JOURNAL OF NATURAL PRODUCTS

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Antitubercular Chromones and Flavonoids from *Pisonia aculeata*

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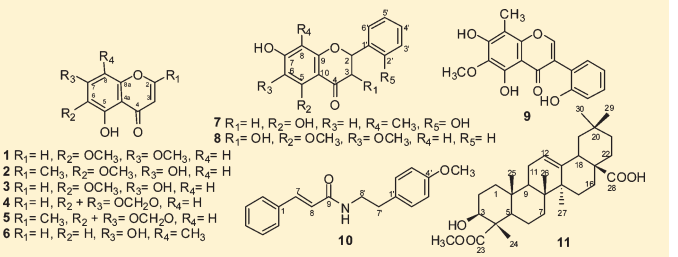
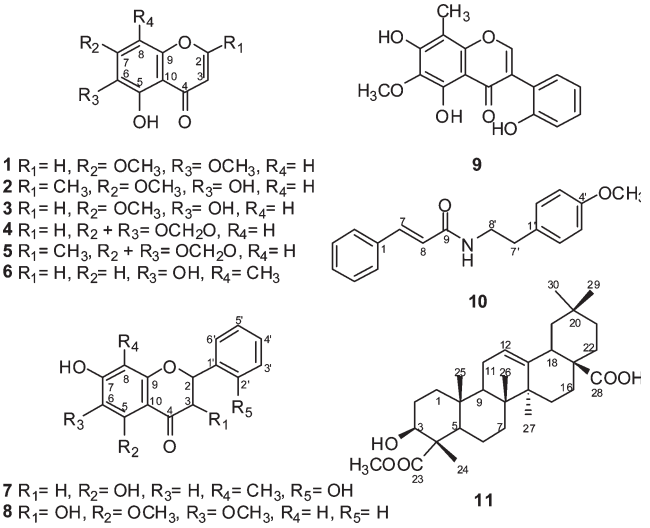
Supporting Information

ABSTRACT: Three new chromones, pisonins A (**1**), B (**2**), and D (**4**), two new flavonoids, pisonivanone [(2*S*)-5,7,2′-trihydroxy-8-methylflavanone] (**7**) and pisonivanol [(2*R*,3*R*)-3,7-dihydroxy-5,6-dimethoxyflavanone] (**8**), one new isoflavonoid, pisonianone (5,7,2′-trihydroxy-6-methoxy-8-methylisoflavone) (**9**), and five compounds first isolated from nature, namely, pisonins C (**3**), E (**5**), and F (**6**), pisoniamide (**10**), and pisonolic acid (**11**), together with 18 known compounds have been isolated from the methanol extract of the combined stem and root of *Pisonia aculeata*. Among these isolates, **2**, **7**, **14**, **16**, and **19** exhibited antitubercular activities (MICs ≤ 50.0 μg/mL) against *Mycobacterium tuberculosis* H37Rv in vitro.



Pisonia aculeata L.[1] (Nyctaginaceae), a scandent shrub, is distributed in south China, the Ryukyus, India, Malaysia, Indonesia, and Taiwan. Its sticky seeds are dispersed by small mammals and birds, which allowed the plant's broad distribution from southeastern Asia throughout the world including Australia, Africa, Florida, and Texas.[2,3] Its leaves have been reported to have hepatoprotective and antioxidant activities.[4] However, an analysis of its chemical constituents has never been undertaken. *P. aculeata* was found to be one of the active species in an in vitro screening program for antitubercular activity against *Mycobacterium tuberculosis* strain H37Rv. Phytochemical investigation of this plant afforded 29 compounds, including six new (**1**, **2**, **4**, **7**−**9**) and five known compounds (**3**, **5**, **6**, **10**, **11**) isolated from natural sources for the first time. The isolation and elucidation of the structures of these compounds and the assessment of their antitubercular activity are described herein.



RESULTS AND DISCUSSION

Compounds **1**−**6** were obtained as colorless needles. Their UV absorptions showed maxima at about 225, 255, and 292 nm[5,6] and a bathochromic shift upon the addition of KOH, which suggests the presence of the phenol moiety of a chromone skeleton.

The molecular formula of **1** was established to be $C_{11}H_{10}O_5$ by ESIMS (*m*/*z* 245 $[M + Na]^+$) and HRESIMS (*m*/*z* 245.0428 $[M + Na]^+$). The IR absorptions revealed a hydroxy group at 3436 cm^{-1} and a conjugated carbonyl group at 1661 cm^{-1}. The ^1H NMR spectrum (Table 1) was similar to that of 5-hydroxy-6,7-dimethoxy-2-methylchromone (**12**),[7] except that the olefinic proton at C-2 of **1** replaced the methyl group of **12**. The long-range HMBC correlations (Figure 1) from the proton at δ_H 7.76 (1H, d, *J* = 6.0 Hz) to C-3 (δ_C 111.1), C-4 (δ_C 182.1), and C-8a (δ_C 153.7) led to the designation of this proton as H-2. Similarly, the long-range HMBC correlations from the proton at δ_H 6.23 to C-2 (δ_C 155.5), C-4 (δ_C 182.1), and C-4a (δ_C 107.3) designated this proton as H-3. H-8 (δ_H 6.45) showed HMBC correlations with C-4a (δ_C 107.3), C-6 (δ_C 132.7), C-7 (δ_C 158.9), and C-8a (δ_C 153.7) to confirm this designation. The HMBC correlations from OH-5 (δ_H 12.52) to C-4a (δ_C 107.3), C-5 (δ_C 153.1), and C-6 (δ_C 132.7), from OCH_3-6 (δ_H 3.91) to C-6 (δ_C 132.7), and

