R^3 were obtained when $\phi = 123°$ and $\phi' = 313°$ ($\psi = 133°$), as given by Arnott and Dover.[19]

The results of the calculation, given in Table III, show that an upfield shift of about 0.1 ppm should occur in going from helix to coil. The largest uncertainty in the calculation is in the choice of where to locate the origin of the induced dipole in eq 7. When allowed to vary by 0.4 Å about our center of charge, it was found that the shift changed from a 0.05-ppm upfield shift to a 0.18-ppm upfield shift.

Table III. The Calculated α-Proton Magnetic Shielding in the Helical and Random-Coil Forms of Poly(L-alanine) Due to the Induced Magnetic Moment of the Amide Group Using the Measured Magnetic Susceptibility Anisotropy of Formamide[a]

	ϕ	ϕ'	Shielding
Helical form	123°	312°	−0.43 ppm
Random-coil form	22–127°	92–179° and 300–320°	−0.33 ppm

[a] The angles and limits are illustrated in Figure 1.

Conclusion

The molecular Zeeman effect has been observed in formamide-^{15}N, and the relevant magnetic parameters and molecular quadrupole moments are reported.

The chemical shift at the α proton in the random-coil and helix forms of poly(L-alanine) was calculated using the measured magnetic susceptibility anisotropy of the amide group.

Our calculation predicts a small upfield shift of approximately 0.1 ppm due to the reorientation of the amide planes when poly(L-alanine) undergoes the helix to coil transition. Experimentally, a downfield shift of 0.3–0.4 ppm is observed. We therefore conclude that the shift is not caused by the reorientation of these amide planes and support the view that solvation of the amide planes causes the observed shift. Tam and Klotz[6] have measured the shift of the α proton in poly(L-alanine) (helix) and poly(DL-alanine) (random coil) in $CDCl_3$ and found them to be the same which agrees quite well with our prediction of a small shift.

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An Approximate Treatment of the Conformational Characteristics of the Cyclic Hexa-L-peptides (Pro-Ser-Gly-Pro-Ser-Gly) and (Ser-Pro-Gly-Ser-Pro-Gly)

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Abstract: Several conformations for the cyclic hexa-L-peptides (Pro-Ser-Gly-Pro-Ser-Gly) and (Ser-Pro-Gly-Ser-Pro-Gly), which are consistent with conformation dependent information obtained in recently reported nmr investigations of these same cyclic hexapeptides, are presented. Deduction of these several conformations from the myriad of possible cyclic conformations is achieved by eliminating from consideration all conformations having a high intramolecular conformational energy. The intramolecular conformational energies of both cyclic hexapeptides are estimated by summing the independent residue energies which have been calculated previously by others using approximate potential functions to account for the intrinsic torsional potentials about the backbone bonds and the nonbonded steric (6–12 potential) and electrostatic (monopole–monopole) interactions solely dependent upon one or both of the backbone residue rotations φ and ψ about the N–C^α and C^α–C bonds, respectively. A search for the presence of intramolecular hydrogen bonds is made in each cyclic conformation generated, and, when present, their stabilizing effect is accounted for by adding their negative energies to the previously determined sum of residue energies. Comparisons of the magnitudes of the vicinal coupling constants between N–H and C^α–H^α in the serine and glycine residues and comparison of the presence of the appropriate intramolecular hydrogen bonds with the nmr findings are used to test the consistency of the generated conformations with experiment. The averaging of a "Karplus-like" relation connecting the dihedral angle φ' and vicinal coupling $J_{N\alpha}$ between N–H and C^α–H^α over all of the low-energy cyclic conformations generated makes the comparison between the predicted and experimentally observed vicinal coupling constants possible. The hexa-L-peptide (Pro-Ser-Gly-Pro-Ser-Gly) flips *via* small rotations about φ_{Gly} between two Gly-Gly hydrogen-bonded conformations, both of which have all-trans peptide bonds and minimum C prolines ($\varphi_{Pro} \approx 300°$). Cyclic (Ser-Pro-Gly-Ser-Pro-Gly) adopts at least two conformations, one with trans, the other with cis imide bonds, but both containing minimum C prolines and non-α-helical serines ($\varphi_{Ser} \approx 330°$). The trans peptide bond conformation possesses strong internal Ser-Ser hydrogen bonds.

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

(1) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, 87, 2791 (1965).

