Cyclic Peptides. 14. Conformational Energy and Circular Dichroism of Proline-Containing Cyclic Dipeptides[1]

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Abstract: Computations of intramolecular potential energies indicate that the peptide backbone is confined to a single conformational region for a series of cyclic dipeptides which contain proline, but the side chain of the nonprolyl residue has a number of sterically reasonable rotamers. A choice among the various side chain conformers is possible from analysis of experimental spectra. Circular dichroism spectra, in conjunction with nuclear magnetic resonance data (reported elsewhere), show that *cyclo*(L-Pro-D-Tyr) and *cyclo*(L-Pro-D-Phe), in both polar and nonpolar solvents, assume a common conformer in which the aromatic ring is folded over the diketopiperazine ring. Spectral data also indicate that *cyclo*(L-Pro-L-Tyr) and *cyclo*(L-Pro-L-Phe) have a novel folded-type conformation in polar solvents such as water.

Statistical analysis of crystal structures of numerous globular proteins has shown that each amino acid shows a definite frequency of occurrence in the established types of secondary structure.[2] These conformational preferences depend to some extent on neighboring residues but are also highly dependent on the individual amino acid residue. Presumably, the physical basis of an amino acid's conformational preference is the mutual forces between its side chain and the peptide groups formed by its amino and carboxyl termini—tempered by interactions with neighboring residues and the surrounding medium.

The forces between an amino acid side chain and peptide groups can be explored by incorporating the amino acid into a molecule in which the side chain can assume only a limited number of orientations relative to the peptide groups—then determining the conformational distribution under a variety of environmental conditions. In the present study, the peptide backbone is held in a nearly constant conformation by the bicyclic ring system of cyclic dipeptides consisting of proline and various amino acids. The conformational preferences of the side chains of the second amino acid residue are then determined relative to this fixed peptide backbone. First, low-energy conformers are identified by computing intramolecular potential energies over the range of conceivable conformations. The experimental population of each of the low-energy conformers is then determined by the analysis of spectroscopic data [primarily nuclear magnetic resonance (NMR) and circular dichroism (CD) spectra].

Computed conformational energies and analysis of CD spectra are presented herein. Due to the fact that alkyl side chains possess no electronic transitions in the solution ultraviolet region, CD spectra of cyclic dipeptides with only alkyl side chains reflect the conformation of the peptide backbone rather than that of the side chain. However, since the electronic transitions of the aromatic amino acids provide a sensitive means of monitoring side-chain conformers via CD spectra, conformational inferences are presented for the cyclic dipeptides which contain aromatic groups. Discussion of alkyl side-chain conformers is deferred to the following paper.[3]

Computational and Experimental Methods

Conformational Energy. For the bi- and tricyclic systems of *cyclo*(L-Pro-Gly), *cyclo*(L-Pro-L-Pro), and *cyclo*(L-Pro-D-Pro), conformational energies were minimized using the consistent force field (CFF) method developed by Lifson and co-workers.[4] This method utilizes a complete intramolecular potential which includes terms for bond angle and length deformations as well as torsional, van der Waals, and electrostatic potentials. The parameters utilized were those employed in previous work on amides (e.g., see ref 5). The starting geometry for the prolyl residue was taken from a crystal structure of Leung and Marsh,[6] while the glycyl residue had standard Pauling–Corey geometry.[7] In order to reduce computation time, the initial dihedral angles were chosen to produce approximate closure of the diketopiperazine ring prior to minimization of the structure's energy with the CFF program. All computations were performed on the Harvard-MIT IBM 370/165 computer.

For subsequent calculations of side-chain potential energy, the bicyclic backbone was fixed in the minimum energy conformation computed for *cyclo*(L-Pro-Gly) with the CFF program. The side-chain bond lengths and bond angles were also fixed at standard values.[8] [The one exception to this procedure was *cyclo*(L-Pro-L-Leu) for which the initial geometry of the entire molecule's heavy atoms was taken from a crystal structure.[9] The hydrogen atoms were positioned relative to the heavy atoms using standard bond lengths and angles.] Intramolecular potential energies were then computed at 10° increments of the side-chain dihedral angle(s), (χ_1,χ_2).[10] To avoid the elimination of any reasonable conformers and the introduction of additional variables, the hydrogen atoms were omitted from terminal methyl groups. For the side chains the intramolecular potential energy was computed as the sum of a Lennard-Jones 6–12 (van der Waals) term with parameters from Karplus and Lifson[5b] and a torsional term for orbital interactions with a barrier height of 2.8 kcal/mol for the threefold barrier of the alkyl chains and 0.6 kcal/mol for the sixfold barrier of the benzene ring.[11] A negligible contribution was found for the coulombic interaction energy between the permanent dipoles of the relatively nonpolar alkyl or aromatic side chains with the peptide groups.

Theoretical Circular Dichroism. Optical properties were calculated using the configuration interaction theory, parameters, and computer programs developed by Schellman and co-workers.[12] The well-characterized $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions for each peptide group and the four lowest energy transitions for the tyrosine chromophore were included. (According to Platt's notation for benzene,[13] these are the ^1L_b, ^1L_a, ^1B_b, and ^1B_a transitions which occur at about 280, 223, 194, and 192 nm, respectively.) The monopole representations of the tyrosine transition moments were kindly supplied by Dr. Thomas Hooker from his molecular orbital calculations (see ref 12b). The transition energies and approximate band widths were taken from data on model compounds.[12b,14]

Experimental Circular Dichroism. The synthesis, characterization, and NMR spectra of the series of proline-containing cyclic dipeptides are reported in the accompanying paper.[3] CD measurements were performed on a Cary 60 spectropolarimeter with Model 6001 CD attachment. Most measurements were made at 20°, but additional measurements were performed at temperatures between 0 and 80° by means of a thermostated cell holder. The peptide concentration range was $0.1\text{–}2.0 \times 10^{-3}$ M. A Cary 15 spectrophotometer was used for absorption spectra. Spectral grade solvents and distilled, deionized water were employed.

In the case of *cyclo*(D-Pro-L-Tyr) the experimental CD spectra were multiplied by −1 to obtain the spectra expected for the enantiomer, *cyclo*(L-Pro-D-Tyr). This manipulation facilitates comparison of these spectra with those of the other compounds which all contain L-proline.

