Antitubercular Dihydroagarofuranoid Sesquiterpenes from the Roots of *Microtropis fokienensis*

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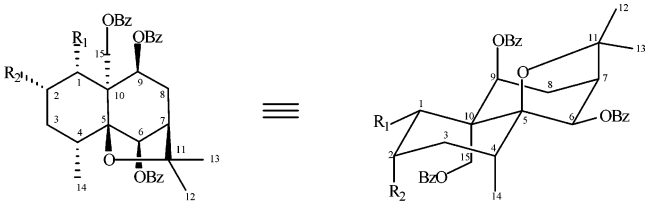
Four new dihydroagarofuranoid sesquiterpenes (**1**−**4**) and a new hydroxybenzylsalicylaldehyde, forkienin (**5**), together with nine known compounds have been isolated from the roots of *Microtropis fokienensis*. The structures of the new compounds were determined through analyses of physical data. Compounds **3**, **4**, **7**, and **8** exhibited potent antitubercular activities (MICs ≤ 26.0 μM) against *Mycobacterium tuberculosis* 90-221387 *in vitro*.

Microtropis fokienensis Dunn (Celastraceae) is a small shrub that grows in high-altitude forests throughout southern China and Taiwan.[1] Various triterpenes,[2,3] sesquiterpene alkaloids,[4] and dihydroagarofuranoid sesquiterpenes[5,6] are widely distributed in plants of the family Celastraceae. Many of these compounds exhibit antitumor,[2,3] antiinflammatory,[6] insecticidal,[5] and anti-AIDS[2] activities. In our search for compounds with antitubercular activities, four new β-dihydroagarofuranoid sesquiterpenes (**1**−**4**), a new hydroxybenzylsalicylaldehyde, forkienin (**5**), and nine known compounds (**6**−**14**) have been isolated and identified from the root of *M. fokienensis*. Their antitubercular activities against *M. tuberculosis* 90-221387 were evaluated *in vitro*.

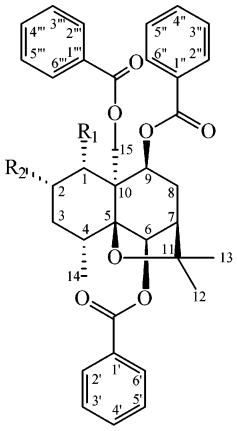


1 $R_1 = R_2 = OAc$
2 $R_1 = OAc, R_2 = H$
3 $R_1 = OAc, R_2 = OH$
4 $R_1 = OH, R_2 = OAc$

Results and Discussion

Extensive chromatographic purification of the EtOAc-soluble fraction of the roots of *M. fokienensis* on a silica gel column and preparative TLC afforded five new (**1**−**5**) and nine known compounds (**6**−**14**).

Compound **1** was isolated as an amorphous powder, $[\alpha]_D^{25}$ +34.7. The FABMS of **1** afforded an $[M + H]^+$ ion at *m*/*z* 699, implying a molecular formula of $C_{40}H_{43}O_{11}$, which was confirmed by HRFABMS. UV absorptions at 231, 274, and 281 nm were similar to those of mutangin[7] and suggested the presence of aromatic moieties. Ester carbonyl groups in the molecule were indicated by the bands at 1746, 1721, and 1714 cm^{-1} in the IR spectrum and were confirmed by resonances at δ 165.2, 165.4, 166.8, 169.5, and 170.0 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **1** was also similar to that of mutangin[7] except that a C-6 benzoyloxy group [δ 8.04 (2H, d, J = 7.5 Hz, H-2′ and H-6′), 7.46 (2H, t, J = 7.5 Hz, H-3′ and H-5′), and 7.59 (1H, t, J = 7.5 Hz, H-4′)] of **1** replaced a C-6 acetoxy group [δ 2.12 (3H, s)] of mutangin.[7] This was supported by the HMBC correlations between H-6 (δ 6.32) and Ph*C*O_2-5 (δ 165.4). In the ^1H NMR spectrum of **1**, resonances due to acylated oxymethine protons at δ 5.84 (1H, d, J = 3.5 Hz), 5.67 (1H, q, J = 3.5 Hz), 6.32 (1H, s), and 5.55 (1H, d, J = 7.0 Hz) were assigned to H_{ax}-1, H_{eq}-2, H_{ax}-6, and H_{eq}-9, respectively, by $^1H-^1H$ COSY and NOESY (Figure 1) spectra. The axial orientation of the C-9 benzoate moiety was supported by NOESY experiments (Figure 1), which showed the interactions between H-2″,6″ (δ 8.06) of the C-9 benzoate and the C-12 methyl (δ 1.51) and H-1 (δ 5.84). NOESY correlations between the C-14 methyl and the AcO-2, H-6, and H-15 groups confirmed their axial orientations. The stereochemical assignments, which were based on the splitting patterns and coupling constants of H-1 [δ 5.84 (d, J = 3.5 Hz)], H-2 [δ



