Glycosides from the Stem Bark of *Fraxinus sieboldiana*

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A norditerpene glucopyranoside with a novel carbon skeleton (**1**), eight new aromatic glycosides (**2**−**9**), and 25 known glycosides have been isolated from a H_2O-soluble portion of an ethanolic extract of the stem bark of *Fraxinus sieboldiana*. Their structures were determined by spectroscopic and chemical methods. Based on analysis of the NMR data of *threo*- and *erythro*-arylglycerols in different solvents, an application of $\Delta\delta_{C8-C7}$ values to distinguish *threo*-arylglycerol and *erythro*-arylglycerol isomers was proposed. In the *in vitro* assays, compound **5** displayed TNF-α secretion inhibitory activity with an IC_{50} value of 1.6 μM, compound **6** showed antioxidative activity inhibiting Fe^{+2}-cystine-induced rat liver microsomal lipid peroxidation with an IC_{50} value of 0.9 μM, and plantasioside (**10**) showed selective activity against the human colon cancer cell line (HCT-8) with an IC_{50} value of 3.4 μM.

Fraxinus sieboldiana Blume (Oleaceae) is widely distributed in eastern Asia, especially in southern China. Its dried bark has long been used as a folk medicine "Qin Pi" in China and Japan, as it is reported to have diuretic, antifebrile, analgesic, and antirheumatic activities.[1,2] Many coumarins,[3,4] lignans,[5] secoiridoid glucosides,[6−8] and phenylethanoids[9] have been reported from species of this genus. As part of a program to assess the chemical and biological diversity of traditional Chinese medicines, we undertook investigation of the stem bark of *F. sieboldiana* and describe herein isolation and structural elucidation of a new norditerpene glucopyranoside (**1**) and eight new aromatic glycosides (**2**−**9**), along with 25 known glycosides. Some biological assay results are also reported.

Results and Discussion

The ethanolic extract of the stem bark of *F. sieboldiana* was partitioned between H_2O and EtOAc. The H_2O phase was subjected to separation using various column chromatographic techniques to afford nine new glycosides (**1**−**9**).

Compound **1** was obtained as a colorless gum, and the presence of hydroxyl (3389 cm^{-1}) and carbonyl (1747 cm^{-1}) groups was evident in its IR spectrum. The positive mode ESIMS of **1** gave a quasi-molecular ion peak at *m*/*z* 505 $[M + Na]^+$. The molecular formula $C_{25}H_{38}O_9$, with seven degrees of unsaturation, was indicated by HRESIMS. The ^1H NMR spectrum of **1** in MeOH-d_4 showed two quaternary methyl singlets at δ 0.91 (H_3-8′) and 0.95 (H_3-9′), two olefinic methyl singlets at δ 1.63 (H_3-9), and 1.75 (H_3-7′), and an olefinic methyl triplet at δ 1.85 (J = 2.0 Hz, H_3-10). The ^1H NMR spectrum also displayed signals attributed to two olefinic methines at δ 7.21 (J = 2.0 Hz, H-3) and 5.72 (J = 9.6 Hz, H-6), two oxygen-bearing methines at δ 5.30 (dt, J = 9.4 and 1.6 Hz, H-4) and 3.32 (dd, J = 10.8 and 2.8 Hz, H-5′), and one oxygen-bearing methylene at δ 3.90 (s, H-8). It also had a double doublet attributed to a deshielded methine at δ 2.96 (H-5) and partially overlapped multiplets due to two methylenes between δ 1.75 and 2.20, together with characteristic signals due to a β-glucopyranosyl unit (Table 1). In addition to protonated carbon signals corresponding to the above protons, the ^{13}C NMR and DEPT spectra of **1** showed six quaternary carbons that were identified as a carboxylic carbon, four sp^2 olefinic carbons, and a sp^3 carbon (Table 1). These data suggested that **1** was a glycosidic norditerpene containing a carboxylic acid group, two rings, and three double bonds. This was confirmed by spectroscopic data of the aglycone (**1a**) obtained from enzymatic hydrolysis of **1** with β-glucosidase (Table 1 and Experimental Section). The glucose isolated gave a positive optical rotation, $[\alpha]_D^{20}$ +46.2 (*c* 0.11, H_2O), indicating that it was D-glucose.[13]

Table 1. NMR Data (δ) for Compounds **1** and **1a**[a]

	1 (MeOH-d_4)		**1a** (MeOH-d_4)	
no.	H	C	H	C
1		176.6		176.5
2		130.4		130.5
3	7.21 t (2.0)	152.0	7.20 s	151.9
4	5.30 dt (9.4, 1.6)	86.0	5.30 d (9.6)	86.0
5	2.96 dd (9.6, 9.4)	45.8	2.97 t (9.6)	46.0
6	5.72 d (9.6)	124.8	5.71 (10.0)	124.8
7		138.7		138.8
8	3.90 s	68.5	3.90 s	68.4
9	1.63 s	14.5	1.63 s	14.5
10	1.85 t (2.0)	10.5	1.82 s	10.5
1′		136.2		136.5
2′		131.2		131.0
3′	2.06 m	33.1	2.06 m	33.0
4′	1.99 m, 1.75 m	26.8	1.67 m	27.7
5′	3.32 dd (10.8, 2.8)	87.5	3.31 dd (14.0, 7.2)	76.6
6′		42.3		42.2
7′	1.75 s	22.0	1.75 s	21.7
8′	0.91 s	22.5	0.82 s	22.0
9′	0.95 s	25.6	0.88 s	25.6
1″	4.26 d (7.6)	106.5		
2″	3.13 dd (8.4, 7.6)	75.7		
3″	3.20 t (8.4)	78.3		
4″	3.24 t (8.4)	71.7		
5″	3.26 m	77.8		
6″a	3.79 dd (12.0, 2.0)	62.8		
6″b	3.60 dd (12.0, 5.2)			

[a] NMR data (δ) were measured in MeOH-d_4 for **1** and **1a** at 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on DEPT, $^1H-^1H$ COSY, HSQC, HMBC, and phase-sensitive $^1H-^1H$ COSY experiments.

