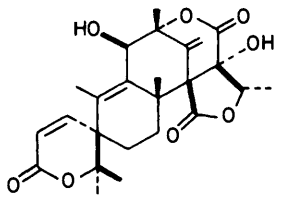
The Structures of Some Metabolites of *Penicillium diversum*: α- and β-Diversonolic Esters

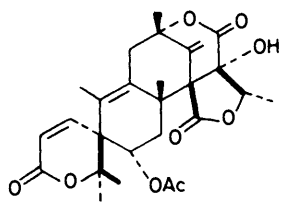
John S. E. Holker * and Eugene O'Brien
Robert Robinson Laboratories, University of Liverpool, P.O. Box 147, Liverpool L69 3BX
Thomas J. Simpson *
Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ

The metabolites of *Penicillium diversum* include the meroterpenoids austinol (1) and isoaustin (2), together with the known poly-β-ketides lichenxanthone (3), alternariol monomethyl ether (4), and two new compounds, α- and β-diversonolic esters, (5) and (6) respectively. The structure elucidation of the latter compounds is based essentially on extensive ^1H and ^{13}C n.m.r. spectroscopic studies. The biogenetic significance of these compounds is discussed.

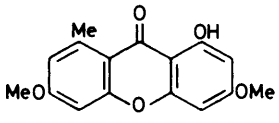
In a recent paper [1] W. B. Turner reported the structure determination of the fungal metabolite diversonol (7) from *Penicillium diversum*. In an attempt to isolate this compound for biosynthetic studies we have re-investigated the metabolites produced by this organism.† Although we have been unable to isolate diversonol, we have found that this organism produces a wide range of metabolites, some of which we have purified and characterised. Of these we have already reported [2] the isolation and structure elucidation of the meroterpenoids austinol (1) and isoaustin (2) and we now add the known compounds lichenxanthone (3) [3] and alternariol monomethyl ether (4),[4] together with two new optically active isomeric compounds, α- and β-diversonolic esters, $C_{16}H_{16}O_7$. The purpose of this paper is to report the structure elucidation of these compounds as (5) and (6) respectively.



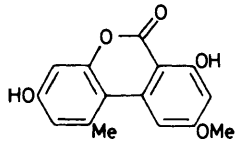
(1)



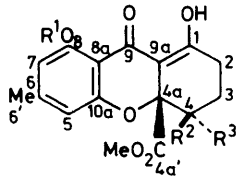
(2)



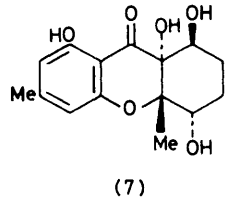
(3)



(4)



(5) $R^1 = R^3 = H$, $R^2 = OH$
(6) $R^1 = R^2 = H$, $R^3 = OH$
(8) $R^1 = Me$, $R^2 = OH$, $R^3 = H$
(9) $R^1 = Me$, $R^2 = H$, $R^3 = OH$
(10) $R^1 = Ac$, $R^2 = OAc$, $R^3 = H$
(11) $R^1 = Ac$, $R^2 = H$, $R^3 = OAc$



(7)

The principal structural features of α- and β-diversonolic esters became apparent from the spectroscopic properties of the parents and their derivatives. Both compounds give monomethyl ethers, (8) and (9) respectively, on treatment with diazomethane and are presumably monohydric phenols. Since the parents give strong chelate ferric reactions and show hydroxylic protons at low field in their ^1H n.m.r. spectra (δ 11.85 and 11.92 in the α- and β-compounds respectively) it is clear that this phenolic hydroxy group is *ortho* to a carbonyl group. This is confirmed by the shift of the i.r. frequency of this carbonyl group to longer wavelength on methylation, *e.g.* 1 655 to 1 700 cm^{-1}, in the case of the α-compound.[5] The presence of a second hydroxy group in the parent compounds is shown by the formation of di-*O*-acetyl derivatives, (10) and (11) respectively. Since this acetylation is accompanied by a downfield shift of *ca.* 1.1 p.p.m. of one proton in the ^1H n.m.r. spectra of the compounds, characteristic of the value associated with that of a geminal proton on acetylation of a secondary alcohol,[6] it is clear that both these compounds contain this structural feature. Both diacetates show a proton at *ca.* δ 4.0, exchangeable with D_2O, and i.r. bands at *ca.* 3 480 cm^{-1}. There must therefore be a third hydroxy group in the parents, which is not derivatised in either the methylation or acetylation reactions. The nature of this is discussed in the sequel.

