Carbon-13 Nuclear Magnetic Resonance Studies of the Conformations of Cyclic Dipeptides

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Abstract: Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra of a number of cyclic dipeptides have been examined in dimethyl-d_6 sulfoxide solution. The ^{13}C chemical shifts of cyclic dipeptides containing aromatic amino acid residues do not always reflect the ring-current effects caused by folding of aromatic amino acid side chains over the diketopiperazine ring. This may be a consequence of conformational changes in the diketopiperazine. ^{13}C spin-lattice relaxation times (T_1) have been used as monitors of over-all molecular motion as well as monitors of intramolecular motion in the diketopiperazines. Using either an isotropic diffusion model or an anisotropic diffusion model to describe over-all molecular motion, cyclic dipeptides containing one glycine residue have T_1 values which are consistent with enhanced intramolecular motion of the α carbon of the glycyl residue when compared with that of the α carbon of the second residue in the same cyclic dipeptide. ^{13}C spin-lattice relaxation times in diketopiperazines are sensitive to the presence of solvent molecules which are strongly hydrogen bonded to the diketopiperazine.

The molecular structures and conformations of cyclic dipeptides containing the diketopiperazine ring (Figure 1) have been studied by a number of physical techniques such as X-ray crystallography,[3-7] infrared,[8-10] nuclear magnetic resonance,[11-21] and ORD and CD spectroscopy.[22,23] Quantum mechanical calculations have also been done on the cyclic dipeptides.[23-25] From these studies general rules relating to the conformations of diketopiperazines are emerging. The rules apply particularly well to cyclic peptides containing aromatic residues. Webb and Lin[3a] propose that avoidance of interference between the amino acid side chains appears to have a great influence on the diketopiperazine ring conformation. In cyclic dipeptides containing an aromatic amino acid residue maximal overlap between the diketopiperazine ring and the aromatic ring will occur.[3a] Peptides such as cyclo(Gly-X), where X is an aromatic amino acid residue, would be in the flagpole-boat conformation (Figure 1).[3a] Introduction of an amino acid residue other than glycine in such a diketopiperazine (i.e., cyclo(L-Y-L-X)) prevents the flagpole conformation and, if Y is not too bulky, an unfolded, possibly buckled, diketopiperazine ring is found.[3a] This conformation still allows interaction of X with the diketopiperazine ring without steric interference between X and Y. In cyclo(L-Y-L-Z) where Y and Z are nonaromatic, a boat form is suggested which is subject to Y-Z steric effects.

We have undertaken carbon-13 nuclear magnetic resonance (NMR) studies on a number of cyclic dipeptides. We wish to investigate the molecular flexibility of these cyclic peptides in solution by measuring the ^{13}C spin-lattice relaxation times (T_1) and to correlate these data with that obtained by ^1H NMR and X-ray crystallography. Cyclo(L-Leu-L-Trp), one of the peptides included in this study, produces the taste "bitter". Conformational studies on this compound may lead to a greater understanding of the conformational requirements of the molecular receptor for bitter taste.

Experimental Section

NMR spectra of ^{13}C in natural abundance were obtained at 25.16 MHz on a Varian XL-100-15 spectrometer and at 20 MHz on a Varian CFT-20 spectrometer operating in the pulsed Fourier transform mode with complete proton noise decoupling. The XL-100-15 spectrometer operates with a Varian 620-L computer with 16K memory. The probe temperatures were 32°C. Samples run at 25.16 MHz were contained in 12 mm o.d. tubes. Samples run at 20 MHz were contained in 10 mm o.d. tubes.

Spin-lattice relaxation times (T_1) were measured with a ±15% accuracy using the CFT-20 spectrometer using the inversion-recovery method of Freeman and Hill[26] with a (180°-τ-90°-t) pulse sequence. τ is a variable delay time and t is at least five times longer than the longest T_1 value measured. The width of a 90° pulse is 18 μsec. T_1 values were determined from a nonlinear two-parameter regression using

$$M(\tau) = M(o)(1 - 2\exp^{-\tau}/T_1)$$

where $M(o)$ is the equilibrium value of the magnetization. $M(\tau)$ is the value of the magnetization resulting from a given value of τ in the 180°-τ-90°-t sequence. Nuclear Overhauser enhancements (N.O.E.) were measured in order to indicate the extent to which the relaxation of a given carbon nucleus is caused by dipole-dipole interactions with protons. The nuclear Overhauser enhancements were measured by comparing the integrated intensities of the ^{13}C resonances in proton noise decoupled spectra and in coupled spectra. When dipole-dipole interactions provide the dominant relaxation mechanism for carbons bonded to protons, the ratio of the integrated intensities of the ^1H noise-decoupled ^{13}C resonances and the proton-coupled ^{13}C resonances should be 2.99.[27,28] The ^{13}C chemical shifts are referenced to tetramethylsilane ($(CH_3)_4Si$), contained in a capillary tube present in the sample tube.

