electrophoretic mobility or give a positive ninhydrin test. Amino acid analysis showed only phenylalanine to be present with a trace amount of aspartic acid. The esr spectra were characteristic of a single nitronyl nitroxide species and showed distinctive coupling constants in water at pH 6.9, α_N 8.35; $\alpha_{\beta CH}$ 2.34; $\alpha_{\beta CH'}$ 1.80.

N-Acetyl-β-(1,3-dioxy-4,4,5,5-tetramethyldihydroimidazol-2-yl)-DL-alanyl-L-phenylalanine (4). The dipeptide was prepared by the standard procedure where the N-acetyl free radical amino acid **2** was coupled to the phenylalanyl resin. After cleavage from the resin the dipeptide was purified by thin-layer chromatography on silica gel using 5% formic acid in ethyl acetate (R_f 0.53). Elution and evaporation of the single red product gave an oil which showed no mobility on electrophoreses nor a positive ninhydrin reaction. Amino acid analysis showed only phenylalanine. The esr spectra indicated that a single nitronyl nitroxide radical species was present and gave the distinctive coupling constants α_N 8.47; $\alpha_{\beta CH}$ 2.18; $\alpha_{\beta CH'}$ 1.92 G at pH 7.3.

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Angiotensin II Analogs. IX.[1] Conformational Studies of Angiotensin II by Proton Magnetic Resonance

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Abstract: Proton magnetic resonance spectra of [Asn[1],Val[5]]angiotensin II and five analogs were determined at 20° and 50° in deuterated dimethyl sulfoxide with a 220-MHz instrument. Spectra of the Gly-Val-Tyr-Ile-His-Pro-Phe analog were taken at 100 MHz in D_2O at different pH values and temperatures. Some observed manifestations of conformational stability included upfield shifts of the Pro β-, γ-, and δ-methylene hydrogens; line broadening of the His C-2 and C-4 hydrogens with increasing temperature; and the presence of a single intramolecular hydrogen bound amide hydrogen. These results have been interpreted to indicate the presence of a stable conformer of angiotensin in solution which includes an intramolecular ion–dipole bond between the Phe carboxylate ion and the imidazole ring of histidine. This conformation holds the imidazole ring in the vicinity of the proline ring causing the observed upfield shifts of the proline methylene hydrogens and line broadening of the imidazole hydrogens. This conformer is stabilized by an intramolecular hydrogen bond between the Phe amide NH and the His carbonyl function and has an activation energy of interconversion of at least 13–15 kcal/mole. Further evidence indicates that the remaining amino acids in the side chain of the intramolecularly bound ring maintain a preferred conformation which is lost on increasing the temperature beyond 40° in D_2O. These results are in agreement with previously indicated conformational properties derived from esr spectra of free radical containing angiotensin analogs.

Angiotensin II is a biologically important octapeptide which has been the subject of numerous studies on the relationship between chemical modification and biological activity.[2] In view of the large number of publications dealing with angiotensin, relatively little is known about its tertiary structure. Optical rotatory dispersion and thin-film dialysis studies indicate that angiotensin has conformational stability, but the studies differ greatly in their description of the structural properties of the peptide in solution. Nmr data concerning angiotensin have not been previously reported.

Three groups have reported different descriptions of the conformational properties of angiotensin derived from thin-film dialyses data. Craig and coworkers[3] reported dialysis rate changes of [β-Asp[1],Val[5]]angiotensin II with pH. A compact structure with high dialysis rates is observed in acidic solution and attributed to a loose association of the positively and negatively charged groups along the peptide chain. A more extended and possibly more rigid conformer was indicated in basic media. Ferreira, *et al.*,[4] reported that two different conformations of [Asn[1],Ile[5]]angiotensin II exist between pH 2 and 11. The relative concentration of these conformations is said to be independent of pH but dependent on the ionic strength of the solution. Fernandez, *et al.*,[5] reported rates of dialysis for [Asn[1],Ile[5]]angiotensin II which are pH independent between 5 and 9 but which increase at pH 10 where the phenolic hydroxyl group is ionized.

