Conformations of Peptides in Solution by Nuclear Magnetic Resonance Spectroscopy. Part IV.[1] Conformations of Valinomycin determined from Homoallylic Proton Coupling across Peptide Bonds

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100 MHz Proton magnetic resonance measurements have been made on valinomycin in the complexed and uncomplexed form in polar [CD_3OD, $(CD_3)_2N{\cdot}CDO$] and non-polar ($CDCl_3$) solutions. A small five-bond long-range coupling was observed between α-CH groups across the peptide bonds of the L-Lac-L-Val and D-HyIv-D-Val molecular fragments. Observation of ^5J(HH) enables the L- and D-Val α-CH proton signals to be assigned conveniently. Analysis of ^5J(HH) in terms of homoallylic coupling provides information on peptide conformational angles ϕ and ψ, *i.e.* for valinomycin ψ (L-Lac), ϕ (L-Val), ψ (D-HyIv), ϕ (D-Val). The results for both complexed and uncomplexed valinomycin are compared with those of recent crystal structure analyses and with conformational models of these systems in polar and non-polar solvents.

THE conformational properties of the cyclododecadepsipeptide antibiotic valinomycin (VM) † have been studied extensively in the solid state by *X*-ray crystallography,[2-5] ‡ in solution by many physical techniques,[6-17] and by potential energy calculations.[13,18,19] There is

$$\overline{\text{[(-L-Lac-L-Val-D-HyIv-D-Val-)}_3\text{]}}$$

general agreement that the symmetrical, crystal conformation [2,3] of the K^+ complex of valinomycin (VMK^+) is maintained in solution. In this conformation the six ester carbonyl groups (from L- and D-Val residues) are involved in ion–dipole interactions and all amide protons are involved in 1,4-type hydrogen bonding with the six peptide carbonyl groups (from the hydroxy-acid residues) shown by the partial structure in Figure 1. Proton magnetic resonance studies taken in conjunction with potential energy calculations indicate that the crystal conformation of VMK^+ is maintained in CD_3OD solution in agreement with previous work,[13] whereas different conformational properties have been proposed for VMK^+ in dimethylformamide (DMF) solution.[13]

† Abbreviations used: L-Lac L-lactic acid; L-Val L-valine; D-HyIv D-α-hydroxyisovaleric acid; D-Val D-valine; VM valinomycin; VMK^+ valinomycin–K^+ complex.

‡ *Note added in proof:* An independent *X*-ray crystallographic investigation of valinomycin has been published recently (I. Karle, *J. Amer. Chem. Soc.*, 1975, **96**, 4379). The structure is similar to that determined by Smith *et al.*[5] with four intramolecular hydrogen bonds of the 1,4-type and two possible intramolecular bonds of the 1,5-type generating an overall shape of a flattened oval. The conformational angles of the cyclododecadepsipeptide ring [average values for ψ(D-HyIv) −5, −12.5; ϕ(D-Val) 65, 61, 106; ψ(L-Lac) −9, 10, 25; ϕ(L-Val) −109, −66, −65] and calculated ^5J magnitudes for the D-HyIv, D-Val (0.20, 0.25, 0.01 Hz) and L-Lac, L-Val (0.01, 0.35, 0.40 Hz) molecular fragments are similar to those listed for modication A in Table 3. As ^5J (calc) values for the time-averaged conformations of valinomycin [^5J(LL) = 0.25 Hz, ^5J(DD) = 0.15 Hz] are the same as those listed in Table 3 for modifications A, B_1 and B_2, the similarity of the two independent crystal structure determinations of valinomycin adds weight to the present analysis of ^5J(HH) of valinomycin in solution in terms of a flexible backbone structure described by a dynamic equilibrium between 1,4- and 1,5-intramolecular hydrogen-bonded forms.

Although two forms of uncomplexed valinomycin have been crystallised [5] (monoclinic and triclinic),

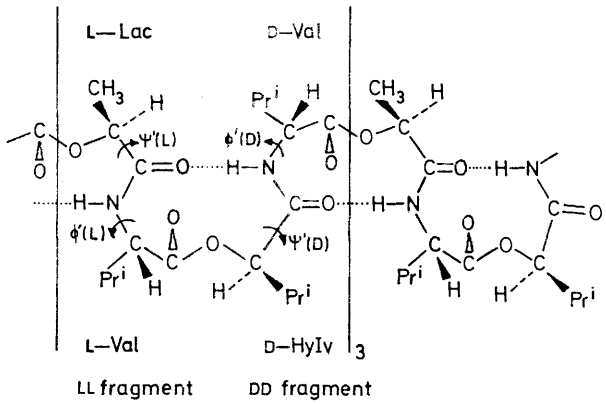

FIGURE 1 Schematic representation of 1,4-hydrogen bonding in valinomycin, including homoallylic torsional angles (ϕ' and ψ') for LL and DD molecular fragments

analysis shows that the conformation of the independent molecules is essentially the same and this conformation is different from the complexed form and from models

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proposed from solution studies. Each structure of VM shows that four amide protons (two L- and D-Val residues) are involved in the usual 1,4-hydrogen bonding of β-turns but that two amide protons (one L- and one D-Val which are centrically related) form weaker 1,5-hydrogen bonds, which distort the doughnut-shaped molecule into an oval.[5] Based on this observed conformation, a mechanism of co-ordinating a potassium ion was postulated.[5] Since the conformation of valinomycin in polar and non-polar media may influence its capacity for binding and release of metal ions, it is necessary to study the solvent-dependent conformations in more detail and to compare the results with the crystal conformation.

