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The Crystal and Molecular Structure of the Triclinic and Monoclinic Forms of Valinomycin, $C_{54}H_{90}N_6O_{18}$

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Abstract: Two forms of uncomplexed valinomycin have been crystallized and their structures determined by X-ray diffraction. The monoclinic form crystallizes in space group $P2_1$ with the following cell dimensions: $a = 23.144$ Å, $b = 10.347$ Å, $c = 14.526$ Å, $\beta = 99.57°$, and $Z = 2$. The other form crystallizes in the triclinic space group $P1$ with cell dimensions $a = 22.281$ Å, $b = 10.333$ Å, $c = 14.507$ Å, $\alpha = 90.05°$, $\beta = 105.28°$, $\gamma = 93.31°$, and $Z = 2$. The two structures were refined by full-matrix least-squares to residuals of 0.16 and 0.14, respectively. An analysis of the conformations of the three independent molecules as determined in this study indicates that the gross features of all three are the same. However, minor differences in the conformations, as much as 12° in the torsion angles, are observed. Two different kinds of hydrogen bonds are found in all three molecules. Each molecule has four hydrogen bonds involved in β turns (one amino acid residue hydrogen bonding to a carbonyl group of an ester residue three groups removed). Each molecule also has two previously undescribed hydrogen bonds in which the amino acid is hydrogen bonded to a carbonyl group of an amino acid residue four groups away. A mechanism of coordinating potassium ion is postulated based on the observed conformation.

Valinomycin is a cyclic dodecadepsipeptide that selectively transports potassium ion across natural and synthetic membranes.[1,2] Extensive studies of this transport process have been conducted to determine, if possible, the basis for the potassium ion selectivity of valinomycin and to ascertain whether it acts as a channel or a carrier in membranes.[2] Knowledge of the conformation of complexed and uncomplexed valinomycin facilitates the development of models for its complexation, transport, and release mechanisms.

In valinomycin the sequence (L-Val-D-HyIv-D-Val-L-Lac) is repeated three times giving a cyclic molecule with the possibility of threefold symmetry (Figure 1). Infrared, NMR, Raman, and X-ray crystallographic studies have shown that the potassium complex exists in solid and solution as a doughnut shaped molecule possessing threefold symmetry in which all amide protons are hydrogen bonded to neighboring residues in β-turn type conformations.[3-14] The potassium ion in the center of the doughnut has sixfold coordination to the amino carbonyls (Figure 2).[13,14]

The conformation of uncomplexed valinomycin is considerably more complex. By means of ir, NMR, and ORD, it has been found that the hydrogen bonding scheme in valinomycin is a function of solvent polarity.[4,9,10,12,15] It has been proposed that the conformation in nonpolar solvents is similar to that found for the potassium ion complex, i.e., six intramolecular amide–carbonyl hydrogen bonds. An open conformation has been proposed in which there is no intramolecular hydrogen bonding, but rather an interaction between solute and solvent when an aqueous–dioxane solvent system was used. For solvent polarities between these two extremes, it has been suggested that there are three intramolecular amide–carbonyl hydrogen bonds, the nitrogens belonging to either the D-Val or to the L-Val residues, so that threefold symmetry is retained.

Two different crystals, obtained from different solvent systems, have been studied and a single conformation has been found for the three independent molecules whose structure was determined in this study. This conformation is quite different from that proposed on the basis of spectral data.

Experimental Section

Two forms of uncomplexed valinomycin have been crystallized. Modification A was obtained from *n*-octane and has disordered solvent present in the monoclinic lattice. Modification B is a triclinic form obtained from ethanol–water solutions. Relevant crystal data are presented in Table I.[16]

Modification A. The monoclinic crystals were mounted in glass capillaries to prevent solvent loss. Data suitable for least-squares refinement of both positional and thermal parameters were collected by the θ–2θ scan technique on an Enraf Nonius CAD-4 diffractometer using Ni-filtered Cu Kα radiation. No significant changes were observed in the intensities of two standard reflections which were measured after every 96 intensities were recorded. Intensities were corrected for Lorentz and polarization factors but not for extinction or absorption. Atomic scattering factors were taken from the literature; both real and imaginary dispersion corrections were applied.[17] Weights were calculated according to a modified Hughes scheme:[18,19] $w = a/|F_o|$ for $a < |F_o|$; $w = |F_o|/a$ for $|F_o| < a$; the value of "a" was 27.0.

