

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 H₂O-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*-arylgllycerols in different solvents, an application of $\Delta\delta_{C8-C7}$ values to distinguish *threo*-arylgllycerol and *erythro*-arylgllycerol isomers was proposed. In the *in vitro* assays, compound **5** displayed TNF- α secretion inhibitory activity with an IC₅₀ value of 1.6 μ M, compound **6** showed antioxidative activity inhibiting Fe²⁺-cystine-induced rat liver microsomal lipid peroxidation with an IC₅₀ value of 0.9 μ M, and plantasioside (**10**) showed selective activity against the human colon cancer cell line (HCT-8) with an IC₅₀ value of 3.4 μ M.

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 phenylethanoids⁹ 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 H₂O and EtOAc. The H₂O 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 C₂₅H₃₈O₉, 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 (H₃-8') and 0.95 (H₃-9'), two olefinic methyl singlets at δ 1.63 (H₃-9), and 1.75 (H₃-7'), and an olefinic methyl triplet at δ 1.85 (*J* = 2.0 Hz, H₃-10). 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 ¹³C NMR and DEPT spectra of **1** showed six quaternary carbons that were identified as a carboxylic carbon, four sp² olefinic carbons, and a sp³ 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

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

no.	1 (MeOH- <i>d</i> ₄)		1a (MeOH- <i>d</i> ₄)	
	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)			

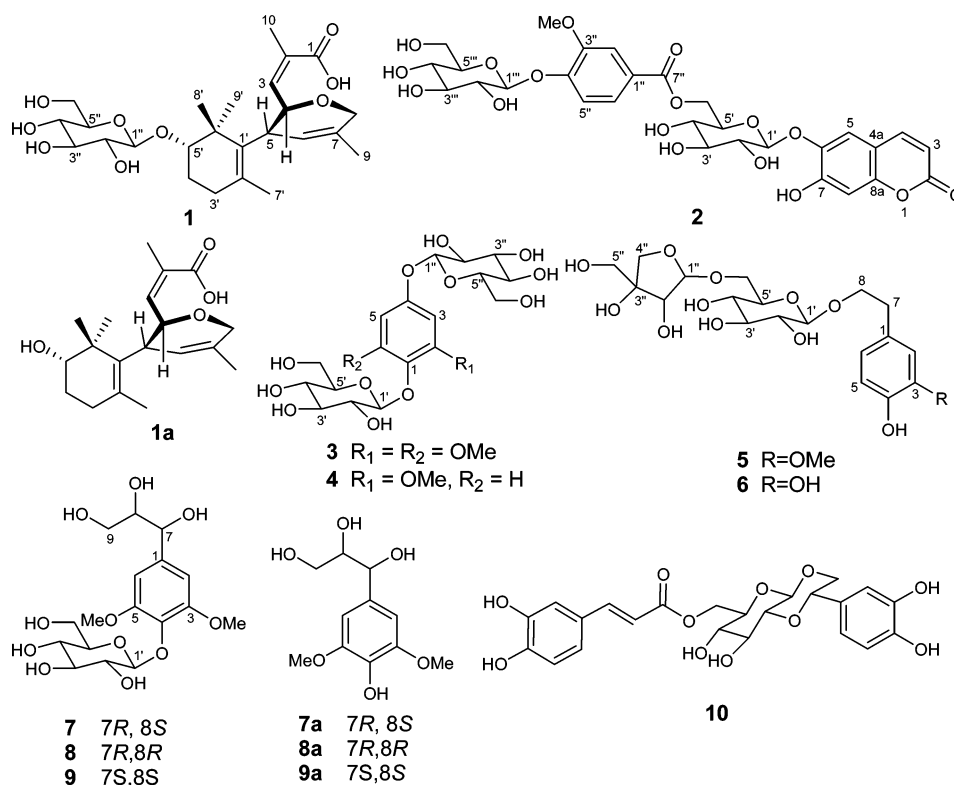
^a NMR data (δ) were measured in MeOH-*d*₄ for **1** and **1a** at 400 MHz for ¹H NMR and at 100 MHz for ¹³C NMR. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on DEPT, ¹H–¹H COSY, HSQC, HMBC, and phase-sensitive ¹H–¹H COSY experiments.