The diketopiperazines were prepared according to the general method of Fischer[29] by cyclization of the appropriate dipeptide methyl ester in the presence of ammonia. Cyclo(L-Leu-Gly), cyclo(L-Leu-L-Leu), cyclo(L-Leu-L-Trp), and cyclo(L-Trp-Gly) were those prepared by Shiba and Nunami.[17] In addition, we prepared cyclo(L-Phe-Gly), mp 267-268° (lit.[30] mp 261-265°); cyclo(L-Tyr-Gly), mp 286-288° (lit.[31] mp 287-288.5°); and cyclo(L-Phe-L-Val). Calcd for $C_{14}H_{18}N_2O_2$: C, 68.3; H, 7.37; N, 11.4. Found: C, 68.4; H, 7.30; N, 11.6; mp 271-272°; single spot on thin-layer chromatography in the solvent systems isopropyl ether-$CHCl_3$-AcOH (6:3:1, v/v/v) and $CHCl_3$:MeOH:AcOH (14:2:1, v/v/v). ^{13}C spectra of peptide samples were run in deuterated dimethyl sulfoxide, $(CD_3)_2SO$, at concentrations of 50 mg/ml when solubility permitted. When samples were not sufficiently soluble, saturated solutions were used (i.e., cyclo(L-Leu-L-Leu), cyclo(L-Phe-L-Val)).

Results

1. ^{13}C Chemical Shifts. The ^{13}C chemical shifts of the diketopiperazines of L-Trp-L-Trp, Trp-L-Gly, L-Leu-L-Leu, L-Leu-L-Trp, L-Tyr-Gly, L-Phe-Gly, and L-Phe-L-Val in $(CD_3)_2SO$ are given in Table I. The assignments of the resonances are based on those of the free amino acids dissolved

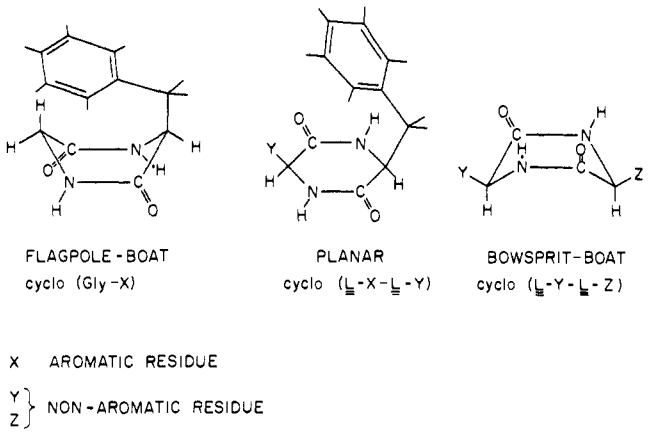

Figure 1. Possible conformations of diketopiperazines.

in $(CD_3)_2SO$;[32] the assignments of the indole ring of tryptophan are based on those of Parker and Roberts.[33] Resonances C-5 and C-6 have been interchanged[34] based on studies of indoles deuterated in position 5 and position 6. The numbering used for the indole ring of tryptophan is that of indole itself. The resonances of the indole ring of tryptophan show no influence of the nature of the second residue in the diketopiperazine. However, in cyclo(L-Trp-L-Trp) the C_β resonance of L-tryptophan is shifted downfield 0.8 ppm when compared with the same resonance in cyclo(L-Trp-Gly) or cyclo(L-Leu-L-Trp). In the diketopiperazines containing glycine and an aromatic amino acid, cyclo(L-Trp-Gly), cyclo(L-Tyr-Gly), and cyclo(L-Phe-Gly), the C_α resonance of glycine is shifted downfield ca. 1.5 ppm when compared with the same resonance in cyclo(L-Leu-Gly). In the L-leucine-containing diketopiperazines of L-Leu-Gly, L-Leu-L-Leu, and L-Leu-L-Trp, distinction can also be made between the presence or absence of an aromatic residue. In cyclo(L-Leu-L-Trp), the C_γ and one C_δ resonance of leucine are shifted upfield 0.7 and 0.4 ppm, respectively, when compared with the same resonances in cyclo(L-Leu-L-Leu) and cyclo(L-Leu-Gly). There appears to be no *consistent* direction in the effect of the presence of an aromatic amino acid residue on the ^{13}C chemical shifts of residues in diketopiperazines. It does appear, however, that the presence of an aromatic residue perturbs ^{13}C chemical shifts and that these perturbations may be due to conformational changes of the diketopiperazine ring (vide infra).

2. J_{CH} Coupling Constants. Couplings of ^{13}C to ^1H through one bond are given in Table II. One bond ^{13}C-^1H coupling constants in acyclic hydrocarbons vary as a function of s character of carbon hybridization.[35] Values of 125 and 160 Hz are expected for sp^3 and sp^2 hybridized carbons, respectively. Other factors also contribute to the observed couplings. $^1J_{CH}$ depends on the C-H bond length as

Table I. ^{13}C Chemical Shifts[a] of Diketopiperazines

	Cyclo(L-Trp-L-Trp)	Cyclo(L-Trp-Gly)	Cyclo(L-Leu-Gly)	Cyclo(L-Leu-L-Leu)	Cyclo(L-Leu-L-Trp)
Trp C-α	56.8	57.0			57.1
C-β	31.5	30.7			30.7
C-2	125.9	126.1			126.2
C-3	110.4	109.9			110.1
C-4	119.9	120.0			119.8
C-5	119.9	120.2			120.5
C-6	122.4	122.4			122.3
C-7	112.8	112.7			112.6
C-8	128.9	129.1			129.3
C-9	137.6	137.5			137.5
C=O	168.2	169.5[b]			168.7[b]
Gly C-α		45.4	43.7		
C=O		167.2[b]	167.8[b]		
Leu C-α			54.4	54.2	53.9
C-β			45.8	45.2	45.2
C-γ			25.1	25.2	24 4
C-δ			24.3	24.5	24.2
C-δ			23.3	23.3	22.9
C=O			170.2[b]	169.9	169.1[b]

	Cyclo(L-Tyr-Gly)	Cyclo(L-Phe-Gly)	Cyclo(L-Phe-L-Val)
Tyr C-α	57.3		
C-β	39.8		
C-1	127.3		
C-2	132.5		
C-3	116.5		
C-4	157.8		
C=O	169.0[b]		
Gly C-α	45.2	45.2	
C=O	167.3[b]	167.2[b]	
Phe C-α		57.0	56.7
C-β		40.3	41.0
C-1		137.5	139.6
C-2		131.6	131.8
C-3		129.6	129.5
C-4		128.2	128.0
C=O		168.7[b]	168.0[b]
Val C-α			60.8
C-β			32.6
C-γ			19.7
C-γ			17.9
C=O			168.0[b]

[a] Measured in parts per million downfield from internal tetramethylsilane. [b] Assignments could be reversed.

