Valinomycin–Cation Complex. Conformational Energy Aspects[1]

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Abstract: Using, as the starting point, the secondary structure of the valinomycin–potassium ion complex as determined by infrared and proton magnetic resonance studies, conformational energy calculations were carried out to determine the complete solution conformation and to assess the possible role of conformational energy in ion selectivity. The lowest energy conformation, for the analog in which all R groups were methyls, was found to be the conformer in which the L-Lac methyl moiety is axial and in which the ester C–O moieties are directed inward. This structure contains a polar core binding of the bare cation. Changes in conformational energy as a function of the size of the polar core reflected the observed ion selectivity. Further means of discerning between the two lowest energy conformations were achieved by calculating the angular distribution functions for the isopropyl R groups of the valyl residues and using the distribution function to calculate the expected α-CH–β-CH coupling constant. Comparison with the experimental coupling constants and their temperature dependence strikingly confirmed the solution valinomycin–potassium ion complex to be as previously proposed.

Valinomycin is a cyclic dodecadepsipeptide, which is to say that it contains 12 residues of alternating ester and peptide linkage. It is of particular interest, as it is known to selectively transport potassium ion across membranes.[2–6] The sequence, –L-Lac-L-Val-D-HyV-D-Val–, is repeated three times giving a cyclic molecule with threefold symmetry.[7] The primary structure is indicated in Figure 1. If one considers the optical isomeric sequence, it is apparent on completing the structure that there is, in an approximation which includes the β carbons, an inversion element of symmetry. On the basis of infrared[8] and nmr[9] studies, the secondary structure of the valinomycin–K^+ complex has been shown to be one in which all of the peptide moieties are intramolecularly hydrogen bonded. This secondary structure has been characterized as a series of β turns.[10,11]

An interesting representation of the backbone conformation is given in Figure 2a. In the approximation of identical R groups (or considering only the β carbons), it is seen that the structure belongs to the S_6 symmetry group (equivalent notation is C_{3i} or $C\bar{3}$). Rotation of the shaded area by 120 and 240°, *i.e.*, the C_3 and C_3^2 symmetry operations, followed by inversion through the center, generates the entire molecule with the proper optical isomeric sequence maintained. It is significant to note that the R group is either axial or radial and that this feature is maintained on generating the whole structure. Two structures may be differentiated on the basis of the R group disposition. When the chain direction is as indicated in Figure 2b, the R group of the hydroxy acid is radial and that of the amino acid is axial. When the chain direction is as indicated in Figure 2c, the amino acid R group is radial and that of the hydroxy acid is axial. Using the distinguishable methyl of the L-Lac residue we can refer to the structure in Figure 2b as radial, R(CH_3), and to that in Figure 2c as axial, A(CH_3). There is another permutation possible. The C–O moieties may be directed inward or outward. Accordingly, once the secondary structure is determined for the valinomycin–cation complex, solving the solution conformation requires distinguishing among four possible conformers: I, that of Figure 2b with the C–O in; II, that of Figure 2b with the C–O out; III, that of Figure 2c with the C–O in; and IV, that of Figure 2c with the C–O out.

Previous efforts[10,11] extrapolated from conformational energy considerations of β turns involving end peptide moieties (the peptide forms from residues 1 and 2 in Figure 2) to β turns with ester moieties in place of the end peptide assumed that these energy considerations applied to specific β turns required in the cyclic structure in Figure 2 and presented an argument for a nonhydrated ion in arriving at the solution conformation for the valinomycin–K^+ complex to be structure I.[10,11] In the present communication, using the secondary structure as the point of departure, the backbone conformations are generated for the structures in Figure 2, and the conformational energies are calculated. In accordance with the general β-turn consideration,[10,11] the conformation I (in Figure 2b with the C–O directed inward) and the conformation IV (in Figure 2c with the C–O directed outward) are much lower (400 kcal/mol) in energy than the other two conformers, and the former is 6.1 kcal/mol lower in energy than the latter. In connection with ion selectivity, the size of the polar core

(1) Presented in part at the 161st National Meeting of the American Chemical Society, Alkali Metal Ion Symposium, Los Angeles, Calif., March 28–April 2, 1971.

