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Structures of Quinoxaline Antibiotics

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Abstract

The crystal structures of three quinoxaline antibiotics – echinomycin 2QN, triostin C and the $C222_1$ form of triostin A – have been determined, and the structure of the $P2_12_12_1$ form of triostin A has been re-refined against our previously reported data. The molecular conformations are compared with those deduced from NMR data and those reported for two complexes of triostin A with oligonucleotides. Although the depsipeptide ring conformations are basically similar, the effective twofold molecular symmetry is violated by the folding of one of the quinoxaline chromophores in echinomycin 2QN and by a rotation of one of the ester planes with the formation of an intramolecular hydrogen bond in triostin C. In the oligonucleotide complexes of triostin A the chirality of the disulfide bridge is inverted. The alanine NH groups are involved in intermolecular hydrogen bonds in all four structures, and (except in echinomycin 2QN) the stacking of the chromophores in the crystal emulates the intercalation involved in DNA complex formation. In echinomycin 2QN, the antibiotic molecules are hydrogen bonded to form a helix along the crystallographic 6_5 screw axes, with a channel of disordered solvent running through the middle of the helix. Crystal data: (1), echinomycin 2QN, $C_{53}H_{66}N_{10}O_{12}S_2.2.5(C_3H_6O).2.5(H_2O)$, $M_r = 1289.5$, hexagonal, $P6_5$, $a = b = 22.196\,(15)$, $c = 24.64\,(2)$ Å, $V = 10\,513\,(13)$ Å^3, $Z = 6$, $D_x = 1.222$ Mg m^{-3}, $\lambda(\mathrm{Cu}\,K\alpha) = 1.5418$ Å, $\mu = 1.275$ mm^{-1}, $T = 193$ K, $R = 9.0\%$ for 4828 $I > 2\sigma(I)$ and 11.8% for all 7102 unique reflections; (2), triostin C, $C_{54}H_{70}N_{12}O_{12}S_2.0.67(CHCl_3).0.67(H_2O)$, $M_r = 1234.2$, orthorhombic, $P2_12_12_1$, $a = 16.054\,(8)$, $b = 17.128\,(9)$, $c = 22.706\,(12)$ Å, $V = 6244\,(6)$ Å^3, $Z = 4$, $D_x = 1.313$ Mg m^{-3}, $\lambda(\mathrm{Mo}\,K\alpha) = 0.71073$ Å, $\mu = 0.239$ mm^{-1}, $T = 188$ K, $R = 7.7\%$ for 4678 $I > 2\sigma(I)$ and 14.0% for all 7260 unique reflections; (3), triostin A, $C_{50}H_{62}N_{12}O_{12}S_2.2(C_7H_{14}O_2)$, $M_r = 1347.6$, orthorhombic, $P2_12_12_1$, $a = 20.94\,(2)$, $b = 18.53\,(2)$, $c = 18.80\,(2)$ Å, $V = 7292\,(13)$ Å^3, $Z = 4$, $D_x = 1.228$ Mg m^{-3}, $\lambda(\mathrm{Cu}\,K\alpha) = 1.5418$ Å, $\mu = 1.245$ mm^{-1}, $T = 293$ K, $R = 6.8\%$ for 2116 $I > 2\sigma(I)$ and 9.3% for all 2928 unique reflections; (4), triostin A, $C_{50}H_{62}N_{12}O_{12}S_2.HCl.2(C_3H_7NO)$, $M_r = 1269.9$, monoclinic, $C222_1$, $a = 10.622\,(10)$, $b = 17.035\,(17)$, $c = 35.21\,(3)$ Å, $V = 6371\,(10)$ Å^3, $Z = 4$, $D_x = 1.324$ Mg m^{-3}, $\lambda(\mathrm{Mo}\,K\alpha) = 0.71073$ Å, $\mu = 0.199$ mm^{-1}, $T = 153$ K, $R = 7.5\%$ for 2164 $I > 2\sigma(I)$ and 13.2% for all 3402 unique reflections. Extensive use was made of restraints on the geometrical and displacement parameters in the successful anisotropic refinement of these structures against weak data.

Introduction

The quinoxaline antibiotics have made a significant contribution to our understanding of the molecular recognition of DNA (Waring, 1979, 1990, 1993; Gale, Cundliffe, Reynolds, Richmond & Waring, 1981). The best known member of the group, echinomycin, was the first ligand shown to bind to DNA by the mechanism of bis-intercalation (Waring & Wakelin, 1974) and now stands as a paradigm for that mode of interaction with the double helix (Wakelin, 1986; Wakelin & Waring, 1990). Yet, paradoxically the structure of echinomycin itself has never been determined by X-ray crystallography, despite years of effort in various laboratories to crystallize the antibiotic.

Interest in the quinoxalines stems from their identification as anti-cancer drugs in the 1950s (Katagiri, Yoshida & Sato, 1975). Echinomycin is currently in

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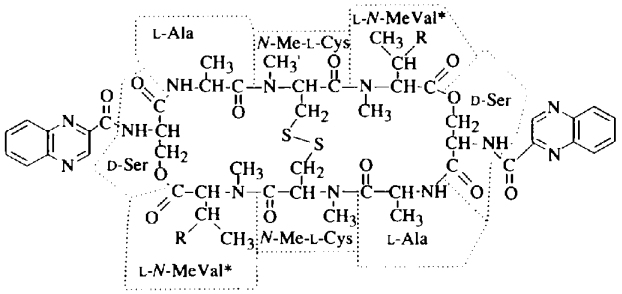
phase II clinical trials (Muss, Blessing & Malfetano, 1990; Muss, Blessing, Baker, Barnhill & Adelson, 1990), but is probably too toxic for widespread use in therapy. Early chemical work established the broad outlines of their structure (Keller-Schierlein, Mihailovic & Prelog, 1959), but it was much later that the exact chemical constitution of echinomycin was determined by spectroscopic methods (Dell, Williams, Morris, Smith, Feeney & Roberts, 1975; Martin, Mizsak, Biles, Stewart, Baczynski & Meulman, 1975). The antibiotics fall into two series, which differ in the nature of the sulfur-containing cross-bridge which links the two halves of their depsipeptide ring. Echinomycin belongs to the quinomycin series, having a thioacetal cross-bridge, whereas the homologous series of triostin antibiotics have a disulfide cross-bridge at the same position. The antibiotic triostin A is the strict homologue (and biosynthetic precursor) of echinomycin. Other members of the two series of antibiotics have conservative amino acid replacements at the positions of the two valine residues: in triostin C the valine residues of triostin A are replaced by moieties of the unusual amino acid *N*-γ-dimethyl-*allo*-isoleucine. All the antibiotics which have been tested share the property of bis-intercalative binding to DNA (Lee & Waring, 1978*a*; Fox, Cornish, Williams & Waring, 1983) and seem to bind preferentially to sites containing the critical CpG element (Low, Olsen & Waring, 1984). In addition to the natural antibiotics, a series of synthetic quinoxaline depsipeptides exist based on the des-methylated octapeptide ring (Olsen, 1983). These molecules generally bind less well to DNA (Lee & Waring, 1978*b*), but one, the des-*N*-tetramethyl derivative of triostin A designated by the acronym TANDEM, binds selectively to the TpA step in DNA, in contrast to the natural antibiotics (Lee & Waring, 1978*b*; Fox, Olsen & Waring, 1982).



NMR studies (Blake, Kalman & Williams, 1977; Kawano, Higuchi & Kyogoku, 1977; Kalman, Blake, Williams, Feeney & Roberts, 1979) have shown that triostin A exists in two conformations in solution, both of which appear to possess twofold symmetry. The conformation denoted '*p*' predominates in polar solvents, whereas the '*n*' conformation is favoured by non-polar solvents. These two components can be resolved by reversed-phase HPLC (Alfredson, Maki, Adaskaveg, Excoffier & Waring, 1990). The similarity of the UV and NMR spectra of the two forms suggests that their differences in conformation may be rather subtle. The activation energy for the interconversion has been estimated at *ca* 20 [84 kJ mol^{-1} (Kawano, Higuchi & Kyogoku, 1977)] and 22 kcal mol^{-1} [92 kJ mol^{-1} (Blake, Kalman & Williams, 1977)]. It appears that only the *p* form binds to DNA (Kyogoku, Yu, Akutsu, Watanabe & Kawano, 1978; Kyogoku, Higuchi, Watanabe & Kawano, 1981).

Crystal structures have been determined for *TANDEM* (Viswamitra, Kennard, Cruse, Egert, Sheldrick, Jones, Waring, Wakelin & Olsen, 1981; Hossain, van der Helm, Olsen, Jones, Sheldrick, Egert, Kennard, Waring & Viswamitra, 1982) and for triostin A (Sheldrick, Guy, Kennard, Rivera & Waring, 1984), but not for any members of the quinomycin series of antibiotics. However, the structures of three complexes between triostin A or echinomycin and short self-complementary deoxyoligonucleotides have been reported by Rich and his collaborators (Wang, Ughetto, Quigley, Hakoshima, van der Marel, van Boom & Rich, 1984; Ughetto, Wang, Quigley, van der Marel, van Boom & Rich, 1985; Quigley, Ughetto, van der Marel, van Boom, Wang & Rich, 1986; Wang, Ughetto, Quigley & Rich, 1986), who have provided a wealth of information, albeit at a relatively low resolution, indicating the likely molecular basis of sequence recognition in DNA. Here we report the crystal structures of two triostin antibiotics and a biosynthetic bis-quinoline derivative of echinomycin designated 2QN (Fox, Gauvreau, Goodwin & Waring, 1980; Low, Fox & Waring, 1986), which define the structural characteristics of the uncomplexed antibiotics much more precisely. We have also rerefined our original triostin A data (Sheldrick *et al.*, 1984) with the more sophisticated techniques now available.