The proton and protonated carbon signals of **1** were assigned unambiguously by the HSQC experiment. $^1H-^1H$ COSY correlations from H-5′ through H_2-4′ to H_2-3′ and HMBC correlations from H_3-7′ to C-1′, C-2′, and C-3′ and from both H_3-8′ and H_3-9′ to C-1′, C-5′, and C-6′, together with their chemical shift values, indicated the presence of a 5′-oxygen-bearing 2′,6′,6′-trimethylcyclohexen-1′-yl moiety in **1**. HMBC correlation of the anomeric proton (H-1″) to C-5′ indicated that the β-D-glucopyranosyl unit was located at C-5′. This was supported by comparison of the chemical shifts of C-5′ between **1** and **1a** (Table 1). The $^1H-^1H$

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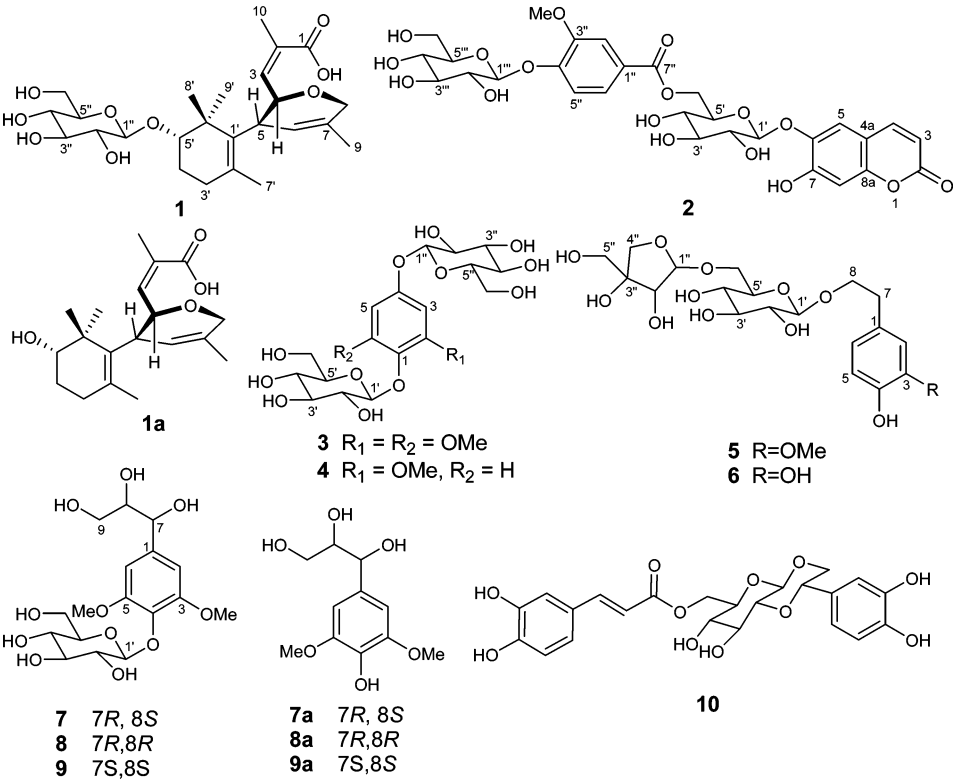
Chart 1



COSY correlations from H-6 through H-5 to H-4 and H-3, in combination with two- and three-bond HMBC correlations from H-5 to C-4 and C-6, from H-6 to C-5, C-8, and C-9, from H_2-8 to C-4, C-6, C-7, and C-9, from H-4 to C-2, C-3, C-5, and C-6, from H-3 to C-1, C-2, C-4, and C-10, from H_3-10 to C-1, C-2, and C-3, and from H_3-9 to C-6, C-7, and C-8, together with their chemical shifts, indicated a 5-substituted 4,8-epoxy-2,7-dimethyl-$\Delta^{2,6}$-octadienoic acid moiety in **1**. This was supported by long-range homonuclear correlations of H-6 with both H_2-8 and H_3-9 and of H_3-10 with both H-3 and H-4 in the $^1H-^1H$ COSY spectrum. In addition, HMBC correlations from H-5 to C-1′, C-2′, and C-6′, and from H-6 to C-1′ indicated unambiguously a connection between C-5 and C-1′. Therefore, the planar structure of **1** was elucidated as 5-(5-*O*-*β*-D-glucopyranosyloxy-2,6,6-trimethylcyclohexen-1-yl)-4,8-epoxy-2,7-dimethyl-$\Delta^{2,6}$-octadienoic acid.

The stereochemistry including the absolute configuration of **1** was elucidated by a combination analysis of the coupling constants in the ^1H NMR spectrum and enhancements in the NOE difference experiments, as well as the empirical rule of *β*-D-glucosylation-induced shifts of the ^{13}C NMR data.[10−12] The splitting pattern and coupling constants of H-5′ (dd, $J_{5',4'a} = 10.8$ Hz, $J_{5',4'e} = 2.8$ Hz) indicated that H-5′ had a pseudoaxial orientation. In the NOE difference experiment of **1**, NOE enhancement of H_3-9′ by irradiation of H-1″ indicated that H_3-9′ and the *β*-D-glucopyranosyl unit at C-5′ were oriented on the same side of the cyclohexene ring system. The splitting patterns and coupling constants of H-5 (dd, $J_{5,6} = 9.6$ Hz and $J_{4,5} = 9.4$ Hz) suggested that H-4 and H-6, opposite H-5, were oriented on the same side of the dihydropyran ring. This was confirmed by a NOE enhancement of H-6 by irradiation of H-4, which, in turn, was enhanced by irradiation of H-6. A *cis*-configuration of the double bond between C-2 and C-3 was indicated by a NOE enhancement of H_3-10 when H-3 was irradiated. In addition, in the NOE difference experiments, H_3-8′ and H_3-9′ were enhanced by irradiation of H-5, but H_3-7′ was not enhanced, whereas irradiation of H-4 caused an enhancement of H_3-7′. These NOE effects indicated that H-5 was close to both H_3-8′ and H_3-9′, and H-4 to H_3-7′, suggesting that free rotation of the bond connecting the two rings, in the solution state of **1**, was restricted due to the bulky 2-methylacrylic acid unit. Therefore, **1** was assigned as an atropisomer possessing the relative configuration illustrated in the structure drawing. On the basis of the empirical rule of *β*-D-glucosylation-induced shifts,[10−13] the deshielded chemical shift of the anomeric carbon of **1** (δ 106.5) and chemical shift differences of C-4′ ($\Delta\delta$ −0.9), C-5′ ($\Delta\delta$ +10.9), and C-6′ ($\Delta\delta$ +0.1) between the ^{13}C NMR data of **1** and **1a** indicated a 5′*S* configuration for **1**. Accordingly, the structure of **1** was determined as (−)-(a*S*)-(4*R*,5*S*,2*Z*)-5-[(5*S*)-5-*O*-*β*-D-glucopyranosyloxy-2,6,6-trimethylcyclohexen-1-yl]-4,8-epoxy-2,7-dimethyl-$\Delta^{2,6}$-octadienoic acid and was named fraxinuacidoside.

