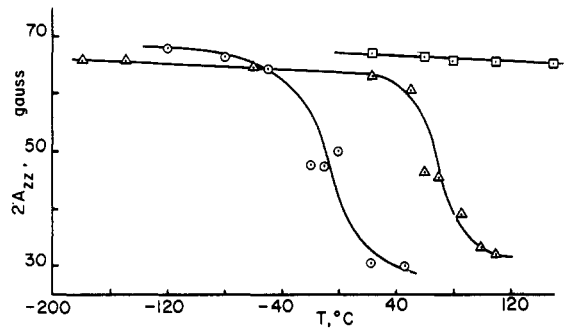

Figure 13. Separation of outer extrema of the esr spectra of MDI-PE-TMP (△), MDI-EG-GLY (□), and swollen MDI-PE-TMP samples (⊙).

asymptote was very close to 31.8 G, a value determined from Figure 2 for free nitroxide in solution. Thus a flexibly structured cage was experienced by nitroxides above 120° for the MDI-PE-TMP, above 23° when the same polymer is swollen, but not below 150° for MDI-EG-GLY.

The spin label was noted to be always adjacent to MDI units in the MDI-EG-GLY polymer. One would expect the spin label to produce spectra indicating the changing participation of these MDI units in microdomains with altering macroscopic states. Even with the perturbation of the bulky nitroxide the strongly cooperative interactions of such a solid and the very low spin concentrations suggest that the observed transitions in Figure 12 are associated with a physical phenomena of the unlabeled polymer. A key point to be made is the absence of any esr spectral changes in the MDI-PE-TMP lower than 70°. This must be contrasted with the mechanical and thermal data which show changes, but ones associated with the soft poly(ether) segment. It is tempting to associate this elastomer transition at 70° with the DTA peak at 119° based on T_w to T_g and to melting temperature correlations found in ref 4. Because of the statistical distribution of labels and the necessarily heterogeneous character of polymeric microdomains the width of the transition can be rationalized. Exact identification of the transitions reported in this study depends on further work. It is sufficient to note here the indications of transitions in an amorphous polyether urethane having a very low "hard segment" composition.

Acknowledgment. A grant from the Research Corp., without which this work would not have been done, is gratefully acknowledged.

References and Notes

(1) T. J. Stone, T. Buckman, P. L. Nordio, and H. M. McConnell *Proc. Nat. Acad. Sci. U. S.*, 54, 1010 (1965).
(2) K. W. Butler, I. C. P. Smith, and H. Schneider, *Biochim. Biophys. Acta*, 219, 514 (1970).
(3) O. H. Griffith and A. S. Waggoner, *Accounts Chem. Res.*, 2, 17 (1969).
(4) G. P. Rabold, *J. Polym. Sci., Part A-1*, 7, 1203 (1969).
(5) A. T. Bullock, J. H. Butterworth, and G. C. Cameron, *Eur. Polym. J.*, 7, 445 (1971).
(6) G. P. Safanov, Y. A. Ol'khov, and S. G. Entelis, *Vysokomol. Soedin., Ser. A*, 12, 2379 (1970).
(7) Pertti Tormala, J. Martinmaa, K. Silvennoinen, and K. Vaahtera, *Acta Chem. Scand.*, 24, 3066 (1970).
(8) S. C. Gross, *J. Polym. Sci., Part A-1*, 9, 3327 (1971).
(9) R. C. McCalley, F. J. Shimshick, and H. M. McConnell, *Chem. Phys. Lett.*, 13, 115 (1972).
(10) H. M. McConnell and B. G. McFarland, *Quart. Rev. Biophys.*, 3, 91 (1970).
(11) G. Pozzi and C. S. Johnson, *J. Magn. Resonance*, 3, 436 (1970).
(12) A. N. Kuznetsov, A. M. Wasserman, A. V. Volkov, and N. N. Korst, *Chem. Phys. Lett.*, 12, 103 (1971).
(13) W. Snipes and A. Keith, *Res./Develop.*, 21, 22 (1970).
(14) J. H. Freed, G. V. Bruno, and C. F. Polnaszek, *J. Phys. Chem.*, 75, 3385 (1971).
(15) A. S. Waggoner, O. H. Griffith, and C. R. Christensen, *Proc. Nat. Acad. Sci. U. S.*, 57, 1198 (1967).
(16) N. Edelstein, A. Kwok, and A. H. Maki, *J. Chem. Phys.*, 41, 179 (1964).
(17) R. W. Seymour and S. L. Cooper, *Macromolecules*, 6, 48 (1973).
(18) L. Morbitzer and H. Hespe, *J. Appl. Polym. Sci.*, 16, 2697 (1972).
(19) S. L. Samuels and G. L. Wilkes, *J. Polym. Sci., Part C*, 11, 807 (1973).
(20) L. L. Harrell in "Block Copolymers," S. L. Aggarual, Ed., Plenum Press, New York, N. Y., 1970, p 213.
(21) E. G. Rozantzev and L. A. Krinitzkaya, *Tetrahedron*, 21, 491 (1969).
(22) P. Ferruti, D. Gill, M. P. Klein, H. H. Wang, G. Entine, and M. Calvin, *J. Amer. Chem. Soc.*, 92, 3704 (1970).
(23) T. C. Ward, Ph.D. Thesis, Princeton University, 1966.
(24) D. J. David and H. B. Staley, "Analytical Chemistry of the Polyurethanes," Interscience, New York, N. Y., p 287.
(25) J. L. Illinger, N. S. Schneider, and F. E. Karasz, *Polym. Eng. Sci.*, 12, 25 (1972).
(26) Polarity effects due to the $CHCl_3$ diluent were small. Values of τ_c calculated using b and $\Delta\gamma$ (see ref 1). Values obtained for both swollen and unswollen polymers were almost identical.
(27) M. Nagura and E. Wada, *Polym. J.*, 4, 115 (1973).

Theoretical Analysis of Pyrrolidine Ring Puckering and the Conformational Energies of Proline and 5-Methylproline Dimers[1]

C. M. Venkatachalam,* B. J. Price, and S. Krimm

Harrison M. Randall Laboratory of Physics, Biophysics Research Division and Macromolecular Research Center, University of Michigan, Ann Arbor, Michigan 48104. *Received October 3, 1973*

ABSTRACT: The stable geometries of the pyrrolidine ring are obtained from semiempirical energy calculations. The puckering of the ring is studied as a function of the dihedral angle θ associated with the atoms C^{δ}, N, C^{α}, and C^{β}. For an isolated prolyl ring, for each value of θ, two energy minima are observed corresponding to two positions of the C^{γ} atom. While the global energy minimum occurs at $\theta = -10°$, it is found that θ can vary from -20 to $+20°$ within 0.5 kcal/mol from the minimum energy. The effect of this flexibility on the conformational characteristics of an internal Pro-Pro dimer is studied by calculating the conformational energies of the dimer. It is shown that while trans dimer prefers γ^+ and γ^- puckered rings, the cis dimer prefers β^+ and γ^- puckered rings. These studies are extended to the case of 5-methylproline dimers.

