Crystal Structure and Absolute Configuration of a Derivative of Dothistromin, a Fungal Toxin implicated in Pine-needle Blight

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The bromoethyl ether tetra-acetate derivative of dothistromin crystallizes in space group $P2_12_12_1$ with $Z = 4$ in a unit cell of dimensions: $a = 6{\cdot}050 \pm 0{\cdot}003$, $b = 38{\cdot}194 \pm 0{\cdot}016$, and $c = 12{\cdot}204 \pm 0{\cdot}005$ Å. The structure was solved from *X*-ray diffractometer data by Patterson and Fourier methods and refined by block-diagonal least-squares techniques to *R* 0·075 for 840 independent reflections. The analysis shows a triacetoxyanthraquinone moiety joined to a *cis*-fused difuran system to give a linear arrangement of five- and six-membered rings. The absolute configuration of the molecule is also established.

INVESTIGATIONS into the causes of ' pine-needle blight ' in commercial pine forests in New Zealand have led to the isolation of the fungal pathogen *Dothistroma pini* Hulbary and subsequently to the isolation of a red pigment. Mass spectrometry has shown that this is a mixture of two closely related compounds of molecular formulae $C_{18}H_{12}O_9$ and $C_{18}H_{12}O_8$ and the name dothistromin has been given to the former which is present in the greater amount.[1] From u.v. spectral studies on dothistromin the presence of a tri-α-hydroxyanthraquinone group as well as a fused difuran system was suspected. The relationship between these two moieties could not be determined unequivocally but by analogy with similar molecules, the aflatoxins, fusion resulting in a linear arrangement of five- and six-membered rings was suggested.[1] To reveal the exact mode of fusion and to establish the absolute configuration of the molecule a single-crystal *X*-ray analysis of the bromoethyl ether tetra-acetate derivative was undertaken.

EXPERIMENTAL

Crystal Data.—$C_{28}H_{23}O_{13}Br$, $M = 647{\cdot}4$. Orthorhombic, $a = 6{\cdot}050 \pm 0{\cdot}003$, $b = 38{\cdot}194 \pm 0{\cdot}016$, $c = 12{\cdot}204 \pm 0{\cdot}005$ Å, $U = 2820$ Å,^{3} $D_m = 1{\cdot}52$, $Z = 4$, $D_c = 1{\cdot}53$, $F(000) = 1720$. Space group $P2_12_12_1$. Mo-K_α radiation: $\lambda = 0{\cdot}7107$ Å; $\mu(\text{Mo-}K_\alpha) = 16{\cdot}2$.

Single crystals, of a size suitable for automatic data-collection on a Hilger Watts four-circle diffractometer were difficult to grow and all showed slightly ' split spots ' in some regions of exploratory photographs. The ω-scan technique was used to record 840 independent reflections with $I > 3\sigma$. Absorption corrections were applied.[2]

The bromine atom was located from the Patterson synthesis, the light atoms from subsequent electron-density maps. Refinement was undertaken by the block-diagonal least-squares method with isotropic thermal motion being assumed initially, but later an anisotropic model for the bromine atom was introduced. Finally hydrogen atom positions were calculated, confirmed from a difference synthesis, and their values as fixed parameters were included in the refinement model with *B* 5·0 Å^2. A residual of 0·094 was obtained at this point. In view of the ratio of observations to adjustable parameters no attempt

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[1] C. Bassett, M. Buchanan, R. T. Gallagher, and R. Hodges, *Chem. and Ind.*, 1970, 1659.

[2] W. R. Busing and H. A. Levy, *Acta Cryst.*, 1957, **10**, 180.

was made to refine hydrogen atom positions nor to allow an anisotropic model of thermal vibration for any of the other light atoms.

