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THE BACKBONE AND SIDE CHAIN CONFORMATIONS OF THE CYCLIC TETRAPEPTIDE HC-TOXIN

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Summary: A study of the conformational parameters of HC-toxin and its diacetyl derivative in chloroform solution has been carried out. Two-dimensional NMR spectroscopy and the nuclear Overhauser effect have been used in order to determine connectivities (assignments and sequence) and approximate torsion angles and interproton distances. The results are consistent with a bis-γ-turn conformation previously reported for dihydrochlamydocin. Model building based upon NMR data supports a D configuration for Ala^2 and Pro^4 residues.

HC-toxin 1a, a metabolite of Helminthosporium carbonum (1) has been shown to have the cyclic structure (L-Ala^1-D-Ala^2-L-AEO^3-D-Pro^4) (2-5). The unusual amino acid, AEO, 2-amino-9,10-epoxy-8-oxodecanoic acid, has also been found in the cyclic tetrapeptides, chlamydocin and Cyl-2 (6,7). It has been postulated on the basis of hydrogen bonding studies (8) that the toxin possesses the same bis-γ-turn conformation as dihydrochlamydocin (9). We report (i) that this hypothesis is fully consistent with NOEs, scalar coupling constants and proton and carbon chemical shifts, (ii) all ϕ, ψ, ω angles and χ rotamer populations, (iii) that the toxin and its diacetyl derivative 1b have identical conformations in chloroform solution except for the terminus of the AEO side chain, (iv) that the NMR data support a L-D-L-D sequence and configuration for the molecule.

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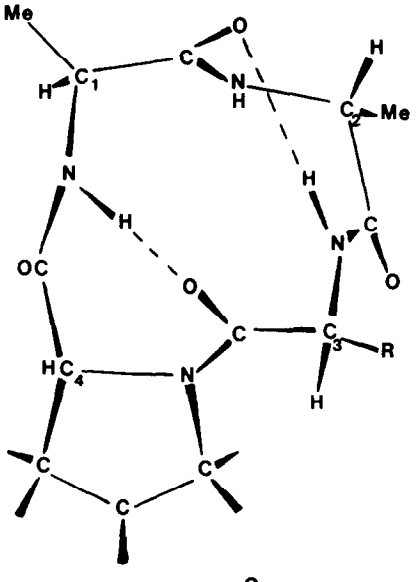
Materials and Methods

The HC-toxin purification and derivatization to 1b and 1c have been previously described (10). The NMR experiments were performed on a 200 MHz Nicolet spectrometer and on the 600 MHz instrument of the Carnegie Mellon Institute, Pittsburg, Pa. The pulse sequence, $T-(\pi/2)-t_1-(\pi/2)-t_2$, was used for the $^1H-^1H$ shift correlation; 256 increments were recorded; the number of data points in F_2 was 512. The applied pulse sequence for the $^1H-^{13}C$ shift correlation was $(\pi/2,^1H)-(t/2)-(\pi,^{13}C)-\Delta1(\pi/2,^1H;\pi/2,^{13}C)-\Delta2$, with $\Delta1$=3.6 ms, and $\Delta2$=2.6 ms; 64 increments of 1024 data points each were recorded.

Results and Discussion

1. ^1H and ^{13}C resonance assignments. The ^1H assignments were obtained by two-dimensional $^1H-^1H$ shift correlation spectroscopy (11) and by difference double resonance (12,13) at 200, and 600 MHz. Figure 1 shows a 200 MHz two-dimensional $^1H-^1H$ shift correlation map of 1a in chloroform-d. The ^3J coupling constants of 1a, 1b and 1c are listed in Table I. The ^{13}C shift assignments were achieved



1a: R=$(CH_2)_5$–CO–CH(O)–CH_2

1b: R=$(CH_2)_5$–CO–CH(OAc)–CH_2(OAc)

1c: R=$(CH_2)_5$–CO–CH(OH)–CH_2(OH)

Figure 1. 200 MHz COSY spectrum of 1a in $CDCl_3$ (55 mM).