Several theoretical studies on the conformational stability of polyproline and peptides containing proline have been reported.[2-7] In many of these, the pyrrolidine ring itself has been kept rigid either in a planar structure or in a puckered conformation. Accordingly, in these studies, the torsion angle φ[8] about the bond N-C^{α} was kept constant at a suitable value to ensure ring closure. Recently, however, it is becoming evident that the puckering of the pro-

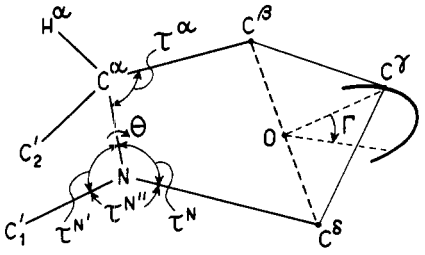

Figure 1. The geometry of the C^{γ} arcing. O is the center of the Γ circle and lies on the line C^{δ}- - -C^{β}. The dihedral angle θ is associated with the atom C^{δ}–N–C^{α}–C^{β} and is related to φ. The methylene hydrogens are not shown here, though they have been included in the energy calculations (see text).

line ring and its effects on backbone conformations of the molecule must be considered in these calculations. The proline ring is capable of adopting different kinds of puckered geometries depending on the nature of the environment it meets within a molecule. This flexibility of the ring gives rise to two effects. A range of values for φ may be available at the prolyl residue, and for a given value of φ the ring can adopt different kinds of structures.

In an earlier study by Ramachandran *et al.*[9] the proline ring energies were theoretically obtained for various ring structures. Only a small range of φ near $-60°$ was explored in that study and the resulting low energy conformations of the proline ring listed in Table III of their paper[9] were essentially γ puckered. In this paper, we extend such a treatment to other kinds of puckering undoubtedly available to the proline ring. The stable ring geometries so obtained are used in calculating conformational energies of proline dimer. The later part of the paper is concerned with a related molecule, 5-methylproline. The methyl group attached to the C^{δ} atom of the ring in this case modifies the puckering of the ring. The conformational energies of the 5-methylproline dimer are also obtained.

Ring Closure. The method of computing the coordinates of the atoms of the pyrrolidine ring follows that of Ramachandran *et al.*[9] All the bond lengths are held constant. The three bonds at the nitrogen are kept in the same plane (Figure 1) and the atoms C^{α} and C^{δ} are fixed by the bond angles $\tau^{N'}$ and τ^{N} at the nitrogen. C^{β} is then determined by the bond angle N–C^{α}–C^{β} (referred to as τ^{α}) and the dihedral angle θ associated with C^{δ}–N–C^{α}–C^{β}. Atoms C_2' and H^{α} are fixed in a plane bisecting the angle τ^{α}. This fixes the dihedral angle[8] φ associated with the atoms C_1'–N–C^{α}–C_2'. There is a linear relation between φ and θ. While the relation depends on τ^{α}, within the range of meaningful variation of τ^{α} considered in this analysis it is found that $\varphi \approx \theta - 60°$.

The atom C^{γ} has to be fixed such that the bond lengths C^{β}–C^{γ} and C^{δ}–C^{γ} are equal to the constant values assumed for them. The locus of all positions of C^{γ} corresponding to the constant bond lengths C^{β}–C^{γ} and C^{δ}–C^{γ} is obviously a circle with its center O lying on the line C^{β}- - -C^{δ}. Various positions of C^{γ} on this circle may be obtained by rotating O- - -C^{γ} about the line C^{β}- - -C^{δ}. Γ is the angle of this rotation. However, only a portion of the Γ circle that produces reasonable values for the bond angles at C^{β} and C^{δ} is of interest here. Choosing a value for Γ completely determines the conformation of the ring. The hydrogens on C^{β}, C^{γ}, and C^{δ} are fixed such that the plane HCH bisects the appropriate C–C–C or C–C–N angle.

Classification of Ring Geometries. The procedure of C^{γ} arcing illustrated in Figure 1 is merely to ensure ring closure, and it does not in any way restrict the types of puckered rings that will be included in the energy calculations. The method is also convenient since it permits a classification of ring geometries in terms of θ (and hence in terms of φ) rather than in terms of any other torsion angle. For a given θ, as the angle Γ varies, ring geometries of various kinds of puckering are produced in a specific sequence. Those geometries corresponding to puckering at a single atom are particularly interesting owing to their simplicity of structure. The ring geometry is perfectly X puckered if the torsion angle about the bond opposite to the atom X is zero or nearly so, so that all the ring atoms except X lie in a plane. Following Torchia,[10] we shall refer to X^{-} puckering if the atom X lies on the same side of the ring plane as the carbonyl carbon C_2' (see Figure 1), and X^{+}puckering if X lies on the other side of the ring plane.

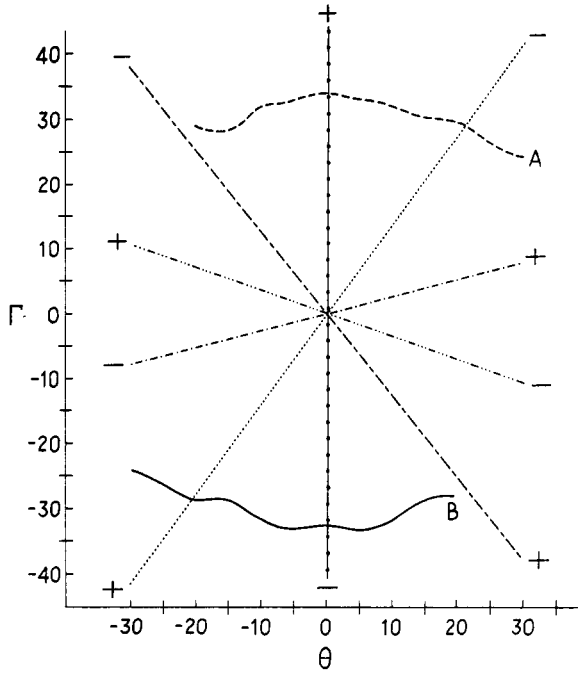

Figure 2. Γ–θ plot for various types of puckered geometries: (- - -) type A minimum energy geometry for proline; (——) type B minimum energy geometry for proline; (· · · · ·) C^{β} puckered; (-·-·-) C^{α} puckered; (-·····-·····-) N puckered; (— — — —) C^{δ} puckered; (-·-·-·-·-) C^{γ} puckered.

For each value of θ, a geometry corresponding to a puckering at a single atom is associated with a certain value of the angle Γ. The value of Γ for a given θ, corresponding to the five kinds of X-puckered geometries, is given in Figure 2. Γ–θ plots for γ-, δ-, N-, α-, and β-puckered geometries are all seen to be straight lines of different slopes; all the lines pass through the point $\Gamma = 0$, $\theta = 0$, which is the perfectly planar ring. Every point on the Γ–θ plane defines a ring geometry, but only those points lying on the five straight lines correspond to geometries that are puckered at a single atom. The sign of the puckering has been marked at the ends of each straight line and it may be noted that the sign reverses at $\theta = 0$ for each kind of puckering. $\Gamma = 0°$ is defined by the position of C^{γ} in the Γ circle corresponding to the condition $\chi^{N\delta} = \chi^{\alpha\beta}$.

The classification of ring geometries that are not puckered at a *single* atom will be more involved. In general, it may be useful to broadly divide the set of geometries into two categories based on the sign of a certain dihedral angle in the ring. Accordingly, those having negative values for $\chi^{\delta\gamma}$ will be referred to as type A and those with positive values for $\chi^{\delta\gamma}$ as type B.[9] (This classification applies to puckering of any type except for those ring

structures for which the dihedral angle χ^{γ} is zero, that is, C^{α}-puckered geometries. Ordinarily this presents no problem since, as is shown in a later section, minimum energy prolyl ring structures are quite unlikely to be C^{α} puckered.) Thus, all points lying above the α straight line in the Γ-θ plot are geometries of type A while those lying below the α line are of type B.