Received: November 24, 2010
Published: May 04, 2011

Table 1. ^{1}H NMR Data of Compounds 1−6[a]

	δ_H (J in Hz)					
position	1	2	3	4	5	6
2	7.76, d (6.0)		8.08, d (5.8)	7.77, d (5.8)		8.14, d (5.8)
3	6.23, d (6.0)	6.02, q (0.6)	6.22, d (5.8)	6.24, d (5.8)	6.04, s	6.21, d (5.8)
6						6.33, s
7						
8	6.45, s	6.46, s	6.48, s	6.49, s	6.44, s	
OH-5[b]	12.52, s	12.97, s	12.97, s	12.53, s	12.67, s	12.69, s
OH-7[b]		6.53, br s	9.29, br s			9.65, br s
OCH_3-6	3.91, s	4.01, s	3.86, s			
OCH_3-7	3.94, s					
CH_3-2		2.35, d (0.6)			2.35, s	
CH_3-8						2.15, s
OCH_2O				6.09, s	6.07, s	

[a] ^{1}H NMR data were measured at 400 MHz in $CDCl_3$ for **1**, **2**, **4**, and **5** and in acetone-d_6 for **3** and **6**. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. [b] D_2O exchangeable.

from OCH_3-7 (δ_H 3.94) to C-7 (δ_C 158.9) verified the locations of the hydroxy and methoxy groups. These data established that **1** had a 5-hydroxy-6,7-dimethoxychromone moiety. Thus, the structure of **1** was determined as 5-hydroxy-6,7-dimethoxychromone, named pisonin A.

With HRESIMS analysis (m/z 245.0427 $[M + Na]^+$), the molecular formula of **2** was determined to be $C_{11}H_{10}O_5$. The ^{1}H NMR spectrum (Table 1) resembled that of **1**, except that H-2 and OCH_3-7 of **1** were replaced by CH_3-2 and OH-7 of **2**. The long-range HMBC correlations (Figure 1) from OH-7 (δ_H 6.53) to C-7 (δ_C 154.8) and C-8 (δ_C 93.2) provided additional structural confirmation. The location of the methyl group (δ_H 2.35, 3H, d, J = 0.6 Hz) at C-2 was corroborated by the HMBC experiments. Consequently, **2** was identified as 5,7-dihydroxy-6-methoxy-2-methylchromone, named pisonin B.

The structure of **3**, pisonin C, was determined as 5,7-dihydroxy-6-methoxychromone, which was isolated from nature for the first time.[8]

The molecular formula of **4**, $C_{10}H_6O_5$, was established by HRESIMS (m/z 229.0112 $[M + Na]^+$). The ^{1}H NMR spectrum (Table 1) resembled that of **3**, except that the methylenedioxy [δ_H 6.09 (2H, s); δ_C 102.7; IR: 1029 and 933 cm^{-1}] group of **4** replaced the 6-methoxy and 7-hydroxy groups of **3**. The methylenedioxy group was attached at C-6 and C-7, which was further confirmed by the HMBC experiments (Figure 1). Thus, **4** was elucidated as 5-hydroxy-6,7-methylenedioxychromone, named pisonin D.

Pisonins E (5-hydroxy-6,7-methylenedioxy-2-methylchromone) (**5**)[7] and F (5,7-dihydroxy-8-methylchromone) (**6**)[8,9] were isolated from nature for the first time.

Compound **7** was isolated as pale yellow needles. Its molecular formula, $C_{16}H_{14}O_5$, was determined by HRESIMS (m/z 309.0741 $[M + Na]^+$) and DEPT. The UV absorptions showed maxima at 238 (sh), 289, and 335 nm and a bathochromic shift upon the addition of an alkaline solution, indicating the presence of a phenolic moiety. The IR absorption showed a conjugated carbonyl group at 1642 cm^{-1}. The ^{1}H NMR spectrum (Table 3) resembled that of (2*S*)-2′-hydroxydemethoxymatteucinol (**14**),[12] except that **7** lacked a methyl group at C-6. The long-range HMBC correlations (Figure 2) of the aromatic proton at δ_H 6.05

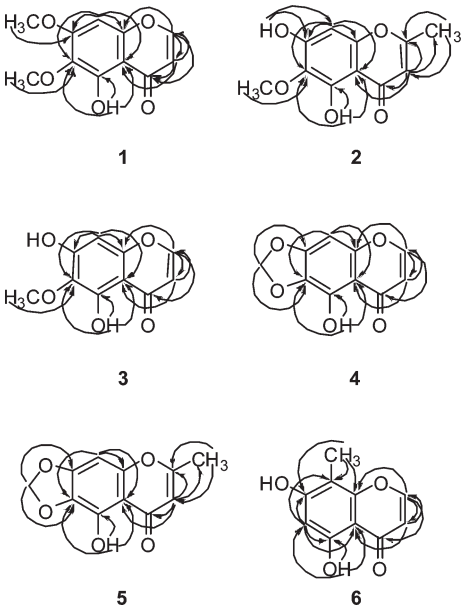


Figure 1. Key HMBC (H→C) correlations of **1**−**6**.

with C-5 (δ_C 163.5), C-7 (δ_C 165.8), C-8 (δ_C 104.6), and C-10 (δ_C 103.8) led to the designation of this proton as H-6. The proton at δ_H 5.78 displayed HMBC correlations with C-4 (δ_C 198.3), C-1′ (δ_C 127.4), C-2′ (δ_C 155.4), and C-6′ (δ_C 128.0) and was designated as H-2. The HMBC correlations confirmed the methyl (δ_H 2.00) group located at C-8. A comparison of the specific rotation values of **7** ($[\alpha]^{25}_D$ −102) and **14** ($[\alpha]^{25}_D$ −98) established the 2*S* absolute configuration.[12,13] Consequently, **7** was determined as (2*S*)-5,7,2′-trihydroxy-8-methylflavanone, named pisonivanone.