(2) (a) B. C. Pressman, *Proc. Nat. Acad. Sci. U. S.*, **53**, 1076 (1965); (b) E. J. Harris and B. C. Pressman, *Nature*, **216**, 918 (1967).
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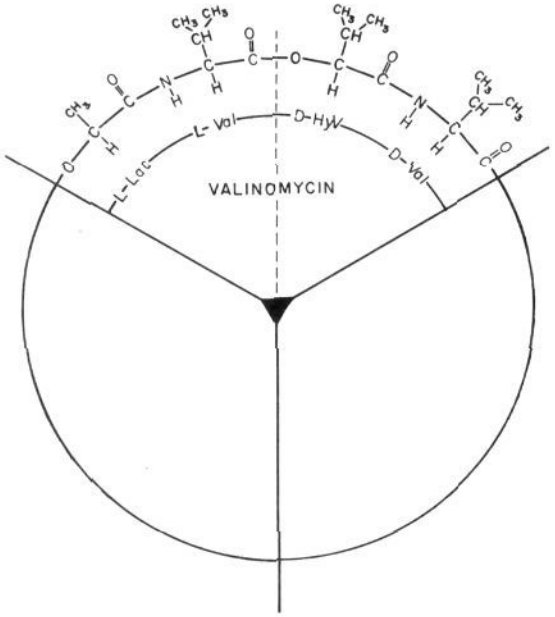

Figure 1. The primary structure of valinomycin[7] showing threefold symmetry in the primary sequence.

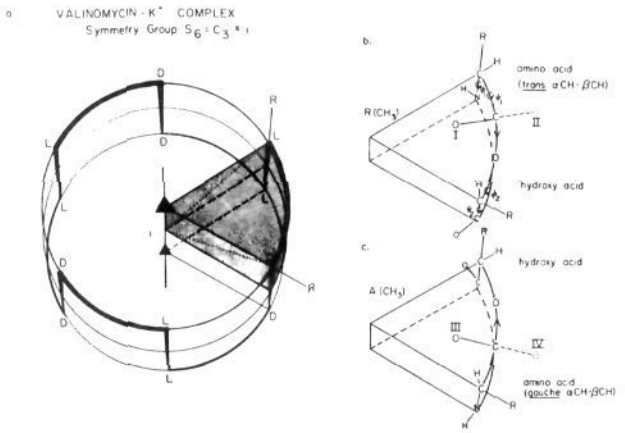

Figure 2. Representation of the valinomycin–potassium ion complex (a) showing that, in the approximation of identical R groups, the molecular system belongs to the S_6 symmetry group. (Equivalent notation is C_{3i} or $C\bar{3}$.) Two chain directions (indicated in b and c) are possible. They are distinguishable by the positions of the residues and the orientation of the R groups. In b, the amino acid residue of the repeating unit is in the upper corner and has an axially oriented R group, while the hydroxy acid residue is in the lower corner with a radially oriented R group. In this case the distinguishable methyl group of the L-Lac residue is directed radially allowing ready identification with a radial methyl group, *i.e.*, $R(CH_3)$. In c, the hydroxy acid is at the top corner with an axial R group and the amino acid is in the lower corner with a radial R group. This conformation can be identified by the axial orientation of the L-Lac methyl, *i.e.*, $A(CH_3)$. In both the b and c ester, which forms the end of the β turns, it may have its C–O moiety directed inward or outward giving four conformers, I, II, III, and IV, as designated in the figure. Also included in b and c is the preferred orientation of the valyl side chains for conformers I and IV.

can be related to the conformational energy by varying the angle with which the C–O is directed toward the center. In addition, the angular distribution functions for rotations about the α-C–β-C bond for the amino-acid side chains are calculated for the low energy structures I and IV and used to calculate $J_{\alpha CH-\beta CH}$ and its