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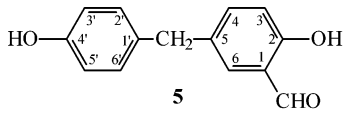

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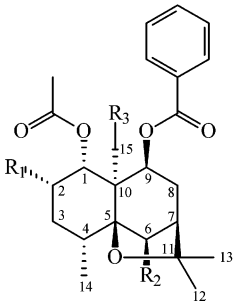

6 $R_1 = R_2 = OAc, R_3 = OBz$
7 $R_1 = R_2 = OBz, R_3 = H$
8 $R_1 = R_2 = R_3 = H$

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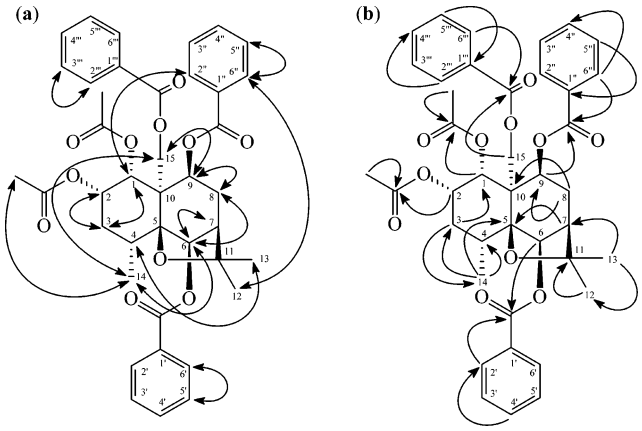

Figure 1. NOESY (a) and HMBC (b) correlations of **1**.

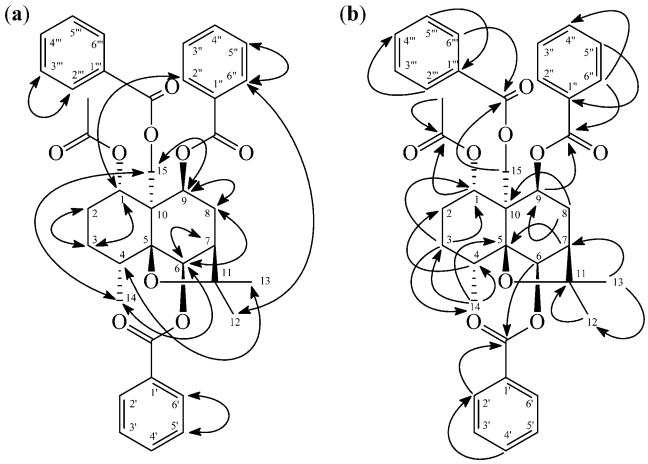

Figure 2. NOESY (a) and HMBC (b) correlations of **2**.

