Dihydroagarofuranoid Sesquiterpenes, a Lignan Derivative, a Benzenoid, and Antitubercular Constituents from the Stem of *Microtropis japonica*

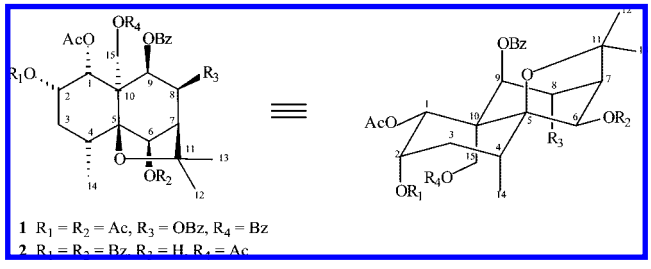
Jih-Jung Chen,*[,†] Chang-Syun Yang,[†] Chien-Fang Peng,[‡] Ih-Sheng Chen,[§] and Chang-Ling Miaw[⊥]

Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung 907, Taiwan, Republic of China, Faculty of Biomedical Laboratory Sciences, College of Health Science, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China, Graduate Institute of Pharmaceutical Sciences, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China, and Department of Nursing, Tajen University, Pingtung 907, Taiwan, Republic of China

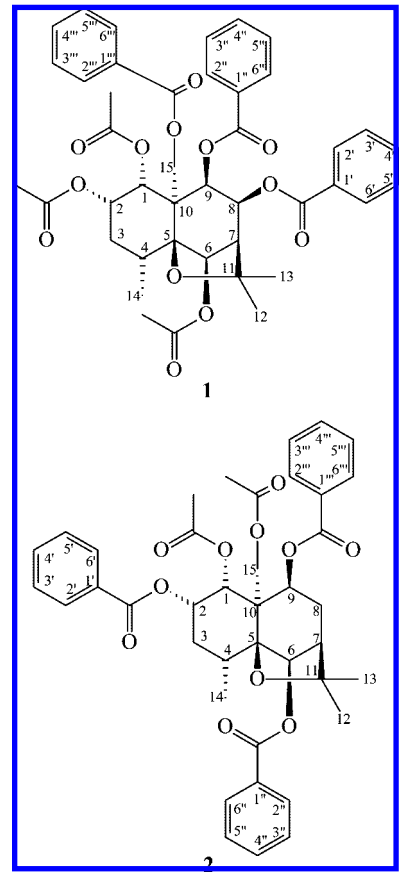
Received February 12, 2008

Four new compounds, including two new dihydroagarofuranoid sesquiterpenes, 8-benzoyloxymutangin (**1**) and 15-acetoxyorbiculin G (**2**), a new lignan derivative, 9,9′-*O*-di-(*Z*)-feruloyl-(−)-secoisolariciresinol (**3**), and a new benzenoid, 5′-methoxyevofolin B (**4**), have been isolated from the stem of *Microtropis japonica*, together with 20 known compounds (**5**−**24**). 3-Ethoxy-4-hydroxybenzaldehyde (**5**) was identified from a natural source for the first time. The structures of these new compounds were determined through analyses of physical data. 15-Acetoxyorbiculin G (**2**), celahin C (**6**), and salasol A (**7**) exhibit antituberculosis activities (MICs ≤ 39.6 μM) against *Mycobacterium tuberculosis* $H_{37}Rv$ *in vitro*.

Microtropis japonica (Fr. & Sav.) Hall. f. (Celastraceae) is a small shrub distributed in Japan, Ryukyus, and Taiwan (Lanyu Island and Nanjenshan, Pingtung).[1] Various dihydroagarofuranoid sesquiterpenes,[2–4] benzenoids,[3,4] and triterpenes[5,6] are widely distributed in plants of the genus *Microtropis*. Many of these compounds exhibit cytotoxic[2,5,6] and antitubercular[3,4] activities. In our studies on the antitubercular constituents of Formosan plants, many species have been screened for *in vitro* antitubercular activity, and *M. japonica* has been found to be one of the active species. Investigation of the EtOAc-soluble fraction of the stem of *M. japonica* has led to the isolation of four new compounds, including two dihydroagarofuranoid sesquiterpenes, 8-benzoyloxymutangin (**1**) and 15-acetoxyorbiculin G (**2**), a lignan derivative, 9,9′-*O*-di-(*Z*)-feruloyl-(−)-secoisolariciresinol (**3**), and a benzenoid, 5′-methoxyevofolin B (**4**), along with 20 known compounds (**5**−**24**). Among the known isolates, 3-ethoxy-4-hydroxybenzaldehyde (**5**) was identified from a natural source for the first time. This paper describes the structural elucidation of **1**−**5** and the antitubercular activities of the isolates.



1 $R_1 = R_2 = Ac$, $R_3 = OBz$, $R_4 = Bz$
2 $R_1 = R_3 = Bz$, $R_2 = H$, $R_4 = Ac$



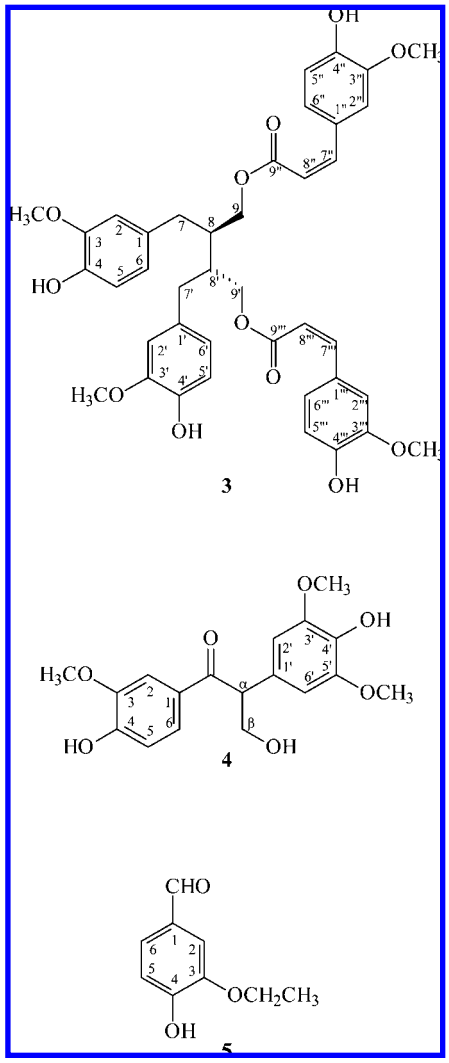
Results and Discussion

Extensive chromatographic purification of the EtOAc-soluble fraction of the stem of *M. japonica* on silica gel column and preparative thin-layer chromatography (TLC) afforded four new (**1**−**4**) and 20 known compounds (**5**−**24**).