The 214.1 Å^3 difference in the unit cell volumes of the two crystal forms is in very good agreement with the volume reported for one *n*-octane molecule (208.5 Å^3).[20] Because of the symmetry requirements in space group $P2_1$, this one solvent molecule must be disordered.

Modification B. The triclinic cell is nearly congruent with the

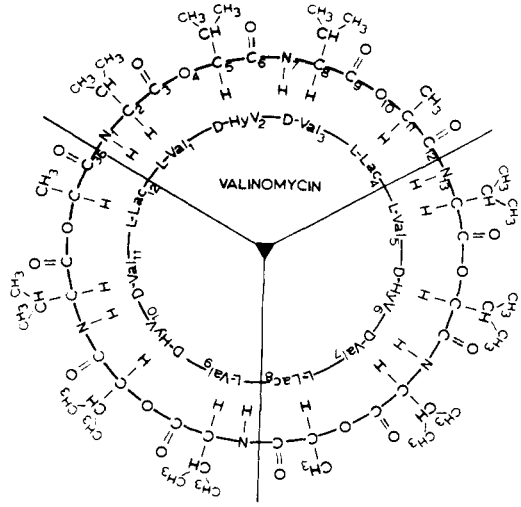

Figure 1. Structural formula for valinomycin.

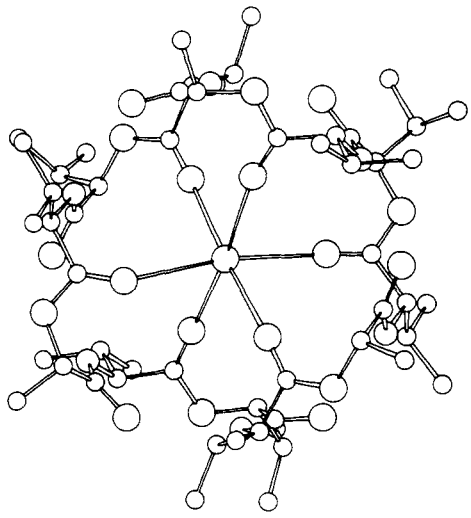

Figure 2. Structure of the potassium ion complex of valinomycin.[14]

monoclinic cell and the triclinic lattice was observed to have pseudo-2/m symmetry. The original X-ray intensity data for modification B were collected from a crystal twinned on the $hk0$ plane. The diffraction data were measured on a G.E. XRD-5 diffractometer by means of the stationary crystal–stationary counter method. Those data for which $l = 4n \neq 0$ were not used since the two lattices were difficult to resolve on these layers. The intensity contribution to the $hk0$ data from each twin was assumed to be proportional to their resolved equivalent intensities. A total of 3268 independent intensities were recorded.

An untwinned crystal of the triclinic form was finally obtained and suitable data were recollected in the manner described for the monoclinic form. The intensities of the two standard reflections remained constant. The data were reduced in the manner previously described; the value of "a" used in the modified Hughes scheme[18,19] was 30.0.

Structure Determination and Refinement. Modification A. The details of the solution of the phase problem of the monoclinic form have been reported elsewhere.[21,22] The 78 nonhydrogen atoms were refined by full-matrix least squares, minimizing $\sum w\Delta^2$, to a residual of 0.17 ($R = \sum|F_o - F_d|/\sum|F_d|$). A difference map was calculated and one minor peak was observed in the vicinity of each of the atoms, C5A and C32A (β-carbon atoms). Since the isotropic thermal parameter for one methyl group (a γ carbon) on each of these residues was nearly twice as large as the thermal parameters for the rest of the γ carbons, it was thought that these isopropyl groups might be disordered by a 120° rotation about the C^{α}–C^{β} bond. Therefore, the two atoms corresponding to the peaks observed on the difference map were included in the calculations as carbon atoms with occupation factors of 0.5; the two carbon atoms with large thermal parameters were also given occupation factors of 0.5. Several cycles of least-squares refinement failed to reduce the residual and, in fact, the weighted residual increased. The thermal parameters of the atoms located from the difference map had refined to values of approximately 14. As a result, the two extra atoms were removed from subsequent least-squares refinement. The refinement converged at a residual of 0.16 for the observed data only and a weighted residual of 0.19 ($R_w = (\sum w|F_o - F_d|^2/\sum wF_o^2)^{1/2}$). A residual of 0.21 was calculated for both the observed and unobserved data. The inclusion of theoretical hydrogen atom positions in the calculations produced a residual of 0.20. The standard deviation of an observation of unit weight, S, was calculated to be 3.16 ($S = (\sum w\Delta^2/(m - n))^{1/2}$ where m is the number of observations and n is the number of parameters).

