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Raman Spectroscopy of Uncomplexed Valinomycin. 2. Nonpolar and Polar Solution

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Abstract: The molecular conformations of uncomplexed valinomycin in CCl_4, CS_2, CH_2Cl_2, $CHCl_3$, CH_3OH, C_2H_5OH, C_3H_7Cl, *p*-dioxane ($C_4H_8O_2$), and dioxane/D_2O have been studied using laser Raman spectroscopy. The stretching frequencies of the ester and amide carbonyl groups are found to be affected by both the polarity of the solvent and its ability to form hydrogen bonds. Results in nonpolar solvents are consistent with the presence of hydrogen bonding ester carbonyl groups, reopening the question of whether the conformation found in valinomycin recrystallized from *n*-octane can exist in nonpolar solution. In polar solvents, a conformation is detected that contains fewer hydrogen bonds. As the dielectric constant of the solvent increases, the stretching frequency of the amide carbonyl groups increases (perhaps reflecting a reduction of intramolecular hydrogen bonding), while the stretching frequency of the ester carbonyl groups decreases.

The macrocyclic dodecadepsipeptide valinomycin (hereafter abbreviated VM; Figure 1a) was among the first ion-specific antibiotics to be used as a model of ionic transport in biological membranes.[2-4] Although the three-dimensional structure of crystals can often be obtained with x-ray or neutron diffraction, these techniques cannot provide information about whether conformations found in the solid state persist in solution. In contrast, Raman spectroscopy can be applied to both solids and liquids, permitting one to compare structural characteristics of molecules in a variety of environments. Raman spectroscopic investigations of the molecular conformations of VM in the solid state have recently been reported,[5] and in this work we report studies of VM in a variety of solvents. These studies, which include deuteration of the NH groups, reveal new information about the dependence of VM conformation on environment.

X-ray methods have been used to reveal[6,7] the complete structure of one form of uncomplexed VM; all six NH groups are intramolecularly hydrogen bonded, four to amide C=O groups and two to ester C=O groups (conformation D, Figure 1b). Structural similarities between crystalline uncomplexed VM and the VM-K^+ complex have led to the suggestion[6] that this form may be involved in ion complexation at the membrane–water interface. However, nuclear magnetic resonance (NMR), infrared absorption (IR), and optical rotatory dispersion (ORD) studies[4,8-11] reveal no evidence of conformation D in solution. One question we address is whether or not there is Raman spectroscopic evidence that conformation D persists in solution.

A mixture of several VM conformations exists in solution.[4,8-11] It is believed that the predominant conformation of VM in nonpolar solvents contains six hydrogen-bonded amide C=O groups and six unbonded ester C=O groups (conformation A, Figure 1c), while the predominant conformation in polar solvents is believed to contain only three hydrogen bonded amide C=O groups (conformation B, Figure 1d). These conclusions are based primarily on NMR data,[9] which indicate a threefold equivalence of L- and D-valine protons. Conformation D lacks this symmetry; however, Patel and Tonelli[9] have pointed out that asymmetric structures could exist in solution if they were in "rapid" equilibrium with each other (rapid on an NMR time scale) thereby appearing to be symmetric in NMR measurements.

The Raman spectra of VM recrystallized from *n*-octane (known[7] to be in conformation D) exhibit a hydrogen-bonded ester C=O group mode near 1742 cm^{-1}, 25 cm^{-1} lower in frequency than the corresponding mode of the free C=O groups.[5] The discovery of a similar downshift (or splitting) in solution would support the presence of hydrogen bonded ester C=O groups (and thus conformation D) in such environments.

Materials and Methods

Valinomycin (VM) was obtained from Calbiochem (San Diego, Calif.) and Sigma Chemicals (St. Louis, Mo.) and was used without

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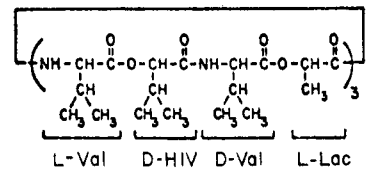
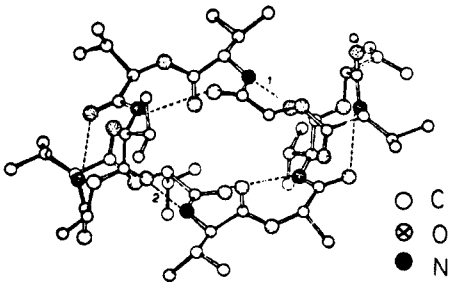
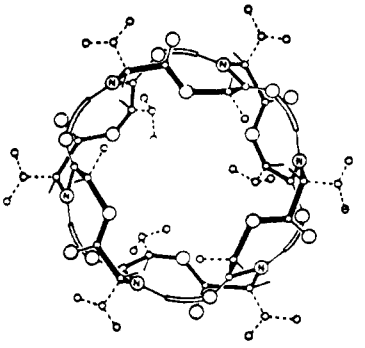


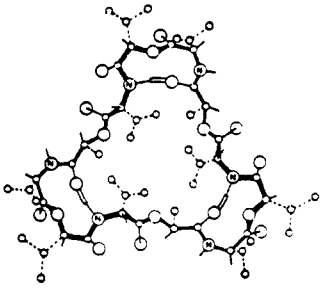
a) Valinomycin (VM)



b) Conformation D



c) Conformation A



d) Conformation B

Figure 1. (a) Primary structure of valinomycin. Here L-Val denotes L-valine; D-HIV denotes D-hydroxyisovaleric acid; D-Val, denotes D-valine; and L-Lac denotes L-lactic acid. (b) Structure of uncomplexed valinomycin crystallized from *n*-octane (from ref 6). (c) Predicted predominant conformation of valinomycin in nonpolar solvents (from ref 4). (d) Predicted predominant conformation of valinomycin in polar solvents (from ref 4).

further purification. Solutions (0.02–0.5 M) were injected into 1.0-mm i.d. capillaries and centrifuged for 5 min to eliminate dust and dissolved air. Deuteration was accomplished directly in the capillary tube by exchange across a D_2O/CCl_4 interface; Raman spectra were recorded at regular intervals over a 24-hr period and subsequently checked over periods of several months.

