seco-Abietane Diterpenoids, a Phenylethanoid Derivative, and Antitubercular Constituents from *Callicarpa pilosissima*

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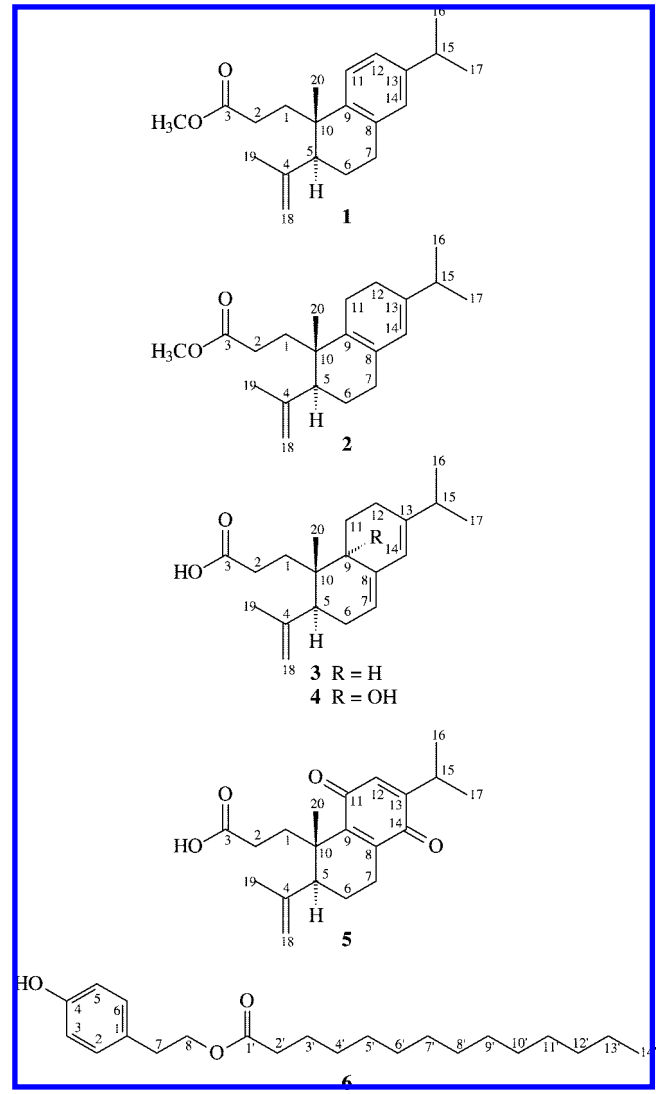
Received November 11, 2008

Six new compounds, including five new *seco*-abietane diterpenoids, 12-deoxy-*seco*-hinokiol methyl ester (**1**), 12-deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**), callicarpic acid A (**3**), 9α-hydroxycallicarpic acid A (**4**), and callicarpic acid B (**5**), and a new phenylethanoid derivative, 4-hydroxyphenethyl tetradecanoate (**6**), have been isolated from the leaves and twigs of *Callicarpa pilosissima*, together with 14 known compounds (**7**−**20**). The structures of these new compounds were determined through analyses of physical data. 12-Deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**), callicarpic acid B (**5**), and α-tocopherol trimer B (**15**) exhibit antitubercular activities (MICs ≤ 63.6 μM) against *Mycobacterium tuberculosis* $H_{37}Rv$ *in vitro*.

Callicarpa pilosissima (Verbenaceae) is an endemic evergreen shrub that grows in low- to medium-altitude forests throughout Taiwan.[1] Diterpenoids,[2] lignanoids,[3] flavones,[4] phenylethanoid glycosides,[5] and their derivatives are widely distributed in plants of the genus *Callicarpa.* Many of these compounds exhibit cytotoxic,[2] fish-killing,[4] and antiamnesic[5] activities. In our studies on the antitubercular constituents of Formosan plants, many species have been screened for *in vitro* antitubercular activity, and *C. pilosissima* has been found to be an active species. Investigation of the EtOAc-soluble fraction of the leaves and twigs of *C. pilosissima* has led to the isolation of six new compounds, including five *seco*-abietane diterpenoids, 12-deoxy-*seco*-hinokiol methyl ester (**1**), 12-deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**), callicarpic acid A (**3**), 9α-hydroxycallicarpic acid A (**4**), and callicarpic acid B (**5**), and a phenylethanoid derivative, 4-hydroxyphenethyl tetradecanoate (**6**), along with 14 known compounds (**7**−**20**). This paper describes the structural elucidation of **1**−**6** and the antitubercular activities of the isolates.



Results and Discussion

Extensive chromatographic purification of the EtOAc-soluble fraction of a MeOH extract of leaves and twigs of *C. pilosissima* on silica gel column (CC) and preparative thin-layer chromatography (TLC) afforded six new (**1**−**6**) and 14 known compounds (**7**−**20**).

