Synthesis and Solution Structure of [Val^3]-HC Toxin by ^{1}H and ^{13}C Nuclear Magnetic Resonance Relaxation Parameters

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The [Val^3] analogue of the fungal tetrapeptide HC toxin has been synthesised. Conformational parameters of the peptide in solution have been measured by a variety of n.m.r. techniques. The ^{1}H and ^{13}C spectral features were assigned by using two-dimensional n.m.r. methods. Accurate backbone distances (proton–proton and proton–carbon) and those relative to the acceptors and donors of the hydrogen bonds were measured by using homo- and hetero-nuclear Overhauser effects and ^{1}H and ^{13}C relaxation parameters. The peptide conformation thus derived was identical with that of the parental toxin.

Modern proton and heteronuclear n.m.r. techniques are now sufficiently advanced and accurate to be used to define the structure of natural products and biological macromolecules in solution. The use of homonuclear nuclear Overhauser effect (n.O.e.) difference spectroscopy[1] and heteronuclear n.O.e. difference spectroscopy,[2] and of selective proton relaxation rates[3] and cross-relaxation rates has permitted conformational analysis by proton–proton[4] and proton–carbon distance measurements.[5] The latter technique has proved very effective for delineating donor and acceptor groups of individual hydrogen bonds.[5]

Here we demonstrate the use of proton–proton distance measurements combined with proton–carbon relaxation studies of hydrogen bonds to delineate the conformation of synthetic [Val^3]-HC toxin, a cyclic tetrapeptide of sequence L-Ala^1-D-Ala^2-L-Val^3-D-Pro^4. All interatomic distances are shown to agree within $\pm$0.15 Å with those determined for analogous peptides by crystallography and n.m.r. measurements.[6,7]

Experimental

Synthesis of (Val^3)-HC *Toxin.*—The protected amino acids were either purchased from Bachem Co. or synthesised according to conventional methods described in the literature.[8] M.p.s and optical rotations were in agreement with those reported. The mixed anhydride method[9] was employed in the synthesis of linear peptides.

Each peptide was purified by recrystallization or chromatography on silica gel after the coupling step, and was shown to be homogenous by t.l.c. and n.m.r.

The linear tetrapeptide Boc-D-Pro-L-Ala-D-Ala-L-Val-OMe was saponified with 3 equiv. of N-KOH in EtOH–H_2O and then coupled to *N*-hydroxysuccinimide with dicyclohexylcarbodiimide. The *N*-deprotected tetrapeptide active ester was then cyclised in high dilution (700 mg in 1.2 l of pyridine). The usual work-up and purification by silica gel column chromatography yielded the desired product.

N.m.r. Measurements.—All the n.m.r. measurements were carried out with a Varian XL-300 instrument; the probe was maintained at 25 $\pm$ 1 °C throughout all the experiments.

Two-dimensional (2*D*) *N.m.r. Spectroscopy.*—The classical pulse sequence 90°–τ–90°–acq was employed for the ^{1}H–^{1}H chemical-shift-correlation experiments;[10] 256 increments of 1 K each were recorded. The second domain was then zero-filled to 512 after acquisition. The same matrix size was used for the NOESY experiment.[11] The mixing time was set to 0.3 s. The ^3J coupling constants were measured from selected 'slices' of a 64 × 2 K, 2D *J* experiment.[12] For the heterocorrelation spectrum we used the phase cycling described by Bax *et al.*[13]

Homonuclear Overhauser Effects.—For these measurements we used a 10mM-solution in order to reduce aggregation. Protons were saturated using a low-power 5 s pulse. The selective relaxation rates were measured by using a selective 180° pulse generated from the decoupler coil and monitoring the recovery of the magnetization as a function of time.[3] Non-selective relaxation times (T_1) were calculated from inversion recovery experiments.

Selective Heteronuclear Overhauser Effects.—Concentrated samples (0.8M) were used for this type of experiment. Selected resonances in the proton domain were irradiated with a low-power pulse for 4 s prior to acquisition in the carbon domain. The BB decoupler was switched on during the acquisition. A relaxation delay of 15 s was allowed between acquisitions; 120 scans were accumulated for the on- and off-resonance spectra.