5.67 (q, $J = 3.5$ Hz)], H-6 [δ 6.32 (s)], and H-9 [δ 5.55 (d, $J = 7.0$ Hz)], are in agreement with the relative configurations observed at these positions in this class of natural products.[8,9] Thus, the structure of **1** was elucidated as 1α,2α-diacetoxy-6β,9β,15-tribenzoyloxy-β-dihydroagarofuran. The location of the ester groups was confirmed by the HMBC spectrum, which exhibited cross-peaks between H-1 (δ 5.84), AcO-1 (δ 1.58), and the carbonyl of one acetate ester (δ 169.5); H-2 (δ 5.67), AcO-2 (δ 2.12), and the carbonyl of the second acetate ester (δ 170.0); H-6 (δ 6.32), H-2′/6′ (δ 8.04), and the carbonyl of one benzoate ester (δ 165.4); H-9 (δ 5.55), H-2″/6″ (δ 8.06), and the carbonyl of the second benzoate ester (δ 165.2); and H-15 (δ 4.96, 5.12), H-2‴/6‴ (δ 8.27), and the carbonyl of the third benzoate ester (δ 166.8). Assignments of carbon resonances are shown in Table 2 based on HSQC and HMBC (Figure 1) techniques.

Compound **2** was isolated as an amorphous powder. The ESIMS of **2** afforded an $[M + Na]^+$ ion at *m/z* 663, implying a molecular formula of $C_{38}H_{40}O_9Na$, which was confirmed by the HRESIMS. The UV absorptions of **2** at 231, 274, and 281 nm were similar to those of **1**. The presence of ester carbonyl groups was revealed by the bands at 1748 and 1722 cm^{-1} in the IR spectrum and was confirmed by resonances at δ 165.3, 165.4, 166.7, and 169.7 in the ^{13}C NMR spectrum. The ^1H and ^{13}C NMR (Tables 1 and 2) spectra of **2** were similar to those of **1** except that a hydrogen replaced the *O*-acetyl group (δ 2.12) at C-2 of **1**. From the $^1H-^1H$ COSY and NOESY spectra of **2**, the resonances at δ 5.69 (1H, dd, $J = 12.5$, 4.5 Hz), 6.24 (1H, s), and 5.57 (1H, d, $J = 7.5$ Hz) were assigned as H_{ax}-1, H_{ax}-6, and H_{eq}-9, respectively. The axial orientation of the C-9 benzoate was supported by NOESY experiments (Figure 2), which showed the interactions between H-2″,6″ (δ 8.08) of the C-9 benzoate and the C-12 methyl (δ 1.50) and H-1 (δ 5.69). NOESY correlations between the C-14 methyl and H-6 and H-15 confirmed their axial orientations. The splitting patterns and coupling constants of H-1 [δ 5.69 (dd, $J = 12.5$, 4.5 Hz)], H-6 [δ 6.24 (s)], and H-9 [δ 5.57 (d, $J = 7.5$ Hz)] are in agreement with the relative configuration observed at these positions in other members of this class of natural products,[9] and thus, the configurational assignments of **2** were also established. On the basis of the above data, the structure of **2** was elucidated as 1α-acetoxy-6β,9β,15-tribenzoyloxy-β-dihydroagarofuran. Assignment of the ^{13}C NMR resonances was confirmed by the DEPT, HSQC, and HMBC (Figure 2) techniques.

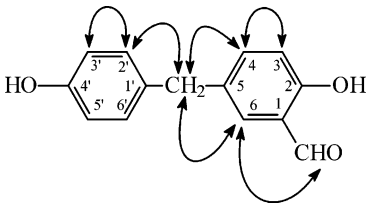

Figure 3. NOESY correlations of **5**.