Table II. $^1J_{^{13}C-^1H}$ Values[a] of Diketopiperazines

	Cyclo(L-Trp-L-Trp)	Cyclo(L-Trp-Gly)	Cyclo(L-Leu-Gly)	Cyclo(L-Leu-L-Trp)	Calcd
Trp C-α	142	142		139	139
C-β	129	130		129	127
C-2	179	175		181	184
C-3					
C-4	158	159		N.O.[b]	159
C-5	158	159		158	159
C-6	158	158		N.O.[b]	159
C-7	159	N.O.[b]			159
C-8					
C-9					
Gly C-α		139	139		138
Leu C-α			142	141	138
C-β			143	139	127
C-γ			124	129	128
C-δ			125	121	124
C-δ			120	N.O.[b]	124

[a] In Hz. [b] N.O.: not observed.

well as on substituent electronegativity.[36] Malinowski[37] has proposed an expression to calculate the $^1J_{CH}$ values of substituted methanes

$$^1J_{CH} = \zeta_X + \zeta_Y + \zeta_Z \quad (1)$$

where ζ_X, ζ_Y, and ζ_Z are parameters for each substituent in the molecule CHXYZ. Using (1) we can estimate $^1J_{CH}$ values for individual amino acid residues (Table II). The following values of ζ were used: H, 41.7 Hz; NH_2, 49.6 Hz; COOH, 47.1 Hz; C_6H_5, 42.6 Hz; CH_3, 42.6 Hz.[26] A value of 42.6 Hz was used for secondary and tertiary carbon substituents (e.g., a $CH(CH_3)_2$ substituent). The $^{13}C-^1H$ couplings through one bond of the indole residue were estimated from those of benzene (159 Hz)[38] and the C-3 carbon of pyrrole (184 Hz).[39] The values observed in the diketopiperazines are consistent with the calculated values.

Couplings through two and three bonds between protons and the carbonyl carbons were detected (5-7 Hz); however, the breadth of the lines in $(CD_3)_2SO$ rendered accurate measurement difficult.

3. ^{13}C Spin-Lattice Relaxation Times. The NT_1 values of the diketopiperazines are given in Table III; N is the number of hydrogens directly bonded to each carbon. The data reveal two general trends. First, the NT_1 values of the diketopiperazines correlate with their molecular weights. Second, the presence of a glycyl residue in a diketopiperazine results in the NT_1 values of glycine up to 2 times as long as those of the α carbons of optically active amino acid residues. Furthermore, a single NT_1 value characterizes the relaxation behavior of the aromatic amino acid residues in the diketopiperazines. There is no large gradation in the NT_1 values of the resonances in the L-leucine and L-valine residues with the exception of the CH_3 groups which are free to rotate rapidly.

The nuclear Overhauser enhancements[40] were determined in cyclo(L-Leu-Gly), cyclo(L-Tyr-Gly), and cyclo(L-Leu-L-Trp). Complete Overhauser enhancements were obtained for all the proton-bearing carbons, indicating that the relaxation of these carbons is dominated by dipole-dipole interactions with protons.[41,42]

Discussion

1. ^1H Chemical Shifts. Two parameters have generally been used to deduce the conformations of cyclic dipeptides in solution from ^1H NMR data; the $^1H-N-C_\alpha-^1H_\alpha$ coupling constants[43,44] and ^1H chemical shift. Cyclic dipeptides containing an aromatic residue (e.g., cyclo(L-Tyr-Gly)) can assume a conformation in which the aromatic ring folds over the diketopiperazine. This conformation results in an upfield shift[45,46] of the protons in the second residue forming the diketopiperazine. The upfield shift is greatest for the proton which is cis to the aromatic rings.[15] A number of cyclic dipeptides have been studied by ^1H NMR, and these studies should correlate with data gained by ^{13}C NMR.

Cyclo(L-Tyr-Gly), Cyclo(L-Phe-Gly), and Cyclo(L-Trp-Gly). In the solid state, cyclo(L-Tyr-Gly) assumes a boat conformation of the diketopiperazine ring. The aromatic ring of the L-tyrosine side chain is folded over the diketopiperazine ring.[3a,7] In solution, ^1H NMR studies have shown that the glycine protons in cyclo(L-Tyr-Gly), cyclo(L-Phe-Gly), and cyclo(L-Trp-Gly) are shifted upfield when compared with glycine in diketopiperazines containing no aromatic residue. These observations are consistent with a conformation in which the aromatic residue is folded over the diketopiperazine ring.[14-16,47] The $^3J_{NH-CH}$ value of the tyrosine residue is consistent with a boat conformation of the diketopiperazine ring in cyclo(L-Tyr-Gly).