The difference map also indicated the location of the single n-octane molecule. A cylindrical shaped cloud of electron density coincident with the b axis was plainly visible. However, distinct peaks could not be resolved and attempts to fit a chemically reasonable n-octane molecule into the volume of high electron density observed on the difference map produced an inordinate increase of about 0.15 in the residual.

Modification B. Because of the fact that the unit cell constants for the two modifications of valinomycin were so remarkably similar, it was expected that the two forms would be nearly isomorphous. However, the weighted reciprocal lattices with $l = 3n$ could be brought into coincidence only after a rotation about the b^* axis of the triclinic lattice. This rotation, which demonstrated a structural relationship between the two modifications, corresponded to transforming the Miller indices of the triclinic lattice from (hkl) to ($h + 2l/3$,k,l). From this relationship a transformation matrix was calculated which would generate the atomic coordinates of the triclinic modification from those of the monoclinic modification. Since the triclinic reciprocal lattice exhibited pseudo-twofold symmetry, the relative orientation of both molecules was known. Structure factor residuals, calculated independently for molecules B1 and B2, were 0.35 and 0.36, respectively. Each valinomycin molecule possesses a pseudo center and the triclinic cell has pseudo-twofold symmetry, and thus, the two independent molecules should be nearly translationally related. Therefore, a translation function[23] was executed to determine the position of the second molecule relative to the first. The resultant vector from molecule B1 to B2 was 0.7 Å from the point ½,½,0 which packing considerations had dictated. The residual calculated for all 156 atoms was 0.26. Two Fourier refinement cycles reduced the residual to 0.23.

Table I. Crystal Data for Both Modifications[a]

Formula	Modification A $C_{54}H_{90}N_6O_{18}\cdot$ ½C_8H_{18}	Modification B $C_{54}H_{90}$-N_6O_{18}	Modification B Reduced[b] cell
a, Å	23.144	22.281	22.281
b, Å	10.347	10.333	10.333
c, Å	14.526	14.507	14.507
α, deg	90	89.95	90.05
β, deg	99.57	105.28	105.28
γ, deg	90	86.69	93.31
V, Å^3	3430.1	3216.0	
F(000)	1266	1200	
ρ_o, g cm^{-3}	1.125		
ρ_c, g cm^{-3}	1.131	1.148	
Space group	$P2_1$	$P1$	
Z	2	2	
$(\sin\theta/\lambda)_{max}$, Å^{-1}	0.60	0.60	
Total independent intensities	6189	10534	
Observed intensities (3σ)	3800	7861	
μ(Cu Kα), cm^{-1}	9.96	7.19	

[a] Two sets of unit cell constants are reported for modification B. The second set is the reduced cell; the first set is the working cell and all results reported pertain to it. [b] The transformation matrix relating the working cell to the reduced cell is $\begin{bmatrix} \bar{1} & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & \bar{1} \end{bmatrix}$

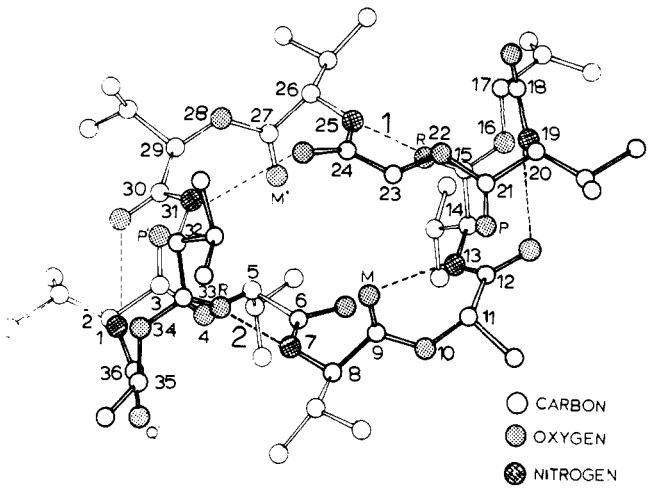

Figure 3. Structure of uncomplexed valinomycin.

