Oblongolides from the Endophytic Fungus *Phomopsis* sp. BCC 9789

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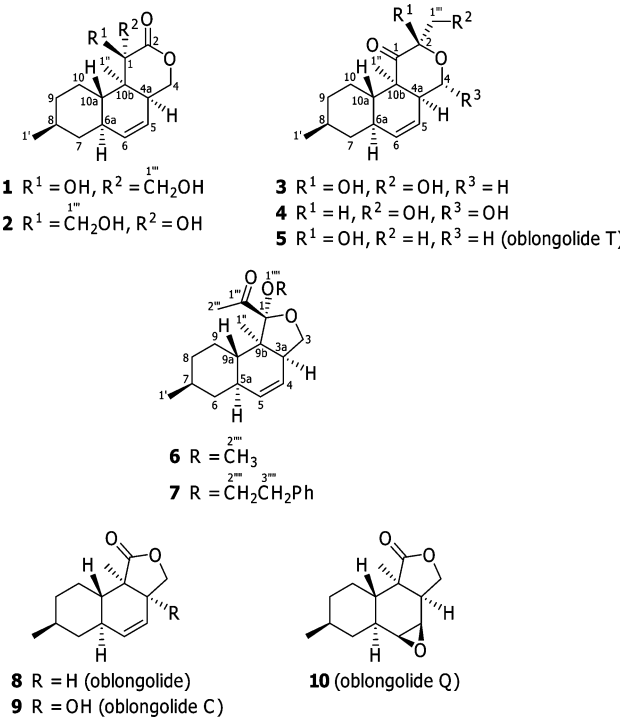
Six new oblongolides, W1, W2, X, Y, and Z (**1**−**3**, **6**, **7**) and 2-deoxy-4α-hydroxyoblongolide X (**4**), and the known compounds oblongolide (**8**), oblongolides T, C, and Q (**5**, **9**, **10**), and (−)-5-methylmellein were isolated from the endophytic fungus *Phomopsis* sp. BCC 9789. Compound **7** showed anti-HSV-1 activity (IC_{50} = 14 μM) and cytotoxic activities against KB, BC, NCI-H187, and nonmalignant (Vero) cell lines with respective IC_{50} values of 37, 26, 32, and 60 μM. Cytotoxic activity against the BC cell line was also observed for compound **6**, with an IC_{50} value of 48 μM.

Fungi in the genus *Phomopsis* are rich sources of secondary metabolites including phomopsins, hexapeptides containing unnatural amino acids, from *P. leptostromiformis*;[1] phomopsolides, α,β-unsaturated δ-lactones, from *P. oblonga*;[2] dicerandrols, xanthone dimers, from *P. longicolla*;[3] and phomodiol,[4] phomopsichalasin,[5] phomoxanthones,[6] phomopsidin,[7] phomol,[8] mycoepoxydienes,[9] phomoenamide,[10] and phomonitroester[10] from *Phomopsis* species. The oblongolides are also known metabolites from this genus.[11–13] Oblongolide is a hexaketide γ-lactone, first isolated from *Phomopsis oblonga* found in the outer bark of an elm tree (*Ulmus* sp.).[11] Oblongolides B−M and oblongolides N−V have since been isolated from *Phomopsis* sp. 6654 and *Phomopsis* sp. XZ-26, endophytes of the halotolerant plant *Melilotus dentata*[12] and *Camptotheca acuminata*,[13] respectively. In our ongoing research on novel bioactive compounds from fungi found in Thailand, the crude extract of *Phomopsis* sp. BCC 9789 isolated from a wild banana (*Musa acuminata*) leaf showed antituberculosis activity with an MIC value of 50 μg/mL. Fractionation of the extract led to the isolation and structure elucidation of six new oblongolides, W1, W2, X, Y, and Z (**1**−**3**, **6**, **7**) and 2-deoxy-4α-hydroxyoblongolide X (**4**), and the known compounds oblongolide (**8**),[11] oblongolides T, C, and Q (**5**, **9**, **10**),[12,13] and (−)-5-methylmellein.[14]