Evidence for conformational stability has also been obtained from optical rotatory dispersion curves. Spectra of [β-Asp[1],Val[5]]angiotensin II[6] and [Ile[5]]angiotensin II[7] show deep minima at 217 and 235 nm at pH 11 but relatively shallow curves when taken at pH 5.67.[6] These data are consistent with a helical model but do not exclude other stable conformers. In other work [Asn[1],Val[5]]angiotensin II was interpreted to have a nonhelical random coil configuration.[8]

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In preceding papers[9–11] we described the use of stable free radical amino acids and peptides as models of histidine and histidine-containing peptides. The nitronylnitroxylalanine free radical was shown to be an effective analog of histidine[10] as it approximates the size and shape of the natural amino acid and parallels the ion–dipole binding observed in histidine derivatives.[9] The esr spectral characteristics of the nitronyl nitroxide function were sensitive to solvational changes and steric interactions and thus could be used as an effective system to report on its environment and, by analogy, the environment of its histidine-containing analog. With this in mind we prepared some free radical containing peptide models of the carboxyl-terminal region of angiotensin II.[11] The free radical models which retained the His-Pro-Phe-OH features of angiotensin showed an intramolecular ion–dipole bond between the carboxylate anion of phenylalanine and the polar nitronyl nitroxide ring of the histidine analog. The energy of activation for conformer interconversion of the intramolecularly bound conformer in the tripeptide was 2.4 kcal/mole.

These results were used[11] to indicate a conformation of angiotensin II which would involve an ion–dipole bond between the phenylalanine carboxylate ion and the imidazole ring of histidine. This cyclic conformation would be further stabilized by the proline ring, the trans amide bonds, the sterically preferred conformations of the His CO–C_α and C_α–C_β bonds, and a hydrogen bond between the phenylalanine amide NH and histidine carbonyl groups.

This paper reports 220-MHz nmr spectra of [Asn[1],-Val[5]]angiotensin II and five analogs in deuterated dimethyl sulfoxide. The heptapeptide Gly-Val-Tyr-Ile-His-Pro-Phe was examined in acidic and basic solutions in D_2O and at different temperatures with a 100-MHz instrument. The results are interpreted as confirming the previous description[11] of the conformation of the carboxyl-terminal region of angiotensin and offer some information involving the environment of the other five amino acids.

Experimental Section

Proton magnetic resonance spectra were taken on a Varian Associates HR-220 MHz instrument with a superconducting solenoid and on a JEOLCO-JMN-4H-100 spectrometer equipped with double resonance and variable temperature accessories. The 220-MHz spectra of the six peptides listed in Table I were taken at 20 and 50° in deuterated dimethyl sulfoxide using tetramethylsilane as an internal standard. These spectra were taken using a 500-Hz sweep width and a 500-sec sweep time. The 100-MHz spectra were taken in D_2O containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Chemical shifts of all spectra are expressed as parts per million (ppm) downfield from the internal standard (0 ppm).

The peptides (**1–5**) employed in this study have been previously reported.[12,13] [Asn[1],Val[5]]angiotensin II was provided by CIBA Pharmaceuticals, Inc., and had a proton-containing contaminant which appeared as a broad peak from 3.1 to 3.7 ppm (approximately 40 protons per mole). These peptides contained 2 equiv of acetic acid which appeared in the 220-MHz spectra. The acetic acid was replaced by deuterated acetic acid in the 100-MHz spectra by repeatedly adding deuterated acetic acid to the peptide and evaporating the solvent until the proton signal disappeared. The same procedure was used in exchanging the amide NH protons using D_2O. The pD of the 100-MHz spectra were measured on a Radiometer pH-M-26.

Table I. Pressor Activities of Angiotensin II Analogs

No.	Peptide	Activity	Relative duration
1	Val- Tyr-Ile- His- Pro-Phe	0.3	
2	Gly-Val- Tyr-Ile- His- Pro-Phe	10	30–40
3	L-Abu-Val- Tyr-Ile- His- Pro-Phe	1	20–40
4	D-Abu-Val- Tyr-Ile- His- Pro-Phe	23	100
5	Asn-Arg-Val- Tyr-Val-His- Pro-Phe	100	100
6	Asn-Arg-Val- Tyr-Ile- His-D-Pro-Phe	0.1	

Results

Pmr spectra of six biologically active angiotensin II analogs have been obtained at 220 MHz. The structures and pressor activities of these compounds are given in Table I. These compounds were tested for pressor activity in nephrectomized, pentolinium-treated, male rats anesthetized with pentobarbital.[14] The hexapeptide **1** contains the essential structural units required for biological activity. The first two amino acids, Asn[1]-Arg[2], serve to enhance this activity of the hexapeptide, but they do not have rigid structural or functional requirements as shown by the relatively high activity of a variety of analogs with simpler groups in place of Asn[1] and Arg[2].[12] It is therefore thought that the hexapeptide section acts at the drug receptor site while the first two amino acids either facilitate the transportation of the hormone to the active site[15] or aid the binding of the molecule at the active site in a favorable orientation.[12,16] Another function postulated for the N-terminal dipeptide unit, but not completely supported by our data, is that it stabilizes the conformation of the peptide backbone giving the octapeptide a rigid, favorable structure.[7,17]

Table II gives the chemical shifts of side chain protons for the six angiotensin analogs taken in deuteriodimethyl sulfoxide (DMSO-d_6). Random coil resonance positions for side-chain functions have been reported in D_2O by McDonald and Phillips[18] and by Mandel.[19] The random coil resonance positions given in Table II for DMSO-d_6 solution are those reported in D_2O solution corrected for solvent shifts.

