New Oblongolides Isolated from the Endophytic Fungus *Phomopsis* sp. from *Melilotus dentata* from the Shores of the Baltic Sea[‡]

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Thirteen new metabolites, namely oblongolides B–M (**2**–**13**) and 4-[5-(1-hydroxyethyl)furan-2-yl]-4-oxobutanoic acid (**14**), together with the six known compounds phomopsolide B (**15**), alternariol dimethyl ether (**16**), alternariol monomethyl ether (**17**), the mycotoxin alternariol (**18**), ergosterol (**19**), and 5α,8α-epidioxyergosterol (**20**) were isolated from the endophytic fungus *Phomopsis* sp. The new biologically active norsesquiterpene γ-lactones differ in their degree of substitution, saturation, and substituent pattern from the known oblongolide **1**. Their structures were determined by means of spectroscopic data including HREIMS, ^{1}H NMR, ^{13}C NMR, 2D NMR (HMQC, HMBC, NOESY) and X-ray single crystal analysis.

Introduction

Marine fungi have proven to be a rich source of novel biologically active organic compounds. This is presumably due to the fact that the fungi are subjected to multiple stresses in the marine habitat, but also because this biotope is relatively unexplored.[2–4] In our screening program for new fungal biologically active secondary metabolites, we investigated the metabolites of an endophytic strain of *Phomopsis* sp. (internal strain no. 6654), which was isolated from the halotolerant plant *Melilotus dentata* from the shores of the Baltic Sea, near Ahrenshoop, Germany. Twelve new norsesquiterpene γ-lactones (**2**–**13**), oblongolides B–M, along with 4-[5-(1-hydroxyethyl)furan-2-yl]-4-oxobutanoic acid (**14**; Scheme 1) and the six known compounds phomopsolide B (**15**), alternariol dimethyl ether (**16**), alternariol monomethyl ether (**17**), the mycotoxin alternariol (**18**), ergosterol (**19**), and 5α,8α-epidioxyergosterol (**20**) were isolated from the ethyl acetate extract of the cultures. Here we describe the isolation, structural elucidation, and herbicidal, antifungal, and antibacterial activities of these new compounds.

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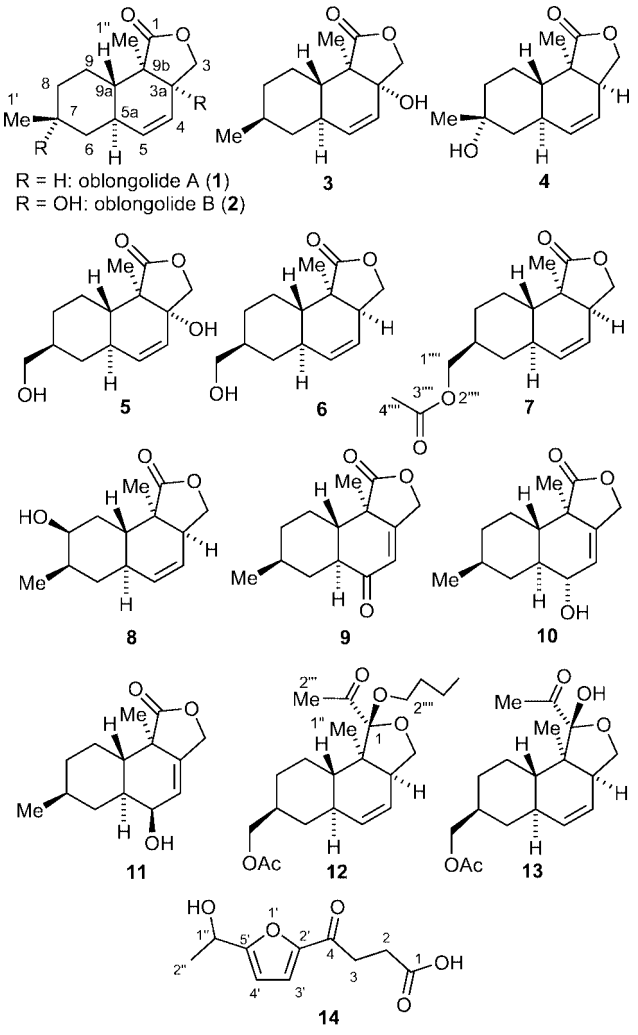
Results and Discussion

The first isolated compound **2** was obtained as colorless crystals, and gave a molecular ion at *m*/*z* = 252.13646 in the HREI mass spectrum, indicating a molecular formula of $C_{14}H_{20}O_4$. Its IR spectra show strong absorptions for hydroxyl groups ($\tilde{\nu}$ = 3415 cm^{-1}) and a γ-lactone moiety ($\tilde{\nu}$ = 1765 cm^{-1}). The presence of the γ-lactone was also supported by the signal at δ = 180.6 ppm in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum (Table 1) suggests the presence of two methyl groups (δ = 1.13 and 1.22 ppm) and two protons on oxygenated carbons at δ = 4.35 (d, J = 9.8 Hz) and δ = 4.20 ppm (d, J = 9.8 Hz). The ^{13}C NMR spectrum (Table 2) shows signals for 14 carbons, and the DEPT spectrum indicates the presence of two methyls, four methylenes, four methines, and four quaternary carbon atoms.

