Solvent-Dependent Conformations of Valinomycin in Solution†

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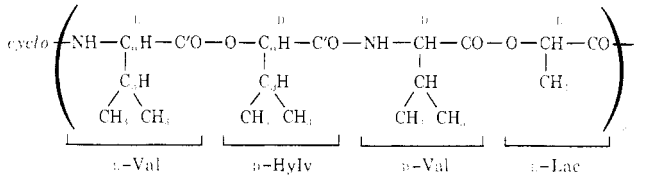
ABSTRACT: Proton nuclear magnetic resonance (nmr), circular dichroism (CD), and conformational calculations were combined to investigate valinomycin conformational changes with solvent polarity. The proton nmr studies elucidated those peptide residues participating in type 1–4 intramolecular hydrogen bonds and gave an estimate of the peptide dihedral angle φ from the $J_{H^N H^\alpha}$ proton–proton coupling constant. The change of valinomycin H^α proton chemical shifts as the solvent was varied from hydrocarbon to dioxane to water permitted the identification of those sections of the depsipeptide involved in conformational changes. The solvent-dependent valinomycin CD $n-\pi^*$ band confirmed a conformational change from hydrocarbon to aqueous media. The nmr data were used to initiate conformational calculations in a search for low-energy structures consistent with the magnetic resonance information in these solvents. Comparison of the nmr spectral parameters in the presence and absence of potassium ion in the solvents dimethylformamide and methanol allowed the derivation of valinomycin conformations in these solvents using the known conformation of the complex derived previously from nmr and X-ray investigations as a reference. The conformations derived from the theoretical calculations in this manuscript for valinomycin in hydrocarbon, dioxane, dimethylformamide, methanol, and water are defined in terms of the backbone rotation angles φ, ψ, and ω. Corey–Pauling–Koltun models of these structures are presented.

Valinomycin is a cyclic 12-membered depsipeptide composed of alternating amino acid ($NC^\alpha(C^\beta)C'$) and ester ($OC^\alpha(C^\beta)C'$) residues with both L and D configuration.



Following an earlier report on valinomycin and its metal ion complexes in solution (Haynes *et al.*, 1969), a conformation for the K^+ complex of valinomycin was reported by Ivanov *et al.* (1969) based on solution optical rotatory dispersion (ORD), nuclear magnetic resonance (nmr), and infrared (ir) spectral information. The conformational conclusions of the latter study were verified and extended further using additional nmr investigations (Ohnishi and Urry, 1969; Urry and Ohnishi, 1970) and energy calculations (Ramachandran and Chandrasekaran, 1970; Mayers and Urry, 1972). An independent X-ray crystallographic investigation (Pinkerton *et al.*, 1969) of the complex yielded the crystal structure, there being good agreement with the proposed conformation in solution (Ivanov *et al.*, 1969; Urry and Ohnishi, 1970).

Ivanov *et al.* (1969) used spectroscopic methods to elucidate the solvent-dependent conformations of valinomycin in solution. These early qualitative attempts suggested that all peptide nitrogen protons were intramolecularly hydrogen bonded in hydrocarbon media, but became selectively exposed with increasing solvent polarity. More recent studies by these workers (Ivanov *et al.*, 1971) coupled the experimental data with theoretical calculations to derive conformations for valinomycin in solution in terms of the backbone rotation angles. Urry and Ohnishi (1970) combined spectroscopic studies with model building to derive an all intramolecularly hydrogen-bonded "pore" conformation for valinomycin in solution. They further proposed that the conformation for valinomycin in dimethyl sulfoxide was stabilized by three intramolecular hydrogen bonds (Ohnishi and Urry, 1969). The crystal structure of uncomplexed valinomycin reported by Duax *et al.* (1972) does not possess the threefold symmetry exhibited by the chemical structure of valinomycin. The conformation is stabilized by four type 1–4 and two 13-membered ring intramolecular hydrogen bonds. It differs significantly from all proposed models for the solvent-dependent conformations of valinomycin in solution.

Since the conformation of valinomycin in nonpolar, hydrogen-bond acceptor, and polar media may influence its capacity for binding and release of metal ions, a systematic and detailed investigation of the solvent-dependent conformations of this depsipeptide was initiated. The coupling of nmr parameters with conformational calculations (Bovey *et al.*, 1972) were used to define the solvent-dependent conformations of valinomycin in solution and the conformations so derived are defined explicitly in terms of the rotation angles φ, ψ, and ω.

Experimental Section

Valinomycin was purchased in crystalline form from Calbiochem and was used without further purification.

Nuclear magnetic resonance spectra were run on a HA-100 Varian spectrometer equipped with a variable-temperature unit. Sample concentrations were in the 10–20-mg/ml range. Chemical shifts are referenced relative to internal tetramethylsilane.

CD studies were carried out at room temperature on a Cary 61 instrument. Sample concentrations were 2.7 mg/ml.

All capacitance measurements were performed in a dielectric constant cell Model NFL1/ms obtained from Kahl Scientific Co. placed in a General Radio Corp. Model 1620-A capacitance measuring assembly. The apparatus was checked by measuring the known dipole moment of *p*-chlorotoluene dissolved in benzene. Measurements of the capacitance of benzene solutions of valinomycin (C = 0.0433, 0.0289, 0.0217, and 0.0173 g per ml) were conducted at 25°. Extraction of the

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dipole moment of valinomycin from the capacitance measurements performed in benzene solutions was accomplished according to the procedure outlined by Smyth (1955).

Results and Discussion

Solution studies (Haynes *et al.*, 1969) indicate that valinomycin and its K^+ complex exhibit threefold symmetry, thereby limiting the investigation to the unit (-L-Lac-L-Val-D-HyIv-D-Val).

I. Spectral Studies

The proton chemical shifts and coupling constants of the ester residues L-Lac and D-HyIv can be readily identified and assigned (Haynes *et al.*, 1969; Ivanov *et al.*, 1969; Ohnishi and Urry, 1969). An essential part of our study was to distinguish the amino acid residues L-Val and D-Val from each other and assign their chemical shifts and coupling constants. Ovchinnikov *et al.* (1971) incorporated ^{15}N L-Val into valinomycin, thus differentiating the H^N resonances of D- and L-Val (L-Val exhibits a ^{15}N–^1H coupling) and permitting rigorous assignments in carbon tetrachloride and dimethyl sulfoxide. In our study, the changes in H^N chemical shifts were followed on addition of Me_2SO to valinomycin in dioxane, dimethylformamide, and methanol. Since the assignments in Me_2SO were established (Ovchinnikov *et al.*, 1971) the D- and L-Val valinomycin H^N resonances in these solvents could also be determined. These resonances were assigned in dioxane–octane and dioxane–water from studies of the gradual addition of octane and water respectively to valinomycin in dioxane. Addition of KSCN to valinomycin in dimethylformamide permitted assignment of the valyl residues in the complex. The H^N and H^α chemical shifts were related to each other by spin decoupling experiments.

Table I summarizes the proton nmr spectral parameters (δ_{H^N} and δ_{H^α} proton chemical shifts in parts per million, $J_{H^NH^\alpha}$ and $J_{H^\alpha H^\beta}$ proton–proton coupling constants in hertz and the temperature coefficients of δ_{H^N} in parts per million per degree centigrade) and Figure 1 outlines the temperature dependence of δ_{H^N} for D- and L-Val in valinomycin in dioxane–octane (1:13), dioxane and dioxane–water (4:1). The depsipeptide was also investigated in tetrahydrofuran, a solvent similar to dioxane, over a larger temperature range. The proton nmr spectral parameters at +28 and −28° are summarized in Table I and the temperature dependence of δ_{H^N} is outlined in Figure 2. The n–π^* band at ~215 nm for valinomycin in solution exhibits variation with solvent polarity (Figure 3). The molar ellipticities in dioxane–octane (1:10), dioxane, and dioxane–water (4:1) are $+3.1 \times 10^4$, $+1.0 \times 10^4$ and -1.05×10^4 (deg cm²)/dmol, respectively.

Table I summarizes the proton nmr spectral parameters for valinomycin and its potassium complex in dimethylformamide while Figures 4 and 5 outline the temperature dependence of δ_{H^N} and δ_{H^α} for valinomycin residues in this solvent.

II. Structural Constraints

This section analyzes the above experimental data and suggests an approach to the search for low-energy conformations.

