Eremophilane-Type Sesquiterpenes from the Fungus *Xylaria* sp. BCC 21097

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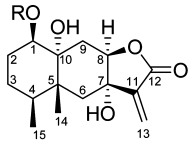
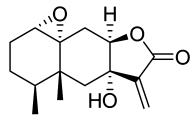
Seven new eremophilane-type sesquiterpenoids (**1**−**5**, **7**, and **8**) and the known mairetolide F (**6**) were isolated from the endophytic *Xylaria* sp. BCC 21097. A new furofurandione (**9**) was also isolated from the fungal extract. The structures of the new compounds were elucidated by analyses of NMR spectroscopic and mass spectrometry data in combination with chemical reaction studies. Eremophilanolides possessing an α-methylene-γ-lactone (**1**−**3**) exhibited moderate cytotoxic activities, while related analogues bearing an *endo* double bond (**6** and **7**) were inactive.

Fungi belonging to the genus *Xylaria* have been the source of a wide range of bioactive compounds. Examples include xyloketals and xyloallenolide from *Xylaria* sp. (No. 2508),[1,2] multiplolides from *X. multiplex* BCC 1111,[3] xylactam from *X. euglossa*,[4] xylobovide and cytochalasins from *X. obovata* ADA-228,[5] integric acid from a *Xylaria* sp.,[6] and xylarenals from *X. persicaria*.[7] As part of our research program on the utilization of fungal sources in Thailand, we investigated secondary metabolites of the endophytic *Xylaria* sp. BCC 21097, as an extract of this fungus showed moderate cytotoxic activity against several cancer cell lines. Scale-up fermentation and chemical studies resulted in the isolation of eight elemophilane-type sesquiterpenes (**1**−**8**), one of which was assigned as the known mairetolide F (**6**). Furofurandione **9** was also isolated from the extract. We report herein the detailed isolation, structure elucidation, and biological activities of these compounds.



1 R = H
2 R = CH_3



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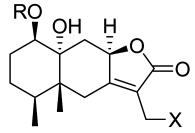
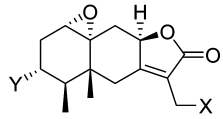
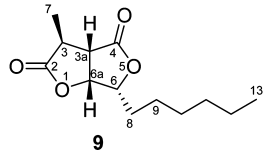


4 R = H, X = OH
5 R = CH_3, X = OH
6 R = H, X = H
11 R = CH_3, X = OCH_3



7 X = OH, Y = H
8 X = H, Y = OH



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Results and Discussion

Compound **1** was the most abundant sesquiterpene constituent of the broth extract and had the molecular formula $C_{15}H_{22}O_5$, as determined by HRESIMS. The IR spectrum exhibited absorption bands at ν_{max} 3526, 3456, and 1766 cm^{-1}, which suggested the presence of OH and ester groups. The ^1H and ^{13}C NMR, DEPT135, and HMQC spectroscopic data revealed that **1** contained a carbonyl carbon (δ_C 169.7), an exomethylene group (δ_C 142.7, qC; δ_C 122.1, CH_2), two oxyquaternary carbons, two oxymethines, an aliphatic quaternary carbon, a methine, four methylenes, and two methyl groups (Table 1). The decalin unit was deduced from HMBC correlations of the methyl protons (H_3-14, s) to quaternary carbons at δ_C 39.1 (C-5) and 74.3 (C-10), a methine carbon (δ_C 35.3, C-4), and a methylene carbon (δ_C 42.0, C-6), and from an OH proton at δ_H 3.57 (s) to C-10, an oxymethine (δ_C 74.7, C-1), and a methylene carbon (δ_C 38.1, C-9). HMBC correlations from H_2-6, H-8, and the OH at δ_H 4.54 (s) to the quaternary olefinic carbon (δ_C 142.7) indicated attachment of the exomethylene group (C-11/C-13) to C-7 (δ_C 72.9). The α-methylene-γ-lactone, joined to the decalin at C-7 and C-8, was established by HMBC correlations from the exomethylene protons (H_2-13; δ_H 5.94, s, and 6.24, s) and H-8 to the carbonyl carbon (δ_C 169.7, C-12). The relative configuration of **1** was based on NOESY correlations (Figure 1). The axial methyl H_3-14 exhibited cross-peaks with H_β-3, H_β-6, and H_β-9. On the other hand, 10-OH showed correlations to H-1 and H_α-6. H-1 showed NOESY correlations with H_α-2 and H_β-2 with similar cross-peak intensity, and it also exhibited an intense cross-peak with H-9$_\alpha$; therefore, H-1 was equatorial. These data indicated a *trans* ring junction of the decalin unit. One of the exomethylene protons (H_2-13) resonated at δ_H 5.94 and showed NOESY correlation to H_β-6, whereas 7-OH exhibited weak cross-peaks with H_α-6 and H-8. The NOESY correlation between H-8 and H_α-9 was much stronger than that between H-8 and H_β-9. The large vicinal coupling constant