Calculation of the Energy of the Prolyl Ring. The total self-energy E of the prolyl ring is a function of τ^{α}, τ^{N}, $\tau^{N'}$, θ, and Γ

$$E = E(\tau^{\alpha}, \tau^{N}, \tau^{N'}, \theta, \Gamma) \quad (1)$$

The self-energy E was evaluated by varying τ^{α} from 99 to 107°, τ^{N} from 109 to 117°, $\tau^{N'}$ from 119 to 127°, in steps of 2°. θ went from −40 to +40° in steps of 5°, while Γ was varied in steps of 2°. The total energy E was calculated as a sum of electrostatic and van der Waals interactions, intrinsic torsional potentials, and bond angle deformation energies.[9] Only interactions involving the ring atoms (including the hydrogens), C_1', H^{α}, and C_2' (see Figure 1) were considered in the ring energy calculations. In particular, C_1^{α} and O_1 attached to C_1' have not been included in the energy calculations. Thus, the self-energies obtained here are applicable to both *cis*- and *trans*-prolyl systems. Also, the interactions of C_1^{α} and O_1 with the ring atoms do not seem to affect the stable ring structures. The electrostatic energy was computed from suitable monopole charges[11] located on the atoms, the dielectric constant being set equal to 4.[12] The nonbonded interaction energy was evaluated using an interatomic potential of the Lennard-Jones type with the values of the parameters given by Scott and Scheraga.[13] Interactions between third- and higher neighboring pairs of atoms were included in computing the nonbonded energy. The bond-angle strain energy V_{τ} was taken to be of the form[9,12]

$$V_{\tau} = K_{\tau}(\tau - \tau_0)^2 \quad (2)$$

The force constant K_{τ} was taken as 72 kcal/mol and the equilibrium bond angle τ_0 was set at 112° for bond angles at the carbon atoms in the ring. However, the parameters K_{τ} and τ_0 for the three angles at the nitrogen atom were assigned values obtained for the nitrogen angles of *N,N*-dimethylacetamide from normal coordinate analysis.[14] Accordingly, τ_0 was taken to be 120° for all the three angles at the nitrogen; for the angles $\tau^{N'}$ and $\tau^{N''}$ the force constant K_{τ} was taken to be 72 kcal/mol while for the angle τ^{N} a value of 107 kcal/mol was employed.

The intrinsic torsional potential[12,15] is calculated as

$$V_{\chi} = (1/2)V_0(1 + \cos 3\chi) \quad (3)$$

for the C-C bonds in the ring with V_0 = 3.4 kcal/mol.[9] V_0 was, however, set at a lower value of 1 kcal/mol for the bonds N-C^{α} and N-C^{δ}.

Stable Ring Geometries. The total self-energy of the prolyl ring is a function of five parameters (eq 1). The *puckering characteristics* of low-energy geometries are, to a large extent, governed by Γ, θ, τ^{α}, and τ^{N}, while the effect due to variation in the angle $\tau^{N'}$ is relatively minor. The smaller the bond angles τ^{α} and τ^{N}, the larger are the torsion angles of the minimum energy structures. However, bond angles for the minimum energy structures are near τ^{α} = 103°, τ^{N} = 113°, and $\tau^{N'}$ = 123° and these are found not to vary appreciably with θ. Figure 3a shows the ring energy as a function of θ for various single atom puckered ring geometries. For each θ, the three bond angles have been fixed at values that yield lowest energy. For a given set of bond angles τ^{N}, $\tau^{N'}$, and τ^{α}, for each θ, and as Γ is varied from −40 to +40°, it is found that there are two well-separated values of Γ for which the self-energy becomes a minimum; one gives a type A structure and the other a type B structure. The energy of these two

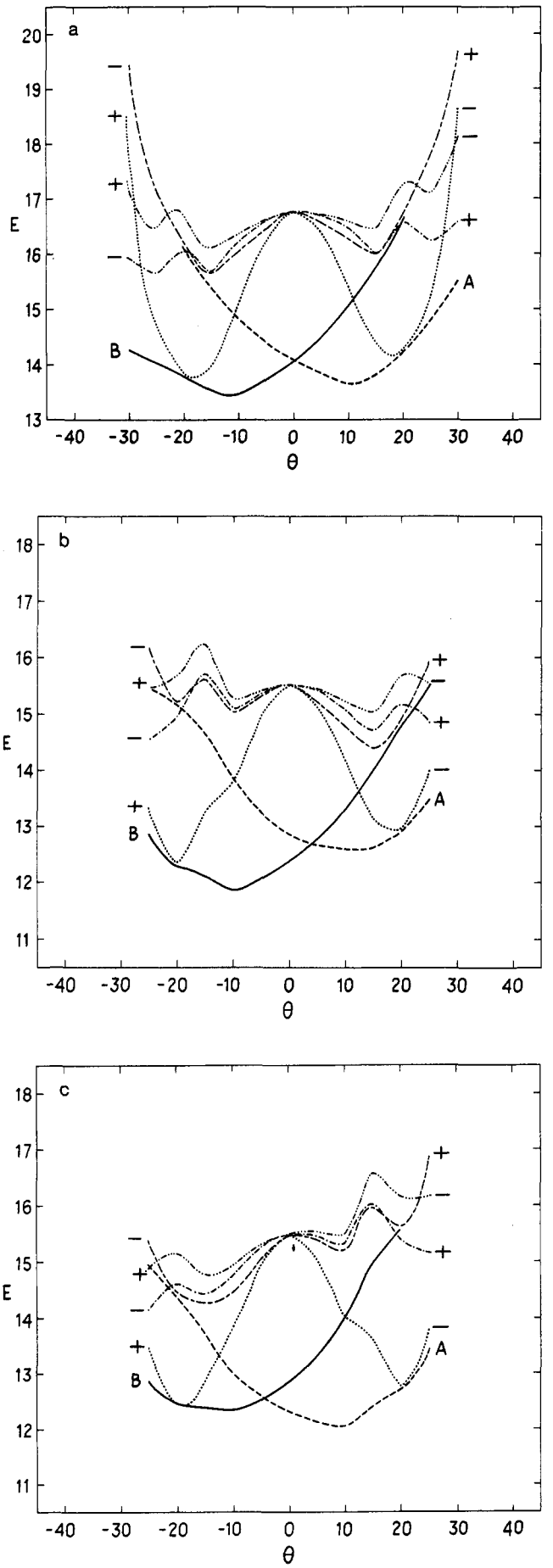

Figure 3. Self-energy of various rings as a function of θ (symbols are the same as in Figure 2): (a) proline; (b) *trans*-5-methylproline; (c) *cis*-5-methylproline.

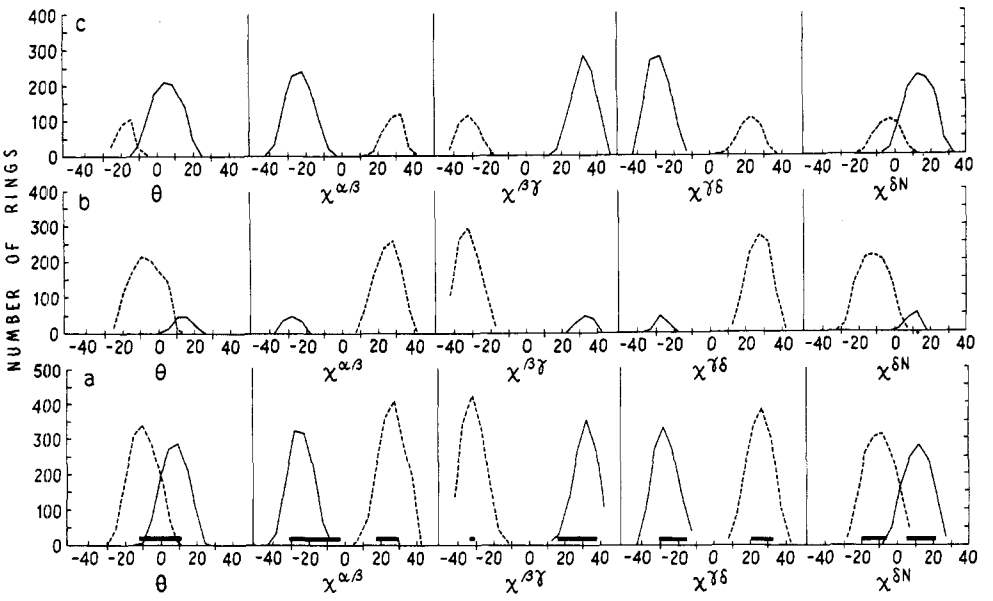

Figure 4. The distribution of the dihedral angles in the set of proline rings of energy within 1.5 kcal/mol from the global minimum energy: (———) type A; (- - -) type B ring geometries; (a) proline; (b) *trans*-5-methylproline; (c) *cis*-5-methylproline.