Analysis of the 2D ^{1}H-^{1}H COSY and HMQC spectra of compound **2** suggested the presence of the fragment –CH_2(8)–CH_2(9)–CH(9a)–CH(5a)[CH_2(6)]–CH(5)–CH(4)–. In the HMBC spectrum, ^{13}C-^{1}H long range correlation signals were found between C-1 and H-3, H-9a, and H-1′′; between C-9b and H-1′′, H-9a, and H-4; between C-3a and H-3, H-4, H-5, H-1′′, and H-9a; as well as between C-7 and H-1′, H-5a, H-6, and H-8. The clear NOE correlations between 1′′-CH_3 and H-5a and H-9α, and between H-9a and H-8β and H-6β, as well as between 1′-CH_3 and H-8β and H-6β indicated that the relevant correlated protons are on the same side of the molecule. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of our first compound with the data of oblongolide **1**, a norsesquiterpene γ-lactone previously isolated from *Phomopsis oblonga*,[5] showed that H-3a and H-7 in **1** are replaced by hydroxyl groups in



Scheme 1. Secondary metabolites isolated from the culture broth of *Phomopsis* sp.

our compound, named oblongolide B (**2**).[6] The structure of **2** was further unambiguously confirmed by X-ray diffraction analysis of a single crystal obtained from methanol (Figure 1).

Despite the relatively simple structure, the norsesquiterpene γ-lactone oblongolide (**1**) has only once been recorded as a natural product. The occurrence of the different oblongolides B–M (**2**–**13**, respectively) reported in this paper is another example of the ability of nature to create structurally diverse molecules starting from one basic skeleton.

The absolute configuration of oblongolide (**1**) was initially assigned as *ent*-**1** based on a circular dichroism study of its dihydro derivative according to the lactone sector rule.[5] This assignment was subsequently proven to be incorrect by an unambiguous total synthesis of *ent*-**1**, identical with the natural oblongolide except for the opposite sign of the optical rotation,[7] and of the natural isomer itself, starting from (–)-citronellol.[8] The optical rotation of oblongolide (**1**) was determined to be –190.[5] Since the optical rotations of the entire family of oblongolides B–M (**2**–**13**) isolated in this investigation are mostly of the same order of magnitude and sign (see Exp. Sect.), the absolute configurations of the new metabolites were assumed to be as established for oblongolide (**1**) and as shown in Scheme 1.

Oblongolide (**1**), isolated from *Phomopsis oblonga*, has been shown to be a boring and feeding deterrent for the Elm bark beetles.[5,9] The fungus *P. oblonga*, which is frequently found in the outer bark of healthy elm trees, can invade the phloem of stressed trees, primarily those infected by *Ceratocystis ulmi*, the causative agent of Dutch elm disease. Elm bark beetles, the insect vector of the disease, reject *P. oblonga*-invaded phloem as being unsuitable for breeding so that such trees do not become brood trees.

Compound **3** was found to have a molecular formula of $C_{14}H_{20}O_3$ due to its molecular ion of 236.14103 in the

Table 1. ^{1}H NMR spectroscopic data of compounds **2**–**7** (500 MHz, $CDCl_3$; **2** in CD_3OD, chemical shift values are in ppm from TMS; *J* values (in Hz) are presented in parentheses).

H atom	**2**	**3**	**4**	**5**	**6**	**7**
3α	4.20 (d, 9.8)	4.27 br. s	3.76 (dd, 11.0, 8.8)	4.29 br. s	3.83 (dd, 11.0, 8.8)	3.86 (dd, 11.0, 8.8)
3β	4.35 (d, 9.8)		4.34 (t, 8.8)		4.39 (t, 8.8)	4.42 (t, 8.8)
3a			2.68 (dd, 11.0, 8.8)		2.73 m	2.76 m
4	5.59 (d, 10.2)	5.56 (d, 10.1)	5.52 br. s	5.60 (d, 10.1)	5.57 m	5.61 m
5	5.61 (d, 10.2)	5.63 (d, 10.1)	5.52 br. s	5.69 (d, 10.1)	5.63 (d, 10.1)	5.65 (d, 10.1)
5a	2.44 (dd,11.4, 10.5)	2.0 m	2.29 (dd, 11.8, 11.4)	2.05 m	1.94 m	1.94 m
6α	1.82 (dd, 13.3, 6.0)	1.88 m	1.75 (d, 13.4)	0.83 m	0.83 (t, 12.2)	0.86
6β	1.21 (dd, 13.3, 10.5)	0.78 m	1.12 m overlap	2.03 m	2.00 m overlap	1.95 m overlap
7		1.50 m		1.64 m	1.62 m	1.80 m
8α	1.75 m overlap	0.89 m	1.68 (dd, 2.1, 13.7)	1.00 m	0.97 m	1.27 m
8β	1.37 (ddd, 13.7, 13.3, 3.9)	1.80 m overlap	1.30 (ddd, 13.1, 13.7, 4.4)	1.87 m	1.88 m	1.90 m
9α	1.54 m overlap	1.30 m	1.54 m	1.40 m	1.25 m	1.65 m
9β	1.73 m overlap	1.80 m	1.60 m	1.92 m	1.88 m	1.92 m
9a	1.56 m overlap	1.45 m	1.23 (ddd, 11.4, 10.6, 2.7)	1.51 m	1.33 m	1.35 m
1′	1.22 s	0.90 (d, *J* = 6.5)	1.18 s	3.51 m	3.46 m	3.93 m
1′′	1.13 s	1.13 s	1.10 s	1.17 s	1.13 s	1.16 s
4′						2.19 s

Table 2. ^{13}C NMR spectroscopic data of compounds **2**–**13** (500 MHz, $CDCl_3$; **2** in CD_3OD).