The magnitude of the solvent shift was determined by comparing spectra of several amino acids and derivatives in D_2O and DMSO-d_6. The 0.09-ppm upfield shift used in Table II appears to hold for all side-chain functions presented in Table II except for histidine and tyrosine where specific solvational interactions occur in

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Table II. Chemical Shifts of Angiotensin Analogs in DMSO-d_6 at 20° Taken at 220 MHz

Proton type	Random coil resonance position	Angiotensin II Analogs, ppm[a] 1	2	3	4	5	6
Ile γ,δ-CH_3, Val 2 γ-CH_3	0.74–0.83	0.74–0.82	0.74–0.81	0.75–0.82	0.75–0.80	0.74–0.75	0.74–0.80
Ile γ-CH	1.05	1.04	1.03	1.04	1.04		0.95
γ-CH′	1.35	1.31	1.34	1.31	1.39		1.33
Pro β,γ-CH_2's	1.96 m	1.72 m	1.72 m	1.72 m	1.71 m	1.75 m[b]	1.63 m[b]
	2.08 sh	1.57 sh	1.59 sh	1.57 sh	1.52 sh	1.45 m[b]	1.33 m[b]
His β-CH_2, Tyr β-CH_2, Phe β-CH_2	3.00–3.18	2.72–3.17	2.82–3.12	2.80–3.10	2.79–3.12	2.82–3.19	2.78–3.05
Pro δ-CH_2	3.34	<3.17	<3.12	<3.10	<3.12	<3.19	3.36
His C-4 H	6.85	6.85	6.84	6.85	6.85	6.85	6.78
C-2 H	7.4–7.6	7.45	7.45	7.45	7.45	7.46	7.42
Tyr ortho H's	6.65	6.73	6.75	6.74	6.64	6.60	6.55
meta H's	6.99	6.98	7.00	6.98	7.00	7.00	6.98
Phe C_6H_5	7.20	7.20	7.19	7.18	7.17	7.13	7.20, 7.23

[a] The abbreviations m and sh refer to the resonance position of the peak maximum and shoulder, respectively. [b] The proline β,γ-methylene hydrogens are overlapped with the arginine β,γ-methylene hydrogens.

DMSO. The observed shifts for these groups were 0.12 and 0.16 ppm upfield for the tyrosine ortho and meta hydrogens, respectively, and 0.3 to 0.5 and 0.15 for the histidine C-2 and C-4 hydrogens.

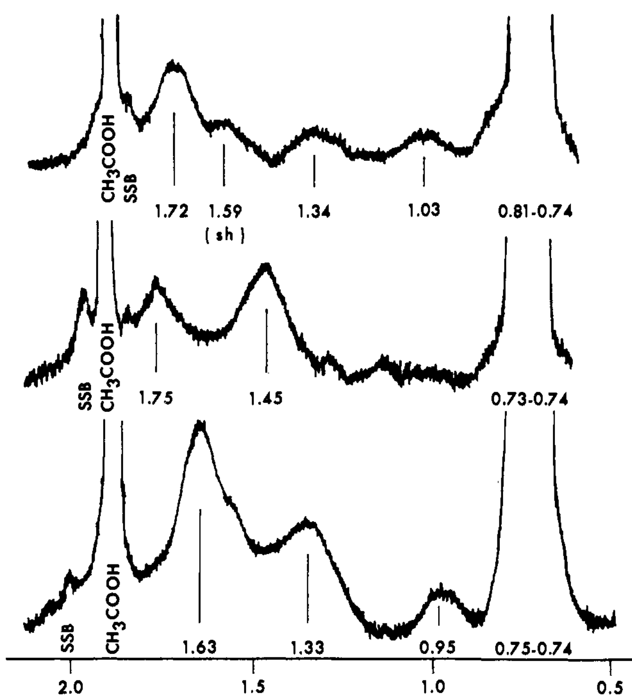

Figure 1. The 220-MHz pmr spectra of the glycyl heptapeptide (2, top); [Asn[1],Val[5]]angiotensin II (5, center), and (Asn[1],Ile[5],-D-Pro[7])angiotensin II (6, bottom) in DMSO-d_6 at 20°.