The secondary alcoholic function is shown to be present in the residue $CH_2CH_2CH(OH)$ by the ^1H n.m.r. spectra of the parents, which are closely similar to each other (Table 1) and which will be illustrated in the following discussion by reference to the β-compound. Three multiplets are present at δ 4.03 (1 H), 2.83 (2 H), and 2.11 (2 H) respectively. Double irradiation of the 220 MHz spectrum at δ 2.11 causes both the other signals to collapse to singlets, not only establishing that the irradiated signal is due to the C-3 methylene group,‡ but also that both the C-2 methylene hydrogens (δ 2.83) and the C-4 methine hydrogen (δ 4.03) are isolated from further spin–spin couplings. Double irradiation at δ 2.83 simplifies the remaining two signals to a typical ABX pattern. On measurement of the ^1H n.m.r. spectrum of the α-compound at 360 MHz full resolution of all lines of the above three multiplets was achieved, leading to the following analysis: δ 4.07, *J* 10.8 and 3.9 Hz ($CHOHCH_2CH_2$), 2.17, *J* 3.9, 5.9, 6.4, or 4.9, 16 Hz and 2.26, *J* 6.4 or 4.9, 8.9, 10.8, 16 Hz ($CHOHCH_2CH_2$),

† We thank Dr. W. B. Turner, Pharmaceuticals Division, Imperial Chemical Industries Ltd., for providing this strain (ATCC 10437, number 946 in I.C.I. collection) of *P. diversum*.

‡ The numbering of the carbon atoms in the diversonolic esters, is that previously adopted for the related compound diversonol (7).[1]

Table 1. ^{1}H N.m.r. chemical shifts of α- and β-diversonolic esters and derivatives (p.p.m. with couplings in Hz)

Proton(s)	Compound (5) *	(6) †	(8) †	(9) †	(10) †	(11) †
1-OH	4.76 s	4.57 s	5.27 s	5.15 s	4.60 s	4.00 s
2	2.82 ($J_{2,3}$ 5.9 and 8.9, $J_{2,2}$ 18.7) 2.88, ($J_{2,3}$ 4.9 and 6.4, $J_{2,2}$ 18.7)	2.83 m	2.82 m	2.80 m	2.83 m	2.73 m
3	2.17 ($J_{2,3}$ 5.9 and 6.4 or 4.9, $J_{3,4}$ 3.9, $J_{3,3}$ 16.0)					
	2.26 ($J_{2,3}$ 6.4 or 4.9, 8.9, $J_{3,4}$ 10.8, $J_{3,3}$ 16.0)	2.11 m	*ca.* 2.2 m	2.07 m	*ca.* 2.25 m	*ca.* 2.3 m
4	4.08 ($J_{3,4}$ 3.9 and 10.8)	4.03 ($J_{3,4}$ 5 and 6.5)	4.11 ($J_{3,4}$ 5 and 11)	4.00 ($J_{3,4}$ 5 and 6.5)	5.19 ($J_{3,4}$ 5 and 6.5)	5.11 ($J_{3,4}$ 11 and 4)
4-OH	3.30 s	2.65 s	3.05 s	2.75 s		
5 and 7	6.67	6.66 d	6.79 d	6.78 d	7.12 d	7.10 d
	6.60 ($J_{5,7}$ 2.4)	6.59 d	6.59 d	6.58 d	6.81 d	6.80 d
6′	2.38 s	2.39 s	2.43 s	2.40 s	2.45 s	2.35 s
8-OH	11.85 s	11.92 s				
4a′-OMe	3.85 s	3.81 s	3.79 s	3.79 s	3.76 s	3.70 s
Others			3.96 s (8-OMe)	3.91 s (8-OMe)	2.03, 2.37 (8- and 4-OAc)	2.10, 2.43 (8- and 4-OAc)

* Measured at 360 MHz. † Measured at 220 MHz.

