Metabolic Products of *Phomopsis oblonga*. Part 2.[1] Phomopsolide A and B, Tiglic Esters of Two 6-Substituted 5,6-Dihydro-5-hydroxypyran-2-ones

John Frederick Grove
School of Molecular Sciences, University of Sussex, Brighton, Sussex BN1 9QJ

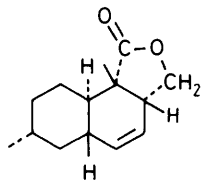
Phomopsolide A and B, two boring/feeding deterrents for elm bark beetles, produced *in vitro* by the fungus *Phomopsis oblonga*, are shown to be (5*S*,6*S*)-5,6-dihydro-6-(4-hydroxy-3-oxopent-1*Z*-enyl)-5-(2-methylbut-2*E*-enoyloxy)-2*H*-pyran-2-one and (5*S*,6*S*)-5,6-dihydro-6-[(3*S*,4*S*)-3,5-dihydroxypent-1*E*-enyl]-5-(2-methylbut-2*E*-enoyloxy)-2*H*-pyran-2-one, respectively. Another active compound, (−)-6-(4-hydroxy-3-oxopent-1*E*-enyl)-2*H*-pyran-2-one, was also isolated but is considered to be an artefact.

Phomopsis oblonga (Desm.) Trav. produces *in vitro* a number of boring/feeding deterrents for Scolytid beetles.[1,2] One of these deterrents, oblongolide, produced in surface culture on malt extract medium by a strain, number 118, of *P. oblonga* Group 1 was shown [1] to be the novel norsesquiterpene γ-lactone (**1**). This paper describes the isolation and structure determination of a further two active compounds, the C_{15} dihydropyrones (phomopsolide A (**2**) and phomopsolide B (**3a**), produced by the same strain on the same medium but in shake as well as in surface culture. An active C_{10} pyrone (**7**) was also isolated but was shown to be an artefact.

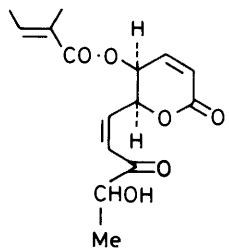
Column chromatography of the neutral portion of the ethyl acetate extract of the culture filtrate from a shake fermentation furnished two fractions which showed activity in the bioassay.[2] Further chromatographic purification of these fractions yielded three active compounds of composition $C_{10}H_{10}O_4$, $C_{15}H_{18}O_6$ (30—42 mgl^{-1}) and $C_{15}H_{20}O_6$ (21—35 mgl^{-1}), but appreciable variation in the yield of the C_{15} compounds was obtained in replicate fermentations.

The $C_{10}H_{10}O_4$ compound contained two C=O groups ($\nu_{max.}$ 1 720, 1 690 cm^{-1}) and a secondary hydroxy group [$\nu_{max.}$ 3 450 cm^{-1}; δ_C 72.8; δ_H 4.48q (*J* 7.1 Hz)]. One C=O group ($\nu_{max.}$ 1 690 cm^{-1}; δ_C 200.5) was conjugated with a *trans* disubstituted ethylenic double bond [δ_H 7.16AB (*J* 15.4 Hz); δ_C 124.4, 133.0]. The partial structure (**13**) suggested by the ^{1}H n.m.r. spectrum (Table 1) was confirmed by the formation of acetaldehyde, isolated as the dinitrophenylhydrazone, on oxidation with periodate. This oxidation also yielded a C_8 fission product, the carboxylic acid (**10**). The u.v. spectrum of the $C_{10}H_{10}O_4$ compound [$\lambda_{max.}$ 240, 342 nm (log ε 4.11, 4.23)] suggested an α-pyrone ring (C=O, $\nu_{max.}$ 1 720 cm^{-1}; δ_C 160.4) conjugated with the ethylenic linkage. These assignments account for all four oxygen atoms in the molecule which is represented by structure (**7**). In the ^{1}H n.m.r. spectrum (Table 1) the chemical shifts and coupling constants were in agreement with those recorded for analogous 6-substituted α-pyrones, *e.g.* sibirinone (**11**)[3] and 6-pent-1-enyl-α-pyrone (**12**).[4] The ^{13}C resonances (Table 2) likewise generally agreed with those recorded[5] for analogous structures. Although the optical rotation at 589 nm was close to zero, the pyrone (**7**) was optically active.

