# The Conformations of Triostin A in Solution

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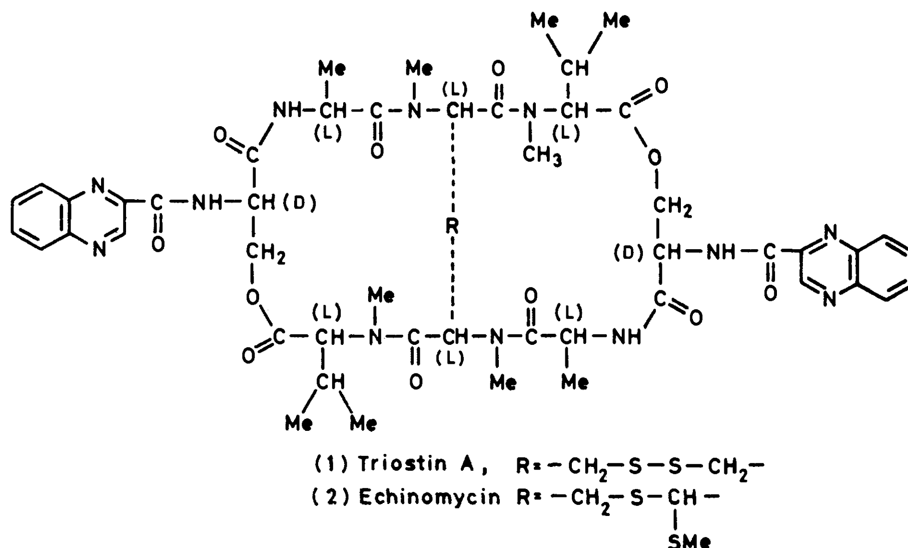
The two symmetrical conformations of triostin A in deuteriochloroform have been investigated by $^1H$ n.m.r. spectroscopy. The occurrence of two conformations is rationalised as resulting from the reversal of chirality of the disulphide bond, which is postulated to exist as a rapidly interconverting mixture of several rotameric states. Analysis of the spectrum of the least polar conformer is consistent with the presence of an intramolecular hydrogen bond.

TRIOSTIN A (1) is a quinoxaline antibiotic [1,2] which has been shown to intercalate bifunctionally between the base-pairs of double-helical DNA.[3] The cyclic octadepsipeptide structure (1) differs from that of echinomycin [4,5] (2) only in the nature of the cross-bridge.




It has already been shown [6] that in deuteriochloroform, the antibiotic exists as a mixture of two conformations separated by an energy barrier of *ca.* 22 kcal $mol^{-1}$, and that the conformer labelled 'triostin p' (for polar) is favoured over 'triostin n' (non-polar) by an increase in solvent polarity. We now wish to report an n.m.r. study of the two conformers, the results of which have been used as a basis for model construction.

## RESULTS AND DISCUSSION

*Hydrogen*-1 *Shifts and Three-bond Couplings*.—The n.m.r. spectrum of triostin A (3mM) in deuteriochloroform is shown in Figure 1. Both triostin p and triostin n are symmetrical in solution, enabling both spectra to be assigned readily by spin-decoupling experiments. Only the two pairs of *N*-methyl resonances remain to be rigorously assigned. Three bond-coupling constants were obtained from ABX analyses of the α-CH-β-$CH_2$ multiplets of the serine and cystine residues. The Cys-β-$CH_2$ resonances of triostin p were overlapped by other resonances and it was necessary to add 0.2 mol equiv. of [Eu(fod)$_3$] to shift them clear of the overlapping signals. Since none of the other coupling constants show a change on addition of [Eu(fod)$_3$], it is reasonable to assume that no significant conformational change has taken place. The measured values of $J_{\alpha\beta1}$ and $J_{\alpha\beta2}$ for the Cys residue of triostin p are therefore assumed to approximate closely to the couplings in $CDCl_3$ solution [these values also agree closely with those for the same couplings in $(CD_3)_2SO$ solution].

The $^1H$ chemical shifts for triostin p and triostin n are given in Table 1 and the coupling constants in Table 2. The chemical-shift values observed are close to those observed for simple linear peptides,[7-9] except than Ala-βMe in triostin n is found at higher field, and Cys-αCH, Val-αCH in triostin p, Ala-NH in triostin n and both Ser-NH resonance are at low field. Comparison of the shift data of each conformer in turn with those of echinomycin under the same conditions shows a close similarity between echinomycin and triostin p. This parallel also extends to the results of the temperature- and solvent-variation experiments discussed below, strongly suggesting that triostin p and echinomycin have very similar conformations. The chemical shifts of triostin n are less similar to those of echinomycin but the [Eu(fod)$_3$] binding studies reveal that the metal binds in the