C atom	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	180.6	179.3	180.2	178.7	180.3	180.0	180.3	177.1	179.0	178.9	110.1	97.2
3	77.7	77.1	70.3	77.2	70.3	70.2	70.3	68.1	68.9	68.9	71.5	61.6
3a	77.7	78.7	44.8	78.7	44.9	44.8	45.0	161.5	140.6	141.0	45.7	44.9
4	126.2	125.7	121.8	126.0	121.6	121.9	121.5	121.9	123.6	122.7	124.4	123.2
5	134.1	135.5	134.1	135.4	134.2	133.9	134.1	198.3	73.3	66.3	130.9	133.9
5a	31.6	36.2	31.5	35.8	35.7	35.6	36.4	45.4	41.6	38.1	37.8	37.3
6	44.2	41.1	45.0	35.4	35.9	35.9	34.5	34.9	39.8	36.6	36.7	36.0
7	68.7	32.8	69.6	40.4	40.4	37.1	36.0	32.4	32.1	32.5	37.3	37.1
8	37.9	34.7	38.9	28.9	29.3	29.4	70.3	33.6	33.9	34.2	29.7	29.5
9	21.2	25.8	21.0	25.3	24.9	24.8	33.2	25.9	25.8	25.6	27.6	26.0
9a	43.2	43.8	39.3	44.1	39.8	39.6	32.1	44.3	42.3	35.4	40.5	39.4
9b	49.6	49.5	42.9	49.5	43.1	43.0	42.7	43.5	42.7	43.2	51.6	48.8
1′	30.1	22.2	31.5	67.9	67.9	69.0	18.1	22.4	22.5	22.6	69.0	69.2
3′						171.1					171.1	171.3
4′						20.9					20.9	20.9
1′′	8.3	8.9	16.2	8.9	16.1	16.1	15.9	16.8	17.6	16.8	13.2	16.0
1′′′											207.7	206.0
2′′′											29.9	25.0
2′′′′											61.6	
3′′′′											32.0	
4′′′′											19.5	
5′′′′											13.9	

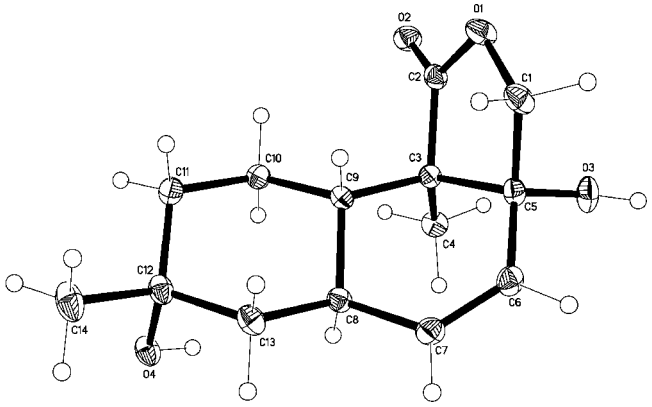


Figure 1. The molecular structure of oblongolide B (**2**) in the crystalline state.

HREI mass spectrum. The ^1H NMR spectrum of **3** has almost the same signal pattern as that found for **2**, except for the appearance of a methine group (δ = 1.50 ppm). The ^{13}C NMR spectrum of **3** shows almost the same chemical shifts as those of **2**, as shown in Table 2, except for the disappearance of an oxygenated quaternary carbon and appearance of a methine group (δ = 32.8 ppm) at C-7. The structure of **3** was confirmed by 2D NMR spectroscopic analysis. The ^1H-^1H COSY and HMQC spectra of **3** suggested the presence of the fragment –CH(9a)[CH(5a)]–CH_2(9)–CH_2(8)–CH(7)[CH_3(1′)]–CH_2(6)–CH(5a)-[CH(9a)]–CH(5)–CH(4)–. The NOESY spectrum exhibits clear correlations between H-7, H-5a, and H-9α, showing that these protons are on the same side of the molecule. The compound was thus assigned structure **3** and named oblongolide C.

The molecular formula of compound **4** was determined as $C_{14}H_{20}O_3$ by HREIMS and ^{13}C NMR spectroscopic data, which are the same as for compound **3**. The 1D (^1H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra of **4** (Tables 1 and 2) are similar to those of **2**, except for the spectral changes due to the absence of the hydroxyl group at C-3a. The ^1H-^1H COSY and HMQC spectra of **4** suggested the presence of the fragment –CH_2(8)–CH_2(9)–CH(9a)–CH(5a)[CH_2(6)]–CH(5)–CH(4)–CH(3a)–CH_2(3)–. The NOESY spectrum exhibits a clear correlation between 1′′-CH_3 and H-3a and H-5a, showing that these protons are on the same side of the molecule. Therefore, the structure was assigned as oblongolide D (**4**).

Oblongolide E (**5**) has the same molecular formula as **2**, as deduced from its NMR and HREI mass spectra. However, the 1D (^1H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra of **5** are more closely related to those of compound **3**. In the structure of **3**, the methyl group at C-7 has been replaced by an oxygenated methylene group, as deduced from the lower field signals at δ = 3.51 ppm. This information led to the identification of the structure of **5** as shown in Scheme 1, which differs from **2** only in the position of the hydroxy group. The HMBC spectrum shows a correlation between C-7/H-1′, H-6, and H-8, thereby confirming the assignment.

The HREI mass spectrum of compound **6** shows a molecular ion at *m/z* = 236.14154 corresponding to the molecular formula $C_{14}H_{20}O_3$ (calcd. 236.14125). The 1D (^1H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra of **6** are very similar to those of **5**, except for the absence of a hydroxyl group at C-3a. Analysis of the COSY and HMQC spectrum of **6** enabled the deduction of the fragment –CH(9a)[CH(5a)]–CH_2(9)–CH_2(8)–CH(7)[CH_3(1′)]–CH_2(6)–CH(5a)[CH(9a)]–CH(5)–CH(4)–. Therefore, the structure of **6** was determined to be that of oblongolide F, as shown in Scheme 1.