Results and Discussion

Compounds **1**−**10** and (−)-5-methylmellein were obtained from the crude EtOAc extract of a culture broth of BCC 9789. Spectroscopic data and specific optical rotations of oblongolide T (**5**),[13] oblongolide (**8**),[11] oblongolide C (**9**),[12] oblongolide Q (**10**),[13] and (−)-5-methylmellein[14] were consistent with those reported in the literature. The absolute configuration of **8** as depicted had been described on the basis of stereoselective synthesis.[15]

Oblongolide W1 (**1**) was isolated as a white powder, possessing the molecular formula $C_{16}H_{24}O_4$ as deduced from HRMS (ESI-TOF). Its IR spectrum revealed absorption bands at 3550, 3460 (OH) and 1722 (C=O) cm^{-1}. Data from ^{13}C NMR, DEPT, and HMQC indicated the presence of a lactone carbonyl (δ_C 173.3), a disubstituted olefin (δ_C 134.5 and 122.2), two oxymethylenes (δ_C 68.5 and 62.2), an oxygenated quaternary carbon (δ_C 78.6), three methylenes, four methines, a quaternary carbon, and two methyl groups. The sequences of C-4−C-4a−C-5−C-6−C-6a−C-7−C-8−C-9−C-10−C-10a, C-6a−C-10a, and C-8−C-1′ were assigned on the basis of COSY cross-signals. Key HMBC correlations from H-4α to C-10b and C-2, from H_3-1″ to C-10a, C-10b, C-4a, and C-1, from 1-OH to C-1, and from H-1‴b to C-1, C-2, and C-10b established the planar structure of **1**. The relative configuration of **1** was deduced on the basis of $^3J_{H-H}$ coupling constants and NOESY spectroscopic data. The vicinal coupling constants, $J_{6a,7\beta}$ = 12.5 Hz, $J_{7\beta,8}$ = 12.5 Hz, $J_{8,9\beta}$ = 12.9 Hz, $J_{9\beta,10\alpha}$ = 12.9 Hz, $J_{10\alpha,10a}$ = 11.0 Hz, and $J_{10a,6a}$ = 12.5 Hz, suggested a chair conformation of the cyclohexane ring, the *trans*-diaxial relationship between H-6a and H-10a, and equatorial orientation of the methyl group (CH_3-1′). NOESY cross-peaks from H_3-1″ to H-4a, H-6a, and H_2-1‴ indicated a *cis*-ring junction through C-4a and C-10b and α-orientation of the hydroxymethyl (1-CH_2OH) side chain (Figure 1).

The molecular formula of oblongolide W2 (**2**), $C_{16}H_{24}O_4$, was identical to that of oblongolide W1 (**1**) as established by HRESIMS. Analysis of ^1H NMR, ^{13}C NMR, COSY, HMQC, and HMBC spectra revealed the same planar structure for both compounds. The relative configuration of **2**, assigned on the basis of $^1H-^1H$ *J*-values and NOESY data, differed from **1** at one stereogenic center. NOESY correlations from H_3-1″ to H-4a and H-6a and from H_2-1‴ to H-10β indicated that the hydroxymethyl group (1-CH_2OH) in **2** was on the β-face of the molecule, opposite of that of **1**.

Oblongolide X (**3**) had the same molecular formula, $C_{16}H_{24}O_4$, as compound **1**, although **3** lacked the lactone carbonyl (δ_C 173.3)

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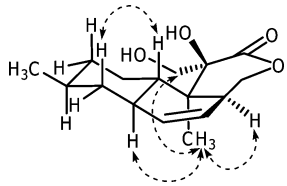


Figure 1. Selected NOESY correlations for **1**.

and oxygenated quaternary carbon (δ_C 78.6) but instead contained a ketone carbonyl (δ_C 208.1) and a hemiacetal carbon (δ_C 94.9). COSY spectroscopic data and HMBC correlations from H_3-1″ to C-1, C-4a, C-10a, and C-10b, from H_2-4 to C-2, C-4a, C-5, and C-10b, and from H_2-1‴ to C-1 and C-2 established the planar structure of **3**. The relative configuration of **3** was deduced from $^3J_{H-H}$ coupling constants and NOESY spectroscopic data. Key NOESY correlations from H-4β to 2-OH and H-10a (observed in acetone-d_6) suggested β-orientation of 2-OH.

