Crystal and Molecular Structure of the DNA-binding Antitumour Antibiotic Triostin A

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Crystals of the polar conformer of the quinoxaline antibiotic triostin A are orthorhombic, space group $P2_12_12_1$, with a = 20.940(5), b = 18.528(4), and c = 18.795(4) Å. There are two molecules of isoamyl acetate solvent per triostin A molecule. The cyclic octadepsipeptide has approximate two-fold symmetry. The quinoxaline chromophores and the disulphide cross-bridge project from opposite sides of the peptide ring. The conformation is compared with that found for the synthetic analogue TANDEM, and with the results of n.m.r. investigation. The observed structure accounts for the higher binding constants of the natural antibiotic, as well as its capacity to bind at GC-rich sequences in DNAs.

Triostin A is a naturally occurring quinoxaline antibiotic[1,2] which is active against Gram-positive bacteria[2] and certain animal tumours.[3] It has been shown to bind strongly to DNA[4,5] and thereby function as a potent inhibitor of RNA synthesis.[6] It is believed to intercalate bifunctionally between the base-pairs in the minor groove of double-helical DNA.[7,8] N.m.r. investigations have shown[9,10] that triostin A exists in solution as a mixture of two conformations separated by an energy barrier of *ca.* 92 kJ mol^{-1}. We have grown crystals from isoamyl acetate of the conformer favoured in polar solvents (triostin p) and report here the *X*-ray structure determination, the first of a naturally occurring quinoxaline antibiotic. We had previously determined[11] the structure of the related synthetic cyclic octadepsipeptide TANDEM, which differs from triostin A by the absence of *N*-methyl groups on the L-Cys and L-Val residues.

Experimental

Needle-shaped crystals were grown by slow evaporation of isoamyl acetate and sealed in Lindemann-glass capillaries to prevent solvent loss. 2 928 Unique date were measured for $2\theta < 85°$ with graphite-monochromated Cu-K_α radiation on a Syntex $P2_1$ four-circle diffractometer, of which 2 450 with $F > 2.5\sigma(F)$ were used for all calculations. After exhaustive investigation using all available direct and Patterson methods over a period of eight years had proved fruitless, the structure was solved at the first attempt by a new multiple random start single-solution program (SHELX-84). The *E*-map revealed about two-thirds of the structure, including both sulphur atoms. Successive difference electron density syntheses located the remaining non-hydrogen atoms, including two isoamyl acetate solvent molecules, one of which was ordered, the other disordered with two superimposed conformations. In contrast to the high degree of hydration observed in the structure of TANDEM, there was no evidence for the presence of solvent water molecules. Because of the relatively low resolution of the data, only the two sulphur atoms could be refined anisotropically. To minimise the number of parameters for solvent refinement, the same six common overall anisotropic thermal motion parameters were employed for all solvent atoms. The CH, CH_2, and amide hydrogens were included using a riding model with C–H 0.96 Å, U(H) = 1.2U(C), N–H 0.90 Å, and U(H) = 1.2U(N). The refinement converged to R = 0.150, $R_w = \Sigma w^{\frac{1}{2}}|\Delta|/\Sigma w^{\frac{1}{2}}|F_o|$ = 0.141 with the weighting scheme $w^{-1} = \sigma^2(F) + 0.006F^2$ for 412 least-squares parameters. The bond lengths in the solvent molecules were restrained to standard values by the method of additional observational equations.[12] Final co-ordinates are given in Table 1, bond lengths and angles in Table 2. Hydrogen and solvent atom co-ordinates and structure factors are in Supplementary Publication No. SUP 50027 (18 pp.).† All programs used were written by G. M. S.

Crystal Data.—$C_{50}H_{56}N_{12}O_{12}S_2 \cdot 2C_7H_{14}O_2$, M = 1 341.6. Orthorhombic, space group $P2_12_12_1$, a = 20.940(5), b = 18.528(4), c = 18.795(4) Å, U = 7 292.0 Å^3, Z = 4, F(000) = 2 848, D_c = 1.222 Mg m^{-3}, λ(Cu-K_α) = 1.5418 Å, μ = 12.1 cm^{-1}.