The nonpolar environment is represented by the solvent system dioxane–octane (1:10) while dioxane–water (4:1) represents an aqueous environment. (Further addition of water to valinomycin in dioxane–water, 4:1, had no effect on the spectral parameters.)

The three nmr spectral parameters reflecting important

TABLE I: Spectral Parameters (Chemical Shifts, Coupling Constants and Temperature Coefficients) for Valinomycin in Dioxane–Octane, Dioxane, Dioxane–Water, H_4furan, and Dimethylformamide.

	D-Val					L-Val				L-Lac			D-HyIv	
	δ_{H^N} (ppm)	$J_{H^NH^\alpha}$ (Hz)	Temp Coef[a] (ppm/°C)	δ_{H^α} (ppm)	$J_{H^\alpha H^\beta}$ (Hz)	δ_{H^N} (ppm)	$J_{H^NH^\alpha}$ (Hz)	Temp Coef[a] (ppm/°C)	δ_{H^α} (ppm)	$J_{H^\alpha H^\beta}$ (Hz)	δ_{H^α} (ppm)	$J_{H^\alpha H^\beta}$ (Hz)	δ_{H^α} (ppm)	$J_{H^\alpha H^\beta}$ (Hz)
Dioxane–octane (1:13), +28°	8.00	7.6	0.0029	*4.04*	11.0	7.87	6.1	0.0031	*3.92*	10.5	5.28	6.5	5.02	3.0
Dioxane, +28°	7.64	8.4	0.0012	*4.27*	8.0	7.60	6.4	0.0028	*4.08*	9.5	5.23	7.0	4.97	3.2
Dioxane–water (4:1), +28°	7.90	8.0	0.0070	4.26	8.5	8.05	7.5	0.0080	4.28	8.0	5.27	6.7	4.96	4.0
H_4furan, +28°	7.70	8.5	−0.001	*4.28*	9.5	7.86	6.5	0.004	4.06	10.0	*5.33*	6.7	5.00	3.5
H_4furan, −28°	7.58	9.5	−0.002	*4.79*	5.0	8.36	7.0	0.010	4.11	10.0	*5.59*	6.5	5.01	3.0
Dimethylformamide, +84°	7.62	8.0	0.0039	4.32		7.86	7.7	*0.0104*	4.37		5.25		4.97	
Dimethylformamide, −34°	8.24	~8.0	0.0106	4.46		8.90	~7.5	*0.0047*	4.39		5.21		4.89	
Dimethylformamide, +28°	7.85	8.5		*4.32*	~8.0	8.45	7.5		*4.44*	6.5	5.26	6.8	4.93	4.0
K complex, dimethylformamide, 28°	8.37	5.5	0.0047	*4.05*	3.2	8.46	5.5	0.0043	*3.95*	3.0	5.13	7.0	4.80	4.0

[a] Temperature coefficients of *N*-methylacetamide in dioxane–octane, dioxane, dioxane–water, H_4furan, and dimethylformamide are 0.0066, 0.0099, 0.0093, 0.0106, and 0.0062 ppm per °C, respectively.

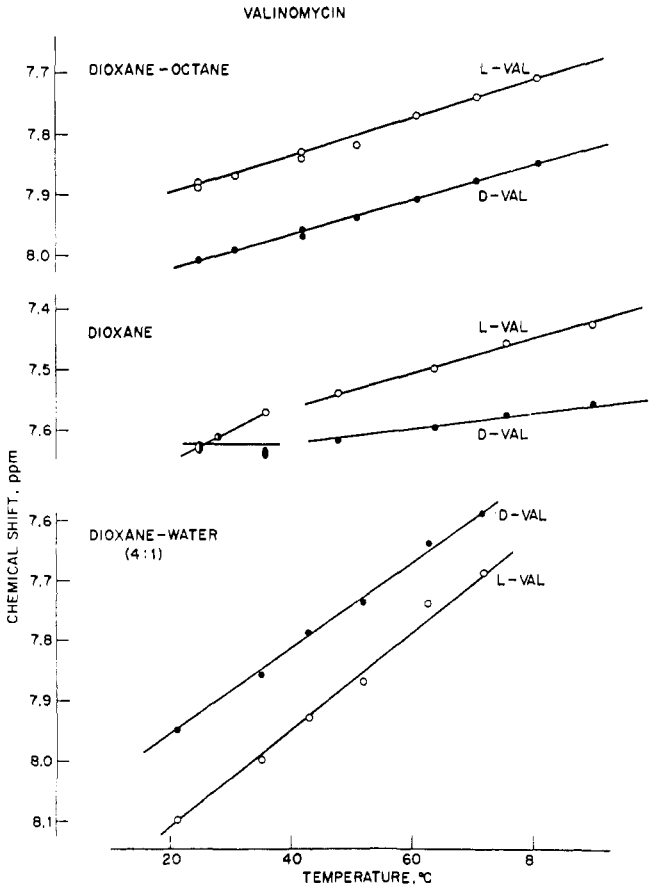


FIGURE 1: Temperature dependence of the D- and L-Val peptide N protons of valinomycin in dioxane–octane (1:13), dioxane, and dioxane–water (4:1).

aspects of the conformations of the depsipeptide are $J_{H^NH^\alpha}$, temperature coefficients of δ_{H^N} and the chemical shift δ_{H^α}. The coupling $J_{H^NH^\alpha}$ can be related to the dihedral angle φ (Barfield and Karplus, 1969). Temperature coefficients are a measure of the accessibility of H^N to solvent (Kopple *et al.*, 1969a,b). The temperature coefficient of *N*-methylacetamide in the solvent system of interest is used as a standard. Peptide

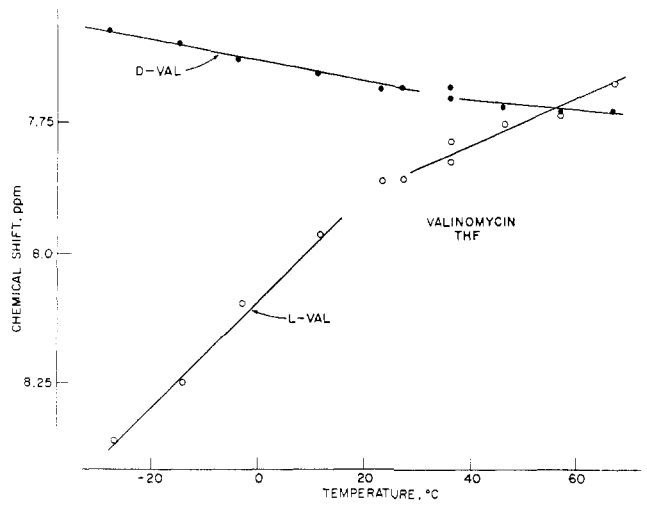


FIGURE 2: Temperature dependence of the D- and L-Val peptide N protons of valinomycin in H_4 furan.

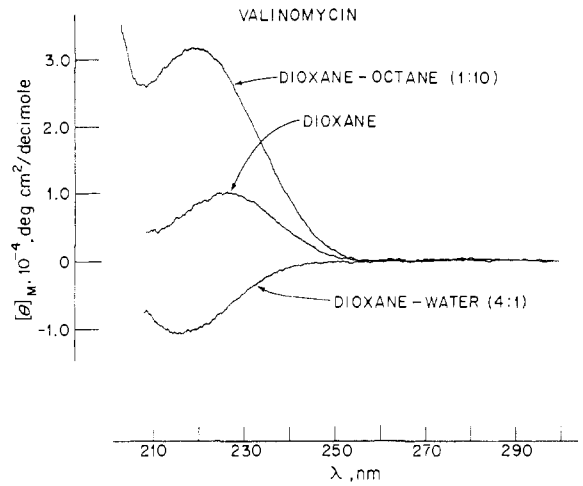
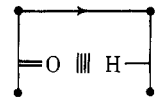


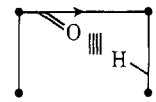
FIGURE 3: The CD spectrum of valinomycin in dioxane–octane (1:10), dioxane, and dioxane–water (4:1).

N proton slopes comparable to $CH_3CONHCH_3$ are designated solvent exposed while those showing reduced slopes indicate solvent shielded and/or intramolecularly hydrogen-bonded protons. The parameters mentioned above need interpretation with caution if minor conformation(s) in addition to the predominant one are in fast exchange in the solvent system of choice. While both δ_{H^N} and δ_{H^α} are a function of conformation and solvent, the former chemical shift is very sensitive to solvent and is a poor indicator of conformational variations.