12-Deoxy-*seco*-hinokiol methyl ester (**1**) was isolated as an amorphous powder, $[\alpha]^{25}_{D}$ +74.6. The ESIMS of **1** afforded an $[M + Na]^+$ ion at *m*/*z* 337, implying a molecular formula of $C_{21}H_{30}O_2$, which was confirmed by HRESIMS. An ester carbonyl group was indicated by the band at 1739 cm^{-1} in the IR spectrum and was confirmed by the resonance at δ 174.5 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **1** was similar to that of *seco*-hinokiol methyl ester[6] except that H-12 [δ 7.01 (1H, dd, J = 8.0, 2.0 Hz)] of **1** replaced a C-12 hydroxy group of *seco*-hinokiol methyl ester.[6] This was supported by the NOESY correlations between H-12 (δ 7.01) and both H-11 (δ 7.17) and H-15 (δ 2.82). With regard to the *seco*-abietane derivatives that co-occur in natural sources,[6−9] the β-orientation of C-20 and the α-orientation of H-5 could be proposed. The configuration of **1** was the same and was evidenced by NOESY experiments (Figure 1), which showed interactions between H-5 (δ 2.42) and both H-1 (δ 2.11) and H-2 (δ 1.91, 2.21) and between H-20 (δ 1.21) and both H-18 (δ 4.71)

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10.1021/np800721f CCC: $40.75
Published on Web 02/04/2009

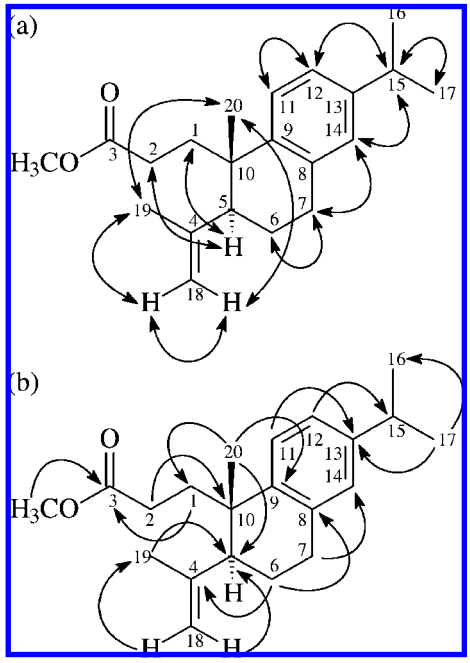

Figure 1. NOESY (a) and HMBC (b) correlations of **1**.

and H-19 (δ 1.79). Furthermore, the absolute configuration of **1** was confirmed to be that shown by comparing specific rotation data ($[\alpha]^{25}_D$ +74.6) of **1** with those reported for *seco*-hinokiol ($[\alpha]^{25}_D$ +77.2)[6] and (+)-hinokiol ($[\alpha]_D$ +66.2),[10] for which complete stereoselective and enantioselective syntheses have been performed.[10] On the basis of the evidence above, the structure of **1** was elucidated as methyl 3-[(1*S*,2*S*)-6-isopropyl-1-methyl-2-(prop-1-en-2-yl)-1,2,3,4-tetrahydronaphthalen-1-yl]propanoate, named 12-deoxy-*seco*-hinokiol methyl ester. This was further confirmed by ^{1}H−^{1}H COSY and NOESY (Figure 1) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 1) techniques.

12-Deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**) was isolated as an optically active colorless oil ($[\alpha]^{25}_D$ +71.5). Its molecular formula, $C_{21}H_{32}O_2$, was determined on the basis of the positive HRESIMS at *m*/*z* 339.2302 $[M + Na]^+$ (calcd 339.2300) and supported by the ^{1}H, ^{13}C, and DEPT NMR data. The presence of an ester carbonyl group was revealed by the band at 1740 cm^{-1} in the IR spectrum and was confirmed by the resonance at δ 174.7 in the ^{13}C NMR spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of **1** suggested that their structures are closely related except that a single bond between C-11 and C-12 of **2** replaced the double bond of **1**. This was supported by HMBC correlations between H-12 (δ 2.01, 2.10) and both C-15 (δ 34.2) and C-9 (δ 131.9) and NOESY correlations between H-12 (δ 2.01, 2.10) and both H-11 (δ 1.79) and H-15 (δ 2.30). The β-orientation of the C-5 prop-1-en-2-yl group was supported by NOESY experiments (Table 1), which showed the interactions between H-20 (δ 0.95) and both H-18 (δ 4.68) and H-19 (δ 1.77). NOESY correlations between H-5 (δ 2.22) and both H-1 (δ 1.80) and H-2 (δ 2.10, 2.36) of the 3-methoxy-3-oxopropyl group at C-10 confirmed its α-orientation. Compound **2** showed a similar specific rotation value when compared to **1** and analogous *seco*-abietane diterpenoids,[6−9] and the configurational assignments of **2** were thus established as shown. On the basis of the above data, the structure of **2** was elucidated as methyl 3-[(1*S*,2*S*)-6-isopropyl-1-methyl-2-(prop-1-en-2-yl)-1,2,3,4,7,8-hexahydronaphthalen-1-yl]propanoate, named 12-deoxy-11,12-dihydro-*seco*-hinokiol methyl ester. Assignment of the ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Table 1) techniques.