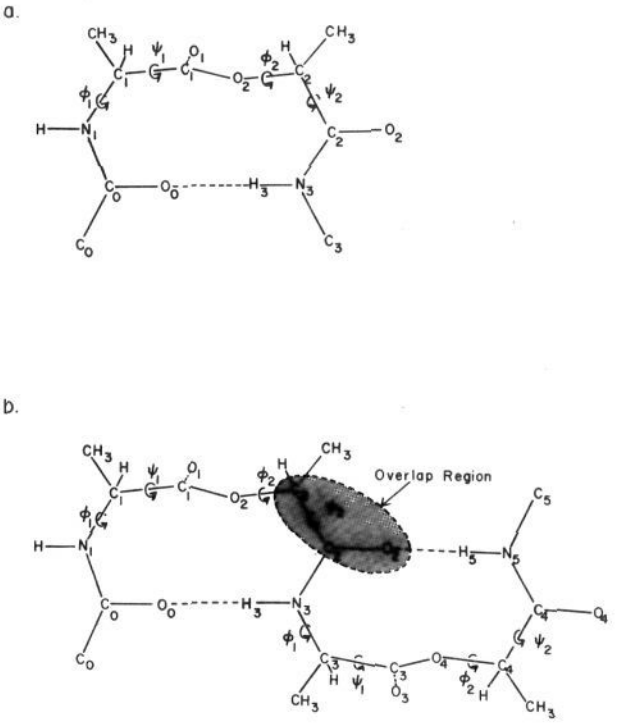

Figure 3. Structure of a β turn (a) in which the peptide at the end of the turn (top of structure as drawn) has been replaced by an ester moiety. The structure in a is duplicated and the two structures are combined with the atoms in the shaded area of b, being made coincident. This is the first step in generating the closed, cyclic structure represented in Figure 2a (see text for discussion). The figure defines the bonds to which the angles ϕ_1, ψ_1, ϕ_2, and ψ_2 are associated.

temperature dependence. When compared to the nmr data on the magnitude and temperature dependence of $J_{\alpha CH-\beta CH}$ for the valyl residues, the result is conclusive that the solution conformation for the valinomycin–K^+ complex is conformer I.

Generation of the Secondary Structure. As a starting point in generating the secondary structure of the valinomycin–cation complex, we utilized the β-turn coordinates of Geddes, *et al.*[12] To the atoms reported were added hydrogen atoms as outlined by Scott and Scheraga.[13] The end peptide moiety of the β turn was replaced by an ester moiety (see Figure 3 legend for the definition of the end ester moiety of a β turn). The bond lengths and angles for the ester moiety were taken from Ooi, *et al.*[14] Also, β-methyl groups were placed with hydrogens staggered relative to α-C substituents. The result is the structure given in Figure 3a. This unit constitutes slightly more than one-sixth of the structure. The important angles are ϕ_1, ψ_1, ϕ_2, and ψ_2, and changes in these angles are carried out in such a way that positions of atoms at higher indices are varied while those of lower indices are held constant.

In generating the structure there are two constraints: one, that of C_3 symmetry is necessarily imposed; the second is the assumption of an O···H hydrogen bond length in C–O···H–N of 1.80 Å. The latter assumption is stated as an O···H distance because the hydro-

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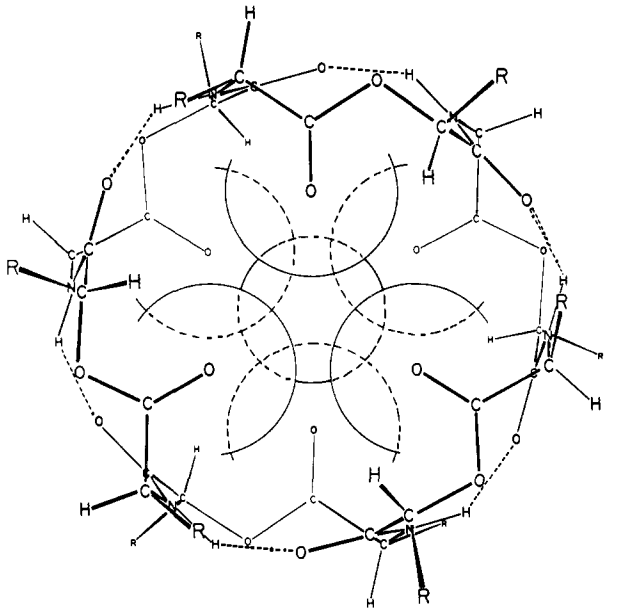