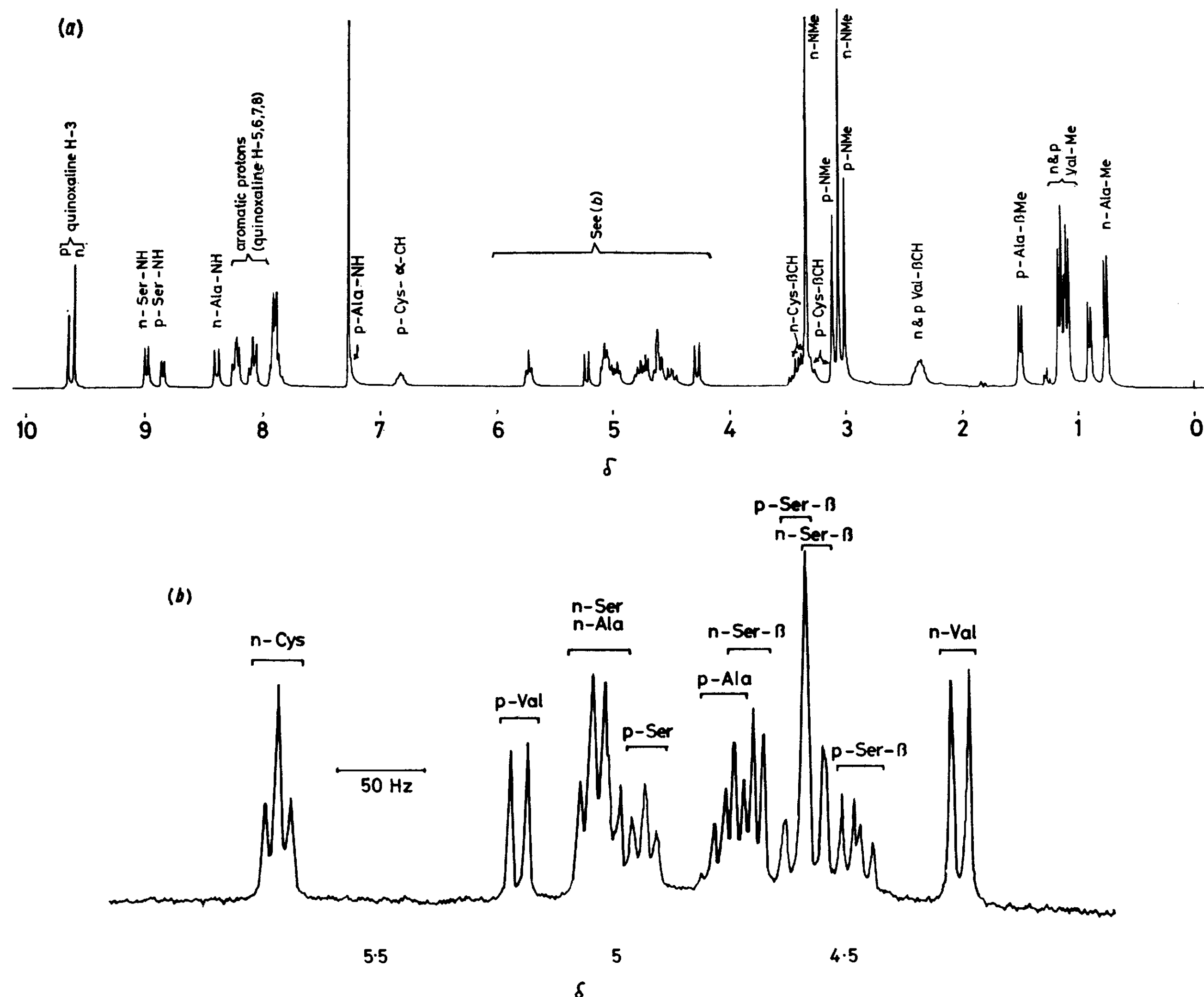



FIGURE 1 (*a*) The 270 MHz $^1H$ n.m.r. spectrum of triostin A in $CDCl_3$ solution. (*b*) Expansion of (*a*) in the δ 4—6 p.p.m. region. In both (*a*) and (*b*) n and p refer to triostin n and triostin p

same manner to each compound and gives very similar lanthanide-induced shifts (LIS) values in each case.

*Temperature and Solvent Effects.*—Table 3 summarises the solvent dependence of the NH chemical shifts, and Table 4 their temperature dependence in chloroform and pyridine solutions. On addition of pyridine to the $CDCl_3$ solution, or on changing the solvent to $(CD_3)_2SO$, the Ala-NH proton of triostin p is considerably deshielded, which can be attributed to the formation of hydrogen bonds to the solvent. This conclusion is confirmed by the high temperature coefficient of the triostin-p Ala-NH proton chemical shift in pyridine solution. In contrast, the chemical shift of the triostin-n Ala-NH proton is virtually the same in all solvents used (except $CDCl_3$–trifluoroethanol) suggesting that this proton is not exposed to the solvent. The triostin-p Ser-NH resonance is at lower field in $CDCl_3$ than in DMSO or $CDCl_3$–pyridine, showing that this NH proton is also inaccessible to these hydrogen-bonding solvents. This proton shows a significantly larger coupling to the α-CH proton in $(CD_3)_2SO$ than in chloroform (*J* 9.1 and 6.4 Hz respectively) suggesting some change in conformation which may be responsible for the upfield shift in DMSO. For the triostin-n Ser-NH resonance a downfield shift is observed on addition of either pyridine or $(CD_3)_2SO$ indicating that this NH proton is moderately accessible to these solvents. Unfortunately this cannot be confirmed by measurements of the temperature coefficients in pyridine or DMSO since only triostin p is present in these solvents. Information about the accessibility of carbonyl groups to the solvent can be obtained by observing the effect on the shift of the neighbouring NH proton of adding trifluoroethanol (TFE) to the sample.[10] Thus the upfield shift of the triostin-p Ala-NH resonance resulting from the addition of TFE indicates that the Ser carbonyl groups are inaccessible to the solvent. By the same criterion, the Ser carbonyl groups of triostin n appear to be accessible to the solvent.

*Conformational Structure Determination of Triostin*

*A*.—Both conformers of triostin A show many similarities to echinomycin in various aspects of their n.m.r. characteristics. In a previous study[5] we used n.m.r. measurements and results of potential-energy calculations to determine the conformation of echinomycin in $CDCl_3$ solution, and this is shown in Figure 2. The structure can be represented diagrammatically as in

TABLE 1

Hydrogen-1 chemical shifts for triostin and echinomycin in $CDCl_3$ solution

| | Triostin | | Echinomycin [a] | |
|---|---|---|---|---|
| | p-conformer | n-conformer | I | II |
| Val-γ | 0.87 | 1.06 | 1.09 | 0.89 |
| | 1.09 | 1.13 | 1.09 | 0.93 |
| Val-β | 2.34 | 2.34 | 2.33 | 2.36 |
| Val-α | 5.22 | 4.27 | 5.14 | 5.18 |
| NMe | 2.99 | 3.05 | 3.00 | 3.01 |
| | 3.10 | 3.33 | 3.11 | 3.18 |
| Ala-β | 1.46 | 0.73 | 1.38 | 1.41 |
| Ala-α | 4.78 | 5.05 | *ca.* 4.82 | *ca.* 4.95 |
| Ala-NH | 7.24 | 8.41 | 6.84 | 6.98 |
| Ser-$\beta_1$ | 4.49 | 4.58 | 4.6—4.75 | |
| Ser-$\beta_2$ | 4.62 | 4.72 | | |
| Ser-α | 4.96 | 5.06 | *ca.* 4.83 | *ca.* 4.97 |
| Ser-NH | 8.86 | 8.99 | 8.83 | 8.64 |
| Cys-$\beta_1$ | *ca.* 3.05 | 3.33 | 2.85 [b] | 4.91 [c] |
| Cys-$\beta_2$ | *ca.* 3.3 | 3.43 | 3.44 [b] | |
| Cys-α | 6.82 | 5.73 | 6.12 [b] | 6.47 [c] |
| H-3 [d] | 9.68 | 9.61 | 9.62 | 9.62 |
| H-6,7 [d] | 7.89 | 7.89 | 7.77 | 7.86 |
| H-5,8 [d] | 8.09 | 8.06 | 7.89 | 7.95 |
| | 8.21 | 8.18 | 8.16 | 8.19 |

[a] From ref. 5. [b] $>CH^{\alpha}–CH_2^{\beta}–S–$. [c] $>CH^{\alpha}–CH^{\beta}<$(S–)(S–).
[d] Quinoxaline aromatic protons.

