to tacticity. We also conclude that comparisons of experimental and calculated values is at present not a good way of studying the tacticity of polypropylene.[31]

Energetics of Internal Rotation

An energy profile for bond rotation in trimethyheptane starting from the most stable conformation appropriate for isotactic polymer (TGTG) was generated by the method previously described.[29] For each position of the bond, ϕ_2 (see 3), the rest of the internal coordinates of the molecule are in a minimum-energy configuration. Thus the path generated is a least energy or "reaction coordinate" path. This profile is shown in Figure 1. The response of ϕ_1 and ϕ_3 to changes in ϕ_2 along the least energy path are plotted in Figure 2. Similarly, a path for trimethylheptane starting from the conformation appropriate to the most stable one of syndiotactic polymer (TTGG, labeled as syndiotactic) was also generated. The bond ϕ_3 was the "driving" bond. This profile is shown in Figure 3 and the response of ϕ_2 and ϕ_4 along the path to changes in ϕ_2 is shown in Figure 4. There is apparently considerable geometrical flexibility in some of the ω interaction containing states, since the TTG'T_ state of the syndiotactic polymer was found in the bond rotation profile to have another state of equal energy nearby. However, this was the only such case to show up, and in general the states generated by bond rotation agreed very well with the state found by initial guesses based on inspection of models. We defer comment on the significance of these profiles to the dynamics of chain motion in bulk polymers to a later time.

(31) One of the reviewers has suggested that since the constant-volume entropy of fusion may be sensitive to the conformational properties,[32-34] a comparison with the conformational entropy based on our conformational energy parameters might be in order. It appears that the two are in agreement, but owing to the theoretical uncertainties in the comparison and the experimental uncertainties in the constant-volume entropy of fusion, the comparison is not highly meaningful.

(32) H. W. Starkweather and R. H. Boyd, *J. Phys. Chem.*, **64**, 410 (1960).

(33) A. E. Tonelli, *J. Chem. Phys.*, **52**, 4749 (1970).

(34) A. E. Tonelli, *ibid.*, **54**, 4637 (1971).

Acknowledgment. The authors are indebted to the U. S. Air Force Office of Scientific Research (Project Themis contract with the University of Utah) and the U. S. Army Research Office (Durham) for financial support of this work.

Solution Conformation of Evolidine

A. E. Tonelli

Bell Laboratories, Murray Hill, New Jersey 07974. *Received January 27, 1972*

ABSTRACT: A computer search for low-energy cyclic conformations has been conducted for the cyclic heptapeptide evolidine *cyclo*-Ser-Phe-Leu-Pro-Val-Asn-Leu. All of the cyclic conformations generated possess individual low-energy residue conformations which are consistent with the vicinal amide to α-proton coupling constants reported by Kopple in a recent solution nmr study of evolidine. The two lowest energy cyclic conformations generated are both consistent with the nmr evidence, but are distinguishable from each other by the cis or trans conformation of the Leu–Pro peptide bond. The conformation containing the cis peptide bond is similar to one proposed by Kopple on the basis of building space-filling molecular models. Infrared spectroscopy is suggested as an experimental means of choosing between these two proposed conformations.

Kopple[1] has recently reported a 220-MHz proton nmr study of the naturally occurring[2] cyclic heptapeptide evolidine[3] in dimethyl sulfoxide. He was able to associate each amide proton resonance with a specific residue (save differentiating between the two Leu NH's) and measure its coupling constant to the α proton in the same residue, the temperature coefficient of its chemical shift and its rate of exchange with deuterium. (Kopple employed homonuclear spin-decoupling techniques to assign the observed resonances to individual amino acid residues. N–H resonances were related to those of the α protons, and the α-proton resonances to those of the β protons. Because the β protons of both the Phe_2 and Asn_6 residues appear near 3 ppm, it is not possible to use the chemical shifts of the β protons to differentiate between these two residues. Instead, Kopple relied on the difference in the absolute values of the geminal β–β proton couplings observed in phenylalanine peptides (13–14 Hz) and in asparagine and aspartic acid peptides (15–16 Hz). On the basis of the greater relative uncertainty in the assignment of resonances to the Phe_2 and Asn_6 residues, we allowed for the possibility that the correct assignment of resonances to these two residues may be the reverse of Kopple's assignment, when searching for cyclic evolidine conformations.)

