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# Intramolecular $^1$H Nuclear Overhauser Effect Study of the Solution Conformation of Valinomycin in Dimethyl Sulfoxide†

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ABSTRACT: Determination of the mechanism of intramolecular nuclear Overhauser effects (NOE) in peptides and depsipeptides is essential to the use of this technique in conformational analysis of these and related biomolecules. Towards this end, $^1$H NMR double-resonance studies were conducted on valinomycin in $(CD_3)_2SO$ at 90 MHz (FT mode) and 250 MHz (correlation mode). The NOE's are positive at the lower frequency and negative at the higher frequency. Consideration of the theoretical dependence of the NOE on the proton–proton internuclear correlation time and on the resonance frequency indicates that these results are explained by a predominantly dipolar relaxation mechanism. It is demonstrated that exchange modulation of scalar coupling does not contribute significantly to the NOE. A formalism for the NOE's of loosely coupled spin systems is presented which takes into account the effects of high magnetic-field strengths and long correlation times. An approximate analysis of the NOE data assuming a single correlation time for the entire molecule and ignoring cross-relaxation effects was used to evaluate various models that have been proposed for the conformation of valinomycin. The III-1 model of Patel and Tonelli (Patel, D. J., and Tonelli, A. E. (1973), *Biochemistry 12,* 486) fits the NOE and peptide N*H*C$^\alpha$*H* coupling constant data and is probably a preferred orientation in dimethyl sulfoxide. These experiments illustrate how intramolecular NOE data provide a valuable auxiliary method to other techniques for delineating the preferred solution conformation of peptides, depsipeptides, and other biomolecules.

The intramolecular nuclear Overhauser effect (NOE,[1] change in the intensity of one resonance when another resonance is irradiated) is a sensitive probe of molecular geometry which has yielded information about the detailed conformation of small molecules (Noggle and Schirmer, 1971). Few comparable $^1$H NOE studies of peptide hormones, peptide and depsipeptide antibiotics, and proteins have been attempted because the small effects anticipated for these relatively large molecules require a precision not attainable with previously available instrumentation. The recent development of Fourier transform (reviewed by Farrar and Becker, 1971) and correlation (Dadok and Sprecher, 1974; Gupta et al., 1974) techniques for efficient signal to noise enhancement now makes possible both intramolecular (Sykes et al., 1974; Campbell et al., 1974) and intermolecular (Balaram et al., 1972a,b; Pitner et al., 1974, 1975a,b; Glickson, 1975; Glickson et al., 1976) homonuclear $^1$H NOE conformational studies of complex

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[1] Abbreviations used are: NOE, nuclear Overhauser effect; Hyv, hydroxyvaleric acid; Lac, lactic acid; $T_1$, spin–lattice relaxation time; NMR, nuclear magnetic resonance; rf, radiofrequency.

molecules. Gibbons et al. (1975) recently reported 250 MHz $^1$H NOE measurements of the decapeptide antibiotic gramicidin-S in $(CD_3)_2SO$. The observation by these investigators of negative Overhauser effects (decreased resonance intensity) for spatially proximal backbone nuclei on adjacent residues of this decapeptide suggests that the intramolecular NOE holds considerable promise for elucidating the secondary structure of polypeptides.

Any meaningful interpretation of such Overhauser measurements requires that the detailed mechanism of these phenomena first be elucidated unambiguously. The suggestion by Gibbons et al. (1975) that the negative NOE's observed originate from exchange modulation of scalar coupling does not appear plausible in view of the availability of only trace quantities of exchangeable protons in dimethyl sulfoxide and the small magnitude of the relevant coupling constants (vide infra). Dipole–dipole interactions appear to be a much more likely cause of this effect, in view of the predominance of the dipolar mechanism in the relaxation of macromolecules (Allerhand et al., 1973). The long rotational correlation times of such molecules can yield negative dipolar NOE's at sufficiently high resonance frequencies (Balaram et al., 1972a,b). It is important to distinguish between these mechanisms. If dipole–dipole interactions predominate, much structural and dynamic information can be extracted from NOE experiments; interpretation of scalar mechanisms yields much less useful information. For macromolecules of intermediate size, the theory of dipole–dipole relaxation (Solomon, 1955) predicts that a negative NOE should be observed at high resonance frequencies (e.g., 200 MHz or more), but at lower frequencies (e.g., 100 MHz or less) positive NOE's (increase in resonance intensity) should occur. By contrast, exchange modulation of scalar coupling always yields a negative NOE. Consequently, for molecules of appropriate size dipolar and scalar mechanisms may be distinguished by the frequency dependence of the NOE.

This approach has been employed in this study of valinomycin, a dodecadepsipeptide antibiotic, which was chosen for this investigation for the following reasons: (1) it has an appropriate correlation time to exhibit NOE's of different sign at 90 and 250 MHz, respectively, in $(CD_3)_2SO$, as indicated in a preliminary investigation (Pitner et al., 1976); (2) its $^1$H NMR spectrum consists of relatively simple, well resolved resonances, which have been unambiguously assigned to specific protons (Haynes et al., 1969; Shemyakin et al., 1969; Ohnishi and Urry, 1969; Urry and Ohnishi, 1970; Ovchinnikov et al., 1971; Patel and Tonelli, 1973); and (3) valinomycin is one of the most documented depsipeptides available. Detailed quantitative evaluation of the NOE data is facilitated by the availability of precise descriptions (torsion angles or coordinates) of specific models proposed for the conformation of this antibiotic.

*cyclo* (−NHC$^{\alpha}$HC′OO−C$^{\alpha}$HC′O−NHC$^{\alpha}$HC′OO−C$^{\alpha}$HCO−)$_3$

| | | | |
|---|---|---|---|
| $H_3CC^{\beta}HCH_3$ | $H_3CC^{\beta}HCH_3$ | $H_3CC^{\beta}HCH_3$ | $CH_3$ |
| L-Val | D-Hyv | D-Val | L-Lac |

The present study demonstrates that at 250 MHz valinomycin exhibits negative NOE's similar to those observed by Gibbons et al. (1975) for gramicidin-S, while at 90 MHz valinomycin yields positive NOE's, clearly indicating a dipolar relaxation mechanism. Various models for the preferred solution conformation of valinomycin in dimethyl sulfoxide have been evaluated semiquantitatively in terms of an approximate equation applicable to nuclei relaxing by a dipolar mechanism in which only the first nearest-neighbor interactions between methine C*H* or peptide N*H* protons are significant. This first-order analysis, which is justified, at least approximately, by an examination of the NOE data, permits a comparison of the observed NOE's with NOE's predicted for each of the proposed conformations. In this preliminary analysis, conformational averaging has been ignored. A rigorous computer analysis of the data, taking into account all the dipolar interactions as well as conformational averaging, is now in progress in our laboratory. The present study, however, illustrates that even a first-order analysis can, in conjunction with other spectral data, particularly peptide N*H*–C$^{\alpha}$*H* coupling constants ($^3J_{NHCH}$), serve as a valuable diagnostic tool for testing proposed structures, as well as a guide for developing new models.