A five-bond, long-range, proton spin coupling has been observed between peptide C_α protons of adjacent residues of linear [20,21] and cyclic peptides.[1,22] For a number of cyclic dipeptides ^5J(HH) has been rationalised in terms of homoallylic coupling according to the relation (i), where

$$^5J(\mathrm{HH}) = n\mathrm{A}\sin^2\phi'\sin^2\psi' \qquad \text{(i)}$$

n equals the number of equivalent coupling paths and A is a constant that depends on solvent and the *syn*/*anti* disposition of groups across peptide bonds.[22] The torsional angles ϕ' and ψ' are related to the peptide conformational angles ϕ(N–C_α) and ψ(C_α–C') by the following relationships: $\phi = 240 - \phi'(\mathrm{L}) = 120 - \phi'(\mathrm{D})$; $\psi = \psi'(\mathrm{L}) - 240 = \psi'(\mathrm{D}) - 120$. In this work ^5J(HH) has been observed between α-CH groups of L-Lac-L-Val and D-HyIv-D-Val peptide bond residues of valinomycin. Measurements have been made for both complexed and uncomplexed forms in different solvents (CD_3OD, DMF, and $CDCl_3$). Observation of such coupling can be readily used for assignment of α-CH signals of L- and D-Val, which has previously relied on ^{15}N incorporation of one residue [18] and extensive mixed solvent studies.[13] Assuming that the valinomycin–K^+ complex maintains the same conformation in solution as in the solid state, the observed ^5J(HH) values for VMK^+ are used to calibrate A in equation (i) for depsipeptides; ^5J(HH) values for uncomplexed valinomycin in different solvents are then analysed in terms of different conformational properties in solution. The results are compared with those values predicted from the crystal conformation [5] and various conformational models for valinomycin in different solvents.[13,17]

TABLE 1

N.m.r. parameters of valinomycin in free and complexed forms [a-c]

	Valinomycin			Valinomycin-K^+ complex		
	$CDCl_3$	CD_3OD	[$^2H_7$]DMF	$CDCl_3$	CD_3OD	[$^2H_7$]DMF
D-Val						
δ(NH)	7.83	8.09 [c]	8.00	8.28	8.43 [c]	8.29
^3J(HNCH)	8.0	7.6	7.6	5.0	5.0	5.5
δ(Hα)	4.15	4.29	4.32	3.82	3.84	3.97
^3J(HαHβ)	9.8	9.5	7.5	11.2	10.9	10.0
L-Val						
δ(NH)	7.72	8.18 [c]	8.32	8.40	8.35 [c]	8.41
^3J(HNCH)	6.0	7.8	7.2	5.0	5.2	5.5
δ(Hα)	4.01	4.37	4.38	3.86	3.88	4.00
^3J(HαHβ)	10.0	9.2	6.8	11.1	10.9	10.0
D-HyIv						
δ(Hα)	5.02	4.94	4.94	4.60	4.68	4.76
^3J(HαHβ)	3.2	4.4	4.1	4.0	3.8	4.0
L-Lac						
δ(Hα)	5.30	5.21	5.26	4.94	4.96	5.07
δ(Hβ)	1.46	1.40	1.39	1.54	1.55	1.52
^3J(HαHβ)	6.8	6.8	6.8	7.0	6.8	7.0

[a] 100 MHz ^1H N.m.r. measurements on 0.1M-solutions at 305 K. [b] For D-Val, L-Val, and D-HyIv residues δ(Hβ) ≃ 2.3, δ(Hα) ≃ 1.0. [c] Assignment of NH signals not possible by spin-decoupling experiments in CD_3OD solutions. Assignments made by comparison with other solvents where downfield NH signal is coupled to downfield α-CH(Val) signal.

EXPERIMENTAL

Valinomycin (Sigma) was used without further purification. The valinomycin–K^+ complex was formed by dissolving a slight molar excess of potassium chloride with valinomycin in methanol. The residue from evaporation of the solution was dissolved in $CDCl_3$, and this solution was filtered and degassed before n.m.r. measurements were made; this same solution was evaporated, the residue dissolved in CD_3OD (DMF on the next cycle), and the resulting solution degassed to obtain spectra with narrowest line-widths.

100 MHz ^1H N.m.r. spectra were observed with a JEOL MH100 spectrometer (Me_4Si as lock signal). Spectra were calibrated by using a Racal 801R frequency counter with chemical shifts being referred to internal Me_4Si (Table 1). Internal lock double resonance experiments were performed by using a JEOL SD100 spin decoupler. Long-range proton spin coupling constants, which were determined from at least ten measurements of the differences in line-widths of coupled and decoupled signals observed at 54 Hz sweep width (*i.e.* 1.5 Hz cm^{-1}), are summarised, with error limits, in Table 2.

Signal Assignment.—The 100 MHz ^1H n.m.r. spectrum of VMK^+ (NH deuteriated) in [$^2H_7$]DMF is shown in Figure 2 together with the results of decoupling experiments.

[20] D. B. Davies and M. A. Khaled, *Tetrahedron Letters*, 1973, 2928.

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[22] D. B. Davies and M. A. Khaled, *J.C.S. Perkin II*, 1976, 187.

The assignment of the hydroxy-acid residues of VM follows directly from the coupling patterns of the Lac and HyIv side chains, whereas assignment of the L- and D-Val α-CH residues at about δ 4 needs careful analysis. Unequivocal assignment of the latter signals has been made by ^{15}N-enrichment of one L-Val residue: it was shown [18] that the DMF [D-Val, downfield α-CH, $^3J(\alpha\beta)$ 3.2 Hz; L-Val, upfield, $^3J(\alpha\beta)$ 3.0 Hz]; the difference in peak assignment might account for the discrepancy in the subsequent conclusions regarding the conformations of VMK^+ in DMF solution.