Compound **3** was also isolated as an amorphous powder. The molecular formula of **3** was established as $C_{38}H_{40}O_9$ by HRESIMS. The IR spectrum of **3** showed absorption bands at 3530, 1752, and 1725 cm^{-1} ascribable to the hydroxy and carbonyl functions. The ^1H NMR spectrum of **3** showed that it was the 2-*O*-deacetyl derivative of **1**. Thus, compound **3** is 1α-acetoxy-2α-hydroxy-6β,9β,15-tribenzoyloxy-β-dihydroagarofuran. This was confirmed by $^1H-^1H$ COSY and NOESY experiments. The assignments of ^{13}C NMR resonances of **3** were confirmed by DEPT, HSQC, and HMBC techniques.

Compound **4** was obtained as an amorphous powder. The molecular formula $C_{38}H_{40}O_{10}$ was deduced from the pseudomolecular ion at *m/z* 679.2523 $[M + Na]^+$ in the HRESI mass spectrum. The IR spectrum of **4** showed absorption bands at 3442, 1750, and 1726 cm^{-1} ascribable to hydroxyl and carbonyl functions. The ^1H NMR spectrum of **4** showed that it was the 1-*O*-deacetyl derivative of **1**. According to the above data, the structure of **4** was elucidated as 2α-acetoxy-1α-hydroxy-6β,9β,15-tribenzoyloxy-β-dihydroagarofuran, which was further confirmed by $^1H-^1H$ COSY, NOESY, DEPT, HSQC, and HMBC experiments.

Compound **5** was isolated as an amorphous powder. The HREIMS gave an $[M]^+$ ion at *m/z* 228.0781 (calcd 228.0781), consistent with a molecular formula of $C_{14}H_{12}O_3$. The presence of hydroxy groups was revealed by the band at 3469 (br) cm^{-1} in the IR spectrum, which was confirmed by the resonances at δ 4.85 (br s, D_2O exchangeable, OH-4′) and 10.89 (s, D_2O exchangeable, OH-2) in the ^1H NMR spectrum. The ^1H NMR spectrum of **5** showed an AA′BB′ spin system at δ 6.78 (2H, d, $J = 8.4$ Hz, H-3′ and H-5′) and 7.04 (2H, d, $J = 8.4$ Hz, H-2′ and H-6′) and an ABX spin system at δ 6.92 (1H, d, $J = 8.4$ Hz, H-3), 7.31 (1H, d, $J = 2.0$ Hz, H-6), and 7.35 (1H, dd, $J = 8.4$, 2.0 Hz, H-4), along with a benzylic methylene group at δ 3.90 (2H, s). The hydrogen-bonded hydroxy group resonated at δ 10.89 (1H, s, OH-2), and the adjacent formyl group at δ 9.83 (1H, s, CHO-1). According to the above data, the structure of **5** is 2-hydroxy-5-(4-hydroxybenzyl)benzaldehyde, named forkienin. This was further confirmed by the $^1H-^1H$ COSY and NOESY (Figure 3) experiments.

The known compounds including three β-dihydroagarofuranoid sesquiterpenes, mutangin (**6**),[7] orbiculin G (**7**),[10] and triptogelin G-2 (**8**),[8] four benzenoids, syringic acid (**9**),[11] vanillic acid (**10**),[12] *p*-hydroxybenzoic acid (**11**),[13] and *p*-hydroxybenzaldehyde (**12**),[14] and two steroids, β-sitosterol (**13**)[15] and β-sitostenone (**14**),[16] were readily identified by comparison of physical and spectroscopic data with corresponding authentic samples or literature values.

The antitubercular effects of the isolates from the roots of *M. fokienensis* were tested *in vitro* against *M. tuberculosis* 90-221387. The antitubercular activity data are shown in Table 3. The clinically used antitubercular agent ethambutol was used as the positive

Table 1. ^{1}H NMR Data of Compounds **1**−**4**[a]

