al.[12] the rotation of a benzene ring cannot be entirely independent of rotations in neighboring residues and no doubt this fact together with the packing problems for ω helices discussed above prevents an exact analysis of the diffraction data.

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Calculation of the Conformation of the Pentapeptide *cyclo*(Glycylglycylglycylprolylprolyl). III. Treatment of a Flexible Molecule[1]

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ABSTRACT: In previous conformational energy calculations on this cyclic pentapeptide, the hard variables [bond lengths, bond angles, and dihedral angles around peptide bonds (ω's)] were kept constant; in this paper, the restriction of constancy of these variables is removed. Flexibility is introduced by assuming that a Urey–Bradley-type force field is applicable to the hard variables. Starting with the thirteen minimum-energy conformations (obtained previously in the rigid-molecule treatment), and using the conjugate gradient method, the total conformational energy was minimized in the extended space corresponding to the flexible-molecule treatment. Eight local minima were thus obtained, some of them arising from more than one (rigid molecule) initial conformation. In a strained molecule such as this one (*i.e.*, one with a few close interatomic contacts), energy barriers between local minima of the rigid-molecule treatment often disappear when the hard variables are allowed to vary. While the dihedral angles (φ's, ψ's, χ's) change very drastically from those of the initial conformations in many cases, the changes of bond lengths, bond angles, and ω's are within the range of X-ray crystallographic data.

In the first two papers[3,4] of this series, conformational energy calculations were carried out for the cyclic pentapeptide *cyclo*(Gly-Gly-Gly-Pro-Pro), which has eight single bonds in the backbone about which rotation can take place. The hard variables[5] [bond lengths, bond angles, and dihedral angles around the peptide bonds (ω's)] were maintained constant[3,4] (rigid molecule), and six of the backbone dihedral angles were computed as functions of the two (remaining) independent backbone dihedral angles. The complete energy surface of the rigid molecule was calculated, and thirteen local minima were located in that region of the energy surface which is within 100 kcal of the global minimum.[3] Using the assumption of small conformational fluctuations,[5] the statistical weights of the three lowest-energy local minima were computed[4] in terms of det **G** and det **F** (quantities that have been defined previously[5]), thereby providing information as to the most stable conformation of this cyclic pentapeptide (in the absence of solvation and crystalline forces).

The purpose of the present paper is to remove the restriction of constancy of the hard variables, and thereby obtain a more complete description of the cyclic pentapeptide; *i.e.*, every atom of the molecule is now allowed to move independently. The treatment of the molecule as a flexible one is the last step of a theoretical framework proposed earlier[5,6] for conformational energy calculations in general. In order to allow the hard variables to change, appropriate potential functions are required. By including such potential functions, together with those used[3] in the treatment of the rigid molecule, we may start from the conformations obtained in the rigid-molecule treatment and minimize the total conformational energy.[5,6] Thus, we require appropriate potential functions for treatment of the hard variables, and also a suitable energy-minimization procedure in order to carry out the computations for a flexible molecule.

In the rigid-molecule treatment, the problem of ring closure was a nontrivial one.[3] However, in the flexible-molecule treatment, no such problem arises since the potential functions for the hard variables serve to maintain the independently moving atoms in a closed ring.

As far as the potential functions are concerned, we may assume that the displacements of the hard variables from their equilibrium positions obey a simple harmonic law, with force constants obtained from infrared and thermodynamic data on simple molecules. While more work has to be done to be assured that force constants (determined in this way) can be used for more complicated molecules like *cyclo*(Gly_3Pro_2), we follow present practice,[7,8] and use them as a first approximation.

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(2) On leave of absence from the Department of Physics, Faculty of Science, University of Tokyo, Tokyo, Japan, 1967–1970, and the summer of 1971, and from the Department of Physics, Kyushu University, Fukuoka, Japan, the summer of 1972.
(3) N. Gō and H. A. Scheraga, *Macromolecules*, 3, 188 (1970) (hereafter referred to as paper I).
(4) N. Gō, P. N. Lewis, and H. A. Scheraga, *Macromolecules*, 3, 628 (1970) (hereafter referred to as paper II).
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(7) K. D. Gibson and H. A. Scheraga, *Biopolymers*, 4, 709 (1966).
(8) A. Warshel, M. Levitt and S. Lifson, *J. Mol. Spectrosc.*, 33, 84 (1970).

Concerning a suitable energy-minimization procedure, a variety of methods[6] that use some sort of systematic search technique are available to locate the local minima of an unconstrained function f of n variables. We have used a method known as *function minimization by conjugate gradients* (FMCG),[9] for reasons stated in section IC. In this method, it is assumed that the value of a function $f(\mathbf{x})$ and its gradient vector $\mathbf{g}(\mathbf{x})$ can be computed at any given point $\mathbf{x}$.

I. Procedures

A. Geometry and Energy Functions. In the flexible-molecule treatment we use the *total* conformational energy E_{tot}, in which the hard variables as well as the dihedral angles (soft variables) can change. The form of E_{tot} is the one used by Warshel *et al.*[8] which was obtained by modification of that of Urey and Bradley,[10] and is expressed as follows

$$E_{tot} = E_{bs} + E_{bab} + E_{opd} + E_{ci} + E_{tor} + E_{nb} \quad (1)$$

where

$$E_{bs} = (1/2)\Sigma K_b(b - b_0)^2 \quad (2)$$

$$E_{bab} = (1/2)\Sigma K_\tau(\tau - \tau_0)^2 \quad (3)$$

$$E_{opd} = (1/2)\Sigma K_\lambda \lambda^2 \quad (4)$$

all in quadratic (or simple harmonic form) and

$$E_{ci} = (1/2)\Sigma K_l(l - l_0)^2 + \Sigma K_l'(l - l_0) \quad (5)$$

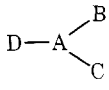
$$E_{tor} = (1/2)\Sigma K_\theta(1 \pm \cos m\theta) \quad (6)$$

$$E_{nb} = \Sigma_{i>j}[(d_{ij}/R_{ij}^{12}) - (e_{ij}/R_{ij}^{6}) + (q_iq_j/DR_{ij})] \quad (7)$$

The abbreviations bs, bab, opd, ci, tor, and nb indicate bond stretching, bond angle bending, out-of-plane deformation, corner interaction, torsional, and nonbonded, respectively.

