Metabolic Products of *Phomopsis oblonga*. Part 1. 3a,5a,6,7,8,9,9a,9b-Octahydro-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide)

Michael J. Begley
Chemistry Dept., University of Nottingham, Nottingham NG7 2RD
John Frederick Grove*
School of Molecular Sciences, University of Sussex, Brighton, Sussex BN1 9QJ

Oblongolide, one of several boring/feeding deterrents for elm bark beetles produced *in vitro* by the fungus *Phomopsis oblonga*, is shown by physical methods to be the $C_{14}H_{20}O_2$ γ-lactone (3a*R*,5a*S*,7*R*,9a*R*,9b*R*)-3a,5a,6,7,8,9,9a,9b-octahydro-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one.

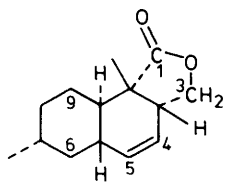
Phomopsis oblonga (Desm) Trav., a fungus frequently found in the outer bark of healthy *Ulmus* sp., particularly wych elm (*U. glabra*), can invade the phloem of stressed trees, principally those infected by *Ceratocystis ulmi*, the causative agent of Dutch elm disease.[1,2] Bark beetles of *Scolytus* sp., the insect vector of the disease, reject *P. oblonga*-invaded phloem as being unsuitable for breeding, and such trees do not become brood trees.[3] Using a laboratory bioassay in which *S. scolytus* beetles are offered a choice between treated and untreated elm bark it has been shown[4] that *P. oblonga* produces *in vitro* a number of boring/feeding deterrents for Scolytid beetles. The present paper describes the isolation and structure determination of one of these compounds, a novel norsesquiterpene γ-lactone, to which we have assigned the trivial name oblongolide.

Two morphologically distinct Groups of *P. oblonga* are found on elm.[2] Ethyl acetate extracts of the culture filtrate of a strain, number 118, of the more common Group 1, grown in surface culture on malt extract medium, were active in the bioassay.[4] Column chromatography of the extracts furnished a number of active compounds including oblongolide, a γ-lactone (**1**) ($\nu_{max.}$ 1 763 cm^{-1}, δ_C 180.3) of composition $C_{14}H_{20}O_2$, obtained only in low yield (1—2 mgl^{-1}).

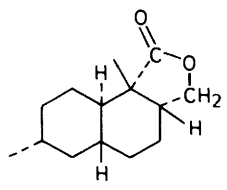
Oblongolide contained one (disubstituted) ethylenic double bond [δ_H 5.56 m, 5.61 d ($J_{4,5}$ 10 Hz), δ_C 134.6(1), 121.4(1)] and therefore three rings, including the lactone ring. Catalytic hydrogenation gave a dihydro derivative (**2**). Oblongolide contained one CMe (δ_H 1.14) and one CHMe group (δ_H 0.91). The oxygen bridge of the lactone was attached to a CH_2 group [δ_H 3.84, 4.38 (J_{AB} 8.7 Hz); δ_C 70.3(2)] contained in the partial structure $R_3CO_2CH_2CHR{\cdot}CH{=}CH-$ as revealed by the appropriate decoupling experiments. In the mass spectrum, after the initial loss of CO_2H to give a base peak of composition $C_{13}H_{19}^+$, the fragmentation pathway showed only the further sequential loss of CH_2.

No further reliable structural information could be obtained from these techniques and so an *X*-ray crystallographic analysis was carried out which established the structure (**1**) (relative configuration). The 5-hydrogen appears as a doublet in the ^1H n.m.r. spectrum because the dihedral angle between the 5- and 5a-hydrogens is 90.4°, giving $J_{5,5a}$ = 0 Hz.