Table 1. ^{13}C NMR Data (δ) for Eremophilanolides **1**−**8** (125 MHz)

position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[b]	**8**[b]
1	74.7	85.0	59.7	74.9	85.2	74.9	60.1	58.5
2	28.8	22.9	22.0	29.0	23.0	28.9	22.2	33.0
3	25.1	25.2	23.9	25.7	25.7	25.7	24.9	67.3
4	35.3	35.3	34.1	35.0	34.8	34.9	33.6	40.7
5	39.1	39.2	35.4	42.8	42.9	42.9	39.0	39.4
6	42.0	41.7	41.4	34.5	34.3	34.7	34.9	35.2
7	72.9	72.8	74.0	165.5	165.2	162.1	163.3	159.3
8	85.1	84.8	83.6	79.1	78.8	79.1	78.9	78.2
9	38.1	38.0	35.7	40.2	40.0	40.2	38.4	38.0
10	74.3	74.1	63.8	74.6	74.4	74.8	63.4	63.2
11	142.7	142.7	141.0	124.8	124.8	120.7	125.1	123.0
12	169.7	169.5	169.6	173.0	172.9	174.0	173.7	174.4
13	122.1	122.1	124.4	53.5	53.5	7.3	54.7	8.2
14	16.5	16.2	16.2	14.2	14.0	13.9	15.2[c]	16.1
15	15.1	14.9	15.3	15.0	14.9	15.0	15.2[c]	10.2
1-OCH_3		56.8			56.8			

[a] Acquired in acetone-d_6. [b] Acquired in $CDCl_3$. [c] Carbon signals were overlapped.

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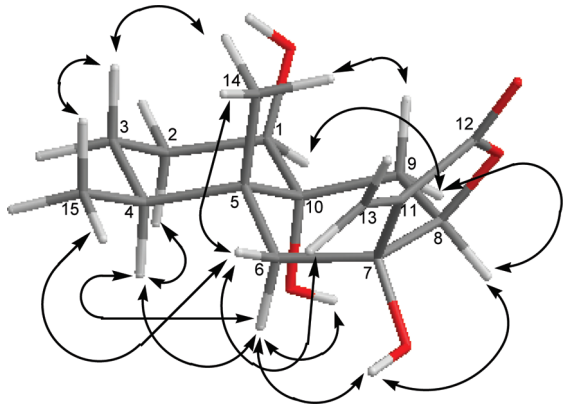

Figure 1. Key NOESY correlations for **1**.

(J = 10.7 Hz) of H-8 and H_β-9 indicated the antiperiplanar relationship. On the basis of these data, the *cis* junction of the γ-lactone with the α-orientation of 7-OH and H-8 was established.

The molecular formula of compound **2** was determined by HRESIMS as $C_{16}H_{24}O_5$. The NMR spectroscopic data of **2** were similar to those of **1**, except for the presence of a methoxy group (δ_C 56.8; δ_H 3.33, 3H, s) and the lack of the 1-O*H* proton resonance. The HMBC correlations of the methoxy protons to C-1 and from H-1 to the methoxy carbon indicated the location of the OCH_3 group. H-1 resonated as a triplet with small coupling constants (J = 2.5 Hz) and exhibited an intense NOESY cross-peak with H_α-9; therefore, it was equatorial.

Compound **3** possessed the molecular formula $C_{15}H_{20}O_4$ (HRESIMS). Significant differences in the ^{1}H and ^{13}C NMR spectroscopic data, when compared to those of **1**, were the upfield shift of H-1 (δ_H 3.01, br d, J = 3.9 Hz) and the oxygenated carbons C-1 (δ_C 59.7) and C-10 (δ_C 63.8). These data implied that the 1,10-diol moiety of **1** was replaced by the 1,10-epoxy functionality in **3**.

Compound **4** possessed the same molecular formula as **1**, $C_{15}H_{22}O_5$ (HRESIMS). The ^{1}H NMR spectroscopic data lacked the resonances of the exomethylene protons and 7-O*H*; instead a hydroxymethyl group was present at δ_H 4.28 (2H, d, J = 5.7 Hz, H_2-13; δ_C 53.5) and 3.94 (t, J = 5.7 Hz, 13-O*H*). These data indicated a rearranged allylic alcohol (C-7−C-11−C-13) moiety with an *endo* olefinic bond. The proposed structure **4** was confirmed by the HMBC correlations: from H_α-9 to C-7 (δ_C 165.5), from H_2-6 and H-8 to C-11, and from H_2-13 to C-7, C-11, and C-12. Compound **5**, possessing the molecular formula $C_{16}H_{24}O_5$ (HRESIMS), was identified as the 1-*O*-methyl analogue of **4**. Compound **6** was assigned as the 13-deoxy analogue of **4**. This compound is identical to mairetolide F, previously isolated from the higher plant *Senecio mairetianus*.[8]

Compound **7** possesses a 1,10-epoxy functionality, as indicated by the similarity of the NMR spectroscopic data with those of **3**, while the γ-lactone moiety was identical to **4**. Compound **8** also possessed the 1,10-epoxy functionality, and its γ-lactone moiety was identical to **6**. The secondary alcohol functionality at C-3 (δ_C 67.3; δ_H 3.48, m) was assigned on the basis of COSY data. The NOESY correlations of H-3 to H_3-14 and H_3-15 established its β-orientation.