Figure 4. Calculated structure of the lowest energy conformation of a valinomycin–cation complex in which the R groups were taken as methyls. The constraints imposed in calculating the structure were the standard dimension of the residues involved, C_3 symmetry, and an $O_i \cdots H_{i+3}$ hydrogen bond distance of 1.80 Å. The center circle corresponds to the K^+ drawn to scale. It can be seen that the size of the polar core containing the ion is controlled by the tilt of the ester moiety.

gen bonding angle is not included as a constraint. In the valinomycin–cation complex, the NH–OC angle is necessarily in the neighborhood of 120°. In the next step the mirror image of the structure in Figure 3a is obtained, and a transformation is carried out which makes coincident atoms C_0^α, C_0', and O_0 of the new set mirror image with atoms C_2^α, C_2', and O_2 of the original set. This results in the structure given in Figure 3b, which constitutes just over one-third of the structure. The structure in Figure 3b is then duplicated twice and transformations are carried out on the duplicate structures to bring them into coincidence at the appropriate overlap regions. Rotations of the four angles, ϕ_1, ψ_1, ϕ_2, and ψ_2, are involved achieving closure with ψ_2 being the dominant angle in effecting closure and C_3 symmetry. For perfect closure the angle subtending lines connecting atoms N_9, N_1, and N_5 should be 120°. An iterative approach was used in which three to four iterations were required to achieve 120 ± 0.05°. The various angles were then printed out along with the $O \cdots H$ hydrogen bond distance.

The above procedures yield a structure with threefold symmetry. The next concern is achieving the assumed $O_i \cdots H_{i+3}$ hydrogen bond distance of 1.80 Å. The hydrogen bond length is most sensitive to ϕ_2. Accordingly, plots of ϕ_1 *vs.* ϕ_2 were made at constant ψ_1 with ψ_2 maintaining C_3 symmetry. The plots were a set of points forming an ellipse which achieved the $O_i \cdots H_{i+3}$ of 1.80 ± 0.005 Å. The plots are repeated with different values of ψ_1. The result is a surface representing the set of structures which satisfy the constraints of symmetry and hydrogen bond length.

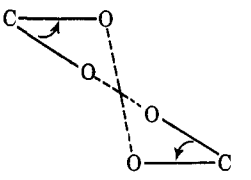
Conformational Energies. Conformational energies for points on the surface are obtained by using the potential functions as outlined by Ooi, *et al.*[14] All nonbonding interactions were calculated using the Lennard-Jones 6–12 potential function; coulombic interactions were calculated assuming a dielectric constant of one, and torsional energies were included for the four angles, ϕ_1, ψ_1, ϕ_2, and ψ_2. The lowest energy conformation is plotted in Figure 4. It is equivalent to that represented by Figure 2b with the C–O moiety directed inward, *i.e.*, conformer I. The next lowest conformation is conformer IV. The difference in energy is 6.1 kcal/mol, and inclusion of the cation at the center of the molecule would further favor the conformation given in Figure 4. An independent and conclusive means of deciding between conformers I and IV is given below. The other conformations, *i.e.*, conformers II and III, are more than 400 kcal/mol higher in energy. The coordinates of the structure in Figure 4 are reported in Table I. It may be noted that the α-CH–NH dihedral angle as calculated from $J_{\alpha CH-NH}$, using the Karplus-type relationship of Ramachandran, *et al.*,[15] would be approximately 125°, whereas the angle for the structure given in Figure 4 is 113°.

Table I. Calculated Coordinates[a] for the Valinomycin–K^+ Complex

Atom	Radius, Å	Angle, deg	Height, Å
N_1	4.27	16.2	0.15
H_1	4.52	3.2	0.07
C_1^α	4.36	23.4	1.51
H_1^α	5.34	28.9	1.61
C_1^β	4.43	8.4	2.53
C_1'	3.37	40.6	1.78
O_1'	2.14	40.0	1.88
O_2	4.28	55.8	1.92
C_2^α	4.08	75.1	2.22
H_2^α	3.23	80.0	2.83
C_2^β	5.39	78.6	2.98
C_2'	4.12	86.1	0.92
O_2'	4.32	102.9	0.94

[a] The origin is at the center of inversion, *i.e.*, the position of the cation.