Cyclo(L-Trp-L-Trp). The ^1H NMR spectra of cyclo(L-Tyr-L-Tyr) in CF_3COOH and alkaline D_2O are consistent with a conformation in which each aromatic ring is face to face, a finding in line with results of Edelhoch et al.[48] who observed energy transfer to occur with this diketopiperazine as well as with cyclo(L-Trp-L-Trp). The aromatic rings of the Tyr residues then share the space over the diketopiperazine ring. The same conclusion has been applied to cyclo(L-Phe-L-Phe).[15] By analogy with these data a similar conformation could be predicted for cyclo(L-Trp-L-Trp), which also would support the energy transfer seen by Edelhoch et al.[39] A planar diketopiperazine above which the indole rings of each tryptophan residue face each other would allow π-π interaction between the indole rings.

Cyclo(L-Phe-L-Val). The ^1H NMR chemical shifts and coupling constants for cyclo(L-His-L-Phe) dissolved in $(CD_3)_2SO$ are consistent with a planar conformation of the diketopiperazine ring.[13] Cyclo(L-Val-L-Tyr) has been studied in $(CD_3)_2SO$[15] and the conformation in which the aromatic residue is folded over the ring is less prevalent in cyclo(L-Val-L-Tyr) than in cyclo(L-Tyr-Gly) due to the steric interference of the valyl side chain. Based on the above findings, a planar conformation of diketopiperazine and a folded conformation of the aromatic residue is predicted for cyclo(L-Phe-L-Val).

2. ^{13}C Chemical Shifts. Ring stacking of aromatic residues should produce the same shielding effects in the carbon-13 spectra as in the ^1H spectra.[49] However, stacking of aromatic residues is proving difficult to detect by ^{13}C chemical shifts. In some instances, no stacking shift is observed[50,51] and in others the values are larger than those ob-

Table III. NT_1 Values[a] of Carbons in Diketopiperazines

	Cyclo(L-Trp-L-Trp)	Cyclo(L-Trp-Gly)	Cyclo(L-Leu-Gly)	Cyclo(L-Leu-L-Trp)
Trp C-α	0.18	0.38		0.31
C-β	0.20	0.56		0.36
C-2	0.20	0.48		0.31
C-3	1.06	N.O.		
C-4	0.20	0.63[b]		0.35
C-5	0.20	0.63[b]		0.31
C-6	0.20	0.41		0.29
C-7	0.18	0.44		0.34
C-8				
C-9				
C=O				
Gly C-α		0.80	1.30	
C=O				
Leu C-α			0.90	0.26
C-β			1.14	0.42
C-γ			1.12	0.44
C-δ			3.60	2.43
C-δ			2.88	2.43
C=O				

	Cyclo(L-Tyr-Gly)	Cyclo(L-Phe-Gly)	Cyclo(L-Phe-L-Val)
Tyr C-α	0.30		
C-β	c		
C-1	N.O.		
C-2	0.32		
C-3	0.32		
C-4			
C=O	N.O.		
Gly C-α	0.58	0.97	
C=O			
Phe C-α		0.64	0.51
C-β		c	c
C-1		N.O.	N.O.
C-2		0.68	0.60
C-3		0.66	0.54
C-4		0.52	0.48
C=O			
Val C-α			0.55
C-β			0.51
C-γ			1.98
C-γ			2.13
C=O			

[a] In seconds. [b] Resonances overlapped. [c] Partially obscured by solvent.

served in the corresponding ^{1}H NMR spectra.[52,53] In mono- and dinucleotides[52,53] the ^{13}C shifts attributed to ring stacking are up to twice as large as the maximum value theoretically predicted.[54,55] The reason for the discrepancies between the anisotropic shielding (ring-current) effects on ^{1}H and ^{13}C nuclei may lie in the fact that the anisotropic shielding effect provides a large contribution to the proton chemical shift but for the carbon-13 nucleus a much smaller contribution to the chemical shift, and this contribution can be totally overshadowed by other effects.[49,56] du Vernet and Boekelheide[57] have made a correlation between anisotropic shielding effects on ^{1}H and ^{13}C chemical shifts. When factors such as geometry, hybridization, and charge are identical, ring-current effects can be monitored by comparing the ^{13}C chemical shifts of aromatic and nonaromatic systems. Furthermore these anisotropic shielding effects are of the same magnitude for ^{1}H and ^{13}C nuclei. However, if two systems which are to be compared differ in any way, other than possessing or lacking aromatic character, the contribution from the anisotropic shielding effect may not be observed in the ^{13}C chemical shift.

In cyclo(L-Trp-L-Trp) no chemical shift change is detected in the indole ring as a consequence of proximity of the two aromatic residues. The downfield shift of the β carbon may result from flattening of the diketopiperazine ring. A planar ring is necessary to allow the two tryptophan residues to lie face-to-face above the diketopiperazine. In cyclo(L-Trp-Gly), cyclo(L-Tyr-Gly), and cyclo(L-Phe-Gly), when compared with cyclo(L-Leu-Gly), downfield shifts of 1.5 ppm in the glycyl residues are observed. These shifts are in the opposite direction from those observed by ^{1}H NMR. In cyclo(L-Leu-L-Trp) the ^{13}C chemical shifts of L-leucine are shifted upfield when compared with the same resonances in cyclo(L-Leu-Gly). These shifts follow the pattern of ^{1}H chemical shifts.[17] The above observations may be the result of alterations in the geometry of diketopiperazine rings which occur when changing the nature of the side-chain substituent. The change in geometry may oppose the anisotropic shielding effect on the ^{13}C chemical shift. Consequently, the net result on the chemical shift may be less than expected from the anisotropic shielding effect or even in the opposite direction.