Compound **2** was obtained as a yellowish, amorphous powder, and HRESIMS indicated the molecular formula to be $C_{29}H_{32}O_{17}$. The IR spectrum of **2** exhibited absorption bands at 3396, 1712, 1612, 1570, and 1510 cm^{-1}, indicating the presence of hydroxyl, carbonyl, and aromatic ring functional groups. The ^1H NMR spectrum of **2**, in DMSO-d_6, had signals indicating that it was a 6,7-disubstituted coumarin, which was confirmed by HMBC correlations from H-3 to C-2 and C-4a, from H-4 to C-2, C-5, C-4a, and C-8a, from H-5 to C-4, C-6, C-7, C-4a, and C-8a, and from H-8 to C-6, C-7, C-4a, and C-8a. The ^1H NMR spectrum showed signals attributed to a 1,3,4-trisubstituted aromatic ring at δ 7.15, 7.44, and 7.54 and a methoxy group at δ 3.74. Two doublets attributed to anomeric protons (δ 4.88 and 5.04), together with coupling patterns of oxymethylene and oxymethine protons between δ 3.00 and 4.61, as well as seven exchangeable OH protons, indicated the presence of two *β*-glucopyranosyl units in **2**. This was confirmed by acidic hydrolysis of **2**, which produced D-glucose as the sole sugar, identified by TLC comparison and optical rotation. The ^{13}C NMR and DEPT spectra of **2** had signals corresponding to the above units (Table 3). An additional carbonyl carbon (δ 165.1, C-7″) in the ^{13}C NMR spectrum, together with the chemical shifts of the protons and carbons assigned to the 1,3,4-trisubstituted aromatic ring (Tables 2 and 3), suggested that there was a 4-oxygen-substituted 3-methoxybenzoyl unit in **2**.

This was confirmed by the NOE difference experiment of **2** showing enhancement of H-2″ by irradiation of the methoxy protons. In addition, H-5 was enhanced by irradiation of H-1′, while

Table 2. ^{1}H NMR Data (δ) for Compounds **2**−**9**[a]

no.	**2** (DMSO-d_6)[b]	**3** (DMSO-d_6)	**4** (DMSO-d_6)	**5** (DMSO-d_6)	**6** (DMSO-d_6)	**7** (D_2O)	**8** (D_2O)	**9** (D_2O)
2				6.83 brs	6.60 brs	6.68 s	6.67 s	6.67 s
3	6.13 d (9.5)	6.37 s	6.70 d (3.0)					
4	7.27 d (9.5)							
5	7.22 s	6.37 s	6.52 dd (9.0, 3.0)	6.64 d (8.0)	6.61 d (8.5)			
6			6.98 d (9.0)	6.61 brd (8.0)	6.47 dd (8.5, 2.0)	6.68 s	6.67 s	6.67 s
7				2.73 t (7.0)	2.67 m	4.51 d (7.0)	4.54 d (6.0)	4.53 d (6.0)
8	6.79 s			3.86 m, 3.59 m	3.81 m, 3.54 m	3.76 m	3.74 m	3.73 m
9a						3.65 dd (12.0, 2.0)	3.43dd (12.0, 2.0)	3.43 dd (12.0, 2.0)
9b						3.49 dd (12.0, 6.5)	3.34 dd (12.0, 7.0)	3.35 dd (12.0, 6.4)
1′	4.88 d (7.5)	4.74 d (7.0)	4.73 d (7.5)	4.16 d (8.0)	4.15 d (7.5)	4.91 d (7.0)	4.90 d (7.0)	4.90 d (7.2)
2′	3.35 m	3.19 ddd (8.5, 7.5, 5.0)[c]	3.19 ddd (8.5, 7.5, 5.0)[c]	2.95 dd (8.0, 8.5)	2.93 dd (8.0, 8.5)	3.44 dd (8.5, 7.0)	3.42 dd (8.5, 7.0)	3.42 dd (8.4, 7.2)
3′	3.36 m	3.16 ddd (8.5, 8.5, 5.0)[c]	3.15 ddd (8.5, 5.0) [c]	3.12 dd (8.5, 9.5)	3.12 dd (8.5, 9.5)	3.42 dd (9.5, 8.5)	3.41 dd (9.5, 8.5)	3.41 dd (9.6, 8.4)
4′	3.30 m	3.10 ddd (9.5, 8.5, 5.0)[c]	3.10 ddd (9.0, 8.5, 5.0)[c]	2.97 dd (9.0, 9.5)	2.97 dd (9.0, 9.5)	3.36 t (9.5)	3.36 t (9.5)	3.36 t (9.6)
5′	3.80 m	3.00 m	3.19 m	3.25 m	3.26 m	3.22 m	3.23 m	3.22 m
6′a	4.60 br d (12.0)	3.58 ddd (11.5, 5.5, 2.0)[c]	3.70 ddd (11.5, 5.5, 2.0)[c]	3.84 m	3.81 m	3.68 brd (13.0)	3.69 brd (12.5)	3.69 brd (12.4)
6′b	4.32 dd (12.0, 7.0)	3.39 ddd (11.5, 6.0, 5.0)[c]	3.43 ddd (11.5, 6.0, 5.0)[c]	3.39 dd (11.0, 7.0)	3.40 dd (11.0, 7.0)	3.58 dd (13.0, 5.5)	3.59 dd (12.5, 5.5)	3.59 dd (12.4, 5.2)
1″		4.78 d (7.5)	4.75 d (7.5)	4.89 d (3.0)	4.84 d (3.0)			
2″	7.44 d (2.0)	3.20 ddd (8.5, 7.5, 5.0) [c]	3.19 ddd (8.5, 7.5, 5.0)[c]	3.73 d (3.0)	3.73 d (3.0)			
3″		3.24 ddd (8.5, 8.5, 5.0) [c]	3.23 ddd (8.5, 8.5, 5.0)[c]					
4″a		3.09 ddd (9.5, 8.5, 5.0)[c]	3.21 ddd (9.5, 8.5, 5.0)[c]	3.83 d (9.5)	3.83 d (9.5)			
4″b				3.56 d (9.5)	3.57 d (9.5)			
5″	7.15 d (9.0)	3.35 m	3.21 m	3.32 s	3.32 s			
6″a	7.54 dd (9.0, 2.0)	3.70 ddd (11.5, 5.0, 2.0) [c]	3.65 ddd (11.5, 5.0, 2.0)[c]					
6″b		3.41ddd (11.5, 6.0, 5.0)[c]	3.44 ddd (11.5, 6.0, 5.0)[c]					
OMe	3.74 s	3.71 s	3.73 s	3.73 s		3.76 s	3.75 s	3.75 s

[a] ^{1}H NMR data (δ) were measured in DMSO-d_6 for **2**−**6** and D_2O for **7**−**9** at 500 or 400 MHz. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b] Data of the terminal glucopyranosyl unit of **2**: δ 5.04 (1H, d, J = 7.5 Hz, H-1‴), 3.30 (2H, m, H-2‴, and H-3‴), 3.18 (1H, m, H-4‴), 3.35 (1H, m, H-5‴), 3.64 (1H, dd, J = 10.0 and 5.0 Hz, H-6‴a), and 3.46 (1H, ddd, J = 10.0, 7.0, and 5.0 Hz, H-6‴b). [c] Coupling with adjacent hydroxyl proton was included.

