Proc. Nat. Acad. Sci. USA
Vol. 68, No. 6, pp. 1203–1207, June 1971

Approximate Treatment of the Conformational Characteristics of a Cyclic Nonapeptide, Cyclolinopeptide A

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Communicated by H. Mark, March 17, 1971

ABSTRACT **Possible conformations for a cyclic nonapeptide that are consistent with conformation-dependent information obtained from an NMR investigation of the peptide in solution are presented. These several conformations are deduced from the myriad of possible conformations by eliminating from consideration all cyclic species having one or more residues in a conformation that does not correspond to the vicinal coupling constants observed by NMR between the amide and α-protons. A Karplus-like relation connecting the dihedral angle φ' and the vicinal coupling $J_{N\alpha}$ between N—H and C^α—H^α is used to test this correspondence. A further reduction in the number of cyclic conformations under consideration is made possible by rejecting the conformations that have a high intramolecular conformational energy. The intramolecular conformational energy of the cyclic nonapeptide is estimated by summing the independent residue energies. These have been calculated by others with approximate potential functions to account for the intrinsic torsional potentials and the nonbonded steric (6–12 potential) and electrostatic (monopole–monopole) interactions solely dependent upon one or both of the residue rotations, φ and ψ, about the N—C^α and C^α—C bonds, respectively.**

The conformational characteristics of polypeptides in solution and in the crystal have been successfully described by approximate intramolecular potential energy calculations (1–8). Residues separated by planar *trans* amide or imide bonds (1, 6) render the potential energy of rotations φ and ψ, $E(\varphi,\psi)$, about the N–C^α and C^α–C bonds (see Fig. 1) in a given residue independent of the corresponding rotations in neighboring residues. Consequently, the total conformational energy of a polypeptide may be estimated by summing the independent residue energies, which include (6) the intrinsic 3-fold torsional potentials about the N–C^α and C^α–C bonds, the nonbonded steric repulsions and London dispersion energies (6–12 potential), and the nonbonded monopole–monopole electrostatic interactions.

If a Karplus-like relation (11) connecting the vicinal NMR coupling $J_{N\alpha}$ and the dihedral angle φ' between N–H and C^α–H^α in a peptide residue is averaged over all conformations found to be energetically favorable by the approximate energy calculations mentioned above, the correct vicinal coupling constants observed for random-coil polypeptides (9) and dipeptides (10) in solution are obtained. The agreement lends further support to these conformational energy estimates and indicates as valid the extension of a Karplus-like relation to the vicinal coupling between amide and α- protons in peptides. A combination of these two approximate theoretical tools may be useful in the conformational analysis of small proteins or polypeptides, such as the synthetic cyclic nonapeptide (12) considered in the present investigation and depicted schematically in Fig. 2.

Briefly, the conformations of each residue are restricted by allowing only those values of the rotation angle* φ (rotation about the N–C^α bond) that reproduce the measured coupling according to a Karplus-like relation (11):

$$J_{N\alpha} = \begin{bmatrix} 8.5\cos^2\varphi' & (0° \leq \varphi' \leq 90°) \\ 9.5\cos^2\varphi' & (90° \leq \varphi' \leq 180°) \end{bmatrix} \quad (1)$$

or (13)

$$J_{N\alpha} = 8.9\cos^2\varphi' - 0.9\cos\varphi' + 0.9\sin^2\varphi'. \quad (2)$$

For each value of φ, only those values of ψ corresponding to energetically favorable residue conformations, as determined by the approximate potential energy calculations, are chosen. The range of conformations is further reduced by eliminating those that do not form a closed ring or cyclic nonapeptide.

DETAILS OF CALCULATION

All amide and imide bonds are assumed to be planar *trans*, and the residue bond-lengths and valence angles used in the conformational energy calculations (1) are adopted. The φ angles in Pro_1 and Pro_2 are assigned† the values 102° and 122°, respectively, according to the crystallographic analysis of poly(L-proline) (16, 17) and L-leucyl-L-prolyl-glycine (18). ψ_{Pro_1} = 310° and ψ_{Pro_2} = 125° or 325° were chosen for the prolyl residues. These values of ψ correspond to the calculated energy minima (7, 8) for an L-prolyl residue succeeded by another L-prolyl residue, and for an L-prolyl residue succeeded by any residue other than prolyl, respectively.