Although long-range coupling constants are used to assign L- and D-Val α-CH signals and also to obtain conformational information, there is no circularity in this approach as a definitive assignment of the L-Val and D-Val α-CH signals has been made by using one ^{15}N-enriched L-Val residue in synthetic valinomycin.[18] Observations of ^5J(HH) giving the same assignment depend on the assumption that no five-bond coupling occurs across the ester bond for the molecular fragments L-Val-D-HyIv and D-Val-L-Lac rather than on a conformational model of the molecule.

TABLE 2

Homoallylic coupling constants (Hz) in valinomycin [a-c]

	Valinomycin–K^+ complex		Valinomycin	
Solvent	^5J(L-Lac, L-Val)	^5J(D-HyIv, D-Val)	^5J(L-Lac, L-Val)	^5J(D-HyIv, D-Val)
$CDCl_3$	0.22 (±0.11)	0.24 (±0.10)	0.25 (±0.10)	0.10 (±0.05)
CD_3OD	0.20 (±0.09)	0.18 (±0.10)	0.07 (±0.09)	0.09 (±0.07)
[$^2H_7$]DMF	0.23 (±0.11)	0.17 (±0.11)	<0.05	<0.05

[a] 100 MHz ^{1}H N.m.r. measurements on 0.1M-solutions at 305 K. [b] Coupling constants determined from line-width changes of at least ten measurements of coupled and decoupled signals (measured under off-resonance and double-resonance conditions, respectively). [c] It has been pointed out by a referee that small coupling constants can be ' decoupled out ' for larger molecules where protons have short relaxation times, and this phenomenon may lead to observation of J values that are smaller than the actual values. In the absence of appropriate relaxation time measurements, the fact that coupling (^5J *ca.* 0.2 Hz) is observed in the K^+-complexed form, which is expected to be in a more rigid conformation than found for uncomplexed valinomycin (^5J *ca.* 0.1 Hz), suggests that the measured spin-coupling constants correspond to the correct values.

upfield NH (and α-CH signals) of VM in CCl_4 correspond to the L-Val residue. Further assignments of VM in different solvents have been made by extensive mixed solvent studies,[13] though this method may lead to incorrect assignment as shown by the present measurements on VMK^+ in [$^2H_7$]DMF. Incorrect assignment also leads to incorrect designation of Val $^3J(\alpha\beta)$, as shown by comparison of 100 and 220 MHz spectra of VM in methanol: it was found [14] that the larger value of peak separations (11.0 Hz) corresponds to $^3J(\alpha\beta)$ instead of the smaller peak separation of 3.2—4.0 Hz.

Assignment of L- and D-Val α-CH signals for VM (complexed or uncomplexed) in different solvents was made by observation of long-range coupling between α-CH groups across the peptide bond, *i.e.* in the L-Lac-L-Val and D-HyIv-D-Val residues. For example, by observation of ^5J(HH) between α-CH L-Lac and L-Val of VM in $CDCl_3$ (0.25 Hz; Table 2), the upfield α-CH signal is assigned to L-Val (δ 4.01; Table 1), in agreement with previous results from ^{15}N-enrichment studies.[18] The assignment was also confirmed by observation of ^5J(HH) between α-CH groups of D-HyIv and D-Val residues. Similar measurements have been made on VM and VMK^+ in different solvents and the results are summarised in Tables 1 (δ, J) and 2 [^5J(HH)]. For two of the three solvents ($CDCl_3$ and DMF) it was found by spin-decoupling that the downfield NH is coupled to the downfield Val α-CH, with the latter signal being assigned by ^5J(HH) observations. In CD_3OD solutions NH exchange precluded their assignment by spin-decoupling experiments and in CD_3OH solutions the residual OH signal obscured the α-CH region, so NH assignments were made by analogy with those in $CDCl_3$ and DMF solutions. The Val α-CH assignments for VMK^+ in [$^2H_7$]DMF differ from those made previously.[13] The expanded spectrum of the Val α-CH region (1·5 Hz cm^{-1}; Figure 2) shows the expected two doublets with $^3J(\alpha\beta)$ either 10.0 or 3.4 Hz depending on the assignment. Irradiation at the frequency of the α-CH(D-HyIv) at δ 4.76 sharpens two peaks of the quartet (separation 10 Hz, D-Val, upfield) whereas irradiation at the frequency of α-CH(L-Lac) at δ 5.07 sharpens the other two peaks (J 10 Hz; L-Val, downfield). This α-CH peak assignment and the $^3J(\alpha\beta)$ value of 10 Hz for L- and D-Val residues differs from that observed previously [13] for VMK^+ in [$^2H_7$]-