Raman spectra were measured using a Spex Ramalog 4 system and a Spectra-Physics Model 164-03 Ar^+ laser (488.0- and 514.5-nm excitations). The incident power level was typically 150–200 mW, resolution 3 cm^{-1}, and scanning speed 3–30 cm^{-1}/min. For polarization measurements, an analyzer was placed in front of the spectrometer; the incident laser light was polarized perpendicular to the scattering plane. Further details of the Raman spectroscopy system are presented in ref 5.

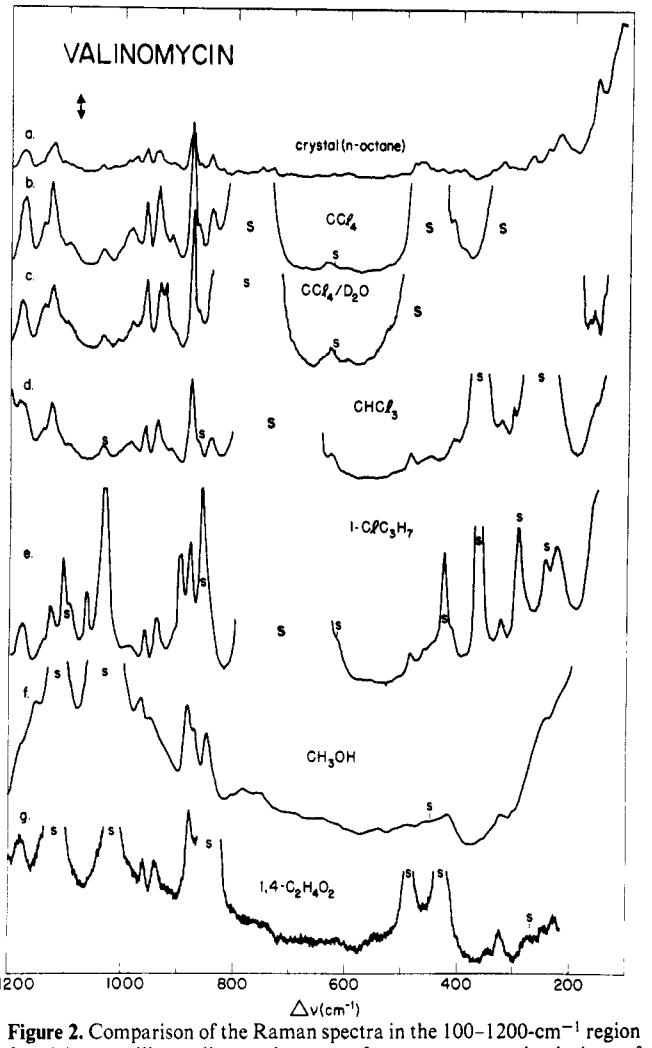


Figure 2. Comparison of the Raman spectra in the 100–1200-cm^{-1} region for: (a) crystalline valinomycin grown from *n*-octane and solutions of valinomycin dissolved in (b) CCl_4, (c) CCl_4 with D_2O in contact as a second phase, (d) $CHCl_3$, (e) 1-ClC_3H_7, (f) CH_3OH and, (g) 1,4-dioxane (*p*-dioxane). Spectra were recorded with incident powers, 20–200 mW, exciting frequencies 457.9, 488.0, 514.5 nm, spectral resolution 3 cm^{-1}, scanning speed 0.1–0.5 cm^{-1}/s. Vertical arrow represents 300 counts/s in (b–g) and 1000 counts/s in (a). No analyzer in scattered beam; incident light polarized perpendicular to the scattering plane. S denotes solvent peak.

Results

(A) CCl_4 (Nonpolar) Solution. We begin by comparing the Raman spectrum of VM powder recrystallized from *n*-octane (Figures 2a and 3a) with that of VM dissolved in CCl_4 (Figures 2b and 3b). Although some spectral regions (denoted by S) are obscured by solvent peaks, most regions of the crystalline and solution spectra are similar. This is not surprising since many peaks are due to residue vibrations which should be relatively insensitive to conformational change. However, spectral changes are observed in the conformationally sensitive 1600–1800 cm^{-1} (C=O stretch), 1220–1320 cm^{-1} (amide III), and 3300–3450 cm^{-1} (NH stretch) spectral regions. In addition, the 981- and 1020-cm^{-1} peaks of crystalline VM shift somewhat in CCl_4 solution.

(1) The C=O Stretch Region (1600–1800 cm^{-1}). Prominent amide and ester C=O stretch vibrations occur in the 1600–1700-cm^{-1} and the 1700–1800-cm^{-1} regions, respectively.[5] An analysis of these regions of VM powder (Figure 4a) and