The first four terms on the right-hand side of eq 1 pertain to changes in the hard variables, and are specific to the flexible-molecule treatment. In eq 2, 3, and 4, b, τ, and λ denote bond lengths, bond angles, and out-of-plane deformations, respectively; b_0 and τ_0 are the values of b and τ at which their respective energy contributions (*viz.*, E_{bs} and E_{bab}, respectively) are a minimum (whose values are taken as zero). In the flexible-molecule treatment, the nonplanarity of the peptide group (with minimum energy in the planar trans form) is taken into account as a torsional energy (eq 6) and as out-of-plane deformations (eq 4).

The angle λ is defined as follows. In the grouping



A is designated as the apex atom, B and C as the first and second anchor atoms, respectively, and D as the end atom.[11] Then λ is the angle between the A–D bond and its projection on the ABC plane. The sign of λ is positive or negative depending on whether D is above or below the ABC plane, when an observer (in the ABC plane) looking from D toward

(*i.e.*, D is closer than A to the observer) sees B on the left and C on the right. In the molecule being considered here, two types of out-of-plane deformations (λ^1 and λ^2) occur. The corresponding end, apex, first anchor and second anchor atoms in these deformations are O, C′, N, C^α, and H (or C^δ), N, C^α, C′, respectively. The force constants for these out-of-plane deformations are taken from the paper by Warshel *et al.*[8,12] In eq 5, l denotes a corner (or so-called 1-3) distance—*i.e.*, that between atoms bonded to a common atom, as between B and C in

l_0 is a parameter which assumes a value approximately equal to the value of l at which the three atoms in such a corner arrangement are so disposed that the bond lengths and bond angles are b_0 and τ_0, respectively. The corner interaction energy is included in eq 1 in order to take into account the interactions between atoms bonded to a common atom, which are quite different from those between atoms separated by more intermediate atoms, because they are essentially interactions between the bonding orbitals. K is an energy parameter (force constant) whose subscript indicates the type of interaction. In the rigid-molecule treatment the values of bond lengths, bond angles, and other hard variables are held fixed, and they are given as geometrical parameters. In the flexible-molecule treatment the values of the hard variables are determined by minimization of E_{tot} with respect to both the hard and the soft variables. In general, the equilibrium values of the bond lengths and bond angles are not equal to b_0 and τ_0, respectively. In fact, the values of b_0, τ_0, and l_0 as well as the force constants K were determined[8,13] by requiring that the calculated equilibrium values of bond lengths and bond angles come close to the observed values, and that the computed frequencies and thermodynamic data agree closely with experimental values. The values of the parameters used in eq 2, 3, 4, and 5 are taken mainly from the papers of Lifson and Warshel[13] and of Warshel *et al.*[8] except for the following changes. The value of b_0 for the C–H bond is taken as 1.000 Å instead of 1.099 Å used by Lifson and Warshel[13] because the smaller bond length of 1.000 Å was used in papers I and II. In the paper of Warshel *et al.*[8] the values of K_l' in eq 5 were determined by

$$K_l' = -FK_ll_0 \quad (8)$$

where F was taken as 0.1, while, in the earlier paper of Lifson and Warshel,[13] the values of K_l' were determined independently of K_l and smaller (absolute) values of K_l' than those given by eq 8 with $F = 0.1$ were obtained; the values obtained[13] correspond to a value of F in the range between 0.004 and 0.01. In the present paper, the values

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(10) H. C. Urey and C. A. Bradley, Jr., *Phys. Rev.*, **38**, 1969 (1931).
(11) E. B. Wilson, Jr., J. C. Decius, and P. C. Cross, "Molecular Vibrations," McGraw-Hill, New York, N. Y., 1955, p 59.
(12) Values which are three-fourths of those of Warshel *et al.*[8] were used inadvertently in the computer program. However, considering the cost of computation, the calculations were not repeated because the energy contribution from this term is very small (see section III), and no significant changes from the results obtained in this paper would be expected to occur.
(13) S. Lifson and A. Warshel, *J. Chem. Phys.*, **49**, 5116 (1968).

Table I
Geometry and Energy Parameters[a]

A. Bond Stretching

Bond	$K_b/2$	b_0
N-H[b]	405	0.980
C′-N[b]	403	1.278
N-C[b]	261	1.457
C-H[c]	287	1.000[d]
C-C′[b]	187	1.470
C-C[c]	111	1.455
C′-O[b]	595	1.200

B. Bond Angle Bending and Corner Interaction

Bond Angle (B-A-C)	$K_\tau/2$	τ_0[e]	$K_l/2$	l_0 (B...C)
C-N-C[f]	54.5	Tri	16.2	2.524[j]
C′-N-C[b]	54.5	Tri	16.2	2.400
C-N-H[b]	31.4	Tri	26.0	1.791
C′-N-H[b]	26.6	Tri	27.9	2.000
C-C′-N[b]	33.1	Tri	50.5	2.229
N-C′-O[b]	48.5	Tri	90.0	2.186
C-C′-O[b]	40.9	Tri	52.0	2.400
C-C-C[c]	22.0	Tetra	37.3	2.450[j]
C-C-C′[g]	22.0	Tetra	37.3	2.450
N-C-C[h]	22.0	Tetra	37.3	2.282[j]
N-C-C′[h]	22.0	Tetra	37.3	2.235[j]
H-C-C[i]	26.8	Tetra	38.4	1.975
H-C-C′[b]	26.8	Tetra	38.4	1.975
H-C-N[b]	30.1	Tetra	41.0	1.900
H-C-H[c]	38.1	Tetra	2.9	1.800

C. Out-of-Plane Deformation

End Atom	Apex Atom	First Anchor Atom	Second Anchor Atom	$K_\lambda/2$
O	C′	N	C^α	4.04[k]
H (or C^δ)	N	C^α	C′	0.69[k,l]

D. Torsional A(X-Y)B

A	X-Y	B	$K_\theta/2$	m	Sign
O, C^α	C′-N	C^α, H^N (or C^δ)	2.500[m]	2	−
N, H^α, H^α (or C^β)	C^α-C′	N	0.017[n]	3	+
N, H^α, H^α (or C^β)	C^α-C′	O	0.017	3	−
H^N (or C^δ)	N-C^α	C′, H^α, H^α (or C^β)	0.048[o]	3	+
	N-C^α	C′, H^α, H^α (or C^β)	0.048	3	−
C′	N-C^δ	C^γ, H^δ, H^δ	0.048	3	+
C^α	N-C^δ	C^γ, H^δ, H^δ	0.048	3	−
C′	C^α-C^β	C^γ, H^β, H^β	0.156[p]	3	+
N, H^α, C′ C^α, H^β, H^β	C^β-C^γ	C^δ, H^γ, H^γ	0.156	3	+
C^β, H^γ, H^γ	C^γ-C^δ	N, H^δ, $H^{\prime\delta}$	0.156	3	+