TABLE 2

Spin–spin coupling constants (Hz) for triostin in $CDCl_3$

| Residue | Coupled protons | Triostin p | Triostin n |
|---|---|---|---|
| Val | β–γ | 6.6, 6.5 | 6.5, 6.8 |
| | α–β | 10.0 | 10.5 |
| Cys | $\beta_1$–$\beta_2$ | 14.8 | 15.0 |
| | α–$\beta_1$ | 5.3 | 6.7 |
| | α–$\beta_2$ | 9.5 | 7.9 |
| Ser | $\beta_1$–$\beta_2$ | 11.0 | 11.2 |
| | α–$\beta_1$ | 7.4 | 5.9 |
| | α–$\beta_2$ | 1.0 | 1.4 |
| | α–NH | 6.4 | 8.2 |
| Ala | α–β | 6.9 | 6.4 |
| | α–NH | 5.7 | 9.0 |

TABLE 3

Solvent effects [a] on the NH chemical shifts of triostin A and echinomycin

| | Triostin n | | Triostin p | | Echinomycin | |
|---|---|---|---|---|---|---|
| Solvent | Ala | Ser | Ala | Ser | Ala | Ser |
| $CDCl_3$–pyridine (1 : 1) | 0.08 | 0.56 | 1.55 | −0.13 | | |
| [$^2H_5$]pyridine | | | | | 1.83, 1.60 | 0.23, 0.20 |
| $(CD_3)_2SO$ | | | 0.96 | −0.32 | 1.05, 0.97 | −0.45, −0.42 |
| $CDCl_3$–DMSO (4 : 1) | −0.01 | 0.13 | 0.26 | −0.10 | | |
| $CDCl_3$–TFE [b] | −0.15 | −0.06 | 0.08 | −0.07 | | |

[a] Difference from shifts in $CDCl_3$ (p.p.m., positive shifts are downfield). [b] Units: $10^3 \times$ p.p.m./10% TFE.

TABLE 4

Temperature coefficients * of the NH proton chemical shifts in triostin A and echinomycin

| | Triostin n | | Triostin p | | Echinomycin [a] | |
|---|---|---|---|---|---|---|
| Solvent | Ala | Ser | Ala | Ser | Ala | Ser |
| $CDCl_3$ | −1.6 | −0.9 | −3.2 | −1.3 | +0.3, −1.1 | −2.0, 0 |
| Pyridine | | | −12.5 | 0 | −8.2, −8.6 | −0.4, +0.6 |

* $10^3 \times$ p.p.m./°C. Data for echinomycin from ref. 5.

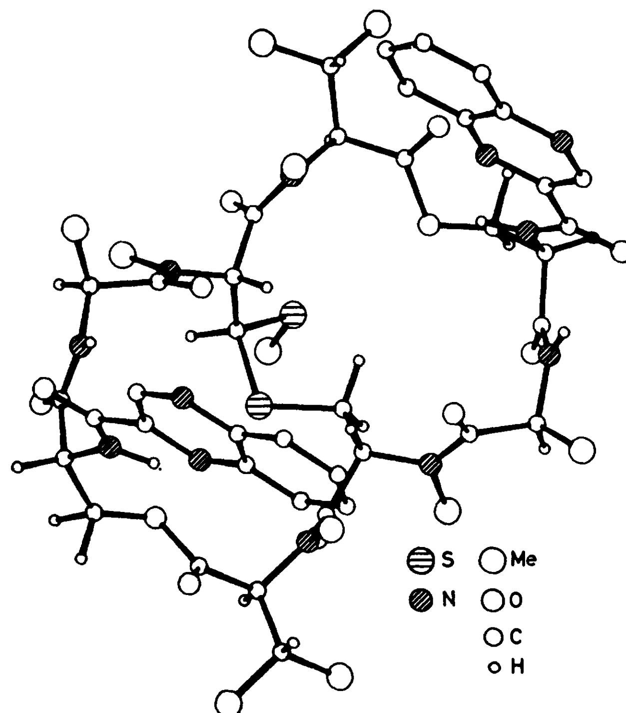
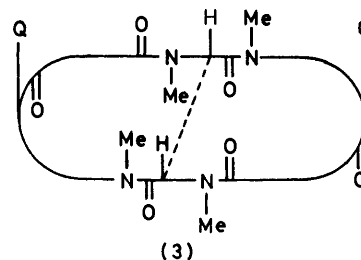



FIGURE 2 The conformational structure of echinomycin as determined from n.m.r. measurements and potential energy calculations [5]

(3) where the peptide ring is seen to form an oval disk with the quinoxaline rings and the Cys-αCH protons all on the same side of the ring (referred to as the ' upper side ').




All the peptide bonds are in the *trans* configuration, the Ala carbonyl groups are on the ' upper ' side and the Ser and Cys carbonyls are on the ' lower ' side of the ring as shown in the structure.

We have analysed the triostin A data using a similar method to that employed for echinomycin.[5] The three-bond coupling constants have been used to provide partial conformational information, which is then combined with results of potential-energy calculations on model compounds as a basis for model construction. A consideration of $^{1}H$ chemical shifts and LIS data, and comparisons with echinomycin, allows the selection of the most likely overall ring conformation consistent with these model building studies.