H	1	2	3	4
1	5.84 d (3.5)	5.69 dd (12.5, 4.5)	5.76 d (3.2)	5.83 d (3.6)
2	5.67 q (3.5)	1.67 m	4.49 q (3.2)	5.40 q (3.6)
		2.02 m		
3 (ax)	1.89 dd (15.0, 2.0)	1.60 m	1.97 br d (15.2)	1.92 br d (14.8)
3 (eq)	2.60 ddd (15.0, 6.8, 3.5)	2.39 m	2.48 m	2.48 ddd (14.8, 6.4, 3.6)
4	2.66 m	2.59 m	2.62 m	2.61 m
6	6.32 s	6.24 s	6.36 s	6.17 s
7	2.57 dd (3.5, 3.0)	2.52 dd (3.5, 2.5)	2.54 dd (3.2, 2.8)	2.54 dd (3.6, 2.8)
8 (ax)	2.31 dd (16.0, 3.0)	2.31 dd (16.0, 2.5)	2.28 (16.0, 2.8)	2.38 dd (16.0, 2.8)
8 (eq)	2.74 ddd (16.0, 7.0, 3.5)	2.69 ddd (16.0, 7.5, 3.5)	2.70 ddd (16.0, 7.2, 3.2)	2.74 ddd (16.0, 7.2, 3.6)
9	5.55 d (7.0)	5.57 d (7.5)	5.53 d (7.2)	5.64 d (7.2)
12	1.51 s	1.50 s	1.49 s	1.55 s
13	1.50 s	1.49 s	1.49 s	1.49 s
14	1.30 d (7.5)	1.10 d (7.5)	1.36 d (7.6)	1.35 (7.6)
15	4.96 d (13.7)	4.74 d (12.5)	5.12 d (12.8)	4.92 d (12.8)
	5.12 d (13.7)	5.06 d (12.5)	5.18 d (12.8)	5.03 d (12.8)
OAc-1	1.58 s	1.54 s	1.54 s	
OAc-2	2.12 s			2.10 s
2′/6′	8.04 d (7.5)	8.05 d (7.5)	8.05 d (7.5)	8.04 d (7.5)
3′/5′	7.46 t (7.5)	7.47 t (7.5)	7.47 t (7.5)	7.48 t (7.6)
4′	7.59 t (7.5)	7.59 t (7.5)	7.59 t (7.5)	7.58 t (7.6)
2″/6″	8.06 d (7.6)	8.08 d (7.5)	8.07 d (7.5)	8.09 d (7.6)
3″/5″	7.46 t (7.6)	7.46 t (7.5)	7.46 t (7.5)	7.48 t (7.6)
4″	7.59 t (7.6)	7.59 t (7.5)	7.59 t (7.5)	7.59 t (7.6)
2‴/6‴	8.27 d (7.5)	8.28 d (7.5)	8.29 d (7.5)	8.21 d (7.6)
3‴/5‴	7.58 t (7.5)	7.58 t (7.5)	7.58 t (7.5)	7.55 t (7.6)
4‴	7.66 t (7.5)	7.65 t (7.5)	7.65 t (7.5)	7.63 t (7.6)

[a] Recorded in $CDCl_3$ at 400 MHz. Values in ppm (δ). J (in Hz) in parentheses.

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a]

C	1	2	3	4
1	71.7	73.6	74.4	69.6
2	69.6	22.6	68.4	73.2
3	30.9	26.5	32.4	30.8
4	33.7	34.0	33.5	33.6
5	89.4	89.7	89.4	89.5
6	79.4	79.6	79.3	79.4
7	48.8	48.7	48.7	48.7
8	34.7	34.6	34.7	34.7
9	69.2	69.6	69.2	69.3
10	53.7	53.5	53.5	54.5
11	82.8	82.6	82.8	82.8
12	26.0	26.0	26.0	26.0
13	30.6	30.6	30.6	30.6
14	18.1	16.9	18.0	18.2
15	66.0	65.6	65.8	65.8
1′	129.7[b]	129.6[b]	129.7[b]	129.6[b]
2′/6′	129.6	129.5	129.6	129.5
3′/5′	128.8	128.8	128.8	128.7
4′	133.4	133.4	133.4	133.4
1″	129.9[b]	129.8[b]	129.9[b]	129.8[b]
2″/6″	130.1	130.2	130.2	130.1
3″/5″	128.3	128.3	128.3	128.3
4″	133.5	133.5	133.5	133.5
1‴	129.1[b]	129.2[b]	129.1[b]	129.2[b]
2‴/6‴	130.0	129.9	130.0	129.7
3‴/5‴	128.7	128.7	128.7	128.5
4‴	133.4	133.3	133.4	133.3
CO_2-1	169.5	169.7	169.4	
CO_2-2	170.0			171.1
CO_2-6	165.4	165.4	165.4	165.5
CO_2-9	165.2	165.3	165.3	165.3
CO_2-15	166.8	166.7	166.8	166.7
MeCO_2-1	20.4	20.8	20.7	
MeCO_2-2	21.4			21.4