Experimental

Crystal data and details of the structure refinements are presented in Table 1. All the structures were solved by direct methods. The solution of the $P2_12_12_1$ form of triostin A and of echinomycin 2QN proved difficult at the time and involved the expansion of the structures from small fragments containing sulfur, but as a result of improved algorithms (Sheldrick, 1990) and the enormous increase in computer power, all four structures can now be solved relatively routinely. They were refined against F^2 using the new refinement program *SHELXL*93 (Sheldrick, 1995). H atoms were refined using a riding model starting from geometrically calculated positions, and the *N*-methyl groups were also allowed to rotate about their local threefold axes. In all refinements rigid-bond restraints (with e.s.d.'s 0.02 Å^2) were applied to the anisotropic displacement parameters of all 1,2- and 1,3-atom pairs (*i.e.* the components along the vector joining the two atoms were restrained to be equal),

Table 1. *Crystal data and refinement data*

Compound	Echinomycin 2QN + 2.5(C_3H_6O) + 2.5H_2O	Triostin C + 0.67$CHCl_3$ + 0.67H_2O	Triostin A + 2($C_7H_{14}O_2$)	Triostin A + HCl + 2(C_3H_7NO)
Formula	$C_{60.5}H_{86}N_{10}O_{17}S_2$	$C_{54.7}H_{72}Cl_2N_{12}O_{12.7}S_2$	$C_{64}H_{90}N_{12}O_{16}S_2$	$C_{56}H_{77}ClN_{14}O_{14}S_2$
M_r	1289.5	1234.2	1347.6	1269.9
Crystal system	Hexagonal	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P6_5$	$P2_12_12_1$	$P2_12_12_1$	$C222_1$
Temperature (K)	193	188	293	153
Wavelength (Å)	1.5418	0.71073	1.5418	0.71073
a (Å)	22.196 (15)	16.054 (8)	20.94 (2)	10.622 (10)
b (Å)	22.196 (15)	17.128 (9)	18.53 (2)	17.035 (17)
c (Å)	24.64 (2)	22.706 (12)	18.80 (2)	35.21 (3)
V (Å^3)	10512.8	6243.5	7292.0	6371.3
Z	6	4	4	4
D_x (Mg m^{-3})	1.222	1.313	1.228	1.324
No. of reflections for cell	25	60	25	25
2θ range for cell (°)	40–45	20–25	40–45	20–25
Index ranges*	−19–19, −20–19, −24–24	−16–16, −18–18, −23–23	0–18, 0–16, 0–16	−11–11, −18–18, −33–17
Maximum 2θ (°)	100.2	44.0	85.4	45.1
Crystal size (mm)	0.2 × 0.2 × 0.2	0.08 × 0.15 × 0.23	0.2 × 0.2 × 0.7	0.2 × 0.2 × 0.3
μ (mm^{-1})	1.275	0.239	1.245	0.199
Total no. of reflections	15965	9028	2928	5578
Unique reflections	7102	7268	2928	3413
Unique reflections with $I > 2\sigma(I)$	4828	4678	2116	2164
R_{int}	0.097	0.096	—	0.091
Parameters refined	852	780	933	404
Absolute structure factor†	−0.01 (5)	−0.2 (2)	0.14 (6)	0.5 (3)
No. of restraints	834	720	1108	367
Weight parameters‡	0.15, 0.0	0.025, 16.0	0.13, 0.5	0.095, 0.0
Mean shift/e.s.d.	0.004	0.000	0.003	0.000
Max. shift/e.s.d.	0.035	0.000	0.053	0.000
Final $R_1[I > 2\sigma(I)]$	0.090	0.077	0.068	0.075
Final R_1 (all data)	0.118	0.140	0.093	0.132
Final wR_2 (all data)	0.255	0.163	0.182	0.208
S (on all F^2)	1.079	1.111	1.059	1.068
Maximum peak (e Å^{-3})	0.31	0.27	0.19	0.25
Minimum hole (e Å^{-3})	−0.37	−0.24	−0.20	−0.25

* Not all redundant reflections in these ranges were measured.

† Flack (1983).

‡ $w = [\sigma^2(F_o^2) + (g_1P)^2 + g_2P]^{-1}$, where parameters g_1 and g_2 are given in the table and $P = (F_o^2 + 2F_c^2)/3$.

$R_{int} = |F_o^2 - F_o^2(\text{mean})|/\sum |F_o^2|$; $R_1 = \sum ||F_o| - |F_c||/\sum |F_o|$; $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.

and weak similarity restraints (equal U_{ij}) were applied to all atoms closer than 1.7 Å to each other, including disorder components (with e.s.d.'s 0.2 Å^2 for terminal or isolated atoms and 0.1 Å^2 for others). For the $P2_12_12_1$ form of triostin A, similarity restraints were applied to the 1,2- and 1,3-distances (with e.s.d.'s 0.03 Å), so that the distances related by the non-crystallographic twofold axes were restrained to be equal; in the $C222_1$ form these distances are related by a crystallographic twofold axis. These restraints were not applied to the echinomycin 2QN and triostin C structures, because they exhibit significant deviations from such non-crystallographic symmetry. Planarity restraints were applied to the chromophores of the two triostin A structures, and also to the non-H atoms of the dimethylformamide molecules in the $C222_1$ form. These chemically reasonable restraints enabled a full anisotropic refinement of all four structures, despite rather weak data (especially for the two triostin A structures). Imposition of the restraints led to a considerable improvement of the quality of the difference Fourier syntheses for all four structures, enabling a more detailed and satisfactory interpretation of the solvent structure, which in turn led to improved models. The known absolute configuration was assumed for all four structures, but consistent values of the absolute structure parameter (Flack, 1983) were obtained in each case (Table 1). All figures were drawn using the *XP* program in the *SHELXTL* system. Details specific to the individual structures follow.*

Echinomycin 2QN

Colourless tapered hexagonal prisms were obtained by liquid diffusion of diisopropyl ether into an acetone solution. Subsequently, we have succeeded in growing much larger crystals (up to 2 mm^3) by vapour diffusion using the same solvents. The crystals lose solvent rapidly in air and so were coated with silicone grease and mounted in capillaries (the oil drop mounting technique used for the remaining two data collections is, however, demonstrably superior for low-temperature data collection and for avoiding solvent loss). Intensity data were collected in the ω–2θ scan mode on a Siemens (then

* Lists of structure factors, anisotropic displacement parameters, H-atom coordinates and complete geometry have been deposited with the IUCr (Reference: HE0091). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Nicolet) R3 diffractometer at 193 K. One solvent acetone molecule accepts a hydrogen bond from N(3)—H(3), and a further acetone does not appear to take part in specific interactions. In addition, one partial molecule of acetone and five partially occupied water molecules were located with difficulty in spiral channels along the 6_5 screw axes; the partial acetone and the five waters were all assigned occupation factors of 0.5, and these atoms were also restrained to be approximately isotropic.

Triostin C

After many attempts, crystals in the form of colourless prisms were obtained as follows. Triostin C (0.2 mg) was dissolved in 0.2 ml of chloroform at 313 K, mixed with 0.2 ml of isopropanol at room temperature, cooled to 277 K and covered with a layer of diisopropylether. After 10 d at this temperature, the crystals had reached their maximum size. They lose solvent instantly in air, and so were transferred to a drop of a viscous perfluorinated ether (RS-3000 oil from Riedel de Haën) on the end of a glass fibre, and quickly cooled to 188 K. Data were collected on a Stoe–Siemens AED at this temperature in the ω–2θ scan mode, with real-time profile fitting (Clegg, 1981). The structure was found to contain a chloroform molecule, assigned occupancy p, which refined to 0.661 (7), occupying the same region as two water molecules (both assigned occupancies $1 - p$).

$P2_12_12_1$ form of triostin A

The same data were used as in our previous refinement, and were not optimal for refinement against F^2 because they had been preserved (they were collected in 1975) only in the form of F and $\sigma(F)$, which involves unnecessary approximations in the calculation of $\sigma(F)$ from the experimental $\sigma(F^2)$ and then converting back again to $\sigma(F^2)$. There were also fewer data for this structure than for the other three because the resolution limit is lower and because the data do not include Friedel opposites. Further experimental details have been given by Sheldrick *et al.* (1984). The standard deviations in the unit-cell parameters have been increased from their previous unduly optimistic estimates. The structure contains one ordered molecule and one disordered molecule [two overlapping components with occupancies $p = 0.570$ (13) and $1 - p$] of isoamyl acetate, which were refined with their bond lengths and 1,3-distances restrained to standard values (with e.s.d.'s 0.03 Å). Although this structure gave the lowest R_1 values, these are purely an artefact caused by the much poorer data-to-parameter ratio; the e.s.d.'s on unrestrained parameters such as torsion angles are consistently higher than for the other three structures.

$C222_1$ form of triostin A

Crystals were grown by cooling concentrated solutions in acetone/dimethylformamide (DMF) mixtures, which had been covered with a layer of diisopropylether, to 279 K. Data were collected as for triostin C, but at 153 K. The structure determination revealed one molecule of DMF and one unexpected half chloride ion, which makes a suitable hydrogen-bonding distance to a quinoxaline nitrogen. Two symmetry-equivalent chloride positions are too close to be occupied simultaneously, so the chloride occupancy was fixed at 0.5 and a H atom with the same occupancy was attached to the corresponding nitrogen. The HCl was presumably formed from chloroform used in an earlier purification stage.

Results and discussion

The diffraction and refinement conditions are summarized in Table 1, and the resulting atomic coordinates are given in Tables 2–5. Fig. 1 shows the structure of the triostin A molecule in the $C222_1$ form, with the numbering of the atoms which is consistent for all the structures discussed here. The important torsion angles are presented in Table 6 and compared with the values calculated from the published coordinates (Wang *et al.*, 1986) for the triostin A molecule in its complexes with the hexanucleotide d(CGTACG) and the octanucleotide d(GCGTACGC). The coordinates for the complex of echinomycin with d(CGTACG) do not appear to have been published or deposited. No restraints were applied to the torsion angles in the four refinements of the uncomplexed antibiotics, so their e.s.d.'s should be realistic. Although standard bond length and angle (distance) restraints were employed in the refinements of the complexes, it appears that the torsion angles were also unrestrained. The refinement method used for the complexes precluded the estimation of standard deviations, but the much lower resolution and higher R-factors [1.67 Å and $R_1 = 0.186$ for triostin A plus d(CGTACG) and *ca* 2.25 Å and $R_1 = 0.200$ for triostin A plus d(GCGTACGC)] would inevitably lead to very much larger e.s.d.'s than those reported here for the uncomplexed antibiotics.

A comparison of the $P2_12_12_1$ triostin A refinement with our previously published structure provides a good illustration of the advantages of restrained anisotropic refinement against F^2 on all data, rather than conventional unrestrained mixed isotropic/anisotropic refine-

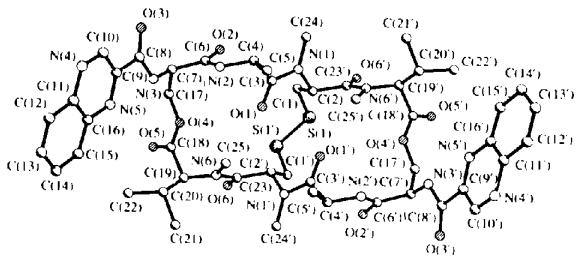


Fig. 1. The $C222_1$ form of triostin A, showing the numbering scheme. The primed atoms are symmetry equivalents in this structure.

