Anisotropic Rotational Diffusion and Intramolecular Motion in Cyclic Amino Acids and Peptides. An Interpretation of ^{13}C Spin–Lattice Relaxation Data[1a]

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Abstract: A nonlinear least-squares method was used to fit the observed and calculated ^{13}C relaxation times (T_1) in cyclic amino acids and peptides. The calculations of the T_1's were based on Woessner's model of rigid overall anisotropic reorientation of the molecules via rotational diffusion as the mechanism of the relaxation process. The fitting was accomplished with rotational diffusion constants and Euler angles as the adjustable parameters. For proline and acetylprolinamide the best fits lead to good agreement with the observed T_1 values but give correlation times τ which are not physically meaningful. For *cyclo*-triprolyl the correlation times are more plausible; however, the calculated T_1's for the α and δ carbons are 25% too large. Furthermore, $\tau_A/\tau_B \approx 3.5$, too large when symmetry arguments are taken into account. For *cyclo*-(L-Ser-L-Tyr) and *cyclo*-(Gly-L-Tyr) very good agreement with the observed data (using a reasonable set of τ's) can be obtained with an assumption of pseudorigid anisotropic reorientation, except for the β carbon of the seryl residue and the α carbon of glycine, respectively. For these carbons internal motion which occurs at a greater rate than overall molecular reorientation has to be premised. For *cyclo*-(L-Pro-L-Leu) the errors in the best calculated T_1's suggest an interpretation in terms of intracyclic motion of the prolyl residue, segmental motion of the leucyl side chain, and fast internal rotation of the methyl groups. Internal motion, rather than anisotropic overall tumbling, is indicated as the relation mechanism responsible for the unequal NT_1 values observed in these molecules.

In the course of studies on the motional characteristics of cyclic peptides and peptide hormones in solution,[2a] nonequivalent carbon-13 (^{13}C) spin–lattice relaxation times (T_1) have been observed for proton-bearing carbons. This nonequivalence can be interpreted in terms of isotropic overall molecular motion and additional internal flexibility for those carbons which possess the larger T_1 values.[2b-6] The purpose of this study is to ascertain whether it is possible to reproduce and interpret the observed relaxation times of cyclic amino acids, such as proline, and peptides, such as the diketopiperazines, postulating *rigid* overall motion only, either isotropic or anisotropic, but *optimized* with respect to the physical parameters of whatever model of relaxation seems most suitable.

The choice of compounds was dictated by the following considerations:

(a) Low molecular weight compounds, amino acids, and dipeptides containing "rigid" cyclic moieties, were chosen in order to restrict the number of allowed conformations. Furthermore, in cyclic dipeptides interactions resulting from charges on terminal amino and carboxyl groups are eliminated.

(b) In these compounds the effects of overall anisotropic motion could be monitored more readily than in larger, linear peptides which have a much greater range of conformational flexibility.

(c) The availability of x-ray structural data for the chosen compounds was an important factor since the atomic coordinates for a given molecular conformation were needed in the calculations.

We have assumed that the x-ray crystallographic data correspond to a *plausible* molecular conformation in solution. This is not necessarily the case (vide infra). We have also assumed that the molecules did not aggregate in solution. Again, this may or may not occur; however, considering only monomers helps to emphasize the effects of motional anisotropy. The presence of aggregates would diminish any influence the anisotropic motion may have on the measured relaxation times when internal motion takes place. Quite specific forms of aggregates, such as the stacking of a large number of molecules, would be required to produce measurable effects of anisotropic reorientation of the aggregate.

The following procedure was adopted in the calculations:

The x-ray data were used to calculate the moments of inertia of the molecules under investigation. The results enabled us to compute the expected correlation times for free rotor models of the molecules and thus test the validity of describing molecular reorientation as a rotational diffusion process. Invariably, rotational diffusion was found to be the appropriate mechanism.

We then determined the principal axes of the moment of inertia tensor for each of the molecules and made the initial assumption that these axes coincided with the corresponding principal axes of the rotational diffusion tensor. Assuming that the *effective* correlation time, τ_J^{eff} about a given axis J was proportional to the corresponding moment of inertia I_J ($\tau_J \propto I_J$), $J = A, B, C$, and that $\tau_A^{eff}:\tau_B^{eff}:\tau_C^{eff} = I_A:I_B:I_C$, the "best" values of corresponding diffusion constants D_J were found by fitting the shortest T_1 value in the molecule. Woessner's model[7] of anisotropic rotational diffusion was employed for the T_1 calculations. Trial values of the D_J's were obtained from the relation $D_J = (6\tau_J^{eff})^{-1}$. The "best" D_J's ($\mathcal{R}_K$, $K = 1, 2, 3$ in Woessner's notation) were then used in calculating the other ^{13}C–H T_1's. Note that τ_J^{eff} are *not* the τ_J of Woessner.

Next, we treated the D_J's in Woessner's model as independent, unknown parameters. A nonlinear least-squares method was used to find those values of the D_J's which would minimize the deviation between observed and calculated T_1 values.

The coincidence of the principal axes of the moment of inertia and rotational diffusion tensors is a convenient, first approximation. By allowing the two axis systems to be different, additional flexibility could be introduced into the calculated T_1's; the three Euler angles (α, β, γ)[8] that characterize the nonalignment were treated as parameters, optimizable together with the D_J's via the least-squares fitting procedure.

The results of these calculations show that the reorientation of the peptides and amino acids we studied can be well described as isotropic overall molecular motion with internal motion of particular molecular fragments. An alternate model, that of anisotropic overall molecular motion of a rigid body, can also reproduce observed T_1 values; however, the optimal D_J's are often not physically meaningful. For the molecules studied the overall shape appears to be the major factor in de-

termining the anisotropy of molecular motion. The same approach could be used in studying more polar, acyclic peptides and could yield information on solute-solvent interactions in peptides and peptide hormones.

In the cyclic dipeptides we have found good agreement between the relative ratios of the correlation times for reorientation about the principal axes and the corresponding ratios of the moments of inertia calculated about these same axes.

Materials and Experimental Methods

The physical characteristics and ^{13}C spin-lattice relaxation times of proline,[2b] acetylprolinamide,[2b] *cyclo*-(Gly-L-Tyr),[3] and *cyclo*-triprolyl[9] have ben reported. *cyclo*-(L-Pro-L-Leu) and *cyclo*-(L-Ser-L-Tyr) were gifts of Dr. Z. Grzonka (Institute of Chemistry, University of Gdansk, Poland). The dipeptides containing proline and leucine were run in $(CD_3)_2SO$ and D_2O. *cyclo*-(L-Ser-L-Tyr) was studied in $(CD_3)_2SO$.

