Solution Conformations of Two Synthetic Analogues of Quinoxaline Antibiotics

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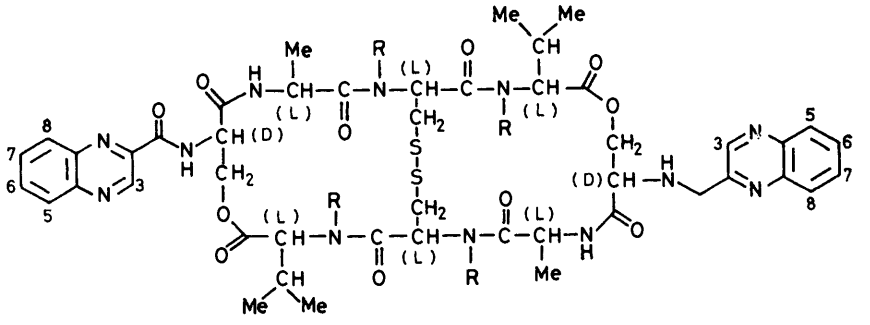
The solution conformations of two synthetic quinoxaline antibiotics (TANDEM and ELSERTA) have been determined by proton n.m.r. The parameters utilised include chemical shifts, coupling constants, and the temperature variation of NH chemical shifts (all in the solvents [^{2}H]chloroform, [$^2H_5$]pyridine, and [$^2H_6$]dimethyl sulphoxide). The conclusions have then been checked and amplified by the measurement of nuclear Overhauser effects in $CDCl_3$ solution to indicate proximities of the various protons. The structures deduced are compared with other conformationally detailed structures of quinoxaline antibiotics, and their relevance to DNA-binding studies is discussed.

QUINOXALINE antibiotics are derived from various species of *Streptomyces*. The history of their discovery and biological properties has been thoroughly reviewed.[1] The basic skeleton of the quinoxaline antibiotics is characterised by two quinoxaline-2-carboxamide chromophores linked to a cross-bridged dilactone cyclic peptide. These antibiotics bind strongly to double stranded DNA *via* the minor groove with bifunctional intercalation of the quinoxaline rings.[2]

Triostin A (1) is a member of the above family. Its de-*N*-tetramethyl analogue, TANDEM (2), has recently been synthesised,[3] as has also ELSERTA, the bis-L-seryl analogue of TANDEM. ELSERTA does not bind to DNA, whereas TANDEM does bind and, most importantly, binds approximately 10^4 times more tightly to poly(dA-dT) than to poly(dG-dC).[4] In order to examine the basis for these effects, we have extended our



(1) R = Me
(2) R = H

^{1}H n.m.r. studies of the quinoxaline antibiotics [5-7] to TANDEM and ELSERTA. We report here our conclusions on the solution conformations of these compounds in the absence of DNA.

DISCUSSION

(*A*) *Solution Conformation of TANDEM*

(1) *Proton Chemical Shifts and Coupling Constants.*—The ^{1}H n.m.r. chemical shifts of the proton resonances of TANDEM are given in Table 1 for three different solvents {[^{2}H]chloroform ($CDCl_3$), [$^2H_5$]pyridine, and [$^2H_6$]dimethyl sulphoxide ([$^2H_6$]-DMSO)}; the corresponding coupling constants are given in Table 2. The spectrum in $CDCl_3$ is reproduced in Figure 1a. The effect of temperature on the NH chemical shifts is expressed as a series of temperature coefficients in Table 3.

Since each pair of amino acids in TANDEM (2) gives rise to only one set of resonances (Table 1), TANDEM adopts a conformation which is symmetrical on the n.m.r. time scale.

(i) *Coupling constants.* The large values of $J_{\alpha,NH}$ for Cys and Val show that their NH and α-CH protons are *trans* to each other, (3), while the small values of Ser J_{α,β_1} and J_{α,β_2} show that the α-CH is *gauche* to both the β-CH protons, (4). For cysteine, J_{α,β_1} is large whereas J_{α,β_2} is small; this indicates a rigid geometry in which one β-proton is *trans* to the α-CH, and the other *gauche*, (5) or (6).

In contrast to the large $J_{\alpha,NH}$ values found for Cys and Val, those for Ala and Ser are smaller (Table 2). Thus for the Ala and Ser residues, more than one α-CH–NH dihedral angle is theoretically possible even if only a single conformer were highly populated; or several conformers could be populated. In view of the variation of the Ser $J_{\alpha,NH}$ coupling constant with solvent (Table 2), a low barrier to rotation about the α-CH–NH bond is

TABLE 1

Proton chemical shifts (δ) for TANDEM (2) in various solvents [a]

	Proton	$CDCl_3$	[$^2H_5$]Pyridine	[$^2H_6$]-DMSO
Val	γ	1.13, 1.17	1.05, 1.15	0.93, 1.08
	β	2.54	2.53	2.30
	α	4.84	5.09	4.13
	NH	8.43	8.84	8.20
Ala	β	1.38	1.31	1.24
	α	4.45	5.03	4.28
	NH	6.30	8.83	8.13
Ser	β_1	4.66	4.85	4.13
	β_2	5.00	5.00	4.64
	α	4.87	5.33	4.76
	NH	8.81	8.88	8.66
Cys	β_1	2.92	3.09	2.91
	β_2	2.95	3.26	2.69
	α	5.68	6.14	5.28
	NH	6.57	10.26	8.71
Quinox	H-3	9.68	9.61	9.50
	H-6,7	*ca.* 7.90	7.64	8.03
Other aromatics	H-5	8.10	7.75	8.13
	H-8	8.23	8.05	8.23

[a] The concentrations of the TANDEM were *ca.* 4 mM.

