Flavanone and Diphenylpropane Glycosides and Glycosidic Acyl Esters from *Viscum articulatum*

Yu-Jen Kuo,[†,‡] Yu-Ching Yang,[†,§] Li-Jie Zhang,[†] Ming-Der Wu,[⊥] Li-Ming Yang Kuo,[†,‡] Yuh-Chi Kuo,[||] Syh-Yuan Hwang,[||] Cheng-Jen Chou,[†] Kuo-Hsiung Lee,[△] Hsiu-O Ho,[*,‡] and Yao-Haur Kuo[*,†,§]

Division of Herbal Drugs and Natural Products, National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China, School of Pharmacy, Taipei Medical University, Taipei 110, Taiwan, Republic of China, Institute of Life Science, National Taitung University, Taitung, 900, Taiwan, Republic of China, Food Industry Research and Development Institute, Hsinchu 300, Taiwan, Republic of China, Endemic Species Research Institute, Council of Agriculture, Nantou County 552, Taiwan, Republic of China, and Laboratory of Natural Products, School of Pharmacy, University of North Carolina, North Carolina 27599

Received July 15, 2009

Seven new compounds including three flavanone glycosides, visartisides A−C (**1**−**3**), three glycoside acyl esters, visartisides D−F (**4**−**6**), and one diphenylpropane glycoside, (4′-hydroxy-2′,3′,6′,3″-tetramethoxy-1,3-diphenylpropane)-4″-*O*-*β*-D-glucopyranoside (**7**), along with four known flavanone glycosides (**8**−**11**) were isolated from the leaves and stems of *Viscum articulatum*. The structure elucidation of **1**−**7** was based on spectroscopic data analysis. Biological evaluation showed that **1**, **2**, and **10** exhibited antioxidant activity using a DPPH method and that compounds **1**, **3**, and **11** were active in a lipopolysaccharide-induced nitric oxide assay.

The leaves and stems of *Viscum articulatum* Burm. (*Aspidixia articulata* Burm. f.) (Viscaceae) are used traditionally in Chinese medicine for the treatment of diseases such as hemorrhage, pleurisy, gout, heart disease, arthritis, and hypertension.[1] Previous phytochemical investigations have revealed that flavonoids, triterpenoids, and organic acids are the major secondary metabolites of this genus.[2] Antioxidative and apoptosis-inducing activities of plants in the genus *Viscum* have also been reported in recent years.[3,4] Free radicals are involved in many disorders such as neurodegenerative diseases, cancer, and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. 1,1-Diphenyl-2-picryl hydrazyl (DPPH) is used as a substrate to evaluate free-radical scavenging activity of antioxidants of a specific compound or plant extracts.[5] Using the DPPH assay, it was found that an EtOH extract of *V. articulatum* has antioxidative activity, and further investigation of the partitioned EtOAc layer of *V. articulatum* by column chromatography and/or HPLC led to the isolation and characterization of three new flavanone glycosides (**1**−**3**), three glycoside acyl esters (**4**−**6**), and a diphenylpropane glycoside (**7**), along with four known flavanone glycosides, pinocembrin 7-*O*-*β*-D-glucopyranoside (**8**), naringenin 7-*O*-*β*-D-glucopyranoside (**9**), eriodictyol 7-*O*-*β*-D-glucopyranoside (**10**), and homoeriodictyol 7-*O*-*β*-D-glucopyranoside (**11**). The structure elucidation of **1**−**7** was based on spectroscopic data analysis, mainly using HRESIMS and 1D- and 2D-NMR experiments. The isolated compounds (**1**−**11**) were assayed for their antioxidant and anti-inflammatory activities.

Results and Discussion

Compound **1** was obtained as a yellow powder. The HRESIMS of **1** showed a quasimolecular ion $[M + Na]^+$ at *m/z* 603.1544, indicating the elemental formula $C_{30}H_{28}O_{12}$. The IR spectrum exhibited absorption bands at 1576, 1524, and 1445 cm^{-1} (benzyl) and strong absorption bands at 3445, 1714, and 1635 cm^{-1} (hydroxy group, carbonyl, and double bonds, respectively). This evidence, together with UV absorption bands at 281 nm, suggested the presence of a flavanone skeleton.[7] The ^1H NMR spectrum showed resonances suggesting a 5,7-disubstituted aromatic ring. In the ^1H and ^{13}C NMR spectra of **1** (Table 1), two phenolic carbons (δ_C 145.9 and 145.4) and three aromatic protons [δ_H 7.01 (1H, s), 6.85 (2H, brs)] in the B ring of the flavanone moiety were found, which were assigned as C-3′, C-4′, H-2′, H-5′, and H-6′, respectively. A conjugated carbonyl carbon at δ_C 197.4 and an intramolecularly bonded hydroxy group at δ_H 12.05 (1H, s), together with two broad singlet signals at δ_H 6.10 (1H, s) and 6.12 (1H, s) that were assigned to H-6 and H-8, respectively, were also observed. Accordingly, the flavanone moiety in **1** was identified as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromanone, in agreement with published data of eriodictyol.[8]

