

Fungal Products. Part 22.¹ X-Ray and Molecular Structure of the Mono-acetate of Colletotrichin

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The structure and relative stereochemistry (1) for colletotrichin, a metabolite of *Colletotrichum capsici*, previously isolated under the name acetylcolletotrichin, is deduced from an X-ray crystallographic study of the mono-acetate (2). The crystals are orthorhombic $P2_12_12_1$, $a = 16.370(12)$, $b = 11.919(3)$, $c = 15.506(9)$ Å, $Z = 4$. Using 1 554 independent 'observed' intensities, the refinement converged to R 0.058 (R' 0.074). The metabolite consists of a tetra-substituted γ -pyrone, containing all non-hydroxylic oxygens, and linked to an unusual bicyclic terpene.

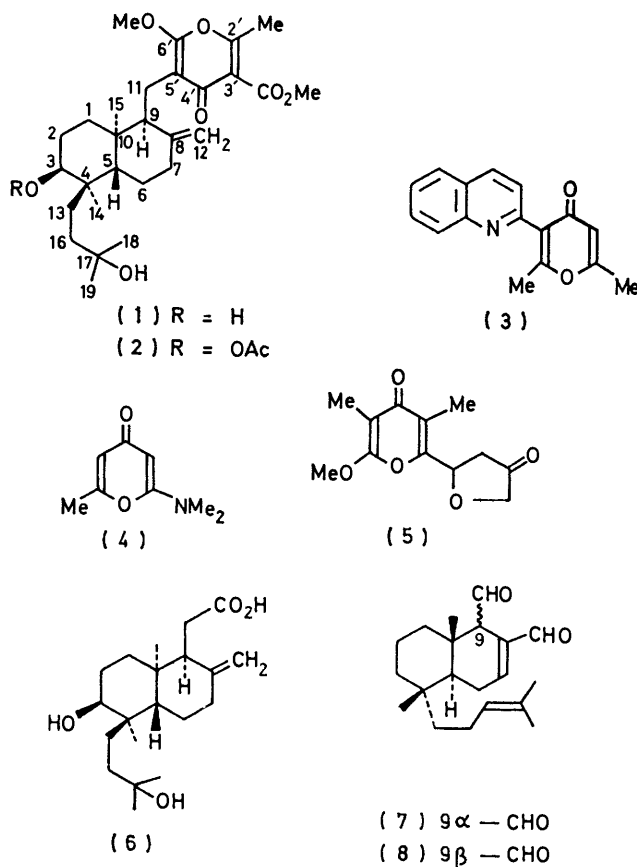
THE plant pathogen, *Colletotrichum capsici*, Syd Butler and Busby, was shown by Grove *et al.*² to produce phytotoxic culture filtrates from which an inhibitor of intact green plants was isolated. This metabolite named acetylcolletotrichin was partially characterised as a neutral acetate, $C_{28}H_{42}O_7$, containing two methoxy, one carbonyl, one secondary hydroxy, and four tertiary methyl groups.

For further structural studies we have re-isolated this metabolite in low yield (0.20–30.0 mg l⁻¹ culture filtrate); its molecular formula and spectroscopic data correspond to those published by Grove *et al.*² However, in addition, the presence of a tertiary hydroxy-group was revealed by the formation of a bis-trimethylsilyl (TMSi) ether from the metabolite and of a mono-trimethylsilyl ether from the ketone, obtained² by oxidation of the secondary hydroxy-function in the metabolite. The mass spectra of these bis- and mono-TMSi ethers contained an intense ion at m/e 131 indicating the partial structure, $Me_2C(OH)$. Further evidence for this grouping was obtained from the mass spectrum of the metabolite which showed an ion at $M^+ - C_5H_{11}O$ indicating the presence of the terpenoid moiety, $Me_2C(OH)CH_2CH_2$. An intense ion, $C_{10}H_{12}O_5^+$ was present in the mass spectra of both the metabolite and its bis-TMSi ether suggesting that the five-non-hydroxylic oxygen functions were situated in a self-contained structural unit.

