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Affirmation of Critical Proton Magnetic Resonance Data on the Solution Conformation of the Valinomycin–Potassium Ion Complex†

D. W. Urry* and N. G. Kumar

ABSTRACT: Two hundred and twenty MHz proton magnetic resonance spectra are presented for the valinomycin–potassium ion complex in methanol-d_4 which demonstrate the valyl α-CH–β-CH coupling constants to be 11.0 ± 0.3 Hz. Data reported at 100 MHz are consistent with the 220-MHz data, but the $^3J_{\alpha CH-\beta CH}$ values of 3.5–4.0 Hz, interpreted from the 100-MHz data, are incorrect. Accordingly, proposed conformations which are based on the valyl α-CH–β-CH coupling constants of 3.5–4.0 Hz require revision. Data are presented which show the 11-Hz coupling constant to be maintained over the temperature range from −35 to 74°. This demonstrates the conformation of the complex to be very stable, but more importantly, the results require a conformation in which the α-CH–β-CH dihedral angle of each valyl residue is constrained in a predominantly trans orientation. The proposal of such a conformation is already in the literature.

The solution conformation of the valinomycin–potassium ion complex, (L-Lac-L-Val-D-OHVal-D-Val)$_3$–K^+, has generated a great deal of interest over the last several years. The result is a general consensus on the secondary structure, yet important details required for a complete solution description remain in question.

Starting with a secondary structure of a series of six β turns in which all of the peptide NH protons are hydrogen bonded, the α-CH–β-CH dihedral angle of the valyl residues have been presented as a criterion for delineating between solution conformations (Mayers and Urry, 1972). The vicinal coupling constant, $^3J_{\alpha CH-\beta CH}$, was reported by Ivanov *et al.* (1969) and by Patel and Tonelli (1973) to be 3.5–4.0 Hz whereas a value of 11 Hz was reported by Ohnishi and Urry (1970) and Urry and Ohnishi (1970). This is the difference between predominantly gauche and predominantly trans orientations of the valyl α-CH–β-CH bonds. As indicated by Patel and Tonelli (1973) the observation of a small coupling constant would reverse the choice of conformation made by Mayers and Urry (1972). The point of basic concern is, of course, the correct magnitude of the coupling constant.

Fundamental to the correct interpretation of the experimental data in CD_3OD is the question of whether the valyl α-CH doublets in the proton magnetic resonance spectra are separate or overlapping. The results of Ivanov *et al.* (1969) and of Patel and Tonelli (1973) were at 100 MHz and they took the doublets to be nonoverlapping, whereas those of Ohnishi and Urry (1970) and Urry and Ohnishi (1970) were obtained at 220 MHz and they took the doublets to be overlapping. However, none of the workers published the spectra; each reported only the interpreted value of J.

Experimental Section

Spectra were recorded on a Varian Associates HR-220 spectrometer. Chemical shifts were measured relative to an internal standard of tetramethylsilane. The spectra were carefully calibrated by introducing side bands which were generated by modulation of the tetramethylsilane reference. The probe temperature was measured by determining chemical shift differences between resonances of ethylene glycol or of methanol.

The valinomycin–potassium ion complex was formed by dissolving valinomycin (Calbiochem, San Diego, Calif., Lot No. 010172 and 860031) and a 10% molar excess of KBr in a sample tube using a CD_3OD–H_2O (9:1 v/v) solvent mixture. The sample was then dried under high vacuum and twice dissolved in CD_3OD and redried under high vacuum. This assures complete exchange of the peptide NH proton. The complex was redissolved in CD_3OD and the sample tube was sealed. The same procedure was followed when using KSCN.

Results and Discussion

Magnetic resonance spectra for the α-proton region of the valinomycin–potassium ion complex formed with KBr and KSCN salts are given in Figures 1 and 2. Bracketing the spectra on the left and on the right are modulation side bands precisely positioning the indicated frequencies. In both figures the α-CH proton of the L-lactic acid residue is near 1100 Hz; the α-CH of the D-hydroxyisovaleric acid residue is near 1030 Hz, and near 850 Hz are the α-CH resonances of the L- and D-valine residues. At 74° the solvent peak is near 950 Hz whereas at 3 and 6° it overlaps with the α-CH of the L-lactic acid residue. That the chemical shifts are so similar over a 70° range of temperature (compare Figures 1 and 2) is indicative of a stable complex. This is to be contrasted with the dramatic changes in chemical shift observed in the absence

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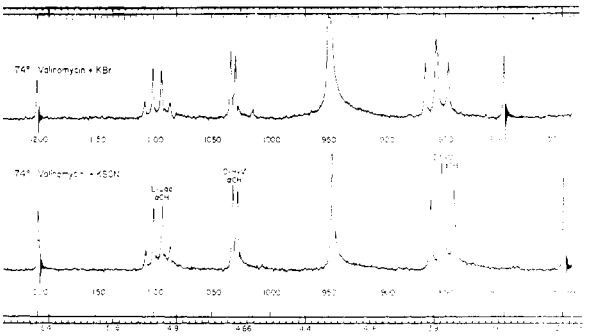