Results and Discussion

Synthesis of [Val^3]-HC *Toxin.*—The synthesis of the linear tetrapeptide Boc-D-Pro-L-Ala-D-Ala-L-Val-OMe was carried out in solution in a stepwise fashion. Dilution in pyridine of the derived succinimide 'active ester' afforded, over 40 h, the [Val^3]-HC toxin in 40% yield.

^{1}H *N.m.r. Assignments.*—Figure 1 shows the ^{1}H spectrum of the synthetic peptide, compared with that of the parental HC-toxin. The combined use of 2D proton–proton chemical shift correlation and 2D-NOESY measurements led to the complete assignment of the proton spectrum. The magnitudes of the coupling constants were determined from the proton–proton 2D *J* spectrum. The chemical shift and coupling constant values are listed in Table 1.

Complete ^{13}C *N.m.r. Assignments.*—The straightforward assignment of all methine, methylene, and methyl carbon signals followed the interpretation of the proton–carbon chemical-shift

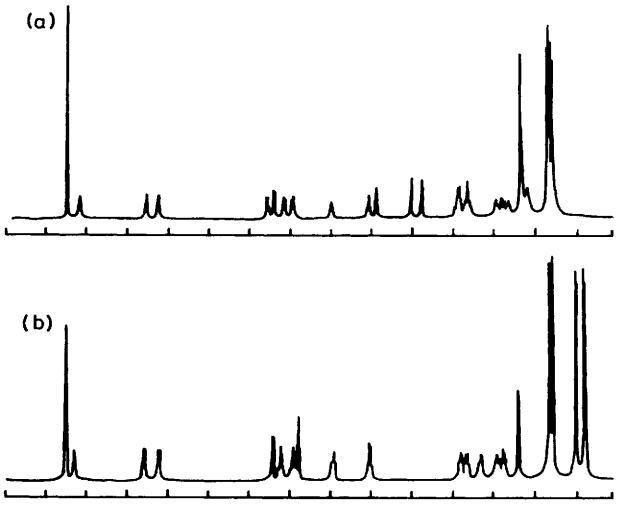

Figure 1. Comparison of the ^{1}H n.m.r. spectra at 500 MHz of (a) HC-toxin and (b) [Val^3]-HC toxin in $CDCl_3$ (25 ± 1 °C; 10mM)

correlation spectrum. The ^{13}C shifts are in Table 5. For the assignment of the carbonyl signals several pulse sequences are now available (*e.g.* carbon–carbon 2D connectivities[14] and long-range heterocorrelation[15]). We relied upon selective heteronuclear Overhauser effect measurements[2] since, besides sequential and assignment information, conformational parameters can also be obtained by use of this technique. The assignment of the carbonyl signals was based on geometric considerations and on the fact that n.O.e.s depend largely upon the distance separating dipolarly coupled spins.

Thus in the sequence $-C_iH-CO-NH-C_{i+1}H-CO-$ a large n.O.e. is expected at the carbonyl of residue *i* upon selective irradiation of the NH proton of the residue $i + 1$. This is illustrated in Figure 2, where the difference between ^{13}C-decoupled spectra obtained in the presence and absence of selective saturation of NH in the proton domain is shown. The largest intensity difference in each spectrum corresponds to the Overhauser effect at the carbonyl carbon adjacent to the irradiated NH, thus affording the assignment of the carbonyl signals (see Table 5).

The magnitude of the other n.O.e.s of Figure 2 and their relationship to conformational parameters will be discussed in the following sections.

Interproton Distances from Nuclear Overhauser Effect Measurements.—Interproton distances for systems exhibiting isotropic motion can be calculated using the n.O.e. ratio method[16,17] [equation (1)] and/or from equations (2) and (3).[4,18] Here, $\sigma_{i,j}$ is the cross-relaxation rate representing the

$$E_k(j)/E_k(i) = r_{k,i}^6/r_{k,j}^6 \quad (1)$$

$$E_i(j) = \sigma_{i,j}/R_i - \sum_{k \neq i,j}(\sigma_{i,k}/R_i)(I_{z,k} - I_{o,k})/I_{o,i} \quad (2)$$

$$\sigma_{i,j} = (\text{const.})(1/r_{i,j}^6)(\tau_c^{i,j}) \quad (3)$$

mutual effect of the two spins, *j* and *i*, on each other's relaxation; R_i in the proton case is simply the spin–lattice relaxation rate that would be observed in a selective inversion recovery experiment on spin *i*. The second term on the right of equation (2) accounts for the indirect contributions to an observed n.O.e.