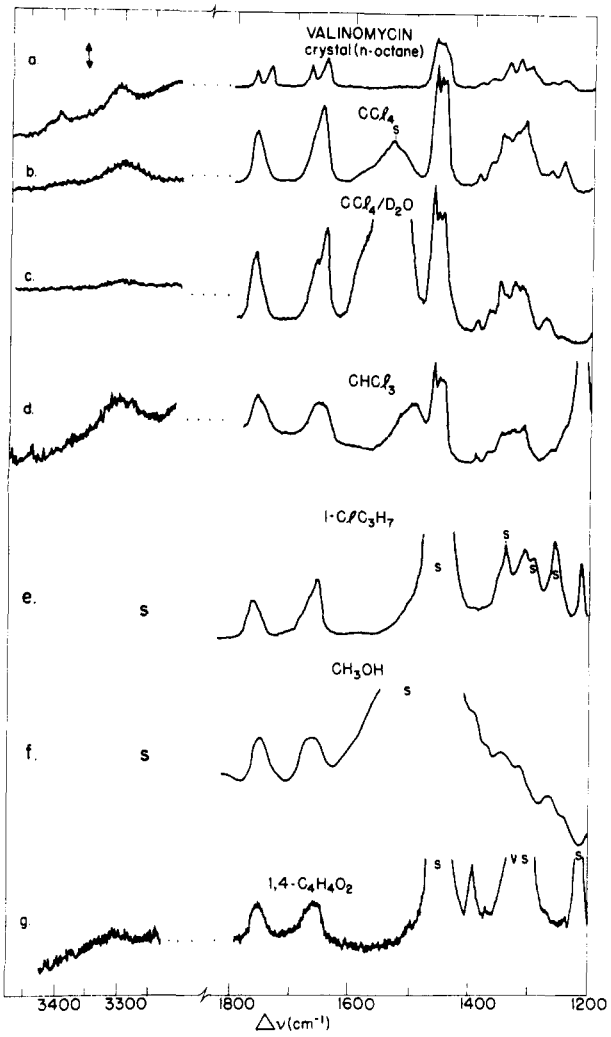


Figure 3. Continuation of Figure 2 to the 1200–1800 and 3200–3500-cm^{-1} regions.

$VM\text{-}CCl_4$ solution (Figure 4b) provides information on the extent of hydrogen bonding. For example, the frequency of the broad 1654-cm^{-1} peak of $VM\text{-}CCl_4$ is near that of the narrower 1649-cm^{-1} peak observed in the solid state (hydrogen-bonded amide C=O stretch). The 1675-cm^{-1} amide C=O peak of crystalline VM (free amide C=O stretch) does not appear in $VM\text{-}CCl_4$, although the marked upward asymmetry of the amide C=O band suggests that there still exist relatively "free" C=O groups.

In the *ester* C=O stretch region, VM/CCl_4 exhibits a broad asymmetric peak at 1760 cm^{-1} which is 7 cm^{-1} below the free ester C=O stretch frequency of crystalline VM. The low-frequency asymmetry may represent contributions from intramolecularly hydrogen-bonded ester C=O groups (a characteristic feature of conformation D), or from the presence of unbonded ester C=O groups exposed in different degrees to the solvent. Carbonyl groups exposed to solvent interactions typically have a lower stretch frequency and larger peak width than unexposed C=O groups;[12] however, such interactions should be weak in CCl_4 (a nonpolar solvent) compared to the effects of intramolecular VM hydrogen bonding.

One can further decompose the C=O stretch bands of VM by using a polarization analyzer to separate scattered light with polarization perpendicular (⊥) and parallel (∥) to the scattering plane. The incident laser light is polarized ⊥ to the scattering plane, so that Raman spectra taken with (incident, scattered) polarizations (⊥, ∥) will contain only "depolarized" peaks, lacking Raman scattering which retains its original polarization ("polarized" peaks).

