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FUNGAL METABOLITES PART 9.[1] ISOLATION AND X-RAY STRUCTURE DETERMINATION OF ALTERNARIAN ACID FROM *ALTERNARIA MALI* Sp.

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A novel metabolite, alternarian acid, has been isolated from cultures of *Alternaria mali*. Spectroscopic data provided a partial structure for alternarian acid and the complete structure was determined by X-ray diffractometry. A possible biosynthetic route to alternarian acid is presented.

Static culture of a strain of *Alternaria mali* yielded the known compounds, alternariol monomethyl ether,[2] altenuene,[3] dehydroaltenusin,[4] ergosterol and a new acid, alternarian acid, of molecular formula, $C_{15}H_{12}O_8$.

Alternarian acid gave a strong purple colour with aqueous ferric chloride, indicating the possible presence of an ortho-hydroxy acid function.[5] Esterification with diazomethane gave a dimethyl ester, $C_{17}H_{16}O_8$, for which the ^{1}H-nmr spectrum showed an exchangeable O$\underline{H}$ peak at δ 11.53, confirming that one OH group was strongly H-bonded (ortho-hydroxy ester).

The ^{1}H-nmr spectrum (220 MHz) of alternarian acid showed peaks at δ 2.06 (singlet, $C\underline{H}_3$), 3.86 (singlet $OC\underline{H}_3$), 6.35 (doublet, 1 $\underline{H}$; J 0.3 Hz), 6.52 (doublet, 1 $\underline{H}$; J 0.3 Hz) and 7.15 (singlet, 1H). These data and similar ones for the dimethyl ester suggested a tetrasubstituted aromatic ring (meta-coupled hydrogens). The upfield positions of the aromatic protons indicated they were ortho or para to phenolic hydroxyl groups and suggested a partial structure (A; Scheme). The ^{13}C-nmr spectrum supported this assignment.

Although several possible structures could be drawn for alternarian acid, including the one finally determined (structure B in the Scheme), the spectroscopic data did not indicate any one structure unequivocally. Consequently, the dimethyl ester was studied by X-ray diffraction methods.

Bond lengths, bond angles and torsion angles are listed in Table 1. The atomic numbering system, molecular and crystal structures are depicted in Figure 1. Dimethyl alternarianate contains an α-pyrone moiety and a search of the Cambridge Crystallographic Database for such structures, not fused to another ring, yielded twenty tallies. In three of these,[6] the pyrone ring bears only one alkyl-type substituent which would not be expected to perturb the ring geometry greatly. The data from these structures were averaged to yield a "standard" α-pyrone ring geometry (Figure 2a) with which the structure found for dimethyl alternarianate (Figure 2b) may be compared. Although the structure of α-pyrone itself, complexed to $Fe(CO)_3$, has been determined,[7] it cannot be used in this comparison because complexation leads to substantial electronic and, therefore, structural changes. Both structures in Figure 2a and 2b display the expected pattern of bond length variation reflecting substantial bond fixation. However, there are significant differences between them, particularly at the lactone carbonyl (1.197Å in 2b and 1.240Å in 2a) and at the adjacent C-C double bond (1.451Å in 3b and 1.394Å in 2a). These differences presumably stem from the electron-withdrawing effect of the methoxycarbonyl group attached to the