Results and Discussion

The triostin A molecule (Figure 1) exhibits approximate two-fold symmetry, only the quinoxaline residues deviating appreciably: the root mean square difference in ring torsion angles for the two halves of the molecule is 8.6°. The depsipeptide ring takes the form of a slightly twisted disk; the mean deviation of the 26 ring atoms from their mean plane is 0.7 Å, with a maximum deviation of 1.4 Å. Similar values were found[11] in TANDEM. However, the extra *N*-methyl groups in triostin A prevent the formation of the two intramolecular hydrogen bonds (between the valyl amides and alanyl carbonyls) observed in TANDEM, with the result that the triostin A ring is slightly wider [C(2)···C(2′) 4.14 Å, *cf.* 3.8 and 3.9 Å in TANDEM] but less elongated [C(7)···C(7′) 11.34 Å, *cf.* 11.9, 12.2 Å]. As predicted[10] from n.m.r. data and also observed in TANDEM, the ester and amide linkages are all *trans*, and the quinoxaline rings lie in the corresponding serine peptide planes.

The conformation of the disulphide bridge is different in triostin A and TANDEM, with opposite chiralities and rotations of *ca.* 120° about the C–C bonds. There are also significant differences in the torsion angles in the Ser-Ala fragment. The major such differences between triostin A and TANDEM are summarised in Table 3, which also includes the torsion angles for which n.m.r. predictions could be made. The n.m.r. values are in error by 40° or more for the torsion angles about the Ser C_α–N bonds and the Cys C_α–C_β bonds. The

† For details of Supplementary Publications see Instructions for Authors in *J. Chem. Soc., Perkin Trans. 2*, 1984, Issue 1.

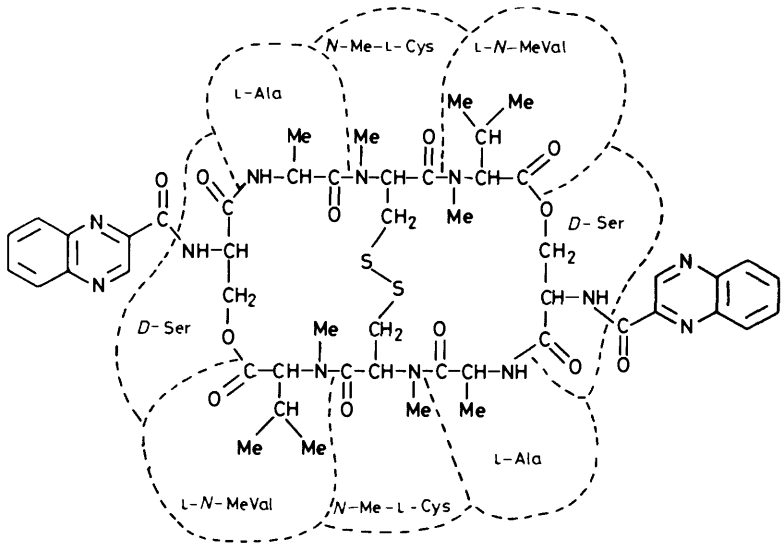

Table 1. Atomic co-ordinates ($\times 10^4$) and isotropic thermal parameters (Å^2 $\times 10^3$) with estimated standard deviations in parentheses