Table I. Minimum Energy Conformer Computed for *cyclo*(L-Pro-Gly)

Angles, deg	L-Pro[b]	Gly[b]
ϕ	−14 (−24)	−14 (−27)
ψ	26 (20)	26 (23)
ω^a	−11 (5)	−9 (2)
χ_1	−32 (−33)	
χ_2	33 (33)	
χ_3	−23 (−21)	
χ_4	3 (1)	

[a] $C_{\alpha_1}C'NC_{\alpha_2}$ dihedral angle. [b] The minimum energy conformer utilized herein was computed employing the parameters of ref 5b. The angles in parentheses are from the minimum energy conformer computed if the minima in the torsional potential for the ϕ angles are shifted from −120°,0°,120° (ref 5b) to −60°,60°,180° (ref 5a). The latter torsional potential seems in better accord with experiment. However, the small differences between the two minimum energy conformers are not critical to this work.

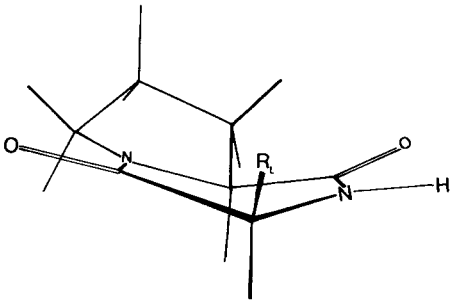

Figure 1. Schematic representation of the minimum energy boat conformer of *cyclo*(L-Pro-Gly).

Results and Discussion

Conformational Energy. Peptide Backbone. As a starting point in the study of a series of proline-containing cyclic dipeptides, the minimum energy conformation and the conformational flexibility were computed for *cyclo*(L-Pro-Gly). In the minimum energy conformer, each of the peptide groups is nearly planar, but each peptide plane is folded relative to the other so that the diketopiperazine ring is in a boat conformation (Table I and Figure 1). The conformations predicted for the pyrrolidine and diketopiperazine rings in *cyclo*(L-Pro-Gly) are similar to those previously observed in crystals of *cyclo*(L-Pro-L-Leu),[9] as well as those inferred for *cyclo*(L-Pro-L-Pro)[15] from NMR data. In contrast to the highly flexible monocyclic diketopiperazines,[5b] the bicyclic *cyclo*(L-Pro-Gly) is restricted to a narrow region of conformational space (Figure 2). Both the pyrrolidine and diketopiperazine rings are restricted to a single minimum. The dihedral angles within both the pyrrolidine and diketopiperazine rings are highly interdependent so that specification of one dihedral angle within either of the rings virtually determines the conformation of that ring. The tricyclic systems, *cyclo*(L-Pro-L-Pro) and *cyclo*(L-Pro-D-Pro), are computed to have conformational flexibility virtually identical with that of *cyclo*(L-Pro-Gly), Figure 2 (see ref 15 for the minimum energy conformers of the former two compounds).

X-Ray diffraction studies[16] on a variety of proline-containing cyclic dipeptides together with the above computations show that the proline and diketopiperazine ring conformations are essentially invariant. Thus, for subsequent calculations of side-chain conformational energy, the bicyclic system was fixed in the minimum energy conformer computed for *cyclo*(L-Pro-Gly). Side-chain bond lengths and bond angles were also fixed so that the computed van der Waals and torsional energies are functions solely of the side-chain dihedral angles. For each compound the lowest energy rotamer was arbitrarily placed at 0 kcal/mol.

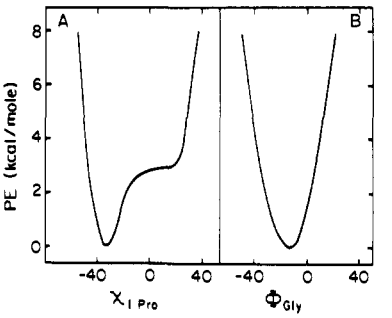

Figure 2. Variation of *cyclo*(L-Pro-Gly) potential energy about the global minimum. These graphs are indicative of the degree of conformational flexibility for the pyrrolidine ring (A) and the diketopiperazine ring (B). The potential energies were minimized on the CFF program subject to the constraint of one dihedral angle to a series of specified values: (A) χ_1 in the pyrrolidine ring constrained; (B) Φ_{Gly} (in the diketopiperazine ring) constrained.

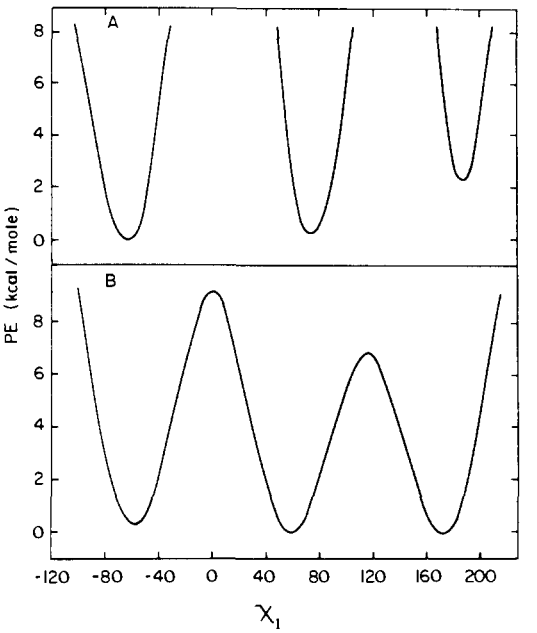

Figure 3. *cyclo*(Pro-Val) intramolecular potential energies computed as a function of the valine side-chain conformation. In this and subsequent figures the bonded geometry of the entire molecule was fixed; only side-chain dihedral angles were varied: (A) *cyclo*(L-Pro-L-Val); (B) *cyclo*(L-Pro-D-Val).

Side Chains. *Cyclo*(L-Pro-L-Val) exhibits three local energy minima as a function of χ_1 (Figure 3, A) at dihedral angles −60, 75, and 190° near those characteristic of alkyl chains which have threefold torsional barriers. While the two extended rotamers[17] (χ_1 = −60 and 75°) have comparable energy, the unfolded rotamer[17] (χ_1 = 190°) is predicted to be more than 2 kcal/mol higher. In *cyclo*(L-Pro-D-Val) the side chain is less sterically restricted as indicated by the fact that the three local minima all occur within 0.3 kcal/mol and that the regions of low energy are broader than in the case of *cyclo*(L-Pro-L-Val) (Figure 3, B).