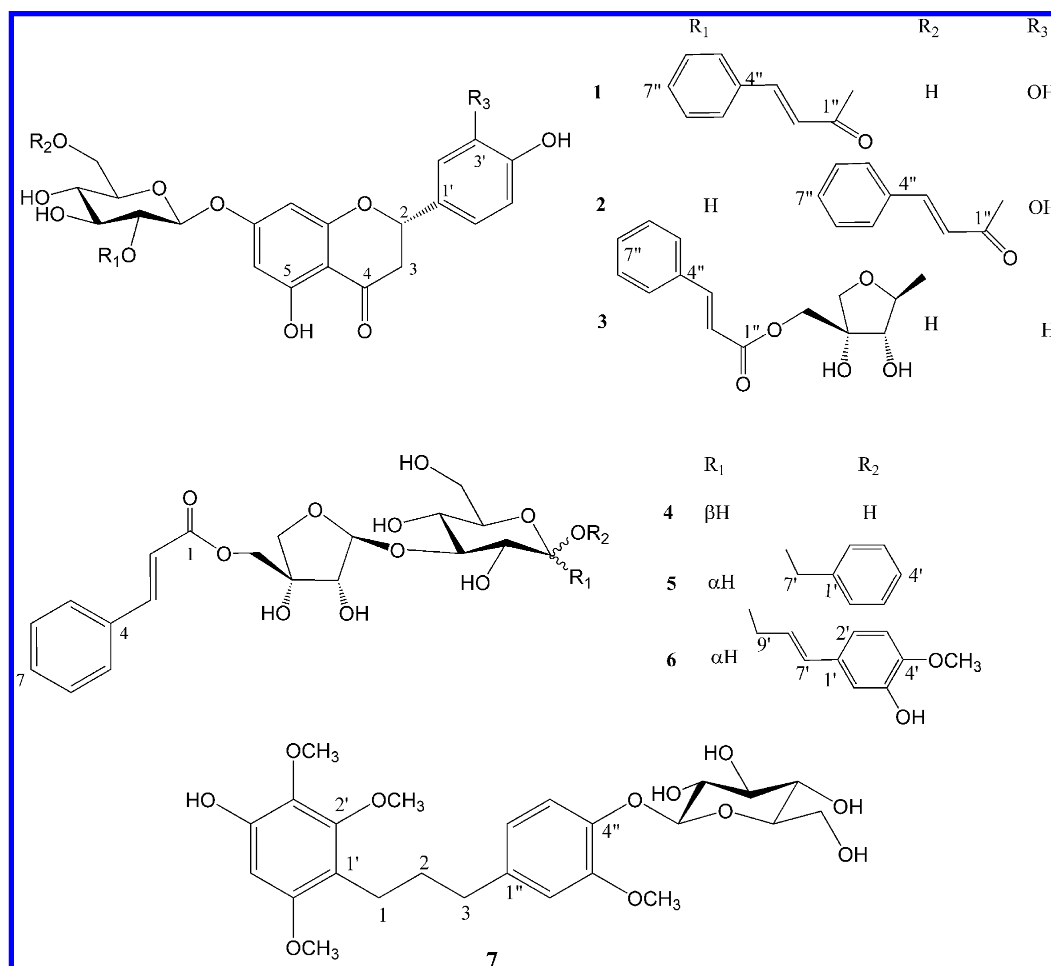
In addition, proton signals for an aromatic moiety [δ_H 7.66 (2H, brs), 7.42 (3H, brs)] and one pair of *trans*-vinyl protons [δ_H 7.73 (1H, d, $J = 16.0$ Hz) and 6.56 (1H, d, $J = 16.0$ Hz)], as well as a carboxyl carbon signal at δ_C 165.5, were obtained, which gave evidence for **1** as possessing a *trans*-cinnamoyl moiety. Characteristic proton signals for a hexose moiety [δ_H 5.36 (1H, d, $J =$ 8.0), 5.13 (1H, dd, $J = 9.0$, 8.0), 3.40−3.90 (5H)] and an anomeric proton at δ_H 5.36 (1H, d, $J = 8.0$ Hz) were also observed. The C-1 of the hexose should be in a *β*-configuration, due to the coupling constant of the anomeric proton[9] and by comparing the ^1H and ^{13}C NMR data with those of glucose.[10] Furthermore, on acid hydrolysis of **1** with 2 N HCl, the released sugar unit was identified as D-glucose by GC analysis ($t_R = 21.41$ min) comparing with an authentic sample (D-glucose $t_R = 21.69$ min; L-glucose $t_R = 23.28$ min). Therefore, the structure of **1** was deduced as having an eriodictyol skeleton with a *trans*-cinnamoyl moiety and a *β*-D-glucose moiety.[11,12]

A long-range correlation between δ_H 5.36 (Glc-H-1) and δ_C 165.4 (C-7) of the aglycon was observed in the HMBC spectrum (Figure 1), revealing the glucose moiety connection to C-7 of the flavanone. Also, the long-range correlation between δ_H 5.13 (Glc-H-2) and the carboxyl carbon (δ_C 165.5, C-1″) confirmed the connectivity of the *trans*-cinnamoyl group with C-2 of the *β*-D-glucose unit. The circular dichroism (CD) spectrum of **1** exhibited a negative Cotton effect at 293 nm. Therefore, C-2 in **1** was determined to be in the *S*-configuration.[12] On the basis of the above, the structure of

* To whom correspondence should be addressed. Tel: 886-02-28201999, ext. 7051; 886-02-27361661, ext. 6126. E-mail: kuoyh@nricm.edu.tw; hsiuoho@tmu.edu.tw.
† National Research Institute of Chinese Medicine (NRICM).
‡ Taipei Medical University (TMU).
§ National Taitung University (NTU).
⊥ Food Industry Research and Development Institute (FIRDI).
|| Council of Agriculture.
△ University of North Carolina.

10.1021/np9004294 © 2010 American Chemical Society and American Society of Pharmacognosy
Published on Web 02/01/2010

Chart 1



1 was elucidated as (2*S*)-eriodictyol 7-*O*-(2-*O*-*trans*-cinnamoyl)-β-D-glucopyranoside, and this compound has been named visartiside A.

Compound **2** was obtained as a yellow powder, and its molecular formula was determined as $C_{30}H_{28}O_{12}$ by HRESIMS analysis, which showed a quasimolecular ion $[M + Na]^+$ at *m/z* 603.1535. The UV, IR, ^1H NMR, and ^{13}C NMR spectroscopic data of **2** were very similar to those of **1**. The proton signals of H_2-Glc-6 of **2** were shifted downfield at δ_H 4.60 (1H, dd, $J = 12.0$, 11.5 Hz, Glc-H-6a) and 4.33 (1H, d, $J = 12.0$ Hz, Glc-H-6b), when compared with these same signals at δ_H 3.93 (m) and 3.76 (m) in **1**. These data, together with the HMBC spectrum of **2** showing correlations between the carboxyl carbon δ_C 166.4 (C-1″) and δ_H 4.60 (Glc-H-6a) and 4.33 (Glc-H-6b), were consistent with a linkage between a *trans*-cinnamoyl moiety and C-6 of a β-glucose unit. Acid hydrolysis of **2** afforded a β-D-glucose moiety. Also, a linkage between the glucose and C-7 of flavanone was indicated, due to the correlation between δ_H 5.15 (1H, d, $J = 7.5$ Hz, Glc-H-1) and δ_C 165.8 (C-7). From these findings, together with the CD spectrum of **2**, which exhibited a similar negative Cotton effect at 293 nm to that of **1**, the structure of **2** (visartiside B) was determined to be (2*S*)-eriodictyol 7-*O*-(6-*O*-*trans*-cinnamoyl)-β-D-glucopyranoside.