Atom	*x*	*y*	*z*	*U*
S	3 669(4)	4 140(4)	1 641(4)	132(4)*
C(1)	3 510(11)	5 079(12)	1 436(13)	111(9)
C(2)	3 796(9)	5 585(11)	1 957(11)	73(7)
N(1)	3 601(8)	6 322(9)	1 806(9)	136(6)
C(24)	3 909(12)	6 659(14)	1 106(13)	120(9)
C(3)	3 111(11)	6 600(13)	2 134(13)	96(8)
O(1)	2 888(7)	6 302(8)	2 677(8)	67(5)
C(4)	2 878(10)	7 363(11)	1 935(12)	96(8)
C(5)	3 258(13)	7 965(14)	2 293(15)	138(10)
N(2)	2 190(8)	7 352(9)	2 025(9)	147(7)
C(6)	1 779(11)	6 872(12)	1 734(14)	96(8)
O(2)	2 000(7)	6 519(8)	1 255(8)	66(5)
C(7)	1 091(9)	6 955(10)	1 913(10)	66(6)
N(3)	930(7)	7 241(8)	2 648(8)	111(5)
C(8)	554(11)	7 837(12)	2 761(12)	98(8)
O(3)	480(8)	8 316(10)	2 285(9)	100(6)
C(9)	297(11)	7 829(13)	3 473(13)	111(9)
C(10)	−51(11)	8 430(13)	3 629(13)	110(9)
N(4)	−348(8)	8 453(9)	4 323(9)	133(6)
C(11)	−296(11)	7 892(12)	4 768(12)	100(8)
C(12)	−612(13)	7 881(15)	5 445(14)	145(11)
C(13)	−482(12)	7 329(13)	5 866(14)	125(10)
C(14)	−90(11)	6 691(13)	5 708(13)	115(9)
C(15)	220(12)	6 759(14)	5 043(14)	126(10)
C(16)	134(10)	7 351(11)	4 618(11)	76(7)
N(5)	418(8)	7 313(9)	3 927(8)	122(6)
C(17)	787(10)	6 261(11)	1 843(11)	89(7)
O(4)	954(7)	5 796(7)	2 467(7)	54(4)
C(18)	725(11)	5 152(11)	2 517(11)	93(8)
O(5)	498(8)	4 858(10)	2 015(10)	104(6)
C(19)	898(8)	4 790(10)	3 198(9)	63(6)
C(20)	428(11)	5 068(14)	3 771(13)	121(9)
C(21)	570(11)	4 584(13)	4 502(13)	113(9)
C(22)	−291(14)	4 896(17)	3 514(16)	166(12)
N(6)	1 545(7)	4 897(8)	3 390(8)	122(6)
C(25)	1 819(10)	5 595(11)	3 677(11)	82(7)
C(23)	1 968(10)	4 314(11)	3 325(11)	75(7)
O(6)	1 753(7)	3 701(8)	3 075(8)	73(5)
S′	2 887(3)	3 754(4)	2 121(3)	119(3)*
C(1′)	3 080(10)	3 871(11)	3 040(11)	94(8)
C(2′)	2 678(8)	4 459(10)	3 394(9)	52(6)
N(1′)	2 807(7)	4 428(8)	4 191(8)	107(5)
C(24′)	2 582(11)	3 817(12)	4 650(12)	102(8)
C(3′)	3 296(10)	4 890(11)	4 347(11)	84(7)
O(1′)	3 486(6)	5 403(7)	4 028(7)	48(4)
C(4′)	3 535(10)	4 784(12)	5 185(11)	87(7)
C(5′)	3 281(9)	5 401(11)	5 642(11)	76(7)
N(2′)	4 247(8)	4 914(9)	5 114(9)	122(6)
C(6′)	4 593(12)	4 382(15)	4 694(14)	127(10)
O(2′)	4 296(8)	3 832(10)	4 471(9)	98(6)
C(7′)	5 319(10)	4 443(13)	4 753(12)	102(8)
N(3′)	5 572(8)	5 153(10)	5 006(9)	147(7)
C(8′)	5 879(12)	5 171(13)	5 698(13)	112(9)
O(3′)	5 898(8)	4 653(9)	6 091(9)	89(6)
C(9′)	6 252(11)	5 819(13)	5 706(13)	102(8)
C(10′)	6 520(13)	6 030(15)	6 356(16)	150(11)
N(4′)	6 868(8)	6 594(10)	6 474(10)	136(6)
C(11′)	7 000(12)	7 033(14)	5 871(13)	101(8)
C(12′)	7 378(11)	7 624(12)	5 954(12)	95(8)
C(13′)	7 528(11)	8 167(13)	5 392(12)	109(9)
C(14′)	7 226(11)	7 914(12)	4 738(13)	104(8)
C(15′)	6 842(11)	7 335(12)	4 634(12)	100(8)
C(16′)	6 703(9)	6 892(10)	5 254(10)	62(6)
N(5′)	6 334(8)	6 310(9)	5 199(8)	125(6)
C(17′)	5 566(12)	4 313(14)	4 021(12)	125(10)
O(4′)	5 445(8)	4 889(9)	3 560(8)	80(5)
C(18′)	5 694(20)	5 035(24)	2 953(23)	229(17)
O(5′)	5 924(18)	4 370(24)	2 735(21)	282(18)
C(19′)	5 607(13)	5 603(15)	2 559(15)	156(11)
C(20′)	5 976(14)	6 367(17)	2 704(17)	182(13)
C(21′)	5 824(21)	6 916(24)	2 296(26)	261(20)
C(22′)	6 559(23)	6 048(26)	2 866(27)	297(23)
N(6′)	4 887(9)	5 723(10)	2 536(10)	154(7)
C(25′)	4 565(11)	6 161(13)	3 139(12)	109(9)
C(23′)	4 519(13)	5 522(16)	1 863(15)	151(11)
O(6′)	4 790(8)	5 203(9)	1 423(9)	96(6)