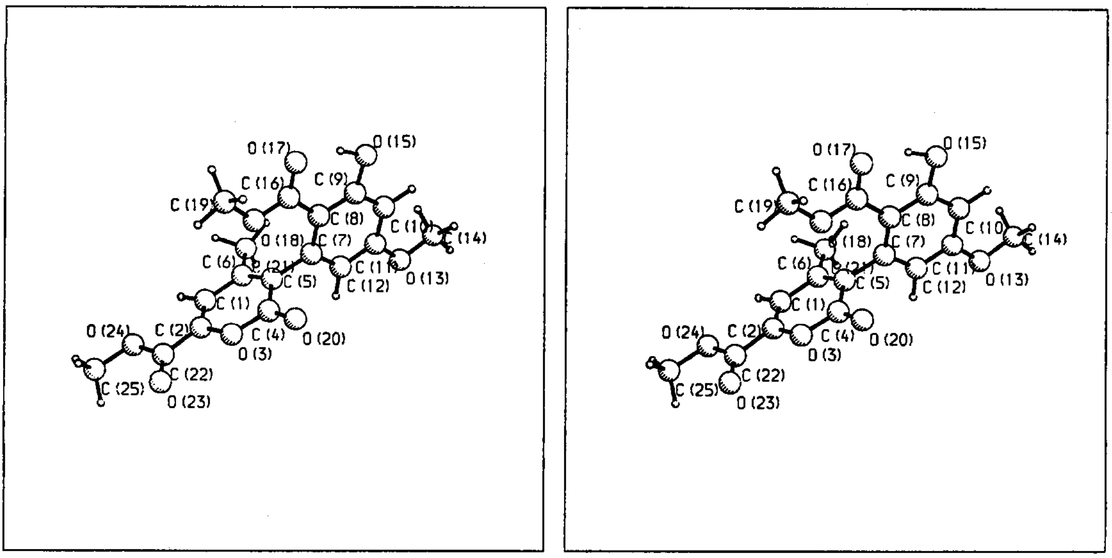


FIGURE 1 Stereoview of dimethyl alternarianate with C,O atom numbering. The view was drawn by the program, PLUTO 78 (W.D.S. Motherwell, Cambridge Crystallographic Data Centre).

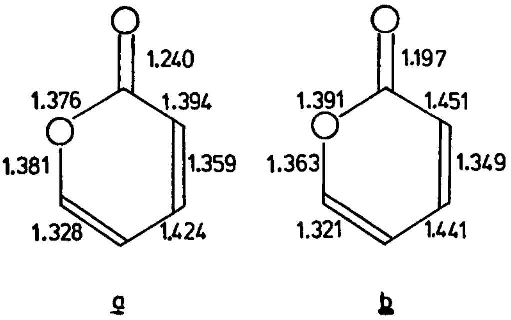
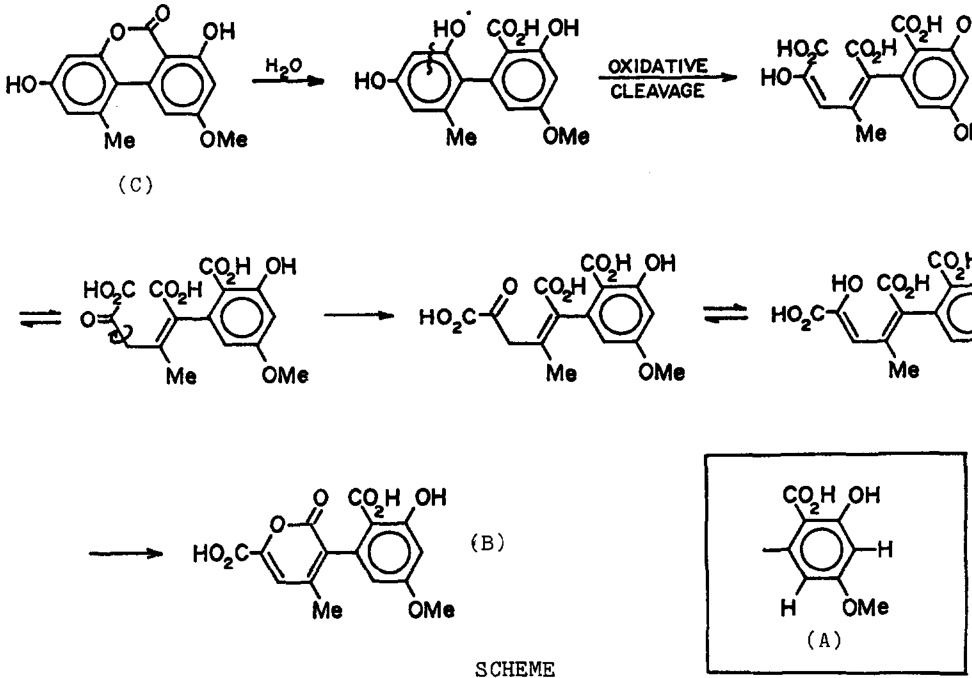


FIGURE 2 (a) Averaged bond lengths for the α-pyrone ring from three structures. (b) Bond lengths found for the α-pyrone ring in the present structure determination.