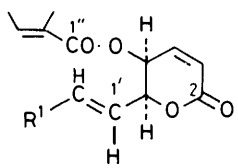
The $C_{15}H_{20}O_6$ compound, phomopsolide B, contained two >CHOH groups (δ_H 3.62, 3.93; δ_C 76.2, 70.6) and formed a diacetate (>CHOAc: δ_H 5.05, 5.37). The remaining four oxygen atoms were contained in two ester (or lactone) groups ($\nu_{max.}$ 1 728, 1 710 cm^{-1}, δ_C 162.6, 166.7). A feature of the ^{1}H n.m.r. spectrum was (disregarding allylic and homoallylic couplings, see below) the number of one-hydrogen double doublets (see Table 1); taking into account both the chemical shift for each resonance and the coupling constants between olefinic hydrogens, the appropriate decoupling experiments established

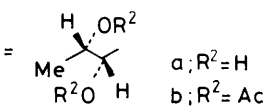


(**1**)



(**2**)



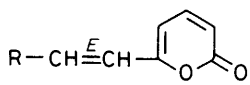
(**3**) ; R^1 =  a ; R^2 = H, b ; R^2 = Ac

c ; R^2 = *p*-MeOC$_6$H$_4$CO

(**4**) ; R^1 = MeCHOHCO

(**5**) ; R^1 = MeCOCO

(**6**) ; R^1 = CHO



(**7**) R = MeCHOHCO

(**8**) R = MeCOCHOH

(**9**) R = MeCOCO

(**10**) R = CO_2H

(**11**) R = Me

(**12**) R = Pr^n

the presence of the partial structure (**14**). The remaining seven hydrogens were contained in one CMe and one CHMe group, and their chemical shifts indicated the presence of the partial structure (**15**). In this structure the β-olefinic hydrogen is deshielded by 0.5—0.9 p.p.m. by the *cis*-alkoxycarbonyl group,[6] and the chemical shifts indicated a tiglate (*E*) rather than an angelate residue. This was confirmed by the isolation of tiglic acid (**15**; R = H) on mild alkaline hydrolysis. Partial structures (**14**) and (**15**) contain all the carbon atoms of phomopsolide B which is monocyclic. They can be combined in only two ways; the i.r. spectrum is consistent only with the six-membered lactone (dihydropyrone) ring structure (**3a**). The ^{1}H n.m.r. spectrum (Table 1) showed allylic coupling between the hydrogens at positions 1′ and 3′ (1.1 Hz) and 2′ and 6 (1.1 Hz), and homoallylic coupling (0.9 Hz) between positions 6 and 3′. There was also allylic coupling to 3″-H in the tiglate residue. The ^{13}C resonances (Table 2) were in close agreement with those recorded for the model compounds (**15**; R = Me) and (**17**). As expected, oxidation of the α-diol (**3a**) with periodate gave acetaldehyde and the αβ-unsaturated C_{13} aldehyde (**6**), $\lambda_{max.}$ 215 nm.

Table 1. ^{1}H N.m.r. resonances (δ, *J* in parentheses[a]) for compounds (**2**), **3a**), and (**7**) and their derivatives

Compound	Solvent (MHz)	Position 3	4	5	6	1′	2′	3′	4′	5′	2″-Me	3″	4″	Other
(**3a**)	$CDCl_3$ (360)	6.23d (9.7)	7.00dd (9.7, 5.6)	5.37dd (5.6, 3.0)	5.10dd (3.0, 5.7)[b,c]	5.89dd (5.7, 15.7)[d]	6.01dd (15.7, 5.6)[b]	3.93t (6.0)[c,d]	3.62dq (6.3)[e]	1.17d (6.3)	1.81s	6.90q (7.5)[f]	1.80d (7.5)	2.3, 2.4 (OH)
(**3a**)	CD_3OD (360)	6.22d (9.7)	7.10dd (9.7, 5.6)	5.40dd (5.6, 3.0)	5.22dd (3.0, 6.3)[b,c]	5.88dd (6.3, 16.0)[d]	6.02dd (16.0, 5.7)[b]	3.91dd (5.7, 6.3)[c,d]	3.60dq (6.3)[e]	1.08d (6.3)	1.81s[f]	6.89q (7.5)[f]	1.80d (7.5)	
(**3c**)	CD_3OD (360)	6.22d (9.7)	7.05dd (9.7, 5.6)	5.43dd (5.6, 3.0)	5.25m	6.00dd (5.4, 15.7)[d]	6.15dd (15.7, 6.2)[b]	5.69t (6.3)	5.34dq (6.4)[e]	1.38d (6.4)	1.62s[f]	6.70q (7.1)[f]	1.54d (7.1)	3.83, 3.84 (2 OMe) 7.92, 8.95 (2AA′BB′)
(**3b**)	$CDCl_3$ (90)	6.25d	7.02dd	5.37m	5.05m	5.90m	5.90m	5.37m	5.05m	1.16d	1.80s	6.91m	1.78d	2.0 (2 OAc)
(**2**)	$CDCl_3$ (360)	6.25d (9.7)	7.10dd (9.7, 5.9)	5.65dd (5.9, 2.8)	5.97dd (2.8, 4.1)[b]	6.43dd (4.4, 11.8)[g]	6.41d (11.8)[g]		4.36q (7.1)	1.39d (7.1)	1.79s	6.8q (7.3)[f]	1.80d (6.3)	3.4 (OH)
(**4**)	$CDCl_3$ (90)	6.26d (9.6)	7.05dd (9.7, 5.4)	5.52dd (5.4, 3.4)	5.30m	6.95dd (2.8, 11.5)	6.70d (15.5)		4.45q (7.0)	1.39d (7.0)	1.81s	*ca*6.9m	1.80d (6.0)	4.3 (OH)
(**7**)	$CDCl_3$ (360)	6.43d (9.0)	7.44dd (9.0, 6.9)	6.43d (6.9)		7.22d (15.4)	7.11d (15.4)		4.48q (7.1)	1.45d (7.1)				3.5 (OH)