Callicarpic acid A (**3**) was obtained as a colorless oil. The molecular formula $C_{20}H_{30}O_2$ was deduced from a sodium adduct ion at *m*/*z* 325.2143 $[M + Na]^+$ (calcd 325.2143) in the HRESI mass spectrum. The presence of a carbonyl group was revealed by a band at 1707 cm^{-1} in the IR spectrum and was confirmed by the resonance at δ 171.1 in the ^{13}C NMR spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **3** with those of **2** suggested that their structures are closely related, except that OH-3, C-3 (δ 171.1), and the C-7,8 double bond of **3** replaced OMe-3 (δ 3.66), C-3 (δ 174.7), and the C-8,9 double bond of **2**. This was supported by NOESY correlations between H-7 (δ 5.41) and both H-6 (δ 2.16, 2.36) and H-14 (δ 5.82) and HMBC correlations between H-7 (δ 5.41) and both C-5 (δ 48.8) and C-9 (δ 51.2). The α-orientation of H-9 was supported by NOESY experiments (Figure 2), which showed the interactions between H-9 (δ 1.93) and both H-1 (δ 1.80) and H-5 (δ 2.84). In addition, NOESY correlations between H-20 (δ 1.00) and both H-18 (δ 4.79) and H-19 (δ 1.81) confirmed the β-orientation of CH_3-20. On the basis of the evidence above, the structure of **3** was elucidated as 3-[(1*S*,2*S*,8a*R*)-6-isopropyl-1-methyl-2-(prop-1-en-2-yl)-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]propanoic acid, named callicarpic acid A. This was further confirmed by ^{1}H−^{1}H COSY and NOESY (Figure 2) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 2) techniques.

9α-Hydroxycallicarpic acid A (**4**) was obtained as colorless needles, and the molecular formula was confirmed to be $C_{20}H_{30}O_3$ from the sodiated ion peak at *m*/*z* = 341.2092 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2093) obtained by HRESIMS. The IR spectrum indicated that OH (3433 cm^{-1}) and carbonyl (1708 cm^{-1}) groups were present. Comparison of the ^{1}H NMR data of **4** with those of **3** suggested that their structures are closely related, except that the 9-hydroxy group of **4** replaced H-9 of **3**. This was supported by HMBC correlations between H-7 (δ 5.67), H-14 (δ 5.82), H-20 (δ 1.00), and C-9 (δ 84.7). The β-orientation of the C-5 prop-1-en-2-yl group was evidenced by NOESY experiments, which showed interactions between H-20 (δ 1.00) and both H-18 (δ 4.91) and H-19 (δ 1.85). NOESY correlations observed between H-5 (δ 2.83) and both H-1 (δ 1.92) and H-2 (δ 2.51, 2.66) confirmed the α-orientation of H-5. Furthermore, the presence of an α-hydroxy group at C-9 induces shieldings of 4−8 ppm on the signals for C-5 and C-12 and deshielding of about 7 ppm on the signal for C-11. This result can be explained by the different contributions of the γ- and β-effects of the hydroxy group, respectively.[11] According to the above data, the structure of **4** was elucidated as 9α-hydroxycallicarpic acid A, which was further confirmed by the ^{1}H−^{1}H COSY, NOESY (Table 2), DEPT, HSQC, and HMBC experiments (Table 2).

Callicarpic acid B (**5**) was obtained as a yellowish oil, and the sodium adduct ion $[M + Na]^+$ (*m*/*z* 353.1725) in HRESIMS was consistent with the formula $C_{20}H_{26}O_4Na$. The presence of carbonyl groups was revealed by the bands at 1708 and 1655 cm^{-1} in the IR spectrum and was confirmed by the resonances at δ 170.6 (C-3), 187.3 (C-11), and 187.5 (C-14) in the ^{13}C NMR spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **5** with those of candesalvoquinone[7] suggested that their structures are closely related, except that OH-3, C-3 (δ 170.6), H-12 (δ 7.09), and C-12 (δ 133.3) of **5** replaced OMe-3 (δ 3.64), C-3 (δ 172.9), OH-12 (δ 7.09), and C-12 (δ 150.7) of candesalvoquinone.[7] This was supported by NOESY correlations between H-12 (δ 7.09) and H-15 (δ 3.39) and HMBC correlations between H-12 (δ 7.09) and both C-14 (δ 187.5) and C-15 (δ 33.6) and between H-1 (δ 1.86, 2.04) and C-3 (δ 170.6). The β-orientation of the C-5 prop-1-en-2-yl group was evidenced by NOESY experiments, which showed interactions between H-20 (δ 1.19) and both H-18 (δ 4.74) and H-19 (δ 1.82). NOESY correlations observed between H-5 (δ 2.20) and both H-1 (δ 1.86, 2.04) and H-2 (δ 2.58, 2.89) confirmed their α-orientations. Compound **5** showed a similar specific rotation value when compared to **1**, **2**, and analogous *seco*-abietane diterpenoids,[6−9] and the configurational assignments of **5** were thus established as

Table 1. ^{1}H NMR Data of **1** and **2**[a]