Fifteen Friedel pairs of reflections for which $I_{hkl} - I_{\bar{h}\bar{k}\bar{l}}| > \frac{I_{hkl}}{10}$ were then selected and their intensities compared with calculated values incorporating the anomalous scattering of the bromine atom. Twelve of these pairs gave a clear indication that the correct hand had been determined for the molecule. As a further check, structure factors for the two mirror images were calculated assuming anomalous scattering by the bromine; the R over all observed reflections was 0·091 for the established hand, 0·097 for the alternative. Inclusion of anomalous scattering effects for the bromine atom then led to further least-squares refinement and a final residual of 0·075. Atomic scattering factors were taken from ref. 3 with appropriate corrections for anomalous dispersion applied to that for bromine. The weights applied to individual reflections were based on counting statistics and took the form, $w = 4F_o^2/[\sigma(F_o^2) + F_o^2]^2$.

TABLE 1

Atomic co-ordinates and isotropic thermal parameters for non-hydrogen atoms with standard deviations in parentheses

Atom	x/a	y/b	z/c	$B/\text{Å}^2$
Br	0·3476(7)	0·25636(8)	0·9082(2)	
C(1)	0·186(5)	0·2113(6)	0·581(2)	4·4(6)
O(2)	−0·020(2)	0·2151(3)	0·524(1)	2·9(3)
C(3)	−0·146(4)	0·1855(5)	0·546(2)	3·0(5)
O(4)	−0·301(3)	0·1934(4)	0·639(1)	3·5(3)
C(5)	−0·249(4)	0·1697(6)	0·722(2)	3·7(6)
C(6)	−0·363(4)	0·1671(5)	0·822(2)	2·8(5)
C(7)	−0·304(4)	0·1411(5)	0·891(2)	2·8(5)
C(8)	−0·418(4)	0·1371(5)	0·995(2)	3·1(5)
C(9)	−0·389(4)	0·1036(5)	1·064(2)	2·3(5)
C(10)	−0·551(4)	0·0941(6)	1·140(2)	3·5(6)
C(11)	−0·546(4)	0·0608(6)	1·193(2)	4·5(6)
C(12)	−0·371(4)	0·0385(6)	1·167(2)	4·2(6)
C(13)	−0·201(4)	0·0478(5)	1·092(2)	3·3(5)
C(14)	−0·216(4)	0·0801(6)	1·038(2)	3·7(6)
C(15)	−0·049(4)	0·0916(5)	0·951(2)	3·0(5)
C(16)	−0·112(4)	0·1189(5)	0·873(2)	2·1(5)
C(17)	−0·006(4)	0·1241(5)	0·773(2)	3·2(5)
C(18)	−0·076(4)	0·1484(5)	0·695(2)	2·2(5)
C(19)	0·014(4)	0·1571(5)	0·579(2)	3·4(5)
C(20)	0·239(4)	0·1724(5)	0·572(2)	3·4(5)
O(21)	0·164(3)	0·2208(3)	0·688(1)	2·9(3)
C(22)	0·114(4)	0·2560(6)	0·706(2)	4·8(6)
C(23)	0·086(4)	0·2644(6)	0·825(2)	4·9(6)
O(24)	−0·559(3)	0·1610(4)	1·025(1)	4·8(4)
O(25)	−0·740(3)	0·1139(4)	1·167(1)	5·0(4)
C(26)	−0·700(5)	0·1431(6)	1·234(2)	5·3(7)
O(27)	−0·532(3)	0·1483(5)	1·278(2)	6·8(5)
C(28)	−0·896(5)	0·1651(7)	1·235(2)	5·4(7)
O(29)	−0·031(3)	0·0260(4)	1·078(1)	4·6(4)
C(30)	−0·006(5)	0·0086(7)	0·976(2)	5·2(7)
O(31)	−0·161(3)	0·0087(4)	0·915(1)	5·7(4)
C(32)	0·200(5)	−0·0088(7)	0·968(2)	7·1(8)
O(33)	0·126(3)	0·0768(3)	0·949(1)	3·5(3)
O(34)	0·206(3)	0·1070(3)	0·752(1)	3·2(3)
C(35)	0·204(5)	0·0719(6)	0·722(2)	4·6(7)
O(36)	0·042(3)	0·0568(4)	0·701(1)	5·9(5)
C(37)	0·435(5)	0·0570(6)	0·732(2)	5·4(7)
O(38)	0·009(3)	0·1254(4)	0·509(1)	4·0(4)
C(39)	−0·182(5)	0·1080(6)	0·495(2)	4·4(6)
O(40)	−0·353(3)	0·1160(4)	0·536(1)	4·8(4)
C(41)	−0·140(5)	0·0767(6)	0·419(2)	5·5(7)