## Experimental Procedures

Sample concentrations were 4% (w/v) valinomycin (Sigma Chemical Co., St. Louis, Mo.) dissolved in $(CD_3)_2SO$ (Stohler Isotopes, Inc., Waltham, Mass.). NMR spectra were measured with 5-mm sample tubes at probe temperatures of 29 ± 1 °C; chemical shifts have been referenced to the $^1$H resonance of internal $Me_4Si$.

$^1$H NMR spectra (90 MHz) were measured with a Bruker HX90/18″ spectrometer operating in the pulse Fourier transform mode. NOE's were determined by applying a 1.5-s low-power saturating pulse at the appropriate peak position, followed immediately by a high power 90° observing pulse. Since all the $^1$H $T_1$'s of valinomycin are less than 0.2 s, as determined by the inversion-recovery technique (Vold et al., 1968; Allerhand et al., 1971), the 1.5-s presaturating pulse is long enough to allow Overhauser equilibrium to establish. Off-resonance control spectra were measured in the same manner, except that the presaturating pulse was offset 1000 Hz to low field so that no solute resonances were perturbed. On-resonance and off-resonance spectra are the sums of 32 scans. Difference spectra were obtained by subtracting 256 off-resonance free induction decays from 256 on-resonance free induction decays followed by Fourier transformation. This mode of operation allows the observation of NOE's of coupled protons without the complications produced by coherent double-resonance effects (Kumar and Gordon, 1971; Krishna et al., 1973).

At 250 MHz, spectra were acquired in the correlation mode (Dadok and Sprecher, 1974; Gupta et al., 1974) employing the Carnegie–Mellon superconducting spectrometer (Dadok et al., 1970). NOE measurements were made with continuous saturation of the appropriate resonance. All spectra at this frequency (including those used in difference spectra) are the sum of 15 scans. The 250 MHz NOE's of coupled protons are unreliable because the observing and saturating rf fields are on simultaneously. This complication may be seen in the 250 MHz spectra of Figure 2 for the D-Val C$^{\alpha}$H signal (vide infra).

## Theoretical

*General.* In the NOE experiment, the steady-state NMR spectrum is obtained while a group of protons are saturated by a strong rf field. The fractional increase in intensity of the signal of proton, *i*, due to saturation of a group of protons, S, is denoted by $f_i$(S), and is related to $\langle I_{zi} \rangle$, the average value of the spin of *i* when S is saturated, by

$$f_i(S) = \frac{\langle I_{zi} \rangle - I_{oi}}{I_{oi}} \quad (1)$$

where $I_{oi}$ is the equilibrium value of $\langle I_{zi}\rangle$ in the absence of saturation. For a coupled system of protons, $\langle I_{zi}\rangle$ satisfies the equation of motion (Noggle and Schirmer, 1971)

$$d\langle I_{zi}\rangle/dt = -\sigma_{ii}(\langle I_{zi}\rangle - I_{oi}) - \sum_j{}' \sigma_{ij}(\langle I_{zj}\rangle - I_{oj}) \quad (2)$$

where

$$\sigma_{ij} = W_2(ij) - W_0(ij),\ i \neq j \quad (3)$$

$$\sigma_{ii} = \sum_j{}' \rho_{ij} + \rho_i^* \quad (4)$$

$$\rho_{ij} = 2W_1(ij) + W_0(ij) + W_2(ij) \quad (5)$$

$W_0$, $W_1$, and $W_2$ are the thermal transition probabilities for zero-, single-, and double-quantum transitions, respectively, and the prime on the summations in eq 2 and 4 means $j \neq i$.

The quantity $\sigma_{ii}$ is a measure of the relaxation rate of nucleus $i$, and, in fact, equals $T_1^{-1}$ for nucleus $i$ if all $\langle I_{zj}\rangle$ for which $j \neq i$ are constant during the relaxation process. The quantity $\rho_i^*$ is equal to the relaxation rate of proton $i$ due to internal mechanisms (e.g., spin rotation) or effectively internal mechanisms (e.g., intermolecular dipolar interactions with solvent or dissolved paramagnetic impurities, or dipolar interactions with directly bonded $N^{14}$). The $\sigma_{ij}$, $i \neq j$, produce cross-relaxation and are responsible for the NOE's. It should be noted that the indices in eq 2 refer to individual protons and that terms due to magnetically equivalent protons have not been lumped together.

The steady-state form of eq 2 may be written in terms of the $f_i(S)$ as

$$\sum_j \sigma_{ij} f_j(S) = 0 \quad (6)$$

where

$$f_s(S) = -1 \quad (7)$$

and the subscript s refers to an individual spin in the set S. In an NOE experiment, the $f_i(S)$ are measured and eq 6 allows the computation of the $\sigma_{ij}$. If there are $N$ nonequivalent protons, there are $N(N-1)$ independent $f_i(S)$. The number of $\sigma_{ij}$ unknowns is $(N(N+1)/2) - 1$ (only the relative values of $\sigma_{ij}$ can be determined). Similar considerations apply when there are groups of equivalent spins. Therefore, in principle, the NOE experiment can completely determine the relative values of the $\sigma_{ij}$. Equation 6 may be solved for $f_i(S)$ to give

$$f_i(S) = \sum_s \frac{\sigma_{is}}{\sigma_{ii}} - \sum_n \frac{\sigma_{in} f_n(S)}{\sigma_{ii}} \quad (8)$$

where $n$ refers to protons which are neither irradiated nor observed. The first term or the right side of eq 8 originates from direct interactions between irradiated and observed protons, whereas the second term on the right is caused by indirect spin-polarization effects. This equation reduces to eq 3.6 of Noggle and Schirmer (1971) for dipolar interactions in the limit of short correlation times.

*Dipolar Relaxation.* Direct dipole–dipole interaction between observed and irradiated protons is the most probable cause of negative NOE's in the NMR spectra of macromolecules. The thermal transition probabilities for this mechanism are given by (Noggle and Schirmer, 1971):

$$W_0(ij) = \frac{\gamma_i^2\gamma_j^2\hbar^2}{10 r_{ij}^6} \frac{\tau_c(ij)}{1 + (\omega_i - \omega_j)^2\tau_c(ij)^2} \quad (9a)$$

$$W_1(ij) = \frac{3}{20} \frac{\gamma_i^2\gamma_j^2\hbar^2}{r_{ij}^6} \frac{\tau_c(ij)}{1 + \omega_i^2\tau_c(ij)^2} \quad (9b)$$

and

$$W_2(ij) = \frac{3}{5} \frac{\gamma_i^2\gamma_j^2\hbar^2}{r_{ij}^6} \frac{\tau_c(ij)}{1 + (\omega_i + \omega_j)^2\tau_c(ij)^2} \quad (9c)$$

where $\gamma_i$ is the magnetogyric ratio for nucleus $i$, $\omega_i$ is the Larmor frequency of nucleus $i$, $\hbar = h/2\pi$, where $h$ is Planck's constant, $\tau_c(ij)$ is the rotational correlation time of the internuclear vector between $i$ and $j$ nuclei, and $r_{ij}$ is the length of this vector.