FIGURE 1: The α-proton region of the valinomycin–potassium ion complex in CD_3OD at 74° formed using KBr (above) and KSCN (below). The spectra were taken at 220 MHz and modulation side bands relative to tetramethylsilane were introduced at 1200 and 800 Hz (above) and at 1200 and 750 Hz (below). The complex is essentially the same whether the anion is Br^- or SCN^-. Of specific interest are the resonances near 850 Hz which are the overlapping doublets of the L-Val and D-Val residues with α-CH–β-CH coupling constants near 11 Hz.

of potassium ion (Urry and Ohnishi, 1970). In CD_3OD over the temperature range of −50 to 50° one of the valyl α-CH resonances shifts by 180 Hz. Also demonstrated in Figures 1 and 2 is the similarity of the complex formed whether using KBr or KSCN.

At low temperature (Figure 2) four distinct lines are observed near 850 Hz which are due to the two valyl α-CH doublets. By the individual spectra at 3 and 6° it is not possible to decide which way the lines pair. The line pairs could be separated either by 7.5 Hz in which case the doublets are not overlapping, or by 11 Hz with overlapping. At 74°, however, two of the lines coincide and it becomes apparent that the paired lines forming the doublet are separated by 11 Hz. This is seen more clearly in Figure 3 for the KBr complex.

Again, in Figure 3 the spectra are bracketed with modulation side bands and the temperature is varied stepwise from −35 to 74°. At −35° the lines exhibit slightly different widths; the two nuclei have discernibly different transverse relaxation times. On this basis lines 1 and 3 form one pair and lines 2 and 4 form a second pair. The doublets appear to overlap.

On raising the temperature the separation between the lines, paired on the basis of line width, remains constant whereas the separation between lines 1 and 2, and lines 3 and 4, steadily increases from 6 Hz at −35° to 10 Hz at 74°. At 74° lines 2

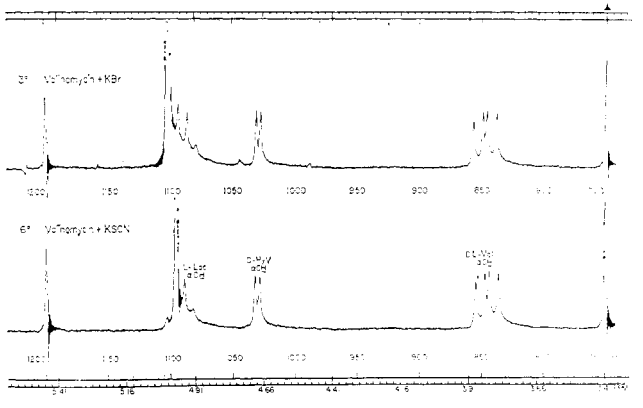

FIGURE 2: The α-proton region of the valinomycin–potassium ion complex in CD_3OD at 3° for the complex formed with KBr and at 6° for the complex formed with KSCN. Modulation side bands were introduced at 1200 and 750 Hz relative to tetramethylsilane. A solvent peak overlaps with the α-CH resonance of the lactic acid residue. (See Figure 3 and text for discussion.)

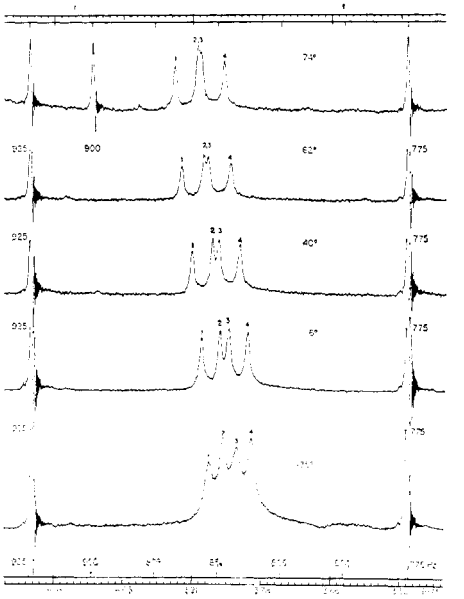

FIGURE 3: Temperature dependence of the chemical shift and coupling constants of the D- and L-Val α-CH resonances. Modulation side bands are at 775 and 925 Hz relative to tetramethylsilane and at 74° a side band is also introduced at 900 Hz. This provides an accurate calibration of the scale and allows ready determination of the magnitude of line separation in the pair of doublets. At −35° lines 1 and 3 and lines 2 and 4 appear to pair on the basis of line width. On raising the temperature the separation of lines 1 and 3 and lines 2 and 4 remains essentially constant whereas the separation of lines 1 and 2 and of lines 3 and 4 steadily increases. At 74° lines 2 and 3 nearly coincides giving a coupling constant for each doublet of between 10 and 11 Hz. See text for further discussion. The dependence of chemical shift is exceedingly small for such a large temperature range and particularly for a cyclododecapeptide. The average shift for the 109° range is less than 0.128 Hz/deg or 0.00058 ppm/deg. This indicates a very stable conformation.

and 3 become nearly superimposed. One either has the unprecedented situation in an organic solvent of a steady increase in the average α-CH–β-CH dihedral angle until it becomes nearly fixed in a trans orientation at 74° or the situation of an α-CH–β-CH angle which is quite rigidly held in a near trans orientation even at 74°. We choose the latter interpretation. Mayers and Urry (1972) described a conformation that calculated to have an approximate 13-Hz coupling constant which exhibited no temperature dependence over the range of interest. For a molecular model approximating that conformation see Figure 5.