[a] Recorded in $CDCl_3$ at 100 MHz. Values in ppm (δ). [b] Values superscripted with *b* are interchangeable in every column.

Table 3. Antitubercular Effects

compound	MICs (μM)[a]
1	96.0
2	78.1
3	19.5
4	15.8
5	276
6	51.8
7	14.6
8	26.0
9	318
10	375
11	362
12	>400
13	>400
14	>400
ethambutol[b]	30.6

[a] Data were means of 3 or 4 replicates. [b]Ethambutol was used as a positive control.

control. From the results of our antitubercular tests, the following conclusions can be drawn: (a) Among the β-dihydroagarofuranoid sesquiterpene analogues (**1**−**8**), compounds **3**, **4**, **7**, and **8** exhibited potent antitubercular activities (MICs $\leq$ 26.0 μM) against *M. tuberculosis* 90-221387 *in vitro*. (b) Among the analogues (**1**−**4**), compound **3**, with a 1-acetoxy-2-hydroxy moiety, and **4**, with a 2-acetoxy-1-hydroxy group, exhibited more effective antitubercular activities than **1**, with a 1,2-diacetoxy group, and **2**, with a 1-acetoxy functionality, against *M. tuberculosis* 90-221387 *in vitro*. (c) Orbiculin G (**7**) is the most effective among the isolates, with an MIC of 14.6 μM against *M. tuberculosis* 90-221387 *in vitro*. (d) The benzenoids **9**−**12** and the steroids **13** and **14** showed no antitubercular activities against *M. tuberculosis* 90-221387 *in vitro*.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter in $CHCl_3$. UV spectra were obtained on a Jasco UV-240 spectrophotometer. IR spectra (KBr or neat) were recorded on a Perkin-Elmer system 2000 FT-IR spectrometer. NMR spectra, including COSY, NOESY, HMBC, and HSQC experiments, were recorded on a Varian Unity 400 or a Varian Inova 500 spectrometer operating at 400 and 500 MHz (^{1}H) and 100 and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using TMS as an internal standard. EI, ESI, and HRESI mass spectra were recorded on a Bruker APEX II mass spectrometer. HREI, FAB, and HRFAB mass spectra were recorded on a JEOL JMX-HX 110 mass spectrometer. Silica gel (70−230, 230−

400 mesh) (Merck) was used for CC. Silica gel 60 F-254 (Merck) were used for TLC and preparative TLC.

Plant Material. The roots of *M. fokienensis* were collected from Chunrih, Pingtung County, Taiwan, in November 2004 and identified by Dr. I. S. Chen. A voucher specimen (Sheng-Zehn Yang 023531) was deposited in the herbarium of the Department of Forest Resource, Management and Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan.