A convenient expression for $f_i(S)$ may be obtained by substituting eq 9 into eq 8. The contributions of the indirect spin-polarization terms are usually small, as appears to be true for valinomycin in dimethyl sulfoxide (vida infra), and can be neglected. In addition, it is assumed that the only contribution to $\rho_i^*$ is the dipolar NH contributions from directly bonded $^{14}$N. The relaxation times for valinomycin in dimethyl sulfoxide are less than a few hundred milliseconds and, assuming no paramagnetic impurities, the other contributions to $\rho_i^*$ should be negligible. Further simplification is obtained if a single correlation time is assumed for all internuclear radius vectors. Under these assumptions, eq 8 becomes

$$f_i(S) = \frac{f_H(H) \sum_s r_{is}^{-6}}{\sum_s r_{is}^{-6} + \sum_n r_{in}^{-6} + \rho_{iN}/\Omega} \quad (10)$$

where

$$f_H(H) = \frac{5 + \omega^2\tau_c^2 - 4\omega^4\tau_c^4}{10 + 23\omega^2\tau_c^2 + 4\omega^4\tau_c^4} \quad (11)$$

and

$$\Omega = \frac{\gamma^4\hbar^2\tau_c}{10} \frac{10 + 23\omega^2\tau_c^2 + 4\omega^4\tau_c^4}{1 + 5\omega^2\tau_c^2 + 4\omega^4\tau_c^4} \quad (12)$$

In eq 10, $\rho_{iN}$ is defined by eq 5, $i$ is an observed proton, $s$ refers to the saturated protons, $n$ to the remaining protons, and N to a directly bonded $^{14}$N. Equation 10 demonstrates that the NOE's are very sensitive to molecular geometry through their dependence on $r^{-6}$. The magnetic field dependence is contained in the expressions for $f_H(H)$, $\rho_{iN}$ and $\Omega$. The ratio $\rho_{iN}/\Omega$ has only a weak magnetic field dependence. The expressions is particularly simple for protons bonded to carbon since then $\rho_{iN} = 0$.

The maximum possible value of $f_i(S)$ for a given $\tau_c$ and magnetic field strength is $f_H(H)$. The enhancement is reduced below $f_H(H)$ because of contributions of the $n$ protons and directly bonded $N^{14}$. Thus, neighboring aliphatic groups can considerably reduce the value of $f_i(S)$ below $f_H(H)$.

A plot of $f_H(H)$ vs. $\tau_c$ is given in Figure 1 for 90 and 250 MHz. As $\tau_c$ increases, $f_H(H)$ goes from a maximal value of 0.5 to $-1$, passing through zero when $\omega^2\tau_c^2 = 5/4$. The range of $\tau_c$ values for which $f_H(H)$ is of opposite sign at 90 and 250 MHz is depicted by the shaded area in Figure 1. For correlation times in the range of $7.1 \times 10^{-10}$ to $2.0 \times 10^{-9}$ s, positive NOE's are observed at 90 MHz and negative NOE's at 250 MHz.

*Scalar Relaxation.* If the saturated and observed protons are coupled by spin–spin interactions, and if one of the protons is undergoing chemical exchange, another mechanism is available for spin–lattice relaxation. For this relaxation mechanism (exchange modulation of scalar coupling) to be effective, the chemical exchange rate must be comparable to

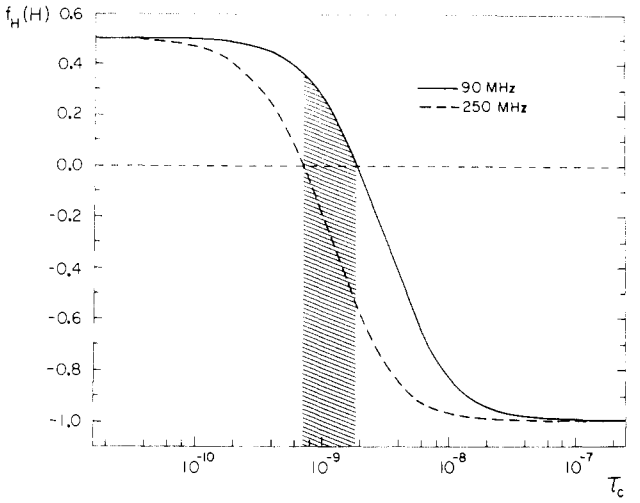


FIGURE 1: Dependence of the homonuclear NOE on correlation time ($\tau_c$) at 90 and 250 MHz. The fractional increase in intensity, $f_H$(H), is indicated on the $y$ axis. The shaded area indicates values of $\tau_c$ for which the NOE has a different sign at the two resonance frequencies.

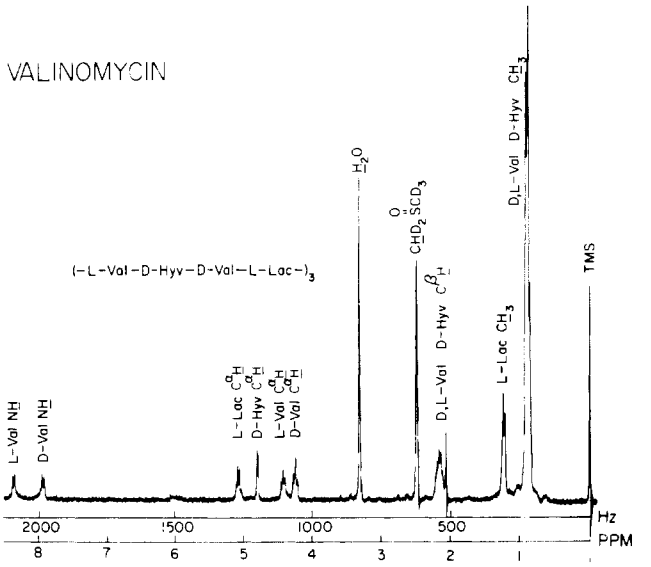


FIGURE 2: 250 MHz $^1$H NMR spectrum of valinomycin in $(CD_3)_2$-SO.