[a] First-order approximations from line separations unless stated otherwise. [b] $J_{6,2'}$ 1.1 Hz. [c] $J_{6,3'}$ 0.9 Hz. [d] $J_{1',3'}$ 1.1 Hz. [e] $J_{3',4'} = J_{4',5'} = 6.3$—6.4 Hz. [f] $J_{3',2''\text{-Me}}$ 1.0—1.5 Hz. [g] By computer simulation, $\Delta\delta_{1',2'} = 0.02$.

MeCHOHCOCH $\overset{E}{=}$ CH—

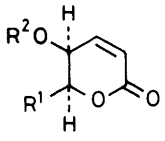
(**13**)

MeCHOHCHOHCH $\overset{E}{=}$ CHCH—CHCH $\overset{Z}{=}$ CHCO$_2$R
[O] [O]

(**14**)

MeCH $\overset{E}{=}$ C(Me)CO$_2$R

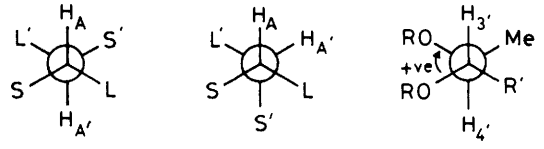
(**15**)



(**16**) R^1 = MeCH=CH, R^2 = H

(**17**) R^1 = MeCH—CH, R^2 = Ac

(**18**) R^1 = Me, R^2 = H



(**19**) (**20**) (**21**)

R = *p*-MeOC$_6$H$_4$CO

Both *cis*- and *trans*-5,6-disubstituted 5,6-dihydro-α-pyrones occur in nature and can readily be distinguished by the ^{1}H n.m.r. spectra on the basis of the values of the coupling constants for the hydrogens at positions 4, 5, and 6. The assignment of the *cis* configuration to phomalactone (**16**)[7] and asperline (**17**)[8] was confirmed by an *X*-ray crystallographic analysis of a derivative of the latter compound.[8] The values (Table 1) for $J_{4,5}$ and $J_{5,6}$ in compound (**3a**) were in close agreement with those reported for phomalactone and asperline, from which a *cis* arrangement of the 5- and 6-substituents may be deduced. On the reasonable assumption that the bulky 6-substituent is quasi-equatorial, the absolute configuration of 6-substituted 5,6-dihydropyrones can be assigned by use of the lactone sector rule.[9,10] Like phomalactone,[7] phomopsolide B showed a positive Cotton effect in the c.d. curve at 264 nm indicative of 6β-substitution.

Successful application of the exciton chirality method[11] to the determination of the absolute configuration of acyclic α-glycols turns on (*a*) the correct identification of the large (LL′) and small (SS′) groups in the generalised Newman projections, (**19**) and (**20**) for the rotamers which, respectively, from a consideration of non-bonding interactions, make the greatest contribution to the *erythro*- and *threo*-isomers; and (*b*) the interpretation attached to the value of the coupling constant $J_{AA'}$. The latter should be determined for the benzoate derivative selected and in the same solvent that is used for the c.d. measurements.

In this series of compounds, replacing $CDCl_3$ by CD_3OD as solvent and converting the glycol (**3a**) into the bis-anisyl derivative (**3c**) did not alter $J_{3',4'}$. The value (6.3 Hz) for $J_{3',4'}$ in the derivative (**3c**) indicated that the hydrogens were *trans* rather than *gauche*.[12] The derivative (**3c**), $\lambda_{max.}$ 260 nm, showed two intense Cotton effects, of similar amplitude but of opposite sign, at 268 nm (+ve) and 248 nm (−ve) in the c.d. curve. Only the rotamer (**21**) (or its mirror image) has *trans* hydrogens and is capable of showing Cotton effects separated by Davidov splitting. Whilst this rotamer falls into category (**19**), it is derived from a *threo*-α-glycol, and the positive chirality[11] indicates a (3′*S*,4′*S*) configuration.