	1	**2**		
position	δ_H *J* (Hz)	δ_H *J* (Hz)	NOE	HMBC
1	2.11 br t (8.5)	1.80 m	2, 5, 20	3, 5, 9
2	1.91 m	2.10 m	1, 5	10
	2.21 ddd (15.5, 8.5, 8.5)	2.36 ddd (15.4, 8.2, 8.2)	1, 5	10
5	2.42 dd (12.0, 3.0)	2.22 dd (12.8, 3.0)	1, 2, 6	1, 7, 18
6	1.79 m	1.58 m	5, 7	4, 8, 10
	1.93 m	1.74 m	7	4, 8
7	2.78 dd (8.5, 4.0)	2.03 m	6, 14	5, 9, 14
11	7.17 d (8.0)	1.79 m	12	8, 10, 13
12	7.01 dd (8.0, 2.0)	2.01 m	11, 15	9, 15
		2.10 m	11, 15	9, 15
14	6.87 d (2.0)	5.43 s	7, 15	7, 12, 15
15	2.82 septet (7.0)	2.30 septet (7.0)	12, 14, 16, 17	12, 14
16	1.22 d (7.0)	1.03 d (7.0)	15, 17	13, 17
17	1.22 d (7.0)	1.03 d (7.0)	15, 16	13, 16
18	4.71 br s	4.68 br s	20	5, 19
	4.95 br s	4.91 br s	19	5, 19
19	1.79 s	1.77 s	18, 20	5, 18
20	1.21 s	0.95 s	1, 18, 19	1, 5, 9
OMe	3.60 s	3.66 s		3

[a] Recorded in $CDCl_3$ at 500 MHz. Values in ppm (δ). *J* (in Hz) in parentheses.

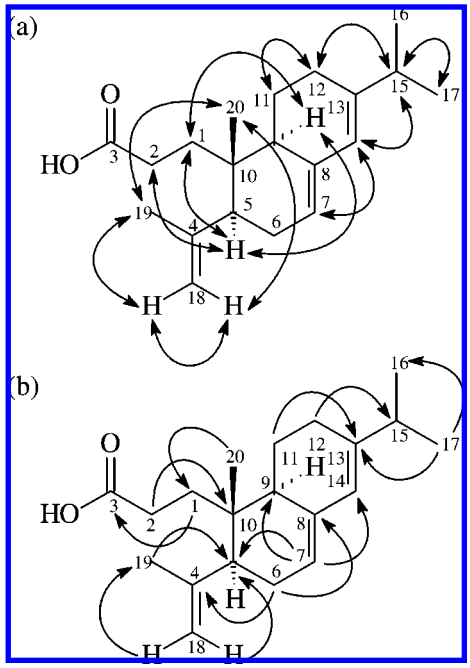

Figure 2. NOESY (a) and HMBC (b) correlations of **3**.

shown. On the basis of the evidence above, the structure of **5** was elucidated as 3-[(1*S*,2*S*)-6-isopropyl-1-methyl-5,8-dioxo-2-(prop-1-en-2-yl)-1,2,3,4,5,8-hexahydronaphthalen-1-yl]propanoic acid, named callicarpic acid B. This was further confirmed by ^{1}H−^{1}H COSY and NOESY (Figure 3) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 3) techniques.

4-Hydroxyphenethyl tetradecanoate (**6**) was isolated as a white powder with molecular formula $C_{22}H_{36}O_3$ as determined by the HREIMS, showing an $[M]^+$ ion at *m/z* 348.2667 (calcd for $C_{22}H_{36}O_3$, 348.2664). The presence of OH and ester carbonyl groups was revealed by the bands at 3370 and 1737 cm^{-1}, respectively, in the IR spectrum. The ^{1}H NMR spectrum showed the presence of a 4-hydroxyphenethoxy moiety, which was confirmed by a prominent peak at *m/z* 120 (base peak) in the EI mass spectrum. This peak, attributable to a vinylphenol fragment, is generated through a McLafferty rearrangement.[12,13] The aromatic region of the NMR spectrum of **6** showed the typical AA′XX′ system of a *p*-disubstituted benzene ring at δ 7.08 (2H, d, J = 8.5 Hz, H-2 and H-6) and δ 6.76 (2H, d, J = 8.5 Hz, H-3 and H-5). The resonances for the phenethyloxy unit were observed at δ 2.86 (2H, t, J = 7.0 Hz, H-7) and δ 4.23 (2H, t, J = 7.0 Hz, H-8). The resonances of the acid moiety included those of the ester carbonyl [δ 173.9 (C-1′)], the methylenes α and β to the ester function [δ 2.28 (2H, t, J = 7.5 Hz, H-2′) and δ 1.59 (2H, m, H-3′)], a terminal methyl group [δ 0.88 (3H, t, J = 7.0 Hz, H-14′)], and those for the remaining methylenes of the aliphatic chain [δ 1.25−1.31 (20H, br s, H-4′−H-13′)]. According to the above data, the structure of **6** was elucidated as 4-hydroxyphenethyl tetradecanoate. This was confirmed by ^{1}H−^{1}H COSY and NOESY (Figure 4) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 4) techniques.