Compound **8** was isolated as colorless needles. The HRESIMS afforded an $[M + Na]^+$ ion peak at m/z 339.0843, consistent with a molecular formula of $C_{17}H_{16}O_6$. The UV absorptions showed maxima at 236 (sh), 279, and 318 nm and a bathochromic shift upon the addition of an alkaline solution, indicating the presence of a phenolic moiety. The presence of

dx.doi.org/10.1021/np1008575 |J. Nat. Prod. 2011, 74, 976–982

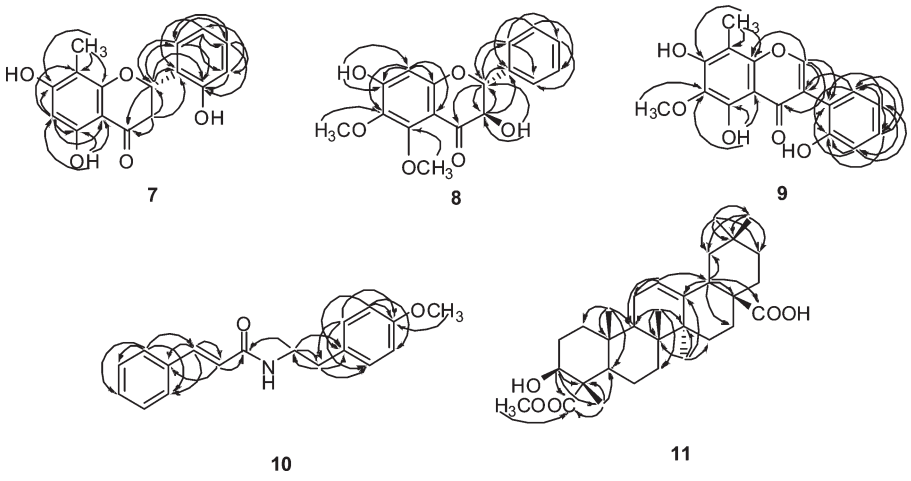


Figure 2. Key HMBC (H→C) correlations of **7**−**11**.

Table 2. ^{13}C NMR Data of Compounds 1−6[a]

position	1	2	3	4	5	6
2	155.5	167.0	158.5	155.6	166.8	158.4
3	111.1	108.2	111.7	111.0	108.5	111.9
4	182.1	183.0	183.8	182.4	183.0	183.7
4a	107.3	105.4	107.6	108.8	107.2	107.2
5	153.1	152.1	154.8	142.2	142.2	161.5
6	132.7	130.2	132.9	130.1	129.8	99.9
7	158.9	154.8	158.6	154.1	153.8	163.3
8	90.6	93.2	95.4	89.5	89.2	103.8
8a	153.7	153.5	155.1	153.6	153.6	157.3
OCH_3-6	60.8	60.8	61.3			
OCH_3-7	56.3					
CH_3-2		20.5			20.4	
CH_3-8						8.2
OCH_2O				102.7	102.5	

[a] ^{13}C NMR data were measured at 100 MHz in $CDCl_3$ for **1**, **2**, **4**, and **5** and in acetone-d_6 for **3** and **6**. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra.

conjugated carbonyl (IR: 1676 cm^{-1}; ^{13}C NMR: δ 191.3) and hydroxy [IR: 3391 (br) cm^{-1}; ^{1}H NMR: δ 3.96 and δ 6.44 (each 1H, br s, exchangeable with D_2O)] groups was observed. The ^{1}H NMR spectrum (Table 3) of **8** was similar to that of (2*R*,3*R*)-3-hydroxy-5-methoxy-6,7-methylenedioxyflavanone (**15**),[14] except that the 6-methoxy and 7-hydroxy groups of **8** replaced the 6,7-methylenedioxy group of **15**. The absence of a downfield-shifted hydrogen-bonding hydroxy resonance in the ^{1}H NMR spectrum of **8** indicated the lack of a C-5 OH group. The location of the methoxy (δ_H 3.98, 3H, s) group at C-5 was confirmed by an HMBC experiment (Figure 2). Usually, the carbon shifts of OCH_3 groups *ortho* to an aromatic proton appear between δ 55.0 and 56.6, but with a *o*-OCH_3 group, they are downfield shifted between δ 59.5 and 63.6.[15] Two methoxy groups of **8**, which showed no NOESY correlations with H-8 (Figure 3), resonated downfield at δ_C 61.6 (Table 3) due to the effects of the C-5 and C-6 substituents.[15] Furthermore, the aromatic singlet at δ 6.39 (H-8) exhibited long-range HMBC correlations with C-6 (δ_C 135.5), C-7 (δ_C 156.7), C-9 (δ_C 159.8), and C-10 (δ_C 106.4). The HMBC correlations from OH-3 (δ_H 3.96) to C-2 (δ_C 83.3) and C-3 (δ_C 73.1); from OH-7 (δ_H 6.44) to C-7 (δ_C 156.7) and C-8 (δ_C 99.5), and from OCH_3-6 (δ_H 3.94) to C-6 (δ_C 135.5) further supported the structural confirmation. The absolute configuration of **8** was established as 2*R*,3*R* by the CD Cotton effects ($[\theta]_{341}$ +12 213, $[\theta]_{326}$ 0, $[\theta]_{308}$ −13 309) and specific rotation ($[\alpha]^{24}_D$ +27).[13,16] Thus, **8** was identified as (2*R*,3*R*)-3,7-dihydroxy-5,6-dimethoxyflavanone, named pisonivanol, which was confirmed by DEPT, HSQC, and NOESY (Figure 3) experiments.