The spin-lattice relaxation times were measured on a Varian CFT-20 spectrometer using the inversion-recovery method of Freeman and Hill.[10] Experimental conditions have been described.[3]

Theory and Computational Techniques

The moments of inertia were calculated from the x-ray crystallographic data, using the computer program XYZ (QCPE 178, modified by W. M. Murphy). The figures of molecular models were drawn from the x-ray coordinates, using the ORTEP thermal-ellipsoid plot program for crystal structure illustrations.[11]

The ^{13}C spin-lattice relaxation times (T_1) and correlation times (τ) for molecular reorientation of a rigid molecule are related by[12]

$$\frac{1}{T_1} = \frac{N}{10}\hbar^2\gamma_H^2\gamma_C^2\left\langle\frac{1}{r^6}\right\rangle[f(\omega_H - \omega_C) + 3f(\omega_C) + 6f(\omega_H + \omega_C)] \quad (1)$$

where $\langle r^{-6}\rangle$ is the vibrationally averaged inverse sixth power of the internuclear distance, γ_H and γ_C are the magnetogyric ratios of hydrogen and carbon, respectively, ω_H and ω_C are the resonance frequencies of H and C in radians/second, $\hbar$ is Planck's constant divided by 2π, and N is the number of directly bonded hydrogens. The exact functional form of the spectral density function $f(\omega)$ depends on the details of the relaxation model. For isotropic motion $f(\omega) = \tau/(1 + \omega^2\tau^2)$, for Woessner's model of anisotropic reorientation $f(\omega) = f(\omega,D_1,D_2,D_3,\lambda_1,\lambda_2,\lambda_3)$ where the D_J's are the diagonal elements of the rotational diffusion tensor in its principal axis system, and $\lambda_1,\lambda_2,\lambda_3$ are direction cosines relative to this coordinate system for the appropriate ^{13}C-H vector. The function f is of the form

$$f(\omega,D_1,D_2,D_3,\lambda_1,\lambda_2,\lambda_3) = \sum_{j=1}^{5}\frac{C_j b_j}{b_j^2 + \omega^2} \quad (2)$$

where the five b_j's are functions of the D_J's only, while the C_j's depend on either the direction cosines only or on both $\{D_J\}$ and $\{\lambda_i\}$.

The rotational correlation time of a particle has been related to both its reorientation time if considered as a free rotor (τ_{FR}) and to its rotational diffusion constant (D) if it is assumed to rotate in a continuous viscous medium. If reorientation is inertial (free rotor)[13,14]

$$\tau_{FR} \approx \frac{2\pi}{9}\left(\frac{I}{kT}\right)^{1/2} \quad (3)$$

where I is the moment of inertia, k is Boltzmann's constant, and T is the absolute temperature. Thus, if reorientation is inertial, we expect a square-root correspondence between the moments of inertia about the principal axes of the molecule and the correlation times for molecular reorientation about these same axes.

For a particle undergoing small-step Brownian rotational diffusion,[15] the relation between the correlation time for molecular reorientation and the diffusion constant is

$$\tau = \frac{1}{6D} = \frac{\beta}{6kT} \quad (4)$$

where β is the molecular friction constant. For a sphere of radius r, $\beta = 8\pi r^3\eta$, where η is the viscosity of the solution. The ratio $\psi = \tau_{eff}/\tau_{FR}$ has been proposed[16,17] as an approximate criterion for deciding whether reorientation is best described as an inertial process or as a diffusional one. Using (3) and (4)

$$\psi = \left(\frac{kT}{I}\right)^{1/2}/6D_{eff} \quad (5)$$

If $\psi \gg 1$, rotational diffusion is assumed to be dominant.

We have calculated the moments of inertia for each of the compounds studied and found the predicted values of τ_{FR} to be one or two orders of magnitude smaller than the observed "effective" values of τ. The latter were obtained directly from eq 1, using the smallest T_1 value observed for the molecule in question, and assuming isotropic, rigid overall motion and no internal motion. Thus it appears that the rotational diffusion model should work better and consequently one cannot expect a simple proportionality between the τ values calculated for rotation about each of the principal axes and the moments of inertia about these same axes. We have, however, assumed such a relation for our initial calculations and calculated the "best" values of the D_J, while maintaining the proportionality $D_A:D_B:D_C = I_A^{-1}:I_B^{-1}:I_C^{-1}$. (Inverse instead of inverse square-root dependence was assumed in order to amplify the anisotropy.) These "best" D_J's were obtained by fitting to the *shortest* T_1 in the molecule. All other ^{13}C-H T_1's were calculated with these D_J's, using Woessner's model[7] of anisotropic rotational diffusion.

If there are at least three carbon atoms with experimentally observed T_1's, and if the directional cosines of the corresponding C-H bonds are known, one can determine an optimal set of D_J's via minimization methods. In particular, nonlinear least-squares fitting procedures are most appropriate, especially since for most of the molecules we considered the T_1 data set is overdetermined.

The least-squares fitting program we developed uses the x-ray crystallographic atom coordinates to calculate the principal axis system (A, B, C) of the moment of inertia tensor. In this coordinate system the relevant direction cosines λ_1^{ij}, λ_2^{ij}, λ_3^{ij} of all C_i-H_j vectors are computed. (Since the x-ray data may have large errors in the atom positions, the relation $\Sigma_{k=1,3}[\lambda_k^{ij}]^2 = 1$ is used to check and adjust one of the λ_k^{ij}'s.) Assuming that the principal axes of the rotational diffusion tensor coincide with corresponding axes of the moment of inertia tensor, we minimize the sum F of squared deviations between experimental and calculated T_1's:

$$\min_{\{D_J\}} F = \min_{\{D_J\}}\sum_{j=1}^{N_C} W_j\left[\left\{T_1^j(\text{exptl}) - \left[\sum_{i=1}^{n_j}(T_1^{ij})^{-1}\right]^{-1}\right\}\Big/ T_1^j(\text{exptl})\right]^2 \quad (6)$$

where N_C is the number of proton-bearing carbon atoms for which T_1 values were measured, n_j is the number of C-H bonds of the jth C atom, T_1^j(exptl) is the experimentally measured relaxation time of the jth carbon, W_j is an input weight, $0 \le W_j \le 1$, and T_1^{ij} is the calculated T_1 for the $^{13}C_i$-H_j vector. The T_1^{ij} were calculated from eq 1, using Woessner's model for $f(\omega)$, eq 2.