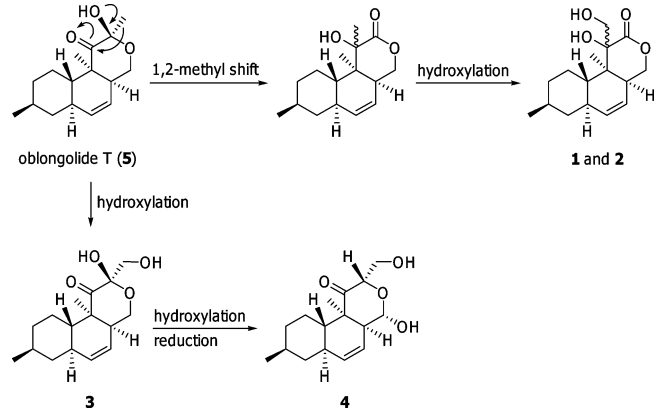
2-Deoxy-4α-hydroxyoblongolide X (**4**) had the same molecular formula as **3**. NMR data of **4** were similar to those of **3**, except that the oxymethylene [δ_H 4.20 (1H, t, J = 11.9 Hz), δ_H 3.67 (1H, dd, J = 11.9, 5.1 Hz), and δ_C 61.9, CH_2-4] and quaternary hemiacetal (δ_C 94.9, C-2) carbons in **3** were replaced by hemiacetal methine [δ_H 5.16 (1H, dd, J = 7.8, 6.0 Hz) and δ_C 96.8, CH-4] and oxymethine [δ_H 4.20 (1H, dd, J = 6.2, 4.7 Hz) and δ_C 76.5, CH-2] carbons. Key HMBC correlations from H_3-1″ to C-1, C-4a, C-10a, and C-10b, from H-2 to C-4, from 4-OH to C-4 and C-4a, and from H-1‴b to C-2 supported the planar structure of **4**, with an OH group attached to C-4 instead of C-2 as observed in **3**. The relative configuration of **4** was deduced from chemical shift comparisons with those of **3**, ^{1}H−^{1}H coupling constants, and NOESY data. Key NOESY cross-peaks observed from H_3-1″ to H-4a and from H-10a to H-2 and H-4 indicated α-orientation of 4-OH and 2-CH_2OH.

Oblongolides Y and Z (**6** and **7**) possessed the same basic skeleton as **8**. The molecular formula of **6** was established as $C_{17}H_{26}O_3$ on the basis of HRESIMS. ^{1}H and ^{13}C NMR data of **6** were similar to those of **8** except that the lactone carbonyl signal (δ_C 180.3) was absent,[11] and quaternary acetal (δ_C 110.4, C-1), acetyl [δ_C 206.6; δ_H 2.23 (3H, s) and δ_C 29.6], and OCH_3 [δ_H 3.02 (3H, s) and δ_C 48.7] groups were present. Attachment of the acetyl and OCH_3 groups to C-1 and the gross structure of **6** were established by COSY data and HMBC correlations from H_3-1″ to C-1, C-3a, C-9a, and C-9b, from H-3β, H_3-2‴, and H_3-2⁗ to C-1, from H-3α to C-3a and C-9b, and from H_3-2‴ to C-1‴. The relative configuration of **6** was determined on the basis of key NOESY correlations from H_3-1″ to H-5a and H-3a, from H-9a to H-3β, from H_3-2⁗ to H-3α, and from H_3-2‴ to H-9β, which indicated α-orientation of 1-OCH_3.