Compound **9** was obtained as yellowish needles. Its molecular formula was determined to be $C_{17}H_{14}O_6$ by the HRESIMS at *m*/*z* 337.0690 $[M + Na]^+$ and DEPT. The UV absorptions resembled those of 6,8-dimethylisogenistein,[17] and a bathochromic shift in alkaline solution suggested the presence of a phenolic moiety. The IR absorption exhibited a conjugated carbonyl group at 1652 cm^{-1}. The ^{1}H NMR spectrum (Table 3) was similar to that of 6,8-dimethylisogenistein, except that **9** had a 6-methoxy [δ_H 4.06 (3H, s)] instead of a 6-methyl group. The OCH_3 (δ_H 4.06) group at C-6 was further confirmed by the HMBC experiments (Figure 2). Thus, the structure of **9** was established as 5,7,2′-trihydroxy-6-methoxy-8-methylisoflavone, named pisonianone, which was further corroborated by DEPT, HSQC, and NOESY (Figure 3) experiments.

The structure of **10**, pisoniamide, was similar to that of *N-trans*-feruloyltyramine[17,18] and was elucidated as *N*-(4-methoxyphenethyl)cinnamamide, which was isolated from a natural source for the first time.[19] Compound **11** was determined to be oleananic acid 23α-methylcarboxylate, a synthetic intermediate,[20] which was isolated from nature for the first time and termed pisonolic acid.

The additional compounds, including 5-hydroxy-6,7-dimethoxy-2-methylchromone (**12**),[7] leptorumol (**13**),[10,11] (2*S*)-2′-hydroxydemethoxymatteucinol (**14**),[12] (2*R*,3*R*)-3-hydroxy-5-methoxy-6,7- methylenedioxyflavanone (**15**),[14] (*R*)-*N-trans*-feruloyloctopamine (**16**),[18,21] eugenin (**17**),[22] noreugenin (**18**),[23] dihydrooroxylin A (**19**),[24] 6,8-dimethylisogenistein,[17] *N-trans*-feruloyltyramine,[17,18] *N-trans*-feruloyl-4′-*O*-methyldopamine,[25] *trans*-methylferulate,[26] vanillin,[27] 4-hydroxybenzaldehyde,[28] syringaldehyde,[29] ergosterol peroxide,[30] and a mixture of β-sitosterol and stigmasterol,[31] were identified by comparing their physical and spectroscopic data ($[\alpha]_D$, UV, IR, ^{1}H NMR, and MS) with reported values.

dx.doi.org/10.1021/np1008575 |*J. Nat. Prod.* 2011, 74, 976–982

Table 3. ^{1}H and ^{13}C NMR Data of Compounds 7−9[a]

	7		8		9	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2	5.78, dd (12.8, 3.1)	75.9	5.03, d (12.4)	83.3	8.11, s	155.9
3	2.88, dd (17.1, 3.1)	43.1	4.47, dd (12.4, 1.9)	73.1		122.7
	3.04, dd (17.1, 12.8)					
4		198.3		191.3		182.6
5		163.5		152.9		149.6
6	6.05, s	96.9		135.5		130.7
7		165.8		156.7		154.2
8		104.6	6.39, s	99.5		102.3
9		162.0		159.8		151.3
10		103.8		106.4		105.6
1′		127.4		136.4		119.9
2′		155.4	7.54−7.56, m	127.5		156.0
3′	6.95, dd (8.0, 1.0)	116.9	7.26−7.48, m	128.7	7.10, dd (7.8, 1.2)	119.6
4′	7.22, td (8.0, 1.7)	130.7	7.26−7.48, m	129.2	7.36, td (7.8, 1.8)	130.7
5′	6.96, td (8.0, 1.0)	121.4	7.26−7.48, m	128.7	7.00, td (7.8, 1.2)	121.2
6′	7.59, dd (8.0, 1.7)	128.0	7.54−7.56, m	127.5	7.17, dd (7.8, 1.8)	129.8
OH-3[b]			3.96, d (1.9)			
OH-5[b]	12.13, s				12.41, s	
OH-7[b]	8.80 or 9.57, br s		6.44, br s		6.81, br s	
OH-2′[b]	9.57 or 8.80, br s				8.20, br s	
CH_3-8	2.00, s	8.4			2.29, s	7.6
OCH_3-5			3.98, s	61.6		
OCH_3-6			3.94, s	61.6	4.06, s	60.9

[a] ^{1}H NMR data were measured at 400 MHz in $CDCl_3$ for **8** and **9** and in acetone-d_6 for **7**. ^{13}C NMR data were measured at 100 MHz in $CDCl_3$ for **8** and **9** and in acetone-d_6 for **7**. The assignments are based on DEPT, $^{1}H-^{1}H$ COSY, HSQC, and HMBC spectra. [b] D_2O exchangeable.