TABLE 2

Couplings constants (Hz) for TANDEM (2) in various solvents

		$CDCl_3$	[$^2H_5$]Pyridine	[$^2H_6$]-DMSO
Val	$J_{\beta,\gamma}$	6.6, 6.9	~6.5	6.5
	$J_{\alpha,\beta}$	4.5	6.1	7.8
	$J_{\alpha,NH}$	9.6	10.0	9.3
Ala	$J_{\alpha,\beta}$	7.1	~7	7.4
	$J_{\alpha,NH}$	6.0	6.1	6.6
Ser	J_{β_1,β_2}	11.0	10.8	11.2
	J_{α,β_1}	2.0	2.4	2.7
	J_{α,β_2}	2.5	3.2	3.3
	$J_{\alpha,NH}$	6.7	7.7	8.4
Cys	J_{β_1,β_2}	15.0	14.5	14.0
	J_{α,β_1}	12.8	11.1	11.9
	J_{α,β_2}	2.8	3.5	3.6
	$J_{\alpha,NH}$	10.0	9.9	9.9

TABLE 3

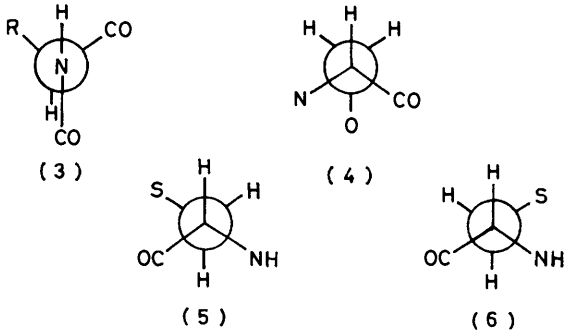
Temperature coefficients (p.p.m./°C) for NH resonances of TANDEM (2) in various solvents [a]

	Temperature coefficient × 10^3		
Proton	$CDCl_3$	[$^2H_5$]Pyridine	[$^2H_6$]-DMSO
Val-NH	−2.2	−3.8	−1.5
Ser-NH	−2.8	−0.6	−0.5
Ala-NH	+0.6	−10.9	−5.0
Cys-NH	−4.6	−8.7	−4.3

[a] A negative value indicates an upfield shift with increasing temperature.

indicated. The value of 4.5 Hz for Val $J_{\alpha,\beta}$ shows that the dihedral angle between Val-α-CH and -β-CH protons is no longer constrained to 180°, as it is in echinomycin [5] and triostin A.[7]

(ii) *Chemical shifts.* In $CDCl_3$ solution, the NH resonances of Ser and Val are at much lower field than those of Ala and Cys. The chemical shift (δ = 8.81 p.p.m. in $CDCl_3$) of the Ser-NH is similar to the shifts observed for this resonance in echinomycin (δ = 8.64 and 8.83 p.p.m., in an unsymmetrical molecule [5]) and triostin A (δ = 8.86 and 8.99 p.p.m., in two conformers [7]). In all three cases, the low-field shift of the Ser-NH may primarily be accounted for by the fact that the NH is held in the plane of the quinoxaline ring (and, therefore, in its deshielding region [5,7]). For the Val-NH resonance, we conclude that the low-field shift is indicative of intramolecular hydrogen bond formation; and, conversely, that the Ala-NH and Cys-NH are not involved in such bonds. These conclusions are strongly supported by the large downfield shifts of the Ala- and Cys-NH resonances



on changing the solvent to pyridine or DMSO, while the Ser- and Val-NH resonances are relatively unaffected (Table 1). Thus, the former pair form hydrogen bonds to the nitrogen atom of pyridine and to the oxygen atom of DMSO, whereas the latter pair are prevented from doing so either by steric hindrance (Ser-NH) or by involvement in intramolecular hydrogen bonding (Val-NH).[8] As expected,[8] the solvent-exposed NH protons of Ala and Cys show relatively large negative temperature coefficients in pyridine and DMSO (Table 3), since hydrogen bonding to the solvent is disrupted with increasing temperature. That the relatively small temperature coefficients of the Val-NH resonance are consistent with its involvement in an intramolecular hydrogen bond is seen by comparison with corresponding values for the rigid model alumichrome C[8] (Table 4).