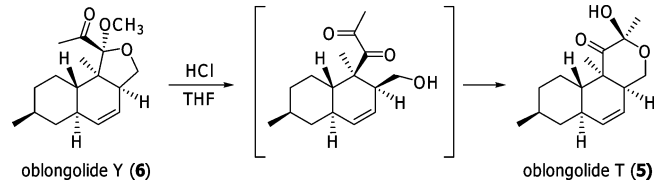
Compound **7** had the molecular formula $C_{24}H_{32}O_3$, as established by HRESIMS. Analysis of its ^{1}H and ^{13}C NMR data indicated a close structural relationship to **6**, except for the lack of the OCH_3 group at δ_H 3.02 (3H, s) and δ_C 48.7 in **6**, but including signals corresponding to a 2-phenylethoxy side chain [phenyl (δ_H 7.17−7.29, 5H, m and δ_C 139.7, 2 × 129.1, 2 × 128.0, 126.0), methylene (δ_H 2.80, 2H, m and δ_C 36.2, CH_2-3⁗), and diastereotopic oxymethylene (δ_H 3.56, 1H, ddd, J = 8.9, 7.6, 6.5 Hz, δ_H 3.30, 1H, dt, J = 8.9, 5.9 Hz, and δ_C 62.4, CH_2-2⁗)]. HMBC correlations from H_3-1″ to C-1, C-3a, C-9a, and C-9b, from H-3β to C-1, C-3a, and C-4, from H_3-2‴ to C-1, and from H_2-2⁗ to C-1, C-3⁗, and the quaternary carbon of phenyl group (δ_C 139.7) suggested the gross structure of **7**. The relative configuration is likely to be the same as that of **6** by comparison of NMR data. α-Orientation of the 2-phenylethoxy was indicated by the NOESY cross-signal between H_3-2‴ and H-9β.

The biosynthetic pathway to oblongolides has been proposed by Shen and co-workers.[13] Acetyl- and malonyl-CoA precursors were

Scheme 1. Proposed Biosynthetic Relationships of Oblongolides **1**−**5**



Scheme 2. Conversion of Oblongolide Y (**6**) to Oblongolide T (**5**)



catalyzed by polyketide synthase and methyltransferase to afford hexa- and heptaketide intermediates.[13] The hexaketide intermediate could convert to **8**−**10**.[13] The heptaketide could transform to the diketone intermediate and finally undergo a six-membered cyclization to obtain **3**−**5** or a five-membered cyclization to afford **6** and **7**.[13] Compounds **1** and **2** may be derived from **5** via a 1,2-methyl shift and subsequent hydroxylation (Scheme 1). Hydrolysis of **6** using 3 M HCl in THF (rt, overnight) yielded a product whose spectroscopic data were identical to those of the known **5**, supporting the existence of the diketone intermediate (Scheme 2) in the proposed biosynthetic pathway of oblongolides.[13]

The results of antituberculosis, anti-HSV-1, and cytotoxic tests of the isolated compounds are shown in Table 3. Compounds **1** and **8** exhibited no significant activity against *Mycobacterium tuberculosis* H37Ra (MICs = 178 and 227 μM, respectively) when compared to the standard antituberculosis drug (isoniazid, MIC = 0.36 μM). Compound **7** had anti-herpes simplex virus type 1 activity (IC_{50} = 14 μM), but unfortunately it also showed comparable cytotoxic activities against KB, BC, NCI-H187, and nonmalignant (Vero) cell lines (IC_{50}'s = 37, 26, 32, and 60 μM, respectively). The anti-HSV-1 activity of **4** (IC_{50} = 76 μM) was about 5 times less than that of **7**. Compound **6** exhibited weak cytotoxic activity against BC cells (IC_{50} = 48 μM) when compared to the positive control (doxorubicin, IC_{50} = 0.30 μM). Herbicidal, antifungal, and antibacterial activities of the known oblongolides have been reported in the literature.[12,13]

Experimental Section

General Experimental Procedures. Optical rotations were determined using a JASCO P-1030 digital polarimeter. IR spectra were recorded on a Bruker Vector 22 spectrometer. NMR spectra were taken on a Bruker AV500D spectrometer. Chemical shift values were reported with respect to the residual solvent signals (δ_H 7.26/δ_C 77.0 for $CDCl_3$ and δ_H 2.05/δ_C 28.9 for acetone-d_6). ESI-TOF mass spectra were recorded on Micromass LCT and Bruker micrOTOF spectrometers. HPLC was performed with a Waters 600 pump and controller, a Waters delta 600 injector, and a Waters 2996 photodiode array detector. TLC was carried out using silica gel 60 F_{254} on aluminum sheets (Merck) and visualized by spraying with anisaldehyde-H_2SO_4 reagent. Sephadex LH-20 (GE Healthcare) and silica gel 60 (230−400 mesh, Merck) were used for column chromatography (CC).