α-pyrone ring and the consequent reduction in π-electron delocalisation from the C-C bond into the lactone carbonyl (the pyrone ring and the ester group are close to co-planarity, the angle between their mean least-squares planes being only 6.3°). Conjugation between the π-systems of the pyrone and benzene rings is of minor significance since the angle between their planes is 81.5°, probably a consequence of ortho-interactions between the methyl group and the lactone carbonyl group of the pyrone ring and the methoxycarbonyl group on the benzene ring. In keeping with the earlier spectroscopic and colour tests, the hydrogen of the hydroxyl group, H(15), is intramolecularly H-bonded to the carbonyl oxygen of the adjacent ester group H(15) ... O(17); 1.576Å. The crystal packing is noteworthy, exhibiting several short, intermolecular contacts e.g., O(13) ... C(22), 3.082Å; O(17)...C(6), 3.155Å, and O(23)...C(21), 3.177Å. Analysis of the data on α-pyrones in the Cambridge Database reveals that this is not unusual for substituted (and, presumably, often substantially dipolar) α-pyrones.

The biosynthesis of several metabolites from *Alternaria* species has been investigated and discussed.[8] Thus, the monomethyl ether of the dibenzopyrone, alternariol, can be transformed readily into another known metabolite, altenusin, and hence to altenuic acid, dehydroaltenusin, altenuene and altertenuol. The biosynthesis of the first of these compounds, altenuic acid, requires oxidation of alternariol methyl ether followed by oxidative cleavage of one of the benzene rings. We suggest that



SCHEME

direct oxidative cleavage of one of the benzene rings of alternariol methyl ether (structure C in the Scheme) can give rise to alternarian acid (B in the Scheme).

EXPERIMENTAL

ISOLATION OF ALTERNARIAN ACID.

Alternaria mali (Roberts)[9] was grown on a sterile, static, potato dextrose culture medium prepared from dextrose (15% w/v), potato extract (5% w/v) and distilled water. After 28 days at 25°, the aqueous liquors (25 l) were filtered from the pelt and concentrated to about 3 l. This aqueous solution was extracted with ethyl ethanoate (6 x 500 ml). The combined ethyl ethanoate extracts were concentrated to about 1 l and then extracted with aqueous $NaHCO_3$ (8% w/v; 4 x 500 ml). The combined alkaline extracts were made acidic (pH 2.5) with dil. HCl and extracted with ethyl ethanoate (4 x 500 ml). These combined ethyl ethanoate extracts were concentrated to about 200 ml and the solution left to stand at 0° for 18 hours to give a brown oily precipitate containing some crystalline material. The semi-crystalline oil was triturated with small amounts of chloroform to give alternarian acid, m.p. 144 - 149° (methanol). Found: C,52.4; H, 4.3. $C_{15}H_{12}O_8$ requires C, 56.2; H, 3.8%. $\lambda_{max.}$ nm (log ε) : 220 (4.53), 260 (4.11), 305 (4.16); $\nu_{max.}$ 3270, 2650, 1752, 1710, 1618 cm^{-1}; ^{13}C-nmr ($CDCl_3$): δ 19.822 (q), 56.150 (q), 101.757(d), 105.317 (s), 110.873 (d), 132.988 (s), 139.407(s), 147.093 (s), 147.795(s), 160.605(s), 160.956(s), 165.649(s), 166.835(s), 172.742(s); ms, m/z (% relative abundance): 320 (41.4), 302 (100), 276 (68.3), 275 (66), 274 (21.6), 248 (64.6), 231 (36.2), 219 (24.5), 203 (51.5), 201 (25.3), 175 (35.5), 77 (13.1); $M^{+\cdot}$, m/z 320.0486.

Reaction of alternarian acid (74.4 mg) with an excess of diazomethane in diethyl ether for 30 min. and evaporation of the solvent gave dimethyl alternarianate (58.7 mg, after preparative scale tlc on silica gel with CH_2Cl_2 as eluent), m.p. 153 - 155° (EtOAc). Found: C, 57.1; H, 4.8. $C_{17}H_{16}O_8$ requires C, 58.6; H, 4.6%. $\lambda_{max.}$, nm (log ε) : 215 (4.48), 260 (4.18), 306 (4.16). $\nu_{max.}$ 1725, 1710, 1655, 1605 cm^{-1}; ^1H-nmr ($CDCl_3$) : δ 1.98 (s, 3H), 3.66 (s, 3H), 3.79 (s,

Table 1 Bond Lengths (Å), Bond Angles (°) and Torsion Angles (°)* not involving H Atoms for the Structure Shown in Figure 1.