The hexapeptide **1** and the glycyl (**2**), L-aminobutyric (**3**), and D-aminobutyric (**4**) heptapeptides show very similar side chain chemical shifts. All show line broadening of the histidine C-2 and C-4 hydrogens which increases on increasing temperature. They also show upfield shifts of 0.25 ppm for the proline β- and γ-methylene hydrogens and a shift of at least 0.17 ppm for the δ-methylene hydrogens relative to the predicted proline chemical shifts in DMSO-d_6. The α-CH protons could not be assigned from our spectral data but do have a similar pattern of chemical shifts in these analogs. Each of these four peptides shows three groups of α-CH peaks which, with slight variations, lie within the limits 3.41–3.59, 4.13–4.29, and 4.43–4.60 ppm. These data suggest that the four analogs have similar conformational properties involving structural stability in the carboxyl-terminal region and random coil or time-averaged configuration of the amino-terminal segment.

The L- and D-proline angiotensin analogs, **5** and **6**, respectively, show more complicated pmr spectra due to overlapping peaks. It was not possible to assign the proline and arginine β- and γ-methylene hydrogens from the spectral data that we have but it can be shown that there are significant differences in the conformations when the proline is changed (Figure 1). The L-prolyl peptide **5** could have a proline maximum at 1.75 ppm with a shoulder at 1.57 ppm; the arginine hydrogens would then be from 1.45 to 1.75 ppm (expected values are from 1.54 to 1.75).[18] Neither of these assignments can be maintained in the D-prolyl analog (**6**) where peaks at 1.63 and 1.33 ppm in a ratio of 6 to 2 were observed. A peak assigned to the proline δ-methylene hydrogens appears at 3.36 ppm in the D-prolyl analog while no peaks distinguishable from the His, Tyr, and Phe β-methylene protons were observed in the L-proline analogs (**1–5**). Small chemical shift differences were observed throughout the nmr spectra of the D and L peptides as shown in Table I with a unique feature of the D-proline analog **6** being the presence of two phenylalanine peaks in the aromatic region. It seems, therefore, that inversion of the proline α-carbon significantly alters the conformational properties of the carboxy terminal region of angiotensin II.

The glycyl heptapeptide **2** was examined in more detail using a 100-MHz instrument. The spectral data for the analogs taken at 220 MHz indicate that the peptides containing the L-proline enantiomer have similar conformational properties and the high order of pressor activity of the glycyl heptapeptide **2** indicates that it has the conformational properties required for biological activity. The pmr spectra of this peptide show less peak overlapping than that of [Asn[1],Val[5]]angiotensin II (**5**), therefore making possible a more detailed spectral analysis. The only unresolved portion of the spectrum of **2** is in the 2.95- to 3.25-ppm region where the β-

Table III. Chemical Shifts[a] of Gly-Val-Tyr-Ile-His-Pro-Phe at 100 MHz and 20°

Proton type	Solvent DMSO-d_6	D$_2$O (pD 8.5)	D$_2$O (pD 2)
Ile δ-CH_3	0.77	0.82	0.85
γ-CH_3	0.79 sh	0.82	0.85
γ-CH	1.06	1.16	1.10
γ-CH	1.36 (dr 0.79)[c]	1.38	1.40
β-CH	2.00 sh	~2.00	~2.00
α-CH	4.29 (dr 1.01, 1.32, 0.79, 7.94)	*b*	*b*
N-H	7.94, J_{NHCH} = 8.5		
Val 2 γ-CH_3	0.77	0.82	0.85
β-CH	2.00 (dr 0.77, 4.27)	~2.00	~2.00
α-CH_2	4.27 (dr 2.00)	*b*	*b*
N-H	7.94, J_{NHCH} = 7.5		
Pro β-CH_2	1.59 and 1.73	1.82	1.98 and 2.08
γ-CH_2	1.73	1.82	1.98
δ-CH_2	2.85 (dr 1.75)	<3.26	3.53
α-CH	4.45 (dr 1.75, 1.62)	*b*	*b*
Gly α-CH_2	3.36	3.36	3.99
His β-CH_2	2.87, 2.97, or 3.06 (dr 3.60)	2.95–3.26	2.95–3.18
α-CH	3.60, 4.19, or 4.38	*b*	*b*
C_4-H	6.74	7.05	7.40
C_2-H	7.44	7.77	8.70
N-H	8.33 (s), 8.39 (s), or 7.91, J_{NHCH} < 3		
Tyr β-CH_2	2.87, 2.97, or 3.06 (dr, 3.60)	2.95–3.26	2.95–3.18
α-CH	3.60, 4.19, or 4.38	*b*	*b*
ortho-H	6.61, J_{CHCH} = 8 Hz	6.78	6.84
meta-H	6.99, J_{CHCH} = 8 Hz	7.11	7.16
N-H	8.33 (s), 8.39 (s), or 7.91, J_{NHCH} < 3		
Phe β-CH_2	2.87, 2.97, or 3.06 (dr 3.60)	2.95–3.26	2.95–3.18
α-CH	3.60, 4.19, or 4.38	*b*	*b*
C_6H_5	7.16	7.38	7.40
N-H	8.33 (s), 8.39 (s), or 7.91, J_{NHCH} = 6		

[a] Parts per million downfield from the standard at 0 Hz; coupling constants are in hertz. [b] Under HOD resonance. [c] Abbreviations dr, m, and sh refer to a resonance position associated through double resonance and the positions of the peak maximum and shoulder, respectively.

methylene hydrogens of the aromatic amino acids and the δ-methylene hydrogens of proline overlap. This area is further complicated as each of the methylene hydrogens may have a complex splitting pattern.