TABLE 4

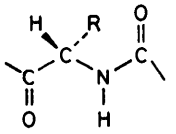
Comparison of temperature coefficients (p.p.m./°C × 10^3) of NH resonances in TANDEM (2) and alumichrome C

Compound	Residue	In DMSO solution	In pyridine solution
Alumichrome C	Orn-3 [a]	−1.7	−5.8
	Gly-3 [a]	−1.1	−3.7
	Gly-1 [b]	−5.4	−11.8
	Ala-2 [b]	−4.7	−10.8
TANDEM	Val	−1.5	−3.8
	Ala	−5.0	−10.9
	Cys	−4.3	−8.7

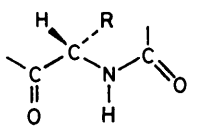
[a] NH Intramolecularly hydrogen bonded, or buried, in alumichrome C. [b] NH Solvent exposed in alumichrome C.

In the search for evidence for the site to which the Val-NH is hydrogen bonded, we consider firstly the likely geometry of the amide bonds in TANDEM and, secondly, the geometry of analogous models. Peptide amide bonds are normally *trans*, (7). A rare exception is a single *cis*-amide, (8), found in the glycopeptide antibiotic vancomycin;[9] the chemical shift of the α-CH is then at relatively high field (4.22 p.p.m.).[10] Presumably, this is largely because the α-CH proton is remote [9] from the carbonyl group of the adjacent amino-

acid, (8). In contrast, in a favoured conformer of a *trans*-amide, (7), the α-CH proton is nearly eclipsed with the carbonyl group with a resulting deshielding effect. The chemical shifts of the Val-, Ser-, and Cys-α-CH protons of TANDEM [4.84, 4.87, and 5.68 p.p.m. (Table 1)] are all consistent with adjacent *trans*-amide bonds. The chemical shift (4.45 p.p.m.) of the Ala-α-CH proton is ambiguous in terms of the above criterion. Here, we assume the Ala-NH–Ser-CO amide bond to be *trans* by analogy to the crystal conformation established by a recent *X*-ray study of TANDEM.[11,12] In the crystal, all of the amide bonds are indeed *trans*.

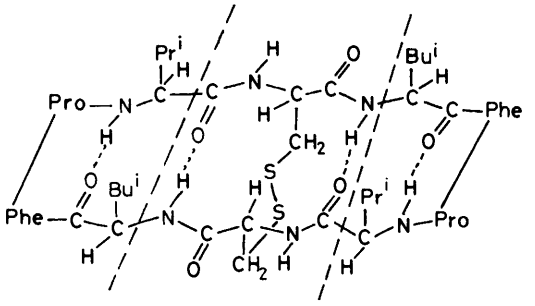


(7)



(8)

Since the above arguments make a general assumption as to the conformation of the peptide backbone [(7), which is consistent with the coupling constants shown in Table 2], it is important to check whether the finally deduced, overall conformation is consistent with this assumption. It will be seen subsequently that it is, as is also the crystal conformation.[11,12]



(9)

The peptides [2,7-dicysteine]-gramicidin S and *SS*′-bis(Cbz-L-Ala-L-Cys-L-Ala-OMe) have both been studied by ^{1}H n.m.r. Relevant details of the anti-parallel arrangements deduced for the conformations of these compounds are reproduced in (9) and (10), respectively. As reproduced, each structure contains a central portion (bounded by dashed lines) which is common with TANDEM (1). Chemical-shift and coupling-constant comparisons are made between (9) [13] and (2) (Table 5) and (10) [14] and (2) (Table 6).

The relatively close agreement between the parameters quoted for the cysteine residues in (2) and (9) in [$^2H_6$]-DMSO solution (Table 5), and between (2) and (10)

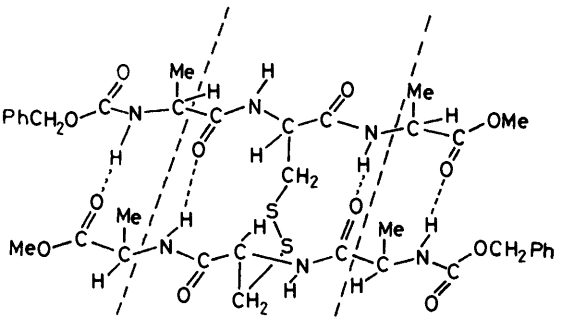
TABLE 5

Comparison of some ^{1}H n.m.r. parameters for TANDEM (2) and [2,7-dicysteine]-gramicidin S (9) [a]

Compound or standard value	δ(Cys α-CH)	δ(Cys-NH)	*J*(α-CH, NH)/Hz
TANDEM (2)	5.26	8.71	9.9
(9)	5.3	8.78	8.5
'Typical' values [15]	4.64	8.29	—

[a] Measured in [$^2H_6$]-DMSO solution, chemical shifts in p.p.m.

in $CDCl_3$ solution (Table 6), strongly support a similar conformation in TANDEM to those shown between the dashed lines in (9) and (10). In particular, the Cys-α-CH chemical shifts are quite different in *SS*′-bis(Cbz-L-Ala-L-Cys-OMe) and *SS*′-bis(Cbz-L-Cys-L-Ala-OMe)



(10)

(Table 6); evidence has been presented [14] to show that these tetrapeptides are too small to form extensively the specific conformations shown in (9) and (10). We conclude that, in analogy to (9) and (10), the Val-NH of TANDEM is hydrogen-bonded to the carbonyl of Ala [see (2)].