Table 2. *Atomic coordinates* ($\times 10^4$) *and equivalent isotropic displacement parameters* ($Å^2 \times 10^3$) *for echinomycin 2QN*

$$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j .$$

	x	y	z	U_{eq}
S(1)	2748 (2)	9231 (2)	9152 (1)	69 (1)
C(1)	4636 (6)	9458 (7)	9316 (4)	72 (3)
C(2)	4038 (5)	9384 (6)	8838 (4)	59 (3)
N(1)	3768 (4)	8658 (4)	8683 (3)	56 (2)
C(24)	3780 (6)	8185 (6)	9106 (4)	74 (3)
C(3)	3449 (5)	8435 (6)	8154 (4)	54 (3)
O(1)	3441 (4)	8837 (4)	7855 (3)	66 (2)
C(4)	3219 (5)	7692 (5)	8027 (4)	48 (2)
C(5)	3838 (6)	7632 (6)	7861 (5)	72 (3)
N(2)	2752 (4)	7534 (4)	7577 (3)	45 (2)
C(6)	2134 (6)	6955 (6)	7537 (4)	55 (3)
O(2)	1840 (4)	6524 (4)	7888 (3)	60 (2)
C(7)	1764 (5)	6853 (5)	6963 (5)	62 (3)
N(3)	2245 (4)	7278 (4)	6535 (3)	54 (2)
C(8)	2802 (6)	7227 (6)	6389 (3)	52 (2)
O(3)	2843 (4)	6698 (4)	6492 (3)	71 (2)
C(9)	3382 (5)	7861 (5)	6182 (4)	59 (3)
C(10)	3733 (6)	7868 (6)	5675 (5)	69 (3)
C(27)	4278 (6)	8487 (6)	5511 (4)	67 (3)
C(11)	4511 (6)	9100 (6)	5815 (4)	64 (3)
C(12)	5074 (6)	9752 (7)	5670 (5)	74 (3)
C(13)	5263 (6)	10301 (6)	6007 (5)	66 (3)
C(14)	4905 (6)	10237 (6)	6493 (5)	63 (3)
C(15)	4348 (5)	9620 (5)	6644 (4)	53 (2)
C(16)	4125 (6)	9026 (5)	6312 (4)	55 (3)
N(5)	3569 (4)	8427 (4)	6466 (3)	52 (2)
C(17)	1165 (5)	6977 (5)	7016 (4)	53 (2)
O(4)	1446 (3)	7713 (3)	7100 (3)	52 (2)
O(5)	366 (4)	7516 (4)	7287 (4)	89 (3)
C(18)	994 (6)	7920 (5)	7217 (4)	54 (2)
C(19)	1282 (5)	8713 (5)	7236 (4)	52 (2)
C(20)	1219 (6)	8987 (6)	6677 (5)	79 (3)
C(21)	478 (7)	8585 (8)	6456 (6)	106 (5)
C(22)	1391 (7)	9749 (6)	6760 (6)	92 (4)
N(6)	1991 (4)	9099 (4)	7438 (3)	51 (2)
C(25)	2572 (5)	9258 (5)	7084 (4)	58 (3)
C(23)	2070 (6)	9356 (5)	7965 (5)	55 (3)
O(6)	1559 (4)	9175 (4)	8264 (3)	68 (2)
C(28')	3312 (11)	10822 (8)	9663 (4)	137 (7)
S(1')	3555 (2)	10801 (2)	8973 (1)	99 (1)
C(1')	2821 (6)	9964 (6)	8778 (4)	69 (3)
C(2')	2820 (5)	9852 (5)	8150 (4)	49 (2)
N(1')	3106 (4)	10512 (4)	7844 (3)	50 (2)
C(24')	2709 (5)	10887 (5)	7856 (5)	64 (3)
C(3')	3756 (5)	10773 (5)	7659 (4)	45 (2)
O(1')	4072 (3)	10459 (3)	7685 (3)	54 (2)
C(4')	4095 (5)	11480 (5)	7364 (4)	48 (2)
C(5')	3829 (6)	11408 (6)	6788 (4)	67 (3)
N(2')	4839 (4)	11773 (4)	7364 (4)	56 (2)
C(6')	5213 (6)	11980 (5)	7828 (6)	63 (3)
O(2')	4937 (4)	12008 (4)	8258 (4)	77 (2)
C(7')	5983 (5)	12231 (6)	7807 (5)	67 (3)
N(3')	6167 (4)	11906 (4)	7370 (4)	59 (2)
C(8')	6532 (6)	12234 (6)	6923 (5)	70 (3)
O(3')	6669 (4)	12826 (5)	6809 (3)	79 (2)
C(9')	6796 (6)	11858 (7)	6596 (5)	73 (3)
C(10')	7045 (7)	12085 (8)	6065 (5)	87 (4)
C(27')	7274 (7)	11729 (9)	5775 (6)	99 (4)
C(11')	7276 (7)	11173 (9)	6002 (5)	90 (4)
C(12')	7554 (8)	10781 (9)	5719 (6)	99 (4)
C(13')	7578 (10)	10297 (11)	5994 (8)	127 (5)
C(14')	7365 (10)	10117 (9)	6513 (6)	125 (5)
C(15')	7042 (10)	10418 (8)	6769 (7)	114 (4)
C(16')	7031 (7)	10984 (8)	6526 (5)	84 (3)
N(5')	6767 (5)	11319 (6)	6825 (4)	70 (2)
C(17')	6244 (6)	12128 (6)	8357 (4)	68 (3)
O(4')	5994 (4)	11385 (4)	8414 (3)	78 (2)
C(18')	6251 (7)	11166 (7)	8791 (6)	87 (4)
O(5')	6668 (8)	11601 (6)	9098 (6)	169 (6)
C(19')	6019 (6)	10420 (5)	8780 (4)	58 (3)
C(20')	6466 (6)	10194 (8)	8485 (6)	90 (3)
C(21')	6151 (10)	9415 (9)	8463 (7)	124 (5)
C(22')	7180 (8)	10526 (12)	8773 (8)	153 (7)
N(6')	5298 (5)	10026 (4)	8627 (3)	58 (2)
C(25')	5121 (6)	9987 (6)	8025 (3)	60 (3)
C(23')	4800 (7)	9724 (6)	9003 (5)	75 (3)
O(6')	4953 (4)	9691 (5)	9492 (3)	102 (3)
O(30)	1915 (5)	8201 (5)	5868 (4)	103 (3)
C(31)	2109 (8)	8552 (8)	5454 (7)	103 (4)
C(32)	2839 (8)	8895 (10)	5283 (7)	133 (6)
C(33)	1629 (8)	8683 (8)	5141 (6)	122 (5)
O(40)	2198 (9)	3650 (10)	346 (8)	211 (7)
C(41)	2611 (11)	4129 (13)	35 (9)	202 (10)
C(42)	3308 (15)	4599 (16)	216 (12)	411 (34)
C(43)	2386 (17)	4201 (18)	−505 (11)	291 (17)
O(50)	1163 (18)	320 (20)	779 (25)	331 (29)
C(51)	1385 (22)	961 (23)	811 (28)	298 (26)
C(52)	2002 (26)	1367 (26)	1138 (38)	329 (30)
C(53)	918 (28)	1229 (26)	707 (32)	281 (31)
O(60)	1226 (41)	98 (40)	−269 (39)	343 (33)
O(61)	1088 (45)	1076 (33)	1492 (42)	395 (38)
O(62)	407 (60)	−328 (57)	1393 (50)	432 (49)
O(63)	−799 (57)	−1124 (45)	2388 (46)	393 (40)
O(64)	−817 (34)	−570 (34)	3502 (34)	312 (29)

Table 3. *Atomic coordinates* ($\times 10^4$) *and equivalent isotropic displacement parameters* ($Å^2 \times 10^3$) *for triostin C*

$$U_{eq} = (1/3)\sum_i \sum_j a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j .$$

	x	y	z	U_{eq}
S(1)	9456 (2)	268 (2)	120 (1)	58 (1)
C(1)	9626 (5)	55 (5)	−655 (4)	47 (2)
C(2)	8968 (5)	411 (4)	−1039 (4)	32 (2)
N(1)	9139 (4)	264 (4)	−1667 (3)	37 (2)
C(24)	9936 (5)	540 (5)	−1913 (5)	57 (3)
C(3)	8657 (6)	−264 (5)	−1960 (4)	36 (2)
O(1)	8017 (4)	−520 (3)	−1729 (3)	48 (2)
C(4)	8860 (6)	−488 (5)	−2588 (4)	43 (2)
C(5)	8370 (7)	−5 (5)	−3030 (4)	61 (3)
N(2)	8663 (5)	−1322 (4)	−2668 (3)	41 (2)
C(6)	9082 (6)	−1822 (5)	−2334 (4)	37 (2)
O(2)	9624 (4)	−1649 (4)	−1994 (3)	52 (2)
C(7)	8870 (5)	−2709 (5)	−2435 (4)	39 (2)
N(3)	8009 (4)	−2835 (4)	−2598 (3)	42 (2)
C(8)	7765 (6)	−3266 (5)	−3063 (5)	42 (2)
O(3)	8224 (4)	−3474 (4)	−3459 (3)	60 (2)
C(9)	6853 (6)	−3461 (5)	−3062 (5)	42 (2)
C(10)	6468 (6)	−3778 (5)	−3564 (4)	48 (3)
N(4)	5662 (5)	−3953 (5)	−3568 (4)	55 (2)
C(11)	5231 (6)	−3817 (6)	−3068 (5)	49 (3)
C(12)	4381 (7)	−3997 (7)	−3046 (6)	71 (3)
C(13)	3964 (8)	−3881 (8)	−2543 (7)	90 (4)
C(14)	4316 (7)	−3567 (9)	−2045 (6)	97 (5)
C(15)	5145 (6)	−3359 (7)	−2048 (5)	76 (4)
C(16)	5603 (6)	−3498 (6)	−2562 (5)	53 (3)
N(5)	6447 (5)	−3308 (4)	−2568 (4)	47 (2)
C(17)	9075 (6)	−3169 (5)	−1882 (4)	41 (2)
O(4)	8421 (4)	−2995 (3)	−1474 (3)	44 (2)
C(18)	8467 (5)	−3321 (5)	−945 (4)	36 (2)
O(5)	9046 (4)	−3716 (4)	−793 (3)	66 (2)
C(19)	7700 (5)	−3163 (5)	−579 (4)	37 (2)
C(20)	6930 (5)	−3619 (5)	−794 (4)	40 (2)
C(21)	6174 (5)	−3404 (6)	−421 (5)	61 (3)
C(22)	7095 (6)	−4513 (5)	−820 (4)	48 (3)
N(6)	7533 (4)	−2302 (4)	−541 (3)	37 (2)
C(25)	7166 (6)	−1917 (5)	−1053 (4)	48 (3)
C(23)	7711 (5)	−1954 (5)	−29 (5)	40 (2)
O(6)	8009 (4)	−2272 (4)	404 (3)	51 (2)
C(32)	7142 (7)	−4870 (6)	−214 (4)	58 (3)
C(31)	6448 (6)	−4898 (5)	−1202 (5)	59 (3)
S(1')	9076 (2)	−757 (2)	478 (2)	68 (1)