Table III shows the chemical shifts of the heptapeptide **2** in deuterated dimethyl sulfoxide and in D_2O at pH values of 8.5 and 2.0. Double resonance was used to make chemical shift assignments when possible. The overlapping in the 2.95- to 3.95-ppm region made complete assignment impossible with a 100-MHz instrument. The amide hydrogens exchange in D_2O and are not observed, and the α-CH peaks are masked by the broad proton resonance from the HOD in the sample. The chemical shifts observed in DMSO-d_6 and in basic D_2O solutions show parallel values indicating that the peptide conformations are similar in both solvents. The upfield shifts of the proline β-, γ-, and δ-hydrogens are not observed in acidic D_2O solution. The observed values of 1.98, 2.08 sh, and 3.53 ppm correlate with values of 1.96, 2.08 sh, and 3.47 ppm observed in a spectrum of *tert*-Boc-proline taken in D_2O. Other spectral differences between the acidic and basic solutions result from changes in the ionic states of the histidine imidazole and glycine amino groups.

The pmr spectra of the amide NH region in DMSO-d_6 changed with temperature as shown in Figure 2. The rate of the upfield shift of these protons is dependent on the nature of the hydrogen-bonding interaction.[20,21] The slope of the amide hydrogen shift with temperature for those protons bound by the solvent should parallel a standard slope such as observed with *N*-methylacetamide. Those amide hydrogens involved in an intramolecular hydrogen bond show a smaller change in chemical shift with temperature. One amide hydrogen, number 4, shows this small rate of change indicative of an intramolecular bond. The other amide NH resonances show varying degrees of deviation from the expected linear relation. The amide peaks associated by double resonance with valine, number 3, and isoleucine, number 5, show increased rates of upfield shifts with temperature between 35 and 45°. The amide peak, number 2, shows a slight change in this temperature range and peak number 1 is linear and parallel to the standard line. The slopes of these lines are nearly parallel to that of *N*-methylacetamide outside of the 35–45° temperature range.

The magnitude of the J_{NHCH} coupling constant has been shown to be dependent on the dihedral angle θ (Figure 3).[22,23] Large J_{NHCN} values may correspond to either cis or trans dihedral arrangement of protons. Correlation of the stereochemical dependence of the J_{NHCH} coupling constants gives eq 1.[24]

$$J_{NHCH} = 9.68 \cos^2 \theta - 0.42 \cos \theta + 0.12 \sin^2 \theta \quad (1)$$

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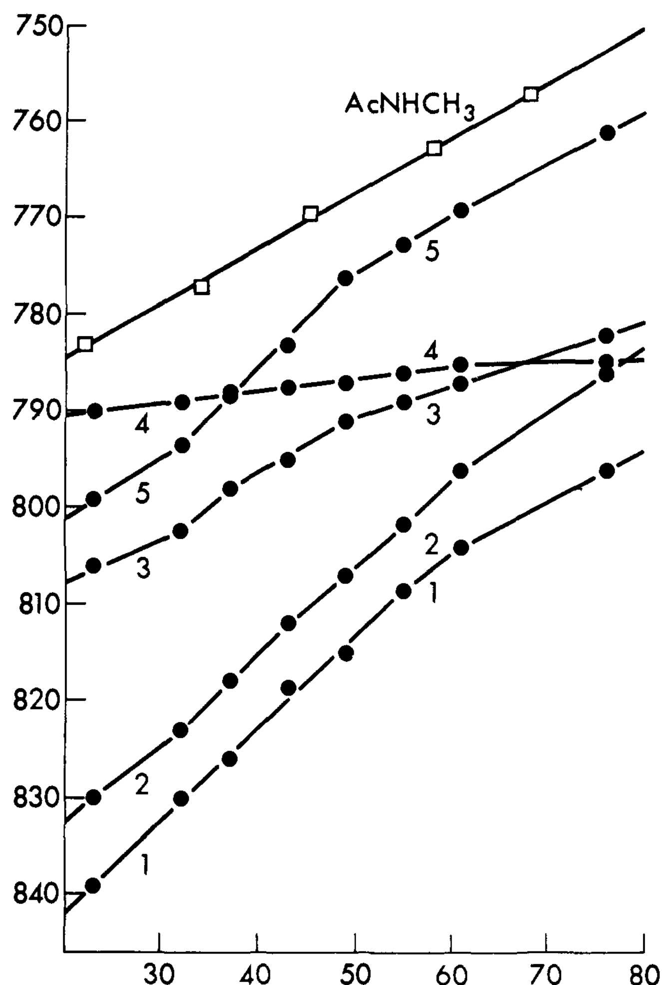


Figure 2. A plot of chemical shift against temperature for the amide protons of **2** in DMSO-d_6 at 100 MHz; (x axis) temperature, degree Celsius, (y axis) chemical shift, hertz.