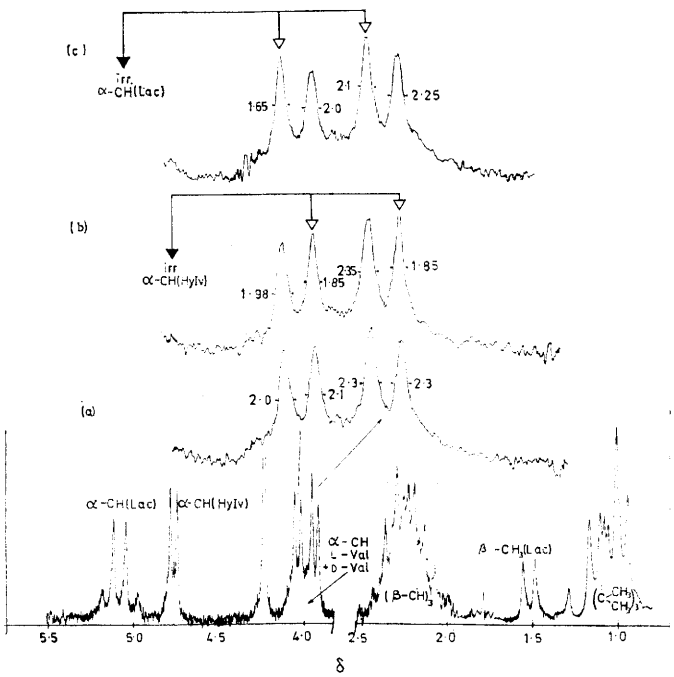

FIGURE 2 100 MHz ^{1}H N.m.r. spectrum of valinomycin–K^+ complex in [$^2H_7$]DMF; inset spectra show (a) Val α-CH region expanded to 1.5 Hz cm^{-1}, and the results of double-resonance experiments with irradiation of (b) α-CH(HyIv) and (c) α-CH(Lac) signals

DISCUSSION

The results in Table 1 show that chemical shifts and spin-coupling constants of valinomycin–K^+ complex

(VMK^+) in chloroform and in methanol are similar; δ(α-CH) values are slightly greater in the more polar solvent (by *ca.* 0.03 p.p.m.) and side chain ^3J values are slightly smaller (*ca.* 0.3 Hz), whereas ^3J(HNCH) values are about the same. In comparison with the other solvents the results for VMK^+ in dimethylformamide (DMF) show significant chemical shift differences (*ca.* 0.13 p.p.m.) for each α-CH signal, which is most likely caused by the preferential interaction of DMF (solvent exhibits magnetic anisotropy) with amide and ester groups. It is also found that ^3J(HNCH) values are slightly greater (*ca.* 0.5 Hz) and side chain ^3J(HH) values slightly smaller (*ca.* 1 Hz) for L- and D-Val residues of VMK^+ in DMF than in the other solvents. Greater variation in δ and J occurs for VM than for VMK^+ in the same three solvents, as shown in Table 1; *e.g.* ^3J(αβ,D-HyIv) varies between 3.2 and 4.5 Hz, ^3J(HNCH) varies between 6.0 and 8.0 Hz, and δ(α-CH) does not show a consistent pattern between $CDCl_3$ and CD_3OD on the one hand and DMF on the other. However, in each case, α-CH signals of VM are downfield (0.2—0.5 p.p.m.) in comparison with VMK^+. These results suggest that the conformation of valinomycin differs from that of the complexed form and that VM exhibits different conformations in the different solvents.

In this work five-bond long-range proton spin-coupling has been observed by spin-decoupling between α-CH groups of L-Lac and L-Val, ^5J(LL), and between α-CH groups of D-HyIv and D-Val, ^5J(DD), in both the free and the complexed form of VM in different solvents as shown in Table 2. Within experimental error ^5J(LL) = ^5J(DD) for VMK^+ in each solvent, but values of ^5J(LL) and ^5J(DD) differ for VM in different solvents. The results indicate that the conformation of VMK^+ is approximately constant in each solvent but that VM exhibits different conformations in the different solvents. The magnitudes of ^5J(HH) for VM and VMK^+ listed in Table 2 can be interpreted in terms of the conformational properties (φ and ψ) of L-Lac-L-Val and D-HyIv-D-Val molecular fragments, if we assume that equation (i) applies to homoallylic torsional angles φ′ and ψ′ of α-CH groups across *trans* peptide * bonds, as found for ^5J(HH) of *cis* peptide bonds in cyclic dipeptides.[22] As it is necessary to calibrate the constant A in equation (i) for α-CH groups in antiperiplanar arrangements across depsipeptide bonds, the results for VMK^+ (^5J in Table 2 and φ,′ ψ′ from the crystal conformation [3]) are used to calculate A^a for LL and DD molecular fragments. The conformational properties of VM in different solvents are then compared with the crystal structure and various solution conformational models.

(a) *Valinomycin–K^+ Complex.*—The crystal structure [2,3] of VMK^+ shows a three-fold symmetry in which L- and D-Val NH groups form intramolecular 1,4-hydrogen bonds with L-Lac and D-HyIv carbonyl groups shown schematically in Figure 1. All six ester carbonyl groups (from L- and D-Val residues) are involved in ion–dipole interactions with K^+ to form a stable complex which is maintained in solution. The conformational angles relevant to homoallylic coupling in L-Lac-L-Val and D-HyIv-D-Val molecular fragments are combinations of ψ(L-Lac) with φ(L-Val) and of ψ(D-HyIv) with φ(D-Val). The magnitudes of φ and ψ in Table 3 † taken from the recent crystal structure of VMK^+ by Neupert-Laves and Dobler [3] show that there is little variation in φ for the three L- or D-Val residues (*i.e.* ±1), but greater variation in ψ for the L-Lac and D-HyIv residues (*i.e.* ±5). The corresponding homoallylic torsion angles are listed in Table 3.