The absolute configuration of **1** was addressed by application of the modified Mosher's method.[9,10] Initial attempts to prepare the Mosher esters of **1** were unsuccessful, giving a complex mixture of products. These results may be due to the allylic alcohol functionality. When compound **1** was treated with $LiBH_4$ in THF, originally planning to achieve the reduction of the lactone, mairetolide F (**6**) was obtained as the major product. This unexpected conversion allowed us to obtain an additional quantity of **6**, which was used for the preparation of the Mosher esters. The $\Delta\delta$ values of the (*S*)- and (*R*)-MTPA esters **10a** and **10b** indicated

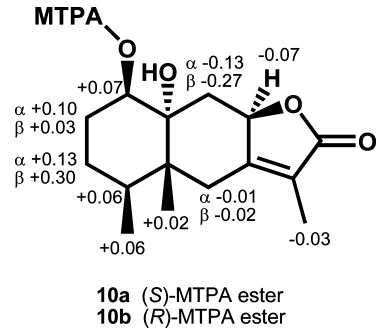

Figure 2. $\Delta\delta$ values ($\delta_S - \delta_R$) of the Mosher esters **10a** and **10b**.

the 1*R* configuration (Figure 2). The remaining question was the configuration of the epoxide moiety of **3**, **7**, and **8**. Since the NMR spectroscopic data did not provide conclusive evidence of the configuration, we examined chemical means. Treatment of **3** with concentrated H_2SO_4/MeOH gave **11** as the sole product. The ^{1}H NMR spectroscopic data of **11** in acetone-d_6 resembled those of **5** except for the presence of an additional methoxy group at δ_H 3.29 (3H, s). Similar regioselective epoxide cleavage by acidic methanolysis of mairetolides was reported previously.[8] Furthermore, **2** was converted to the same compound (**11**) under the same reaction conditions, which confirmed the α-epoxide configuration of **3**. We propose that **7** and **8** are also α-epoxide derivatives on the basis of the similarity of the NMR spectroscopic data with those of **3** and their co-occurrence with **1**−**6**. Structural relations and the reactivities mentioned above suggested that **1** and **2** were derived from **3**, respectively, by hydrative epoxide cleavage and methanolysis. Similarly, **7** may be the precursor of **4** and **5**. The ^{1}H NMR spectrum of the crude EtOAc extract from culture broth (pH 4.23) indicated the presence of the major eremophilanolides **1** and **3**. On the other hand, the minor methoxy analogues **2** and **5** were possibly produced during the chromatographic procedures. The β-epoxy isomer of **7** was previously isolated from the aerial part of *Ondetia linearis* along with a number of eremophilanolides, eudesmanolides, and guaianolides.[11]

Compound **9** was isolated from a fermentation batch of extended incubation. The molecular formula of **9** was determined by HRESIMS as $C_{13}H_{20}O_4$. The IR spectrum exhibited a broad and intense ester(s) band at ν_{max} 1767 cm^{-1}. Analysis of the ^{1}H and ^{13}C NMR, DEPT135, and HMQC data revealed that **9** contained two carbonyls at δ_C 176.7 and 174.6, two oxymethines at δ_C 82.4 (δ_H 4.55, ddd, J = 8.0, 6.3, 3.9 Hz) and 78.3 (δ_H 5.11, dd, J = 6.0, 3.9 Hz), two methines at δ_C 49.0 (δ_H 3.15, dd, J = 6.0, 1.3 Hz) and 38.3 (δ_H 3.08, dq, J = 1.3, 7.7 Hz), five methylenes, and two methyl groups at δ_C 17.1 (δ_H 1.45, d, J = 7.7 Hz) and 14.0 (δ_H 0.89, t, J = 6.9 Hz). The planar structure was established on the basis of COSY and HMBC correlations, and the relative configuration was addressed by NOESY correlations. Intense NOESY cross-peaks were observed from H-6a to H-6, H-3a, and H_3-7. H-3a exhibited intense and weak NOESY correlations respectively to H_3-7 and H-6. These data strongly suggested the *cis* ring junction and that H_3-7, H-3a, H-6, and H-6a are coplanar. Compound **9** is a 3,7-dihydro analogue of sporothriolide, which was previously isolated from the fungus *Sporothrix* sp. (strain No. 700).[12] Canadensolide and dihydrocanadensolide, isolated from *Penicillium canadense*,[13] possess an *n*-butyl substituent instead of the *n*-hexyl group in **9**. These known fungal metabolites are reported to have the (3*S*),3a*S*,6*R*,6a*R* configuration.[12,14,15] The negative optical rotation of **9** isolated in the present study ($[\alpha]^{25}_D$ −33, *c* 0.13, $CHCl_3$) was similar to those of the natural and synthetic (3*S*,3a*S*,6*R*,6a*R*)-dihydrocanadensolide ($[\alpha]_D$ −31; $[\alpha]^{20}_D$ −31, *c* 0.26, MeOH)[13,15] and opposite those reported for the synthetic enantiomer, (3*R*,3a*R*,6*S*,6a*S*)-dihydrocanadensolide ($[\alpha]^{23}_D$ +30.3, *c* 1.9, $CHCl_3$; $[\alpha]_D$ +29.8, *c* 0.35, $CHCl_3$).[16,17]