* The angles of rotation φ and ψ (see Fig. 1) are taken (14) as zero in the *trans* or planar zig-zag conformation and are measured in a right-handed sense. The dihedral angle φ' between N–H and C^α–H^α is directly related to the rotation angle φ. Recently, a new convention defining the rotations about the N–C^α and C^α–C bonds has been proposed (15). I fail to see any rational improvement in the new convention, which assigns $\varphi=\psi=180°$ in the planar zig-zag conformation. I therefore retain the definitions described above.

† Pro_1 is succeeded by Pro_2, and its pyrrolidine ring should therefore have the poly(L-proline) (II) geometry (16, 17) (see ref. 7). Pro_2 is not succeeded by a prolyl residue, so that its pyrrolidine ring should have the geometry of an isolated prolyl residue (18) (see ref. 8).

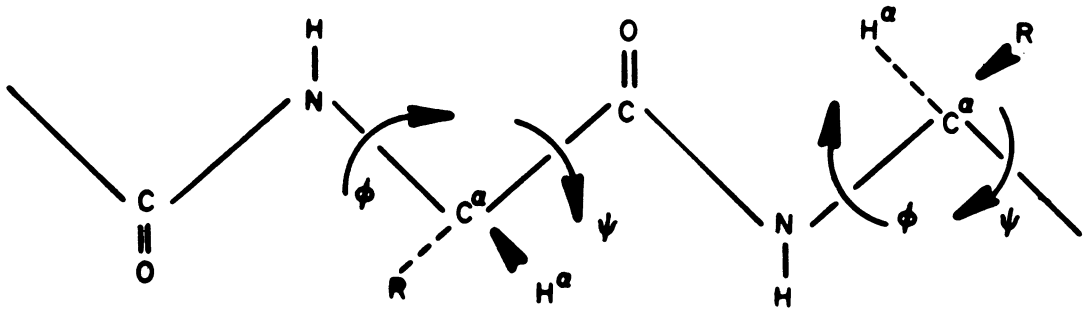


FIG. 1. A schematic representation of a portion of a poly(L-peptide) in the planar *trans* conformation.

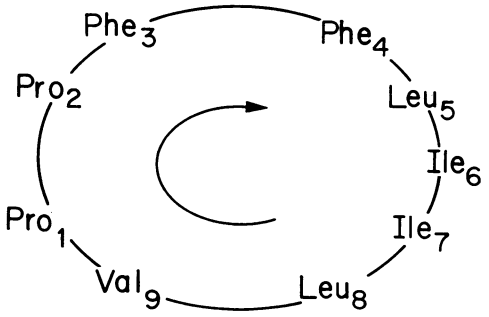


FIG. 2. A schematic representation of cyclolinopeptide A, where the sense of the arrow indicates movement from N to C^{α} in each residue.

Five of the seven residues with amide protons are observed (12) to have amide to α-proton vicinal-couplings in the range $J_{N\alpha} = 7.0$–9.0 Hz in solution, while the two remaining residues show a coupling of 6.0 Hz. Spin-decoupling analyses limit the number of residues with the smaller couplings to the following six pairs: Phe_3 or Phe_4 and Val_9, Phe_3 or Phe_4 and Ile_6, and Phe_3 or Phe_4 and Ile_7. Consequently, six different sets of conformations are tested for ring closure.

In each of the six sets, one of the above-mentioned pairs of residues is assigned $\varphi = 100°$ ($\varphi' = 140°$), while in the five remaining residues $\varphi = 90°$ ($\varphi' = 150$) is adopted. According to Eqs. **1** and **2**, $\varphi = 100°$ corresponds to $J_{N\alpha} = 5.6$ and 6.4 Hz, respectively, and $\varphi = 90°$ yields $J_{N\alpha} = 7.2$ and 7.8 Hz, respectively. $\varphi = 20°$, 200°, and 280° ($\varphi' = 140°$,