Conformational Energy *vs.* Pore Size. The ester C–O moieties, which are related by the inversion element of symmetry, control the size of the polar core. When the ester C–O bond axis is pointed toward the center of the molecule, the size of the polar core is smaller; as it is rotated outward the size of the polar core increases, *i.e.*



The size of the polar core determines the size of the ion which is sequestered by valinomycin. Accordingly, a plot of conformational energy *vs.* size of polar core is of interest in connection with the ion selectivity of valinomycin. This plot is given in Figure 5 where the distance r is the distance of the ester acyl oxygen from the center of the molecule. As the size of the polar core increases, the conformational energy is seen to

(15) G. N. Ramachandran, R. Chandrasekaran, and K. D. Kopple, *Biopolymers*, in press.

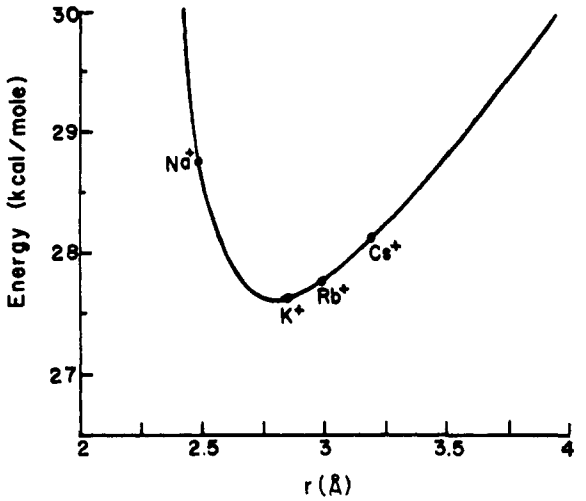


Figure 5. The distance of the acyl oxygen from the center of the structure drawn in Figure 4 plotted as a function of conformational energy. The points on the curve are distances for the sum of the ionic radius and the van der Waals radius of the oxygen atom for the cations indicated. See text for discussion.

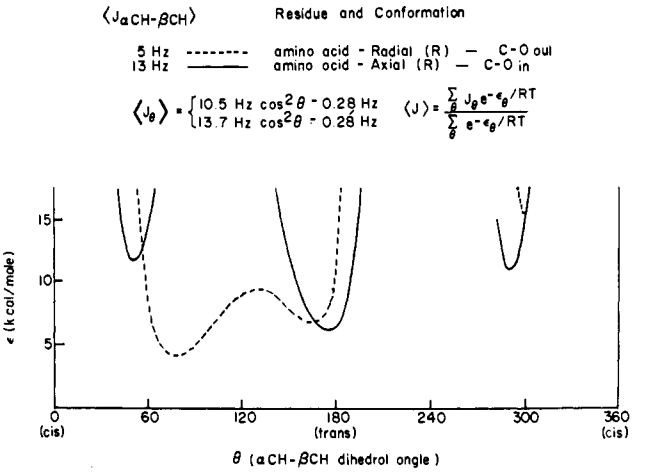


Figure 6. Angular distribution function for rotations about the α-C-β-C bond of ———, the axially oriented isopropyl group of the valine residue in structure I (see Figure 2a with the C–O directed inward), and of - - - -, the radially oriented isopropyl group of the valine residue in structure IV (see Figure 2b with the C–O directed outward). The expected α-CH–β-CH coupling constant at 25° for the valyl residues in structure I is 13 Hz, whereas for structure IV it is 5 Hz. The experimental $J_{\alpha CH-\beta CN}$ is 11 Hz with no temperature dependence in the −50 to 30° range. As structures I and IV have greatly lower conformational energies than do structures II and III, this demonstrates the solution conformation of the valinomycin–K^+ complex to be structure I, *i.e.*, that given in Figure 4. See text for discussion.

drop precipitously and then rise more slowly with the minimum near 2.80 Å.