Acetylation of the secondary hydroxy-group with acetic anhydride-pyridine at room temperature yielded a mono-acetate, m.p. 167–168 °C, in which the hydroxymethine proton signal in the n.m.r. spectrum of the metabolite had moved to lower field by 1.0 p.p.m. The structure (2) (or enantiomer) for the mono-acetate, and hence structure (1) (or enantiomer) for the metabolite itself, was established by a single crystal X-ray diffraction study.

The structure was determined from diffraction data (3006 reflections) by direct methods and refined (using observed reflections only—1554 data) by least-squares and difference-Fourier methods to R 0.058. The results are illustrated in Figure 1 which shows a view of the mono-acetate in projection down the crystallographic a axis. Structural parameters are listed in Tables 1 and 2 and selected torsion angles in Table 3. Figure 2 shows the arrangement of molecules in the unit cell.

In the crystal structure the molecules of the mono-acetate (2) (see Figure 1) have both alicyclic rings in chair-like conformations in which the angular C(10)-methyl, the C(4)-methyl, the C(3)-acetoxy, and the C(9)-pyronyl-methyl groups are axial while the C(4) isopentanyl group



is equatorial. The C–C and C–O single-bond lengths (Table 2) are consistent with the values predicted from covalent radii of 0.77, 0.74, and 0.66 Å for $C(sp^3)$, $C(sp^2)$, and O respectively. The values for the C=C bonds between C(2') and C(3'), C(5') and C(6'), and C(8)–C(12), and the C=O bonds between C(3'-1) and O(3'-1) and C(3-1) and O(3-2) fall within the usual ranges. The carbon–oxygen bond at C(4')–O(4'-1) (1.248 Å) is longer than a normal C=O bond but is part of the planar γ -pyrone ring [mean deviation from the least-square

plane through O(1'), C(2'), C(3'), C(4'), O(4'-1), C(5'), and C(6') equals 0.02 Å]. The elongation of the C=O bond length at C(4')-O(4'-1) is thus a measure of the aromatic character of the pyrone ring, and is in agreement with the $\nu_{\text{C=O}}$ at 1 662 and 1 663 cm^{-1} in the acetate (2) and compound (3) which also indicates considerable single-bond character. The plane of the C(3')-methoxy-carbonyl group makes an angle of 14.2° with the pyrone

TABLE 1

Atomic positional fractional co-ordinates, with estimated standard deviations in parentheses

Atom	<i>x</i>	<i>y</i>	<i>z</i>
C(1)	0.523 6(5)	0.314 7(6)	1.077 5(5)
C(2)	0.488 8(5)	0.235 6(6)	1.145 1(5)
C(3)	0.496 4(5)	0.114 2(6)	1.118 1(5)
O(3-1)	0.582 8(3)	0.087 7(4)	1.108 7(3)
C(3-1)	0.622 4(6)	0.030 3(7)	1.173 9(5)
O(3-2)	0.591 2(6)	0.010 3(7)	1.240 2(4)
C(3-2)	0.705 7(7)	-0.002 3(10)	1.146 1(8)
C(4)	0.454 7(4)	0.087 9(5)	1.030 2(5)
C(5)	0.483 6(4)	0.175 5(5)	0.960 9(4)
C(6)	0.442 7(5)	0.162 6(6)	0.872 5(5)
C(7)	0.483 2(5)	0.235 8(6)	0.804 1(5)
C(8)	0.490 0(4)	0.356 6(6)	0.831 7(5)
C(9)	0.528 9(4)	0.374 0(5)	0.919 8(4)
C(10)	0.482 1(4)	0.301 9(5)	0.989 8(4)
C(11)	0.623 4(4)	0.354 1(5)	0.914 4(5)
C(12)	0.463 9(6)	0.440 3(7)	0.784 0(6)
C(13)	0.480 3(5)	-0.029 3(5)	0.999 5(5)
C(14)	0.361 7(5)	0.086 4(7)	1.045 3(6)
C(15)	0.395 8(4)	0.353 7(6)	0.994 4(6)
C(16)	0.467 2(5)	-0.127 3(6)	1.061 6(5)
C(17)	0.508 6(4)	-0.237 3(6)	1.034 8(4)
O(17-1)	0.595 4(3)	-0.214 2(4)	1.033 3(4)
C(18)	0.491 9(6)	-0.326 6(7)	1.099 3(6)
C(19)	0.480 7(6)	-0.274 8(7)	0.947 0(5)
O(1')	0.733 2(3)	0.514 1(5)	0.740 5(3)
C(2')	0.743 9(4)	0.616 6(7)	0.775 6(5)
C(2'-1)	0.791 8(6)	0.690 0(9)	0.714 5(6)
C(3')	0.719 1(4)	0.639 5(6)	0.855 6(4)
C(3'-1)	0.737 7(4)	0.749 4(6)	0.896 9(5)
O(3'-1)	0.772 8(3)	0.760 7(5)	0.964 1(4)
O(3'-2)	0.707 3(3)	0.835 0(4)	0.851 0(4)
C(3'-2)	0.721 0(7)	0.946 2(8)	0.887 4(7)
C(4')	0.678 5(4)	0.552 7(6)	0.907 0(4)
O(4'-1)	0.657 5(3)	0.569 8(4)	0.983 3(3)
C(5')	0.664 9(4)	0.445 5(6)	0.865 3(5)
C(6')	0.694 1(4)	0.431 7(6)	0.785 0(5)
O(6'-1)	0.686 9(4)	0.337 4(5)	0.741 6(4)
C(6'-1)	0.724 0(7)	0.321 9(10)	0.660 2(6)