The *X*-ray structure was determined by direct methods using diffractometer data. Least squares refinement converged to *R* 5.93% over the 673 independent observed reflections. The structure and relative stereochemistry were confirmed as structure (**1**) while the Figure shows the conformation of the molecule. Bond lengths and angles are listed in Tables 1 and 2 respectively, together with their standard deviations. A study of the torsion angles within the ring systems (Table 3) confirmed the expected conformations with the cyclohexane ring as a chair, and the cyclohexene ring as a half chair with C(9b) 0.21 Å above and C(9a) 0.54 Å below the plane of the four remaining



(**1**)



(**2**)

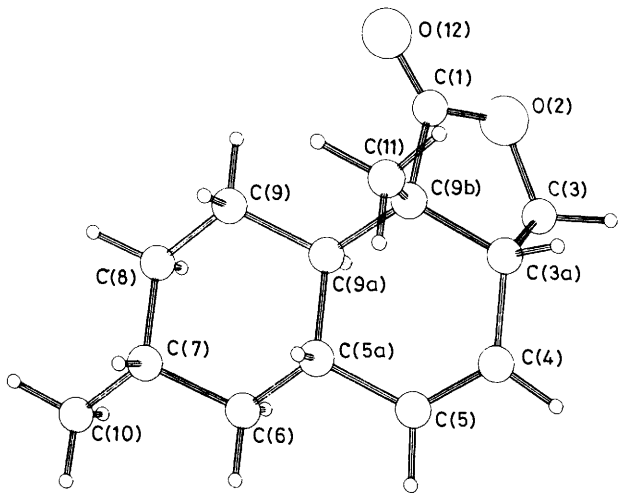


Figure. The conformation of the lactone (**1**) in the crystal structure showing the crystallographic numbering scheme.

atoms. The lactone ring adopts the envelope conformation with C(3a) 0.49 Å out of the plane of the other four atoms. No intermolecular contacts were discovered less than the sum of the Van der Waals radii.

The dihydro derivative (**2**) showed a negative Cotton effect at 225 nm in the c.d. spectrum as predicted by the lactone sector rule[5] for the enantiomer of absolute configuration (**2**). It is concluded that structure (**1**) represents the absolute configuration of oblongolide.

Oblongolide was not produced in surface culture on elm phloem medium nor in shake culture on this or on malt extract medium. It was not produced at all by a strain, number 119, of the less common *P. oblonga* Group 2. *Phomopsis* spp. very similar to those found on elm are also associated with ash and sycamore. On the malt medium, strains from both sources (numbers 123 and 124 respectively) produced oblongolide in

Table 1. Bond lengths (Å) for oblongolide (**1**) with e.s.d.s in parentheses

C(1)–O(2)	1.339(8)	C(5a)–C(6)	1.522(9)
C(1)–C(9b)	1.529(9)	C(5a)–C(9a)	1.541(8)
C(1)–O(12)	1.203(7)	C(6)–C(7)	1.526(9)
O(2)–C(3)	1.454(8)	C(7)–C(8)	1.518(9)
C(3)–C(3a)	1.530(8)	C(7)–C(10)	1.507(9)
C(3a)–C(4)	1.505(9)	C(8)–C(9)	1.523(10)
C(3a)–C(9b)	1.542(7)	C(9)–C(9a)	1.526(8)
C(4)–C(5)	1.305(8)	C(9a)–C(9b)	1.554(8)
C(5)–C(5a)	1.511(9)	C(9b)–C(11)	1.539(8)