*Quinoxaline–Serine Fragment.*—Carbon-13 n.m.r. data for triostin A have already been reported.[11] The chemical-shift values of the quinoxaline ring carbons are summarised in Table 5, together with the corresponding data for the model compound quinoxaline-2-carboxamide (4). This compound is already known [5] to adopt the conformation shown, rather than that with the carbonyl oriented away from H-3. The close similarities between the $^{13}C$ shift values quoted in Table 5, and also

TABLE 5

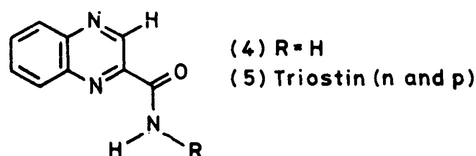
Aromatic $^{13}C$ chemical shifts of quinoxaline-2-carboxamide, triostin n, and triostin p in $CDCl_3$ solution

| Atom | Triostin n [a] | Triostin p [a] | Quinoxaline-2-carboxamide [b] |
|---|---|---|---|
| C-3 | 143.7 | 143.7 | 143.8 |
| C-5 | 132.0 | 132.0 | 132.1 |
| C-6 | 131.2 | 130.9 | 131.1 |
| C-7 | 129.6 | 129.6 | 130.0 |
| C-8 | 129.4 | 129.4 | 129.8 |

[a] From ref. 11. [b] From ref. 5.

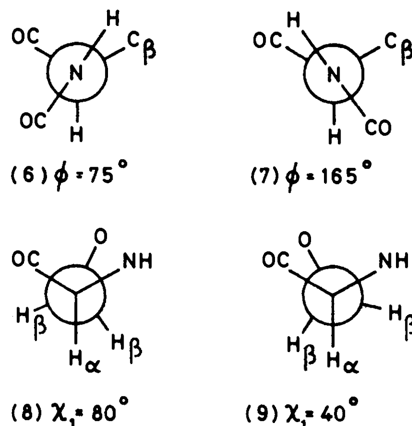
between the $^{1}H$ shifts of H-3 [9.63 p.p.m.[5] for (4) in $CDCl_3$; 9.61 and 9.68 p.p.m. for triostin n and p respectively], indicate that in both triostin conformers the quinoxaline fragment adopts this same orientation. Assuming a *trans* quinoxaline–serine peptide bond (5), the position of the serine NH, in the deshielding region of the quinoxaline ring, accounts for the low-field position of this resonance in both triostin conformers.

An indication of the relative orientation of the depsi-




peptide and quinoxaline rings, as defined by the torsion angle ($\phi$) about the serine Cα–N bond, can be obtained[12] from the Ser $J_{\alpha,NH}$ values. The measured coupling for Ser $J_{\alpha,NH}$ of 6.4 Hz in triostin p, is that expected from a rigid structure with approximate torsion angles $\phi = -90, -30, 75$, or $165°$, or from a flexible structure with $\phi$ angles centred around one of these values. Model-building studies reveal that the serine α-CH and $\beta$-$CH_2$ groups can only point outwards from the depsipeptide ring. It follows that only for $\phi = 75$ or $165°$ [(6) and (7)], does the serine-NH proton satisfy the criterion that it be shielded from the solvent. The Ser $J_{\alpha,N}$ coupling constant in triostin n (8.4 Hz) indicates a significant difference in this $\phi$-angle conformation between the two isomers: in this case the possible $\phi$ torsion angles are $\phi = -55, -65$, 155, and 85°.

The values for triostin p Ser $J_{\alpha\beta}$ and triostin n Ser $J_{\alpha\beta}$ (Table 2) define distorted gauche geometries about the serine Cα–Cβ bonds. Using the Kopple formula,[13] $J = 11.0 \cos^2\theta - 1.4 \cos\theta + 1.6 \sin^2\theta$, for the angular dependence of $J_{\alpha\beta}$, approximate values for both conformers of 40 and 80° (corresponding to calculated values of 6.5 and 1.3 Hz) are obtained for the dihedral angles θ. The two geometries (8) and (9) are possible since the serine β protons cannot be distinguished.




D-Ser–N-Me–Val. Ester groups are known generally to prefer the *trans* orientation,[14] and have been assumed to do so in the following.

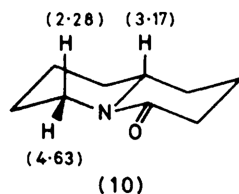
The chemical shifts of the serine $\beta$-$CH_2$ protons would be expected to be strongly influenced by their position relative to the ester carbonyl group.[14b] Therefore not only changes due to rotation about the $CH_2$–O bond, but also any in *cis,trans* geometry should be evident as alterations in the serine $\beta$-$CH_2$ chemical-shift values. Since these protons have comparable shifts in triostin p, triostan n, and echinomycin, it is probable that the conformation of the Ser–Val ester linkage is similar in each case.

Since both the valine and cystine residues are *N*-methylated, no $J_{\alpha,NH}$ is available as a guide to the torsion angle $\phi$. An additional complication introduced by the *N*-methylation is the possibility of *cis* amide bonds, which are approximately equal in energy to *trans* amide bonds in the absence of the proton on nitrogen.[15] An empirical method for distinguishing between *cis* and *trans* *N*-methyl peptide bonds, based on the α-CH chemical shifts, has been described by Mauger.[9] In various diketopiperazines and linear peptides (designed as models for fragments of actinomycin), chemical shifts of 3.7—3.9 p.p.m. were found for the α-CH of *N*-methylvaline when the nitrogen was part of a *cis* amide bond; the corresponding range for *trans* amides was 4.7—4.9 p.p.m. On this basis, the Cys–Val peptide bond of triostin p is predicted to be *trans*. Although linear peptides are not ideal models, since the anisotropic

effect of the peptide group is averaged over a number of conformations, this conclusion is supported by data for the rigid model systems (10)—(12) shown below; [16,17] in each case the position of the most deshielded proton relative to the amide group is the same as that in a *trans* peptide. This geometry cannot be achieved α to a *cis* peptide.

It is therefore proposed that, to account for the low-field valine α-CH resonance, the Cys–Val peptide bond in triostin p is *trans*, with Val $\phi$ close to −120° where the Val-αCH and the Cys-CO are in eclipsed positions. In triostin n, the Val-αCH is less deshielded (4.27 p.p.m. in $CDCl_3$) than in the triostin p but is still deshielded compared with the *cis* amide bond values. This suggests that the Cys–Val peptide bond is also *trans* in the triostin n but that the Val $\phi$ angle is such that the Val-αCH and Cys–CO are no longer in wholly eclipsed positions.