Bond lengths

O(3)	C(2)	1,363	O(3)	C(4)	1,391	O(13)	C(11)	1,360
O(13)	C(14)	1.438	O(15)	C(9)	1,365	O(17)	C(16)	1,227
O(18)	C(16)	1,317	O(18)	C(19)	1,460	O(20)	C(4)	1,197
O(23)	C(22)	1,186	O(24)	C(22)	1,338	O(24)	C(25)	1,460
C(1)	C(2)	1,321	C(1)	C(6)	1,441	C(2)	C(22)	1,485
C(4)	C(5)	1,451	C(5)	C(6)	1,349	C(5)	C(7)	1,490
C(6)	C(21)	1,503	C(7)	C(8)	1,431	C(7)	C(12)	1,383
C(8)	C(9)	1,380	C(8)	C(16)	1,476	C(9)	C(10)	1,403
C(10)	C(11)	1,387	C(11)	C(12)	1,380			

Bond angles

C(2)	O(3)	C(4)	120.8	C(11)	O(13)	C(14)	118,3	C(16)	O(18)	C(19)	115,6
C(22)	O(24)	C(25)	115.1	C(2)	C(1)	C(6)	120,5	O(3)	C(2)	C(1)	122,2
O(3)	C(2)	C(22)	110.8	C(1)	C(2)	C(22)	126,9	O(3)	C(4)	O(20)	116,1
O(3)	C(4)	C(5)	117,0	O(20)	C(4)	C(5)	127,0	C(4)	C(5)	C(6)	121,0
C(4)	C(5)	C(7)	116,9	C(6)	C(5)	C(7)	121,9	C(1)	C(6)	C(5)	118,2
C(1)	C(6)	C(21)	117.9	C(5)	C(6)	C(21)	123,9	C(5)	C(7)	C(8)	123,8
C(5)	C(7)	C(12)	117.1	C(8)	C(7)	C(12)	118,9	C(7)	C(8)	C(9)	118,0
C(7)	C(8)	C(16)	123,7	C(9)	C(8)	C(16)	118,3	O(15)	C(9)	C(8)	123,2
O(15)	C(9)	C(10)	113.7	C(8)	C(9)	C(10)	123,1	C(9)	C(10)	C(11)	117,4
O(13)	C(11)	C(10)	123.4	O(13)	C(11)	C(12)	115.4	C(10)	C(11)	C(12)	121,2
C(7)	C(12)	C(11)	121.4	O(17)	C(16)	O(18)	120,9	O(17)	C(16)	C(8)	122,0
O(18)	C(16)	C(8)	117,1	O(23)	C(22)	O(24)	124,6	O(23)	C(22)	C(2)	125,2
O(24)	C(22)	C(2)	110,1								