* Equivalent isotropic *U* defined as one third of the trace of the orthogonalised U_{ij} tensor.

crystal structure provides no support for the suggestion[10] (in order to account for the Cys $J_{\alpha,\beta}$ values) of a mixture of rapidly interconverting conformations of the Cys-Cys cross-linkage, or for the presence of unsymmetrical conformers. Nevertheless, the overall conformation deduced from the n.m.r. data is essentially correct, and there seems no reason to doubt the deduction that the main difference between triostin n (the conformation in non-polar solvents) and triostin p is an intramolecular hydrogen bond between the quinoxaline carbonyl and the Ala NH in the former, possibly associated with a reversal of the chirality of the disulphide bridge. There are

Table 2. Bond distances (Å) and angles (°)

(a) Distances

S–C(1)	1.81(2)	S–S′	2.00(1)
C(1)–C(2)	1.48(3)	C(2)–N(1)	1.45(3)
C(2)–C(23′)	1.53(3)	N(1)–C(24)	1.59(3)
N(1)–C(3)	1.30(3)	C(3)–O(1)	1.25(3)
C(3)–C(4)	1.54(3)	C(4)–C(5)	1.53(3)
C(4)–N(2)	1.45(3)	N(2)–C(6)	1.35(3)
C(6)–O(2)	1.20(3)	C(6)–C(7)	1.49(3)
C(7)–N(3)	1.52(2)	C(7)–C(17)	1.44(3)
N(3)–C(8)	1.37(3)	C(8)–O(3)	1.27(3)
C(8)–C(9)	1.44(3)	C(9)–C(10)	1.36(3)
C(9)–N(5)	1.31(3)	C(10)–N(4)	1.45(3)
N(4)–C(11)	1.34(3)	C(11)–C(12)	1.44(4)
C(11)–C(16)	1.38(3)	C(12)–C(13)	1.32(4)
C(13)–C(14)	1.47(4)	C(14)–C(15)	1.41(4)
C(15)–C(16)	1.37(3)	C(16)–N(5)	1.43(3)
C(17)–O(4)	1.50(3)	O(4)–C(18)	1.29(2)
C(18)–O(5)	1.19(3)	C(18)–C(19)	1.49(3)
C(19)–C(20)	1.55(3)	C(19)–N(6)	1.42(2)
C(20)–C(21)	1.67(4)	C(20)–C(22)	1.61(4)
N(6)–C(25)	1.51(3)	N(6)–C(23)	1.40(2)
C(23)–O(6)	1.31(2)	C(23)–C(2′)	1.52(3)
S′–C(1′)	1.79(2)	C(1′)–C(2′)	1.53(3)
C(2′)–N(1′)	1.52(2)	N(1′)–C(24′)	1.50(3)
N(1′)–C(3′)	1.37(2)	C(3′)–O(1′)	1.19(3)
C(3′)–C(4′)	1.66(3)	C(4′)–C(5′)	1.53(3)
C(4′)–N(2′)	1.52(3)	N(2′)–C(6′)	1.46(3)
C(6′)–O(2′)	1.27(3)	C(6′)–C(7′)	1.53(3)
C(7′)–N(3′)	1.50(3)	C(7′)–C(17′)	1.49(3)
N(3′)–C(8′)	1.45(3)	C(8′)–O(3′)	1.21(3)
C(8′)–C(9′)	1.43(3)	C(9′)–C(10′)	1.40(4)
C(9′)–N(5′)	1.33(3)	C(10′)–N(4′)	1.29(3)
N(4′)–C(11′)	1.42(3)	C(11′)–C(12′)	1.36(3)
C(11′)–C(16′)	1.34(3)	C(12′)–C(13′)	1.49(3)
C(13′)–C(14′)	1.46(3)	C(14′)–C(15′)	1.35(3)
C(15′)–C(16′)	1.45(3)	C(16′)–N(5′)	1.33(2)
C(17′)–O(4′)	1.40(3)	O(4′)–C(18′)	1.28(5)
C(18′)–O(5′)	1.38(6)	C(18′)–C(19′)	1.30(5)
C(19′)–C(20′)	1.64(4)	C(19′)–N(6′)	1.52(3)
C(20′)–C(21′)	1.31(6)	C(20′)–C(22′)	1.39(6)
N(6′)–C(25′)	1.55(3)	N(6′)–C(23′)	1.53(3)
C(23′)–O(6′)	1.16(3)		