The large vicinal α-CH–β-CH couplings (triostin p, Val $J_{\alpha\beta}$ 10.0 Hz; triostin n Val $J_{\alpha\beta}$ 10.5 Hz) show that the valine side-chain is in both cases fixed in conformation (13). In valine itself, $J_{\alpha\beta}$ 4.5 Hz (*cf.* ref. 7), indicating a preponderance of the other rotamers about the $C_\alpha$–$C_\beta$ bond in which the α-CH is *gauche* to the β-CH. The existence of conformation (13) in triostin can be explained as being due to steric interference in the other



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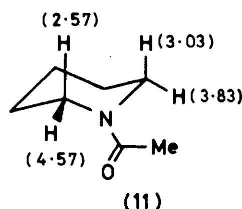

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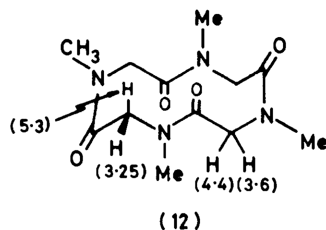

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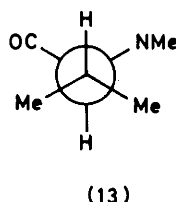
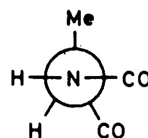


(13)

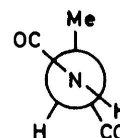
rotamers between the Val γ groups and the Val-*N*-methyl *provided* that the latter is on the 'upper' side of the ring (as in echinomycin).

The downfield positions of the triostin p Cys-αCH resonances in both conformers by the same argument used above, suggest a *trans*-Ala–NMeCys peptide bond in each case.

*The Ser–Ala Fragment.*—The Ala-αCH protons are deshielded in both conformers which is consistent with a *trans* Ser–Ala peptide bond with the Ser-CO near to the Ala-αCH. In triostin n where the Ala-αCH deshielding is 0.5 p.p.m., the Ser-CO is much nearer the Ala-αCH than in triostin p (deshielding 0.2 p.p.m.): this is also consistent with the $\phi$ angles deduced from the $J_{\alpha,NH}$ values. For triostin p the Ala $J_{\alpha,NH}$ value is 5.7 Hz. and for this the Bystrov relationship [8] gives four possible solutions for the Ala $\phi$ angle as indicated in structures (14)—(17). The Ala $J_{\alpha,NH}$ value for triostin n is much larger than that for triostin p ($J_{\alpha,NH}$ 9.0 Hz) and thus restricts the $\phi$ torsion angles to those shown in structures

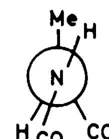


(14) $\phi$ = 27°



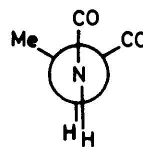
(15) $\phi$ = −164°

(16) $\phi$ = 94°



(17) $\phi$ = −76°

(18)—(20): of these only (19) and (20) could give the deshielding effect observed for Ala-αCH in this isomer.



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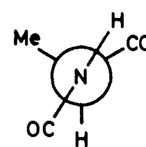

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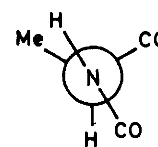
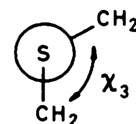


(20)

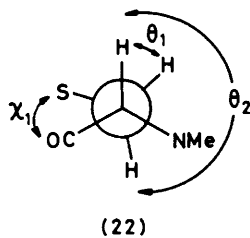
*The Disulphide Cross-bridge.*—The dihedral angle $\chi_3$ in (21) cannot be determined by the methods used in this work and it is assumed to be 80—90° in accordance with numerous studies [18,19] of the preferred conformation of disulphides. Likewise the two possible chiralities ($\chi_3$ = −90 and 90°) cannot be distinguished by n.m.r. methods. For each conformer only one set of signals was observed for the two Cys-αCH–β$CH_2$ fragments indicating that they are equivalent within each conformer. In each case the CyS $J_{\alpha,\beta}$ coupling constants were calculated from an ABX analysis of the multiplets. For triostin p the values were found to be 5.3 and 9.5 Hz. If the cross-

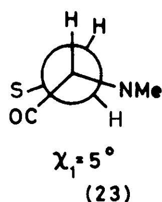


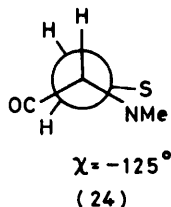
(21)

bridge is assumed to be rigid, the torsional angles $\theta_1$ and $\theta_2$ [such that $\theta_2 - \theta_1 = 120°$ (22)] can be calculated from the Kopple form [13] of the Karplus equation. The observed coupling constants are most closely approximated when $\theta_1 = 5°$, $\theta_2 = 125°$ [calculated values of $J_{\alpha\beta}$ 9.5 and 5.3 Hz, (23) or (24)]. However, both (23) and (24) have an unfavourable interaction between the sulphur atom and a large Cα substituent. For this reason it seems likely that the cross-bridge is flexible in this conformer, resulting in averaged values for the observed coupling constants and chemical shifts. For

the triostin n the Cys $J_{\alpha,\beta}$ values were found to be 6.7 and 7.9 Hz. Using the Kopple form of the Karplus equation no solutions could be found for which $\theta_1$ and $\theta_2$ differed by 120°, which indicates that again there is not a fixed

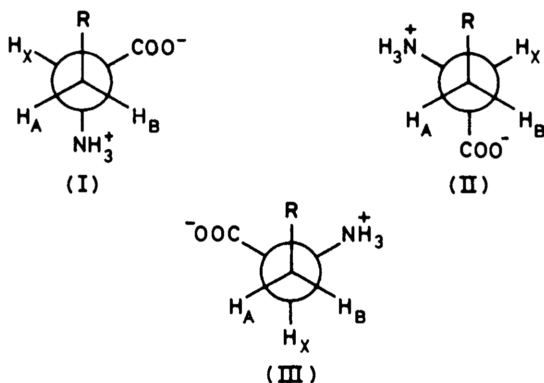


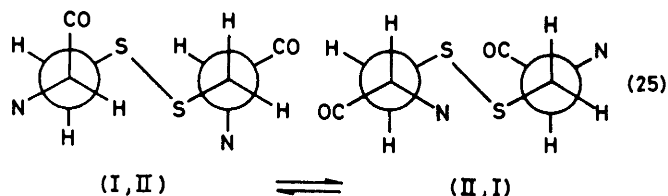




conformation about the Cys $C_\alpha$–$C_\beta$ bond. The best pair of solutions for $\theta_1$ and $\theta_2$ (26 and 130°) differ by 104° and for this conformation there would again be considerable steric interactions. Once again it seems more reasonable to expect the cross-bridge to exist as a rapidly interconverting mixture of conformers. In fact studies of molecular models indicate that interconversion between rotameric states should occur readily.