The known isolates were readily identified by a comparison of physical and spectroscopic data (UV, IR, ^{1}H NMR, $[\alpha]_D$, and MS) with corresponding authentic samples or literature values. These compounds included two diterpenoids, 3-oxo-abieta-8,11,13-triene (**7**)[14] and 7-oxo-abieta-8,11,13-triene (**8**),[15] five flavones, casticin (**9**),[16] penduletin (**10**),[16] acacetin (**11**),[17] artemetin (**12**),[18] and 3,5,6,7,3′,4′-hexamethoxyflavone (**13**),[19] a lignan, (+)-glaberide I (**14**),[20] a tocopherone, α-tocopherol trimer B (**15**),[21] a triterpenoid, squalene (**16**),[22] a fatty acid, stearic acid (**17**),[23] and three steroids, β-sitostenone (**18**)[24] and a mixture of β-sitosterol (**19**)[25] and stigmasterol (**20**).[25]

The antitubercular effects of the isolates from the leaves and twigs of *C. pilosissima* were tested *in vitro* against *M. tuberculosis* $H_{37}Rv$. The antitubercular activity data are shown in Table 3. The clinically used antitubercular agent ethambutol was used as the positive control. From the results of our antitubercular tests, the following conclusions can be drawn: (a) 12-Deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**), callicarpic acid B (**5**), and α-tocopherol trimer B (**15**) exhibited antitubercular activities (MICs ≤ 63.6 μM) against *M. tuberculosis* $H_{37}Rv$ *in vitro*, (b) 12-deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**) (with a single bond at C-11,12) exhibited more effective antitubercular activity than 12-deoxy-*seco*-hinokiol methyl ester (**1**) (with a double bond at C-11,12) against *M. tuberculosis* $H_{37}Rv$, (c) 9α-hydroxycallicarpic acid A (**4**) with a 9α-hydroxy group exhibited stronger antitubercular activity than its analogue, callicarpic acid A (**3**) with a 9α-proton, (d) among the flavone analogues (**9**−**13**), penduletin (**10**) with 5,4′-dihydroxy-3,6,7-trimethoxy groups exhibited stronger antitubercular activity than **9** and **11**−**13** against *M. tuberculosis* $H_{37}Rv$, (e) compound **15** is the most effective among the isolates, with an MIC of 31.2 μM against *M. tuberculosis* $H_{37}Rv$, and (f)

Table 2. ^{1}H NMR Data of **3** and **4**[a]

	3	**4**		
position	δ_H *J* (Hz)	δ_H *J* (Hz)	NOE	HMBC
1	1.80 m	1.79 m	2, 5, 20	3, 5, 9
	1.92 m	1.92 m	2, 5	3, 9
2	2.50 ddd (19.0, 8.0, 1.0)	2.51 ddd (19.2, 8.1, 1.1)	1, 5	10
	2.65 ddd (19.0, 11.5, 9.0)	2.66 ddd (19.2, 11.5, 9.0)	1, 5	10
5	2.84 dd (12.0, 5.5)	2.83 dd (12.0, 5.3)	1, 2, 6	1, 7, 18
6	2.16 ddd (19.0, 12.0, 5.0)	2.17 ddd (19.2, 12.0, 5.0)	7	4, 8, 10
	2.36 dd (19.0, 5.5)	2.38 dd (19.2, 5.3)	5, 7	4, 10
7	5.41 dd (5.0, 2.0)	5.67 dd (5.0, 2.2)	6, 14	5, 9, 14
9	1.93 m			
11	1.59 m	1.74 m	12	8, 10
	1.84 m	1.99 ddd (13.5, 4.5, 2.0)	12	10, 13
12	2.06 dd (17.2, 5.0)	2.06 dd (17.2, 5.1)	11, 15	9, 14
	2.40 m	2.41 m	11, 15	9, 15
14	5.82 s	5.82 s	7, 15	7, 9, 15
15	2.26 septet (7.0)	2.28 septet (6.8)	12, 14, 16, 17	12, 14
16	1.02 d (7.0)	1.05 d (6.8)	15, 17	13, 17
17	1.02 d (7.0)	1.05 d (6.8)	15, 16	13, 16
18	4.79 br s	4.91 br s	20	5, 19
	4.86 br s	4.97 br s	19	5, 19
19	1.81 s	1.85 s	18, 20	5, 18
20	1.00 s	1.00 s	1, 18, 19	1, 5, 9, 10

[a] Recorded in $CDCl_3$ at 500 MHz. Values in ppm (δ). *J* (in Hz) in parentheses.

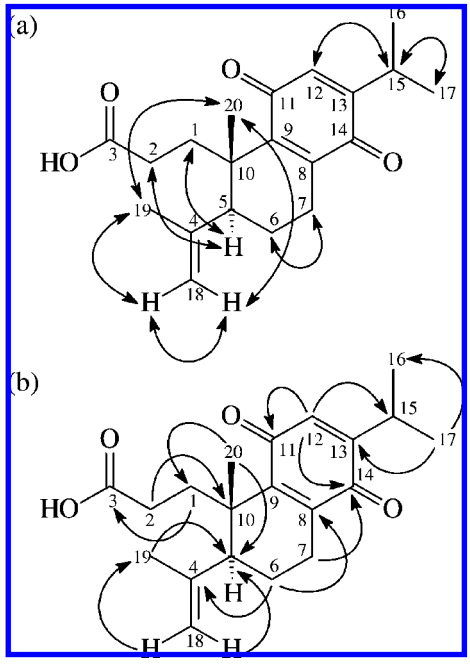

Figure 3. NOESY (a) and HMBC (b) correlations of **5**.

compounds **6**, **8**, and **17**−**20** showed no antitubercular activities (MICs > 300 μM) against *M. tuberculosis* H_{37}Rv.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter. 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. 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.

