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^{13}C NMR chemical shifts of the triclinic and monoclinic crystal forms of valinomycin

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Abstract

Two different crystalline polymorphs of valinomycin, the triclinic and monoclinic forms, have been studied by high resolution, solid state ^{13}C CP-MAS NMR spectroscopy. Although the two polymorphs of the crystal are remarkably similar, there are distinct differences in the isotropic chemical shifts between the two spectra. For the triclinic form, the carbon chemical shift tensor components for the alpha carbons adjacent to oxygen in the lactic acid and hydroxyisovaleric acid residues and the ester carbonyls of the valine residue were obtained using the FIREMAT experiment. From the measured components, it was found that the behavior of the isotropic chemical shift, δ_{iso}, for valine residue ester carbonyl carbons is predominately influenced by the intermediate component, δ_{22}. Additionally it was found that the smallest shift component, δ_{33}, for the *L*-lactic acid (*L*-Lac) and *D*-α-hydroxyisovaleric acid (*D*-Hyi) C_α-O carbon was significantly displaced depending upon the nature of individual amino acid residues, and it is the δ_{33} component that governs the behavior of δ_{iso} in these alpha carbons.

Introduction

Valinomycin ($C_{54}H_{90}N_6O_{18}$), a cyclic dodecadepsipeptide depicted in Figure 1, has been the subject of extensive research since it was shown to be a mobile carrier to facilitate ion transport across living and model membranes (Harris and Pressman, 1967; Mueller and Rudin, 1967; Tosteson et al., 1967; Duax et al., 1996). Numerous studies of the conformation of the free peptide and its complexes with a variety of ions have been reported (Haynes et al., 1969; Ivanov et al., 1969; Ohnishi and Urry, 1969; Ohnishi et al., 1972; Grell et al., 1973; Patel and Tonelli, 1973; Davis and Tosteson, 1975; Smith et al., 1975; Karle, 1975; Bystrov et al., 1977; Karle and Flippen-Anderson, 1988). Three distinct crystal forms have been characterized. In 1975, Smith et al. (1975) and Karle (1975) independently obtained X-ray crystal structures of the triclinic polymorph. Smith et al. (1975) also reported the structure of the monoclinic polymorph. The triclinic form shows the existence of two independent conformations in a unit cell. The monoclinic structure includes a disordered octane solvent molecule. An analysis of the conformations of the three independent molecules in these two forms indicates that the gross features of all three are the same, with only minor conformational differences observed (Karle, 1975). Karle (Karle and Flippen-Anderson, 1988) subsequently characterized a third orthorhombic form, obtained from DMSO, which shows a distinctly different structure possessing nearly three-fold symmetry. This orthorhombic structure also includes a DMSO solvent molecule in the crystal.

High resolution solid-state ^{13}C nuclear magnetic resonance (CP-MAS NMR) has been demonstrated to be a very powerful tool for structural analyses of organic solids, especially in the study of poly-

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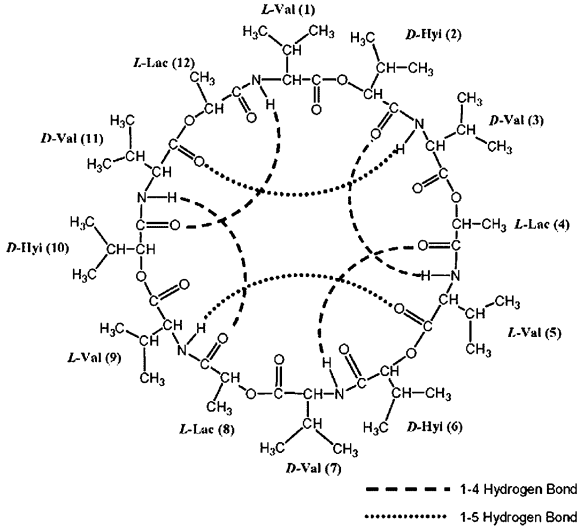

Figure 1. Structural formula of valinomycin. The hydrogen bonding discussed in the text is indicated by the dashed lines.

morphism (Threlfall, 1995; Harper and Grant, 2000). It has also been demonstrated that CP-MAS NMR spectroscopy is a very powerful tool for the structural analysis of peptides in the solid state (Ando and Kuroki, 1996). However, only one high-resolution solid-state NMR study of valinomycin has been reported (Tabeta and Saito, 1985). Consequently, little progress has been made in understanding the relationship between the secondary structure of valinomycin and its corresponding NMR chemical shifts.