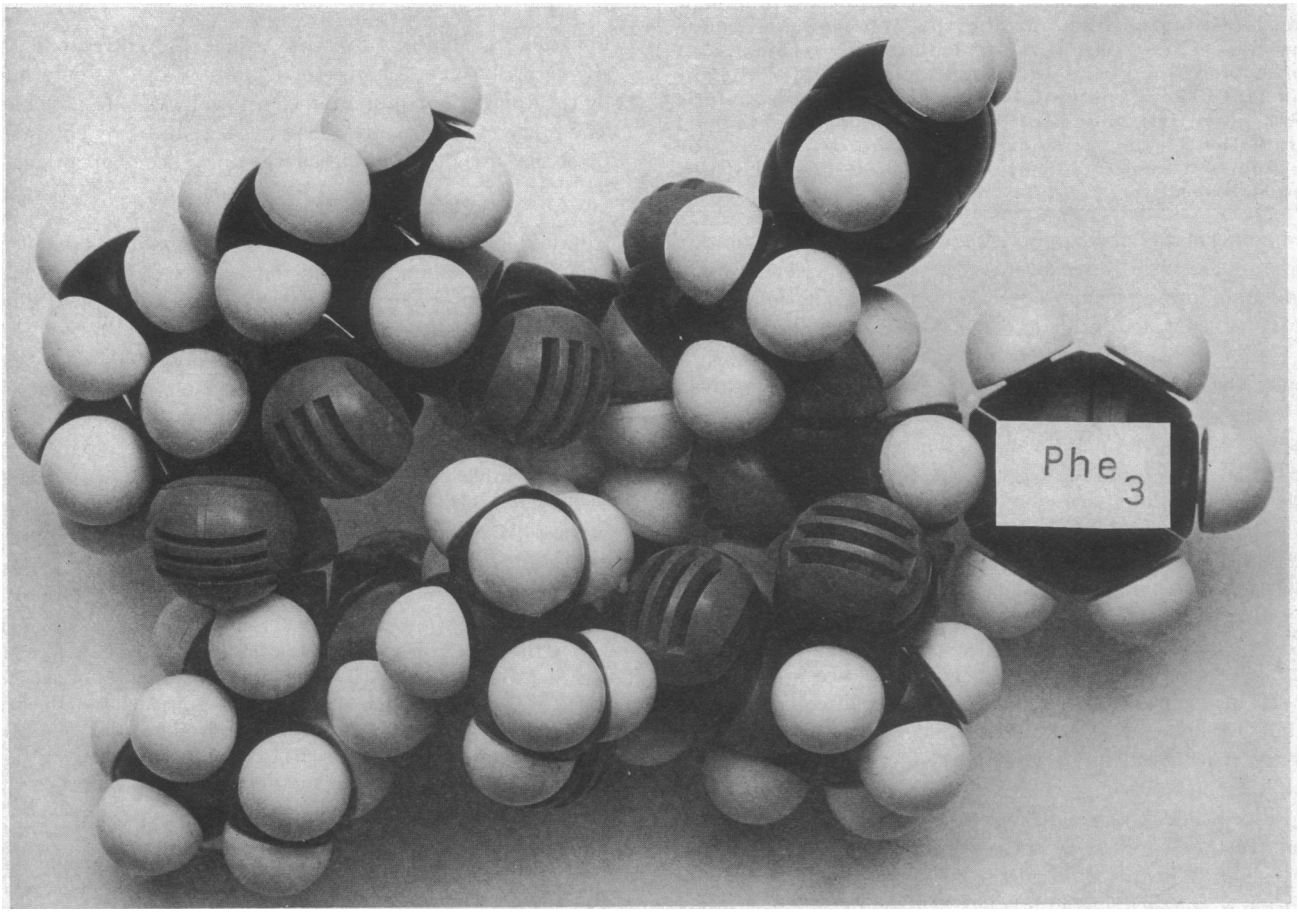


FIG. 3. Two photographs (see also opposite page) of the space-filling molecular model of cyclolinopeptide A in the lowest-energy class-I conformation (see Table 2).

TABLE 1. *Classes of conformations generated for cyclolinopeptide A with* $\psi_{Pro_2} = 125°$
(ψ, degrees)