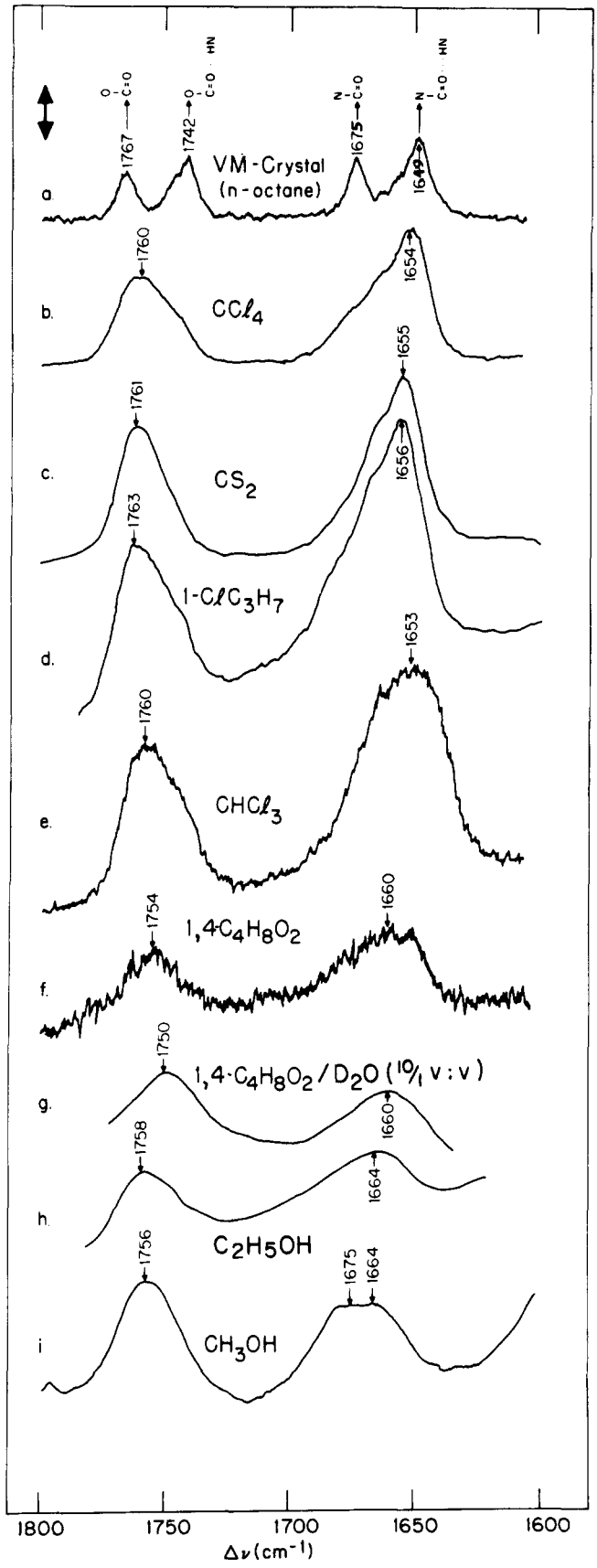


Figure 4. Comparison of Raman spectra in the C=O stretch vibration region of: (a) VM grown from *n*-octane, and solutions of VM dissolved in (b) CCl_4, (c) CS_2, (d) $1\text{-}ClC_3H_7$, (e) $CHCl_3$, (f) 1,4-dioxane, (g) 1,4-dioxane/D_2O (10:1 v/v), (h) C_2H_5OH, (i) CH_3OH. Spectra were recorded with incident power 20–200 mW, exciting frequency 457.9, 488.0, 514.5 nm, spectral resolution 3 cm^{-1}, scanning speed 0.1–0.5 cm^{-1}/s. Vertical arrow represents 300 counts/s. No analyzer in scattered beam, incident light polarized. S denotes solvent peak.

When decomposed in this manner, the *ester* C=O stretch region of VM-CCl_4 (Figure 5) consists of a symmetric depolarized ($\perp$, $\parallel$) peak near 1755 cm^{-1} and a polarized ($\perp$, $\perp$) peak at 1760 cm^{-1} with a distinct shoulder near 1745 cm^{-1}. The frequency of the latter is close enough to the 1742 cm^{-1} hydrogen bonded C=O stretch frequency of crystalline VM (Figure 4a) to suggest that some hydrogen-bonded C=O groups may be present in CCl_4 solution (as in conformation D).

The *amide* C=O stretch region consists of a depolarized ($\perp$, $\parallel$) band at 1667 cm^{-1} and a highly polarized ($\perp$, $\perp$) band at 1650 cm^{-1} (Figure 5); the latter is near the frequency expected for hydrogen-bonded amide C=O groups. The frequency of the 1667-cm^{-1} band, while not as high as in crystalline VM (1675 cm^{-1}), is nonetheless consistent with the presence of unbonded amide C=O groups.

Substituting deuterium for hydrogen facilitates assignments of vibrations that involve NH groups.[13] Since D_2O does not mix with CCl_4, a D_2O phase is placed in contact with the VM-CCl_4 solution; after about 24 h deuterium exchange reaches completion as determined by Raman observations of the VM in the CCl_4 phase. Upon deuteration, the amide I regions show a peak at 1645 cm^{-1} with a clear shoulder near 1660 cm^{-1}. In contrast, the shoulder on the ester carbonyl vibration, which is most apparent in the ($\perp$, $\perp$) spectrum, seems to disappear upon deuteration (cf. Figure 5).

(2) The Amide III Region. The amide III vibration consists primarily of NH bend and C=O stretch of the peptide linkage.[13] The frequency of this vibration varies between 1230 and 1330 cm^{-1} in most polypeptides and is sensitive to hydrogen bonding.[14-20] Although several C-C and CH vibrations also occur in this spectral region, the amide III vibrations can usually be identified by their sensitivity to conformational change and by their marked frequency shift upon deuteration of the NH group.

Deuteration of the NH group typically shifts amide III modes from the 1220-1330-cm^{-1} region to the 850-950-cm^{-1} region.[21] The 1248-cm^{-1} peak of VM-CCl_4 disappears upon deuteration (Figure 3c), and a new peak appears near 920 cm^{-1}. There is also a reduction of intensity near 1313 cm^{-1}, which indicates that both peaks involve amide vibrations.

The 1307-cm^{-1} peak of crystalline VM (Figure 3) is shifted to 1313 cm^{-1} in VM-CCl_4 solution, and its intensity increases (perhaps partially due to the upward shift of its low-frequency components). The 1252-cm^{-1} peak of crystalline VM is shifted to 1248 cm^{-1} in CCl_4 solution.

(3) The NH Stretch Region. Unbonded NH stretch vibrations are observed near 3312 cm^{-1} in crystalline VM; a single broad peak is observed near 3305 cm^{-1} in CCl_4 solution (Figure 3a,b). Upon deuteration this band is reduced in intensity (Figure 3d) and a new peak emerges near 2470 cm^{-1}, as might be expected for an ND stretch mode.

(B) CS_2 (Nonpolar) and $CHCl_3$ (Weakly Polar) Solution. Like CCl_4, CS_2 has a zero dipole moment, and Raman spectra of VM in the two solvents are very similar. In particular, similar asymmetries appear in the amide and ester C=O stretch bands. Shoulders near 1675 and 1745 cm^{-1} are apparent even in the unpolarized spectrum and again are more pronounced in the depolarized and polarized spectra.

$CHCl_3$ has a small dipole moment (D = 1.02 D) with excess positive charge on the hydrogen atom; thus $CHCl_3$ can interact with VM C=O groups. The Raman spectrum of VM-$CHCl_3$ (Figure 2d) is similar to that of VM-CCl_4 and VM-CS_2, although in this solvent the *amide* C=O stretch band shifts to higher frequency and becomes broader and more symmetric. The *ester* C=O stretch band remains unchanged (Figure 4e).

Dichloromethane (CH_2Cl_2) has a larger dipole moment (D = 1.54 D). The VM spectrum still appears similar to VM dissolved in nonpolar solvents, although the amide C=O appears shifted to a higher frequency, 1656 cm^{-1}, and the ester C=O to a lower frequency, 1760 cm^{-1}.