8-Benzoyloxymutangin (**1**) was isolated as an amorphous powder, $[\alpha]^{25}_D$ +83.5. The ESIMS of **1** afforded an $[M + Na]^+$ ion at *m/z* 779, implying a molecular formula of $C_{42}H_{44}O_{13}$, which was confirmed by HRESIMS. 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 were indicated by the bands at 1747 and 1721 cm^{-1} in the IR spectrum and were confirmed by resonances at δ 164.8, 165.7, 166.7, 169.8, and 170.5 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **1** was similar to that of 8-acetoxymutangin[4] except that a C-8 benzoyloxy group [δ 8.00 (2H, d, J = 7.6 Hz, H-2′ and H-6′), 7.46 (1H, t, J = 7.6 Hz, H-4′), and 7.29 (2H, t, J = 7.6 Hz, H-3′ and H-5′)] of **1** replaced a C-8 acetoxy group [δ 1.83 (3H, s)]. This was supported by the HMBC correlations between H-8 (δ 5.60) and Ph*C*O_2-8 (δ 165.7). In the ^1H NMR spectrum of **1**, resonances

* To whom correspondence should be addressed. Tel: +886-8-7624002, ext. 332. Fax: +886-8-7625308. E-mail: jjchen@mail.tajen.edu.tw.
† Graduate Institute of Pharmaceutical Technology, Tajen University.
‡ College of Health Science, Kaohsiung Medical University.
§ College of Pharmacy, Kaohsiung Medical University.
⊥ Department of Nursing, Tajen University.

10.1021/np800097t CCC: $40.75
Published on Web 05/10/2008



due to acylated oxymethine protons at δ 5.81 (1H, d, J = 3.6 Hz), 5.66 (1H, q, J = 3.6 Hz), 6.27 (1H, s), 5.60 (1H, d, J = 3.2 Hz), and 6.11 (1H, s) were assigned to H_{ax}-1, H_{eq}-2, H_{ax}-6, H_{ax}-8, 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 interactions between H-2″,6″ (δ 8.06) of the C-9 benzoate and both C-12 methyl (δ 1.67) and H-1 (δ 5.81). NOESY correlations observed between the C-14 methyl and AcO-2, H-6, and H-15 confirmed their axial orientations. The stereochemical assignments, which were based on the splitting patterns and coupling constants of H-1 [δ 5.81 (d, J = 3.6 Hz)], H-2 [δ 5.66 (q, J = 3.6 Hz)], H-6 [δ 6.27 (s)], H-8 [δ 5.60 (d, J = 3.2 Hz)], and H-9 [δ 6.11 (1H, s)], are in agreement with the relative configurations observed at these positions in this class of natural products.[4,8,9] The CD spectrum of **1** showed a split first Cotton effect at 237 nm ($\Delta\epsilon$ = −14.2) and a second one at 221 nm ($\Delta\epsilon$ = +5.02), very similar to that of (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-8,9-dibenzoyloxy-1,2,6,15-tetraacetoxydihydro-β-agarofuran,[10] indicating identical absolute configurations. The location of the ester groups was confirmed by the HMBC spectrum, which exhibited cross-peaks between an acetyl C═O group (δ 169.8) and both H-1 (δ 5.81) and AcO-1 (δ 1.36); between an acetyl C═O group (δ 170.5) and both H-2 (δ 5.66) and AcO-2 (δ 2.03); between an acetyl C═O group (δ 169.8) and both H-6 (δ 6.27) and AcO-6 (δ 2.11); between a benzoyl C═O group (δ 165.7) and both H-8 (δ 5.60) and H-2′/6′ (δ 8.00); between a benzoyl C═O group (δ 164.8) and both H-9 (δ 6.11) and H-2″/6″ (δ 8.06); and between a benzoyl C═O group (δ 166.7) and both H-15 (δ 4.85, 5.60) and H-2‴/6‴ (δ 8.15). The full assignment of the carbon resonances are based on HSQC and HMBC techniques. According to the above data, the structure of **1** was identified as 8-benzoyloxymutangin, i.e., (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,2,6-triacetoxy-8,9,15-tribenzoyloxy-β-dihydroagarofuran.

15-Acetoxyorbiculin G (**2**) was isolated as an amorphous powder. Its molecular formula, $C_{40}H_{42}O_{11}$, was determined on the basis of the positive HRESIMS at m/z 721.2622 $[M + Na]^+$ (calcd 721.2625) and supported by the ^1H, ^{13}C, and DEPT NMR data. The presence of ester carbonyl groups was revealed by the bands at 1742 and 1720 cm^{-1} in the IR spectrum. Comparison of the ^1H and ^{13}C NMR data of **2** with those of fokienagarofuran D (**7**)[2] suggested that their structures are closely related except that the 15-acetoxy group (δ 2.34) of **2** replaced the 15-benzoyloxy group of fokienagarofuran D. This was supported by HMBC correlations between MeCO_2-15 (δ 170.8) and both *Me*CO_2-15 (δ 2.34) and H-15 (δ 4.48, 5.39). From the $^1H-^1H$ COSY and NOESY spectra of **2**, the resonances at δ 5.89 (1H, d, J = 4.0 Hz), 5.91 (1H, q, J = 4.0 Hz), 6.29 (1H, s), and 5.54 (1H, d, J = 7.2 Hz) were assigned as H_{ax}-1, H_{eq}-2, 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 1.52) and H-1 (δ 5.89). NOESY correlations between the C-14 methyl and both H-6 and H-15 confirmed their axial orientations. The splitting patterns and coupling constants of H-1 [δ 5.89 (d, J = 4.0 Hz)], H-2 [δ 5.91 (q, J = 4.0 Hz)], H-6 [δ 6.29 (s)], and H-9 [δ 5.54 (d, J = 7.2 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,15-diacetoxy-2α,6β,9β-tribenzoyloxy-β-dihydroagarofuran, named 15-acetoxyorbiculin G. Assignment of the ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 2) techniques.

