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TWO SYMMETRICAL CONFORMATIONS OF THE TRIOSTIN ANTIBIOTICS IN SOLUTION.

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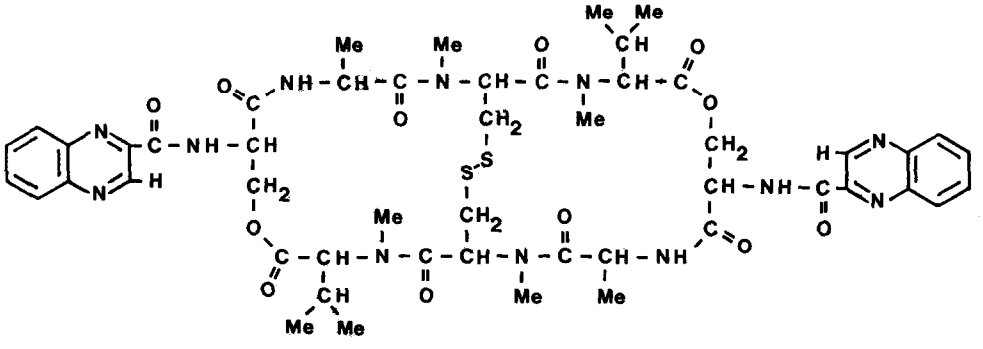
Triostin A,[1,2] 1, a member of the quinoxaline group of antibiotics, has been shown [3] to bind strongly to double-helical DNA by the simultaneous intercalation of both quinoxaline groups between base pairs. In order to understand the molecular basis for this biological activity, some knowledge of the solution conformation of the antibiotic is necessary. Here we report our preliminary nmr results which, with supporting chromatographic evidence, lead us to propose that triostin A has two discrete conformations.



1 Triostin A

The two halves of the triostin A molecule 1 are related by a two-fold axis of symmetry. Whereas the reported ^{1}H nmr spectrum of 1 in d_6-DMSO is in accord with this symmetry[4], its spectrum in $CDCl_3$ (Fig. 1; Table 1) shows two resonances for each pair of symmetry related protons. The spectrum in $CDCl_3$ has been interpreted [4] in terms of a conformation in which the two halves of

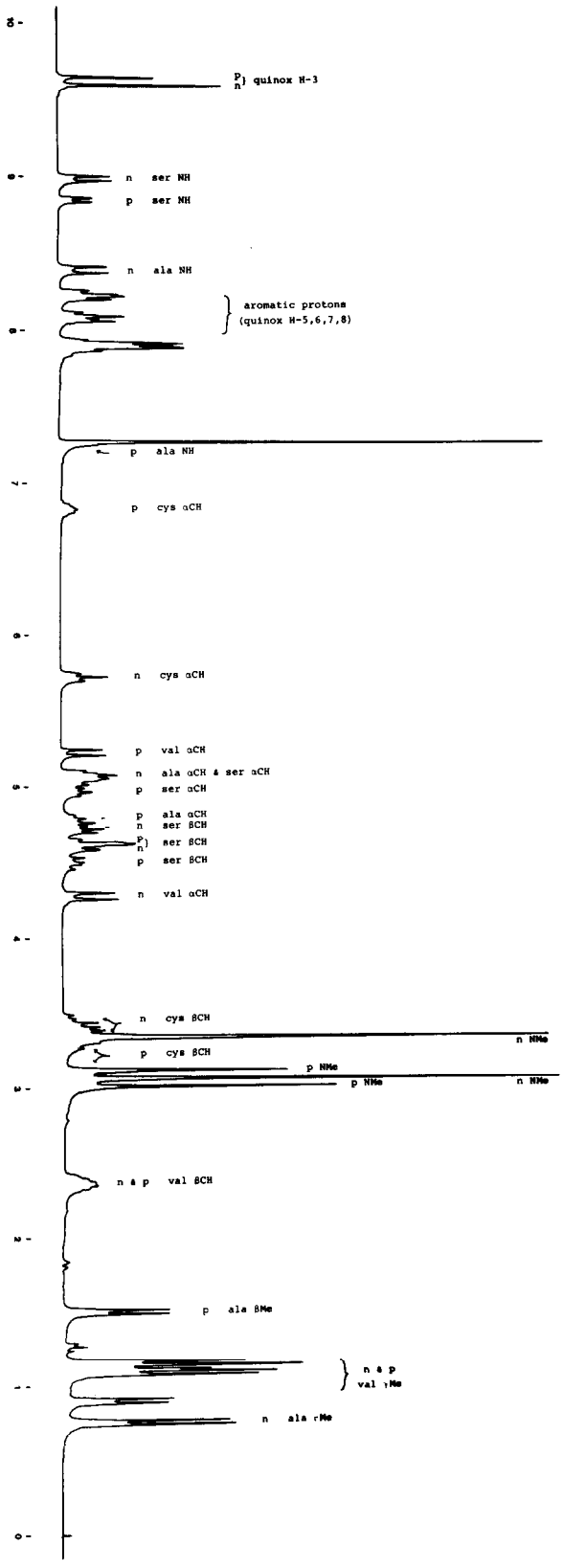


Fig. 1. 270 MHz nmr spectrum of Triostin A in $CDCl_3$ solution.