Some years ago, it was proposed [8] that the relatively acidic alcohol trifluoroethanol (TFE) may be used to protonate an exposed oxygen atom of an amide carbonyl group. In such cases, the resulting downfield shifts of the associated NH resonance are rationalised in terms of a reduction in electron density on nitrogen [(11) ⟶ (12)]. On the assumption that this provides a valid criterion for an exposed carbonyl group, we added 10% TFE to a solution of TANDEM (2) in $CDCl_3$, in the

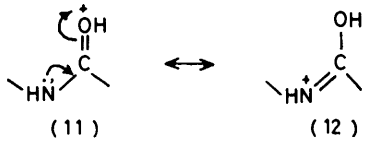
TABLE 6

Comparison of some ^{1}H n.m.r. parameters for TANDEM (2) and *SS*′-bis(Cbz-L-Ala-L-Cys-L-Ala-OMe) (10) [a] and analogues

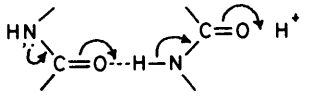
Compound	δ(Cys-α-CH)	δ(Cys-NH)	δ(Cys-β-CH_2)	*J*(α-CH, NH)/Hz
TANDEM (2)	5.68	6.57	2.92, 2.95	10.0
(10)	5.49	6.88	2.9	9.6
SS′-bis(Cbz-L-Ala-L-Cys-OMe)	5.12	—	—	—
SS′-bis(Cbz-L-Cys-L-Ala-OMe)	4.85	—	—	—

[a] Measured in $CDCl_3$ solution; chemical shifts in p.p.m.

expectation that the Cys-NH resonance would not suffer a downfield shift (since the Cys-NH group forms an amide bond with the sequestered carbonyl group of Ala). In this experiment, the expectation is not fulfilled; the downfield shifts are 0.18 (Val-NH), 0.14 (Ser-NH), 0.12 (Ala-NH), and 0.5 p.p.m. (Cys-NH). In view of the clear evidence for a Val-NH to Ala-CO intramolecular hydrogen bond cited earlier (and supported by the presence of the same hydrogen bond in the crystal structure [11,12]), we conclude that the criterion is not reliable in this case (and perhaps in other cases also, although we have used it in previous work [7]). Possible reasons for the failure of the criterion in the present case include (*i*) a conformational change upon addition of TFE and, perhaps more likely, (*ii*) transmission of polarisation through the proposed hydrogen bond, (13).



(11) (12)



(13)

The above proposal, (13), gains credence if the Cys-carbonyl group is a relatively good electron donor. The shift reagent $Eu(fod)_3$ is a Lewis acid [16] and should bind preferentially either to an exposed carbonyl which is a good electron donor, or in multidentate chelation. The ^1H n.m.r. spectrum of TANDEM in $CDCl_3$ solution after addition of 0.1 equivalents of $Eu(fod)_3$ is reproduced in Figure 1b. Marked broadening and downfield shifts of the α-CH and NH resonances of Val and Cys, and of the β-CH_2 resonances of Cys occur; the resonances associated with Ala and Ser are only slightly affected. That this broadening is due to a chemical exchange phenomenon, rather than to the paramagnetism of the lanthanide, is shown by lowering the temperature to 6 °C, whereupon the broadened signals sharpen and move back towards their normal resonance positions. It is clear that a complex is formed between TANDEM and $Eu(fod)_3$, with an energy barrier to the dissociation of this complex. The recorded spectra (*e.g.* Figure 1b) are near the slow exchange limit between free antibiotic and complex. Since the amount of complex is relatively small (*ca.* 10%), and its signals probably broadened by paramagnetism, signals due to complex are not detected. The resonances most shifted in the complex, and hence those most broadened in the slow exchange limit, are anticipated to be those nearest the Eu atom. Since the protons corresponding to the broadened resonances (Figure 1b) are all in the vicinity of the Cys-carbonyl group [see (2)], we conclude that this group is exposed and is a relatively good electron donor [as required to support the proposal in (13)].