[a]Units: energy in kcal/mol, length in Å, angle in radian. All carbon atoms other than C′ are sometimes designated simply as C. [b]Taken from ref 8. [c]Taken from ref 13. [d]This value is taken (instead of 1.099 as suggested in ref 13), because the smaller bond distance of 1.000 Å for C-H was used in papers I and II. [e]Tri designates the trigonal angle 120° and tetra the tetrahedral angle 109.47°. [f]Taken to be the same as those for C′-N-C, except for l_0, whose value was determined as indicated in footnote *j*. [g]Taken to be the same as those for C-C-C. [h]Taken to be the same as those for C-C-C, except for l_0, whose value was determined as indicated in footnote *j*. [i]The values suggested[13] for this case are 26.8, 43.6, and 2.2 for $K_\tau/2$, $K_l/2$ and l_0, respectively, but the values for H-C-C′ were used inadvertently in the computer program. However, we did not redo the calculation because of the cost of computation and because essentially no changes were expected to occur in the results obtained in this paper. [j]Calculated as $l_0 = [(b_0)_{AB}^2 + (b_0)_{AC}^2 - 2(b_0)_{AB}(b_0)_{AC} \cos \tau_{B\text{-}A\text{-}C}]^{1/2}$. [k]The definitions of the angle of out-of-plane deformation in this paper and in ref 8 are different. The factor of 4/3, by which the numerical values in ref 8 must be multiplied in order to obtain the corresponding force constants in the new definition, was inadvertently not included in the computer program. Therefore, these values are smaller than those used in ref 8 by a factor of 3/4. See footnote 12 of the text. [l]The same force constant is used in both cases, *i.e.*, when the end atom is C^δ and when it is H. [m]This value corresponds to a barrier height of 20.0 kcal in the rigid-molecule treatment. Warshel *et al.*[8] determined the values of $(1/2)K_\theta$ which differ for the different ways of defining the dihedral angles. However, we used the same value of $(1/2)K_\theta$ for all possible ways of defining a dihedral angle for the sake of simplicity in programming. [n]This corresponds to the value of 0.20 kcal/mol, used for the barrier height in I.[3] [o]This corresponds to the value of 0.58 kcal/mol, used for the barrier height in I.[3] [p]This corresponds to a barrier height of 2.80 kcal.[14]

of K_l' were determined by eq 8 with an intermediate value of $F = 0.0425$. With this value of F, the corner interaction energies were neither strongly positive nor negative for the initial conformations.

The torsional energy function (eq 6) and the nonbonded, electrostatic and hydrogen-bonding energy functions (eq 7) are essentially the same as those used previously in the rigid molecule treatment,[3,4] except as discussed below. In eq 6, m is the number of minima in the torsional potential in the range between 0 and 2π, and the sign of the cosine term indicates whether the torsional energy would make its maximum (+) or minimum (−) contribution at zero dihedral angle.[14] The values of these parameters are listed in Table I. Special consideration is required in the calculation of the torsional energy in the flexible-molecule treatment. The torsional angle around a bond between atoms X and Y is defined as the angle between the two planes formed by atoms A, X, Y and X, Y, B, respectively, where atoms A and B are connected to atoms X and Y, respectively. When there is more than one atom, A_1, A_2, ..., A_p and B_1, B_2, ..., B_q, connected to X and Y, respectively, there are pq ways of defining the dihedral angle, all of which are equivalent (except for constant additive factors) as long as the bond lengths and bond angles are maintained constant (rigid-molecule treatment). The situation is different in the flexible-molecule treatment, in which the bond lengths and bond angles can vary. In this case, the value of the dihedral angle depends on which atoms are chosen from A_1, A_2, ..., A_p and from B_1, B_2, ..., B_q to define the angle. The form of eq. 6 for the torsional energy (with m the same as in the rigid-molecule treatment) is assumed for all possible ways of defining the dihedral angle. Warshel *et al.*[8] used different values of K_θ for different ways of defining the same torsional angle. However, we have used the same value of K_θ for all possible ways of defining a dihedral angle for the sake of simplicity in programming. Therefore, for example, when there are six ways of defining a dihedral angle (as for bonds between N and C^α and between C^α and C′), the value of K_θ used in eq 6 (and given in Table ID) in the flexible-molecule treatment is one-sixth of the value used in the rigid-molecule treatment. In this manner, all the

(14) H. A. Scheraga, *Advan. Phys. Org. Chem.*, 6, 103 (1968).

Table II
Characterization of Eight Local Energy Minima for the Flexible Molecule[a]