The isoleucines and *allo*-isoleucines are β-branched as are the valines. Thus, the conformational energy of the isoleucines as a function of χ_1 is similar to that of the corresponding valine isomer. Table II shows that, for the L-Pro-L compounds, rotamers with one C_γ extended toward either the carbonyl oxygen or the amide nitrogen (i.e., $\chi_1 \simeq -60$, −60, and +60° or 60, 60, and 180° for L-Val, L-*allo*-Ile, and L-Ile, respectively)

Table II. Local Energy Minima Computed for the X Side Chain in *cyclo*(L-Pro-X)[a]

X	E_I[b]	χ_1,χ_2[c]	E_{II}	χ_1,χ_2	E_{III}	χ_1,χ_2	E_{IV}	χ_1,χ_2
L-Val[d]	0.0	−60	0.3	75	2.3	190		
L-Ile	0.0	60,170	1.7	200,160	1.8	50,60	2.5	210,70
L-*allo*-Ile	0.0	70,180	0.2	−70,190	0.7	−70,−60	1.6	70,−80
L-Phe[e]	0.0	60,90	0.8	−70,110	0.9	−160,60		
L-Leu	0.0	−70,180	0.6	−160,70	1.1	70,170	1.7	50,70
	1.7	−80,70	2.2	−80,−60	2.5	−150,170	2.7	−140,−60
D-Val[d]	0.0	60	0.0	170	0.3	−60		
D-Ile	0.0	170,190	0.3	60,180	0.4	170,−60	0.9	60,60
	1.0	−60,180	1.9	60,−90				
D-*allo*-Ile	0.0	60,170	0.3	180,190	0.5	60,60	0.5	−70,180
	0.8	180,−60	1.7	180,90	3.0	70,−80		
D-Phe[e]	0.0	−60,90	0.2	175,105	0.7	65,75		
D-Leu	0.0	70,70	0.1	170,180	1.3	170,90	1.3	80,160

[a] The potential energy of the lowest energy rotamer is arbitrarily placed at 0.0 kcal/mol. All rotamers within 3.0 kcal/mol of the minimum are listed in order of increasing energy. [b] E is the intramolecular potential energy in kcal/mol. [c] The side chain dihedral angles in degrees. [d] For the valines χ_1 alone specifies the side-chain conformation. [e] Conformational energies of the tyrosine compounds are virtually identical with those of the corresponding phenylalanine compound.

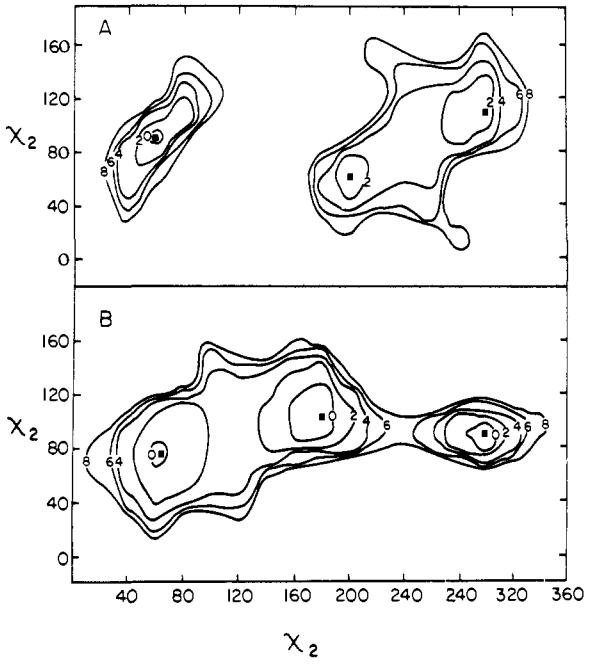

Figure 4. *cyclo*(Pro-Phe) intramolecular potential energy contours vs. side-chain dihedral angles. Contour lines are at 2 kcal/mol intervals. Local minima are indicated by ■: (A) *cyclo*(L-Pro-L-Phe); (B) *cyclo*(L-Pro-D-Phe).

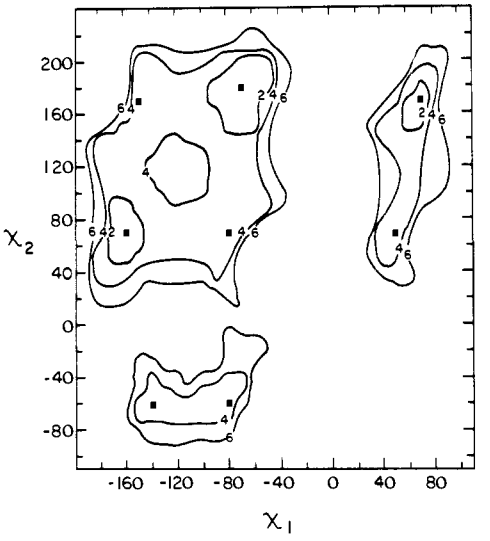

Figure 5. *cyclo*(L-Pro-L-Leu) intramolecular potential energy contours vs. side-chain dihedral angles. Contour lines are at 2 kcal/mol intervals. Local minima are indicated by ■. For this compound only, coordinates were taken from a crystal structure (ref 9).

have considerably lower energy than the unfolded rotamer ($\chi_1 \simeq 180$, 180, and −60° for the respective compounds). This latter rotamer is unstable, since one of the bulky β substituents eclipses the amide nitrogen while the other β substituent simultaneously eclipses the carbonyl group.

For the L-Pro-D compounds, the three types of χ_1 rotamers are of comparable energy with the possible exception of *cyclo*(L-Pro-D-Ile), for which the rotamer with the methyl group extended toward nitrogen and the ethyl group folded over the diketopiperazine ring ($\chi_1 = -60°$) is 1 kcal/mol above the minimum (Table II). For both the L- and D-isoleucines, the potential energy varies less rapidly as a function of χ_2 than χ_1.

The remaining compounds are branched at the γ carbon of the side chain. When the substituent is the planar benzene ring, steric interactions are rather similar for the three χ_1 rotamers for both *cyclo*(L-Pro-L-Phe) and *cyclo*(L-Pro-D-Phe) (Table II and Figure 4). For both compounds the rotamer with the aromatic ring folded over the diketopiperazine ring has slightly lower energy ($\chi_1 = 60°$ for L-Phe and −60° for D-Phe). As the *p*-hydroxy group of tyrosine in *cyclo*(Pro-Tyr) compounds is a considerable distance from the diketopiperazine and pyrrolidine rings, the *cyclo*(Pro-Tyr) compounds have computed intramolecular potential energy virtually identical with that of the corresponding *cyclo*(Pro-Phe) molecule.