Table II. Averaged Bond Distances and the Standard Deviations from the Mean for the Four Residues

Atoms	L-Val	D-Hylv[a]	D-Val	L-Lac[a]
C_{i-1}–N	1.34 (5)[b]	1.35 (3)	1.35 (6)	1.35 (2)
N–C^{α}	1.44 (3)	1.45 (4)	1.47 (4)	1.47 (5)
C^{α}–C^{β}	**1.56** (7)	1.57 (3)	1.57 (4)	1.55 (5)
C^{β}–$C^{\gamma 1}$	1.52 (6)	1.48 (7)	1.59 (5)	
C^{β}–$C^{\gamma 2}$	1.58 (3)	1.52 (4)	1.49 (14)	
C^{α}–C_i	1.54 (4)	1.50 (6)	1.50 (5)	1.53 (4)
C_i–O_i	1.20 (3)	1.26 (4)	1.21 (3)	1.24 (4)

[a] Atom names pertain to an amino acid residue;[25,28] N must be replaced by O for the ester residues. [b] The value in parentheses is the standard deviation from the mean.

Positional and isotropic thermal parameters for the nonhydrogen atoms were then refined by full-matrix least squares to a residual of 0.16 using the recollected data. Thermal parameters, bond distances, and bond angles of three γ-carbon atoms in each molecule were considered to be unreasonable. These six isopropyl methyl atoms were removed from the calculations and a difference map was calculated. A broad peak was found in the positions which had been occupied by each of these six atoms; in addition, a second broad peak in each of the two residues (C5 and C32) was also observed as well as a smaller peak in a third residue C(17). This again suggested that the isopropyl groups are disordered by a 120° rotation about the C^{α}–C^{β} bond. The refinement was continued by placing three methyl groups on two of the residues (C5 and C32) in each molecule and assigning occupation factors of 0.5 for two of the methyl carbons and 1.0 for the third. The isopropyl group attached to C17 was left unaltered since the difference map did not indicate a significant amount of disorder. The refinement converged at a residual for the observed data of 0.14 and a weighted residual of 0.12. The standard deviation of an observation of unit weight was calculated to be 1.88. The residual for both the unobserved and observed data was 0.17. Theoretical hydrogen atom positions were calculated and included in a structure factor calculation from which the residual, including both observed and unobserved data, was calculated to be 0.15.

Results

A drawing of one molecule of valinomycin,[21] illustrating the observed conformation of the three independent molecules, is shown in Figure 3. Mean values of the bond distances and angles of the four residues (L-Val, D-HyIv, D-Val, and L-Lac), averaged over all three independent molecules, are listed in Tables II and III[24] along with the standard deviation from the mean.[25]

Half-normal probability plots[26,27] were prepared in order to compare all three molecules of valinomycin (Figure 4). Distances and standard deviations were calculated for all intramolecular contacts; the calculated hydrogen atom positions were not included. The first plot compares the two independent molecules in modification B (triclinic form). The equation of the straight line, fitted by least squares, has a slope of about 2.2 and an intercept of −0.04. However, the points do indeed deviate from linearity, indicating that the errors do not follow a normal distribution. Of the final 22 points on the plot, it is observed that three atoms (C8, C24, and O27A) are involved in all of the contacts.

The second and third plots compare each of the independent triclinic molecules with that of the monoclinic determination. The deviations from linearity are much more pronounced in the case of molecule B1, although in both cases the points deviate from the least-squares straight line by a sinusoidal variation, i.e., δ(real) is too small when δ(expected) is less than 1.8 and too large when δ(expected) is greater than 1.8. The slopes and intercepts for the lower portion of the line for which δ(real) is less than 3.0 are 2.18, −0.10 and 1.96, −0.09 for B1 and B2, respectively. The slopes of all three plots indicate that the standard deviations are probably underestimated by a factor of about 2.

The torsion angles which describe the conformation of the three independent molecules of valinomycin are given in

Table III. Averaged Bond Angles and the Standard Deviations from the Mean for the Four Residues

Atoms	L-Val	D-Hylv[a]	D-Val	L-Lac[a]
C_{i-1}–N–C^{α}	119 (1)	117 (3)	117 (2)	115 (2)
N–C^{α}–C^{β}	109 (2)	107 (3)	109 (2)	106 (2)
N–C^{α}–C_i	108 (3)	110 (3)	108 (3)	108 (5)
C^{β}–C^{α}–C_i	110 (3)	112 (2)	110 (2)	109 (2)
C^{α}–C^{β}–$C^{\gamma 1}$	110 (3)	112 (3)	107 (2)	
C^{α}–C^{β}–$C^{\gamma 2}$	106 (3)	111 (4)	112 (3)	
$C^{\gamma 1}$–C^{β}–$C^{\gamma 2}$	109 (2)	109 (5)	109 (3)	
C^{α}–C_i–O	111 (3)	120 (2)	111 (3)	120 (2)
C^{α}–C_i–O_i	126 (3)	118 (3)	127 (1)	117 (2)
O_i–C_i–O	122 (3)	122 (4)	122 (4)	122 (2)

[a] Atom names pertain to an amino acid residue;[25] N and O must be interchanged for the ester residues.