Table 2. ^{13}C N.m.r. chemical shifts of α- and β-diversonolic esters and derivatives (p.p.m.) with multiplicities for off-resonance decoupled spectra, where obtained (spectra at 25.19 MHz)

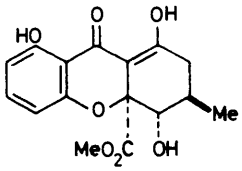
Carbon	(5)	(6)	(8)
1	166.7 s	166.9 s	163.4
2	26.1 t	26.7 t	26.0
3	24.4 t	25.3 t	24.4
4	72.6 d	70.4 d	70.4
4a	76.3 s	72.8 s	73.0
4a′	172.7 s	173.9 s	172.5
5	107.1 d	107.2 d	107.6
6	147.5 s	147.6 s	145.7
6′	22.4 q	22.4 q	22.3
7	112.0 d	112.1 d	111.3
8	160.0 s	160.1 s	159.3
8a	108.0 s	108.0 s	109.8
9	181.8 s	181.1 s	177.6
9a	116.6 s	116.7 s	119.0
10a	155.9 s	155.8 s	157.8
OMe	53.2 q	53.5 q	52.8
8-OMe			56.3

2.82, *J* 5.9, 8.9, 18.7 Hz and 2.88, *J* 4.9, 6.4, 18.7 Hz (CHOH-CH_2CH_2).

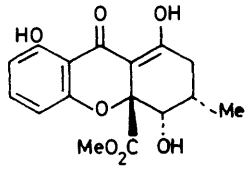
The ^{13}C n.m.r. spectra of the two compounds are very similar (Table 2), the chemical shift values and multiplicities in the off-resonance decoupled spectra being entirely compatible with the proposed structures. The fully proton coupled ^{13}C spectrum of the α-compound (5) measured at 90.6 MHz was particularly informative in establishing the substitution pattern of the aromatic ring. It showed the following features. The signal at δ 160.0 p.p.m. due to C-8, showed a large upfield shift (22 Hz, 0.24 p.p.m.) and sharpened on addition of D_2O, confirming this as the carbon carrying the phenolic hydroxy group.[7] The multiplet at δ 108 p.p.m. due to C-8a also moved upfield slightly with D_2O (2.5 Hz, 0.03 p.p.m.) and sharpened to a triplet (*J ca.* 7 Hz) due to equal coupling to both 5-H and 7-H, as shown by irradiation of the aromatic protons when the triplet collapsed to a singlet. The doublet of multiplets centred on 112.0 p.p.m. also showed a small upfield shift and sharpened with D_2O, allowing its assignment to C-7; and on irradiation of the protons of the aromatic methyl this resonance collapsed to a doublet of doublets (*J* 160 Hz, due to 1-bond coupling to 7-H) due to the removal of the 3-bond coupling to the methyl hydrogens, the residual coupling (*J ca.* 7 Hz) being the 3-bond coupling to 5-H. Similarly the doublet of multiplets due to C-5 (δ 107.1 p.p.m.) collapsed to a doublet of doublets (*J* 160 and 6 Hz) on irradiation of the 6′-methyl protons. This irradiation also sharpened the quartet (*J* 6 Hz) at 147.5 p.p.m. to an intense singlet and it must be due to C-6. Irradiation of the aromatic hydrogens also caused a sharpening and enhancement of the C-8 resonance at 160 p.p.m., with removal of the 2-bond coupling (*J* 2.4 Hz) to 7-H and enhancement of the resonance at 155.9 p.p.m. due to C-10a. Finally, the C-9 carbonyl singlet resonance at 181.8 p.p.m. also showed a small upfield shift on addition of D_2O (2.5 Hz, 0.03 p.p.m.). This evidence leaves no doubt that the orientation of substituents around the aromatic ring is as defined by structure (5).