Table 3 (*cont.*)

	x	y	z	U_{eq}
C(1')	7958 (5)	−694 (5)	525 (5)	51 (3)
C(2')	7516 (5)	−1049 (5)	6 (4)	38 (2)
N(1')	6612 (4)	−936 (4)	59 (3)	39 (2)
C(24')	6184 (5)	−1307 (5)	545 (4)	50 (3)
C(3')	6287 (5)	−327 (5)	−249 (4)	36 (2)
O(1')	6663 (4)	−28 (3)	−663 (3)	46 (2)
C(4')	5418 (5)	−44 (5)	−87 (4)	38 (2)
C(5')	4745 (5)	−421 (5)	−452 (4)	48 (2)
N(2')	5396 (4)	802 (4)	−181 (3)	36 (2)
C(6')	5862 (5)	1240 (5)	167 (4)	34 (2)
O(2')	6303 (4)	990 (4)	556 (3)	48 (2)
C(7')	5782 (5)	2147 (5)	90 (4)	31 (2)
N(3')	5412 (4)	2406 (4)	−446 (3)	32 (2)
C(8')	4594 (6)	2481 (5)	−538 (4)	35 (2)
O(3')	4049 (4)	2169 (4)	−225 (3)	48 (2)
C(9')	4351 (5)	2940 (5)	−1072 (4)	32 (2)
C(10')	3513 (5)	3165 (5)	−1185 (4)	44 (2)
N(4')	3277 (4)	3487 (4)	−1673 (4)	43 (2)
C(11')	3867 (6)	3619 (5)	−2086 (5)	42 (2)
C(12')	3666 (6)	3922 (6)	−2636 (4)	54 (3)
C(13')	4269 (7)	4062 (6)	−3047 (5)	66 (3)
C(14')	5103 (7)	3912 (7)	−2912 (5)	70 (3)
C(15')	5335 (6)	3623 (6)	−2379 (4)	60 (3)
C(16')	4718 (5)	3446 (5)	−1962 (4)	39 (2)
N(5')	4948 (4)	3105 (4)	−1446 (3)	38 (2)
C(17')	6604 (5)	2543 (5)	207 (4)	36 (2)
O(4')	7258 (3)	2307 (4)	−190 (3)	40 (2)
C(18')	7386 (5)	2721 (5)	−675 (4)	37 (2)
O(5')	6892 (4)	3215 (4)	−842 (3)	52 (2)
C(19')	8199 (5)	2544 (4)	−985 (4)	33 (2)
C(20')	8302 (5)	3001 (5)	−1559 (4)	36 (2)
C(21')	8992 (6)	2636 (6)	−1934 (4)	56 (3)
C(22')	8459 (5)	3877 (5)	−1446 (4)	41 (2)
N(6')	8247 (4)	1688 (4)	−1070 (3)	36 (2)
C(25')	7545 (5)	1343 (5)	−1379 (4)	46 (3)
C(23')	8952 (6)	1295 (5)	−938 (4)	37 (2)
O(6')	9561 (4)	1632 (3)	−729 (3)	52 (2)
C(32')	8339 (6)	4351 (5)	−2006 (4)	57 (3)
C(31')	9304 (6)	4050 (6)	−1179 (5)	69 (3)
C(40)	7297 (7)	−3405 (8)	1355 (7)	63 (5)
Cl(1)	6246 (2)	−3367 (3)	1388 (2)	71 (2)
Cl(2)	7753 (3)	−2818 (3)	1884 (2)	94 (2)
Cl(3)	7667 (4)	−4375 (4)	1420 (3)	99 (2)
O(40)	8060 (28)	−3571 (19)	1112 (18)	179 (16)
O(41)	7048 (50)	−4275 (55)	1480 (38)	386 (44)

Table 4. *Atomic coordinates* ($\times 10^4$) *and equivalent isotropic displacement parameters* ($Å^2 \times 10^3$) *for the* $P2_12_12_1$ *form of triostin A*

$$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j .$$

	x	y	z	U_{eq}
S(1)	3679 (2)	4124 (2)	1639 (2)	134 (2)
C(1)	3509 (7)	5049 (7)	1446 (7)	120 (4)
C(2)	3802 (6)	5587 (7)	1962 (7)	101 (4)
N(1)	3630 (6)	6322 (6)	1794 (5)	104 (3)
C(24)	3895 (7)	6652 (8)	1142 (7)	135 (5)
C(3)	3131 (7)	6597 (7)	2168 (8)	95 (4)
O(1)	2897 (5)	6292 (5)	2673 (5)	122 (3)
C(4)	2874 (7)	7341 (8)	1935 (7)	111 (4)
C(5)	3235 (7)	7964 (7)	2277 (9)	145 (6)
N(2)	2199 (6)	7363 (6)	2089 (6)	102 (3)
C(6)	1804 (7)	6915 (9)	1752 (8)	101 (4)
O(2)	1979 (4)	6515 (6)	1270 (5)	125 (3)
C(7)	1088 (6)	6952 (7)	1921 (6)	89 (3)
N(3)	946 (5)	7230 (5)	2634 (5)	91 (3)
C(8)	615 (6)	7821 (8)	2766 (7)	99 (4)
O(3)	468 (6)	8267 (5)	2329 (5)	153 (5)
C(9)	318 (6)	7849 (8)	3513 (7)	92 (4)
C(10)	−68 (7)	8435 (7)	3702 (8)	116 (4)
N(4)	−360 (5)	8455 (7)	4319 (7)	109 (4)
C(11)	−263 (7)	7873 (9)	4767 (8)	109 (4)

Table 4 (*cont.*)

	x	y	z	U_{eq}
C(12)	−578 (7)	7871 (10)	5448 (9)	129 (5)
C(13)	−489 (8)	7333 (11)	5876 (8)	138 (6)
C(14)	−84 (8)	6759 (10)	5715 (8)	135 (5)
C(15)	224 (7)	6743 (8)	5076 (9)	124 (5)
C(16)	147 (6)	7309 (9)	4591 (8)	104 (4)
N(5)	420 (5)	7294 (6)	3934 (6)	93 (3)
C(17)	751 (6)	6222 (7)	1839 (6)	100 (4)
O(4)	942 (4)	5803 (5)	2456 (4)	96 (3)
C(18)	744 (6)	5138 (8)	2499 (8)	95 (4)
O(5)	514 (6)	4870 (6)	2001 (6)	167 (5)
C(19)	889 (5)	4790 (6)	3197 (7)	84 (3)
C(20)	424 (6)	5030 (7)	3786 (7)	101 (4)
C(21)	565 (7)	4652 (8)	4489 (7)	135 (5)
C(22)	−266 (6)	4909 (10)	3554 (9)	154 (6)
N(6)	1557 (5)	4896 (6)	3386 (5)	75 (3)
C(25)	1789 (6)	5585 (6)	3679 (6)	88 (4)
C(23)	1956 (6)	4338 (8)	3292 (6)	77 (3)
O(6)	1763 (4)	3727 (5)	3077 (5)	111 (3)
S(1')	2889 (2)	3749 (2)	2123 (2)	125 (1)
C(1')	3062 (6)	3810 (7)	3058 (6)	108 (4)
C(2')	2678 (5)	4413 (6)	3427 (6)	82 (3)
N(1')	2809 (5)	4437 (6)	4184 (5)	83 (3)
C(24')	2578 (7)	3825 (8)	4619 (7)	124 (5)
C(3')	3274 (7)	4897 (9)	4386 (7)	93 (4)
O(1')	3481 (4)	5388 (6)	4020 (5)	110 (3)
C(4')	3556 (6)	4821 (7)	5154 (6)	93 (4)
C(5')	3264 (6)	5408 (7)	5629 (6)	106 (4)
N(2')	4239 (5)	4917 (6)	5115 (5)	93 (3)
C(6')	4575 (7)	4433 (10)	4746 (9)	120 (5)
O(2')	4318 (5)	3900 (6)	4469 (6)	136 (4)
C(7')	5308 (6)	4449 (8)	4728 (8)	122 (4)
N(3')	5566 (5)	5132 (6)	4989 (6)	113 (3)
C(8')	5871 (6)	5192 (8)	5619 (8)	99 (4)
O(3')	5897 (5)	4710 (6)	6065 (6)	132 (4)
C(9')	6264 (6)	5860 (8)	5718 (8)	96 (4)
C(10')	6544 (7)	6040 (8)	6374 (7)	111 (4)
N(4')	6879 (5)	6622 (7)	6469 (6)	108 (4)
C(11')	6984 (6)	7058 (8)	5872 (8)	99 (4)
C(12')	7366 (6)	7678 (8)	5927 (7)	101 (4)
C(13')	7478 (7)	8088 (7)	5368 (9)	108 (4)
C(14')	7188 (8)	7922 (8)	4727 (8)	122 (5)
C(15')	6805 (7)	7341 (9)	4649 (7)	112 (5)
C(16')	6698 (6)	6870 (8)	5224 (8)	90 (4)
N(5')	6331 (5)	6281 (7)	5156 (6)	96 (3)
C(17')	5581 (8)	4297 (10)	3978 (9)	154 (6)
O(4')	5437 (5)	4938 (7)	3551 (7)	148 (4)
C(18')	5715 (10)	4975 (12)	2940 (11)	218 (8)
O(5')	5950 (10)	4432 (11)	2702 (8)	312 (10)
C(19')	5557 (7)	5610 (11)	2497 (8)	195 (7)
C(20')	5931 (9)	6299 (13)	2785 (14)	240 (9)
C(21')	5805 (14)	6935 (14)	2294 (16)	333 (15)
C(22')	6621 (8)	6113 (18)	2831 (17)	369 (18)
N(6')	4874 (5)	5745 (7)	2513 (7)	127 (4)
C(25')	4557 (7)	6088 (9)	3119 (6)	133 (5)
C(23')	4534 (8)	5491 (9)	1965 (9)	124 (5)
O(6')	4800 (4)	5187 (7)	1447 (6)	144 (4)
C(31)	5250 (13)	7272 (17)	9827 (13)	284 (12)
O(32)	4141 (12)	7677 (15)	9928 (11)	346 (13)
C(33)	4614 (14)	7524 (20)	9548 (13)	292 (14)
O(34)	4569 (10)	7623 (10)	8852 (9)	254 (8)
C(35)	3944 (12)	7861 (13)	8548 (10)	223 (9)
C(36)	4029 (11)	7791 (14)	7736 (10)	252 (10)
C(37)	3462 (11)	8072 (14)	7317 (11)	241 (10)
C(38)	3495 (11)	7842 (17)	6558 (11)	299 (13)
C(39)	3364 (17)	8858 (14)	7392 (18)	360 (17)
C(41)	7672 (28)	5326 (38)	9113 (31)	428 (30)
O(42)	6888 (29)	5674 (35)	8268 (27)	391 (29)
C(43)	7108 (33)	5756 (38)	8884 (29)	429 (28)
O(44)	6862 (33)	6214 (35)	9351 (26)	448 (27)
C(45)	7157 (45)	6439 (32)	9998 (32)	449 (26)
C(46)	6887 (47)	5989 (31)	10611 (27)	455 (27)
C(47)	6895 (34)	5174 (31)	10510 (36)	450 (27)
C(48)	7267 (38)	4805 (40)	11083 (51)	441 (27)
C(49)	6233 (34)	4875 (51)	10431 (44)	419 (30)
C(51)	6787 (51)	3784 (30)	9635 (34)	561 (50)

