Secondary Metabolites from the Roots of *Litsea hypophaea* and Their Antitubercular Activity

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Bioassay-guided fractionation of the roots of *Litsea hypophaea* led to the isolation of seven new butanolides, namely, litseakolides H−N (**1**−**7**), all with the 3*R*,4*S* configuration, as well as three new biarylpropanoids, hypophaone (**8**), hypophaol (**9**), and hypophane (**10**), and 15 known compounds. The structures of **1**−**10** were determined by means of spectroscopic analysis. Litseakolide L (**5**) and *N-trans*-feruloylmethoxytyramine (**11**) showed antitubercular activity against *Mycobacterium tuberculosis* strain H_{37}Rv, with MIC values of 25 and 1.6 μg/mL, respectively.

Litsea hypophaea Hay.[1] (*Actinodaphne pedicellata* Hay.,[2] *Litsea kostermansii* Chang) (Lauraceae) is an endemic evergreen tree that grows abundantly in broadleaf forests throughout Taiwan. Analysis of its chemical constituents has not been conducted, except for constituents of the volatile oil of the leaves and stems.[3] Recently, about 1000 species of Formosan plants were screened in our laboratory for antitubercular activity against *Mycobacterium tuberculosis* strain H_{37}Rv, and *L. hypophaea* was found to be one of the active leads. Bioassay-guided investigation of the roots of this species led to the isolation of eight new compounds (**1**−**8**), together with 17 other isolates, of which the biphenylpropanoids hypophaol (**9**) and hypophane (**10**) have not been obtained previously from a natural source. The isolation and structure elucidation of these compounds and an assessment of their in vitro antitubercular activity are described herein.

Results and Discussion

Compound **1** was obtained as an optically active colorless oil, with $[\alpha]^{23}_{D}$ +18.7 (*c* 0.04, $CHCl_3$). The molecular formula was established as $C_{18}H_{32}O_4$ by ESIMS and HRESIMS analysis (335.2201 $[M + Na]^+$). The IR spectrum showed absorption bands for a hydroxy group at 3427 cm^{-1} and an α,β-unsaturated-γ-lactone moiety at 1745 and 1680 cm^{-1}. The ^{1}H NMR spectrum (Table 1) of **1** was similar to that of litseakolide F[4] and showed the same α-alkylidene-β-hydroxy-γ-methoxy-γ-methyl-γ-lactone unit and the same *E*-geometry of the trisubstituted double bond [δ_H 6.99 (1H, td, J = 7.8, 1.8 Hz)], and also a methoxy group [δ_H 3.38 (3H, s, OCH_3-4)]. The major difference was that two additional methylenes of the side chain in **1** were evident when compared with litseakolide F.[4] Thus, the planar structure of **1** was elucidated as (2*E*)-2-dodecylidene-3-hydroxy-4-methoxy-4-methylbutanolide. The literature indicates the 3*R*,4*S* absolute configuration for compounds with a β-hydroxy-γ-methoxy-γ-methyl-α,β′-unsaturated-γ-lactone moiety, such as isodihydromahubanolide A,[5] and the 3*R*,4*R* absolute configuration for compounds with a β-hydroxy-γ-methoxy-γ-methyl-α,β′-unsaturated-γ-lactone unit, such as litseakolides G and F,[4] subamolides A and B,[6] and (2*E*,3*R*,4*R*)-2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide,[7] all of which show dextrorotatory specific rotations. The latter four $[\alpha]_D$-positive compounds have a 3*R*,4*R* configuration and showed a NOESY correlation between H-3 and OCH_3-4. However, as no NOESY correlation (Figure 1) was observed in **1**, the absolute configuration between H-3 and OCH_3-4 [δ_H 3.88 (1H, s)] of **1** could be deduced as 3*R*,4*S*. An effort to confirm the absolute configuration at C-3 using the Mosher ester method was unsuccessful due to the lablilty of compound **1**. On the basis of the above evidence, the structure of **1** was elucidated as (2*E*,3*R*,4*S*)-2-dodecylidene-3-hydroxy-4-methoxy-4-methylbutanolide, which has been designated as litseakolide H.

Compound **2** was isolated as a colorless oil with $[\alpha]^{24}_{D}$ +22.3 (*c* 0.02, $CHCl_3$). ESIMS and HRESIMS data were used to determine the molecular formula as $C_{18}H_{32}O_4$, the same as that of **1**. The IR spectrum showed absorption bands for a hydroxy group at 3418 cm^{-1} and an α,β-unsaturated-γ-lactone at 1746 and 1680 cm^{-1}. From its ^{1}H NMR spectroscopic data (Table 1), compound **2** was found to be similar to **1**, with the same β-hydroxy-γ-methoxy-γ-methyl-α,β′-unsaturated-γ-lactone partial structure. A difference involving H-6 at δ_H 6.57 (1H, td, J = 7.6, 0.7 Hz) in **2** vs δ_H 6.99 (1H, td, J = 7.8, 1.8 Hz) for **1** suggested a *Z* configuration for $\Delta^{2(6)}$. The dextrorotatory optical activity of **2** and lack of a NOESY correlation (Figure 1) between H-3 and OCH_3-4 again indicated that the absolute configuration of **2** is 3*R*,4*S*,[5] as in the case of **1**. Thus, the structure of **2** (litseakolide I) was deduced as (2*Z*,3*R*,4*S*)-2-dodecylidene-3-hydroxy-4-methoxy-4-methylbutanolide.

Compounds **3** and **4** were obtained as colorless oils. The UV, IR, ^{1}H NMR (Table 1), and ^{13}C NMR (Table 2) data were similar to those of **1** and **2**, but **3** and **4** showed two fewer methylenes in the alkyl side chain connected to C-6, with both demonstrating a molecular formula of $C_{16}H_{28}O_4$ by HRESIMS. Compounds **3** and **4** were assigned as a pair of geometric isomers at C-6, with **3** an *E* isomer, showing H-6 at δ_H 7.00 (1H, td, J = 8.0, 1.5 Hz), and **4** a *Z* isomer, showing H-6 at δ_H 6.56 (1H, td, J = 7.8, 1.8 Hz). Compound **3** was therefore proposed as (2*E*)-2-decylidene-3-hydroxy-4-methoxy-4-methylbutanolide, and **4** was determined as (2*Z*)-2-decylidene-3-hydroxy-4-methoxy-4-methylbutanolide. Compounds **3** and **4** showed dextrorotatory specific rotations, with $[\alpha]^{25}_{D}$ +22.3 (*c* 0.028, $CHCl_3$) and $[\alpha]^{25}_{D}$ +16.1 (*c* 0.016, $CHCl_3$), respectively. Neither compound **3** nor compound **4** showed any NOESY correlation (Figure 1) between H-3 and OCH_3-4. The absolute configurations of **3** and **4** consequently were proposed to be 3*R* and 4*S*. The structures of compounds **3** and **4** (litseakolides J and K) were confirmed by DEPT, COSY, HSQC, and HMBC NMR (Figure 2) experiments.