	Flexible Local Energy Minima[b]							
	$J_{II,I}$	$J_{I,I}$	$J_{II,II}$	$K_{II,I}$	$K_{I,I}$	$L_{II,I}$	$M_{I,I}$	$N_{I,I}$
	Initial Rigid Local Energy Minima[c]							
	E H D′[d] E′	G	C′	A′	D	A[d] B′	F	B[d] C
	Principal dihedral angles[e]							
ϕ_1	82.3	83.6	82.6	179.7	174.6	−71.8	173.2	−103.5
ψ_1	−58.8	−54.6	−59.1	117.8	112.3	75.3	143.6	−50.4
ω_1	166.1	163.5	165.0	−171.7	−173.8	−174.0	−167.1	172.2
ϕ_2	−65.2	−71.8	−75.6	82.0	83.3	132.1	47.4	−77.2
ψ_2	71.9	73.8	71.8	−63.4	−66.3	67.9	39.1	74.3
ω_2	−164.9	−163.6	−164.2	155.8	154.0	−178.4	−156.8	−178.8
ϕ_3	89.1	74.1	92.6	−79.9	−75.2	106.8	139.8	89.8
ψ_3	−143.1	−129.8	−148.9	−174.1	−179.8	−126.9	−177.9	−117.3
ω_3	175.4	−175.3	173.7	−179.0	−179.1	168.0	−172.0	−175.2
ϕ_4	−47.3	−51.1	−49.4	−41.0	−36.0	−56.1	−43.7	−53.0
χ_4^1	−24.3	8.1	−22.7	−28.2	5.8	−21.7	7.0	7.7
χ_4^2	33.9	−17.0	31.8	35.2	−22.3	32.8	−21.7	−16.6
χ_4^3	−28.3	18.4	−26.5	−26.4	28.8	−29.2	26.6	18.2
χ_4^4	−165.4	169.1	−164.6	−170.4	151.4	−159.5	159.1	170.9
ψ_4	−43.2	−50.7	−38.2	−46.6	−50.8	−41.2	−52.9	−42.2
ω_4	178.9	177.6	174.7	178.8	179.8	179.4	176.6	176.5
ϕ_5	−90.5	−88.4	−80.8	−76.1	−73.7	−93.2	−79.4	−89.3
χ_5^1	36.1	34.8	−13.1	30.0	28.0	38.7	30.7	31.7
χ_5^2	−38.8	−40.3	31.1	−37.0	−36.8	−38.3	−36.7	−33.3
χ_5^3	23.8	27.7	−35.0	27.4	28.9	20.5	26.1	19.6
χ_5^4	−172.5	−175.7	−139.4	175.3	173.6	−169.8	178.0	−155.8
ψ_5	44.7	46.2	33.2	−33.8	−35.3	−52.6	−34.7	−97.4
ω_5	−176.9	−178.7	−171.8	178.2	179.5	167.1	−179.1	169.8
	Out-of-plane deformation							
λ_1^1	3.8	3.9	4.7	−6.4	−5.3	−2.4	−6.7	1.9
λ_1^2	8.2	8.7	8.5	−8.3	−7.7	−4.0	−5.5	4.4
λ_2^1	−5.3	−6.2	−5.3	6.6	7.1	0.0	−3.9	2.4
λ_2^2	−13.5	−14.3	−14.1	17.5	18.8	−5.5	−14.4	−5.2
λ_3^1	2.8	0.3	2.5	4.4	4.0	3.1	−1.0	0.0
λ_3^2	−1.0	−2.9	−2.6	−0.9	1.7	−3.8	−2.5	−4.5
λ_4^1	4.6	4.7	−7.6	−0.9	−1.2	−3.7	−0.2	−2.5
λ_4^2	−7.0	−8.7	−10.5	−3.4	−4.5	−4.8	−4.2	−12.2
λ_5^1	−1.1	0.5	−2.7	0.1	−1.4	5.5	−1.7	−2.4
λ_5^2	−4.3	−3.1	−6.2	6.4	5.7	6.4	2.7	1.1
	Conformational energy[f]							
E_{tot}	−28.7	−27.2	−25.5	−17.9	−16.7	−16.2	−11.8	−9.2
ΔE_{tot}	0	1.5	3.2	10.9	12.1	12.5	17.0	19.5
E_{tor}	11.1	14.6	13.5	11.9	14.3	12.4	14.8	16.0
E_{nb}	−28.3	−28.3	−27.1	−26.1	−26.1	−27.9	−25.4	−27.4
E_{es}	−19.0	−19.5	−18.9	−11.3	−11.6	−8.4	−7.4	−3.1
E_{bs}	5.2	5.1	5.3	5.0	5.0	5.2	4.9	4.9
E_{bab}	8.4	7.8	8.0	8.3	8.1	9.0	8.2	7.9
E_{ci}	−6.4	−7.0	−6.6	−6.0	−6.5	−6.6	−7.1	−7.7
E_{opd}	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1
	Hydrogen bonding[g]							
	No. of Pairs							
	4	4	4	2	2	1	1	1
	Residues Whose O and H^N Atoms, Respectively, Are Hydrogen Bonded							
	5-2	5-2	5-2	1-3	1-3	5-2	3-1	1-3
	1-3	1-3	1-3	3-1	3-1			
	3-1[h]	3-1[h]	3-1[h]					
	4-1[h]	4-1[h]	4-1[h]					

Footnotes to Table II

[a]Units: energy in kcal/mol, angle in deg. [b]The same name (J through N) is given to conformations with similar backbone dihedral angles. Roman numeral subscripts (I and II) distinguish the puckered states of the pyrrolidine rings in residues 4 and 5. See text for further details. [c]Taken from paper I.[3] [d]The data listed pertain to the minimum-energy conformation obtained by starting from this initial conformation. Corresponding data for minimum-energy conformations obtained by starting from the other initial conformations in this same group deviate at most by: ±2° for dihedral angles, ±0.5° for out-of-plane deformations, and ±0.08 kcal/mol for conformational energies. The FMCG minimizer stopped moving at these conformations, because the gradient vectors vanished in the single-precision machine calculation. [e]The definition of the dihedral angles conforms to the recent IUPAC–IUB convention;[19] in order to convert the values given in paper I[3] (which are given in an older convention[20]) to the new convention, 180° should be subtracted from the old[3] values. [f]E_{tot} = total conformational energy; $\Delta E_{tot} = E_{tot} + 28.7$; E_{tor} = sum of torsional energy; E_{nb} = nonbonded energy (not including E_{es}); E_{es} = electrostatical energy, with $D = 1$; E_{bs} = bond stretching energy; E_{bab} = bond angle bending energy; E_{ci} = corner interaction energy; and E_{opd} = out-of-plane deformation energy. [g]A hydrogen bond is considered to be formed if the distance between O and H^N falls in a range between 1.90 and 2.40 Å, provided that O and H^N are separated by five or more bonds. [h]A bifurcated hydrogen bond[18] is formed for these two pairs of interactions, 3–1 and 4–1, with 1 being common to both pairs.

value of K_θ used in eq 6 (and given in Table ID) in the rigid-molecule treatment.[3,4] In eq 7, R_{ij} is the distance between atoms i and j which are separated by more than one intermediate covalently bonded atom; in such cases the interactions are designated 1–4, 1–5, etc. The coefficients d_{ij} and e_{ij} of the Lennard–Jones potential for the nonbonded interactions, and the partial charges q_i and q_j for the electrostatic interactions E_{es}, are the same as in paper I;[3] also, as in paper I,[3] the hydrogen-bonding energy is treated by eq 7, *i.e.*, by a combination of a modified Lennard–Jones potential and an electrostatic interaction. A dielectric constant D of 1 is used instead of 4 as in paper I.[3] The reason for this change is the desire to treat the molecule here as an isolated one (in vacuum) since no solvation or crystalline forces are included. This change in the value of D does not involve any inconsistency between the results obtained here and those of paper I since the only purpose of carrying out the computations of paper I was to obtain good starting conformations for the energy minimizations of the present paper. Since the choice of starting conformations is arbitrary to some extent, the energy parameters used in the first computation[3] do not have to be identical with those used here.