Table 4 (*cont.*)

	x	*y*	*z*	U_{eq}
O(52)	6665 (26)	4441 (32)	10728 (24)	469 (28)
C(53)	6775 (34)	4456 (30)	10077 (28)	488 (27)
O(54)	7072 (35)	5011 (31)	9757 (26)	482 (23)
C(55)	7448 (39)	5519 (30)	10170 (33)	466 (24)
C(56)	7575 (39)	6181 (29)	9697 (30)	475 (25)
C(57)	7471 (32)	6906 (27)	10086 (32)	516 (31)
C(58)	6776 (29)	6936 (32)	10338 (45)	471 (32)
C(59)	7920 (34)	6939 (31)	10725 (38)	459 (30)

Table 5. *Atomic coordinates* ($\times 10^4$) *and equivalent isotropic displacement parameters* ($Å^2 \times 10^3$) *for the* $C222_1$ *form of triostin A*

$$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j .$$

	x	*y*	*z*	U_{eq}
S(1)	1643 (2)	5516 (2)	5147 (1)	53 (1)
C(1)	511 (7)	6140 (5)	4894 (3)	48 (3)
C(2)	−780 (7)	6164 (5)	5096 (2)	34 (2)
N(1)	−1699 (6)	6562 (4)	4853 (2)	41 (2)
C(24)	−1730 (9)	7403 (5)	4826 (3)	52 (3)
C(3)	−2262 (10)	6064 (6)	4594 (3)	56 (3)
O(1)	−1996 (8)	5383 (4)	4574 (2)	94 (3)
C(4)	−3340 (8)	6383 (6)	4339 (3)	51 (2)
C(5)	−4577 (9)	6210 (7)	4542 (3)	68 (3)
N(2)	−3303 (7)	5994 (5)	3980 (2)	48 (2)
C(6)	−2243 (10)	6045 (6)	3758 (3)	49 (2)
O(2)	−1345 (6)	6457 (4)	3858 (2)	68 (2)
C(7)	−2202 (7)	5586 (5)	3403 (3)	43 (2)
N(3)	−3361 (7)	5213 (4)	3277 (2)	47 (2)
C(8)	−4251 (9)	5612 (7)	3088 (2)	50 (3)
O(3)	−4238 (6)	6297 (5)	3006 (2)	62 (2)
C(9)	−5332 (8)	5079 (6)	2955 (2)	36 (2)
C(10)	−6458 (9)	5427 (6)	2814 (2)	48 (2)
N(4)	−7408 (6)	4993 (6)	2703 (2)	59 (2)
C(11)	−7275 (9)	4197 (6)	2717 (2)	52 (3)
C(12)	−8261 (10)	3687 (8)	2596 (3)	70 (3)
C(13)	−8107 (12)	2920 (8)	2615 (3)	76 (3)
C(14)	−6986 (10)	2567 (8)	2748 (2)	70 (3)
C(15)	−6039 (10)	3032 (7)	2864 (3)	65 (3)
C(16)	−6157 (8)	3853 (7)	2856 (2)	47 (2)
N(5)	−5174 (7)	4325 (5)	2985 (2)	45 (2)
C(17)	−1169 (8)	4973 (6)	3414 (3)	54 (3)
O(4)	−1586 (6)	4355 (4)	3664 (2)	45 (2)
C(18)	−1021 (9)	3673 (6)	3631 (3)	45 (2)
O(5)	−260 (7)	3524 (4)	3393 (2)	74 (2)
C(19)	−1510 (9)	3047 (6)	3913 (3)	46 (2)
C(20)	−2701 (9)	2615 (6)	3763 (3)	52 (3)
C(21)	−3039 (10)	1949 (7)	4028 (4)	79 (4)
C(22)	−2501 (10)	2314 (7)	3363 (3)	81 (4)
N(6)	−1688 (7)	3376 (4)	4288 (2)	45 (2)
C(25)	−2889 (7)	3755 (6)	4378 (3)	48 (3)
C(23)	−681 (9)	3431 (5)	4520 (3)	44 (2)
O(6)	351 (6)	3132 (4)	4419 (2)	51 (2)
O(7)	−5376 (10)	4892 (10)	3892 (3)	162 (7)
C(26)	−5846 (15)	4309 (13)	3868 (3)	118 (6)
N(7)	−6953 (9)	4086 (7)	3803 (3)	89 (3)
C(27)	−7905 (11)	4703 (7)	3741 (4)	90 (4)
C(28)	−7323 (15)	3299 (8)	3779 (4)	123 (6)
Cl(1)	−9818 (8)	5808 (5)	2687 (2)	123 (3)

ment against F for $F > 2.5\sigma(F)$. The chemically more realistic model for the molecular geometry and displacement parameters led to better phases and hence a more precise interpretation of the disordered solvent, which in turn improved the overall precision of the structure determination. The standard deviations in the torsion angles (which were not restrained in either refinement) were reduced by a factor of two, and the conventional R-index (on F!) was halved. There were also changes of up to three times the original e.s.d.'s in the torsion angles.

The thermal ellipsoids for the $C222_1$ form of triostin A (Fig. 2) show that restrained anisotropic refinement can produce chemically sensible results despite extremely weak data. For example, virtually all the carbonyl oxygens are vibrating most strongly in the expected direction at right angles to the carbonyl plane.

General comparison of the molecular structures

Although there are significant variations in the torsion angles for the different molecules (Table 6), for example, the mean difference in the 26 torsion angles in the main ring is 10.1° between the two modifications of triostin A, the ring conformations shown in Fig. 3 are in general rather similar. All peptide bonds are *trans*, the Cys—αCH and Cys—C═O units point into the ring, and the valine and alanine peptide planes are approximately at right angles to the ring, with their carbonyl oxygens on the same side of the ring as the bridge containing the S atoms. There is appreciable variability in the orientation of the lactone (ester) plane relative to the ring plane, and this may be associated with a low torsional barrier. The conformation of all four molecules is similar to that deduced for the *p* form of triostin A by Kalman *et al.* (1979) from NMR data, except that they predicted a more open structure with the Cys—αCH and Cys—C═O units almost at right angles to the ring plane (on the opposite side to the bridge).

The chromophores all adopt a conformation in which a N atom [N(5)] in the quinoxaline or quinoline ring is *cis* to the amide nitrogen [N(3)], as predicted by Kalman *et al.* (1979). With the exception of the 'twisted' quinoline in echinomycin 2QN (see below), the maximum angle between the aromatic and amide planes is 12°. In echinomycin 2QN, one quinoline chromophore is oriented perpendicular to the main peptide ring, but the other is twisted by *ca* 50°, primarily by rotating the

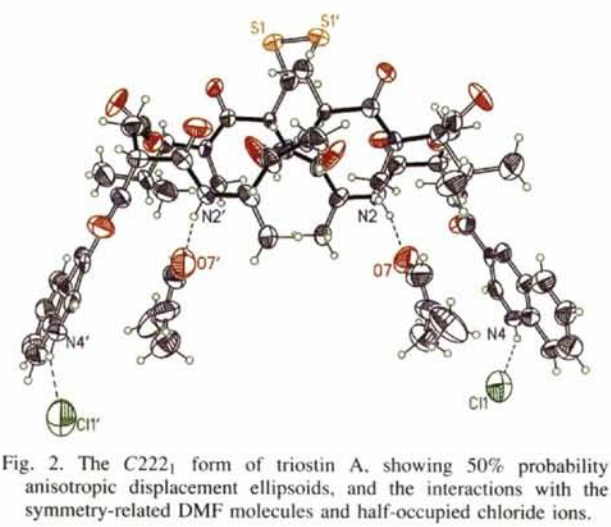


Fig. 2. The $C222_1$ form of triostin A, showing 50% probability anisotropic displacement ellipsoids, and the interactions with the symmetry-related DMF molecules and half-occupied chloride ions.

Table 6. *Selected torsion angles* (°) *and interchromophore distances* (Å)

	Echinomycin 2QN	Triostin C	Triostin A $P2_12_12_1$	Triostin A $C222_1$	Triostin A d(CGTACG)	Triostin A d(GCGTACGC)
(*a*) Torsion angles in depsipeptide ring						
C(4)—C(3)—N(1)—C(2)	177.4 (8)	−174.3 (7)	−172.0 (11)	175.0 (8)	−161	−174
ω (Ala)	−176.8 (8)	−163.7 (8)	−165.4 (10)		174	−160
N(2)—C(4)—C(3)—N(1)	163.7 (8)	144.4 (8)	149.2 (11)	147.7 (9)	162	168
ψ (Ala)	160.8 (8)	146.6 (8)	138.9 (11)		177	144
C(6)—N(2)—C(4)—C(3)	−134.5 (9)	−61.4 (10)	−64.3 (14)	−59.5 (11)	−99	−77
φ (Ala)	−66.5 (11)	−67.0 (9)	−64.1 (14)		−63	−100
C(7)—C(6)—N(2)—C(4)	−171.7 (8)	−179.1 (7)	−179.6 (10)	175.4 (8)	−156	−155
ω (D-Ser)	175.3 (9)	−175.7 (7)	−174.5 (12)		−160	−163
C(17)—C(7)—C(6)—N(2)	−107.5 (9)	−155.5 (7)	−149.5 (11)	−116.2 (9)	−138	−153
	−154.5 (9)	−146.5 (7)	−138.5 (14)		−177	−111
O(4)—C(17)—C(7)—C(6)	71.1 (10)	75.6 (8)	75.0 (10)	73.8 (10)	64	84
χ (D-Ser)	73.9 (11)	61.5 (9)	71.3 (16)		76	4
C(18)—O(4)—C(17)—C(7)	−172.7 (9)	−178.8 (7)	−176.6 (11)	160.8 (8)	162	−164
	166.4 (11)	92.6 (9)	169.0 (14)		112	−84
C(19)—C(18)—O(4)—C(17)	−172.7 (8)	−173.9 (7)	−171.9 (9)	179.2 (8)	−167	158
ω (Val) (ester)	−173.9 (11)	166.5 (7)	178.3 (14)		166	−172
N(6)—C(19)—C(18)—O(4)	−34.2 (12)	−54.4 (9)	−49.5 (14)	−42.7 (11)	−56	−48
ψ (Val) (ester)	−37.3 (15)	51.6 (10)	−43.9 (22)		34	−81
C(23)—N(6)—C(19)—C(18)	−105.5 (9)	−105.6 (9)	−102.6 (12)	−83.1 (10)	−66	−96
φ (Val)	−98.0 (12)	−133.5 (8)	−98.1 (17)		−116	−116
C(2′)—C(23)—N(6)—C(19)	−171.4 (8)	−179.0 (7)	175.8 (10)	174.6 (8)	162	−173
ω (Cys)	172.7 (9)	−179.6 (8)	177.5 (14)		−169	−172
N(1′)—C(2′)—C(23)—N(6)	68.5 (10)	74.6 (9)	74.2 (13)	60.8 (9)	78	72
ψ (Cys)	88.3 (12)	81.4 (9)	75.4 (17)		73	65
C(3′)—N(1′)—C(2′)—C(23)	−136.3 (9)	−140.8 (8)	−141.4 (11)	−151.4 (8)	−139	−121
φ (Cys)	−125.2 (9)	−133.0 (8)	−140.6 (12)		−133	−128
(*b*) Torsion angles in serine–quinoxaline (quinoline) unit						
N(3)—C(7)—C(6)—N(2)	18.3 (12)	−32.6 (11)	−26.1 (15)	10.2 (12)	−32	−31
ψ (D-Ser)	−29.5 (13)	−17.3 (11)	−14.9 (20)		−51	8
C(8)—N(3)—C(7)—C(6)	59.4 (12)	129.9 (9)	118.7 (13)	81.3 (11)	106	99
φ (D-Ser)	110.9 (11)	85.4 (10)	108.8 (14)		103	154
C(9)—C(8)—N(3)—C(7)	−150.5 (9)	166.7 (8)	158.0 (11)	175.1 (7)	176	−166
ω (D-Ser)	166.8 (9)	165.7 (7)	163.3 (11)		−177	114
N(5)—C(9)—C(8)—N(3)	47.6 (13)	−11.4 (12)	−0.2 (16)	−10.5 (10)	−10	−15
	−12.9 (15)	11.5 (12)	−7.4 (16)		−4	−30
(*c*) Torsion angle of disulfide bridge						
C(1′)—S(1′)—S(1)—C(1)	—	−99.2 (5)	−96.9 (7)	−94.6 (6)	81	110
(*d*) Interchromophore distance						
N(3)···N(3′)	9.804 (13)	11.039 (10)	11.325 (17)	12.157 (18)	10.6	10.8

chromophore out of the amide plane (Fig. 4 and Table 6). This enables the amide N(3)—H(3) to hydrogen bond to a solvent molecule, in contrast to the other chromophore amide nitrogens in the structures reported here.