Kim et al. isolated actinolide B as a new compound from *Actinodaphne lancifolia* and proposed its structure as (2*Z*,3*R*,4*R*)-

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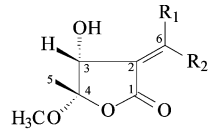
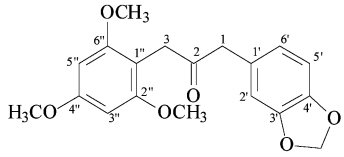
Chart 1



	R_1	R_2
1	$(CH_2)_{10}CH_3$	H
2	H	$(CH_2)_{10}CH_3$
3	$(CH_2)_8CH_3$	H
4	H	$(CH_2)_8CH_3$
5	$(CH_2)_{12}CH_3$	H
6	H	$(CH_2)_{12}CH_3$
7	$CH_2(CH_2)_8CH{=}CH_2$	H



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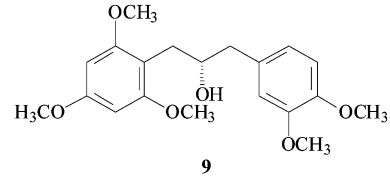

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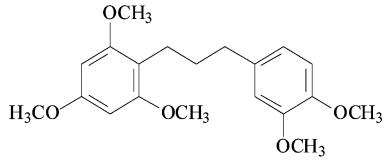

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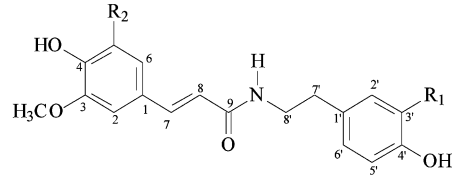

11 $R_1 = OCH_3$, $R_2 = H$
12 $R_1 = R_2 = H$
13 $R_1 = H$, $R_2 = OCH_3$

Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**−**7**[a]

	δ_H (J in Hz)						
position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
3	4.54, brdd (6.9, 1.2)	4.41, brd (5.5)	4.54, brs	4.40, brd (5.4)	4.52, brs	4.40, brd (4.8)	4.54, brd (6.0)
4							
5	1.61, s	1.56, s	1.61, s	1.55, s	1.60, s	1.55, s	1.61, s
6	6.99, td (7.8, 1.8)	6.57, td (7.6, 0.7)	7.00, td (8.0, 1.5)	6.56, td (7.8, 1.8)	6.97, td (7.8, 1.3)	6.56, td (7.7, 1.5)	7.00, td (7.8, 1.4)
7	2.38, m	2.76, q (7.6)	2.39, m	2.75, m	2.38, m	2.75, q (7.7)	2.38, m
8	1.52, m	1.47, m	1.52, m	1.48, m	1.51, m	1.46, m	1.51, m
9	1.26, brs	1.26, brs	1.27, brs	1.25, brs	1.25, brs	1.25, brs	1.28, brs
10	1.26, brs	1.26, brs	1.27, brs	1.25, brs	1.25, brs	1.25, brs	1.28, brs
11	1.26, brs	1.26, brs	1.27, brs	1.25, brs	1.25, brs	1.25, brs	1.28, brs
12	1.26, brs	1.26, brs	1.27, brs	1.25, brs	1.25, brs	1.25, brs	1.28, brs
13	1.26, brs	1.26, brs	1.27, brs	1.25, brs	1.25, brs	1.25, brs	1.28, brs
14	1.26, brs	1.26, brs	1.27, brs	1.25, brs	1.25, brs	1.25, brs	1.28, brs
15	1.26, brs	1.26, brs	0.89, t (7.0)	0.88, t (7.0)	1.25, brs	1.25, brs	2.04, brq (6.9)
16	1.26, brs	1.26, brs			1.25, brs	1.25, brs	5.81, ddt (17.0, 10.4, 6.9)
17	0.88, t (7.2)	0.89, t (6.8)			1.25, brs	1.25, brs	4.93, dtd (10.4, 1.8, 1.2, H-17$_a$) 4.99, dtd (17.0, 1.8, 1.4, H-17$_b$)
18					1.25, brs	1.25, brs	
19					0.87, t (6.8)	0.88, t (6.8)	
OCH_3	3.38, s	3.41, s	3.39, s	3.40, s	3.37, s	3.40, s	3.38, s
OH-3[b]	1.78, brd (6.9)	1.83, brd (5.5)	1.71, brs	1.78, br d (5.4)	1.71, brs	1.94, brd (4.8)	1.69, brd (6.0)

[a] ^{1}H NMR data (δ) were measured in $CDCl_3$ at 600 MHz for **1** and **4**, at 500 MHz for **2** and **3**, and at 400 MHz for **5**−**7**. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. [b] D_2O exchangeable.

2-decylidene-3-hydroxy-4-methoxy-4-methylbutanolide.[8] The 4*R* configuration of actinolide B was suggested by a NOESY correlation between H-3 and CH_3-4. According to the NOESY spectra of litseakolides D−G[4] and a computer-assisted 3D structure, which was obtained using the molecular modeling program CS CHEM 3D Ultra 10.0, with MM2 force-field calculations for energy minimization between H-3 and CH_3-4 (Figure 3), the calculated distances between H-3/CH_3-4 (*cis* form) (2.151 Å) and H-3/CH_3-4 (*trans* form) (3.469 Å) are all less than 4 Å. The NOESY correlation (Figure 1) between H-3 and CH_3-4 is found in either the *cis* or *trans* orientation of a five-membered ring like butanolide. The determination of the C-4 configuration of 3-hydroxy-4-methoxy-4-methylbutanolide should be confirmed by a NOESY correlation between H-3 and OCH_3-4. However, as actinolide B[8] showed no NOESY correlation between H-3 and OCH_3-4, its assigned absolute configuration of 3*R*,4*R* may be revised to 3*R*,4*S*, as in litseakolides J and K.