(b) Angles

C(1)–S–S′	106.8(9)	S–C(1)–C(2)	113.1(16)
C(1)–C(2)–N(1)	110.5(17)	C(1)–C(2)–C(23′)	106.1(18)
N(1)–C(2)–C(23′)	109.1(18)	C(2)–N(1)–C(24)	114.6(16)
C(2)–N(1)–C(3)	120.0(18)	C(24)–N(1)–C(3)	123.7(18)
N(1)–C(3)–O(1)	120.3(21)	N(1)–C(3)–C(4)	119.8(20)
O(1)–C(3)–C(4)	119.1(20)	C(3)–C(4)–C(5)	113.5(19)
C(3)–C(4)–N(2)	105.8(17)	C(5)–C(4)–N(2)	118.4(19)
C(4)–N(2)–C(6)	126.4(18)	N(2)–C(6)–O(2)	114.5(20)
N(2)–C(6)–C(7)	117.1(20)	O(2)–C(6)–C(7)	126.7(21)
C(6)–C(7)–N(3)	117.2(16)	C(6)–C(7)–C(17)	108.3(17)
N(3)–C(7)–C(17)	107.3(15)	C(7)–N(3)–C(8)	123.3(16)
N(3)–C(8)–O(3)	121.5(20)	N(3)–C(8)–C(9)	110.5(19)
O(3)–C(8)–C(9)	128.0(21)	C(8)–C(9)–C(10)	113.0(21)
C(8)–C(9)–N(5)	122.7(21)	C(10)–C(9)–N(5)	124.2(22)
C(9)–C(10)–N(4)	116.7(21)	C(10)–N(4)–C(11)	120.3(18)
N(4)–C(11)–C(12)	121.8(21)	N(4)–C(11)–C(16)	119.5(20)
C(12)–C(11)–C(16)	118.2(21)	C(11)–C(12)–C(13)	116.6(25)
C(12)–C(13)–C(14)	128.1(25)	C(13)–C(14)–C(15)	111.3(22)
C(14)–C(15)–C(16)	121.8(23)	C(11)–C(16)–C(15)	123.4(21)
C(11)–C(16)–N(5)	119.6(18)	C(15)–C(16)–N(5)	115.8(19)
C(9)–N(5)–C(16)	118.4(18)	C(7)–C(17)–O(4)	109.8(16)
C(17)–O(4)–C(18)	120.2(16)	O(4)–C(18)–O(5)	120.9(20)
O(4)–C(18)–C(19)	112.9(18)	O(5)–C(18)–C(19)	125.0(20)
C(18)–C(19)–C(20)	107.2(17)	C(18)–C(19)–N(6)	112.9(16)
C(20)–C(19)–N(6)	112.6(16)	C(19)–C(20)–C(21)	106.4(18)
C(19)–C(20)–C(22)	108.5(19)	C(21)–C(20)–C(22)	107.9(19)
C(19)–N(6)–C(25)	124.9(25)	C(19)–N(6)–C(23)	118.3(16)
C(25)–N(6)–C(23)	116.7(15)	N(6)–C(23)–O(6)	118.8(17)
N(6)–C(23)–C(2′)	118.4(16)	O(6)–C(23)–C(2′)	121.4(17)
S–S′–C(1′)	102.0(8)	S′–C(1′)–C(2′)	112.5(14)
C(23)–C(2′)–C(1′)	112.1(16)	C(23)–C(2′)–N(1′)	104.5(14)
C(1′)–C(2′)–N(1′)	107.7(14)	C(2′)–N(1′)–C(24′)	122.6(15)
C(2′)–N(1′)–C(3′)	108.7(15)	C(24′)–N(1′)–C(3′)	125.8(16)