(2) *Model-building Studies.*—In the following discussion, the dissection of the peptide backbone of TANDEM (2) will be considered as shown for the analogues (9) and (10). Construction of CPK models then shows that a cross-ring hydrogen bond of Val-NH

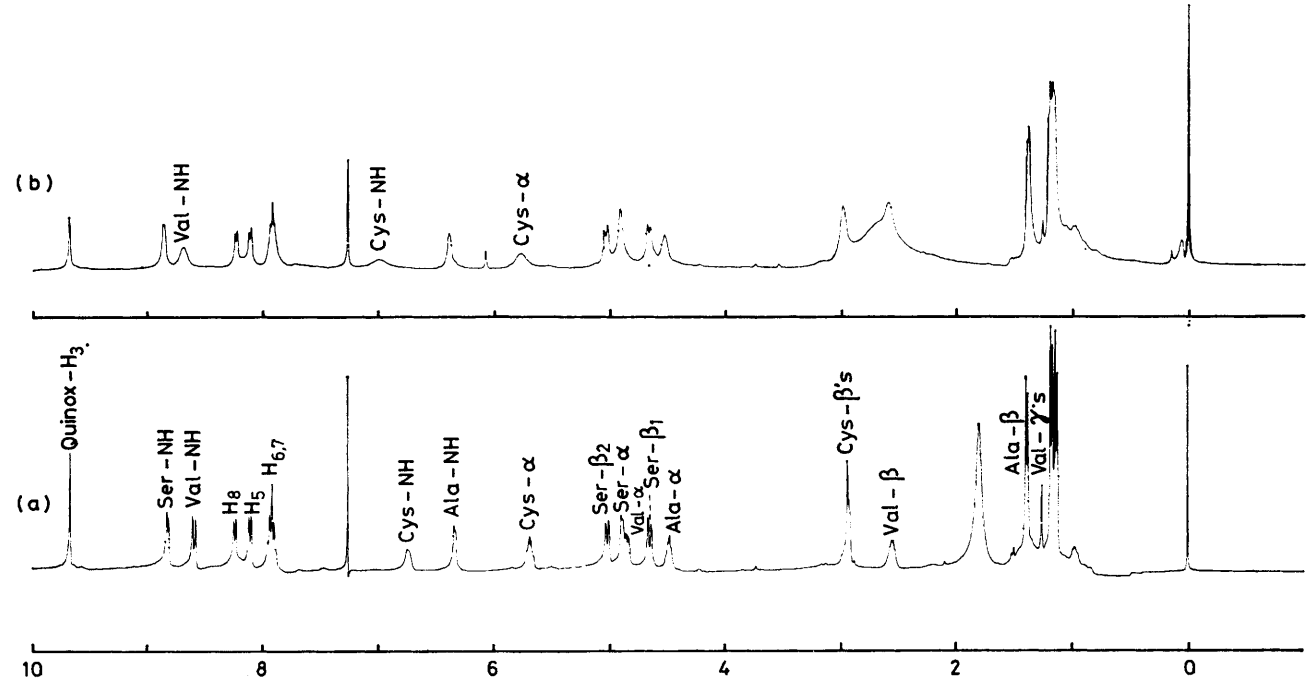
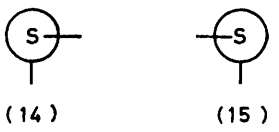


FIGURE 1 (a) 360 MHz ^1H Spectrum of TANDEM (4mM) in $CDCl_3$ solution measured at 360 MHz; and (b) the same spectrum after addition of $Eu(fod)_3$ (0.10 equivalents)

to Ala-CO is possible only if the disulphide bridge is 'down', *i.e.* as shown in (9) and (10). The disulphide groups may be either P-helical or M-helical, (14) and (15) respectively, which represent views along the S–S bond, and recognise that the most stable dihedral angle of the CSSC group is restricted to *ca.* 90°.[17] The models establish that a P-helical structure is obtained if the cross-bridge adopts the allowed conformation (6); and, conversely, that an M-helical structure is obtained if the cross-bridge adopts the allowed conformation (5).

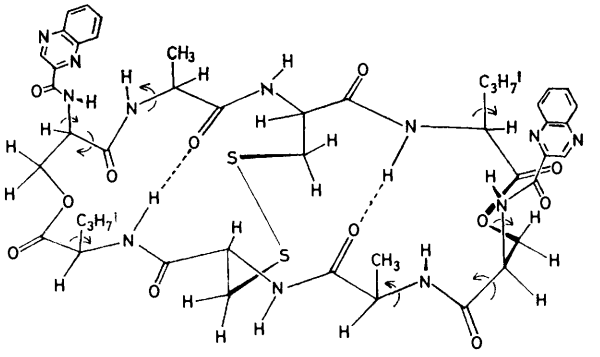


(14) (15)

The barrier to interconversion of P- and M-helical structures is expected to lie in the range 50—90 kJ mol^{-1}.[7,18] If both were significantly populated, we would expect their interconversion to be slow on the n.m.r. time-scale within the range of available temperatures. Since no such dynamic behaviour is observed for TANDEM, we conclude that only one of the helical structures is occupied. In the following section, we show by the use of nuclear Overhauser effects (n.O.e.s), that this structure is P-helical in conjunction with conformation (6).