The molecular formulas of compounds **5** and **6** (litseakolides L and M) were determined as $C_{20}H_{36}O_4$ by HRESIMS, which showed them to be two methylenes larger than **1** and **2**, with $C_{18}H_{32}O_4$ in

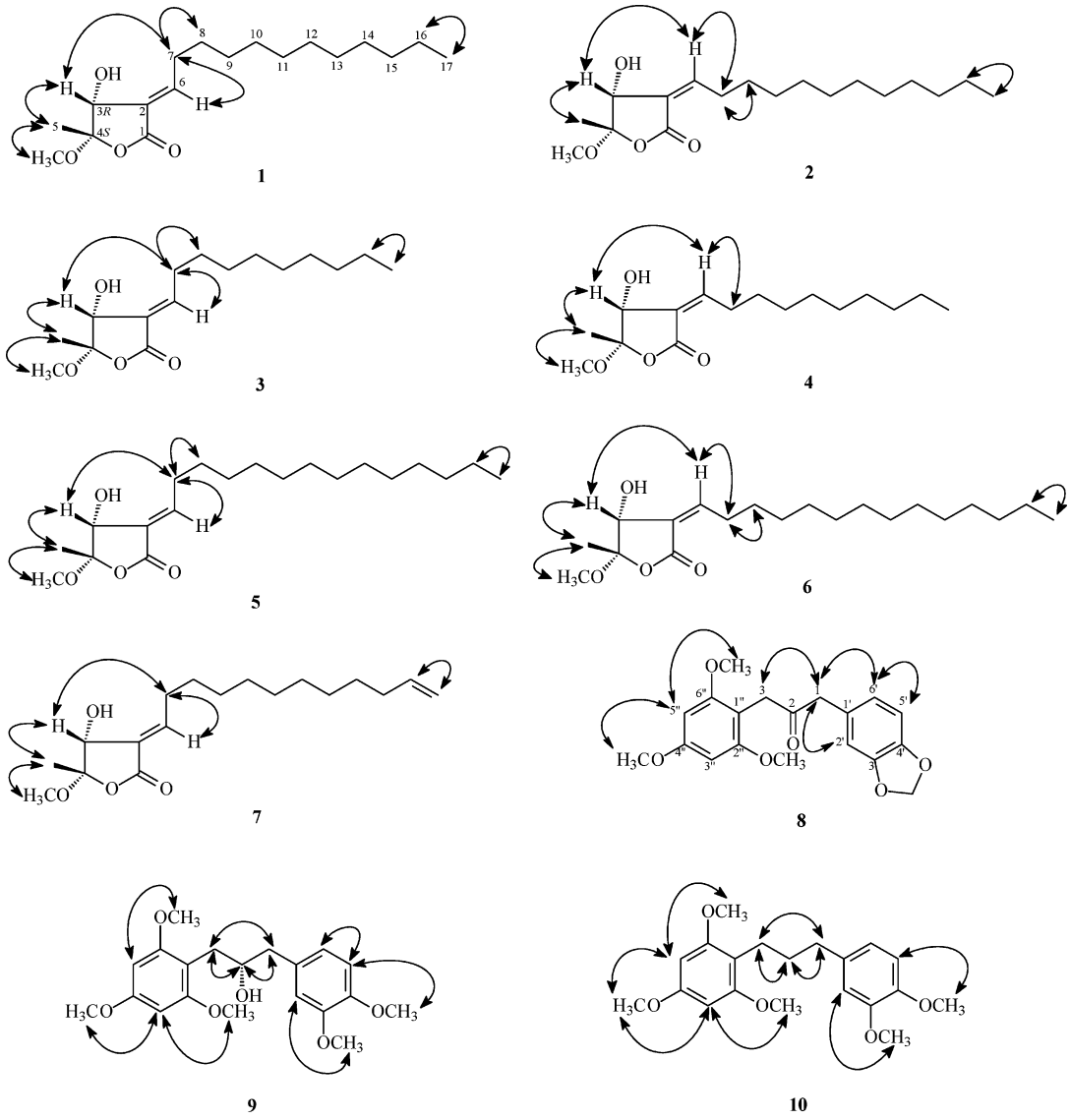

Figure 1. Key NOESY (H↔H) correlations of **1**−**10**.

Table 2. ^{13}C NMR Data for Compounds **1**−**3** and **5**−**7**[a]

position	1	2	3	5	6	7
1	168.8	168.0	168.9	169.2	167.2	168.3
2	130.0	128.9	130.0	130.0	128.8	130.0
3	72.5	75.8	72.5	72.5	75.8	72.5
4	109.3	108.7	109.3	109.5	108.8	109.2
5	16.1	16.3	16.1	16.1	16.2	16.1
6	148.4	150.3	148.4	148.5	150.4	148.4
7	29.8	29.8	29.8	29.8	28.1	29.8
8	28.4	28.4	28.4	28.4	28.7	28.4−29.4
9	29.3−29.6	29.3−29.6	29.2−29.4	29.3−29.7	29.2−29.7	28.4−29.4
10	29.3−29.6	29.3−29.6	29.2−29.4	29.3−29.7	29.2−29.7	28.4−29.4
11	29.3−29.6	29.3−29.6	29.2−29.4	29.3−29.7	29.2−29.7	28.4−29.4
12	29.3−29.6	29.3−29.6	29.2−29.4	29.3−29.7	29.2−29.7	28.4−29.4
13	29.3−29.6	29.3−29.6	29.2−29.4	29.3−29.7	29.2−29.7	28.4−29.4
14	29.3−29.6	29.3−29.6	29.2−29.4	29.3−29.7	29.2−29.7	28.4−29.4
15	31.9	31.9	31.8	29.3−29.7	29.2−29.7	33.8
16	22.7	22.7	22.6	29.3−29.7	29.2−29.7	139.2
17	14.1	14.1	14.1	31.9	31.9	114.1
18				22.7	22.7	
19				14.1	14.1	
OCH_3	50.3	50.4	50.3	50.3	50.4	50.3

[a] ^{13}C NMR data (δ) were measured in $CDCl_3$ at 150 MHz for **1**, at 125 MHz for **2** and **3**, and at 100 MHz for **5**−**7**. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra.