The presence of a methoxycarbonyl group in these compounds is compatible with an i.r. absorption at *ca.* 1 740 cm^{-1} an OMe signal in the ^{1}H n.m.r. spectra at δ *ca.* 3.8 p.p.m. and ^{13}C signals at *ca.* 173 and 53.5 p.p.m., due to the carbonyl and methoxy carbon atoms respectively. In the fully coupled spectrum of the α-compound the signal at 173 p.p.m. is broad, presumably due to coupling to the methoxy hydrogens. Although specific decoupling was not carried out none of the other irradiations affected it, so by default the coupling must be to OMe.

Apart from the carbon resonances due to the CH(OH)-CH_2CH_2 fragment, which have the expected chemical shift values and multiplicities in the off-resonance decoupled spectrum, the only remaining ^{13}C-resonances are singlets at δ *ca.* 167 and 116.5 p.p.m. in the off-resonance decoupled spectrum. These chemical shift values are entirely consistent with those expected for the residue C=C(OH). Furthermore, in the fully coupled spectrum of the α-compound the C-1 resonance at δ 166.7 p.p.m. appeared as a pseudo-quartet (*J* 5 Hz); this is due to coupling to the C-2 protons and one of the C-3 protons, as irradiation of the allylic methylene protons collapsed the C-1 signal to a doublet, and irradiation of the 3-methylene protons collapsed it to a triplet. Irradiation of the C-2 protons also sharpened and enhanced the broadish signal at δ 116.6 p.p.m. due to C-9a. This work, together with previous considerations, completely defines the structural unit $-CO-C=C(OH)CH_2CH_2CH(OH)$ in the metabolites. Hence, the proposed structures (5) and (6) for the diversonolic esters are entirely compatible with all the evidence. They also bear an obvious structural and biosynthetic relationship to diver-



(12)



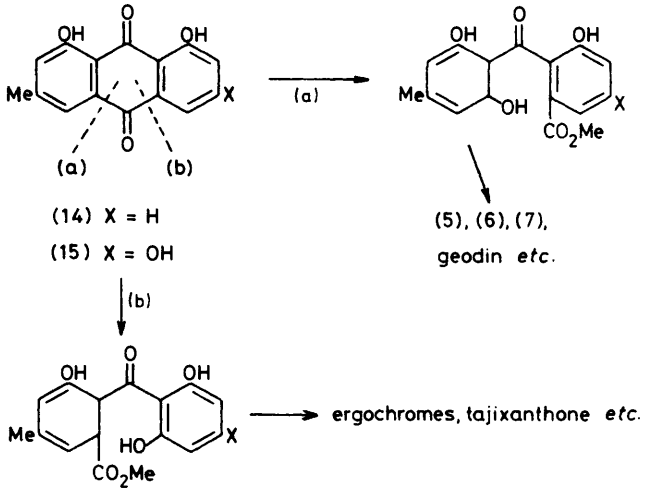
(13)

sonol (7), previously isolated from this organism, and for which the absolute configuration has been established by *X*-ray crystallographic evidence.[1]

Although there seems little doubt that structures (5) and (6) uniquely satisfy the spectroscopic properties of the α- and β-diversonolic esters, there are some anomalies in the chemistry which are worthy of comment. In particular, the properties of the enolic β-diketone system (C_1, C_{9a}, C_9) appear to be exceptional. Thus the chemical shift of the exchangeable enolic proton [4.76 and 4.57 p.p.m. in compounds (5) and (6) respectively] and the absence of a colouration with ferric chloride in the monomethyl ethers (8) and (9) show the absence of hydrogen bonding in this group. This is in direct contrast to those ergochromes [8] which contain the structural units (12) and (13) in their dimeric structures, and therefore possess an analogous enolised β-diketone system. The chemical shift of the enolic proton in these compounds is typically at δ 11.5 p.p.m. and the dimethyl ethers, prepared with diazomethane, give positive ferric reactions. Furthermore, these enolic hydroxy groups can normally be methylated by use of an excess of diazomethane or acetylated with acetic anhydride, although the isolated yields of these derivatives are low.[8,9]