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data for **1**, **2**, and **4** in Acetone-d_6 and **3** in $CDCl_3$

		1		**2**		**3**		**4**	
position	mult.	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	qC		78.6		80.5		208.1		209.3
2	qC or CH		173.3 (qC)		175.1 (qC)		94.9 (qC)	4.20, dd (6.2, 4.7)	76.5 (CH)
4	CH or CH_2	4.49, t (10.6), H-4β 4.11, dd (10.6, 4.4),H-4α	68.5 (CH_2)	4.33, t (11.9), H-4β 4.23, dd (11.9, 5.0), H-4α	68.9 (CH_2)	4.20, t (11.9), H-4β 3.67, dd (11.9, 5.1), H-4α	61.9 (CH_2)	5.16, dd (7.8, 6.0)	96.8 (CH)
4a	CH	2.14, dtt (10.6, 4.4, 2.3)	47.0	2.26, m	45.8	2.52, dtt (11.9, 5.1, 1.5)	46.9	2.21, ddt (7.8, 4.9, 1.4)	53.2
5	CH	5.42, ddd (9.9, 4.4, 2.5)	122.2	5.40, ddd (10.0, 3.3, 2.7)	121.7	5.42, ddd (10.0, 5.1, 2.5)	122.3	5.70, ddd (10.1, 4.9, 2.3)	124.6
6	CH	5.56, br d (9.9)	134.5	5.60, br d (10.0)	134.6	5.60, br d (10.0)	134.7	5.62, br d (10.1)	132.9
6a	CH	1.84, m[a]	36.8	1.82, m[b]	36.5	1.82, m[c]	37.8	1.86−1.75, m[d]	37.3
7	CH_2	1.81, m,[a] H-7α 0.77, q (12.5), H-7β	42.3	1.84, m,[b] H-7α 0.77, q (12.5), H-7β	42.6	1.82, m,[c] H-7α 0.87, q (12.4), H-7β	41.6	1.86−1.75, m,[d] H-7α 0.85, q (12.0), H-7β	41.5
8	CH	1.46, m	32.5	1.49, m	32.7	1.46, m	33.0	1.47, m	32.6
9	CH_2	1.74, ddq (12.9, 2.4, 3.2), H-9α 0.96, dq (3.2, 12.9), H-9β	35.5	1.74, ddq (12.8, 2.0, 3.2), H-9α 0.97, dq (3.2, 12.8), H-9β	35.0	1.75, ddq (12.7, 1.8, 3.2), H-9α 1.00, dq (3.2, 12.7), H-9β	35.2	1.71, ddq (12.8, 2.0, 3.2), H-9α 0.98, m, H-9β	35.0
10	CH_2	2.10, dq (12.9, 3.2), H-10β 1.21, ddt (11.0, 3.2, 12.9), H-10α	27.8	2.03, m, H-10β 1.27, ddt (10.5, 3.2, 12.8), H-10α	28.7	1.23, dq (12.7, 3.2), H-10β 1.12, ddt (11.0, 3.2, 12.7), H-10α	26.7	1.09, m	26.1
10a	CH	1.61, ddd (12.5, 11.0, 3.2)	40.4	1.11, ddd (12.5, 10.5, 3.2)	41.1	2.12, ddd (12.4, 11.0, 3.2)	39.3	1.86−1.75, m[d]	41.2
10b	qC		40.4		41.8		49.4		48.7
1′	CH_3	0.88, d (6.6)	21.9	0.89, d (6.5)	21.8	0.92, d (6.6)	22.4	0.89, d (6.6)	21.7
1″	CH_3	0.87, s	14.8	0.92, s	19.8	1.03, s	15.5	0.95, s	14.7
1‴	CH_2	4.10, dd (11.4, 5.2), H-1‴a 3.70, dd (11.4, 8.2), H-1‴b	62.2	4.07, dd (11.6, 8.5), H-1‴a 3.80, dd (11.6, 4.0), H-1‴b	64.7	3.88, d (12.0), H-1‴a 3.51, d (12.0), H-1‴b	65.6	3.84, ddd (11.9, 6.5, 4.7), H-1‴a 3.60, dt (11.9, 6.2), H-1‴b	60.8
1-OH		4.50, s		4.24, s					
4-OH								5.93, d (6.0)	
1‴-OH		3.86, dd (8.2, 5.2)		4.13, dd (8.5, 4.0)				3.49, dd (6.5, 6.2)	

[a-d] Proton signals were overlapped.