TABLE 2

Anisotropic thermal parameters for the bromine atom with standard deviations in parentheses

	10^3b_{11}	10^5b_{22}	10^4b_{33}	10^4b_{12}	10^3b_{13}	10^4b_{23}
Br	68(1)	139(2)	97(2)	15(5)	−16(1)	−8(2)

The scattering factor is of the form $f = f_0 \exp[-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)]$.

TABLE 3

Atomic parameters for hydrogen atoms, which are numbered according to the carbon atom to which they are attached

	x/a	y/b	z/c
H(1)	−0·31	0·228	0·54
H(3)	−0·25	0·178	0·47
H(6)	−0·51	0·186	0·84
H(11)	−0·67	0·053	1·25
H(12)	−0·38	0·014	1·21
H(20a)	0·34	0·165	0·50
H(20b)	0·35	0·163	0·64
H(22a)	−0·05	0·261	0·65
H(22b)	0·23	0·273	0·66
H(23a)	−0·07	0·248	0·85
H(23b)	0·01	0·291	0·82
H(28a)	−1·00	0·158	1·17
H(28b)	−1·00	0·160	1·30
H(28c)	−0·88	0·191	1·25
H(32a)	0·26	−0·009	0·89
H(32b)	0·32	0·001	1·02
H(32c)	0·19	−0·036	0·99
H(37a)	0·47	0·042	0·66
H(37b)	0·45	0·041	0·80
H(37c)	0·55	0·076	0·74
H(41a)	−0·25	0·058	0·43
H(41b)	−0·16	0·085	0·34
H(41c)	0·01	0·068	0·43

TABLE 4

Bond lengths (Å) with standard deviations in parentheses

Br–C(23)	1·91(3)	C(14)–C(15)	1·53(3)
C(1)–O(2)	1·43(3)	C(15)–C(16)	1·46(3)
C(1)–C(20)	1·53(3)	C(15)–O(33)	1·20(3)
C(1)–O(21)	1·37(3)	C(16)–C(17)	1·39(3)
O(2)–C(3)	1·39(3)	C(17)–C(18)	1·40(3)
C(3)–O(4)	1·51(3)	C(17)–O(34)	1·46(3)
C(3)–C(19)	1·51(3)	C(18)–C(19)	1·56(3)
O(4)–C(5)	1·40(3)	C(19)–C(20)	1·49(3)
C(5)–C(6)	1·40(3)	C(19)–O(38)	1·48(3)
C(5)–C(18)	1·36(3)	O(21)–C(22)	1·39(3)
C(6)–C(7)	1·35(3)	C(22)–C(23)	1·50(3)
C(7)–C(8)	1·45(3)	O(25)–C(26)	1·41(3)
C(7)–C(16)	1·45(3)	C(26)–O(27)	1·16(3)
C(8)–C(9)	1·54(3)	C(26)–C(28)	1·45(4)
C(9)–O(24)	1·30(3)	O(29)–C(30)	1·43(3)
C(9)–C(10)	1·40(3)	C(30)–O(31)	1·19(3)
C(9)–C(14)	1·41(3)	C(30)–C(32)	1·41(4)
C(10)–C(11)	1·42(3)	C(34)–C(35)	1·39(3)
C(10)–O(25)	1·41(3)	C(35)–O(36)	1·16(3)
C(11)–C(12)	1·40(4)	C(35)–C(37)	1·52(4)
C(12)–C(13)	1·42(3)	O(38)–C(39)	1·35(3)
C(13)–C(14)	1·40(3)	C(39)–O(40)	1·19(3)
C(13)–O(29)	1·33(3)	C(39)–C(41)	1·53(3)