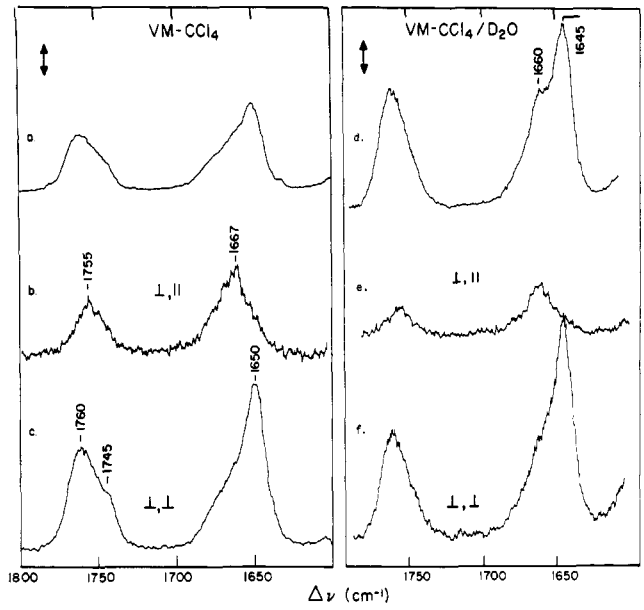


Figure 5. Raman spectra of valinomycin-CCl_4 solution in the 1600-1800-cm^{-1} region recorded with (a) no analyzer in scattered beam, (b) analyzer parallel to scattering plane, and (c) analyzer perpendicular to scattering plane. Spectra a, b, c were recorded with incident power 150 mW, exciting frequency 488.0 nm, spectral resolution 3 cm^{-1}, scanning speed 0.2 cm^{-1}/s. The vertical arrow represents 1000, 100, and 300 counts in a, b, and c, respectively. The spectra of parts (d)-(f) correspond to the spectra of parts (a)-(c) except that the samples are deuterated. Spectra (d, e, f) were recorded with incident power 200 mW, exciting frequency 457.9 nm, spectral resolution 3 cm^{-1}, scanning speed 0.5 cm^{-1}/s. The vertical arrow represents 300 counts/s.

(C) C_3H_7Cl Solution (Polar). Although VM is not sufficiently soluble in nonpolar hydrocarbons such as propane to provide quality Raman spectra, it dissolves readily in the polar hydrocarbon 1-chloropropane (C_3H_7Cl). Interestingly, its spectrum in that solvent does not differ significantly from that of VM in CCl_4 (Table I and Figure 6). In particular the amide C=O region is unchanged (except for a 2 cm^{-1} upshift in frequency), suggesting that increasing the dipole moment of the solvent from D = 0 to 2.0 D is, by itself, not sufficient to significantly change the equilibrium of VM conformations. At all concentrations of VM between 0.02 and 0.50 M a polarized 1745-cm^{-1} contribution to the ester C=O stretch band was evident (cf. Figure 7). (Measurements at concentrations below 0.01 M were not possible because of decreasing signal-to-noise ratio.)

(D) CH_3OH and C_2H_5OH Solution (Polar and Hydrogen Bonding). Although ethanol has a smaller dipole moment (D = 1.69 D) than 1-chloropropane, it has a greater effect on the conformationally sensitive C=O stretch band of VM (Figure 4). The amide I band is broadened and shifted upward in frequency to above 1660 cm^{-1}, and a slight splitting can be discerned in the polarized ($\perp$, $\parallel$) spectrum. A symmetric ester C=O stretch peak is observed, slightly downshifted, near 1758 cm^{-1}. Ethanol has little observable effect on the rest of the VM spectrum; the amide III region cannot be studied due to the presence of solvent peaks.

In methanol (D = 1.70 D) the amide and ester C=O stretch bands appear near 1665 and 1756 cm^{-1}, respectively. The broad amide I band appears to contain a second component at higher frequency, ~1675 cm^{-1}. The amide III region is observable in methanol (Figure 3f) and shows significant changes from the corresponding region in CCl_4 (Figure 3b). The intensity of the 1250-cm^{-1} peak is reduced, and there is increased

Table I. Raman Spectral Peaks from Valinomycin Crystals and Valinomycin Solutions[a]

Crystal *n*-Octane	Dioxane	CCl_4	CS_2	C_3H_7Cl	$CHCl_3$	CH_2Cl_2	C_2H_5OH	CH_3OH	Dioxane	Assignments
	141									
158	155				155				150	
	175	178								
223	225			228		230			225	
246	245							242	246	
274	275	278			274				270	
(298-318)										
326	324		326	325	326			375	325	Skeletal deformation region
346	348		353					375	345	
398	(396)	392-398				397				
412	412	412		411	410	418		420		
435	435									Ester OCC bend (?); $C-CH_3$ rock
(454)			446B	447	451b					
466	465		460 sh	463	462	456				
474										
484	486		490	490	488	487		488		
509										
531		530						544		
597 B		595				573				Amide VI
610 B						605 B				Amide VI; ester deformation
631		635								Amide IV; C=O out-of-plane bend
		677								
736	740-745							747		Amide V
757	762							782		
804 B								802		
(826)										
847	847	843			845	846		845	852	Ester COC symmetric stretch + CC stretch
867	867		870	856		867 sh		868		
880	880	879	881	880	879	880		880	878	CH_2 rock:CC stretch + NC=O bend
						897 sh				
912	912	915	915		918	914				$C-CH_3$ stretch
940	940	940	940	940	911	940		944	939	Ester O-CHR-C symmetric stretch + CH_3 symmetric rock
(945)										
962	959	960	961	958	960	962		964	960	
981	986	988	991	985	988	988			982 sh	
993				989	995					
1020		1010								Isopropyl symmetric stretch
1039	1033	1038	1040			1036				
1096 s		1097	1098		10958	1100 sh				Ester O-CHR-C asymmetric stretch
1127		1128	1130	1130	1130	1130				Isopropyl asymmetric stretch
1140 sh		1142			1145 sh					
(1150)									1150 sh	CH_3 asymmetric rock
1178	1175	1178	1179	1180	1180		1180	1183 sh	1171	OCC asymmetric stretch (?)
1252	1245	1248	1250		1250 sh	1248		1248		Amide III
1271	1267	1269	1271	1264 sh	1270			1272		CH vibration
(1294) sh		1282								
1307	1310	1313	1316		1312	1315	1311			Amide mode
1325	1328	1330 sh	1332 sh		1332	1330 sh		1330		$C^{\alpha}H$ bend
1344										$C^{\beta}H$ bend
1353 sh	1351	1351	1353		1350	1352		1352		
1372	1367	1368	1370		1373	1374		1377		CH_3 symmetric bend
(1391)	1391	1390	1391		1392			1400		
					1448					
1454	1450	1450	1450		1457					CH_3 asymmetric bend
(1461)										
1467	1466	1462	1467		1466					
1649	1650	1654	1655	1656	1654	1656	1664	1665	1660	Amide I (H bonded)
1657 sh										Amide I
1675	1663							(1675)		Amide I (unbonded)
1742										Ester C=O stretch (H bonded)
1747 sh										
							1758	1756	1754	Ester C=O stretch (solvated)
	1757 sh									
1767	1763	1760	1761	1763	1760	1760				Ester C=O stretch (unbonded)
2723			2728		2725					Combination frequencies
2731		2728	2771							