N(1′)–C(3′)–O(1′)	129.9(19)	N(1′)–C(3′)–C(4′)	110.7(16)
O(1′)–C(3′)–C(4′)	118.1(18)	C(3′)–C(4′)–C(5′)	109.9(16)
C(3′)–C(4′)–N(2′)	101.1(15)	C(5′)–C(4′)–N(2′)	105.9(16)
C(4′)–N(2′)–C(6′)	115.4(17)	N(2′)–C(6′)–O(2′)	118.7(22)
N(2′)–C(6′)–C(7′)	113.9(20)	O(2′)–C(6′)–C(7′)	124.9(23)
C(6′)–C(7′)–N(3′)	116.2(19)	C(6′)–C(7′)–C(17′)	105.4(19)
N(3′)–C(7′)–C(17′)	108.2(18)	C(7′)–N(3′)–C(8′)	117.5(17)
N(3′)–C(8′)–O(3′)	122.8(21)	N(3′)–C(8′)–C(9′)	105.7(19)
O(3′)–C(8′)–C(9′)	129.8(23)	C(8′)–C(9′)–C(10′)	117.5(23)
C(8′)–C(9′)–N(5′)	129.5(22)	C(10′)–C(9′)–N(5′)	112.5(22)
C(9′)–C(10′)–N(4′)	127.0(26)	C(10′)–N(4′)–C(11′)	115.8(21)
N(4′)–C(11′)–C(12′)	118.9(21)	N(4′)–C(11′)–C(16′)	119.1(21)
C(12′)–C(11′)–C(16′)	121.7(23)	C(11′)–C(12′)–C(13′)	125.8(21)
C(12′)–C(13′)–C(14′)	106.8(19)	C(13′)–C(14′)–C(15′)	129.2(22)
C(14′)–C(15′)–C(16′)	116.7(20)	C(11′)–C(16′)–C(15′)	119.3(19)
C(11′)–C(16′)–N(5′)	119.7(19)	C(15′)–C(16′)–N(5′)	120.7(17)
C(9′)–N(5′)–C(16′)	125.0(18)	C(7′)–C(17′)–O(4′)	112.7(20)
C(17′)–O(4′)–C(18′)	129.6(25)	O(4′)–C(18′)–O(5′)	102.6(35)
O(4′)–C(18′)–C(19′)	128.2(37)	O(5′)–C(18′)–C(19′)	127.0(40)
C(18′)–C(19′)–C(20′)	122.7(30)	C(18′)–C(19′)–N(6′)	105.8(27)
C(20′)–C(19′)–N(6′)	110.2(21)	C(19′)–C(20′)–C(21′)	117.3(30)
C(19′)–C(20′)–C(22′)	94.8(29)	C(21′)–C(20′)–C(22′)	132.1(37)
C(19′)–N(6′)–C(25′)	119.1(19)	C(19′)–N(6′)–C(23′)	119.1(20)
C(25′)–N(6′)–C(23′)	120.9(18)	C(2)–C(23′)–N(6′)	112.7(20)
C(2′)–C(23′)–O(6′)	127.0(24)	N(6′)–C(23′)–O(6′)	117.9(23)

however only two weak hydrogen bonds in the triostin p crystals; both involve the Ala NH groups: N(2′)···N(4) (a quinoxaline ring N, obtained by the transformation: $0.5 + x$, $1.5 - y$, $1 - z$) 3.32 Å, and N(2)···B(7′) (a solvent carbonyl; $x - 0.5$, $1.5 - y$, $1 - z$) 3.00 Å.