enzymatic hydrolysis of **1** with β -glucosidase (Table 1 and Experimental Section). The glucose isolated gave a positive optical rotation, [α]_D²⁰ +46.2 (c 0.11, H₂O), indicating that it was D-glucose.¹³

The proton and protonated carbon signals of **1** were assigned unambiguously by the HSQC experiment. ¹H–¹H COSY correlations from H-5' through H₂-4' to H₂-3' and HMBC correlations from H₃-7' to C-1', C-2', and C-3' and from both H₃-8' and H₃-9' 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 H₂-8 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 H₃-10 to C-1, C-2, and C-3, and from H₃-9 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 H₂-8 and H₃-9 and of H₃-10 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 ¹³C 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 H₃-9' by irradiation of H-1'' indicated that H₃-9' 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 H₃-10 when H-3 was irradiated. In addition, in the NOE difference experiments, H₃-8' and H₃-9' were enhanced by irradiation of H-5, but H₃-7' was not enhanced, whereas irradiation of H-4 caused an enhancement of H₃-7'. These NOE effects indicated that H-5 was close to both H₃-8' and H₃-9', and H-4 to H₃-7', 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 ¹³C 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 fraxinuacidiside.

Compound **2** was obtained as a yellowish, amorphous powder, and HRESIMS indicated the molecular formula to be C₂₉H₃₂O₁₇. 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 ¹³C 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 ¹³C 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. ^1H 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 $_2$ O)	8 (D $_2$ O)	9 (D $_2$ O)
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 ^1H NMR data (δ) were measured in DMSO- d_6 for 2–6 and D $_2$ O for 7–9 at 500 or 400 MHz. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^1H – ^1H 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'''), and 3.46 (1H, ddd, J = 10.0, 7.0, and 5.0 Hz, H-6'''). ^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 $\Delta\delta_{\text{H}}$ 0.96 (H-6'a) and 0.86 (H-6'b), and $\Delta\delta_{\text{C}}$ 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 $[\text{M} + \text{Na}]^+$, and the molecular formula $\text{C}_{20}\text{H}_{30}\text{O}_{14}$ was indicated by HRESIMS. The ^1H 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 $[\text{M} + \text{Na}]^+$ in its ESIMS. The molecular formula $\text{C}_{19}\text{H}_{28}\text{O}_{13}$ 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 $[\text{M} + \text{Na}]^+$ and 501 $[\text{M} + \text{K}]^+$ in the ESIMS. Its molecular formula, $\text{C}_{20}\text{H}_{30}\text{O}_{12}$, 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 ₂ O)	8 (D ₂ O)	9 (D ₂ O)
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 D₂O for 7–9 at 125 and 100 MHz. The assignments were based on DEPT, ^1H – ^1H 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 H₂-7, 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 $\rightarrow$ 6)- β -D-glucopyranoside].

Compound 6 was obtained as a colorless gum, and HRESIMS at m/z 471.1498 [$M + \text{Na}$]⁺ indicated the molecular formula to be C₁₉H₂₈O₁₂. 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 $\rightarrow$ 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 [$M + \text{Na}$]⁺, and HRESIMS indicated the molecular formula to be C₁₇H₂₆O₁₁. The ^1H NMR spectrum of 7 in D₂O 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, H₂O). The NMR data of 7a (Experimental Section) were in good agreement with those of *erythro*-1-C-syringylglycerol, 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-glucopyranoside 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*-aryl glycerols 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, H₂O). 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, ^1H and ^{13}C NMR) with those reported in the literature as fraxin,³ esculin,⁴ 6,7-di-*O*-glucopyranosylaesuletin,¹⁸ (+)-syringaresinol *O*- β -D-glucopyranoside, liri-odendrin,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-apiofuran-

syl-(1 $\rightarrow$ 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 $\rightarrow$ 6)-*O*- β -D-glucopyranoside;³⁰ linarionoside B, (9S)-linarionoside B,^{31,32} and (3R,9R)-3-hydroxy-7,8-dihydro- β -ionol 9-*O*- β -D-apiofuranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside.³³ (In the literature,^{29,33} different nomenclature was used.)