The pure isolates were evaluated for their in vitro antitubercular effects against *M. tuberculosis* H37Rv with the clinical drug ethambutol (MIC 6.25 μg/mL) as a positive control (Table 5). The results showed moderate to weak antitubercular activity, indicating that **2**, **7**, **14**, **16**, and **19** had MIC values of 25.0, 12.5, 20.0, 50.0, and 50.0 μg/mL, respectively. The antitubercular activity of these isolates was weaker than ethambutol (Table 5). The results showed **2** to be more potent than **3**, and **5** better than **4**, indicating that the methyl group at C-2 of the chromone analogues (**1**−**5**, **12**, **13**, **17**, **18**) possibly plays an important role in the antimycobacterial activity. Compound **2**, with a C-7 hydroxy group, was more potent than **12**, with the C-7 methoxy group. Furthermore, isolates **4** and **5** showed less potency than others, possibly due to the methylenedioxy group at C-6 and C-7. Among the flavonoid analogues **7**, **9**, **14**, **15**, and **19**, the flavanone **14** was more potent than the isoflavonoid 6,8-dimethylisogenistein.[17] The flavanone moiety may serve an important role in antitubercular activity superior to the isoflavonoid moiety with the same functional groups.[32] In addition, compound **14** exhibited weaker antitubercular activity than compound **7**, possibly due to the presence of the methyl group at C-6 of the 5,7,2′-trihydroxy-8-methylflavanone moiety.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Yanaco micromelting apparatus and are uncorrected. Optical rotations were measured using a Jasco P-1020 polarimeter, UV spectra were recorded using a Jasco V-530 UV/vis spectrophotometer, and IR spectra (KBr or neat) were obtained with a Genesis II FTIR spectrophotometer. 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, NOESY, HMQC, HMBC) NMR spectra using $CDCl_3$ or acetone-d_6 as solvents were recorded on Varian Unity-plus 400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) and Varian Unity Inova-600 (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR) spectrometers. Chemical shifts were internally referenced to the solvent signals in $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0) or acetone-d_6 (^{1}H, δ 2.05; ^{13}C, δ 205.1), with TMS as the internal standard. Low-resolution MS data were obtained using a Micromass Trio-2000 GC/MS, VG Biotech Quattro 5022, and JEOL-JMS-HX 100 mass spectrometer. HRMS spectra were recorded using a JEOL JMS-SX102A GC/LC/MS and a Finnigan MAT-95XL high-resolution mass spectrometer. Column chromatography (CC) was performed using 70−230 or 230−400 mesh silica gel (Merck) and Spherical C18 100A reversed-phase silica gel (RP-18) (particle size: 20−40 μm) (SILICYCLE). Preparative TLC was conducted with silica gel 60 F-254 (Merck) and RP-18 F_{254S} (Merck). MPLC (EYELA-VSP-3050) was used for chromatography.

Plant Material. The stems and roots of *P. aculeata* were collected from Mudan, Pingtung County, Taiwan, in September 2007, and the plant was identified by Dr. I. S. Chen. A voucher specimen (no. Chen 6310) has been deposited in the herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. Dried stems and roots (9.1 kg) were extracted with cold MeOH (3 × 10 L) at room temperature to yield an MeOH extract (500 g), which was partitioned between EtOAc−H_2O (1:1) to obtain an EtOAc-soluble (80 g) and an H_2O-soluble fraction. The H_2O-soluble fraction was partitioned between *n*-BuOH−H_2O

dx.doi.org/10.1021/np1008575 |J. Nat. Prod. 2011, 74, 976–982

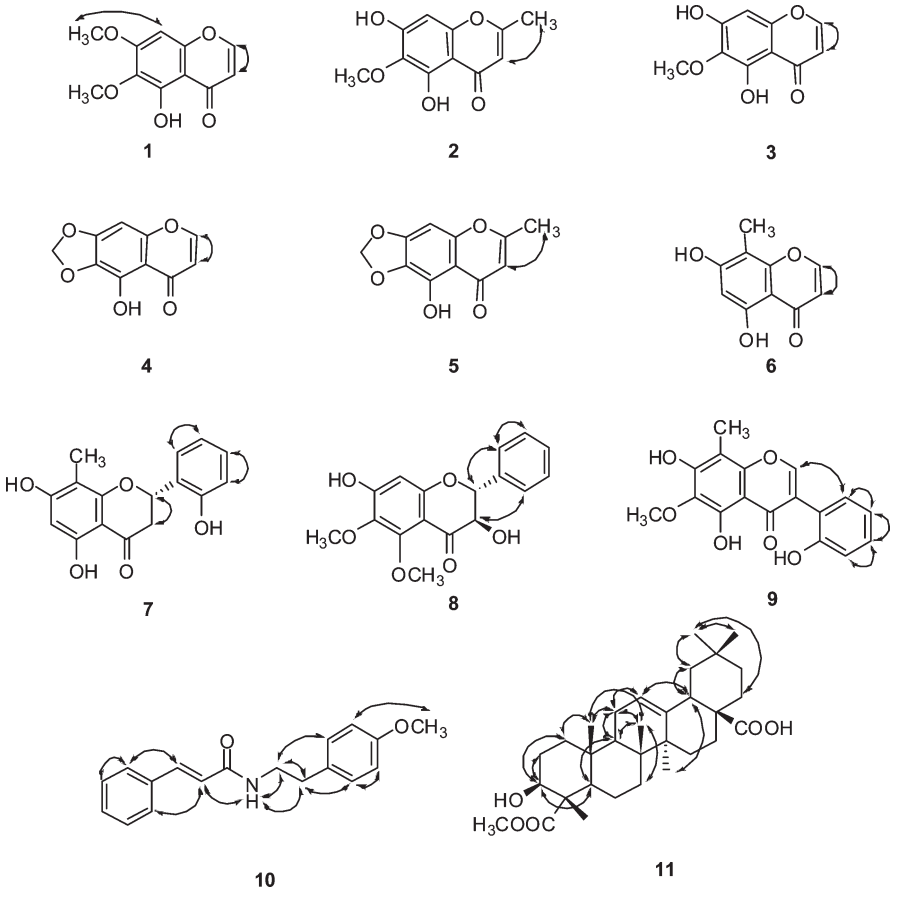


Figure 3. Key NOESY (H⇀H) correlations of **1**−**11**.