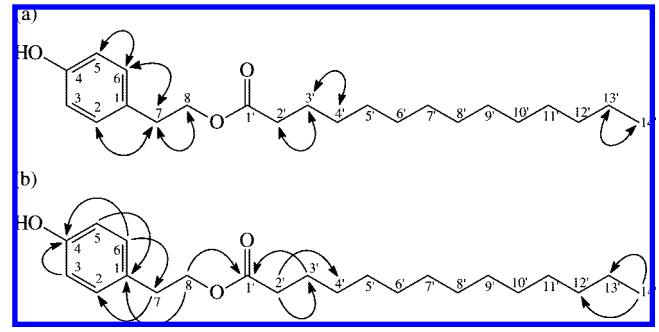

Figure 4. NOESY (a) and HMBC (b) correlations of **6**.

Table 3. Antitubercular Effects of **1**−**20** against *Mycobacterium tuberculosis* H_{37}Rv

compound	MIC (μM)[a]
12-deoxy-*seco*-hinokiol methyl ester (**1**)	152.9
12-deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**)	38.0
callicarpic acid A (**3**)	238.4
9α-hydroxycallicarpic acid A (**4**)	113.2
callicarpic acid B (**5**)	63.6
4-hydroxyphenethyl tetradecanoate (**6**)	>300
3-oxo-abieta-8,11,13-triene (**7**)	147.9
7-oxo-abieta-8,11,13-triene (**8**)	>300
casticin (**9**)	213.9
penduletin (**10**)	157.0
acacetin (**11**)	281.7
artemetin (**12**)	185.6
3,5,6,7,3′,4′-hexamethoxyflavone (**13**)	179.1
(+)-glaberide I (**14**)	257.1
α-tocopherol trimer B (**15**)	31.2
squalene (**16**)	122.0
stearic acid (**17**)	>300
β-sitostenone (**18**)	>300
mixture of β-sitosterol (**19**) and stigmasterol (**20**)	>300
ethambutol[b]	30.6

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

Plant Material. The leaves and twigs of *C. pilosissima* were collected from Wutai, Pingtung County, Taiwan, in October 2006 and identified by one of the authors (I.S.C.). A voucher specimen (68373) 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 leaves and twigs of *C. pilosissima* (2.34 kg) were pulverized and extracted three times with MeOH (10 L each) for three days. The MeOH extracts were concentrated under reduced pressure at 35 °C, and then the residue (253 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc layer was concentrated to give a residue (fraction A, 115 g). The water layer was further extracted with *n*-BuOH, and the *n*-BuOH-soluble part (fraction B, 62 g) and the water-solubles (fraction C, 71 g) were separated. Fraction A (115 g) was chromatographed on silica gel (70−230 mesh, 4.5 kg), eluting with CH_2Cl_2, gradually increasing the polarity with MeOH to give 12 fractions: A1 (8 L, CH_2Cl_2), A2 (9 L, CH_2Cl_2/MeOH, 100:1), A3 (6 L, CH_2Cl_2/MeOH, 98:1), A4 (6 L, CH_2Cl_2/MeOH, 95:1), A5 (5 L, CH_2Cl_2/MeOH, 90:1), A6 (5 L, CH_2Cl_2/MeOH, 80:1), A7 (4 L, CH_2Cl_2/MeOH, 60:1), A8 (4 L, CH_2Cl_2/MeOH, 30:1), A9 (4 L, CH_2Cl_2/MeOH, 10:1), A10 (5 L, CH_2Cl_2/MeOH, 5:1), A11 (5 L, CH_2Cl_2/MeOH, 1:1), A12 (7 L, MeOH). Fraction A1 (6.2 g) was chromatographed further on silica gel (230−400 mesh, 250 g) eluting with *n*-hexane/CH_2Cl_2 (5:1) to give 12 fractions (each 1 L, A1-1−A1-12). Fraction A1-2 (210 mg) was purified further by preparative TLC (silica gel, *n*-hexane/$CHCl_3$, 3:2) to obtain **5** (4.5 mg) (R_f = 0.73). Fraction A1-4 (196 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 20:1) to yield **15** (3.8 mg) (R_f = 0.71). Fraction A1-10 (187 mg) was purified further by preparative TLC (silica gel, *n*-hexane/CH_2Cl_2, 100:1) to obtain **16** (3.8 mg) (R_f = 0.69). Fraction A2 (6.8 g) was chromatographed further on silica gel (230−400 mesh, 250 g) eluting with *n*-hexane/EtOAc (20:1) to give 10 fractions (each 1.2 L, A2-1−A2-10). Fraction A2-4 (205 mg) was purified further by preparative TLC (silica gel, *n*-hexane/acetone, 25:1) to obtain **1** (4.5 mg) (R_f = 0.73). Fraction A2-6 (185 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 15:1) to obtain **2** (3.3 mg) (R_f = 0.80) and **3** (4.2 mg) (R_f = 0.75). Fraction A2-7 (312 mg) was washed with MeOH and filtered to afford **7** (68 mg) after recrystallization ($CHCl_3$/MeOH). Fraction A2-9 (190 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 20:1) to obtain **8** (4.1 mg) (R_f = 0.55). Fraction A2-10 (208 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/MeOH, 30:1) to obtain **17** (3.5 mg) (R_f = 0.57). Fraction A3 (5.7 g) was chromatographed on silica gel (230−400 mesh, 225 g) eluting with $CHCl_3$/acetone (4:1) to give nine fractions (each 1.0 L, A3-1−A3-9). Fraction A3-3 (348 mg) was purified by MPLC (13.5 g silica gel, 230−400 mesh, *n*-hexane/acetone, 4:1) to give 15 fractions (each 50 mL, A3-3-1−A3-3-15). Fraction A3-3-4 (45 mg) was washed with MeOH and filtered to afford **6** (9.0 mg) after recrystallization ($CHCl_3$/MeOH). Fraction A3-7 (355 mg) was washed with MeOH and filtered to afford a mixture of **19** and **20** (27.5 mg). Fraction A4 (6.3 g) was chromatographed on silica gel (230−400 mesh, 255 g) eluting with *n*-hexane/acetone (2:1) to give 10 fractions (each 500 mL, A4-1−A4-10). Fraction A4-3 (207 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 5:1) to yield **12** (5.4 mg) (R_f = 0.60). Fraction A4-9 (188 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 25:1) to afford **18** (5.4 mg) (R_f = 0.41). Fraction A5 (7.5 g) was chromatographed on silica gel (230−400 mesh, 305 g) eluting with *n*-hexane/EtOAc (3:1) to give 11 fractions (each 500 mL, A5-1−A5-11). Fraction A5-6 (185 mg) was purified further by preparative TLC (silica gel, *n*-hexane/EtOAc, 1:1) to obtain **4** (4.4 mg) (R_f = 0.62). Fraction A5-7 (208 mg) was purified further by preparative TLC (silica gel, $CHCl_3$/acetone, 5:1) to yield **11** (3.2 mg) (R_f = 0.67). Fraction A8 (5.9 g) was chromatographed on silica gel (230−400 mesh, 235 g) eluting with *n*-hexane/acetone (3:1) to give 10 fractions (each 500 mL, A8-1−A8-10). Fraction A8-4 (198 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/MeOH, 30:1) to obtain **9** (4.1 mg) (R_f = 0.55) and **13** (2.5 mg) (R_f = 0.45). Fraction A8-7 (203 mg) was purified further by preparative TLC (silica gel, $CHCl_3$/acetone, 5:1) to afford **10** (6.5 mg) (R_f = 0.68). Fraction A9 (6.7 g) was chromatographed on silica gel (230−400 mesh, 265 g) eluting with $CHCl_3$/acetone (4:1) to give 12 fractions (each 750 mL, A9-1−A9-12). Fraction A9-6 (192 mg) was purified further by preparative TLC (silica gel, CH_2Cl_2/EtOAc, 2:1) to obtain **14** (3.6 mg) (R_f = 0.50).