Compared with the phomopsolide B (**3a**), phomopsolide A contained an additional C=O group (δ_C 202.1) but only one >CHOH group (δ_C 73.2; δ_H 4.36q, *J* 7.1 Hz). Although the ^{13}C n.m.r. spectrum (Table 2) was consistent with a close

Table 2. ^{13}C N.m.r. resonances for compounds (**2**), (**3a**), and (**7**) and some analogues in $CDCl_3$

Compound	Position														
	2	3	4	5	6	1′	2′	3′	4′	5′	1″	2″	3″	4″	2″-Me
(**3a**)	162.6	124.8[a]	141.1	63.4	78.7	135.0	124.6[a]	70.6	76.2	18.8	166.7	127.5	139.8	14.6	12.0
(**2**)	162.0	124.5[a]	141.1	63.2	77.0	143.0	124.1[a]	202.1	73.2	19.6	166.5	127.5	139.6	14.5	12.0
(**15**; R = Me)											168.2	128.2	136.7	13.8	11.5
(**17**)[13]	161.5	124.7	140.6	62.1	78.3										
(**7**)	160.4	118.7	142.5	110.9	156.4	133.0	124.4	200.5	72.8	19.7					
2-Pyrone[5]	162.0	116.7	144.3	106.8	153.3										

[a] Assignments may be reversed.

relationship between the two metabolites, the ^1H n.m.r. spectrum revealed a large (0.8 p.p.m.) deshielding of the 6-H resonance by the C=O group, consistent only with a *cis* configuration of the 1′-ene ($J_{1',2'}$ 11.8 Hz) as in structure (**2**). Despite this structural difference revealed by the spectroscopic data, oxidation of both dihydropyrones with the chromic oxide–sulphuric acid reagent[14] gave the same gummy α-diketone (**5**) characterised, after preparative t.l.c., as the pyrone (**9**). Although the α-glycol (**3a**) was stable in chloroform in the presence of silica gel, both 3′-oxo compounds (**2**) and (**5**) were converted, partially in the case of (**3**) and quantitatively in the case of (**5**), into the corresponding pyrones (**7**) and (**9**) by the elimination of the elements of tiglic acid. It follows that pyrone (**7**) is unlikely to be a genuine metabolic product of *P. oblonga*. The formation of the pyrone (**7**) from phomopsolide A appeared to be preceded by isomerisation of the 1′-ene to the *trans*-olefin (**4**), $J_{1',2'}$ 15.5 Hz, which was sometimes isolated. The mechanism of the formation of the racemic pyrone (**8**), isolated on only one occasion following oxidation of the diol (**3a**) and chromatographic purification of the product, is uncertain, but may involve an enediol intermediate.

Electron impact mass spectra at 70 ev of the 5-hydroxy-5,6-dihydropyrones esterified with tiglic acid showed base peaks at *m/z* 83 ($C_5H_7O^+$), but molecular ions were absent. These were obtained (as MH^+ peaks) by chemical ionisation using ammonia as reagent gas. The fragmentation pathway for the 6-substituted pyrones was straightforward (see Experimental section) combining loss of CO from the pyrone ring with sequential fission of the C_5 side chain. After the initial loss of the elements of tiglic acid, the esterified 5-hydroxy-5,6-dihydropyrones showed spectra very similar to those given by the corresponding pyrones.

Phomopsolide A and B were also produced in surface culture by strain 118, but in much lower yield (14 and 3 mgl^{-1} respectively). Phomopsolide B was produced by *Phomopsis* sp. strain 124 (*ex.* sycamore)[1] in shake (8 mgl^{-1}) but not in surface culture on malt extract medium. With strain 123 (*ex.* ash) the reverse situation was found and phomopsolide B was produced in high yield (63 mg ml^{-1}) in surface but not at all in shake culture. Neither of these two strains made phomopsolide A. Neither phomopsolide A nor phomopsolide B was produced by the less-common Group 2 (strain 119)[1] of *P. oblonga* under any of these culture conditions.

6-Pentyl-2-pyrones and the corresponding 5-hydroxy-5,6-dihydro-2-pyrones derived from a pentaketide precursor frequently occur among the secondary metabolic products of fungi, but esterification (of the 5-hydroxy compounds) with carboxylic acids other than acetic is unusual. Indeed, although esters of tiglic acid are commonly isolated from higher plants they have not hitherto been obtained from fungi. As the free acid was isolated[15] from crude penicillin, it is presumed to be a metabolic product of *Penicillium notatum*.