9,9′-*O*-Di-(*Z*)-feruloyl-(−)-secoisolariciresinol (**3**) was obtained as a pale yellow, amorphous powder, and the molecular formula was confirmed to be $C_{40}H_{42}O_{12}$ from the sodiated ion peak at m/z = 737.2572 $[M + Na]^+$ (calcd for $C_{40}H_{42}O_{12}Na$, 737.2574) obtained by HRESIMS. The presence of a carbonyl group was revealed by a band at 1710 cm^{-1} in the IR spectrum, which was confirmed by the resonance at δ 166.6 in the ^{13}C NMR spectrum. Comparison of the ^1H NMR data (Table 1) of **3** with those of 9,9′-*O*-di-(*E*)-feruloyl-(−)-secoisolariciresinol (**11**)[11] suggested that their structures are closely related, except that the 9,9′-*O*-di-(*Z*)-feruloyl moiety of **3** replaced the 9,9′-*O*-di-(*E*)-feruloyl moiety of 9,9′-*O*-di-(*E*)-feruloyl-(−)-secoisolariciresinol (**11**).[11] This was supported by the coupling constant (J = 12.8 Hz) for both H-7″/H-8″ and H-7‴/H-8‴, confirming the *Z*-configuration of both feruloyl moieties of **3**. Compound **3** showed a similar CD curve when compared to (−)-secoisolariciresinol, and the absolute configuration of **3** has to be 8*R*,8′*R*.[12] On the basis of the above data, the structure of **3** was elucidated as 9,9′-*O*-di-(*Z*)-feruloyl-(−)-secoisolariciresinol, which was further confirmed by the $^1H-^1H$ COSY, NOESY (Table 1), DEPT, HSQC, and HMBC (Table 1) experiments.

5′-Methoxyevofolin B (**4**) was obtained as a pale yellow oil. The molecular formula $C_{18}H_{20}O_7$ was deduced from the sodiated ion at m/z 371.1108 $[M + Na]^+$ (calcd 371.1107) in the HRESI mass spectrum. The IR spectrum showed the presence of hydroxy (3439 cm^{-1}) and carbonyl (1660 cm^{-1}) functions. Comparison of the ^1H (Table 2) and ^{13}C NMR data of **4** with those of evofolin B (**12**)[13] suggested that their structures are closely related, except that the 5′-methoxy group (δ 3.87) of **4** replaced H-5′ (δ 6.87) of **12**. This was supported by NOESY correlations (Table 2) between OMe-5′ (δ 3.87) and H-6′ (δ 6.41). On the basis of the evidence above, the structure of **4** was elucidated as 3-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)propan-1-one, named 5′-methoxyevofolin B. This was further confirmed by $^1H-^1H$ COSY and NOESY (Table 2) experiments. The assignment of ^{13}C

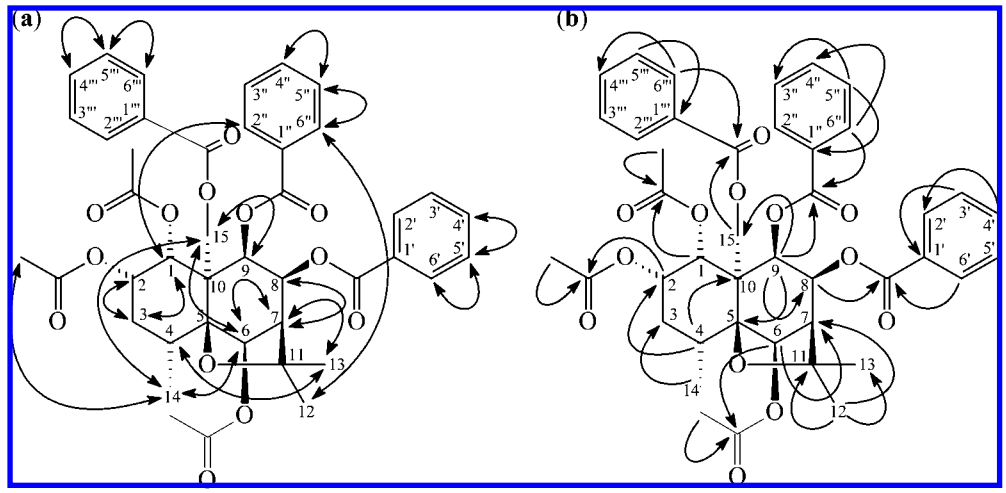


Figure 1. NOESY (a) and HMBC (b) correlations of **1**.

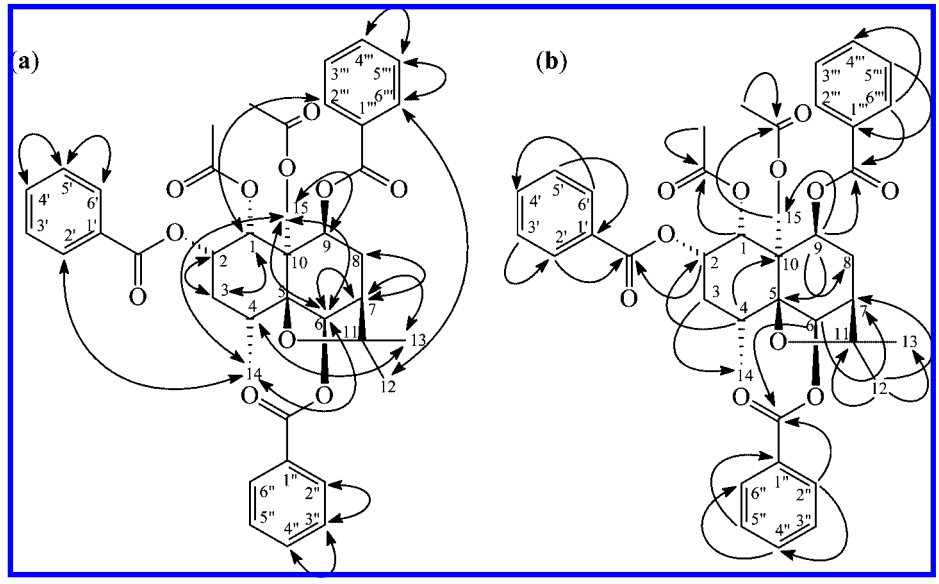


Figure 2. NOESY (a) and HMBC (b) correlations of **2**.

NMR resonances was confirmed by DEPT, HSQC, and HMBC (Table 2) techniques.