Compound **7**, which analyzed for $C_{16}H_{22}O_4$ according to the HREI mass spectrum, contains one acetyl group more

than **6**. The 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra of **7** are similar to those of **6**, except for the proton signals assignable to C-1′. The shifts observed for the signals of C-1′ in **7**, of between 3.46 and 3.93 ppm with respect to those in **6**, are consistent with the proposed structure in which the acetyl group is located at C-1′. Therefore, compound **7** was deduced to be oblongolide G (**7**), as shown in Scheme 1.

Compound **8** has the molecular formula $C_{14}H_{20}O_3$, as deduced from the HREI mass and ^{13}C NMR spectroscopic data. The 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra of **8** show some similarity to those of **6**. However, the absence of the hydroxyl group at C-1′ causes some signal differences and the appearance of a new hydroxy carbon at C-8 is made clear by the shift of H-8 to δ = 3.96 ppm. The stereochemistry was unambiguously deduced from the magnitude of the coupling constant between H-7 and H-8 (J = 2.1 Hz). The absence of a 7,8-diaxial coupling constant indicates an axially orientated hydroxyl group at C-8 with the equatorial methyl group on the same side of the molecule. The 2D NMR spectra further supported the assigned structure. The ^{1}H-^{1}H COSY and HMQC spectra of **8** suggested the presence of the fragment –CH(9a)[CH(5a)]–CH_2(9)–CH(8)–CH(7)[CH_3(1′)]–CH_2(6)–CH(5a)[CH(9a)]–CH(5)–CH(4)–. The NOESY spectrum exhibits a clear correlation between H-7 and H-5a and H-9α as well as between H-8 and H-6α, thus showing that the respective correlated protons are on the same side of the molecule. Therefore, oblongolide H was assigned structure **8** (Scheme 1).

Compound **9** has the molecular formula $C_{14}H_{18}O_3$, as deduced from HREI mass and ^{13}C NMR spectroscopic data. Its ^{13}C NMR spectrum (Table 2) is, in part, quite different from the previous ones as it exhibits signals for two methyls, four methylenes, and four methines, as well as four quaternary carbons. Moreover, the ^{1}H NMR spectrum (Table 3) exhibits the presence of two methyl groups, of which one is a singlet (δ = 1.46 ppm) and one is a doublet (δ = 0.98 ppm, J = 6.5 Hz), two protons on oxygenated carbons at δ = 5.08 (dd, J = 14.3, 2.2 Hz) and δ = 4.88 ppm (dd, J = 14.3, 1.2 Hz), and one alkene proton at δ = 5.97 ppm (broad s). The ^{1}H-^{1}H COSY and HMQC spectra of **9** show the presence of its coupled proton systems as –CH(9a)–CH_2(9)–CH_2(8)–CH(7)[CH_3(1′)]–CH_2(6)–CH(5a)–CH(9a)–. In the HMBC spectrum of **9**, ^{13}C-^{1}H long range correlation signals are found between C-5 and H-5a, H-9a, and H-6; between C-3a and H-4, H-3, H-1′′, and H-9a; as well as between C-9b and H-1′′, H-9a, and H-4. Therefore, oblongolide I was assigned structure **9** (Scheme 1). The assignment of **9** was further confirmed unambiguously by X-ray diffraction analysis of a single crystal obtained from methanol solution (Figure 2).

Compound **10** has the molecular formula $C_{14}H_{20}O_3$, as deduced from HREI mass and ^{13}C NMR spectroscopic data. The 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra of **10** are related to those of **9** except for the changes induced by the absence of the ketone on C-5, and changes caused by the appearance of a new hydroxy carbon at δ = 73.3 ppm. The NOESY spectrum exhibits a clear correlation between H-5 and H-9a, thus proving that H-5 is positioned in the β-orientation. Therefore, oblongolide J was assigned the structure **10** (Scheme 1).

Compound **11** has the molecular formula $C_{14}H_{20}O_3$, as deduced from HREI mass and ^{13}C NMR spectroscopic data. The 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC

Table 3. ^{1}H NMR spectroscopic data of compounds **8**–**13** (500 MHz, $CDCl_3$, chemical shift values are in ppm from TMS; J values (in Hz) are presented in parentheses).

Proton	**8**	**9**	**10**	**11**	**12**	**13**
3α	3.89 (dd, 11.0, 8.6)	4.88 (dd, 14.3, 1.2)	4.57 (d, 14.0)	4.67 (d, 11.8)	3.68 (dd, 8.9, 8.1)	3.60 (dd, 12.0, 5.0)
3β	4.39 (t, 8.6)	5.08 (dd, 14.3, 2.2)	4.82 (d, 14.0)	4.87 (d, 11.8)	4.04 (dd, 9.7, 8.1)	4.17 (t, 12.0)
3a	2.75 m				2.82 m	2.52 m
4	5.58 m	5.97 br. s	5.57 br. s	5.81 br. s	5.45 (d, 9.9)	5.42 m
5	5.63 (d, 10.0)		4.09 (d, 7.2)	4.03 s	5.61 m	5.56 (d, 10.0)
5a	1.90 m	2.23 (dt, 11.9, 3.7)	1.50 m	1.80 m	1.80 m	1.81 m
6α	1.33 (t, 12.4)	0.83 m	2.19 m	1.25 m	0.90 overlap	0.89 (t, 12.2)
6β	1.56 m	2.41 m	0.79 m	1.80 m	1.80 m	1.88 m
7	1.65 m	1.47 m	1.38 m	1.30 m	1.75 m	1.72 m
8α	3.96 (d, 2.1)	0.91 m	0.88 m	1.00 m	1.20 m	1.03 m
8β		1.82 m	1.73 m	1.83 m	1.75 m	1.80 m
9α	1.16 (dt, 11.8, 2.1)	1.40 m	1.20 m	1.30 m	1.12 m	1.13 m
9β	1.97 m	2.57 (ddd, 10.1, 6.7, 3.3)	2.42 m	2.49 m	1.45 m	1.26 m
9a	1.89 m	1.93 (dt, 12.1, 3.3)	1.50 m	1.59 m	1.30 m	2.10 (ddd, 11.8, 11.3, 2.7)
1′	1.00 (d, 6.9)	0.98 (d, 6.5)	0.87 (d, 6.7)	0.91(d, 6.8)	3.89 m	3.88 m
1′′	1.13 s	1.46 s	1.25 s	1.24 s	1.15 s	1.02 s
4′					2.06 s	2.04 s
2′′′					2.30 s	1.45 s
2′′′′a					3.11 m	
2′′′′b					3.43 m	
3′′′′					1.54 m	
4′′′′					1.29 m	
5′′′′					0.94 (t, 7.4)	