Fungal Material. The fungus was isolated from a wild banana (*Musa acuminata*) leaf collected at Doi Suthep Pui National Park, Chiang Mai Province, Thailand.[16] The fungus was deposited at the BIOTEC Culture Collection as BCC 9789 on November 14, 2001. The CTAB extraction method was used to obtain total genomic DNA from lyophilized mycelia.[17] The ITS1-5.8S-ITS2 rDNA region was PCR amplified using the universal primers ITS4 and ITS5.[18,19] PCR products were purified with a QIAquick PCR purification kit (QIAGEN) and sequenced by Macrogen Inc., Korea. Phylogenetic analysis of the sequence data using BioEdit 7.0.5 and PAUP programs indicated the genus *Phomopsis* for BCC 9789. The fungus sequences were submitted to GenBank with accession number GU086404.

Fermentation, Extraction, and Isolation. BCC 9789 was maintained on potato dextrose agar at 25 °C. The agar blocks (4 × 1 cm^2) were finely chopped and transferred into Erlenmeyer flasks (4 × 250 mL), each containing 25 mL of potato dextrose broth (PDB). After incubation on a rotary shaker (200 rpm) for 9 days, the primary seed culture (4 × 25 mL) was transferred into Erlenmeyer flasks (4 × 1 L), each containing 250 mL of PDB, and then fermented on a rotary shaker (200 rpm) for 9 days. The secondary seed culture (40 × 25 mL) was subsequently transferred into Erlenmeyer flasks (40 × 1 L), each containing 250 mL of malt extract broth, then cultured at 25 °C for 5 days on rotary shakers (200 rpm). The culture was filtered to separate broth and mycelia. The culture broth was extracted three times with EtOAc (3 × 10 L). The organic layer was then concentrated under reduced pressure to obtain a brown gum (2.69 g). The crude extract was fractionated by Sephadex LH-20 CC (elution with 100% MeOH) to provide fractions 1−12. Fraction 6 (632 mg) was subjected to silica gel CC (step gradient, elution with 0−10% MeOH in CH_2Cl_2) to afford nine fractions (6-1−6-9). Fraction 6-2 (90 mg) was separated by preparative HPLC using a reversed-phase column (Nova-Pak HR C_{18}, 6 μm, 25 × 100 mm; MeOH−H_2O (85:15); flow rate 8 mL/min) to yield **6** (16 mg, t_R 12 min) and **7** (4 mg, t_R 29 min). Fraction 7 (1.37 g) was subjected to silica gel CC (step gradient, elution with 0−10% acetone in CH_2Cl_2) to obtain 12 fractions (7-1−7-12). Fraction 7-3 (168 mg) was separated by preparative HPLC using MeOH−H_2O (70:30) to furnish **10** (7 mg, t_R 7 min) and **5** (71 mg, t_R 21 min). Fractions 7-2 (96 mg), 7-5 (48 mg), 7-7 (74 mg), and 7-10 (68 mg) were purified by preparative HPLC using MeOH−H_2O (65:35) to yield (−)-5-methylmellein (20 mg, t_R 9 min) and **8** (20 mg, t_R 29 min) from fraction 7-2, **9** (10 mg, t_R 10.5 min) and **1** (25 mg, t_R 23 min) from fraction 7-5, **2** (12 mg, t_R 19 min) and **3** (39 mg, t_R 25 min) from fraction 7-7, and **4** (27 mg, t_R 15 min) from fraction 7-10.

Oblongolide W1 (1): white powder; mp 118−120 °C; $[\alpha]^{27}_D$ −12.9 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3550, 3460, 2909, 1722, 1397, 1033, 735 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRMS (ESI-TOF) *m/z* 303.1576 [M + Na]$^+$ (calcd for $C_{16}H_{24}O_4Na$, 303.1572).

Oblongolide W2 (2): white, amorphous solid; $[\alpha]^{28}_D$ +1.6 (*c* 0.1, MeOH); IR (film, $CHCl_3$) ν_{max} 3454, 2923, 1736, 1384, 1053, 758 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRMS (ESI-TOF) *m/z* 303.1562 [M + Na]$^+$ (calcd for $C_{16}H_{24}O_4Na$, 303.1572).