Table 2. ^{1}H NMR Data for Eremophilanolides **1**−**3** (500 MHz)

position	**1**[a]	HMBC for **1**	**2**[a]	**3**[b]
1	3.62 m	C-3,5,10	3.11 t (2.5)	3.01 br d (3.9)
2	α 2.14 m		α 1.94 m	α 1.94 m
	β 1.50 m	C-10	*β* 1.73 m	*β* 1.89 m
3	α 1.17 m		α 1.17 m	α 1.19 m
	β 1.58 dq (4.2, 12.8)	C-2,4	*β* 1.40 dq (4.1, 12.7)	*β* 1.17 m
4	1.98 m		1.98 m	1.74 m
6	α 2.17 d (13.6)	C-4,5,7,11,14	α 2.16 d (13.6)	α 1.90 d (14.4)
	β 2.24 d (13.6)	C-5,7,8,10,11,14	*β* 2.24 d (13.6)	*β* 2.42 d (14.4)
8	4.77 dd (10.7, 7.5)	C-7,9,11,12	4.76 dd (10.8, 7.4)	4.67 dd (10.7, 7.2)
9	α 2.02 dd (14.1, 7.5)	C-1,5,7,8	α 2.00 dd (14.1, 7.4)	α 1.73 dd (14.2, 7.2)
	β 1.89 dd (14.1, 10.7)	C-8,10	*β* 1.84 dd (14.1, 10.8)	*β* 1.88 dd (14.2, 10.7)
13	5.94 s	C-7,11,12	5.94 s	5.93 s
	6.24 s	C-7,11,12	6.23 s	6.46 s
14	0.94 s	C-4,5,6,10	0.83 s	0.84 s
15	0.81 d (6.9)	C-3,4,5	0.80 d (6.9)	0.77 d (6.9)
1-O*H*	3.84 br d (3.4)			
1-OCH_3			3.22 s	
7-O*H*	4.54 s	C-6,7,8,11	4.53 s	3.16 s
10-O*H*	3.57 s	C-5,9,10	3.72 s	

[a] Acquired in acetone-d_6. [b] Acquired in $CDCl_3$.

The ^{1}H and ^{13}C NMR ($CDCl_3$) spectroscopic data of the bicyclic bis-lactone core of **9** showed an excellent match with those reported for synthetic dihydrocanadensolide.[15−17] Therefore, the absolute configuration of **9** was assigned as 3*S*,3a*S*,6*R*,6a*R*. Sharma reported[18] the enantioselective synthesis of (3*S*,3a*S*,6*R*,6a*R*)-dihydrocanadensolide, (3*S*,3a*S*,6*R*,6a*R*)-dihydrosporothrioride (**9**), and their C-3 epimers (3*R*,3a*S*,6*R*,6a*R*); however, the reported optical rotation data were opposite and inconsistent with those of all other reports[15−17] on the synthesis of dihydrocanadensolides. In addition, the ^{13}C NMR ($CDCl_3$) spectroscopic data of the synthetic (3*S*,3a*S*,6*R*,6a*R*)-dihydrosporothrioride[18a] were significantly different from those of our natural product (**9**).

Eremophilanolides **1**−**3**, **6**, and **7** were subjected to our biological assay protocols, inclusive of cytotoxicity against cancer cell lines (KB, MCF-7, and NCI-H187) and nonmalignant Vero cells and antimalarial (*Plasmodium falciparum* K1), antituberculosis (*Mycobacterium tuberculosis* H37Ra), and antifungal (*Candida albicans*) activities. Eremophilanolides possessing an α-methylene-γ-lactone (**1**−**3**) exhibited moderate cytotoxic activities in the range of IC_{50} 3.8−21 μM. Compounds **2** and **3** also displayed antimalarial activity with respective IC_{50} values of 8.1 and 13 μM. Only **3** was active against *Candida albicans* (IC_{50} 7.8 μM), which suggested that the epoxide functionality may play an important role. Related analogues possessing an *endo* double bond (**6** and **7**) were inactive in these assays.