Antitubercular Activity Assay. The antitubercular activities were evaluated and minimal inhibitory concentration (MIC) values were determined using the *Mycobacterium tuberculosis* $H_{37}Rv$ strain. Middlebrook 7H10 agar was used to determine the MICs as recommended by the proportion method.[26] Briefly, each test compound was added to Middlebrook 7H10 agar supplemented with OADC (oleic acid-albumin-dextrose-catalase) at 50−56 °C by serial dilutions 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 *C. pilosissima* 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.

12-Deoxy-*seco*-hinokiol methyl ester (1): amorphous powder; $[\alpha]^{25}_D$ +74.6 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 231 (3.54), 277 (3.04) nm; IR (neat) ν_{max} 1739 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.9 (C-19), 23.9 (C-16), 23.9 (C-17), 24.8 (C-6), 28.1 (C-20), 29.6 (C-2), 30.5 (C-7), 33.4 (C-15), 34.6 (C-1), 40.7 (C-10), 47.1 (C-5), 51.5 (OMe-3), 114.2 (C-18), 124.5 (C-12), 126.6 (C-11), 126.8 (C-14), 136.7 (C-8), 140.3 (C-9), 145.7 (C-13), 146.7 (C-4), 174.5 (C-3); ESIMS *m/z* 337 $[M + Na]^+$; HRESIMS *m/z* 337.2145 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_2Na$, 337.2143).

12-Deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (2): colorless oil; $[\alpha]^{25}_D$ +71.5 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 242 (3.52), 250 (3.50), 265 (3.43) nm; IR (neat) ν_{max} 1740 (C═O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.1 (C-16), 21.1 (C-17), 22.8 (C-19), 23.2 (C-20), 23.5 (C-11), 24.6 (C-6), 26.2 (C-7), 29.8 (C-12), 29.9 (C-2), 31.3 (C-1), 34.2 (C-15), 41.2 (C-10), 46.7 (C-5), 51.5 (OMe-3), 113.9 (C-18), 120.6 (C-14), 129.1 (C-8), 144.1 (C-13), 147.1 (C-4), 174.7 (C-3); ESIMS *m/z* 339 $[M + Na]^+$; HRESIMS *m/z* 339.2302 $[M + Na]^+$ (calcd for $C_{21}H_{32}O_2Na$, 339.2300).