H-5″ was enhanced by irradiation of H-1‴. These enhancements indicated that the two β-D-glucopyranosyl units were located at C-6 of the coumarin moiety and C-4 of the 3-methoxybenzoyl unit, respectively. In the NMR spectrum, the proton and carbon signals attributed to the hydroxymethylene of one β-D-glucopyranosyl unit were deshielded by $\Delta\delta_H$ 0.96 (H-6′a) and 0.86 (H-6′b), and $\Delta\delta_C$ 3.5 ppm, respectively. This indicated that the benzoyl unit was located at C-6 of the sugar unit that was connected to the coumarin moiety. The above deductions were confirmed by gHSQC and gHMBC experiments of **2**. Thus, compound **2** was determined to be 6-[6-(4-*O*-β-D-glucopyranosyloxy-3-methoxybenzoyl)]-*O*-β-D-glucopyranosyloxy-7-hydroxycoumarin.

Compound **3** showed IR absorption bands for hydroxyl (3398 cm^{-1}) and aromatic ring (1601 and 1508 cm^{-1}) functional groups. Its positive mode ESIMS gave a quasi-molecular ion peak at *m*/*z* 517 $[M + Na]^+$, and the molecular formula $C_{20}H_{30}O_{14}$ was indicated by HRESIMS. The ^{1}H NMR spectrum of **3** in DMSO-d_6 showed a six-proton methoxy singlet at δ 3.71 and a two aromatic proton singlet at δ 6.37, in addition to signals attributed to two β-glucopyranosyl units. Enzymatic hydrolysis of **3** produced β-D-glucose as the sole sugar. In addition to a methoxy carbon signal and two sets of carbon signals due to β-glucopyranosyl moieties, the ^{13}C NMR spectrum of **3** showed four signals in the aromatic region (Table 3). These data suggested that **3** was a phenolic diglucoside substituted symmetrically by the two β-D-glucopyranosyl and two methoxy groups. The separation of signals of the two β-D-glucopyranosyls indicated that the magnetic environments of the two were different. This suggested a 2,5-diglucosyl-1,3-dimethoxy substitution pattern for **3**, which was supported by the NOE difference experiment showing enhancement of H-1″ by irradiation of the two overlapped aromatic protons and further confirmed by the HMBC experiment showing correlations from H-1′ to C-1 and H-1″ to C-4. Therefore, **3** was determined to be 2,5-di-*O*-β-D-glucopyranosyloxy-1,3-dimethoxybenzene.

Compound **4** exhibited a quasi-molecular ion peak at *m*/*z* 487 $[M + Na]^+$ in its ESIMS. The molecular formula $C_{19}H_{28}O_{13}$ was indicated by HRESIMS. The IR and NMR spectra of **4** resembled those of **3** except that the NMR signals of the symmetrically tetrasubstituted benzene moiety and two methoxys of **3** were replaced by signals attributed to a 1,2,4-trisubstituted benzene moiety and one methoxy of **4** (Tables 2 and 3). These data indicated that **4** was a demethoxy derivative of **3**, which was further confirmed by enzymatic hydrolysis and HMBC experiments of **4**. Therefore, **4** was determined to be 1,4-di-*O*-β-D-glucopyranosyloxy-2-methoxybenzene.

Compound **5** showed quasi-molecular ion peaks at *m*/*z* 485 $[M + Na]^+$ and 501 $[M + K]^+$ in the ESIMS. Its molecular formula, $C_{20}H_{30}O_{12}$, was indicated by HRESIMS. The NMR spectra of **5** were similar to those of the co-occurring osmanthuside H,[14] except that resonances of the *para*-disubstituted aromatic moiety of osmanthuside H were replaced by resonances ascribed to a 1,2,4-trisubstituted aromatic moiety and an additional methoxy in **5**

Table 3. ^{13}C NMR Data (δ) for Compounds **2**−**9**[a]

no.	**2** (DMSO-d_6)[b]	**3** (DMSO-d_6)	**4** (DMSO-d_6)	**5** (DMSO-d_6)	**6** (DMSO-d_6)	**7** (D_2O)	**8** (D_2O)	**9** (D_2O)
1		129.3	141.4	129.4	129.2	138.4	138.5	138.5
2	160.4	153.0	149.6	113.0	115.4	105.0	104.5	104.5
3	112.0	95.1	102.5	147.3	143.5	152.5	152.6	152.5
4	143.8	153.9	152.8	144.7	144.9	133.1	133.0	133.0
4a	110.5							
5	113.7	95.1	107.3	115.2	116.3	152.5	152.6	152.5
6	142.5	153.0	116.4	120.9	119.5	105.0	104.5	104.5
7	151.1			35.1	35.1	74.1	74.2	74.2
8	103.2			69.8	70.0	74.8	75.6	75.6
8a	150.3							
9						62.7	62.7	62.7
1′	99.4	102.9	100.9	102.7	102.8	103.1	103.1	103.1
2′	73.1	74.1	73.3	73.3	73.3	73.8	73.8	73.8
3′	75.8	76.5	76.8	76.6	76.6	75.9	75.9	75.9
4′	70.1	70.0	69.9	70.2	70.2	69.5	69.5	69.5
5′	74.0	77.1	77.1	75.5	75.4	76.5	76.5	76.5
6′	64.0	60.9	60.8	67.7	67.6	60.6	60.7	60.6
1″	122.9	100.9	101.3	109.2	109.2			
2″	112.7	73.2	73.3	75.8	75.8			
3″	148.7	76.8	76.7	78.8	78.8			
4″	150.8	70.0	69.7	73.2	73.2			
5″	114.3	77.2	76.9	63.1	63.1			
6″	122.8	60.8	60.7					
7″	165.1							
OMe	55.7	56.2	55.6	55.5		56.4	56.4	56.4

[a] ^{13}C NMR data (δ) were measured in DMSO-d_6 for **2**−**6** and D_2O for **7**−**9** at 125 and 100 MHz. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b] Data of the terminal glucopyranosyl unit of **2**: δ 101.4 (C-1‴), 73.1 (C-2‴), 76.8 (C-3‴), 69.5 (C-4‴), 77.1 (C-5‴), 60.5 (C-6‴).