In leucine the γ carbon has tetrahedral geometry and two methyl substituents. The favored χ_1 rotamers are extended, placing the C_γ adjacent to the amide nitrogen or the carbonyl oxygen. In *cyclo*(L-Pro-L-Leu) eight of the nine possible rotamers are within 3 kcal/mol of the minimum (Table II, Figure 5). However, only two of these rotamers, both of which are extended, lie within 1 kcal/mol of the minimum. As steric restrictions are more severe for *cyclo*(L-Pro-D-Leu), only four rotamers lie within 3 kcal/mol of the minimum (Table II, Figure 6). For the D-Leu compound the rotamer with C_γ folded over the diketopiperazine ring ($\chi_1 = -60°$) is not a reasonable alternative. In fact, only two of the extended rotamers are within 1 kcal/mol of the minimum.

For the compounds with alkyl side chains in the LL series, β-branching destabilizes the unfolded rotamers, while the methyl groups of the γ-branched L-leucine side chain are

Table III. Experimental CD Extrema for *cyclo*(L-Pro-X)

X	Solvent	Transition: n → π* M_θ[a]	n → π* λ[b]	M_θ[e]	λ[e]	π → π*$_1$ M_θ	π → π*$_1$ λ	π → π*$_2$ M_θ	π → π*$_2$ λ
Gly	H_2O	24 000	215					−110 000	189
	CH_3CN	11 000	228					−100 000	192
L-Ala	H_2O	5 300[c]	222			10 200	211	−58 000	189
	CH_3CN	1 800	240	−4 200	223	5 800	211	−43 000	193
L-Leu	H_2O	5 200[c]	225			9 000	212	−68 000	190
	CH_3CN			−9 000	224	5 600	209	−48 000	193
L-Val	H_2O	16 400	220					−92 000	190
	CH_3CN	7 000	232					−76 000	193
L-Pro[d]	H_2O	−10 000	220			4 800	206	−60 000	186
D-Ala	H_2O	11 200[c]	220			14 400	210	−92 000	189
	CH_3CN	7 000	233	1 600	220	11 000	209	−84 000	191
D-Leu	H_2O	3 000[c]	225			19 200	206	−64 000	189
	CH_3CN	2 800	236	−2 600	223	18 400	208	−60 000	192
D-Val	H_2O					20 000	209	−78 000	190
	CH_3CN	3 200	235			16 600	210	−74 000	192
D-Ile	H_2O					19 400	209	−70 000	190
	CH_3CN	2 200	235			17 800	211	−71 000	192
D-*allo*-Ile	H_2O					17 000	210	−55 000	190
	CH_3CN	3 300[c]	230			13 500	211	−62 000	192

[a] Molar ellipticity in degrees. [b] Wavelength in nanometers. [c] Shoulder. [d] From ref 18. [e] Assignment uncertain.

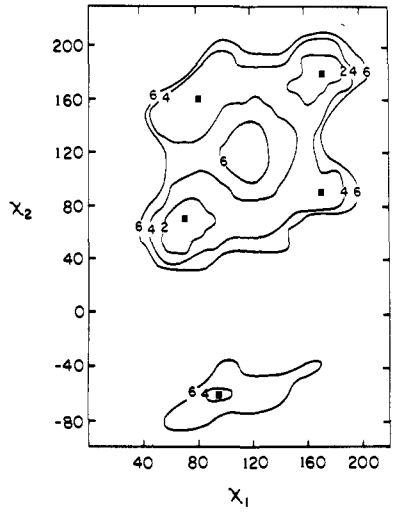

Figure 6. *cyclo*(L-Pro-D-Leu) intramolecular potential energy contours vs. side-chain dihedral angles. Contour lines are at 2 kcal/mol intervals. Local minima are indicated by ■. Cartesian coordinates for the conformation with $(\chi_1,\chi_2) = (0°,0°)$ were taken from CFF calculations on *cyclo*(L-Pro-Gly) along with standard bond lengths and angles (see Computational and Experimental Methods).

sufficiently distant from the bicyclic backbone so that all three χ_1 rotamers are predicted to have comparable stability.

In contrast for the LD diastereomers, all three χ_1 rotamers of β-branched side chains have low energies, since the side chain is folded away from the peptide backbone. Surprisingly for *cyclo*(L-Pro-D-Leu), the folded rotamer is not sterically reasonable, even though the leucine methyl groups are separated from the peptide backbone by an additional chemical bond compared to those of the β-branched compounds. Examination of molecular models shows that, while χ_2 can be adjusted to eliminate short steric contacts between the β-branched side chains and the bicyclic backbone, the γ branching of the D-leucine side chains prevents such adjustments.

Circular Dichroism. For the compounds with alkylamino acids, the observed circular dichroism should reflect the conformation of the peptide backbone. The alkyl side chains are nonpolar and have their electronic transitions in the vacuum ultraviolet region of the spectrum so that they should not contribute significantly to the CD in the 185–250-nm region. The large negative band observed near 190 nm is seen to be a constant feature of the L-proline-containing cyclic dipeptides (Table III). The average magnitude of this band is −72 000° molar ellipticity with a standard deviation less than 14 000°. For all the compounds there is a positive band to the red of the 190-nm band. In the case of the molecules containing a D-amino acid, this positive band near 210 nm has molar ellipticity of about 17 000°. These invariant features of the CD spectra are consonant with the prediction that the diketopiperazine ring in the bicyclic molecules has very little conformational freedom (Figure 2). The red shift of the longest wavelength CD band in acetonitrile, compared to water solutions, is due to the well-known solvent shift of the n → π* transition and is observed for all of the compounds [except for *cyclo*(L-Pro-L-Leu) which may have the n → π* obscured by the negative 224-nm band].

Theoretical CD predicted for the proposed boat conformation of the diketopiperazine ring (Figure 1, Table I) agrees qualitatively with that observed for *cyclo*(L-Pro-L-Pro) and *cyclo*(L-Pro-L-Leu) in nonpolar solvents (cf. Hooker et al.[18]). As pointed out above, a number of the spectral features remain constant throughout the series of compounds and these features, the negative band near 190 nm as well as the positive band near 210 nm, are in accord with theoretical predictions.

The variable features of the CD spectra (Table III) are difficult to reconcile with the rigidity of these bicyclic compounds and the CD predictive scheme utilized herein. For example, in many cases the longest wavelength CD band is observed to be positive, while a negative peptide n → π* band is predicted for the proposed boat conformation. Further, for the compounds containing alanine and leucine, there is a positive CD band near 222 nm in water solutions, but this band becomes negative in acetonitrile solutions. For *cyclo*(L-Pro-L-Ala) and *cyclo*(L-Pro-D-Leu) in acetonitrile, there seem to be four CD bands (Table III) rather than the three predicted if only two transitions are considered for each amide group (the two n → π* transitions are usually degenerate).