Table I. Comparison of Scalar Coupling Constants in $CDCl_3$ solution (J_{ij},Hz)[a]

		1a	1b	1c
Ala[1]	$^3J_{\alpha-NH}$	11.2[b]	11.3[b]	11.0[b]
	$^3J_{\alpha-\beta}$	7.0_4	6.6_2	8.0_3
Ala[2]	$^3J_{\alpha-NH}$	10.6[b]	10.8[b]	10.6[b]
	$^3J_{\alpha-\beta}$	7.0_4	6.6_2	6.9_2
AEO[3]	$^3J_{\alpha-NH}$	11.3[b]	11.2[b]	11.3[b]
	$^3J_{\alpha-\beta(u)}$	7.6_8	7.7_2	7.7_8
	$^3J_{\alpha-\beta(d)}$	7.6_8	7.7_2	7.7_8
	$^3J_{\varepsilon-\xi(u)}$		7.3_5	7.2_7
	$^3J_{\varepsilon-\xi(d)}$		7.3_5	7.2_7
	$^3J_{\xi-\xi}$		-18.0_1	-17.2_8
	$^3J_{\theta-\iota(u)}$	3.9_2	3.3_1	3.3_0
	$^3J_{\theta-\iota(d)}$	3.6_6	5.5_5	3.3_0
	$^3J_{\iota-\iota}$	5.6_4	-12.3_3	-11.8_5
Pro[4]	$^3J_{\alpha-\beta(u)}$	7.8_1	8.2_3	7.8_1
	$^3J_{\alpha-\beta(d)}$	2.1_9	2.1_1	1.8_6
	$^3J_{\beta-\beta}$	-12.0_1	-12.8_3	-12.0_2
	$^3J_{\beta(u)-\gamma(d)}$	8.0_1	8.4_9	8.3_1
	$^3J_{\beta(u)-\gamma(u)}$	8.0_1	8.4_9	8.3_1
	$^3J_{\beta(d)-\gamma(d)}$	7.5_1	7.9_9	8.0_1
	$^3J_{\beta(d)-\gamma(u)}$	4.0_1	4.3_5	4.3_1
	$^3J_{\gamma-\gamma}$	-12.0_1	-12.6_6	-12.0_1
	$^3J_{\gamma(u)-\delta(u)}$	6.6_4	8.0_4	7.5_1
	$^3J_{\gamma(u)-\delta(d)}$	4.9_3	4.9_9	4.5_1
	$^3J_{\gamma(d)-\delta(u)}$	6.6_4	7.8_4	7.5_1
	$^3J_{\gamma(d)-\delta(d)}$	8.5_5	8.2_8	8.0_1
	$^3J_{\delta-\delta}$	-9.0_5	-10.1_0	-10.0_1

[a] u = upfield signal, d = downfield signal. [b] Corrected coupling constant

by two-dimensional ^{13}C-^1H shift correlation (14) of 1a in chloroform-d (Fig.2). Empirical formulae (15) were used for γ, δ and ε ^{13}C signals of the AEO[3] residue in order to solve the ambiguities arising from overlap in the upfield