The numbering system for the molecule is shown in the formula. Positional and isotropic thermal parameters with their standard deviations are listed in Table 1, anisotropic thermal parameters for the bromine atom are given in Table 2, atomic parameters for hydrogen atoms in Table 3

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[3] 'International Tables for X-Ray Crystallography,' vol. 3, Kynoch Press, Birmingham, 1962, p. 202.

and bond distances and angles for atoms other than hydrogen are listed in Tables 4 and 5.*

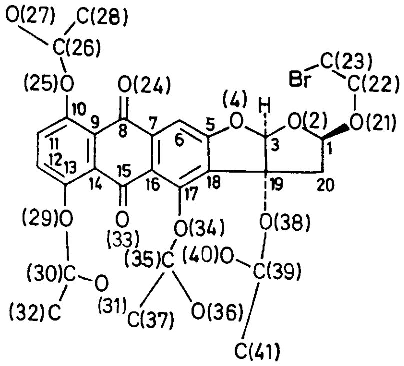

TABLE 5

Bond angles (deg.) with standard deviations in parentheses

O(2)–C(1)–C(20)	104(1)	C(7)–C(16)–C(15)	122(1)
O(2)–C(1)–O(21)	111(1)	C(7)–C(16)–C(17)	115(1)
C(20)–C(1)–O(21)	110(1)	C(15)–C(16)–C(17)	124(1)
C(1)–O(2)–C(3)	107(1)	C(16)–C(17)–C(18)	124(1)
O(2)–C(3)–O(4)	109(1)	C(16)–C(17)–O(34)	120(1)
O(2)–C(3)–C(19)	107(1)	C(5)–C(18)–C(17)	118(1)
O(4)–C(3)–C(19)	110(1)	C(5)–C(18)–C(19)	111(1)
C(3)–O(4)–C(5)	106(1)	C(17)–C(18)–C(19)	131(1)
O(4)–C(5)–C(6)	124(1)	C(3)–C(19)–C(18)	100(1)
O(4)–C(5)–C(18)	113(1)	C(3)–C(19)–C(20)	107(1)
C(6)–C(5)–C(18)	123(1)	C(3)–C(19)–O(38)	115(1)
C(5)–C(6)–C(7)	117(1)	C(18)–C(19)–C(20)	117(1)
C(6)–C(7)–C(8)	120(1)	C(18)–C(19)–O(38)	110(1)
C(6)–C(7)–C(16)	123(1)	C(20)–C(19)–O(38)	108(1)
C(8)–C(7)–C(16)	117(1)	C(1)–C(20)–C(19)	101(1)
C(7)–C(8)–C(9)	120(1)	C(1)–O(21)–C(22)	115(1)
C(7)–C(8)–O(24)	119(1)	O(21)–C(22)–C(23)	112(1)
C(9)–C(8)–O(24)	121(1)	Br–C(23)–C(22)	113(1)
C(8)–C(9)–C(10)	120(1)	C(10)–O(25)–C(26)	115(1)
C(8)–C(9)–C(14)	120(1)	O(25)–C(26)–O(27)	123(2)
C(10)–C(9)–C(14)	120(1)	O(25)–C(26)–C(28)	109(2)
C(9)–C(10)–C(11)	121(1)	O(27)–C(26)–C(28)	128(2)
C(9)–C(10)–O(25)	126(1)	C(13)–O(29)–C(30)	119(1)
C(11)–C(10)–O(25)	113(1)	O(29)–C(30)–O(31)	117(2)
C(10)–C(11)–C(12)	117(1)	O(29)–C(30)–C(32)	112(2)
C(11)–C(12)–C(13)	123(1)	O(31)–C(30)–C(32)	131(2)
C(12)–C(13)–C(14)	119(1)	C(17)–O(34)–C(35)	118(1)
C(12)–C(13)–O(29)	119(1)	O(34)–C(35)–O(36)	123(2)
C(14)–C(13)–O(29)	122(1)	O(34)–C(35)–C(37)	109(2)
C(13)–C(14)–C(15)	123(1)	O(36)–C(35)–C(37)	127(2)
C(9)–C(14)–C(13)	120(1)	C(19)–C(38)–C(39)	120(1)
C(9)–C(14)–C(15)	117(1)	O(38)–C(39)–O(40)	125(2)
C(14)–C(15)–C(16)	119(1)	O(38)–C(39)–C(41)	109(2)
C(14)–C(15)–O(33)	118(1)	O(40)–C(39)–C(41)	127(2)
C(16)–C(15)–O(33)	123(1)		
C(18)–C(17)–O(34)	116(1)		