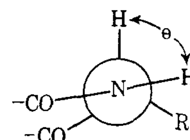


Figure 3. The dihedral angle of an NHCH bond in the gauche position.

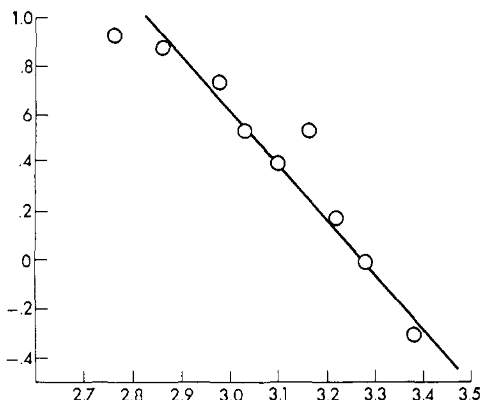


Figure 4. A plot of the log of line width increase against the reciprocal of temperature for the C-4 hydrogen of the His[6] imidazole of **2**; (x axis) $1/T$, °K^{-1}, (y axis) $\log(1/T_2 - 1/T_2')$.

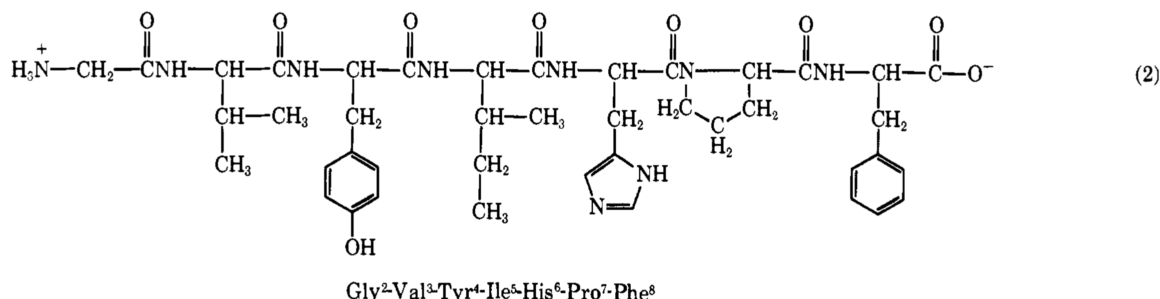
This relationship gives J_{NHCH} values of 6–8 Hz for nearly cis dihedral angles (θ = 0–30°) and values of 8–10 Hz for nearly trans angles (θ = 150–180°) while very small coupling constants, J_{NHCH} = 0.5–2.5 Hz, result from the nearly perpendicular arrangement (θ = 60–120°). The freely rotating NHCH bond gives a coupling constant value of 4–5 Hz.[23]

The J_{NHCH} coupling constants associated with Val and Ile (numbers 3 and 5, respectively, in Figure 2) have values of 7.5 and 8.5 Hz. Correction for electronegativity of substituents[22] gives values of 8.1 and 9.1 Hz. These correspond to a trans dihedral angle of θ = 160°. The coupling constant value of the intramolecularly hydrogen bound amide hydrogen (number 4, Figure 2) is 6.0 Hz which could be correlated to dihedral angles of 30 or 140° if it were held in a rigid conformation. The other two amide hydrogens which are associated with two of the aromatic amino acids (numbers 1 and 2, Figure 2) appear as singlets ($J_{NHCH} < 3$ Hz) in the pmr spectrum and would therefore be expected to be in a preferred conformation with a nearly perpendicular dihedral angle or a gauche position (θ = 60–120°).

Another feature observed on increasing the temperature of the glycyl heptapeptide **2** in DMSO-d_6 was the line width increase of the imidazole C-2 and C-4 hydrogens. The rate of increase of the line width at half-height ($1/T_2$) for the C-4 hydrogen was linear between 25 and 80°. The line width approached a minimum ($1/T_2'$) of 5.25 Hz at lower temperatures. One set of peaks for the C-2 and C-4 hydrogens was visible at the lower temperatures, suggesting that a single preferred conformer was present. Above 80° the rate of line broadening slowed; measurements taken above 90° were hindered by overlapping with the tyrosine meta hydrogen peaks. The C-2 proton showed a parallel line width increase from 25 to 60° where overlapping with the amide protons occurred. A plot of the log of the line width increase against the reciprocal of temperature is shown in Figure 4. The activation energy for conformation interconversion may be calculated from the slope ($k = -E_a/2.3R$). Values of 13 and 15 kcal/mole were obtained from the plots of C-4 and C-2 line broadening, respectively. This calculation neglects changes in molecular rotation rates and viscosity which tend to decrease line width with increasing temperature so that the above values represent a minimum activation energy estimate.