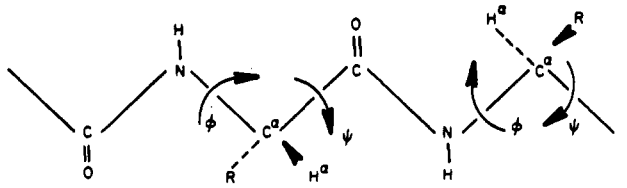

Figure 1. A schematic representation of a portion of a poly(L-peptide) in the planar trans conformation.

trans amide or imide bonds[1,6] render the potential energy of rotations φ and ψ, $V(\varphi,\psi)$, about the N–C^α and C^α–C bonds (see Figure 1) in a given residue independent of the corresponding rotations in neighboring residues. Consequently, the total intramolecular conformational energy of a polypeptide exclusive of hydrogen bonding may be estimated by summing the independent residue energies, which include[6] the intrinsic threefold torsional potentials about the N–C^α and C^α–C backbone bonds, the nonbonded steric repulsions and London dispersion energies (6–12 potential), and the nonbonded monopole–monopole electrostatic interactions.

It has recently been shown[9,10] that averaging 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 over all conformations found to be energetically favorable by the approximate energy calculations mentioned above leads to the correct vicinal couplings observed for random coil polypeptides[9] and dipeptides[10] in solution. This agreement lends further support to the 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. The combination of these two approximate theoretical tools has proved useful in the conformational analyses of a synthetic cyclic nonapeptide[12] and the cyclic decapeptide antamanide,[13] which is an antidote to mushroom toxins,[14] as well as in the present study of the synthetic cyclic hexa-L-peptides[15,16] depicted schematically in Figure 2.

Briefly, testing for ring closure is performed only on those conformations whose individual residues have rotation angles[17a] φ and ψ corresponding to intramolecular energies within 5.0 kcal/mol of residue of the minimum energy conformation for the residue in question. Then the presence of intramolecular hydrogen bonds is tested[19] for in those conformations which form a closed ring structure. Finally, the residue energies are summed together with any hydrogen bonding contributions to obtain an estimate of the total intramolecular conformational energy, and a space-filling model is constructed in a search for any steric overlaps longer in range than those considered in the previously mentioned residue energy calculations.[1,6]

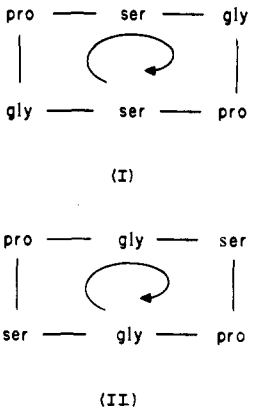

Figure 2. A schematic representation of the cyclic L-hexapeptides (Pro-Ser-Gly-Pro-Ser-Gly) (I) and (Ser-Pro-Gly-Ser-Pro-Gly) (II), where the sense of the arrow indicates movement from N to C^α in each residue.

Details of Calculation

All amide and imide bonds are assumed to be planar and trans, and the residue bond lengths and valence angles used previously in the conformational energy calculations[1] are adopted. The φ angles of rotation in both proline residues are restricted by the pyrrolidine rings and are assigned the value 122° appropriate for an isolated L-prolyl residue according to the crystallographic analysis[20] of L-leucyl-L-prolylglycine.

The conformation of each pair of like residues (Pro, Ser, and Gly) are assumed to be simultaneously identical based on the observation[15,16] of a single resonance for the same protons in each pair of like residues and the twofold symmetry of both cyclic hexapeptides. This assumption precludes the existence of rapidly interconverting asymmetric conformations and reduces the total number of conformations n, which must be considered in the ring closure calculations, to $n^{1/2}$. The rotation angles φ and ψ in each of the residues are varied in 30° increments over all residue conformations (φ,ψ) whose energies are less than 5.0 kcal/mol of residue above the minimum energy conformation of the residue