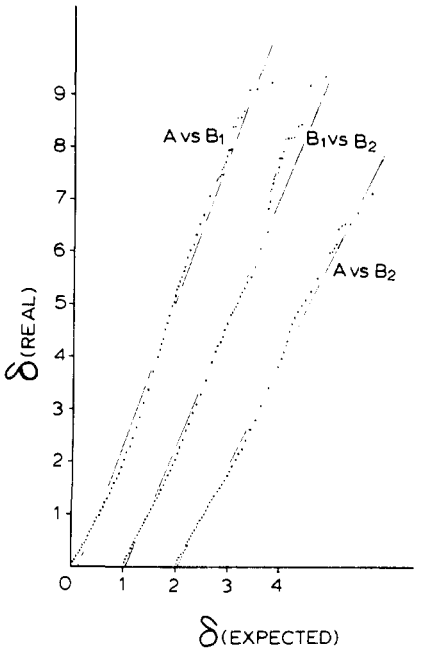

Figure 4. Half-normal probability plots comparing the three independently determined molecules of valinomycin in paris. For clarity, not all points in the lower portions of the plots are included. Two of the plots have been shifted by 1.0 and 2.0 in δ(expected).

Table IV. Torsion Angles[a]

	Modification B1					Modification B2					Modification A				
	ω	ϕ	ψ	χ_1	χ_2	ω	ϕ	ψ	χ_1	χ_2	ω	ϕ	ψ	χ_1	χ_2
L-Val	174	−110	80	176	−68	174	−108	78	179	−61	173	−102	74	180	−58
	174	−67	132	174	−70	180	−66	132	169	−68	174	−64	131	178	−64
	177	−71	130	178	−66	180	−59	129	−178	−62	−179	−63	128	177	−62
D-Hylv	173	147	−6	(69, 166)[b]	−58	179	147	−10	(62, 162)[b]	−61	174	145	−8	63	−59
	179	81	3	74	−50	179	78	7	68	−54	180	80	2	66	−47
	177	98	−7	166	−74	176	94	−5	164	−71	174	98	−4	162	−69
D-Val	173	54	−133	63	−178	171	64	−134	68	−174	169	65	−135	72	−173
	−171	105	−68	58	−178	−172	108	−68	64	−175	−170	106	−71	63	−178
	179	67	−136	61	(−71, −174)[b]	−177	67	−136	57	(−84, 178)[b]	−178	65	−134	64	177
L-Lac	−171	−100	13			−172	−97	7			−173	−98	10		
	−176	−165	31			−173	−166	22			−174	−160	21		
	179	−71	−11			177	−71	−9			180	−77	−7		

[a] The convention of defining and naming the torsional angles is that of Kendrew et al.[28] [b] Disordered isopropyl groups; the torsional angles for two atoms with occupation factors of 0.5 are included together in parentheses.

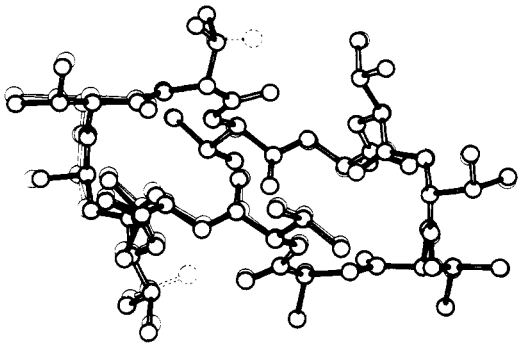

Figure 5. Comparison of the differences in the overall structure of molecules A (heavy line) and B1 (light line).

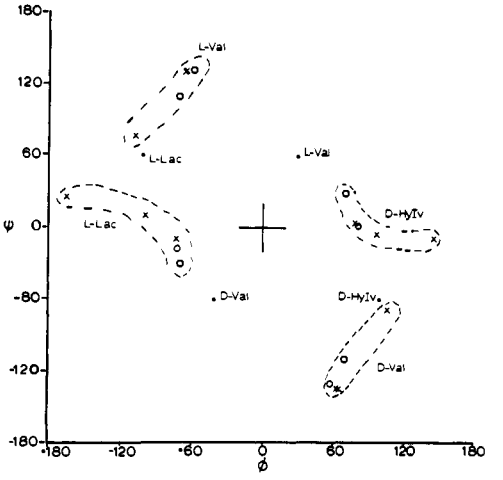

Figure 6. ϕ-ψ plot for valinomycin: (X) torsion angles observed in this study, (O) observed torsion angles for the crystalline potassium complex,[14] (□) calculated angles from NMR data for the potassium complex of valinomycin in methanol,[4] and (●) calculated angles for uncomplexed valinomycin in a dioxane-water solvent system.[4]