Table I *(Continued)*

Crystal *n*-Octane	Dioxane	CCl_4	CS_2	C_3H_7Cl	$CHCl_3$	CH_2Cl_2	C_2H_5OH	CH_3OH	Dioxane	Assignments
(2765)	2772	2770			2775					
2774										
2875	2874	2875	2874		2872					Isopropyl C^{β} H stretch
		2900 sh	2900 sh		2900 sh					
2913	2913	2912	2912		2910					$C^{\alpha}H$ stretch
				2930						
2938	2936	2940	2940		2942					CH_3 symmetric stretch
2966	2972	2967	2970		2972					CH_3 asymmetric stretch
2984										
3312	3301 B	3305	3300	3304	3305					NH stretch (H bonded)
					3390					NH stretch (unbonded)
3406										Combination frequency (?)
(3426)										

[a] B = broad, sh = shoulder, ? = uncertain assignment.

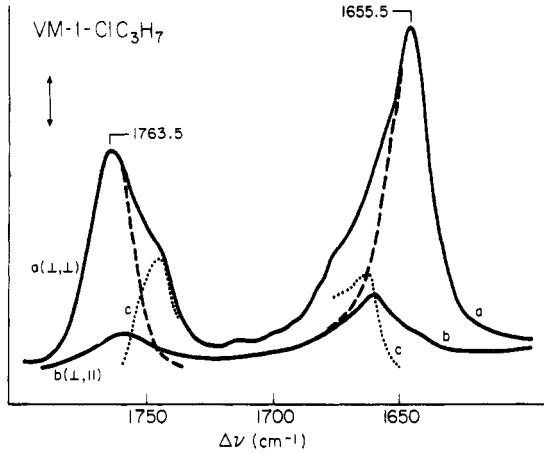


Figure 6. Raman spectra of 0.3 M valinomycin-1-ClC_3H_7 (a) recorded with analyzer ⊥ to scattering plane curve, (b) with analyzer ∥ to scattering plane, (c) and with decomposition of a shoulder found by subtracting symmetric component (dashed line) from (a). Spectra recorded with incident power 200 mW, exciting frequency 488.0 nm, spectral resolution 3 cm^{-1}, scanning speed 0.1 cm^{-1}/s. The vertical arrow represents 100 counts/s.

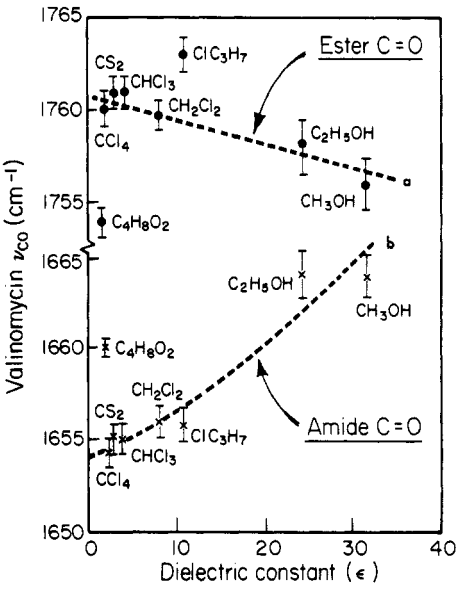


Figure 7. Plot of valinomycin amide C=O and ester C=O stretching frequencies as a function of the dielectric constant ϵ of the solvent. The dotted lines are only included to help visualize the general trend in the data and are not meant to imply any specific dependence of ν_{CO} on ϵ (such as a linear relationship).

activity near 1275 cm^{-1}. The 1313-cm^{-1} peak totally disappears. There is also an increase in the intensity of the 868-cm^{-1} peak relative to the 880-cm^{-1} peak. Methanol has a similar effect on the 865- and 800-cm^{-1} peaks of the macrotetrolide nactins in which the 865-cm^{-1} peak has been assigned to C-O-C symmetric stretch vibrations.[22]

(E) Dioxane and Dioxane/D_2O Solution (Nonpolar and Hydrogen Bonding). Dioxane is a hydrogen bond acceptor with zero dipole moment. Proton magnetic resonance studies[9] of VM in *p*-dioxane and dioxane/D_2O indicate that in these hydrogen bonding environments VM has a significantly different conformation than in CCl_4. The Raman spectrum of VM dissolved in pure dioxane and dioxane/D_2O (Figures. 2 and 3; Table I) confirms this conclusion. The broad amide C=O band of VM-dioxane, centered near 1660 cm^{-1}, is only slightly asymmetric; in 10:1 v/v dioxane/D_2O it is symmetric and centered near 1662 cm^{-1}. This band can be decomposed into a polarized (⊥, ⊥) component near 1655 cm^{-1} and a depolarized (⊥, ∥) component near 1665 cm^{-1}. The ester C=O stretch mode appears as a broad symmetric band centered near 1750 cm^{-1} in both dioxane and dioxane/D_2O. In contrast the ester C=O stretch frequency of VM recrystallized from dioxane[5] is significantly higher.

