# Raman Spectroscopic Evidence for Two Conformations of Uncomplexed Valinomycin in the Solid State

Abstract. *Raman spectroscopy is applied for the first time to elucidate the different conformations of the carrier transport molecule, valinomycin. Splitting of the ester and amide carbonyl stretch vibrations is observed in the Raman spectrum of crystals of valinomycin grown from both* n*-octane and acetone. These observations support the contention that some ester carbonyl groups are intramolecularly hydrogen bonded. The Raman spectrum of valinomycin grown from* o*-dichlorobenzene does not display this feature.*

Valinomycin, a cyclic depsipeptide, has been extensively studied because of its ion-selective carrier properties in biological and artificial membranes (*1*, *2*). Elucidating the conformations of valinomycin in well-defined media is an essential preliminary to understanding the detailed mechanism of selective transport of $K^+$ (or $Rb^+$) across membranes of the lipid bilayer type.

The primary structural unit of valinomycin consists of L-valine, D-hydroxyisovaleric acid, D-valine, and L-lactic acid, linked alternately by peptide and ester bonds; this unit is repeated three times (Fig. 1a) (*2*).

A direct determination by x-ray scattering of the three-dimensional structure of an uncomplexed valinomycin crystal grown from *n*-octane has been reported by Duax *et al.* (*3*) (Fig. 1b). One outstanding feature of this model is the intramolecular hydrogen bonding of two out of the six ester carbonyl groups in the molecule, a feature which suggests a particularly plausible mechanism for the complexing of ions by valinomycin (*3*). However, studies of valinomycin in different solvents have not revealed evidence for such a feature (*4*–*6*), although the interpretation of these studies has been questioned (*3*, *5*).

We report here the first application of Raman spectroscopy to the study of different conformations of valinomycin; evidence is found for at least two different crystalline forms of uncomplexed valinomycin. The study was done with a Coherent Radiation (CR) model 52 $Ar^+$ laser with a prism wavelength selector, and a Spex 1401 double monochromator (*7*). The spectra were taken at room temperature with incident wavelengths 5145 and 4880 Å and moderate power (50 to 150 mw); additional wavelengths were provided by a CR model 52G-K $Kr^+$ laser. Spike filters were inserted into the laser beam to eliminate other wavelengths. The scattered light was detected at 90° to the incident beam. A polarization scrambler before the spectrometer entrance slit assured equal response of the spectrometer to all polarizations of scattered light. The problem of grating ghosts and plasma lines was obviated by comparing spectra at different exciting wavelengths and by checking our results on a new Spex Ramalog 4 system.

Valinomycin powder recrystallized from a slowly cooled *n*-octane solution and valinomycin crystals (2 by 1 by 1 mm) were obtained commercially (*8*). The powder was dissolved in acetone and *o*-dichlorobenzene, and allowed to recrystallize inside loosely sealed glass capillaries. The capillaries were aligned with their axes perpendicular to the plane of scattering. Other crystals were mounted on microscope slides.

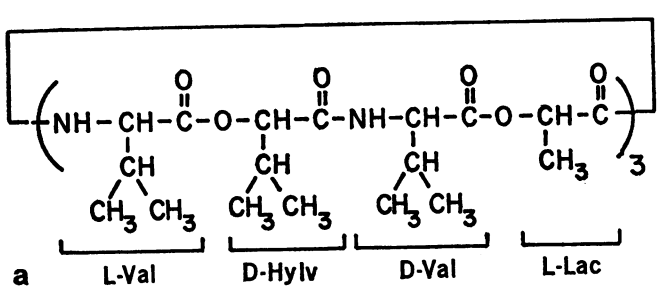
Figure 2 shows the Raman spectra in the region 1400 to 1800 $cm^{-1}$ for valinomycin crystals and powder grown in *n*-octane. The spectra display peaks at virtually identical frequencies, although in the crystalline samples (Fig. 2, curves a and b) the Raman intensities are dependent on polarization. Such data are introduced here only to emphasize the need for caution in analyzing intensity information. The doublet near 1460 $cm^{-1}$, due to methyl group deformations, is observed in valine and poly-L-valine (*9*, *10*). It is used as an internal reference.