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a GBC Cintra 404 spectrophotometer. FTIR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESITOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

Fungal Material. The fungus used in this study was isolated from the palm *Licuala spinosa* in Trang Province, Thailand, and it was deposited in the BIOTEC Culture Collection as BCC 21097 on April 10, 2006. This fungus was identified as a *Xylaria* sp. on the basis of the gene sequence data of the 18S rDNA and ITS genes by one of the authors (N.R.).

Fermentation and Isolation (Batch 1). The fungus BCC 21097 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 2 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 4 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 4 days on a rotary shaker (200 rpm). These secondary cultures were pooled, and each 25 mL portion was transferred into 20 × 1 L Erlenmeyer flasks containing 250 mL of malt extract broth (MEB; malt extract 6.0 g, yeast extract 1.2 g, maltose 1.8 g, dextrose 6.0 g, per liter). Final fermentation was carried out at 25 °C for 15 days under static conditions. The cultures were filtered to separate broth (filtrate, pH 4.23) and mycelia (residue). Culture broth was extracted with EtOAc (3 × 4.5 L), and the combined organic phase was concentrated to obtain a brown gum (broth extract; 1.90 g). The broth extract was subjected to column chromatography (CC) on Sephadex LH-20 (3.8 × 50 cm, MeOH) to obtain seven pooled fractions: fractions 1 (44 mg), 2 (89 mg). 3 (362 mg), 4 (1.20 g), 5 (147 mg), 6 (56 mg), and 7 (6 mg). Fraction 4 was subjected to CC on silica gel (3.2 × 14 cm, EtOAc/CH_2Cl_2, step gradient elution) to obtain 20 fractions (fraction 4-1−4-20). Fraction 4-5 (8 mg) contained 2-phenylethyl alcohol. Fraction 4-7 (49 mg) was further purified by CC on silica gel (EtOAc/hexane, 40:60) to furnish **3** (30 mg). Fraction 4-8 (8 mg) was separated using a short silica gel column (EtOAc/hexane, 40:60) to afford **3** (2 mg) and **7** (4 mg). Fractions 4-9 (12 mg), 4-11 (140 mg), and 4-12 (29 mg) were purified by CC on silica gel (EtOAc/hexane, 60:40) to furnish **4** (5 mg), cytochalasin E (8 mg), and **2** (8 mg), respectively. Fraction 4-13 (37 mg) was subjected to preparative HPLC using a reversed-phase column (Phenomenex Luna 10u C18(2) 100A, 21.2 × 250 mm, 10 μm; mobile phase MeOH/H_2O, 50:50, flow rate 15 mL/min) to furnish **6** (4 mg, t_R 21 min), **5** (2 mg, t_R 26 min), and **2** (2 mg, t_R 37 min). Fraction 4-15 (200 mg) was purified by CC on silica gel (EtOAc/hexane, 50:50) to afford **1** (100 mg). Fraction 4-17 (29 mg) was purified by preparative HPLC (MeOH/H_2O, 45:55) to furnish **4** (10 mg, t_R 8 min). Fraction 5 was fractionated by CC on silica gel (EtOAc/CH_2Cl_2, step gradient elution) to afford mellein (26 mg) and 3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone (6 mg). Chromatographic fractionations of the MeOH extract from mycelia (400 mg) afforded cytochalasin E (7 mg) and mellein (12 mg).

Fermentation and Isolation (Batch 2). The fermentation and isolation was repeated to obtain additional quantities of the minor metabolites. The fungus BCC 21097 was fermented using the same procedure as described above, but the final fermentation was extended to 30 days using 14 L (64 × 250 mL) of MEB medium. The EtOAc extract from culture broth (6.0 g) was fractionated by chromatographic methods as described above to furnish eremophilanes **7** (3 mg) and **8** (3 mg), furofurandione **9** (50 mg), cytochalasins E (223 mg) and K (9 mg), mellein (29 mg), 3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone (6 mg), 2-phenylethyl alcohol (43 mg), and 2-(4-hydroxyphenyl)-ethyl alcohol (9 mg).

1*β*,7α,10α-Trihydroxyeremophil-11(13)-en-12,8*β*-olide (1): colorless solid; mp 194−196 °C; $[\alpha]^{24}_D$ −65 (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log *ε*) 205 (3.94) nm; IR (KBr) ν_{max} 3526, 3456, 1766 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6), Tables 1 and 2; HRESIMS *m*/*z* 305.1366 [M + Na]$^+$ (calcd for $C_{15}H_{22}O_5Na$ 305.1359).