Extraction and Separation. The dried roots (3.5 kg) were extracted with cold MeOH, and the extract was concentrated under reduced pressure. The MeOH extract (362 g), when partitioned between H_2O−EtOAc (1:1), afforded an EtOAc-soluble fraction (fraction A, 41 g). The H_2O-soluble fraction was further extracted with *n*-BuOH to afford an *n*-BuOH-soluble fraction (B, 88 g) and an H_2O-soluble fraction (C, 173 g). Fraction A (41 g) was chromatographed on silica gel (70−230 mesh, 1.65 kg), eluting with CH_2Cl_2, gradually increasing the polarity with MeOH to give 15 fractions: A1−3 (each 6 L, CH_2Cl_2), A4 and A5 (each 12 L, CH_2Cl_2−MeOH, 20:1), A6−8 (each 4 L, CH_2Cl_2−MeOH, 10:1), A9−11 (each 2 L, CH_2Cl_2−MeOH, 5:1), A12 and A13 (each 2 L, CH_2Cl_2−MeOH, 2:1), A14 (3 L, CH_2Cl_2−MeOH, 1:1), A15 (5 L, MeOH). Fraction A2 (2.5 g) was chromatographed further on silica gel (230−400 mesh, 76 g) eluting with *n*-hexane−EtOAc (10:1) to give 13 fractions. Fraction A2-4 (223 mg) was purified further by preparative TLC ($CHCl_3$−EtOAc, 30:1) to obtain **14** (23.4 mg). Fraction A2-5 (202 mg) was purified further by preparative TLC ($CHCl_3$−acetone, 50:1) to obtain **8** (3.3 mg). Fraction A2-6 (211 mg) was purified further by preparative TLC (*n*-hexane−acetone, 3:1) to obtain **2** (4.0 mg). Fraction A2-8 (245 mg) was purified further by preparative TLC (CH_2Cl_2) to obtain **7** (3.5 mg). Fraction A3 (3.5 g) was chromatographed further on silica gel (230−400 mesh, 108 g) eluting with *n*-hexane−EtOAc (10:1) to give 11 fractions. Fraction A3-3 (151 mg) was purified further by preparative TLC (CH_2Cl_2−EtOAc, 20:1) to obtain **5** (2.1 mg). Fraction A3-4 (142 mg) was purified further by preparative TLC (CH_2Cl_2−acetone, 30:1) to obtain **1** (3.2 mg). Fraction A3-7 (267 mg) was purified further by preparative TLC (CH_2Cl_2−acetone, 30:1) to obtain **6** (5.2 mg). Fraction A3-10 (155 mg) was purified further by preparative TLC (*n*-hexane−EtOAc, 3:2) to obtain **3** (3.3 mg), **4** (2.4 mg), and **12** (2.6 mg). Fraction A5 (2.5 g) was chromatographed further on silica gel (230−400 mesh, 74 g) eluting with *n*-hexane−EtOAc (10:1) to give eight fractions. Fraction A5-4 (141 mg) was purified further by preparative TLC (*n*-hexane−EtOAc, 30:1) to obtain **13** (14.1 mg). Fraction A9 (2.45 g) was chromatographed further on silica gel (230−400 mesh, 86 g) eluting with CH_2Cl_2−MeOH (10:1) to give nine fractions. Fraction A9-5 (180 mg) was purified further by preparative TLC (EtOAc−MeOH, 8:1) to obtain **9** (4.5 mg) and **10** (2.3 mg). Fraciton A10 (2.33 g) was chromatographed further on silica gel (230−400 mesh, 81 g) eluting with CH_2Cl_2−MeOH (10:1) to give 10 fractions. Fraction A10-7 (180 mg) was purified further by preparative TLC (EtOAc−MeOH, 8:1) to obtain **11** (3.7 mg).