Table 1. Proton chemical shifts and scalar coupling constants (Hz) of [Val^3]-HC toxin in $CDCl_3$ solution[a]

	Ala^1	D-Ala^2	Val^3	D-Pro^4
NH	7.1_5	6.1_0	6.2_7	
H_α	4.4_6	4.6_0	4.3_9	4.7_0
H_β^D	1.3_1	1.2_8	2.1_4	2.3_8
H_β^U	1.3_1	1.2_8	2.1_4	1.8_7
H_γ^D			0.9_9	2.3_1
H_γ^U			0.8_9	1.9_4
H_δ^D				3.9_5
H_δ^U				3.5_1
^3J(NH–α)	11.1_6[b]	10.4_2[b]	11.3_4[b]	
$^3J(\alpha-\beta)^D$	6.8_6	6.9_0	10.4_0	2.2_0
$^3J(\alpha-\beta)^U$	6.8_6	6.9_0		7.8_2
$^3J(\beta^D-\gamma^D)$			6.7_2	7.6_8
$^3J(\beta^U-\gamma^D)$			6.7_2	8.1_9
$^3J(\beta^D-\gamma^U)$			6.6_0	6.7_9
$^3J(\beta^U-\gamma^U)$			6.6_0	8.1_9
$^3J(\beta-\beta)$				-12.9_0
$^3J(\gamma^D-\delta^D)$				8.5_2
$^3J(\gamma^U-\delta^D)$				4.7_1
$^3J(\gamma^D-\delta^U)$				7.5_1
$^3J(\gamma^U-\delta^U)$				7.5_1
$^3J(\gamma-\gamma)$				-12.8_0
$^3J(\delta-\delta)$				-10.5_0

[a] U = upfield signal; D = downfield signal. [b] Corrected coupling constants.[22]

Table 2. Non-selective and monoselective proton spin–lattice relaxation rates (s^{-1}) of [Val^3]-HC toxin in $CDCl_3$ solution

	R_1^N	R_1^S	F_i
Ala^1 NH	0.9_0	0.8_0	$1.1_3(1.2_2)$[a]
Val^3 NH	0.9_5	0.8_0	$1.2_0(1.3_3)$[a]
Ala^2 NH	0.9_8	0.8_0	$1.2_2(1.4_0)$[a]
Pro^4 H_α	0.7_6	0.5_4	1.4_1
Ala^2 H_α	0.7_3	0.5_2	1.4_0
Ala^1 H_α	0.7_6	0.5_4	1.4_1
Val^3 H_α	0.9_3	0.6_6	1.4_1
Pro^4 H_δ^D	1.4_5	1.0_0	1.4_5
Pro^4 H_δ^U	1.4_7	1.0_0	1.4_7

[a] Corrected for ^{14}N–^{1}H dipolar relaxation.

In our case, this was within the calculated experimental error and not taken into account.

The use of the first method is confined to the analysis of dipolar couplings modulated by the same correlation time τ_c, whereas for the distances to be measured from equations (2) and (3), an *a priori* knowledge of τ_c is needed and the extreme narrowing conditions, $\omega_0^2\tau_c^2 \ll 1$, must also be satisfied.

To prove that the latter limit applied to our system, preliminary studies on relaxation mechanisms and solution dynamics were carried out. Thus, F_i ratios[3] of selective relaxation rates (R^S) to non-selective rates (R^N), which approximate to 1.5, indicate that the intramolecular dipole–dipole relaxation mechanism dominates the proton relaxation pathway and that molecular motions within the extreme motional narrowing range effectively modulate such interactions.[3]

In Table 2, F_i ratios, R^S, and R^N for selected protons of [Val^3]-HC toxin are listed. Apart from NH, all the other investigated protons exhibited F_i ratios of 1.4. The deviation of the amide proton F_i values from the 1.5 limit was not totally unexpected, since the interaction with the ^{14}N nucleus may contribute largely to the N–H proton relaxation rate.[19] When this interaction was considered, an R_{NH} relaxation contribution of