The region 1600 to 1700 $cm^{-1}$ is characteristic of the C=O stretch vibration (the amide I vibration) (*11*, *12*); we observed two peaks, centered at 1651 and 1675 $cm^{-1}$. In comparison, poly-L-valine

$$(-NH-\underset{\displaystyle C_3H_7}{\underset{|}{CH}}-\overset{\displaystyle O}{\overset{\|}{C}}-)_n$$

has a single strong peak near 1666 $cm^{-1}$ (*9*, *10*). The appearance of two distinctly resolved peaks in the amide I region of valinomycin suggests the existence of two kinds of amide carbonyl groups (presumably hydrogen bonded and free). It has long been known that the infrared amide I vibration frequencies are shifted downward by hydrogen bonding to the C=O groups (*12*), and such effects have also been observed in the Raman spectra of several proteins (*9*). Both hydrogen bonded and free amide groups are found in the model proposed by Duax *et al.* (*3*) (Fig. 1b) and in certain conformations of valinomycin proposed by Ivanov *et al.* (*4*) and Patel and Tonelli (*5*). Inspection reveals a reproducible shoulder around 1659 $cm^{-1}$ for several orientations of the valinomycin crystal. This may indicate the existence of two different types of amide carbonyl hydrogen bonds.

The split doublet at 1742 and 1767



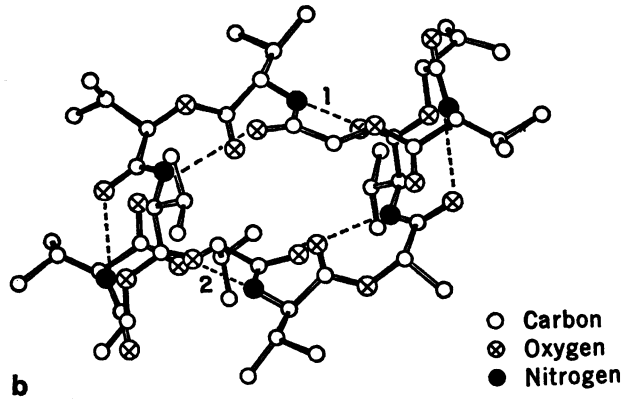




Fig. 1. (a) Primary structure of valinomycin. The abbreviations are: L-Val, L-valine; D-HyIv, D-hydroxyisovaleric acid; D-Val, D-valine; and L-Lac, L-lactic acid. (b) Three-dimensional valinomycin model. The dashed lines represent hydrogen bonds; the numerals indicate hydrogen bonded ester carbonyl groups. [Courtesy of Duax *et al.* (*3*)]

$cm^{-1}$ is similarly interpreted as representing strongly hydrogen bonded and semifree stretch vibrations of the ester carbonyl groups (*13*). The infrared absorption spectrum (*8*) of the powder does not resolve this splitting. A single strong peak appears near 1740 $cm^{-1}$ in the Raman spectra of the model compound methyl acetate (*10*)

$$(CH_3-O-\overset{\overset{O}{\|}}{C}-CH_3)$$

Hydrogen bonded ester carbonyls have appeared so far only in the Duax model; two ester carbonyls are intramolecularly bonded and four are free. While the observed predominance of the peak at 1742 $cm^{-1}$ seems to conflict with the 2 : 1 ratio of free to bonded ester carbonyls in the Duax model, this discrepancy may be explainable in terms of orientation and polarization effects (Fig. 2, curves a and b) or inherent differences in Raman activity of these modes. Alternatively, but less likely, we may be observing forms of valinomycin in which more than two ester carbonyls are hydrogen bonded (for example, intermolecular bonding).

The splitting of both the amide and ester carbonyl doublets is approximately 25 $cm^{-1}$, which suggests comparable hydrogen bond strengths. This hypothesis is consistent with the three-dimensional x-ray model derived by Duax (*14*); however, a computer energy calculation for that model by Patel and Tonelli (*5*) indicates that the ester carbonyl hydrogen bonds are much weaker than those expected on the basis of our observation.

A broad peak near 3312 $cm^{-1}$ indicates the existence of hydrogen-bonding NH groups, as would be expected on the basis of the Duax model, in which all NH groups are bonded. A weaker peak appearing as a doublet at 3407 and 3426 $cm^{-1}$ may suggest free NH groups, although a single peak has been detected around 3425 $cm^{-1}$ in L-hydroxyisovaleric acid, which does not contain NH groups; a more complete analysis (150 to 3600 $cm^{-1}$) of this form of valinomycin crystal will appear elsewhere (*10*).