B. Selection of Independent Variables. The molecular formula of the cyclic pentapeptide is given in Figure 1 together with the definition of the dihedral angles ϕ's, ψ's, ω's, and χ's, and out-of-plane deformations λ's.

The number of internal degrees of freedom in a nonlinear molecule consisting of N atoms is $3N - 6$, which means that the conformational energy of such a molecule is a function of $3N - 6$ independent varibles if the molecule is assumed to be flexible. However, the conformational energy of a molecule, given by eq 1, is expressed as a function of more than $3N - 6$ internal quantities. Therefore, a proper set of $3N - 6$ independent variables must be chosen, and the other redundant internal quantities must be expressed as functions of these. It is easy[4] to choose a set of internal variables (bond lengths, bond angles, dihedral angles, etc.) that are nonredundant. However, the formulation of the dependence of the other (redundant) variables on the selected independent variables is a rather complicated problem. Therefore, we take the cartesian coordinates (instead of the internal quantities) of a molecule as the independent variables. In order to treat only $3N - 6$ cartesian coordinates as the independent variables, six of the $3N$ cartesian coordinates are held fixed as zero; these are: the x, y, and z coordinates of one of the atoms, the y and z coordinates of another atom, and the z coordinates of a third atom. In other words, the first, second and third atoms are held at the origin, on the x axis and in the xy plane, respectively. Any atoms in the molecule may be taken as the first, second and third atoms. In the present paper, these are chosen as C^α, C', and O, respectively, of Pro_5 (see Figure 1). The internal

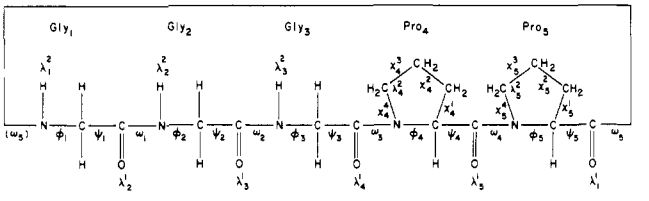

Figure 1. Definition of dihedral angles (ϕ's, ψ's, ω's, and χ's) and out-of-plane deformations (λ's) in *cyclo*(Gly_3Pro_2).

quantities appearing in eq 1 can be calculated readily from the cartesian coordinates.

In the process of energy minimization (described in section IC), use has been made of the gradient of the energy. The gradient vector $\mathbf{g}(\mathbf{x})$ can be calculated by means of eq 9, where $\mathbf{x}$ refers to the cartesian coordinates, and r_i are the internal quantities which appear in eq 1. The analytical form of $\partial V/\partial r_i$ can be obtained from eq 1, and $\partial r_i/\partial \mathbf{x}$ is the Wilson **s** vector.[15]

$$\mathbf{g}(\mathbf{x}) \equiv \partial V/\partial \mathbf{x} = \Sigma_i(\partial V/\partial r_i)(\partial r_i/\partial \mathbf{x}) \qquad (9)$$

C. Minimization of Conformational Energy. There are 49 atoms in the molecule under consideration here. Hence, energy minimization must be carried out in a 141 (= 49×3 − 6)-dimensional space. The Fletcher–Powell method,[16] often used for minimization, requires a large working space in the computer [$n(n + 7)/2$ words,[16] where n is the dimensionality of the problem] whereas the FMCG method of Fletcher and Reeves[9] requires much less ($2n$ words).[9] These two methods[9,16] were tested by minimizing the energy of one conformation of the cyclic pentapeptide, and found to require approximately the same computer time. Therefore, the FMCG method, which requires much less storage space, was used for energy minimization.

In the rigid-molecule treatment,[3] a complete (two-dimensional) energy contour map was calculated, and all local minima were located on it. However, it is impossible to provide a complete energy contour map in a 141-dimensional space. Therefore, in the flexible-molecule treatment, we have to proceed by picking a small number of starting conformations, from which to carry out energy minimization. The starting conformations were taken as the thirteen minimum-energy ones obtained from the rigid-molecule treatment,[3] according to the justification provided previously[5] (primarily the anticipation that the conformations obtained in the rigid-molecule treatment would not change considerably when flexibility was intro-

(15) See ref 11, chapter 4.
(16) R. Fletcher and M. J. D. Powell, *Computer J.*, 6, 163 (1963).

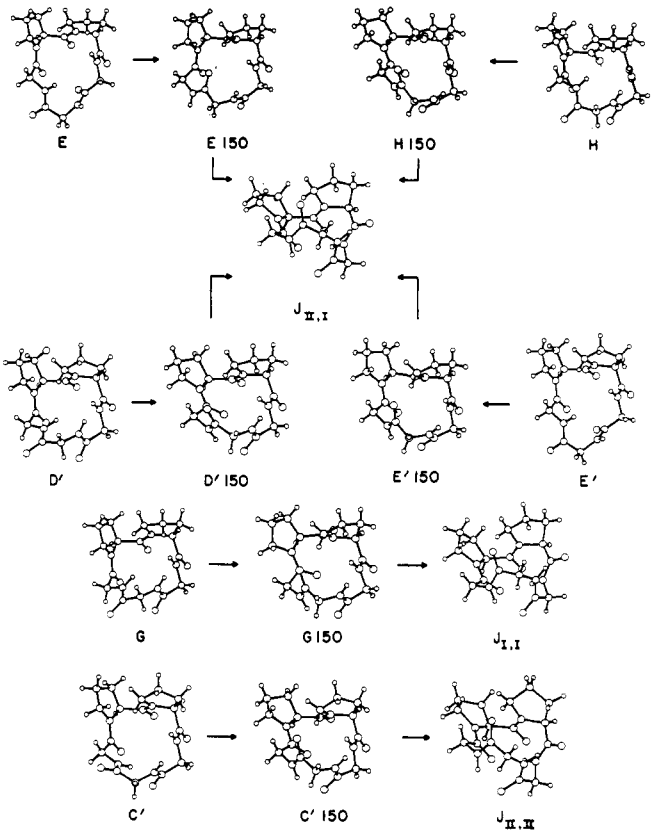

Figure 2. Illustration of change in conformation during energy minimization, starting with the (rigid) minimum-energy structures of paper I,[3] passing through an intermediate structure (at the 150th cycle of minimization), and ending with the minimum-energy structures (for the flexible molecule). The final structures are those of lowest energy (J series).

duced). However, as will be described in section III, significant changes in conformation do occur in extending the treatment from the rigid-molecule one to the flexible-molecule one. This is partly due to the fact that energy barriers between local minima in the rigid-molecule treatment sometimes disappear in this crowded and strained molecule, when flexibility is introduced. Nonetheless, as will be described in section III, the results obtained in this paper support the assumption that the set of local minimum-energy conformations in the rigid-molecule treatment is a good one to use as starting points to reach *all* local minima in the flexible-molecule treatment. This is true even though the possibility exists that a local minimum in the rigid-molecule treatment with an energy in excess of 100 kcal might become a low-energy minimum during energy minimization in the flexible-molecule treatment. However, the existence of a local minimum in the high-energy region is not very likely (and indeed was not considered in paper I,[3] because it was considered to be a physically unrealistic situation) since the energy is dominated by repulsions between very few pairs of atoms in such a high-energy region; and it is unlikely that these few repulsions would compensate each other to produce a local minimum in the high-energy region.