3. ^{13}C Spin–Lattice Relaxation Times of the Diketopiperazine Ring. Isotropic Rotational Diffusion. Spin-lattice relaxation times in liquids are influenced by fluctuating local magnetic fields of neighboring spins.[58] In the case where carbons relax via dipole-dipole interactions with protons in the same molecule, the fluctuating field is produced by molecular reorientation. Assuming over-all isotropic molecular motion, T_1 and molecular motion are related by[40,42,58]

$$\frac{1}{NT_1} = \left\langle \frac{1}{r^6} \right\rangle \hbar^2 \gamma_C^2 \gamma_H^2 \tau_{eff} \qquad (2)$$

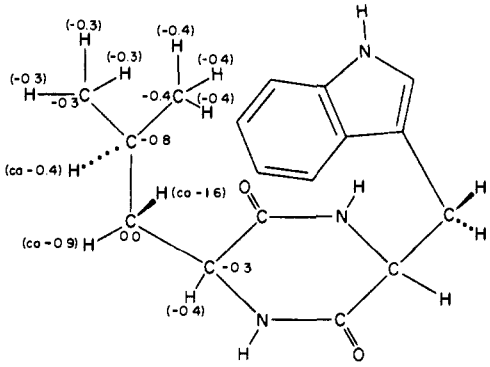

Figure 2. ^1H and ^{13}C chemical shift differences (ppm) between cyclo(L-Leu-L-Leu) and cyclo(L-Leu-L-Trp). ^1H chemical shift data obtained from ref 17. Complete ^1H spectral analysis was not performed on the cyclic dipeptides, therefore only approximate values are cited. A minus sign indicates an upfield shift.

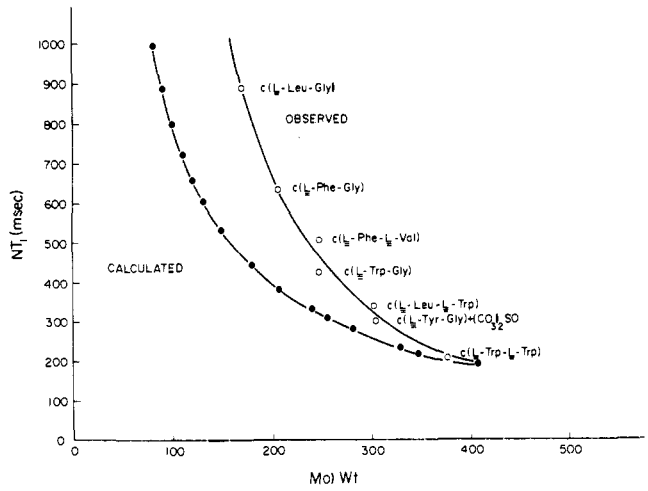

Figure 3. Observed (O) correlation between molecular weight of diketopiperazines and T_1 values of α carbons of optically active amino acids in various diketopiperazines. T_1 values calculated (●) using eq 2 with $f_r = 1$. $T = 305°$, $\rho = 1.0$, $\eta = 0.020$.

where $\langle r^{-6} \rangle$ is the vibrationally averaged inverse sixth power of the ^1H-^{13}C internuclear distance, $\hbar$ is Planck's constant divided by 2π, γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^1H, respectively, and τ_{eff} is the effective correlation time for over-all molecular reorientation. τ_{eff} can be related to the rotational diffusion constant D and the molecular friction constant β (for a sphere) by

$$\tau_{eff} = 1/6D = \beta/6kT \tag{3}$$

where k is Boltzmann's constant, T is the absolute temperature, and

$$\beta = 8\pi\eta r_0^3 f_r \tag{4}$$

η is the viscosity of the solution in poise, r_0 is the radius of the solute in ångström, and f_r is a microviscosity factor which is equal to $1/6$[59] or $1/12$[60] for pure solutes and 1 for larger molecules undergoing Brownian rotational diffusion. Rearranging (3) and (4) yields

$$\tau_{eff} = \frac{8\pi\eta r_0^3 f_r}{6kT} = \frac{V_m \eta f_r}{kT} \tag{5}$$

V_m is the molecular volume. The molecular volume is estimated to equal

$$V_m = 0.74 M_w / N_0 \rho \tag{6}$$

where N_0 is Avogadro's number, ρ is the density of the solute, M_w is the molecular weight of the solute. The factor 0.74 is introduced by the assumption that pure solute is hexagonally close packed. Equations 2, 5, and 6 predict a relationship between NT_1 values and molecular weights of the form

$$\frac{1}{NT_1} = \left\langle \frac{1}{r^6} \right\rangle \frac{\hbar^2 \gamma_C^2 \gamma_H^2 \eta 0.74 f_r M_w}{kTN_0\rho} \tag{7}$$