TABLE 2

Bond lengths (Å) and angles (°)

(a) Distances			
C(10)-C(1)	1.528(10)	C(8)-C(12)	1.314(11)
C(1)-C(2)	1.520(10)	C(8)-C(9)	1.522(10)
C(2)-C(3)	1.512(10)	C(9)-C(10)	1.583(9)
C(3)-O(3-1)	1.458(9)	C(10)-C(15)	1.543(10)
O(3-1)-C(3-1)	1.383(10)	C(9)-C(11)	1.566(9)
C(3-1)-O(3-2)	1.172(11)	C(6')-O(1')	1.361(9)
C(3-1)-C(3-2)	1.482(15)	O(1')-C(2')	1.349(10)
C(3)-C(4)	1.556(10)	C(2')-C(2'-1)	1.509(13)
C(4)-C(13)	1.534(9)	C(2')-C(3')	1.333(10)
C(4)-C(14)	1.540(11)	C(3')-C(3'-1)	1.491(10)
C(13)-C(16)	1.530(10)	C(3'-1)-O(3'-1)	1.196(9)
C(16)-C(17)	1.533(10)	C(3'-1)-O(3'-2)	1.339(9)
C(17)-O(17-1)	1.448(9)	O(3'-2)-C(3'-2)	1.458(11)
C(17)-C(18)	1.486(11)	C(3')-C(4')	1.466(10)
C(17)-C(19)	1.504(11)	C(4')-O(4'-1)	1.248(9)
C(4)-C(5)	1.571(10)	C(4')-C(5')	1.449(10)
C(5)-C(6)	1.534(10)	C(5')-C(11)	1.493(10)
C(5)-C(10)	1.572(9)	C(5')-C(6')	1.344(10)
C(6)-C(7)	1.525(11)	C(6')-O(6'-1)	1.315(10)
C(7)-C(8)	1.506(10)	O(6'-1)-C(6'-1)	1.413(12)

TABLE 2 (Continued)