The anomalous optical activity discussed in the previous paragraph could be significant in refining prediction of peptide

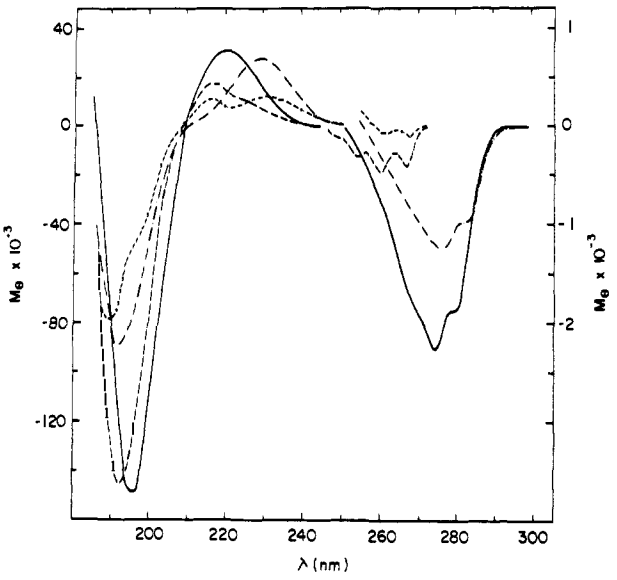

Figure 7. Experimental CD spectra of *cyclo*(L-Pro-D-Tyr) and *cyclo*(L-Pro-D-Phe) at 20°. The ordinate values are molar ellipticities. The scale on the right is for the ^1L_b band (~250–300 nm), while the scale on the left applies to the lower wavelength portion of the spectra. The spectra for the two compounds and solvents are indicated: *cyclo*(L-Pro-D-Tyr) in water, pH 3 (—); *cyclo*(L-Pro-D-Tyr) in acetonitrile (– • – -); *cyclo*(L-Pro-D-Phe) in water (– – – –); *cyclo*(L-Pro-D-Phe) in acetonitrile (- - -). Note that for each compound the longest wavelength CD bands (^1L_b) in chloroform solutions are identical with those shown for acetonitrile solution.

conformation from CD spectra. As dimerization of the molecules is unlikely under the conditions employed (peptide concentration 0.1–2.0 × 10^{-3} M, 20°) for the spectra of Table III, the anomalous CD must reflect the properties of individual peptide molecules, including any asymmetrically bound solvent molecules. It is possible that the peptide electronic transitions are altered in the strained cyclic dipeptides relative to the monoamide models upon which the CD theory is based. The CD changes observed for the cyclic dipeptides could be due to *subtle* conformational changes, especially if the planarity of the peptide groups is altered, or, alternatively, to nonconformational effects such as differing states of specific solvation. Effects such as these could result in nondegenerate n → π* transitions. Further discussion of possible sources for the anomalous optical activity of cyclic dipeptides appears in the work of Hooker et al.[18] on *cyclo*(L-Ala-L-Ala) and *cyclo*(L-Pro-L-Pro).

The above discussion of refinements to the CD theory and the conformational state of the cyclic dipeptides should not obscure the fact that the energy calculations, as well as NMR and CD spectra, all imply that the conformations of both the pyrrolidine and diketopiperazine rings remain essentially constant throughout the series.

The aromatic amino acids have optically active transitions which reflect the population of side-chain rotamers. For example, Hooker and co-workers,[12b,19a] as well as Chen and Woody,[19b] have shown that the CD of the ^1L_b transition of tyrosine (~280 nm) is sensitive to conformation and arises from dipole-dipole coupling (Kirkwood mechanism) with other electrically allowed transitions. The pattern of CD bands for *cyclo*(L-Pro-D-Phe) and *cyclo*(L-Pro-D-Tyr) is similar both in water and acetonitrile solutions (Figure 7). This resemblance of the CD spectra is a consequence of similar conformations for the two compounds and the fact that both aromatic chromophores are substituted benzenes with the same symmetry so that their electronic transition moments are in the same direction.

The CD magnitude (rotatory strength) of an electric transition will be proportional to its absorption extinction coefficient and approximately inversely proportional to the energy difference between the given transition and the one with which it interacts most strongly. In *cyclo*(L-Pro-D-Tyr) the ^1L_b transition acquires optical activity primarily through interaction with one (or both) of the amide π → π* transitions. The expression for the rotatory strength, R_1, generated from the interaction of two transitions via the Kirkwood mechanism is[20]

$$R_1 = \frac{-V_{12}\epsilon_1\epsilon_2}{\hbar c(\epsilon_2^2 - \epsilon_1^2)} [\bar{\mathbf{R}}_{21} \cdot \bar{\mu}_2 \times \bar{\mu}_1]$$

where V_{12} is the interaction potential between the two transitions, $\hbar$ is Planck's constant divided by 2π, c is the velocity of light, ϵ_1 and ϵ_2 are the transition energies, $\bar{\mathbf{R}}_{21}$ is the vector from transition moment one to two ($\bar{\mathbf{R}}_{21} = \bar{\mathbf{R}}_2 - \bar{\mathbf{R}}_1$), and $\bar{\mu}_1$ and $\bar{\mu}_2$ are the electric transition moments. In the point dipole approximation, V_{12} is proportional to the product of the transition moment magnitudes.[20] With the assumptions of a common conformation and interaction with the tertiary amide π → π*, the ratio of the ^1L_b rotatory strength of *cyclo*(L-Pro-D-Tyr) relative to that of *cyclo*(L-Pro-D-Phe) can be calculated

$$\frac{R_{\text{Tyr}}}{R_{\text{Phe}}} = \frac{\mu^2_{\text{Tyr}}\epsilon_{\text{Tyr}}/(\epsilon^2_{\pi\to\pi^*} - \epsilon^2_{\text{Tyr}})}{\mu^2_{\text{Phe}}\epsilon_{\text{Phe}}/(\epsilon^2_{\pi\to\pi^*} - \epsilon^2_{\text{Phe}})}$$

or

$$\frac{R_{\text{Tyr}}}{R_{\text{Phe}}} \simeq 0.91(\mu^2_{\text{Tyr}}/\mu^2_{\text{Phe}}) \simeq 6.8$$

using approximate transition energies of 35 700, 38 400, and 50 000 cm^{-1} (280, 265, and 200 nm) for ϵ_{Tyr}, ϵ_{Phe}, and $\epsilon_{\pi\to\pi^*}$, respectively, and approximating $\mu^2_{\text{Tyr}}/\mu^2_{\text{Phe}}$ as 7.5, the ratio of extinction coefficients at the absorption maxima.