(1:1) to provide an *n*-BuOH-soluble (120 g) and an H_2O-soluble fraction (200 g). The active EtOAc-soluble fraction was subjected to silica gel CC eluting with *n*-hexane while gradually increasing the polarity with EtOAc and MeOH to furnish eight fractions (A-1−A-8). Fraction A-4 (8.75 g) was recrystallized from *n*-hexane to give a mixture of *β*-sitosterol and stigmasterol (0.8 g). Fraction A-5 (4.65 g) was subjected to silica gel CC eluting with *n*-hexane while gradually increasing the polarity with acetone to give 10 fractions (A-5-1−A-5-10). Fraction A-5-3 (739 mg) was applied to silica gel MPLC eluting with *n*-hexane and acetone to afford **17** (6.48 mg). Fraction A-5-5 was applied to silica gel CC eluting with *n*-hexane and acetone to afford four fractions (A-5-5-1−A-5-5-4). Fraction A-5-5-3 (460 mg) was subjected to RP-18 gel MPLC, eluting with acetone−H_2O (1:1), to give five fractions (A-5-5-3-1−A-5-5-3-5), of which fraction A-5-5-3-1 afforded **19** (19.8 mg) and ergosterol peroxide (23.1 mg). Fraction A-5-5-3-2 (35.6 mg) was purified by preparative TLC (*n*-hexane−CH_2Cl_2, 1:5) to produce **1** (3.5 mg), vanillin (3.2 mg), and 4-hydroxybenzaldehyde (0.6 mg). Fraction A-5-5-3-3 (34.5 mg) was combined with fraction A-5-5-3-4 (10.9 mg) and purified by preparative TLC (*n*-hexane−$CHCl_3$, 1:10) to afford **12** (5.6 mg), **4** (1.5 mg), **5** (0.6 mg), **13** (3.4 mg), and *trans*-methylferulate (1.3 mg). Fraction A-5-6 (297 mg) was subjected to silica gel CC eluting with CH_2Cl_2 while gradually increasing the polarity with MeOH to give 10 fractions (A-5-6-1−A-5-6-10). Fraction A-5-6-3 (43.3 mg) was subjected to RP-18 CC, eluting with acetone and H_2O, to afford **15** (1.9 mg), **9** (6.8 mg), and 6,8-dimethylisogenistein (5.8 mg). Fraction A-5-6-4 (46.9 mg) was purified by preparative RP-18 TLC (MeOH−H_2O, 1:1) to give **2** (2.4 mg) and **3** (1.0 mg). Fraction A-5-6-5 (23.7 mg) was purified by preparative RP-18 TLC (acetone−H_2O, 1:1) to afford **6** (1.7 mg). Fraction A-5-7 (1.03 g) was applied to silica gel CC eluting with *n*-hexane while gradually increasing the polarity with acetone to produce 11 fractions (A-5-7-1−A-5-7-11). Fraction A-5-7-5 (572 mg) was subjected to silica gel CC eluting with *n*-hexane while gradually increasing the polarity with EtOAc to obtain 13 fractions (A-5-7-5-1−A-5-7-5-13). Fraction A-5-7-5-3 (41.2 mg) was purified by preparative RP-18 TLC (MeOH−H_2O, 3:1) to afford **14** (10.9 mg). Fraction A-5-7-5-6 (21.0 mg) was purified by preparative RP-18 TLC (MeOH−H_2O, 1:1) to give **18** (2.1 mg). Fraction A-5-7-5-8 (21.5 mg) was purified by preparative TLC (CH_2Cl_2−EtOAc, 30:1) to afford **8** (2.5 mg), **10** (1.3 mg), and syringaldehyde (4.7 mg). Fraction A-5-7-5-9 (51.5 mg) was purified by preparative TLC (CH_2Cl_2−EtOAc, 20:1) to afford **11** (7.7 mg). Fraction A-5-8 (435 mg) was subjected to silica gel CC eluting with CH_2Cl_2 while gradually increasing the polarity with MeOH to produce five fractions (A-5-8-1−A-5-8-5). Fraction A-5-8-2 (59.6 mg) was purified by preparative TLC (CH_2Cl_2−MeOH, 30:1) to give **7** (5.6 mg). Fraction A-6 (6.40 g) was subjected to silica gel CC eluting with CH_2Cl_2 while gradually increasing the polarity with EtOAc to give 15 fractions (A-6-1−A-6-15). Fraction A-6-15 (120.7 mg) was subjected to silica gel CC eluting with CH_2Cl_2 while gradually increasing the polarity with EtOAc to give 10 fractions (A-6-15-1−A-6-15-10). Fraction A-6-15-4 (53.2 mg) was purified by preparative TLC ($CHCl_3$−MeOH, 20:1) to give *N-trans*-feruloyltyramine (22.1 mg) and *N-trans*-feruloyl-4′-*O*-methyldopamine (23.6 mg). Fraction A-6-15-7 (35.1 mg) was purified by preparative TLC ($CHCl_3$−MeOH, 20:1) to afford **16** (12.5 mg).