(b) Angles			
C(10)-C(1)-C(2)	112.6(6)	C(7)-C(8)-C(9)	114.6(6)
C(1)-C(2)-C(3)	111.8(6)	C(7)-C(8)-C(12)	122.9(7)
C(2)-C(3)-C(4)	113.6(6)	C(12)-C(8)-C(9)	122.6(7)
C(2)-C(3)-O(3-1)	108.3(6)	C(8)-C(9)-C(10)	109.8(5)
C(4)-C(3)-O(3-1)	107.2(5)	C(8)-C(9)-C(11)	110.2(5)
C(3)-O(3-1)-C(3-1)	119.3(6)	C(11)-C(9)-C(10)	115.7(5)
O(3-1)-C(3-1)-O(3-2)	122.5(9)	C(9)-C(11)-C(5')	111.5(5)
O(3-1)-C(3-1)-C(3-2)	110.3(8)	C(9)-C(10)-C(1)	109.9(5)
O(3-2)-C(3-1)-C(3-2)	127.2(9)	C(9)-C(10)-C(15)	105.0(5)
C(3)-C(4)-C(5)	109.4(5)	C(15)-C(10)-C(1)	109.0(6)
C(3)-C(4)-C(13)	109.6(6)	C(6')-O(1')-C(2')	120.7(6)
C(3)-C(4)-C(14)	107.6(6)	O(1')-C(2')-C(2'-1)	109.8(7)
C(5)-C(4)-C(13)	108.0(6)	C(2'-1)-C(2')-C(3')	128.6(8)
C(5)-C(4)-C(14)	114.2(6)	O(1')-C(2')-C(3')	121.4(7)
C(13)-C(4)-C(14)	107.9(6)	C(2')-C(3')-C(3'-1)	121.2(7)
C(4)-C(13)-C(16)	117.5(6)	C(3')-C(3'-1)-O(3'-1)	124.8(7)
C(13)-C(16)-C(17)	114.9(6)	C(3')-C(3'-1)-O(3'-2)	111.4(6)
C(16)-C(17)-O(17-1)	105.9(5)	O(3'-1)-C(3'-1)-O(3'-2)	123.7(7)
C(16)-C(17)-C(18)	110.4(6)	C(3'-1)-O(3'-2)-C(3'-2)	115.4(7)
C(16)-C(17)-C(19)	111.4(6)	C(3'-1)-C(3')-C(4')	118.6(6)
O(17-1)-C(17)-C(18)	109.2(6)	C(2')-C(3')-C(4')	120.0(7)
O(17-1)-C(17)-C(19)	109.9(6)	C(3')-C(4')-O(4'-1)	121.7(6)
C(18)-C(17)-C(19)	109.9(6)	O(4'-1)-C(4')-C(5')	121.6(6)
C(4)-C(5)-C(6)	114.4(6)	C(3')-C(4')-C(5')	116.7(6)
C(4)-C(5)-C(10)	115.9(5)	C(4')-C(5')-C(11)	119.1(6)
C(5)-C(10)-C(1)	110.0(5)	C(11)-C(5')-C(6')	123.0(6)
C(5)-C(10)-C(9)	108.5(5)	C(4')-C(5')-C(6')	117.8(6)
C(5)-C(10)-C(15)	114.3(5)	C(5')-C(6')-O(6'-1)	123.2(7)
C(5)-C(6)-C(7)	112.0(6)	C(6')-O(6'-1)-C(6'-1)	122.0(7)
C(6)-C(5)-C(10)	110.1(5)	C(5')-C(6')-O(1')	123.3(7)
C(6)-C(7)-C(8)	112.5(6)	O(6'-1)-C(6'-1)-O(1')	113.6(6)

TABLE 3

Selected torsion angles

C(10)-C(1)-C(2)-C(3)	58.4(8)
C(2)-C(1)-C(10)-C(5)	-52.8(8)
C(2)-C(1)-C(10)-C(9)	-172.3(6)
C(1)-C(2)-C(3)-C(4)	-57.1(8)
C(2)-C(3)-C(4)-C(5)	50.1(8)
C(3)-C(4)-C(5)-C(6)	-176.6(6)
C(3)-C(4)-C(5)-C(10)	-46.7(7)
C(4)-C(5)-C(6)-C(7)	-170.2(6)
C(10)-C(5)-C(6)-C(7)	57.2(7)
C(4)-C(5)-C(10)-C(1)	48.6(7)
C(11)-C(5)-C(10)-C(9)	169.0(5)
C(6)-C(5)-C(10)-C(1)	-179.5(6)
C(6)-C(5)-C(10)-C(9)	-59.2(7)
C(5)-C(6)-C(7)-C(8)	-52.2(8)
C(6)-C(7)-C(8)-C(9)	51.5(8)
C(7)-C(8)-C(9)-C(10)	-54.1(8)
C(7)-C(8)-C(9)-C(11)	74.3(7)
C(8)-C(9)-C(10)-C(1)	177.2(6)
C(8)-C(9)-C(10)-C(5)	56.9(7)
C(11)-C(9)-C(10)-C(1)	51.8(7)
C(11)-C(9)-C(10)-C(5)	-68.5(7)
C(8)-C(9)-C(11)-C(5)	70.3(7)
C(10)-C(9)-C(11)-C(5')	-164.4(5)
C(9)-C(11)-C(5')-C(4')	77.0(8)
C(9)-C(11)-C(5')-C(6')	-106.4(8)
C(6')-O(1')-C(2')-C(2'-1)	177.7(7)
C(6')-O(1')-C(2')-C(3')	2.2(10)
C(2')-O(1')-C(6')-C(5')	-0.8(10)
O(1')-C(2')-C(3')-C(4')	-0.6(10)
C(2')-C(3')-C(4')-C(5')	-2.2(10)
C(3')-C(4')-C(5')-C(11)	-179.7(6)
C(3')-C(4')-C(5')-C(6')	3.4(10)
C(11)-C(5')-C(6')-O(1')	-178.8(6)
C(4')-C(5')-C(6')-O(1')	-2.1(11)