The longest wavelength CD band (Figure 7) is due to the ^1L_b transition of the aromatic ring in both *cyclo*(L-Pro-D-Tyr) and *cyclo*(L-Pro-D-Phe). In water this negative band is five times more intense for the D-Tyr compound than for the D-Phe compound, which is comparable to the expected intensity ratio (6.8) if the two compounds exist in a common conformer.

The remaining CD bands are comparable in magnitude for the two compounds but occur at slightly different wavelengths. The ^1L_a transition of tyrosine occurs at ca. 225 nm and that of phenylalanine at ca. 210 nm.[21] Studies of model compounds have shown that the amide n → π* transition occurs at ca. 215 nm in water and near 230 nm in acetonitrile.[14] Examination of Figure 7 leads to the conclusion that both the ^1L_a and n → π* transitions have positive rotatory strengths in all four spectra. The aromatic ^1B and amide π → π* transitions occur between 180 and 205 nm. The blue shift of the minima near 190 nm in the D-Phe compound relative to those of the D-Tyr compound is similar to that observed in absorption spectra.[21]

The experimental CD data lead to the conclusion that *cyclo*(L-Pro-D-Tyr) and *cyclo*(L-Pro-D-Phe) have virtually identical conformations in each of the solvents studied (water, acetonitrile, and chloroform). In addition, it is likely that the conformations in acetonitrile are identical with those in chloroform and quite similar to those in water.

Theoretical CD was calculated in order to determine which conformational regions would be consistent with the observed CD spectra. The rotatory strengths computed for the ^1L_b transition of *cyclo*(L-Pro-D-Tyr) (Figure 8, B) show that a large negative band (as is observed) is predicted only for two regions with (χ_1,χ_2) near (40°,60°) or (300°,90°). For the region near (χ_1,χ_2) = (40°,60°) both the aromatic ^1L_a and amide n → π* transitions are predicted to have positive rotatory strength in agreement with experiment, but the predicted pattern of rotatory strengths for the higher energy transitions (−,+,+,− for the tertiary amide π → π*, the two aromatic ^1B, and the secondary amide π → π* transitions, respectively) does not agree with the experimental spectrum in the 185–205-nm region. In contrast for the region near (χ_1,χ_2) = (300°,90°)

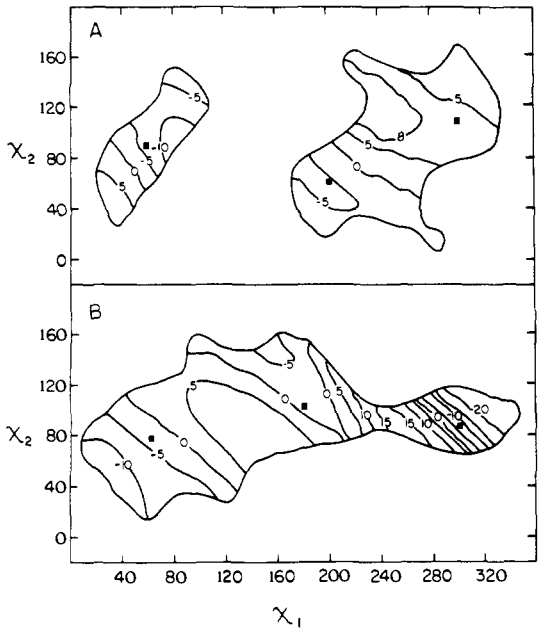

Figure 8. Theoretical rotatory strength contours for tyrosyl ^1L_b transition. The rotatory strengths are in units of Debye magnetons (μ_D) and have been multipled by 1000 in the figure. The outline enclosing the contours is the highest intramolecular potential energy contour (8 kcal/mol, see Figure 4). Local energy minima are indicated by ■: (A) *cyclo*(L-Pro-L-Tyr); (B) *cyclo*(L-Pro-D-Tyr).

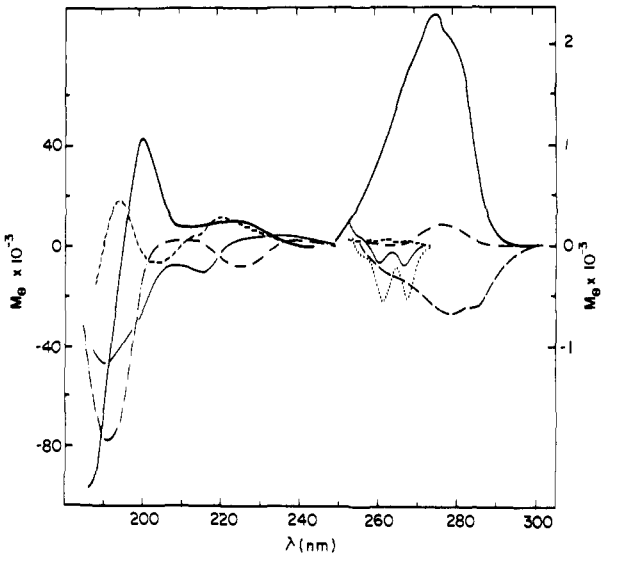

Figure 9. Experimental CD spectra of *cyclo*(L-Pro-L-Tyr) and *cyclo*(L-Pro-L-Phe) at 20°. The molar ellipticity scale on the right is for the ^1L_b band (250–300 nm), while the scale on the left applies to the remaining lower wavelength portion of the spectra. For *cyclo*(L-Pro-L-Tyr) the spectra for each of the solvents are indicated: water, pH 3 (—); acetonitrile (- · - ·); chloroform (- - - -). For *cyclo*(L-Pro-L-Phe) spectra the solvents are water (· · · ·); acetonitrile (—); chloroform (· · · ·).

in which the aromatic ring is folded over the diketopiperazine ring, the predicted rotatory strength pattern for the higher energy transitions (−,−,+,−) agrees well with the corresponding portion of the experimental spectrum. However, for this folded conformer the ^1L_a and n → π* transitions are predicted to have opposite signs (experimentally both bands appear to be positive). As a result of the strong interactions between the ^1L_a and n → π* transitions in the folded conformation, the sign of each transition's rotatory strength depends on its energy relative to the other; the lower energy transition has positive rotatory strength regardless of which transition is assigned the lower energy. Since each of the two conformers gives partial agreement between theoretical and experimental CD spectra, a choice between the two is not possible from CD data alone. NMR evidence in favor of the folded conformer (300°,90°) is presented in a companion paper.[3] Note that in the neighborhood of the folded conformer the ^1L_b rotatory strength changes rapidly with conformation (Figure 8, B). Thus, a minor conformational adjustment could produce the observed twofold reduction in the ellipticity of the 280-nm band (Figure 7) in acetonitrile relative to water solution.