(Tables 2 and 3). In the HMBC spectrum of **5**, correlations of C-3 with H-5 and the methoxy protons, and C-2 and C-6 with H_2-7, demonstrated that the methoxy group was located at C-3. HMBC correlations from H-1′ to C-8 and from H-1″ to C-6′ confirmed that the connection among the 4-hydroxy-3-methoxyphenylethyl and the two sugar moieties of **5** was identical to that of osmanthuside H. Therefore, **5** was determined to be 2-(4-hydroxy-3-methoxyphenyl)ethanol 1-*O*-[*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside].

Compound **6** was obtained as a colorless gum, and HRESIMS at *m*/*z* 471.1498 $[M + Na]^+$ indicated the molecular formula to be $C_{19}H_{28}O_{12}$. The IR and NMR spectra of **6** were very similar to those of **5** (Tables 2 and 3), except for the absence of methoxy resonances in the NMR spectra of **6**. In addition, as compared to those of **5**, C-3 and C-6 of **6** were shielded by $\Delta\delta$ 3.8 and 1.4 ppm, respectively, while C-2 and C-5 were deshielded by $\Delta\delta$ 2.4 and 1.1 ppm, respectively. These changes revealed that **6** was a demethyl derivative of **5**. Thus, **6** was determined to be 2-(3,4-dihydroxyphenyl)ethanol 1-*O*-[*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside].

Compound **7** showed IR absorption bands for OH (3277 cm^{-1}) and aromatic ring (1601 and 1512 cm^{-1}) functional groups. ESIMS gave a quasi-molecular ion peak at *m*/*z* 429 $[M + Na]^+$, and HRESIMS indicated the molecular formula to be $C_{17}H_{26}O_{11}$. The ^{1}H NMR spectrum of **7** in D_2O showed a two-proton aromatic singlet at δ 6.68, a six-proton methoxy singlet at δ 3.76, two deshielded oxymethine doublets at δ 4.91 and 4.51, and partially overlapped oxymethylene and/or oxymethine multiplets integrated for nine protons between δ 3.20 and 3.75 (Table 2). The ^{13}C NMR and DEPT spectra of **7** displayed characteristic signals for 1-*C*-syringylglycerol and *β*-glucopyranosyl moieties (Table 3). Enzymatic hydrolysis of **7** with *β*-glucosidase yielded **7a** with $[\alpha]_D^{20}$ −19.8 (*c* 0.11, MeOH) and *β*-D-glucose with $[\alpha]_D^{20}$ +39.8 (*c* 0.55, H_2O). The NMR data of **7a** (Experimental Section) were in good agreement with those of *erythro*-1-*C*-syringylglycerol,[15] indicating that **7** was (−)-*erythro*-1-*C*-syringylglycerol *β*-D-glucopyranoside. Comparison of the NMR data of **7** and **7a** indicated that C-1 and C-3/C-5 (overlapped) of **7** were significantly deshielded by $\Delta\delta$ 4.3 and 5.1 ppm, respectively. This suggested that *β*-D-glucopyranosyl was located at C-4 of (−)-*erythro*-1-*C*-syringylglycerol in **7**, which was confirmed by correlations from H-7 and H-8 to C-1 and from H-1′ to C-4 in the HMBC spectrum. Since *erythro*-arylglycerols with 7*R*,8*S* configuration were reported to have negative $[\alpha]_D$ values,[16,17] the absolute configuration at C-7 and C-8 of **7a** was assigned as 7*R*,8*S*. Thus, the structure of **7** was determined to be (−)-(7*R*,8*S*)-*erythro*-1-*C*-syringylglycerol 4-*O*-*β*-D-glucopyranoside.

The spectroscopic data of **8** (Tables 2 and 3 and Experimental Section) were similar to those of **7**. Comparison of the NMR data of **7** and **8** indicated that H-7 of **8** was deshielded by $\Delta\delta$ 0.03 ppm and that H-9a and H-9b were shielded by $\Delta\delta$ 0.22 and 0.15 ppm, respectively, while C-8 of **8** was deshielded by $\Delta\delta$ 0.8 ppm. This suggested that it was a *threo*-isomer of **7**, which was further confirmed by enzymatic hydrolysis and 2D NMR experiments of **8**. The enzymatic hydrolysis of **8** gave **8a** with $[\alpha]_D^{20}$ −22.0 (*c* 0.15, MeOH) and *β*-D-glucose with $[\alpha]_D^{20}$ +41.0 (*c* 0.38, H_2O). The NMR data of **8a** were consistent with those of *threo*-1-*C*-syringylglycerol.[15] The negative optical rotation of **8a** indicated that the configuration of the glycerol moiety of **8** and **8a** was 7*R*,8*R*.[16,17] Therefore, **8** was determined to be (−)-(7*R*,8*R*)-*threo*-1-*C*-syringylglycerol 4-*O*-*β*-D-glucopyranoside.

Compound **9** showed IR, ESIMS, and NMR spectroscopic data completely identical to those of **8** (Tables 2 and 3 and Experimental Section). However, **8** and **9** were separable by reversed-phase HPLC with retention times of 27.9 and 29.8 min (Supporting Information), respectively. Enzymatic hydrolysis of **9** yielded **9a** and *β*-D-glucose. The spectroscopic data of **9a** were identical to those of **8a** except that the optical rotation of **9a** was opposite that of **8a**, indicating that **9a** was (+)-*threo*-syringylglycerol. Thus, **9** was determined to be (−)-(7*S*,8*S*)-*threo*-1-*C*-syringylglycerol 4-*O*-*β*-D-glucopyranoside.

The known compounds were identified by comparison of spectroscopic data (UV, IR, ESIMS, ^{1}H and ^{13}C NMR) with those reported in the literature as fraxin,[3] esculin,[4] 6,7-di-*O*-glucopyranosylaesculetin,[18] (+)-syringaresinol *O*-*β*-D-glucopyranoside, liriodendrin,[19] (+)-1-hydroxypinoresinol 4′-*O*-*β*-D-glucopyranoside, (+)-1-hydroxypinoresinol 4″-*O*-*β*-D-glucopyranoside;[20] 4-(2-hydroxyethyl)-2-methoxyphenyl *β*-D-glucopyranoside,[21] 2-(4-hydroxyphenyl) ethyl *β*-D-glucopyranoside,[22] 2-(3,4-dihydroxyphenyl) ethyl *β*-D-glucopyranoside,[23] 2-hydroxy-5-(2-hydroxyethyl) phenyl *β*-D-glucopyranoside,[24] 2-(4-hydroxyphenyl) ethyl *β*-D-apiofurano-

syl-(1→6)-β-D-glucopyranoside (osmanthuside H),[14] calceolariosides A and B, chiritoside C,[25] ferruginoside A,[26] acteoside,[27] plantasioside;[28] 2,6-dimethoxy-*p*-hydroxyquinone 1-*O*-β-D-glucopyranoside,[15] 2,6-dimethoxy-*p*-hydroxyquinone 4-*O*-β-D-glucopyranoside,[29] 4-hydroxy-3-methoxyphenyl β-D-glucopyranoside, 4-hydroxy-3-methoxyphenyl β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside;[30] linarionoside B, (9*S*)-linarionoside B,[31,32] and (3*R*,9*R*)-3-hydroxy-7,8-dihydro-β-ionol 9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.[33] (In the literature,[29,33] different nomenclature was used.)