The sign convention is such that the sign is positive if a clockwise rotation is required of atom (1) to eclipse atom (4) whilst looking down the (2)-(3) bond.

ring, and there is little multiple-bond character in the C(3')-C(3'-1) bond length which approximates to twice the covalent radius of an sp^2 -carbon.

In the crystal packing (Figure 2) all the intermolecular distances are larger than the sums of the relevant van der Waals radii but there is a slight interaction between the C(17) tertiary hydroxy-group and the C(4'-1) carbonyl oxygen of the γ -pyrone in molecules which are

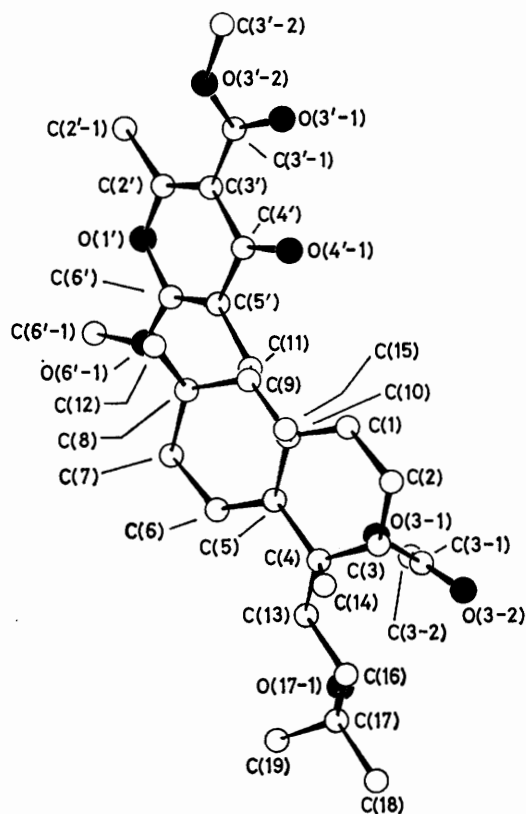


FIGURE 1 Projection down the crystallographic a axis

related by a translation of y [$O(4'-1) \cdots O^*(17-1) = 2.87(1) \text{ \AA}$ where $*$ denotes the symmetry operation $x, 1 + y, z$].

The derived structure (1) for the parent metabolite contains no acetyl group. Hence it is suggested that the name acetylcolletotrichin be replaced by colletotrichin. The ^1H n.m.r. signal at τ 7.65 (s, 3 H), originally assigned² to an acetyl group, can now be assigned to the C(2')-methyl group on the γ -pyrone [cf. the C-2 and C-6 methyl signals of compounds (3)³ which occur at τ 7.61 and 7.77 respectively and the C-methyl signal of compound (4)⁴ at τ 7.8]. The $\nu_{\text{C=O}}$ band at 1736 cm^{-1} , also assigned² to an acetate carbonyl, can now be re-assigned to the methoxycarbonyl group at C(3') of the γ -pyrone ring. The i.r. absorption at 1660 cm^{-1} agrees with that observed⁴ (1662 cm^{-1}) for the carbonyl of the γ -pyrone (4) and the bands at 1607 and 1588 cm^{-1} are the $\nu_{\text{C=C}}$ absorptions of the pyrone. The u.v. spectrum, λ_{max} 261 (ϵ 7000), agrees with the data⁵ for the γ -pyrone chromophore in compound (5). All other spectroscopic assignments made by Grove *et al.*² fit structure (1). Structure (1) also explains the observed² instability of colletotrichin to basic conditions

and the alkaline hydrogen peroxide oxidation to an acid for which structure (6) can now be considered.