Table 3. ^{1}H NMR Data for Eremophilanolides **4**−**8** (500 MHz)

position	**4**[a]	**5**[a]	**6**[a]	**7**[b]	**8**[b]
1	3.71 m	3.19 t (2.6)	3.70 br s	3.08 d (4.0)	3.06 d (4.4)
2	α 2.17 m	α 1.97 m	α 2.17 m	α 2.00 m	α 1.91 dd (15.6, 8.7)
	β 1.56 m	*β* 1.79 ddt (14.2, 4.1, 2.5)	*β* 1.55 m	*β* 1.96 m	*β* 2.47 ddd (15.6, 7.9, 4.5)
3	α 1.25 m	α 1.24 m	α 1.25 m	α 1.25 m	*β* 3.48 m
	β 1.62 dq (4.1, 12.8)	*β* 1.43 dq (4.1, 13.6)	*β* 1.62 dq (4.1, 13.2)	*β* 1.27 m	
4	2.15 m	2.13 m	2.15 m	1.90 m	1.82 m
6	α 2.52 d (12.9)	α 2.50 br d (12.9)	α 2.49 br d (12.8)	α 2.26 d (13.6)	α 2.25 br d (13.6)
	β 2.83 d (12.9)	*β* 2.83 d (12.9)	*β* 2.52 d (12.8)	*β* 2.93 d (13.6)	*β* 2.78 d (13.6)
8	5.09 dd (10.9, 7.2)	5.08 dd (11.0, 7.3)	5.05 m	4.98 dd (11.1, 7.0)	4.91 dd (11.0, 7.0)
9	α 2.19 dd (13.0, 7.2)	α 2.18 dd (12.9, 7.3)	α 2.16 dd (12.7, 7.5)	α 1.91 dd (12.8, 7.0)	α 1.86 dd (12.8, 7.0)
	β 2.06 dd (13.0, 10.9)	*β* 2.00 dd (12.9, 11.0)	*β* 2.01 dd (12.7, 10.8)	*β* 2.07 dd (12.8, 11.1)	*β* 1.97 dd (12.8, 11.0)
13	4.28 d (5.7)	4.28 br s	1.75 t (1.5)	4.41 s	1.85 t (1.6)
14	0.94 s	0.83 s	0.91 s	0.86 s	0.84 s
15	0.85 d (6.9)	0.84 d (6.9)	0.87 d (7.0)	0.82 d (6.9)	0.98 d (6.9)
1-O*H*	3.97 d (4.2)		3.96 br s		
1-OC*H*$_3$		3.26 s			
3-O*H*					1.37 br d (6.1)
10-O*H*	3.76 s	3.92 s	3.73 br s		
13-O*H*	3.94 t (5.7)	3.94 br s		not observed	

[a] Acquired in acetone-d_6. [b] Acquired in $CDCl_3$.

7α,10α-Dihydroxy-1*β*-methoxyeremophil-11(13)-en-12,8*β*-olide (2): colorless solid; mp 181−184 °C; $[\alpha]^{25}_D$ −61 (*c* 0.11, MeOH); UV (MeOH) end absorption; IR (KBr) ν_{max} 3484, 3357, 1740, 1019 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6), Tables 1 and 2; HRESIMS *m*/*z* 319.1527 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_5Na$ 319.1521).

1α,10α-Epoxy-7α-hydroxyeremophil-11(13)-en-12,8*β*-olide (3): colorless solid; mp 150−152 °C; $[\alpha]^{25}_D$ −112 (*c* 0.11, MeOH); UV (MeOH) end absorption; IR (KBr) ν_{max} 3436, 1765, 1745, 1629, 1001 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), Tables 1 and 2; HRESIMS *m*/*z* 287.1251 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$ 287.1254).

1*β*,10α,13-Trihydroxyeremophil-7(11)-en-12,8*β*-olide (4): colorless solid; mp 147−150 °C; $[\alpha]^{24}_D$ −65 (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log *ε*) 223 (4.19) nm; IR (KBr) ν_{max} 3516, 3429, 1708, 1662, 1012 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6), Tables 1 and 3; HRESIMS *m*/*z* 305.1349 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_5Na$ 305.1359).

10α,13-Dihydroxy-1*β*-methoxyeremophil-7(11)-en-12,8*β*-olide (5): colorless amorphous; $[\alpha]^{27}_D$ −52 (*c* 0.145, MeOH); UV (MeOH) λ_{max} (log *ε*) 222 (3.99) nm; IR (KBr) ν_{max} 3424, 1731, 1674 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6), Tables 1 and 3; HRESIMS *m*/*z* 297.1698 $[M + H]^+$ (calcd for $C_{16}H_{25}O_5$ 297.1697).

1*β*,10α-Dihydroxyeremophil-7(11)-en-12,8*β*-olide (mairetolide F, 6): colorless solid; mp 164−165 °C; $[\alpha]^{27}_D$ −84 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log *ε*) 223 (4.09) nm; IR (KBr) ν_{max} 3425, 1725, 1678, 1034 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6), Tables 1 and 3; HRESIMS *m*/*z* 289.1414 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_4Na$ 289.1410).