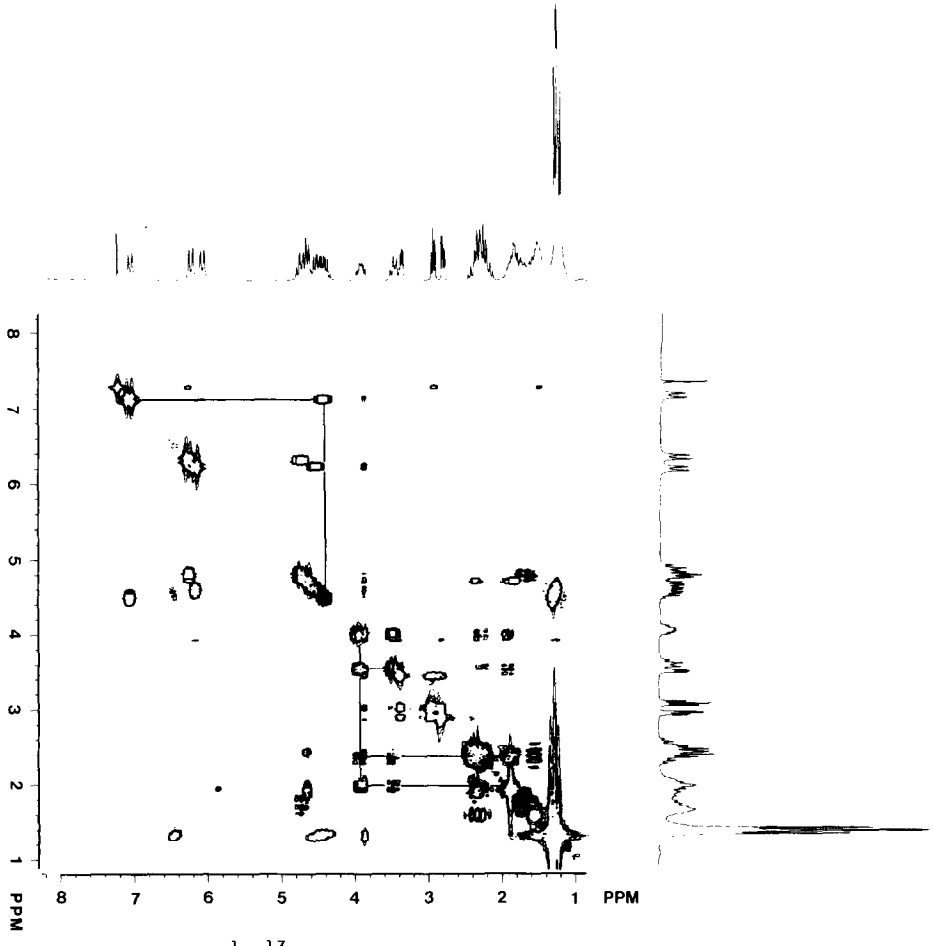


Figure 2. ^{1}H-^{13}C shift correlation of the high field region of a 55mM solution of HC-toxin in $CDCl_3$ at 298°K.

region of the proton spectrum. The upfield shift of the Pro^{4} C^{β} resonance supports a trans X-Pro peptide bond where X = AEO^{3} (16).

2. rϕ distances and ϕ angles from NOEs and $^{3}J_{\phi}$ coupling constants. Karplus relationships (17) were used to correlate $^{3}J_{\phi}$ coupling constants with the torsion angles ϕ. The corrected $^{3}J_{\phi}$ values (17) of 11.2 and 11.3 Hz observed for Ala^{1} and AEO^{3} respectively, correspond to ϕ angles of 120° ± 20° and hence to rϕ interproton distances of 3.0 ± 0.2 Å (18). The relatively small NOEs (less than 1 %) observed at $H^{1}\alpha$ and $H^{3}\alpha$ when the appropiate N-H protons were irradiated and the observation of intraresidue NH↔CH_3 for Ala^{1} support this

hypothesis. The $Ala^2\phi$ angle $110^0 \pm 20^0$ ($^3J_\phi$ = 10.6 Hz) is also consistent with $r\phi = 3.0 \pm 0.2$ Å.

3. rψ distances and ψ angles from NOEs. With certain assumptions, rψ and hence ψ angles can be calculated from NOE measurements using the NOE ratio method (18,19). Thus for the Ala^1 residue

$$\frac{NOE\phi^+(1)}{NOE\psi^-(1)} = \frac{[r\psi^-(1)]^6}{[r\phi^+(1)]^6} \leq \frac{1}{7}$$

Using $r\phi$ (1) = 3.0 ± 0.2 Å (vide supra) the interproton distance rψ (1) is 2.2 ± 0.2 Å which corresponds to an $Ala^1\psi$ torsion angle of $95^0 \pm 20^0$. An approximate value for the $\psi(Pro^4)$ torsion angle can be attained from the ^{13}C spectrum of 1a in chloroform-d. Thus the upfield shift of the Pro^4 C^β resonance indicates a $\psi(Pro^4) = -60^0$ (20), and the existence of an inverse γ turn (21,28). This conformational moiety has already been proposed (8). The observation of strong NOEs between the Phe Hα and the Pro Hδ_d and Hδ_u protons led Jones et al (22) to the determination of the ψ(Phe) torsion angle of the D-Phe-Pro sequence in gramicidin S, which was later confirmed by crystallography (23). Using the NOE values from Table II and the appropriate equations the distance between AEO^3 Hα and Pro^4 Hδ_d is 2.0 ± 0.2 Å and the distance between $AEO^3\alpha H$ and Pro^4 Hδ_u is 2.1 ± 0.2 Å. Molecular modelling incorporating these distances and torsional angle $\omega_3 = 180^0$ (vide supra) yielded $\psi(AEO^3) = 90^0 \pm 20^0$.

4. Side chain structure: rotamer populations. The $^3J_{\alpha\beta}$ coupling constants from Table I were used to determine the rotamer populations for the AEO^3 residue (24). The results indicate that the χ_i=180° and χ_i=+60° rotamers of the Cα-Cβ bond of AEO^3 have equal population and an essentially zero population for the χ_i=-60° rotamer (Table III). The non-classical χ_i rotation in the prolyl residue of 1a was analyzed by the Karplus relationships (25, 26). From the observed vicinal coupling constants $^3J\alpha\beta$ = 2 and 8 Hz two possible values of χ_1 satisfy the Karplus relations: approximately -30°and -80°. Only the former value must be chosen on the basis of energetically allowed conformations of proline (26,27). Values of $\chi_2 = 30^0$ and $\chi_3 = -30^0$

Table II. Proton-proton Nuclear Overhauser Effect measurements[a].