Included on the curve in Figure 5 are points representing the sum of the ionic radii and the acyl oxygen van der Waals radius for the cations Na^+, K^+, Rb^+, and Cs^+. This would be the longest expected O–Me^+ distance. The X-ray data of Kilbourn, *et al.*,[16] on the nonactin–K^+ complex give O–K^+ distances of 2.75 and 2.79 Å rather than the 2.83 Å which would be the sum of the potassium ion radius and the oxygen van der Waals radius; therefore, it can be expected that the points included in Figure 5 should be shifted to smaller values by 0.04–0.08 Å. This places K^+ and Rb^+ in positions straddling the minimum. These results correlate surprisingly well with the reported selectivities of valinomycin in which the order of selectivity is $Rb^+ > K^+ > Cs^+ > Na^+$.[4]

In the constraints utilized in generating the secondary structure we assumed an O···H distance of 1.80 Å. While this is the preferred length (actually N–H···O of 2.80 Å with N–H of 1.00 Å), variations have been seen in crystals from 1.75 to 2.00 Å.[17] Increasing the 1.80-Å value would have the effect of shifting the curve in Figure 5 to slightly larger values of *r*. The magnitude of the shift would be small, less than 0.1 Å, but it may function in placing Rb^+ in the bottom of the curve. An additional factor which has been emphasized by Prestegard and Chan[18] is that of hydration energy which for Na^+, K^+, Rb^+, and Cs^+ is 97, 77, 70, and 63 kcal/mol, respectively. This may also be involved in the slight favoring of Rb^+ over K^+.[4] We wish to emphasize here that the present work elucidates the significance of conformational energy in the ion specificity exhibited by valinomycin.

Calculation of $\langle J_{\alpha CH-\beta CH}\rangle$ for the Valyl Amino Acid Residues. The conformational energy calculations provided evidence for conformer I as the lowest energy conformation where the R groups are all methyls. This may be checked in quite an independent way due to the very different angular distribution functions for the amino acid R groups when comparing conformers I and IV, which, in the absence of the ion and with β-methyl groups, differ by 6.1 kcal/mol. The angular dependence of $J_{\alpha CH-\beta CH}$ for amino acids has been suggested by Abraham and McLauchlan[19] to be

$$J_{\theta,\alpha CH-\beta CH} = \begin{cases} 10.5\ \text{Hz}\cos^2\theta - 0.28\ \text{Hz} & (0\text{–}90°) \\ 13.7\ \text{Hz}\cos^2\theta - 0.28\ \text{Hz} & (90\text{–}180°) \end{cases} \quad (1)$$

Attaching the isopropyl R groups for the valyl residues and using the Lennard-Jones nonbonding potential functions and the torsional potential functions as outlined by Ooi, *et al.*,[14] the angular distribution functions for rotation about the α-C–β-C bond are given in Figure 6. The angular distribution functions are seen to be very different for the two conformers. In conformer I the hydrogens on the α and β carbons are locked in the trans position, whereas in conformer IV the orientation is primarily gauche but could open up with increasing temperature to populate states with larger angles.

Using the calculated angular distribution function and the above equation for the α-CH–β-CH coupling constant for amino acids as a function of dihedral angle, the expected coupling constant, $J_{\alpha CH-\beta CH}$, can be calculated, *i.e.*

$$J_{\alpha CH-\beta CH} = \frac{\sum_\theta J_\theta e^{-\epsilon_\theta/RT}}{\sum e^{-\epsilon_\theta/RT}} \quad (2)$$

(16) B. T. Kilbourn, J. D. Dunitz, L. A. R. Pioda, and W. Simon, *J. Mol. Biol.*, **30**, 559 (1967).

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where J_θ is as given in eq 1. The $J_{\alpha CH-\beta CH}$ for structure I is 13 Hz with no significant temperature dependence, whereas for structure IV, a value of 5 Hz is obtained at 25°. The experimental value is 11 Hz for the valyl residues of the valinomycin–K^+ complex with no change in temperature over the range of −50 to 30°. The experimental result for the side chain of hydroxyisovaleric acid is 2.5 Hz at −50° and 4 Hz at 30°. This result quite conclusively defines the solution conformation of the valinomycin–K^+ complex to be conformer I. This result is the same as that reported from the crystal studies for the backbone atoms.[20] The X-ray coordinates have not yet been reported for the backbone atoms nor have positions of the side chains been reported. It will be of interest when the X-ray coordinates are reported to compare them with those given in Table I.