Threo- and *erythro*-arylglycerols either in optically pure forms or enantiomeric mixtures have been reported from several plants,[15,17,20,23,24,34−36] and coupling constants of the deshielded benzylic proton (H-7) signal in the ^{1}H NMR spectra of their acetates were used to distinguish *threo* ($J_{7,8}$ > 7.0 Hz) and *erythro* ($J_{7,8}$ < 6.5 Hz) isomers.[20,23,34,36] A systematic analysis of the ^{13}C NMR data of the reported *threo*- and *erythro*-arylglycerols in different solvents indicated that the chemical shift difference of C-7 and C-8 ($\Delta\delta_{C8-C7}$) of the *threo*- and *erythro*-arylglycerols may be directly applicable to distinguish *threo*- and *erythro*-arylglycerols without substituent(s) at C-7 or/and C-8 of the glycerol moiety. In order to confirm the validity of the $\Delta\delta_{C8-C7}$ for distinguishing *threo*- and *erythro*-arylglycerols, the ^{13}C NMR data of **7**−**9** in DMSO-d_6, pyridine-d_5, and D_2O, as well as **7a**−**9a** in DMSO-d_6, pyridine-d_5, MeOH-d_4, and Me_2CO-d_6, were obtained. Without exception, the $\Delta\delta_{C8-C7}$ values of *erythro*-arylglycerols **7** and **7a** in the tested solvents were smaller than those of *threo*-arylglycerols **8**, **9**, **8a**, and **9a** (Tables 1 and 2, Supporting Information), which were consistent with literature reports.

Compounds **1**−**9** were tested for their TNF-α secretion inhibitory activities of mouse peritoneal macrophages, as well as antioxidant activities inhibiting Fe^{+2}-cystine-induced rat liver microsomal lipid peroxidation. At a concentration of 10^{-5} M, compounds **2**−**9** showed inhibition rates of 30.8%, 25.2%, 16.3%, 44.3%, 28.8%, 26.6%, 27.5%, and 28.2%, respectively, to TNF-α secretion of mouse peritoneal macrophages. The inhibition rate of **5** was higher than the positive control indomethacin, which gave an inhibition rate of 33.2% at the same concentration. Compound **6** showed antioxidant activity with an IC_{50} value of 0.9 μM, which was stronger than the positive control vitamin E, with an IC_{50} value of 4.6 μM, while others gave IC_{50} values larger than 10 μM. In addition, in the *in vitro* cytotoxic assay against human cancer cell lines including ovary (A 2780), colon (HCT-8), hepatoma (Bel-7402), stomach (BGC-823), and lung (A549), plantasioside (**10**) showed selective activity against the human colon cancer cell line (HCT-8) with an IC_{50} value of 3.4 μM. The other compounds were inactive to all tested cell lines (IC_{50} > 5 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. NMR spectra were obtained at 500 or 400 MHz for ^{1}H and 125 or 100 MHz for ^{13}C, respectively, on Inova 500 and 400 MHz spectrometers in DMSO-d_6, MeOH-d_4, pyridine-d_5, Me_2CO-d_6, or D_2O with solvent peaks (or TMS, in the case of D_2O) being used as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo ionspray source) spectrometer. HRESIMS data were measured using an AccuToFCS JMS-T100CS spectrometer. Column chromatography was performed using silica gel (200−300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual-λ absorbance detector with an Alltima (250 × 10 mm) preparative column packed with C_{18} (5 μm). TLC was carried out on precoated silica gel GF_{254} plates. Spots were visualized under UV light (254 or 356 nm) or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. Stem bark of *F. sieboldiana* (20 kg) was collected at Lu Mountain, Jiangxi Province, China, in August 2004. Plant identification was verified by Prof. Lin Ma (Institute of Materia Medica). A voucher specimen (No. ZH02272) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Beijing 100050, China.

Extraction and Isolation. The air-dried stem bark of *F. sieboldiana* (20 kg) was powdered and extracted with 11.0 L of 95% EtOH at room temperature for 3 × 48 h. The EtOH extract was evaporated under reduced pressure to yield a residue (428.6 g). The residue was suspended in H_2O (1500 mL) and then partitioned with EtOAc (5 × 1000 mL). The aqueous phase was applied to a HDP100 macroporous adsorbent resin (1000 g) column. Successive elution of the column with H_2O, 30% EtOH, 50% EtOH, and 95% EtOH (5000 mL each) yielded four corresponding fractions after removing solvents. The fraction (82.2 g) eluted by H_2O was separated by MPLC over reversed-phase silica gel eluting with a gradient of increasing MeOH (0−50%) in H_2O to give four fractions (A−D) on the basis of TLC analysis. Separation of fraction B (5.52 g) on normal silica gel CC, eluting with a gradient of increasing MeOH (0−100%) in $CHCl_3$, afforded five fractions (B_1−B_5). Fraction B_3 (1.98 g) was subjected to CC over Sephadex LH-20, using MeOH−H_2O (70:30) as the eluting solvent, to give three subfractions (B_{3-1}−B_{3-3}). Subfractions B_{3-2} (0.32 g) and B_{3-3} (0.24 g) were separately purified by reversed-phase preparative HPLC, using MeOH−H_2O (20:80), to afford **3** (85.0 mg), **4** (61.6 mg), **5** (73.0 mg), and **6** (56.9 mg). Fraction B_4 (0.61 g) was chromatographed over Sephadex LH-20 eluting with MeOH and then separated by reversed-phase preparative HPLC, using MeCN−H_2O (0.8:99.2), to afford **7** (12.1 mg), **8** (7.3 mg), and **9** (11.0 mg).

The fraction eluted by 30% EtOH (73.0 g) was subjected to MPLC over reversed-phase silica gel (C-18), with a gradient of increasing MeOH (0−100%) in H_2O, to give five fractions (E−I). Fraction G (1.25 g) was subjected to CC over Sephadex LH-20 (MeOH) to give **2** (75.2 mg). Fraction H (2.15 g) was chromatographed over silica gel, with a gradient of increasing MeOH (10−50%) in $CHCl_3$, to afford subfractions H_1−H_3. Subfraction H_3 (0.42 g) was purified by reversed-phase preparative HPLC, using MeCN−H_2O (16:84), to yield **1** (3.1 mg).