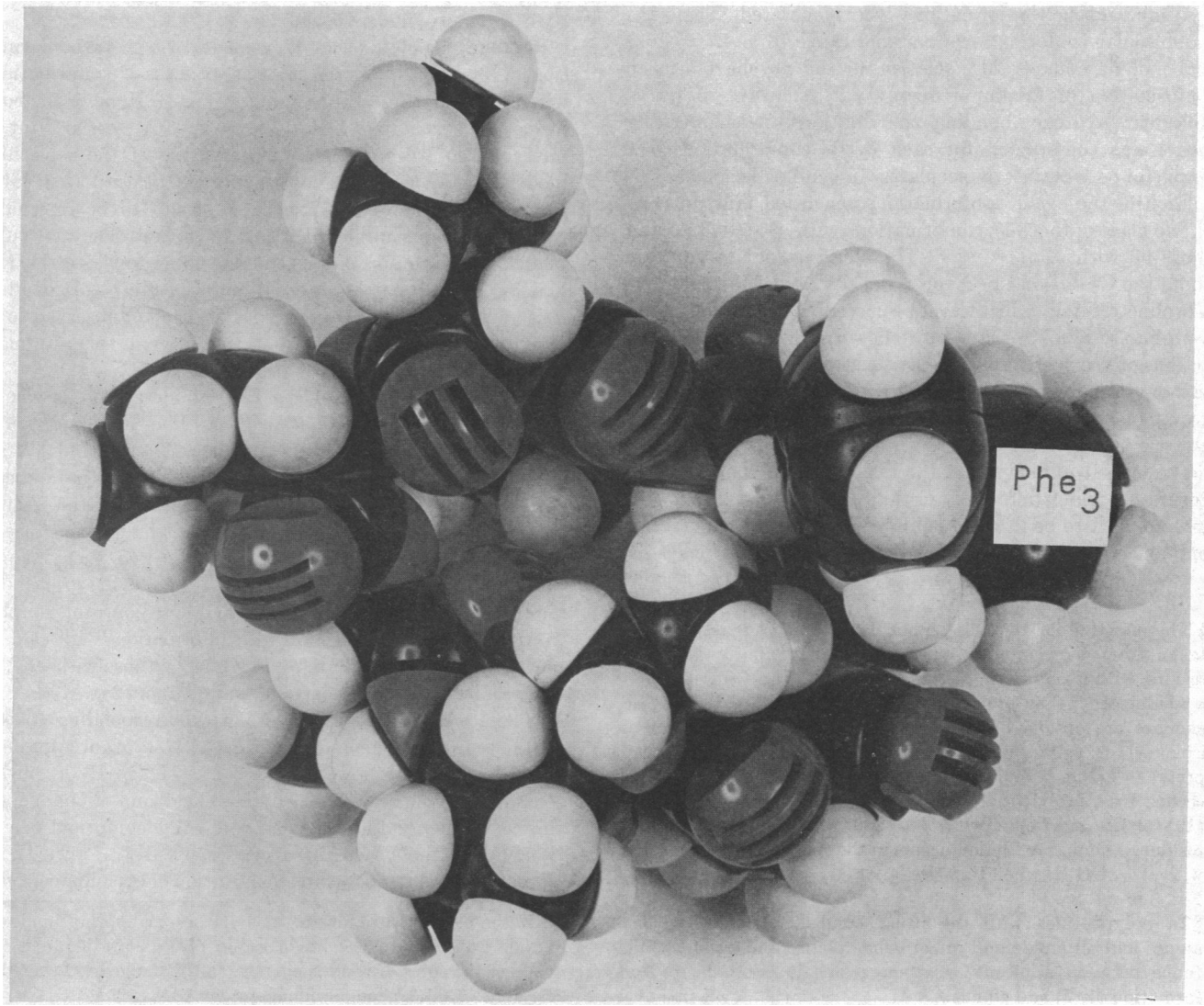
Class	Phe_3	Phe_4	Leu_5	Ile_6	Ile_7	Leu_8	Val_9
I*	330, 0	270	330, 0	120	120	270, 300, 330	310, 340
II*	300, 330	120	120	120	330	0	280, 310, 340
III†	300, 330	120	120	120	270, 300	120	280, 310, 340
IV†	120	270, 300, 330	120	120	120	270, 300, 330	280, 310, 340

* All nonprolyl φ's = 90° except $\varphi_{Phe_3} = \varphi_{Phe_4} = \varphi_{Ile_6} = \varphi_{Val_9} = 90°$ or 100°.
† All nonprolyl φ's = 90° except $\varphi_{Phe_3} = \varphi_{Phe_4} = \varphi_{Ile_6} = 90°$ or 100°.

TABLE 2. *Lowest energy cyclic conformations generated*
(ψ, degrees)

Class of conformations	Phe_3	Phe_4	Leu_5	Ile_6	Ile_7	Leu_8	Val_9	E_{conf}
I* (N—H_{Leu_5} buried)	330	270	0	120	120	330	310	5.1
II* (N—H_{Phe_3} buried)	330	120	120	120	330	0	310	7.6
III† (N—H_{Phe_3} buried)	330	120	120	120	330	120	310	8.5
IV† (N—H_{Phe_3} buried)	120	270	120	120	120	330	310	7.0

* $(\varphi,\psi)_{Pro_1} = 102°, 310°$; $(\varphi,\psi)_{Pro_2} = 122°, 125°$; and all other φ's = 90° except φ_{Phe_4} and φ_{Ile_6} or $\varphi_{Val_9} = 100°$.
† Same as *, except all other φ's = 90° except $\varphi_{Phe_4} = \varphi_{Ile_6} = 100°$.