Construction of space-filling molecular models of cyclic conformations with H–N–C^α–H^α dihedral angles φ' which are consistent with the measured coupling constants according to a "Karplus-like" relation[4]

$$J_{N\alpha} = 7.5 \cos^2 \varphi' - 1.9 \cos \varphi' + 1.7 \sin^2 \varphi' \quad (1)$$

resulted in Kopple's proposal of a cis Leu–Pro peptide bond conformation for evolidine in solution. He was unable to find any all-trans peptide bond conformations which were also consistent with his nmr findings.

Because the conformation proposed by Kopple possesses a cis Leu–Pro peptide bond and is of relatively high intramolecular conformational energy, it was decided to conduct a more extensive search for cyclic conformations which are not only consistent with his nmr data, but which also have low intramolecular conformational energies. Instead of molecular model building, we used a computer in the search for low-energy cyclic conformations.

(1) K. D. Kopple, *Biopolymers*, **10**, 1139 (1971).

(2) F. W. Eastwood, G. K. Hughes, E. Ritchie, and R. M. Curtis, *Aust. J. Chem.*, **8**, 552 (1955).

(3) R. O. Studer and W. Lergier, *Helv. Chim. Acta*, **48**, 460 (1965).

(4) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); *J. Amer. Chem. Soc.*, **85**, 2870 (1963); M. Barfield and M. Karplus, *ibid.*, **91**, 1 (1969).

TABLE I
PROPOSED SOLUTION CONFORMATIONS OF EVOLIDINE

Conformations[a]	E_{conf},[b] kcal/mol	$(\varphi, \psi)_{Ser_1}$	$(\varphi, \psi)_{Phe_2}$	$(\varphi, \psi)_{Leu_3}$	$(\varphi, \psi)_{Pro_4}$	$(\varphi, \psi)_{Val_5}$	$(\varphi, \psi)_{Asn_6}$	$(\varphi, \psi)_{Leu_7}$
Kopple (ref 1)	21.8	70, 220	30, 340	120, 330	110, 130	30, 260	10, 0	135, 140
Kopple like	5.1	60, 240	30, 330	110, 300	120, 125	90, 270	10, 330	120, 120
All-trans peptide bonds	7.5	240, 0	90, 300	0, 300	120, 125	90, 120	10, 120	110, 330

[a] The Kopple and Kopple-like conformations possess a cis Leu_3-Pro_4 peptide bond. [b] Sum of residue energies from random-coil conformational energy maps[12-14] relative to the acyclic, non-hydrogen-bonded conformation of minimum energy.

The following Karplus-like relation proposed by Bystrov, *et al.*,[5] which connects the dihedral angle φ' and the coupling $J_{N\alpha}$ between the amide and α proton, was employed.

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

Only those low-energy conformations consistent with the measured coupling constants according to eq 2 were considered in the ring search. These conformations[6a] are (Figures 1 and 2): $(\varphi, \psi)_{Ser_1}$ = 60°, 0°; 60°, 240°; 60°, 270°; 60°, 300°; 60°, 330°; 240°, 0°; 240°, 240°; and 240°, 270°; $(\varphi, \psi)_{Phe_2}$ or $(\varphi, \psi)_{Asn_6}$ = 30°, 0°; 30°, 120°; 30°, 240°; 30°, 270°; 30°, 300°; 30°, 330°; 90°, 0°; 90°, 120°; 90°, 270°; 90°, 300°; 90°, 330°; 240°, 0°; 240°, 240°; and 240°, 270°; $(\varphi, \psi)_{Pro_4}$ = 120°, 125°; 120°, 270°; 120°, 300°; 120°, 330°; 120°, 0°; and 120°, 30°; $(\varphi, \psi)_{Val_5}$ = 30°, 120°; 30°, 240°; 30°, 270°; 30°, 300°; 30°, 330°; 90°, 120°; 90°, 270°; 90°, 300°; 90°, 330°; 240°, 240°; and 240°, 270°; $(\varphi, \psi)_{Asn_6}$ or $(\varphi, \psi)_{Phe_2}$ = 10°, 0; 10°, 120°; 10°, 240°; 10°, 270°; 10°, 300°; 10°, 330°; 110°, 0°; 110°, 120°; 110°, 270°; 110°, 300°; and 110°, 330°; $(\phi, \psi)_{Leu_{3 \text{ or } 7}}$ = $(\varphi, \psi)_{Asn_6}$ or $(\varphi, \psi)_{Phe_2}$; and $\varphi_{Leu_{3 \text{ or } 7}}$ = 0° or 120° instead of $\varphi_{Leu_{3 \text{ or } 7}}$ = φ_{Asn_6} or φ_{Phe_2} = 10° or 100°. All peptide bonds were assumed to be trans except the Leu_3-Pro_4 peptide bond, which was allowed to adopt the cis conformation as well.