The similarity in δ and J values for VMK^+ in polar (CD_3OD) and non-polar ($CDCl_3$) solvents (Table 1) and the similarity of ^5J(HH) for LL and DD molecular fragments in these solvents (Table 2) indicates that VMK^+ exists in a similar conformation in each solvent. It is assumed that the crystal conformation is maintained in solution and that the torsion angles observed in the solid state characterise the conformation in solution.‡ By using the average value of ^5J(HH) = 0.21 Hz and the values of φ′ and ψ′ for VMK^+ listed in Table 3, A^a was calibrated for the depsipeptide molecular fragment according to equation (i) with $n = 1$. The resulting magnitudes of A^a differed for the molecular fragments [A^a(LL) = 0.60 (±0.12) Hz; A^a(DD) = 0.37(±0.04) Hz] and the difference is outside the error limits calculated from the range of angles observed for the crystal conformation of VMK^+ (Table 3). At present there is no obvious reason for the discrepancy in A^a for LL and DD molecular fragments other than that the crystal conformation is not precisely maintained in solution. The values of A^a were used to analyse observed ^5J(HH) values of valinomycin in different solvents and to compare the results with various conformational models.

(ii) *Valinomycin.*—It has been suggested that VM exists in solution as an equilibrium mixture of three major conformers which are predominant in non-polar, medium-polar, and polar media and which possess six, three, and zero 1,4-type hydrogen bonds, respectively.[26] The existence of other hydrogen-bonded forms (one, two, four, and five) has been suggested by ultrasonic relaxation studies.[27] Supporting evidence for the assumption

* It is assumed that the *trans* peptide bonds observed in the crystal forms [VM, ω = 178 (±6);[5] VMK^+, ω = 178 (±3) [3]] are maintained in solution.

† The values differ from those inferred [5,13] from the *X*-ray structure determination by Pinkerton *et al.*,[2] *i.e.* conformation C-I in ref. 13 and those shown in Figure 6 of ref. 5.

‡ The assumption must be viewed with some caution as the magnitudes of φ (L-Val −58.6; D-Val 57.8) and the corresponding dihedral angles θ(HNCH) (L-Val 118.6; D-Val 117.8) do not predict the observed ^3J(HNCH) of L- and D-Val residues (5.0—6.0 Hz; Table 1) when any of the various Karplus relations are used for this molecular fragment,[23-25] *i.e.* 3.0—3.5 Hz.

[23] M. T. Cung, M. Marraud, and J. Neel, *Macromolecules*, 1974, **7**, 606.
[24] V. F. Bystrov, V. T. Ivanov, S. L. Portnova, T. A. Balashova, and Yu. A. Ovchinnikov, *Tetrahedron*, 1973, **29**, 873.
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[26] Yu. A. Ovchinnikov and V. T. Ivanov, *Tetrahedron Report*, 1975, 2177, and references therein.
[27] E. Grell, F. Eggers, and Th. Funk, *Chimia* (*Switz.*), 1972, **26**, 632.

that VM exists in solution as a rapidly interconverting equilibrium mixture of hydrogen-bonded forms is given by observation of one NH signal and one ^3J(NHCH) value for L- and D-Val residues rather than three individual δ and J values, but contrary evidence [13] is provided by the temperature independence of ^3J(HNCH) for VM in solution, arguing against an equilibrium between conformers with different ^3J(HNCH), and by comparison of the calculated (6.9 D) and observed dipole moments (CCl_4, 3.5 ± 0.1 D; [6] benzene, 3.4 D [13]). The equilibrium properties of VM in solution are difficult to substantiate by n.m.r. spectroscopy as, in the rapid exchange condition, observed n.m.r. parameters depend not only on the conformations available but also on the relative proportions of each. However for those solvent systems where it was concluded that one conformation predominates,[13] such conformations are investigated by comparison of observed and predicted ^5J(HH) magnitudes. The results are also compared with predictions of ^5J(HH) from the crystal conformation.

From a combination of n.m.r. observations and potential energy calculations, Patel and Tonelli [13] have characterised the predominant conformations of VM in different solvents. Other conformations have recently been reviewed.[26,28] For comparison with the results of crystal structures the torsion angles ϕ and ψ of these different conformations have been transformed from the convention suggested by Edsall *et al.*[29] to a standard system.[30] The peptide torsion angles listed in Table 3 indicate quite different conformations for VM in the crystal form and in solvents of different polarity. The corresponding homoallylic torsional angles ϕ' and ψ' were used to calculate ^5J(HH) for the L-Lac-L-Val and D-HyIv-D-Val molecular fragments. The results for VM (Table 3), which predict that ^5J(HH) varies between 0.05 and 0.40 Hz for crystal conformation A and between 0 and 0.11 Hz for different solution conformations, show significant differences from the observed values (Table

TABLE 3

Conformational properties and ^5J(HH) values for crystal and solution forms of valinomycin