If we assume that the averaging takes place between the three staggered conformations (I)—(III) with component coupling constants $J_g$ 2.56 and $J_t$ 13.6 Hz as suggested by Pachler[20] then the observed coupling constants $J_{AX}$ and $J_{BX}$ can be fitted to a weighted average of these conformations (fractional populations




$P_I$, $P_{II}$, and $P_{III}$); $J_{AX} = 2.56\ P_I + 13.6\ P_{II} + 2.56\ P_{III}$; and $J_{BX} = 13.6\ P_I + 2.56\ P_{II} + 2.56\ P_{III}$. The fractional populations calculated in this manner are triostin p ($J_{\alpha,\beta}$ 5.3, 9.5); $P_I$ 0.25; $P_{II}$ 0.63; $P_{III}$ 0.12 (Errors $\pm$0.10): triostin n ($J_{\alpha,\beta}$ 6.7, 7.9); $P_I$ 0.38; $P_{II}$ 0.48; $P_{III}$ 0.14 (Errors $\pm$0.10). It is seen that the predominant rotamers in each case are the g/t rotamers (I) and (II) with very small contributions from rotamer (III). If we consider the complete cross-linkage consisting of the two Cys residues, then for each chirality of the disulphide bond we have nine conformations, each corresponding to a different combination of the two Cys rotameric states [(I,I), (I,II), (I,III), (II,I), (II,II), (II,III), (III,I), (III,II) and (III,III)]. There are many ways in which mixtures of such interconverting conformations could give the averaged coupling constants and chemical shift equivalence seen in the spectra. These include not only mixtures of (I,I), (II,II), and (III,III) conformers but also mixtures such as (I,II) and (II,I) as shown in (25). In fact it is apparent from consideration of molecular models that interconversions of this type where there is concerted motion of the two halves of the cross-linkage could occur particularly readily.




Clearly rotations of the type (I,II) ⇌ (II,I) will lead to the required equivalence of the two Cys-αCH-β$CH_2$ fragments in the averaged situation.

*Lanthanide-induced Shifts*.—The changes in chemical shift produced by addition of 0.5 mol equiv. of [Eu(fod)$_3$] to the triostin conformers are given in Table 6. Much

TABLE 6

Shifts * produced in the $^1$H n.m.r. spectrum of triostin A on addition of [Eu(fod)$_3$]

| Hydrogen | Triostin n | Triostin p |
|---|---|---|
| Val-γ | 0.14 | 0.05 |
| | 0.10 | 0.04 |
| Val-β | 0.16 | 0.16 |
| Val-α | 0.56 | 0.63 |
| NMe | 0.30 | 0.11 |
| | 0.72 | 0.26 |
| Ala-β | 0.82 | 0.12 |
| Ala-α | 1.57 | 0.22 |
| Ala-NH | 1.28 | 0.21 |
| Ser-β$_1$ | 0.48 | 0.21 |
| Ser-β$_2$ | 0.34 | 0.15 |
| Ser-α | 1.59 | 0.25 |
| Ser-NH | 0.85 | 0.21 |
| Cys-β$_1$ | 0.55 | *ca.* 0.17 |
| Cys-β$_2$ | 0.44 | *ca.* 0.19 |
| Cys-α | 0.75 | 0.30 |
| Quinoxaline H-3 | 0.28 | 0.15 |

* In p.p.m., for 0.5 mol equiv. [Eu(fod)$_3$] in $CDCl_3$.

larger LIS values are observed for triostin n than for triostin p, indicating that the binding to the n-conformer is stronger than to the p-conformer. Further evidence for this is found in the displacement of the equilibrium between the two conformers in favour of the n-conformer upon the addition of [Eu(fod)$_3$]. There is a marked similarity in the LIS data observed for triostin n and echinomycin, as indicated in Table 7: in each case it is clear that the major site of complexation is at the Ser-CO groups, very large shifts being observed for the protons

of the alanine and serine residues. The similarity in the LIS values indicates that there is no major difference in the overall ring conformation between the two compounds. Thus it seems unlikely that triostin n and echinomycin could differ to the extent of *cis–trans* isomerisation of a peptide bond or rotation of a complete peptide bond through the ring. Because there is also weaker complexation at sites other than Ser-CO it is difficult to treat the LIS data in a quantitative manner and this has not been attempted.

TABLE 7

Comparison of LIS data for echinomycin and triostin n *

| Hydrogen | Echinomycin | Triostin n |
|---|---|---|
| Ser-α | 1.00 | 1.00 |
| Ser-NH | 0.60 | 0.54 |
| Ser-$\beta_1$ | 0.32 | 0.30 |
| Ser-$\beta_2$ | 0.19 | 0.22 |
| Ala-α | 1.00 | 1.00 |
| Ala-β | 0.41 | 0.52 |
| Ala-NH | 0.70 | 0.81 |
| Cys-α | 0.44 | 0.47 |
| Cys-$\beta_1$, $\beta_2$ | 0.32 | 0.31 |
| Val-α | 0.29 | 0.35 |
| Val-β | 0.19 | 0.10 |
| Val-γ | 0.10 | 0.08 |
| NMe | 0.41 | 0.46 |
|  | 0.14 | 0.19 |
| Quinoxaline H-3 | 0.41 | 0.18 |

* Expressed relative to Ala-αCH ≃ Ser-αCH = 1.00. Data for echinomycin from ref. 5.