an alkyl side chain and a pair of geometric isomers at C-6. The UV, IR, ^{1}H NMR (Table 1), and ^{13}C NMR (Table 2) data of **5** and **6** were similar to those of **1** and **2**. The compounds were established, respectively, as the *E* isomer **5**, with H-6 at δ_H 6.97 (1H, td, J =

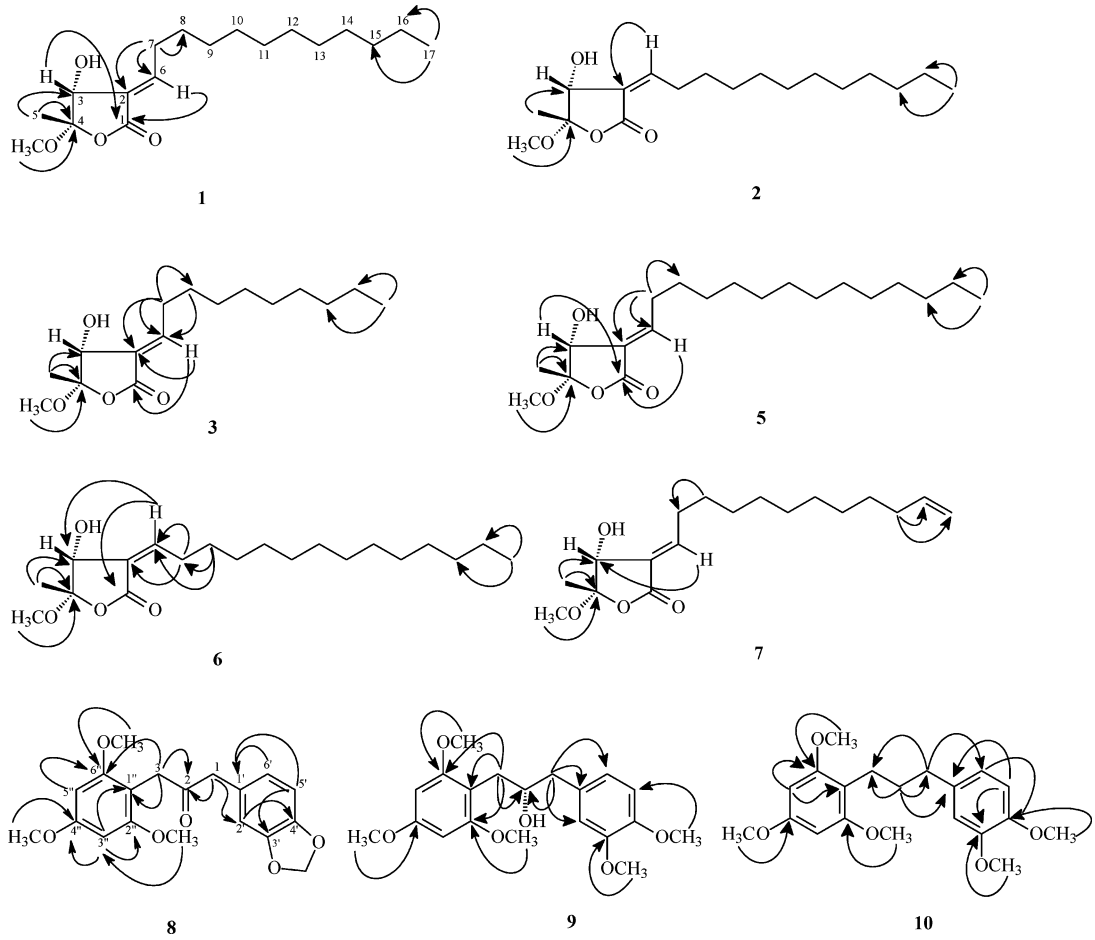

Figure 2. Key HMBC (H→C) correlations of **1**−**3** and **5**−**10**.

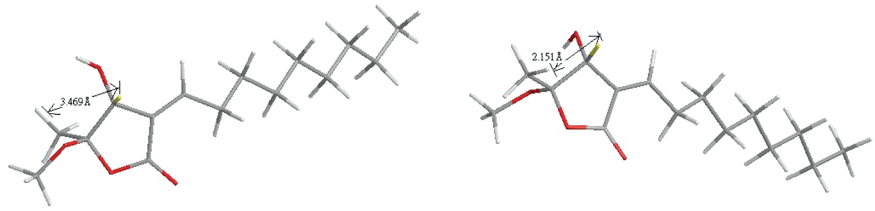

Figure 3. Most stable conformation for β-hydroxy-γ-methoxy-γ-methyl-α,β'-unsaturated-γ-lactone as predicted by molecular mechanics (MM2) calculations.

7.8, 1.3 Hz), and the *Z* isomer **6**, with H-6 at δ_H 6.56 (1H, td, J = 7.7, 1.5 Hz). Thus, compounds **5** and **6** were determined, in turn, as (2*E*)-2-tetradecylidene-3-hydroxy-4-methoxy-4-methylbutanolide and (2*Z*)-2-tetradecylidene-3-hydroxy-4-methoxy-4-methylbutanolide. Compounds **5** and **6** both showed a dextrorotatory optical activity, with $[\alpha]^{25}_D$ +19.7 (*c* 0.03, $CHCl_3$) and $[\alpha]^{25}_D$ +20.4 (*c* 0.14, $CHCl_3$), respectively. Therefore, the structures of these substances were elucidated as (2*E*,3*R*,4*S*)-2-tetradecylidene-3-hydroxy-4-methoxy-4-methylbutanolide (**5**) and (2*Z*,3*R*,4*S*)-2-tetradecylidene-3-hydroxy-4-methoxy-4-methylbutanolide (**6**). The two structures were confirmed by DEPT, COSY, NOESY (Figure 1), HSQC, and HMBC NMR (Figure 2) experiments.