Raman spectra in the region 1400 to 1800 $cm^{-1}$ of valinomycin grown from *o*-dichlorobenzene and acetone are shown in Fig. 3, curves a and b; the spectrum of valinomycin grown from octane (Fig. 3, curve c) is repeated for comparison. The peaks for the crystals grown from acetone and octane are at the same frequencies, whereas striking differences appear in the spectra of valinomycin crystals grown from *o*-dichlorobenzene (*15*). A single strong peak appears at 1762 $cm^{-1}$ in the ester carbonyl stretch region, with no appreciable activity near 1742 $cm^{-1}$; at higher resolution, there appears to be a shoulder near 1757 $cm^{-1}$. The amide I doublet remains, but the upper member (1675 $cm^{-1}$, free amide carbonyl) is shifted downward in frequency; for several crystal orientations it increases

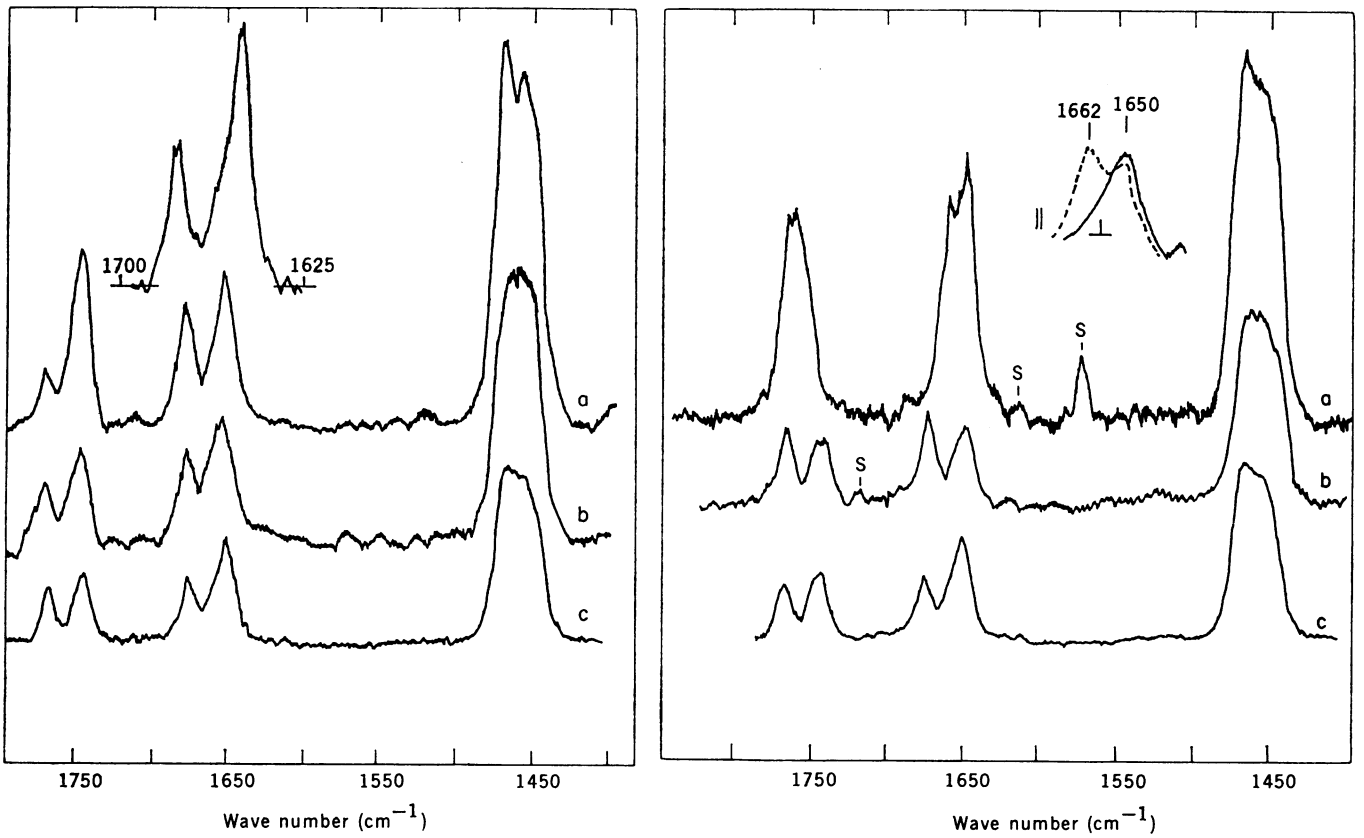



Fig. 2 (left). Raman spectra of valinomycin grown from octane. Spectra were obtained at a scanning speed of 20 $cm^{-1}$/min and a slit width of 4 $cm^{-1}$. (a) Crystal; incident light (5208 Å) polarized perpendicular to the plane of scattering. (b) Crystal, incident light (5208 Å) polarized parallel to the plane of scattering. (c) Powder; incident light, 5145 Å. (Inset) Higher-resolution spectrum of the crystal with incident light (4880 Å) polarized parallel to the plane of scattering; scanning speed, 5 $cm^{-1}$/min; slit width, 2 $cm^{-1}$. Fig. 3 (right). Raman spectra, obtained at a scanning speed of 20 $cm^{-1}$/min and a slit width of 4 $cm^{-1}$, of (a) valinomycin crystal grown from *o*-dichlorobenzene (5145 Å); (b) valinomycin crystal grown from acetone (4880 Å); (c) valinomycin powder from octane (5145 Å). (Inset) Effect of rotating the polarization of the incident light on the amide I peaks of valinomycin crystals grown from *o*-dichlorobenzene. The symbols are: *S*, solvent peaks; ⊥, light perpendicular to the scattering plane; ||, light parallel to the scattering plane. The scanning speed was 10 $cm^{-1}$/min and the slit width 2 $cm^{-1}$.