(3) *Nuclear Overhauser Effects.*—Nuclear Overhauser effects (n.O.e.s) can be used to obtain information about the relative proximities of protons. With the advent of n.O.e.-difference spectroscopy, these measurements can be carried out with great reliability.[19] Therefore, we have irradiated in turn each proton in the TANDEM spectrum, using an appropriate sequence (see Experimental section), then recorded the FID after switching off the irradiating frequency. N.O.e.-difference spectra were obtained by subtracting n.O.e.-enhanced and control spectra; these were acquired alternately to minimise the effect of spectrometer drift. At ambient temperature in $CDCl_3$ solution, no n.O.e.s were found. In DMSO or voltalef–CD_2Cl_2 (25 : 75) mixtures (using viscous solvents to reduce the rotational correlation time of TANDEM), small negative n.O.e.s were found.[20] At 40 °C in $CDCl_3$ solution, small positive n.O.e.s (<5%) were observed. These positive enhancements were utilised so that steady-state n.O.e.s could be employed with no spin-diffusion anomalies. The data are summarised in Table 7.

We now use the data of Table 7, in conjunction with earlier conclusions, to derive an overall conformation [(16) Figure 2] for TANDEM.



(16)

FIGURE 2 Solution conformation(s) (16) of TANDEM as derived from ^1H n.m.r. data

Val-NH shows a n.O.e. to Cys-α-CH, but not to Val-α-CH. The first observation indicates a *cis*-orientation of the Cys-α-CH and Val-NH protons [analogous to the situations in the models (9) and (10)], whereas the second observation is in accord with a *trans*-orientation of the Val-α-CH and Val-NH protons (as required by the large vicinal coupling between these protons). Similarly, Cys-NH shows a n.O.e. to Ala-α-CH, but not to Cys-α-CH, again in accord with the geometry given in (16), and the large vicinal CH-NH coupling in the Cys residue. Val-α-CH gives a n.O.e. to both Val-β and Val-γ. These results preclude a situation where the Val-α and Val-β protons are held in a rigid *trans*-geometry (as in echinomycin [5] and triostin A [7]). The observation of both n.O.e.s indicates that free rotation is possible about

TABLE 7

N.O.e.s observed in the ^1H n.m.r. spectrum of TANDEM (2) in $CDCl_3$ at 40 °C [a]

^1H signal enhanced	^1H peak irradiated													
	Ser-NH	Val-NH	Cys-NH	Ala-NH	Cys-α	Ser-β_2	Ser-α	Val-α	Ser-β_1	Ala-α	Cys-β	Val-β	Ala-β	Val-γ
Ser-NH	—	*b*		×		×								
Val-NH		—			×						×			
Cys-NH			—	*b*						×	×			
Ala-NH	×		*b*	—						×			×	
Cys-α		×			—						×			
Ser-β_2	×			×		—	*b*	*b*	*b*					
Ser-α	×			×		*b*	—	*b*	*b*	*b*				
Val-α						*b*	*b*	—	*b*	*b*		×		×
Ser-β_1						*b*	*b*	*b*	—	*b*				
Ala-α			×	×				*b*	*b*	—			×	
Cys-β					×						—			
Val-β								×				—		×
Ala-β				×						×			—	
Val-γ	×	×		×				×				×	*b*	—

[a] N.O.e.s are indicated in the Table by a cross (×). [b] Resonance perturbed by its proximity to the irradiating frequency.

the Cys α–β bond. This is in accord with the relatively low and solvent-variable coupling constant (Table 2), and with the *X*-ray structure which shows two different rotamers about the Cys α–β bond.[11,12] The possibility for the Val-γ protons to occupy the space over the inside perimeter of the peptide backbone is also indicated by the n.O.e.s Val-NH ⟶ Val-γ and Ser-NH ⟶ Val-γ.

Given the conformation of the peptide backbone so far deduced, the position of the Val-carbonyl carbon is approximately located; this carbonyl group must be oriented with its oxygen atom outside the depsipeptide ring (16) in order to allow completion of the ring structure. As in earlier work,[5,7] the ester linkages can be assumed to be *trans* (Syn-planar) as in many crystal studies of esters,[21] including the recent *X*-ray structure of TANDEM.[11,12] Model-building studies reveal that the Ser-α-CH and -β-CH_2 groups can only point outwards from the depsipeptide ring, and in conjunction with the Ser-α,β coupling constants (Table 2), allow this ring to be completed as shown in (16) (Figure 2).

The problems of the conformations about the Ser(α-CH–NH) and Ala(α-CH–NH) bonds are now considered. The mutual n.O.e.s between Ala-β and Ala-NH, and the n.O.e. Ala-NH ⟶ Val-γ indicate the population of conformations in which the Ala-NH is on the top face of the molecule, as shown in (16). However, the mutual n.O.e.s between Ala-α and Ala-NH indicate the population of conformations in which the Ala-NH is near to the plane of the outer perimeter of the depsipeptide ring. Similarly, the n.O.e. Ser-NH ⟶ Val-γ indicates the population of conformations in which the Ser-NH is on the top face, as shown in (16). Clearly there are also one or more conformations in which the Ser-NH is near to the plane of the depsipeptide ring since the n.O.e. Ser-NH ⟶ Ser-α and the mutual n.O.e.s between Ser-β_2 and Ser-NH are observed. These data indicate conformational mobility about the Ala(α–NH), Ser(α–NH) and Ser(α–CO) bonds denoted by curved arrows in (16). It is noteworthy that precisely this conformational mobility had been considered possible from the consideration of coupling constants (Table 2). Despite rotation about the Ser(α–NH) bond, the Ser-NH remains poorly accessible to solvent since the quinoxaline-amide unit rotates as a planar unit in which the Ser-NH is buttressed against N-1 of the heterocycle.[5] We do not feel that the remaining n.O.e.s Ala-NH ⟶ Ser-β_2 and Ala-NH ⟶ Ser-α can be securely interpreted to indicate further flexibility in the vicinity of the Ser and Ala residues since these could arise *via* transfer of saturation from Ala-NH to Ser-NH, these protons being proximate in some conformations.