The form of eq 6 is different from that used by Berger et al.[18] Our form (without the weight W_j) ensures that the relative errors in the calculated T_1's are comparable, irrespective of

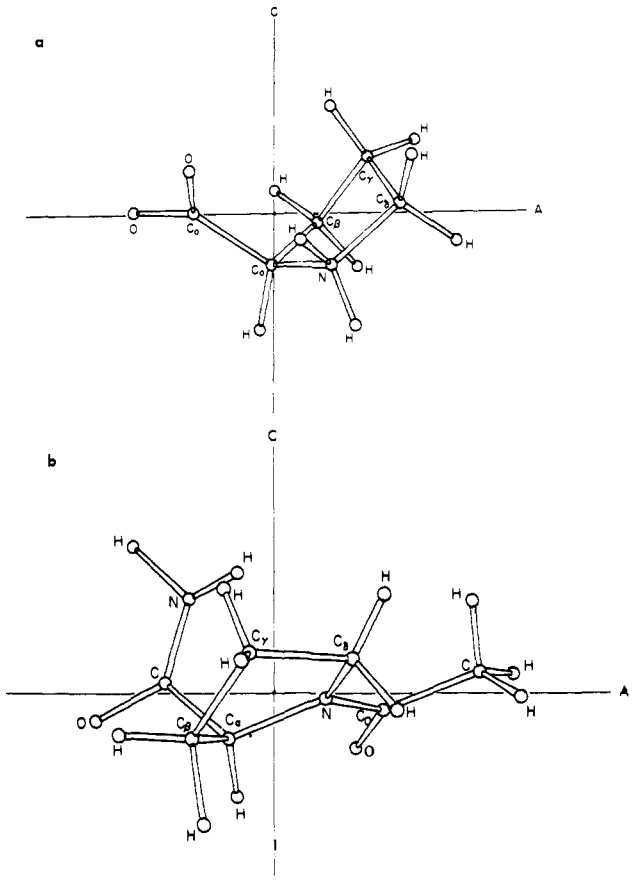

Figure 1. Molecular models of proline (a) and acetylprolinamide (b) showing principal axes of moment of inertia tensor. Models are viewed along the B axis.

the relative magnitudes of the T_1(exptl). Introducing the W_j's enabled us to remove any number of C atoms from the set to be fitted (e.g., CH_3 groups).

To minimize F of eq 6 with respect to the D_J's, it was essential to scale and constrain these variables. Physical considerations compelled us to limit the D_J's to the range $10^{13}/6$–$10^9/6$ (i.e., $10^{-13} \leq \tau_J \leq 10^{-9}$). A nonlinear transformation to new variables D_J^* leads to the equivalent but computationally more appropriate $0 \leq D_J^* \leq \pi/2$ range. This is particularly important for the next stage of the calculation.

At this stage we relax the reasonable first approximation that the principal axes of the moment of inertia tensor and the rotational diffusion tensor coincide. This approximation is less acceptable for polar molecules that can interact strongly with the solvent. The three Euler angles α, β, γ relate the principal axis system (A, B, C) of the moment of inertia tensor to the new principal axis system (A', B', C') of the rotational diffusion tensor. The alignment of the two-axes systems can be accomplished by three successive rotations: (1) about the C axis through α, (2) about the B axis through β, (3) about the C axis again through γ. The nine elements of the matrix formed from the products of these three rotation matrices are given by eq 14.10-20 of ref 8. The Euler angles are considered optimizable parameters, $-2\pi \leq \alpha, \beta, \gamma \leq 2\pi$, increasing the total number of adjustable parameters to six.

The minimization method used was that of Powell.[19] It does not require the analytical evaluation of derivatives. The minimization strategy we followed consisted of the optimization of the D_J's first, with $\alpha = \beta = \gamma = 0$, and a reasonably large set of starting values for $\{D_J\}$. The suboptimal D_J's so obtained were kept fixed, while the Euler angles were optimized. Finally all six parameters were optimized simultaneously, starting both from the sequentially obtained D_J's and angles and also from a wide range of more arbitrary initial points in the six-dimensional parameter space. Note that in the case of the six-dimensional parameter optimization care must be taken not to choose the initial set of the parameters such that $D_1 \approx D_2 \approx D_3$, since for this near-isotropic case F becomes very insensitive to the values of α, β, and γ. Only by the previously described scaling and transformation of variables could we make the minimization program work; the ten orders of magnitude disparity in angle and original D_J values would have led to premature termination of the minimization. The calculations were carried out in double precision on an IBM 360/67.

Results and Discussion

Cyclic Amino Acids. Proline and Acetylprolinamide. Molecular models of proline and acetylprolinamide are shown in Figures 1a and 1b, respectively. The axes are drawn along the principal axes of the moment of inertia tensor. The observed and calculated T_1 values are given in Tables I and II. In both cases we find that fitting the calculated T_1 value of the most restricted, the α carbon does not lead to a good fit for the β, γ, and δ carbons. These results are expected if the β, γ, and δ carbons of the ring system possess additional degrees of freedom compared with the α carbon. Calculations were also carried out using the C–H bond lengths which were deduced from the x-ray analysis.[20,21] In general these values show a probable error of ∓ 0.10 Å which in turn leads to a large error in the calculated T_1-values because of the $\langle r^{-6} \rangle$ dependence of $1/T_1$. Using the r values obtained from the x-ray data produces a larger spread in the calculated T_1 values; however, it *does not* improve the fit between calculated and observed T_1 values. It must be emphasized that these first calculations assumed the correlation time about each axis to be proportional to the moment of inertia about the same axis.

In order to obtain a better fit, we then allowed the relative value of the correlation times about the axes of the moments of inertia to be independently adjustable. For proline, the best fit is obtained for $\tau_A = 5.4 \times 10^{-10}$, $\tau_B = 5.0 \times 10^{-12}$, and $\tau_C = 2.8 \times 10^{-12}$ s. Furthermore, only small (10–12°) angular deviations from the moment of inertia axes were required for optimum fit, suggesting weak *effective* interaction with the solvent. Good (within experimental error) agreement was found between observed and calculated T_1 values assuming only overall rigid anisotropic molecular motion; however, the relative magnitudes of the best correlation times (i.e., $\tau_A \approx 100\tau_B \approx 200\tau_C$) do not seem realistic and it appears physically more meaningful to interpret the T_1 values in terms of overall (isotropic or anisotropic) reorientation and additional internal motion for the β, γ, and δ carbons. The same general results were obtained for acetylprolinamide (Table II), and the same general conclusions apply.