Oblongolide X (3): white, amorphous solid; $[\alpha]^{28}_D$ −165.1 (*c* 0.1, MeOH); IR (film, $CHCl_3$) ν_{max} 3434, 2947, 1714, 1456, 1378, 1042, 759 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRMS (ESI-TOF) *m/z* 303.1574 [M + Na]$^+$ (calcd for $C_{16}H_{24}O_4Na$, 303.1572).

2-Deoxy-4α-hydroxyoblongolide X (4): white powder; mp 142−144 °C; $[\alpha]^{25}_D$ −75.9 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3422, 2950, 1704, 1110, 1031, 734 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRMS (ESI-TOF) *m/z* 303.1570 [M + Na]$^+$ (calcd for $C_{16}H_{24}O_4Na$, 303.1572).

Oblongolide T (5): white solid; mp 148−150 °C; $[\alpha]^{27}_D$ −184.8 (*c* 0.1, MeOH); lit.,[13] $[\alpha]^{25}_D$ −186.4 (*c* 0.5, $CHCl_3$); HRMS (ESI-TOF) *m/z* 287.1614 [M + Na]$^+$ (calcd for $C_{16}H_{24}O_3Na$, 287.1623). IR and NMR spectroscopic data were identical to those reported in the literature.[13]

Oblongolide Y (6): white, amorphous solid; $[\alpha]^{27}_D$ −9.2 (*c* 0.05, MeOH); IR (film, $CHCl_3$) ν_{max} 2948, 1721, 1455, 1354, 1075, 750 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRMS (ESI-TOF) *m/z* 301.1782 [M + Na]$^+$ (calcd for $C_{17}H_{26}O_3Na$, 301.1774).

Oblongolide Z (7): colorless oil; $[\alpha]^{25}_D$ −33.6 (*c* 0.21, MeOH); IR (film, $CHCl_3$) ν_{max} 2948, 1720, 1454, 1354, 1087, 752 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRMS (ESI-TOF) *m/z* 391.2255 [M + Na]$^+$ (calcd for $C_{24}H_{32}O_3Na$, 391.2244).

Oblongolide (8): white solid; mp 99−102 °C; $[\alpha]^{25}_D$ −186 (*c* 0.20, $CHCl_3$); lit.,[11] $[\alpha]^{20}_D$ −190 (*c* 0.0475, MeOH); HRMS (ESI-TOF) *m/z*

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data for **6** and **7** in acetone-d_6

		6		**7**	
position	mult.	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	qC		110.4		110.0
3	CH_2	4.03, dd (9.7, 7.9), H-3α; 3.59, dd (8.8, 7.9), H-3*β*	71.5	3.60, dd (9.6, 7.7), H-3α; 3.43, dd (8.9, 7.7), H-3*β*	71.4
3a	CH	2.71, dddt (9.7, 8.8, 5.1, 1.5)	45.7	2.57, m	45.7
4	CH	5.60, ddd (10.0, 5.1, 2.7)	124.1	5.50, ddd (9.9, 5.1, 2.6)	124.0
5	CH	5.42, br d (10.0)	131.0	5.38, br d (9.9)	131.0
5a	CH	1.84, m	38.2	1.82, m	38.2
6	CH_2	1.80, ddt (12.5, 2.1, 3.6), H-6α; 0.81, q (12.5), H-6*β*	42.6	1.78, m, H-6α; 0.78, q (12.5), H-6*β*	42.5
7	CH	1.48, m	32.8	1.46, m	32.9
8	CH_2	1.69, ddq (12.9, 2.1, 3.1), H-8α; 0.92, dq (3.1, 12.9), H-8*β*	35.6	1.67, m, H-8α; 0.87, qd (12.9, 3.1), H-8*β*	35.5
9	CH_2	1.34, dq (12.9, 3.1), H-9*β*; 1.10, dddt (11.1, 3.1, 1.7, 12.9), H-9α	28.3	1.31, dq (12.9, 3.1), H-9*β*; 1.08, ddt (10.6, 3.1, 12.9), H-9α	28.2
9a	CH	1.30, dt (3.1, 11.1)	40.6	1.26, dt (3.1, 10.6)	40.5
9b	qC		51.4		51.5
1′	CH_3	0.87, d (6.6)	21.7	0.85, d (6.6)	21.7
1″	CH_3	1.02, s	13.0	1.04, s	13.0
1‴	qC		206.6		206.5
2‴	CH_3	2.23, s	29.6	2.16, s	29.4
2⁗	CH_3 or CH_2	3.02, s	48.7(CH_3)	3.56, ddd (8.9, 7.6, 6.5), H-2⁗a; 3.30, dt (8.9, 5.9), H-2⁗b	62.4(CH_2)
3⁗	CH_2			2.80, m	36.2
Ph	qC			7.17−7.29, m	139.7
	CH				2 × 129.1
	CH				2 × 128.0
	CH				126.0