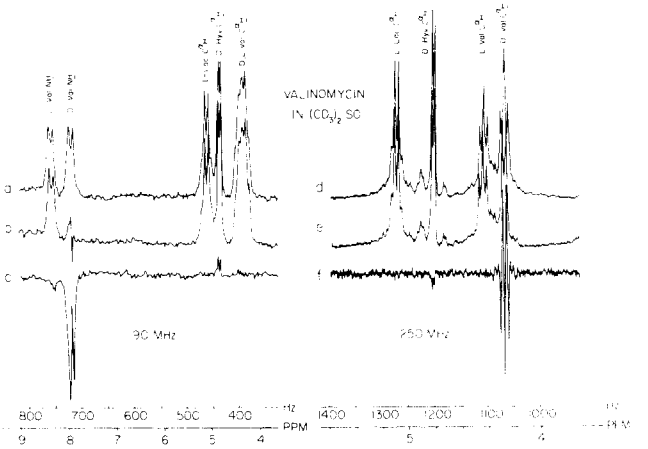


FIGURE 3: NOE measurements of valinomycin in $(CD_3)_2SO$ at 90 MHz by Fourier transform NMR: (a) control spectrum, (b) spectrum obtained after low-power presaturation of D-Val N*H* resonance, (c) difference spectrum (spectrum b minus spectrum a multiplied by a factor of 2.0). NOE measurements at 250 MHz by correlation NMR: (d) control spectrum (secondary rf 1000 Hz to low-field of D-Val N*H* resonance), (e) spectrum obtained with simultaneous saturation of D-Val N*H* resonance, (f) difference spectrum (spectrum e minus spectrum d multiplied by a factor of 3.1).

the difference between the Larmor frequencies of the coupled protons (Noggle and Schirmer, 1971). For this relaxation mechanism,

$$W_1^{\text{exch}}(ij) = W_2^{\text{exch}}(ij) = 0$$

and

$$W_0^{\text{exch}}(ij) = \frac{\pi^2 J_{ij}^2}{2} \frac{\tau_{\text{exch}}}{1 + (\omega_i - \omega_j)^2 \tau_{\text{exch}}^2}$$

where $J_{ij}$ is the scalar coupling constant between protons $i$ and $j$, and $\tau_{\text{exch}}$ is the chemical exchange lifetime of the exchanging proton. Since the relaxation times are in the range of 100 msec, $W_0^{\text{exch}}$ must at least equal 1 for the scalar mechanism to make a significant contribution (e.g., 10%) to $\sigma_{ij}$ for valinomycin in dimethyl sulfoxide in the frequency range 90 to 250 MHz. Typical values of $\tau_{\text{exch}}$ in dimethyl sulfoxide–water are about 100 min (Glickson et al., 1972). Three or four bond coupling constants should be no larger than about 10 Hz. Therefore, at 90 MHz, $W_0^{\text{exch}} \sim 10^{-13}$, and an order of magnitude smaller at 250 MHz. If $\tau_{\text{exch}}$ were equal to $|\omega_i - \omega_j|^{-1}$, the value which maximizes $W_0^{\text{exch}}$, then $W_0^{\text{exch}}$ would equal 0.13 at 90 MHz and 0.04 at 250 MHz, which is still too small, and would require unrealistic exchange lifetimes of $5.3 \times 10^{-4}$ s at 90 MHz and $1.6 \times 10^{-4}$ s at 250 MHz. Looking at this in another way, $J_{ij}$ would have to equal about $1.3 \times 10^3$ Hz at 90 MHz and about $3.5 \times 10^4$ Hz at 250 MHz in order for the scalar coupling mechanism to be effective for $\tau_{\text{exch}}$ equal to 100 min. Even if we assume that $\tau_{\text{exch}}$ equals $|\omega_i - \omega_j|^{-1}$ (the value which maximizes $W_0^{\text{exch}}$), $J_{ij}$ would have to equal ~30 Hz at 90 MHz or ~50 Hz at 250 MHz for the scalar coupling mechanism to be significant for valinomycin. These considerations illustrate why a scalar mechanism cannot make a significant contribution to the observed NOE of valinomycin in the frequency range 90–250 MHz.

## Results and Discussion

*Mechanism of NOE.* The $^1$H NMR spectrum of valinomycin in $(CD_3)_2SO$ at 250 MHz is presented in Figure 2 with assignments previously determined by other investigators cited above. Typical NOE experiments with valinomycin at 90 and 250 MHz are shown in Figure 3. At both proton resonance frequencies, the D-Val N*H* is saturated. At 90 MHz a positive NOE ($f_{\text{D-Hyv C}^{\alpha}\text{H}}$(D-Val NH) = 0.04) is observed for the D-Hyv C$^{\alpha}$*H* without measurable perturbations of other nonirradiated resonances; at 250 MHz intensity changes of observed resonances are again limited to D-Hyv, but at this frequency the NOE is negative ($f_{\text{D-Hyv C}^{\alpha}\text{H}}$(D-Val NH) = −0.02). Table I summarizes NOE data obtained at 90 MHz by successively saturating all the resonances of valinomycin. For the resonances examined at 250 MHz the NOE's are qualitatively similar to those observed at 90 MHz, except for a decrease in magnitude and a reversal in sign at the higher frequency.

These observations can be explained only by the intramolecular dipolar mechanism. Another mechanism which, in principle, might produce negative NOE's is scalar coupling modulated by chemical exchange. However, as shown in the preceeding section, for reasonable values of NH exchange rates and three and four bond NH coupling constants, this mechanism makes a completely negligible contribution to relaxation for valinomycin in $(CD_3)_2SO$. In addition, the scalar coupling

TABLE I: Nuclear Overhauser Enhancements $f_i$(S) of Peptide NH and C$^\alpha$H Protons at 90 MHz.[a]

| Resonance Saturated | Resonance Observed | | | | |
|---|---|---|---|---|---|
| | L-Val N*H* | D-Val N*H* | L-Lac C$^\alpha$*H* | D-Hyv C$^\alpha$*H* | D,L-Val C$^\alpha$*H* |
| L-Val N*H* | | *c* | $0.06_8$ | 0 | 0 |
| D-Val N*H* | *c* | | 0 | $0.04_0$ | 0 |
| L-Lac C$^\alpha$*H* | $0.05_8$ | 0 | | *c* | *c* |
| D-Hyv C$^\alpha$*H* | 0 | 0 | *c* | | *c* |
| D,L-Val C$^\alpha$*H* | 0 | 0 | *c* | *c* | |
| D,L-Val, D-Hyv C$^\beta$H[b] | $0.04_6$ | $0.04_6$ | 0 | $0.05_4$ | $0.09_9$ |
| Lac C*H*$_3$ | 0 | 0 | $0.09_0$ | 0 | 0 |
| Val, Hyv C*H*$_3$ | <0.02 | <0.02 | 0 | $0.07_6$ | $0.06_5$ |

[a] The fractional increase in intensity (±10%) of the observed resonance upon saturation of specific resonances. [b] Peaks overlap; cannot be distinguished. [c] Peaks are too close to saturated resonance to be measured accurately.

mechanism would not predict the observed magnetic field dependence of the $f_i$(S).