Table 2. Bond angles (°) for oblongolide (**1**) with e.s.d.s in parentheses

O(2)–C(1)–C(9b)	110.8(6)	C(6)–C(7)–C(8)	108.6(5)
O(2)–C(1)–C(12)	121.8(7)	C(6)–C(7)–C(10)	112.6(7)
C(9b)–C(1)–O(12)	127.4(7)	C(8)–C(7)–C(10)	111.2(7)
C(1)–O(2)–C(3)	111.1(5)	C(7)–C(8)–C(9)	112.6(6)
O(2)–C(3)–C(3a)	104.0(5)	C(8)–C(9)–C(9a)	111.7(6)
C(3)–C(3a)–C(4)	113.6(6)	C(5a)–C(9a)–C(9)	109.9(5)
C(3)–C(3a)–C(9b)	103.0(5)	C(5a)–C(9a)–C(9b)	110.0(5)
C(4)–C(3a)–C(9b)	114.9(5)	C(9)–C(9a)–C(9b)	115.4(5)
C(3a)–C(4)–C(5)	123.7(6)	C(1)–C(9b)–C(3a)	101.1(5)
C(4)–C(5)–C(5a)	123.1(6)	C(1)–C(9b)–C(9a)	109.4(5)
C(5)–C(5a)–C(6)	112.4(6)	C(1)–C(9b)–C(11)	110.8(5)
C(5)–C(5a)–C(9a)	111.5(5)	C(3a)–C(9b)–C(9a)	109.6(5)
C(6)–C(5a)–C(9a)	109.9(6)	C(3a)–C(9b)–C(11)	112.3(5)
C(5a)–C(6)–C(7)	112.5(6)	C(9a)–C(9b)–C(11)	113.0(4)

yields of 0.51—1 mgl^{-1} in surface culture and strain 124 produced it in shake culture (1 mgl^{-1}).

Experimental

M.p.s were taken on a Kofler hot-stage apparatus and are corrected. 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 $CDCl_3$ with $SiMe_4$ as internal standard. Molecular weights and compositions were taken from the high resolution mass spectra. In analytical t.l.c. Merck silica gel F_{254} was used with chloroform–methanol (95:5) and spots were detected in iodine vapour. Merck silica gel 7734 was used in column chromatography. Light petroleum had b.p. 60—80 °C. Fermentation details are described elsewhere.[4]

Extraction and Isolation of the Metabolite (**1**).—In a typical fermentation, the culture filtrate (11.1 l; pH 5.3) from a surface culture of *P. oblonga* strain 118, grown on 2% malt extract and harvested after 35 days, was extracted with ethyl acetate. A portion (10 mg) of the extract, a brown oil (508 mg), was retained for bioassay and the remainder, in benzene (5 ml), was chromatographed on a column (25 × 1.2 cm) of silica gel (12 g) made up in benzene. After gums (6 mg) had been eluted with benzene (100 ml), benzene–methanol (200:1; 150 ml) eluted a colourless oil (21 mg) which solidified on trituration with light petroleum. Further elution of the column with benzene containing increasing proportions of methanol yielded other active products which will be described elsewhere.[6]

The solid crystallised from light petroleum in needles (12 mg), m.p. 105—106 °C, R_F 0.64, of (3aR,5aS,7R,9aR,9bR)-3a,5a,6,7,8,9,9a,9b-*octahydro*-7,9b-*dimethylnaphtho*[1,2-c]-*furan*-1(3H)-*one* (**1**) (*oblongolide*) (Found: C, 76.5; H, 9.3%; *M* 220.1469. $C_{14}H_{20}O_2$ requires C, 76.3; H, 9.2%; *M* 220.1463); $\nu_{max.}$ 1 763, 760, 725 cm^{-1}; u.v. end absorption only; δ_H (360 MHz) 0.91 (d, *J* 6.5 Hz, 3 H), 1.14 (s, 3 H), 0.9—2.0 (9 H), 2.73 (m, 3a-H), 3.84 (dd, 3β-H, J_{gem} 8.7 Hz, $J_{3a3\beta}$ 11 Hz), 4.38 (t, 3α-H, $J_{gem} = J_{3a3\alpha}$ = 8.7 Hz), 5.56 (m, 4-H), and 5.61 (d, 5-H, *J* 10 Hz); δ_C (number of bonded H) 180.3 (0), 134.6 (1), 121.4 (1), 70.3 (2), 45.1 (1), 43.1 (0), 41.7 (2), 39.6 (1), 36.2 (1), 35.1 (2), 32.8 (1), 25.5 (2), 22.3 (3), and 16.1 (3); *m/z* (% base peak) 220 (10), 205 (3), and 175.1505 (100) ($C_{13}H_{19}$ requires 175.1486), 147 (54), 133 (13), 119 (74), 105 (66), and 91 (53); $[\alpha]_D^{20}$ −190° (*c* 0.0475); c.d., λ 212 nm, Δε −1.42.