3-Ethoxy-4-hydroxybenzaldehyde (**5**) was isolated as colorless needles with a molecular formula of $C_9H_{10}O_3$, as determined by positive-ion HRESIMS, showing an $[M + Na]^+$ ion at *m/z* 189.0530 (calcd for $C_9H_{10}O_3Na$, 189.0528). The presence of OH and carbonyl groups was revealed by the bands at 3350 and 1675 cm^{-1}, respectively, in the IR spectrum. The ^1H NMR spectrum showed the presence of a formyl group, an ethoxy group, a hydroxy group, and three ABX-coupled protons similar to resonances described previously for vanillin (**15**),[14] except for the resonance of EtO-3 [δ 1.49 (3H, t, $J = 7.0$ Hz, OCH_2CH_3) and δ 4.21 (2H, q, $J = 7.0$ Hz, OCH_2CH_3)] in the spectrum of **5**, replacing that of OMe-3 (δ 3.97) of **15**. This was supported by NOESY correlations between OCH_2CH_3-3 (δ 4.21) and H-2 (δ 7.40). According to the above data, the structure of **5** was elucidated as 3-ethoxy-4-hydroxybenzaldehyde. This was confirmed by $^1H-^1H$ COSY and NOESY (Figure 3) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 3) techniques. This is the first report of the occurrence of **5** in a natural source, although it was produced by the alkaline cupric oxide (CuO) oxidation of lignin.[15]

The known isolates were readily identified by a comparison of physical and spectroscopic data (UV, IR, ^1H NMR, $[\alpha]_D$, and MS) with corresponding authentic samples or literature values. These compounds included five dihydroagarofuranoid sesquiterpenes, fokienagarofuran A (**6**),[2] fokienagarofuran D (**7**),[2] 1α,2α-diacetoxy-6β,9β,15-tribenzoyloxy-β-dihydroagarofuran (**8**),[3] salasol A (**9**),[16] and celahin C (**10**),[17] a lignan derivative, 9,9′-*O*-di-(*E*)-feruloyl-(−)-secoisolariciresinol (**11**),[11] six benzenoids, evofolin-B (**12**),[13] β-hydroxypropiovanillone (**13**),[18] 4-hydroxy-3-methoxycinnamaldehyde (**14**),[19] vanillin (**15**),[14] vanillic acid (**16**),[20] and syringaldehyde (**17**),[21] a flavanone, 7-*O*-methylnaringenin (**18**),[22] a coumarin, aesculetin dimethyl ether (**19**),[23] four steroids, β-sitosterol (**20**),[24] 3β-hydroxystigmast-5-en-7-one (**21**),[25] and a mixture of β-sitostenone (**22**)[26] and stigmasta-4,22-dien-3-one (**23**),[26] and a 2-pyrrolidone, *N*-methyl-2-pyrrolidone (**24**).[27]

The antitubercular effects of the isolates from the stems of *M. japonica* 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) 8-Benzoyloxymutangin (**1**), 15-acetoxyorbiculin G (**2**), fokienagarofuran A (**6**), 1α,2α-diacetoxy-6β,9β,15-tribenzoyloxy-β-dihydroagarofuran (**8**), salasol A (**9**), and celahin C (**10**) exhibited antitubercular activities (MICs ≤ 95.0 μM) against *M. tuberculosis* $H_{37}Rv$ *in vitro*. (b) Among the β-dihydroagarofuranoid sesquiterpene analogues (**1**, **2**, and **6**−**10**), salasol A

Table 1. ^{1}H NMR Data of **3** and **11**[a]

	3			**11**
position	δ_H *J* (Hz)	NOE	HMBC	δ_H *J* (Hz)
2	6.49 d (2.0)	OMe-3, 7	4, 6, 7	6.53 d (2.0)
5	6.87 d (8.0)	6	1, 3	6.81 d (8.0)
6	6.58 dd (8.0, 2.0)	5, 7	2, 4, 7	6.62 dd (8.0, 2.0)
7	2.70 dd (13.8, 7.2)	2, 6, 8	2, 8, 9	2.70 dd (13.8, 7.2)
	2.75 dd (13.8, 7.2)	2, 6, 8	2, 9	2.75 dd (13.8, 7.2)
8	2.16 m	7, 9	1, 9	2.22 m
9	4.16 dd (11.4, 5.4)	8	7, 9″	4.22 dd (11.4, 5.4)
	4.34 dd (11.4, 5.6)	8	7, 9″	4.40 dd (11.4, 5.6)
2′	6.49 d (2.0)	OMe-3′, 7′	4′, 6′, 7′	6.53 d (2.0)
5′	6.87 d (8.0)	6′	1′, 3′	6.81 d (8.0)
6′	6.58 dd (8.0, 2.0)	5′, 7′	2′, 4′, 7′	6.62 dd (8.0, 2.0)
7′	2.70 dd (13.8, 7.2)	2′, 6′, 8′	2′, 8′, 9′	2.70 dd (13.8, 7.2)
	2.75 dd (13.8, 7.2)	2′, 6′, 8′	2′, 9′	2.75 dd (13.8, 7.2)
8′	2.16 m	7′, 9′	1′, 9′	2.22 m
9′	4.16 dd (11.4, 5.4)	8′	7′, 9‴	4.22 dd (11.4, 5.4)
	4.34 dd (11.4, 5.6)	8′	7′, 9‴	4.40 dd (11.4, 5.6)
2″	7.78 d (2.0)	OMe-3″, 7″	4″, 6″, 7″	7.01 d (2.0)
5″	6.88 d (8.4)	6″	1″, 3″	6.91 d (8.0)
6″	7.12 dd (8.4, 2.0)	5″, 7″	2″, 4″, 7″	7.07 dd (8.0, 2.0)
7″	6.90 (12.8)	2″, 6″, 8″	2″, 6″, 9″	7.59 (15.8)
8″	5.84 (12.8)	7″	1″, 9″	6.29 (15.8)
2‴	7.78 d (2.0)	OMe-3‴, 7‴	4‴, 6‴, 7‴	7.01 d (2.0)
5‴	6.88 d (8.4)	6‴	1‴, 3‴	6.91 d (8.0)
6‴	7.12 dd (8.4, 2.0)	5‴, 7‴	2‴, 4‴, 7‴	7.07 dd (8.0, 2.0)
7‴	6.90 (12.8)	2‴, 6‴, 8‴	2‴, 6‴, 9‴	7.59 (15.8)
8‴	5.84 (12.8)	7‴	1‴, 9‴	6.29 (15.8)
OMe-3	3.78 s	2	3	3.78 s
OH-4	5.50 br s			5.51 br s
OMe-3′	3.78 s	2′	3′	3.78 s
OH-4′	5.50 br s			5.51 br s
OMe-3″	3.93 s	2″	3″	3.93 s
OH-4″	5.50 br s			5.51 br s
OMe-3‴	3.93 s	2‴	3‴	3.93 s
OH-4‴	5.50 br s			5.51 br s