Pisonin A (***1***): colorless needles (MeOH); mp 123−124 °C; UV (MeOH) λ_{max} (log ε) 226 sh (3.97), 256 (3.92), 295 (3.70) nm; UV (MeOH + KOH) λ_{max} (log ε) 263 (3.92), 334 (3.82) nm; IR (KBr) ν_{max} 3436 (OH), 1661 (C═O), 1618, 1571, 1500 (benzene ring) cm^{-1};

dx.doi.org/10.1021/np1008575 |*J. Nat. Prod.* 2011, 74, 976–982

Table 4. ^{1}H and ^{13}C NMR Data of Compound 10[a]

position	δ_H (J in Hz)	δ_C
1		134.8
2	7.48, m	127.8
3	7.36, m	128.8
4	7.36, m	129.7
5	7.36, m	128.8
6	7.48, m	127.8
7	7.62, d (15.6)	141.1
8	6.31, d (15.6)	120.5
9		165.8
1′		130.8
2′, 6′	7.14, d (8.7)	129.7
3′, 5′	6.87, d (8.7)	114.1
4′		158.3
7′	2.83, t (6.8)	34.7
8′	3.63, q (6.8)	40.9
OH-7′[b]		
OCH_3-3		
OCH_3-4′	3.80, s	55.3
NH[b]	5.57, br s	

[a] ^{1}H NMR data were measured at 600 MHz, and ^{13}C NMR data were measured at 150 MHz in $CDCl_3$. The assignments of **10** are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. [b] D_2O exchangeable.

^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; ESIMS m/z 245 $[M + Na]^+$; HRESIMS m/z 245.0428 $[M + Na]^+$ (calcd for $C_{11}H_{10}O_5Na$, 245.0426).

Pisonin B (**2**): colorless needles (acetone); mp 164−166 °C; UV (MeOH) λ_{max} (log ε) 231 (4.32), 251 (4.22), 296 (4.02) nm; UV (MeOH + KOH) λ_{max} (log ε) 226 (4.36), 264 (4.12), 336 (4.08) nm; IR (KBr) ν_{max} 3431 (OH), 1645 (C═O), 1559, 1486 (benzene ring) 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 245 $[M + Na]^+$; HRESIMS m/z 245.0427 $[M + Na]^+$ (calcd for $C_{11}H_{10}O_5Na$, 245.0426).

Pisonin C (**3**): colorless needles (MeOH); mp 184−186 °C; UV (MeOH) λ_{max} (log ε) 228 (3.97), 257 (3.92), 289 (3.68) nm; UV (MeOH + KOH) λ_{max} (log ε) 263 (3.82), 351 (3.09) nm; IR (KBr) ν_{max} 3426 (OH), 1651 (C═O), 1610, 1543, 1496 (benzene ring) cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz), see Table 2; ESIMS m/z 231 $[M + Na]^+$; HRESIMS m/z 231.0271 $[M + Na]^+$ (calcd for $C_{11}H_{10}O_5Na$, 231.0269).

Pisonin D (**4**): colorless needles ($CHCl_3$); mp 155−157 °C; UV (MeOH) λ_{max} (log ε) 233 (3.80), 262 (3.65), 289 (3.48), 326 (3.20) nm; UV (MeOH + KOH) λ_{max} (log ε) 236 sh (3.73), 269 (3.67), 339 (3.06) nm; IR (KBr) ν_{max} 3423 (OH), 1686 (C═O), 1628, 1571, 1465 (benzene ring), 1029, 933 (OCH_2O) 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 229 $[M + Na]^+$; HRESIMS m/z 229.0112 $[M + Na]^+$ (calcd for $C_{10}H_6O_5Na$, 229.0113).

Pisonin E (**5**): colorless needles (CH_2Cl_2); mp 172−174 °C; UV (MeOH) λ_{max} (log ε) 237 (4.12), 259 (3.89), 289 (3.78), 321 (3.45) nm; UV (MeOH + KOH) λ_{max} (log ε) 239 (4.03), 265 (3.94), 343 (3.30) nm; IR (KBr) ν_{max} 3416 (OH), 1682 (C═O), 1629, 1576, 1472 (benzene ring), 1033, 929 (OCH_2O) 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 243 $[M + Na]^+$; HRESIMS m/z 243.0268 $[M + Na]^+$ (calcd for $C_{11}H_8O_5Na$, 243.0269).

Table 5. Antitubercular Activities of Isolates from *P. aculeata* on *M. tuberculosis* H37Rv

compound	MIC (μg/mL)
pisonin A (**1**)	≥100
pisonin B (**2**)	25.0
pisonin C (**3**)	80
pisonin D (**4**)	≥236
pisonin E (**5**)	≥190
pisonivanone (**7**)	12.5
pisonianone (**9**)	≥175
leptorumol (**13**)	82.9
(2*S*)-2′-hydroxydemethoxymatteucinol (**14**)	20.0
(2*R*,3*R*)-3-hydroxy-5-methoxy-6, 7-methylenedioxyflavanone (**15**)	≥100
(*R*)-*N*-*trans*-feruloyloctopamine (**16**)	50.0
eugenin (**17**)	103
noreugenin (**18**)	110
dihydrooroxylin A (**19**)	50.0
ethambutol[a]	6.25

[a] Positive control.

Pisonin F (**6**): colorless needles (MeOH); mp >197 °C (dec); UV (MeOH) λ_{max} (log ε) 222 sh (3.96), 259 (4.15), 299 (3.54), 321 (3.36) nm; UV (MeOH + KOH) λ_{max} (log ε) 222 sh (4.03), 270 (4.18), 329 (3.68) nm; IR (KBr) ν_{max} 3432 (OH), 1663 (C═O), 1610, 1557, 1495 (benzene ring) cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 100 MHz), see Table 2; ESIMS m/z 215 $[M + Na]^+$; HRESIMS m/z 215.0319 $[M + Na]^+$ (calcd for $C_{10}H_8O_4Na$, 215.0320).