The determination of the relative configurations of the α- and β-diversonolic esters is based on LIS studies on the parent compounds. It was anticipated by analogy with previous work [10] that the principal site of co-ordination of Eu(fod)$_3$ would be the secondary alcoholic hydroxy group at C-4. Examination of the LIS induced ^{13}C-shifts shows that this is a correct conclusion since the largest values for both compounds are associated with the carbon atoms in this region of the molecules, *i.e.* C-4, C-4a, and C-4a'. Furthermore, since the ester carbonyl (C-4a') experiences a much larger induced shift in the α-component (13.4 p.p.m.) as compared with that in the β-component (6.2 p.p.m.), it is concluded that the 4-hydroxy and 4a'-methoxycarbonyl groups are *cis*-related in α-diversonolic ester, as in structure (5) and *trans*-related in β-diversonolic ester, structure (6). The relative configurations of the corresponding groups in the ergochromes have usually been determined by comparison of the mass spectral intensities of the molecular ions and the base peaks due to loss of CO_2Me. It has been concluded [11] that the more prominent loss is facilitated by the less stable *cis*-orientation of the hydroxy and methoxycarbonyl groups, type (12), as compared with the *trans*-orientation, type (13). In our compounds although this prominent fragmentation is observed, the ratios for the α- and β-compounds are very similar and no stereochemical conclusions can be drawn from this evidence.

The alicyclic ring of the diversonolic esters would be expected to be relatively conformationally mobile. In the case of the parent *cis*-α-compound (5) and its monomethyl ether (8) the values of the *vic*-^{1}H-couplings between the C-3 methylene protons and the C-4 proton (*ca.* 11 and 4 Hz) are consistent with diaxial and axial : equatorial couplings of a pseudo-axial C-4 proton, and hence a pseudo-equatorial hydroxy group. However, in the corresponding diacetate (10) the corresponding couplings (*ca.* 5 and 6.5 Hz) indicate a pseudo-equatorial C-4 proton, and hence a pseudo-axial



Scheme.

acetoxy group. In the corresponding *trans*-β-compound (6) and its monomethyl ether (9) the observed C-3 to C-4 proton couplings are both small (*ca.* 5 and 6.5 Hz), suggesting a pseudo-equatorial C-4 proton and hence a pseudo-axial hydroxy group. However, on acetylation the corresponding couplings are again changed (*ca.* 4 and 11 Hz), similarly suggesting a conformational change in the alicyclic ring. Although the reasons for this are not entirely clear, they are probably associated with intramolecular hydrogen bonding between the C-4 hydroxy and C-4a' methoxycarbonyl groups in the parent *cis*-α-alcohol (5), which would be removed on acetylation.

It has been pointed out that diversonol (7) appears to be related biosynthetically to the sulochrin group of fungal metabolites,[2] which are derived by cleavage of an anthraquinone intermediate. In this case the 4a'-methyl group of diversonol must result from complete reduction of a carbonyl intermediate. Since in the diversonolic esters (5) and (6) the corresponding 4a'-carbon is unreduced and present as a methoxycarbonyl group, the anthraquinone derivation of these compounds is supported. It is interesting that such derivation would involve oxidative fission of an anthraquinone or anthrone precursor, *e.g.* chrysophanol (14) at (a) as shown in the Scheme. This corresponds with the biosynthesis of geodin, where emodin (15) has been shown to be an intermediate.[12] On the other hand alternative oxidative fission at (b) has been demonstrated in the biosynthesis of the ergochromes [13] and has also been proposed to account for the results of feeding experiments on the fungal xanthone, tajixanthone, and related compounds.[10]