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

Table 2. ^{1}H NMR Data of **4** and **12**[a]

	4			**12**
position	δ_H *J* (Hz)	NOE	HMBC	δ_H *J* (Hz)
2	7.54 d (2.0)	OMe-3	4, 6, C=O	7.54 d (2.0)
5	6.86 d (8.8)	6	1, 3	6.85 d (8.8)
6	7.53 dd (8.8, 2.0)	5, α	2, 4, C=O	7.53 dd (8.8, 2.0)
α	4.67 dd (8.4, 4.8)	6, *β*, 2′, 6′	1, 2′, 6′	4.66 dd (8.4, 4.8)
β	3.86 dd (11.5, 4.8)	α	1′, C=O	3.86 dd (11.6, 4.8)
	4.24 dd (11.5, 8.4)	α	1′, C=O	4.23 dd (11.6, 8.4)
2′	6.41 s	α, OMe-3′	α, 4′, 6′	6.71 d (1.6)
5′				6.87 d (8.0)
6′	6.41 s	α, OMe-5′	α, 2′, 4′	6.80 dd (8.0, 1.6)
OMe-3	3.91 s	2	3	3.91 s
OH-4	5.52 br s			5.56 br s
OMe-3′	3.87 s	2′	3′	3.85 s
OH-4′	5.45 br s			5.56 br s
OMe-5′	3.87 s	6′	5′	

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

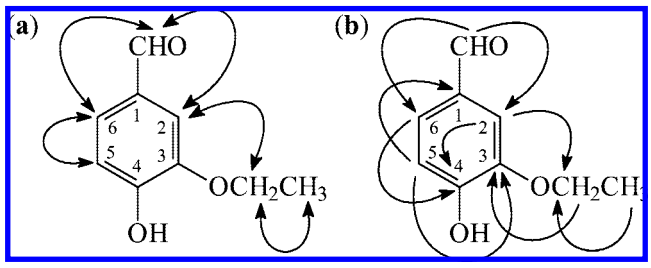


Figure 3. NOESY (a) and HMBC (b) correlations of **5**.

(**9**) and celahin C (**10**), with a hydroxy group at C-2 or C-1, exhibited stronger antituberculosis activity than **1**, **2**, and **6**−**8** against *M. tuberculosis* H_{37}Rv. (c) Compounds **9** and **10** are the most effective among the isolates, with MICs of 28.2 and 31.3 μM against *M. tuberculosis* H_{37}Rv, respectively. (d) The benzenoids **5**, **14**, **15**, and **17** and the steroids **20**, **22**, and **23** showed no antitubercular activities (MICs > 400 μM) against *M. tuberculosis* H_{37}Rv.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micromelting point apparatus and were uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter. CD spectra were recorded on a Jasco J-810 spectropolarimeter. 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

Table 3. Antitubercular Effects of **1**−**24** against *Mycobacterium tuberculosis* $H_{37}Rv$

compound	MICs (μM)[a]
8-benzoyloxymutangin (**1**)	79.3
15-acetoxyorbiculin G (**2**)	39.6
9,9′-*O*-di-(*Z*)-feruloyl-(−)-secoisolariciresinol (**3**)	122
5′-methoxyevofolin B (**4**)	215
3-ethoxy-4-hydroxybenzaldehyde (**5**)	>400
fokienagarofuran A (**6**)	85.9
fokienagarofuran D (**7**)	105
1α,2α-diacetoxy-6β,9β,15-tribenzoyloxy-β-dihydroagarofuran (**8**)	95.0
salasol A (**9**)	28.2
celahin C (**10**)	31.3
9,9′-*O*-di-(*E*)-feruloyl-(−)-secoisolariciresinol (**11**)	126
evofolin B (**12**)	235
β-hydroxypropiovanillone (**13**)	153
4-hydroxy-3-methoxycinnamaldehyde (**14**)	>400
vanillin (**15**)	>400
vanillic acid (**16**)	238
syringaldehyde (**17**)	>400
7-*O*-methylnaringenin (**18**)	209
aesculetin dimethyl ether (**19**)	388
β-sitosterol (**20**)	>400
3β-hydroxystigmast-5-en-7-one (**21**)	128
mixture of β-sitostenone (**22**) and stigmasta-4,22-dien-3-one (**23**)	>400
N-methyl-2-pyrrolidone (**24**)	>400
ethambutol[b]	30.6

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

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.