Table 3. Biological Activities of Compounds **1**−**10** and (−)-5-Methylmellein

			cytotoxicity, IC_{50} (μM)			
compound	anti-TB MIC (μM)	anti-HSV-1 IC_{50} (μM)	KB cells	BC cells	NCI-H187 cells	Vero cells
oblongolide W1 (**1**)	178	>178	>71	>71	>71	>178
oblongolide W2 (**2**)	>178	>178	>71	>71	>71	>178
oblongolide X (**3**)	>178	>178	>71	>71	>71	96
2-deoxy-4α-hydroxyoblongolide X (**4**)	>178	76	>71	>71	>71	>178
oblongolide T (**5**)	>189	>189	>76	>76	>76	>189
oblongolide Y (**6**)	>180	>180	>72	48	>72	>180
oblongolide Z (**7**)	>136	14	37	26	32	60
oblongolide (**8**)	227	>227	>91	>91	>91	>227
oblongolide C (**9**)	>212	>212	>85	>85	>85	>212
oblongolide Q (**10**)	>212	>212	>85	>85	>85	>212
(−)-5-methylmellein	>260	>260	>104	>104	>104	>260
isoniazid[a]	0.36					
acyclovir[b]		13.0				
doxorubicin[c]			0.24	0.30	0.08	
ellipticine[c]						1.75

[a] Standard antituberculosis drug. [b] Standard compound for anti-herpes simplex virus type 1 activity. [c] Reference compounds for cytotoxicity assays.

221.1539 $[M + H]^+$ (calcd for $C_{14}H_{21}O_2$, 221.1542); IR and NMR spectroscopic data were identical to those reported in the literature.[11]

Oblongolide C (9): white solid; mp 110−113 °C; $[\alpha]^{27}_D$ −197 (*c* 0.20, CH_2Cl_2); lit.,[12] $[\alpha]^{25}_D$ −184 (*c* 0.2, CH_2Cl_2); HRMS (ESI-TOF) *m*/*z* 237.1488 $[M + H]^+$ (calcd for $C_{14}H_{21}O_3$, 237.1491); IR and NMR spectroscopic data were identical to those reported in the literature.[12]

Oblongolide Q (10): white solid; mp 146−148 °C; $[\alpha]^{26}_D$ −65.5 (*c* 0.05, MeOH); lit.,[13] $[\alpha]^{25}_D$ −66.8 (*c* 2.1, $CHCl_3$); HRMS (ESI-TOF) *m*/*z* 259.1320 $[M + Na]^+$ (calcd for $C_{14}H_{20}O_3Na$, 259.1305); IR and NMR spectroscopic data were identical to those reported in the literature.[13]

Hydrolysis of Oblongolide Y (6). To a solution of **6** (1.0 mg, 3.6 μmol) in THF (500 μL) was added aqueous HCl (3 M, 50 μL). The resulting mixture was stirred at room temperature overnight and dried under vacuum. The residue was purified by preparative HPLC using a reversed-phase column (LiChroCART C_{18}, 10 μm, 10 × 250 mm; MeOH−H_2O (80:20); flow rate 4 mL/min) to yield **5** (0.7 mg, 2.7 μmol, 75%).

Biological Assays. The microplate Alamar-Blue assay (MABA) was used to determine growth inhibition against *Mycobacterium tuberculosis* H37Ra.[20] The MIC was defined as the minimum inhibition concentration at which ≥90% of growth was inhibited. Anti-herpes simplex virus type 1 (HSV-1) and cytotoxicity assays against oral human epidermal carcinoma (KB) cells, human breast cancer (BC) cells, human small-cell lung cancer (NCI-H187) cells, and African green monkey kidney fibroblast (Vero) cells were assessed employing a colorimetric method.[21,22]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**4**, **6**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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