All the chromophores could clearly be rotated into the parallel orientations required for bis-intercalation. Since this rotation would leave N(3) unmoved, we can use the N(3)···N(3′) distance (Table 6) as a rough estimate of the distance between the chromophore planes in the parallel conformation. The distance in triostin C is closest to those observed in the complexes.

There is a curious asymmetry in the structure of triostin C (Fig. 5), which derives from the rotation of the lactone (ester) linkage in one half of the depsipeptide ring so that a strong hydrogen bond is formed between the ester carbonyl group and the nearby serine N(3)—H(3). This is associated with a further weak hydrogen bond linking the quinoxaline carbonyl with the alanine NH (Table 7). This same alanine also forms an intermolecular hydrogen bond with the quinoxaline ring nitrogen of a neighbouring antibiotic molecule in the quasi-intercalated packing arrangement.

Intermolecular interactions and packing

Triostin C and the $P2_12_12_1$ form of triostin A (Figs. 6*a* and *b*) adopt a rather similar stacking of their chromophores. Both structures are also held together by intermolecular (alanine)N(2)—H(2*A*)···N(4′)(quinoxaline) and N(2′)—H(2′*A*)···N(4) hydrogen bonds (Table 7). In the $C222_1$ form of triostin A, the alanine NH groups make hydrogen bonds to the carbonyl groups of DMF molecules. The DMF molecules lie parallel to the chromophore planes (Fig. 2) and a chromophore of another antibiotic molecule stacks on the other side of each quinoxaline ring (Fig. 6*a*). Thus, in all three structures the packing emulates the intercalative binding of the antibiotics to DNA. In echinomycin 2QN one chromophore has folded into the molecule and the corresponding alanine NH hydrogen bonds

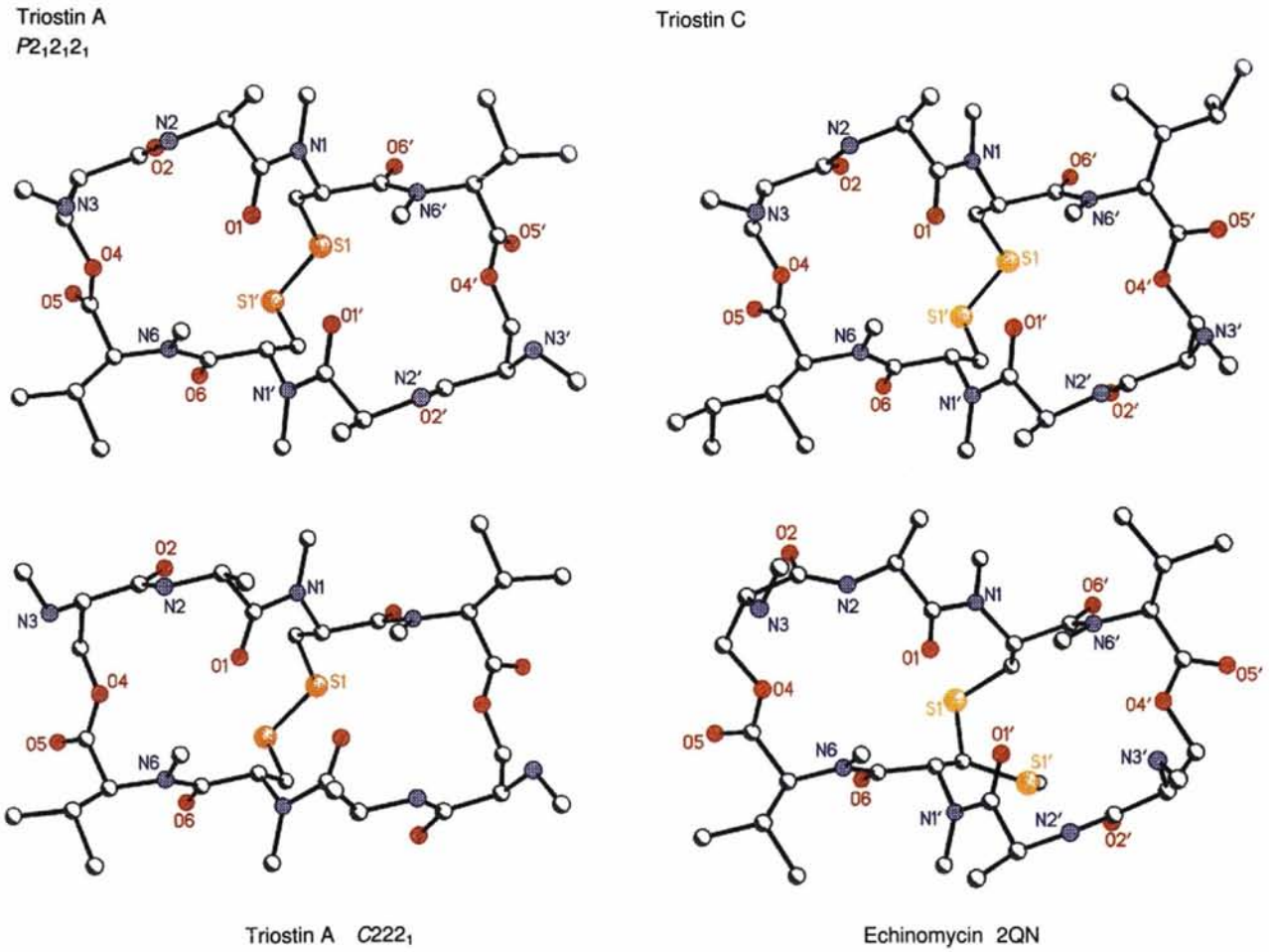


Fig. 3. The depsipeptide ring conformations, with unique heteroatoms labelled.

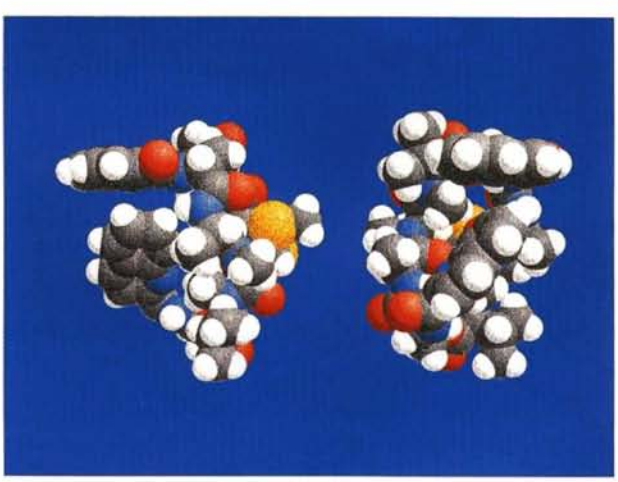


Fig. 4. Two space-filling views of echinomycin 2QN.

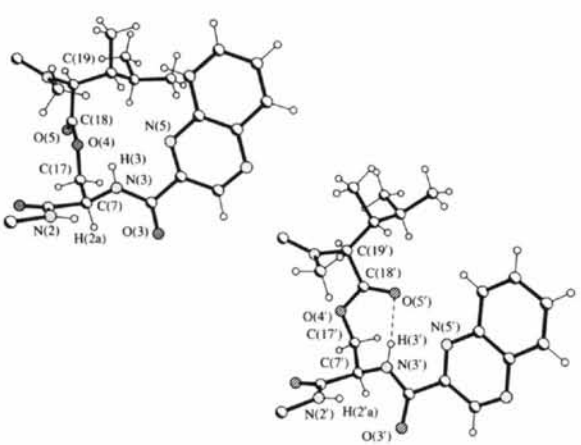


Fig. 5. The two sides of the depsipeptide ring in triostin C, showing the rotation of the ester plane and the internal hydrogen bond on one side of the molecule only.

Table 7. *Hydrogen bond distances* (Å) *and angles* (°)

(*a*) Echinomycin 2QN

N(2′)···O(2′)	2.986 (12)	∠NHO	165.1 (3)	
N(3)···O(30)	2.991 (13)	∠NHO	171.8 (3)	(acetone)

There are also a number of distances in the hydrogen-bonding range within the disordered solvent channels.

(*b*) Triostin C

N(3′)···O(5′)	2.894 (9)	∠NHO	136.1 (2)	
N(2′)···O(3′)	3.190 (9)	∠NHO	121.1 (2)	
N(2)···N(4^{ii})	3.471 (11)	∠NHN	151.2 (2)	
N(2′)···N(4^{iii})	3.336 (11)	∠NHN	155.6 (2)	
C(40)···O(6)	3.121 (16)	∠CHO	150.4 (4)	(chloroform)

(*c*) Triostin A in $P2_12_12_1$

N(2)···N(4^{iv})	3.366 (15)	∠NHN	162.8 (4)	
N(2′)···N(4^{v})	3.308 (15)	∠NHN	158.8 (4)	

(*d*) Triostin A in $C222_1$

N(2)···O(7)	2.910 (13)	∠HNO	160.3 (4)	(DMF)
N(4)···Cl(1)	2.913 (12)	∠NHCl	154.5 (3)	($NH^+\cdots Cl^-$)

Symmetry codes: (i) $1+x-y, 1+x, z-\frac{1}{6}$; (ii) $1-x, y-\frac{1}{2}, -z-\frac{1}{2}$; (iii) $1-x, y+\frac{1}{2}, -z-\frac{1}{2}$; (iv) $x-\frac{1}{2}, \frac{3}{2}-y, 1-z$; (v) $x+\frac{1}{2}, \frac{3}{2}-y, 1-z$.