Plant Material. The stem of *M. japonica* was collected from Lanyu Island, Taitung County, Taiwan, in September 2006 and identified by Dr. I. S. Chen. A voucher specimen (Chen 6187) was deposited in the herbarium of the Faculty of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Separation. The dried stem of *M. japonica* (11.3 kg) was pulverized and extracted three times with MeOH (35 L each) for 3 days. The MeOH extracts were concentrated under reduced pressure at 35 °C, and then the residue (289 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc layer was concentrated to give a residue (fraction A, 65.5 g). The water layer was further extracted with *n*-BuOH, and the *n*-BuOH-soluble part (fraction B, 81 g) and the water-solubles (fraction C, 116 g) were separated. Fraction A (65.5 g) was chromatographed on Si gel (70−230 mesh, 2.9 kg), eluting with CH_2Cl_2, gradually increasing the polarity with MeOH to give 12 fractions: A1 (10 L, CH_2Cl_2), A2 (20 L, CH_2Cl_2/MeOH, 100:1), A3 (4 L, CH_2Cl_2/MeOH, 70:1), A4 (6 L, CH_2Cl_2/MeOH, 60:1), A5 (3.5 L, CH_2Cl_2/MeOH, 50:1), A6 (3 L, CH_2Cl_2/MeOH, 30:1), A7 (3 L, CH_2Cl_2/MeOH, 20:1), A8 (8 L, CH_2Cl_2/MeOH, 10:1), A9 (3 L, CH_2Cl_2/MeOH, 5:1), A10 (3.5 L, CH_2Cl_2/MeOH, 3:1), A11 (3.5 L, CH_2Cl_2/MeOH, 1:1), A12 (10 L, MeOH). Fraction A2 (9.7 g) was chromatographed further on Si gel (230−400 mesh, 410 g) eluting with *n*-hexane/acetone (15:1) to give 15 fractions (each 1.2 L, A2-1−A2-15). Fraction A2-2 (1.3 g) was washed with MeOH and filtered to yield **20** (875 mg) after recrystallization (MeOH). Fraction A2-5 (550 mg) was washed with MeOH and filtered to afford **21** (13.2 mg) after recrystallization (CH_2Cl_2/MeOH). Fraction A2-7 (230 mg) was purified further by preparative TLC (Si gel, *n*-hexane/acetone, 5:1) to obtain **9** (3.6 mg) (R_f = 0.50) and **10** (3.4 mg) (R_f = 0.76). Fraction A2-8 (212 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/acetone, 70:1) to yield **6** (6.5 mg) (R_f = 0.76) and **7** (5.3 mg) (R_f = 0.94). Fraction A2-9 (230 mg) was purified further by preparative TLC (Si gel, *n*-hexane/acetone, 70:1) to obtain a mixture of **22** and **23** (23.5 mg) (R_f = 0.29). Fraction A3 (5.3 g) was chromatographed further on Si gel (230−400 mesh, 210 g) eluting with *n*-hexane/acetone (10:1) to give 12 fractions (each 600 mL, A3-1−A3-12). Fraction A3-4 (186 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/acetone, 70:1) to afford **2** (3.1 mg) (R_f = 0.59). Fraction A3-5 (228 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/acetone, 70:1) to yield **8** (13.4 mg) (R_f = 0.53) and **19** (2.9 mg) (R_f = 0.47). Fraction A3-7 (186 mg) was purified further by preparative TLC (Si gel, *n*-hexane/acetone, 2:1) to obtain **1** (5.6 mg) (R_f = 0.71). Fraction A3-8 (208 mg) was purified further by preparative TLC (Si gel, *n*-hexane/acetone, 2:1) to afford **14** (3.8 mg) (R_f = 0.61) and **17** (2.7 mg) (R_f = 0.60). Fraction A3-10 (192 mg) was purified further by preparative TLC (Si gel, *n*-hexane/acetone, 2:1) to obtain **18** (2.4 mg) (R_f = 0.52). Fraction A6 (5.4 g) was chromatographed further on Si gel (230−400 mesh, 200 g) eluting with *n*-hexane/acetone (2:1) to give 13 fractions (each 500 mL, A6-1−A6-13). Fraction A6-4 (965 mg) was purified by MPLC (35 g Si gel, 40−63 mesh, *n*-hexane/acetone, 1:1, 150 mL fractions) to obtain 15 subfractions: A6-4-1−A6-4-15. Fraction A6-4-5 (88 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/MeOH, 50:1) to obtain **5** (3.3 mg) (R_f = 0.67). Fraction A6-4-6 (73 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/MeOH, 50:1) to afford **15** (3.8 mg) (R_f = 0.50). Fraction A6-5 (210 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/MeOH, 10:1) to yield **24** (2.5 mg) (R_f = 0.50). Fraction A6-6 (185 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/MeOH, 10:1) to afford **13** (4.2 mg) (R_f = 0.56). Fraction A6-8 (198 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/MeOH, 10:1) to yield **16** (3.6 mg) (R_f = 0.47). Fraction A7 (6.6 g) was chromatographed further on Si gel (230−400 mesh, 275 g) eluting with *n*-hexane/acetone (2:1) to give 18 fractions (each 1.0 L, A7-1−A7-18). Fraction A7-9 (225 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/EtOAc, 1:2) to obtain **11** (4.4 mg) (R_f = 0.92). Fraction A7-11 (235 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/EtOAc, 1:2) to afford **3** (2.8 mg) (R_f = 0.61). Fraction A7-13 (198 mg) was purified further by preparative TLC (Si gel, $CHCl_3$/EtOAc, 1:2) to obtain **4** (2.6 mg) (R_f = 0.49) and **12** (3.2 mg) (R_f = 0.47).

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.[28] 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.

8-Benzoyloxymutangin (1): amorphous powder; $[\alpha]^{25}_D$ +83.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (4.16), 274 (3.14), 281 (3.07) nm; CD (MeOH, $\Delta\epsilon$) 237 (−14.2), 221 (+5.02) nm; IR (neat) ν_{max} 1747 (C=O), 1721 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (3H, d, *J* = 7.6 Hz, H-14), 1.36 (3H, s, OAc-1), 1.51 (3H, s, H-13), 1.67 (3H, s, H-12), 1.84 (1H, dd, *J* = 15.0, 1.8 Hz, H_{ax}-3), 2.03 (3H, s, OAc-2), 2.11 (3H, s, OAc-6), 2.49 (1H, m, H-4), 2.57 (1H, ddd, *J* = 15.0, 6.4, 4.0 Hz, H_{eq}-3), 2.71 (1H, d, *J* = 3.2 Hz, H-7), 4.85 (1H, d, *J* = 12.2 Hz, H-15), 5.60 (1H, d, *J* = 3.2 Hz, H-8), 5.60 (1H, d, *J* = 12.2 Hz, H-15), 5.66 (1H, q, *J* = 3.6 Hz, H-2), 5.81 (1H, d, *J* = 3.6 Hz, H-1), 6.11 (1H, s, H-9), 6.27 (1H, s, H-6), 7.29 (2H, t, *J* = 7.6 Hz, H-3′ and H-5′), 7.33 (2H, t, *J* = 7.6 Hz, H-3‴ and H-5‴), 7.46 (1H, t, *J* = 7.6 Hz, H-4′), 7.46 (1H, t, *J* = 7.5 Hz, H-3″ and H-5″), 7.48 (1H, t, *J* = 7.6 Hz, H-4‴), 7.59 (1H, t, *J* = 7.5 Hz, H-4″), 8.00 (2H, d, *J* = 7.6 Hz, H-2′ and H-6′), 8.06 (2H, d, *J* = 7.5 Hz, H-2″ and H-6″), 8.15 (2H, d, *J* = 7.6 Hz, H-2‴ and H-6‴); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.5 (C-14), 20.6 (*Me*CO_2-1), 21.5 (*Me*CO_2-2), 21.5 (*Me*CO_2-6), 26.2 (C-12), 30.9 (C-13), 31.4 (C-3), 33.3 (C-4), 53.1 (C-10), 53.2 (C-7), 66.1 (C-15), 69.6 (C-2), 72.3 (C-1), 72.3 (C-9), 75.6 (C-6), 77.7 (C-8), 82.3 (C-11), 90.0 (C-5), 128.6 (C-3′), 128.6 (C-5′), 128.6 (C-3‴), 128.6 (C-5‴), 128.7 (C-3″), 128.7 (C-5″), 128.9 (C-1″), 129.6 (C-1‴), 129.7 (C-1′), 130.1 (C-2′), 130.1 (C-6′), 130.2 (C-2‴), 130.2 (C-6‴), 130.5 (C-2″), 130.5 (C-6″), 133.4 (C-4′), 133.4 (C-4‴), 134.0 (C-4″), 164.8 (Ph*C*O_2-9), 165.7 (Ph*C*O_2-8), 166.7 (Ph*C*O_2-15), 169.8 (Me*C*O_2-1), 169.8 (Me*C*O_2-6), 170.5 (Me*C*O_2-2);

ESIMS *m/z* (rel int) 779 ([M + Na]$^+$, 100); HRESIMS *m/z* 779.2679 [M + Na]$^+$ (calcd for $C_{42}H_{44}O_{13}Na$, 779.2681).