	Peptide torsion angles				Homoallylic angles				Predicted coupling constants	
	ϕ(N–C$_\alpha$)		ψ(C$_\alpha$–C′)		ϕ'		ψ'			
	L-Val	D-Val	L-Lac	D-HyIv	L-Val	D-Val	L-Lac	D-HyIv	^5J(LL) (A = 0.60 Hz)	^5J(DD) (A = 0.37 Hz)
Crystal forms										
(i) Valinomycin–K^+ complex [a]	−58.6 (±1.0)	57.8 (±0.5)	−17.8 (±4.9)	2.1 (±4.8)	298.6	62.2	222.2	122.1		
(ii) Valinomycin (modification A) [b]	−64	106	21	2	304	14	261	122	0.40	0.02
	−63	65	−7	−4	303	55	233	116	0.27	0.20
	−102	65	10	−8	342	55	250	112	0.05	0.21
								Mean	0.24	0.14
(modification B_1) [b] Mean									0.20	0.12
(modification B_2) [b] Mean									0.23	0.12
Solution forms										
(i) Valinomycin–K^+ complex										
MeOH (conformer C-I) [c]	−70	70	−30	30	310	50	210	150	0.09	0.05
MeOH [d]	−68	68	−5	5	308	52	235	125	0.25	0.15
DMF (C-II) [c] (Me_2SO)	−160	160	50	−50	40	320	290	70	0.22	0.13
(ii) Valinomycin										
Non-polar [I, octane–dioxan (13 : 1)] [c]	30	−40	60	−60	210	160	300	60	0.11	0.03
Non-polar [(1), $CDCl_3$ with shift reagent] [f]		90		140		30		260		0.09
Non-polar [(2), $CDCl_3$ with shift reagent] [f]		90		−50		30		70		0.08
Cyclic ether [II-1, dioxan, THF, DMF (Me_2SO [e]) <0 °C] [c]	30	140	90	−60	210	340	330	60	0.04	0.03
[II-2, DMF (Me_2SO [e]) > 0 °C] [c]	−140	−40	90	−60	20	160	330	60	0.02	0.03
Polar [III-1; aqueous (dioxan–water, 4 : 1), methanol >30 °C] [c]	−135 [g]	145 [g]	70 [g]	−70 [g]	25 [g]	335 [g]	310 [g]	50 [g]	0.06	0.04
Polar [III-2; aqueous (dioxan–water, 4 : 1), methanol >30 °C] [c]	−90	90	175 [g]	−175 [g]	330	30	55 [g]	305 [g]	0.10	0.06
Polar (methanol <50 °C) [h]	−145	−60	150	−150	25	180	30	330	0.03	0
Polar (methanol <50 °C) [h]	−145	−60	120	−120	25	180	0	0	0	0
Polar (methanol <50 °C) [h]	−95	−60	120	−150	335	180	0	330	0	0

[a] Ref. 3. [b] Ref. 5. [c] Ref. 13. [d] G. N. Ramachandran and R. Chandrasekaran, in ' Progress in Peptide Research,' ed. S. Lande, Gordon and Breach, New York, 1972, p. 195. [e] Conformation in Me_2SO suggested by analogy (ref. 28). [f] Ref. 17; quoted torsional angles (ϕ, ψ) converted to IUPAC–IUB nomenclature for polypeptide conformations (ref. 30) for direct comparison with crystal conformations. [g] Mean values of range. [h] Ref. 28.

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2). For example, in non-polar solvents [I, octane–dioxan (13 : 1)] it is predicted that ^5J(LL) > ^5J(DD), in accordance with observed behaviour, though the calculated values of ^5J(LL) (0.11 Hz) and ^5J(DD) (0.03 Hz) vary markedly from the observed values for VM in $CDCl_3$ solution (*i.e.* 0.25 and 0.10 Hz, respectively).

Recent measurements [5] of the crystal structure of VM have shown that the molecule maintains essentially the same conformation in different crystal habits (*i.e.* monoclinic, one VM per unit cell, modification A, crystallised from n-octane; triclinic, two molecules per unit cell, modifications B_1 and B_2, crystallised from ethanol–water). In contrast to VMK^+ the structure is asymmetric with two D- and L-Val NH groups involved in 1,4-hydrogen bonds and one D- and L-Val NH

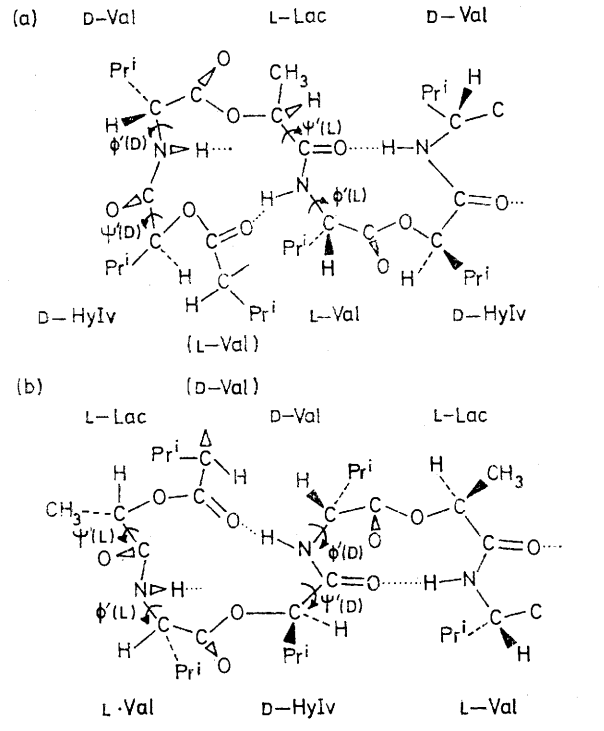

FIGURE 3 Schematic representation of 1,5-hydrogen bonding in valinomycin: (a) L-Val · · · (L-Val); (b) D-Val · · · (D-Val)

group involved in 1,5-hydrogen bonds. The latter are shown schematically for the L- and D-Val residues in Figure 3. This arrangement results in different conformational angles (ϕ, ψ) for each residue in a particular crystal form such that two of the values are markedly different from the third [except ψ(L-Lac) and ψ(D-HyIv)]. Examples of this behaviour are given in Table 3 for L-Val (ϕ −64, −63, and −102) and D-Val (ϕ 65, 106, and 65). The 1,5-hydrogen bonding asymmetric structure is different from all previous models determined by spectroscopic methods.[5] Although different hydrogen bonding properties of the two ester carbonyls were concluded from the splitting of the i.r.[31] and Raman bands [32,33] in the crystal state, the absence of such splitting for VM in non-polar solvents indicated that VM exhibits different conformations in the solid state and in solution.[26] On the other hand, i.r. and n.m.r. studies of VM in CCl_4 solution showed that L- and D-Val NH groups have different hydrogen bonding properties.[16] Hence it is pertinent to compare ^5J(HH) magnitudes predicted from the crystal conformation with those observed in solution.