For the tetrapeptide sequence below, the C^α carbons are represented by dark circles and the peptide backbone by lines. The type 1–4 intramolecular hydrogen bond has been observed



type 1–4
intramolecular hydrogen bond



type 1–3
intramolecular hydrogen bond

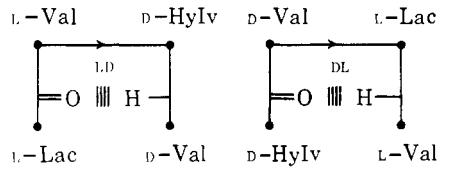
in synthetic (Kopple *et al.*, 1969a,b; Schwyzer and Ludescher, 1969; Torchia *et al.*, 1972) and biologically active cyclic peptides (Stern *et al.*, 1968; Ivanov *et al.*, 1969; Ohnishi and Urry, 1969; Llinas *et al.*, 1970). Their nmr characteristics are slow exchange with water and a decreased temperature coefficient for the hydrogen-bonded peptide N proton relative to *N*-methylacetamide. The C=O||H—N groups in the hydrogen bond are linear and nearly coplanar indicating a relatively strong hydrogen bond. The type 1–3 intramolecular hydrogen bond has been observed in protected dipeptides (Bystrov *et al.*, 1969). Though this intramolecular hydrogen bond can be characterized by its ir spectrum and slow nmr exchange with water, its temperature coefficient is the same as *N*-methylacetamide (Brewster and Bovey, 1972).[1] In the relatively weak 1–3 intramolecular hydrogen bond, the C=O and

[1] Unpublished results.

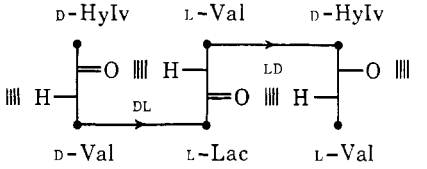
H—N groups are considerably nonlinear and nonplanar (Tonelli, 1971a,b).

Valinomycin in dioxane–octane (1:13) exhibits temperature coefficients of 0.0029 and 0.0031 ppm per °C for D- and L-Val NH resonances, respectively (Table I and Figure 1), compared to 0.0066 ppm/°C observed for *N*-methylacetamide in this solvent. The decreased temperature coefficients suggest that all the valinomycin N protons are either solvent shielded and/or hydrogen bonded in the predominant conformation in this solvent. The NH stretch region in the infrared spectrum of valinomycin in nonpolar CCl_4 was interpreted by Ivanov *et al.* (1969) as being consistent with an all intramolecularly hydrogen-bonded conformation.

The direction of a polypeptide chain can be reversed by forming two types of bends as derived from theoretical calculations (Venkatachalam, 1968; Ramachandran and Chandrasekaran, 1970). Indeed, for valinomycin–K^+, the type II bend is predicted from Venkatachalam's proposals and has been observed in solution (Ivanov *et al.*, 1969; Ohnishi and Urry, 1969) and in the crystal (Pinkerton *et al.*, 1969). Consider bends shown below involving the D-Val N proton in a type 1–4 intramolecular hydrogen bond (designated LD) and the L-Val N proton in a type 1–4 intramolecular hydrogen



bond (designated DL). Bend LD is completely defined by rotation angles (φ,ψ,ω) for L-Val and D-HyIv while bend DL is defined by the corresponding rotations angles for D-Val and L-Lac. The valyl residues of valinomycin in dioxane–octane exhibit $J_{H^NH^\alpha}$ in the range 6.0–7.5 Hz (Table I). The approach taken in this study is to generate bends LD and DL by simply considering those residue conformations that are consistent with the coupling constant observed experimentally (Table I) and have low conformational energies according to the appropriate potential energy estimates (Tonelli *et al.*, 1973). The conformation (structure I) is defined by this procedure provided a cyclic structure can be generated:



I

Valinomycin in dioxane exhibits temperature coefficients of 0.0012 and 0.0028 ppm per °C between 30 and 90° for D- and L-Val residues, respectively, compared to a value of 0.0099 ppm/°C for *N*-methylacetamide in the same solvent (Table I and Figure 1). The data between 20 and 30° do not lie on the slopes of those between 30 and 90° (Figure 1). The lower freezing point of H_4furan (−60°) permitted an investigation over a greater temperature range in this solvent. At low temperatures (+10 to −30°), the temperature coefficients of valinomycin D- and L-Val residues are −0.002 and 0.010 ppm per °C compared to 0.0106 ppm/°C for *N*-methylacetamide in H_4furan (Figure 2 and Table I). These data strongly suggest a predominant valinomycin conformation in H_4furan

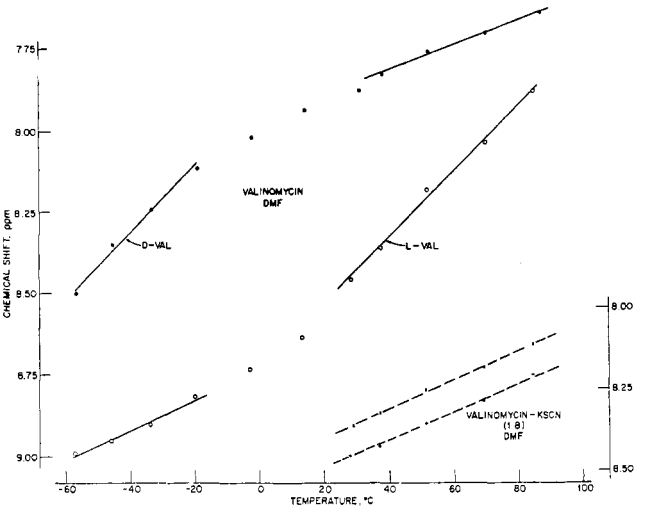
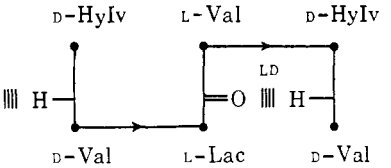


FIGURE 4: The temperature dependence of the D- and L-Val N proton chemical shifts for valinomycin and its K complex in dimethylformamide.

at low temperatures consisting of intramolecularly (type 1–4) hydrogen-bonded D-Val N protons only (II-1). Thus, valinomycin in dioxane or H_4furan exists in an equilibrium between structures I and II-1 with the latter predominant at lower temperatures.



II-1

Bend LD in I and II-1 should be structurally similar since $J_{H^NH^\alpha}$ for L-Val is 6.0–6.5 Hz in dioxane–octane, dioxane, and H_4furan (Tables I and II). Since structures I and II-1 both contain an LD bend, the rotation angles at L-Val and D-HyIv should remain essentially unchanged and this should be reflected in their H^α chemical shifts. On the other hand,

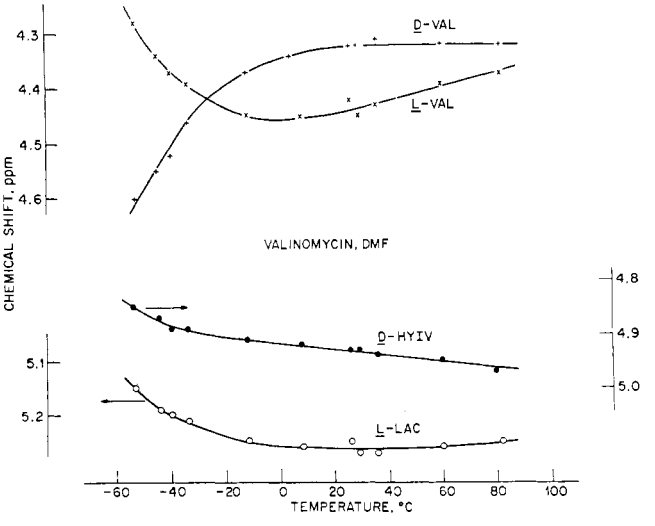


FIGURE 5: The temperature dependence of the H^α chemical shifts for valinomycin in dimethylformamide.

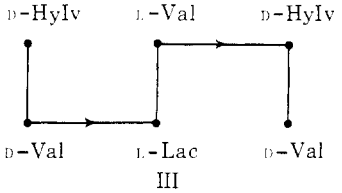
TABLE II: Rotation Angles (φ,ψ), According to the Convention of Edsall *et al.* (1966) for the Solvent-Dependent Valinomycin and Valinomycin-K Conformations Determined in This Investigation.