1α,10α-Epoxy-13-hydroxyeremophil-7(11)-en-12,8*β*-olide (7): colorless solid; mp 121−124 °C; $[\alpha]^{25}_D$ −126 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log *ε*) 220 (4.19) nm; IR (KBr) ν_{max} 3424, 1758, 1011 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), Tables 1 and 3; HRESIMS *m*/*z* 287.1251 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$ 287.1254).

1α,10α-Epoxy-3α-hydroxyeremophil-7(11)-en-12,8*β*-olide (8): colorless solid; mp 202−203 °C; $[\alpha]^{26}_D$ −126 (*c* 0.145, MeOH); UV (MeOH) λ_{max} (log *ε*) 219 (4.06) nm; IR (KBr) ν_{max} 3489, 1727, 1684, 1038 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), Tables 1 and 3; HRESIMS *m*/*z* 287.1251 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$ 287.1254).

Compound 9: colorless solid; mp 92−93 °C; $[\alpha]^{26}_D$ −50 (*c* 0.0975, MeOH), $[\alpha]^{25}_D$ −33 (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 1767 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 5.11 (1H, dd, *J* = 6.0, 3.9 Hz, H-6a), 4.55 (1H, ddd, *J* = 8.0, 6.3, 3.9 Hz, H-6), 3.15 (1H, dd, *J* = 6.0, 1.3 Hz, H-3a), 3.08 (1H, dq, *J* = 1.3, 7.7 Hz, H-3), 1.91 (1H, m, Ha-8), 1.82 (1H, m, Hb-8), 1.50−1.45 (2H, m, H-9), 1.45 (3H, d, *J* = 7.7 Hz, H-7), 1.38−1.35 (2H, m, H-10), 1.32−1.28 (4H, m, H-11 and H-12), 0.89 (3H, t, *J* = 6.9 Hz, H-13); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 176.7 (C, C-4), 174.6 (C, C-2), 82.4 (CH, C-6), 78.3 (CH, C-6a), 49.0 (CH, C-3a), 38.3 (CH, C-3), 31.5 (CH_2, C-11), 28.9 (CH_2, C-10), 28.8 (CH_2, C-8), 25.3 (CH_2, C-9), 22.5 (CH_2, C-12), 17.1 (CH_3, C-7), 14.0 (CH_3, C-13); HRESIMS *m*/*z* 263.1256 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_4Na$ 263.1254).

Reaction of 1 with LiBH$_4$. To a solution of **1** (5.2 mg) was added $LiBH_4$ (10 mg), and the mixture was stirred for 2.5 h. The reaction was terminated by addition of H_2O and extracted three times with EtOAc. The organic layer was concentrated under reduced pressure to leave a colorless solid (5.6 mg), which was purified using a short silica gel column (EtOAc/CH_2Cl_2, 30:70) to furnish the major reaction product (4.0 mg), whose MS and ^{1}H NMR data were identical to those of **6**.

Preparation of the MTPA Esters 10a and 10b. Compound **6** (1.0 mg; obtained by $LiBH_4$ reduction of **1**) was treated with (−)-(*R*)-MTPACl (5 *μ*L) in pyridine (0.2 mL) at room temperature for 16 h. The mixture was diluted with EtOAc and washed with H_2O and 1 M $NaHCO_3$, and the organic layer was concentrated in vacuo. The residue was purified by HPLC (MeCN/H_2O, 40:60) to obtain the (*S*)-MTPA ester **10a** (1.0 mg). Similarly, (*R*)-MTPA ester **10b** was prepared from **6** (0.9 mg) and (+)-(*S*)-MTPACl. Although the conversion of **6** into **10b** was poor, separation of the crude reaction products by HPLC furnished a pure sample of **10b** (0.2 mg). The assignments of protons for **10a** and **10b** were achieved by analysis of COSY and NOESY data.

(*S*)-MTPA Ester 10a: colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.47−7.37 (5H, m, phenyl of MTPA), 4.94 (1H, m, H-8), 4.91 (1H, m, H-1), 3.55 (3H, br s, OC*H*$_3$ of MTPA), 2.39 (1H, d, *J* = 12.8 Hz, H$_\beta$-6), 2.38 (1H, d, *J* = 12.8 Hz, H$_\alpha$-6), 2.16 (1H, m, H$_\alpha$-2), 1.98 (1H, m, H-4), 1.94 (1H, dd, *J* = 13.1, 7.0 Hz, H$_\alpha$-9), 1.78 (1H, m, H$_\beta$-2), 1.75 (3H, br s, H-13), 1.39 (2H, m, H$_\alpha$-3 and H$_\beta$-3), 1.28 (1H, dd, *J* = 13.1, 10.8 Hz, H$_\beta$-9), 0.85 (3H, d, *J* = 6.9 Hz, H-15), 0.45 (3H, s, H-14); HRESIMS *m*/*z* 505.1805 $[M + Na]^+$ (calcd for $C_{25}H_{29}O_6F_3Na$, 505.1808).