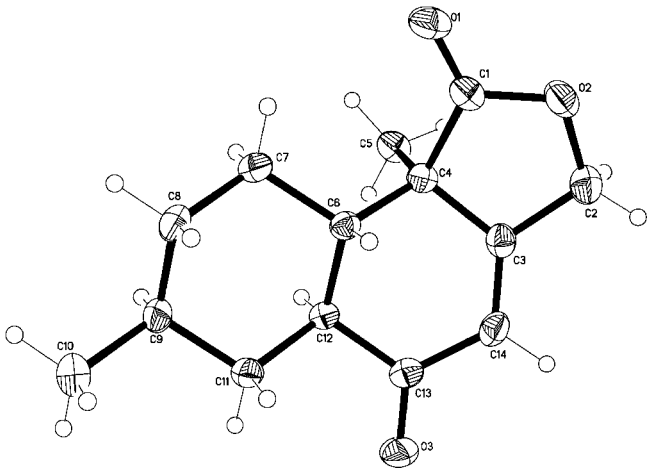


Figure 2. The molecular structure of oblongolide I (**9**) in the crystal.

and HMBC) NMR spectra of **11** are similar to those of **10** except for the differences in chemical-shift values of the signals related to C-5. The NOESY spectrum exhibits a clear correlation between H-5 and H-6α, proving that the hydroxy group on C-5 is in a β-orientation; the structure of oblongolide K was therefore determined to be **11** (Scheme 1). Thus, compounds **10** and **11** are epimeric at the stereogenic center at C-5.

Compound **12** was obtained as a colorless gum with the molecular formula $C_{22}H_{34}O_5$, as deduced from HREI mass and ^{13}C NMR spectroscopic data. The ^1H NMR spectrum (Table 3) indicates the presence of four methyl groups (δ = 0.94, 1.15, 2.06, and 2.30 ppm). The presence of a signal at δ = 2.06 ppm indicates an acetyl group, in addition to the two aliphatic and the acetoxy methyl groups. The ^{13}C NMR spectrum of **12** (Table 2) shows signals for 22 carbons, and the DEPT spectrum indicates the presence of four methyls, eight methylenes, six methines, and four quaternary carbon atoms. The ^1H,^{13}C/DEPT NMR spectra of compound **12** are similar to those of **7** (Tables 1 and 2), in particular those parts related to the "bottom" part of the molecule. However, the signal for C-1 in the ^{13}C NMR spectrum is shifted upfield (δ = 110.5 ppm) relative to the corresponding signal in compound **7** (δ = 180.0 ppm). Analysis of the ^1H-^1H COSY and HMQC spectra of **12** enabled the deduction of the fragments –CH(9a)[CH(5a)]–CH_2(9)–CH_2(8)–CH(7)-[CH_2(1′)]–CH_2(6)–CH(5a)[CH(9a)]–CH(5)–CH(4)–CH(3a)–CH_2(3)– and –CH_2(2′′′′)–CH_2(3′′′′)–CH_2(4′′′′)–CH_3(5′′′′). In the HMBC spectrum of **12**, ^{13}C-^1H long range correlation signals are found between C-1 and H-3, H-1′′, H-2′′′, and H-2′′′′, showing that the latter butyl chain is connected to an oxygen atom attached to C-1. The NOESY spectrum exhibits a clear correlation between H-1′′ and H-2′′′. The acetyl group is thus placed in an α-orientation on the same side as the methyl group at C1′′, as shown in structure **12** for oblongolide L (Scheme 1).

Compound **13** was obtained as a colorless gum with the molecular formula $C_{18}H_{26}O_5$, as deduced from HREI mass and ^{13}C NMR spectroscopic data. Its IR spectrum shows strong absorptions for hydroxy groups (3436 cm^{-1}) and an alkene moiety (1713 cm^{-1}), while the ^1H NMR spectrum, which is closely related to that of compound **12** (Table 3), shows the presence of three methyl groups (δ = 1.02, 1.45, and 2.04 ppm). The ^{13}C NMR spectrum of **13** (Table 2) exhibits signals for 18 carbons, and the DEPT spectra indicate the presence of three methyls, five methylenes, six methines, and four quaternary carbon atoms. A comparison of the NMR spectra of **13** (Tables 2 and 3) with those of **12** revealed that **13** possesses a similar structure, except for the absence of the signal for an oxygenated butyl group at C-1. The EI mass spectrum of **13** shows a molecular ion 56 mass units (corresponding to four methylene groups) lower than found for **12**. ^{13}C-^1H long-range correlation signals were found between C-1′′′ and H-2′′′ and H-1′′, as well as between C-1 and H-3, H-1′′, and H-2′′′. Therefore, structure **13** was assigned to oblongolide M.