Each of the more than 25 × 10^6 low-energy conformations was tested for ring closure on the computer in the manner described in detail elsewhere.[8-11] The independent (the residue energies are independent in the first approximation only if each peptide bond is planar trans) residue energies obtained from the conformational energy maps of Brant, Miller, Flory, *et al.*,[12-14] were summed and a molecular model was built for each of the cyclic conformations generated and checked for interactions (hydrogen bonds and steric overlaps) longer in range than those considered in the energy map calculations.

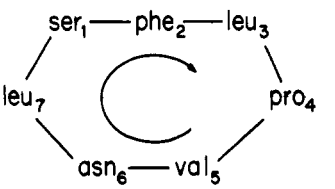

Figure 1. A schematic representation of the cyclic heptapeptide evolidine, where the sense of the arrow indicates movement from N to C^α in each residue.

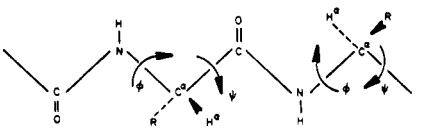

Figure 2. A schematic representation of a poly(L-peptide) in the all-trans or planar-zigzag conformation.

Results and Discussion

The backbone rotation angles of the two lowest energy cyclic conformations generated, whose molecular models indicate possession of the appropriate intramolecular hydrogen bonds,[15a] are listed in Table I together with the conformation proposed by Kopple.[1] It is apparent that the cis Leu_3-Pro_4 peptide bond conformation generated here is similar to but much lower in energy than Kopple's conformation.

All three conformations possess intramolecular hydrogen bonds involving NH_{Asn_6}, NH_{Phe_2}, and $(NH_2)_{Asn_6(side\ chain)}$.

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

(6) (a) See Figures 1 and 2 for the residue numbering scheme and the definition of the rotation angles φ and ψ. The rotation angles φ and ψ are taken[6b] as 0° in the trans or planar-zigzag conformation and adopt positive values for right-handed rotations (φ' = |240° − φ|). A more recently proposed convention,[7] which assigns φ = ψ = 180° to the planar-zigzag conformation, is not adopted here 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).

(7) J. C. Kendrew, W. Klyne, S. Lifson, T. Miyazawa, G. Nemethy, G. Ramachandran, and H. A. Scheraga, *Biochemistry*, **9**, 3471 (1970); *J. Biol. Chem.*, **245**, 6489 (1970); *J. Mol. Biol.*, **52**, 1 (1970).

(8) A. E. Tonelli, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1203 (1971).

(9) A. E. Tonelli, D. J. Patel, M. Goodman, F. Naider, H. Faulstich, and Th. Wieland, *Biochemistry*, **10**, 3211 (1971).

(10) F. A. Bovey, A. I. Brewster, D. J. Patel, A. E. Tonelli, and D. A. Torchia, *Accounts Chem. Res.*, in press.

(11) A. E. Tonelli and A. I. Brewster, *J. Amer. Chem. Soc.*, in press.

(12) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

(13) P. R. Schimmel and P. J. Flory, *ibid.*, **34**, 105 (1968).

(14) W. G. Miller and C. V. Goebel, *Biochemistry*, **7**, 3925 (1968).