Fraxinuacidoside (1): colorless gum; $[\alpha]_D^{20}$ −17.5 (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ε) 215 (1.9), 279 (sh); IR (KBr) ν_{max} 3389, 2921, 2853, 1747, 1654, 1573, 1484, 1364, 1077, 1038 cm^{-1}; ^{1}H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR (MeOH-d_4, 100 MHz) data, see Table 1; ESIMS *m*/*z* 505 $[M + Na]^+$; HRESIMS *m*/*z* 505.2386 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_9Na$, 505.2414).

6-[6-(4-*O*-β-D-Glucopyranosyloxy-3-methoxybenzoyl)]-*O*-β-D-glucopyranosyloxy-7-hydroxycoumarin (2): yellowish, amorphous powder; $[\alpha]_D^{20}$ −82.2 (*c* 0.30, DMSO); UV (MeOH) λ_{max} (log ε) 226 (3.9), 256 (3.8), 296 (2.9), 336 (3.2) nm; IR (KBr) ν_{max} 3396, 2908, 1712, 1612, 1570, 1510, 1419, 1296, 1277, 1219, 1074, 760 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 651 $[M - H]^-$, 675 $[M + Na]^+$, and 691 $[M + K]^+$; HRESIMS *m*/*z* 675.1547 $[M + Na]^+$ (calcd for $C_{29}H_{32}O_{17}Na$, 675.1537).

2,5-Di-*O*-β-D-glucopyranosyloxy-1,3-dimethoxybenzene (3): amorphous powder; $[\alpha]_D^{20}$ −52.1 (*c* 0.08, H_2O); UV (MeOH) λ_{max} (log ε) 226 (3.9), 280 (sh) nm; IR (KBr) ν_{max} 3398, 2939, 2893, 1601, 1508, 1469, 1425, 1242, 1173, 1130, 1078, 816 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 517 $[M + Na]^+$; HRESIMS *m*/*z* 517.1547 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_{14}Na$, 517.1533).

1,4-Di-*O*-β-D-glucopyranosyloxy-2-methoxybenzene (4): amorphous powder; $[\alpha]_D^{20}$ −25.7 (*c* 0.09, H_2O); UV (MeOH) λ_{max} (log ε) 230 (3.8), 280 (sh) nm; IR (KBr) ν_{max} 3402, 2927, 1597, 1506, 1466, 1423, 1333, 1238, 1128, 1074 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 487 $[M + Na]^+$; HRESIMS *m*/*z* 487.1446 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_{13}Na$, 487.1428).

2-(4-Hydroxy-3-methoxyphenyl)ethanol 1-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] (5): colorless gum; $[\alpha]_D^{20}$ −61.8 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.8), 280 (3.4) nm; IR (KBr) ν_{max} 3392, 2935, 2883, 1604, 1518, 1273, 1045, 822 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 485 $[M + Na]^+$ and 501 $[M + K]^+$; HRESIMS *m*/*z* 485.1650 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_{12}Na$, 485.1635).

2-(3,4-Dihydroxyphenyl)ethanol 1-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] (6): colorless gum; $[\alpha]_D^{20}$ −53.5 (*c* 0.54, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (3.6), 278 (3.1) nm; IR (KBr) ν_{max} 3388, 2933, 2885, 1606, 1527, 1282, 1049 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 471 [M + Na]$^+$ and 487 [M + K]$^+$; HRESIMS *m*/*z* 471.1498 [M + Na]$^+$ (calcd for $C_{19}H_{28}O_{12}Na$, 471.1478).

(−)-(7*R*,8*S*)-*erythro*-1-*C*-Syringylglycerol 4-*O*-β-D-glucopyranoside (7): amorphous powder; $[\alpha]_D^{20}$ −32.7 (*c* 0.28, H_2O); UV (MeOH) λ_{max} (log ϵ) 215 (3.3), 230 (sh) nm; IR (KBr) ν_{max} 3277, 2943, 2896, 1661, 1601, 1512, 1461, 1249, 1126, 1027, 839 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 429 [M + Na]$^+$; HRESIMS *m*/*z* 429.1379 [M + Na]$^+$ (calcd for $C_{17}H_{26}O_{11}Na$, 429.1373).

(−)-(7*R*,8*R*)-*threo*-1-*C*-Syringylglycerol 4-*O*-β-D-glucopyranoside (8): amorphous powder; $[\alpha]_D^{20}$ −28.2 (*c* 0.40, H_2O); UV (MeOH) λ_{max} (log ϵ) 215 (3.2), 230 (sh) nm; IR (KBr) ν_{max} 3370, 2958, 2921, 1596, 1504, 1465, 1421, 1335, 1239, 1132, 1066, 834 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 429 [M + Na]$^+$ and 445 [M + K]$^+$.

(−)-(7*S*,8*S*)-*threo*-1-*C*-Syringylglycerol 4-*O*-β-D-glucopyranoside (9): amorphous powder; $[\alpha]_D^{20}$ −21.1 (*c* 0.47, H_2O); UV (MeOH) λ_{max} (log ϵ) 215 (3.3), 230 (sh) nm; IR (KBr) ν_{max} 3377, 2959, 2842, 1645, 1597, 1504, 1465, 1422, 1335, 1240, 1131, 1065, 1005, 835 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) and ^{13}C NMR (D_2O, 100 MHz) data, see Tables 2 and 3; ESIMS *m*/*z* 429 [M + Na]$^+$ and 445 [M + K]$^+$.

Enzymatic Hydrolysis of 1. Compound **1** (3.0 mg) was hydrolyzed with 7.0 mg of β-glucosidase (Almonds Lot 1264252, Sigma-Aldrich) in 1.5 mL of H_2O at 37 °C for 12 h. After removal of solvent under reduced pressure, the residue was extracted with MeCN, and the MeCN extract was chromatographed over silica gel, eluting with $CHCl_3$−MeCN (25:1), to give **1a** (1.6 mg), and then eluting with $CHCl_3$−MeCN (3:1) to yield a sugar with $[\alpha]_D^{20}$ +46.2 (0.11, H_2O). Compound **1a** was a colorless gum: $[\alpha]_D^{20}$ −68.2 (*c* 0.36, MeOH); ^{1}H NMR (MeOH-d_4, 400 MHz) and ^{13}C NMR (MeOH-d_4, 100 MHz) data, see Table 1; ESIMS *m*/*z* 319 [M − H]$^-$, 343 [M + Na]$^+$.