Compound **3** was obtained as a white powder and gave the quasimolecular ion $[M + Na]^+$ at *m/z* 719.2030, indicating an elemental formula of $C_{35}H_{36}O_{15}$. Due to its similar UV and IR spectra to **1**, the structure of **3** was shown to have a flavanone skeleton containing cinnamoyl and sugar units. Although **1** and **3** showed very closely comparable ^1H and ^{13}C NMR data, additional signals for a pentose moiety were shown in the ^1H and ^{13}C NMR spectra of **3**. In addition, the presence of two pairs of aromatic protons at δ_H 6.68 (2H, d, $J = 7.5$) and 7.09 (2H, d, $J = 8.0$), appearing as an A_2B_2 spin−spin system in **3**, instead of three aromatic protons [δ_H 7.01 (1H, s), 6.85 (2H, brs)] in **1**, was also found. These data, together with literature reported values,[13] were in agreement with the structure of **3** containing a narigenin [(2*S*)-2-(4-hydroxyphenyl)-5,7-dihydroxy-4-chromanone] moiety.

On the basis of the chemical shifts of the anomeric protons at δ_H 4.96 (d, $J = 7.0$ Hz) and 5.39 (s), along with the other characteristic ^{13}C NMR data as shown in Table 1, the structure of **3** was deduced as possessing glucose and apiose moieties.[14] Due to the magnitude of the coupling constants of the anomeric proton δ_H 4.96 (1H, d, $J = 7.0$ Hz, Glc-H-1) and δ_H 5.39 (1H, s Api-H-1), the sugars were assigned to have a β-configuration.[9,14] The glucosides were identified as D-glucose and D-apiose by hydrolysis of **3** with 2 N HCl and GC analysis (D-glucose, $t_R = 21.55$ min; D-apiose, $t_R = 21.30$ min). Moreover, in the HMBC spectrum (Figure 1), ^3J correlations between Api-H-1 [δ_H 5.39 (1H, s)] and Glc-C-2 (δ_C 76.5), between Api-H_2-5 [δ_H 4.10 (2H, brs)] and C-1″ [(δ_C 166.9), carboxyl of *trans*-cinnamoyl group], and between Glc-H-1 [δ_H 4.96 (d, $J = 7.0$ Hz)] and C-7 (δ_C 165.4) were found. Thus the linkages between the cinnamic acid, apiose, glucose, and naringenin units in **3** were determined. Therefore, the structure of **3** (visartiside C) was elucidated as (2*S*)-naringenin 7-*O*-[5-*O*-*trans*-cinnamoyl-β-D-apiosyl(1→2)]-β-D-glucopyranoside.

Compound **4** was obtained as a white powder. The molecular formula of **4** was determined to be $C_{20}H_{26}O_{11}$ on the basis of HRESIMS, which exhibited a quasimolecular ion $[M + Na]^+$ at *m/z* 465.1392. The IR spectrum of **4** showed absorption bands at 3382 (OH), 1722 (carboxyl group), 1627 (double bond), and 1587 and 1445 cm^{-1}, ascribable to a benzene ring function. In the ^1H NMR spectrum (Table 2), signals for two sugar moieties at δ_H 5.26 (1H, d, $J = 3.0$ Hz), 5.17 (1H, s), 4.32 (2H, brs), 4.10 (1H, dd, *J*

Table 1. ^{1}H and ^{13}C NMR (CD_3OD, 500 and 125 MHz) Data of **1**−**3**

	1		**2**		**3**	
position	δ_H, mult., Hz	δ_C	δ_H, mult., Hz	δ_C	δ_H, mult., Hz	δ_C
2	5.40 dd (13.0, 3.0)	79.4	5.39 dd (12.5, 3.5)	79.4	5.02 dd (13.0, 3.0)	79.3
3	3.17 dd (17.0, 13.0)(α)	42.8	3.13 dd (17.0, 12.5)(α)	42.9	2.83 dd (16.5, 13.0)(α)	42.7
	2.77 dd (17.0, 3.0)(β)		2.75 dd (17.5, 3.0)(β)		2.40 dd (17.0, 3.0)(β)	
4		197.4		197.2		197.1
5		164.0		164.1		163.8
6	6.10 s	96.8	6.22 s	96.8	6.00 s	96.5
7		165.4		165.8		165.4
8	6.12 s	95.8	6.16 s	96.0	5.97 s	95.5
9		163.4		163.2		163.1
10		104.0		103.9		103.7
1′		130.6		130.7		130.4
2′	7.01 brs	114.1	7.02 s	114.0	7.09 d (8.0)	127.9
3′		145.9		145.8	6.68 d (7.5)	115.2
4′		145.4		145.4		157.9
5′	6.85 brs	115.4	6.84 brs	115.4	6.68 d (7.5)	115.2
6′	6.85 brs	118.6	6.84 brs	118.6	7.09 d (8.0)	127.9
Glc-1	5.36 d (8.0)	98.3	5.15 d (7.5)	100.2	4.96 d (7.0)	98.3
Glc-2	5.13 dd (9.0, 8.0)	73.7	3.53 m	73.8	3.58 m	76.5
Glc-3	3.71 d (9.0)	75.0	3.60 m	77.0	3.30 m	77.0
Glc-4	3.62 m	70.6	3.52 m	70.5	3.29 m	70.0
Glc-5	3.83 m	77.4	3.93 ddd (9.0, 6.5, 2.0)	74.5	3.85 m	77.5
Glc-6	3.93 m	61.6	4.60 d (12.0)	63.7	3.75 m	61.1
	3.76 m		4.33 dd (12.0, 11.5)		3.52 m	
1″		165.5		166.4		166.9
2″	6.56 d (16.0)	118.1	6.57 d (16.0)	118.1	6.23 d (16.0)	117.2
3″	7.73 d (16.0)	145.2	7.68 d (16.0)	145.0	7.44 d (16.0)	145.4
4″		134.7		134.7		134.4
5″	7.66 brs	128.4	7.64 brs	128.4	7.34 brs	128.2
6″	7.42 brs	129.2	7.43 brs	129.1	7.27 brs	128.9
7″	7.42 brs	130.5	7.43 brs	130.5	7.27 brs	129.6
8″	7.42 brs	129.2	7.43 brs	129.1	7.27 brs	128.9
9″	7.66 brs	128.4	7.64 brs	128.4	7.34 brs	128.2
OH-5	12.05 s		12.10 s		11.81 s	
Api-1					5.39 s	109.0
Api-2					3.54 s	77.0
Api-3						78.0
Api-4					4.10 brs 3.77 m	74.0
Api-5					4.10 brs (2H)	66.8