DISCUSSION

The structure shows the expected linear fusion of the anthraquinone and difuran ring systems. The observed bond distances and angles within the basic but non-planar skeleton are as expected. Although a bend in the molecule is expected at the junction of the two five-membered rings where *cis*-fusion occurs there is a further deviation from planarity in the anthraquinone moiety (see Figure). This is apparent from the planes of best fit listed in Table 6 where the plane defined by C(5)—(8), and C(15)—(18) is seen to be inclined to that through the atoms C(9)—(14), at an angle of 157°. In addition to this bend there is also a slight ' twist ' about the long axis through the three six-membered rings which is shown by the non-planarity of C(8), C(9), C(14), and C(15) and of C(7), C(9), C(14), and C(16). As a result the central ring is buckled slightly towards a skew-boat conformation. Such distortions are not seen in anthraquinone itself,[4] nor in a number of its

TABLE 6

Planes of best fit. Equations of planes are referred to the cell axes and are given in the cosine form ($Ax + By + Cz + D = 0$, where A, B and C are the direction cosines). Displacements (Å) of atoms from the plane are in square brackets; α is the probability that a planar set of atoms will have χ^2 as large as that found *

Plane (1):

C(5)—(8), C(15)—(18) $0{\cdot}616x + 0{\cdot}658y + 0{\cdot}434z - 7{\cdot}166 = 0$
C(5) −0·001, C(6) 0·034, C(7) −0·031, C(8) −0·008, C(15) −0·009, C(16) 0·028, C(17) 0·023, C(18) −0·036, C(1) 1·915, O(2) 0·942, C(3) −0·155, O(4) −0·039, C(9) −0·381, C(10) −0·815, C(11) −1·356, C(12) −1·405, C(13) −0·933, C(14) −0·457, C(19) −0·101, C(20) 1·089, O(24) 0·229, O(33) 0·278, O(34) 0·278]

$$\chi^2 = 3{\cdot}11,\ \alpha = 0{\cdot}68$$

Plane (2):

C(9)—(14) $0{\cdot}543x + 0{\cdot}400y + 0{\cdot}738z - 9{\cdot}889 = 0$
[C(9) −0·001, C(10) 0·012, C(11) −0·007, C(12) −0·009, C(13) 0·020, C(14) −0·015, C(5) −1·603, C(6) −1·121, C(7) −0·700, C(8) −0·201, C(15) −0·078, C(16) −0·572, C(17) −1·046, C(18) −1·605, O(25) −0·066, O(29) 0·124]

$$\chi^2 = 0{\cdot}54,\ \alpha = 0{\cdot}87$$

Plane (3):

C(8), C(9), C(14), C(15) $0{\cdot}552x + 0{\cdot}484y + 0{\cdot}679z - 9{\cdot}400 = 0$
[C(8) −0·015, C(9) 0·031, C(14) −0·030, C(15) 0·015]

$\chi^2 = 1{\cdot}45,\ \alpha = 0{\cdot}24$; *i.e.* non-planar

Plane (4):

C(7), C(9), C(14), C(16) $0{\cdot}588x + 0{\cdot}575y + 0{\cdot}569z - 8{\cdot}241 = 0$
[C(7) −0·034, C(9) 0·034, C(14) −0·034, C(16) 0·034]