Figure 3 shows the curve calculated from eq 7 using a value of f_r equal 1. This value implies Brownian motion of the solute. The viscosity of the solution was assumed to be determined mainly by the solvent; therefore, η was set equal to 0.020.[61] Figure 3 also shows a plot of the experimental NT_1 values of the α carbons of nonglycine residues in the diketopiperazines. The observed NT_1 values fit smoothly on a curve, with the exception of cyclo(L-Tyr-Gly). In order to fit this compound to the curve the molecular weight of cyclo(L-Tyr-Gly) must be increased by that of the solvent molecule. This does not appear unreasonable; cyclo(L-Tyr-Gly) is the only compound in this study which has an additional chemical function group available for hydrogen-bond formation. $(CD_3)_2SO$ is a good hydrogen bond accepting solvent. From these data we can postulate that cyclo(L-Tyr-Gly) is tumbling in solution with one solvent molecule which remains hydrogen bonded to the hydroxyl group for a period of time which is long compared with the correlation time for molecular reorientation (10^{-10} sec). Other explanations are also possible: cyclo(L-Tyr-Gly) could self-associate to a certain extent and result in an apparently higher molecular weight for the monomer. In this case T_1 values would be shortened if the lifetime of the aggregate were comparable to τ_{eff}. However, such a phenomenon does not occur with any of the other cyclic dipeptides studied here. In order to test whether $(CD_3)_2SO$ hydrogen bonds strongly to hydroxyl groups, a study of more model compounds is required. The tryptophan-containing cyclic dipeptides do not show any anomalous behavior. This may be due to the greater basicity of the indole ring of tryptophan. Wessels et al.[62] in a ^1H NMR study of luteinizing hormone-releasing hormone (LH-RH), <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, have reported large changes in chemical shift for both the histidine and tyrosine residues in LH-RH when the peptide was dissolved in $(CD_3)_2SO$ rather than in D_2O. This was attributed to specific solvent interactions between the imidazole ring and $(CD_3)_2SO$ as well as between tyrosine and $(CD_3)_2SO$. No anomalies were reported for the tryptophan residue. We are now synthesizing cyclic dipeptides containing histidine and tyrosine as well as serine and threonine in order to test whether these fit the experimental curve of T_1 values vs. molecular weight.

Other peptides dissolved in $(CD_3)_2SO$ are found to fit the experimental curve. For example, the α carbon of L-leucine in L-Pro-L-Leu-Gly-NH_2 (molecular weight 284) shows a T_1 value of 0.29 sec[63] and the α carbon of L-histidine in L-Glu-L-His-L-Pro-NH_2 (molecular weight 362) has a T_1 value of 0.20 sec.[64] These protonated carbons are the most restricted in each of the peptides and represent most accurately the over-all tumbling of the molecule. By extrapolation, the experimental curve in Figure 3 should fit the solvent itself as well as large molecules. We find an experimental NT_1 value of >18 sec for the methyl groups of the solvent. This value can be fitted to the curve because the methyl groups in dimethyl sulfoxide are not believed to undergo rapid internal rotation.[60] In oxytocin, a cyclic peptide hormone of molecular weight close to 1000, we find T_1 values for the most restricted carbons bearing protons to be ca. 0.05 sec.[65] Inspection of Figure 3 shows a good correspondence for large molecules; for smaller molecules the experimental curve predicts longer NT_1 values than those cal-

Table IV. Sensitivity of T_1 Values to Molecular Dimensions Anisotropic Molecular Motion[a]

a_1	a_2	a_1/a_2	θ, deg 0	30	45	60	90
4.8	4.0	1.2	0.28	0.28	0.29	0.29	0.30
5.2	4.0	1.3	0.25	0.25	0.26	0.27	0.27
5.4	4.0	1.35	0.23	0.24	0.25	0.26	0.26
5.6	4.0	1.4	0.22	0.23	0.24	0.24	0.25
6.0	4.0	1.5	0.20	0.21	0.22	0.22	0.23
6.2	4.0	1.55	0.19	0.20	0.21	0.21	0.22
6.4	4.0	1.6	0.18	0.19	0.20	0.21	0.22
7.2	4.0	1.8	0.14	0.15	0.16	0.18	0.19
8.0	4.0	2.0	0.12	0.13	0.14	0.15	0.16
5.4	5.4	1.0	0.14	0.14	0.14	0.14	0.14
5.7	5.4	1.05	0.13	0.13	0.13	0.14	0.14
6.4	3.6	1.8	0.20	0.22	0.23	0.25	0.26

[a] Calculated as in ref 40 and 78 using $\eta = 0.020$, $T = 305$, $\rho = 1.0$, $f_r = 1.0$. a_1 and a_2 are the semiaxes of the ellipsoid of rotation used as a model of cyclic dipeptides. The lengths of the semiaxes (in angstrom) were determined on molecular space-filling models. The range of values used here comprises possible values for both folded and extended conformations of cyclic peptides used in this study. θ is the angle (in degrees) between a C–H internuclear vector and the long axis of the ellipsoid. T_1 values are in seconds.

Table V. Effective Correlation Times (τ_{eff}) for Molecular Reorientation in Cyclic Dipeptides[a]

Dipeptide	τ_{eff}, sec
Cyclo(L-Trp-L-Trp)	2.3×10^{-10}
Cyclo(L-Trp-L-Leu)	1.6×10^{-10}
Cyclo(L-Tyr-Gly)	1.5×10^{-10}
Cyclo(L-Trp-Gly)	1.0×10^{-10}
Cyclo(L-Phe-L-Val)	9.0×10^{-11}
Cyclo(L-Phe-Gly)	7.0×10^{-11}
Cyclo(L-Leu-Gly)	5.0×10^{-11}

[a] Determined from the average T_1 value for both α carbons except in glycine-containing dipeptides where only the T_1 value of the α-carbon of the optically active amino acid was used.

culated using a spherical model. This discrepancy can be removed by introducing an empirical microviscosity correction factor. The microviscosity theory of Gierer and Wirtz[59] suggests for neat liquids a microviscosity factor of 1/6. Glasel[60] using deuterium NMR suggested a value of 1/12 to explain the quadrupolar relaxation in neat liquids. For large molecules, the classical diffusion theory should apply and no correction factor is required. For molecules between these limits, an intermediate value of f_r will be required. Based upon data in Figure 3 an interpolation formula for f_r is suggested

$$f_r = -0.04 + 0.20 \text{ (mol wt solute)/(mol wt solvent)} \quad (8)$$

The discrepancy between observed and calculated values of T_1 can partially be explained by examining the assumptions which are used to establish the correlation between T_1 values and molecular weight (eq 7). It is assumed that the solute molecules are undergoing rotational diffusion in which many small steps are required to reorient the molecule by 1 radian ("small-step Brownian diffusion"). The solute is assumed to be a macroscopic body rotating in a continuous medium of viscosity η.[66,67] This model applies well to translational motions; however, in the case of rotational motions the theory applies mainly to polar highly associated liquids such as water. Rotational motions of spheroidal molecules are often characterized by long correlation times which may depend on inertial effects rather than frictional constants.[68-71]