The 100-400-cm^{-1} region can be observed in dioxane solution. It resembles VM recrystallized from dioxane;[5] for example, the 143-cm^{-1} peak does not appear in spectra of VM recrystallized from *n*-octane. Vibrations in this region may represent hydrogen bond vibrations or delocalized, low-energy skeletal modes.

Discussion

(A) The C=O Stretch Region. Nonpolar Solvents. Before reexamining the question of whether VM conformations other than conformations A (Figure 1c) or B (Figure 1d) exist in nonpolar solution, we will review the experiments through which the presence of those conformations were detected and their structures determined.

(1) Nuclear Magnetic Resonance. Measurements of the proton magnetic resonance temperature coefficient of VM in dioxane-octane[9] suggest that all six nitrogen protons are solvent shielded and/or hydrogen bonded, whereas measurements in more polar solvents indicate a reduction in the number of such bonds. Although no splitting is seen in the chemical shifts of the nitrogen protons of D- and L-valine (implying threefold

symmetry), this does not rule out the existence of rapidly exchanging conformers with lower symmetry (e.g., conformation D) since only an average of the chemical shifts of the nitrogen protons is observed. In fact, evidence for several rapidly exchanging conformers of VM in hexane ($T_1 = 2 \times 10^{-9}$ s, $T_2 = 2 \times 10^{-8}$ s) has been found[10] using ultrasound absorption techniques.

(2) Infrared Spectroscopy. Infrared measurements of valinomycin in CCl_4 and $CHCl_3$ have led to the conclusion that both hydrogen bonded and free amide carbonyl groups are present.[4,8] A high-frequency amide I shoulder is seen at 1678 cm^{-1} and the NH stretch peak is a doublet (3310 and 3390 cm^{-1}). Such a result is consistent with the presence of both conformations A and B. The presence of conformation D could also account for the high-frequency shoulder at 1678 cm^{-1} but would not be expected to give rise to activity near the 3400 cm^{-1} if the latter represented free NH stretch. However, a peak appears near 3406 cm^{-1} in the Raman spectrum of VM powder in conformation D although no free NH groups are present. This band may represent a combination frequency.[5]

(3) Optical Rotatory Dispersion. Although optical rotatory dispersion measurements[4,8] reveal the presence of at least two different conformations of uncomplexed VM in solution, they provide little information on their molecular structures.

(4) Minimum Free Energy Calculations. A recent minimum energy calculation[9] based on conformation D predicts weak ester C=O hydrogen bonds which would tend to be unstable in solution. However, recent Raman measurements[5] on conformation D reveal equal hydrogen-bonding-induced downshifts (25 cm^{-1}) in the C=O stretch frequency for both ester and amide C=O groups, implying similar bond strengths.

Since none of the above mentioned solution studies exclude the existence of the crystal conformation D, it is important to consider the Raman evidence. Raman spectra[5] of crystalline VM in conformation D exhibit hydrogen-bonded ester C=O peaks at 1742 cm^{-1} and nonbonded amide C=O peaks at 1675 cm^{-1}. We note the presence of a low-frequency component of the ester C=O stretch band of VM in CCl_4, CS_2, $CHCl_3$, and C_3H_7Cl solution near 1745 cm^{-1} and a component of the amide carbonyl stretch band near 1667 cm^{-1}. Although the asymmetry of the *amide* C=O stretch band can also be explained by the presence of conformation B (which also contains free amide C=O groups), the asymmetry of the *ester* C=O stretch band cannot.

Strongly coupled C=O groups sometimes exhibit two or more stretch vibrations, corresponding to normal modes of different symmetry.[14] Although this could partially account from the splitting of the ester carbonyl stretch band in nonpolar solutions, the existence of conformation D in such solutions is a possibility which must be seriously reconsidered.

(B) The C=O Stretch Region. Polar and Hydrogen-Bonding Solvents. The C=O stretch frequency is expected to be sensitive to changes which affect either intramolecular hydrogen bonding[12] or interactions with the solvent.[25] Since solvents of different polarity are known to alter the conformation of VM,[4,8-10] VM was studied in a variety of solvents (CCl_4, CS_2, $CHCl_3$, CH_2Cl_2, 1-chloropropane (C_3H_7Cl), C_2H_5OH, CH_3OH, *p*-dioxane ($C_4H_8O_2$), and *p*-dioxane/D_2O) with different dipole moments, dielectric constants, and hydrogen bonding capacities. Figures 7a and 7b display the observed amide and ester C=O stretch frequencies ν_{CO} plotted as a function of dielectric constant ϵ. It is found that the stretching frequency of the *amide* C=O groups tends to increase with ϵ, except for the cases of dioxane, CH_3OH, and C_2H_5OH (where a splitting occurs). In contrast, the stretching frequency of the *ester* C=O groups decreases with increasing ϵ, except for dioxane and 1-chloropropane.

A decrease in C=O stretch frequency with increasing dipole moment of the solvent has also been observed in model compounds.[25,26] Recently, a study of ν_{CO} vs. ϵ for a variety of model compounds was made.[27] In the case of the model compound acetone dissolved in different solvents (at a solvent:acetone molar ratio of 8:1) the ester carbonyl stretch frequency decreases monotonically as a function of ϵ (except for C_3H_7Cl). The similar behavior of the *ester* C=O groups in VM may indicate that they are predominantly exposed to the solvent; this does not, however, rule out the possibility that a minority (e.g., 2 out of 6) of the ester C=O groups are intramolecularly hydrogen bonded.