In contrast to the compounds with D-aromatic amino acids, the CD spectra of both *cyclo*(L-Pro-L-Tyr) and *cyclo*(L-Pro-L-Phe) are highly sensitive to solvent (Figure 9), and in a given solvent there are distinct differences between the spectra of the L-Tyr and L-Phe compounds. For example, the ratios of the magnitudes of the ^1L_b band ellipticities are not simply related to the extinction coefficients of the respective groups (see above discussion). In fact, in acetonitrile the ^1L_b band of *cyclo*(L-Pro-L-Phe) is negative, while that of *cyclo*(L-Pro-L-Tyr) is positive. The differences in CD spectra between the two compounds in a given solvent and the variation of each compound's spectrum with solvent indicate conformational variability. Nevertheless, NMR spectra indicate that differences between the two compounds are minor.

The moderate, negative ^1L_b Cotton effects in chloroform indicate significant populations of the folded and/or extended toward oxygen conformational regions for which negative rotatory strengths are predicted, i.e., (χ_1,χ_2) near (60°,90°) and/or (200°,60°), see Figure 8, A. Analysis of NMR spectra for *cyclo*(L-Pro-L-Phe) in chloroform indicates the presence of both the folded and extended toward nitrogen conformers, (χ_1,χ_2) ≃ (60°,90°) and (310°,110°), with the latter predominating.[3] A positive ^1L_b band is predicted for the rotamer extended toward nitrogen, so that the CD results seem to indicate a slight preponderance of the folded conformer.

In methanol and water solutions the magnitude of the ^1L_b band of *cyclo*(L-Pro-L-Tyr) (Figure 9) is equal to that of *cyclo*(L-Pro-D-Tyr) (Figure 7), indicating a rotatory strength near 0.01 μ_D (Debye magneton), which is the maximum value predicted for the ^1L_b rotatory strength. Thus, the large positive ^1L_b band of *cyclo*(L-Pro-L-Tyr) in water solution indicates a relatively fixed conformer with the aromatic ring in one (or both) of the regions for which a large positive rotatory strength is predicted, (χ_1,χ_2) ≃ (40°,50°), folded, and/or (260°,120°), see Figure 8, A. The match between theoretical and experimental CD spectra is qualitatively good in the 185–210-nm region for either of the above conformational regions. However, a negative band at about 220 nm is predicted for both regions, while a positive band is observed.

Evidence from NMR spectra of *cyclo*(L-Pro-L-Tyr) in methanol favors the folded conformer, (40°,50°). At −30 °C the H_α–H_β coupling constants are 3.8 and 4.4 Hz, indicating via a Karplus-like relationship[22] that χ_1 is near 60°. In addition, one of the proline H_β's is at 0.9 ppm (δ scale) and is about 1 ppm upfield from the other H_β and the two H_γ's. The upfield shift of the proline H_β is probably due to a ring-current shift from the folding of the aromatic ring over the diketopiperazine and pyrrolidine rings.

It has been proposed that the folded conformer in aromatic diketopiperazines is stabilized by interactions between the π systems of the aromatic ring and the peptide groups.[23] In the flexible monocyclic diketopiperazines and in those of L-proline and a D-amino acid, a conformer can be attained in which the aromatic ring is folded over the diketopiperazine ring with the two rings nearly parallel and making van der Waals contacts. In such a conformer, interactions between the two π systems, e.g., from electron exchange or induced dipoles, should be independent of solvent. In contrast, the bicyclic ring system of diketopiperazines formed from L-proline and an L-amino acid seems to constrain the aromatic side chain to positions at appreciable angles and distances from the diketopiperazine ring,

so that the aromatic ring is probably more exposed to solvent in the L-Pro-L compounds than in the L-Pro-D compounds. The above observations are borne out by the fact that the conformational distribution (as inferred from CD spectra) is much more solvent sensitive for *cyclo*(L-Pro-L-Tyr) and *cyclo*(L-Pro-L-Phe) than for the corresponding LD compounds which maintain the folded conformation in both polar and nonpolar solvents.

In an attempt to determine the type of forces which stabilize the folded conformer of *cyclo*(L-Pro-L-Tyr), the temperature dependence of its CD was determined in water and methanol. In water, the maximum ^1L_b Cotton effect is observed at the lowest temperature (6°) with a progressive decrease in ellipticity as the temperature is elevated. At 80° the magnitude of the ^1L_b band is only 50% of the maximum value. This behavior would be expected if the folded conformer is stabilized by forces which release enthalpy upon folding (with relative small entropy changes). Specifically, the inverse temperature dependence of hydrophobic bonding was *not* observed.[24] (Over the limited temperature range employed, the Boltzmann factor would not alter the population of a given conformer by more than 5%.) The temperature dependence in methanol is similar to that in water. At 0° the molar ellipticity at 275 nm is 2400° (equal to that in water at 6°), while at 55° the ellipticity has decreased to 50% of this value. By contrast, the molar ellipticity of *cyclo*(L-Pro-D-Tyr) in water is less sensitive to temperature. For the LD compound the magnitude of the ^1L_b Cotton effect is decreased only 15–20% at 80° relative to the value at 6°.

Considering only intramolecular van der Waals, electrostatic, and torsional potential energies (Table II, Figure 4) of *cyclo*(L-Pro-D-Tyr) and *cyclo*(L-Pro-D-Phe), each of the rotamers about χ_1 would be approximately equally probable. However, the folded conformer $(\chi_1,\chi_2) \simeq (300°,90°)$ predominates over a range of solvents and temperatures. These observations are consistent with an additional stabilizing force (perhaps due to interactions between the π systems of the aromatic and peptide groups) in the folded conformer. On the other hand, for *cyclo*(L-Pro-L-Phe) and *cyclo*(L-Pro-L-Tyr), even though the computed intramolecular potential energies are slightly lower for the folded conformer relative to other rotamers, the conformational distribution is quite sensitive to solvent, temperature, and the addition of the hydroxyl group to the aromatic ring (to form Tyr from Phe). Polar solvents (water, methanol, and dimethyl sulfoxide) seem to favor the folded conformer of *cyclo*(L-Pro-L-Tyr)—the conformer with the lowest van der Waals' energy.