Compound **7** was isolated as a colorless oil. The molecular formula was determined to be $C_{18}H_{30}O_4$ by HRESIMS, and the IR spectrum showed absorption bands for a hydroxy group at 3438 cm^{-1} and an α,β-unsaturated-γ-lactone at 1746 and 1680 cm^{-1}. The ^{1}H NMR spectrum (Table 1) was similar to that of **1**, but a vinyl group [δ_H 4.93 (1H dtd, J = 10.4, 1.8, 1.2 Hz), 4.99 (1H dtd, J = 17.0, 1.8, 1.4 Hz), 5.81 (1H ddt, J = 17.0, 10.4, 0.9 Hz)] was evident in **7** as opposed to a terminal ethyl group [δ_H 0.88 (3H, t, J = 7.2 Hz, H-17), 1.26 (brs, H-16)] in **1**. The ^{13}C NMR spectrum (Table 2) also supported the presence of a vinyl group [δ_C 114.1 (C-17), 139.2 (C-16)]. The dextrorotatory specific rotation {$[\alpha]^{25}_D$ +25.4 (*c* 0.02, $CHCl_3$)} and lack of any NOESY correlation between H-3 and OCH_3-4, when compared to observations made for (2*E*,3*R*,4*R*)-2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide,[7] indicated the structure of **7** (litseakolide N) to be (2*E*,3*R*,4*S*)-2-(11-dodecenylidene)-3-hydroxy-4-methoxy-4-methylbutanolide.

(3*R*,4*S*)-3-Hydroxy-4-methylbutanolides have previously been isolated from the Lauraceous plant *Clinostemon mahuba*,[5] but this is the first reported occurrence of 3-hydroxy-4-methoxy-4-methylbutanolides with a 3*R*,4*S* configuration, as found in litseakolides H−N, having been isolated from a natural source.

Compound **8** was isolated as an amorphous powder, and its molecular formula was established as $C_{19}H_{20}O_6$ by HRESIMS. The UV spectrum exhibited bands at 233 (sh) and 286 nm, suggesting the presence of a benzenoid moiety. A band attributable to a ketone

Table 3. NMR Spectroscopic Data ($CDCl_3$, 600 MHz) for Compounds **8**−**10**

	8		**9**		**10**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	3.56, s	48.0	2.71, dd (13.8, 7.8) 2.76, dd (13.8, 4.2)	43.2	2.59, t (9.0)	35.5
2		207.4	3.99, m	73.1	1.76, m	31.1
3	3.64, s	36.9	2.79, dd (13.8, 7.8) 2.88, dd (13.8, 4.2)	30.2	2.61, t (9.0)	22.5
1′		128.5		132.1		135.9
2′	6.63, d (1.6)	110.0	6.80, d (1.2)	112.6	6.74, brs	120.1
3′		147.5		148.7		148.6
4′		146.3		147.3		146.8
5′	6.72, d (7.9)	108.1	6.81, d (9.0)	111.1	6.74, d (8.0)	111.7
6′	6.57, dd (7.9, 1.6)	122.6	6.79, dd (9.0, 1.2)	121.3	6.78, dd (8.0, 1.8)	111.0
1″		104.3		107.5		111.5
2″		158.8		159.1		158.8
3″	6.12, s	90.4	6.15, s	90.7	6.13, s	90.5
4″		160.5		159.8		159.1
5″	6.12, s	90.4	6.15, s	90.7	6.13, s	90.5
6″		158.8		159.1		158.8
H_3CO-3′			3.87, s	55.9	3.87, s	55.8
H_3CO-4′			3.86, s	55.8	3.85, s	55.9
H_3CO-2″	3.74, s	55.6	3.78, s	55.7	3.78, s	55.6
H_3CO-4″	3.82, s	55.4	3.81, s	55.3	3.81, s	55.3
H_3CO-6″	3.74, s	55.6	3.78, s	55.7	3.78, s	55.6
OCH_2O	5.92, s	100.9				

(1729 cm^{-1}) was observed in the IR spectrum. The ^1H NMR data (Table 3) of **8** showed an ABX system of a benzene ring [δ_H 6.57 (1H, dd, J = 7.9, 1.6 Hz, H-6′), 6.63 (1H, d, J = 1.6 Hz, H-2′), 6.72 (1H, d, J = 7.9 Hz, H-5′)], two symmetrical aromatic protons [δ_H 6.12 (2H, s, H-3″, H-5″)] in another benzene ring, and three methoxy groups [δ_H 3.74 (6H, s, OCH_3-2″, 6″), 3.82 (3H, s, OCH_3-4″)], indicating the presence of 3,4-methylenedioxyphenyl and 2,4,6-trimethoxyphenyl moieties. These two phenyl moieties were connected by an oxopropylene group [δ_H 3.56 (2H, s, H-1) and 3.64 (2H, s, H-3)]. The HMBC spectrum (Figure 2) revealed correlations between H-3 (δ_H 3.64)/C-1″ (δ_C 104.3), H-3″ (δ_H 6.72)/C-1″ (δ_C 104.3), H-3 (δ_H 3.64)/C-2 (δ_C 207.4), C-1″ (δ_C 104.3), C-6″ (δ_C 158.2), and H-1 (δ_H 3.56)/C-2 (δ_C 207.4), C-2′ (δ_C 110.0), C-6′ (δ_C 122.6). Thus, the biphenylpropanoid structure of **8** was elucidated as 1-(3′,4′-methylenedioxyphenyl)-3-(2″,4″,6″-trimethoxyphenyl)propan-2-one, which was confirmed by DEPT, COSY, HSQC, NOESY (Figure 1), and HMBC NMR (Figure 2) experiments, and this compound was named hypophaone.

