DOI: 10.1002/ejoc.200801021

Secondary Metabolites of *Phomopsis* sp. XZ-26, an Endophytic Fungus from *Camptotheca acuminate*

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Keywords: *Camptotheca acuminate* / *Phomopsis* sp. / Polyketides / Natural products / Oblongolide

Eleven new metabolites, including nine lovastatin analogues [oblongolides N–V (**1**–**2** and **5**–**11**), which were defined as naphthalene-type fungal polyketides], one linear furanopolyketide (**13**) and a monoterpene named dihydroxysabinane (**14**), together with four known compounds including oblongolides B (**3**) and C (**4**), one linear furanopolyketide (**12**) and the sesterterpene terpestacin (**15**), were isolated from the endophytic fungal strain *Phomopsis* sp. XZ-26 of *Camptotheca acuminate*. Their structures were elucidated by spectroscopic analyses including HR-ESI-MS, ^{1}H and ^{13}C NMR, 2D NMR (HMQC, HMBC, ^{1}H-^{1}H COSY and NOESY), and X-ray single-crystal analysis. The antimicrobial activities of **1**–**5**, **8**, **10** and **13**–**15** were evaluated, but none showed a substantial effect. Additionally, a hypothetical biosynthetic pathway for oblongolides was proposed.

Introduction

Plant endophytes are a group of microorganisms, including fungi and bacteria, which not only live within plants' internal tissues or organs without causing any apparent symptoms or diseases in the host plants, but also serve as important sources of bioactive compounds, presumably due to the symbiotic relationship with their hosts.[1,2] In the course of our exploration for chemical constituents from the endophytic microorganisms of medicinal plants, a series of new compounds were isolated previously.[3–7] *Camptotheca acuminate*, a medicinal plant specifically distributed in China, is rich in the anticancer compound camptothecin, with the highest distribution in the new leaf of up to 0.4%. In such a special living environment, special endophytic fungi may exist. Meanwhile, endophytic fungi in *C. acuminate* may take part in the synthesis or transformation of camptothecin.[8] Therefore, it should be interesting to study bioactive components of the endophytic fungi isolated from *C. acuminate*. Previously, we isolated approximately 174 endophytic fungal strains.[9] Among these endophytic fungi, 27.6% displayed inhibitory activities against more than one indicator microorganism, and 4.0% showed cytotoxicity against human cancer cells. In order to investigate the bioactive components of these active strains further, four new ten-membered-ring lactones and four new linear furanopolyketides were isolated from the endophytic fungal strains *Phomopsis* sp. NXZ-05[7] and *Diaporthe* sp. XZ-07,[10] respectively. Continuous research on the secondary metabolites from *Phomopsis* sp. XZ-26, an endophytic fungal strain isolated from the surface-sterilized twig tissues of *Camptotheca acuminate* whose extract showed inhibitory activities against human pathogenic microorganisms (data not shown), led to the discovery of 11 lovastatin-analogous oblongolides N–V (**1**–**2** and **5**–**11**), oblongolides B (**3**) and C (**4**),[11,12] two linear furanopolyketides [namely 5-{5-[(*R*)-1-hydroxyethyl]furan-2-yl}dihydrofuran-2(3*H*)-one (**12**) and 5-{5-[(*R*)-1-methoxyethyl]furan-2-yl}dihydrofuran-2(3*H*)-one (**13**)],[10,12] one new monoterpene dihydroxysabinane (**14**)[13] and the sesterterpene terpestacin (**15**).[14] In this paper, we report the isolation and structural characterization of **1**–**15** and the antimicrobial activities of **1**–**5**, **8**, **10** and **13**–**15**.

Results and Discussion

We obtained **1** as a white powder and determined it to have the molecular formula $C_{14}H_{20}O_4$ by HR-ESI-MS and ^{13}C NMR (Table 1). The IR absorptions at 3411 and 1755 cm^{-1} indicated the presence of an OH group and a γ-lactone moiety, respectively. The ^{13}C NMR (DEPT) spectra of **1** revealed 14 signals: two methyl groups, three methylene groups, six methine groups and three quaternary carbon atoms including a carbonyl carbon atom. The HMBC cor-

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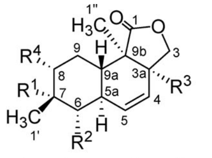
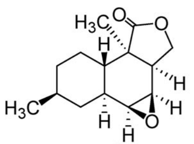


1 $R^1 = R^2 = H, R^3 = R^4 = OH$
2 $R^1 = R^3 = R^4 = H, R^2 = OH$
3 $R^1 = R^3 = OH, R^2 = R^4 = H$
4 $R^1 = R^2 = R^4 = H, R^3 = OH$
5 $R^1 = R^2 = R^3 = H, R^4 = OH$



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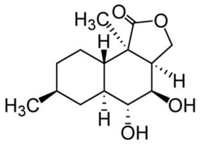

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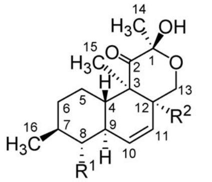
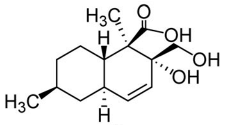


9 $R^1 = R^2 = H$
10 $R^1 = H, R^2 = OH$
11 $R^1 = OH, R^2 = H$



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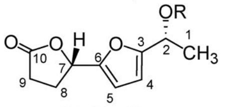
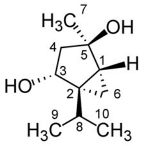


12 R = H
13 $R = CH_3$



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relations from the protons of the two methyl groups (H-1′ to C-6, C-7 and C-8 and H-1" to C-9a, C-9b, C-3a and C-1) established one 4-carbon and one 5-carbon moiety, respectively (Figure 1, in green). The ^1H-^1H COSY correlations between H-8 and H-9, H-9α and H-9a, H-9a and H-5a, H-5a and H-6β, H-5a and H-5, and H-5 and H-4 established the structure of a 7-carbon moiety (Figure 1, in blue), which bridged the two green moieties through C-8/C-9/C-9a and C-6/C-5a/C-9a, respectively. The last carbon atom of **1** to be assigned was an oxymethylene group. Its proton signals at δ = 4.33 (d, J = 9.8 Hz) and 4.19 (d, J = 9.8 Hz) ppm showed HMBC correlations to the carbonyl group (C-1), indicating the presence of a lactone residue. The HMBC correlations from H-3 to C-4 and C-9a revealed the linkage between C-3 and C-3a and between C-3a and C-4, establishing the other two rings in **1**.