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(5) R. Scott and H. Scheraga, *J. Chem. Phys.*, **45**, 209 (1966).
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(12) A. E. Tonelli, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1203 (1971).
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(15) D. A. Torchia, A. di Corato, S. C. K. Wong, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 609 (1971).
(16) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *ibid.*, **94**, 616 (1971).
(17) (a) The angles of rotation φ and ψ (see Figure 1) are taken[17b] as zero in the trans or planar zigzag conformation and are measured in a right-handed sense. Recently, a new convention, which assigns $\varphi = \psi = 180°$ for the planar zigzag conformation, has been proposed.[18] However, the author fails to see any major improvement in the new convention and therefore continues to adopt the former convention to avoid confusion. (b) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966).
(18) J. C. Kendrew, W. Klyne, S. Lifson, T. Miyazawa, G. Nemethy, D. C. Phillips, G. N. Ramachandran, and H. A. Scheraga, *Biochemistry*, **9**, 3471 (1970); *J. Biol. Chem.*, **245**, 6489 (1970); *J. Mol. Biol.*, **52**, 1 (1970).
(19) D. A. Brant, *Macromolecules*, **1**, 297 (1968).
(20) Y. C. Leung and R. E. Marsh, *Acta Crystallogr.*, **11**, 17 (1958).

in question, except ψ_{Pro} = 100–160° is varied in 15° increments. The intramolecular conformational energies are taken from the energy maps appropriate to each residue being in a randomly coiling polypeptide; *i.e.*, the energy maps in Figure 5 of ref 6 and in Figure 3 of ref 8 for the serine residues,[21] in Figure 8 of ref 6 and in Figure 2 of ref 8 for the glycine residues,[21] and in Figure 4 of ref 8 for the proline residues are used.

For each hexapeptide conformation, *i.e.*, for each set of $(\varphi,\psi)_{Pro}$, $(\varphi,\psi)_{Ser}$, and $(\varphi,\psi)_{Gly}$, where φ_{Pro} = 122°, the distance between the terminal α-carbon atoms in the corresponding linear or acyclic hexapeptides (Pro-Ser-Gly-Pro-Ser-Gly) and (Pro-Gly-Ser-Pro-Gly-Ser) is calculated following the transformation of virtual bond vectors method described by Brant and Flory.[1] If this distance is between 3.7 and 3.9 Å, then the hexapeptide conformation under consideration is assumed to be cyclic.[22]

The total intramolecular energy of each cyclic conformation generated is obtained by summing the individual residue energies and adding to this sum any contributions made by Gly-Gly hydrogen bonds in hexapeptide I (Pro-Ser-Gly-Pro-Ser-Gly) or by Ser-Ser hydrogen bonds in hexapeptide II (Ser-Pro-Gly-Ser-Pro-Gly) as suggested by Schwyzer and Ludescher[23] (see companion papers[15,16] in this issue). Any contributions to the total intramolecular conformational energy made by intramolecular hydrogen bonds are evaluated according to the method of Brant.[19] A space-filling molecular model is constructed for each of the lowest energy cyclic conformations generated in a search for steric overlaps longer in range than those considered in the potential energy calculations,[17] for each residue.

The following relations connecting the vicinal nmr coupling $J_N{}^\alpha$ and the dihedral angle[24] φ' between N–H and C^α–H^α are averaged[9,10] at 25° over all of the lowest energy cyclic conformations generated to obtain this coupling in the serine and glycine residues.

$$J_N{}^\alpha = \begin{cases} 8.5\cos^2\varphi' & (0 \le \varphi' \le 90°) \\ 9.5\cos^2\varphi' & (90° \le \varphi' \le 180°) \end{cases} \quad (1)[11]$$

$$J_N{}^\alpha = 8.9\cos^2\varphi' - 0.9\cos\varphi' + 0.9\sin^2\varphi' \quad (2)[25]$$

Calculated Results and Discussion

The cyclic conformations generated for both hexapeptides fall into two distinct classes, those conformations where ψ_{Pro} = 100–160° (minimum A)[8] and those with ψ_{Pro} = 270–360° (minimum C).[8] As implied by Schimmel and Flory's[8] conformational energy map for an isolated L-prolyl residue and as observation of space-filling models indicate, there exists a considerable steric barrier[26a] to rotation about the C^α–C bond between minima A and C in an isolated L-prolyl residue. Steric overlaps involving the N–H group of the succeeding residue with the β-CH_2 group of the pyrrolidine ring and with the carbonyl group of the preceeding residue constitute the major contributions to this high barrier. Consequently, it seems unlikely that hexapeptide conformations whose prolyl residues are in minimum A can rapidly convert or be in dynamic equilibrium[27] with conformations whose prolyls are in minimum C. Since the nmr spectra[15,16] of both cyclic hexapeptides indicate a single average conformation for each pair of like residues, the L-prolyl residues in each hexapeptide in solution are probably in conformations corresponding either to minimum A or to minimum C.