The mass spectrum of colletotrichin can be rationalised by cleavage of the C(9)-C(11) bond to give the base peak m/e 212 ($\text{C}_{10}\text{H}_{12}\text{O}_5$) containing the pyrone ring and m/e 243 ($\text{C}_{18}\text{H}_{27}$) containing the terpenoid portion. In the mass spectrum of the bis-TMSi ether, the three most intense peaks at m/e 212, 199, and 131 probably arise by cleavage of the C(9)-C(11), C(11)-C(5'), and C(16)-C(17) bonds respectively.

Colletotrichin (1) is one of the few natural γ -pyrones and the first which is polyprenylated. Another notable feature is the novel terpenoid ring system though since our preliminary announcement⁶ of structure (1) for colletotrichin, the dialdehydes (7) and (8) have been isolated from liverworts.^{7,8} The interesting biochemical properties⁹ of colletotrichin may be primarily associated with the pyrone ring; the γ -pyrone, aureothin,¹⁰ shows mammalian toxicity and the aurovertins¹¹ are potent inhibitors of ATP-synthesis and ATP-hydrolysis catalysed by mitochondrial enzymes.

Since the publication of a preliminary account⁶ of this work, the single crystal X-ray structure of colletotrichin has been reported in preliminary form¹² and serves to confirm structure (1). More recently the preliminary account of [^{13}C]-biosynthetic studies has been published¹³ indicating that colletotrichin is a mero-norditerpene.

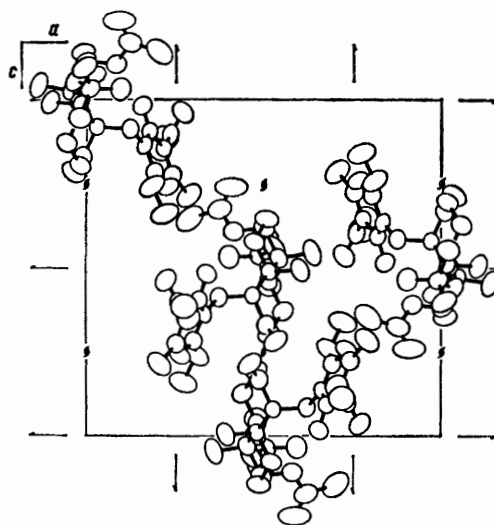


FIGURE 2 Arrangement of molecules in the unit cell

EXPERIMENTAL

General details are given in refs. 1 and 14; i.r. spectra were determined for Nujol mulls except where stated otherwise.

Isolation of Colletotrichin (1).—*Colletotrichum capsici* Syd. Butler and Bisby strain 1287, obtained from I.C.I. (Pharmaceuticals) Ltd., was stored on slopes of 2% malt agar under liquid paraffin. For inoculation a spore suspension was prepared from agar slopes, grown for 7 days in medicine bottles. The spore suspension was dispensed (1 ml per

flask) into penicillin flasks (9 in diameter) each containing 200 ml of a medium consisting of dextrosol (5%), potassium dihydrogen phosphate (1%), ammonium nitrate (0.24%), magnesium sulphate heptahydrate (0.20%), and trace elements solution (1 ml l⁻¹). The fermentations, at pH 4.5, were monitored by g.l.c. [TMSi derivative; 2% OV-17, glass column (152.5 × 0.64 cm); isothermal, 265 °C; R_T 28 min] and t.l.c. [silica gel; ethyl acetate–light petroleum–acetic acid (60 : 35 : 5); R_F 0.2]; yields of colletotrichin reached a maximum at 28–32 days.