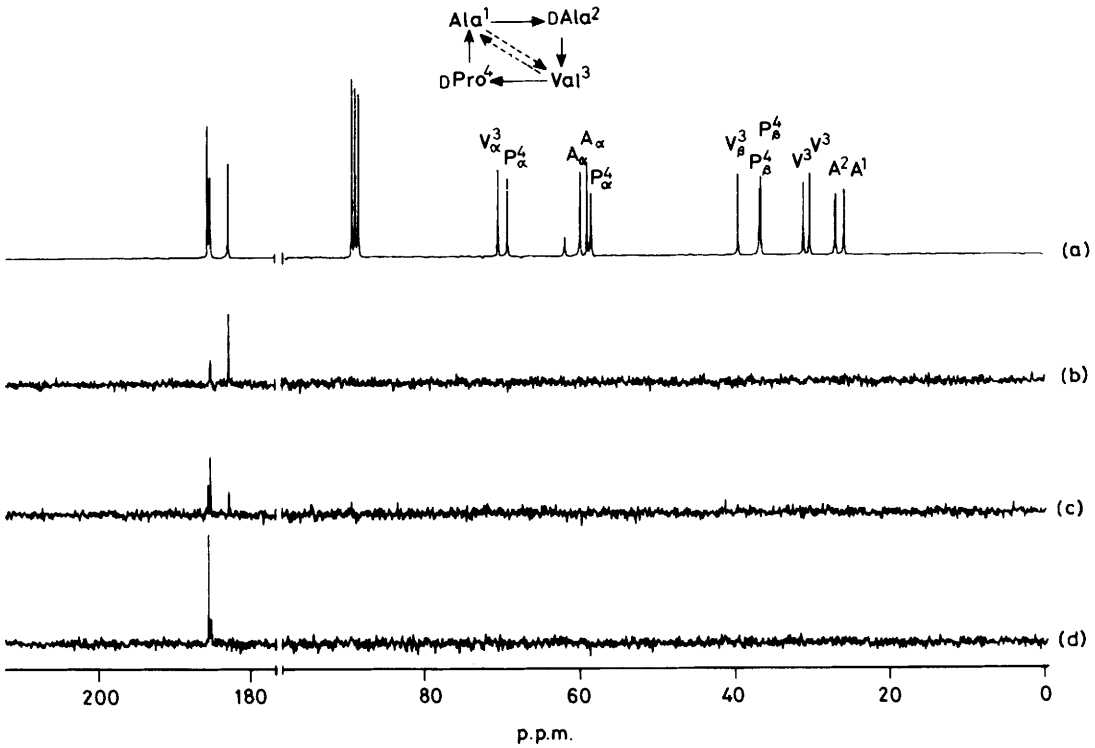

Figure 2. Selective heteronuclear Overhauser effects for [Val³]-HC toxin: (a) ^{13}C spectrum at 75 MHz of the peptide in $CDCl_3$ solution (0.8M; 25 °C); (b) difference spectrum after selective irradiation of the Ala¹ NH proton; (c) difference spectrum after irradiation of the Ala² NH proton; (d) difference spectrum after irradiation of the amide proton Val³ NH

0.35 s^{-1} was calculated, which thus partially accounted for the lower F_i ratios seen for the amide protons (Table 2).

In conclusion, it appears from these results that relaxation mechanisms other than intramolecular dipole–dipole do not contribute significantly to the pathway of the majority of the protons of this peptide [only the Ala¹ NH protons are outside this limit (Table 2)] and that $\omega_0^2\tau_c^2 \ll 1$ holds.

The latter conclusion is also justified by the ^{13}C findings (see later), which showed how a single correlation time governs the motion in solution of the peptide. Thus a virtually constant value for τ_c was found through the backbone of the cyclic tetrapeptide.

The correlation time τ_c was calculated by using equation (3) and the standard interproton distance of 1.8 Å separating the two Pro⁴ geminal δ-protons. Combining this knowledge with the experimentally derived σ_{gem} for the Pro⁴ δ-protons yielded $\tau_c = 4.22 \times 10^{-11}$ s. This value agrees with that of 4.4×10^{-11} s calculated for the cyclic tetrapeptide desdimethylchlamydocin.[4]

The knowledge of τ_c and σ values and Overhauser effects (Table 3) then permitted the calculation of the interproton distances (Table 4), using equations (1) and/or (2) and (3).