Compound **14** was obtained as a colorless gum with the molecular formula $C_{10}H_{12}O_5$, as deduced from HREI mass and ^{13}C NMR spectroscopic data. Its IR spectrum shows strong absorptions for hydroxy groups (3436 cm^{-1}) and a carboxy group (1734 cm^{-1}), while the ^1H NMR spectrum (Table 4) shows the presence of one methyl group at δ = 1.48 ppm (d, J = 6.6 Hz), two olefinic protons (δ = 6.31 and 7.09 ppm, d, J = 3.5 Hz), and one proton on an oxygenated carbon at δ = 4.84 ppm (q, J = 6.6 Hz). The ^{13}C NMR spectrum of **14** (Table 4) shows signals for 10 carbons, and the DEPT spectra indicate the presence of one methyl, two methylenes, three methines, and four quaternary carbon atoms.

Table 4. NMR data for compound **14** (125 and 500 MHz, $CDCl_3$).

Position	δ_C	δ_H
1	177.2	
2	27.6	2.67 (t, 6.5)
3	32.7	3.05 (t, 6.5)
4	187.2	
2′	151.2	
3′	118.6	7.09 (d, 3.5)
4′	107.7	6.31 (d, 3.5)
5′	162.7	
1′′	63.7	4.84 (q, 6.6)
2′′	21.3	1.48 (d, 6.6)

In the HMBC spectrum of **14**, ^{13}C-^1H long range correlation signals are found between C-8 and H-6, H-7, H-9, and H-10; between C-5 and H-6 and H-7; between C-4 and H-2, H-3, and H-6; as well as between C-1 and H-2 and H-3. The structure of compound **14** was assigned to be 4-[5-(1-hydroxyethyl)furan-2-yl]-4-oxobutanoic acid. The current data have not yet allowed the assignment of the absolute configuration.

The known compounds were identified as phomopsolide B (**15**),[10,11] alternariol dimethyl ether (**16**),[12] alternariol monomethyl ether (**17**),[12] the mycotoxin alternariol (**18**),[13] ergosterol (**19**),[14] and 5α,8α-epidioxyergosterol

(**20**)[15,16] by comparison of their physical and spectroscopic data with those reported in the literature.

Previous studies have not reported the cytotoxicity of this kind of norsesquiterpene γ-lactone. In our studies, these new botryane metabolites were all found to be biologically active against some or all of the test organisms: the gram-positive bacterium *Bacillus megaterium*, the fungi *Microbotryum violaceum* and *Septoria tritici*, and the green alga *Chlorella fusca*, as shown in Table 5. Thus, it represents a potential lead structure in particular for herbicidal and antifungal properties. The interesting Ulm bark beetle repellent property of a selected series of the oblongolides is currently under investigation.

Table 5. Relative inhibition by compounds **2**–**14**.[a]

Compound	Chl	Mv	St	Bm
2	25%	63%	12%	80%
3	75%	53%	18%	70%
4	41%	79%	65%	64%
5	0	55%	20%	0
6	44%	51%	39%	48%
7	58%	57%	61%	74%
8	22%	68%	21%	77%
9	60%	68%	69%	71%
10	40%	72%	29%	81%
11	85%	74%	75%	79%
12	16%	59%	35%	76%
13	46%	59%	17%	73%
14	19%	49%	40%	61%

[a] Chl = *Chlorella fusca*, Mv = *Microbotryum violaceum*, St = *Septoria tritici*, Bm = *Bacillus megaterium*.

Experimental Section

General Experimental Procedures: For microbiological methods and culture conditions see refs.[17,18] Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. The IR spectra were taken on a Nicolet-510P spectrometer. NMR spectra were recorded on a Bruker Avance-500 NMR spectrometer with TMS as internal standard. EI mass spectra were obtained on a MAT 8200 mass spectrometer. Silica gel (70-230 mesh) was used for column chromatography. Spots were detected on TLC under UV or by heating after spraying with 0.5 mL of anisaldehyde in 50 mL of HOAc and 1 mL of H_2SO_4.

Extraction and Isolation: The marine fungus *Phomopsis sp.6654* was isolated from the plant *Melilotus dentata* from the shores of the Baltic Sea, near Ahrenshoop, Germany, and was cultivated at room temperature for 28 d on 12 L of biomalt solid agar media (Schulz et al. 1995). The culture media were then extracted with ethyl acetate to afford 15.5 g of residue after removal of solvent under reduced pressure. The extract was separated into three fractions by column chromatography on silica gel (350 g), using gradients of dichloromethane/ethyl acetate (85:15, 50:50, 0:100). The less-polar fraction **1** (5.8 g) contained mainly fatty acids and lipids. The remaining two fractions were each further purified by chromatography [silica gel column chromatography (CC), preparative TLC, Sephadex (LH-20)]. The next fraction (5.0 g) was separated by CC over 150 g of silica gel with hexane/ethyl acetate (10:1, 1500 mL; 5:1, 1000 mL) to give two sub-fractions A and B. Fraction A (2.0 g) was separated by CC over 20 g of silica gel with hexane/ethyl acetate (7:1, 850 mL) to give crude **6**, **7**, **9**, **12**, and **16**. Fraction B (1.5 g) was separated by CC over 18 g of silica gel with hexane/ethyl acetate (5:1, 750 mL) to give crude **3**, **4**, **10**, **11**, **13**, **17**, and **20**. Subsequently, each crude fraction was further purified by preparative TLC chromatography on silica gel (1 mm, Macherey–Nagel) and Sephadex (LH-20) to give compounds **3** (15 mg), **4** (12 mg), **6** (20 mg), **7** (7 mg), **9** (5 mg), **10** (2 mg), **11** (2 mg), **12** (7 mg), **13** (10 mg), **16** (30 mg), **17** (25 mg), and **20** (20 mg). The more-polar fractions (2.5 g) were separated by silica gel column chromatography, eluting with dichloromethane/ethyl acetate (4:1, 1000 mL), to give **2**, **5**, **8**, **14**, **15**, **18**, and **19**, successively, as crude gums. The samples were further subjected to silica gel column chromatography and were eluted with dichloromethane/methanol (15:1, 780 mL) to give pure compounds **2** (25 mg), **5** (18 mg), **8** (15 mg), **14** (30 mg), **15** (20 mg), **18** (35 mg), and **19** (30 mg).