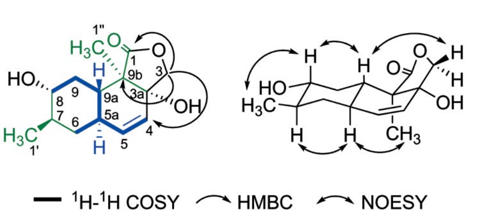

Figure 1. Selected ^1H-^1H COSY, HMBC and NOESY correlations for **1**.

We determined the relative configuration of **1** by analysis of the ROESY spectrum. The NOE correlations between H-1" and H-5a and between H-5a and H-7 established the α-orientations of H-5a, H-7 and C-1′′, whereas the correlations between H-3β and H-9a, H-9a and H-8, and H-8 and H-1′ indicated the β-orientations of H-8, H-9a, C-1′ and C-3 (Figure 1). A comparison of the NMR data of **1** with that of oblongolide C (**4**, Table 1) indicated that **1** was the 8α-hydroxy derivative of **4**. Therefore, we determined the structure of **1** to be 8α-hydroxyoblongolide C or 3a,8α-dihydroxyoblongolide A and named it as oblongolide N for consistency with the literature.[12]

We obtained **2** as a white powder and determined it to have the molecular formula $C_{14}H_{20}O_3$ by HR-ESI-MS ([M + Na]$^+$, m/z = 259.1305; calcd. for $C_{14}H_{20}O_3Na$ 259.1310) and ^{13}C NMR (Table 1). We determined the structure of **2** to be a 6-hydroxy derivative of oblongolide A by a compari-

Table 1. ^1H and ^{13}C NMR data of **1**–**4** [**1**, **3** and **4** at 400 MHz, **2** at 500 MHz, CD_3OD; chemical shift values are in ppm relative to TMS; J values (in Hz) are in parentheses].

No.	**1**		**2**		**3**		**4**	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1		186.1		182.6		182.0		182.0
3α	4.19 (d, 9.8)	79.0	3.88 (dd, 9.0, 10.8)	71.9	4.19 (d, 9.8)	79.1	4.18 (d, 9.7)	79.1
3β	4.33 (d, 9.8)		4.44 (t, 8.6)		4.35 (d, 9.8)		4.33 (d, 9.7)	
3a		78.8	2.81 (dt, 6.3, 9.5)	46.2		79.1		79.1
4	5.58 (dd, 2.4, 10.0)	127.7	5.64 (d, 10.6)	122.8	5.58 (dd, 2.4, 11.4)	127.6	5.56 (dd, 2.6, 10.1)	127.2
5	5.67 (d, 10.1)	134.4	5.61 (d, 10.6)	135.0	5.61 (d, 11.4)	135.4	5.63 (d, 9.4)	135.5
5a	2.03 (m)	37.2	1.96 (dd, 11.9)	37.3	2.44 (dt, 10.9, 11.2)	33.0	2.06 (t, 10.7)	37.6
6α	0.94 (t, 12.6)	40.3		71.3	1.82 (d, 13.3)	45.6	1.90 (dd, 2.1, 10.8)	42.4
6β	1.88 (dd, 3.6, 3.4)		3.86 (dd, 9.0, 10.8)		1.16 (dd, 10.1, 12.6)		0.81 (t, 12.2)	
7	1.41 (m)	41.2	1.62 (d, 6.0)	37.8		70.1	1.48 (m)	34.1
8α		76.4	1.25 (dt, 3.2, 9.4)	35.9	1.73 (m)	39.2	0.98 (dd, 6.8, 3.0)	36.0
8β	3.11 (dt, 4.3, 10.3)		1.57 (ddd, 3.2, 3.4, 9.4)		1.50 (m)		1.82 (m)	
9α	1.35 (dd, 11.2, 12.3)	36.1	1.47 (d, 13.1)	34.1	1.39 (ddd, 3.8, 12.2, 14.0)	22.5	1.35 (dd, 3.3)	27.0
9β	1.96 (dd, 3.2, 9.1)		1.85 (m)		1.67 (m)		1.69 (m)	
9a	1.63 (dt, 10.3)	42.7	1.88 (m)	33.5	1.55 (dt, 9.4, 11.1)	44.5	1.51 (dt, 2.4, 10.1)	44.9
9b		50.8		44.1		51.0		51.0
1'	1.03 (d, 6.4)	18.7	0.96 (d, 6.8)	18.6	1.22 (s)	31.4	0.93 (d, 6.5)	22.7
1"	1.10 (s)	9.6	1.09 (s)	16.0	1.10 (s)	9.6	1.08 (s)	9.5

Table 2. ^{1}H and ^{13}C NMR data of **5**–**8** [**5** in 400 MHz, **6** and **7** in 600 MHz, **8** in 500 MHz, **5** and **8** in CD_3OD, **6** and **7** in $CDCl_3$, chemical shift values are in ppm from TMS; *J* values (in Hz) are presented in parentheses].