For *cyclo*(L-Pro-L-Tyr) interactions of the π systems do not seem to be the dominant force in stabilizing the folded conformer, as this molecule unfolds in relatively inert solvents such as chloroform (i.e., solvents which should favor the conformer which is most stable considering only *intra*molecular forces). However, the increased population in chloroform solution of the rotamer extended toward nitrogen would be consistent with the increased importance of dipole (N-H)-induced dipole (aromatic ring) stabilization of this conformer in a medium of low dielectric constant. Dipole-induced dipole interactions were not included in our computations.

The changes in the conformational distribution with solvent for *cyclo*(L-Pro-L-Phe) are qualitatively similar to those of *cyclo*(L-Pro-L-Tyr). In addition, the NMR spectra of *cyclo*(L-Pro-L-Tyr) and *cyclo*(L-Pro-L-Phe) are similar both in water and in chloroform solutions. Thus, the differences between the CD spectra of the two compounds may indicate subtle shifts in the relative rotamer populations, perhaps due to interactions of the tyrosyl hydroxy group with solvent molecules.

Conclusions

The bicyclic diketopiperazines which contain proline are restricted to a narrow region of conformational space. Due to the rigidity of the bicyclic system and its steric bulk, amino acid side chains can only assume conformations within a limited set. For the LL-β-branched compounds, the extended rotamers are predicted to have lower energy than the unfolded rotamers. In contrast, the three rotamers have comparable energy for the LD diastereomers. A folded conformer for *cyclo*(L-Pro-D-Tyr) and *cyclo*(L-Pro-D-Phe) is affirmed by analysis of CD and NMR spectra. This conformation may be stabilized by interactions between the aromatic and peptide π systems. A related, but novel, folded conformation is assumed by *cyclo*(L-Pro-L-Tyr) and *cyclo*(L-Pro-L-Phe) in polar solvents. However, in this latter case, the solvent dependence of the conformational distribution indicates that other forces are competitive with those stabilizing the folded conformer.

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References and Notes

(1) For the preceeding paper in this series, see C. M. Deber, V. Madison, and E. R. Blout, *Acc. Chem. Res.*, **9**, 106 (1976).
(2) (a) See, for example, B. Robson and R. H. Pain, *J. Mol. Biol.*, **58**, 237 (1971); (b) A. V. Finkelstein and O. B. Ptitsyn, *ibid.*, **62**, 613 (1971); (c) T. T. Wu and E. A. Kabat, *ibid.*, **75**, 13 (1973); (d) J. L. Crawford, W. N. Lipscomb, and C. G. Schellman, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 538 (1973); (e) P. Y. Chou and G. D. Fasman, *Biochemistry*, **13**, 222 (1974).
(3) P. E. Young, V. Madison, and E. R. Blout, *J. Am. Chem. Soc.*, following paper in this issue.
(4) (a) A. Warshel, M. Levitt, and S. Lifson, *J. Mol. Spectrosc.*, **33**, 84 (1970); (b) A. Warshel and S. Lifson, *J. Chem. Phys.*, **53**, 582 (1970); (c) the CFF computer program was obtained through the courtesy of Dr. Arieh Warshel and Dr. Bruce Gelin, who supplied subroutines to compute an energy minimum subject to constraints.
(5) (a) J. A. Schellman and S. Lifson, *Biopolymers*, **12**, 315 (1973); (b) S. Karplus and S. Lifson, *ibid.*, **10**, 1973 (1971).
(6) Y. C. Leung and R. E. Marsh, *Acta Crystallogr.*, **11**, 17 (1958).
(7) L. Pauling in "The Nature of the Chemical Bond", 3rd ed, Cornell University Press, Ithaca, N.Y., 1960, pp 281, 498.
(8) "Tables of Interatomic Distances and Configurations in Molecules and Ions", *Chem. Soc., Spec. Publ.*, **No.** 11 (1958).
(9) I. L. Karle, *J. Am. Chem. Soc.*, **94**, 81 (1972).
(10) The peptide dihedral angle nomenclature and conventions are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970).
(11) T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).
(12) (a) P. M. Bayley, E. B. Nielsen, and J. A. Schellman, *J. Phys. Chem.*, **73**, 228 (1969); (b) T. M. Hooker, Jr., and J. A. Schellman, *Biopolymers*, **9**, 1319 (1970); (c) V. Madison and J. A. Schellman, *ibid.*, 11, 1041 (1972).
(13) J. R. Platt, *J. Chem. Phys.*, 17, 484 (1949).
(14) E. B. Nielsen and J. A. Schellman, *J. Phys. Chem.*, 71, 2297 (1967).
(15) P. E. Young, V. Madison, and E. R. Blout, *J. Am. Chem. Soc.*, **95**, 6142 (1973).
(16) For a summary of the crystal studies, see Table IV in I. L. Karle, H. C. J. Ottenheym, and B. Witkop, *J. Am. Chem. Soc.*, **96**, 539 (1974).
(17) For each staggered χ_1 rotamer, the three positions for the β substituents are referred to as extended toward nitrogen, extended toward oxygen, and folded over the diketopiperazine ring. In naming rotamers, carbons are given precedence over hydrogens, so that unfolded rotamers have no carbons in the folded position. For the β-branched compounds, the so-called extended rotamers have one C_γ extended toward either the amide nitrogen or the carbonyl oxygen, while the other C_γ is folded. See the accompanying paper for a schematic representation (Figure 1 of ref 3) and a further explanation of this rotamer designation.
(18) T. M. Hooker, Jr., P. M. Bayley, W. Radding, and J. A. Schellman, *Biopolymers*, **13**, 549 (1974).
(19) (a) W. J. Goux, D. B. Cooke, R. E. Rodriguez, and T. M. Hooker, Jr., *Biopolymers*, **13**, 2315 (1974); (b) A. K. Chen and R. W. Woody, *J. Am. Chem. Soc.*, **93**, 29 (1971).
(20) J. A. Schellman, *Acc. Chem. Res.*, 1, 144 (1968).
(21) D. B. Wetlaufer, *Adv. Protein Chem.*, 17, 304 (1962).
(22) (a) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); (b) the parameters used in the Karplus equation were those proposed by A. A. Bothner-by, *Adv. Nucl. Magn. Reson.*, **1**, 195 (1965).
(23) (a) K. D. Kopple and D. H. Marr, *J. Am. Chem. Soc.*, **89**, 6193 (1967); (b) J. Vicar, M. Budesinsky, and K. Blaha, *Collect. Czech. Chem. Commun.*, **38**, 1940 (1973).
(24) W. Kauzmann, *Adv. Protein Chem.*, **14**, 1 (1959).