As described by Noggle and Schirmer (1971, chapter 3), negative NOE's are also observed for indirect dipole–dipole interactions involving certain orientations of multispin systems (e.g., a linear array). This effect is due to the second term on the right of eq 8. Such negative NOE's are, however, always accompanied by stronger positive NOE's originating from direct dipole–dipole interaction between irradiated and observed nuclei. Since the NOE's all have the same sign at a given resonance frequency (Table I), the negative NOE's observed at 250 MHz cannot be attributed to indirect dipole–dipole interactions. Negative NOE's resulting from dipolar relaxation and long molecular rotational correlation times have been observed in $^1$H NMR studies of complexes of bovine neurophysin II with lysine-vasopressin analogues (Balaram et al., 1972a,b). The effects of long correlation times on $^{13}$C–H NOE's (Allerhand et al., 1973; Allerhand and Oldfield, 1973) and $^{15}$N–H NOE's (Hawkes et al., 1975) have been described by other investigators.

*Conformational Analysis.* Proposed Models. Valinomycin is of considerable biological interest because of its ability to enhance cation permeability of membranes of mitochondria (Pressman, 1965), erythrocytes (Tosteson et al., 1967; Harris and Pressman, 1967), and synthetic lipid bilayers (Andreoli et al., 1967; Mueller and Rudin, 1967). These properties are manifestations of the ability of valinomycin to sequester $K^+$ and $Rb^+$ selectively in preference to $Na^+$ and to serve as a mobile ionophorous carrier of the former cations across a lipid bilayer membrane (Pressman, 1968). Numerous investigations have been directed at explaining these biological properties of valinomycin in terms of the solution conformation of this antibiotic. The conformations of this antibiotic and its metal complexes have been investigated by various spectroscopic techniques (NMR, IR, ORD, CD), dipole-moment measurements, ultrasonic relaxation, x-ray crystallography, and potential energy calculations (reviewed by Ovchinnikov and Ivanov, 1975; and by Ovchinnikov et al., 1974; Karle, 1975a,b; Smith et al., 1975; Neupert-Laves and Dobler, 1975). These investigations indicate that the preferred conformation of valinomycin is very sensitive to experimental conditions.

The general conformational equilibrium of this antibiotic in solution has been described by the scheme: A ⇄ B ⇄ C, where A refers to two conformation, $A_1$ and $A_2$ (proposed to be most stable in nonpolar media), which contain six 4 → 1 hydrogen bonds (Geddes et al., 1968; Venkatachalam, 1968; Ramachandran and Chandrasekaran, 1970; Chandrasekaran et al., 1973); the B states (believed to be favored in less polar media) consist of a number of structures with three 4 → 1 hydrogen bonds; the C states (reportedly preferred in very polar media) are a class of conformers with no internal hydrogen bonds (see reviews by Ovchinnikov and Ivanov, 1975 and Ovchinnikov et al., 1974). Table II summarizes the backbone torsion angles of various models proposed for the solution conformation of valinomycin. Each of the A, B, and C models has the threefold symmetry indicated by NMR data (reviewed by Ovchinnikov et al., 1974). Approximately equivalent structures proposed by different investigators have been designated by a single symbol in this study in accordance with their resemblance to the A, B, and C structures described above. The "pore", "core", and "bear-trap" models proposed by Urry and Ohnishi (1970), for which coordinates are not available, correspond approximately to models $A_1$, $A_2$, and B (i.e., $B_1$ and $B_2$), respectively. The III-1 and C-II models of Patel and Tonelli (1973) (designated as $C_1$ and $C_2$, respectively, in this study) are very similar and differ significantly from their III-2 model (designated here as $C_3$). A number of closely related conformations of crystalline valinomycin (designated in this study as D) are also listed in Table II. The crystalline models lack the threefold symmetry of the other models.

General Analysis of NOE Data. The observed values of $f_i$(S) obtained at 90 MHz by successively saturating the resolved resonances of valinomycin are given in Table I. A general analysis of these data yields specific conformational constraints which must be satisfied by any suitable model of the antibiotic. The relaxation is dominated by the intramolecular dipolar mechanism, and eq 10 should approximately describe the results. An accurate analysis would utilize the coupled set of eq 6. The generally low values of the $f_i$(S) are due to the relatively large reorientational correlation time of valinomycin. It is seen from eq 10 that the $f_i$(S) for any column of a C$^\alpha$H proton should sum to $f_H$(H). Each of these columns sums to approximately 0.2. Using eq 11, this implies that $\tau_c \approx 1.2 \times 10^{-9}$ s. This value of $\tau_c$ predicts proton relaxation times of the order of 100 ms, in agreement with experiment. The lower $f_i$(S) sums for the N*H* columns (~0.1) are attributed to proton–nitrogen dipolar interactions.

The results for irradiation of the various methyl peaks show that the methyl protons are responsible for a substantial portion of the C$^\alpha$H proton relaxation. The small effect produced by the methyl protons on the NH hydrogens is partly due to the NH dipolar interactions.

Irradiation of the C$^\beta$H hydrogens shows that these protons are an effective source of relaxation for all NH protons and the D-Val, L-Val, and D-Hyv C$^\alpha$H protons. The absence of an NOE for the L-Lac C$^\alpha$H proton is not surprising, since lactate has no $\beta$ hydrogens (other than methyls).

The NOE's involving irradiation of NH and C$^\alpha$H hydrogens show a distinctive pattern. For example, irradiation of the L-Val NH proton produces an NOE on the L-Lac C$^\alpha$H proton, but does not affect the L-Val C$^\alpha$H proton. A similar occurrence is true for irradiation of the D-Val NH and L-Lac C$^\alpha$H protons. In each case, the observed NOE originates from a hydrogen on a residue not containing the irradiated proton. This suggests that the NOE reflects a distinct pattern of folding rather than a highly disordered structure. Furthermore, for each irradiated resonance there is at most only one resonance which exhibits an NOE. This argues against extensive conformational averaging.

TABLE II: Torsion Angles[a] and N*H*-C$^{\alpha}$*H* Vicinal Coupling Constants[b] for Various Proposed Conformations of Valinomycin.

