Results of the bioassays are presented in Table I.

Results and Discussion

The hexapeptide, Val-Tyr-Ile-His-Pro-Phe (**2**), in the guinea pig ileum myotropic assay, showed an activity of 0.1% relative to 100% for [Asn[1],Val[5]]-angiotensin II (**1**). This is much lower than the rat uterus myotropic activity of 2.6% reported[3] for **2**, but is close to the rat pressor value (0.3%)[4] for **2**, or for its analog, Val-Tyr-Val-His-Pro-Phe (<0.1%).[2] The cyclic hexapeptide (**3**) showed a myotropic activity identical with that of the open-chain analog (**2**). Thus an increase in structural order by cyclization resulted in neither loss nor gain in myotropic activity. Removal of the free carboxyl function would normally be expected to decrease activity,[5] unless this group aided in the internal stabilization of a biologically effective cyclic structure. Maintenance of even this low order of activity suggests that the structure of the cyclic hexapeptide stabilizes the positions of side chain groups in a manner favorable to the production of the myotropic effect.

Two side-chain functional groups present in the hexapeptide are the imidazole ring of histidine and the phenolic ring of tyrosine. In angiotensin II, and in its open-chain hexapeptide fragment, these residues are in a 1,3 relationship, separated by a single amino acid residue, either isoleucine or valine. In the cyclic analog, these aromatic residues occupy a 1,3 spacing in the normal direction of describing the peptide linkage, –CO→NH–, and a 1,5 spacing in the reverse direction, –CO←NH–. Of the cyclic peptides tested, three have a 1,4 spacing in both directions of phenolic and imidazole residues (**4**, **6**, **7**); one of these (**7**) contains an unnatural amino acid, D-tyrosine. These peptides, and the mixed diketopiperazine in which the residues are adjacent (**8**), are essentially inactive in the myotropic assay. Only the peptide **5** in which tyrosine and histidine are spaced 1,5 in the –NH→CO– direction, or 1,3 in the –NH←CO– direction, showed appreciable myotropic activity, some 15 times that of either the linear or cyclic hexapeptides (**2**, **3**) related to angiotensin II. However, the nonspecific nature of the myotropic assay is evident, for peptide **5** was essentially inactive in the rat pressor test. Therefore, a simple relationship between a spacing of histidine and tyrosine residues, although apparently of importance in the production of the myotropic response, is not alone a determining factor in the pressor response of angiotensin and its analogs.

Experimental Section[11]

Val-Tyr-Ile-His-Pro-Phe.—To 250 mg (0.24 mmole) of Z-Val-Tyr-Ile-His-Pro-Phe-OBzl(NO_2)[12] in 25 ml of MeOH was added 70 mg of 10% Pd–C suspended in 6 ml of HOAc. The suspension was flushed with N_2 for 5 min, H_2 was bubbled through slowly for 5 hr, followed by N_2 for 5 min. The catalyst was removed and the filtrate was evaporated to dryness *in vacuo*. The oily residue was dissolved in 50 ml of H_2O and lyophilized three times, yielding a white solid, 185 mg (100%). Tlc (BAW) and paper electrophoresis at pH 3.5 and pH 6.5 showed a single major com-

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(11) Melting points were measured in a Thomas–Hoover apparatus and are uncorrected. Amino acid analyses were done on a Spinco 120B analyzer on samples hydrolyzed with twice-distilled hydrochloric acid for 24 hr at 110° in evacuated and sealed tubes. Optical rotations were measured in a Rudolph photoelectric polarimeter. Paper electrophoresis was carried out at 2000 V at pH 3.5 (HOAc–pyridine–H_2O, 10:1:190) or pH 6.5 (0.4:10:190). Electrophoretic mobilities are reported relative to the migration of histidine, E_H = 1.0. Tlc was on silica gel G (Merck) with the solvent system *n*-BuOH–HOAc–H_2O (100:10:30) (BAW) (top layer).

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TABLE I

RELATIVE BIOLOGICAL ACTIVITIES OF PEPTIDES

No.	Compound	Guinea pig ileum, %	Rat pressor, %
1	Asn-Arg-Val-Tyr-Val-His-Pro-Phe[a]	100	100
2	Val-Tyr-Ile-His-Pro-Phe	0.1	
3	*cyclo*-(-Val-Tyr-Ile-His-Pro-Phe-)	0.1	0.03
4	*cyclo*-(-Val-Tyr-Gly-Gly-His-Gly-)[b]	0.005	
5	*cyclo*-(-Gly-Tyr-Gly-Gly-Gly-His-)[b]	1.5	<0.01
6	*cyclo*-(-Gly-Tyr-Gly-Gly-His-Gly-)[b]	0.005	
7	*cyclo*-(-Gly-DTyr-Gly-Gly-His-Gly-)[b]	0.003	
8	*cyclo*-(-Tyr-His-)[b]	0.003	