Compounds **9** and **10** were isolated as colorless needles, and their respective molecular formulas of $C_{20}H_{26}O_6$ and $C_{20}H_{26}O_5$ were established by HRESIMS. The ^1H NMR data of **9** (Table 3) revealed the presence of a diarylpropanoid analogue, with a 3,4-dimethoxyphenyl moiety [δ_H 3.86 (3H, s, OCH_3-4′), 3.87 (3H, s, OCH_3-3′), 6.79 (1H, dd, J = 9.0, 1.2 Hz, H-6′), 6.80 (1H, d, J = 1.2 Hz, H-2′), 6.81 (1H, d, J = 9.0 Hz, H-5′)], a 2,4,6-trimethoxyphenyl unit [δ_H 6.15 (2H, s, H-3″, 5″)], and a 2-hydroxypropylene group [δ_H 2.71 (1H, dd, J = 13.8, 1.8 Hz, H-1a), 2.76 (1H, dd, J = 13.8, 4.2 Hz, H-1b), 3.99 (1H, m, H-2)]. In **10**, the ^1H NMR spectrum showed the occurrence of a 3,4-dimethoxyphenyl moiety [δ_H 6.74 (1H, brs, H-2′), 6.74 (1H, d, J = 8.0 Hz, H-5′), 6.78 (1H, dd, J = 8.0, 1.8 Hz, H-6′), 3.87 (3H, s, OCH_3-3′), 3.85 (3H, s, OCH_3-4′)] and a propylene group [δ_H 2.59 (2H, t, J = 9.0 Hz, H-1), 1.76 (2H, m, H-2), 2.61 (2H, t, J = 9.0 Hz, H-3)]. The HMBC spectrum (Figure 2) supported the planar structure of **9** as being 1-(3′,4′-dimethoxyphenyl)-3-(2″,4″,6″-trimethoxyphenyl)propan-2-ol and that of **10** as 1-(3′,4′-dimethoxyphenyl)-3-(2″,4″,6″-trimethoxyphenyl)propane. Furthermore, compound **9** showed a dextrorotatory optical activity with $[\alpha]^{27}_D$ +8.0 (c 0.09, $CHCl_3$), so the absolute configuration of C-2 was proposed as *S* after comparison with the synthetic substance (2*S*)-1-(3′,4′-dimethoxyphenyl)-3-(2″,4″,6″-trimethoxyphenyl)propan-2-ol.[9] This is the first report of compounds **9** and **10** having been isolated from a natural source, although compound **10** has been synthesized previously.[9−11]

Table 4. Antitubercular Activities of Isolates from the Roots of *Litsea hypophaea* against *M. tuberculosis* Strain $H_{37}Rv$

compound	MIC (μg/mL)
litseakolide L (**5**)	25
hypophaol (**9**)	100
N-trans-feruloylmethoxytyramine (**11**)	1.6
N-trans-feruloyltyramine (**12**)	60
ethambutol[a]	6.25

[a] Positive control. [b] Compound **13** and 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxylphenyl)-N^1,N^2-bis[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide were inactive in the in vitro test system used (MIC > 100 μg/mL).

The known compounds methyl syringate,[12,13] 4-hydroxybenzaldehyde,[14] vanillin,[15,16] methyl 4-hydroxy-3-methoxybenzoate,[17] methyl 4-hydroxybenzoate,[18] *p*-hydroxybenzoic acid,[19] *N-trans*-feruloylmethoxytyramine (**11**),[20,21] *N-trans*-feruloyltyramine (**12**),[22] *N-trans*-sinapoyltyramine (**13**),[23] 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-N^1,N^2-bis[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide,[23] cannabisin D,[24,25] syringaresinol,[26] northalifoline,[27] and a mixture of β-sitosterol and stigmasterol[28] were identified by comparison of their physical and spectroscopic data ($[\alpha]_D$, UV, IR, ^1H NMR, and MS) with values reported in the literature. This is the first time the two lignanamides 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-N^1,N^2-bis[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide[23] and cannabisin D[24,25] have been isolated from a plant in the family Lauraceae.

When the isolates obtained from the roots of *L. hypophaea* were evaluated for their antitubercular activities against *M. tuberculosis* strain $H_{37}Rv$ in vitro, compounds **5** and **11**, with minimal inhibitory concentration (MIC) values of 25 and 1.6 μg/mL, respectively, were the most active compounds obtained (Table 4). Ethambutol (MIC 6.25 μg/mL) was used as the positive control. In comparing the three amides (**11**−**13**), the oxygenated groups at the 3,4,3′,4′ positions of the phenyl groups in **11** were seen to play an important role in mediating the resultant antitubercular activity.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were obtained on a JASCO UV-240

spectrophotometer in MeOH, and IR spectra (KBr or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, NOESY, TOCSY, HSQC, HMBC) NMR spectra using $CDCl_3$ as solvent were recorded on Varian Unity Plus 400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR), Varian INOVA-500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR), and Varian VNMRS-600 (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR) NMR spectrometers. Chemical shifts were internally referenced to the solvent signals in $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0), with TMS as the internal standard. Low-resolution ESIMS were obtained on an API 3000 mass spectrometer (Applied Biosystems) and high-resolution ESIMS on a Bruker Daltonics APEX II 30e mass spectrometer. Low-resolution EIMS were recorded on a Quattro GC/MS spectrometer having a direct inlet system. Silica gel (70−230, 230−400 mesh) (Merck) and Pharmaceutical Biotech Sephadex LH-20 were used for column chromatography, and silica gel 60 F-254 (Merck) was used for analytical and preparative TLC. Medium-pressure liquid chromatography was used for chromatograpy. A spherical C_{18} column (250 × 10 mm, 5 μm), an LDC-Analytical-III apparatus, and an UV−vis detector (SPD-10A, Shimadzu) were used for HPLC.