Torsion Angles

C(4)	O(3)	C(2)	C(1)	3,4	C(4)	O(3)	C(2)	C(22)	-176,7
C(2)	O(3)	C(4)	O(20)	-179,4	C(2)	O(3)	C(4)	C(5)	-.3
C(14)	O(13)	C(11)	C(10)	-10,3	C(14)	O(13)	C(11)	C(12)	171,3
C(19)	O(18)	C(16)	O(17)	-1,1	C(19)	O(18)	C(16)	C(8)	178,3
C(25)	O(24)	C(22)	O(23)	.3	C(25)	O(24)	C(22)	C(2)	-179,2
C(6)	C(1)	C(2)	O(3)	-1,9	C(6)	C(1)	C(2)	C(22)	178.3
C(2)	C(1)	C(6)	C(5)	-2,9	C(2)	C(1)	C(6)	C(21)	176.4
O(3)	C(2)	C(22)	O(23)	-4,3	O(3)	C(2)	C(22)	O(24)	175,2
C(1)	C(2)	C(22)	O(23)	175,6	C(1)	C(2)	C(22)	O(24)	-4,9
O(3)	C(4)	C(5)	C(6)	-4.5	O(3)	C(4)	C(5)	C(7)	-179,7
O(20)	C(4)	C(5)	C(6)	174,6	O(20)	C(4)	C(5)	C(7)	-,7
C(4)	C(5)	C(6)	C(1)	6,0	C(4)	C(5)	C(6)	C(21)	-173,2
C(7)	C(5)	C(6)	C(1)	-179,1	C(7)	C(5)	C(6)	C(21)	1,7
C(4)	C(5)	C(7)	C(8)	-86,2	C(4)	C(5)	C(7)	C(12)	98,3
C(6)	C(5)	C(7)	C(8)	98,6	C(6)	C(5)	C(7)	C(12)	-76,9
C(5)	C(7)	C(8)	C(9)	-174,1	C(5)	C(7)	C(8)	C(16)	5,0
C(12)	C(7)	C(8)	C(9)	1,3	C(12)	C(7)	C(8)	C(16)	-179,6
C(5)	C(7)	C(12)	C(11)	173,4	C(8)	C(7)	C(12)	C(11)	-2,3
C(7)	C(8)	C(9)	O(15)	-179,6	C(7)	C(8)	C(9)	C(10)	-,3
C(16)	C(8)	C(9)	O(15)	1,3	C(16)	C(8)	C(9)	C(10)	-179.4
C(7)	C(8)	C(16)	O(17)	-178,0	C(7)	C(8)	C(16)	O(18)	2,6
C(9)	C(8)	C(16).	O(17)	1,0	C(9)	C(8)	C(16)	O(18)	-178,3
O(15)	C(9)	C(10)	C(11)	179,6	C(8)	C(9)	C(10)	C(11)	,3
C(9)	C(10)	C(11)	O(13)	-179,5	C(9)	C(10)	C(11)	C(12)	-1.2
O(13)	C(11)	C(12)	C(7)	-179,3	C(10)	C(11)	C(12)	C(7)	2,3

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* E.s.d.'s are *ca.* 0.007Å for bond lengths, 0.5° for bond angles and somewhat greater for torsion angles.

3H), 3.93 (s, 3H), 6.18 (d, 1H), 6.49 (d, 1H), 7.06 (s, 1H), 11.53 (s, 1H, exchangeable with D_2O); ms, m/z (% relative abundance) : 348 (69.4), 320 (17.4), 316 (100), 289 (87), 261 (39.1), 245 (43.5), 233 (47.8), 201 (60.9), 158 (22), 59 (91.3); M^+ , m/z 348.0840.

CRYSTALLOGRAPHIC DATA

$C_{17}H_{16}O_8$, Mr = 348.084, monoclinic, $P2_1/c$, a = 8.42(2), b = 13.42(3), c = 14.64 (3)Å, β = 104.7(1)°, U = 1601.6Å^3, Z = 4, D_x = 1.46 Mg. m^{-3}, λ(CuKα) = 1.5418Å, μ = 1.00 mm^{-1}, F (000) = 728, T = ca. 293°K. Final R = 0.071 for 1970 unique reflexions.

CRYSTALLOGRAPHY

Crystals suitable for X-ray analysis were obtained by slow diffusion of light petroleum (b.p. 40 - 60°) into a solution of dimethyl alternarianate in ethyl ethanoate. Reflexion intensities were measured by the SERC Microdensitometer Service, Daresbury Laboratory, from Weissenberg photographs of layers h0l to h11l for a crystal 0.3mm in edge. Equivalents were merged, yielding 1970 unique data which were used for structure solution and refinement. Cell dimensions were determined from precession photographs and interlayer scale factors, initially from exposure times. The structure was solved by direct methods (MULTAN 80)[10] and refined on F-values by full-matrix least squares (Shel X).[11] At an intermediate stage, positions for all hydrogen atoms, except for the hydroxyl hydrogen which was located from a difference map, were calculated geometrically. These atoms were allowed to refine with two common isotropic temperature factors, one for the hydrogens on the methyl groups and the other for all remaining hydrogens, by "riding" on the associated heavy atoms with appropriate bond-length constraints. In the final stages of refinement, interlayer scale factors were refined individually and an extinction correction was applied. Maximum and minimum peak heights in the final difference maps were 0.34 and -0.38 eÅ^{-3} respectively.

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