Table 3. Selected torsion angles around the ring system of oblongolide (**1**)

C(9b)–C(1)–O(2)–C(3)	2.4
C(1)–O(2)–C(3)–C(3a)	−21.3
O(2)–C(3)–C(3a)–C(9b)	30.7
C(3)–C(3a)–C(9b)–C(1)	−28.4
C(3a)–C(9b)–C(1)–O(2)	17.2
C(9b)–C(3a)–C(4)–C(5)	6.6
C(3a)–C(4)–C(5)–C(5a)	2.6
C(4)–C(5)–C(5a)–C(9a)	20.2
C(5)–C(5a)–C(9a)–C(9b)	−50.5
C(5a)–C(9a)–C(9b)–C(3a)	59.0
C(9a)–C(9b)–C(3a)–C(4)	−37.0
C(9a)–C(5a)–C(6)–C(7)	−58.7
C(5a)–C(6)–C(7)–C(8)	57.3
C(6)–C(7)–C(8)–C(9)	−55.3
C(7)–C(8)–C(9)–C(9a)	55.9
C(8)–C(9)–C(9a)–C(5a)	−55.0
C(9)–C(9a)–C(5a)–C(6)	56.0

Table 4. Atomic co-ordinates for the non-hydrogen atoms of oblongolide (**1**) with e.s.d.s in parentheses

	x/a	*y/b*	*z/c*
C(1)	0.432 1(9)	0.372 0(10)	0.240 0(4)
O(2)	0.363 0(6)	0.320 7(6)	0.297 6(2)
C(3)	0.184 2(8)	0.278 6(9)	0.289 0(3)
C(3a)	0.132 3(8)	0.373 4(9)	0.225 8(3)
C(4)	−0.018 3(8)	0.296 6(9)	0.190 6(4)
C(5)	−0.008 7(8)	0.222 6(9)	0.132 6(4)
C(5a)	0.156 8(8)	0.199 3(9)	0.095 3(3)
C(6)	0.163 2(9)	0.032 9(9)	0.057 4(3)
C(7)	0.325 6(10)	0.013 9(9)	0.016 3(3)
C(8)	0.477 7(9)	0.029 7(11)	0.062 6(3)
C(9)	0.476 0(8)	0.193 2(10)	0.102 5(3)
C(9a)	0.311 4(7)	0.213 1(7)	0.142 2(3)
C(9b)	0.298 2(6)	0.376 7(8)	0.184 7(3)
C(10)	0.331 0(12)	−0.149 5(11)	−0.022 1(3)
C(11)	0.315 5(9)	0.538 9(8)	0.143 0(4)
O(12)	0.580 9(6)	0.405 8(7)	0.235 4(3)

Catalytic Hydrogenation of Oblongolide (**1**).—The lactone (**1**) (3 mg) in ethyl acetate (4 ml) took up hydrogen (0.33 ml; 1.08 double bonds) in 5 min in the presence of 5% palladium–charcoal (5 mg). (3aR,5aS,7R,9aR,9bR)-3a,4,5,5a,6,7,8,9,9a,9b-*Decahydro*-7,9b-*dimethylnaphtho*[1,2-c]*furan*-1(3H)-*one* (**2**), was recovered in the usual way and crystallised from light petroleum in rhombs, m.p. 93—94 °C (Found: *M* 222.1600. $C_{14}H_{22}O_2$ requires *M* 222.1620); $\nu_{max.}$ 1 760 cm^{-1}; c.d. 214, 225 nm, Δε −2.14, −2.26 respectively.