***cyclo*-Triprolyl.** Cyclization of three linked proline residues is expected to have a greater effect on the overall motion of the proline residues than on intracyclic motion, although the latter is influenced by the steric constraints imposed by ring closure of the tripeptide.[9] Table III shows the calculated moments of inertia for *cyclo*-triprolyl. In this compound the moments of inertia about the A and B axes are the same, whereas in proline the moments of inertia are more similar for the B and C axes. Table III shows the individual contributions of each C–H group to the observed effective T_1 values. As with the proline monomer, fitting the smallest observed T_1 value, that of the α carbon, does not yield good fits for the β, γ, and δ carbons.

The least-squares approach reduces the average errors between observed and calculated T_1 values from ca. 50 to 17%. We find the calculated T_1 values of the α and δ carbons to be 25% too small whereas the β and γ are 7–8% too large. The best values of τ are $\tau_A = 5.6 \times 10^{-11}$ s, $\tau_B = 1.5 \times 10^{-10}$ s, $\tau_C =$

Table I. Observed and Calculated ^{13}C Spin-Lattice Relaxation Times[a] of L-Proline in D_2O

		Calcd					
		Fit to smallest NT_1[b]			Least-squares fit[c]		
Carbon	Obsd T_1	Effective T_1	Components	% error (obsd − calcd)	Effective T_1	Components	% error (obsd − calcd)
α-CH	4.30	4.4	4.4	(−2.3)	4.36	4.36	−1.4
β-CH_2	3.75	1.9	3.9	49	3.68	8.09	1.9
			3.9			6.76	
γ-CH_2	4.30	1.9	3.6	56	4.25	10.21	1.2
			3.4			7.28	
δ-CH_2	3.30	1.9	3.4	42	3.52	10.21	−6.7
			4.4			5.37	

[a] T_1 values are in seconds. Calculated moments of inertia (I) and correlation times of free rotor (τ_{FR}): I_A = 138.7 amu Å^{2}n I_B = 292.6 amu Å^2, I_C = 340.0 amu Å^2, $\tau_{FR_A} = 5.2 \times 10^{-13}$ s, $\tau_{FR_B} = 7.5 \times 10^{-13}$ s, $\tau_{FR_C} = 8.1 \times 10^{-13}$ s. [b] $\tau_A = 0.7 \times 10^{-11}$, $\tau_B = \tau_C = 1.61 \times 10^{-11}$ s. [c] $\tau_A = 5.4 \times 10^{-10}$, $\tau_B = 5.0 \times 10^{-12}$, $\tau_C = 2.8 \times 10^{-12}$ s, $\alpha = -12.61°$, $\beta = 0.0°$, $\gamma = 0.0°$.

Table II. Observed and Calculated ^{13}C Spin-Lattice Relaxation Times[a] of Acetylprolinamide in D_2O (trans isomer)

		Calcd					
		Fit to smallest NT_1[b]			Least-squares fit[c]		
Carbon	Obsd T_1	Effective T_1	Components	% error (obsd − calcd)	Effective T_1	Components	% error (obsd − calcd)
α-CH	1.8	1.9	1.9	(−5.3)	2.07	2.07	−15.
β-CH_2	1.6	0.8	1.4	50.	1.40	4.94	12.5
			1.9			1.95	
γ-CH_2	1.5	0.9	1.7	40.	1.22	2.57	18.7
			1.8			2.33	
δ-CH_2	1.2	0.9	1.8	25.	1.35	2.31	−12.5
			1.7			3.25	

[a] T_1 values are in seconds. Calculated moments of inertia (I) and correlation times for free rotor (τ_{FR}): I_A = 336.6 amu Å^2, I_B = 489.1 amu Å^2, I_C = 677.8 amu Å^2, $\tau_{FR_A} = 8.0 \times 10^{-13}$ s, $\tau_{FR_B} = 9.7 \times 10^{-13}$ s, $\tau_{FR_C} = 11.4 \times 10^{-13}$ s. [b] $\tau_A = 2.00 \times 10^{-11}$, $\tau_B = 2.88 \times 10^{-11}$, $\tau_C = 4.00 \times 10^{-11}$ s. [c] $\tau_A = 1.00 \times 10^{-9}$, $\tau_B = 1.1 \times 10^{-11}$, $\tau_C = 6.4 \times 10^{-12}$ s, $\alpha = 11.46°$, $\beta = 0.0°$, $\gamma = 0.0°$.

Table III. Observed and Calculated ^{13}C Spin-lattice Relaxation Times[a] of *cyclo*-(Tri-L-prolyl) in $CDCl_3$

		Calcd									
		Fit to smallest NT_1[b]					Least-squares fit[c]				
	Obsd	Effective	Components			% error	Effective	Components			% error
Carbon	T_1	T_1	Pro-1	Pro-2	Pro-3	(obsd − calcd)	T_1	Pro-1	Pro-2	Pro-3	(obsd − calcd)
α-CH	0.620	0.61	0.61	0.61	0.61	(1.6)	0.785	0.806	0.773	0.775	−26
β-CH_2	0.505	0.28	0.50	0.50	0.50	45	0.468	1.355	1.134	1.094	7.3
			0.62	0.60	0.63			0.706	0.884	0.747	
γ-CH_2	0.585	0.27	0.50	0.56	0.53	54	0.537	0.914	1.224	1.001	8.2
			0.58	0.52	0.54			1.313	0.908	1.230	
δ-CH_2	0.545	0.28	0.66	0.50	0.50	49	0.409	1.249	1.274	0.590	−25
			0.50	0.65	0.64			0.641	0.647	1.019	

[a] T_1 values are in s. Calculated moments of inertia (I) and correlation times for free rotor (τ_{FR}): $I_A = I_B$ = 1340.9 amu Å^2, I_C = 2367.4 amu Å^2, $\tau_A = \tau_B = 1.6 \times 10^{-12}$, $\tau_C = 2.1 \times 10^{-12}$ s. [b] $\tau_A = \tau_B = 6.0 \times 10^{-11}$, $\tau_C = 10.8 \times 10^{-11}$ s. [c] $\tau_A = 5.6 \times 10^{-11}$, $\tau_B = 1.5 \times 10^{-10}$, $\tau_C = 1.9 \times 10^{-11}$ s, $\alpha = -0.17°$, $\beta = 2.29°$, $\gamma = 0.0°$.

1.9×10^{-11} s with Euler angles of $\alpha = 0.17°$, $\beta = 2.29°$, and $\gamma = 0.0°$. The τ values range over one order of magnitude, a smaller range than obtained for the proline monomers. *cyclo*-Triprolyl has a threefold axis of symmetry and it thus appears unlikely that τ_A and τ_B should differ by a factor of 3. Another solution from the least-squares fitting gave a worse fit (25%) but with τ_A more nearly equal to τ_B ($\tau_A = 8.2 \times 10^{-11}$, $\tau_B = 6.1 \times 10^{-11}$, $\tau_C = 3.5 \times 10^{-11}$ s), a physically more acceptable result. There the α carbon T_1's were overestimated by ~31% while the β, γ, and γ carbon T_1's were too low by 16-27%. Therefore we prefer to conclude that the overall motional characteristics of proline are not responsible for the differences in T_1 which we observe.