No.	**5**		**6**		**7**		**8**	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1		182.3		179.7		179.7		182.7
3α	3.87 (dd, 8.9, 10.8)	71.8	4.28 (dd, 8.9, 10.7)	66.3	4.21 (dd, 9.7, 11.7)	66.4	3.87 (t, 8.6)	72.0
3β	4.47 (t, 8.7)		4.46 (t, 8.8)		4.38 (dd, 8.4, 9.4)		4.45 (t, 8.6)	
3a	2.83 (dt, 8.0, 8.5)	45.8	2.71 (ddd, 5.2, 8.8, 14.0)	42.6	2.84 (dddd, 1.8, 2.0, 2.9, 6.2, 6.4)	48.8		70.2
4	5.62 (d, 10.3)	123.2	3.20 (dd, 4.1, 5.1)	48.8	3.81 (dd, 6.3, 9.4)	71.6	5.60 (d, 10.2)	123.1
5	5.69 (d, 10.3)	134.1	3.13 (d, 3.9)	56.8	3.39 (t, 9.6)	76.0	5.65 (d, 10.2)	135.2
5a	1.99 (m, overlapped)	37	1.80 (dd, 1.1, 3.1)	34.6	1.37 (m)	40.9	2.80 (m)	32.8
6α	0.92 (q, 12.6)	40.6	1.08 (dq, 6.2, 13.0)	38.6	0.68 (m)	38.1	1.22 (dd, 11.5, 14.7)	46.0
6β	1.92 (dd, 3.7, 13.1)		1.94 (ddd, 3.4, 5.6, 12.9)		2.17 (m)		2.37 (dd, 11.3, 11.4)	
7	1.42 (m)	41.2	1.49 (m)	32.7	1.37 (m)	31.5	1.62 (d, 3.3)	40.6
8α		76.8	0.90 (ddd, 3.5, 12.1, 13.0)	34.6	0.84 (d, 3.4)	34.2	1.37 (ddd, 4.0, 5.3, 8.2)	22.1
8β	3.13 (dt, 4.3, 10.4)		1.78 (dd, 1.6, 3.2)		1.76 (2.3, 3.4, 4.2)		1.81 (dd, 2.9, 13.3)	
9α	1.27 (ddd, 11.1)	35.7	1.23 (ddd, 3.5, 12.1, 13.0)	25.1	1.18 (3.4)	25.8	1.35 (m)	39.6
9β	2.02 (ddd, 2.9, 11.4)		1.84 (ddd, 3.3, 6.5, 13.1)		1.90 (1.7, 2.5)		1.74 (d, 2.8)	
9a	1.47 (dt, 2.7, 10.1)	38.9	1.38 (dt, 3.1, 12.2)	34.6	1.37 (m)	39.9	1.56 (d, 3.1)	46.2
9b		44.3		42.1		45.0		44.5
1'	1.04 (d, 6.4)	18.7	0.93 (d, 6.5)	22.3	0.92 (d, 6.5)	22.3	1.13 (d, 6.4)	31.4
1''	1.13 (s)	16.3	1.13 (s)	17.0	1.21 (s)	16.8	1.20 (s)	16.4

son of its NMR spectra with those of oblongolides B (**3**), C (**4**), P (**5**) and N (**1**) (Tables 1 and 2). We determined the relative configuration of the C-6 hydroxy group to be α based on the coupling pattern of H-6 (δ_H = 3.88, dd, *J* = 10.8, 9.0 Hz) ppm, which indicated that H-5a, H-6 and H-7 were all axial (see Figure 1). Therefore, we determined **2** to be 6α-hydroxyoblongolide A and named it as oblongolide O.

We obtained **5** as colorless crystals and determined it to have the molecular formula $C_{14}H_{20}O_3$ by HR-ESI-MS ($[M + Na]^+$, *m*/*z* = 259.1308; calcd. for $C_{14}H_{20}O_3Na$ 259.1310) and ^{13}C NMR (Table 2). The ^{13}C NMR and DEPT spectra of **5** were similar to those of oblongolide H,[12] except that the proton signal at δ_H = 3.96 (d, *J* = 2.1 Hz) ppm in oblongolide H moved to δ_H = 3.13 (dt, *J* = 4.3, 10.4 Hz) ppm, which established **5** as the 8-epimer of oblongolide H or 8α-hydroxyoblongolide A; we named it oblongolide P.

We obtained **6** as colorless crystals and determined it to have the molecular formula $C_{14}H_{20}O_3$ by HR-ESI-MS ($[M + Na]^+$, *m*/*z* = 259.1761; calcd. for $C_{14}H_{20}O_3Na$ 259.1310) and ^{13}C NMR (Table 2). The ^{13}C NMR and DEPT spectra of **6** showed 14 carbon signals for two methyl groups, four methylene groups, six methine groups, and two quaternary carbon atoms including one carbonyl group. A comparison of the ^{1}H and ^{13}C NMR data of **6** with that of oblongolide O (**2**) revealed that these two compounds had similar structures; however, **6** did not have a hydroxy group at C-6, and the double bond at C-4 and C-5 of **2** was oxidized to an epoxide. Therefore, we determined **6** to be 4,5-dihydro-4,5-epoxyoblongolide A and named it oblongolide Q. We unambiguously confirmed the structure of **6** by X-ray diffraction analysis of a single crystal obtained from methanol (Figure 2).

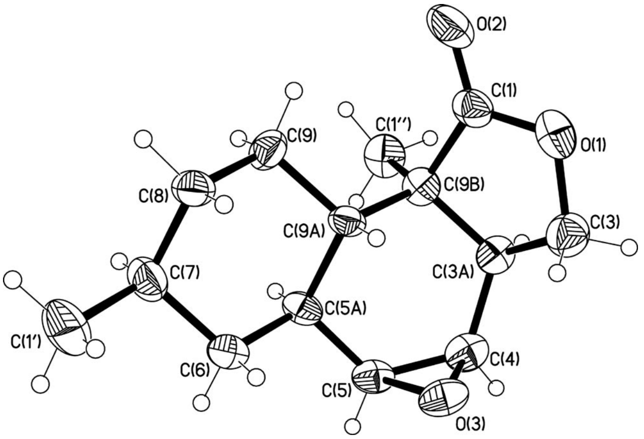

Figure 2. Molecular structure of **6** in the crystalline state.