Callicarpic acid A (3): colorless oil; $[\alpha]^{25}_D$ −77.6 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 240 (3.95) nm; IR (neat) ν_{max} 1707 (C═O) cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.9 (C-20), 21.2 (C-16), 21.2 (C-17), 22.8 (C-19), 22.9 (C-11), 27.5 (C-12), 27.9 (C-1), 28.1 (C-2), 28.6 (C-6), 34.7 (C-15), 36.9 (C-10), 48.8 (C-5), 51.2 (C-9), 114.2 (C-18), 121.1 (C-7), 122.4 (C-14), 135.5 (C-8), 145.3 (C-4), 145.3 (C-13), 171.1 (C-3); ESIMS *m/z* 325 $[M + Na]^+$; HRESIMS *m/z* 325.2143 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_2Na$, 325.2143).

9α-Hydroxycallicarpic acid A (4): colorless needles from CH_2Cl_2/MeOH; mp 127−129 °C; $[\alpha]^{25}_D$ +94.7 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (4.02) nm; IR (neat) ν_{max} 3433 (OH), 1708 (C═O) cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.1 (C-20), 21.3 (C-16), 21.3 (C-17), 22.8 (C-19), 23.1 (C-12), 26.3 (C-2), 27.0 (C-1), 29.0 (C-6), 29.9 (C-11), 34.6 (C-15), 36.0 (C-10), 42.1 (C-5), 84.7 (C-9), 115.7 (C-18), 120.4 (C-14), 126.0 (C-7), 132.5 (C-8), 144.9 (C-13), 145.3 (C-4), 171.2 (C-3); ESIMS *m/z* 341 $[M + Na]^+$; HRESIMS *m/z* 341.2092 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2093).

Callicarpic acid B (5): yellowish oil; $[\alpha]^{25}_D$ +64.5 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 256 (4.08), 334 (3.63), 415 (3.89) nm; IR (neat) ν_{max} 1708 (C═O), 1655 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.19 (3H, s, H-20), 1.25 (6H, d, *J* = 6.8 Hz, H-16 and H-17), 1.80 (1H, m, H-7), 1.82 (3H, s, H-19), 1.86 (1H, ddd, *J* = 12.8, 12.8, 5.2 Hz, H-1), 2.04 (1H, ddd, *J* = 12.8, 5.2, 2.0 Hz, H-1), 2.17 (1H, m, H-7), 2.20 (1H, dd, *J* = 12.8, 2.8 Hz, H-5), 2.57 (1H, m, H-6), 2.58 (1H, ddd, *J* = 18.4, 5.2, 2.0 Hz, H-2), 2.75 (1H, br dd, *J* = 16.8, 6.4 Hz, H-6), 2.89 (1H, ddd, *J* = 18.4, 12.8, 5.2 Hz, H-2), 3.39 (1H, septet, *J* = 6.8 Hz, H-15), 4.74 (1H, br s, H-18), 4.96 (1H, br s, H-18), 7.09 (1H, s, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.0 (C-20), 23.4 (C-6), 23.8 (C-19), 24.9 (C-7), 29.6 (C-16), 29.6 (C-17), 30.7 (C-2), 33.6 (C-15), 35.4 (C-10), 38.2 (C-1), 51.1 (C-5), 113.4 (C-18), 133.3 (C-12), 143.1 (C-8), 145.6 (C-4), 153.0 (C-13), 157.9 (C-9), 170.6 (C-3), 187.3 (C-11), 187.5 (C-14); ESIMS *m/z* 353 $[M + Na]^+$; HRESIMS *m/z* 353.1725 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_4Na$, 353.1729).

4-Hydroxyphenethyl tetradecanoate (6): white powder; UV (MeOH) λ_{max} (log ε) 277 (3.28) nm; IR (KBr) ν_{max} 3370 (OH), 1737 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, *J* = 7.0 Hz, H-14′), 1.25 (8H, br s, H-6′, H-7′, H-8′, and H-9′), 1.26 (4H, br s, H-5′ and H-10′), 1.29 (4H, m, H-11′ and H-12′), 1.31 (4H, m, H-4′ and H-13′), 1.59 (2H, m, H-3′), 2.28 (2H, t, *J* = 7.5 Hz, H-2′), 2.86 (2H, t, *J* = 7.0 Hz, H-7), 4.23 (2H, t, *J* = 7.0 Hz, H-8), 4.80 (1H, br s, D_2O exchangeable, OH-4), 6.76 (2H, d, *J* = 8.5 Hz, H-3 and H-5), 7.08 (2H, d, *J* = 8.5 Hz, H-2 and H-6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (C-14′), 22.7 (C-13′), 24.9 (C-3′), 29.1 (C-4′), 29.3 (C-5′), 29.4 (C-11′), 29.5 (C-10′), 29.6 (C-6′), 29.6 (C-9′), 29.7 (C-7′), 29.7 (C-8′),

31.9 (C-12′), 34.3 (C-7), 34.3 (C-2′), 64.9 (C-8), 115.3 (C-3 and C-5), 130.0 (C-1), 130.0 (C-2 and C-6), 154.2 (C-4), 173.9 (C-1′); EIMS *m/z* (rel int) 348 ($[M]^+$, 5), 137 ($[M - CO(CH_2)_{12}CH_3]^+$, 37), 121 ($[M - OCO(CH_2)_{12}CH_3]^+$, 62), 120 (100), 91 (48); HREIMS *m/z* 348.2667 $[M]^+$ (calcd for $C_{22}H_{36}O_3$, 348.2664).

Acknowledgment. This work was supported by a grant from the National Science Council of the Republic of China.

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NP800721F