Two-thirds of the total number of cyclic conformations generated for hexapeptide I have prolyl residues in conformations corresponding to minimum A. None of these conformations have serine and glycine residues with φ rotations which, according to eq 1 or 2, approximate the experimentally observed[15] vicinal couplings for these residues ($J_N{}^\alpha$(Ser) = 8.5 and $J_N{}^\alpha$(Gly) = 3.5 and 5.0 Hz). Averaging[9,10] the couplings calculated from eq 1 or 2 over all of the lowest energy conformations having prolyl residues in minimum A fails to bring the calculated and experimental coupling constants into agreement. In addition, no intramolecular Gly-Gly hydrogen bonds are found in those cyclic conformations generated whose prolyl residues are in conformations corresponding to minimum A in contradic-

(21) Two different conformational energy maps are required for both the serine and glycine residues, because in hexapeptide I glycine is succeeded by proline and in hexapeptide II serine is succeeded by proline (see Figure 2), which restricts the number of conformations accessible to both residues from those available when they are not succeeded by a proline residue.

(22) In polypeptides with the usual bond lengths and valence angles and all amide and imide bonds trans, the distance between adjacent α-carbon atoms is invariant to conformation,[1,7] (φ,ψ) and equals 3.8 Å. Ring generation is completed by checking the distances between the terminal [Pro and Gly (I) or Ser (II)] N and C (carbonyl) atoms and between the terminal proline δ carbon and glycine or serine carbonyl oxygen to ensure closure of the ring with a planar trans peptide bond.

(23) R. Schwyzer and U. Ludescher, *Helv. Chim. Acta*, **52**, 2033 (1969).

(24) The dihedral angle φ' is 180° when N–H and C^α–H^α are trans and 0° when they are cis and is directly related to the angle of rotation φ about the N–C^α bond.

(25) U. F. Bystrov, S. L. Portnova, U. I. Tsetlin, V. T. Ivanov, and Yu. A. Ovchinnikov, *Tetrahedron*, **25**, 493 (1969).

(26) (a) Recently Maigret, Perahia, and Pullman[26b] have calculated the conformational energy map for an isolated trans L-prolyl residue (succeeded by any residue other than proline) using the PCILO quantum mechanical method. Their map is very similar to that obtained by Schimmel and Flory[8] who employed a semiempirical potential energy function. The only significant difference between the two maps is the smaller barrier to ψ rotation at ψ = 230° (35 kcal/mol), as compared to the barrier at ψ = 60° (75 kcal/mol), in the PCILO map. Maigret, *et al.*,[26b] ascribe this reduction in the barrier at ψ = 230° to the presence of a seven-membered intramolecular hydrogen bond. In fact they say that if ω is set at −40°, the barrier to interconversion between minima A and C at ψ = 230° can be lowered further to 9 kcal/mol (ω is the angle of rotation about the peptide bond). However, we have recently discussed[26c] the validity of the PCILO method with particular reference to the tendency of this method to overestimate hydrogen bond strength. As an example, the seven-membered intramolecular hydrogen bond corresponding to ω = −40° and ψ = 230° (φ = 122°)[20] is characterized by an O···H distance of 2.10 Å, an angle of 112° between C=O and O···H, and an angle of 38° between N—H and N···O. This is a highly nonlinear and nonplanar hydrogen bond and should be quite weak.[19] Furthermore, inspection of molecular models indicates that the severe steric interactions which occur when rotating about the C^α–C backbone bond in L-proline between minima A and C are not appreciably reduced when the imide bond is nonplanar (ω = −40°). Thus, a rotation of −40° about the imide bond leads only to a weak hydrogen bond and does not diminish substantially the steric interactions at ψ = 230°. According to Maigret, *et al.*,[26b] the conformational energy corresponding to ω = −40° and (φ,ψ) = 122, 150 (min A), or 330° (min C) is 8 kcal mol. It is not clear how they can obtain an energy of 9 kcal/mol (only a 1 kcal/mol increase from minima A or C) when ψ is rotated to 230° where severe steric interactions come into play. Consequently, we believe the barrier to interconversion between minima A and C may be greater than 9 kcal/mol and of such a magnitude as to prevent the rapid (rapid on the nmr time scale) dynamic equilibration of conformations corresponding to these minima. (b) B. Maigret, P. Perahia, and B. Pullman, *J. Theor. Biol.*, **29**, 275 (1970); (c) A. E. Tonelli, *Macromolecules*, **4**, 618 (1971).