Conformation, Solvent System	Rotation Angles (φ,ψ)[a]				Calcd[b] Dipole Moments
	L-Val	D-HyIv	D-Val	L-Lac	$(\mu^2)^{1/2}$ (D)
I, hydrocarbon (dioxane–octane)	210, 240	280, 120	140, 120	80, 240	3.9
II-1, cyclic ether (dioxane or H_4-furan), dimethylformamide, <0°	210, 270	280, 120	320, 280	80, 270	2.9
II-2, dimethylformamide, >0°	40, 80	280, 120	140, 120	80, 270	6.3
III-1, aqueous (dioxane–water), methanol, >30°	30–40, 250–320	260–270, 100–120	320–330, 40–110	80–90, 240–260	1.4–5.8
III-2, aqueous (dioxane–water), methanol, >30°	90, 290–310	250–260, 0–10	270, 50–70	110–130, 350–360	0.0–1.9
C-I, methanol	110, 290	250, 210	250, 70	110, 150	6.6
C-II, dimethylformamide	20, 340	290, 130	340, 20	70, 230	2.6

[a] All peptide and ester bonds are trans. The rotation angles are defined according to Edsall *et al.* (1966a–c). [b] Calculated according to the method of Flory (Flory and Schimmel, 1967; Brant *et al.*, 1969).

Since a DL bend is broken, the D-Val and L-Lac residues in structure II-1 can adopt new conformations. Indeed, the D-Val and L-Lac H^α resonances in H_4furan shift 0.5 and 0.26 ppm downfield, respectively (the other two H^α resonances being unaffected), on lowering the temperature from +28° to −28° (Table II). Thus, the procedure adopted is to search for cyclic structures of low conformational energy in which the L-Val and D-HyIv residues exhibit the rotation angles of the LD bend while those of D-Val (consistent with $J_{H^NH^\alpha}$ = 9.5 Hz) and L-Lac are varied.

The temperature coefficients for valinomycin in dioxane–water (4:1) are 0.0070 and 0.0080 ppm per °C for D- and L-Val N protons, respectively, compared to 0.0093 ppm/°C for *N*-methylacetamide in the same solvent (Table I), suggesting that the valinomycin conformation in dioxane–water (4:1) contains no intramolecular (type 1–4) hydrogen bonds (structure III). Comparison of the valinomycin H^α chemical shifts in dioxane–octane (1:13) with those in dioxane–water (4:1) shows that δ_{H^α} for L-Lac and D-HyIv exhibits no change with increasing solvent polarity, while the D-Val and L-Val H^α chemical shifts move downfield by 0.22 and 0.36 ppm, respectively (Table I). Starting with the backbond rotation angles derived for the all type 1–4 hydrogen-bonded conformation (I) the procedure adopted below in the search for cyclic conformations varies the D-Val (consistent with $J_{H^NH^\alpha}$ = 8.0 Hz) and L-Val (consistent with $J_{H^NH^\alpha}$ = 7.5 Hz) rotation angles only and attempts to search for low-energy conformations containing no intramolecular (type 1–4) hydrogen bonds (structure III).



III

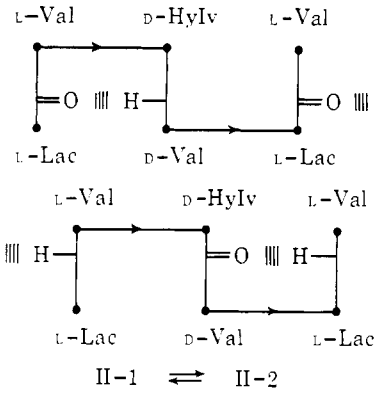
The C^α valyl chemical shifts of valinomycin in dioxane–octane (1:13) at 28° are 4.04 and 3.92 ppm (difference of 0.12 ppm) and in dioxane–water (1:4) at 28° are 4.26 and 4.28 ppm (difference of 0.02 ppm). This suggests that $(\varphi,\psi)_{D\text{-}Val} \simeq (-\varphi,-\psi)_{L\text{-}Val}$ for structure I and structure III-1, the predominant conformations in dioxane–octane and dioxane–water, respectively. Also note that $(J_{H^NH^\alpha})_L \approx (J_{H^NH^\alpha})_D$ in these solvents, consistent with the similarity of $(\varphi,\psi)_{D\text{-}Val}$ and $(-\varphi,-\psi)_{L\text{-}Val}$. The H^α valyl chemical shifts of valinomycin in H_4furan at −28° are 4.79 and 4.11 ppm. The large chemical shift difference of 0.68 ppm suggests that $(\varphi,\psi)_{D\text{-}Val} \neq (-\varphi,-\psi)_{L\text{-}Val}$ for structure II-1, the predominant conformation in H_4furan at low temperatures. [Note that $(J_{H^NH^\alpha})_D \neq (J_{H^NH^\alpha})_L$, consistent with $(\varphi,\psi)_{D\text{-}Val} \neq (-\varphi,-\psi)_{L\text{-}Val}$.]

The conclusions reached from proton nmr studies as solvent polarity is increased from octane to dioxane to water are supported in a qualitative fashion by the CD spectra in these solvents (Figure 3). The 220-nm $n\text{–}\pi^*$ band is assumed to arise from both the amide and ester chromophores, and this large positive molar ellipticity ($\theta = +3.1 \times 10^4$) in hydrocarbon environment changes to a negative rotation ($\theta = -1.05 \times 10^4$) in aqueous media consistent with a large conformational change. No attempt is made to define this conformational change from the CD data.

The conformational characteristics of valinomycin–K^+ in methanol and dimethylformamide (and by analogy Me_2SO) are evaluated below. Compared to a temperature coefficient of 0.0074 ppm/°C for δ_{H^N} of *N*-methylacetamide in methanol, the D- and L-Val peptide H^N resonances of valinomycin–K^+ in methanol ($J_{H^NH^\alpha}$ = 5.2 Hz) exhibit a temperature coefficient of 0.0019 ppm/°C (Ohnishi and Urry, 1969). This decreased temperature coefficient for the complex led Urry and Ohnishi (1970) to suggest a "core" conformation in which all peptide N protons formed type 1–4 intramolecular hydrogen bonds in agreement with the work of others (Ivanov *et al.*, 1969; Pinkerton *et al.*, 1969; Ramachandran and Chandrasekaran, 1970). By contrast, the D- and L-Val peptide N proton resonances of the complex (4 equiv of KSCN) in dimethylformamide ($J_{H^NH^\alpha}$ = 5.5 Hz) exhibit temperature coefficients of 0.0047 and 0.0043 ppm per °C, respectively, relative to 0.0062 ppm/°C for *N*-methylacetamide in dimethylformamide (Table I). The data in dimethylformamide suggest that the complex exhibits either weak intramolecular hydrogen bonds or N protons partially shielded from solvent by the depsipeptide structure. The H^α chemical shifts for valinomycin–KSCN in

methanol and in dimethylformamide show differences for the D- and L-Val, L-Lac, and D-HyIv residues.

The temperature coefficients of the N protons of valinomycin in dimethylformamide are sensitive to the temperature range investigated. At low temperatures the coefficients are 0.0106 and 0.0047 ppm per °C for D- and L-Val residues, respectively, while at high temperatures the coefficients are reversed, namely, 0.0039 and 0.0104 ppm per °C (Table I and Figure 4). The D- and L-Val protons exhibit coupling constants $J_{H^NH^\alpha}$ between 7.5–8.0 Hz over the temperature range investigated (Table I). The temperature coefficient and coupling constant data suggest that the depsipeptide in dimethylformamide exists in conformational equilibrium between structure II-2 containing L-Val type 1–4 intramolecular hydrogen bonds only (predominant at low temperatures) and structure II-1 containing D-Val type 1–4 intramolecular hydrogen bonds only (predominant at high temperatures).



Since average nmr spectra are observed over the entire temperature range, the equilibrium between II-1 and II-2 is fast on the nmr time scale and thus a type 1–4 intramolecularly hydrogen-bonded proton in one conformation would be exposed to solvent in the other, and this is reflected in the temperature coefficient data. Since the H^α chemical shifts of D- and L-Val residues show the greatest temperature dependence (Figure 5) the two conformers differ structurally at these residues while the rotations at the ester residues remain relatively unchanged.