The pmr spectra of the biologically active glycyl heptapeptide **2** show several features which are indicative of a stable conformation in solution. The more prominent examples include (a) the upfield chemical shift of the proline β-, γ-, and δ-methylene hydrogens, (b) the pH dependence of these upfield chemical shifts, (c) the line width increases of the C-2 and C-4 hydrogens on the histidine imidazole ring, (d) the single intramolecularly hydrogen bond amide proton associated with an aromatic amino acid, and (e) the observed NHCH bond angles. Most of these conformational features involve amino acids in the carboxyl terminal region.

In an earlier paper we described a stable conformation of this region of angiotensin II which was indicated by esr data of a free radical angiotensin analog.[11] The pH dependence and line broadening of the esr spectra



(2)

of the nitronyl nitroxide free radical analog of the histidine imidazole ring indicated that it was involved in an ion-dipole bond with the terminal carboxylate ion of phenylalanine. These results were extended by analogy to the His-Pro-Phe portion of angiotensin II.

A stable cyclic structure of this type explains much of the pmr data mentioned above. The 12-membered cyclic structure (Figure 5) would be held by an intramolecular ion-dipole bond between the carboxylate anion and the imidazole ring and by an intramolecular hydrogen bond between the phenylalanine NH and the histidine carbonyl groups. This structure could hold the imidazole ring in an almost stable configuration at 20°. The pmr line widths of the C-2 and C-4 imidazole hydrogens would then broaden at higher temperatures if bending of this folded ring system was initiated. The intramolecularly hydrogen-bound amide proton would then be assigned to phenylalanine.

Examination of a Dreiding model of this cyclic structure shows that the imidazole ring of histidine can be placed face to face with the proline ring, thus accounting for the upfield shifts of the β-, γ-, and δ-methylene hydrogens but not the α-hydrogen. In this model the α-hydrogen is on the far side of the proline ring and farthest removed from the imidazole ring current. It is difficult to predict an accurate resonance position for the α-hydrogens but the proton associated by double resonance with proline (δ 4.45 ppm) is the farthest downfield of those present in this system.

This cyclic structure has a number of favorable features. The most important is that the histidine amide nitrogen group and the attached peptide chain containing five amino residues are in a most open position. The plane perpendicular to and through the histidine CHNH bond is unobstructed by the cyclic system. The histidine CO-C_α and C_α-C_β bonds have a large steric requirement and are in a preferred rotamer configuration in this cyclic conformer. This model also indicates that the phenylalanine CH-NH bond would be in the cis configuration so that the J_{NHCH} value of 6 Hz corresponds to a dihedral angle of 30°.

An ion-dipole bond between the carboxylate anion and the imidazole ring of histidine may be a contributing factor in the cleavage of the labile proline amide bond in the His-Pro-Phe tripeptide. Mazur and Schlatter[25] have shown that the His-Pro-Phe tripeptide gives His-Pro diketopiperazine and phenylalanine after 24 hr in 50% acetic acid at room temperature. The methyl ester His-Pro-Phe-OCH_3, however, does not react under these conditions. In addition to the terminal carboxylate ion, these workers have demonstrated that the histidine and proline units must be present to observe the rapid reaction rate. A mechanism involving a series of hydrogen bonds was used to explain these data, but an alternative explanation involving a cyclic phenylalanine carboxylate-histidine imidazole bond and a cis His C_α-CONH-Pro C_α amide bond may be preferable. This cyclic structure holds the histidine amino group very close and perpendicular to the proline amide carbonyl. The presence of this type of conformer could easily account for the rate increase and the striking influence of the carboxylate functionalization observed in this system.