Acidic Hydrolysis of 2. Compound **2** (6.6 mg) was refluxed in 2 N HCl (5.0 mL) at 80 °C for 3 h. The reaction mixture was extracted with CH_3Cl (3 × 5 mL), and the aqueous phase was neutralized with 1 N NaOH and dried using a stream of N_2. The residue was subjected to CC over silica gel with $CHCl_3$−MeCN (3:1) to yield a sugar (3.2 mg), $[\alpha]_D^{20}$ +40.2 (0.45, H_2O, 24 h after being dissolved in the solvent).

Enzymatic Hydrolyses of 3, 4, and 7−9. A solution of each compound in H_2O (3 mL) was individually hydrolyzed with β-glucosidase (10 mg) at 37 °C for 16 h. The reaction mixtures of **3** and **4** were extracted separately with CH_3Cl (3 × 3 mL). The CH_3Cl extracts were chromatographed over silica gel, eluting with CH_3Cl−MeCN (100:1), for the hydrolyzates from **3** (10.2 mg) and **4** (10.8 mg), to yield 4-hydroxy-2,6-dimethoxyphenol (3.3 mg) and 1,4-dihydroxy-2-methoxyphenol (3.5 mg), respectively. The aqueous phases of the hydrolyzates of **3** and **4** were dried using a stream of N_2 and then subjected to CC over silica gel eluted with $CHCl_3$−MeCN (3:1) to yield glucose (4.3 mg) from **3**, $[\alpha]_D^{20}$ +46.6 (*c* 0.43, H_2O), and glucose (5.1 mg) from **4**, $[\alpha]_D^{20}$ +48.6 (*c* 0.51, H_2O). The hydrolyzates of **7**−**9** were dried under reduced pressure, and the residues were chromatographed over silica gel, eluting with CH_3Cl−MeCN (25:1), to yield **7a** (5.1 mg), **8a** (3.6 mg), and **9a** (4.2 mg), and then eluting with $CHCl_3$−MeCN (3:1) to give a sugar, respectively, from the hydrolyzates of **7** (9.6 mg), **8** (6.2 mg), and **9** (7.8 mg). For the ^{1}H NMR (500 or 400 MHz) and ^{13}C NMR (125 or 100 MHz) data of **7a**, **8a**, and **9a** in different solvents, see Tables 2 and 4 in the Supporting Information. The optical rotations of the sugar obtained from **7**, **8**, and **9** were $[\alpha]_D^{20}$ +39.8 (*c* 0.55, H_2O), $[\alpha]_d^{20}$ +41.0 (*c* 0.38, H_2O), and $[\alpha]_d^{20}$ +38.9 (*c* 0.48, H_2O), respectively. The optical rotations of the sugars were measured after the samples were dissolved in H_2O for 24 h. Solvent systems $CHCl_3$−MeOH (2.5:1) for TLC and the upper layer of *n*-BuOH−AcOH−H_2O (4:1:5) for PC were used in glucose identification.

Cells, Culture Conditions, and Cell Proliferation Assay. See refs 37−39.

TNF-α Secretion Inhibition Assay. Peritoneal macrophages were prepared from male C57BL6J mice (the Experimental Animal Center, Institute of Experimental Animal, Chinese Academy of Medical Sciences & Peking Union Medical College), 4 days after the injection (i.p.) of Brewer's thioglycollate medium, washed twice with D-Hank's buffer, and resuspended in RPMI-1640 (Gibco/BRL, Gaithersburg, MD) at 10^6 cell/mL. The macrophage cells were plated in 48-well tissue culture plates at 2×10^5 cells per well and incubated at 37 °C in 5% (v/v) CO_2 for 2 h, the medium was removed, and the cells were then washed twice with D-Hank's buffer to remove cells not adhered to the well wall. After RPMI 1640 containing test compounds at a final concentration of 10^{-5} M, or stimulator lipopolysaccharide (LPS, 1 μg/mL), and 5% fetal calf serum was supplemented, the adhered macrophage cell line was incubated at 37 °C in 5% (v/v) CO_2 for 24 h. The supernatant was collected and kept for later use.

L929 cells (200 μL, 10^5 cells/mL) were inoculated in 96-well plates and incubated at 37 °C in 5% CO_2 for 24 h, and the supernatant was removed. Then 100 μL of RPMI 1640 containing actinomycin D (0.5 μg/mL) was supplemented, and the supernatant was prepared as described above or RPMI 1640 was added. After incubation at 37 °C in 5% CO_2 for 20 h, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) was added to the wells, and incubation continued for an additional 4 h. The supernatant was removed, and the cells were decomposed with DMSO (100 μL) for 10 min. Absorbance was measured at 570 nm using a MK 3 Wellscan (Labsystem Drogon) plate reader. Each value is the mean of reactions in three wells for a single compound. The absorbance of the wells with only RPMI 1640 added was used as blank. The percent TNF-α secretion inhibition of peritoneal macrophages was calculated as follows, by using a MS Excel 2000 (Microsoft Corp.) based program developed for this purpose:

$$\text{percent inhibition} = [(L - S)/(L - B)] \times 100$$

Here *L*, *S*, and *B* are the absorbances for the stimulator LPS, test samples, and blank, respectively. Indomethacin was used as the reference compound.

Antioxidative Activity Assay. Antioxidative activity was evaluated as the inhibitory activity of compounds against lipid peroxidation in rat liver microsomes according to a modified thiobarbituric acid (TBA) method.[40] In the TBA assay, microsomes were isolated from rat livers and suspended in 100 mM Tris-HCl buffer (pH 7.4). The microsomal suspension (1 mg protein/mL), different concentrations of compound or vehicle, and 0.2 mM cysteine in 0.1 M PBS (pH 7.4) were incubated at 37 °C for 15 min, 50 μM $FeSO_4$ was added, and the reaction mixture was then incubated at 37 °C for 15 min again. An equal volume of 20% TCA was added to terminate the reaction, and the mixture was centrifuged at 3000*g* for 10 min. The supernatant (1 mL) was mixed with 0.67% (w/v) TBA and kept in a boiling water bath for 10 min. After cooling, lipid peroxidation was assessed by measuring the thiobarbituric acid reactive product at 532 nm. Lipid peroxidation inhibitory activity was calculated as follows: $[1 - (T - B)/(C - B)] \times 100$ (%), in which *T*, *C*, and *B* are absorbance values of the sample treated, the control without sample, and the zero time control, respectively. Vitamin E was used as the positive control.

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Supporting Information Available: 1D NMR spectra of compounds **1**−**9** and **1a**; SI Tables 1−4 of the NMR data of **7**−**9** and **7a**−**9a** in different solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

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