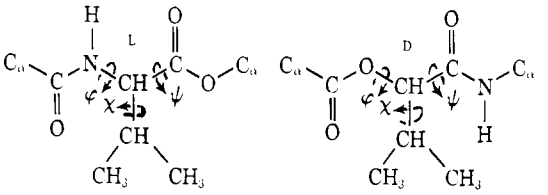
It should be noted that average resonances were observed in the valinomycin proton nmr spectra in the solvent systems studied and this suggests absence of slow exchange between conformations resulting from peptide or ester bond isomerization between cis and trans forms. Since crystallographic analysis of both valinomycin (Duax *et al.*, 1972) and its potassium complex (Pinkerton *et al.*, 1969) exhibited only trans peptide and ester bonds, the same geometry is considered for their solution conformations.

The $J_{H^\alpha H^\beta}$ coupling constants for the valyl residues in valinomycin decrease from 10.5–11.0 Hz to 8.0–8.5 Hz on proceeding from low to high polarity solvents, *i.e.*, from dioxane–octane (1:13) to dioxane–water (1:4). In addition, a further reduction in the $J_{H^\alpha H^\beta}$ coupling constants for the D- and L-Val residues occurs on complexation with K^+ in both methanol and dimethylformamide. According to the Karplus relationship (Karplus, 1959; Pachler, 1964) this suggests a change in the rotamer population about the C^α—C^β bonds in the valyl residues.

III. Generation of Valinomycin Conformations

The potential energy of interaction, $E(\varphi,\psi,\chi)$, of all the atoms in the trans peptide and ester fragments have been calculated (Tonelli *et al.*, 1973) as a function of the rotation angles φ, ψ, and χ (Edsall *et al.*, 1966a–c). Rotation χ about the C^α—C^β bond was restricted to the three staggered positions χ = 60, 180, and 300° (Chart I). Intrinsic torsional potentials, nonbonded van der Waals interactions (6–12 potentials), and electrostatic interactions (monopole–monopole) were considered. Based on the similarity of the L-Ala and L-Val energy maps (Miller and Goebel, 1968), it is assumed that the same ester energy maps can be used to describe the conformational characteristics of the D-HyIv and L-Lac ester residues. This assumption receives further support from a comparison of the D-HyIv and L-Lac ester energy maps calculated by Ivanov *et al.* (1971).

CHART I



These conformational energy maps define the energetically favorable conformations which each of the residues in valinomycin may adopt. The allowed valinomycin conformations are further restricted by requiring each of the amide residues to adopt rotation angles φ about the N—C^α bonds which are consistent with the measured amide to α proton coupling constant according to either of the relationships (Karplus, 1959; Barfield and Karplus, 1969; Bystrov *et al.*, 1969)

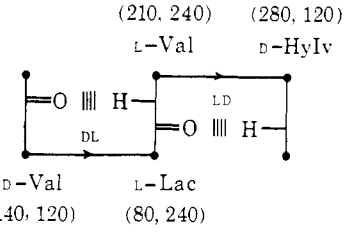
$$J_{H^NH^\alpha} = \begin{bmatrix} 8.5\cos^2\varphi' \ (0° \leq \varphi' \leq 90°) \\ 9.5\cos^2\varphi' \ (90° \leq \varphi' \leq 180°) \end{bmatrix} \quad (1)$$

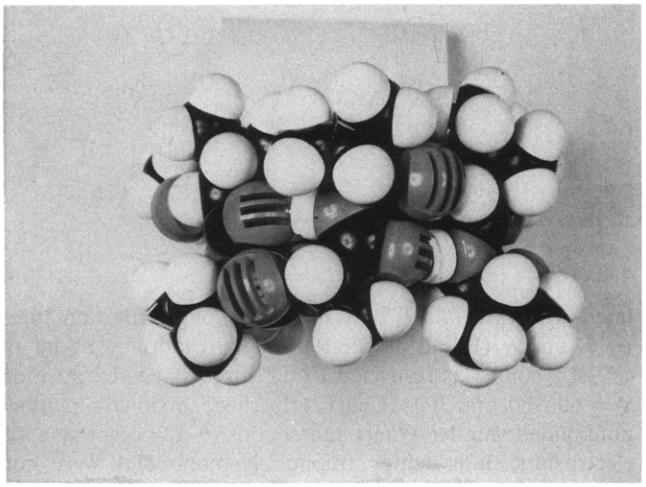
$$J_{H^NH^\alpha} = 8.9\cos^2\varphi' - 0.9\cos\varphi' + 0.9\sin^2\varphi' \quad (2)$$

where the dihedral angle φ' between H—N and C^α—H^α is directly related to the rotation angle φ. The conformations generated must also be consistent with the nmr derived nature of the exchangeable peptide protons (intramolecularly hydrogen bonded, solvent shielded, or exposed to solvent).

The selection of parameters and the mathematical methods utilized to search for cyclic conformations have been presented in earlier studies (see Tonelli *et al.*, 1971, and Tonelli, 1971a,b, 1972).

As outlined in section II, a search was undertaken to define bends, designated LD and DL, involving type 1–4 intramolecular hydrogen bonds with $J_{H^NH^\alpha}$ in the range 6–7.5 Hz. The strengths of the hydrogen bonds were evaluated following the method of Brant (1968). For the case of interest, *i.e.*, $J_{H^NH^\alpha}$ = 6.1 Hz (L-Val) and = 7.6 Hz (D-Val) for valinomycin in dioxane–octane, the bends are defined by the φ,ψ rotation angles summarized below. Note that $(\varphi,\psi)_{D\text{-}Val} \simeq (-\varphi,$





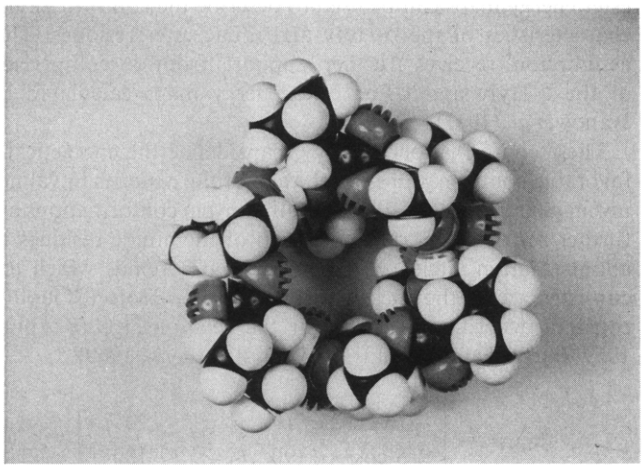
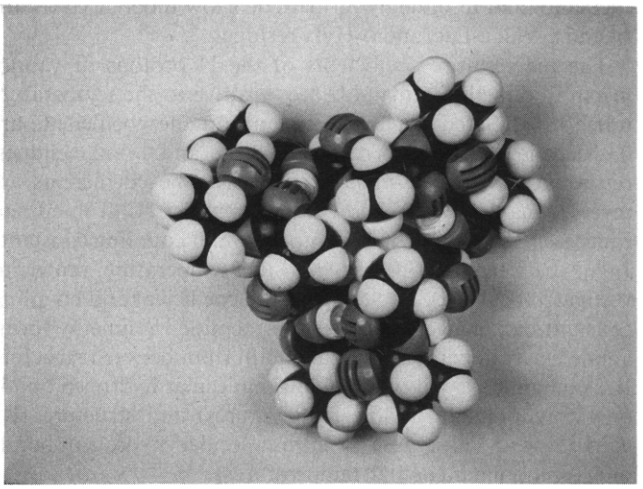


PLATE 1: Two views of a CPK model of the conformation I, predominant structure for valinomycin in hydrocarbon solvents. The top photograph shows two of the six type 1–4 bends stabilized by intramolecular hydrogen bonds. The bottom view shows the cavity with the O-linked carbonyls pointing toward the interior of the cavity.