= 9.5, 3.0 Hz), 4.07 (1H, s), 3.86 (1H, dd, J = 9.5, 2.5 Hz), 3.83 (1H, m), 3.77 (2H, brs), 3.68 (1H, m), and 3.34 (2H, brs) were found in **4**, in addition to the signals of a *trans*-cinnamoyl group. These data, together with the ^{13}C NMR data, supported the presence of a *trans*-cinnamoyl group, a glucose,[9] and an apiose[14] in **4**. The coupling constants of anomeric protons δ_H 5.26 (1H, d, J = 3.0 Hz, Glc-H-1) and 5.17 (1H, s, Api-H-1) suggest that the glycones were α-glucose[9] and β-apiose.[14] In addition, acid hydrolysis with 2 N HCl of **4** and GC analysis identified the sugar units as D-glucose (t_R = 21.96 min) and D-apiose (t_R = 21.40 min). Long-range correlations between the signal of δ_H 5.17 (Api-H-1) and δ_C 80.3 (Glc-C-3) and between δ_H 4.32 (2H, brs, Api-H_2-5) and δ_C 167.2 (C-1) of the carboxyl in the *trans*-cinnamoyl group indicated the linkage of the two sugars by Glc-3-*O*-Api-1 and the connectivity

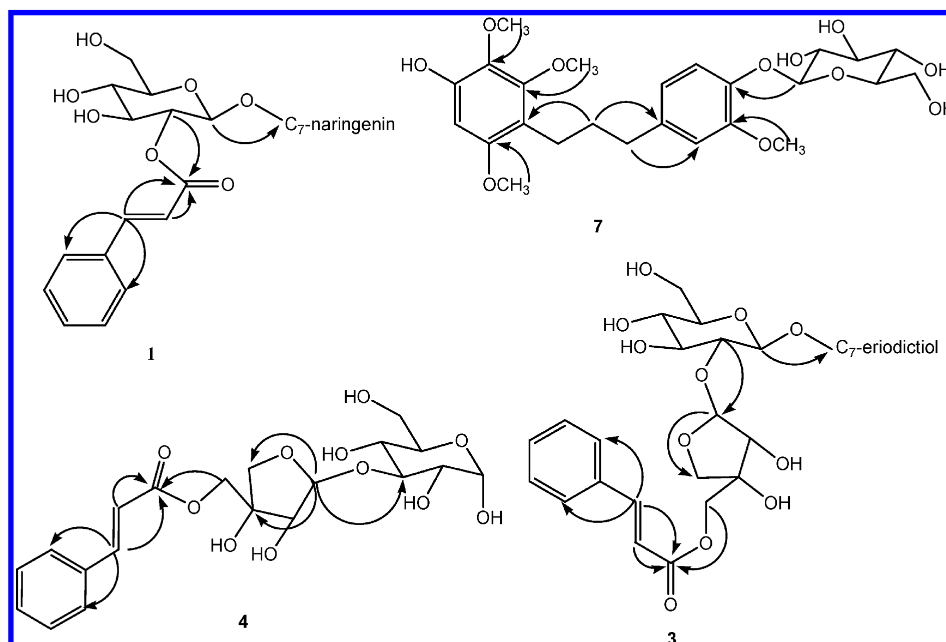


Figure 1. Major HMBC correlations of compounds **1**, **3**, **4**, and **7**.

Table 2. ^{1}H and ^{13}C NMR (CD_3OD, 500 and 125 MHz) Data of **4**−**6**

	4		**5**		**6**	
position	δ_H, mult., Hz	δ_C	δ_H, mult., Hz	δ_C	δ_H, mult., Hz	δ_C
1		167.2		168.4		168.5
2	6.58 d (16.0)	117.4	6.43 d (16.0)	118.6	6.45 d (16.0)	118.5
3	7.77 d (16.0)	145.5	7.61 d (16.0)	146.6	7.67 d (16.0)	146.6
4		134.6		135.7		135.6
5	7.61 brs	128.1	7.49 m	129.3	7.50 t (3.5)	129.3
6	7.41 brs	128.9	7.19 d (7.0)	130.0	7.38 m	130.0
7	7.41 brs	130.4	7.11 t (9.0)	131.6	7.38 m	131.6
8	7.41 brs	128.9	7.19 d (7.0)	130.0	7.38 m	130.0
9	7.61 brs	128.1	7.49 m	129.3	7.50 t (3.5)	129.3
G1	5.26 d (3.0)	92.5	4.34 d (7.0)	102.1	4.40 d (7.5)	102.0
G2	3.77 brs	71.5	3.41 m	78.8	3.51	78.9
G3	3.34 brs	80.3	3.41 m	78.3	3.43	77.9
G4	3.34 brs	70.7	3.41 m	71.7	3.43	71.8
G5	3.77 brs	72.5	3.18 m	77.9	3.51	77.8
G6	3.68 m	61.5	3.60 m	62.8	3.66 dd (6.0, 12.0)	62.8
	3.83 m		3.80 d (10.0)		3.86 dd (1.6, 10.0)	
A1	5.17 s	111.0	5.34 s	110.2	5.45 s	109.9
A2	4.07 s	77.4	3.41 m	78.4		78.3
A3		77.8		79.1		79.3
A4	4.10 dd (9.5, 3.0)	74.0	3.60 m	75.4	3.79 m	75.6
	3.86 dd (9.5, 2.5)		3.90 d (9.5)		4.21 m	
A5	4.32 s (2H)	66.5	4.10 d (11.0)	68.6	4.31 d (11.5)	68.8
			4.20 d (11.0)		4.37 d (11.5)	
1′				139.0		130.3
2′			7.30 d (7.0)	129.2	6.96 s	110.5
3′			7.19 t (7.0)	129.3		149.1
4′			7.29 t (7.0)	128.7		147.7
5′			7.19 t (7.0)	129.3	6.80 dd (8.0, 1.5)	121.2
6′			7.30 d (7.0)	129.2	6.65 d (8.0)	116.2
7′			4.83 d (11.5)	71.7	6.54 d (16.0)	134.3
			4.50 d (11.0)			
8′					6.15 brd (16.0)	123.5
9′					4.21 dd (6.5, 12.0)	70.9
					4.48 dd (6.0, 12.0)	
OMe					3.79 s	56.3