Osmundalactone (**18**),[16] the aglycone of the glycoside osmundalin, from the fern *Osmunda japonica*, has recently[17] been shown to be a feeding deterrent for larvae of the butterfly *Eurema hecabe mandarina*. This suggests that the substituted five-carbon side chain and esterification of the 5-hydroxy group in structure (**3a**) may not be essential for insect feeding deterrent in 5-hydroxy-5,6-dihydro-2-pyrones.

Experimental

M.p.s were taken on a Kofler hot-stage apparatus and are corrected. Unless otherwise stated, i.r. spectra were determined for mulls in Nujol, and u.v. spectra and c.d. measurements were determined in methanol. N.m.r. spectra were obtained in $CDCl_3$ or CD_3OD with $SiMe_4$ as internal standard. Molecular weights were taken from the mass spectra. NH_3 was used to obtain chemical ionisation mass spectra; the fragmentations recorded (% base peak in parentheses) are from the electron impact spectra. In analytical t.l.c., Merck silica gel 60 F_{254} was used with chloroform–methanol (95:5). Merck silica gels 7 739 and 7 734 were used in preparative t.l.c. (0.1-cm layer) and column chromatography respectively. Light petroleum had b.p. 60—80 °C.

Extraction and Isolation of Metabolites from P. oblonga *Strain 118.—A. Shake culture.* From a typical fermentation on 2% malt extract medium harvested after 14 days, the culture filtrate (3.9 l, pH 5.6) was extracted with ethyl acetate. A portion (8 mg) of the extract, a brown oil (797 mg), was retained for bioassay[2] and the remainder, in benzene (10 ml), was chromatographed on a column (50 × 1.2 cm) of silica gel (24 g) made up in benzene. After gummy fractions (total 39 mg) had been eluted with benzene (150 ml), benzene–methanol (200:1; 100 ml), and benzene–methanol (100:1; 100 ml), further elution with benzene–methanol (100:1; 100 ml) brought off a brown band. A portion (201 mg) of the recovered brown oil (351 mg) was subjected to p.l.c. on four pretreated 40 × 20 cm plates developed in chloroform–methanol (20:1) made up from freshly redistilled solvents. The material from two bands, (i) R_F 0.47 and (ii) R_F 0.35, visible in u.v. light, was recovered by extraction with redistilled chloroform. (5S,6S)-5,6-*Dihydro*-6-(4-*hydroxy*-3-*oxopent*-1Z-*enyl*)-5-(2-*methylbut*-2E-*enoyloxy*)-2H-*pyran*-2-*one* (**2**) (*phomopsolide A*) was obtained from (i) as a yellow oil (92 mg, 41 mg/l), R_F 0.47, $[\alpha]_D^{20}$ +491 (*c* 0.185) (Found: C, 60.7; H, 6.9%; MH^+ 295. $C_{15}H_{18}O_6$ requires C, 61.2; 6.2%; *M* 294); $\nu_{max.}$(film) 3 450br, 1 715br, 1 635br, 825, 730 cm^{-1}; $\lambda_{max.}$ 217 nm (log ε 4.19); *m/z* 167.0347 (12), 150.0347 (50), 149.0256 (33), 122.0393 (51), 100.0520 (37), 95.0155 (50), and 83.0488 (100) ($C_8H_7O_4^+$, $C_8H_6O_3^+$, $C_8H_5O_3^+$, $C_7H_6O_2^+$, $C_5H_8O_2^+$, $C_5H_3O_2^+$, and $C_5H_7O^+$ require 167.0344, 150.0317, 149.0239, 122.0368, 100.0524, 95.0133, and 83.0497 respectively). Band (ii) gave a semi-solid gum (37 g) which crystallised from

benzene in citrine prisms, m.p. 129—131 °C, R_F 0.35, of (−)-6-(4-*hydroxy*-3-*oxopent*-1E-*enyl*)-2H-*pyran*-2-*one* (**7**) (Found: C, 62.0; H, 5.3%; *M* 194.0571. $C_{10}H_{10}O_4$ requires C, 61.85; H, 5.2%; *M* 194.0579); $\nu_{max.}$ 3 450, 3 080w, 3 040w, 1 720, 1 690, 1 600, 1 537, 980, and 812 cm^{-1}; $\lambda_{max.}$ 240, 342 nm (log ε 4.11, 4.23). *m/z* 194 (5) 176.0476 (2), 166.0622 (11), 152 (20), 150.0315 (98), 149.0215 (40), 122.0374 (100), 95.0135 (36), 94.0416 (38), and 93.0345 (22) ($C_{10}H_8O_3^+$, $C_9H_{10}O_3^+$, $C_8H_6O_3^+$, $C_8H_5O_3^+$, $C_7H_6O_2^+$, $C_5H_3O_2^+$, $C_6H_6O^+$, and $C_6H_5O^+$ require 176.0473, 166.0630, 150.0317, 149.0239, 122.0368, 95.0133, 94.0419, and 93.0340 respectively); $[\alpha]_{589}^{20}$ −9°, $[\alpha]_{546}^{20}$ −14°, $[\alpha]_{436}^{20}$ −83° (*c*, 0.0915).