We obtained **7** as a yellow powder and determined it to have the molecular formula $C_{14}H_{22}O_4$ by HR-ESI-MS ($[M + Na]^+$, *m*/*z* = 277.1870; calcd. for $C_{14}H_{22}O_4Na$ 277.1416) and ^{13}C NMR (Table 2). The 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR spectra of **7** were similar to those of **6** except for changes at C-4 and C-5 due to the opening of the epoxide to form a vicinal diol moiety. We determined the relative configurations of these two hydroxy groups by analysis of NOESY spectra (Figure 3), and they were supported by the coupling patterns of H-4 and H-5 (Table 2). Therefore, we determined **7** to be 4,5-dihydro-4β,5α-dihydroxyoblongolide A and named it oblongolide R.

We obtained **8** as colorless crystals and determined it to have the molecular formula $C_{14}H_{22}O_4$ by HR-ESI-MS ($[M + Na]^+$, *m*/*z* = 277.1420; calcd. for $C_{14}H_{22}O_4Na$ 277.1415) and ^{13}C NMR (Table 2). The NMR data of **8** and **1** were almost identical except that the chemical shift values of H-3 and C-1 were moved upfield and downfield, respectively (Tables 1 and 2), indicating that the γ-lactone ring in **1** was

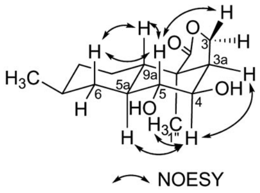

Figure 3. Selected NOESY correlations for **7**.

opened in **8**, which was consistent with the HR-ESI-MS data. Therefore, we determined **8** to be oblongolide A acid and named it oblongolide S.

We obtained **9** as a white powder and determined it to have the molecular formula $C_{16}H_{24}O_3$ by HR-ESI-MS ([M + Na]$^+$, m/z = 287.2123; calcd. for $C_{16}H_{24}O_3Na$ 287.1623) and ^{13}C NMR (Table 3). Its IR absorption at 1715 cm^{-1} and ^{13}C NMR signal at δ_C = 208.9 (C-2) ppm indicated the presence of a ketone carbonyl group. The ^{13}C NMR and DEPT spectra showed 16 carbon signals for three methyl groups, four methylene groups, six methine groups, and three quaternary carbon atoms including one carbonyl group. An inspection of the NMR data (^1H, ^{13}C, HMQC, HMBC and ^1H-^1H COSY) revealed that **9** had the same octahydronaphthalene moiety as **1**–**5** and **8** but lacked the γ-lactone residue. Furthermore, **9** had one more methyl group and one more oxy quaternary carbon atom (Figure 4, in red) than did **1**–**8**. The HMBC correlations from the extra methyl protons (H-14) to C-1 and C-4 and from H-13 to C-1 established a β-pyrone moiety. We determined the relative configurations of **9** by analysis of NOESY spectra. Particularly, the NOE correlations between H-14 and H-15 and between H-14 and H-13α indicated the α-orientation of the C-14 methyl group (Figure 4). Therefore, we determined the structure of **9** and named it oblongolide T. Indeed, **9** and oblongolide M[12] were structurally similar. The main difference was that a hemiketal formed between the 13-hydroxy group and the 2-oxo group to give an α-hydroxyfuran residue in oblongolide M, whereas the hemiketal formed between the 13-hydroxy group and the 1-oxo group afforded an α-hydroxypyran in **9**. Interestingly, the relative configuration of the hemiketal carbon atoms in **9** and oblongolide M was the same.

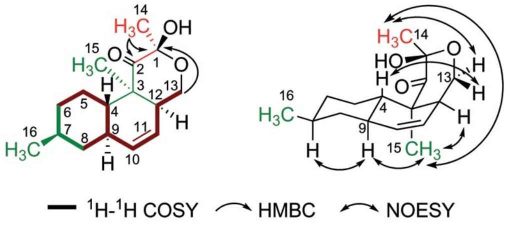

Figure 4. Selected ^1H-^1H COSY, HMBC and NOESY correlations for **9**.

We obtained **10** as colorless crystals and determined it to have the molecular formula $C_{16}H_{24}O_4$ by HR-ESI-MS ([M + Na]$^+$, m/z = 303.1579; calcd. for $C_{16}H_{24}O_4Na$ 303.1572) and ^{13}C NMR (Table 3). The NMR data of **10** were similar to those of **9**, the only difference being that the proton signal at δ = 2.46 ppm (H-12) in **9** was replaced by a hydroxy signal in **10**. Therefore, we determined **10** to be 12α-hydroxyoblongolide S and named it oblongolide U.

We obtained **11** as a white powder and determined it to have the molecular formula $C_{16}H_{24}O_4$ by HR-ESI-MS ([M + Na]$^+$, m/z = 303.2003; calcd. for $C_{16}H_{24}O_4Na$ 303.1572) and ^{13}C NMR (Table 3). A comparison of the NMR data of **11** with those of **9** indicated that **11** was a hydroxy derivative of **9** as well, similar to **10**. However, the hydroxy substitution in **11** was at a methine group rather than at a quaternary carbon atom as in **10**. The HMBC correlations from both the H-16 and H-10 signals to the carbon signal at δ_C =

Table 3. ^1H and ^{13}C NMR data of **9**–**11** [**9** at 500 MHz, **10** and **11** at 400 MHz, **9** and **10** in CD_3OD, **11** in CD_3COCD_3, chemical shift values are in ppm relative to TMS; J values (in Hz) are in parentheses].