40°, and 40°) and $\varphi = 30°$, 210°, and 270° ($\varphi' = 150°$, 30°, and 30°) lead to nearly the same couplings as $\varphi = 100°$ and $\varphi = 90°$, respectively, but they are not as energetically favorable as conformations with $\varphi = 100°$ or $\varphi = 90°$ (see the conformational energy maps in ref. 6, 8, and 19). The following sets of values for the ψ rotation angles, which correspond to the lowest energy conformations with $\varphi = 100°$ and $\varphi = 90°$, are adopted.‡ $\psi_{Phe_3} = \psi_{Phe_4} = \psi_{Leu_5} = \psi_{Leu_8} =$ 0°, 120°, 240°, 270°, 300°, and 330°; $\psi_{Ile_6} = \psi_{Ile_7} = 120°$, 270°, 300°, and 330°; and $\psi_{Val_9} = 280°$, 310°, and 340°.

For each conformation, i.e., for each of the sets of nine pairs of rotation angles φ and ψ, the distance between the α-carbon atoms in Pro_1 and Pro_2 in the corresponding linear nonapeptide terminated by prolyl residues is calculated. The method of transformation of virtual bond vectors (1) is employed in the distance calculations. If the distance between $C^{\alpha}_{Pro_2}$ and $C^{\alpha}_{Pro_1}$ lies in the range 3.7–3.9 Å, then the conformation under consideration is assumed to be cyclic.§ The intramolecular energy of a given cyclic conformation is evaluated by summing the individual residue energies obtained from their random-coil energy maps, (6–8, 19), which do not include energetic contributions made by intramolecular hydrogen-bonds.

CALCULATED RESULTS AND DISCUSSION

Sixty-nine cyclic conformations were generated. Their intramolecular conformational energies range from 4.7 to 10.5 kcal/mol of nonapeptide, relative to the acyclic or linear conformation of minimum energy and exclusive of intramolecular hydrogen-bonding contributions. A space-filling model was constructed for each cyclic conformation in a search for possible intramolecular hydrogen-bonds.

Each of the cyclic conformations generated falls into one of two classes; those 17 conformations with $\psi_{Pro_2} = 325°$ and those 52 with $\psi_{Pro_2} = 125°$. The steric barrier to rotation about the C^{α}–C bond between $\psi = 125°$ and 325° in a *trans* L-proline residue, which is not succeeded by another *trans* L-proline residue, appears (8) to be substantial. Steric interactions of the N–H group of the succeeding residue with the carbonyl group of the preceding residue and with the β-CH_2 group of the pyrrolidine ring constitute the major contributions to this high barrier. The magnitude of this barrier may make it unlikely that the two classes of conformations generated, which differ only in that $\psi_{Pro_2} = 125°$ or 325°, can interconvert to be in rapid equilibrium with each other. Since only one average conformation for each of the residues is detected (12) in the NMR spectrum of cyclolinopeptide A, ψ_{Pro_2} is probably either 125° or 325°, and not a mixture in solution.

Space-filling models of the conformations generated with $\psi_{Pro_2} = 325°$ show that the environments of the α-protons in both proline residues are very similar. The equivalence of the α-proton environments in both proline residues is a consequence of the fact that $(\varphi,\psi)_{Pro_1} = 102°$, 310° and $(\varphi, \psi)_{Pro_2} = 122°$, 325° in each of these conformations. However, in the models of those conformations with $\psi_{Pro_2} = 125°$, the environments of the α-protons in Pro_1 and Pro_2 are dissimilar. In the NMR spectrum of the cyclononapeptide dissolved in [^{2}H]Me_2SO, the α-proton resonances of one proline residue appear 0.5 τ upfield from the α-proton resonances of the other proline residue, indicating different environments for the two sets of α-protons. Only those 52 conformations generated with $\psi_{Pro_2} = 125°$ have dissimilar α-proton environments for the prolines. Thus, the 17 cyclic conformations with $\psi_{Pro_2} = 325°$ are rejected.