Pisonivanone (**7**): colorless needles (EtOAc); mp 192−194 °C; $[\alpha]^{24}_D$ −102 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 238 sh (3.69), 289 (4.12), 335 (3.45) nm; UV (MeOH + KOH) λ_{max} (log ε) 249 sh (3.61), 279 (3.48), 328 (4.30) nm; IR (KBr) ν_{max} 3374 (OH), 1642 (C═O), 1600, 1505, 1457 (benzene ring) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; ESIMS m/z 309 $[M + Na]^+$; HRESIMS m/z 309.0741 $[M + Na]^+$ (calcd for $C_{16}H_{14}O_5Na$, 309.0739).

Pisonivanol (**8**): colorless needles (MeOH); mp 167−169 °C; $[\alpha]^{24}_D$ +27 (c 0.05, MeOH); CD $[\theta]_{341}$ +12 213, $[\theta]_{326}$ 0, $[\theta]_{308}$ −13 309 (c 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 236 sh (4.03), 279 (4.01), 318 (3.54) nm; UV (MeOH + KOH) λ_{max} (log ε) 253 (3.80), 331 (4.21) nm; IR (KBr) ν_{max} 3391 (OH), 1676 (C═O), 1607, 1576, 1474 (benzene ring) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; ESIMS m/z 339 $[M + Na]^+$; HRESIMS m/z 339.0843 $[M + Na]^+$ (calcd for $C_{17}H_{16}O_6Na$, 339.0845).

Pisonianone (**9**): yellowish needles (MeOH); mp 184−186 °C; UV (MeOH) λ_{max} (log ε) 267 (4.31), 340 (3.66), 395 (3.21) nm; UV (MeOH + KOH) λ_{max} (log ε) 279 (4.33), 346 (3.98) nm; IR (KBr) ν_{max} 3352 (OH), 1652 (C═O), 1614, 1569, 1486 (benzene ring) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; ESIMS m/z 337 $[M + Na]^+$; HRESIMS m/z 337.0690 $[M + Na]^+$ (calcd for $C_{17}H_{14}O_6Na$, 337.0688).

Pisoniamide (**10**): colorless needles (MeOH); mp 142−144 °C; UV (MeOH) λ_{max} (log ε) 215 (4.20), 220 (4.19), 273 (4.23), 296 sh (3.88) nm; IR (KBr) ν_{max} 3308 (NH), 1656 (C═O), 1620, 1510 (benzene ring) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 4; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 4; ESIMS m/z 304 $[M + Na]^+$; HRESIMS m/z 304.1314 $[M + Na]^+$ (calcd for $C_{18}H_{19}NO_2Na$, 304.1313).

dx.doi.org/10.1021/np1008575 |*J. Nat. Prod.* 2011, 74, 976–982

Pisonolic acid (***11***): colorless needles (MeOH); mp 257−258 °C; $[\alpha]^{24}_{D}$ +51 (*c* 0.1, $CDCl_3$); IR (KBr) ν_{max} 3448 (OH), 1713, 1697 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.72 (3H, s, CH_3-26), 0.90 (3H, s, CH_3-29), 0.92 (6H, s, CH_3-25, 30), 1.13 (6H, s, CH_3-24, 27), 2.81 (1H, dd, *J* = 13.4, 4.2 Hz, H-18), 3.72 (3H, s, OCH_3-23), 3.99 (1H, dd, *J* = 11.6, 4.4 Hz, H-3), 5.27 (1H, t, *J* = 3.4 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.8 (CH_3-24), 15.7 (CH_3-25), 17.0 (CH_3-26), 21.1 (C-6), 22.8 (C-16), 23.3 (C-11), 23.5 (CH_3-29), 26.0 (CH_3-27), 26.4 (C-2), 27.6 (C-15), 30.7 (C-20), 32.1 (C-7), 32.4 (C-22), 33.0 (CH_3-30), 33.7 (C-21), 36.5 (C-10), 38.0 (C-1), 39.5 (C-8), 40.9 (C-18), 41.6 (C-14), 45.8 (C-19), 46.4 (C-17), 47.6 (C-9), 51.2 (C-5), 52.1 (OCH_3-23), 53.8 (C-4), 75.5 (C-3), 122.3 (C-12), 143.6 (C-13), 178.2 (C-23), 183.4 (C-28); ESIMS *m/z* 523 $[M + Na]^+$; HRESIMS *m/z* 523.3401 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_5Na$, 523.3400).

Antitubercular Activity Assay. The in vitro antitubercular activity of each compound was evaluated using the *M. tuberculosis* strain H37Rv. Middlebrook 7H10 agar was used, and the MIC values of the compounds were determined, as recommended by the agar proportion method.[33] Briefly, each test compound was added to Middlebrook 7H10 agar and supplemented with oleic acid-albumin-dextrose-catalase (OADC) at 50−56 °C by serial dilution to yield final concentrations of 100 to 0.8 μg/mL. Next, 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 were used as the initial inoculum. These inoculums were prepared by diluting the initial inoculum in Middlebrook 7H9 broth until the turbidity was reduced to the equivalent of 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} standardized bacterial suspension dilutions was placed on each quadrant of the agar plates. The agar plates were then incubated at 35 °C with 10% CO_2 for two weeks. The MIC is the lowest concentration of test compound that completely inhibited the growth of the test isolate of *M. tuberculosis*, as detected by eye.

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR and HMBC spectra for compounds **1**−**11** are available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was supported by a grant from the National Science Council of the Republic of China (NSC 96-2320-B-037-015).

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