In principle, the chemical shift is expressed as a second rank tensor (Mehring, 1983). Thus, the chemical shift tensor components, or principal values δ_{11}, δ_{22} and δ_{33}, have potentially more detailed structural information compared with the averaged isotropic chemical shift, δ_{iso}, available from the CP-MAS. Tensor components have been closely associated with the electronic structure. For example, previous studies have shown that the principal values of the amide C=O carbon chemical shift tensors of peptides and polypeptides depend on the secondary structure (Kameda, 1996; Ando, 1997, 1998).

The principal values are available from a powder pattern that can be obtained from a stationary or slowly spinning sample provided the molecule is small enough that the spectrum can be interpreted. Unfortunately, overlap of more than three to four broad powder patterns often prevents the resolution necessary for the identification and measurement of the respective principal values. This problem of spectral overlap has been addressed, however, by a number of 2D techniques which separate the anisotropic chemical shift information on the basis of the isotropic shift (Orendt, 1996), allowing for the simultaneous measurement of all of the shift tensors. One such family of techniques are based on the magic-angle-turning (MAT) method (Gan, 1994), and includes the FIREMAT (Hu et al., 1994; Alderman et al., 1998) experiment. The combination of FIREMAT and the associated TIGER (McGeorge et al., 1997) linear-model technique for processing 2D data used in this work has lead to measurement of chemical shift tensors in compounds with over 50 magnetically inequivalent carbons (J.K. Harper and D.M. Grant, private communication).

In this work, the ^{13}C CP-MAS NMR spectra for the both the triclinic and monoclinic crystalline forms of valinomycin are measured. The assignment of resonances in this spectrum is used to understand the structural correlation to the NMR chemical shifts. In addition, a FIREMAT spectrum was recorded for the triclinic form. From this spectrum, the principal components of the chemical shift tensor were obtained for the ester carbonyl and the alpha carbons adjacent to oxygen (C_α-O) carbons. The relationship of the isotropic shifts to their corresponding chemical shift tensor components for these carbons is explored. Unfortunately, the coupling to the quadrupolar ^{14}N nuclei precludes obtaining the tensor components of the remaining amide carbonyl carbons as well as the remaining C_α carbons in this molecule.

Experimental

Valinomycin was purchased from Sigma Chemical Co. The crystals of the triclinic and monoclinic forms were recrystallized from ethanol-water and *n*-octane solutions, respectively (Smith et al., 1975).

All spectra were recorded at room temperature on a CMX-400 NMR spectrometer with ^1H and ^{13}C Larmor frequencies of 400.122 and 100.620 MHz, respectively. The ^{13}C chemical shift were calibrated indirectly by external adamantane (low frequency line at 29.5 ppm relative to tetramethylsilane $(CH_3)_4Si$). Isotropic chemical shift spectra were obtained using CP-MAS with TOSS (Dixon, 1982) to achieve total suppression of all sidebands, and TPPM (Bennett, 1995) proton decoupling during the acquisition period. The spectra were collected with a spinning speed of 4,000 Hz, a contact time of 2 ms, and a recycle delay of 3 s. Nutation frequencies of 62.5 kHz were em-