Spin–Lattice Relaxation Rates and Proton–Carbon Distance Measurements.—The use of the methods described in the previous section is not restricted to the calculation of interproton distances; it can also be applied to the heteronuclear case.[5] We measured ^{13}C spin–lattice relaxation rates, selective heteronuclear Overhauser effects, and proton–carbon cross-relaxation rates and used equations (1), (2), and (3) to calculate H–C distances of the synthetic peptide. In Table 5 the experimental data are reported.

A feature common to all the protonated carbon atoms is a large non-selective n.O.e. (> 1.63) which indicates that the ^1H–^{13}C dipolar interactions are the main source of the spin–lattice relaxation of these nuclei. It also suggests that all the dipolar couplings are modulated by molecular motions which satisfy (or are very close to) the narrowing limit.

Correlation times for each class of C–H vector of the molecules were calculated by fitting the experimental data from Table 5 and predicted R^{DD} and nuclear Overhauser effect E° (BB) according to equations (4) and (5). Here $f(\tau_c)$ and $f'(\tau_c)$

$$R^{DD} = 1/nT_1{}^{DD} = (\hbar^2\gamma_H{}^2\gamma_c{}^2/10r^6)f(\tau_0) \quad (4)$$

$$E^\circ(BB) = \gamma_H f'(\tau_c)/\gamma_c f(\tau_c) \quad (5)$$

are functions of the effective correlation time. The fractional effectiveness of the dipolar relaxation mechanism (X^{DD}) then correlates predicted and experimental relaxation parameters through the equations (6) and (7).

$$X^{DD} = E(BB)/E^\circ(BB) \quad (6)$$

$$X^{DD} = R^{DD}/R_{exp.} \quad (7)$$

For the C–H vectors $\tau_c = 1.1 \times 10^{-10}$ was derived, which fits the experimental data. Thus this proposed correlation time and the standard C–H distance of 1.09 Å yielded $R = 2.24$ s^{-1} and $E^\circ(BB) = 1.94$, confirming the hypothesis of an entirely dipolar mechanism for the carbon nuclei.

Equations (6) and (7) then gave R^{DD} values that on average were consistent with $R = 2.24$ s^{-1} calculated previously and hence with $\tau_c = 1.1 \times 10^{-10}$. The methyl carbon nuclei exhibited longer spin–lattice relaxation times, presumably because of faster reorientation of their C–H vectors. Using the theoretical $E^\circ(BB) = 1.99$ and the appropriate R^{DD} values from Table 5 gave correlation times of 2.3×10^{-11} s for the CH_3 groups of Ala², Ala¹, and Val³, respectively.

Table 3. Proton–proton n.O.e.s of [Val^3]-HC toxin in $CDCl_3$[a]

	Ala^1 NH	Val^3 NH	Ala^2 NH	Pro^4 H_α	Ala^2 H_α	Ala^1 H_α	Val^3 H_α	Pro^4 H_δ^D	Pro^4 H_α^U
Ala^1 NH	−100			8.8_5		<1			
Val^3 NH		−100			10.3		3.4[c]		
Ala^2 NH			−100		<1	12.3			
Pro^4 H_α	14.0_4			−100					
Ala^2 H_α		9.5_0	3.1_5[b]		−100				
Val^3 H_α						−100	6.7_0	7.6_0	
Pro^4 H_δ^D						3.9_0	−100	27.5_0	
Pro^4 H_δ^U						6.6_0	24.2_0	−100	

[a] Corrections for cross-relaxation of spins k, on the intensity of the observed spin, i, when j is saturated, accounted for less than 10% of the total n.O.e. and hence was ignored. [b] From overlap with Ala^1 H_α. [c] From overlap with Ala^2 H_α.