(27) In support of the absence of a rapid equilibrium between minimum A and minimum C proline conformations is the fact that the amide to α-proton coupling constants calculated by averaging eq 1 or 2 over all of the cyclic conformations generated do not reproduce the measured couplings.

Table I. Summary of Lowest Energy Cyclic Conformations Generated for Hexapeptides I and II

Hexapeptide	ψ_{Pro}	φ_{Ser}	ψ_{Ser}	φ_{Gly}	ψ_{Gly}	V_{sum}[a]	$V_{H\ bond}$[b]	V_{total}[c]
I	290	240	210	340	80	13.0	−6.0	7.0
I	280	240	210	20	350	13.5	−6.0	7.5
II	300	30	330	290	150	13.0	−8.0	5.0

[a] V_{sum} is the sum of residue energies obtained from the conformational energy maps in ref 6 and 8 expressed in kcal/mol of hexapeptide relative to the acyclic, non-hydrogen-bonded conformation of minimum energy. [b] $V_{H\ bond}$ is the Gly-Gly (I) or Ser-Ser (II) hydrogen bond energy in kcal/mol of hexapeptide. [c] $V_{total} = V_{sum} + V_{H\ bond}$.

tion to their experimental[15] observation.[28] On the other hand, several of the cyclic conformations generated for hexapeptide I whose prolyl conformations correspond to minimum C do possess intramolecular Gly-Gly hydrogen bonds. Hence, all-trans peptide bond conformations with minimum A prolyl residues are rejected for hexapeptide I.

In the nmr spectrum of hexapeptide II dissolved in H_2O, two distinct and symmetric conformations or average conformations are observed[16] in the ratio of 4:1. The least abundant conformation does not appear to be intramolecularly hydrogen bonded, while the major component exhibits internal Ser-Ser hydrogen bonds. As Torchia, *et al.*,[16] systematically added DMSO to the aqueous solution of hexapeptide II the proportion of the minor component increased over that observed in the absence of DMSO until in pure DMSO the minor component observed in pure H_2O became the major component in the ratio of 3:1. The presence of Ser-Ser hydrogen bonds in the major component in H_2O and in the minor component in DMSO, together with their nearly identical vicinal coupling constants, strongly suggests the same conformation or average conformation for both components. The identity of the vicinal couplings observed for the minor component in H_2O and for the major component in DMSO and the absence of internal hydrogen bonding in both components similarly suggest the same conformation for this component in both solvents.

None of the cyclic conformations generated for hexapeptide II, whose prolyl residues have minimum A conformations, possess φ rotations which reproduce the experimentally observed vicinal couplings of either component in H_2O or in DMSO. In addition, steric overlaps between the serine carbonyl groups are observed in the molecular models of all minimum A conformations generated for hexapeptide II. For these reasons all-trans peptide bond conformations with minimum A prolyl residues are also rejected for hexapeptide II.

Hence, only those cyclic conformations generated for both hexapeptides whose prolyl residues have conformations corresponding to minimum C are considered further. More specifically, the ring closure and intramolecular hydrogen bond tests are performed again using 10° instead of 30° increments in the rotation angles about all residue conformations whose energies are within 5.0 kcal/mol of residue of the minimum energy conformation for the residue in question, while L-prolyl conformations corresponding to minimum A are ignored. The lowest energy cyclic conformations found for both hexapeptides are listed in Table I.

The Gly-Gly hydrogen bonds in the lowest energy conformations found for hexapeptide I are characterized[29] by O to H distances of 1.89–1.92 Å, angles of 55–66° between C=O and O···H, angles of 6–15° between N—H and N···O, and energies of *ca.* −3.0 kcal/mol of hydrogen bond. Their serine vicinal couplings are $J_{N^\alpha} = 8.0$–8.5 Hz, according to eq 1 and 2, in agreement with experiment[15] (see Table II). The calculated couplings for the glycine residues in the two lowest energy conformations found for hexapeptide I are J_{N^α} [Gly(L)] = 5.6–6.4 and 0.3–1.4 Hz and J_{N^α} [Gly(D)] = 0.3–1.4 and 5.6–6.4 Hz, respectively. If one of these two lowest energy conformations, which are in rapid equilibrium, is favored[30] over the other by 1.0 kcal/mol of hexapeptide or less, then the calculated average glycine couplings are in agreement with the observed values (see column 7 in Table II).