| Designation | | L-Val | | D-Hyv | D-Val | | L-Lac |
|---|---|---|---|---|---|---|---|
| This Paper | Literature | $\phi, \psi$ | $^3J_{NHCH}$ (Hz) | $\phi, \psi$ | $\phi, \psi$ | $^3J_{NHCH}$ (Hz) | $\phi, \psi$ |
| $A_1$ | I[c] | 30°, 60° | 6.0, 3.5 | 100°, -60° | -40°, -60° | 7.0, 4.4 | -100°, 60° |
| | $A_1$[d] | 25°, 70° | 5.3, 3.1 | 100°, -30° | 40°, -70° | 7.0, 4.4 | -100°, 40° |
| | C-I[c] | 70°, 110° | 4.6, 4.9 | 70°, 30° | 70°, -110° | 4.6, 4.9 | -70°, -30° |
| $A_2$ | $A_2$[d] | -60°, 135° | 3.0, 3.2 | 75°, 15° | 55°, -110° | 2.3, 2.5 | -70°, -20° |
| | K-valinomycin[e] | -58.6°, 132.2° | 2.8, 3.0 | 81.7°, 2.1° | 57.8°, -130.9° | 2.8, 3.0 | 71.8°, -17.8° |
| $B_1$ | II-1[c] | 30°, 90° | 6.0, 3.5 | 100°, -60° | 140°, 100° | 8.9, 9.3 | -100°, 90° |
| | B[d] | 50°, 65° | 7.7, 4.9 | 100°, -40° | 120°, 85° | 10.0, 10.4 | -60°, 120° |
| $B_2$ | II-2[c] | -140°, -100° | 8.9, 9.3 | 100°, -60° | -40°, -60° | 7.0, 4.4 | -100°, 90° |
| $C_1$ | III-1[c] | -150–(-140°), 70–140° | 7.7–8.9, 8.1–9.3 | 80–90°, 80–(-60°) | 140–150° 140–(-70°) | 8.9–7.7, 9.3–8.1 | -100–(-90°), 60–80° |
| $C_2$ | C-II[c] | -160°, 160° | 6.2, 6.5 | 110°, -50° | 160°, -160° | 6.2, 6.5 | -110°, 50° |
| $C_3$ | III-2[c] | -90°, 110–130° | 7.7, 8.1 | 70–80°, -180–(-170°) | 90°, -130–(-110°) | 7.7, 8.1 | 70–(-50°), 170–180° |
| | I[f] | -63°, 129° | 3.5, 3.7 | 96°, 3° | 63°, 134° | 3.5, 3.7 | -74°, -6° |
| | | -108°, 78° | 9.6, 10.0 | 146°, -11° | 60°, -135° | 3.0, 3.2 | -98°, 14° |
| | | -67°, 130° | 4.1, 4.4 | 82°, 3° | 108°, -69° | 9.6, 10.0 | -164°, 23° |
| | II[f] | -68°, 130° | 4.3, 4.5 | 99°, -8° | 68°, -134° | 4.3, 4.5 | 75°, -11° |
| | | -110°, 78° | 9.7, 10.1 | 150°, -12° | 63°, -134° | 3.5, 3.7 | -96°, 6° |
| | | -65°, 132° | 3.8, 4.0 | 77°, 8° | 104°, -71° | 9.3, 9.7 | -162°, 27° |
| D | $B_1$[g] | -71°, 130° | 4.7, 5.0 | 98°, -7° | 67°, -136° | 4.1, 4.4 | -71°, -11° |
| | | -110°, 80° | 9.7, 10.1 | 147°, -6° | 54°, -133° | 2.2, 2.3 | -100°, 13° |
| | | -67°, 132° | 4.1, 4.4 | 81°, 3° | 105°, -68° | 9.4, 9.7 | -165°, 31° |
| | $B_2$[g] | -59°, 129° | 2.9, 3.1 | 94°, -5° | 67°, -136° | 4.1, 4.4 | -71°, -9° |
| | | -108°, 78° | 9.6, 10.0 | 147°, -10° | 64°, -134° | 3.6, 3.9 | -97°, 7° |
| | | -66°, 132° | 3.9, 4.2 | 78°, 7° | 108°, -68° | 9.6, 10.0 | -166°, 22° |
| | A[g] | -63°, 128° | 3.5, 3.7 | 98°, -4° | 65°, -134° | 3.8, 4.0 | 77°, -7° |
| | | -102°, 74° | 9.1, 9.5 | 145°, -8° | 65°, -135° | 3.8, 4.0 | -98°, 10° |
| | | -64°, 131° | 3.6, 3.9 | 80°, 2° | 106°, -71° | 9.5, 9.8 | -160°, 21° |

[a] Torsion angles ($\phi, \psi$) are designated in accordance with the convention established by the IUPAC–IUB Commission on Biochemical Nomenclature (Kendrew et al., 1970). [b] The observed values of the vicinal N*H*-C$^{\alpha}$*H* coupling constant corrected for electronegativity differences of the C$^{\alpha}$H carbon substituents are calculated from the equation of Bystrov et al. (1973) (first value shown) and from the equation of Cung et al. (1974) (second value shown). [c] Patel and Tonelli, 1973. [d] Ovchinnikov et al., 1974. [e] Average values of dihedral angles from Neupert-Laves and Dobler, 1975. [f] Karle, 1975a. [g] Smith et al., 1975.

TABLE III: Comparison of Observed and Predicted NOE's for Various Proposed Models of Valinomycin. Tabulated for Each Model is the Closest Methine C*H* or Peptide N*H* to the Saturated Proton(s) (which Yield Resolved Resonances).[a]