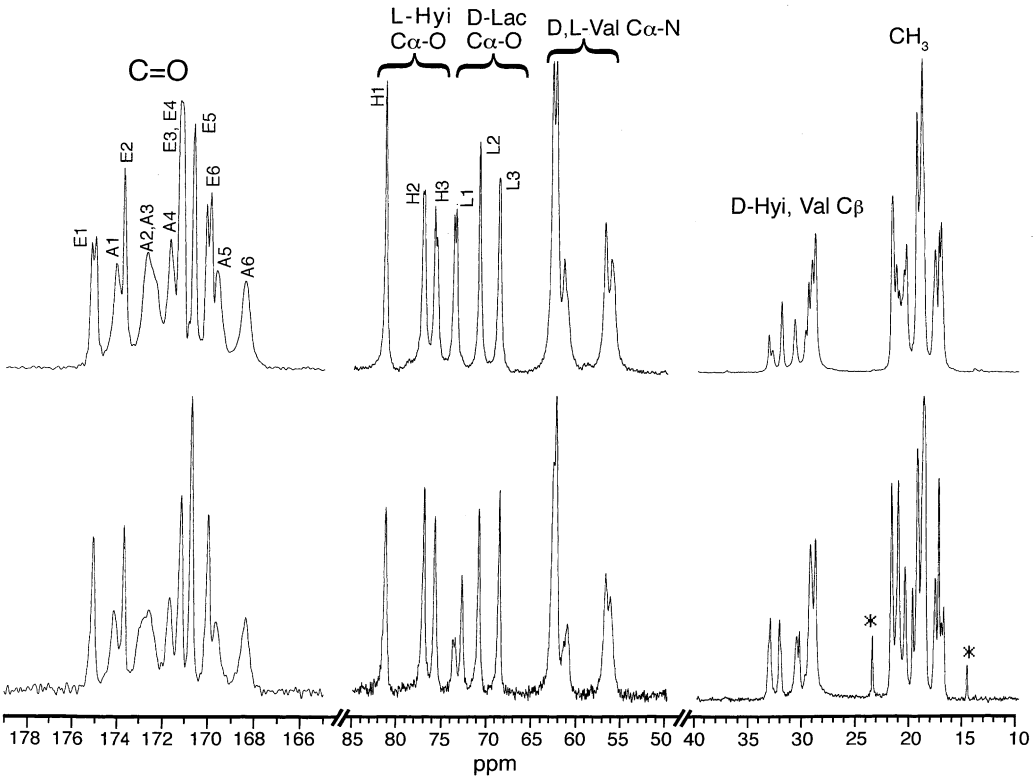

Figure 2. ^{13}C CP-MAS NMR spectrum of valinomycin: (a) triclinic form crystallized from water-ethanol solution, (b) monoclinic form crystallized from *n*-octane solution.

ployed throughout the experiment for the initial proton $\pi/2$ pulses, establishing the Hartman-Hahn matching, and for the carbon π pulses used for encoding the phase information during the evolution dimension. The TPPM decoupling was optimized to a phase angle of 20° and a modulation frequency corresponding to a 180° pulse, again at a nutation frequency of 62.5 kHz. For the acquisition of the FIREMAT data, the spinning rate was stable to 400 Hz $\pm$0.33 Hz with the spectrometer pulses synchronized to the rotor position. The evolution time was equivalent to two rotor periods (5 ms). Additional spectral collection parameters for the FIREMAT dataset included a recycle delay of 3s and a cross polarization contact time of 2 ms. The ^{13}C chemical shift tensor components were obtained from the intensities of the sidebands in the slow MAS spectra by the Herzfeld and Berger method (Herzfeld and Berger, 1980). While an explicit error analysis was not completed on the fitting of this dataset, previous results from our laboratory indicate that the errors in the principal values obtained from the fitting of spinning sideband patterns are typically under 2 ppm (Grant et al., 2000).