The lowest energy, all-trans peptide bond cyclic conformation generated for hexapeptide II has internal Ser-Ser hydrogen bonds which are characterized by an O to H distance of 1.80 Å, an angle of 39° between C=O and O···H, an angle of 13° between N—H and N···O, and an energy of −4.0 kcal/mol of hydrogen bond. The presence of internal Ser-Ser hydrogen bonds and the vicinal coupling constants calculated for this lowest energy conformation are seen in Table II to be in excellent agreement with the corresponding experimental[16] results obtained for the major component of hexapeptide II observed in H_2O which is also the minor component observed in DMSO. All other cyclic conformations generated for hexapeptide II, which have trans peptide bonds and minimum C prolyl conformations, have intramolecular energies at least 3.0 kcal/mol of hexapeptide above the lowest energy conformation generated and are presented in Tables I and II.

Recent nmr studies[15,16,31] of $(CH_3)_3COC(=O)$-$(Pro)_{n=2-6}OCH_2C_6H_5$, poly(L-proline), HBr-Ser-Pro-Gly-$OC_6H_5NO_2$, $(CH_3)_3COC(=O)$-Pro-Ser-Gly-$OCH_2C_6H_5$, $(CH_3)_3COC(=O)$-Tyr, or Gly-Pro-$OCH_2C_6H_5$, etc., have demonstrated the cis–trans isomerization about the urethane and imide bonds in L-proline containing polypeptides. It is also possible to infer[32]

(28) The glycine amide proton chemical shifts in hexapeptide I are observed[15] to be temperature independent, while the amide protons in the serine residues are shifted strongly upfield as the temperature is increased indicating the presence of internal or intramolecular hydrogen bonding between the glycine residues.

(29) See ref 19 for a detailed discussion of hydrogen bond strength criteria.

(30) Owing to the approximate nature of the potential functions[1,6] employed in the conformational energy calculations, it is not possible to say which of these calculated conformations has a lower intramolecular energy.

(31) (a) C. M. Deber, F. A. Bovey, P. Carver, and E. R. Blout, *J. Amer. Chem. Soc.*, **92**, 6191 (1970); (b) D. A. Torchia and F. A. Bovey, *Macromolecules*, **4**, 246 (1971).

(32) As an example, the nmr spectrum of HBr-Ser-Pro-Gly-$OC_6H_4NO_2$ (D. A. Torchia, private communication) shows two amide and α-proton resonances for the serine and glycine residues, while only single resonances are observed in the nmr spectra of $(CH_3)_3COC(=O)$-

Table II. Comparison of Calculated and Experimental Vicinal Coupling Constants between N–H and C^{α}–H^{α} in the Serine and Glycine Residues in Hexapeptides I and II

Hexapeptide	φ_{Ser}	φ_{Gly}	$J_{N^{\alpha}(Ser)}$, Hz Calcd	$J_{N^{\alpha}(Ser)}$, Hz Exptl	$J_{N^{\alpha}(Gly)}$, Hz Calcd[a]	$J_{N^{\alpha}(Gly)}$, Hz Exptl
I	240	340	8.0–8.5	8.5	5.6–6.4 (D), 0.3–1.4 (L)	5.0, 3.5
I	240	20	8.0–8.5	8.5	0.3–1.4 (D), 5.6–6.4 (L)	5.0, 3.5
II	30	290	7.0–7.5	7.0[b]	9.2–9.6 (D),	12.5[b] (sum of couplings)
				6.0[c]	3.5–3.6 (L)	8.0,[c] 3.5[c]

[a] The letters "L" and "D" differentiate between the glycyl α protons; such a differentiation is not possible experimentally. [b] Major component in H_2O and minor component in DMSO. [c] Major component in DMSO and minor component in H_2O.

from the nmr spectra of these linear polypeptides that the proline residues have a single average conformation about the C^{α}–C bond probably corresponding either to minimum A or minimum C. It is highly unlikely that after cyclization[16] of the tripeptide precursor of hexapeptide II the prolyl residues can interconvert between these two minima, so the major and minor components or conformations of hexapeptide II probably both have prolyl residues in conformations corresponding to the same potential energy minimum, A or C. Both components most likely have minimum C proline conformations since the nmr data for the major component in H_2O and the minor component in DMSO are consistent with the generated conformation of lowest energy which has minimum C prolyl residues (see Table I).