Table 4. Distances (Å) of [Val^3]-HC toxin calculated from relaxation parameters and from crystallography[a]

	Ala^1 C_α–H	Ala^2 C_α–H	Val^3 C_α–H	Pro^4 C_α–H
Ala^1 NH	3.1_5 (2.8_0)			2.3_3 (2.2_8)
Ala^2 NH	2.3_0 (2.3_0)	3.0_0 (—)		
Val^3 NH	2.5_7 (2.6_4)	2.2_9 (—)	2.7_6 (2.8_0)	
Pro^4 H_δ^D			2.3_3 (2.3_4)	
Pro^4 H_δ^U			2.5_1 (2.5_0)	

[a] Proton–proton distances for dihydrochlamydocin as determined by crystallography[7] are given in parentheses.

Table 5. ^{13}C Relaxation parameters and ^{13}C chemical shifts for [Val^3]-HC toxin in $CDCl_3$

	p.p.m.	R[a]	n.O.e. (BB)[b]	X^{DD}[c]	R^{DD}[c]
Ala^2 C=O	187.7_8	0.2_7	0.9_6	0.5	0.1_3
Ala^1 C=O	185.4_9	0.4_2	0.7_3	0.3_8	0.1_6
Val^3 C=O	185.4_3	0.3_8	0.6_3	0.3_3	0.1_3
Pro^4 C=O	183.0_9	0.3_2	0.8_1	0.4_2	0.1_3
Val^3 C_α	58.3_3	2.4_5	1.7_0	0.8_8	2.1_5
Pro^4 C_α	57.1_4	2.4_9	1.7_3	0.9_0	2.2_3
Ala^1 C_α	47.7_7	2.6_5	1.6_7	0.8_6	2.2_9
Ala^2 C_α	46.9_0	2.5_3	1.6_3	0.8_4	2.1_3
Pro^4 C_δ	46.3_7	1.7_6	1.7_1	0.8_8	1.5_5
Val^3 C_β	27.4_0	2.2_2	1.6_7	0.8_6	1.9_1
Pro^4 C_β	24.5_4	1.7_5	1.6_5	0.8_5	1.4_9
Pro^4 C_γ	24.4_1	1.4_0	1.6_8	0.8_7	1.2_2
Val^3 C_γ^D	18.8_1	0.5_2	1.8_6	0.9_3	0.4_8
Val^3 C_γ^U	18.0_2	0.5_1	1.8_9	0.9_5	0.4_8
Ala^1 C_β	14.6_3	0.6_1	1.9_3	0.9_7	0.5_9
Ala^2 C_β	13.5_4	0.5_4	1.8_4	0.9_2	0.5_0

[a] ^{13}C Spin-lattice relaxation rates in s^{-1}; rates R for CH_3 and CH_2 groups have been divided by the number of attached protons. [b] Non-selective n.O.e.s calculated as $(I_z - I_0)/I_0$. [c] See text for explanations.

Table 6. Proton–carbon distances (Å) for [Val^3]-HC toxin from relaxation parameters[a] and crystallography

	Ala^1 NH	Ala^2 NH	Val^3 NH
Ala^1 CO	2.6_5 (2.5_7)	2.0_9 (1.7_7)	2.5_7 (2.6_4)
Ala^2 CO		(2.9_8)	2.2_1 (1.9_3)
Val^3 CO	$2.5.^5$ (2.6_3)		2.4_9 (2.6_0)
Pro^4 CO	2.1_6 (1.8_8)		

[a] Data in parentheses are from X-ray data for dihydrochlamydocin.[7]

Table 7. Torsion angles (°) for [Val^3]-HC toxin in $CDCl_3$ solution[a,b]

	Ala^1	D-Ala^2	Val^3	D-Pro^4
φ	120 (−105.5)	100 (71.8)	120 (−105.5)	— (83.0)
ψ	100 (104.7)	100 (−63.7)	100 (94.4)	60 (−72.8)
ω	*transoid* (−163.7)	*transoid* (162)	*transoid* (−165.7)	*transoid* (156.5)

[a] All the angles are ±20°. [b] Values in parentheses are from the crystal structure of dihydrochlamydocin.[7]

For the β, γ, and δ methylene and methine carbon nuclei a behaviour intermediate between that of the α carbon nuclei and that of methyl groups would be expected and, in accord with the experimental results, $1.94 \leqslant E^c(BB) \leqslant 1.99$. If the lower limit was used, R^{DD} values less than those of C_α were found, showing that indeed an additional mobility characterises the dipolar interactions between the protons and these side-chain carbon atoms.