Results and discussions

Isotropic chemical shifts

The ^{13}C CP-MAS NMR spectra of crystalline valinomycin in the triclinic and monoclinic forms are given in Figure 2, with the top spectrum (a) from the triclinic sample and the bottom spectrum (b) from the monoclinic sample. While the secondary structures (main chain conformation and hydrogen-bonded structure etc.) of the triclinic and monoclinic forms are remarkably similar (Smith et al., 1975), a comparison of the spectra for these two forms shows only very minor differences. A number of the carbon peaks in the triclinic form are split due to two crystallographically unrelated molecules in the asymmetric component of the unit cell (Smith et al., 1975; Karle, 1975), indicating the sensitivity of the ^{13}C isotropic chemical shifts to either the secondary structure of the cyclic dodecadepsipeptide or slight changes in the crystal packing of the two different molecules. In addition, one of the peaks in the spectrum of the monoclinic form, assigned to a lactic acid residue, also is split into a 2:1

doublet with a difference of nearly 1 ppm between the two peaks. This splitting possibly could be due to the presence of the disordered octane in the crystal. These small shifts due to the crystallographic inequivalence emphasize the utility of solid NMR as these differences are lost in a solution state analysis. Additional weak signals corresponding to the presence of the *n*-octane solvent molecule appeared in the spectrum of the monoclinic polymorph. Two of the four solvent peaks are clearly identifiable and are indicated by asterisks in Figure 2, with the remaining two solvent peaks being within the set of peaks in the C_β region between 28 and 34 ppm. The resolution of resonances that differ by only 0.2 ppm in these CP-MAS spectra illustrates the higher resolution attainable with the use of both higher magnet field strengths and the TPPM decoupling (Bennett et al., 1995). This resolution can be compared with that obtained in a previous CP-MAS study of triclinic valinomycin taken on a 300 MHz spectrometer without TPPM decoupling (Tabeta and Saito, 1985). However, whether the observed increase in resolution is due to different sample preparation, the higher field strength, and/or higher decoupling strength is unknown. The remainder of this paper will focus on the carbonyl and alpha carbon region, as these are the regions of interest for the backbone structure as well as the regions of the spectra where the most chemical shift information can be extracted from the spectra.

The determination of the exact isotropic chemical shifts for all the carbons is complicated by the presence of ^{14}N nuclei in the molecule. Carbon-13 nuclei that are adjacent to a nitrogen atom are manifested in the CP-MAS spectrum as either broad resonances or asymmetric 2:1 doublets due to incomplete averaging of the ^{13}C-^{14}N dipolar coupling (Hexem et al., 1981; Naito et al., 1981) in contrast to the narrow lines observed for the uncoupled carbons. The isotropic ^{13}C chemical shifts of the six alpha carbons that are adjacent to oxygen (C_α-O) that are found in the L-lactic acid (*L*-Lac) and the *D*-α-hydroxyisovaleric (*D*-Hyi) residues as well as the six ester carbonyl of the valine residues (both *L*-Val and *D*-Val) are well resolved singlets (or doublets for the two molecules in the triclinic form in some cases). These peaks are labeled in Figure 2, with the C_α-O lines labeled H1-H3 and L1-L3 and the ester carbonyls E1-E6. In addition, in the carbonyl region tentative values can be extracted for the isotropic of the 6 amide carbonyls, from the peaks labeled A1-A6, as they appear only as broadened resonances instead of doublets. The remainder of the Cα