structures has also been plotted in Figure 3a for each θ. For positive values of θ, the type A minimum is lower in energy than the type B, while the reverse is the case for negative θ. Figure 3 yields interesting information about the stable ring geometries. The global minimum occurs at $\theta \cong -10°$ and the geometry is of type B and is not single-atom puckered. This fact may also be gleaned from Figure 2 where the values of Γ for the minimum energy A and B structures have also been plotted against θ. If $\theta \cong 0°$, the ring is γ puckered. The ring may be considered to be approximately γ puckered if $|\theta| < 10°$. At $|\theta| \sim 10°$, both $|\theta|$ and $|\chi^{N\delta}|$ are nearly equal and the structure is not single atom puckered. At $|\theta| \sim 20°$, the two minimal energy geometries are almost identical to β-puckered and δ-puckered geometries. At $\theta \sim -20°$, type A is similar to a δ^--puckered structure, while type B is almost identical with a β^+-puckered structure. The reverse is the situation at $\theta \sim 20°$, where A is β^- puckered and B is δ^+ puckered. But, it is seen from Figure 3a that at $\theta \sim -20°$, A is higher in energy than B by nearly 2.5 kcal/mol and at $\theta \sim 20°$, B is higher than A by about 2.5 kcal/mol. Thus we may conclude that at $\theta \sim -20°$ the ring will prefer to be β^+ puckered and at $\theta \sim 20°$ it will prefer to be β^- puckered.

When $|\theta| < 10°$, however, the ring is essentially γ puckered. This region has been studied earlier.[9] Here, for negative θ, the ring is γ^--puckered and for positive θ it is γ^+-puckered. However, the energy difference between γ^- and γ^+ is not appreciable at $\theta \sim 0°$. It may be noted that the planar ring $\theta = 0°$, $\Gamma = 0°$ is about 2.8 kcal/mol higher in energy than an optimum γ-puckered ring with $\theta = 0°$, and is about 3.4 kcal/mol higher than that of the global minimum geometry with $\theta \sim -10°$. This clearly demonstrates the fact that it is unwise to use a planar ring structure in energy calculations in those molecular systems where interactions with prolyl ring atoms are involved.

Figure 3a also shows that the α-, N-, and β-puckered geometries have ring energies that are at least 2 kcal/mol higher than that of the minimum energy structure. This indicates that in a molecule having very little overcrowding the ring geometry will probably not be α, N, or β puckered. In a strained system, however, the ring may adopt any of these geometries and still minimize the total conformational energy of the molecule.

The variation of the self-energy with θ is within 1 kcal/mol in the region $-25° < \theta < +25°$. This will provide a rotational flexibility about the bond N-C$^\alpha$, φ therefore varying from about -85 to $-35°$. Further restrictions on φ may occur in a molecule as a result of other interactions.

The nature of the torsion angles can be studied in greater detail by using Figure 4, where the distribution of the torsion angles for various geometries having ring energies within 1.5 kcal/mol from the global minimum is presented. In (a) the distribution curves for the L-prolyl ring are given. These plots are similar to those obtained by Ramachandran *et al.*[9] The horizontal thick lines show the spread of values of the torsion angles found in a few proline-containing peptides from X-ray structure analysis.[16] There is good agreement between experimental values and peaks in the distribution curves. It is interesting to note that the A and B curves overlap and show an appreciable number of ring structures having zero torsion angle (*i.e.*, single-atom puckered) only for θ and $\chi^{\delta N}$; γ and β puckering are the only single-atom puckering available for the ring within 1.5 kcal/mol. For the other torsion angles, the A and B branches are well separated.

While the results obtained here are in excellent agreement with an earlier study of ring puckering,[9] it is important to consider the ring energy calculations of Nishikawa and Ooi[17] in the context of the present studies. Instead of fixing the C$^\gamma$ atom by energy minimization, they[17] obtain the C$^\gamma$ position by requiring either that $\chi^{N\delta} = 0$ (their $\gamma 1$ position) or $\chi^{\alpha\beta} = 0$ ($\gamma 2$ position). $\gamma 1$ is β puckered and $\gamma 2$ is δ puckered. The values of Γ that they have employed for each θ may be seen from the Γ-θ plot in Figure 2 to be largely different from those of the minimum energy structures obtained by us in the region $-15° < \theta < +15°$. In this region of θ, the ring energies will also be about 1.5 kcal/mol higher, and even higher near $\theta \sim 0°$, where their artificial restrictions to $\gamma 1$ or $\gamma 2$ positions lead inevitably to the use of the planar ring. It is rather difficult to justify the use of these restrictions in a study especially devoted to the understanding of the nature of the ring puckering. In view of this, the use of Nishikawa-Ooi geometries and their ring energies[17] in energy calculations on polymers containing proline[18,19] is undoubtedly questionable, and some of the results so obtained[18,19] could be erroneous and misleading.

Table I
Torsion Angles of the Proline Ring Corresponding to Minimum Energy Ring Puckering

	Type A				Type B			
θ	$\chi^{\alpha\beta}$	$\chi^{\beta\gamma}$	$\chi^{\gamma\delta}$	$\chi^{\delta N}$	$\chi^{\alpha\beta}$	$\chi^{\beta\gamma}$	$\chi^{\gamma\delta}$	$\chi^{\delta N}$
−40		*a*			32	−18	−3	26
−30		*a*			31	−25	8	14
−20	3	14	−25	28	30	−31	19	1
−10	−11	27	−31	26	27	−35	28	−11
0	−21	34	−32	20	20	−32	31	−19
10	−28	35	−28	11	11	−27	31	−26
20	−31	33	−21	0	−2	−16	27	−29
30	−32	27	−10	−13		*a*		
40	−33	20	1	−25		*a*		

[a] The minimum does not exist.

Table II
Comparison with Ring Torsions Experimentally Found in Some Crystals

			Obsd				Calcd[a]			
Crystal	θ	Type	$\chi^{\alpha\beta}$	$\chi^{\beta\gamma}$	$\chi^{\gamma\delta}$	$\chi^{\delta N}$	$\chi^{\alpha\beta}$	$\chi^{\beta\gamma}$	$\chi^{\gamma\delta}$	$\chi^{\delta N}$
Leu-*Pro*-Gly[b]	−11.1	A	−3.4	16.1	−21.8	21.0	−10	26	−31	27
		B	26.1	−31.4	23.8	−8.2	27	−34	26	−9
Gly-*Pro*-Leu-Gly[c]	6.3	A	−17.3	22.1	−17.5	6.2	−24	34	−29	14
cyclo(Pro-Leu)[d]	18.5	A	−31.5	36.0	−25.1	4.5	−31	34	−22	2
Tosyl-*Pro*-Hyp[e]	0.3	B	20.6	−32.4	31.2	−20.0	21	−34	32	−20

[a] These dihedral angles correspond to minimum energy structure for the corresponding value of θ. [b] Reference 20. [c] Reference 21. [d] Reference 22. [e] Unpublished results of M. N. Sabesan and K. Venkatesan reported in ref 9.