As expected, the carbonyl carbon atoms, owing to their unsuitable magnetic environment, had inefficient relaxation mechanisms. Nevertheless, long-range proton–carbon dipolar couplings contributed a minimum of 33% to their nuclear dipolar relaxation, under the reasonable assumption that these couplings are modulated by the molecular correlation time τ_c = 1.1×10^{-10} s. This allowed the use of equations (2) and (3) as structural bases for obtaining the proton–carbon distances reported in Table 5.

It is worth noting that the τ_c value used for these calculations is 3 times that used in the previous section, for the interproton distance measurements. This discrepancy can be explained on the basis of the different conditions under which the two types of experiments were carried out. Thus the peptide concentration for the heteronuclear studies was ten times that used for the homonuclear Overhauser effects. In these concentrated solutions aggregation occurred (as indicated by the large chemical-shift dependence of the free Ala^2 NH upon the peptide concentration[20]), hence justifying the different τ_c values.

Conformational Analysis of [Val^3]-HC *Toxin using Internuclear Distances.*—The experimentally derived proton–carbon distances elucidated the hydrogen-bonding pattern of this tetrapeptide. Thus two N^1H–^{13}CO dipolar couplings, that between Val^3 CO and Ala^1 NH and that between Val^3 NH and Ala^1 CO were ascribed to hydrogen bonds across the cyclic backbone and the corresponding distances (see Table 6) were in agreement with those generated by γ-turns in other similar cyclic tetrapeptides.[4,6,21]

The extent of the NH–CO dipolar interactions between adjacent residues was consistent with this solution. Thus the experimentally measured distances indicated that the NH and CO moieties of Ala^1 and Val^3 residues have a *cis* conformation which can exist only in the presence of the two aforementioned hydrogen bonds (see Figure 3).

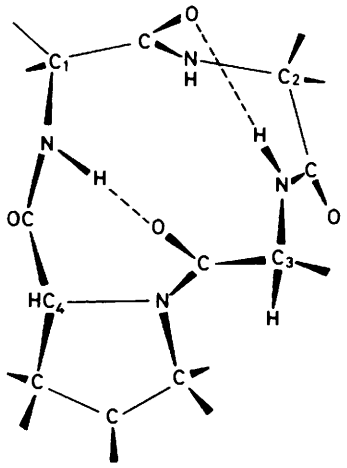

Figure 3. Conformation of [Val³]-HC toxin in chloroform solution

Molecular models incorporating these features yielded interproton and C–H distances fully in agreement with those calculated from the relaxation parameters. Furthermore, these distances were consistent within 0.15 Å with those previously calculated for the peptide desdimethylchlamydocin[4] and, more remarkably, with those from *X*-ray data for dihydrochlamydocin.[7] These two peptides were found to possess the same conformation as proposed for [Val³]-HC toxin.[6,21]

Conformation of [Val³]-HC *Toxin from Torsion Angles.*—Suitable Karplus relationships[22] and the J_{α} values from Table 1 yielded angles φ of 120 ± 20° and 100 ± 20° for the Ala¹, Val³, and Ala² residues, respectively. By using appropriate equations,[23] the r_{ψ} distances from Table 4 were correlated with angles ψ of approximately 100° for residues 1, 2, and 3. The Pro⁴ angles ψ and ω could be measured from ^{13}C parameters. Thus the position of the Pro⁴ C_{β} resonance in the carbon spectrum of peptides has been regarded as diagnostic for both the Pro angles ψ and ω angles.[24,25] Here the upfield shift of Pro⁴ C_{β} to 24.5 is indicative of Pro $\psi = (\pm)60°$ and Pro ω *trans*.

These experimentally derived torsional angles were consistent with the proposed structure for [Val³]-HC toxin. Comparison with the *X*-ray data of dihydrochlamyodocin (Table 7) confirmed the validity of this conclusion: the deviation from the crystal angles is within ±20°, the experimental error.