Experimental

Unless otherwise stated i.r. spectra were measured in Nujol with a Perkin-Elmer 257 instrument and u.v. spectra in ethanol (95%) with a Pye-Unicam SP 8-100 instrument. ^{1}H N.m.r. spectra were measured in $CDCl_3$ with Me_4Si as internal standard either at 220 MHz with a Perkin-Elmer R34 instrument or at 360 MHz with a Bruker WH360 instrument. ^{13}C N.m.r. spectra were similarly measured either at 25.19 MHz with a Varian XL-100 or at 90.6 MHz with a Bruker WH360 instrument. Mass spectra were determined with an AEI MS-12 instrument at 70 eV and accurate mass measurements with an AEI MS-9 instrument. Preparative layer chromatography (p.l.c.) was performed on 1-mm thick layers of Kieselgel 60 PF_{254} (Merck) on glass plates (20 × 20 cm.), activated at 110 °C for 12 h.

Isolation of α- and β-Diversonolic Esters (5) *and* (6).—*Penicillium diversum* (no. 946 in I.C.I. collection) was grown from spore suspensions in static cultures for 29 days at 25 °C in 100 flat vessels (*ca.* 1 l capacity) each containing Raulin-Thom medium (500 ml). After removal of the mycelium the culture broth was exhaustively extracted with chloroform (15 l). After concentration, the resultant extract was washed repeatedly with 2M-sodium hydrogen carbonate, and then water. After drying (Na_2SO_4) the chloroform solution was evaporated and the residue (2.2 g) was subjected to p.l.c. using ethanol–chloroform (1 : 25) as developing solvent. The band, R_F 0.15, was isolated, extracted and further separated by p.l.c., developing with ether, to give two bands. That with higher R_F was separated and extracted to give *α-diversonolic ester* (5) which formed prisms (*ca.* 100 mg) from ethyl acetate, m.p. 182 °C; $[\alpha]_D$ −22.6° (*c* 0.23 in $CHCl_3$), $\nu_{max.}$ (CCl_4) 3 570, 3 490, 1 740, 1 655 and 1 625 cm^{-1}; $\lambda_{max.}$ 328 (ε 3 800), 261 (7 400), and 250 nm (5 000) (Found: C, 59.9; H, 5.2; M^+, 320.089. $C_{16}H_{16}O_7$ requires C, 60.0; H, 5.0%; *M*, 320.090). The lower R_F band from above was re-purified by p.l.c., developing with ethyl acetate, to give *β-diversonolic ester* (6) as a gum (20 mg), $[\alpha]_D$ +40.0° (*c* 0.12 in $CHCl_3$); $\nu_{max.}$ (CCl_4) 3 540, 2 480, 1 750, 1 660, 1 650, 1 620, and 1 600 cm^{-1}; $\lambda_{max.}$ 326 (2 500), 259 (10 500), 250 (10 600), 239 (17 300), and 228 nm (17 000) (Found: M^+, 320.088). Both compounds gave intense red-brown colourations with ferric chloride solution.

Methylation of Diversonolic Esters.—The parent compounds (5) and (6) (60 and 30 mg respectively) were separately methylated in chloroform with an excess of ethereal diazomethane at 0 °C for 48 h. 8-O-*Methyl-α-diversonolic ester* (8) was purified by p.l.c. with ether as developing solvent, and obtained as a gum (35 mg); $\nu_{max.}$ (CH_2Cl_2) 3 660, 3 550, 3 480, 1 725, 1 700, and 1 605 cm^{-1}; $\lambda_{max.}$ 314 (4 300), 254 (11 100), and 233 nm (22 300) (Found: C, 64.1; H, 5.5; M^+, 334.101. $C_{17}H_{18}O_7$ requires C, 64.1; H, 5.4; *M*, 334.095). Similarly obtained, 8-O-*methyl-β-diversonolic ester* (9) was a gum (16 mg); $\nu_{max.}$ (CH_2Cl_2) 3 680, 3 540, 3 520, 3 450, 1 730, 1 695, and 1 610 cm^{-1} (Found: M^+, 334.100 Neither compound gave a colour with ferric chloride solution.