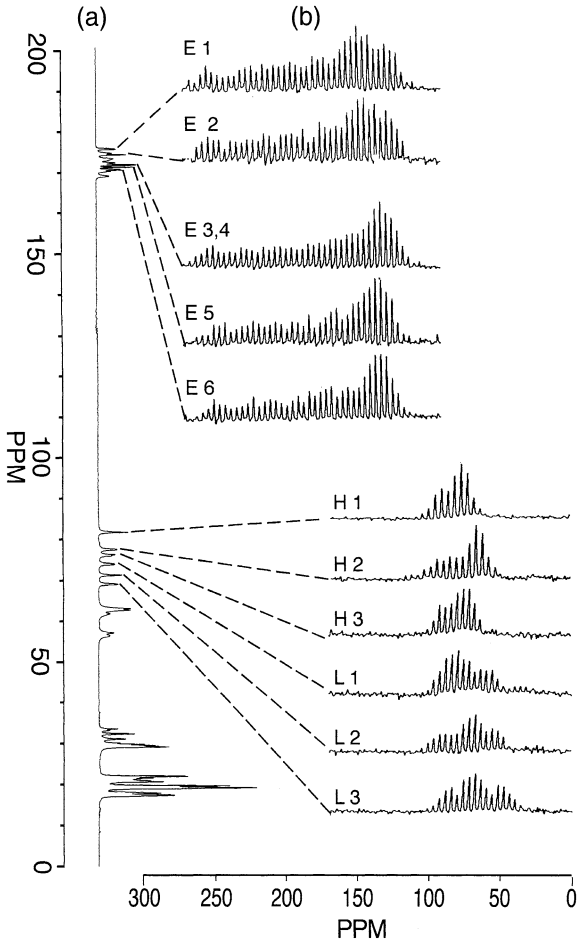

Figure 3. ^{13}C 2D FIREMAT spectrum of triclinic valinomycin. (a) Replicated projection of the 2D spectrum onto the evolution axis (guide spectrum). (b) Acquisition dimension slice corresponding to the eleven peaks for which the tensor components were measured.

isotropic values for those carbons adjacent to nitrogens cannot be determined with any certainty and are therefore not reported.

The six narrow resonances in the alpha carbon region are assigned to the six C_α-O in the *D*-Hyi and *L*-Lac groups. These peaks are split into two sets of three sharp resonances with equal peak intensities. In the earlier CP-MAS paper by Tabeta and Saito (1996) these assignments were based on comparison with solution chemical shifts. The three highest chemical shift resonances of this group, labeled H1-H3 in Figure 2, are assigned to the three *D*-Hyi residues, whereas the three lower chemical shift resonances, labeled L1-L3, are from the three *L*-Lac residues. These assignments are also supported by preliminary calculations of the chemical shifts in valinomycin

Table 1. ^{13}C Chemical shifts for selected carbons in solid state valinomycin

		Monoclinic	Triclinic[a]	Triclinic		
		δ_{iso}	δ_{iso}	δ_{11}	δ_{22}	δ_{33}
C=O						
	D, *L*-Val	175.1	175.2d (E1)	268	146	112
		173.7	173.8 (E2)	269	141	111
		171.2	171.2 (E3)	270	132	112
		170.7	171.2 (E4)			
		170.7	170.7 (E5)	262	135	115
		170.0	170.1d (E6)	263	132	116
	D-Hyi,*L*-Lac	174.2	174.1 (A1)			
		172.7	172.7 (A2,A3)			
		171.7	171.7 (A4)			
		169.7	169.6 (A5)			
		168.4	168.4 (A6)			
C_α-O						
	D-Hyi	81.3	81.4 (H1)	100	78	66
		77.1	77.2 (H2)	105	68	59
		75.9	75.9d (H3)	96	73	58
	L-Lac	72.9d	73.7d (L1)	97	80	45
		71.0	71.0 (L2)	102	68	43
		68.8	68.8 (L3)	98	71	38

[a]The d indicates that this carbon shows a doublet due to the two independent molecules per unit cell. The labels after the chemical shift correspond to those used in Figure 2 and in the text.

(T. Kameda and I. Ando, personal communication); however, further assignments of these resonances to a specific carbon are not possible at this time. It can be commented that the major difference in these isotropic shifts of the *L*-lac and *D*-Hyi residues is most likely due to a substituent effect of the different sidechains.

The carbonyl carbon resonance region also shows six narrow and six broad peaks. Analogous to the arguments made for the alpha carbons, the six narrow resonances, labeled E1 - E6, correspond to ester carbonyl carbons of the six Val residues. Six intramolecular hydrogen bonds are present in the valinomycin molecule (Karle, 1975), as shown in Figure 1. Four of these hydrogen bonds are formed by 'β-turns' ($4 \rightarrow 1$ hydrogen bonds) and are between the N-H of one amino acid residue and the carbonyl oxygen of a residue three groups removed. All of these β-turn hydrogen bonds involve carbonyls adjacent to nitrogens in the *D*-Hyi and *L*-Lac residues. The two remaining hydrogen bonds are the much less common $5 \rightarrow 1$ hydrogen bonds, formed by the ester carbonyl of one *L*- or *D*-Val and the N-H of the second *L*- or *D*-Val, respectively, four amino acid residues away. The $4 \rightarrow 1$ hydrogen bonds are shorter, with an average N···O bond distance of 2.95 Å versus 3.05 Å for the longer and weaker $1 \rightarrow 5$ hydrogen bonds.