in relative intensity, whereas in other crystal orientations the amide I doublet is barely resolved (Fig. 3, inset). A broad peak, characteristic of strongly bonded NH vibrations, is now found near 3290 $cm^{-1}$. These observations suggest that all ester carbonyl groups in the crystals grown from *o*-dichlorobenzene are only weakly hydrogen bonded (perhaps to residual solvent), and that there are still two distinct types of amide carbonyl groups—one strongly and the other weakly hydrogen bonded. Such features agree with the structure in polar solvent proposed independently [conformation B in (*4*) and conformation II-1 in (*5*)] on the basis of nuclear magnetic resonance, infrared, and optical rotatory dispersion measurements and energy calculations; in this conformation valinomycin has six semifree ester carbonyl groups, and three semifree and three intramolecularly hydrogen bonded amide carbonyl groups.

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15. We have obtained Raman spectra of valinomycin recrystallized from *p*-dioxane solution (nonpolar); these spectra resemble the *o*-dichlorobenzene spectra and hence suggest that this new conformation of valinomycin is stable in more than one environment (*10*).
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## Microearthquakes at St. Augustine Volcano, Alaska, Triggered by Earth Tides

Abstract. *Microearthquake activity at St. Augustine volcano, located at the mouth of Cook Inlet in the Aleutian Islands, has been monitored since August 1970. Both before and after minor eruptive activity on 7 October 1971, numerous shallow-foci microearthquake swarms were recorded. Plots of the hourly frequency of microearthquakes often show a diurnal peaking of activity. A cross correlation of this activity with the calculated magnitudes of tidal acceleration exhibited two prominent phase relationships. The first, and slightly more predominant, phase condition is a phase delay in the microearthquake activity of approximately 1 hour from the time of maximum tidal acceleration. This is thought to be a direct microearthquake-triggering effect caused by tidal stresses. The second is a phase delay in the microearthquake activity of approximately 5 hours, which correlates well with the time of maximum oceanic tidal loading. Correlation of the individual peaks of swarm activity with defined components of the tides suggests that it may be necessary for tidal stressing to have a preferential orientation in order to be an effective trigger of microearthquakes.*

Earth tides with amplitudes characteristically of the order of $10^3$ newton $m^{-2}$ are the largest short-period cyclic stresses in the earth's crust. It has long been thought that they may act as a trigger for the release of tectonic or volcanic stress, but a convincing demonstration of such a cause-and-effect relationship has been elusive. The results of most investigations (*1*) designed to show correlations between tectonic or volcanic events and earth tides have been either inconclusive or negative. A possible exception with respect to earthquakes was reported by Ryall *et al.* (*2*) who found a significant correlation between microearthquakes in an aftershock sequence and the principal diurnal tidal component.

With respect to volcanoes, Eggers and Decker (*3*) have suggested that semiannual and annual clustering of worldwide volcanic activity may be related to corresponding tidal periodicities. Johnston and Mauk (*4*), in a more detailed study, found a definite correlation between the periodicity in the eruptions of Mount Stromboli, Italy, and the fortnightly component of the tides. A similar relationship was reported by Mauk and Johnston (*5*) on the basis of an investigation of a nearly complete catalog of volcanic eruptions from 1900 to 1971. The imprecise determination of eruption times, however, precluded an analysis with the use of diurnal or semidiurnal tidal components.

On the basis of these earlier investigations, we believe that three important conditions must be satisfied if tidal triggering of stress release is to occur or to have a reasonable probability of being discovered, or both: (i) the tectonic stress level must be sufficiently great, and the rate of accumulation of tectonic stress must be sufficiently smaller than the periodicity of the tides in order for the small-amplitude tidal perturbations to act as a triggering agent; (ii) the geographic distribution of events should be small so that there is likely to exist a spatially homogeneous mechanism that will respond in a similar manner to the repetitive tidal perturbations; and (iii) the events should be of sufficiently shallow focus so that the brittle fracturing nature of the rock is maximized. All of these conditions may prevail in aftershock zones and volcanic regions.

St. Augustine volcano, located at