Table I lists the torsion angles in the ring for various θ for type A and type B minimum energy structures. In comparing these values with the experimental values for a prolyl residue in a molecule, several points should be noted. The ring puckering can be appreciably influenced by interactions in the molecule not considered in the ring energy calculations. The value of θ is governed not only by the ring puckering, but also by the chain conformation and folding of the polypeptide. It is here that other interactions not involving the ring atoms become important. Such factors as backbone conformational stability, steric interactions in a cyclic peptide, etc., may restrict the value of φ (and hence θ) to a certain value in the range −100 to −20°. For the given value of φ, the torsion angles of the ring may be expected to be close to the values given by Table I only if the ring atoms themselves are not involved in any significant intra- or intermolecular interactions.

The present calculations assume a trigonal planar nitrogen, which is appropriate for a prolyl ring in a peptide with the partial double bond C′–N. In an amino acid, the puckering can be different, as shown by Ramachandran.[9]

The torsion angles calculated from the X-ray structure of peptides are given in Table II. Of the various peptides studied, only those containing proline with a trigonal nitrogen (proline at N terminal has not been included) have been included. In each case, the calculated torsion angles corresponding to the minimum *ring* energy structure for the relevant θ and the type of the ring are also given. In the three cases of Leu-Pro-Gly (type B), *cyclo*(Pro-Leu), and tosyl-Pro-Hyp, there is excellent agreement between calculated and observed torsion angles. In Gly-Pro-Leu-Gly, however, the torsion angles are quite different. The ring energy minimum for θ = 6.3° is calculated to be at Γ = 32° while the observed position of C^γ corresponds to $\Gamma \approx 18°$.

The origin of this discrepancy was suspected to be possible steric conflicts between C^γ and other atoms *in the crystal*. This was verified to be correct by an energy calculation for various positions of C^γ atom in the Γ circle including all intra- and intermolecular interactions in the crystal. The observed symmetry of the crystal was employed to generate neighboring molecules. The minimum in total energy was found to be at Γ = 18°! The position of C^γ atom for Γ = 32° resulted in a net increase of about 1 kcal/mol in total energy. While this agreement explains the apparent discrepancy in Table II and shows that the ring energy calculations are correct, it also shows that the C^γ position can be easily modified from the minimum energy geometry of Table I by interactions in the crystal. This obviously complicates the comparison between observed and calculated proline structures.

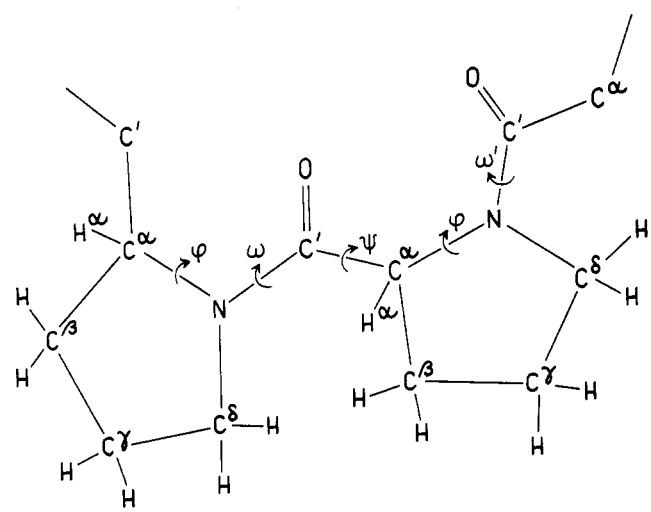

Figure 5. The Pro-Pro internal dipeptide.

Pro-Pro Internal Dipeptide. The prolyl ring geometries associated with Pro-Pro dimers need not be identical to those of the isolated prolyl residue, and we have therefore extended our studies to the dimer system. The chemical structure of the internal dipeptide Pro-Pro is shown in Figure 5. The geometries of both of the rings were kept identical. While ψ was varied from −180 to +180° in steps of 10°, ω was varied in steps of 5°, from −25 to +25° for

the *cis* dimer and from 155 to 205° for the trans dimer. For each (φ, ψ, ω) the angle ω' was varied near 0° for cis and near 180° for trans. Thus the total conformational energy of the dimer was obtained by summing nonbonded, electrostatic and torsional potentials and the self-energy of the two prolyl rings. The torsional energy for ω rotation was taken as

$$V_\omega = (1/2)V_0(1 - \cos 2\omega) \quad (4)$$

with a value of 20 kcal/mol for V_0.

For each value of φ (*i.e.*, for each θ) six ring geometries were selected corresponding to the values of Γ for α-, β-, δ-, and N-puckered rings and two minimum ring energy structures of the types A and B. The conformational energies so obtained were used to prepare plots of energy distribution in the θ-ψ plane; for each θ and ψ the best ring geometry and the values of ω and ω' that lead to the lowest energy have been employed. The θ-ψ energy plots obtained for cis and trans dimers are given in Figure 6. In addition to the energy contours, the ring conformation for which the lowest energy is obtained is symbolically indicated for each θ and ψ. Dots represent the C^γ position corresponding to the lowest ring energy minimum for each θ, while the X indicates the second energy minimum obtained by C^γ arcing. The dot will be of type A for positive θ, and of type B for negative θ, while the reverse is the case for the X.

It is found that for the cis (Figure 6d) and for the trans dimer (Figure 6a), the global *ring energy* minimum geometry is found to give the lowest dimer energy near energy minima in the θ-ψ plots. Therefore, for most of the low-energy conformations of the dimer, only C^γ positions corresponding to the values of Γ given by the A and B curves of the Γ-θ plot are relevant. (Thus, in particular, for most of the dimer conformations, the Nishikawa-Ooi geometries[17,18] are not the preferred ones.)

The energy map for the trans dimer shows two energy minima—the deeper minimum at $\psi = 160°$ and $\theta = -10°$ and the second minimum at $\psi = 120°$ and $\theta = 10°$. Note that a flexibility in θ from -25 to $+20°$ is available for the dimer within 2 kcal/mol of the dimer energy minimum. The range of values of ψ available for the dimer is from 100 to 190°, in agreement with the results of Schimmel and Flory[3] and other earlier calculations.[23,24] The dimer prefers the first minimum C^γ arced conformation. In the region $-10 < \theta < 10$ the ring is essentially γ^- or γ^+ puckered. In the region $-15 < \theta < 10°$, the ring is puckered at more than one atom. At $\theta \sim -20°$, the ring is β^+ puckered. Thus, the internal dimers in a random *trans*-polyproline will contain primarily γ^+- and γ^--puckered rings and to a smaller extent β^+-puckered rings. Only a few of the high-energy conformations near $\psi \sim 190°$ can accommodate δ^-- and N^+-puckered geometries.

The energy map for the cis dimer (Figure 6d) shows that its flexibility is considerably less. The only minimum is at $\psi = 160°$ and $\theta = -15°$. Within 2 kcal/mol from this minimum, θ can vary from -30 to 0°. Thus, the energy minimum is rather asymmetrically located with respect to $\theta = 0°$. Since the first minimum C^γ position is favored for most of the dimer conformations, the cis dimer prefers γ^-- and β^+-puckered geometries. It is interesting to compare the puckering in the cis with that in the trans dimer. The cis dimer will have more β^+-puckered and considerably less γ^+-puckered ring geometries than the trans internal dimer. That the ring puckering is different in forms I and II, is compatible with the results obtained by Torchia[10] who has estimated the torsion angles in the prolyl ring from coupling constants obtained from nmr spectra of polyproline.