Acetylation of Diversonolic Esters.—The parent compounds (5) and (6) (40 mg of each) were each acetylated with acetic anhydride (0.5 ml) and pyridine (2 ml) at room temperature overnight. After removal of the bulk of the reagents under reduced pressure the residues were dissolved in chloroform (10 ml) and the solutions washed with water (3 × 5 ml), dried (Na_2SO_4), and evaporated. The residues each showed one major and one minor component on t.l.c. and were therefore purified by p.l.c. with ether as developing solvent. *Di-O-acetyl-α-diversonolic ester* (10) was an amorphous solid (22 mg), $[\alpha]_D$ −11.2° (*c* 0.16 in $CHCl_3$); $\nu_{max.}$ 3 480br 1 740, and 1 640 cm^{-1} (Found: M^+, 404.113. $C_{20}H_{20}O_9$ requires *M*, 404.111). Di-O-acetyl-β-diversonolic ester (11) was an amorphous solid (16 mg), $[\alpha]_D$ +30.0° (*c* 0.13 in $CHCl_3$); $\nu_{max.}$ 3 480br, 1 780, 1 740, 1 715, and 1 640 cm^{-1} (Found: C, 59.3; H, 4.7. $C_{20}H_{20}O_9$ requires C, 59.4; H, 5.0%). Neither compound gave a colouration with ferric chloride solution.

Isolation and Characterisation of Lichenxanthone and Alternariol Monomethyl Ether.—A portion (40 g) of the dried mycelium (200 g) from the above growth was continuously extracted with chloroform for 24 h. The extract was separated by p.l.c. with chloroform into two principal components. The faster running band on isolation and extraction gave lichenxanthone as cream needles (80 mg) from chloroform, m.p. 190 °C (lit.,[3] m.p. 186—187 °C), with identical i.r. and u.v. spectral bands to those published,[14] δ_H 13.3 (s, 1 H, exchangeable with D_2O, phenolic OH) 6.68 (s, 2 H, ArH), 6.32 (s, 2 H, ArH), 3.90 (s, 3 H, OMe), 3.88 (s, 3 H, OMe), and 2.85 p.p.m. (s, 3 H, Ar*Me*) (Found: C, 67.0; H, 4.85%; M^+, 286.083. Calc. for $C_{16}H_{16}O_5$: C, 67.1; H, 4.9%; *M*, 286.084). Prepared with methyl iodide and potassium carbonate in acetone under reflux for 10 h the methyl ether separated as plates from ether, m.p. 155 °C (lit.,[15] m.p. 155—157 °C); δ_H 6.65 (s, 2 H, Ar*H*), 6.41 (d, 1 H, *J* 2 Hz, ArH), 6.32 (d, 1 H, *J* 2 Hz, ArH), 3.97 (s, 3 H, OMe), 3.89 (s, 3 H, OMe), 3.88 (s, 3 H, OMe), and 2.85 (s, 3 H, Ar*Me*).