Biological Testing: The culture extract was found to be biologically active against the fungal test organisms *Microbotryum violaceum*, *Septoria tritici*, *Botrytis cinerea*, and *Phytophthora infestans*, antibacterial against *Bacillus megaterium*, and algicidal against *Chlorella fusca*. 200 μL of medium, 50 μL of test organism, and 10 μL of substance (10 mg mL^{-1}) were incubated at 20 °C (Chl and St), at 37 °C (Bm), or at room temperature (Mv). Growth was evaluated as percent inhibition of cells per milliliter in comparison to the control. Media: Chl in CP, Mv and Sep in MPY, and Bm in NB.[18]

(3a*R*,5a*R*,7*R*,9a*S*,9b*R*)-3a,5a,6,7,8,9,9a,9b-Octahydro-3a,7-dihydroxy-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide B) (2): Colorless crystals (MeOH), m.p. 190–191 °C. $[\alpha]_D^{25}$ = –130.6 (*c* = 0.06, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3415, 2924, 1765, 1243, 1129, 1020, 917, 731 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2. EIMS: *m*/*z* (%) = 252 (10) [M$^+$], 234 (80), 206 (20), 194 (60), 176 (100), 149 (60), 121 (90), 99 (50), 81 (60), 57 (95), 43 (85). HREIMS (EI): calcd. for $C_{14}H_{20}O_4$ 252.13615; found 252.13646.

Crystal Structure Determination of Oblongolide B (2):[19] $C_{14}H_{20}O_4$, M = 252.3, orthorhombic, space group $P2_12_12_1$, a = 7.7557(5), b = 9.9611(6), c = 17.3889(11) Å, V = 1343.4(2) Å^3, Z = 4, D_c = 1.247 g cm^{-3}, $F(000)$ = 544, T = 120(2) K. Bruker-AXS SMART APEX, graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.09 mm^{-1}, colorless prismatic crystal, size 0.45 × 0.32 × 0.25 mm^3, 14191 intensities collected $2.3 < \theta < 28.2°$, $-10 < h < 10$, $-13 < k < 13$, $-21 < l < 23$. Structure solved by direct methods, full-matrix least-squares refinement based on F^2 and 167 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with U = 1.5 U_{iso}(O and methyl-C) or 1.2 U_{iso}(C). The title compound crystallizes in the non-centrosymmetric space group $P2_12_12_1$; however, in the absence of significant anomalous scattering effects, the Flack[20] parameter is essentially meaningless. Accordingly, Friedel pairs were merged. Refinement converged at R_1(F) = 0.034, wR_2(F^2, all data) = 0.089, S = 1.07, max(δ/σ) < 0.001, min/max height in final ΔF map –0.16/0.31 e Å^{-3}. Figure 2 shows the molecular structure. Program used: SHELXTL.[21]

(3a*R*,5a*R*,7*S*,9a*S*,9b*R*)-3a,5a,6,7,8,9,9a,9b-Octahydro-3a,7-dihydroxy-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide C) (3): Amorphous powder. $[\alpha]_D^{25}$ = –184 (*c* = 0.2, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3446, 2944, 1770, 1243, 1108, 1010, 772, 720 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2. EIMS: *m*/*z* (%) = 236 (15) [M$^+$], 220 (12), 205 (50), 178 (100), 121 (85), 85 (70), 57 (99), 29 (20). HRMS (EI): calcd. for $C_{14}H_{20}O_3$ 236.14125; found 236.14103.

(3a*S*,5a*R*,7*R*,9a*S*,9b*R*)-3a,5a,6,7,8,9,9a,9b-Octahydro-7-hydroxy-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide D) (4): Amorphous powder. $[\alpha]_D^{25}$ = –127.9 (*c* = 0.42, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3472, 2918, 1760, 1465, 1387, 1258, 1000, 741 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 1 and 2. HRMS (EI): calcd. for $C_{14}H_{20}O_3$ 236.14125; found 236.14122.

(3a*R*,5a*R*,7*S*,9a*S*,9b*R*)-3a,5a,6,7,8,9,9a,9b-Octahydro-3a-hydroxy-7-(hydroxymethyl)-9b-methylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide E) (5): Amorphous powder. $[\alpha]_D^{25}$ = –113.5 (*c* = 0.05, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3415, 2918, 1755, 1243, 1015, 720 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 1 and 2. HRMS (EI): calcd. for $C_{14}H_{20}O_4$ 252.13615; found 252.13600.

(3a*S*,5a*R*,7*S*,9a*S*,9b*R*)-7-(Hydroxymethyl)-9b-methyl-3a,5a,6,7,8,9,9a,9b-octahydronaphtho[1,2-*c*]furan-1(9b*H*)-one (Oblongolide F) (6): Amorphous powder. $[\alpha]_D^{25}$ = –205.5 (*c* = 0. 2, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3431, 2924, 1765, 1450, 1377, 1217, 1000, 762, 720 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 1 and 2. HRMS (EI): calcd. for $C_{14}H_{20}O_3$ 236.14125; found 236.14154.