Finally, the n.O.e.s establish a P-helical structure (15) of the cross-bridge in conformation (6). The evidence lies in the n.O.e. Cys-β ⟶ Val-NH; the P-helical structure (16) brings one of the Cys-β protons into the required proximity with Val-NH on the same side of the ring. A M-helical structure in conformation (5) brings one of the Cys-β protons towards the Val-NH on the opposite side of the ring, but still too distant for the expectation of a clear n.O.e. The ratio of distances between the Val-NH and Cys-α, and between Val-NH and Cys-β can be estimated from the ratio of the n.O.e.s. From Cys-α to Val-NH there is a 4% n.O.e.; this effect is 1.3 times larger than from Cys-β. The ratio of the distances is thus $1 : \sqrt[6]{1.3}$, *i.e.* 1.04; this ratio is consistent only with the P-helical structure (16).

(*B*) *The Solution Conformation of ELSERTA*

The ^1H n.m.r. spectra of ELSERTA [(2) but with both serine residues possessing the L-configuration] have been studied in $CDCl_3$, [$^2H_6$]-DMSO, and [$^2H_5$]pyridine. The data are summarised in Tables 8—10.

Comparison of these data with the corresponding data

TABLE 8

Proton chemical shifts (δ) for ELSERTA in various solvents [a]

Proton		$CDCl_3$ at 23 °C	[$^2H_5$]Pyridine at 23 °C	[$^2H_6$]-DMSO at 30 °C
Val	γ	1.0	0.80, 0.96	0.85/0.89
	β	2.32	2.25	2.22
	α	4.69	5.07	4.33
	NH	8.56	8.91	8.32
Ala	β	1.48	1.45	1.23
	α	4.64	5.06	4.30
	NH	6.81	9.61	8.50
Ser	β_1	4.02	4.62	4.17
	β_2	5.02	4.95	4.62
	α	4.90	5.53	4.84
	NH	8.73	9.52	8.72
Cys	β_1	2.91	3.17	2.83
	β_2	2.93	3.33	2.90
	α	5.78	6.19	5.26
	NH	7.11	10.41	8.76
Quinox	H-3	9.61	9.09	9.44
Other aromatics		7.87	7.56	7.96
		8.16	7.79/8.04	8.15

[a] The concentrations of ELSERTA were *ca.* 4mM.

TABLE 9

Coupling constants (Hz) for ELSERTA in various solvents

		$CDCl_3$	[$^2H_5$]Pyridine	[$^2H_6$]-DMSO
Val	$J_{\beta,\gamma}$	*ca.* 6	*ca.* 6	7
	$J_{\alpha,\beta}$	4.0	4.7	5.8
	$J_{\alpha,NH}$	10.1	10.0	10.0
Ala	$J_{\alpha,\beta}$	*ca.* 6	7.6	7
	$J_{\alpha,NH}$	5.6	*ca.* 5	5
Ser	$J_{\beta_1\beta_2}$	10.2	10.0	11.1
	$J_{\alpha\beta_1}$	10.8	9.5	6.9
	$J_{\alpha\beta_2}$	4.5	6.1	4.2
	$J_{\alpha,NH}$	7.5	8	7.5
Cys	$J_{\beta_1\beta_2}$	15.2	14.7	14.5
	$J_{\alpha\beta_1}$	11.5	12.3	12.1
	$J_{\alpha\beta_2}$	2.7	3.3	3.4
	$J_{\alpha,NH}$	*ca.* 9	9—10	10

TABLE 10

Temperature coefficients (p.p.m./°C) for NH resonances of ELSERTA in various solvents [a]

	Temperature coefficient × 10^3		
Proton	$CDCl_3$	[$^2H_5$]Pyridine	[$^2H_6$]-DMSO
Val-NH	−1.6	−3.6	−2.5
Ser-NH	−1.5	−6.2	−1.7
Ala-NH	−4.0	−8.3	−4.7
Cys-NH	−6.5	−11.1	−4.2

[a] A negative value indicates an upfield shift with increasing temperature.

for TANDEM (Tables 1—3) shows that they are very similar except in the following features; (*i*) the chemical shifts of the Ser-β, Ser-NH, and Ala-NH protons are appreciably different, (*ii*) the Ser-α,β coupling constants (Table 9) show that the Ser-α proton is now *trans*- and *gauche*-orientated with respect to the Ser-β protons (in contrast to *gauche–gauche* in TANDEM, and (*iii*) the Ser-NH shows a larger temperature variation in ELSERTA in pyridine solution (Table 10), indicating that it is now somewhat more solvent exposed. These conclusions are consistent with the conformations of TANDEM and ELSERTA being similar except insofar as the positions and orientations of the Ser-α protons and NHCO-quinoxaline residues in (16) have been interchanged.