The short N(3)···N(5) and N(3′)···N(5′) distances (*ca* 2.7 Å) present in all four structures have not been included in this table; they are associated with long H···N distances (*ca* 2.5 Å) and very bent N—H···N angles (*ca* 90°).

to an acetone molecule (Table 7). With the help of intermolecular (alanine)N(2′)—H(2′*A*)···O(2)(alanine) hydrogen bonds, the molecules form a helix about the crystallographic 6_5 screw axis (Fig. 7). A disordered solvent channel runs along the middle of this helix.

Comparison with triostin A in DNA complexes

In Table 6, torsion angles calculated from the coordinates published by Wang *et al.* (1986) are compared with those found here for the four uncomplexed antibiotics. It will be seen (Fig. 8) that the general conformations are similar in all cases, except that the chirality of the disulfide bridge is inverted in the complexes. The complexes deviate more from the approximate twofold molecular symmetry, especially in the region of the disulfide bridge. There are some energetically rather unlikely details in the octanucleotide complex – for example, the eclipsed conformation about the serine C(7)—C(17) bond and the severely twisted serine peptide unit ($\omega = 114°$) – which we attribute to difficulties in obtaining a correct model at low resolution (2.25 Å); indeed Wang *et al.* (1986) themselves warn against

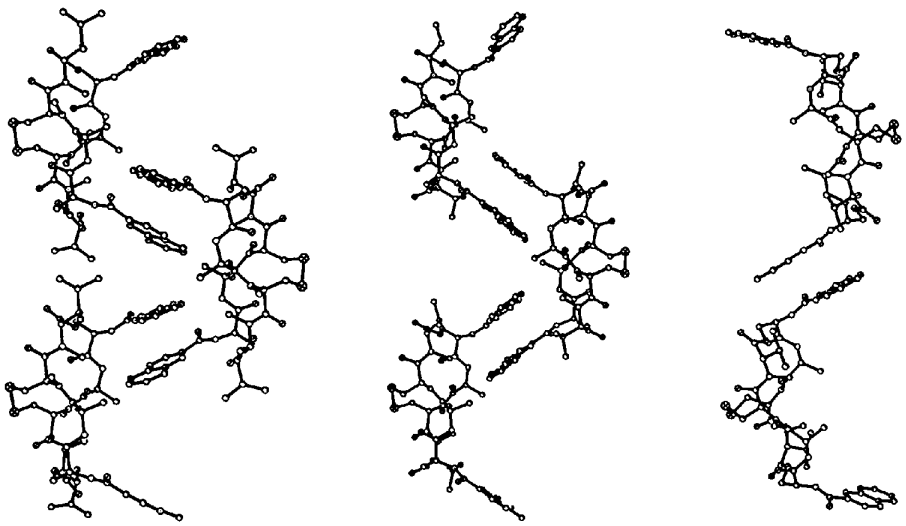


Fig. 6. Chromophore stacking motifs in (*a*) triostin C, (*b*) $P2_12_12_1$ form of triostin A and (*c*) $C222_1$ form of trisostin A.

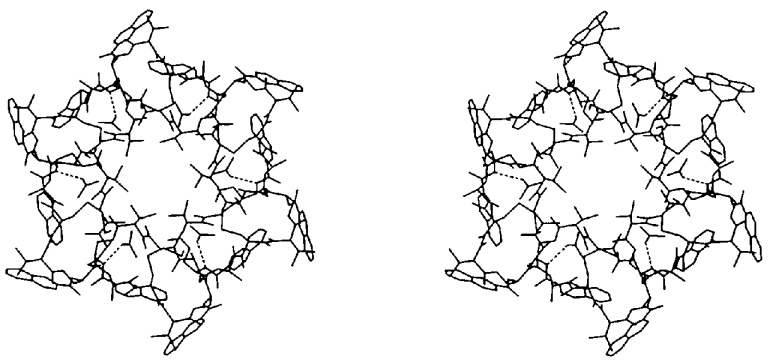


Fig. 7. Stereoview of the helical packing of echinomycin 2QN.

too detailed an interpretation. The resolution of the hexanucleotide complex is better (1.67 Å) and the 26 ring torsion angles are rather close to those in triostin C (mean deviation 15.5°); even the anomalous orientation of the ester plane on only one side of the molecule is observed, although the rotation is smaller. Fig. 9 shows a least-squares fit of the atoms in the depsipeptide ring for triostin C and the hexanucleotide complex; the inversion of the disulfide bridge chirality should be noted. The displacements of the chromophores are caused by relatively small rotations about the bonds, linking them to the depsipeptide ring.

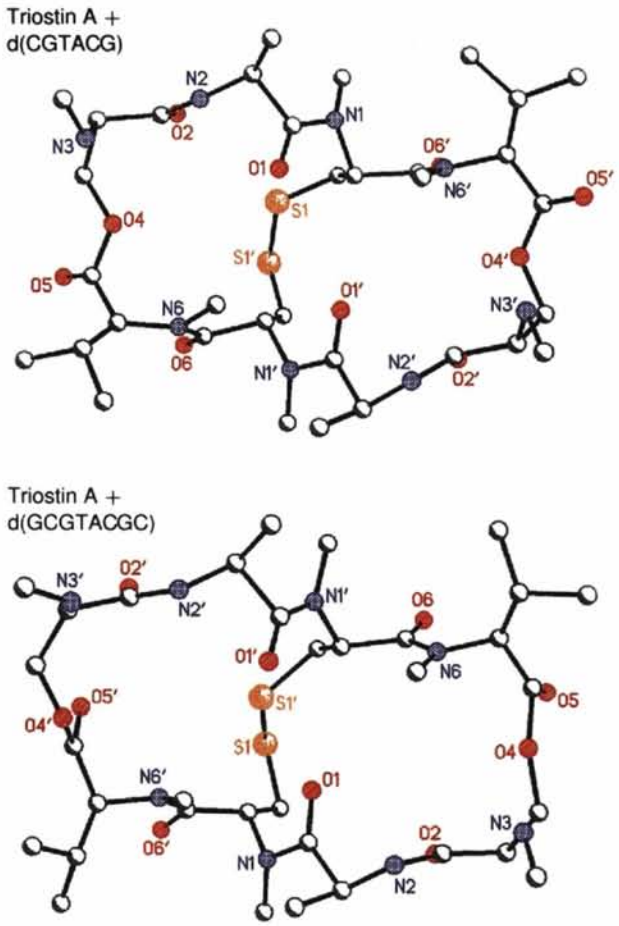


Fig. 8. The depsipeptide ring conformations in the oligonucleotide complexes of triostin A (drawn from the coordinates reported by Wang *et al.*, 1986).

The n and p conformers of triostin A

With the possible exception of triostin C, the *p* form of triostin would be expected to dominate under the crystallization conditions employed in this study, and there is every indication that the four uncomplexed antibiotic structures, and the two complexes described by Wang *et al.* (1986), do indeed correspond closely to the *p* form. It is tempting to deduce that since the solvent used for triostin C was the least polar, the rather asymmetrical structure reported here for triostin C represents an intermediate stage between the *n* and *p* forms; one side of the molecule is stabilized by an internal hydrogen bond which would be favoured in a non-polar solvent. On the other hand, the NMR data of Kalman *et al.* (1979) provide no evidence of a (serine)N(3)—H(3)···O5(ester) hydrogen bond, but they did indicate the presence of an internal (alanine)N(2)—H(2*A*)···O(3)(quinoxaline) hydrogen bond in the *n* form. The conformation of the triostin A molecule in the d(CGTACG) complex is remarkably similar to that of triostin C (see above), and only the *p* form is believed to form such complexes. The crystal structures also indicate that the ester linkage is relatively free to twist, so a conformational change involving only one hydrogen bond on each side of the molecule might not suffice to account for the observed slowness of interconversion and height of the energy barrier, 20–22 kcal mol^{-1} [84–92 kJ mol^{-1} (Kawano *et al.*, 1977; Blake *et al.*, 1977)].

Kalman *et al.* (1979) also considered the possibility that the *n*–*p* conversion might be associated with an inversion of the chirality of the disulfide bridge. However, Higuchi, Kyogoku, Shin & Inouye (1983) found that the di-*S*-benzyl analogue of triostin A, which

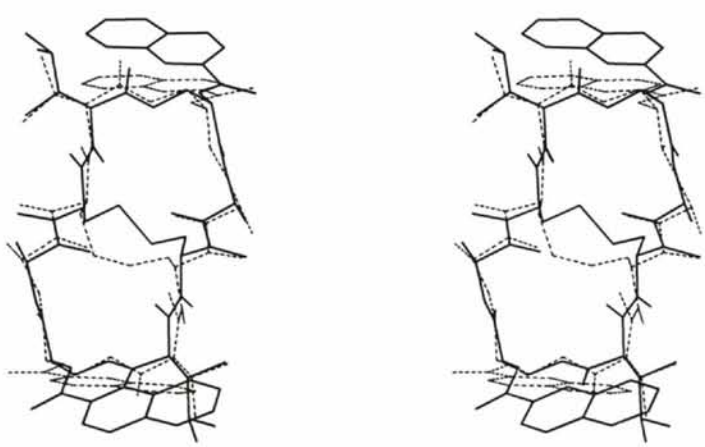


Fig. 9. Stereoview of a least-squares fit of the depsipeptide ring atoms of triostin C (solid) to the corresponding atoms of triostin A (dotted) in its d(CGTACG) complex.

does not possess a disulfide bridge, also shows *n* and *p* isomers. Since the inversion of the chirality of the disulfide bridge on complex formation can occur without much alteration of the ring torsion angles [witness the similarity between the d(CGTACG) complex of triostin A and triostin C], it is not possible to make any conclusions regarding the chirality of the disulfide bridge in the *n* form. Only a crystal structure determination can resolve this question; however, we have never succeeded in obtaining crystals of the *n* form of triostin A. An N(2)—H(2*A*)···O(3) hydrogen bond would fix the chromophore conformation, making it more difficult to stack the aromatic systems in the crystal, and would also prevent stabilization of the structure by intermolecular hydrogen bonds involving the alanine NH (as in all four structures reported here). This might conceivably explain the difficulties in obtaining crystals of the *n* form.

Implications for the molecular recognition of DNA

The striking uniformity of conformation observed among the antibiotics, which is substantially preserved in the oligonucleotide complexes, reinforces the belief that in this instance the complementarity between drug and receptor is dominated by the structure of the ligand. The cost of binding in terms of conformational adjustments is a decidedly one-sided affair; apart from the reversed chirality of the disulfide bridge in the triostins, which seems to be of little consequence, practically all the changes required to optimize steric complementarity are on the part of the nucleic acid 'receptor', which must mould itself to fit its rather rigid peptide guest (Wang *et al.*, 1986; Waring, 1990). The formation of Hoogsteen base pairs in the oligonucleotide complexes (Wang *et al.*, 1984; Quigley *et al.*, 1986) represents a major change. Efforts to detect altered base pairing in echinomycin–DNA complexes using chemical probes have been to no avail (McLean & Waring, 1988; Sayers & Waring, 1993), but Gao & Patel (1988) found NMR evidence for Hoogsteen pairing in the echinomycin–d(ACGT) complex in aqueous solution through only normal (Watson–Crick) pairing in the other echinomycin complexes they studied. In addition, the bases that interact on either side of the antibiotic are forced apart, and the helix is distorted and unwound.