Recently, ^{13}C nmr spectra for molecules containing proline have been reported.[25,26] It has been noted[26] that C^α and C^δ chemical shifts in *trans*-polyproline are almost identical to those in *cis*-polyproline, while the C^β and C^γ chemical shifts are altered as one goes from *cis*- to *trans*-polyproline. It is interesting that chemical shifts of only C^β and C^γ are altered and this is probably due, in part, to the kinds of ring puckering associated with the *cis*- and *trans*-polyproline.[25]

Conformation $\psi \sim -60°$. The compact conformation corresponding to $\psi \sim -60°$, one of the possible conformations for an *isolated* prolyl residue, was found to be precluded by severe steric interactions for the Pro-Pro dimer by Schimmel and Flory,[4] who employed a planar prolyl ring. On the other hand, Gō and Scheraga[27] found that the Pro-Pro dimer has an energy minimum at $\psi \sim -60°$, but the energy at this minimum is about 8 kcal/mol higher than that at the minimum near $\psi \sim 160°$, if both the rings in the dimer are taken to be γ puckered. They further recognized[27] that the actual energy difference between the two minima may be somewhat less than 8 kcal/mol if flexibility of bond lengths and bond angles are considered. Recently, however, it has been contended[18,19] that this energy difference is only about 1 kcal/mol.

A detailed calculation involving an energy minimization at $\psi \sim -60°$ region with respect to the bond angles τ^α, τ^N, $\tau^{N'}$, the torsion angle θ and the different kinds of puckering, shows that an energy minimum occurs at $\theta = 10°$, $\omega = 185°$, and $\psi = -45°$ with γ^+-puckered ring. The energy of this compact conformation is remarkably sensitive to the bond angles, ring puckering, the torsion angle θ and the parameters of the potential functions employed especially the partial charges on the atoms used to calculate the electrostatic energy. One set of partial charges[11] employed here for the other calculations gave an energy for this conformation of about 2.9 kcal/mol higher than that of the extended conformation at $\psi \sim 160°$, while another set of partial charges[28] gave a lower value of 1.7 kcal/mol for this energy difference.

The actual energy difference between the two conformations is a crucial factor in the calculation of the characteristic ratio of polyproline in solution. The calculated characteristic ratio, on the basis of the conformational energy map in Figure 6a, is 44 while it is somewhat lower if the region $\psi \sim -45°$ is included in the calculation. A value of about 14 is obtained if the energy difference between $\psi = -45°$ and $\psi = 160°$ is about 1 kcal/mol.[19] Higher energy differences produce markedly higher characteristic ratio. Mattice and Mandelkern[29] obtained a characteristic ratio of 14 to 20 for polyproline in aqueous and organic solvents on the basis of viscosity measurements. While such a low characteristic ratio indicates that the polymer is in a collapsed state in solution, it also supports the possibility of the occurrence of occasional $\psi = -45°$ conformation in the polymer. The strength of this evidence stems from the fact that the low characteristic ratio of 14 to 20 and the observed temperature coefficient have been theoretically obtained as yet by only one calculation[19] and this calculation took into consideration a 6% population of the $\psi \sim -45°$ conformation. On the other hand, it is likely that cis residues may occur occasionally in the polyproline chain and, indeed, it has been realized earlier[3] that this may also lower the characteristic ratio.[30] Since the energy calculations are inevitably approximate, only sensitive experiments may be able to resolve this question.

5-Methylproline. The detailed study of ring puckering presented here finds an immediate application in the interesting case of a related polymer: poly(5-methylproline). This polymer differs from polyproline only in that there is a methyl group attached to the C^δ atom in place of one of

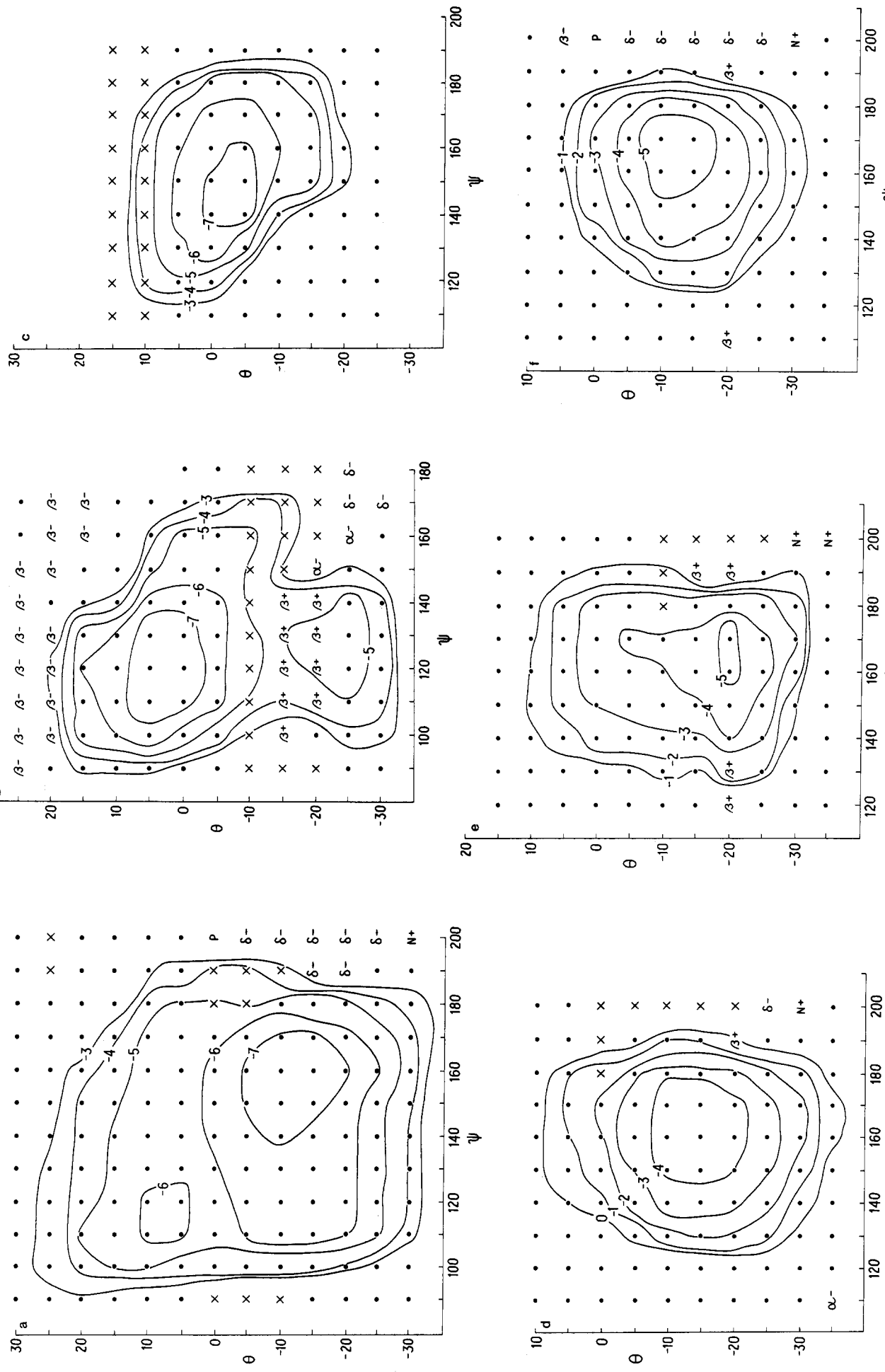

Figure 6. θ–ψ energy plots for dimers: (a) proline trans; (b) *cis*-5-methylproline trans; (c) *trans*-5-methylproline; (d) proline cis; (e) *cis*-5-methylproline cis; (f) *trans*-5-methylproline cis. The best ring geometry for each (θ,ψ) is symbolically represented (see text).

the hydrogens. This methyl substitution may be done in one of two ways. In *cis*-5-methylproline the methyl group is on the same side of the ring as the carbonyl carbon of the same residue, while the methyl group is on the opposite side of the ring in *trans*-5-methylproline (see Figure 7). χ is the angle of rotation of the methyl hydrogens about the C^{δ}–C bond. These polymers have been synthesized and their properties are being studied.[31]

The presence of the methyl group attached to the C^{δ} atom in these polymers influences the puckering of the ring, and also leads to markedly different conformational features in the dimer owing to additional steric interactions involving the methyl group.