II. Results

Starting with the thirteen local-minimum conformations (A–H, A′–E′) obtained in paper I,[3] energy minimization of the flexible molecule led to the eight local-minimum conformations shown in Table II. The reduction in the number of minima arises from the fact that several of the initial conformations converged to common conformations when flexibility was introduced. For example, four different local minima (E, H, D′, and E′) from the rigid-molecule treatment converged to the same minimum $J_{II,I}$ in the flexible-molecule treatment; other convergences are indicated in the first two rows of Table II. The eight local minima fall into five classes, J through N, shown in the first row of Table II, where the conformations in each class are similar to each other except for the puckered states of the two pyrrolidine rings in residues 4 and 5 (see Figure 1 for the numbering of the residues). The letters J through N indicate gross backbone conformations, and the Roman numeral subscripts I and II designate conformations (the same as in paper I[3]) of the pyrrolidine rings (with positive and negative values, respectively, for χ_4^1 or χ_5^1); for example, $J_{II,I}$ means that the conformations of the pyrrolidine rings of residues 4 and 5 are such that χ_4^1 is negative and χ_5^1 is positive. Therefore, the eight local minima are distinguished by appropriate letters and subscripts.

The values of the dihedral angles and the out-of-plane deformations in the eight local minimum-energy conformations are given in Table II. The total energy (and its components) and the existence of hydrogen bonds[17,18] are also given in Table II. The definition of the dihedral angles conforms to the recent IUPAC–IUB convention;[19] in order to convert the values in paper I[3] (which are given in an older convention[20]) to the new convention, 180° should be subtracted from the old[3] values.

(17) In some cases, a bifurcated hydrogen bond[18] is formed.
(18) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 451.
(19) IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970).
(20) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, *J. Biol. Chem.*, **241**, 1004 (1966).

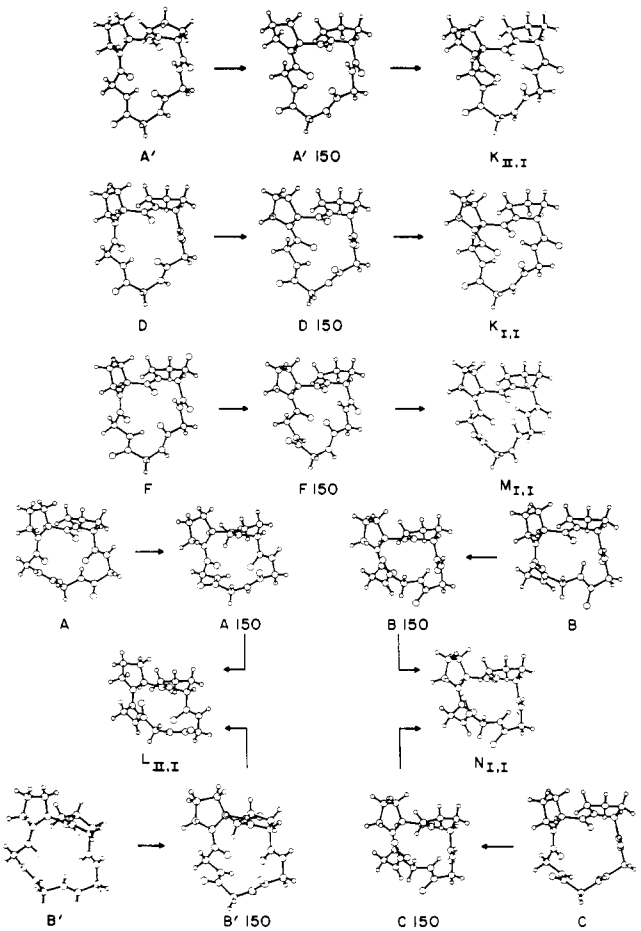

Figure 3. Same as Figure 2, but for the higher-energy structures.

In Figure 2 and 3 the conformations for the initial, intermediate (at the 150th cycle of iteration) and the final minimum-energy structures are illustrated. The change of each energy term during the minimization is shown in Figure 4 for a typical case, *viz.*, E → $J_{II,I}$. Also, data are given in Table III for this typical case to show how the interatomic contacts and hydrogen bonding changed during the energy minimization.

The distributions of bond angles and bond lengths in the minimum-energy conformations, and a comparison with data from the literature[13,21] are shown in Table IV. The distribution of the values of the dihedral angles ω is given in Figure 5.

III. Discussion

It should be noted that the values of the bond lengths and bond angles in the minimum-energy conformations agree very well with those found in an X-ray crystal study of the model compound L-Leu-L-Pro-Gly[21] (see Table IV). The ranges of the distributions of bond lengths, bond angles (see Table IV) and the dihedral angle ω (see Table II and Figure 5) are reasonable. These observations provide evidence for the reasonableness of the energy parameters.

As can be seen from Table II, the *global minimum* in the flexible-molecule treatment is at conformation $J_{II,I}$. However, in the J group, $J_{I,I}$ and $J_{II,II}$ are low-energy local minima. Thus, it is quite natural to suspect that we may have missed another local minimum ($J_{I,II}$) because

(21) Y. C. Leung and R. E. Marsh, *Acta Crystallogr.*, 11, 17 (1958).