Antitubercular Activity Assay. The antitubercular activity of each test compound was evaluated and compared with the minimal inhibitory concentration (MIC) using the clinical susceptible isolate of *M. tuberculosis* (90-221387). Middlebrook 7H10 agar was used to determine the MICs as recommended by the proportion method.[17] Briefly, each test compound was added to Middlebrook 7H10 agar supplemented with OADC (oleic acid-albumin-dextrose-catalase) at 50−56 °C by a serial dilution to yield a final concentration of 100 to 0.8 μg/mL. Ten milliliters of each concentration of test compound-containing medium was dispensed into plastic quadrant Petri dishes. The inoculum of test isolate of *M. tuberculosis* was prepared by diluting the initial inoculum in Middlebrook 7H9 broth until the turbidity was reduced to that of an equivalent of McFarland no. 1 standard. Final suspensions were performed by adding Middlebrook 7H9 broth and preparing 10^{-2} dilutions of the standardized suspensions. After solidification of the Middlebrook 7H10 medium, 33 μL portions of the dilutions were placed on each quadrant of the agar plates, and the agar plates were incubated at 35 °C with 10% CO_2 for 2 weeks.

Compound 1: amorphous powder; $[\alpha]^{25}_D$ +34.7 (*c* 0.2, $CDCl_3$); UV (MeOH) λ_{max} (log ϵ) 231 (4.12), 274 (3.13), 281 (3.06) nm; IR (KBr) ν_{max} 1746 (C═O), 1721 (C═O), 1714 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m*/*z* (rel int) 699 ($[M + H]^+$, 100); HRFABMS *m*/*z* 699.7729 $[M + H]^+$ (calcd for $C_{40}H_{43}O_{11}$, 699.7731).

Compound 2: amorphous powder; $[\alpha]^{25}_D$ +45.5 (*c* 0.18, $CDCl_3$); UV (MeOH) λ_{max} (log ϵ) 231 (4.17), 274 (3.14), 281 (3.08) nm; IR (KBr) ν_{max} 1748 (C═O), 1722 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m*/*z* (rel int) 663 ($[M + Na]^+$, 100); HRESIMS *m*/*z* 663.2567 $[M + Na]^+$ (calcd for $C_{38}H_{40}O_9Na$, 663.2570).

Compound 3: amorphous powder; $[\alpha]^{25}_D$ +40.6 (*c* 0.14, $CDCl_3$); UV (MeOH) λ_{max} (log ϵ) 231 (4.18), 275 (3.13), 281 (3.05) nm; IR (KBr) ν_{max} 3530 (OH), 1752 (C═O), 1725 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m*/*z* (rel int) 663 ($[M + Na]^+$, 100); HRESIMS *m*/*z* 663.2572 $[M + Na]^+$ (calcd for $C_{38}H_{40}O_9Na$, 663.2570).

Compound 4: amorphous powder; $[\alpha]^{25}_D$ +41.8 (*c* 0.16, $CDCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (4.12), 275 (3.12), 281 (3.04) nm; IR (KBr) ν_{max} 3442 (OH), 1750 (C═O), 1726 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m*/*z* (rel int) 679 ($[M + Na]^+$, 84); HRESIMS *m*/*z* 679.2523 $[M + Na]^+$ (calcd for $C_{38}H_{40}O_{10}Na$, 679.2519).

Fokienin (5): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 225 (4.46), 284 (4.04), 335 (3.58) nm; IR (KBr) ν_{max} 3469 (OH), 1653 (C═O) cm^{-1}; ^1H NMR (400 MHz) δ 4.85 (br s, D_2O exchangeable, OH-4′), 3.90 (2H, s, $-CH_2-$), 6.78 (2H, d, J = 8.4 Hz, H-3′/5′), 6.92 (1H, d, J = 8.4 Hz, H-3), 7.04 (2H, d, J = 8.4 Hz, H-2′/6′), 7.31 (1H, d, J = 2.0 Hz, H-6), 7.35 (1H, dd, J = 8.4, 2.0 Hz, H-4), 9.83 (1H, s, CHO-1), 10.89 (1H, s, D_2O exchangeable, OH-2); EIMS *m*/*z* (rel int) 228 ($[M]^+$, 100), 199 (50), 181 (28), 178 (51), 162 (250, 149 (30), 107 (82), 91 (95), 77 (38); HREIMS *m*/*z* 228.0781 $[M]^+$ (calcd for $C_{14}H_{12}O_3$, 228.0781).

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