(3a*S*,5a*R*,7*S*,9a*S*,9b*R*)-9b-Methyl-1-oxo-1,3,3a,5a,6,7,8,9,9a,9b-decahydronaphtho[1,2-*c*]furan-7-yl)methyl Acetate (Oblongolide G) (7): Colorless gum. $[\alpha]_D^{25}$ = –73.3 (*c* = 0.08, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 2918, 2355, 1765, 1450, 1382, 1227, 1010 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 1 and 2. HRMS (EI): calcd. for $C_{16}H_{22}O_4$ 278.15181; found 278.15184.

(3a*S*,5a*R*,7*R*,8*S*,9a*S*,9b*R*)-3a,5a,6,7,8,9,9a,9b-Octahydro-8-hydroxy-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide H) (8): Amorphous powder. $[\alpha]_D^{25}$ = –135 (*c* = 0. 2, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3487, 2918, 1760, 1460, 1377, 1206, 1000 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 2 and 3. HRMS (EI): calcd. for $C_{14}H_{20}O_3$ 236.14125; found 236.13970.

(5a*S*,7*S*,9a*S*,9b*R*)-3,5a,6,7,8,9,9a,9b-Octahydro-7,9b-dimethylnaphtho[1,2-*c*]furan-1,5-dione (Oblongolide I) (9): Colorless crystals (MeOH), m.p. 156–157 °C. $[\alpha]_D^{25}$ = –64.8 (*c* = 0.05 CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 2929, 1775, 1672, 1460, 1382, 1243, 1020 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 2 and 3. HRMS (EI): calcd. for $C_{14}H_{18}O_3$ 234.12560; found 234.12561.

Crystal Structure Determination of Oblongolide I (9):[19] $C_{14}H_{18}O_3$, *M* = 234.3, orthorhombic, space group $P2_12_12_1$, *a* = 6.4624(4), *b* = 9.2294(6), *c* = 20.5565(14) Å, *V* = 1226.1(1) $Å^3$, *Z* = 4, D_c = 1.269 $g\,cm^{-3}$, *F*(000) = 504, *T* = 120(2) K. Bruker-AXS SMART APEX, graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.09 mm^{-1}, colorless prismatic crystal, size 0.40 × 0.12 × 0.10 mm^3, 11331 intensities collected $2.0 < \theta < 28.2°$, $-8 < h < 8$, $-12 < k < 12$, $-27 < l < 27$. Structure solved by direct methods, full-matrix least-squares refinement based on F^2 and 156 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with $U = 1.5\,U_{iso}$(methyl-C) or $1.2\,U_{iso}$(C). The title compound crystallizes in the non-centrosymmetric space group $P2_12_12_1$; however, in the absence of significant anomalous scattering effects, the Flack[20] parameter is essentially meaningless. Accordingly, Friedel pairs were merged. Refinement converged at $R_1(F)$ = 0.037, $wR_2(F^2$, all data) = 0.060, *S* = 0.88, $\max(\delta/\sigma) < 0.001$, min/max height in final ΔF map –0.19/0.16 e $Å^{-3}$. Figure 2 shows the molecular structure. Program used: SHELXTL.[21]

(5*R*,5a*S*,7*S*,9a*S*,9b*R*)-5,5a,6,7,8,9,9a,9b-Octahydro-5-hydroxy-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide J) (10): Colorless gum. $[\alpha]_D^{25}$ = –5.1 (*c* = 0.1, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3420, 2929, 1770, 1450, 1382, 1077, 1005 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 2 and 3. HRMS (EI): calcd. for $C_{14}H_{20}O_3$ 236.14125; found 236.14231.

(5*S*,5a*S*,7*S*,9a*S*,9b*R*)-5,5a,6,7,8,9,9a,9b-Octahydro-5-hydroxy-7,9b-dimethylnaphtho[1,2-*c*]furan-1(3*H*)-one (Oblongolide K) (11): Colorless gum. $[\alpha]_D^{25}$ = –90 (*c* = 0.02, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 2924, 2375, 1770, 1465, 1077, 1010, 663 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 2 and 3. HRMS (EI): calcd. for $C_{14}H_{20}O_3$ 236.14125; found 236.14097.

{(1*R*,3a*S*,5a*R*,7*S*,9a*S*,9b*R*)-1-Acetyl-1-butoxy-1,3,3a,5a,6,7,8,9,9a,9b-decahydro-9b-methylnaphtho[1,2-*c*]furan-7-yl} Methyl Acetate (Oblongolide L) (12): Colorless gum. $[\alpha]_D^{25}$ = –68.3 (*c* = 0.09, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 2929, 2375, 1739, 1446, 1248, 1036 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 2 and 3. HRMS (EI): calcd. for $C_{22}H_{34}O_5$ 378.24063; found 378.24029.

{(1*R*,3a*S*,5a*R*,7*S*,9a*S*,9b*R*)-1-Acetyl-1-hydroxy-1,3,3a,5a,6,7,8,9,9a,9b-decahydro-9b-methylnaphtho[1,2-*c*]furan-7-yl} Methyl Acetate (Oblongolide M) (13): Colorless gum. $[\alpha]_D^{25}$ = –102.5 (*c* = 0.19, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3436, 2934, 1713, 1372, 1237, 1077, 1031, 901 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Tables 2 and 3. HRMS (EI): calcd. for $C_{18}H_{26}O_5$ 322.17801; found 322.17802.

4-[5-(1-Hydroxyethyl)furan-2-yl)-4-oxobutanoic Acid (14): Colorless gum. $[\alpha]_D^{25}$ = –3.8 (*c* = 0.3, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3436, 2924, 1734, 1662, 1512, 1398, 1206, 1041, 808 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data see Table 4. EIMS: *m*/*z* (%) = 212 (50) [M^+], 179 (95), 169 (30), 151 (90), 111 (100), 100 (35), 83 (90), 55 (95). HRMS (EI): calcd. for $C_{10}H_{12}O_5$ 212.06847; found 212.06829.

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