Although the dihydropyrone (**2**) gave only one spot on analytical t.l.c., p.l.c., particularly in bench (AnalaR) chloroform resulted both in isomerisation to the pent-1*E*-enyl compound (**4**) and in the formation of the pyrone (**7**), R_F 0.35. Yields were not reproducible, but in one such experiment the dihydropyrone (**2**) (224 mg) gave the isomer (**4**) (147 mg) and the crude pyrone (**7**) (50 mg). The (1*E*)-isomer (**4**) was a gum R_F 0.45 characterised by the ^1H n.m.r. spectrum (Table 1). The i.r. spectrum (film) was identical with that of the (1*Z*)-isomer (**2**).

Further elution of the column with benzene–methanol (100:1, 100 ml; 50:1, 200 ml; and 20:1, 100 ml) gave intractable gums (total 84 mg) but benzene–methanol (20:1, 100 ml) then furnished a resin (196 mg) which crystallised from benzene in felted needles, m.p. 93—95 °C (138 mg, 35 mgl^{-1}). Recrystallisation from ethyl acetate afforded (5S,6S)-5,6-*dihydro*-6-[(3S,4S)-3,4-*dihydroxypent*-1E-*enyl*]-5-(2-*methylbut*-2E-*enoyloxy*)-2H-*pyran*-2-*one* (**3a**) (*phomopsolide B*) as felted needles, m.p. 97 °C, R_F 0.19 (Found: C, 60.9; H, 6.8%; MH^+ 297. $C_{15}H_{20}O_6$ requires C, 60.8; H 6.8%; *M* 296); $\nu_{max.}$ 3 555, 3 340br, 1 725, 1 710, 1 650, 1 625, 990, 828, and 733 cm^{-1}; $\nu_{max.}$($CHCl_3$) 1 728, 1 710 cm^{-1}. End absorption in u.v., ε 18 000 at 210 nm; $[\alpha]_D^{20}$ +250° (*c*, 0.202); c.d., λ 214, 264 nm; Δε 35.9, 1.41; *m/z* 179 (35), 169 (12), 152.0458 (65), 151.0380 (55), 134.0361 (22), 123.0432 (22), 107.0493 (38), 95.0118 (35), and 83.0517 (100) ($C_8H_8O_3^+$, $C_8H_7O_3^+$, $C_8H_6O_2^+$, $C_7H_7O_2^+$, $C_7H_7O^+$, $C_5H_3O_2^+$, and $C_5H_7O^+$ require 152.0473, 151.0395, 134.0368, 123.0446, 107.0487, 95.0133, and 83.0497 respectively). It was recovered unchanged after stirring in AnalaR chloroform for 24 h at room temperature with silica gel 7739.

The *diacetate* (**3b**), prepared in pyridine with acetic anhydride during 3 days at room temperature, was a gum R_F 0.64 (Found: C, 60.0, H, 5.9%. $C_{19}H_{24}O_8$ requires C, 60.0; H, 6.4%); $\nu_{max.}$(film) OH absent; 1 720, 1 709, 1 645, 825, and 735 cm^{-1}. The *dianisate* (**3c**), prepared in benzene (1 ml), from the diol (**3a**) (15 mg), anisyl chloride (18 mg), and pyridine (30 mg), during 4 days at room temperature, followed by elution from a column of alumina (2 g, Woelm, neutral, grade III) with benzene–methanol (98:2), was a gum, R_F 0.67 (Found: MH^+ 565. $C_{31}H_{32}O_{10}$ requires *M* 564); $\nu_{max.}$(film) OH absent; 1 710br, 1 605, and 1 510; $\lambda_{max.}$ 211, 260 (log ε 4.52, 4.43); c.d., λ 218, 248, 268 nm; Δε +24.7, −6.7, +5.7.

B. Surface culture. After elution of oblongolide (**1**),[1] further elution of the silica gel column [1] with benzene–methanol (100:1) gave gums (150 ml, 18 mg) followed by a yellow oil (100 ml, 153 mg, 13.8 mgl^{-1}) identified by its i.r. and ^1H n.m.r. spectra as phomopsolide A (**2**). After intractable gums (60 mg) had been eluted with benzene–methanol (50:1; 200 ml), benzene–methanol (20:1; 100 ml) furnished a resin (79 mg) which gave phomopsolide B (**3a**) (28 mg) on crystallisation from benzene followed by ethyl acetate.