15-Acetoxyorbiculin G (2): amorphous powder; $[\alpha]^{25}_D$ +36.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (4.12), 274 (3.14), 280 (3.06) nm; IR (neat) ν_{max} 1742 (C=O), 1720 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33 (3H, d, J = 7.2 Hz, H-14), 1.52 (6H, s, H-12 and H-13), 1.54 (3H, s, OAc-1), 2.02 (1H, m, H_{ax}-3), 2.31 (1H, m, H_{ax}-8), 2.34 (3H, s, OAc-15), 2.43 (1H, dd, J = 3.5, 3.0 Hz, H-7), 2.62 (1H, m, H-4), 2.65 (1H, m, H_{eq}-3), 2.67 (1H, ddd, J = 16.0, 7.2, 3.5 Hz, H_{eq}-8), 4.48 (1H, d, J = 12.4 Hz, H-15), 5.39 (1H, d, J = 12.4 Hz, H-15), 5.54 (1H, d, J = 7.2 Hz, H-9), 5.89 (1H, d, J = 4.0 Hz, H-1), 5.91 (1H, q, J = 4.0 Hz, H-2), 6.29 (1H, s, H-6), 7.47 (2H, t, J = 7.6 Hz, H-3‴ and H-5‴), 7.48 (2H, t, J = 7.5 Hz, H-3″ and H-5″), 7.49 (2H, t, J = 7.5 Hz, H-3′ and H-5′), 7.57 (1H, t, J = 7.5 Hz, H-4″), 7.59 (1H, t, J = 7.5 Hz, H-4′), 7.61 (1H, t, J = 7.6 Hz, H-4‴), 8.04 (2H, d, J = 7.5 Hz, H-2″ and H-6″), 8.08 (2H, d, J = 7.6 Hz, H-2‴ and H-6‴), 8.11 (2H, d, J = 7.5 Hz, H-2′ and H-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2 (C-14), 20.4 (*Me*CO_2-1), 21.3 (*Me*CO_2-15), 26.0 (C-12), 30.6 (C-13), 31.9 (C-3), 33.4 (C-4), 35.0 (C-8), 49.0 (C-7), 53.3 (C-10), 66.0 (C-15), 69.4 (C-2), 69.4 (C-9), 71.5 (C-1), 78.7 (C-6), 82.8 (C-11), 89.7 (C-5), 128.4 (C-3‴), 128.4 (C-5‴), 128.6 (C-3′), 128.6 (C-5′), 128.7 (C-3″), 128.7 (C-5″), 129.1 (C-1‴), 129.5 (C-2″), 129.5 (C-6″), 129.7 (C-1′), 129.7 (C-1″), 129.8 (C-2′), 129.8 (C-6′), 130.2 (C-2‴), 130.2 (C-6‴), 133.3 (C-4′), 133.3 (C-4″), 133.5 (C-4‴), 165.3 (Ph*C*O_2-9), 165.5 (Ph*C*O_2-6), 166.1 (Ph*C*O_2-2), 169.4 (Me*C*O_2-1), 170.8 (Me*C*O_2-15); ESIMS *m/z* (rel int) 721 ([M + Na]$^+$, 100); HRESIMS *m/z* 721.2629 [M + Na]$^+$ (calcd for $C_{40}H_{42}O_{11}Na$, 721.2625).

9,9′-*O*-Di-(*Z*)-feruloyl-(−)-secoisolariciresinol (3): pale yellow, amorphous powder; $[\alpha]^{25}_D$ −42.6 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (4.46), 290 (4.36), 326 (4.48) nm; CD (MeOH, $\Delta\epsilon$) 229 (−0.35), 287 (−0.27) nm; IR (neat) ν_{max} 3421 (OH), 1710 (C=O) cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 35.5 (C-7 and C-7′), 40.1 (C-8 and C-8′), 56.0 (OMe-3 and OMe-3′), 56.2 (OMe-3″ and OMe-3‴), 64.5 (C-9 and C-9′), 111.5 (C-2 and C-2′), 113.1 (C-2″ and C-2‴), 114.1 (C-5″ and C-5‴), 114.4 (C-5 and C-5′), 116.5 (C-8″ and C-8‴), 122.0 (C-6 and C-6′), 126.0 (C-6″ and C-6‴), 127.3 (C-1″ and C-1‴), 131.9 (C-1 and C-1′), 144.1 (C-4 and C-4′), 144.9 (C-7″ and C-7‴), 146.2 (C-3″ and C-3‴), 146.7 (C-3 and C-3′), 147.4 (C-4″ and C-4‴), 166.6 (C-9″ and C-9‴); ESIMS *m/z* (rel int) 737 ([M + Na]$^+$, 100); HRESIMS *m/z* 737.2572 [M + Na]$^+$ (calcd for $C_{40}H_{42}O_{12}Na$, 737.2574).

5′-Methoxyevofolin B (4): pale yellow oil; $[\alpha]^{25}_D$ −16.2 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 229 (4.61), 278 (4.31), 307 (4.12) nm; IR (neat) ν_{max} 3439 (OH), 1660 (C=O) cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.1 (C-α), 56.2 (OMe-3), 56.3 (OMe-3′), 56.3 (OMe-5′), 65.6 (C-β), 105.3 (C-2′), 105.3 (C-6′), 110.8 (C-2), 114.1 (C-5), 124.7 (C-6), 129.4 (C-1), 131.1 (C-1′), 134.0 (C-4′),146.7 (C-3), 147.1 (C-3′), 147.1 (C-5′), 150.7 (C-4), 198.8 (C=O); ESIMS *m/z* (rel int) 371 ([M + Na]$^+$, 100); HRESIMS *m/z* 371.1108 [M + Na]$^+$ (calcd for $C_{18}H_{20}O_7Na$, 371.1107).