Molecular recognition itself, *i.e.* the interplay of forces which drive the antibiotics to bind preferentially to sequences containing the dinucleotide step CpG, has long been attributed to hydrogen bonding between the alanine residues of the peptide ring and guanine-specific substituents in the minor groove of DNA, particularly the exocyclic 2-amino group (Waring, 1979, 1993). Indeed, shifting the purine 2-amino group onto AT base pairs alters the echinomycin binding sites, as predicted (Bailly, Marchand & Waring, 1993). Hydrogen bonds are evident in the antibiotic–oligonucleotide crystal structures (Ughetto *et al.*, 1985; Wang *et al.*, 1986) and have been detected by NMR (Gao & Patel, 1988; Addess, Gilbert & Feigon, 1991). They involve the same alanine NH groups which we find hydrogen bonded to various acceptors, together with one (but not both) of the alanine carbonyl oxygens. These substituents are positioned very similarly in all four structures reported here and lie in a diagonal array across the face of the peptide ring, which must be closely apposed to the GC base pairs in the DNA complex. As such they are ideally placed to serve as the critical determinants of sequence recognition. Indeed, the propensity of the alanine NH group to engage in hydrogen bonding has already been noted, and in one case (that of the unusual half of triostin C) it even engages in bifurcated hydrogen bonding to two different acceptors. By contrast, in TANDEM and its analogues the alanine carbonyl O atoms are located differently, being engaged in internal hydrogen bonding to the valine NH groups (Viswamitra *et al.*, 1981; Hossain *et al.*, 1982; Addess *et al.*, 1991). Consequently, the main GC-specific recognition element is missing and the ligand binds to TpA steps by default (Lee & Waring, 1978*b*; Fox *et al.*, 1982). Replacement of the D-serine by L-serine would position the chromophores on the wrong side of the depsipeptide ring for binding, and it has been shown that the bis L-serine analogue of TANDEM interacts only very weakly (if at all) with DNA (Lee & Waring, 1978*b*).

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References

ADDESS, K. J., GILBERT, D. E. & FEIGON, J. (1991). *Structure and Function: Proceedings of the Seventh Conversation in Biomolecular Stereodynamics*, edited by R. H. SARMA AND M. H. SARMA. Vol. 1, pp. 147–164. New York: Adenine Press.

ALFREDSON, T. V., MAKI, A. H., ADASKAVEG, M. E., ENCOFFER, J. L. & WARING, M. J. (1990). *J. Chromatogr.* **507**, 277–292.

BAILLY, C., MARCHAND, C. & WARING, M. J. (1993). *J. Am. Chem. Soc.* **115**, 3784–3785.

BLAKE, T. J., KALMAN, J. R. & WILLIAMS, D. H. (1977). *Tetrahedron Lett.* **30**, 2621–2624.

CLEGG, W. (1981). *Acta Cryst.* A**37**, 22–28.

DELL, A., WILLIAMS, D. H., MORRIS, H. R., SMITH, G. A., FEENEY, J. & ROBERTS, G. C. K. (1975). *J. Am. Chem. Soc.* **97**, 2497–2502.

FLACK, H. D. (1983). *Acta Cryst.* A**39**, 876–881.

FOX, K. R., CORNISH, A., WILLIAMS, R. C. & WARING, M. J. (1983). *Biochem. J.* **211**, 543–551.

FOX, K. R., GAUVREAU, D., GOODWIN, D. C. & WARING, M. J. (1980). *Biochem. J.* **191**, 729–742.

Fox, K. R., Olsen, R. K. & Waring, M. J. (1982). *Biochim. Biophys. Acta*, **696**, 315–322.
Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H. & Waring, M. J. (1981). *The Molecular Basis of Antibiotic Action*, 2nd ed. Wiley: London.
Gao, X. & Patel, D. J. (1988). *Biochemistry*, **27**, 1744–1751.
Higuchi, N., Kyogoku, Y., Shin, M. & Inouye, K. (1983). *Int. J. Protein Res.* **21**, 541–545.
Hossain, M. B., van der Helm, D., Olsen, R. K., Jones, P. G., Sheldrick, G. M., Egert, E., Kennard, O., Waring, M. J. & Viswamitra, M. A. (1982). *J. Am. Chem. Soc.* **104**, 3401–3408.
Kalman, J. R., Blake, T. J., Williams, D. H., Feeney, J. & Roberts, G. C. K. (1979). *J. Chem. Soc. Perkin Trans.* 1, pp. 1313–1321.
Katagiri, K., Yoshida, T. & Sato, K. (1975). *Antibiotics, Mechanism of Action of Antimicrobial and Antitumour Agents*, edited by J. W. Corcoran & F. E. Hahn, Vol. I, pp. 234–251. Berlin, Heidelberg, New York: Springer.
Kawano, K., Higuchi, N. & Kyogoku, Y. (1977). *Peptide Chemistry* 1976, edited by A. Nakajima, English ed., pp. 93–96. Peptide Research Foundation.
Keller-Schierlein, W., Mihailovic, M. Lj. & Prelog, V. (1959). *Helv. Chim. Acta*, **42**, 305–322.
Kyogoku, Y., Higuchi, N., Watanabe, M. & Kawano, K. (1981). *Biopolymers*, **20**, 1959–1970.
Kyogoku, Y., Yu, B. S., Akutsu, H., Watanabe, M. & Kawano, K. (1978). *Biochem. Biophys. Res. Commun.* **83**, 172–179.
Lee, J. S. & Waring, M. J. (1978*a*). *Biochem. J.* **173**, 115–128.
Lee, J. S. & Waring, M. J. (1978*b*). *Biochem. J.* **173**, 129–144.
LePage, Y. (1987). *J. Appl. Cryst.* **20**, 264–269.
Low, C. M. L., Fox, K. R. & Waring, M. J. (1986). *Anti-Cancer Drug Des.* **1**, 149–160.
Low, C. M. L., Olsen, R. K. & Waring, M. J. (1984). *FEBS Lett.* **176**, 414–420.
Martin, D. G., Mizsak, S. A., Biles, C., Stewart, J. C., Baczynski, L. & Meulman, P. A. (1975). *J. Antibiot.* **28**, 332–336.
McLean, M. J. & Waring, M. J. (1988). *J. Mol. Recognit.* **1**, 138–151.
Muss, H. B., Blessing, J. A., Baker, V. V., Barnhill, D. R. & Adelson, M. D. (1990). *Am. J. Clin. Oncol.* **13**, 299–301.
Muss, H. B., Blessing, J. A. & Malfetano, J. (1990). *Am. J. Clin. Oncol.* **13**, 191–193.
Olsen, R. K. (1983). *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, edited by B. Weinstein, Vol. 7, pp. 1–33. New York: Marcel Dekker.
Quigley, G. J., Ughetto, G., van der Marel, G. A., van Boom, J. H., Wang, A. H. J. & Rich, A. (1986). *Science*, **232**, 1255–1258.
Sayers, E. W. & Waring, M. J. (1993). *Biochemistry*, **32**, 9094–9107.
Sheldrick, G. M. (1990). *Acta Cryst.* A**46**, 467–473.
Sheldrick, G. M. (1995). *J. Appl. Cryst.* In preparation.
Sheldrick, G. M., Guy, J. J., Kennard, O., Rivera, V. & Waring, J. (1984). *J. Chem. Soc. Perkin Trans.* 2, pp. 1601–1605.
Ughetto, G., Wang, A. H. J., Quigley, G. J., van der Marel, G. A., van Boom, J. H. & Rich, A. (1985). *Nucleic Acids Res.* **13**, 2305–2323.
Viswamitra, M. A., Kennard, O., Cruse, W. B. T., Egert, E., Sheldrick, G. M., Jones, P. G., Waring, M. J., Wakelin, L. P. G. & Olsen, R. K. (1981). *Nature (London)*, **289**, 817–819.
Wakelin, L. P. G. (1986). *Med. Res. Rev.* **6**, 275–340.
Wakelin, L. P. G. & Waring, M. J. (1990). *Comprehensive Medicinal Chemistry*, edited by P. G. Sammes, Vol. II, pp. 703–724. Oxford: Pergamon Press.
Wang, A. H. J., Ughetto, G., Quigley, G. J., Hakoshima, T., van der Marel, G. A., van Boom, J. H. & Rich, A. (1984). *Science*, **225**, 1115–1121.
Wang, A. H. J., Ughetto, G., Quigley, G. J. & Rich, A. (1986). *J. Biomol. Struct. Dyn.* **4**, 319–342.
Waring, M. J. (1979). *Antibiotics*, Vol. V, Part 2, *Mechanism of Action of Antieukaryotic and Antiviral Compounds*, edited by F. E. Hahn, pp. 173–194. Heidelberg: Springer.
Waring, M. J. (1990). *Molecular Basis of Specificity in Nucleic Acid–Drug Interactions*, 23rd Jerusalem Symposium on Quantum Chemistry and Biochemistry, edited by B. Pullman & J. Jortner, pp. 225–245. Dordrecht: Kluwer Academic Publishers.
Waring, M. J. (1993). *Molecular Aspects of Anticancer Drug–DNA Interactions*, edited by S. Neidle & M. J. Waring, Vol. 1, pp. 213–242. London: Macmillan.
Waring, M. J. & Wakelin, L. P. G. (1974). *Nature (London)*, **252**, 653–657.

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Different Crystal Modifications of 3a*β*,4*α*-Dihydro-4*β*,10-dimethyl-2-phenyl-1*H*,3*H*,5*H*-pyrrolo[3,4-*b*]carbazol-1,3-dione – Crystal Data and Theoretical Calculations

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Abstract

Two crystal modifications of $C_{22}H_{18}N_2O_2$ were investigated. Crystal data: $M_r = 342.34$, $\lambda(Cu K\alpha) = 1.5418$ Å, $T = 298$ K; crystal (I): monoclinic, $C2/c$, $a = 14.9853$ (5), $b = 10.1607$ (5), $c = 23.729$ (2) Å, $\beta = 108.680$ (4)°, $V = 3422.7$ (3) Å^3, $Z = 8$, $D_x = 1.333$ g cm^{-3}, $\mu = 6.04$ cm^{-1}, $F(000) = 1440.0$, $R = 0.069$, $wR = 0.081$ for 2628 observed unique reflections; crystal (II): monoclinic, $C2/c$, $a = 14.977$ (9), $b = 10.177$ (1), $c = 23.663$ (2) Å, $\beta = 108.17$ (2)°, $V = 3426$ (2) Å^3, $Z = 8$, $D_x = 1.333$ g cm^{-3}, $\mu = 6.04$ cm^{-1}, $F(000) = 1440.0$, $R = 0.076$, $wR = 0.084$ for 1984 observed unique

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