Conclusions

We have described a conformational analysis of a cyclic tetrapeptide by using ^{1}H and ^{13}C relaxation parameters such as spin–lattice relaxation rates and n.O.e.s. A comparison between the conformational features experimentally derived from n.m.r. and the crystal structure of a related compound confirms that a structural investigation of biomolecules in solution can be carried out by using relaxation parameters of proton and carbon nuclei. Furthermore, the close similarity between distances found in the crystal and in solution suggests that the tetrapeptide exists predominantly in a single conformation. As a corollary, the rigidity of the proposed structure can be inferred although slow motions occurring within the backbone cannot be ruled out. However, the experimental relaxation data indicate that these motions do not significantly affect the effective correlation times.

References

1 W. A. Gibbons, D. Crepaux, J. Delayre, J. J. Dunand, G. Hajdukovic, and H. R. Wyssbrod, Proc. 4th American Peptide Symposium, eds. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, 1975, pp. 127—137.
2 J. J. Ford, W. A. Gibbons, and N. Niccolai, *J. Magn. Reson.*, 1982, **47**, 522.
3 R. Freeman, H. D. Hill, B. L. Tomlinson, and L. D. Hall, *J. Chem. Phys.*, 1974, **61**, 4466.
4 P. Mascagni, W. A. Gibbons, D. H. Rich, and N. Niccolai, *J. Chem. Soc., Perkin Trans. 1*, 1985, 245.
5 (*a*) N. Niccolai, C. Rossi, V. Brizzi, and W. A. Gibbons, *J. Am. Chem. Soc.*, 1984, **106**, 5732; (*b*) N. Niccolai, C. Rossi, P. Mascagni, W. A. Gibbons, and V. Brizzi, *J. Chem. Soc., Perkin Trans. 1*, 1985, 239; (*c*) N. Niccolai, C. Rossi, P. Mascagni, P. Neri, and W. A. Gibbons, *Biochem. Biophys. Res. Commun.*, 1984, **124**, 739.
6 P. Mascagni, M. Pope, W. A. Gibbons, L. M. Ciuffetti, and H. W. Knoche, *Biochem. Biophys. Res. Commun.*, 1983, **113**, 10.
7 J. Flippen and I. Karle, *Biopolymers*, 1976, **15**, 1081.
8 M. Bodanszky, 'Principles of Peptide Synthesis,' Springer-Verlag, Berlin, 1984.
9 T. Wieland, W. Kern, and R. Sehring, *Justus Liebigs Ann. Chem.*, 1950, **569**, 117.
10 W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, 1976, **64**, 2229.
11 S. Macura and R. R. Ernst, *Mol. Phys.*, 1980, **41**, 95.
12 W. P. Aue, J. Karhan, and R. R. Ernst, *J. Chem. Phys.*, 1976, **64**, 4226.
13 A. Bax and G. A. Morris, *J. Magn. Reson.*, 1981, **42**, 501.
14 A. Bax, R. Freeman, and S. P. Kempsell, *J. Am. Chem. Soc.*, 1980, **102**, 4839.
15 A. Bax, *J. Magn. Reson.*, 1983, **52**, 330.
16 J. H. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect,' Academic Press, New York, 1971.
17 (*a*) C. R. Jones, C. T. Sikakana, S. P. Hehir, M. Kuo, and W. A. Gibbons, *Biophys. J.*, 1978, **24**, 815; (*b*) M. Kuo and W. A. Gibbons, *ibid*, 1980, **32**, 807.
18 N. Niccolai, H. K. Schnoes, and W. A. Gibbons, *J. Am. Chem. Soc.*, 1980, **102**, 1513.
19 H. E. Bleich, K. R. K. Easwaran, and J. A. Glasel, *J. Magn. Reson.*, 1978, **31**, 517.
20 P. Mascagni, unpublished results.
21 M. Kawai and D. J. Rich., *Tetrahedron Lett.*, 1983, **24**, 5309.
22 V. F. Bystrov, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1976, **10**, 41.
23 M. Kuo and W. A. Gibbons, Proc. 6th American Peptide Symposium, eds. E. Gross and J. Meinhofer, Pierce Chemical Company, 1979, pp. 229—232.
24 I. Siemion, T. Wieland, and K. Pook, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 702.
25 L. Pease and J. C. Watson, *J. Am. Chem. Soc.*, 1978, **100**, 1279.

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