In contrast, the increase of ν_{CO} with ϵ for the *amide* C=O groups of VM indicates that the effect of the solvent on these groups differs from that on the exposed free C=O groups in acetone. A similar increase was found for intermolecular hydrogen bonded model compounds such as *N*-methylacetamide.[27] This supports the contention that most of the amide C=O groups of VM are hydrogen bonded. The increase in solvent polarity (and its ability to form hydrogen bonds) may shift the conformational equilibrium of the system toward conformations with fewer intramolecularly bonded amide C=O groups (e.g., conformation B, Figure 1d) with a consequent rise in the average frequency of the amide C=O stretch bond. A second possibility is that more polar solvents weaken the hydrogen bonds, thereby raising ν_{CO}. Raman spectroscopy alone cannot readily distinguish between these alternatives.

The anomalous effect of 1-chloropropane on the ester C=O stretch frequency of VM (Figure 7) and acetone may be explained by the inability of 1-chloropropane to form hydrogen bonds. Nonetheless, 1-chloropropane may disrupt or weaken VM hydrogen bonds by interacting electrostatically with the NH groups. This may account for its "normal" position on the curve for VM amide C=O groups in Figure 7. Dioxane, which disrupts hydrogen bonds due to its hydrogen bond accepting ability, also gives rise to an anomalously high VM amide ν_{CO} and anomalously low ester ν_{CO} (Figure 6).

In general, plots of ν_{CO} vs. the dielectric constant ϵ may be an effective way to detect intramolecular hydrogen bonding. A decrease of ν_{CO} with ϵ would indicate semifree C=O groups, while an increase in ν_{CO} with ϵ would indicate hydrogen-bonded C=O groups. Further, comparisons with model compounds such as acetone may help provide information on the microenvironment of C=O groups inside molecules like valinomycin.[27]

Deuteration of VM in CCl_4 (by exchange with a D_2O phase) results in several changes in the Raman spectrum (Figures 2, 3, and 5). The observed change of *shape* of the amide C=O stretch peak may arise from incomplete deuteration of the NH groups; this is supported by residual activity at 3320 (NH stretch) and 1250 cm^{-1} (amide III). The inability of those NH groups to exchange H for D may reflect strong hydrogen bonding. The shoulder at 1660 cm^{-1} could also arise from the presence of free amide C=O groups whose stretch frequency ν_{CO} is expected to be higher than that of hydrogen-bonded C=O groups.

The reduction of the low-frequency shoulder of the ester carbonyl peak upon NH deuteration is more difficult to explain, unless one assumes that some of the ester C=O groups are hydrogen bonded (as in conformation D).

(C) The Amide III Region. The amide III vibration is known to be extremely sensitive to hydrogen bonding and the conformation of polypeptides.[15-19] In model homopolypeptides, Chen and Lord[20] locate the amide III modes of α-helices near 1265-1300 cm^{-1}, β antiparallel-pleated sheets near 1229-1235 cm^{-1} (with a second weaker band near 1289-1295 cm^{-1}), and random coil structures near 1243-1253 cm^{-1}. No corresponding empirical rules are available for depsipeptides like VM.

The amide III peaks of VM in CCl_4 and in the solid state

(conformation D) are similar, which suggests similarity in their conformations. In contrast, the amide III region of VM-CH_3OH is different. In particular, the disappearance of the high-frequency component of the amide III vibration may reflect the loss of hydrogen bonds in the more polar solvent.

Deuteration of VM in CCl_4 solution (Figure 4) corroborates the assignment of the 1248- and 1313-cm^{-1} peaks of VM-CCl_4 to amide III vibrations; these vibrations appear at 1252 and 1307 cm^{-1} in crystalline VM (conformation D). The upward shift of the 1307-cm^{-1} peak to 1313 cm^{-1} may account for its apparent increase in intensity in solution, since it is superposed on a second peak between 1325 and 1330 cm^{-1}. In CH_3OH (Figure 3d) the intensity of the 1250-cm^{-1} peak is reduced to roughly half that of the 1275-cm^{-1} peak, and no amide III peak appears above 1300 cm^{-1}.

Conclusion

This study has focussed on the effect of different solvents on the amide and ester C=O stretch frequencies of valinomycin. Using the Raman spectrum of the solid-state conformation D as a reference, tentative conclusions were drawn about the extent of hydrogen bonding of the amide and ester C=O groups. We find evidence suggesting that hydrogen-bonded ester C=O groups may be present in VM dissolved in nonpolar solvents; this in turn may indicate that conformer D is among the conformers present.

Increasing the polarity of the solvent shifts the conformational equilibrium of VM to forms containing fewer hydrogen bonds; these results are consistent with findings using other techniques.[8-11] A plot of ν_{CO} vs. dielectric constant of the solvent reveals an *increase* in ν_{CO} for amide C=O groups and a *decrease* in ν_{CO} for ester C=O groups. This may reflect weakening or rupture of *amide* C=O hydrogen bonds induced by polar or hydrogen bonding solvents and a concomitant increase in solvent interaction with *ester* C=O groups.

Further Raman studies will be made of VM incorporated into bilayers, as has recently been done for the membrane protein opsin.[27]

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Environmental Effects on Vibronic Band Intensities in Pyrene Monomer Fluorescence and Their Application in Studies of Micellar Systems

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Abstract: The fluorescence intensities for various vibronic fine structures in the pyrene monomer fluorescence show strong solvent dependence. In the presence of polar solvents, there is a significant enhancement in the intensity of the 0-0 vibronic band at the expense of other bands. This strong perturbation in the vibronic band intensities is more dependent on the solvent dipole moment than on the bulk solvent dielectric constant. This suggests the operation of some specific solute-solvent dipole-dipole interaction mechanism. The strong perturbation of the vibronic band intensities has been used as a probe to accurately determine critical micelle concentrations and also to investigate the extent of water penetration in micellar systems.

Fluorescence probe analysis is becoming an important area in biophysical studies of multimolecular aggregates such as micelles[2] and membranes.[3] Studies with pyrene as a fluorescence probe have received special consideration.[4,5] Pyrene has several interesting photophysical properties which make it suitable for use as an effective probe, notably the long life-