between C-5 of β-D-apiose and C-1 of the *trans*-cinnamoyl moiety. Accordingly, the structure of **4** (visartiside D) was elucidated as 5-*O*-*trans*-cinnamoyl β-D-apiosyl (1→3)-α-D-glucopyranoside.

Compound **5** was isolated as a yellow powder, and its UV and IR spectra were very close to those of **4**. The quasimolecular ion $[M + Na]^+$ at *m/z* 555.1836 indicated an elemental formula of $C_{27}H_{32}O_{11}Na$, which suggested an additional C_7H_6 unit when compared with **4**. The ^{1}H and ^{13}C NMR data of **5** were similar to those of **4**, except for the presence of signals for a benzyl group (C_7H_6) in **5**. The benzyl group was further assigned at the C-1 position of glucose, due to the long-range correlation between Glc-H-1 and C-1′ of the benzyl unit. These findings deduced **5** to possess *trans*-cinnamoyl, glucose, and apiose moieties with a benzyl unit. In addition, the higher coupling constant value (J = 7.0 Hz) of Glc-1 in **5** than that of **4** (J = 3.0 Hz) suggested that glucose was β-configured in **5**. On the basis of the above data analysis, the structure of **5** (visartiside E) was elucidated as benzyl-7-*O*-(5-*O*-*trans*-cinnamoyl-β-D-apiosyl)-(1→3)-β-D-glucopyranoside.

Compound **6** had similar UV and IR spectra to **5**. On inspection of the HRESIMS of **6**, the quasimolecular ion $[M + Na]^+$ at *m/z* 627.2067 indicated an elemental formula of $C_{30}H_{36}O_{13}Na$. When comparing the ^{1}H and ^{13}C NMR spectra of **6** with **5**, they showed similar signals for the *trans*-cinnamoyl, β-apiose, and β-glucose moieties, except for the signals for a benzyl group in **5**, rather than a phenylpropanoid moiety in **6**. The signals for three protons [δ_H 6.96 (1H, brs), 6.80 (1H, dd, J = 8.0, 1.5 Hz), and 6.65 (1H, d, J = 8.0 Hz)] coupled by an ABX spin−spin system, two protons of a *trans*-ethene group at δ_H 6.54 (1H, d, J = 16.0 Hz) and 6.15 (1H, dt, J = 16.0, 6.0 Hz), two oxygenated methylene protons at δ_H 4.48 (1H, dd, J = 12.0, 6.0 Hz) and 4.21 (1H, dd, J = 12.5, 6.5 Hz), and one methoxy group at δ_H 3.79 (3H, s) were observed. Together with the ^{13}C NMR spectrum, this suggested that the phenylpropanoid moiety is 3-hydroxy-4-methoxycinnamic alcohol, equivalent to coniferyl alcohol.[15] Moreover, due to the long-range correlation between H-9 of phenylpropanoid and C-1 of Glc in the HMBC spectrum, the phenylpropanoid moiety was deduced at C-1 of the glucose in **6**. According to the above evidence, **6** was determined as coniferyl 9-*O*-(5-*O*-*trans*-cinnamoyl-β-D-apiosyl)-(1→3)-β-D-glucopyranoside and has been named visartiside F.

Compound **7** was obtained as yellow crystals. The molecular formula of **7** was determined to be $C_{25}H_{34}O_{11}$ on the basis of HRESIMS, which exhibited a quasimolecular ion $[M + Na]^+$ at *m/z* 533.2053. The IR spectrum of **7** showed absorption bands at 3394, 1592, 1508, and 1465 cm^{-1} ascribable to hydroxy and benzene ring functions.[10] In the ^{1}H NMR spectrum of **7**, the aromatic signals at δ_H 6.24 (1H, s), 7.05 (1H, d, J = 8.0 Hz), 6.82 (1H, d, J = 2.0 Hz), and 6.71 (1H, dd, J = 8.0, 2.0 Hz) displayed by an AMX splitting pattern, together with the ^{13}C NMR spectrum, which showed resonances at δ_C 154.3, 152.1, 148.7, 134.6, 115.2, 95.3 (A ring) and δ_C 149.4, 144.7, 120.8, 117.0, 112.8, 138.1 (B ring), and indicated two aromatic moieties in **7**. In addition, proton signals for four aromatic methyl groups [δ_H 3.83 (3H, s), 3.77 (3H, s), 3.75 (3H, s), 3.69 (3H, s)] and three mutually coupled methylene groups [δ_H 2.56 (2H, m), 2.53 (2H, m), 1.74 (2H, dd, J = 8.0, 7.0 Hz)], as well as the characteristic signals for a β-glucose unit [δ_H 4.84 (1H, d, J = 7.0 Hz, Glc-1), 3.49 (1H, m, Glc-2), 3.39 (2H, m, Glc-3 and 4), 3.46 (1H, m, Glc-5), 3.85 (1H, m, Glc-6), and 3.70 (1H, brs, Glc-6)], were also found. This glucose unit was identified as D-glucose by GC analysis (t_R = 21.67 min) after acid hydrolysis of **7** with 2 N HCl. These data were consistent with **7** possessing a diphenylpropane glycoside skeleton.[16,17]

Inspection of the HMBC spectrum of **7** (Figure 1) showing long-range correlations between δ_H 3.77 (3H, s) and δ_C 152.1 (C-2′), δ_H 3.69 (3H, s) and δ_C 154.2 (C-6′), δ_H 3.75 (3H, s) and δ_C 134.6 (C-3′), and δ_H 3.83 (3H, s) and δ_C 149.4 (C-3″) suggested that the OMe groups were located at C-2′, C-3′, and C-6′ of the A ring and C-3″ of the B ring, respectively. Also, on the basis of the HMBC spectrum, the propane moiety linking the two phenyl units was

Table 3. DPPH Radical-Scavenging Activity of Compounds **1**−**11**[a]

compound	ED_{50} (μM)
1	37.6
2	34.1
10	63.3
α-tocopherol[b]	28.4

[a] Compounds **3**−**9** and **11** were inactive in this assay (ED_{50} >100 μM). [b] α-Tocopherol was used as the positive control.