(15) (a) Kopple[1] found the chemical shifts of the Phe_2 and Asn_6 amide protons to be nearly temperature independent, which is indicative[15b-20] of their participation in intramolecular hydrogen bonds or of their partial internal burial resulting in their limited accessibility to solvent. On the other hand, while the Asn_6 NH did exchange slowly with deuterium ($\tau_{1/2} \approx$ 500 min, the same as $\tau_{1/2}$ for Val_5), NH_{Phe_2} exhibited a deuterium exchange rate more than an order of magnitude faster ($\tau_{1/2}$ = 38 min). Kopple attributes[15b] this difference in exchange rates to differences in the local conformational flexibility of the Asn_6 and Phe_2 residues. (b) A. Stern, W. Gibbons and L. D. Craig, *Proc. Nat. Acad. Sci. U. S.*, **61**, 734 (1968).

(16) M. Ohnishi and D. W. Urry, *Biochem. Biophys. Res. Commun.*, **36**, 194 (1969).

(17) K. D. Kopple, M. Ohnishi, and A. Go, *J. Amer. Chem. Soc.*, **91**, 4087, 4264 (1969).

(18) N. Llinas, M. C. Klein, and J. B. Neilands, *J. Mol. Biol.*, **52**, 399 (1970).

(19) D. A. Torchia, A. diCorato, S. C. K. Wong, and C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 609 (1972).

(20) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *ibid.*, **94**, 616 (1972).

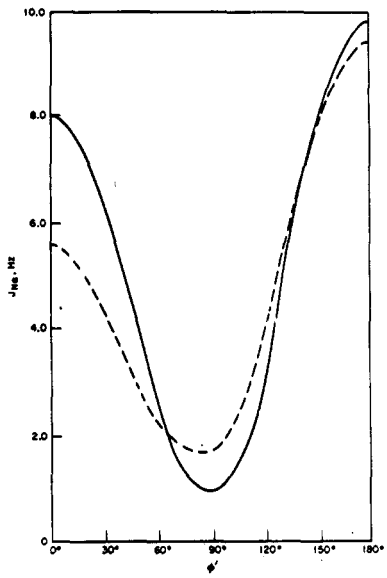

Figure 3. The "Karplus-like" relation of the vicinal coupling $J_{N\alpha}$ and dihedral angle φ' between N–H and C^{α}–H^{α}: (---) eq 1, (——) eq 2.

However, whereas these protons are bonded to CO_{Phe_2}, CO_{Asn_6}, and CO_{Leu_3}, respectively, in the cis peptide bond Kopple and Kopple-like conformations of Table I, they are bonded to CO_{Leu_3}, $CO_{Ser_1(side\ chain)}$ and CO_{Val_5}, respectively, in the lowest energy all-trans peptide bond conformation found. (In the all-trans conformation, $(NH_2)_{Asn_6(side\ chain)}$ forms a hydrogen bond with CO_{Val_5} only if the rotation angle x_1 about the C^{α}–C^{β} bond in the Asn_6(side chain) is 300°. The Asn_6 α to β proton coupling observed by Kopple indicates a 20% probability of $x_1 = 300°$ and 80% $x_1 = 60°$.)

The Val_5 amide proton appears to be partially buried by the Pro_4 pyrrolidine ring and the Val_5 side chain in both types of conformations, and might therefore be relatively inaccessible to solvent, as manifested[15-20] by its slow exchange with deuterium. Also, in both conformations NH_{Phe_2} is shielded (by the Leu_7–Ser_1 peptide bond in the Kopple-like conformation and by the Phe_2 phenyl ring in the all-trans conformation), and its resonance should and does appear[1] at higher field than other amide protons.

The curves in Figure 3 describe the Karplus-like relationship of the vicinal coupling and the dihedral angle between the amide and α protons and are obtained from eq 1 and 2. Clearly, the most striking differences in these relationships (eq 1 (proposed and used by Kopple[1]) and eq 2 (Bystrov, *et al.*[5]) used here) occur in the neighborhood of $\varphi' = 0°$, *i.e.*, when the amide and α protons are cis to each other. Equation 1 predicts $J_{N\alpha} = 5.6$ Hz and eq 2 $J_{N\alpha} = 8.0$ Hz for $\varphi' = 0°$ ($\varphi = 240°$).