The slower moving band from above on isolation and extraction gave alternariol monomethyl ether (4), needles (45 mg) from chloroform, m.p. 270—272 °C (decomp.) [lit.,[4] m.p. 267 °C (decomp.)], with identical i.r. and u.v. bands to those published,[14] δ_H ([$^2H_6$]acetone), 9.27 (s, 1 H, exchangeable with D_2O, H-bonded O*H*), 7.31, 6.82, 6.73, 6.58 (all d, each 1 H, *J* 1.5 Hz, Ar*H*), 3.98 (s, 3 H, OMe), and 2.78 (s, 3 H, Ar*Me*) (Found: M^+, 272.067. Calc. for $C_{15}H_{12}O_5$: *M*, 272.071). Methylation of this compound (50 mg) in chloroform with an excess of ethereal diazomethane at 0 °C for 12 h gave a mixture of two components, separated by p.l.c. in chloroform into di-*O*-methylalternariol, needles (15 mg) from chloroform m.p. 183 °C (lit.,[16] m.p. 186°), δ_H 11.9 (s, 1 H, exchangeable with D_2O, H-bonded OH), 7.24 (d, 1 H, J 2 Hz, ArH), 6.72 (s, 2 H, ArH), 6.53 (d, 1 H, *J* 2 Hz, ArH), 3.90 (s, 3 H, OMe), 3.85 (s, 3 H, OMe), and 2.78 (s, 3 H, Ar*Me*) (Found: M^+, 286. Calc. for $C_{16}H_{14}O_5$: *M*, 286) and tri-*O*-methylalternariol, needles (26 mg) from ethanol, m.p. 142 °C, then 165 °C after cooling and re-melting (lit.,[4] m.p. 140 °C, then 162.5—164 °C), δ_H 7.28 (s, 1 H, Ar*H*), 6.70 (s, 1 H, Ar*H*), 6.69 (d, 1 H, *J* 1.5 Hz, Ar*H*), 6.51 (d, 1 H, *J* 1.5 Hz, ArH), 3.90 (s, 3 H, O*Me*), 3.93 (s, 3 H, O*Me*), 3.82 (s, 3 H, O*Me*), and 2.78 p.p.m. (s, 3 H, Ar*Me*). An authentic sample of alternariol, kindly supplied by Professor R. Thomas, University of Surrey, was similarly methylated to give di- and tri-*O*-methylalternariol with identical m.p.s and ^1H n.m.r. spectra.

References

1 W. B. Turner, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1621.
2 T. J. Simpson, D. J. Stenzel, A. J. Bartlett, E. O'Brien, and J. S. E. Holker, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2687.
3 Y. Asahina and H. Nogami, *Bull. Chem. Soc. Jpn.*, 1942, **17**, 202.
4 H. Raistrick, C. E. Stickings, and R. Thomas, *Biochem. J.*, 1953, **55**, 421.
5 L. J. Bellamy, 'The Infra-red Spectra of Complex Molecules,' Methuen, London, 1958, p. 143.
6 D. H. Williams and I. Fleming, 'Spectroscopic Methods in Organic Chemistry,' McGraw-Hill, London, 1980, p. 136.
7 F. W. Wehrli, *J. Chem. Soc., Chem. Commun.*, 1975, 663.
8 P. S. Steyn, *Tetrahedron*, 1970, **26**, 51; J. W. Hooper, W. Marlow, W. B. Whalley, A. D. Borthwick, and R. Bowden, *J. Chem. Soc. Perkin Trans. 1*, 1971, 3580, and references cited therein.
9 C. H. Howard and R. A. W. Johnstone, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2440.
10 J. S. E. Holker, R. D. Lapper, and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2135.
11 B. Franck, E. M. Gottschalck, U. Ohnsorge, and F. Hüper, *Chem. Ber.*, 1966, **99**, 3842.
12 H. Fujimoto, H. Flasch, and B. Franck, *Chem. Ber.*, 1975, **108**, 1224.
13 B. Franck, *Angew. Chem., Int. Ed. Engl.*, 1969, **8**, 258; D. Gröger, D. Erge, B. Franck, U. Ohnsorge, H. Flasch, and F. Hüper, *Chem. Ber.*, 1968, **101**, 1970.
14 T. M. Harris and J. V. Hay, *J. Am. Chem. Soc.*, 1977, **99**, 1631.
15 P. K. Grover, G. D. Shah, and R. C. Shah, *J. Sci. Ind. Res.*, 1956, **15B**, 629.
16 G. G. Freeman, *Phytochemistry*, 1965, **5**, 719.

Received 27*th September* 1982; *Paper* 2/1637