(*R*)-MTPA Ester 10b: colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.44−7.38 (5H, m, phenyl of MTPA), 5.01 (1H, m, H-8), 4.84 (1H, m, H-1), 3.47 (3H, br s, OC*H*$_3$ of MTPA), 2.41 (1H, d, *J* = 13.0 Hz, H$_\beta$-6), 2.39 (1H, d, *J* = 13.0 Hz, H$_\alpha$-6), 2.07 (1H, dd, *J* = 12.9, 7.0 Hz, H$_\alpha$-9), 2.06 (1H, m, H$_\alpha$-2), 1.92 (1H, m, H-4), 1.78 (3H, br s, H-13), 1.75 (1H, m, H$_\beta$-2), 1.55 (1H, m, H$_\beta$-9), 1.26 (1H, m, H$_\alpha$-3), 1.09 (1H, dq, *J* = 4.1, 13.7 Hz, H$_\beta$-3), 0.79 (3H, d, *J* = 6.8 Hz, H-15), 0.43 (3H, s, H-14); HRESIMS *m*/*z* 505.1318 $[M + Na]^+$ (calcd for $C_{25}H_{29}O_6F_3Na$, 505.1308).

Synthesis of 11. Compound **3** (1.4 mg) was dissolved in concentrated H_2SO_4/MeOH, 1:9 (0.4 mL), and the solution was stirred at room temperature for 16 h. The mixture was evaporated without heating, and the residue was diluted with EtOAc (5 mL) and washed with H_2O (3 × 3 mL). The organic layer was concentrated under reduced pressure, and the residue was purified by CC on silica gel to obtain **11** (0.5 mg).

1*β*,13-Dimethoxy-10α-hydroxyeremophil-7(11)-en-12,8*β*-olide (11): colorless, amorphous; ^{1}H NMR (400 MHz, acetone-d_6) *δ* 5.12 (1H, dd, *J* = 10.8, 7.3 Hz, H-8), 4.10−4.08 (2H, m, H-13), 3.92 (1H, s, 10-OH), 3.29 (3H, s, 13-OC*H*$_3$), 3.26 (3H, s, 1-OC*H*$_3$), 3.20 (1H, m, H-1), 2.75 (1H, d, *J* = 13.0 Hz, H$_\beta$-6), 2.53 (1H, br d, *J* = 13.0 Hz, H$_\alpha$-6), 2.19 (1H, dd, *J* = 12.8, 7.3 Hz, H$_\alpha$-8), 2.13 (1H, m, H-4), 2.01 (1H, dd, *J* = 12.8, 10.8 Hz, H$_\beta$-8), 1.97 (1H, m, H$_\alpha$-2), 1.79 (1H, m,

H_β-2), 1.43 (1H, dq, $J = 4.1$, 13.0 Hz, H_β-3), 1.25 (1H, m, H_α-3), 0.84 (3H, d, $J = 6.9$ Hz, H-15), 0.81 (3H, s, H-14); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (1H, t, $J = 9.1$ Hz, H-8), 4.17 (1H, d, $J = 12.2$ Hz, Ha-13), 4.15 (1H, d, $J = 12.2$ Hz, Hb-13), 3.38 (3H, s, 13-OCH_3), 3.27 (3H, s, 1-OCH_3), 3.07 (1H, m, H-1), 2.72 (1H, d, $J = 13.0$ Hz, H_β-6), 2.47 (1H, br d, $J = 13.0$ Hz, H_α-6), 2.13 (2H, d, $J = 9.1$ Hz, H_α-8 and H_β-8), 1.91 (1H, m, H-4), 1.86 (1H, m, H_β-2), 1.79 (1H, m, H_α-2), 1.47 (1H, dq, $J = 4.5$, 13.1 Hz, H_β-3), 1.29 (1H, m, H_α-3), 0.88 (3H, d, $J = 6.8$ Hz, H-15), 0.82 (3H, s, H-14); HRESIMS *m/z* 333.1674 $[M + Na]^+$ (calcd for $C_{17}H_{26}O_5Na$ 333.1672).

Biological Assays. Assay for activity against *Plasmodium falciparum* (K1, multidrug-resistant strain) was performed using the microculture radioisotope technique.[19] Growth inhibitory activity against *Mycobacterium tuberculosis* H37Ra and cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed using the green fluorescent protein microplate assay (GFPMA).[20] Antifungal activity against *Candida albicans* and anticancer activities against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) were evaluated using the resazurin microplate assay.[21]

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Supporting Information Available: NMR spectra of **1**−**9** and biological activities of **1**−**3**, **6**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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