Alms et al.[72] in a study of the ^{13}C relaxation behavior of benzene, toluene, and *p*-xylene in solutions of carbon tetrachloride, isopentane, cyclohexanol, and *tert*-butyl alcohol found the relaxation time of the solute was a function only of solution viscosity and not of the molecular nature of the solvent. Both inertial and viscous effects contributed to the relaxation of the solutes. It was proposed that in systems where solute and solvent molecules are roughly the same size, the solvent molecules at the surface of the solute do not rotate with the solute. This was termed the "slip" boundary condition for rotational diffusion (as opposed to the "stick" boundary condition which applies when the solute is much larger than the solvent[73]). This approach would yield slightly larger values of the diffusion constant and consequently larger predicted T_1 values. A recent study of molecular motion in aromatic molecules has combined depolarized Rayleigh light scattering and ^{13}C NMR data to obtain reorientation times for motion about different molecular axes.[74] In benzene the reorientation time $\tau_\perp$, for rotation about an axis in the plane of the ring (perpendicular to the symmetry axis), is consistent with the "slip" model of rotational diffusion. The value of $\tau_\parallel$, for rotation about the symmetry axis, is dominated by inertial effects. Studies on toluene and nitrobenzene have shown that rotations which take place almost within the volume of the molecule, and thus do not appreciably disturb solvent, are likely to be dominated by inertial effects.[74]

Use of the classical Stokes-Einstein equation (eq 4) for rotational diffusion predicts T_1 values which are of the same order of magnitude as those observed (Figure 3). However, deviations are found when the ratio of molecular weights of solute and solvent is less than 5. Under these conditions the classical diffusion model may not apply ("stick" model for solvent-solute interaction) and the "slip" model for solvent-solute interaction appears necessary.[75]

Anisotropic Rotational Diffusion. For diketopiperazines having large substituents rotation about the C_α–C_β bond would allow the molecule to assume an elongated shape where the ratio between the long and short axis would be greater than 2 (e.g., cyclo(L-Trp-L-Trp)). In order to calculate the effect of molecular shape on T_1 values, a model for anisotropic rotational diffusion is required.[40] Any molecule undergoing anisotropic diffusion will show smaller NT_1 values than a molecule of similar volume undergoing isotropic motion.[76] Because the observed values are appreciably larger than the predicted values for the spherical model, assuming an elongated molecular shape would lead to an even worse fit between the observed and calculated NT_1 values.

^{13}C Spin–Lattice Relaxation Times of Glycine. In the glycine-containing diketopiperazines the NT_1 value for the α carbon of glycine is consistently greater than the T_1 value observed for the other α carbons in the diketopiperazines. This fact can *only* be explained in the isotropic diffusion model by rapid internal motion.[77-79] The anisotropic diffusion model of a rigid ellipsoid would not account for differences in T_1 values of the magnitude observed for the glycine α carbons of the glycine-containing dipeptides.[40,76] However, it would predict different NT_1 values for carbons bearing one proton compared with carbons bearing two protons if the C–H vectors had different orientations with respect to the principal axes of the ellipsoid (Table IV).

The increased NT_1 values of the α carbon of glycine in peptides relative to α carbons of other amino acids may be a consequence of the lack of a sterically bulky side chain in glycine. This would allow greater conformational flexibility for glycine residues in peptides.[81] However, an alternate explanation which should not be neglected is that the average C–H bond length is longer in glycine than in other, optically active, amino acids.

^{13}C Spin–Lattice Relaxation Times of Side Chains. The side chains of the residues in the cyclic dipeptides studied here do not undergo internal motion at a greater rate than over-all molecular motion. Only the CH_3 groups show rapid internal motion.[80] Table V gives the effective correlation times for over-all molecular reorientation in the cyclic dipeptides (eq 2). In cyclo(L-Trp-L-Trp), cyclo(L-Trp-Gly), and cyclo(L-Leu-L-Trp), the side-chain carbons of tryptophan show the same NT_1 values as the α carbons. This implies that the rate of internal motion of tryptophan in each dipeptide must *not be greater* than the rate of over-all molecular motion. The aromatic rings in cyclo(L-Tyr-Gly), cyclo(L-Phe-Gly), and cyclo(L-Phe-L-Val) do not undergo internal motion at a much greater rate than over-all molecular motion. In cyclo(L-Leu-Gly), cyclo(L-Leu-L-Trp), and cyclo(L-Phe-L-Val), the CH_3 groups undergo rapid internal motion; i.e., internal motion is of the order 10^2 times faster than over-all molecular motion.