Table 1: NMR Spectral Parameters of Triostin A

Chemical shifts $\delta(CDCl_3)$ ex TMS

residue	n	p	residue	n	p
val γMe	1.06,1.13	0.87,1.09	ala βMe	0.73	1.46
β	2.34	2.34	α	5.05	4.78
α	4.27	5.22	NH	8.41	7.24
cys β_1	3.33	~3.05	ser β_1	4.58	4.49
β_2	3.43	~3.3	β_2	4.72	4.62
α	5.73	6.82	α	5.06	4.96
NMe	3.05	2.99	NH	8.99	8.86
NMe	3.33	3.10	quinox H-3	9.61	9.68
			quinox H-5,6,7,8	7.7-8.3	7.7-8.3

Coupling Constants

		n	p			n	p
val	$J_{\beta\gamma}$	6.5,6.8Hz	6.6,6.5 Hz	ala	$J_{\alpha\beta}$	6.4 Hz	6.9 Hz
	$J_{\alpha\beta}$	10.05 Hz	10.0 Hz		$J_{\alpha,NH}$	9.0 Hz	5.7 Hz
ser	$J_{\beta_1\beta_2}$	11.2 Hz	11.0 Hz	cys	$J_{\beta_1\beta_2}$	15.0Hz	14.8 Hz
	$J_{\alpha\beta_1}$	1.4 Hz	7.4 Hz		$J_{\alpha\beta_1}$	6.7 Hz	5.3 Hz
	$J_{\alpha\beta_2}$	5.9 Hz	1.0 Hz		$J_{\alpha\beta_2}$	7.9 Hz	9.5 Hz
	$J_{\alpha,NH}$	8.2 Hz	6.4 Hz				

the molecule are non-equivalent and in slow chemical exchange, while it was proposed that in DMSO the same asymmetric conformation is in rapid exchange, or else a different, symmetrical conformation is present. Close inspection of Fig. 1, however, reveals that two sets of resonances (labelled n and p for reasons to become apparent below) are unequal in intensity, being approximately in the ratio 3:2. On addition of $\underline{d}_6$-DMSO to the solution in $CDCl_3$, the resonances labelled p increase in intensity at the expense of the n resonances; once the amount of $\underline{d}_6$-DMSO in the solvent exceeds 25%, only the p resonances remain.

These observations suggest that in $CDCl_3$ solution there are two symmetrical conformations of triostin A which interconvert slowly on the nmr time scale. The presence of an unsymmetrical conformation is excluded. The effect of solvents on the equilibrium between the conformers, summarised in Table 2, shows that n is favoured in non-polar solvents, and p in polar solvents.

Table 2: Solvent Dependence of the Conformer Equilibrium

Solvent	%n	%p
2:1 $CCl_4/CDCl_3$	80	20
1:1 $\underline{d}_6$-benzene/$CDCl_3$	65	35
$CDCl_3$	60	40
1:1 $CDCl_3/\underline{d}_5$-pyridine	25	75
$\underline{d}_5$-pyridine	0	100
$\underline{d}_6$-DMSO	0	100

At temperatures above 100°, the spectrum of triostin A in $CDCl_3$ shows chemical exchange broadening; a coalescence temperature of 130°, found for the quinoxaline H-3 proton, corresponds to an energy barrier of about 22 kcal $mole^{-1}$ between the conformers.

Both the high energy barrier and the difference in polarity of the conformers is illustrated by a simple t.l.c. experiment. At -11°, triostin A separates cleanly into two components, R_f 0.58 and 0.66, on Merck silica gel plates developed with 9:1 chloroform/methanol. This experiment was performed on two 10x10 cm plates with samples of triostin applied at one corner. Plate I was dried for 90 minutes at -11° and plate II for a similar time at 22°, both plates were then developed, under the same conditions as before, at right angles to the first direction of development. The results, illustrated in Fig. 2, show that after 90 mins. the separated conformers have equilibrated only very slightly at -11°, but to a considerable extent at 22°.

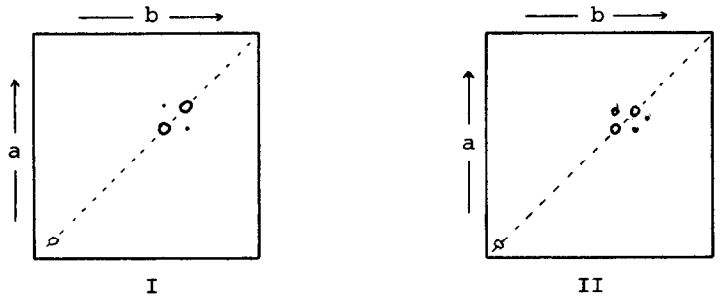


Fig. 2. Two dimensional separation of triostin A conformers on silica gel. 90 mins allowed between development in directions a and b for equilibration, I at -11° and II at 22°.

Two analogous symmetrical conformations (n:p ≃ 4:3) are adopted by Triostin C (in which the N-methylvalines of Triostin A are replaced by N,γ-dimethylalloisoleucines [2]), as evidenced by its ^1H nmr spectrum in $CDCl_3$ solution.

A detailed analysis of both conformations of triostin A, based on ^1H nmr evidence, is the subject of a manuscript now in preparation.

References and Footnotes

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2. H. Otsuka and J. Shoji, Tetrahedron, 1967, 23, 1535.
3. M. J. Waring, L.P.J. Wakelin and J.S. Lee, Biochim. Biophys. Acta., 1975, 407, 200.
4. H. Otsuka and J. Shoji, J. Antibiotics, 1976, 29, 107.
5. Cys βCH_2 protons of polar conformer are obscured by NMe resonances. Values quoted were obtained from a spectrum in the presence of $Eu(fod)_3$.