Isolation of Oblongolide from Other Phomopsis *Strains.*—Column chromatography as described above, of the extracts (231 mg; 500 mg) of filtrates (6.0 l, 25 days; 7.1 l, 31 days) from surface cultures on malt extract of strains 123 (ash) and 124 (sycamore) furnished oblongolide (**1**) (3 mg and 4 mg respectively), identified by the i.r. spectrum. By the same procedure, oblongolide (**1**) (4 mg) was obtained from the filtrate (4.1 l, 21 days) from strain 124 grown in shake culture on the same medium.

Crystallographic Analysis of Oblongolide (**1**).—The space group and preliminary cell parameters were determined photographically. For the intensity measurement the crystal was mounted on an Enraf-Nonius CAD4 diffractometer. Accurate lattice parameters were obtained by least-squares refinement of the positions of 25 reflections measured on the diffractometer with θ *ca.* 25°. Intensity data were collected with Cu-K_α radiation using an ω—2θ scan for $1° \leqslant \theta \leqslant 66°$. A total of 1 289 independent reflections were measured of which 673 had $I > 3\sigma(I)$ and were considered observed and used in the subsequent refinement. The data were corrected for Lorentz and polarisation factors, but no absorption corrections were applied. Data reduction and subsequent crystallographic calculations were performed using the CRYSTALS[7] system of programs.

Crystal data. $C_{14}H_{20}O_2$, M = 220.31. Orthorhombic, a = 7.854(1), b = 7.920(1), and c = 20.058(4) Å, U = 1 247.8 Å^3, Z = 4, D_c = 1.17 g cm^{-1}, $F(000)$ = 480. Space group $P2_12_12_1$ from systematic absences, Cu-K_α radiation, λ = 1.541 78 Å, μ(Cu-K_α) = 6.12 cm^{-1}.

Structure solution and refinement. The structure was solved by direct methods using the MULTAN[8] program. 140 Reflections with $E > 1.4$ were used and the E map based on the best set of phases revealed the positions of all 16 non-hydrogen atoms in the molecule as the largest peaks in the map. Full-matrix isotropic least-squares refinement of these positions gave an R value of 13.4%. Refinement was continued with anisotropic thermal parameters for all non-hydrogen atoms. A difference map next revealed the positions of many of the hydrogen atoms. Geometric considerations were then used to calculate the accurate positions of all of the hydrogen atoms which were then included in the structure factor calculations, but without refinement. Analysis of the agreement between F_o and F_c suggested the adoption of a weighting scheme based on a Chebyshev polynomial. Refinement finally converged with the largest parameter shifts 0.1σ after 17 cycles of least-squares refinement. The final R value at convergence was 5.93% with R_w 0.0607. A final difference map was calculated which showed no peaks or depressions >0.2 e Å^{-3}. Final atomic co-ordinates are listed in Table 4. H-Atom co-ordinates and temperature factors are available as a Supplementary Publication (SUP. No. 56151, 3 pp.).* Observed and calculated structure factors are available on request from the editorial office.

* For details of the Supplementary Publications Scheme, see Instructions for Authors (1985) in *J. Chem. Soc., Perkin Trans. 1*, 1985, Issue 1.

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References

1 J. N. Gibbs and M. E. Smith, *Ann. Appl. Biol.*, 1978, **89**, 125.
2 J. F. Webber and J. N. Gibbs, *Trans. Brit. Mycol. Soc.*, 1984, **82**, 348.
3 J. Webber, *Nature*, 1981, **292**, 448.
4 N. Claydon, J. F. Grove, and M. Pople, *Phytochemistry*. Paper 2769, in the press.
5 J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 1965, 7211.
6 J. F. Grove, Part 2. *J. Chem. Soc., Perkin Trans. 1*, 1985, following paper.
7 J. R. Carruthers, 'CRYSTALS User Manual,' Oxford University Computing Laboratory, 1975.
8 P. Main, G. L. Fiske, S. E. Hull, L. Lessinger, E. German, J. P. Declerq, and M. M. Woolfson, 'MULTAN a system of computer programs for the automatic solution of crystal structures from *X*-ray diffraction data,' Universities of York, England and Louvain, Belgium, 1980.

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