Table IV. As expected, all peptide and ester linkages are trans. The average value of the torsion angle ω was calculated to be 179.4°; the largest deviation of this angle from 180° was 11°. When ω, ϕ, ψ and χ values[28] for corresponding residues in the three conformers are compared, the ranges in observed values of individual torsion angles vary from 1 to 12°. Closer inspection of the eight angles for which these ranges in observed values are 8° or more reveals that in all cases one observation is significantly different from the average of the other two and seven of these outliers are observed in molecule B1. These eight torsion angles are underlined in Table IV. An indication of the significance of the deviations of these torsion angles in molecule B1 is provided by the ratio of $\Delta_{B1\text{-}AVER}$ to $\Delta_{B2\text{-}A}$ ($\Delta_{B1\text{-}AVER}$ is the difference between the B1 torsion angle and the average of the B2 and A angle; $\Delta_{B2\text{-}A}$ is the difference between the B2 and A torsion angle): 8/3, 10/4, 7/2, 10/1, 7/2, 8/1, and 10/1. The deviation in these values between molecules A and B2 (denominator) is clearly minor compared to the discrepancies between molecule B1 and the average of A and B2. Figure 5 illustrates the differences in the overall structure of molecules A and B1.

Examination of Table IV shows that, on the basis of the ϕ and ψ torsion angles, the hydroxy acid residues fall into three distinct conformational groups while the amino acid residues have only two conformational groups in each of the three molecules. Average values of the ϕ and ψ torsion angles for each of these groups are plotted on a conformational map in Figure 6 along with the average value of each torsion angle from each type of residue observed in the crystalline potassium ion complex;[14] calculated values based upon solution spectra for both the uncomplexed and potassium ion complex are also included.[3,4,29] As can be seen from Figure 6, two of the points for each amino acid residue are quite close to that observed in the crystalline potassium ion complex and are consistent with the values one would expect for a β turn (1–4 hydrogen bonding). The remaining point for each amino acid residue corresponds to the conformation obtained in a 13-membered, hydrogen-bonded ring (1–5 hydrogen bonding). Thus, the C_{i-1}, C_i and the N_i, O_{i+1} atoms of the amino acid residues are either both syn clinal[30] or both anti clinal. Only one residue of each type of hydroxy acid has the same conformation in the uncomplexed form as is observed in the potassium ion complex of valinomycin. Both hydroxy acid residues are observed to have the C_{i-1}, C_i atoms in syn clinal or anti clinal conformations with the exception of one L-Lac residue which is anti perpendicular. The O_i, N_{i+1} atoms have a syn perpendicular conformation in both hydroxy acid residues.

The χ torsion angles which describe the disposition of the side chains are also given in Table IV. These side chains are all in a nearly staggered conformation. The C^α–C^β protons of the amino acid residues are trans while the D-Hylv protons are gauche.

Six intramolecular hydrogen bonds are observed in all three molecules of valinomycin. Four of these hydrogen bonds are from amide groups to neighboring carbonyl oxygens forming four "β turns" (1–4 hydrogen bond). Nitrogen-oxygen distances range from 2.81 to 3.10 Å with an av-

Table V. Hydrogen Bonded Distances and Angles

	N···O			H···O			N–H···O angle		
	B1	B2	A	B1	B2	A	B1	B2	A
N_1–H···O_{30A}	2.81	2.82	2.83	1.86	1.87	1.90	144	145	142
N_7–H···O_{33A}[a]	3.07	3.18	3.11	2.38	2.41	2.33	120	128	127
N_{13}–H···O_{6A}	2.96	2.98	2.98	1.93	1.95	1.97	157	158	155
N_{19}–H···O_{12A}	2.88	2.87	2.85	1.95	1.92	1.89	142	145	146
N_{25}–H···O_{15A}[a]	3.04	2.98	3.06	2.15	2.17	2.24	138	129	132
N_{31}–H···O_{24A}	3.10	3.06	3.06	2.03	2.01	2.03	170	161	159

[a] Non-β hydrogen bond.