As discussed in the literature, the isotropic chemical shift of the carbonyl carbon of a valine residue is predicted by theory to move to a higher chemical shift value when a hydrogen bond is introduced (Tsuchiya et al., 1995). This allows for the tentative assignment of the two furthest downfield peaks (E1 and E2) to these two valine residues. The remaining peaks of (E3-E6) correspond to the carbonyls of the remaining four valine residues that do not participate in hydrogen bonding. As the span of the isotropic chemical shifts is less than 13 ppm, this precludes more specific assignments based on the isotropic shifts at this time.

Principal values of the chemical shift tensor

The 2D FIREMAT dataset of the triclinic sample is shown in Figure 3. The slow-spinning-sideband patterns in the acquisition dimension are separated by the isotropic shift of the resonances in the evolution dimension. Slow spinning sideband patterns are resolved for the six C_α-O (H1-H3 and L1-L3) and five ester carbonyls (E1-E6, with E3 and E4 not resolved).

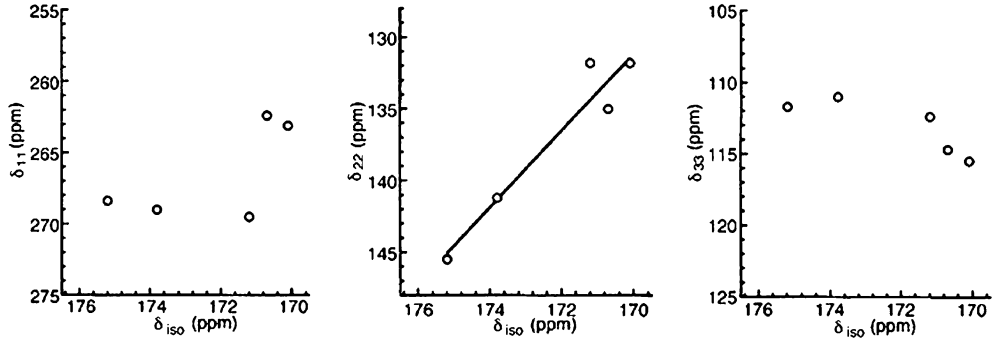

Figure 4. Plots of the observed ^{13}C chemical shift tensor components for the ester carbonyl carbon of the *L*- and *D*-Val residues in Valinomycin against the isotropic chemical shift: (a) δ_{11}, (b) δ_{22}, and (c) δ_{33}.

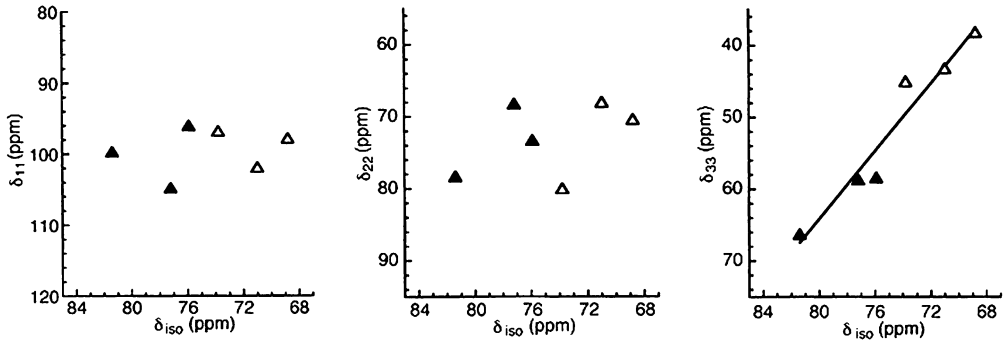

Figure 5. Plots of the observed ^{13}C chemical shift tensor components, δ_{11} (a), δ_{22} (b) and δ_{33} (c) for the C_α-O carbon in *L*-Lac (△) and *D*-Hyi (▲) residues in Valinomycin against the isotropic chemical shift (δ_{iso}).