From a large-scale fermentation, carried out at M.R.E. Porton Down, the culture filtrates (50 l) were extracted with chloroform to yield a crude product (12 g) which was adsorbed on silica gel (25 g) and placed on top of a column (70 × 5 cm) of silica gel (500 g). The column was eluted with 500-ml portions of 2, 5, 10, 25, 50, and 75% diethyl ether in light petroleum, then with ether (1 l) followed by 1-l fractions of 1, 2, 5, 10, and 25% methanol in ether. The last fraction yielded 1.27 g of material which was crystallised from ethyl acetate–light petroleum to give colletotrichin (1.0 g). Further purification by p.l.c. on silica gel layers (0.8 mm), developed with ethyl acetate–light petroleum–acetic acid (9 : 10 : 1) gave colletotrichin (R_F 0.5) which crystallised from ethyl acetate–light petroleum as small needles, m.p. 181–184 °C (lit.,² 186–187 °C) (Found: M⁺ 490.290. C₂₈H₄₂O₇ requires M⁺ 490.293); ν_{max.} 3 300, 3 145, 1 748, 1 663, 1 620, 1 583, 1 265, 1 145, and 892 cm⁻¹; τ(CDCl₃) 9.22 (s, 15-H₃), 9.05 (14-H₃), 8.78 and 8.74 (both s, 18-H₃ and 19-H₃), 7.66 (s, 2'-H₃), 6.34 (m, 3α-H), 6.13 and 6.10 (both s, CO₂Me and OMe), 5.74 br and 5.46 br (12-H₂); m/e 490 (M⁺, 3%), 475 (6), 472 (11), 459 (8), 457 (14), 441 (26), 403.210 (9; C₂₃H₃₁O₆ requires 403.212), 385.201 (10; C₂₃H₂₉O₅ requires 385.202), 261.222 (2; C₁₈H₂₉O requires 261.222), 243.211 (25; C₁₈H₂₇ requires 243.211), 212.068 (100; C₁₀H₁₂O₅ requires 212.068), 197 (27), 180 (16), 179 (13), 165 (32), 113 (57), 67 (55), and 43 (44); m/e (bis-TMSi ether) 634 (M⁺, 1%), 619 (9), 603 (2), 544 (7), 529 (4), 513 (12), 475 (11), 439 (11), 385 (12), 243 (16), 212 (60), 199 (63), 131 (65), 75 (100), 73 (92), 55 (165), and 43 (64).

Jones Oxidation.—Colletotrichin (15 mg) in acetone (1 ml) was treated with Jones reagent (3 drops) at 0 °C. After 2 min methanol (2 ml) was added and the mixture was filtered through a short column of silica. Removal of the solvent gave 3-oxocolletotrichin which crystallised from ethyl acetate–light petroleum as prisms (8 mg), m.p. 186–188 °C (lit.,² 187–188 °C); λ_{max.} (EtOH) 262 nm (ε 7 600); ν_{max.} (CHCl₃) 1 746, 1 672, and 1 591 cm⁻¹; τ(CDCl₃) 8.97 (s, 15-H₃), 8.91 (s, 18- and 19-H₃), 7.64 (s, 2'-H₃), 5.67br and 5.38br (12-H₂); m/e 488 (M⁺, 8%), 470 (34), 455 (31), 401 (22), 212 (36), 211 (30), 113 (47), and 43 (100); m/e (mono-TMSi ether) 560 (M⁺, 5%), 545 (5), 529 (2), 439 (15), 212 (24), 210 (26), 199 (100), 139 (39), 131 (48), 97 (40), 75 (40), 73 (57), 43 (90), and 41 (75).

Colletotrichin monoacetate (2).—Colletotrichin (40 mg) was stirred for 36 h at room temperature in acetic anhydride (2 ml) and pyridine (12 drops). The mixture was acidified with dilute hydrochloric acid and extracted with ethyl acetate (3 × 10 ml). The solvent was removed *in vacuo* to give an oil which was purified by p.l.c. on 0.8-mm layers of silica gel, developed with ethyl acetate–light petroleum–acetic acid (9 : 10 : 1). Elution of the band at R_F 0.2 gave the *mono-acetate* (2) as prisms (22 mg) m.p. 167–168 °C (from ethyl acetate–light petroleum) (Found: M⁺ 532.303 C₃₀H₄₄O₇ requires M⁺ 532.304); ν_{max.} 3 410, 1 743, 1 662, 1 618, 1 583, 1 410, 1 240, 887, and 800 cm⁻¹; τ(CDCl₃)

9.14 (s, 15-H₃), 9.02 (s, 14-H₃), 8.83 (s, 18- and 19-H₃), 7.95 (s, OCOMe), 7.65 (s, 2'-H₃), 6.12 and 6.10 (both s, CO₂Me and OMe), 5.75 (m, 3-H), 5.44br and 5.27br (12-H₂); m/e 532 (M⁺, 9%), 517 (11), 514 (11), 501 (8), 483 (11), 445 (7), 439 (13), 385 (11), 212 (100), 197 (19), 165 (22), 113 (46), 67 (37), and 43 (37).