The cyclic conformations generated with $\psi_{Pro_2} = 125°$ can be divided into four classes, each with a different overall shape. Table 1 contains a summary of the individual residue conformations corresponding to the overall conformations in each of these four classes. The sum of the residue energies, E_{conf}, for the generated conformations in each of the four classes presented in Table 1 spans the range¶ from 5.1 to 10.6 kcal/mol of nonapeptide.

Photographs of a space-filling model of a single conformation of the peptide in the low-energy class I conformation, with $\psi_{Pro_2} = 125°$, are presented in Fig. 3. None of the conformations generated with $\psi_{Pro_2} = 125°$ possess any intramolecular hydrogen bonds. Observation of the molecular models of conformations corresponding to class I (see Table 1 and Fig. 3) shows the Leu_5 N–H group to be internally buried, resulting in limited access to solvent. The molecular models of the conformations corresponding to classes II, III, and IV indicate partial internal burial of the N—H of Phe_3. The amide protons of Leu_5 and of Phe_3 are not observed to be simultaneously protected from the solvent, or internally buried, in any of the conformations generated.

The observed burial of the Leu_5 amide-proton in class I conformations, and the burial of the Phe_3 amide-proton in conformations corresponding to classes II, III, and IV, are consistent with deuterium-exchange studies (12) that indicate that one of the leucine amide-protons and one of the phenylalanine amide-protons exchange at about half of the rate of the remaining five amide-protons. Of the two phenylalanine residues, the one whose amide proton is found (12) to exchange more slowly has the larger vicinal amide to α-proton coupling constant. On this basis then, all conformations with $\varphi_{Phe_3} =$ 90° and $\varphi_{Phe_4} = 100°$ are retained, while those with $\varphi_{Phe_3} =$ 100° and φ_{Phe_4} 90° are rejected (see Eqs. 1 and 2).

The lowest-energy conformations, corresponding to each of the four classes generated with $\psi_{Pro2} = 125°$, are presented in Table 2. The lowest-energy class-I ($N–H_{Leu_5}$ buried) conformation is between 2.0 and 3.0 kcal/mol of nonapeptide lower in energy than the lowest-energy conformations belonging to the other three classes ($N–H_{Phe_3}$ buried). An increase in the temperature of a solution of cyclolinopeptide A

‡ The energies for the Phe_3, Phe_4, Leu_5, and Leu_8 residues are taken from the conformational energy map appropriate to a residue with a side chain of the type R = CH_2R' succeeded by a residue other than prolyl (see Fig. 5 in ref. 6). The Ile_6 and Ile_7 energies are obtained from the energy map appropriate to an isoleucyl or valyl residue succeeded by a residue other than prolyl (see Fig. 5 in ref. 19). Because the Val_9 residue is succeeded by a prolyl residue, only the portion of the energy map (19) for Ile_6 and Ile_7 between $\psi = 280°$ and 340° is used for this residue. A similar reduction occurs in the energy maps for residues with R = CH_2R' when they are followed by a prolyl residue (see Fig. 3 in ref. 8).

§ In polypeptides with the usual bond lengths and valence angles and all amide and imide bonds planar and *trans*, the distance between adjacent α-carbon atoms is invariant to conformation (1, 7) and equals 3.8 Å.

¶ If the electrostatic interactions of the neighboring imide and amide groups with the imide group of Pro_2 are ignored, then each of the sums of residue energies in this range are lowered (8) by about 1.0 kcal/mol of nonapeptide.

should result in an increase in the fraction of conformations corresponding to classes II, III, and IV, at the expense of the class I conformations. Hence, a rise in temperature should increase the probability or number of conformations with $(N-H)_{Phe3}$ buried, while decreasing the number or fraction of conformations with $(N-H)_{Leu5}$ buried. This may explain the anomalous negative temperature-coefficient of the chemical shift observed (12) for the amide proton of the slowly-exchanging phenylalanine residue.

Based on the present comparison of predicted conformations with experimental (12) NMR observations, it appears that cyclolinopeptide A assumes at least four types of conformations in solution, none of which possess intramolecular hydrogen bonds. Thus, this cyclic nonapeptide is believed to exhibit considerable conformational flexibility when dissolved.

I am indebted to Dr. F. A. Bovey and Mrs. A. I. Brewster for many enlightening discussions of the conformational behavior of cyclolinopeptide A in solution as manifested by NMR spectroscopy.

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