For comparison of data obtained at 220 MHz with data obtained at 100 MHz one should be aware that the splitting of lines, *i.e.*, the coupling constants, do not vary with observing frequency but the chemical shifts in terms of ppm do. At 20° the four lines comprising two valine α-CH doublets in CD_3OD occur at 859, 851, 848, and 840 Hz. Depending on the pairing of lines, coupling constants of 8 Hz or of 11 Hz would be indicated. If, as seen in Figure 3, we take 1 and 3 as one pair and 2 and 4 as the second doublet, the centers occur at 853.5 and 845.5 Hz. At 100 MHz these band centers would be at 388 and 384.3 Hz, *i.e.* multiply by 100/220. When the lines are split into the appropriate doublets, *i.e.*, 388 ± 5.5 and 384.3 ± 5.5 Hz, one obtains the four lines of the 100-MHz spectrum at 393.5, 389.8, 382.5, and 378.8 Hz. The splitting of lines 1 and 3, and 2 and 4 are still 11 Hz. If at 100 MHz one takes the doublets to be nonoverlapping, *i.e.*, pairs 1 with 2 and 3 with 4, the coupling constants would be 3.7 Hz. This is indicated in Figure 4. Accordingly the reported values of 3.5–4.0 Hz, while properly giving the distance between

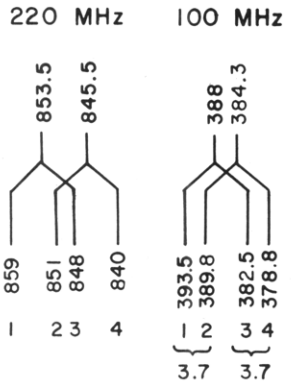

FIGURE 4: Conversion of 220-MHz data on the four lines comprising the two α-CH doublets of D- and L-Val to 100-MHz data under the assumption of the pairing of lines 1 and 3 and lines 2 and 4, *i.e.*, taking the coupling constant to be 11 Hz. At 100 MHz lines 1 and 2 and lines 3 and 4 are each separated by 3.7 Hz whereas the coupling constant remains 11 Hz. This explains previous reports for $^3J_{\alpha CH-\beta CH}$ of 3.5–4.0 Hz based on 100-MHz data as being due to an incorrect assignment of lines comprising the doublet. (See text for detailed discussion.)

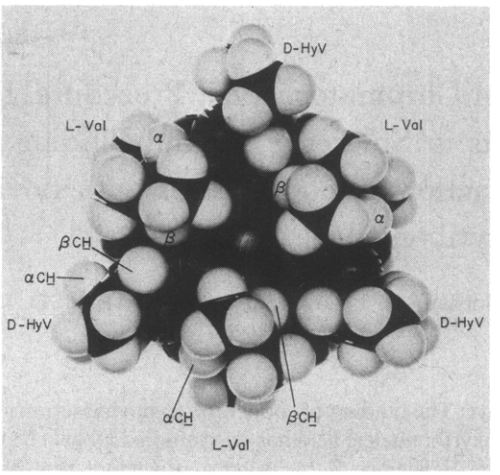

FIGURE 5: Model (L-Val, D-OHV view) of the valinomycin–potassium ion complex as proposed by Ohnishi and Urry (1970). The α-CH–β-CH dihedral angle of Val residues is seen to be predominantly trans. In the CPK model the restriction to rotation is sufficiently striking that a preferred angle of near 160° is apparent, giving an expected coupling constant of 12 Hz.

lines 1 and 2 and between lines 3 and 4, represent an incorrect choice of lines for the doublets. From this analysis it is seen that 100- and 220-MHz data are consistent and the correct $^3J_{\alpha CH-\beta CH}$ (the value which is constant between 100 and 220 MHz) is 11 Hz.

Construction of the molecular model as given in Figure 5 shows the α-CH–β-CH dihedral angle of the valyl residues to be constrained in a predominantly trans orientation. From the CPK model a preferred angle of near 160° is approximated, giving an approximate coupling constant of 12 Hz (Abraham and McLauchlan, 1963). This is consistent with the observed $^3J_{\alpha CH-\beta CH}$ of 11.0 ± 0.3 Hz over the entire temperature range. The α-CH–β-CH dihedral angle of the L-hydroxyisovaleric acid residue exists with only limited constraint in a gauche orientation, consistent with a coupling constant of 4 Hz (see Figures 1 and 2).

It is apparent from Figures 1–4 that conformations of the valinomycin–potassium ion complex in which $^3J_{\alpha CH-\beta CH}$ of the valyl residues would be 3.5–4.0 Hz are inconsistent, in a very significant manner, with the proton magnetic resonance data of the complex in CD_3OD.

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