| Saturated Proton[b] | Observed NOE | Closest Nonmethyl Proton(s)[c,d] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $A_1$ | $A_2$ | $B_1$ | $B_2$[e] | $C_1$ | $C_2$ | $C_3$ |
| L-$Val_1$ N*H* | L-Lac C$^{\alpha}$*H* | **(*)L-$Val_1$ C$^{\alpha}$*H* | *L-$Val_1$ C$^{\beta}$*H* | *{L-$Val_1$ C$^{\alpha}$*H*, L-$Lac_{12}$ C$^{\alpha}$*H* | L-$Val_1$ C$^{\beta}$*H*, L-$Val_1$ C$^{\alpha}$*H* | L-$Lac_{12}$ C$^{\alpha}$*H* | {L-$Lac_{12}$ C$^{\alpha}$*H*, L-$Val_1$ C$^{\alpha}$*H*, L-$Val_1$ C$^{\beta}$*H* | **{L-$Val_1$ C$^{\beta}$*H*, L-$Val_{12}$ C$^{\alpha}$*H* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D-Val$_3$ N*H* | D-Hyv C$^\alpha$*H* | *(*)D-Val$_3$ C$^\alpha$*H* | **D-Val$_3$ C$^\beta$*H* | *(**) {D-Val$_3$ C$^\beta$*H*, D-Hyv$_2$ C$^\alpha$*H*} | {D-Val$_3$ C$^\beta$*H*, D-Hyv$_2$ C$^\alpha$*H*} | D-Hyv$_2$ C$^\alpha$*H* | {D-Hyv$_2$ C$^\alpha$*H*, D-Val$_3$ C$^\alpha$*H*, D-Val$_3$ C$^\beta$*H*} | {D-Hyv$_2$ C$^\beta$*H*, D-Val$_3$ C$^\beta$*H*, D-Hyv$_2$ C$^\alpha$*H*} |
| L-Lac$_4$ C$^\alpha$*H* | L-Val N*H* | *L-Val$_5$ N*H* | *D-Val$_7$ C$^\beta$*H* | *(*)L-Val$_5$ N*H* | *L-Val$_5$ N*H* | L-Val$_5$ N*H* | *L-Val$_5$ N*H* | *L-Val$_5$ N*H* |
| D-Hyv$_2$ C$^\alpha$*H* | None | *D-Hyv$_2$ C$^\beta$*H* | {*D-Hyv$_2$ C$^\beta$*H*, *L-Val$_5$ C$^\beta$*H*} | *D-Hyv$_2$ C$^\beta$*H* | ** D-Hyv$_2$ C$^\beta$*H* | {*D-Hyv$_2$ C$^\beta$*H*, *D-Val$_3$ N*H*} | *D-Hyv$_2$ C$^\beta$*H* | *D-Hyv$_2$ C$^\beta$*H* |
| L-Val$_1$ C$^\alpha$*H* | None | ***(*) {L-Val$_1$ N*H*, (D-Hyv$_{10}$ C$^\beta$*H*)} | {L-Val$_1$ N*H*, L-Val$_1$ C$^\beta$*H*} | **L-Val$_1$ N*H* | {L-Val$_1$ C$^\beta$*H*, L-Val$_1$ N*H*} | {L-Val$_1$ C$^\beta$*H*, L-Val$_1$ N*H*} | {L-Val$_1$ C$^\beta$*H*, L-Val$_1$ N*H*} | {L-Val$_1$ C$^\beta$*H*, L-Val$_1$ N*H*} |
| D-Val$_3$ C$^\alpha$*H* | None | ***(*)D-Val$_3$ N*H* | {D-Val$_3$ N*H*, D-Val$_3$ C$^\beta$*H*} | ****(*) {D-Val$_3$ C$^\beta$*H*, D-Val$_3$ N*H*} | {D-Val$_3$ C$^\beta$*H*, D-Val$_3$ N*H*} | {D-Val$_3$ C$^\beta$*H*, D-Val$_3$ N*H*} | {D-Val$_3$ C$^\beta$*H*, D-Val$_3$ N*H*} | {D-Val$_3$ C$^\beta$*H*, D-Val$_3$ N*H*} |
| L-Lac$_4$ C*H*$_3$ | L-Lac C$^\alpha$*H* | *(*) {D-Val$_7$ C$^\alpha$*H*, (L-Lac$_4$ C$^\alpha$*H*)} | (**)L-Lac$_4$ C$^\alpha$*H* | **L-Lac$_4$ C$^\alpha$*H* | L-Lac$_4$ C$^\alpha$*H* | L-Lac$_4$ C$^\alpha$*H* | L-Lac$_4$ C$^\alpha$*H* | |

[a] The D, L-Val C$^\alpha$–C$^\beta$ orientations are trans and the D-Hyv C$^\alpha$–C$^\beta$ orientations are gauche ($\chi = -60°$) (Urry and Kumar (1974)). Parentheses denote a discrepancy between data derived from different sets of coordinates corresponding to a given structure. [b] Closest neighbors are indicated for the residue with sequence position indicated by the subscript. Residues are numbered from the amino terminus starting with L-Val. Entries appear in the approximate order of proximity to the irradiated proton. [c] Methine CH and peptide NH hydrogens within about 0.2 Å from the proton nearest to the irradiated proton(s) are indicated. [d] Asterisks denote the number of methyl groups which are approximately as close to or closer to the irradiated proton as is the indicated proton. [e] Distances were calculated using slightly different coordinates than those indicated for model $B_2$ in Table II: $\phi$, $\psi = -140°, -120°; 100°, -60°; 60°, -120°; -100°, 20°$ for L-Val, D-Hyv, and L-Lac, respectively. The angles indicated in Table II failed to produce closure of the dodecadepsipeptide ring.

Evaluation of Specific Models. The presence or absence of NOE's on irradiation of the NH and C$^\alpha$H hydrogens provides a means for testing proposed solution structures for valinomycin. From eq 10, the absence of an NOE may be explained by a large distance between the observed and irradiated protons (decrease $\Sigma r_{is}^{-6}$) or the proximity of methyl groups to the observed proton (increases $\Sigma r_{in}^{-6}$). NOE's can be predicted for each of the models in Table II by simply determining which methine CH or peptide NH proton is closest to the saturated hydrogen(s). For this purpose, a computer was employed to generate Cartesian coordinates of each of the structures whose torsion angles are summarized in Table II. Standard bond lengths and bond angles were employed. Internuclear distances were computed and confirmed by referring to x-ray data available for some of the structures (Neupert-Laves and Dobler, 1975; Karle, 1975a,b; Smith et al., 1975) and by constructing CPK models. Table III compares the observed NOE's with values predicted for the A, B, and C models. Shown for each conformation are the specific hydrogens (other than methyls) which are closest to the irradiated proton. In a number of cases, two or more hydrogens were within 0.2 Å of the shortest internuclear distance to the irradiated proton. These nuclei are shown in their relative order of proximity. In these cases, cross-polarization effects would have to be taken into account in predicting NOE's.

The $C_1$ model (the III-1 structure of Patel and Tonelli, 1973) is in remarkably good agreement with the observed NOE's. When only a single methine C*H* or peptide N*H* of this model is in closest proximity to the site of saturation, this nearest neighbor corresponds to the proton whose resonance exhibits the NOE. For each case for which no NOE is observed, this model predicts that more than one proton (or methyl group) is in very close proximity to the saturated hydrogen. As noted above, interaction with methyl groups would tend to attenuate the NOE exhibited by a proton close to the saturation site, whereas proximity to other protons would produce cross-polarization effects, which would also diminish the magnitude of the intensity enhancement predicted for the closest methine C*H* or peptide N*H* to the irradiated hydrogen. For these reasons, the $C_1$ model is also consistent with those cases for which no NOE was observed.

None of the other models predicts NOE's similar to those observed for valinomycin in dimethyl sulfoxide solution. Both the $A_1$ and $A_2$ models are clearly at variance with the experimental NOE data. This is not surprising in view of the conclusion of other investigators that neither of these structures is favored in dimethyl sulfoxide (Ovchinnikov et al., 1974; Urry and Ohnishi, 1970; Patel and Tonelli, 1973).