For triostin p not only is the pattern of the LIS shifts different but they are much smaller, indicating weaker binding. This suggests that the major binding site in triostin n and echinomycin involving the Ser carbonyl is no longer available in triostin p: this is supported by the effects of TFE on the Ala-NH, which indicate that the Ser-CO is not solvent-accessible in triostin p. The predominant binding site for [Eu(fod)$_3$] in triostin p is at the Cys carbonyl groups but there is significant binding to other carbonyls as shown by the LIS values for the various α-CH protons in the molecule. The presence of multiple binding sites prevents any quantitative evaluation of the data. Furthermore, because the predominant binding site for triostin p is not the same as for triostin n or echinomycin, one cannot extract even qualitative information about similarities or differences between the various structures.

*Conformational Differences between Triostin p and n.*—We must now consider the various possible conformational or configurational structural differences which could lead to the two solution conformations of triostin A separated by an energy barrier of 22 kcal mol$^{-1}$. The following possibilities must be considered. (*a*) Rotation of a *trans-N*-methylated peptide unit through the ring. The energy barrier for this process in cyclopentasarcosyl [21] (a 15-membered ring) has been measured as 16 kcal mol$^{-1}$. To maintain the symmetry of the triostin structure *two* such interconversions would be required. (*b*) Conversion of a *trans-N*-methyl amide bond to *cis*. The energy required for such an isomerisation in a number of cyclic peptides has been found [21] to be *ca.* 19—23 kcal mol$^{-1}$ (the higher value being for cyclotetrasarcosyl, having a 12-membered ring). Again *two* such interconversions are required to maintain the symmetry of the structure. In fact all models containing *cis*-peptide bonds proved to be difficult to construct. (*c*) Reversal in chiralty of the disulphide bond. This process requires 11.7—16.3 kcal mol$^{-1}$ in a range of compounds.[22,23]

To decide which of these differences is responsible for the existence of the two conformers of triostin A we must consider the n.m.r. data which gives information about the overall ring conformations of the two isomers.

*Overall Ring Conformation.*—Molecular models have been constructed using the partial conformational information derived earlier and including the additional constraints imposed by cyclisation and cross-bridge formation. This leads to a limited number of possible structures, all of which have certain features in common. Thus, just as in the case of echinomycin,[5] the depsipeptide ring in the triostin models is a rigid disc as in (3), with the quinoxaline groups and the Cys-αCH on the same (' upper ') side. The ' overall ' ring conformations are defined by specifying the configuration of the peptide bonds and the orientation of the peptide carbonyl groups. In the case of echinomycin it has been shown that all the peptide bonds are *trans*, the Ala carbonyl groups are on the ' upper ' side of the ring and the Ser and Cys carbonyls are on the ' lower ' side of the ring as shown in (3). It will now be shown, from a detailed comparison of the n.m.r. data that the overall ring conformation for both conformers of triostin A is essentially the same as that found for echinomycin, *i.e.* the configuration and orientation of the peptide bonds are the same in all three cases.

*Triostin p and Echinomycin.*—Examination of the n.m.r. data in Table 1 shows that there is general agreement between the shift values of all the α-CH and NH protons for triostin p and echinomycin. In each case a large deshielding effect is experienced by the Cys and Val α-CH protons indicating that a carbonyl group is immediately adjacent to each proton.

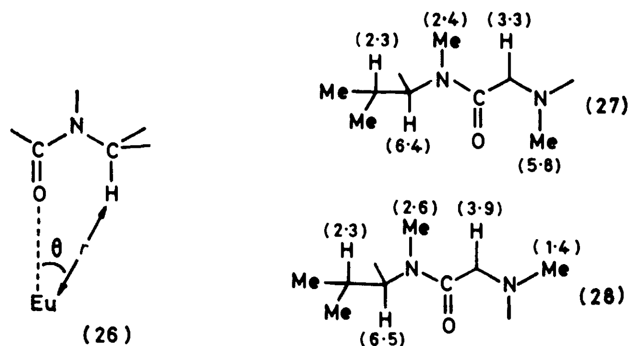
The differences in vicinal coupling constants between the two compounds can be readily explained in terms of small changes ($<20°$) in dihedral angles. Clearly the overall ring conformation is similar in triostin p and echinomycin: this is illustrated in the CPK model shown in Figure 3.

The differences in the LIS values for triostin p and echinomycin are mainly due to the fact that the Ser carbonyl is not accessible for [Eu(fod)$_3$] binding in triostin p. Because the metal is not binding in the same way to triostin p and echinomycin it is not possible to use the LIS data to compare their overall ring conformations.

*Triostin n and Echinomycin.*—The chemical shift data for these molecules show close agreement only for the Ser and quinoxaline residues. Substantial differences

in chemical shift are observed for the Val and Cys α-CH protons which show more nearly normal chemical shifts in triostin n (although they are still somewhat deshielded compared with the values in linear peptides). Again the vicinal coupling constants do not imply large differences in dihedral angles between the two compounds.

In spite of these differences in chemical shifts the LIS data for triostin n and echinomycin are almost identical (see Table 7). This must imply that [Eu(fod)$_3$] is binding similarly to the same sites in both compounds and furthermore that the overall ring conformation is the same in each case. A major change in ring conformation such as a rotation of a peptide bond through 180° or a change from a *trans* to a *cis* peptide bond would readily be detected in the LIS experiments. For example, if the Ser–Ala peptide bond were rotated through the ring such that the Ser carbonyl appeared on the ' upper ' side, [Eu(fod)$_3$] binding to this carbonyl would give very different LIS values for the Ser and Ala α-CH protons. Similarly, if we consider a change from *trans* to *cis* at the Cys–NMeVal peptide bond it is easy to demonstrate that this would also give very different LIS values. We have estimated the relative LIS values for each configuration by using a simple model where the metal is assumed to be located 3 Å from the Cys-CO along the C=O bond (26). The angles θ and distances *r* were




measured for each proton from a three-dimensional model. The relative shifts were calculated [24] from the expression LIS $\propto (3\cos^2\theta - 1)/r^3$. From the results shown in (27) and (28) it is apparent that a very different pattern of relative LIS values is obtained for *trans* and *cis* configurations. In view of the similarity of the observed LIS values for Val-αCH, Val-βCH, Cys-αCH, and the NMe protons of echinomycin and triostin n, it is clear that they have the same configurations for the NMe–Cys–Val peptide bonds. Similar arguments can be used to show that the Ala–NMeCys peptide bond has the same configuration in the two compounds. Thus the LIS data clearly show that the overall ring conformation is the same in echinomycin and triostin n.