(C) Comparison with other Structural Studies on Quinoxaline Antibiotics and Implications for DNA Binding

The conformation of TANDEM in the crystal as determined by *X*-ray analysis is reproduced in Figure 3.[11] Although the n.m.r. analysis cannot produce a structure

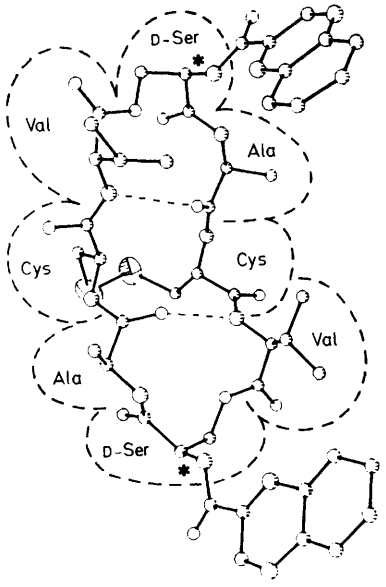
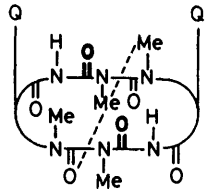


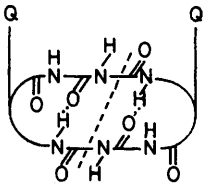
FIGURE 3 Crystal structure of TANDEM determined by *X*-ray analysis at room temperature to a resolution of 0.85 Å [11]

with the same precision as *X*-ray analysis, a comparison of Figures 2 and 3 clearly shows that the conformations in the crystal and solution are very similar; the disulphide bridge is P-helical in each case. It has been noted [11] that in the crystal structure, the mean planes of the two quinoxaline chromophores are inclined at an angle of 14°. However, these chromophore planes are expected to lie parallel to allow for bifunctional intercalation to DNA.[22] The present n.m.r. study establishes that the necessary rotations of *ca.* 20° about the Ser C_α-N bonds can occur with energy barriers that are surmountable at room temperature in solution (see Figure 2). Additionally, the ready population of conformers possessing other than a *trans*-orientation of Val-α and Val-β protons, established by n.m.r. in solution, is also indicated in the crystal structure at room temperature (Figure 3) where two different orientations of the Val side-chain occur within the TANDEM molecule.

The most fundamental differences in the solution conformations of echinomycin [5] and triostin A (P-conformation) [7] relative to TANDEM are that the former pair have their Ala-Cys and Cys-Val amide bonds oriented roughly perpendicular to the plane of the depsipeptide ring (17) whereas the latter has these amide bonds near to the plane of the depsipeptide ring (18). In the case of echinomycin and triostin A, conformation (18) is precluded by methylation of the Cys- and Val-nitrogens (17).



(17)



(18)

It has been suggested [11] that TANDEM may show strong binding to poly(dA-dT) but much weaker binding to poly(dG-dC) because the alanine-NH protons [underlined in (18)] may form a hydrogen-bond to the C-2 oxygen of thymine in the narrow groove. Further, that echinomycin and triostin A may show stronger binding to poly(dG-dC) because the alanine-carbonyl group [heavy outline in (17)] is now available to form a hydrogen-bond to the 2-amino-groups of guanine nucleotides in the minor groove.[11] The present, and earlier,[5,7] findings by n.m.r. are consistent with these proposals. In future work, we plan to test these proposals by direct examination of antibiotic–DNA-duplex complexes by n.m.r. It is clear that ELSERTA does not bind to DNA because the quinoxaline chromophores are not oriented as in TANDEM [Q-groups in (18)], but lie close to the plane of the depsipeptide ring.

EXPERIMENTAL

TANDEM and ELSERTA were synthesised by the previously published procedure.[3] N.m.r. spectra were recorded on Varian XL-100, and Bruker WH-270 and WH-400 instruments. Spin-decoupling experiments were performed on the WH-270 instrument using a power level of −20 dB. Steady state n.O.e. experiments were carried out on the WH-400 instrument in $CDCl_3$ at 40 °C using the sequence (D_1—D_2—τ—90°—AT), where D_1 = 5 s (recovery of the spin system); D_2 = 1.5 s (decoupler on for n.O.e. build-up); τ = 2 ms delay; 90° = 90° pulse; and AT = 1 s acquisition time.

Control and enhanced spectra were acquired alternately in separate memory blocks to minimise spectrometer drift, and data then displayed as n.O.e. difference spectra.

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