Irradiated proton	Observed proton	Actual NOE[b](%)
Ala^1 NH	Pro^4 CαH	12-13
	Ala^1 Me	2
	Ala^1 CαH	<1
Ala^2 NH	Ala^1 CαH	10
	Ala^2 Me	2
	Ala^2 CαH	<1
AEO^3 NH	Ala^2 CαH	10
AEO^3 CαH	Pro^4 δH(d)	10
	Pro^4 δH(u)	7
Pro^4 δH(d)	Pro^4 δH(u)	22.6
	AEO^3 CαH	13
Pro^4 δH(u)	Pro^4 δH(d)	21
	AEO^3 CαH	8

[a] u = upfield signal, d = downfield signal

[b] Corrections for cross relaxation were less than 10% and henceignored

were determined by similar methods. Comparison of these χ_i angles with those of the D-Pro residue of dihydrochlamydocin shows a close similarity between the conformation of the two systems. Since the latter was determined crystallographically this lends strength to the present arguments.

5. ω angles. It is generally difficult to determine ω angles in solution by NMR, but model building based upon several pieces of data forced the conclusion that $\omega_1 \simeq \omega_2 \simeq \omega_3 \simeq \omega_4 \simeq$ trans. The data used for molecular modelling were: (a) L-Ala^1 NH and L-AEO^3 NH are hydrogen bonded whereas Ala^2 NH is solvent exposed (8), (b) the $r\phi$ and $r\psi$ values of Table III, (c) the ^{13}C shift evidence for $\omega_4 \simeq 180^0$ and $\psi_4 \simeq -60^0$, (d) the χ_1, χ_2 and χ_3 angles of Pro^4 and χ_i for L-AEO^3. The results was that HC-toxin has a conformation with four transoid amide bonds virtually identical to that of dihydrochlamydocin and of other cyclic tetrapeptides (28) (Fig.3).

Table III. Approximate Torsion Angles[a] and Interproton Distances[b] for HC-toxin and its Diacetyl derivative in Chloroform[c].

	L-Ala[1] (L-AEO)	D-Ala[2] (Aib)	L-AEO[3] (L-Phe)	D-Pro[4] (D-Pro)
ϕ	-120^0 (-105.5^0)	110^0 (72^0)	-120^0 (-105.5^0)	-- (83^0)
$r\phi$	3 Å	3 Å	3 Å	--
ψ	95^0 (104.7^0)	-- (-63.7^0)	90^0 (94.4^0)	-60^0 (-72.8^0)
$r\psi$	2.2 Å	--	--	--
ω	transoid (-163.7^0)	transoid (162^0)	transoid (-165.7^0)	transoid (165.5^0)
χ_i			$P^{\alpha\beta}_{180}$ 0.41	-30^0 (-30^0)
			$P^{\alpha\beta}_{60}$ 0.41	30^0 (26.2^0)
			$P^{\alpha\beta}_{\cdots 60}$ 0.08	-30^0 (-11.4^0)

[a]Values of angles ± 20°. [b]Values of distances are ± 0.2 Å. [c]Values in parenthesis refer to dihydrochlamydocin crystal structure.

More comprehensive and quantitative proton relaxation and CMR studies of the conformation and the motion of these and the other tetrapeptides are necessary before we have complete confidence in the proposed solution structure. But

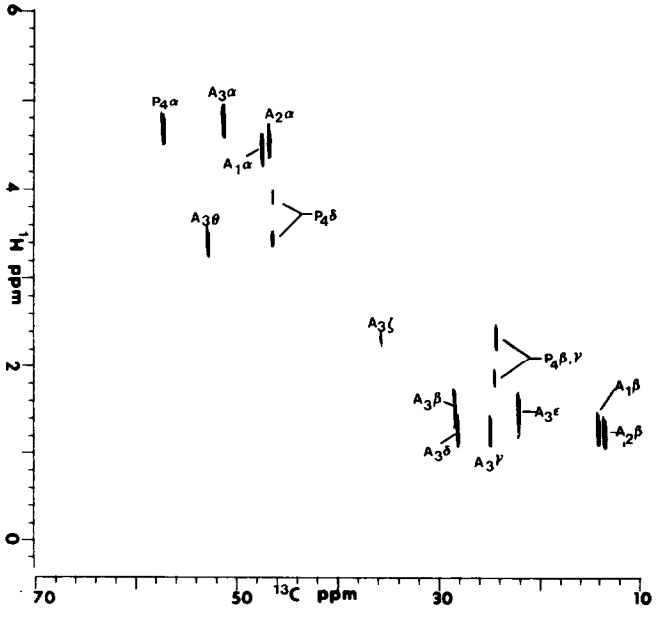


Figure 3. Proposed conformation for HC-toxin and its diacetyl derivative in $CDCl_3$.

the present conformational hypothesis is reasonable and fits all the known facts including the amino acid sequence and configurations.

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