The conformation of the antibiotic is in good accord with that predicted[4] on the basis of its known activity to bind to DNA by a process of bifunctional intercalation, *i.e.* simultaneous insertion of both its quinoxaline chromophores between the stacked base-pairs of the DNA helix. Even before the n.m.r. conformational studies and the elucidation of the TANDEM and triostin A crystal structures, the following criteria had been adduced[13] to define an acceptable model for a quinoxaline antibiotic structure which would fit the established facts about DNA binding: (a) the chromophores should lie on the same side of the peptide ring; (b) their planes should be approximately parallel; (c) the vertical distance between those planes should be an integral multiple (x) of 3.4 Å, in order to enclose ($x - 1$) base pairs; (d) the space between the chromophores should be essentially free from obstruction by any other substituents attached to the peptide ring. A space-filling drawing of triostin A is shown in Figure 2; the molecule takes the form of a cradle, with the quinoxaline chromophores, Ala methyls, and Val *N*-methyls projecting upwards from the edge. Granted that there is essentially free rotation about the Ser N–C_α bonds, it is possible to render the planes of the quinoxaline chromophores parallel and to adjust their separation to 10.2 Å, sufficient to accommodate a two-base-pair sandwich as required by the simple calculation above. Because the distance between the Ser α-C atoms is 11.34 Å, there is sufficient latitude in adopting the idealised 10.2 Å centre-to-centre spacing to allow for the helical twist of the DNA at the same time as providing good overlap between the van der Waals contours of the chromophores and base-pairs.

Triostin A binds well to all natural DNAs tested with a tight binding constant which is, if anything, slightly enhanced with

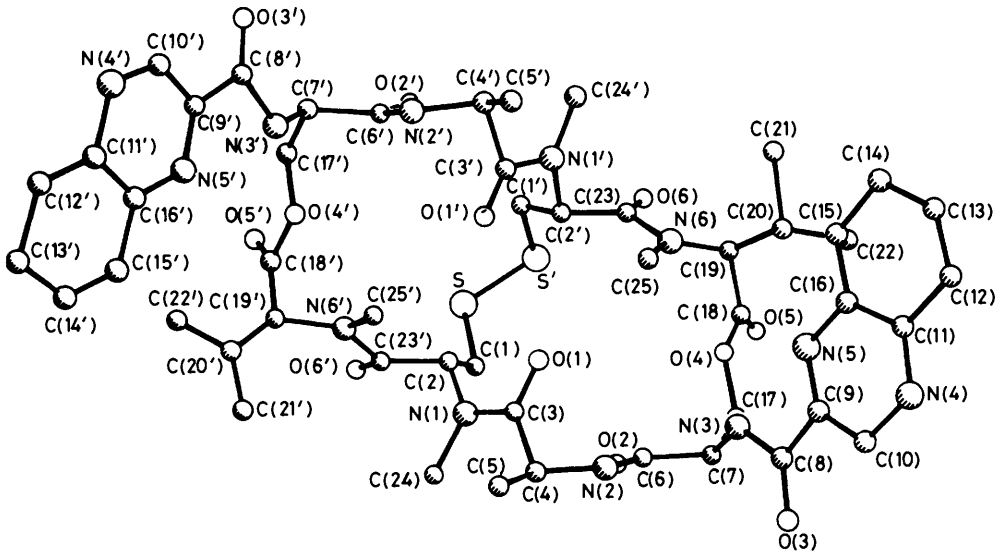

Figure 1. The triostin A molecule, showing the atom labelling

Table 3. Selected dihedral angles (°)