$\chi^2 = 2{\cdot}91,\ \alpha = 0{\cdot}09$; *i.e.* non-planar

Plane (5):

C(3), O(4), C(5), C(18), C(19) $0{\cdot}637x + 0{\cdot}671y + 0{\cdot}380z - 6{\cdot}743 = 0$
[C(3) −0·021, O(4) 0·016, C(5) −0·004, C(18) −0·009, C(19) 0·018, C(1) 2·081, O(2) 1·121, C(20) 1·250, O(21) 2·735, C(22) 3·528, C(23) 4·187, Br 5·376]

$$\chi^2 = 0{\cdot}79,\ \alpha = 0{\cdot}68$$

Plane (6):

O(2), C(3), C(19), C(20) $-0{\cdot}072x + 0{\cdot}281y + 0{\cdot}957z - 8{\cdot}431 = 0$
[O(2) 0·003, C(3) −0·005, C(19) 0·005, C(20) 0·003, C(1) 0·539]

$$\chi^2 = 0{\cdot}05,\ \alpha = 0{\cdot}84$$

Interplanar angles (deg.)

Plane (1)–Plane (2)	157
Plane (1)–Plane (5)	177

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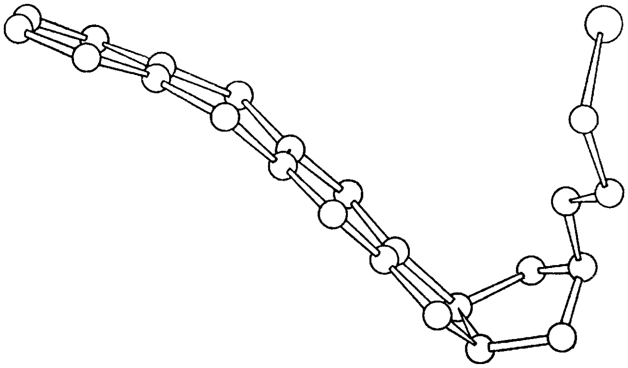
* Observed and calculated structure factors are listed in Supplementary Publication No. SUP 20526 (4 pp., 1 microfiche). For details see Notice to Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20 (items less than 10 pp. are sent as full size copies).

* See G. H. Stout and L. H. Jensen, ' *X*-Ray Structure Determination: A Practical Guide,' Macmillan, New York, 1968, p. 424.

[4] A. Prakesh, *Acta Cryst.*, 1967, **22**, 439.

derivatives[5-7] but in 1,5-dichloroanthraquinone[8] buckling of the central ring into a chair conformation is observed as are distortions in the angles subtended at those carbon centres between the chlorine and the quinonoid oxygen atoms. Similar distortions in the angles between the acetate substituents and the quinonoid oxygens are observed in the dothistromin derivative, the different conformation of the middle ring probably arising from the asymmetric substitution of acetate groups. In dothistromin itself (the pentahydroxy-parent compound) therefore, the anthraquinone moiety is presumed to be planar. The five-membered ring directly attached to the anthraquinone system appears not to be coplanar with the end ring [O(4), C(3), C(19) lie —0·039, —0·155, —0·101 Å respectively from plane (1)] but the angle one plane makes with the other is only 3°. The bromoethyl ether side-chain curls back over this ring system so that the bromine atom lies above the furan ring fused directly to the anthraquinone at a distance of 5·38 Å.



A schematic representation of the absolute configuration is shown in the formula. A complete stereochemical correlation between the bromoethyl ether tetra-acetate derivative and the parent pentahydroxy-compound is uncertain. However it is clear that racemisation is not likely at C(19), nor is any but *cis*-fusion likely between the furan rings, so that the configurations at two of the three optical centres [C(3) and C(19)] should be the same in dothistromin and the bromo-derivative. (These configurations are the same as those found in the similar molecules, the aflatoxins,[9] by degradative studies and in sterigmatocystin by *X*-ray diffraction.[10]) However the ready epimerisation which can be expected at C(1) precludes specification of this centre in dothistromin.

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