Conclusion

The effects of aromatic "ring currents" on the ^{13}C and ^{1}H chemical shifts of diketopiperazines have been compared. The absolute magnitude of anisotropic shielding effects should be the same for ^{13}C and ^{1}H chemical shifts. However, anisotropic shielding effects in the ^{13}C spectra appear overshadowed by the changes in geometry which occur in diketopiperazines upon substitution of aliphatic residues by aromatic residues. Diketopiperazines containing a glycyl residue show enhanced intramolecular mobility of the α carbon of the glycyl residue when compared with that of the α carbon of the second residue in the diketopiperazine. The increased mobility of glycine in peptides with respect to other, optically active, residues may be a direct consequence of the absence of a bulky side chain. A correlation has been found between the molecular weights of diketopiperazines and the T_1 values of the α carbons of the optically active residues. When the molecular weight of a solute molecule is less than five times that of the solvent, the correlation observed between T_1 values and molecular weights is different from that predicted for molecules undergoing Brownian rotational diffusion with so-called "stick" boundary conditions. The T_1 values of cyclo(L-Tyr-Gly) are sensitive to hydrogen bonding of the hydroxyl group to the solvent. This leads us to predict that T_1 values should be sensitive monitors of solvation in other diketopiperazines.

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Vacuum Ultraviolet Circular Dichroism of Poly(L-proline) I and II[1]

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Abstract: The vacuum ultraviolet circular dichroism of helical poly(L-proline) I and II was measured. The circular dichroism of poly(L-proline) II in trifluoroethanol was measured to 162 nm; the ellipticity is negative below 220 nm, indicating that exciton interactions are not the dominant source of circular dichroism for the π–π^* transition in this conformation. The circular dichroism of a film of poly(L-proline) II cast from aqueous solution was measured to 135 nm. In the vacuum ultraviolet region two strong negative circular dichroism bands were observed at 146 and 178 nm, and the positive band near 230 nm was observed to be more intense than it is in solution. The observation of negative ellipticity between 135 and 230 nm indicates that the π–π^* transition must be strongly coupled to transitions below 135 nm. The interconversion of poly(L-proline) I → II was studied by casting a series of films from trifluoroethanol solution 3 min, 88 min, 179 min, 291 min, and 52 hr after preparation of the solutions and measuring the circular dichroism of each film to 135 nm. A negative band was observed in poly(L-proline) I (3 min film) at 150 nm and a broad negative plateau between 165 and 175 nm indicates the presence of another band in that region. A treatment of the spectra depicting the interconversion process (poly(L-proline) I → II in trifluoroethanol) yields a first-order rate constant of 7.8×10^{-3} min^{-1} ($t^{1/2}$ = 89 min) in agreement with previous workers.

The vacuum ultraviolet circular dichroism spectra of α helical poly(γ-methyl L-glutamate) and poly(L-alanine) have been reported,[3,4] the latter from this laboratory. In this paper we report the vacuum ultraviolet circular dichroism (VUCD) of the helical conformations of poly(L-proline) I and II (PPI, PPII). In the near-ultraviolet (180–240 nm) circular dichroism (CD) of PPI a pair of oppositely signed bands are observed within the region of the π–π^* transition.[5-7] These have been understood as arising largely from exciton interactions, and calculations of the exciton contribution to π–π^* CD in this conformation are in qualitative agreement with experiment.[8-10] On the other hand, the near-ultraviolet CD of PPII is predominantly negative,[5-7] indicating either that exciton interactions are not dominant in this loosely wound helix[8-10] or that there is a positive exciton component CD band outside the range of measurement accessible with commerical instruments.[11] One purpose of this work is to show that the first of these two descriptions is the correct one. In the course of doing so, we have observed two new optically active transitions in the vacuum ultraviolet region for both PPI and PPII and have studied the interconversion of PPI to PPII in trifluoroethanol.

Experimental Section

Our instrument is the same as that used in our earlier work on poly(L-alanine)[3,12] with the exception that here we used an improved light source.[13] With this instrument the low wavelength limit is determined, in the case of solution studies, by solvent absorption and, in the case of film studies, by the combined low transmittance of the CaF_2 optical elements and of the polymer film itself. All spectra reported here were taken with a spectral slit width of 1.66 nm, a time constant of 10 sec, and a scan rate of 2 nm/min.

Poly(L-proline) obtained from Miles Laboratories, Elkhart, Ind. (Lot PR-17,MW 6730) was in form I by CD criteria.[5,6] After several days in trifluoroethanol solution conversion to form II is complete.[5] Our solution cell consisted of two CaF_2 disks separated by aluminum foil spacers. The thin layer of solution was protected from evaporation by sealing the edges with O-rings under light pressure. With such a cell we were able to obtain spectra to 162 nm, below which absorption by the solvent became excessive.

To prepare films of PPII from aqueous solution, PPI as purchased was suspended in water and periodically shaken for several days. Films cast from the clear filtrate displayed the near-ultraviolet CD characteristic of form II.[5,6] With our instrument we are able to obtain VUCD spectra of films 1000–3000 Å thick to 135 nm routinely and occasionally to 127 nm. It is known that the circular dichroism of polymer films can show birefringence effects, in that the signal obtained with such films depends upon the orientation of the film in the light path. If we observed such an orientational dependence with a film, that sample was discarded.

For the time study of the poly(L-proline) I → II interconversion process,[5] 20 mg of PPI as purchased was suspended in 4 ml of trifluoroethanol at 25°. With this procedure dissolution was complete in 4 min, which allowed us to define a t_0 for the interconversion process as being 2 min after addition of the polymer, and having an uncertainty of ±2 min. Small samples of this solution were withdrawn periodically and placed onto 1-mm thick CaF_2 disks in a nitrogen filled glove box. Evaporation of the solvent was complete within 1 min, which allowed us to define a t_f as the time at which solvent evaporation was complete and having an uncertainty of ±1 min. We adopted $t = t_f - t_0$ as the time available to the polymer for mutarotation.

Each film was further dried under a stream of dry nitrogen and