X-Ray Data Collection.—The *mono-acetate* (2) grew, from ethyl acetate–light petroleum, as colourless needles elongated in the *c* direction. Oscillation and Weissenberg photographs were taken to establish unit-cell parameters and space group. Diffracted intensities were collected with Mo-K_α radiation to 2θ_{max.} = 50°, as previously described,¹⁵ using a crystal (0.2 × 0.83 × 0.35 nm), mounted on a Syntex P₂ four-circle diffractometer linked to a Novo 1200 computer. The scan rate varied from 0.049° s⁻¹ to 0.490° s⁻¹, linearly coupled to a preliminary 2-s peak count which varied from 100 to 800. Of 3 006 independent reflections measured, 1 554 satisfied the criterion I > 2.5σ(I) where σ(I) is the estimated standard deviation based on counting statistics, and only these were used in the least-squares refinement of the structure. Three standard reflections were re-measured every 40 reflections to ensure the stability of the crystal and apparatus. The crystal showed no appreciable decay. No absorption corrections were made.

The structure was solved by direct methods using the MULTAN program¹⁶ run at the University of Glasgow computer centre, and refinement was carried out with the 'X-Ray System of Programs'¹⁷ available on the CDC 7600 computer at the University of London.

Crystal Data.—C₃₀H₄₄O₈, M = 532.6, orthorhombic, a = 16.370(12), b = 11.919(3), c = 15.506(9) Å, U = 3 025(5) Å³ at T = 293 K, D_m (floatation) = 1.17 g cm⁻³, Z = 4, D_c = 1.169 g cm⁻³, F(000) = 1 152. Space group P₂₁2₁2₁, Mo-K_α X-radiation, λ = 0.710 69 Å; μ(Mo-K_α) = 0.78 cm⁻¹.

The structure was determined by the multi-solution method of crystal structure solution, deduced by Germain and Woolfson.¹⁸ The origin and enantiomorph defining reflections were chosen and their phases fixed according to normal criteria. It was decided to include two special reflections (*i.e.* those whose phases were restricted to one of two values by the space group symmetry) and two general reflections, and these were chosen by the MULTAN program.¹⁶ Finally for each of the 64 sets of phases generated by the tangent formula, figures of merit were calculated. One set of phases had a combined figure of merit 3.0 and from the subsequent *E* map based on these phases, 28 atoms were located at chemically reasonable positions. After 2 cycles of isotropic least-squares refinement, R = 0.26. The remaining 10 non-hydrogen atoms were located from successive electron-density difference syntheses.

The data proved insensitive to atom type and so it was only during the final cycles of refinement that the chemical nature of the molecule was confirmed (Figure 1). The hydrogen atoms were set at calculated positions (C–H = 0.95 Å) except for the methyl and hydroxy-hydrogens, which were located from difference-Fourier maps. All the parameters for the hydrogen atoms were fixed during refinement (U_{iso} = 0.076 Å²). In the final cycles, refinement was by blocked-matrix least-squares with anisotropic thermal parameters for all non-hydrogen atoms. The weighting scheme used for refinement was w⁻¹ = (3.25 + 0.410|F_o| + 0.022|F_o|² - 0.0003|F_o|³), devised to minimise the variation of ⟨w(ΔF)²⟩ with |F_o|. The refinement converged at R = 0.058 (R' = 0.074) with a mean shift/error value in

the final cycle of 0.05. A final electron-density difference-Fourier synthesis showed no peaks $>0.3 \text{ e } \text{\AA}^{-3}$ or $<-0.4 \text{ e } \text{\AA}^{-3}$. Atomic scattering factors for carbon and oxygen were taken from ref. 19, and for hydrogen from ref. 20. Final atomic co-ordinates are listed in Table 1; temperature factors and observed and calculated structure factors are listed in Supplementary Publication No. 22484 (12 pp.).*

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* For details of the Supplementary publications scheme see Notice to Authors No. 7, *J.C.S. Perkin I*, 1978, Index issue.

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