This conclusion is not altered when we assume ±15% error in the observed T_1 values. The optimum τ_J's will differ quantitatively; however, the "best" values obtained are still meaningless from the physical point of view.

Cyclic Peptides. *cyclo*-(L-Ser-L-Tyr), *cyclo*-(Gly-L-Tyr). We have previously reported that cyclic dipeptides containing one glycyl residue show unequal NT_1 values for the α carbons of the two amino acids forming the diketopiperazine ring.[3] It has been suggested that this difference in NT_1 values is the result

Table IV. Observed and Calculated ^{13}C Spin-Lattice Relaxation Times[g] in *cyclo*-(Gly-L-Tyr) in $(CD_3)_2SO$

Carbon	Obsd T_1	Fit to smallest NT_1[c,d] Effective T_1	Components	% error (obsd − calcd)	Least-squares fit[e,f] Effective T_1	Components	% error (obsd − calcd)
			Calcd ("Unsolvated")[a]				
Gly α-CH_2	0.29	0.14	0.32	52	0.19	0.40	34
			0.25			0.37	
Tyr α-CH	0.30	0.30	0.30	(0)	0.33	0.33	−10
β-CH_2	N.O.	0.36	0.36				
			0.36				
δ-C-H	0.32	0.33	0.31	−3.0	0.34	0.34	−6.3
			0.36			0.33	
ε-C-H	0.32	0.33	0.36	−3.0	0.33	0.34	−3.0
			0.31			0.32	
			Calcd ("Solvated")[b]				
Gly α-CH_2	0.29	0.14	0.41	52	0.18	0.36	38
			0.23			0.37	
Try α-CH	0.30	0.30	0.30	(0)	0.34	0.34	−13
β-CH_2	N.O.	0.43	0.43				
			0.43				
δ-CH	0.32	0.40	0.38	−25	0.32	0.33	0.0
			0.42			0.32	
ε-CH	0.32	0.40	0.42	−25	0.32	0.32	0.0
			0.37			0.33	

[a] Calculated moments of inertia (I) and correlation times for free rotor (τ_{FR}): I_A = 602.4 amu Å^2, I_B = 1280.3 amu Å^2, I_C = 1414.8 amu Å^2, $\tau_{FR_A} = 1.1 \times 10^{-12}$ s, $\tau_{FR_B} = 1.6 \times 10^{-12}$ s, $\tau_{FR_C} = 1.6 \times 10^{-12}$ s. [b] I_A = 628.5 amu Å^2, I_B = 2407.9 amu Å^2, I_C = 2522.4 amu Å^2. [c] Values for unsolvated: $\tau_A = 8.7 \times 10^{-11}$, $\tau_B = \tau_C = 2.0 \times 10^{-10}$ s. [d] Values for solvated: $\tau_A = 5.5 \times 10^{-11}$, $\tau_B = \tau_C = 2.2 \times 10^{-10}$ s. [e] Values for unsolvated: $\tau_A = 1.14 \times 10^{-10}$, $\tau_B = 1.90 \times 10^{-10}$, $\tau_C = 1.09 \times 10^{-10}$ s, $\alpha = -4.8°$, $\beta = 00°$, $\gamma = -0.1°$. [f] Values for solvated: $\tau_A = 1.28 \times 10^{-10}$, $\tau_B = 1.86 \times 10^{-10}$, $\tau_C = 1.09 \times 10^{-10}$ s, $\alpha = 11.5°$, $\beta = -2.9°$, $\gamma = 0.0°$. [g] T_1 values in s.

Table V. Observed and Calculated ^{13}C Spin-Lattice Relaxation Times[i] of *cyclo*-(L-Ser-L-Tyr) in $(CD_3)_2SO$

Carbon	Obsd T_1	Fit to T_1 of Ser Cα[c,d] Effective T_1	Components	% error (obsd − calcd)	Fit to T_1 of Tyr Cα[e,f] Effective T_1	Components	% error (obsd − calcd)	Least-squares fit[g,h] Effective T_1	Components	% error (obsd − calcd)
					Calcd ("Unsolvated")[a]					
Ser α-CH	0.260	0.26	0.26	(0)	0.23	0.23	−12	0.272	0.272	−4.6
β-CH_2	0.210	0.18	0.36	14	0.15	0.31	29	0.155	0.328	26
			0.36			0.31			0.294	
Tyr α-CH	0.290	0.34	0.34	−17	0.29	0.29	(0)	0.288	0.288	0.7
β-CH_2	0.145	0.14	0.37	3.4	0.16	0.32	−10	0.155	0.326	−6.9
			0.37			0.32			0.297	
δ-CH	0.280	0.35	0.33	−25	0.30	0.29	−7.1	0.290	0.288	−3.6
			0.37			0.32			0.292	
ε-CH	0.290	0.35	0.37	−21	0.30	0.32	−3.4	0.290	0.294	0.0
			0.33			0.29			0.287	
					Calcd ("Solvated")[b]					
Ser α-CH	0.260	0.26	0.26	(0)	0.21	0.21	19	0.273	0.273	−5.0
β-CH_2	0.210	0.21	0.43	0.0	0.18	0.35	14	0.161	0.350	23.0
			0.44			0.35			0.298	
Tyr α-CH	0.290	0.35	0.35	−21	0.28	0.28	(3.4)	0.284	0.284	2.1
β-CH_2	0.145	0.21	0.41	−45	0.17	0.33	−17	0.160	0.335	−10.
			0.44			0.36			0.307	
δ-CH	0.280	0.41	0.39	−46	0.34	0.31	−21	0.285	0.279	−1.8
			0.43			0.35			0.293	
ε-CH	0.290	0.41	0.44	−41	0.34	0.35	−17	0.285	0.296	1.7
			0.38			0.31			0.278	