No cyclic conformations with conformational energies within 1.0 kcal of the lowest energy conformation generated, containing all-trans peptide bonds, and with prolyl conformations corresponding to minimum C are found for hexapeptide II, which reproduce the vicinal serine and glycine coupling constants measured for the major component in DMSO and the minor component in H_2O. Several conformations which do reproduce the measured couplings were found, but their intramolecular energies are more than 3.0 kcal/mol of hexapeptide above the lowest energy conformation generated (see Table I) precluding their substantial presence (25–75%) in equilibrium with the lowest energy conformation as is observed. In addition, the barrier to interconversion between these conformations and the lowest energy conformation generated is not nearly high enough to explain the persistent appearance of distinct resonances observed[16] from 7 to 80°.

One final possibility for the conformation of the major component in DMSO and the minor component in H_2O was explored. Recently it has been suggested[33] that residues containing a β-carbon side chain may adopt the α-helical conformation when succeeded by a prolyl residue despite, as pointed out by Schimmel and Flory,[8] the severe steric interactions of the side-chain β carbon with the δ-CH_2 group of the pyrrolidine ring in this conformation. However, when the pyrrolidine ring is allowed some flexibility[33b] the β-carbon side chain containing residue preceding a proline residue is allowed to assume an α-helical conformation with an energy *ca.* 7.0 kcal/mol of residue higher than non-α-helical conformations. Consequently, cyclic hexapeptide II conformations containing all-trans peptide bonds, minimum C proline conformations, and α-helical serine conformations were generated. All had intramolecular energies 16.0 kcal/mol of hexapeptide greater than the lowest energy conformation found, and none could reproduce the vicinal couplings measured for the major component in DMSO and the minor component in H_2O. Furthermore, according to the conformational energy map[33b] for a β-carbon side chain containing residue preceding a proline residue with a flexible pyrolidine ring, the barrier to rotation about the C^{α}–C bond between α-helical ($\psi \approx 120°$) and non-α-helical ($\psi \geq 240°$) conformations appears to be on the same order (50 kcal/mol) as the barrier[8] between minima A and C in an isolated L-proline residue. Hence, the same arguments that suggest both the major and minor components of hexapeptide II have minimum C prolyl residues also apply to the serine residues and strongly suggest that both components have non-α-helical serine conformations.

In view of the recently reported nmr evidence[16] and the arguments based on approximate conformational energy estimates presented above, it seems resonable to conclude that the conformation(s) of the major component of hexapeptide II in DMSO and its minor component in H_2O contain cis imide bonds with minimum C prolines and non-α-helical serine residues. Torchia, *et al.*,[16] have proposed just such a conformation on the basis of a molecular model study, but the absence of conformational energy estimates for cis peptide residues precludes the kind of search for low-energy cyclic conformations which was conducted and described above for the all-trans peptide bond hexapeptides I and II.

In the nmr spectra of hexapeptide I, the presence of a minor component (*ca.* 10%) is observed, which unlike the major component, is not twofold symmetric. The proportions of major and minor component appear to be independent of solvent. Following the same evidence and reasoning applied to hexapeptide II, it seems most reasonable that the minor component of hexapeptide I has a single cis imide bond and minimum C prolyl residues.

In light of the calculated[8,33] conformational energies for an isolated L-proline residue and for L-residues with a β carbon in their side chains succeeded by an L-prolyl residue, and the nmr[15,16] and present investigations of

Ser-Gly-$OCH_2C_6H_5$ indicating cis–trans isomerization about the imide bond in the former polypeptide. There would be a quadrupling of resonances instead of the observed doubling in the spectrum of the proline containing polypeptide if conformations corresponding to minima A and C were simultaneously present with cis and trans imide bonds and if the barrier between minima A and C is substantial (*ca.* 15 kcal/mol).

(33) (a) A. Damiani, P. De Santis, and A. Pizzi, *Nature (London)*, **226**, 542 (1970); (b) M. Maigret, B. Pullman, and J. Caillet, *Biochem. Biophys. Res. Commun.*, **40**, 808 (1970).

the cyclic hexapeptides I and II, it appears that the conformations of an isolated L-proline residue and residues with a β carbon in the side chain which precede L-proline may be locked into single conformations or average conformations, after incorporation into a growing protein. The steric barriers to inter conversion between minimum A and C in an isolated L-proline residue and between α-helical and non-α-helical conformations in a β-carbon containing residue preceding L-prolyl would seem sufficient to maintain these residues in the conformations in which they were synthesized into the protein. On the other hand, the barrier to cis–trans isomerization[34a] of the imide bond in an L-proline residue does not appear to be as formidable.[16,31] There are no such severe steric barriers present in the calculated[1-6] conformational energy maps for nonprolyl L residues which are not succeeded by L-proline.