No.	**9**		**10**		**11**	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1		98.1		98		97.7
2		208.9		208.5		206.8
3		50.0		55.7		49.2
4	2.16 (dt, 2.3, 8.8)	40.9	2.38 (t, 8.6)	45.4	2.33 (dt, 2.1, 10.8)	38.2
5α	1.11 (ddd, 3.2, 8.9)	27.9	1.16 (dd, 3.1, 12.0)	28.0	1.10 (dd, 2.4, 6.9)	27.0
5β	1.22 (d, 2.8)		1.25 (m)		1.19 (ddd, 2.8, 3.3, 6.1)	
6α	0.96 (dd, 3.8, 12.7)	36.5	0.96 (d, 3.6)	36.2	1.02 (m, overlapped)	33.8
6β	1.73 (d, 12.9)		1.72 (d, 12.4)		1.70 (m, overlapped)	
7	1.45 (m)	34.2	1.43 (m)	34.3	1.37 (m, overlapped)	41.3
8α	0.81 (dd, 12.0)	43.1	0.79 (d, 11.9)	42.7	2.80 (dd, 9.6, 16.8)	79.1
8β	1.81 (d, 11.1)		1.85 (d, 9.9)			
9	1.79 (m, overlapped)	39.1	1.89 (d, 13.2)	39.2	1.71 (m)	45.7
10	5.58 (d, 9.9)	135.3	5.62 (d, 9.9)	136.0	6.22 (d, 10.1)	131.2
11	5.46 (dd, 2.2, 9.9)	124.2	5.32 (dd, 2.3, 10.1)	129.1	5.53 (d, 9.8)	124.4
12	2.46 (t, 5.7)	49.0		79.2	2.45(ddd, 3.9, 4.4, 6.0)	48.2
13α	3.57 (dd, 5.0, 11.9)	62.4	3.38 (d, 12.6)	66.9	3.58 (dd, 5.1, 11.9)	61.7
13β	4.19 (t, 11.8)		4.54 (d, 12.6)		4.17 (dd, 11.8)	
14	1.32 (s)	24.3	1.37 (s)	24	1.33 (s)	24.6
15	0.98 (s)	16.6	1.01 (s)	11.5	0.97 (s)	16.4
16	0.91 (d, 6.5)	21.8	0.92 (d, 6.5)	22.8	1.00 (d, 6.4)	19.1

79.1 ppm located the hydroxy substitution at C-8. NOESY spectra indicated that the relative configurations of **11** were similar to **9**, too. Additionally, H-8 had NOE correlations with H-4 and H-16, which established the α-orientation of the C-8 hydroxy group. Therefore, we determined **11** to be 8α-hydroxyoblongolide T (**9**) and named it oblongolide V.

Besides 11 oblongolides including 8 new ones, we isolated three more linear, small polyketides having a furan residue. We readily determined the structure of **12** to be 5-[5-(1-hydroxyethyl)furan-2-yl]dihydrofuran-2(3*H*)-one by a comparison of NMR data, and it was isolated from another endophytic fungal strain *Diaporthe* sp. XZ-07 of *C. acuminate* previously.[10] Meanwhile, HR-ESI-MS and NMR data (Table 4) indicated that **13** was the methyl ether of **12**, namely 5-{5-[(*R*)-1-methoxyethyl]furan-2-yl}dihydrofuran-2(3*H*)-one.

Table 4. ^{1}H and ^{13}C NMR chemical shift assignments for **12** and **13** [**12** at 400 MHz, **13** at 500 MHz, in CD_3OD, chemical shift values are in ppm relative to TMS; *J* values (in Hz) are in parentheses].

No.	**12**		**13**	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	1.49 (d, 6.6)	21.7	1.50 (d, 6.5)	19.4
2	4.82 (q, 6.5)	64.1	4.36 (q, 6.5)	72.1
3		160.7		156.7
4	6.27 (d, 3.1)	106.9	6.24 (d, 2.5)	107.8
5	6.48 (d, 3.1)	111.2	6.37 (d, 2.4)	109.8
6		151.7		151.7
7	5.57 (t, 7.4)	76.4	5.49 (t, 7.2)	74.5
8	2.56 (m)	27.4	2.55 (m)	26.5
9	2.77 (m)	29.7	2.78 (m)	28.6
10		179.4	–	179.4
MeO-2			3.29 (s)	56.3

Additionally, we isolated two terpenoids. We obtained **14** as white needles and determined it to have the molecular formula $C_{10}H_{18}O_2$ by ^{1}H and ^{13}C NMR data. The ^{1}H and ^{13}C NMR data of **14** were similar to those of trihydroxysabinane,[13] except that one ^{1}H NMR AB system in **14** replaced the doublet at δ_H = 3.42 (d, *J* = 6.5 Hz, H-4α) ppm of trihydroxysabinane. Therefore, we determined **14** to be dihydroxysabinane.

We determined **15** to be terpestacin, a sesterterpene, by comparing its NMR data with those in the literature.[14]