On the assumption that an equilibrium exists between the 1,4- (two residues) and 1,5- (one) hydrogen-bonded forms and that interconversion between the three forms is rapid on the n.m.r. time-scale, ^5J(HH) was calculated for LL and DD molecular fragments by using ϕ and ψ observed in the crystal conformation. The results for crystal modification A summarised in Table 3 indicate markedly different ^5J(HH) values for individual molecular fragments, *i.e.* ^5J(LL) of 0.40, 0.27, and 0.05 with A = 0.60 Hz and ^5J(DD) of 0.02, 0.20, and 0.21 Hz with A = 0.37 Hz. However, the averages of these values [rapid equilibrium, equal populations; ^5J(LL) = 0.24 Hz, ^5J(DD) = 0.14 Hz] are close to those observed for VM in $CDCl_3$ solution [Table 2; ^5J(LL) = 0.25 Hz, ^5J(DD) = 0.10 Hz], but quite different from those observed in CD_3OD and DMF solutions. Similar results are found for crystal modifications B_1 and B_2 as shown in Table 3. The ^5J(HH) observations of LL and DD molecular fragments of VM in $CDCl_3$ are consistent with the predictions from the time-averaged asymmetric structure observed in the crystal.

This conclusion must be seen in the light of the error involved in the present observations of ^5J(HH) and the fact that the time-averaged structure does not precisely predict ^3J(HNCH) for L- and D-Val residues of VM in $CDCl_3$ from available Karplus relations.[23-25] For crystal modification A the average ^3J(HNCH) of 5.8 Hz for L-Val is close to the observed value of 6.0 Hz [ϕ(L-Val) values of −64, −63, and −102 correspond to θ(HNCH) of 124, 123, and 162 and ^3J(HNCH) of 3.9, 3.7, and 9.9 Hz, as determined by the Karplus relation of Bystrov *et al.*[24]], but the average ^3J(HNCH) value of 6.1 Hz for the D-Val residue is quite different from the observed value of 8.0 θHz [ϕ(D-Val) values 65, 106, and 65 correspond to (HNCH) of 125, 166, and 125, and ^3J(HNCH) of 4.0, 10.2, and 4.0 Hz]. Similar calculations for the other two crystal modifications yield similar results, *i.e.* ^3J(HNCH) for L-Val of 6.7 Hz (B_1) and 5.9 Hz (B_2) and for D-Val of 5.6 Hz (B_1) and 6.2 Hz (B_2). Small differences in calculated ^3J(HNCH) values occur for the different crystal modifications though the values averaged over all three structures are the same, *i.e.* ^3J(L-Val) = 6.1(±0.4) Hz and ^3J(D-Val) = 6.0(±0.2) Hz. Despite the small discrepancy in observed and calculated ^3J(HNCH), to a first approximation, the n.m.r. parameters of VM in $CDCl_3$ solution are consistent with a time-averaged

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conformation of the asymmetric crystal structure containing one 1,5- and two 1,4-hydrogen bonds.

In a recent study of the solution conformation of VM in $CDCl_3$ using lanthanide shift reagents,[17] Servis and Patel have interpreted large changes in ^1H and ^{13}C chemical shift of D-Val and D-HyIv residues in terms of the conformational properties of the D-HyIv-D-Val molecular fragment. Of the two possible conformations [listed as (1) and (2) in Table 3] it was suggested that conformer (1) is more likely from predicted ^3J(HNCH) magnitudes.[17] Neither predicted conformation resembles the crystal conformation observed by Smith *et al.*,[5] nor the conformation previously suggested [13] for VM in non-polar solvents [*i.e.* conformer I, octane–dioxan (13 : 1)] as seen by comparison of the ϕ(D-Val) and ψ(D-HyIv) values listed in Table 3. The conformations determined from shift reagent studies have to be included as possible structures for VM in $CDCl_3$, as both conformations predict magnitudes of ^5J(DD) of *ca.* 0.08—0.09 Hz, similar to the observed value, *i.e.* 0.10($\pm$0.05) Hz (Table 2). Further analysis of those possible conformations awaits analysis of the L-Lac-L-Val molecular fragment from LIS measurements.[17] Measurements of ^5J(HH) of VM in $CDCl_3$ solution made under the same solution conditions of added lanthanide shift reagent provide a method to check if addition of the shift reagent alters the non-polar conformer equilibrium.