The tensor values obtained from the fitting of these patterns are listed in Table 1. The remaining spinning sideband patterns are not resolved and/or complicated by the coupling to nearby ^{14}N nuclei, just as in the CP-MAS spectrum. There are instances in the literature where the shift tensors along with information on the ^{13}C-^{14}N coupling have been extracted from FIREMAT datasets (Strohmeier et al., 2002); however, in this compound the magnitude of the coupling and the resolution attained did not allow for further information to be extracted from the spectrum.

For carbonyls in amino acid residues it has been previously reported that the δ_{11} is in the plane containing the CO_2 group and lies along the direction perpendicular to the C=O bond, the δ_{22} component lies almost along the amide C=O bond, and the δ_{33} component aligns in the direction normal to the plane containing the CO_2 group (Gu et al., 1994). In the above discussion of the isotropic chemical shift of the ester carbonyl resonance it was mentioned that a previous theoretical study (Tsuchiya et al., 1995) on amide peptide carbonyl carbons found that the isotropic chemical shifts for valine move to higher values with the presence of a hydrogen bond. It was subsequently found, both in further theoretical studies (Kameda et al., 1996) as well as experimentally (Gu et al., 1994), that the δ_{22} tensor component is responsible for the changes observed in the isotropic chemical shift in peptides when hydrogen bonding is introduced. While only small changes in δ_{11} and δ_{33} were observed as the strength of the hydrogen bond is changed, both δ_{22} and δ_{iso} showed a strong, linear dependence on the length of the hydrogen bond. Consequently, the decrease in δ_{iso} as the hydrogen-bond length is shortened correlated with the decrease in δ_{22}. Similar relationships are found in the carbonyl carbon of the valine residues in valinomycin, and are shown graphically as plots of δ_{11}, δ_{22}, and δ_{33} *versus* δ_{iso} for the valine ester carbonyls in Figure 4. While the difference between the average δ_{11} and δ_{33} components of the valine carbonyl carbons involved in the hydrogen bonding and not involved in hydrogen bonding is about -4 and $+3$ ppm, respectively, the variation in the average of the δ_{22} component of the same carbons is -9 ppm. These experimental results agree nicely

with the predicted behavior discussed for these tensor components (Kameda et al., 1996).

A similar analysis is completed on the chemical shift tensor components for the C_α-O carbons of the *D*-Hyi and *L*-Lac residues. The plots of δ_{11}, δ_{22} and δ_{33} against δ_{iso} are shown in Figure 5(a)–(c), with the shifts for the Hyi residue given in solid marks and those of the Lac residue given in open marks. From these figures and the values in the table, it can be seen that the δ_{33} is most sensitive to the nature of individual amino acid residues. The δ_{33} components of the *D*-Hyi residues are found at higher chemical shifts with respect to those of the *L*-Lac residues. It is interesting to note that there is a linear relationship between δ_{iso} and δ_{33}, independent of the nature of residues. Therefore, the chemical shift change in δ_{iso} is predominantly governed by the δ_{33} components in both of these residues. The variations in the δ_{22} components among the three *L*-Lac residues are also large.

Conclusion

The high-resolution ^{13}C CP-MAS NMR spectra for crystalline valinomycin in both the triclinic and monoclinic form have been measured. The chemical shift tensor components for the carbonyl carbon of the six valine residues and C_α-O carbons of the *D*-Hyi and *L*-Lac residues were also obtained from a 2D FIREMAT dataset. It was found that both the isotropic chemical shift and the corresponding tensor components give useful information relating to the molecular structure of valinomycin in the solid-state. It is clear that the general trends previously observed for carboxylic acid groups for individual amino acids are observed for that residue in a polypeptide. This is extremely useful since this demonstrates the utility of using tensors for the understanding of the secondary structure of these peptides. Further investigations of chemical shift tensor components in ion complexes of valinomycin might prove extremely useful for the elucidation of the molecular mechanism of membranes transport.

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