3-Ethoxy-4-hydroxybenzaldehyde (5): colorless needles (MeOH); mp 77−79 °C; UV (MeOH) λ_{max} (log ϵ) 231 (4.34), 278 (4.16), 308 (4.12) nm; IR (neat) ν_{max} 3350 (OH), 1675 (C=O), 1587, 1511, 1442 (aromatic ring C=C stretch) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.49 (3H, t, J = 7.0 Hz, *Me*CH_2O-3), 4.21 (2H, q, J = 7.0 Hz, MeC*H*$_2$O-3), 6.22 (1H, br s, D_2O exchangeable, OH-4), 7.05 (1H, d, J = 8.0 Hz, H-5), 7.40 (1H, d, J = 2.0 Hz, H-2), 7.41 (1H, dd, J = 8.0, 2.0 Hz, H-6), 9.82 (1H, s, CHO-1); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.7 (*Me*CH_2O-3), 64.8 (Me*C*H_2O-3), 109.5 (C-2), 114.3 (C-5), 127.4 (C-6), 129.9 (C-1), 146.4 (C-3), 151.7 (C-4), 190.9 (CHO); ESIMS *m/z* (rel int) 189 ([M + Na]$^+$, 100); HRESIMS *m/z* 189.0530 [M + Na]$^+$ (calcd for $C_9H_{10}O_3Na$, 189.0528).

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

References and Notes

(1) Lu, S. U.; Yang, Y. P. *Celastraceae in Flora of Taiwan*, 2nd ed.; Editorial Committee of the Flora of Taiwan: Taipei, Taiwan, 1993; Vol *3*, pp 640−656,
(2) Chen, J. J.; Chou, T. H.; Duh, C. Y.; Chen, I. S. *J. Nat. Prod.* **2006**, *69*, 685–688.
(3) Chen, J. J.; Chou, T. H.; Peng, C. F.; Chen, I. S.; Yang, S. Z. *J. Nat. Prod.* **2007**, *70*, 202–205.
(4) Chou, T. H.; Chen, I. S.; Sung, P. J.; Peng, C. F.; Shieh, P. C.; Chen, J. J. *Chem. Biodiversity* **2007**, *4*, 1594–1600.
(5) Chen, I. H.; Chang, F. R.; Wu, C. C.; Chen, S. L.; Hsieh, P. W.; Yen, H. F.; Du, Y. C.; Wu, Y. C. *J. Nat. Prod.* **2006**, *69*, 1543–1546.
(6) Wang, K. W.; Ahang, H.; Pan, Y. J. *Helv. Chim. Acta* **2007**, *90*, 277–281.
(7) Tsanuo, M. K.; Hassanali, A.; Jondiko, I. J. O.; Torto, B. *Phytochemistry* **1993**, *34*, 665–668.
(8) Takaishi, Y.; Aihara, F.; Tamai, S.; Nakano, K.; Tomimatsu, T. *Phytochemistry* **1992**, *31*, 3943–3947.
(9) González, A. G.; Nuñez, M. P.; Ravelo, A. G.; Luis, J. G.; Jiménez, I. A. *J. Nat. Prod.* **1990**, *53*, 474–478.
(10) Muñoz-Martínez, F.; Mendoza, C. R.; Bazzocchi, I. L.; Castanys, S.; Jiménez, I. A.; Gamarro, F. *J. Med. Chem.* **2005**, *48*, 4266–4275.
(11) Karikome, H.; Mimaki, Y.; Sashida, Y. *Phytochemistry* **1991**, *30*, 315–319.
(12) Xie, L. H.; Akao, T.; Hamasaki, K.; Deyama, T.; Hattori, M. *Chem. Pharm. Bull.* **2003**, *51*, 508–515.
(13) Wu, T. S.; Yeh, J. H.; Wu, P. L. *Phytochemistry* **1995**, *40*, 121–124.
(14) Chen, J. J.; Duh, C. Y.; Chen, I. S. *Planta Med* **2005**, *71*, 370–372.
(15) Lobbes, J. M.; Fitznar, H. P.; Kattner, G. *Anal. Chem.* **1999**, *71*, 3008–3012.
(16) Morikawa, T.; Kishi, A.; Pongpiriyadacha, Y.; Matsuda, H.; Yoshikawa M. *J. Nat. Prod.* **2003**, *66*, 1191–1196.
(17) Kuo, Y. H.; Chou, C. J.; Kuo, L. M. Y.; Hu, Y. Y.; Chen, Y. C.; Chen, C. F.; Lee, K. H. *Phytochemistry* **1996**, *41*, 549–551.
(18) Lin, R. C.; Skaltsounis, A. L.; Seguin, E.; Tillequin, F.; Koch, M. *Panta Med.* **1994**, *60*, 168–170.
(19) Miyazawa, M.; Hisama, M. *J. Agric. Food Chem.* **2003**, *51*, 6413–6422.
(20) Chen, J. J.; Huang, S. Y.; Duh, C. Y.; Chen, I. S.; Wang, T. C.; Fang, H. Y. *Planta Med* **2006**, *72*, 935–938.
(21) Chen, J. J.; Peng, C. F.; Huang, H. Y.; Chen, I. S. *Planta Med.* **2006**, *72*, 473–477.
(22) Jerz, G.; Waibel, R.; Achenbach, H. *Phytochemistry* **2005**, *66*, 1698–1706.
(23) Afek, U.; Sztejnberg, A.; Carmely, S. *Phytochemistry* **1986**, *25*, 1855–1856.
(24) Chen, J. J.; Chou, E. T.; Peng, C. F.; Chen, I. S.; Yang, S. Z.; Huang, H. Y. *Planta Med* **2007**, *73*, 567–571.
(25) Chen, J. J.; Cho, J. Y.; Hwang, T. L.; Chen, I. S. *J. Nat. Prod.* **2008**, *71*, 71–75.
(26) Chen, J. J.; Chen, P. H.; Liao, C. H.; Huang, S. Y.; Chen, I. S. *J. Nat. Prod* **2007**, *70*, 1444–1448.
(27) Latip, J.; Hartley, T. G.; Waterman, P. G. *Phytochemistry* **1999**, *51*, 107–110.
(28) Inderlied, C. B.; Nash, K. A. *Antibiotics in Laboratory Medicine*, 4th ed.; Lippincott Williams & Wilkins: Philadelphia, 1996; pp 127−175.

NP800097T