The ring energy for the 5-methylprolines is also a function of χ in addition to the five parameters in eq 1. It was, however, found that the lowest ring energies were obtained for $\chi = 60°$, corresponding to the staggered conformation of the methyl hydrogens about the C^{δ}–C bond. A departure from this staggered conformation by 30°, for instance, produced an increase of about 2.3 kcal/mol in ring energy. The value of χ was therefore fixed at 60° for the energy calculations.

Once again, as Γ is varied for each θ, two distinctly separate energy minima are obtained in general, and as in proline, one is for a geometry of type A and the other for type B. The ring energy is plotted against θ for various puckered geometries and for the types A and B minimum energy geometries in Figure 3. For *cis*-5-methylproline (Figure 3c) the global ring energy minimum occurs at $\theta = 10°$ for a type A geometry. For *trans*-5-methylproline (Figure 3b), on the other hand, the global ring energy minimum is obtained at $\theta = -10°$ for a type B geometry. In this respect, it is the *trans*-5-methylproline that is similar to the proline ring. However, the energy difference between the minima at $\theta \sim -10°$ and $\theta = 15°$ is higher in *trans*-5-methylproline than in proline or *cis*-5-methylproline. In both the 5-methylprolines, the planar ring is about 3.5 kcal/mol higher in energy than the global minimum ring geometry. In other respects also, the general features of ring puckering in 5-methylprolines are similar to those in proline. At $\theta \sim 0°$, the ring is γ puckered, while at $|\theta| \sim 20°$, it is β puckered. All the other types of puckering are of higher energy.

The distribution of torsion angles in a set of geometries of energy within 1.5 kcal/mol from the global minimum is given in Figure 4b for trans and in Figure 4c for *cis*-5-methylproline. The cis form prefers type A while the trans prefers the type B geometry. These preferences are markedly greater in the 5-methylprolines than in proline.

There are however, some differences in detail. For proline, type B is preferred for $\theta < 0°$ and type A for $\theta > 0°$. For *cis*-5-methylproline, the change from A to B occurs at $\theta = -5°$ and for *trans*-5-methylproline it occurs at $\theta = 5°$. In particular, this means that near $\theta = 0°$ cis will prefer γ^+ puckering while trans will prefer γ^- puckering.

(5-Methylproline)$_2$ Internal Dipeptide. The energy distributions in the θ–ψ plane have been shown in Figure 6 for the two types of 5-methylprolyl dimers with cis and trans imide bonds. The ring puckering that produces the lowest dimer energy for each θ and ψ has been symbolically indicated.

Considering first the case of trans imide bond, there are two energy minima for *cis*-5-methylproline (Figure 6b), the deeper one at $\theta = 0°$, $\psi = 120°$ with γ^+ puckering and the other at $\theta = -20°$, $\psi = 130°$ with β^+ puckering. At $\theta = -10°$, the dimer prefers the second energy minimum C^{γ} position, which leads to type A geometry. Thus, in this region, the puckering is similar to γ^+ rather than γ^-. The conformational flexibility of the *trans*-5-methylprolyl dimer is seen to be rather restricted (Figure 6c). The only

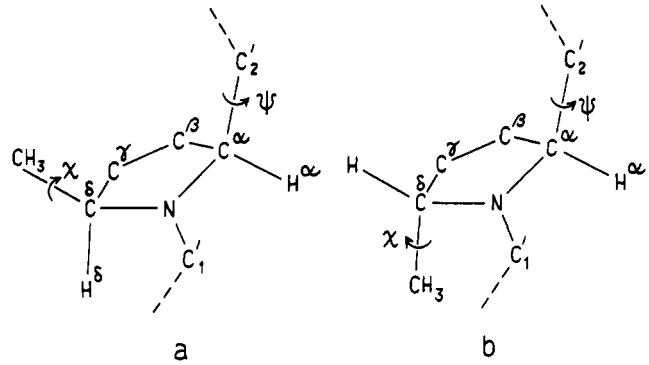

Figure 7. 5-Methylprolines: (a) cis; (b) trans.

minimum occurs at $\theta = -5°$, $\psi = 150°$ with γ^--puckered ring geometry.

No energy minimum is observed in the region $\psi \sim -60°$ for both the 5-methylproline dimers in contrast to the prolyl dimer. Using the energy maps in Figure 6b,c, characteristic ratios are found to be 246 and 174 for *cis*- and *trans*-5-methylprolines, respectively. Experimental determination of unperturbed dimensions of 5-methylprolines is in progress.

For the cis conformation of the imide bond, the θ–ψ maps for both the 5-methylprolines (Figure 6e,f) are similar to that of the proline dimer (Figure 6d) and the energy minimum is at $\theta = -15°$ and $\psi = 160°$. Thus, the methyl substitution at the C^{δ} atom seems to have very little effect on the dimer conformation with the cis imide bond unlike the case for the trans imide bond.

Acknowledgments. We thank Mr. Sidney Meier for help in computer calculations.

References and Notes

(1) This work was supported in part by Research Grant GB-15682 from the National Science Foundation. B. J. P. acknowledges N. I. H. Training Grant GM-1355.
(2) P. DeSantis, E. Giglio, A. M. Liquori, and A. Ripamonti, *Nature (London)*, **206**, 456 (1965).
(3) P. R. Schimmel and P. J. Flory, *Proc. Nat. Acad. Sci. U. S.*, **58**, 53 (1967).
(4) P. R. Schimmel and P. J. Flory, *J. Mol. Biol.*, **34**, 105 (1968).
(5) A. J. Hopfinger and A. G. Walton, *J. Macromol. Sci. Phys.* **3**(1), 171 (1969).
(6) A. E. Tonelli, *J. Amer. Chem. Soc.*, **92**, 6187 (1970).
(7) C. M. Venkatachalam, *Biochim. Biophys. Acta*, **168**, 397 (1968).
(8) IUPAC-IUB Abbreviations and Symbols for the Description of the Conformation of Polypeptide Chains, *J. Mol. Biol.*, **52**, 1 (1970).
(9) G. N. Ramachandran, A. V. Lakshminarayanan, R. Balasubramanian, and G. Tegoni, *Biochim. Biophys. Acta*, **221**, 165 (1970).
(10) D. A. Torchia, *Macromolecules*, **4**, 440 (1971).
(11) G. Holzwarth and R. Chandrasekharan, *Macromolecules*, **2**, 245 (1969).
(12) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, **23**, 283 (1968).
(13) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2091 (1966).
(14) S. Krimm and N. Johnston, private communication.
(15) H. A. Scheraga, *Advan. Phys. Org. Chem.*, **6**, 103 (1968).
(16) R. Balasubramanian, A. V. Lakshminarayanan, M. N. Sabesan, G. Tegoni, K. Venkatesan, and G. N. Ramachandran, *Int. J. Protein Res.*, **3**, 25 (1971).
(17) K. Nishikawa and T. Ooi, *Progr. Theoret. Phys.*, **46**, 670 (1971).
(18) K. Nishikawa and T. Ooi, Bull. *Inst. Chem. Res., Kyoto Univ.*, **50**, 94 (1972).
(19) W. L. Mattice, K. Nishikawa, and T. Ooi, *Macromolecules*, **6**, 443 (1973).
(20) Y. C. Leung and R. E. Marsh, *Acta Crystallogr.*, **11**, 17 (1958).
(21) T. Ueki, T. Ashida, M. Kakudo, Y. Sasada, and Y. Katsube, *Acta Crystallogr., Ser. B*, **25**, 1840 (1969).
(22) I. L. Karle, *J. Amer. Chem. Soc.*, **94**, 81 (1972).
(23) S. Krimm and C. M. Venkatachalam, *Proc. Nat. Acad. Sci. U. S.*, **68**, 2468 (1971).
(24) C. M. Venkatachalam and S. Krimm in "Conformation of Biological Molecules and Polymers," The Israel Academy of Sciences and Humanities, Jerusalem, 1973, p 141.
(25) W. A. Thomas and M. K. Williams, *J. Chem. Soc., Chem. Commun.*, 994 (1972).