[a] [Asn[1],Val[5]]-angiotensin II, provided by CIBA Pharmaceuticals, Inc. [b] Provided by Dr. K. D. Kopple, see ref 8.

ponent, no starting material, and four minor components. A 170-mg portion was dissolved in MeOH–H_2O (1:1), applied in bands on five sheets of Whatman No. 3MM filter paper, and subjected to electrophoresis at pH 3.5. The major band at E_H 0.50 was eluted with water and lyophilized to yield 63 mg of white powder: single spot on paper electrophoresis at pH 3.5 (E_H 0.53) and pH 6.5 (E_H 0.51); on paper chromatography, R_f 0.74; ninhydrin and Pauly +; amino acid analysis; Val 1.00, Tyr 0.57, Ile 0.91, His 1.02, Pro 0.98, Phe 0.94.

cyclo-(**-Val-Tyr-Ile-His-Pro-Phe-**).—To a stirred solution of 16 mg (0.084 mmole) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in 2 ml of DMF was added dropwise over 14 hr 32 mg (0.04 mmole) of Val-Tyr-Ile-His-Pro-Phe dissolved in 14 ml of DMF. After 24 hr at room temperature in the dark, paper electrophoresis showed no starting material at E_H 0.50 (pH 3.5), and in addition to several minor spots, a single major component at E_H 0.40 (pH 3.5); Pauly +, ninhydrin −. The solvent was removed *in vacuo* and the residue was dissolved in MeOH, applied in bands on sheets of Whatman No. 3MM filter paper. Following electrophoresis, the band at E_H 0.40 was eluted with water and lyophilized to yield 15 mg (47%) of a white powder: single spot on paper electrophoresis at pH 3.5, E_H 0.43; Pauly +, ninhydrin −; amino acid analysis, Val 1.07, Tyr 0.91, Ile 0.89, His 1.01, Pro 1.04, Phe 1.08.

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Hypotensive, Antiadrenergic, and Antihistaminic 3-Substituted 2-Methyl- (or 2-Phenyl-) 4(3H)-quinazolones

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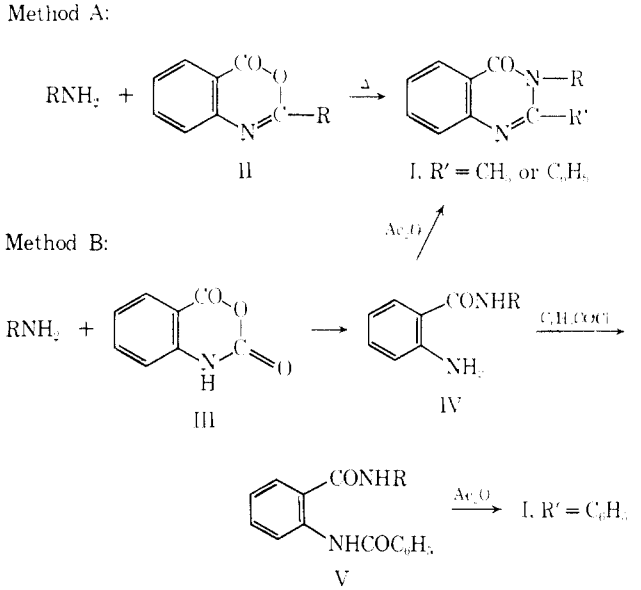
3-Aryl-2-methyl-4(3H)-quinazolones[2] are known to possess hypnotic, sedative, and anticonvulsant activities. Also, 3-ω-dialkylaminoalkyl-2-methyl-4(3H)-quinazolones[3] are reported to have similar activities. Our previous experience with N-arylpiperazine derivatives[4] having sedative, hypotensive, and antiadrenergic activities led us to study certain 3-ω-(4-aryl-1-piperazinyl)alkyl-2-methyl- (or 2-phenyl-) 4(3H)-quinazolones (I) (Tables I and II).

These compounds were readily prepared by heating 2-methyl- (or 2-phenyl-) 4-oxo-4H-3,1-benzoxazine (II) with appropriate primary amines (method A) or by treating isatoic anhydride (III) with the amines to give *o*-amino-N-substituted benzamides (IV) which were then benzoylated and cyclized with Ac_2O (method B) (Scheme I). I (R′ = CH_3) is also prepared by heating IV in Ac_2O. The details of the preparative chemistry have been described in a recent patent.[5]

SCHEME I



Pharmacology.—The activity of compounds of this series was evaluated as follows: antiadrenergic action

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