Urry and Ohnishi (1970) have proposed that in dimethyl sulfoxide a dynamic equilibrium exists between the $B_1$ and energetically equivalent structures (e.g., $B_2$), which interconvert by way of the A structures. This hypothesis is not supported by the NOE data, which is incompatible with either of the two B structures. For these models, the proton which exhibits the experimental NOE is generally predicted to be in close proximity to the irradiated proton, but numerous other hydrogens are also in the immediate vicinity of the saturation site. Consequently, much more complex NOE's with considerably diminished magnitudes would be predicted for these structures. In addition, Ovchinnikov et al. (1974) have noted that the large N*H*C$^\alpha$*H* coupling constant ($^3J_{NHCH}$) observed for L-Val (7–8 Hz) is inconsistent with the value predicted for the $B_1$ structure. Examination of Table II indicates that this conclusion depends on the choice of equations employed to calculate $^3J_{NHCH}$. The equation of Bystrov et al. (1973) yields

TABLE IV: Comparison of Observed NOE'S for Valinomycin with Values Predicted for Conformation D (see Table III for Details).[a]

| Saturated Proton | Observed NOE | Closest Nonmethyl Proton(s) |
|---|---|---|
| L-Val$_1$ N*H* | L-Lac C$^\alpha$*H* | *L-Val$_1$ C$^\beta$*H* |
| L-Val$_5$ N*H* | | L-Val$_5$ C$^\beta$*H* |
| L-Val$_9$ N*H* | | *L-Val$_9$ C$^\beta$*H* |
| D-Val$_3$ N*H* | D-Hyv C$^\alpha$*H* | *D-Val$_3$ C$^\beta$*H* |
| D-Val$_7$ N*H* | | *D-Val$_7$ C$^\beta$*H* |
| D-Val$_{11}$ N*H* | | D-Val$_{11}$ C$^\beta$*H* |
| L-Lac$_4$ C$^\alpha$*H* | L-Val N*H* | ***(L-Val$_5$ N*H*, D-Val$_7$ C$^\beta$*H*, D-Val$_7$ N*H*) |
| L-Lac$_8$ C$^\alpha$*H* | | *L-Val$_9$ N*H* |
| L-Lac$_{12}$ C$^\alpha$*H* | | *L-Val$_1$ N*H* |
| D-Hyv$_2$ C$^\alpha$*H* | None | *D-Hyv$_2$ C$^\beta$*H* |
| D-Hyv$_6$ C$^\alpha$*H* | | *(*)D-Hyv$_6$ C$^\beta$*H* |
| D-Hyv$_{10}$ C$^\alpha$*H* | | *D-Hyv$_{10}$ C$^\beta$*H* |
| D-Val$_3$ C$^\alpha$*H* | None | **(D-Val$_3$ N*H*, D-Val$_3$ C$^\beta$*H*) |
| D-Val$_7$ C$^\alpha$*H* | | **(D-Val$_7$ N*H*, D-Val$_7$ C$^\beta$*H*) |
| D-Val$_{11}$ C$^\alpha$*H* | | **(D-Val$_{11}$ C$^\beta$*H*, D-Val$_{11}$ N*H*) |
| L-Val$_1$ C$^\alpha$*H* | None | **(L-Val$_1$ N*H*, L-Val$_1$ C$^\beta$*H*) |
| L-Val$_5$ C$^\alpha$*H* | | **(L-Val$_5$ C$^\beta$*H*, L-Val$_5$ N*H*) |
| L-Val$_9$ C$^\alpha$*H* | | **(L-Val$_9$ N*H*, L-Val$_9$ C$^\beta$*H*) |
| L-Lac$_4$ C*H*$_3$ | L-Lac C$^\alpha$*H* | L-Lac$_4$ C$^\alpha$*H* |
| L-Lac$_8$ C*H*$_3$ | | L-Lac$_8$ C$^\alpha$*H* |
| L-Lac$_{12}$ C*H*$_3$ | | *L-Lac$_{12}$ C$^\alpha$*H* |

[a] Distances refer to structure I of Karle (1975a) which is representative of all the crystal structures of metal-free valinomycin (conformation D).

values of 6.0 and 7.7 Hz for the two slightly different $B_1$ orientations given in Table II. This is roughly consistent with the experimental value. The equation of Cung et al. (1974) yields corresponding values of 3.5 and 4.9 Hz, respectively, which are clearly too low. Equations derived by Ramachandran et al. (1971) and Barfield and Karplus (1969) also yield low values of $^3J_{NHCH}$. The temperature dependence of N*H* chemical shifts cited in support of the B structures in dimethyl sulfoxide (Urry and Ohnishi, 1970) is not very conclusive; temperature coefficients are only slightly less than those of *N*-methylacetamide, the model for a solvent-bonded N*H*. Furthermore, these authors noted that the deuterium exchange rates of D-Val and L-Val NH protons were comparable. These data are therefore consistent with the $C_1$ structure, which has no internal hydrogen bonds. For these reasons, the proposal of an equilibrium between B structures interconverting through A structures can probably be rejected.

The $C_2$ and $C_3$ models are considerably less compatible with the experimental NOE data than is the $C_1$ structure. The major discrepancy is with respect to predicted NOE's for the two Val N*H* resonances. In addition to the L-Lac C$^\alpha$H, and the D-Hyv C$^\alpha$H protons, which, as required by the NOE data, are close to the L-Val and D-Val NH protons, respectively, the $C_2$ and $C_3$ models place at least two additional CH hydrogens in close proximity to the two Val N*H*'s. Relatively much smaller NOE's would therefore be expected for these protons. Thus, while there may be some conformational averaging between the various C structures, the equilibrium would be expected to strongly favor the $C_1$ structure.

Table IV summarizes the predicted NOE's for the I structure reported by Karle (1975a,b), which was chosen as representative of the various D structures. Because of the absence of threefold symmetry, each of the 12 residues has been considered separately in this analysis. The D structure can be excluded not only because it lacks the required symmetry, but because for this model predicted NOE's and coupling constants (Table II) are clearly in disagreement with experimental values.

*Conclusions.* The frequency dependence of the NOE clearly indicates that dipole–dipole interactions constitute the predominant mechanism causing intramolecular NOE's of valinomycin in dimethyl sulfoxide. It is likely that this mechanism applies quite generally to peptides and depsipeptides in solution. This is fortunate because the dipolar mechanism yields the most information about molecular geometry and dynamics. The preliminary analysis presented here is based on simplifying approximations that appear to apply to valinomycin under these conditions, but are not expected to be generally valid. This analysis indicates that the $C_1$ model (the III-1 model of Patel and Tonelli, 1973) is most consistent with all the spectral data—NOE and $^3J_{NHCH}$—in dimethyl sulfoxide solution. Computer analysis of each of the proposed models in terms of eq 6 is now in progress in our laboratory, and should provide a definitive test of the proposed models and indicate how they may be refined to give maximal agreement with spectral data. Specific deuteration, particularly of methyl groups, would also increase the magnitude of NOE's and greatly simplify subsequent analysis. It is seen from eq 10 or Figure 1 that the values of NOE's for biomolecules are sensitive to the magnetic field strength. For favorable values of $\tau_c$, high field strengths can lead to larger NOE's (although negative) than can be obtained at low field strengths. It is apparent from these considerations that $^1$H NMR NOE measurements in combination with $^1$H $T_1$ measurements should play a critical role in future studies of the solution conformation of peptides and other biomolecules. The NOE formalism presented here, while applicable to long correlation times in high magnetic fields, is strictly valid only for loosely coupled spin systems. A density matrix description (Abragam, 1961) of NOE's applicable to tightly coupled spin systems with long correlation times will be presented in a separate paper.

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