Plant Material. The roots of *L. hypophaea* were collected from Mudan, Pingtung County, Taiwan, in June 2007, and identified by one of the authors (I.-S.C.). A voucher specimen (Chen 2183) was deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. Dried roots (6.4 kg) of *L. hypophaea* were sliced and extracted with cold MeOH (30 L) three times. On concentration, the MeOH extract was partitioned between EtOAc−H_2O (1:1) to obtain an EtOAc-soluble fraction (70 g) and an H_2O-soluble fraction (209 g). The EtOAc fraction (70 g) was applied to a silica gel column (230−400 mesh, 1.8 kg), eluting with a gradient of *n*-hexane−acetone, to give 25 fractions (1−25). Fraction 5 (2.37 g) was recrystallized from *n*-hexane to give a mixture of β-sitosterol and stigmasterol (53 mg). Fraction 6 (0.83 g) was applied to a column containing silica gel (230−400 mesh, 20 g), eluting with a gradient of *n*-hexane−acetone, to give eight fractions (6-1−6-8). Fraction 6-2 (179 mg) was chromatographed on a Sephadex LH-20 column (MeOH) to give five fractions (6-2-1−6-2-5). Fr. 6-2-3 (119 mg) was applied to a RP-C_{18} column (10 g), eluting with acetonitrile−H_2O (2.5:1), to obtain 10 fractions (6-2-3-1−6-2-3-10). Fr. 6-2-3-10 (99.4 mg) was chromatographed on a silica gel column (230−400 mesh, 3 g), eluting with $CHCl_3$−MeOH (20:1), to give seven fractions (6-2-3-10-1−6-2-3-10-7). Fr. 6-2-3-10-3 was applied to a RP-C_{18} column (10 g), eluting with acetonitrile−H_2O (3:1), to obtain three fractions (6-2-3-10-1−6-2-3-10-3). Fr. 6-2-3-10-3-2 (3.8 mg) was purified by preparative TLC ($CHCl_3$−EtOAc, 20:1) to obtain **1** (1.9 mg, R_f 0.25) and **2** (0.8 mg, R_f 0.32). Fr. 6-2-3-10-3-3 (22.1 mg) was purified by preparative TLC (CH_2Cl_2−EtOAc, 50:1) to afford **5** (1.4 mg, R_f 0.23) and **6** (14.8 mg, R_f 0.31). Fr. 6-2-5 (3.2 mg) was further purified by preparative RP-18 TLC (MeOH−H_2O, 5:1) to afford **10** (0.8 mg, R_f 0.30). Fr. 7 (1.20 g) was applied to a silica gel column (230−400 mesh, 30 g), eluting with CH_2Cl_2, to obtain seven fractions (7-1−7-7). Fraction 7-3 (105 mg) was chromatographed on an RP-C_{18} column (2 g), eluting with MeOH−H_2O (2.5:1), to afford methyl 4-hydroxy-3-methoxybenzoate (1.5 mg, R_f 0.83). Fraction 7-6 (39.4 mg) was applied to a RP-C_{18} column (10 g), eluting with MeOH−H_2O (2.5:1), and purified further by preparative TLC ($CHCl_3$−EtOAc, 15:1) to obtain methyl 4-hydroxybenzoate (2.0 mg, R_f 0.7), **7** (0.8 mg, R_f 0.38), **3** (0.8 mg, R_f 0.61), and **4** (1.4 mg, R_f 0.53). Fraction 8 (2.25 g) was chromatographed on a silica gel column (230−400 mesh, 60 g), eluting with a gradient of *n*-hexane−acetone, to give 12 fractions (8-1−8-12). Fr. 8-5 (88.6 mg) was chromatographed on a RP-18 column (4 g), eluting with acetone−H_2O (2.5:1), to obtain six fractions (Fr. 8-5-1−8-5-6). Fr. 8-5-1 (1.2 mg) was subjected to preparative RP-18 TLC (MeOH−H_2O, 10:1) to afford **8** (0.8 mg, R_f 0.39). Fr. 8-7 (168 mg) was applied to a silica gel column, eluting with CH_2Cl_2−acetone (50:1), to afford vanillin (2.8 mg, R_f 0.65). Fr. 8-8 (274.1 mg) was subjected to a silica gel column (230−400 mesh, 7 g), eluting with *n*-hexane−EtOAc (2:1), to give six fractions (8-8-1−8-8-6). Fr. 8-8-3 (53.6 mg) was applied to a silica gel column (230−400 mesh, 1.5 g), eluting with CH_2Cl_2−acetone (50:1), and purified further by preparative TLC (CH_2Cl_2−acetone, 20:1) to obtain 4-hydroxybenzaldehyde (3.1 mg, R_f 0.60) and methyl syringate (2.3 mg, R_f 0.67). Fr. 8-9 (216 mg) was applied to a silica gel column (230−400 mesh, 6 g), eluting with CH_2Cl_2−acetone (20:1), to give eight fractions (8-9-1−8-9-8). Fr. 8-9-5 (5.8 mg) was subjected to preparative HPLC (acetonitrile−H_2O, 5:1) to afford **9** (2.5 mg, t_R 8.26 min, 2 mL/min). Fraction 12 (1.56 g) was chromatographed on a silica gel column (230−400 mesh, 40 g), eluting with a gradient of CH_2Cl_2−acetone, to obtain 12 fractions (12-1−12-12). Fr. 12-3 (54.4 mg) was passaged over Sephadex LH-20 (MeOH) to give three fractions (12-3-1−12-3-3). Fr. 12-3-2 (12.3 mg) was subjected to preparative RP-18 TLC (MeOH−H_2O, 2:1) to afford syringaresinol (7.9 mg, R_f 0.40). Fr. 12-5 (102 mg) was subjected to a Sephadex LH-20 column (MeOH) to give seven fractions (12-5-1−12-5-7). Fr. 12-5-3 (67.3 mg) was recrystallized from MeOH to give **11** (9.4 mg, R_f 0.40). Fr. 12-7 (98.6 mg) was subjected to Sephadex LH-20 column chromatography (MeOH) to give four fractions (12-7-1−12-7-4). Fr. 12-7-1 (81.8 mg) was further purified by preparative TLC ($CHCl_3$−MeOH, 20:1) to give **12** (42.5 mg, R_f 0.20). Fr. 12-9 (46.2 mg) was chromatographed on a silica gel column (230−400 mesh, 1.2 g), with a gradient of $CHCl_3$−MeOH, to obtain four fractions (12-9-1−12-9-4). Fr. 12-9-2 (22.4 mg) was further purified by preparative RP-18 TLC (isopropyl alcohol−H_2O, 1:2) to afford **13** (15.5 mg, R_f 0.34). Fr. 12-11 (128 mg) was applied to a Sephadex LH-20 column (MeOH) to give five fractions (12-11-1−12-11-5). Fr. 12-11-2 (43 mg) was chromatographed on an RP-C_{18} column (1.5 g), eluting with acetonitrile−H_2O (1:1), to give eight fractions (12-11-2-1−12-11-2-8). Fr. 12-11-2-3 (37 mg) was chromatographed on a silica gel column (230−400 mesh, 1.5 g), eluting with a gradient of $CHCl_3$−MeOH, and purified further by preparative TLC (CH_2Cl_2−acetone−EtOAc, 10:1:1) to give 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-*N*^{1},*N*^{2}-bis[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (2.2 mg, R_f 0.23). Fr. 12-11-3 (48 mg) was further purified by preparative RP-18 TLC (acetone−H_2O, 1:1) to give *p*-hydroxybenzoic acid (3.8 mg, R_f 0.67). Fr. 15 (2.52 g) was chromatographed on a RP-C_{18} column (10 g), eluting with acetonitrile−H_2O (1:1), to give eight fractions (15-1−15-8). Fr. 15-1-3 (7.8 mg) was purified by preparative TLC ($CHCl_3$−EtOAc−MeOH, 3:0.3:0.3) to obtain northalifoline (1.6 mg, R_f 0.26). Fr. 15-2 (75.3 mg) was applied to a silica gel column (230−400 mesh, 3.8 g), eluting with a gradient of CH_2Cl_2−MeOH, to give five fractions (15-2-1−15-2-5). Fr. 15-2-4 (6.7 mg) was further purified by preparative TLC (*n*-hexane−EtOAc, 1:7) to obtain cannabisin D (1.8 mg, R_f 0.25).