Table 4. LPS-Induced NO Production and Cell Viability in RAW264.7 Macrophages of **1**−**11**

compound	IC_{50} (μM)[a]	cell viability (% control)
1	15.6	100
2	25.1	100
3	14.9	99.7
4	32.3	80.2
5	39.6	88.1
6	27.8	85.2
7	30.2	77.0
8	19.2	74.5
9	42.6	100
10	41.2	100
11	23.4	88.7
quercetin[b]	32.1	100

[a] IC_{50}: half maximal inhibitory concentration. [b] Quercetin was used as the positive control.

assigned as shown. Compound **7** was found to contain a 4′,4″-dihydroxy-2′,3′,6′,3″-tetramethoxy-1,3-diphenylpropane moiety, as reported for viscolin.[17] Due to a cross-peak of anomeric protons [δ_H 4.84 (d, J = 7.0 Hz)] and C-4″ of the B ring found in the HMBC spectrum, a β-Glc unit connecting with C-4″ of the B ring was deduced. Accordingly, the diphenylpropane glycoside **7** was determined as (4′-hydroxy-2′,3′,6′,3″-tetramethoxy-1,3-diphenylpropane)-4″-*O*-β-D-glucopyranoside and has been named viscolin-4″-*O*-β-D-glucopyranoside.

The other isolated compounds, **8**−**11**, were identified as pinocembrin 7-*O*-β-D-glucopyranoside (**8**),[18] naringenin 7-*O*-β-D-glucopyranoside (**9**),[19] eriodictyol 7-*O*-β-D-glucopyranoside (**10**),[20] and homoeriodictyol 7-*O*-glucopyranoside (**11**),[21] respectively, by comparison with their literature data.

After assaying the antioxidant abilities of **1**−**11** using DPPH, flavanones **1**, **2**, and **10** had the highest levels of radical-scavenging activity (ED_{50} 37.6, 34.1, and 63.3 μM, respectively) (Table 3), whereas all other compounds were inactive (ED_{50} > 100 μM). Notably, flavanones **1**, **2**, and **10** possess a 3′,4′-dihydroxyphenyl group, compared with the inactive flavanone derivatives **8**, **9**, and **11**, which either lack or have only one OH group in the B ring, suggesting that the number of OH groups in the B ring of flavanones plays a crucial role in radical-scavenging activity.

Recently, phenolic compounds including flavonoids were demonstrated to have inhibitory activity for LPS-induced NO production.[22] Compounds **1**−**11** were evaluated for their anti-inflammatory activities (Table 4), using RAW264.7 cells supplemented with lipopolysaccharide (LPS) to induce cell inflammation and cause nitrite accumulation in the medium. Quercetin not only possesses inhibitory activity for the LPS-induced NO production but also shows protective effects in neuronal cells with no cell toxicity.[23,24] Thus, quercetin was used as a positive control. Among the isolates tested, compounds **1** (IC_{50} 15.6 μM), **3** (IC_{50} 14.9 μM), **8** (IC_{50} 19.2 μM), and **11** (IC_{50} 23.4 μM) markedly inhibited NO production in macrophages when compared with quercetin (IC_{50} 32.1 μM). Eriodictyol glucosides such as **1** (IC_{50} 15.6 μM) and **2** (IC_{50} 25.1 μM) showed higher NO inhibition than that of **10** (IC_{50} 41.2 μM). Also, the activity of narigenin glucoside derivative **3**, with a cinnamoyl group (IC_{50} 14.9 μM), had a more potent inhibitory activity than **9** (IC_{50} 42.6 μM), which lacks a cinnamoyl group.

General Experimental Procedures. Optical rotations were recorded on a JASCO P-1020 polarimeter. IR spectra were measured on a Mattson Genesis II spectrophotometer (Thermo Nicolet, Madison, WI). NMR spectra were recorded on a Varian Unity INOVA-500 MHz using CD_3OD as solvent. Chemical shifts are given in δ values (ppm) and coupling constants in Hz. Low-resolution ESIMS were recorded on a VG Quattro 5022 mass spectrometer. High-resolution ESIMS were measured on a MAT-95XL high-resolution mass spectrometer. Sephadex LH-20 (Pharmacia) and silica gel (Merck 70−230 mesh and 230−400 mesh) were used for column chromatography, and precoated silica gel (Merck 60 F-254) plates were used for TLC. TLC plates were detected by spraying with 5% H_2SO_4 and then heating at 110 °C. HPLC separations were performed on a Waters 600 series apparatus with a Waters 996 photodiode array detector, equipped with a 250 × 10 mm i.d. preparative Cosmosil AR-II column (Nacalai, Tesque, Inc.).

Plant Material. *Viscum articulatum* was collected in Qingshui Township, Taichung County, Taiwan, in July 2005. A voucher specimen (No. 2005-07-012) has been identified by one of the authors (C.-J.C.) and deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

Extraction and Isolation. The leaves and stems plant of *V. articulatum* (22.5 kg) were extracted with EtOH (80 L, three times) at 50 °C and concentrated under reduced pressure. The EtOH extract (2.24 kg) was successively partitioned with *n*-hexane−H_2O (1:1) and EtOAc−H_2O (3:1) to give an EtOAc-soluble fraction, A (463.6 g). Fr. A was chromatographed on a silica gel column (120 × 20 cm i.d.) eluting with $CHCl_3$−MeOH (1:0 → 1:1) to give 14 fractions (fr. A-1 to A-14). Using a silica gel 60 column eluted with a gradient system [(CH_2Cl_2−acetone (40:1 → 1:1)], fr. A-10 (37.5 g, $CHCl_3$−MeOH, 3:1) was further purified to 19 fractions (fr. A-10-1 to A-10-19). Compounds **1** (255 mg) and **2** (195 mg) were obtained from fr. A-10-14 by passage over a LH-20 column (94 × 4 cm i.d.), after eluting with $CHCl_3$−MeOH (1:1). Fr.A-10-17 was further separated by passage over a LH-20 column (94 × 4 cm i.d.), eluted with $CHCl_3$−MeOH (1:1), to give eight fractions (fr. A-10-17-1 to A-10-17-8). Fr.A-10-17-4 was repeatedly chromatographed by HPLC (Cosmosil $5C_{18}$-AR II, 250 × 10.0 mm i.d., flow rate: 2.5 mL/min, 65% MeOH) to yield **4** (8.8 mg), **5** (11.9 mg), **6** (4.2 mg), and **7** (13.4 mg). Using preparative TLC (plate: 20 × 20 cm; $CHCl_3$−MeOH, 8:1), fr.A-10-17-7 was purified to afford **3** (33.3 mg).