Thus, based on eq 1, Kopple[1] would, and apparently does, reject conformations having $\varphi' \approx 0°$ ($\varphi \approx 240°$) for the Ser_1 ($J_{N\alpha} = 9.2$ Hz), Phe_2 ($J_{N\alpha} = 7.3$ Hz), and/or Val_5 ($J_{N\alpha} = 7.8$ Hz) residues. We, on the other hand, used eq 2 and allowed $\varphi' = 0°$ ($\varphi = 240°$) for all three residues. Consequently, the disparity in the vicinal couplings predicted by eq 1 and 2 near $\varphi = 0°$ ($\varphi = 240°$) resulted in Kopple's failure to find conformations similar to the all-trans peptide bond conformation generated here (compare φ_{Ser_1} in each of the conformations in Table I).

We believe that the Karplus-like relations[1,5] and the conformational energy maps[1,5,12,21-23] used to test[1,5,21-28] them are not presently precise enough to decide whether or not an observed $J_{N\alpha} = 7.0$–9.0 Hz can correspond to a cis conformation ($\varphi' = 0°$) of the amide and α protons. Until these relations (eq 1 or 2) are refined[24a] further, it would seem a bit risky to ignore the possible correspondence between a large coupling and a cis conformation when searching for solution conformations of cyclic polypeptides which are consistent with their nmr spectra.

Since no attempt has been made to minimize the energy of either of the low-energy conformations generated here and since neither the energies of the intramolecular hydrogen bonds[25] nor the energy of the cis peptide bond[26] when present was included, the difference in the sums of residue energies presented in Table I is not large enough to be used as a criterion for the rejection of either conformation.

One possible experimental means of selecting the correct solution conformation from the two generated here would be measurement of the infrared spectrum of evolidine. The amide III C–N stretch band at ≈ 1350 cm^{-1} has been interpreted[27-30] as being characteristic of cis peptide bonds. If such a band appears in the ir spectrum of evolidine, then the Kopple-like conformation with a cis Leu_3-Pro_4 peptide bond is probably the conformation of evolidine in solution.

(21) A. E. Tonelli and F. A. Bovey, *Macromolecules*, **3**, 410 (1970).
(22) A. E. Tonelli, F. A. Bovey and A. I. Brewster, *ibid.*, **3**, 412 (1970).
(23) G. N. Ramachandran, R. Chandrasekaran, and K. D. Kopple, *Biopolymers*, **10**, 2113 (1971).
(24) (a) Ramachandran, *et al.*,[23] have fitted the constants A, B, and C in the vicinal coupling relation $J_{N\alpha} = A \cos^2 \varphi' + B \cos \varphi' + C \sin^2 \varphi$ by least-squares methods to the couplings calculated and measured for several *N*-methylacetamides and cyclic dipeptides. When $A = 7.5$, $B = -1.9$, and $C = 1.7$, $|\Delta J_{N\alpha}|_{av} = 0.17$ Hz and for $A = 8.9$, $B = -0.9$, and $C = 0.9$, $|\Delta J_{N\alpha}|_{av} \approx 0.20$ Hz according to Ramachandran, *et al.*[23] Thus, it is impossible to choose between the Kopple and Bystrov, *et al.*,[5] relations (eq 1 and 2) on the basis of this comparison. In addition, an example of a large coupling $J_{N\alpha} = 8.0$–8.5 Hz for $\varphi' = 0°$ (*cis*-N(H)–$C^{\alpha}(H^{\alpha})$ conformation) has been observed[19,24b] recently for the serine amide protons in *cyclo*-Pro-Ser-Gly-Pro-Ser-Gly. (b) A. E. Tonelli, *J. Amer. Chem. Soc.*, **94**, 346 (1972).
(25) D. A. Brant, *Macromolecules*, **1**, 297 (1968).
(26) A. E. Tonelli, *J. Amer. Chem. Soc.*, **93**, 7153 (1971).
(27) T. Miyazawa, *J. Mol. Spectrosc.*, **4**, 155 (1960).
(28) K. Blaha, J. Smokikova, and A. Vitek, *Collect. Czech. Chem. Commun.*, **31**, 4296 (1966).
(29) L. Mladenova-Orlinova, K. Blaha, and J. Rudinger, *ibid.*, **32**, 4070 (1967).
(30) T. Isemura, H. Okabazashi, and S. Sakakibara, *Biopolymers*, **6**, 307 (1968).