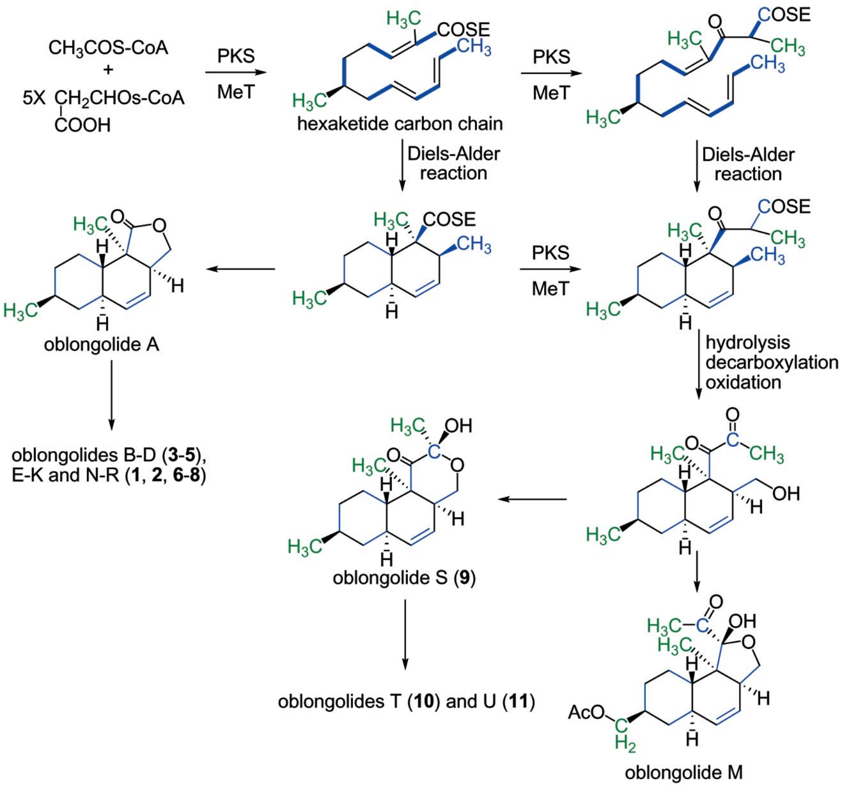

Figure 5. Proposed biosynthetic pathway of oblongolides. The hexaketide carbon chain may be synthesized from one acetate and five malonate molecules (in blue) and then methylated by methyl groups derived from the methyl group of methionine (in green). The open-chain polyketide may undergo intramolecular cyclization catalyzed by a putative Diels–Alderase to produce the octahydronaphthalene intermediate, as proposed for the biosynthesis of dihydromonacolin L.[21] The hexaketide intermediate could be cleaved from the PKS by hydrolysis and oxidized and lactonized to yield oblongolide A. Oblongolide A can readily be transformed into oblongolides B–K and N–R by hydroxylations, and/or epoxidation and hydrolysis. Oblongolides L, M, T and U, having one more extension unit, may be derived from a heptakeitde or from the cyclized hexaketide. Either way, the naphthalene-type heptakeitde intermediate could be easily transformed into diverse end products such as **9**, **10** and oblongolide M. Other fungal metabolites such as versiol,[15] decumbenones A and B[16] and the calbistrins,[17–19] having the same naphthalene ring system as the oblongolides, may be derived on similar biosynthetic pathways. Abbreviations: PKS: polyketide synthase; MeT: methyltransferase.

In contrast to the activities of oblongolides A–M against the gram-positive bacterium *Bacillus megaterium*, the fungi *Microbotryum violaceum* and *Septoria tritici*, and the green algae *Chlorella fusca*, **1**–**5**, **8** and **10** only exhibited very mild inhibitory activities (inhibition rates were $<20\%$ as measured by OD_{560}) against the gram-negative bacterium *Escherichia coli* ATCC 25922, the gram-positive bacterium *Bacillus subtilis* ATCC 9372 and no activity against the gram-positive bacterium *Staphylococcus aureus* ATCC 25923 and the fungi *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* As 2.538 at 50 µg/mL when using gentamycin and amphotericin B as positive controls. Meanwhile, **13**–**15** showed no antimicrobial activities either.

The putative biosynthetic pathway of oblongolides (Figure 5) suggested these compounds to be classified as polyketides rather than sesquiterpenes.[12] This suggestion was supported by the evidence that all oblongolides reported so far have the identical fusion pattern of the two six-membered rings, the octahydronaphthalene skeleton. Actually, this type of carbon skeleton has been found in other fungal metabolites such as versiol,[15] decumbenones A and B[16] and the calbistrins.[17–19] Moreover, the well-known fungal polyketide dihydromonacolin L[20], which is a nonaketide and the first recognized intermediate in lovastatin biosynthesis, also has the same octahydronaphthalene skeleton.[21] By following the known biosynthetic pathway of dihydromonacolin L,[21] it was speculated that the biosynthesis of oblongolides started from acetyl-CoA and proceeded via a hexaketide intermediate, which may undergo intramolecular cyclization catalyzed by a putative Diels–Alderase (Figure 5).[22] This cyclization determines the configuration of the octahydronaphthalene moiety and may be conserved in all naphthalene-type fungal polyketides.

Experimental Section

General: The IR spectra were recorded with a Nicolet AVATAR 330 FT spectrometer. Optical rotations were measured with a Perkin–Elmer 341 automatic polarimeter in methanol. NMR spectra were recorded with Bruker AM-400, Bruker DRX-500 and Avance III-600 NMR spectrometers with TMS as an internal standard. ESI-MS and HR-ESI-MS data were acquired by using Finnigan LCQ-Advantage and VG Auto-Spec-3000 mass spectrometers, respectively. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, P. R. China), silica gel H (10–40 µm, Qingdao), Sephadex LH-20 (40–70 µm, Amersham Pharmacia Biotech AB, Uppsala, Sweden) and Lichroprep reversed-phase RP-18 silica gel (40–63 µm, Merck, Darmstadt, Germany). Preparative TLC (1.0–1.5 mm) was conducted with glass-precoated silica gel GF_{254} (Qingdao), and spots were visualized under UV light.

Fungal Material: The fungus (XZ-26) was isolated from the current-year twig (8–12 × 1–2 cm, length × diameter) of *Camptotheca acuminate* collected from the Jiangshi Natural Reserve, Shaowu, Fujian, China. It was identified as a non-sporulating fungus by traditional morphology. A BLAST search result showed that the internal transcribed spaces (ITS) sequence of XZ-26 (accession number DQ272500 in GenBank) was highly homologous to that of *Phomopsis* species, indicating that XZ-26 belongs to the genus *Phomopsis*.

Culture Conditions and Extraction: The strain was cultivated at 28 ± 1 °C with 8 L of PDA (Potato Dextrose Agar) media for 10 d. The culture media was then extracted with ethyl acetate/methanol/acetic acid (80:15:5) at room temperature overnight. The organic solution was collected through filtration. The remaining agar residue was extracted twice more as described above. The combined filtrates were concentrated under vacuum to remove organic solvents. The aqueous solution was extracted several times with ethyl acetate to afford 4.0877 g of residue after removal of the solvent under reduced pressure.