(26) D. E. Dorman, D. A. Torchia, and F. A. Bovey, *Macromolecules*, 6, 80 (1973).
(27) N. Gō and H. A. Scheraga, *Macromolecules*, 3, 188 (1970).
(28) M. Goodman, V. Chen, E. Benedetti, C. Pedone, and P. Corradini, *Biopolymers*, 11, 1779 (1972).
(29) W. L. Mattice and L. Mandelkern, *J. Amer. Chem. Soc.*, 93, 1769 (1971).
(30) Examination of space-filling models show that there is a large energy barrier between the conformation with $\psi \sim -60°$ and the other low-energy conformations of the dimer. Energy calculations with *planar* residues show that this energy barrier is more than 100 kcal/mol. On the other hand, further energy calculations (B. J. Price, C. M. Venkatachalam, and S. Krimm, in preparation) show that this barrier is only of the order of 18 kcal/mol if the imide bond is allowed to become highly nonplanar in the transition to the $\psi \sim -60°$ conformation. Thus, this energy barrier is of the same order of magnitude as that between cis and trans residues for the conformation with $\psi \sim 160°$.
(31) C. G. Overberger and K. H. David, *Macromolecules*, 5, 373 (1972).

Light Scattering by Chain Molecules Composed of Anisotropic Units

G. D. Patterson

Bell Laboratories, Murray Hill, New Jersey 07974. *Received November 27, 1973*

ABSTRACT: The theory of Rayleigh light scattering by independent isotropic systems composed of anisotropic units developed by Nagai is extended according to the rotational isomeric state model. Calculations for the polymethylene chain indicate that for molecules in the random-coil state in dilute solution the terms arising from the anisotropy of the units should be small in comparison to those arising from the scalar mean polarizability of the molecules. However, for molecules with a regular structure such as a helix or all-trans conformation the anisotropy of the units must be taken into account.

A theory of Rayleigh light scattering by independent isotropic systems composed of anisotropic units has been developed by Nagai.[1] In this paper we take the system to be a chain molecule with n skeletal bonds and assign a vector $\mathbf{l}_i$ to each main-chain bond and a polarizability tensor α_i to each unit of the chain composed of the skeletal bond and the preceding atom or group(s) attached to that atom.[2]

The intensity of Rayleigh scattering in the forward direction depends on the quantities[2]

$$\bar{\alpha}^2 = \sum_{i,j} \bar{\alpha}_i \bar{\alpha}_j \tag{1}$$

$$\langle \gamma^2 \rangle = \frac{3}{2} \sum_{i,j} \langle \hat{\boldsymbol{\alpha}}_i{}^{\mathrm{R}} \hat{\boldsymbol{\alpha}}_j{}^{\mathrm{C}} \rangle \tag{2}$$

where $\bar{\alpha}_i = (1/3)\mathrm{Tr}\alpha_i$ is the scalar mean polarizability of the ith unit, $\bar{\alpha}^2$ is the square of the molecular scalar mean polarizability, $\hat{\alpha}_i = \alpha_i - \bar{\alpha}_i \boldsymbol{E}_3$ is the anisotropy tensor of the ith unit, $\boldsymbol{E}_3$ is the identity matrix of order three, $\hat{\boldsymbol{\alpha}}_i{}^{\mathrm{R}}$ and $\hat{\boldsymbol{\alpha}}_i{}^{\mathrm{C}}$ are the row and column forms of $\hat{\boldsymbol{\alpha}}_i$, respectively, $\langle \gamma^2 \rangle$ is the mean-squared optical anisotropy, and the angular brackets denote an average over all configurations of the chain.

If the intensity of Rayleigh scattering as a function of angle is expanded in a power series in the quantity $\mu = 4\pi/\lambda \sin \theta/2$, where λ is the wavelength of light in the scattering medium and θ is the angle between the incident light and the scattered light in the scattering plane, then to terms of order μ^2 the intensity will also depend on the quantities[1] (see Appendix)

$$\langle Q_1 \rangle = \sum_{i<j} \langle \bar{\alpha}_i \bar{\alpha}_j r_{ij}{}^2 \rangle \tag{3}$$

$$\langle Q_2 \rangle = \sum_{i<j} \langle \bar{\alpha}_i \boldsymbol{r}_{ij}{}^{\mathrm{T}} \hat{\boldsymbol{\alpha}}_j \boldsymbol{r}_{ij} \rangle \tag{4}$$

$$\langle Q_3 \rangle = \sum_{i<j} \langle \boldsymbol{r}_{ij}{}^{\mathrm{T}} \hat{\boldsymbol{\alpha}}_i \boldsymbol{r}_{ij} \bar{\alpha}_j \rangle \tag{5}$$

$$\langle Q_4 \rangle = \sum_{i<j} \langle r_{ij}{}^2 \hat{\boldsymbol{\alpha}}_i{}^{\mathrm{R}} \hat{\boldsymbol{\alpha}}_j{}^{\mathrm{C}} \rangle \tag{6}$$

$$\langle Q_5 \rangle = \sum_{i<j} \langle \boldsymbol{r}_{ij}{}^{\mathrm{T}} \hat{\boldsymbol{\alpha}}_i \hat{\boldsymbol{\alpha}}_j \boldsymbol{r}_{ij} \rangle \tag{7}$$

where

$$\boldsymbol{r}_{ij} = \sum_{k=i+1}^{j} \boldsymbol{l}_k$$

is the vector from the ith unit to the jth unit and the superscript T denotes the transpose.

For a random flight chain, all the above five invariants are zero, except the first. Nagai has evaluated these averages for the Porod-Kratky chain model.[1] In this paper we evaluate the above quantities according to the rotational isomeric state model[2] and carry out calculations for the polymethylene chain.

Theoretical

A cartesian coordinate system is chosen for each unit with the x_i axis along the ith skeletal bond, the z_i axis perpendicular to the plane defined by bonds i and $i-1$, and the y_i axis chosen to complete a right-handed coordinate system. A vector expressed in the frame of reference of bond $i+1$ can be transformed into the coordinate system of bond i by the orthogonal matrix

$$T_i = \begin{bmatrix} \cos\theta & \sin\theta & 0 \\ \sin\theta\cos\varphi & -\cos\theta\cos\varphi & \sin\varphi \\ \sin\theta\sin\varphi & -\cos\theta\sin\varphi & -\cos\varphi \end{bmatrix} \tag{8}$$

where θ_i is the angle between vectors $\mathbf{l}_{i+1}$ and $\mathbf{l}_i$ (the supplement of the normal valence angle) and φ_i is the angle of rotation of bond $i+1$ around bond i relative to bond $i-1$, measured from the trans state for which $\varphi = 0°$.

The quantity of $\bar{\alpha}^2$ depends only on the scalars $\bar{\alpha}_i$ and $\bar{\alpha}_j$ so that no averaging is required. The evaluation of the mean-squared optical anisotropy has been developed elsewhere,[3] but is reproduced here for illustration before proceeding to the more complex averages.