Since we have already shown that triostin p also has a similar overall ring conformation to echinomycin it follows that the two conformers of triostin must be similar in this respect. In both conformers all the peptide bonds are *trans* and the Ala-CO is on the ' upper ' side of the ring while the Cys and Ser carbonyls are on the ' lower ' side. Thus it seems likely that the difference between the two conformers which gives rise to the 22 kcal mol$^{-1}$ energy barrier between them is the difference in chirality of the disulphide bond. Such a change in chirality will produce some conformational changes in the other parts of the molecule and we must now consider whether these changes can account for the differences in the n.m.r. parameters for the two conformers of triostin A.

We have constructed models of triostin in both the chiral forms of the Cys–S–S–Cys fragment. It is apparent from examination of these models that there are significant differences, notably in the steric interactions between residues across the ' disk ', between the two forms. Although the details of these interactions depend upon the conformation about the two Cys $C_\alpha$–$C_\beta$ bonds (and cannot, therefore, be specified precisely, in view of the ambiguities in our description of the rotamer distributions), it is qualitatively clear that effects of this sort could readily account for the differences between the n.m.r. spectra of the two conformers.

For example, both the Val-αCH and the Cys-αCH resonances are 1 p.p.m. further downfield in the spectrum of triostin p than in that of triostin n. This can be explained as arising from a difference in the orientation of the neighbouring carbonyl groups relative to these α-protons, amounting to a difference in the angle between the plane of the peptide bond and that of the ' disk '. Consideration of the shift differences between the α-$CH_2$ protons of glycine residues in rigid cyclic peptides [25] shows that the α-proton chemical shifts are very sensitive to the precise orientation of the adjacent carbonyl group. Only a small change in angle would thus be required to produce a relatively large change in chemical shift, and this could readily arise from the kind of cross-ring steric interactions discussed above. Further clear differences between the two conformers are seen in the Ala–Ser portion of the molecule: the Ala-NH proton resonance appears 1.17 p.p.m. further downfield in triostin n than in triostin p, and this proton is readily accessible to solvent in the latter but not in the former case. There are also significant differences in the chemical shifts of the Ala β-Me protons, in the $J_{\alpha CH-NH}$ values of both Ala and Ser, and in the solvent accessibility of the serine carbonyl group {*cf.* the discussion of the [Eu(fod)$_3$]-induced shifts, above}. All these effects can be rationalised in a qualitative sense by the following model.

We propose that in the triostin n there is an intramolecular hydrogen bond between the quinoxaline carbonyl group and the Ala-NH, forming a seven-membered ring hydrogen-bonded system of the type frequently encountered in cyclic peptides.[26] This would account for the marked deshielding of the Ala-NH proton and for its inaccessibility to solvent, and is consistent with the Ala and Ser $J_{\alpha CH-NH}$ values (Ser $\phi = 85$, Ala $\phi = -148°$). The quinoxaline carbonyl appears to be at least partly accessible to solvent (by

the criterion of the effect of TFE on the chemical shift of the Ser-NH resonance); this would not be irreconcilable with the existence of a (weak) hydrogen bond in the absence of trifluoroethanol. We propose that, just as the change in disulphide chirality to give triostin p leads to a change in the orientation of the Val–Cys and Cys–Ala peptide bonds, so it has a similar effect on the Ala–Ser peptide bond. This rotates at least 20° so that the Ala-NH points out toward the edge of the ' disk ', and the Ser carbonyl becomes ' buried ' in the inside of the ' disk ', thus accounting for the changes in accessibility of these two groups {and notably for the much weaker [Eu(fod)$_3$] binding in triostin p}. This rotation leads to the breaking of the quinoxaline-CO–Ala-NH hydrogen-bond, normalising the chemical shift of the Ala-NH, and hence to an increased freedom of rotation about the Ser $C_\alpha$–N bond. In fact there is only a slight change in the (time-average) Ser $\phi$ angle, the Ser-NH becoming directed more toward the centre of the ' disk ' and thus less accessible to the solvent. This change in conformation will be favoured by solvents such as pyridine or DMSO because the Ala-NH is now available for hydrogen-bonding to the solvent, rather than being involved in an intramolecular bond.

The LIS data show clearly that triostin n and echinomycin have very similar conformations, but some difference must be postulated to account for the substantial difference in chemical shift of the Ala-NH, and the difference in the LIS of the quinoxaline H-3 resonance. It appears that the proposed intramolecular hydrogen bond does not exist in echinomycin. This would explain the difference in Ala-NH, and would also free the quinoxaline-CO for interaction with [Eu(fod)$_3$] in echinomycin, thus explaining the larger LIS of the H-3 resonance. The good agreement of the other LIS between triostin n and echinomycin indicates that the quinoxaline-CO is only a weak binding site for [Eu(fod)$_3$]. However, in echinomycin breakage of the hydrogen bond is accompanied by a much smaller rotation of the Ala–Ser peptide bond, presumably due to the different steric effects of the different cross-bridge of echinomycin.

*Solution Conformation and the Binding of Triostin to DNA*.—The conformation of triostin when bound to DNA may of course be different from either of the conformations present in chloroform solution. Nonetheless, the solution conformation does influence the binding process, and in the case of triostin and echinomycin the apparent rigidity of the peptide ring makes major conformational changes on binding less likely.

For triostin, as for echinomycin, the proposed conformation of the peptide ring places the two quinoxaline chromophores approximately 10 Å apart. This is ideally suited for simultaneous intercalation of the quinoxaline rings to form a two-base-pair ' sandwich '. It is reasonable to suppose that triostin p, which is favoured by polar solvents, will predominate in aqueous solution. We have shown that this conformation differs from that of echinomycin in the orientation of the Ala–Ser peptide bond (apart from the obvious differences in the cross-bridge). It is possible that the Ala-NH is involved in determining the specificity of binding to DNA by hydrogen-bonding to groups in the narrow groove, and the difference in orientation of this group in triostin and echinomycin may thus contribute to the different specificity of these two antibiotics.

We are grateful to Dr. Lila G. Pease for useful discussions.

[8/563 *Received, 29th March*, 1978]

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