	TANDEM (mean *X*-ray)	Triostin A (*X*-ray)	Triostin A (n.m.r.)
C(23')–C(2)–N(1)–C(3) C(23)–C(2')–N(1')–C(3')	−96	−148.8(2.1) −148.7(1.6)	
C(3)–C(4)–N(2)–C(6) C(3')–C(4')–N(2')–C(6')	−86	−52.9(2.8) −65.6(2.1)	27, −164, 94, or −76
N(2)–C(6)–C(7)–N(3) N(2')–C(6')–C(7')–N(3')	1	−32.9(2.7) −20.2(2.9)	
C(6)–C(7)–C(17)–O(4) C(6')–C(7')–C(17')–O(4')	62	76.0(2.0) 72.2(2.5)	40 or 80
C(6)–C(7)–N(3)–C(8) C(6')–C(7')–N(3')–C(8')	122	124.7(2.0) 112.5(2.2)	75 or 165
C(18)–C(19)–N(6)–C(23) C(18')–C(19')–N(6')–C(23')	−99	−107.2(2.0) −104.6(3.1)	−120
N(3)–C(8)–C(9)–N(5) N(3')–C(8')–C(9')–N(5')	−14	−0.1(3.1) −1.8(3.4)	*ca.* 0
C(2)–C(1)–S–S' C(2')–C(1')–S'–S	−88	99.5(1.6) 110.2(1.4)	
C(23')–C(2)–C(1)–S C(23)–C(2')–C(1')–S'	−177	67.5(2.1) 56.7(1.9)	*ca.* 5 or −125
N(1)–C(2)–C(1)–S N(1')–C(2')–C(1')–S'	−58	−174.3(1.4) 171.1(1.2)	*ca.* 125 or −5
C(1)–S–S'–C(1')	101	−94.2(1.1)	

increasing GC-content of the polynucleotide.[4] By contrast, the binding of TANDEM is considerably weaker and is clearly related to the AT-content of the DNA; its binding constant only attains the same levels as seen with triostin A when it interacts with synthetic poly(dA-dT).[14,15] These differences can be explained by the structural alterations which result from the presence of the methylated peptide bonds in triostin A. In particular, the methyl substituents of the *N*-methylvaline residues prevent formation of the internal hydrogen-bonds to the Ala CO groups seen in TANDEM, the result of which is to open out the peptide backbone by rotating the obstructive isopropyl side-chains of the valyl residues outwards, allowing a

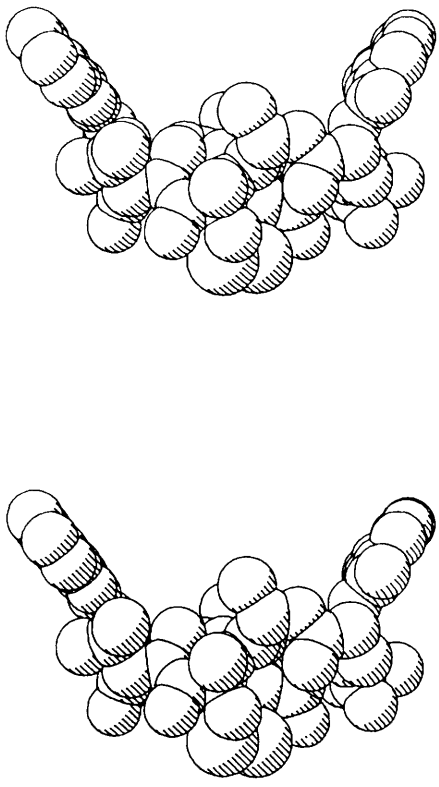

Figure 2. Space-filling stereoview of the triostin A molecule

much closer apposition of the face of the depsipeptide ring to the DNA receptor surface. At the same time the carbonyl oxygen atoms of the alanine residues are exposed, making them readily accessible as potential hydrogen-bond acceptors to interact with donor groups in the DNA. If we accept that triostin A, in common with other quinoxaline antibiotics, probably binds *via* the minor groove of the DNA helix, [7,8] this indicates the 2-amino groups of guanine bases as the likely donors. The facility to form these additional interactions with triostin A, but not with TANDEM, explains the higher binding constants characteristic of the natural antibiotic as well as its capacity to bind at GC-rich sequences in natural DNAs.

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Received 21*st September* 1983; *Paper* 3/1662