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Crystal Structure and Conformation of the Cyclic Dipeptide *cyclo*-L-Prolyl-L-leucyl

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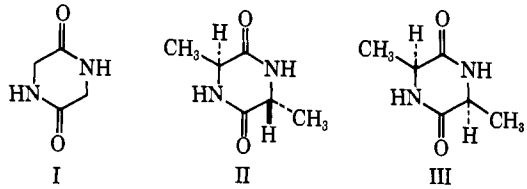
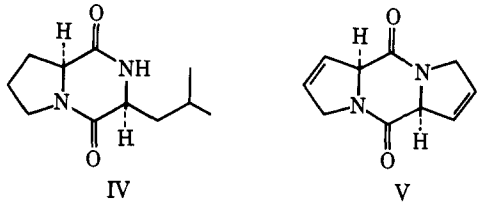
Abstract: A single-crystal X-ray diffraction analysis has been made of the structure of the cyclic dipeptide *cyclo*-L-Pro-L-Leu. The diketopiperazine ring has a folded conformation with a dihedral angle of 143° between the two planar peptide units. In the pyrrolidine ring, C^β is 0.52 Å out of the plane of the other four atoms. The leucyl side chain is fully extended. Molecules form infinite chains parallel to the *a* axis by means of NH···O hydrogen bonds. The material crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters $a = 9.451$, $b = 19.587$, and $c = 6.340$ Å. The X-ray intensity data were collected with an automatic diffractometer and refined to $R = 6.4\%$. The crystal structure was solved by the symbolic-addition procedure for noncentrosymmetric crystals.

Cyclic dipeptides contain the diketopiperazine (DKP) ring which in the crystalline state has been found to be planar when unsubstituted, *i.e.*, in *cyclo*-Gly-Gly[1] (I), and nearly planar in *cyclo*-D-Ala-L-Ala[2] (II).



I II III



IV V

Both of these molecules possess a center of symmetry. In *cyclo*-L-Ala-L-Ala[2] (III), on the other hand, the DKP ring is appreciably puckered and has been described as a twist boat. In the present investigation of *cyclo*-L-Pro-L-Leu (IV), the side groups on the DKP ring are dissimilar and bulkier than those previously studied. In this case, the ring has been found to have a symmetric boat shape, with all parameters almost identical with those found in 3,4-dehydroproline anhydride[3] (V).

In addition to the conformation of the DKP ring, the conformation of the pyrrolidine ring is of interest. In L-proline and other pyrrolidine-related amino acids, the pyrrolidine ring has assumed a number of conformations in the crystalline state as shown in Table I. The five-membered ring has the envelope conformation, with four atoms in a plane and one out of the plane by 0.4–0.6 Å either on the same side as or on the opposite side to the carboxyl group. In the various prolines, atom C^β, C^γ, or N is out of the plane. In the prolyl residues in the linear polypeptides tosyl-L-Pro-L-Pro(OH),[4] L-Leu-L-Pro-Gly,[5] and *p*-bromocarboxy-Gly-L-Pro-L-Leu-Gly(OH),[6] atom C^γ is 0.26–0.60 Å out of the plane of the other four atoms. However, in *cyclo*-L-Pro-L-Leu, it is C^β which is 0.52 Å out of the plane.

Experimental Section

A large colorless crystal of *cyclo*-L-Pro-L-Leu, in the shape of a hexagonal plate, was grown by slow evaporation from ethyl acetate solution by Dr. T. C. McMorris of the New York Botanical Garden. The soft crystal was cut to a more suitable shape, with dimensions 0.3 × 0.5 × 1.0 mm, for the diffraction experiment. X-Ray intensities were measured with copper radiation on a four-circle automatic diffractometer using the θ–2θ scan technique with a 2.4° +

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