[a] Calculated moments of inertia (I) and correlation times of free rotor (τ_{FR}): I_A = 816.2 amu Å^2, I_B = 1569.2 amu Å^2, I_C = 1771.3 amu Å^2, $\tau_{FR_A} = 1.2 \times 10^{-12}$ s, $\tau_{FR_B} = 1.7 \times 10^{-12}$ s, $\tau_{FR_C} = 1.8 \times 10^{-12}$ s. [b] I_A = 868.9 amu Å^2, I_B = 2804.7 amu Å^2, I_C = 3043.9 amu Å^2. [c] Values for unsolvated: $\tau_A = 9.0 \times 10^{-11}$, $\tau_B = \tau_C = 1.8 \times 10^{-10}$ s. [d] Values for solvated: $\tau_A = 6.00 \times 10^{-11}$, $\tau_B = 1.86 \times 10^{-10}$, $\tau_C = 1.98 \times 10^{-10}$ s. [e] Values for unsolvated: $\tau_A = 1.05 \times 10^{-10}$, $\tau_B = \tau_C = 2.1 \times 10^{-10}$ s. [f] Values for solvated: $\tau_A = 7.5 \times 10^{-11}$, $\tau_B = 2.3 \times 10^{-10}$, $\tau_C = 2.48 \times 10^{-10}$ s. [g] Values for unsolvated: $\tau_A = 1.26 \times 10^{-10}$, $\tau_B = 2.05 \times 10^{-10}$, $\tau_C = 1.57 \times 10^{-10}$ s, $\alpha = 10.5°$, $\beta = -0.1°$, $\gamma = 22°$. [h] Values for solvated: $\tau_A = 1.07 \times 10^{-10}$, $\tau_B = 2.54 \times 10^{-10}$, $\tau_C = 1.48 \times 10^{-10}$ s, $\alpha = 2.47°$, $\beta = 0.0°$, $\gamma = 0.0°$. [i] T_1 values in s.

of increased flexibility of the glycyl residue within the diketopiperazine ring when compared to optically active amino acids which bear bulky side chains. Tables IV and V shows the calculated and observed T_1 values for *cyclo*-(Gly-L-Tyr) and *cyclo*-(L-Ser-L-Tyr) in $(CD_3)_2SO$. The principal axes about which rotation was assumed were calculated from the x-ray data[22] and are shown in Figures 2 and 3, respectively. In both sets of calculations we have also attempted to evaluate the

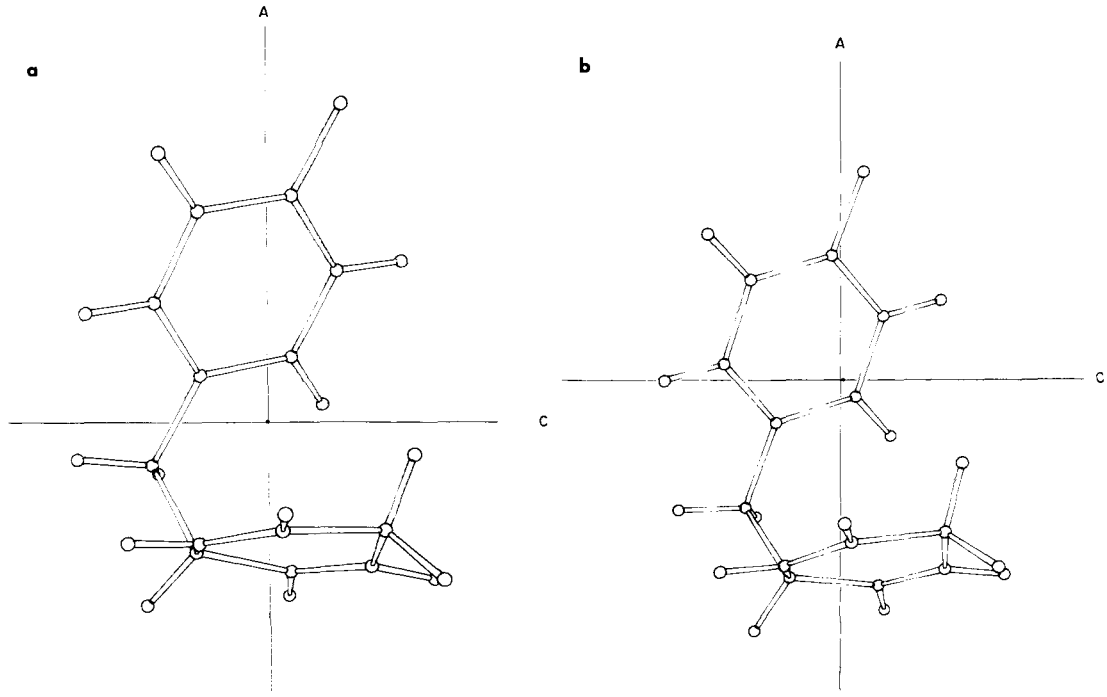

Figure 2. Molecular models of *cyclo*-(Gly-L-Tyr) (a) and "solvated" *cyclo*-(Gly-L-Tyr) showing principal axes of moment of inertia tensor.