Table III
Changes of Interatomic Contacts and Hydrogen Bonding during the Minimization of the Initial Conformation E

	Conformation[a]		
	Initial	Intermediate	Final
Number of pairwise interactions with[b]			
$E_{vdw} > 5.0$	2	0	0
$5.0 > E_{vdw} > 1.0$	3	0	0
$1.0 > E_{vdw} > 0.5$	2	0	0
$0.5 > E_{vdw} > 0.25$	2	1	2
$0.25 > E_{vdw} > 0$	4	10	9
Number of pairs of hydrogen bonds	0	0	4[c]

[a]Initial conformation is the minimum-energy one, E, obtained previously[3] in the rigid-molecule treatment. Intermediate conformation is the one obtained at the 150th cycle of iteration. Final conformation is $J_{II,I}$ of Table II. [b]E_{vdw} is the van der Waals nonbonded interaction energy between a pair of atoms. [c]The pairs of atoms participating in these hydrogen bonds can be seen in Table II. Two of the four hydrogen bonds are bifurcated.

we started the minimization only from the local minima obtained in the rigid-molecule treatment. If such a local minimum exists in the expanded space of the flexible molecule, then it would be one which cannot be reached from any of the local minima of the rigid-molecule treatment. Since we are assuming that all minima in the flexible-molecule treatment can be reached by starting from the local minima of the rigid-molecule treatment (see section IC and also ref 5), it is necessary to examine this question. For this purpose, energy minimization was performed by starting from a point which was artificially made to adopt conformation $J_{I,II}$. This was done by taking conformation $J_{I,I}$ and replacing the coordinates of the atoms in residue Pro_5 by the coordinates of the atoms in residue Pro_5 of conformation $J_{II,II}$. This artificially generated conformation had an initial energy which was quite low (it had no steric overlaps, and E_{tot} = −22.9 kcal/mol). However, after energy minimization, a *new* local minimum was *not* reached; instead, the conformation and energy converged to those of $J_{II,II}$. Therefore, we can conclude that there is no local minimum corresponding to $J_{I,II}$.

One of the most striking results obtained here is that the global minimum $J_{II,I}$ is reached from four different local minima of the rigid-molecule treatment, and obviously through different pathways (see Figure 2). This means that, as soon as flexibility is introduced, the local minima of the rigid-molecule treatment are no longer points of minimum energy, and new pathways are found which lead to the low-energy region. The extended flexible-molecule conformational energy surface is, in a sense, smoother than that of the rigid-molecule treatment. A similar smoothing and lowering of the barriers between local minima of an energy contour map, upon introduction of flexibility, was observed earlier[7] for the dipeptide glycyl-L-alanine. A primary effect of the introduction of flexibility is the relief of steric overlaps by small changes in bond lengths, bond angles, and dihedral angles around peptide bonds. This relief occurs mostly within the first 10 cycles of iteration by the FMCG method, as can be seen in Figure 4. However, no less important an effect of the introduction of flexibility is the elimination of energy barriers between conformations. When flexibility is introduced, the dimensionality of the conformational space is

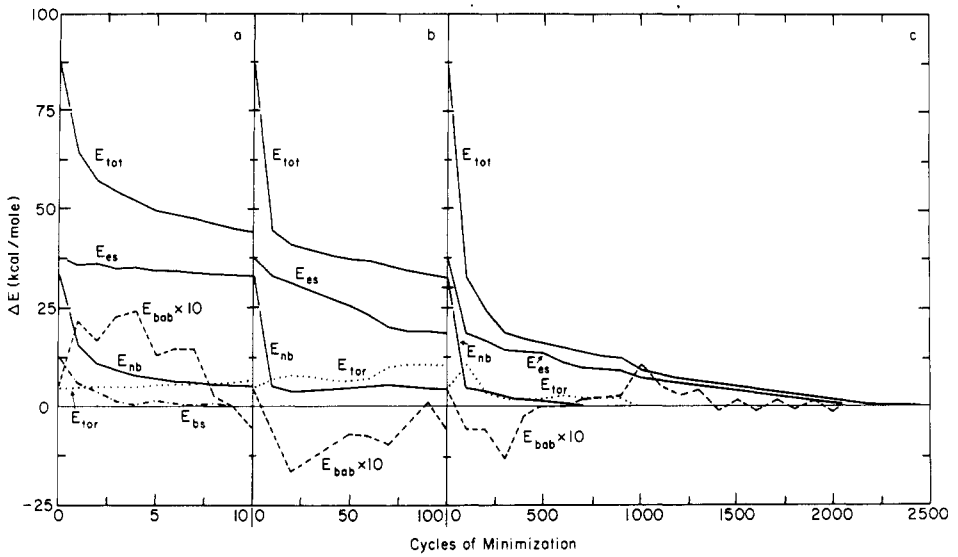

Figure 4. Changes of the total energy, E(current in cycle i) − E(final), and its constituents with cycle of minimization, for the case E → $J_{II,I}$. (a) for cycles 0–10, plotted for every cycle; (b) for cycles 0–100, plotted for every 10 cycles; (c) for cycles 0–2445 cycles, plotted for every 100 cycles. The same abbreviations as in Table II are used for the various energy contributions.

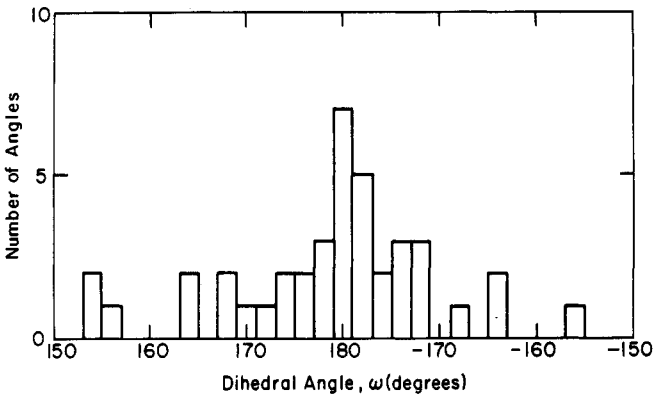

Figure 5. Distribution of the dihedral angles ω around the peptide bonds in the eight minimum-energy conformations.

enlarged, and new paths are often followed; these pass through the newly added dimensions, and avoid the energy barriers which are present in the limited space corresponding to the rigid-molecule treatment. In Figure 4, the changes of total conformational energy E_{tot} and its constituents are plotted against the cycles of minimization for a typical case in which the starting conformation is E. The bond stretching energy E_{bs} approaches a value which differs from the final one by only a few tenths of a kcal within 10 cycles, as shown in Figure 4a. The changes of the bond angle bending energy E_{bab} in the course of minimization are very small (requiring a magnification by a factor of 10 to be visible in Figure 4) and rather irregular; essentially the same behavior was observed for the corner interaction energy E_{ci} and the out-of-plane deformation energy E_{opd}, and are not shown in Figure 4. E_{opd}, especially, remains very small, the maximum value observed in the process of minimization being 0.5 kcal/mol. The torsional energy E_{tor} behaves similarly at the beginning, but approaches the final value steadily toward the end of the minimization. Other energy terms like E_{nb} or E_{es} often decrease at the (small) expense of the energy terms corresponding to the hard variables. The main contribution to the decrease of 88.1 kcal/mol in the total energy E_{tot} comes mainly from the nonbonded interaction energy E_{nb} and the electrostatic interaction energy E_{es}. E_{nb} decreases more rapidly than E_{es} because the former is a sharper function of conformation than the latter.