Isolation: The crude extract (4.0877 g) was separated into nine fractions (Fr. 1–9) by column chromatography on RP-18 silica gel (170 g), eluted by methanol/H_2O (0:100, 30:70, 50:50, 70:30, and 100:0). These fractions were further purified by repeated column chromatography on Sephadex LH-20 and silica gel.

Fr. 1 (699.3 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give four subfractions (Fr. 1a–1d). Fr. 1b (47.6 mg) was separated by column chromatography on silica gel (eluting with chloroform) to afford **13** (7.6 mg) and **12** (6.0 mg).

Fr. 2 (278.7 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give two subfractions (Fr. 2a–2b). Fr. 2b (40.3 mg) was further purified by column chromatography on silica gel [eluting with petroleum ether/ethyl acetate (5:1, v/v)] to afford **1** (5.3 mg) and **4** (13 mg).

Fr. 3 (411.6 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to produce three subfractions (Fr. 3a–3c). Fr. 3a (163.3 mg) was separated by column chromatography on silica gel [eluted by petroleum ether/ethyl acetate (8:1, v/v)] to afford **14** (8.8 mg).

Fr. 5 (408.0 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to afford three subfractions (Fr. 5a–5d). Fr. 5b (110.2 mg) was further purified by column chromatography on silica gel. Elution with chloroform gave **5** (3.6 mg), and elution with a gradient chloroform/acetone and chloroform/methanol afforded **8** (6.6 mg). Fr. 5c (10 mg) was further purified by repeated column chromatography on silica gel [eluted by petroleum ether/chloroform (3:1. 2:1, 1:1, v/v)] to yield **7** (3.5 mg). Fr. 5d (143.6 mg) was separated by column chromatography onr Sephadex LH-20 (eluted with methanol) to yield eight subfractions (Fr. 5d1–5d8). Fr. 5d4 (44 mg) was further purified by column chromatography on silica gel [eluted with petroleum ether/chloroform (2:1, v/v)] to yield **6** (30.0 mg).

Fr. 6 (268.3 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give three subfractions (Fr. 6a–6c). Fr. 6b (82.0 mg) was further purified by column chromatography on silica gel [eluted with chloroform/methanol (160:1, v/v)] to give **2** (10.3 mg).

Fr. 7 (495.0 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give six subfractions (Fr. 7a–7f). Fr. 7d (98.8 mg) was separated by column chromatography on silica gel [eluted with petroleum ether/ethyl acetate (8:1, v/v)] to yield pure **3** (8 mg) and **10** (2 mg). Fr. 7e (10 mg) was subjected to column chromatography on silica gel [eluted with petroleum ether/ethyl acetate (10:1, v/v)] to yield **9** (1 mg) and **11** (1 mg).

Fr. 8 (175.8 mg) was separated by column chromatography on Sephadex LH-20 (140 g, eluted with methanol) to give four subfractions (Fr. 8a–8d). Fr. 8b (96.9 mg) was further purified by column chromatography on silica gel [eluted with chloroform/methanol (80:1, v/v)] to afford **15** (2 mg).

Biological Assay: 100 µL of medium (test microorganism concentration: 10^6 cfu) and 100 µL of compound solution (50 µg/mL final concentration) were incubated at 30 °C for 18 h. Growth was evaluated as the percent decrease of the OD at 560 nm in comparison to that of the negative control with the formula: [OD (negative control) – OD (sample)]/OD (negative control). *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 9372, and *Staphylococcus aureus* ATCC 25923 were inoculated in LB medium; *Saccharomyces cerevisiae* ATCC 9763, and *Candida albicans* As 2.538 were inoculated in Potato Dextrose (PD) medium.

Oblongolide N (1): White powder, $[\alpha]_D^{20}$ = –154.5 (c = 0.067, MeOH). IR (KBr): $\tilde{\nu}$ = 3411, 2925, 1755, 1446, 1242, 1121, 1019, 760 cm^{-1}. ^1H and ^{13}C NMR: see Table 1. HR-ESI-MS: m/z = 275.1252 $[M + Na]^+$ (calcd. 275.1259 for $C_{14}H_{20}O_4Na$).

Oblongolide O (2): White powder, $[\alpha]_D^{20}$ = –51.8 (c = 0.0062, MeOH). IR (KBr): $\tilde{\nu}$ = 3417, 2918, 1755, 1651, 1384, 1094, 1022 cm^{-1}. ^1H and ^{13}C NMR: see Table 1. HR-ESI-MS: m/z = 259.1305 $[M + Na]^+$ (calcd. 259.1310 for $C_{14}H_{20}O_3Na$).

Oblongolide P (5): Colorless crystals. ^1H and ^{13}C NMR: see Table 2. HR-ESI-MS: m/z = 259.1308 $[M + Na]^+$ (calcd. 259.1310 for $C_{14}H_{20}O_3Na$).

Oblongolide Q (6): Colorless crystals, m.p. 141.0–141.2 °C, $[\alpha]_D^{25}$ = –66.8 (c = 2.10, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 2947, 2913, 2848, 1758, 1454, 1376, 1020, 907, 877 cm^{-1}. ^1H and ^{13}C NMR: see Table 2. HR-ESI-MS: m/z = 259.1761 $[M + Na]^+$ (calcd. 259.1310 for $C_{14}H_{20}O_3Na$).