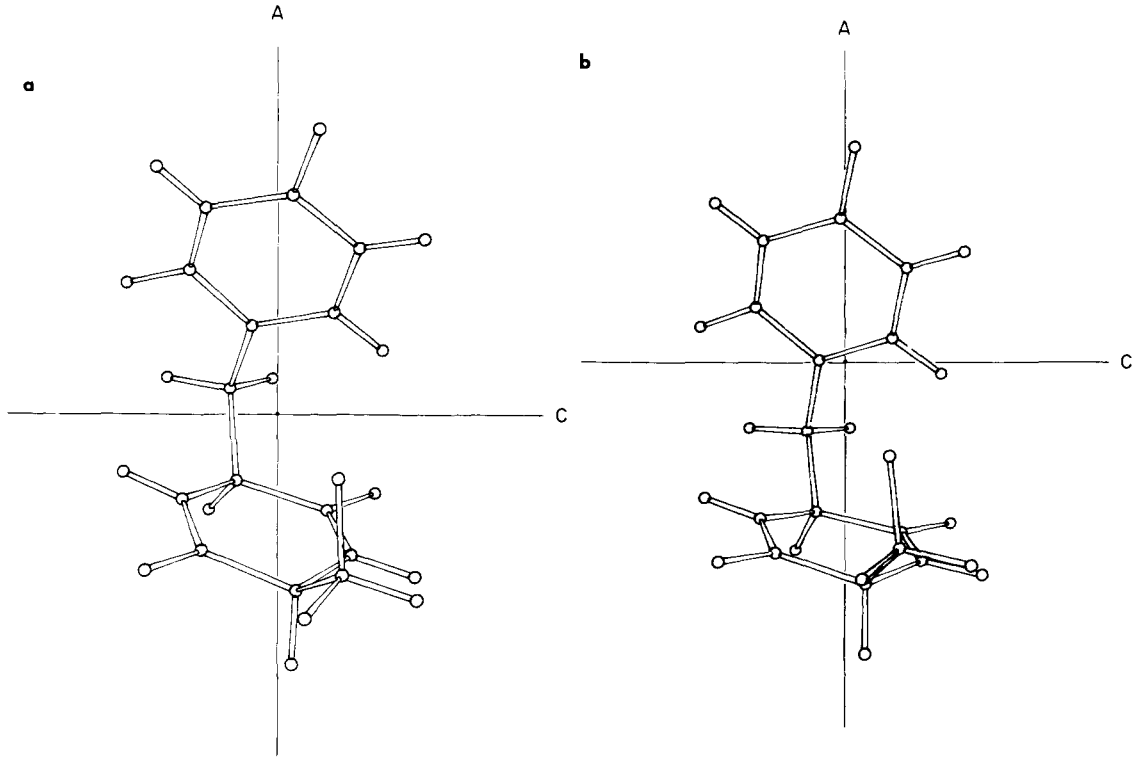

Figure 3. Molecular models of *cyclo*-(L-Ser-L-Tyr) (a) and "solvated" *cyclo*-(L-Ser-L-Tyr) showing principal axes of moment of inertia tensor.

effect of hydrogen-bonded solvent molecules. No x-ray data are available for such systems; therefore, as a first approximation, we increased the molecular weight of the hydroxyl group of tyrosine in both peptides by that of one solvent molecule. This is a very simple approximation, yet it is useful in providing plausible extremes for the values of the moments of inertia.

In a first set of calculations we attempted to reproduce the T_1 behavior of the most restricted α carbon, i.e., that of tyrosine. For "unsolvated" *cyclo*-(Gly-L-Tyr) the calculated and observed T_1 values of tyrosine agree very well. However, the value calculated for glycine is less than half the observed value. Addition of a solvent molecule does not improve the fit. It should be noted that the effect of anisotropic overall molecular motion becomes apparent in the calculated T_1 values for "solvated" *cyclo*-(Gly-L-Tyr). Whereas the α carbons of tyrosine and glycine have equal NT_1 values in the "solvated" and "unsolvated" models, the β, δ, and ϵ carbons of tyrosine show T_1 values which are ca. 25% greater in the "solvated" molecule. Such a difference would be interpreted as due to the presence of segmental motion in an isotropically reorienting molecule. A further point of interest is that the calculated NT_1 value of

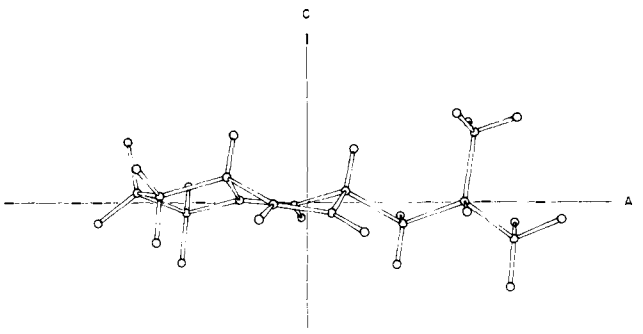

Figure 4. Molecular model of *cyclo*-(L-Pro-L-Leu) showing principal axes of moment of inertia tensor.

the β carbon of tyrosine is greater than the values for the δ and ϵ C-H groups. Such an observation has been made experimentally in the case of gramicidin-S.[23] Calculations of Birdsall et al.[24] have also reproduced this type of behavior in hexane. The calculated "effective" NT_1 values for carbons bearing more than one hydrogen can mask the effects of overall anisotropic molecular reorientation. For instance, the effective NT_1 value of the glycyl residue in "solvated" *cyclo*-(Gly-L-Tyr) is 0.29 s, a value which corresponds quite closely to that observed for the α carbon of tyrosine, 0.30 s. However, the individual contributions to the NT_1 value of the α carbon of glycine are 0.41 and 0.23 s. These data simulate an extreme case of anisotropic molecular motion and should be considered only as illustrative. We have exaggerated the anisotropy of motion by adding a solvent molecule as a point mass to the hydroxyl group which lies close to one of the principal axes of rotation and have made the relative values of the rotational correlation times directly proportional to the moments of inertia rather than the square root of the moment of inertia. We have thus shown that anisotropic molecular reorientation along the axes of the moments of inertia will not yield the different NT_1 values observed for the α carbons of the glycyl and tyrosyl residues in *cyclo*-(Gly-L-Tyr).

We next used the least-squares fit to optimize the agreement between observed and calculated T_1 values for all carbons. The τ values obtained are of the same order of magnitude for rotation about all three axes ($\tau_i = 1\text{-}2 \times 10^{-10}$ s) with only small angular changes (<12°) about the *C* axis of the moment of inertia tensor. Thus *cyclo*-(Gly-L-Tyr) is well described by an isotropic model for rotational diffusion. Again the glycyl residue has the poorest fit. The observed T_1 values of glycine are not adequately reproduced in any of the models of rotational reorientation investigated herein. This lends further support to the proposal that glycine undergoes internal motion within the diketopiperazine ring. The aromatic ring of the tyrosyl residue can rotate with respect to the diketopiperazine ring; however, the equality of the T_1 values of the α, β, ϵ, and δ carbons indicates that the rate of motion is slow compared to the rate of overall molecular reorientation.[3]

In the case of *cyclo*-(L-Ser-L-Tyr) where the α carbons of the seryl and tyrosyl residues show similar T_1 values, we performed two sets of calculations in order to fit the T_1 value of each α carbon. We obtain better correspondence between observed and calculated NT_1 values for the "unsolvated" dipeptide if the tyrosyl α carbon is used to fit the absolute values of the correlation times. The value calculated for the α carbon of the seryl residue is 12% too low. The β CH_2 group is likely to undergo internal motion and a good fit would not be expected. For the "solvated" dipeptide the fit is worse, whether the tyrosyl or the seryl α carbon is used. There is more spread in the moments of inertia and hence in the anisotropy.

The least-squares approach for fitting all the observed T_1 values gave values of τ_i between $1.3\text{-}2.1 \times 10^{-10}$ and $1.1\text{-}2.5 \times 10^{-10}$ s for the "solvated" and "unsolvated" models of *cyclo*-(L-Ser-L-Tyr), respectively. In both cases, the difference between observed and calculated T_1 values for all carbons, except the β carbon of serine, is within the experimental error of the T_1 measurement. Thus we see that for *cyclo*-(L-Ser-L-Tyr) the more isotropic molecular motion provides the best fit between observed and calculated NT_1 values and internal motion need only be postulated to explain the T_1 values of the seryl β carbon. The NT_1 values observed in the tyrosyl residues indicate that any motion of the aromatic ring, e.g., rotation with respect to the diketopiperazine ring, must be slow compared to the rate of overall molecular reorientation.