Since protein synthesis involves[35] the stepwise addition of single amino acids beginning with the N-terminal amino acid or peptide residue, how does the residue with a β-carbon side chain in the process of attachment to the growing protein chain know whether to adopt α-helical or non-α-helical conformations when the following residue to be incorporated is an L-proline? For that matter, how does the newly attached L-prolyl residue know whether to adopt conformations corresponding to minimum A or minimum C, since the interconversion between them would appear to be difficult once the next peptide residue is added? In fact, only conformations corresponding to minimum C are possible[7] if the next residue to be added is also L-prolyl. A possible explanation for the conformational prescience suggested above may lie in the observed[35] degeneracy of the template ribonucleic acid (RNA) codons and the multiplicity of transfer RNA's required in the attachment of several of the amino acids in protein synthesis.

Finally, it appears that the combination of approximate conformational energy calculations and a Karplus-like relation connecting the dihedral angle and vicinal coupling constants between N–H and C^{α}–H^{α} in peptide residues enables the proposal of several conformations for the proline containing cyclic hexapeptides I and II, which are consistent with the conformation-dependent information obtained by nmr measurements performed in solution. Hexapeptide I apparently flips between two Gly-Gly hydrogen-bonded conformations, both of which have all-trans peptide bonds with minimum C prolines, by small rotation in the glycine residues. The minor component of hexapeptide I probably contains a single imide bond in the cis conformation. The proportion of major and minor hexapeptide I conformations appears to be independent of solvent. Hexapeptide II adopts at least two conformations, or average conformations, one with trans and the other with cis imide bonds whose proportions are solvent dependent. The trans imide bond conformation possesses strong internal Ser-Ser hydrogen bonds, while the cis imide bond conformation does not. Both conformations contain proline and serine residues in conformations corresponding to minimum C and non-α-helical regions of their conformational energy maps, respectively.

(34) (a) It is of interest to note that *cis*-L-prolyl imide bonds have been postulated to account for certain conformational features of the proteins subtilisin BPN'[34b] and ribonuclease S.[34c] (b) C. S. Wright, R. A. Alden, and J. Kraut, *Nature (London)*, **221**, 235 (1969); (c) H. W. Wyckoff, K. D. Hardman, N. M. Allewell, T. Inagami, C. M. Johnson, and F. M. Richards, *J. Biol. Chem.*, **242**, 3984 (1967).

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The N–N Torsional Potential Function in Methylhydrazine

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Abstract: The potential function hindering the N–N torsional motion in methylhydrazine has been determined using microwave and far-infrared spectroscopic data. The Fourier coefficients in the potential function, including three sine terms (a_k) and three cosine terms (b_k), are (in cm^{-1}): $a_1 = 73$, $a_2 = 21$, $a_3 = -87$, $b_1 = 569$, $b_2 = 967$, and $b_3 = 339$. These constants lead to a trans barrier height of 1253 ± 25 cm^{-1} (3.58 ± 0.07 kcal/mol) with the maximum at a torsional angle θ of 197° and a cis barrier height of 3028 ± 300 cm^{-1} (8.66 ± 0.86 kcal/mol) with the maximum at a θ value of 359°. The spectra were interpreted using a one-dimensional model in which the only motion is the torsion about the bond connecting the frame ($-NH-CH_3$) and the top ($-NH_2$). The far-infrared spectrum of methylhydrazine has been reinterpreted on the basis of the derived potential function. The spectroscopic entropy of methylhydrazine has been reexamined, and we have found our results to be consistent with the entropy determined experimentally from heat capacity measurements.

Barriers to internal rotation and relative stabilities of rotational isomers have been subjects of interest for a number of years. Although many different techniques have been employed,[1] the most accurate studies of hindered internal rotation have involved either microwave or far infrared spectroscopy, or a combination of these methods. Most of the microwave–far-infrared studies to date have involved molecules in which a symmetric-top group (usually $-CH_3$) is attached to a sym-

(1) J. P. Lowe, *Progr. Phys. Org. Chem.*, **6**, 1 (1968).