The observed ^5J(HH) values for VM in CD_3OD and DMF solutions (Table 2) are sufficiently different from those in $CDCl_3$ solution to suggest that VM exists in different conformations in the different solvents. For valinomycin in dimethylformamide (conformer II-2, >0 °C), both ^5J(LL) and ^5J(DD) are predicted to be small (0.02—0.03 Hz) with ^5J(LL) $\simeq$ ^5J(DD); these values are compatible with the observed small values of ^5J(DMF) <0.05 Hz. For valinomycin in other polar solvents (dioxan–water, methanol, *etc.*) it was concluded [13] that the conformation is not represented by a unique rigid structure but by a rapidly interconverting equilibrium mixture of flexible structures that include conformations III-1 and III-2. As ranges of angles of *ca.* 10° were suggested for these conformers, ^5J(HH) was calculated by using average ϕ' and ψ' values. It is predicted that ^5J(LL) $\simeq$ ^5J(DD) and that the magnitudes of ^5J, although depending on the equilibrium position (III-1 $\rightleftharpoons$ III-2), vary between 0.06 and 0.10 Hz for LL and between 0.04 and 0.06 Hz for DD molecular fragments. Observed ^5J(HH) values (Table 2) are consistent with these predictions, particularly in view of the error involved in the ^5J(HH) measurements and the sensitivity of ^5J(HH) to small changes in angles according to equation (i), *e.g.* a change of 5° in ϕ' and ψ' of conformer III-1 results in variations of ^5J(LL) = 0.06($\pm$0.03) Hz and ^5J(DD) = 0.04($\pm$0.02) Hz. It is concluded that the conformational models for VM proposed by Patel and Tonelli [13] are consistent with observations of ^5J(HH) in DMF (>0 °C; II-2) and CD_3OD (polar; III-1 $\rightleftharpoons$ III-2) solutions but not in $CDCl_3$ (non-polar; I) solutions. It is unlikely that the other polar conformations of VM listed in Table 3 (methanol; <50 °C) are predominant in the conformer equilibrium mixture, as predicted values of ^5J(HH) of 0—0.03 Hz vary considerably from the observed values.

The description of the conformation of VM in CD_3OD and DMF solutions in terms of a rapidly interconverting equilibrium mixture of 1,4- and 1,5-hydrogen-bonded forms can be made in an analogous manner to that for VM in $CDCl_3$. Taking averages over all three crystal modifications (A, B_1, B_2) the two 1,4-hydrogen-bonded forms predict average ^5J(HH) values of 0.32 Hz (LL) and 0.18 Hz (DD) whereas the 1,5-hydrogen-bonded form predicts much smaller values of 0.03 Hz (LL) and 0.02 Hz (DD). Consequently, an observed $^5J(CD_3OD)$ value of *ca.* 0.1 Hz corresponds to *ca.* 75% (LL) and *ca.* 50% (DD) contributions of the 1,5-hydrogen-bonded form; such conformational equilibria correspond to ^3J(L-Val) = 8.7 Hz and ^3J(D-Val) = 7.1 Hz. Although these predicted values are similar in magnitude to the observed values (7.6—7.8 Hz) (Table 2), the expected difference in magnitude, *i.e.* ^3J(L-Val) > ^3J(D-Val) is not observed. Hence further measurements are needed to refine the conformational models of VM in polar solvents, as a number of different models are consistent with observed ^5J(HH) values within the experimental error limits.

Observations of ^5J(HH) values in amides and peptides have been extended to depsipeptides and interpreted in terms of peptide conformational angles ϕ and ψ. Even though limitations of the method result because of the small values so far observed and because the $\sin^2$ dependence in equation (i) means that a number of combinations of ϕ and ψ predict the same ^5J(HH) value, in suitable cases, the magnitudes of ^5J(HH) can be analysed to decide between various conformational models. Taken in conjunction with ^3J(HNCH) measurements (which give information on ϕ), analysis of ^5J(HH) leads to a procedure for limiting the range of the peptide conformational angle ψ. The method can also be applied to give conformational information for peptides in which ^3J(HNCH) cannot be observed, *e.g. N*-substituted peptides and peptides in D_2O solution. In this work it was found that observations of ^5J(HH) served a useful role in assignment of α-CH protons of adjacent residues across peptide bonds, *i.e.* for valinomycin differentiation of L-Lac-L-Val and D-HyIv-D-Val molecular fragments enabled unequivocal assignment of the L- and D-Val α-CH protons under various solution conditions. The method, which is applicable to other linear and cyclic peptides, can be applied in those molecular fragments where combinations of ϕ and ψ lead to an observable ^5J(HH).

Conclusion.—In this work it is suggested from ^5J(HH) measurements (together with similarity in all δ and J values) that the valinomycin–K^+ complex exists in approximately the same conformation in $CDCl_3$, CD_3OD, and DMF solutions and that this conformation is similar to the one observed in the crystal form by Neupert-Laves and Dobler.[3] In agreement with previous suggestions,[13] the present results indicate that valinomycin in solution exists in various conformations depending on the solvent.

We have shown that VM in $CDCl_3$ could exist as the time-averaged crystal conformation of VM (observed by Smith *et al.*[5]) where equal populations of the different intramolecular hydrogen-bonded forms (1,4- and 1,5-) are rapidly interconverting; it is not surprising that the backbone hydrogen-bonded form of VM is maintained in a relatively non-polar environment. We have also found that observed ^5J(HH) values of VM in DMF and CD_3OD solutions are compatible either with the conformational models previously determined from a combination of n.m.r. measurements and potential energy calculations,[13] or with greater contributions of the 1,5-intramolecular hydrogen-bonded fragment rapidly interconverting with the 1,4-form. We have shown that ^5J(HH) observations in the cyclodepsipeptide antibiotic valinomycin have a useful role in determining the conformational properties of the free and complexed form in solution. The method is being extended to other biologically important linear and cyclic peptides.

We thank the British Council for a maintenance award (to A. K.), the University of London Central Research Fund for an equipment grant, and the S.R.C. for providing 220 MHz n.m.r. facilities (P.C.M.U., Harwell).

[6/019 *Received, 5th January,* 1976]