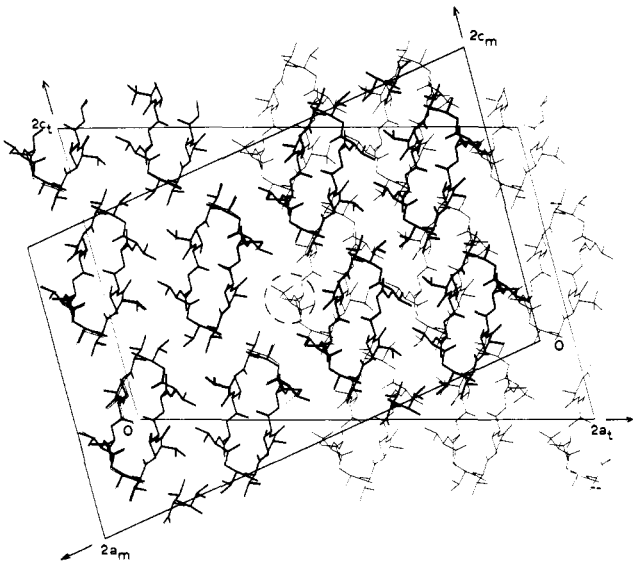

Figure 7. Comparison of the packing of valinomycin in the monoclinic (heavy lines) and triclinic (light lines) modifications.

erage value of 2.93 Å. Based upon the positions of the theoretical hydrogen atoms, the average O···H distance is 1.9 Å while the average N–H···O angle was calculated to be 152°. These distances and angles are listed in Table V.

Each of the two remaining hydrogen bonds produces a previously undescribed[21] 13-membered ring (1–5 hydrogen bond) which distorts the doughnut shaped molecule into an oval; in this case each hydrogen bond acceptor is a carbonyl oxygen belonging to an amino acid residue. These hydrogen bonds are drawn as dotted lines in Figure 3 and are labeled 1 and 2. The nitrogen atoms are numbered 7 and 25; the carbonyl oxygen atoms, labeled R in Figure 3, are bonded to carbon atoms 33 and 15. The average N···O distance was calculated to be 3.07 Å, with values ranging from 2.98 to 3.18 Å. The O···H distances, based upon the theoretical hydrogen atom positions, are considerably longer than those found in the "β turns". These distances ranged from 2.2 to 2.4 with an average value of 2.3 Å. The average N–H···O angle was calculated to be 129°. However, values such as these are not unique in the literature (a neutron diffraction study has reported angles at hydrogen as small as 119° and H···O distances as long as 2.38 Å in $(NH_4)_2SO_4$)[31] and they are still less than the calculated van der Waals contact distance of 2.6 Å.[32] Thus this latter type of hydrogen bond is not as strong as those involved in the "β turns". Solid state Raman spectra support this hydrogen bonding pattern.[6]

The molecular packing in the two crystal forms is compared in Figure 7. There are nearly identical "bilayers" of valinomycin molecules extending parallel to the *bc plane* in both crystal forms. These layers are seen overlapped on the left side of Figure 7 but fail to overlap on the right side of this figure due to differences in the molecular packing in the two crystalline forms. This difference in packing is primarily a result of the solvent which is incorporated into the monoclinic structure. The projection of the cylindrical cavity containing the disordered solvent is illustrated in Figure 7 by the dashed circle. Solvent is excluded in the triclinic structure and thus a more efficient packing of the valinomycin molecules is achieved. The disordered γ-carbon atoms of the triclinic structure are indicated by dotted lines in Figure 7.

Discussion

A pseudocenter of symmetry is present in each valinomycin molecule. L-Val residues are transformed into the D-Val residues by the operation of this pseudosymmetry element; however, an equivalance between the D-HyIv and L-Lac residues is not maintained because of the dissimilar side chains. This pseudosymmetry element is clearly illustrated by noting that a sign change of the ϕ and ψ torsion angles of Table IV transforms one residue into its centrically related one.

All three half-normal probability plots show systematic deviations from linearity. A minor break in the points, fitted to a straight line, is observed at a δ(expected) value of about 1.1 for the two comparisons involving molecule B2. The third plot which compares modification A with B1 shows a definite sinusoidal variation of the points and has a greater deviation from linearity than do the other two plots. This indicates that there are minor differences in the conformations of the three molecules and that molecules A and B2 have the most in common. This finding is consistent with the observation that in seven of the eight torsion angles having the widest range of variability (Table IV), the magnitudes in molecule B1 are significantly at variance with the averages of the corresponding magnitudes in molecules A and B2. It was earlier noted that the final 22 points on one of the half-normal probability plots (B1 and B2) deviated considerably from the remaining points. The three atoms involved in all 22 of these distances are found in the first D-Val residue, the second L-Lac residue, and the third L-Val residue. These are the residues in which the greatest differences in the torsion angles are found.