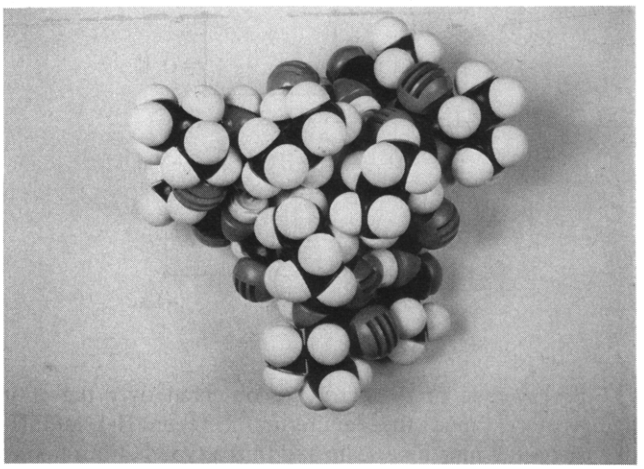


PLATE 2: The top photograph presents a view of conformation II-1 containing type 1–4 intramolecular hydrogen bonds at D-Val only and represents the valinomycin structure predominant in cyclic ether solvents with hydrogen-bond acceptor properties. Conformation II-2 presented in the bottom photograph contains type 1–4 intramolecular hydrogen bonds at L-Val only. Valinomycin in dimethylformamide is represented by a conformational equilibrium between structures II-1 and II-2.

$-\psi)_{\text{L-Val}}$ and $(\varphi,\psi)_{\text{D-HyIv}} = (-\varphi,-\psi)_{\text{L-Lac}}$. A pictorial representation of this sequence is illustrated in Plate 1.

Utilizing the symmetry of the depsipeptide and the rotation angles derived above, a cyclic structure of valinomycin is generated. The lowest energy (intramolecular) conformer so generated, designated I, is defined in terms of the rotation angles summarized in Table II and the CPK models of the structure are presented in Plate 1.

Starting with the rotation angles derived in conformation I, a search was undertaken for the conformation of valinomycin in dioxane or H_4furan at low temperatures by varying only the rotation angles of D-Val and L-Lac as outlined in section II. Additional requirements included a type 1–4 intramolecular hydrogen bond at D-Val only and $(\varphi,\psi)_{\text{L-Val}} \neq (-\varphi,-\psi)_{\text{D-Val}}$. Of the cyclic structures generated, the conformation of lowest intramolecular energy which meets the above criteria is defined by rotation angles summarized in Table II. This structure is designated conformation II-1 and presented in Plate 2.

Starting with the rotation angles derived in conformation I, a search was undertaken for the conformation(s) of valinomycin in dioxane–water by varying only the rotation angles of L-Val and D-Val as outlined in Section II. The nmr temperature coefficient data suggested no type 1–4 intramolecular hydrogen bonds while the nmr H^{α} chemical shift suggested that $(\varphi,\psi)_{\text{L-Val}} \simeq (-\varphi,-\psi)_{\text{D-Val}}$. The rotation angles of the lowest energy cyclic conformation which meets these requirements are summarized in Table II. In another set of conformational calculations, the restriction of conformation I as a starting point was lifted, and structure III-2, the generated conformation of lowest intramolecular energy, exhibited rotation angles outlined in Table II. CPK models of III-1 and III-2 are presented in Plate 3.

Valinomycin structure II-1, predominant in dimethylformamide at low temperatures, exhibits 1–4 type intramolecular hydrogen bonds at D-Val only and $J_{H^N H^{\alpha}} =$ 7.5–8.0 Hz for both valyl residues. The rotation angles defining this conformation were presented above. Valinomycin structure II-2, predominant in dimethylformamide at high temperatures, exhibits 1–4 type intramolecular hydrogen bonds at L-Val only. This conformation is defined by the rotation angles

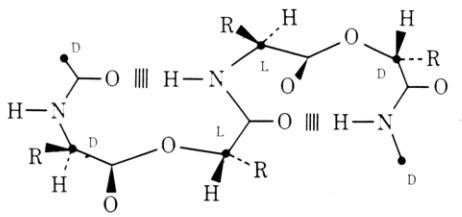
summarized in Table II. CPK models of conformations II-1 and II-2 are presented in Plate 2.

A similar search for low energy valinomycin–K^+ conformations, consistent with $J_{H^NH^\alpha}$ = 5.5 Hz for both valyl residues and exhibiting a cavity lined with carbonyl groups to bind metal ion, was undertaken. The structure of the complex in methanol is stabilized by intramolecular type 1–4 hydrogen bonds for both valyl residues and conformation C-I defined by rotation angles in Table II meets this requirement. It supports conclusions of earlier workers (Ivanov *et al.*, 1969; Ohnishi and Urry, 1969; Pinkerton *et al.*, 1969; Ramachandran and Chandrasekaran, 1970). The structure of the complex in dimethylformamide exhibits either solvent-shielded and/or type 1–3 intramolecular hydrogen bonds for both valyl residues as determined from temperature coefficient analysis in this study. Conformation C-II defined by rotation angles in Table II meets this requirement with the peptide N protons pointing into the interior of the cavity.

In addition, the dipole moment of each conformation generated was estimated according to the method of Flory (Flory and Schimmel, 1967; Brant *et al.*, 1969) and each is reported in Table II.

IV. Comments on Bends and Conformations I, II, and III

The LD and DL bends shown pictorially in Plate 1 with rotation angles outlined in section III have interesting features. Both bends exhibit curvature such that cyclization is possible with twleve residues. The N-linked carbonyl groups are each involved in intramolecular hydrogen bonds with the peptide N proton of the third subsequent residue. The O-linked carbonyl groups are directed to the exterior while the ester oxygen points toward the interior (see Plate 1). The "pore"



conformation of valinomycin put forward by Urry and Ohnishi (1970) corresponds to structure I. The rotation angles derived independently by Ivanov *et al.* (1971) and in this study are in agreement.

Conformation II-1 exhibits a threefold symmetric propeller-like structure with strong type 1–4 intramolecular hydrogen bonds involving D-Val protons only. In addition, the L-Val N protons may form weak type 1–3 intramolecular hydrogen bonds. The center of the conformation has a hydrophobic surface with the polar groups distant from this region. Conformations I and II-1 differ only at rotation angles (φ,ψ) for the D-Val residue and the barrier to interconversion should be low.

In solvents other than water, alcohols and acids (*i.e.*, solvents lacking hydroxyl groups), it is proposed that valinomycin conformations I and II-1 are in rapid equilibrium. In good hydrogen-bond acceptor solvents, *e.g.*, dioxane, H_4furan at low temperatures, II-1 is the predominant conformation while I is probably the predominant conformation in neat hydrocarbon solvents. Since valinomycin is insoluble in hydrocarbons, this study investigated the depsipeptide in hydrocarbon–dioxane mixtures, and thus valinomycin in the

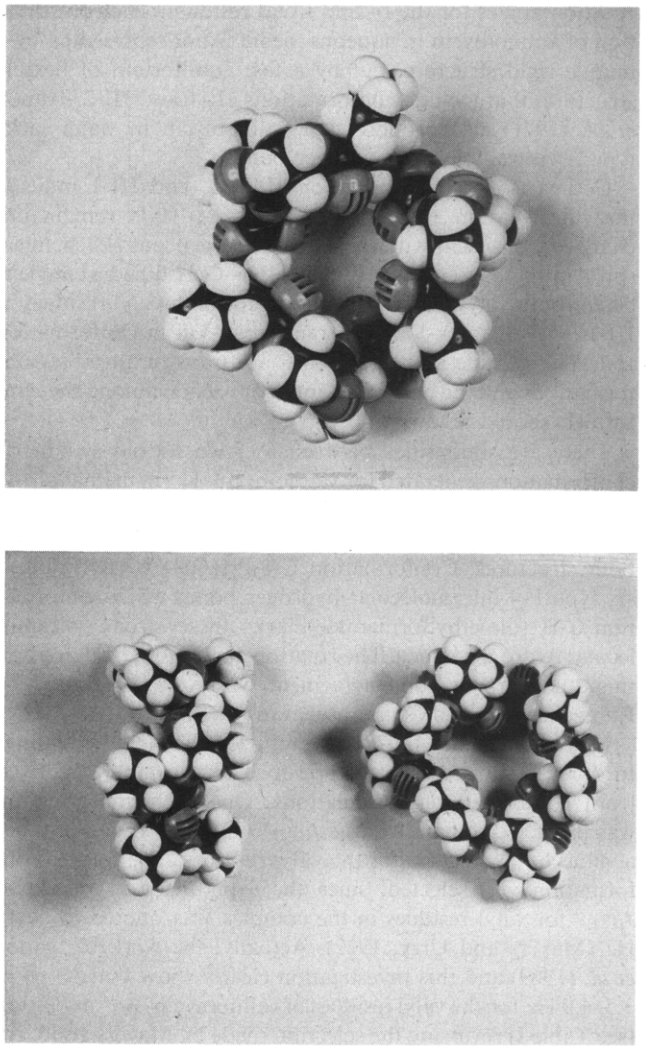


PLATE 3: The top photograph is a CPK model of conformation III-1, while the bottom photograph presents two views of conformation III-2. The conformation of valinomycin in methanol at temperatures >30° and in aqueous media are presented by a set of flexible conformations lacking type 1–4 intramolecular hydrogen bonds of which III-1 and III-2 are low-energy structures.

latter solvent system must be represented as an equilibrium mixture of I and II-1, the equilibrium shifting to II-1 with increasing dioxane content.