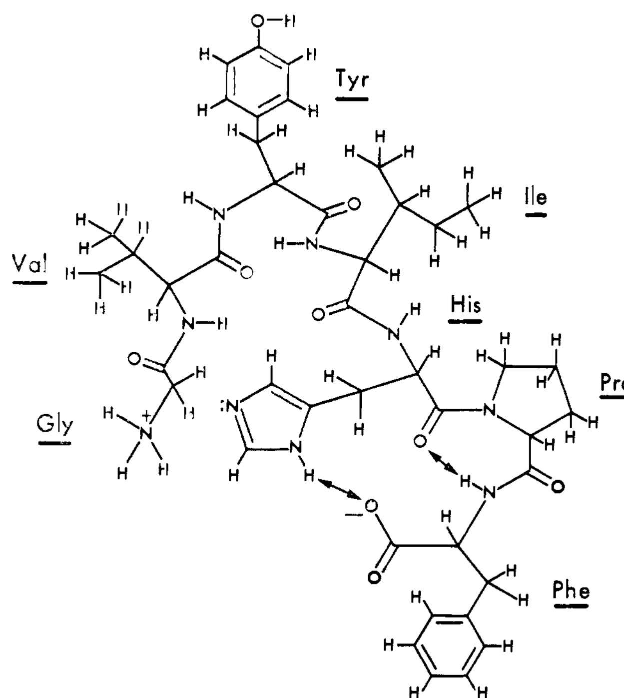


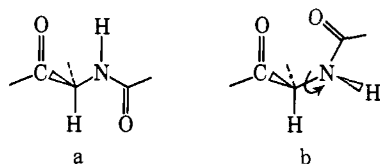
Figure 5. A preferred conformation of Gly-Val-Tyr-Ile-His-Pro-Phe (**2**). In this figure the His imidazole ring has been pulled back from the face of the Pro ring by bending the βC bond angle.

A cyclic conformer with a cis histidine amide bond is not possible when the histidine amino group is substituted with a bulky group. It also shows no possibility of having an intramolecular hydrogen bond.

The pmr data concerning the Gly-Val-Tyr-Ile portion of the glycyl heptapeptide **2** indicates that this chain exists in a preferred time averaged conformation. The J_{NHCH} coupling constants of the Val and Ile units (7.5–8.5 Hz) could result from a preferred trans configuration while the His and Tyr units ($J_{NHCH} < 3$Hz) prefer a gauche rotamer conformation. These dihedral angles do not describe a preferred side chain configuration as rotations about the CO-C_α bond may still occur; they do show that free rotation of the peptide backbone does not occur at lower temperatures.

(25) R. H. Mazur and J. M. Schlatter, *J. Org. Chem.*, **28**, 1025 (1963).

Another indication of conformational preference is seen by the nonlinearity of the lines in Figure 2. An increased rate of change between 35 and 45° for the amide hydrogens 3 and 5 suggests that these protons undergo an environmental change in this temperature range which involves an upfield chemical shift. An amide NH proton in the nearly trans configuration to the α-CH can be deshielded by the adjacent carbonyl function (a). Increased rotational freedom (b) would lower the probability of this interaction and would result in the upfield shift seen in Figure 2.



The amino acids Val and Ile (θ = 150–160°; numbers 3 and 5, Figure 2) would be expected to have the greatest upfield movement with randomization of rotational motion as they are in a nearly trans configuration. The linear temperature dependence of the phenylalanine amide proton, number 4, indicates that the cyclic structure is not opened in this temperature range.

The chemical shift data of Table II clearly show that the angiotensin analogs with an L-proline in the seven position have similar conformations. Each of these peptides (**1–5**) show the upfield shift of the proline β-, γ-, δ-methylene hydrogens and line broadening of the histidine imidazole hydrogens at higher temperatures which is characteristic of the cyclic conformation of Figure 5. The conformational properties of the side-chain amino acids of other angiotensin analogs (**1**, **3–5**) cannot be inferred from the characteristics of the glycyl heptapeptide **2**, but the presence of conformational preference in **2** raises the possibility that some loose structure may exist. The pmr spectrum of the D-proline analog **6**, Table I, also shows features indicative of extensive conformational preference. The upfield region, Figure 1, shows significant differences in the proline and arginine β-, γ-, and δ-methylene protons and the isoleucine γ-CH resonance from those of **5**. Two Phe C_6H_5 peaks of equal intensity are observed in the aromatic region. These differences show that **6** does not have the same conformation of [Asn[1],Val[5]]angiotensin II (**5**) nor does it exist as an unstructured molecule.

The structural requirements involved in the cyclic conformation of Figure 5 show an interesting relation to those features which are necessary for biological activity. The hexapeptide **1** contains all of the structural features necessary for biological activity. The terminal carboxylate ion, the Phe aromatic, proline, and His imidazole rings, and the Tyr hydroxyl group are all necessary for biological activity.[2] Of these five structural features three, the Phe COO^-, His Im, and Pro, are all essential members in the intramolecular ion–dipole bond. The Phe and Tyr aromatic rings are not involved in this conformation and appear, from pmr data, to rotate freely. In spite of the mutual importance of some structural features to conformational and biological properties a correlation between peptide conformation and biological activity has not yet been established.

The importance of these structural features to biological activity may result from their contributions to peptide tertiary structure, rather than through their direct interactions with a biological receptor. It is also possible that they may serve both functions by interacting with the receptor in the stabilized peptide configuration.

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