Visartiside A (1): yellow powder; mp 168 °C; $[\alpha]^{25}_D$ +30.8 (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ε) 281 (2.79) nm; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (MeOD, 125 MHz), see Table 1; HRESIMS *m/z* 603.1544 $[M + Na]^+$ (calcd for $C_{30}H_{28}O_{12}Na$, 603.1478).

Visartiside B (2): yellow powder; mp 162−165 °C; $[\alpha]^{25}_D$ −38.0 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 279 (2.94) nm; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; HRESIMS *m/z* 603.1535 $[M + Na]^+$ (calcd for $C_{30}H_{28}O_{12}Na$, 603.1478).

Visartiside C (3): white powder; mp >295 °C; $[\alpha]^{25}_D$ +72.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 280 (3.00) nm; ^1H NMR (MeOD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; HREIMS *m/z* 719.2030 $[M + Na]^+$ (calcd for $C_{35}H_{36}O_{15}Na$, 719.1952).

Visartiside D (4): white powder; mp 240 °C; $[\alpha]^{25}_D$ +52.6 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ε) 275 (2.62) nm; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; HREIMS *m/z* 465.1392 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_{11}Na$, 465.1373).

Visartiside E (5): yellow powder; $[\alpha]^{25}_D$ −28.6 (*c* 1.40, MeOH); UV (MeOH) λ_{max} (log ε) 278 (2.51) nm; ^1H NMR (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; HREIMS *m/z* 555.1836 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_{11}Na$, 555.1842).

Visartiside F (6): yellow powder; $[\alpha]^{25}_{D}$ −34.8 (*c* 1.35, MeOH); UV (MeOH) λ_{max} (log ε) 275 (2.50) nm; ^{1}H NMR (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; HREIMS *m/z* 627.2067 [M + Na]$^+$ (calcd for $C_{30}H_{36}O_{13}Na$, 627.2054).

Viscolin-4″-*O*-*β*-D-glucopyranoside (7): yellow crystals; mp >295 °C; $[\alpha]^{24}_{D}$ −29.7 (*c* 0.74, MeOH); UV (MeOH) λ_{max} (log ε) 280 (2.25) nm; IR (neat) ν_{max} 3394, 2851, 1592, 1508, 1465, 1089 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ_H 7.05 (1H, d, *J* = 8.0 Hz, H-5″), 6.82 (1H, d, *J* = 2.0 Hz, H-2′), 6.71 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.24 (1H, s, H-5′), 4.84 (1H, d, *J* = 7.0 Hz, H-Glc-1), 3.39−3.85 (H-Glc-2-Glc-6), 3.83 (3H, s, OCH_3-3′), 3.77 (3H, s, OCH_3-2′), 3.75 (3H, s, OCH_3-3′), 3.69 (3H, s, OCH_3-6′), 2.56 (1H, m, H-3), 2.53 (1H, m, H-1), 1.74 (1H, dd, *J* = 8.0, 7.0 Hz, H-2); ^{13}C NMR (CD_3OD, 125 MHz) δ_C 154.3 (C-6′),152.1 (C-2′),149.4 (C-3″), 148.7 (C-4′), 144.7 (C-4″), 138.1 (C-1″), 134.6 (C-3′), 120.8 (C-6″), 117.0 (C-5″), 115.2 (C-1′), 112.8 (C-2″), 101.9 (C-Glc-1), 70.1−76.6 (C-Glc-2-Glc-5), 61.3 (Glc-6), 60.1 (OCH_3-2′), 59.9 (OCH_3-3′), 55.5 (OCH_3-3″), 54.9 (OCH_3-6′), 35.4 (C-3), 31.9 (C-2), 22.9 (C-1); HREIMS *m/z* 533.2053 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{11}Na$, 533.1999).

Acid Hydrolysis. Solutions of compounds **1** and **2** (each 10 mg) and **3**−**7** (each 5 mg) in 2 N HCl (2 mL) were refluxed at 80 °C for 20 min, respectively. After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and filtered. Then the filtrate was evaporated to dryness, and 1-(trimethylsilyl)imidazole and pyridine (0.2 mL) were added. After reacting for 1 h, the mixture was dried by a stream of N_2, and partitioned with *n*-hexane and water. The *n*-hexane layer was analyzed by GC with the following conditions: CP-Chirasil-L-Val column (25 m × 0.25 mm); injection temperature 60 °C; column temperature 60−180 °C; rate 2 °C/min. Peaks of trimethylsilyl derivatives derived from sugar were detected by comparison with retention times of authentic samples [D-glucose (21.69 min) and D-apiose (21.22 min)] treated with 1-(trimethylsilyl)imidazole.

DPPH Radical-Scavenging Activity Determination. The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used for the determination of free radical-scavenging activity of the extracts and compounds.[5] Compounds **1**−**11** (120 μL) were added to 30 μL of DPPH (0.75 mM). After 30 min at room temperature, the absorbance was recorded at 520 nm. The experiment was repeated three times. Radical-scavenging activity (%) = {[Ab − (A − As)]/Ab} × 100 (Ab: the absorbance without sample, A is the absorbance with compound and DPPH, and As is the absorbance with compound only). The ED_{50} value was calculated if the radical-scavenging activity of tested compound was more than 70%.