A rapid equilibrium between threefold symmetric conformations II-1 (predominant at low temperatures) and II-2 (predominant at high temperatures) for valinomycin in dimethylformamide were demonstrated in this study. The two conformations differ at the φ,ψ rotation angles for D- and L-Val residues, and a low barrier to interconversion is predicted, consistent with the observation of average resonances only. Urry and Ohnishi (1970) predicted such an equilibrium from an investigation of valinomycin H^N chemical shift temperature coefficients in Me_2SO. The rotation angles derived independently by Ivanov *et al.* (1971) and in this study for conformations II-1 and II-2 are in agreement.

Conformation III-1 exhibits a flexible bracelet structure in which the peptide N protons of D- and L-Val may form weak, bent type 1–3 intramolecular hydrogen bonds. This conformation exhibits a large cavity lined by the amino acid N—H and C=O groups. Conformations I and III-1 differ at (φ,ψ)

rotation angles for the D- and L-Val residues. The conformation of valinomycin in aqueous media is not represented by a unique rigid structure, but by a fast equilibrium of flexible structures that include conformations III-1 and III-2. Ivanov *et al.* (1971) also propose that valinomycin in water lacks type 1–4 intramolecular hydrogen bonds.

Comparison of conformations I, II-1, and III-1 indicate that the rotation angles for L-Lac and D-HyIv remain unchanged, consistent with the solvent-independent H^{α} chemical shifts of these ester residues. The HN—$C^{\alpha}H$ dihedral angle is cis for both valyl residues in I, trans for both valyl residues in III-1, and cis for L-Val and trans for D-Val in conformation II-1. The peptide and ester bonds are trans in all three conformations and the barriers to interconversion among the conformers should be low.

There are similarities between the two sixfold symmetric conformations generated for valinomycin-K^+ in methanol and dimethylformamide. The size of the metal ion cavity and disposition of the O-linked carbonyl groups are the same in both structures. Conformation C-I (MeOH) is rigid due to six type 1–4 intramolecular hydrogen bonds while conformation C-II (dimethylformamide) lacks these strong intramolecular hydrogen bonds. The rotation angles for C-I derived in this study are in good agreement with values reported by Ramachandran and Chandrasekaran (1970).

Mayers and Urry (1972) have utilized the $J_{H^{\alpha}H^{\beta}}$ couplings to differentiate among structures for valinomycin-K^+ derived from conformational calculations. One such conformation was predicted to have a value $J_{H^{\alpha}H^{\beta}} = 13$ Hz while the other a value $J_{H^{\alpha}H^{\beta}} = 5$ Hz for the valyl residues. The former conformation was selected, since the experimentally measured $J_{H^{\alpha}H^{\beta}}$ for valyl residues in the complex was quoted to be 11 Hz (Mayers and Urry, 1972). Actually, the work of Ivanov *et al.* (1969) and this investigation clearly show that $J_{H^{\alpha}H^{\beta}} = 3.5$–$4.0$ Hz for the valyl residues of valinomycin-K^+ in solution (see Table I) reversing the selection made by Mayers and Urry (1972).

At the end of section II it was noted that the $J_{H^{\alpha}H^{\beta}}$ coupling constants of the valyl residues in valinomycin decreases on passing from dioxane–octane to dioxane–water and upon complexation with K^+ in both methanol and dimethylformamide. This decrease in $J_{H^{\alpha}H^{\beta}}$ implies (Pachler, 1964) a reduction in the H^{α}—H^{β} rotamer population corresponding to $\chi = 60°$ simultaneous with an increase in the population(s) of either or both of the $\chi = 180°$ and $\chi = 300°$ rotamers (the dihedral angle between H^{α} and H^{β} in the valyl residues is 180, 120, and 120° for C^{α}—C^{β} rotations $\chi = 60$, 180, 300°, resulting (Karplus, 1959; Pachler, 1964) in a large $J_{H^{\alpha}H^{\beta}}$ for $\chi = 60°$ and small couplings for $\chi = 180$ and 300°). The conformations proposed for valinomycin in dioxane–octane, dioxane–water, methanol, and dimethylformamide and for the K^+ complex in the last two solvents are consistent with the observed decrease in $J_{H^{\alpha}H^{\beta}}$ for the valyl residues.

As an example, conformation I proposed for valinomycin in dioxane–octane has $(\varphi,\psi)_{\text{L-Val}} = 210°$, 240° and $(\varphi,\psi)_{\text{D-Val}} = 140°$, 120° (see Table II). For these values of φ and ψ, the intramolecular energy $E(\varphi,\psi,\chi)_{\text{D or L-Val}}$ is lowest for $\chi = 60°$. By contrast, in conformations III-1 and III-2 proposed for valinomycin in dioxane–water $(\varphi,\psi)_{\text{L-Val}} = 30$–40°, 250–320° and 90°, 290–310° and $(\varphi,\psi)_{\text{D-Val}} = 320$–330°, 40–110° and 270°, 50–70°, respectively. $E(\varphi,\psi,\chi)_{\text{D or L-Val}}$ is lowest for these sets of (φ,ψ) when $\chi = 60$ or 300°. Thus, the conformations proposed for valinomycin in dioxane–octane and dioxane–water are consistent with the observed $J_{H^{\alpha}H^{\beta}}$ couplings of the valyl residues in these solvents.

V. Limitations of the Methods Used for Conformational Analysis

In this study it has been demonstrated that valinomycin exhibits solvent dependent conformations in solution. The conformation in nonpolar solvents (structure I) was first deduced and the structures in the remaining solvents were determined from observation of the spectral perturbations away from I resulting from variations of the solvent medium. In the determination of structure I it was assumed that the six intramolecular hydrogen bonds are of the 1–4 type. The crystal structure of uncomplexed valinomycin has demonstrated the presence of two relatively weak 13-membered intramolecular hydrogen bonds, and undoubtedly other types of intramolecular hydrogen bonds cannot be ruled out.

The independent studies by Professor Ovchinnikov's group (Ivanov *et al.*, 1971) and this investigation have put forward several solvent-dependent conformations for the cyclic depsipeptide valinomycin. Neither study included the effects of solvent perturbations on the energies of the peptide and ester residue conformational maps utilized to derive structures for the depsipeptide.

The conformational calculations rely heavily on the magnitude of $J_{H^{N}H^{\alpha}}$ to determine limitations on the rotation angle φ about the N—C^{α} bonds. If the observed coupling constant is an average of several values differing greatly in magnitude, the conformational analysis can give erroneous results. Fortunately for valinomycin in solution, $J_{H^{N}H^{\alpha}}$ in a particular solvent exhibited temperature independence, arguing against an equilibrium between conformers with different $J_{H^{N}H^{\alpha}}$ which would result in the observation of conformationally averaged couplings.

Finally, the conformations presented in this study for valinomycin in solution exhibit low intramolecular conformational energies and are consistent with the proton nmr data. This does not unequivocally rule out structures of higher intramolecular conformational energy with favorable depsipeptide–solvent interactions. On the other hand, energy minimization of the structures generated here was not attempted.

VI. Comparison of Valinomycin Conformations in the Crystal and Solution

Recently the preliminary results of an X-ray structure investigation of the crystalline conformation of uncomplexed valinomycin have been reported (Duax *et al.*, 1972). The authors do not give x, y, z atomic coordinates or φ, ψ, ω residue rotation angles, but present a drawing using skeletal models. The uncomplexed crystalline conformation possesses a pseudo center of symmetry, but not threefold symmetry. Eight of the twelve residues are in conformations closely similar to those found in the K^+ ion complex, and four of the six type (1–4) hydrogen bonds are maintained. However, four of the residues (one each of L-Val, D-Val, L-Lac, and D-HyIv) deviate substantially from their K^+ complex conformation resulting in two 13-membered intramolecular hydrogen bonds involving a L-Val and a D-Val peptide proton.