Litseakolide H (1): colorless oil; $[\alpha]^{23}_D$ +18.7 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.74) nm; IR (neat) ν_{max} 3427 (OH), 1745, 1680 (α,β-unsaturated-γ-lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; ESIMS *m*/*z* 335 [M + Na]$^+$; HRESIMS *m*/*z* 335.2201 [M + Na]$^+$ (calcd for $C_{18}H_{32}O_4Na$, 335.2198).

Litseakolide I (2): colorless oil; $[\alpha]^{24}_D$ +22.3 (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.50) nm; IR (neat) ν_{max} 3418 (OH), 1746, 1680 (α,β-unsaturated-γ-lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; ESIMS *m*/*z* 335 [M + Na]$^+$; HRESIMS *m*/*z* 335.2201 [M + Na]$^+$ (calcd for $C_{18}H_{32}O_4Na$, 335.2198).

Litseakolide J (3): colorless oil; $[\alpha]^{25}_D$ +22.3 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.51) nm; IR (neat) ν_{max} 3435 (OH), 1745, 1680 (α,β-unsaturated-γ-lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; ESIMS *m*/*z* 307 [M + Na]$^+$; HRESIMS *m*/*z* 307.1884 [M + Na]$^+$ (calcd for $C_{16}H_{28}O_4Na$, 307.1885).

Litseakolide K (4): colorless oil; $[\alpha]^{25}_D$ +16.1 (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.58) nm; IR (neat) ν_{max} 3425 (OH), 1745, 1679 (α,β-unsaturated-γ-lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ESIMS *m*/*z* 307 [M + Na]$^+$; HRESIMS *m*/*z* 307.1883 [M + Na]$^+$ (calcd for $C_{16}H_{28}O_4Na$, 307.1885).

Litseakolide L (5): colorless oil; $[\alpha]^{25}_D$ +20.4 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.57), nm; IR (neat) ν_{max} 3448 (OH), 1746, 1680 (α,β-unsaturated-γ-lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; ESIMS *m*/*z* 363 [M + Na]$^+$; HRESIMS *m*/*z* 363.2514 [M + Na]$^+$ (calcd for $C_{20}H_{36}O_4Na$, 363.2511).

Litseakolide M (6): colorless oil; $[\alpha]^{25}_D$ +19.7 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (3.58) nm; IR (neat) ν_{max} 3388 (OH), 1739, 1651 (α,β-unsaturated-γ-lactone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; ESIMS *m*/*z* 363 [M + Na]$^+$; HRESIMS *m*/*z* 363.2508 [M + Na]$^+$ (calcd for $C_{20}H_{36}O_4Na$, 363.2511).

Litseakolide N (7): colorless oil; $[\alpha]^{25}_D$ +25.4 (*c* 0.02, $CHCl_3$), UV (MeOH) λ_{max} (log ε) 217 (4.11) nm; IR (neat) ν_{max} 3438 (OH), 1746,

1680 (α,β-unsaturated-γ-lactone) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; ESIMS *m/z* 333 $[M + Na]^+$; HRESIMS *m/z* 333.2040 $[M + Na]^+$ (calcd for $C_{18}H_{30}O_4Na$, 333.2042).

Hypophaone (8): amorphous powder; UV (MeOH) λ_{max} (log ε) 233 sh (4.07), 286 (3.88) nm; IR (KBr) ν_{max} 1729 (C=O), 1602, 1495 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 3; ESIMS *m/z* 367 $[M + Na]^+$; HRESIMS *m/z* 367.1157 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_6Na$, 367.1158).

Hypophaol (9): colorless needles; mp 86−87 °C; $[\alpha]^{25}_D$ +8.0 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (4.02), 278 (3.05) nm; IR (neat) ν_{max} 3529 (OH), 1601, 1512 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 3; ESIMS *m/z* 385 $[M + Na]^+$; HRESIMS *m/z* 385.1625 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_6Na$, 385.1627).

Hypophane (10): colorless needles; mp 88−89 °C; UV (MeOH) λ_{max} (log ε) 225 (4.30), 275 (3.73) nm; IR (KBr) ν_{max} 1569, 1514, 1460 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 3; ESIMS *m/z* 369 $[M + Na]^+$; HRESIMS *m/z* 369.1680 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 369.1678).

Antitubercular Activity Assay. The in vitro antitubercular activity of each tested compound was evaluated using *Mycobacterium tuberculosis* strain $H_{37}Rv$. Middlebrook 7H10 agar was used to determine MIC values, as recommended by the proportion method.[29] Briefly, each test compound was added to Middlebrook 7H10 agar supplemented with OADC (oleic acid−albumin−dextrose−catalase) at 50−56 °C, by serial dilution, to yield a final concentration of 100 to 0.8 μg/mL. Then 10 mL of each concentration of test compound-containing medium was dispensed into plastic quadrant Petri dishes. Several colonies of the test isolate of *M. tuberculosis* were selected to make a suspension with Middlebrook 7H9 broth and used as the initial inoculum. The inoculum of test isolate of *M. tuberculosis* was prepared by diluting the initial inoculum in Middlebrook 7H9 broth until turbidity was reduced to that equivalent to the McFarland no. 1 standard. Final suspensions were prepared by adding Middlebrook 7H9 broth and preparing 10^{-2} dilutions of the standardized bacterial suspensions. After solidification of the Middlebrook 7H10 medium, 33 μL of the 10^{-2} dilution of the standardized bacterial suspensions was placed on each quadrant of the agar plates. The agar plates were then incubated at 35 °C with 10% CO_2 for 2 weeks. The minimal inhibitory concentration is the lowest concentration of test compound that completely inhibited the growth of the test isolate of *M. tuberculosis*, as detected by the unaided eye.

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Supporting Information Available: 1D and 2D NMR spectra for compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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