Determination of NO Production and Cell Viability Assay.[25] The murine macrophage cell line RAW264.7 (BCRC 60001 = ATCC TIB-71) was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and incubated at 37 °C in a humidified 5% CO_2 atmosphere with a 96-well flat-bottomed culture plate. After 24 h, the medium was replaced with fresh DMEM and FBS. Then, compounds **1**−**11** (0, 1, 5, 10, or 20 μg/mL) were each added in the presence of lipopolysaccharide (LPS, 1 μg/mL; Sigma, Cat. No. L-2654) and incubated under the same conditions for 24 h. The cultured cells were then centrifuged and the supernatants used for NO production measurement, with a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay used to determine cell viability.

The supernatant was mixed with an equal volume of the Griess reagent (1% sulfanilamide, 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% phosphoric acid solution) and incubated for 10 min at room temperature. Nitrite concentration was determined by measuring the absorbance at 540 nm using an ELISA plate reader (μ Quant).

The MTT colorimetric assay was modified from that of Mosmann.[26] The test is based upon the selective ability of living cells to reduce the yellow soluble salt, MTT, to a purple-blue insoluble formazan MTT (Merck; dissolved in phosphate-buffered saline at 5 mg/mL). MTT solution was added to the attached cells mentioned above (10 μL per 100 μL culture) and incubated at 37 °C for 4 h. Then, DMSO was added and the amount of colored formazan metabolite formed was determined by absorbance at 550 nm. The optical density of formazan formed in control (untreated) cells was taken as 100% viability.

Acknowledgment. The authors thank Dr. M. J. Don, National Research Institute of Chinese Medicine, for EIMS measurements, and the Instrument Center of National Taiwan University, for the HRESIMS measurements. This work was supported by grants from the National Science Council and the National Research Institute of Chinese Medicine, Taiwan, Republic of China.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**−**7**. This information is available free of charge via the Internet at http://pubs.acs.org

References and Notes

(1) Leu, Y. L.; Kuo, S. M.; Hwang, T. L.; Chiu, S. T. *Chem. Pharm. Bull.* **2004**, *52*, 858–860.
(2) Kong, D. Y.; Li, H. T.; Luo, S. Q. *Acta Pharm. Sin.* **1990**, *25*, 608–611.
(3) Hwang, T. L.; Leu, Y. L.; Kao, S. H.; Tang, M. C.; Chang, H. L. *Free Radical Biol. Med.* **2006**, *41*, 1433–1441.
(4) Yao, H.; Zhou, G. X.; Wu, Q.; Lei, G. Q.; Chen, D. F.; Chen, J. K.; Zhou, T. S. *Molecules* **2007**, *12*, 312–317.
(5) Koleva, I. I.; Van Beek, T. A.; Linssen, J. P. H.; De Groot, A.; Evstatieva, L. N. *Phytochem. Anal.* **2002**, *13*, 8–17.
(6) Amoros, M.; Simoes, C. M. O.; Girre, L.; Sauvager, F.; Cormier, M. *J. Nat. Prod.* **1992**, *55*, 1732–1740.
(7) Harborne, J. B.; Mabry, T. J. *The Flavonoids*; Chapman and Hall: London, 1975; pp 45−77.
(8) Moretti, C.; Sauvain, M.; Lavaud, C.; Massiot, G.; Bravo, J. A.; Munoz, V. *J. Nat. Prod.* **1998**, *61*, 1390–1393.
(9) Xu, R. S.; Ye, Y.; Zhao, W. M. *Natural Product Chemistry*; Science Press: Beijing, 2004; p 505.
(10) Matsuda, H.; Nishida, N.; Yoshikawa, M. *Chem. Pharm. Bull.* **2002**, *50*, 429–431.
(11) Lokvam, J.; Coley, P. D.; Kursar, T. A. *Phytochemistry* **2004**, *65*, 351–358.
(12) Mizuno, M.; Kato, M.; Iinuma, M.; Tanaka, T.; Kimura, A.; Ohashi, H.; Sakai, H. *Phytochemistry* **1987**, *26*, 2418–2420.
(13) Sang, S.; Lapsley, K.; Jeong, W. S.; Lachance, P. A.; Ho, C. T.; Rosen, R. *J. Agric. Food Chem.* **2002**, *50*, 2459–2463.
(14) Leu, Y. L.; Kuo, S. M.; Hwang, T. L.; Chiu, S. T. *Chem. Pharm. Bull.* **2004**, *52*, 858–860.
(15) Sakakibara, N.; Nakatsubo, T.; Suzuki, S.; Shibata, D.; Shimada, M.; Umezawa, T. *Org. Biomol. Chem.* **2007**, *5*, 802–815.
(16) Lin, J. H.; Chiou, Y. N.; Lin, Y. L. *J. Nat. Prod.* **2002**, *65*, 638–640.
(17) Leu, Y. L.; Hwang, T. L.; Chung, Y. M.; Hong, P. Y. *Chem. Pharm. Bull.* **2006**, *54*, 1063–1066.
(18) Hammami, S.; Ben, J. H.; Bergaoui, A.; Ciavatta, L.; Cimino, G.; Mighri, Z. *Molecules* **2004**, *9*, 602–608.
(19) Turner, A.; Chen, S. N.; Joike, M. K.; Pendland, S. L.; Pauli, G. F.; Farnsworth, N. R. *J. Agric. Food Chem.* **2005**, *53*, 8940–8947.
(20) Moretti, C.; Sauvain, M.; Lavaud, C.; Massiot, G.; Bravo, J. A.; Munoz, V. *J. Nat. Prod.* **1998**, *61*, 1390–1393.
(21) Zhao, Y.; Wang, X.; Zhao, Y.; Gao, X.; Bi, K.; Yu, Z. *Biol. Pharm. Bull.* **2007**, *30*, 617–620.
(22) Fang, S. C.; Hsu, C. L.; Yen, G. C. *J. Agric. Food Chem.* **2008**, *56*, 4463–4468.
(23) Heo, H. J.; Lee, C. Y. *J. Agric. Food Chem.* **2004**, *52*, 7514–7517.
(24) Motai, T.; Daikonta, A.; Kitanaka, S. *J. Nat. Prod.* **2004**, *67*, 432–436.
(25) Mosman, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
(26) Johansson, M.; Kopcke, B.; Anke, H.; Sterner, O. *J. Antibiot.* **2002**, *55*, 104–106.

NP9004294