Beginning from the K^+ complex conformation, a space-filling molecular model of crystalline uncomplexed valinomycin was constructed by forming the two 13-membered hydrogen bonds discussed above. The rotation angles (φ,ψ) for each residue were read from the molecular model, with all ester and peptide bonds in the trans conformation. The (φ,ψ) angles of the four residues whose conformations differ from those found in the K^+ complex were allowed to vary ± 30 in 10° increments from

their values read from the molecular model in a computer search for closed ring or cyclic structures. The lowest energy cyclic conformation generated [$(\varphi,\psi)_{\text{L-Val}_1}$ = 100°, 240°; $(\varphi,\psi)_{\text{D-HyIv}_2}$ = 290°, 160°; $(\varphi,\psi)_{\text{D-Val}_{3,11}}$ = 250°, 70°; $(\varphi,\psi)_{\text{L-Lac}_{4,12}}$ = 110°, 160°; $(\varphi,\psi)_{\text{L-Val}_{5,9}}$ = 100°, 290°; $(\varphi,\psi)_{\text{D-HyIv}_{6,10}}$ = 250°, 210°; $(\varphi,\psi)_{\text{D-Val}_7}$ = 260°, 120° and $(\varphi,\psi)_{\text{L-Lac}_8}$ = 70°, 200°] was allowed to vary ±10° in each of its residue rotation angles in an attempt to determine the strength of the two 13-membered hydrogen bonds.

In general, the 13-membered hydrogen bonds could be characterized by short O---H distances (<2.0 Å) and angles of ≈ 40° between C=O and O---H and between N—H and N---O, respectively. Hence, these highly nonplanar hydrogen bonds are most probably weak relative to the four remaining type (1–4) hydrogen bonds.

In terms of the present study, whether or not the crystalline structure of uncomplexed valinomycin is present in significant proportion in solution is of paramount interest. As discussed earlier, the nmr spectrum of valinomycin in hydrocarbon (dioxane–octane 1:13) shows threefold symmetry and $J_{H^NH^\alpha}$ = 6.1 and 7.6 Hz for the L- and D-Val residues, respectively. Hence, either a rigid threefold symmetric conformer exists in hydrocarbon solution or a rapid equilibrium (rapid on the nmr time scale) between several asymmetric structures, such as the crystalline conformation, is possible, where each conformer has all six peptide protons hydrogen bonded.

The crystalline structure does not possess threefold symmetry, and therefore cannot be the only solution conformer unless a rapid equilibrium, where each L- and D-Val residue spends a portion of its time in a 13-membered hydrogen bond, exists. When the amide to α-proton couplings of the L- and D-Val residues are averaged according to eq 1 and 2 over their two different conformations [two-thirds of the time in type 1–4 hydrogen bond and one-third of the time in a 13-membered hydrogen bond] an average coupling of $\langle J_{H^NH^\alpha}\rangle$ (L- or D-Val) = 5.0–5.5 Hz is obtained. This is to be compared with the experimental values of 6.1 and 7.6 Hz for the L- and D-Val residues.

In addition to the disparity between the calculated and measured coupling constants, the dipole moment calculated for the crystalline structure of uncomplexed valinomycin is 6.9 D. Ivanov *et al.* (1969) measured the dipole moment of uncomplexed valinomycin in CCl_4 and reported a value of 3.5 ± 0.1 D while we find a dipole moment of 3.4 D in benzene. Both values are in agreement with the dipole moment (3.9 D) calculated for our proposed solution conformation I of uncomplexed valinomycin in hydrocarbon solvents which differs markedly from the crystalline conformation (see Table II).

Primarily on the basis of the large difference between the dipole moments of uncomplexed valinomycin measured in hydrocarbon solvents and calculated for the crystalline structure, we conclude that the solution and crystalline conformers are substantially different. Valinomycin illustrates once again (see Tonelli and Brewster, 1972) the high probability that the solution and crystalline conformations of small polypeptides are distinct. (It would be of interest to know the solvent from which the valinomycin crystals were grown.)

Acknowledgment

Professor Yu. A. Ovchinnikov kindly brought to our attention the more recent efforts of his group (Ivanov *et al.*, 1971) to expand on earlier studies of the conformation of valinomycin in solution (Ivanov *et al.*, 1969).

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Carbon Framework of Valinomycin and Its Metal Ion Complex in Solution†

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ABSTRACT: The solvent-dependent solution conformations of valinomycin and its K^+ complex were determined using proton nuclear magnetic resonance (nmr) spectroscopy and conformational calculations in the previous manuscript (Patel, D. J., and Tonelli, A. E. (1973), *Biochemistry 12*, 486). An extension of this study utilizes ^{13}C nmr spectroscopy to monitor the carbon framework of this depsipeptide and its complex in solution. The C^α, C^β, and C' carbon chemical shifts were assigned to the amino acid and ester residues and found to be a sensitive function of conformation. The ^{13}C chemical shifts reflect the large conformational changes in the depsipeptide on complex formation. The carbonyl carbon resonances involved in complexation move 5 ppm downfield on coordination with diamagnetic univalent metal ions. Potassium exchange between valinomycin and its complex in the uncertainty broadened slow exchange region could be monitored with the larger range of ^{13}C chemical shifts. The rotational correlation time τ_R for valinomycin and its complex in methanol were derived from the measurement of carbon spin–lattice relaxation times in proton-decoupled spectra.

The solution conformations of several cyclic polypeptides have been derived using proton nuclear magnetic resonance (nmr) spectroscopy and conformational energy maps (Hassal and Thomas, 1971). Recently this analysis has been extended to include a computer search to determine the lowest energy conformation of a cyclic polypeptide consistent with the nmr parameters and conformational energy maps (Bovey *et al.*, 1972).

The cyclic depsipeptide valinomycin occupies a special place in these investigations because of its involvement in potassium ion transport. Proton nmr studies on valinomycin and its K ion complex have been reported from several laboratories (Haynes *et al.*, 1969; Ivanov *et al.*, 1969; Ohnishi and Urry, 1969). In the previous paper from this laboratory the conformations of valinomycin in hydrocarbon, hydrogen-bond acceptor, and aqueous media were elucidated using proton nmr spectroscopy and conformational calculations (Patel and Tonelli, 1973). This study reports on the ^{13}C spectra of valinomycin and its metal ion complex in these same solvent systems.

The ^{13}C nmr spectrum of gramicidin S has been reported and the spectral assignments were made by comparison with the amino acid spectra (Gibbons *et al.*, 1970). The ^{13}C resonances of the carbonyl groups participating in intramolecular hydrogen bonds and those exposed to solvent had similar chemical shifts. A preliminary report on the ^{13}C nmr studies of the binding of metal ions to cyclic crown ethers and depsipeptides has appeared (Ohnishi *et al.*, 1972). This investigation of valinomycin was initiated in the expectation that carbonyl group complexation with the metal ion would be reflected in their ^{13}C chemical shifts.

Experimental Section

^{13}C spectra were run on an XL-100 Varian spectrometer with heteronoise proton spin decoupling and variable-temperature facilities. The instrument was interfaced with an F&H pulse program generator and a Fabri-Tek computer for data collection in the Fourier transform mode (Sternlicht and Zuckerman, 1972).

Valinomycin was purchased from Calbiochem. The sample was dissolved in deuterated solvents and the spectrometer locked on deuterium. The carbon resonances of solvent served as an internal standard and were later referenced relative to CS_2. Sample concentrations were in the range 50–100 mg/ml for all nmr runs except T_1 measurements, where the sample concentration was 200 mg/ml.

T_1 measurements were undertaken using the 180°, τ, 90° pulse sequence method (Vold *et al.*, 1968). Measurements in the carbonyl, C^α and $C^\beta + CH_3$ regions were undertaken in separate runs. Repetition rates were 10 sec for carbonyl runs and 3 sec for all other measurements. The estimated errors in the measurements are <10%.

Results and Discussion

Valinomycin is a cyclic depsipeptide with the sequence *cyclo*-(D-HyIv-D-Val-L-Lac-L-Val)$_3$. The amino acid grouping is defined by the atoms ($NC^\alpha(C^\beta)C'$) and the ester grouping

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