Isolation of Phomopsolides A and B from Other Phomopsis *Strains.*—Column chromatography as described above of the ethyl acetate extract (2.31 g) of the filtrate from a surface culture on malt extract of the ash strain 123 (6.0 l, 25 days) gave phomopsolide B (**3a**) (375 mg, 62.5 mgl^{-1}); but phomopsolide B (**2**) was absent as judged by a careful examination of the eluted fractions by ^1H n.m.r. spectroscopy. Likewise a shake culture of the sycamore strain 124 (4.1 l; 21 days) gave phomopsolide B (**3a**) (31 mg, 7.5 mgl^{-1}) but no phomopsolide A (**2**).

Oxidation of the Dihydropyrones (**2**) *and* (**3a**).—*A. With chromic oxide* (*a*). Phomopsolide B (**3a**) (15 mg) in acetone (1 ml) at 0 °C was treated with the chromic oxide–sulphuric acid reagent [12] (50 μl) during 15 min. After 15 min at room temperature most of the solvent was removed in a stream of N_2, and water (2 ml) was added. The resulting emulsion was extracted with ethyl acetate and the organic layer was washed with sodium hydrogen carbonate. On recovery the diketone (**5**) was obtained as a yellow gum (11 mg), R_F 0.57; $\nu_{max.}$(film) OH absent, 3 070w, 1 735, 1 712, 1 648, 975, 830, and 732 cm^{-1}; $\lambda_{max.}$ 217 nm. Recovery by extraction with chloroform after preparative t.l.c. afforded 6-(3,4-*dioxopent*-1E-*enyl*)-2H-*pyran*-2-*one* (**9**) which crystallised from benzene as orange prisms (5 mg), m.p. 155—156 °C, R_F 0.52 (Found: C, 62.5; H, 4.2%; MH^+ 193. $C_{10}H_8O_4$ requires C, 62.4; H, 4.2%; *M* 192); $\nu_{max.}$ 3 090w, 3 040w, 1 732, 1 710, 1 680, 1 622, 1 580, 1 565, 990, 835, and 808 cm^{-1}; $\lambda_{max.}$ 243, 347 nm (log ε 3.95, 4.13); *m/z* 192 (5), 164 (10), 150 (98), 149 (100), 122 (90), 121 (30), 95 (70), and 93 (85).

On one occasion when the oxidation was incomplete 6-(3-*hydroxy*-4-*oxopent*-1E-*enyl*)-2H-*pyran*-2-*one* (**8**) was also obtained as prisms from benzene–light petroleum, m.p. 114—115 °C, R_F 0.47 (Found: MH^+ 195. $C_{10}H_{10}O_4$ requires *M* 194); $\nu_{max.}$ 3 400br, 3 100w, 3060w, 1 730, 1 662, 1 595, 1 530, 970, and 800 cm^{-1}; $\lambda_{max.}$ 233, 332 nm (log ε 4.15, 4.17); $[\alpha]_{589-436}^{D}$ 0°; *m/z* 150 (85), 122 (75), 121 (52), 95 (100), and 94 (90).

(*b*) Phomopsolide A (**2**) (9 mg) was oxidised as described above with the same reagent (20 μl). The i.r. spectrum of the yellow gummy product (5 mg), R_F 0.57, was indistinguishable from that of the diketone (**5**), and preparative t.l.c. furnished prisms, m.p. 152—155 °C, R_F 0.52, of the pyrone (**9**).

B. With sodium periodate. Sodium metaperiodate (5%; 1 ml) was added to phomopsolide B (**3a**) (15 mg) in methanol (0.5 ml) and water (0.5 ml) at room temperature. A slow stream of N_2 was passed through the reaction mixture and into a trap containing 2,4-dinitrophenylhydrazine in 2M-hydrochloric acid. After 2 h the precipitate was collected, dried and recrystallised from ethanol, giving acetaldehyde 2,4-dinitrophenylhydrazone (4 mg), R_F 0.70, m.p. and mixed m.p. with an authentic specimen 158—160 °C. After standing 18 h at room temperature the reaction mixture had deposited needles (3 mg), m.p. 89—90 °C of 6-(3-*oxoprop*-1E-*enyl*)-5(2-*methylbut*-2E-*enoyloxy*)-5,6-*dihydro*-2H-*pyran*-2-*one* (**6**) (Found: C, 62.5; H, 5.6%; MH^+ 251. $C_{13}H_{14}O_5$ requires C, 62.4; H, 5.6%; *M* 250); $\nu_{max.}$ 3 100w, 3 060w, 1 730, 1 705, 1 678, 1 630, 972, 830, and 730 cm^{-1}; $\lambda_{max.}$ 220 nm (log ε 4.32); *m/z* 166 (12), 151 (33), 150 (30), 122 (20), 100 (40), 95 (42), 94 (35), and 83 (100).