The structure of valinomycin, obtained from both of these crystal structure determinations, is quite different from that found for the potassium ion complex as well as that which has been proposed on the basis of solution spectra. Nearly all interpretations of the NMR spectra of valinomycin, dissolved in a variety of solvent systems, have required the presence of threefold symmetry in the molecule which is not observed in the crystalline state. Peptide folding, producing a 1–5 hydrogen bond, is observed between residues L-Val (9) and L-Val (5) as well as between D-Val (3) and D-Val (11). By permuting these hydrogen bonds around the peptide backbone, these 1–5 interactions would be found in the following residues: L-Val (1), L-Val (9) and D-Val (7), D-Val (3); L-Val (5), L-Val (1) and D-Val (11), D-Val (7). This permutation gives rise to three identical conformers and maintains the pseudocenter of symmetry

observed in the crystal structure. The breaking of a 1-5 hydrogen bond should be rather easy since these are the hydrogen bonds that are the weakest, while the subsequent formation of a 1-4 hydrogen bond requires torsion angle changes of less than 90° in only two residues, neither of which are involved in the donating or accepting of a proton. The net effect of these changes is primarily a redirection of the H-N bond towards the carbonyl oxygen atom since this N-O distance in the 1-5 turn is approximately 3 Å. The breaking of a 1-4 hydrogen bond and subsequent formation of a 1-5 bond would be the reverse of the above. If this interconversion is fast on the NMR time scale, threefold symmetry would in fact be observed. Ultrasonic absorption spectra have shown that even in nonpolar solvents, at least three conformers must be present and the rate constants of the slowest conformational transitions are estimated at 10^7 sec^{-1}.[15]

It should be carefully noted that the gross conformational features of the valinomycin molecule (the peptide folding and hydrogen bonding) are nearly identical in the three observations despite variations in crystallization conditions, crystal packing, the presence of minor disorder in isopropyl groups, and the presence of occluded solvent in the monoclinic lattice. The absence of intermolecular hydrogen bonding is particularly significant. In the absence of strong packing forces and in view of the disorder in the most exposed isopropyl groups, the three perceptibly different conformers can be interpreted as lying in local minima in the potential energy surface. The differences amount to small concerted shifts in the peptide conformations such as those evidenced in molecule B1. The observed conformations certainly approximate the minimum energy form of an isolated molecule. Although the presence of this conformation in solution has not been detected, at least a small amount must be present in the polar as well as nonpolar solutions from which the crystals were obtained. In view of the very weak packing forces, it is unlikely that the crystallization process independently promotes peptide folding. However, because of the competition for hydrogen bonding, this is certainly likely to be a less populated form in polar solvents than in nonpolar environments such as lipid membranes.

The conformation observed in the solid state does suggest a mechanism by which valinomycin may coordinate potassium ion.[21] Four free amino carbonyls (P and M, P′ and M′, Figure 3) are in exposed positions at the surface of the molecule. One of these pairs of carbonyls may initiate complexing with potassium; once this loose complex is formed, hydrogen bonds 1 and 2 (Figure 3) may be disrupted. These are the hydrogen bonds which form the 13-membered rings and on the basis of distances and angles are considered to be the weakest. Once these hydrogen bonds are broken, these amino carbonyls are free to coordinate to the potassium. As the potassium ion is brought into full coordination, the molecule rounds out and the appropriate free carbonyl oxygens (Q and Q′) are brought into position to hydrogen bond to the two free amide protons, giving rise to the observed structure of the potassium complex. For the most part, this mechanism requires minor displacements of the atoms involved and is consistent with the observation that, when a cation is complexed at a membrane-water interface, a conformational change occurs that decreases the surface area by approximately 25-30 Å^2.[33]

In view of the stability of the conformation, its independence of crystal packing forces, and the plausibility of this simple mechanism of complex formation, the observed conformation is proposed as an intermediate in the complexing process. While cation release could proceed by a reversal of the complexing process at a membrane surface of different electronic balance, it is equally possible that the complexed molecule reaches a region where there is competition for the β-turn hydrogen bonds and the molecule unfolds releasing the ion.

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Supplementary Material Available. A complete listing of bond distances, bond angles, positional and thermal parameters, and structure factor amplitudes for both determinations will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JACS-75-7242.

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