***cyclo*-(L-Pro-L-Leu).** The conformation of *cyclo*-(L-Pro-L-Leu) has been examined in the solid state[25] and the solution

Table VI. Observed and Calculated ^{13}C Spin-Lattice Relaxation Times[a] of *cyclo*-(L-Pro-L-Leu)

		Calcd					
		Fit to smallest NT_1[b]			Least-squares fit[c]		
Carbon	Obsd T_1	Effective T_1	Components	% error (obsd − calcd)	Effective T_1	Components	% error (obsd − calcd)
Pro α-CH	0.770	0.670	0.670	13	0.825	0.825	−7.1
β-CH_2	0.535	0.320	0.610	40	0.439	0.940	18
			0.670			0.820	
γ-CH_2	0.660	0.285	0.490	57	0.478	1.097	28
			0.670			0.846	
δ-CH_2	0.435	0.330	0.670	24	0.423	0.820	2.8
			0.650			0.875	
Leu α-CH	0.680	0.670	0.670	(1.5)	0.826	0.826	−21
β-CH_2	0.455	0.330	0.670	27	0.433	0.823	4.8
			0.640			0.912	
γ-CH	0.880	0.640	0.640	27	0.908	0.908	−3.2
δ-CH_3	0.820	0.190	0.670	77	0.314	0.820	62
			0.450			1.176	
			0.640			0.897	
δ-CH_3	0.750	0.193	0.400	74	0.338	1.218	55
			0.580			0.968	
			0.640			0.908	

[a] T_1 values in s. Calculated moments of inertia (I) and correlation times of free rotor (τ_{FR}): $I_A = 434.0$ amu Å^2, $I_B = 1397.4$ amu Å^2, $I_C = 1686.1$ amu Å^2, $\tau_{FR_A} = 9.1 \times 10^{-13}$ s, $\tau_{FR_B} = 1.64 \times 10^{-12}$ s, $\tau_{FR_C} = 1.80 \times 10^{-12}$ s. [b] $\tau_A = 3.8 \times 10^{-11}$, $\tau_B = 1.25 \times 10^{-10}$, $\tau_c = 1.48 \times 10^{-10}$ s. [c] $\tau_A = 4.0 \times 10^{-11}$, $\tau_B = 8.7 \times 10^{-11}$, $\tau_C = 3.3 \times 10^{-11}$ s, $\alpha = 5.7°$, $\beta = 0.0°$, $\gamma = 0.0°$.

conformation of both *cyclo*-(L-Pro-L-Leu) and *cyclo*-(L-Pro-D-Leu) is under study using ^{13}C NMR.[26] Figure 4 and Table VI show the axes and moments of inertia of *cyclo*-(L-Pro-L-Leu) with corresponding T_1 values for carbons reorienting along these axes. The least-squares approach provides a best fit for values of $\tau_A = 4.0 \times 10^{-11}$, $\tau_B = 8.7 \times 10^{-11}$, and $\tau_C = 3.3 \times 10^{-11}$ s, with Euler angles $\alpha = 5.7°$, $\beta = \gamma = 0.0°$. It can be seen that anisotropic overall molecular motion, with correlation times given in Table VI, will not account for the trends in the observed T_1 values. The worst agreements between observed and calculated T_1 values are obtained for the CH_3 groups of leucine (50-60%) with 20-30% errors for the β and γ CH_2 of proline and the α carbon of leucine. The errors reported in the table were obtained without using the experimental T_1's for the CH_3 groups. However, even when included in the observed T_1 set to be fitted, the results did not change qualitatively. We therefore choose to interpret the data in terms of intracyclic motio of the prolyl residue and of segmental motion in the side chain of the leucyl residue with fast internal rotation of the CH_3 groups.

Conclusions

A nonlinear least-squares method has been used in an attempt to fit observed and calculated ^{13}C relaxation times in cyclic amino acids and peptides. The model used was that of overall anisotropic reorientation of a rigid body.

For proline and acetylprolinamide the best fits were within experimental error; however, the correlation times giving these good agreements were not physically meaningful (ranged over two orders of magnitude). An interpretation based on internal flexibility appears more realistic.

For *cyclo*-triprolyl a reasonable set of optimum correlation times can be obtained; however, the calculated T_1's for the α and δ carbons were ~25% too large while the β and γ carbons were underestimated by 7 and 8%, respectively. Symmetry arguments indicate that τ_A should be very similar to τ_B; the optimum values gave $\tau_A/\tau_B \simeq 3.5$. Another solution of the least-squares program provides a physically more reasonable ratio ($\tau_A/\tau_B \approx 1.3$); however, the errors in the calculated T_1's were considerably larger. Again, the existence of internal flexibility might better explain the observed data.

For *cyclo*-(L-Ser-L-Tyr) the assumption of pseudorigid anisotropic motion is sufficient to give very good agreement with the observed data, except for the β carbon of the seryl residue which is expected to have rapid internal motion. Similar conclusions apply to *cyclo*-(Gly-L-Tyr), except in this case the α carbon of glycine appears to possess internal flexibility.

For *cyclo*-(L-Pro-L-Leu) the observed T_1's are best interpreted in terms of intracyclic motion of the prolyl residue and segmental motion of the leucyl side chain, with fast internal rotation of the CH_3 groups.

It is worth mentioning that all optimal fits were produced with very little or no change of the Euler angles, thus suggesting that the overall shape of these molecules is the deciding factor in determining the anisotropy of molecular motion. Related to this is the observation that for the cyclic dipeptides the relative proportions of the optimum τ values and those of the independently calculated $I^{1/2}$'s are similar, even though Brownian rotational diffusion is the dominant reorientational mechanism.

It must be emphasized that because of the intrinsic nature of the nonlinear least squares method (possibility of many minima in the 3- or 6-dimensional parameter space) any result obtained has to be regarded as a purely mathematical solution unless it is corroborated by additional physical evidence.

These studies have shown that, although interpretation of ^{13}C T_1 data on peptides *can* be made in terms of anisotropic overall molecular motion of a rigid body, physically more meaningful results can be obtained using the simpler approach of an isotropically reorienting body which undergoes internal motion. We believe these conclusions to be of importance in the interpretation of T_1 data on larger peptides and proteins since in the systems we have chosen anisotropic effects would have the most pronounced influence on the relaxation time relative to contributions from rapid internal motion. In large systems the contribution of anisotropic overall molecular motion to the observed relaxation time would be of importance only if segmental or internal motion could not occur. In all other cases, it is physically more meaningful to assume overall isotropic motion and varying degrees of internal motion in side chains and near backbone termini.

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