Oxidation of the Pyrone (**7**).—*A. With chromic oxide.* The pyrone (5 mg) in acetone (0.5 ml) at 0 °C was oxidised with the chromic oxide–sulphuric acid reagent (15 μl) as described above. On the addition of water the product separated as needles (2 mg), m.p. 155—156 °C of the pyrone (**9**), identified by the i.r. spectrum.

B. With sodium periodate. The pyrone (2 mg) was oxidised as described above but in a stoppered tube at room temperature. After 24 h a stream of N_2 was passed through the reaction mixture which was kept at 60—70 °C for 20 min. A small precipitate of acetaldehyde 2,4-dinitrophenylhydrazone, R_F 0.70, was collected from the trap and was identified as described above. The cooled reaction mixture was extracted with ethyl acetate. Recovery afforded 3-(6-*oxo*-6H-*pyran*-2-*yl*)*prop*-1E-*enoic acid* (**10**) as prisms, m.p. 204—207 °C (decomp.) R_F 0.06 (Found: *M* 166.0266. $C_8H_6O_4$ requires *M* 166.0266); $\nu_{max.}$

3 200—2 400br, 3 100w, 3 050w, 1 710, 1 680br, 1 595, 1 535, 985, 810, and 670 cm^{-1}; $\lambda_{max.}$ 227, 330 nm (log ε 4.29, 4.28); *m/z* 166 (35), 138 (100), 121 (15), 95 (70), and 94 (32).

Hydrolysis of Phomopsolide B (**3a**).—The dihydropyrone (15 mg) in 0.1N-sodium hydroxide (2.00 ml) was left for 24 h at room temperature. Back titration to pH 6 with 0.1M-hydrochloric acid (1.05 ml, 2 equiv. acid liberated), followed by extraction with ethyl acetate gave a small (1 mg) intractable neutral fraction. The aqueous layer was acidified to pH 3 with concentrated hydrochloric acid and re-extracted with ethyl acetate. The recovered acid fraction (4 mg) showed only one spot (R_F 0.45) in t.l.c. and crystallised from benzene in prisms, m.p. 61—63 °C (lit., 64 °C) of tiglic acid, identified by the i.r. spectrum; $\nu_{max.}$ 3 500—2 500br, 1 690, 1 645, 790, and 740 cm^{-1}.

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References

1 M. J. Begley and J. F. Grove, *J. Chem. Soc., Perkin Trans. 1*, 1985, preceding paper.
2 N. Claydon, J. F. Grove, and M. Pople, *Phytochemistry*, in the press.
3 M. S. R. Nair and S. T. Casey, *Phytochemistry*, 1977, **16**, 1613.
4 M. O. Moss, R. H. Jackson, and D. Rogers, *Phytochemistry*, 1975, **14**, 2706.
5 W. V. Turner and W. H. Pirkle, *J. Org. Chem.*, 1974, **39**, 1935.
6 L. M. Jackman and R. H. Wiley, *J. Chem. Soc.*, 1960, 2886.
7 R. H. Evans, G. A. Ellestad, and M. P. Kunstmann, *Tetrahedron Lett.*, 1969, 1791.
8 K. Fukuyama, Y. Katsube, A. Noda, T. Hamasaki, and Y. Hatsuda, *Bull. Chem. Soc. Jpn*, 1978, **51**, 3175.
9 J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 1965, 7211.
10 G. Snatzke, *Angew. Chem., Int. Ed. Engl.*, 1968, **7**, 14.
11 N. Harada and K. Nakanishi, *Acc. Chem. Res.*, 1972, **5**, 257.
12 W. J. McGahren, G. A. Ellestad, G. O. Morton, M. P. Kunstmann, and P. Mullen, *J. Org. Chem.*, 1973, **38**, 3542.
13 S. Lesage and A. S. Perlin, *Can. J. Chem.*, 1978, **56**, 2889.
14 K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 1946, 39.
15 D. J. Cram and M. Tishler, *J. Am. Chem. Soc.*, 1948, **70**, 4238.
16 K. H. Hollenbeak and M. E. Kuchne, *Tetrahedron*, 1974, **30**, 2307.
17 A. Numata, K. Hokimoto, T. Takemura, and S. Fukui, *Appl. Ent. Zool.*, 1983, **18**, 129.

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