The rate of convergence, *i.e.*, the number of cycles of iteration with the FMCG minimizer until the minimum point is reached, ranges from 258 cycles (for B → $N_{I,I}$) to 2743 cycles (for H → $J_{II,I}$). The quickest minimization (258 cycles) is, in fact, an extremely rapid process in light of the complexity of the function minimized and the number of variables involved (*i.e.*, 141).

Local minimum-energy conformations from the rigid-molecule treatment, which were used as starting conformations, were sometimes found to be close to those obtained in the flexible-molecule treatment (*e.g.*, B → $N_{I,I}$, see Figure 3), but often not so. This means that the energy barriers around local minima in the rigid-molecule treatment are in many cases (at least in the molecule studied here) quite artificial, and arise from the assumption of rigidity of bond lengths, bond angles, and dihedral angles around peptide bonds. When the molecule is treated as flexible, the energy barriers can often be avoided by allowing these hard variables to deviate slightly from the values used in the rigid-molecule treatment. In particular, the energy barriers existing between very similar minimum-energy conformations in the rigid-molecule treatment often disappear and these similar conformations merge into the same minimum-energy one in the flexible-molecule treatment (*e.g.*, B and C → $N_{I,I}$, A and B′ → $L_{II,I}$, E and E′ → $J_{II,I}$, D′ and H → $J_{II,I}$), though sometimes the barriers remain [*e.g.*, A′ and D, which are similar, go to two different (but similar) conformations, *viz.*, $K_{II,I}$ and $K_{I,I}$, respectively]. The effect of introducing flexibility is large in a strained system, and it is observed in this strained molecule of *cyclo*(Gly_3Pro_2). The results obtained in this paper indicate the necessity for carrying out the second minimization in the flexible-molecule treatment, after the first minimization has been performed in the rigid-molecule treatment, especially in strained systems. Even though the conformations of local minima in the rigid-molecule treatment are often not close to those in the flexible-molecule treatment, it seems to be a good idea to use the local minima in the rigid-molecule treatment as starting conformations for the second minimization. The absence of $J_{I,II}$ as a local minimum provides supporting evidence for the assumption that all

Table IV
Distributions of Bond Lengths and Bond Angles in the Minimum-Energy Conformations

Type	Amino Acid Residue[a]	Amino Acid Type	Distribution[b]	Values from Model Compound[c]	Values for Rigid Geometry[d]
$N(C^{\alpha})C'$	1, 2, 3	Gly	108.3 ± 1.8	107.9	109.5
	4, 5	Pro	110.4 ± 5.1	111.2	109.5
$C'(N)C^{\alpha}$	1, 2, 3	Gly	122.8 ± 1.2	122.2	123.0
	4, 5	Pro	121.7 ± 1.3	120.6	121.0
$C^{\alpha}(C')N$	1, 2, 3	Gly	116.8 ± 2.1	115.3	114.0
	4, 5	Pro	118.3 ± 1.6	118.6	119.0
$N(C^{\alpha})C^{\beta}$	4, 5	Pro	101.0 ± 2.4	103.7	104.0
$C^{\alpha}(C^{\beta})C^{\gamma}$	4, 5	Pro	106.0 ± 2.0	106.8	107.0
$C^{\beta}(C^{\gamma})C^{\delta}$	4, 5	Pro	105.1 ± 1.6	105.6	106.0
$C^{\gamma}(C^{\delta})N$	4, 5	Pro	101.8 ± 1.5	103.4	103.0
$C^{\delta}(N)C^{\alpha}$	4, 5	Pro	113.7 ± 0.7	113.3	113.0
C′=O	1, 2, 3	Gly	1.220 ± 0.003	1.236	1.24
	4, 5	Pro	1.221 ± 0.002	1.276	1.24
C′–N	1, 2, 3	Gly	1.300 ± 0.003	1.314	1.32
	4, 5	Pro	1.304 ± 0.003	1.339	1.34
C^{α}–C′	1, 2, 3	Gly	1.494 ± 0.003	1.512	1.53
	4, 5	Pro	1.492 ± 0.006	1.519	1.50
N–C^{α}	1, 2, 3	Gly	1.447 ± 0.003	1.454	1.47
	4, 5	Pro	1.472 ± 0.007	1.452	1.45

[a]The residue numbers are those of Figure 1. The C′–N bond is regarded as belonging to the amino acid residue to which the N belongs. [b]The distribution means that the values found in the eight minimum-energy conformations (described in Table II) are distributed in the range bracketed by the upper (+) and lower (−) limit indicated. Units are degrees for angles and Å for lengths. [c]The values in this column are the X-ray data of Leung and Marsh[21] for the model compound L-Leu-L-Pro-Gly. The uncertainties in the observed values for the bond angles and bond lengths are ±2° and ±0.03 Å, respectively. [d]Taken from ref 14.

local minima in the flexible-molecule treatment can be arrived at by energy minimization starting from local minima in the rigid-molecule treatment.

Free energies rather than energies should be compared to determine the relative stabilities of various conformations.[5] However, we can conclude from the present energy calculation that the most stable conformation is one of the J group in Table II, because the difference in contributions to the free energy from conformational entropy is of the order of 1 kcal/mol,[4] while the difference in energies of conformations in the J and K groups is about 10 kcal/mol. Of the conformations in the J group, $J_{II,I}$ is the most stable energetically. The difference in energies among the conformations in the J group arises mainly from the difference in the conformations of the pyrrolidine rings. The conformational entropies would also expected to differ. But the difference would be expected to be smaller in this case (where the main differences in conformation are confined to the pyrrolidine rings) than in the one in which the backbone conformations differ from each other. Therefore, the order of stability obtained in the energy calculation, *i.e.*, $J_{II,I}$ being the most stable conformation, would not be changed even if entropic contributions were calculated. The conclusion of this paper is that $J_{II,I}$ in Table II and Figure 2 is the most stable all-trans peptide bond conformation of *cyclo*(Gly_3Pro_2).