Crystal Structure Analysis of Oblongolide Q (6): $C_{14}H_{20}O_3$, M = 236.3, monoclinic, space group $P21$, a = 9.9000(10) Å, b = 6.4296(5) Å, c = 10.4510(9) Å, β = 107.476(10)°, V = 634.53(10) Å^3, Z = 2, $D_{calcd.}$ = 1.237 mg/m^3, F(000) = 256, T = 293(2) K. A single crystal of dimensions 0.1 × 0.05 × 0.8 mm was used for X-ray measurements. The intensity data of all the unique reflections within the range $4.01° < \theta < 25.00°$ were collected at 292 K in an Oxford Gemini S Ultra single-crystal diffractometer, by using graphite-monochromated Mo-K_α (λ = 0.71073 Å) radiation. A total of 4019 independent reflections were measured, and 2066 were considered to be observed ($|F|^2 \geq 2\sigma|F|^2$). The data were processed with the software CRYSALIS (CRYSALIS, version 1.171.31.8, Oxford Diffraction Ltd., 2007). The structure was solved by direct methods and refined by full-matrix least squares based on F^2 with the SHELXL program (G. M. Sheldrick, *SHELXL-97: Program for Crystal Structure Solution and Refinements*, University of Göttingen, Germany, 1997.) H atoms were added in ideal positions and refined by using riding models. CCDC-665512 (for **6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via http://www.ccdc.cam.ac.uk/data_request/cif.

Oblongolide R (7): Yellow powder, $[\alpha]_D^{25}$ = –150.0 (c = 0.35, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3424, 2929, 1762, 1458, 1388, 1221, 1104, 1061, 999, 754 cm^{-1}. ^1H and ^{13}C NMR: see Table 2. HR-ESI-MS: m/z = 277.1870 $[M + Na]^+$ (calcd. 277.1416 for $C_{14}H_{22}O_4Na$).

Oblongolide S (8): Colorless crystal, $[\alpha]_D^{20}$ = –47.2 (c = 0.0032, MeOH). IR (KBr): $\tilde{\nu}$ = 3434, 2924, 1765, 1593, 1375, 1139, 1026, 710 cm^{-1}. ^1H and ^{13}C NMR: see Table 2. HR-ESI-MS: m/z = 277.1420 $[M + Na]^+$ (Calcd. 277.1415 for $C_{14}H_{22}O_4Na$).

Oblongolide T (9): White powder, $[\alpha]_D^{25}$ = –186.4 (c = 0.50, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3431, 2945, 2918, 1715, 1454, 1376, 1073, 898, 719 cm^{-1}. ^1H and ^{13}C NMR: see Table 3. HR-ESI-MS: m/z = 287.2123 $[M + Na]^+$ (calcd. 287.1623 for $C_{16}H_{24}O_3Na$).

Oblongolide U (10): Colorless crystals, $[\alpha]_D^{20}$ = –50.8 (c = 0.00347, MeOH). IR (KBr): $\tilde{\nu}$ = 3417, 2947, 2923, 2853, 1714, 1593, 1457, 1375, 1115, 1076, 1039, 907 cm^{-1}. ^1H and ^{13}C NMR: see Table 3. HR-ESI-MS: m/z = 303.1579 $[M + Na]^+$ (calcd. 303.1572 for $C_{16}H_{24}O_4Na$).

Oblongolide V (11): White powder, $[\alpha]_D^{25}$ = –85.5 (c = 0.20, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3296, 2922, 1710, 1650, 1555, 1454, 1374, 1106, 1067, 1036, 905, 699 cm^{-1}. ^1H and ^{13}C NMR: see Table 3. HR-ESI-MS: m/z = 303.2003 $[M + Na]^+$(calcd. 303.1572 for $C_{16}H_{24}O_4Na$).

5-[5-(1-Hydroxyethyl)furan-2-yl]dihydrofuran-2(3*H*)-one (12): Colorless oil, $[\alpha]_D^{20}$ = –3.6 (c = 0.0042, MeOH). IR (KBr): $\tilde{\nu}$ = 3375, 1770, 1176, 1018, 918, 798 cm^{-1}. ^1H and ^{13}C NMR: see Table 4. HR-ESI-MS: m/z = 219.0631 $[M + Na]^+$ (calcd. 219.0633 for $C_{10}H_{12}O_4Na$).

5-{5-[(*R*)-1-Methoxyethyl]furan-2-yl}dihydrofuran-2(3*H*)-one (13): Colorless oil, $[\alpha]_D^{20}$ = +3.9 (c = 0.00513, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3434, 1778, 1598, 1391, 1117, 1021, 912 cm^{-1}. ^1H and ^{13}C NMR: see Table 4. HR-ESI-MS: m/z = 233.0794 $[M + Na]^+$ (calcd. 233.0789 for $C_{11}H_{14}O_4Na$).

Dihydroxysabinane (14): Colorless oil, $[\alpha]_D^{20}$ = –3.8 (c = 0.00373, MeOH). IR (KBr): $\tilde{\nu}$ = 3370, 2976 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.48 (dd, J = 5.1, 7.7 Hz, 1 H, H-6a), 0.59 (dd, J = 4.0, 5.1 Hz, 1 H, H-6b), 1.01 (s, 6 H, 9-CH_3, 10-CH_3), 1.12 (t, J = 4.9 Hz, 1 H, H-1), 1.17 (dd, J = 7.8, 13.5 Hz, 1 H, H-4a), 1.23 (s, 3 H, 7-CH_3), 1.65 (d, J = 6.9 Hz, 1 H, H-8), 1.88 (dd, J = 7.8, 13.5 Hz, 1 H, H-4b), 4.61 (t, J = 8.2 Hz, 1 H, H-3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0 (6-CH_3), 20.4 (10-CH_3), 20.8 (9-CH_3), 25.9 (7-CH_3), 31.8 (C-8), 34.7 (C-1), 38.7 (C-2), 46.3 (C-4), 74.3 (C-3), 78.4 (C-5) ppm.

Supporting Information (see footnote on the first page of this article): ^1H and ^{13}C NMR spectra of **1**, **2**, and **5**–**14**.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (30500632), the National Science Fund for Distinguished Young Scholars to Y.-M. S. (30325044) and the Key Grant of Chinese Ministry of Education (No. 306010).

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Received: October 20, 2008