Threo- and *erythro*-arylgllycerols 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 ¹H 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 ¹³C NMR data of the reported *threo*- and *erythro*-arylgllycerols in different solvents indicated that the chemical shift difference of C-7 and C-8 ($\Delta\delta_{C8-C7}$) of the *threo*- and *erythro*-arylgllycerols may be directly applicable to distinguish *threo*- and *erythro*-arylgllycerols 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*-arylgllycerols, the ¹³C NMR data of 7–9 in DMSO-*d*₆, pyridine-*d*₅, and D₂O, as well as 7a–9a in DMSO-*d*₆, pyridine-*d*₅, MeOH-*d*₄, and Me₂CO-*d*₆, were obtained. Without exception, the $\Delta\delta_{C8-C7}$ values of *erythro*-arylgllycerols 7 and 7a in the tested solvents were smaller than those of *threo*-arylgllycerols 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 Fe²⁺-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 IC₅₀ value of 0.9 μ M, which was stronger than the positive control vitamin E, with an IC₅₀ value of 4.6 μ M, while others gave IC₅₀ values larger than 10 μ M. 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 IC₅₀ value of 3.4 μ M. The other compounds were inactive to all tested cell lines (IC₅₀ > 5 μ M).

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 ¹H and 125 or 100 MHz for ¹³C, respectively, on Inova 500 and 400 MHz spectrometers in DMSO-*d*₆, MeOH-*d*₄, pyridine-*d*₅, Me₂CO-*d*₆, or D₂O with solvent peaks (or TMS, in the case of D₂O) 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 $\times$ 10 mm) preparative column packed with C₁₈ (5 μ m). TLC was carried out on precoated silica gel GF₂₅₄ plates. Spots were visualized under UV light (254 or 356 nm) or by spraying with 7% H₂SO₄ 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 $\times$ 48 h. The EtOH extract was evaporated under reduced pressure to yield a residue (428.6 g). The residue was suspended in H₂O (1500 mL) and then partitioned with EtOAc (5 $\times$ 1000 mL). The aqueous phase was applied to a HDP100 macroporous adsorbent resin (1000 g) column. Successive elution of the column with H₂O, 30% EtOH, 50% EtOH, and 95% EtOH (5000 mL each) yielded four corresponding fractions after removing solvents. The fraction (82.2 g) eluted by H₂O was separated by MPLC over reversed-phase silica gel eluting with a gradient of increasing MeOH (0–50%) in H₂O 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 CHCl₃, afforded five fractions (B₁–B₅). Fraction B₃ (1.98 g) was subjected to CC over Sephadex LH-20, using MeOH–H₂O (70:30) as the eluting solvent, to give three subfractions (B₃₋₁–B₃₋₃). Subfractions B₃₋₂ (0.32 g) and B₃₋₃ (0.24 g) were separately purified by reversed-phase preparative HPLC, using MeOH–H₂O (20:80), to afford 3 (85.0 mg), 4 (61.6 mg), 5 (73.0 mg), and 6 (56.9 mg). Fraction B₄ (0.61 g) was chromatographed over Sephadex LH-20 eluting with MeOH and then separated by reversed-phase preparative HPLC, using MeCN–H₂O (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 H₂O, 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 CHCl₃, to afford subfractions H₁–H₃. Subfraction H₃ (0.42 g) was purified by reversed-phase preparative HPLC, using MeCN–H₂O (16:84), to yield 1 (3.1 mg).

Fraxinuacidoside (1): colorless gum; [α]_D²⁰ –17.5 (c 0.52, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 215 (1.9), 279 (sh); IR (KBr) $\nu_{\max}$ 3389, 2921, 2853, 1747, 1654, 1573, 1484, 1364, 1077, 1038 cm^{–1}; ¹H NMR (MeOH-*d*₄, 400 MHz) and ¹³C 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 C₂₅H₃₈O₉Na, 505.2414).

6-[6-(4-*O*- β -D-Glucopyranosyloxy-3-methoxybenzoyl)]-*O*- β -D-glucopyranosyloxy-7-hydroxycoumarin (2): yellowish, amorphous powder; [α]_D²⁰ –82.2 (c 0.30, DMSO); UV (MeOH) $\lambda_{\max}$ (log ϵ) 226 (3.9), 256 (3.8), 296 (2.9), 336 (3.2) nm; IR (KBr) $\nu_{\max}$ 3396, 2908, 1712, 1612, 1570, 1510, 1419, 1296, 1277, 1219, 1074, 760 cm^{–1}; ¹H NMR (DMSO-*d*₆, 500 MHz) and ¹³C 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 C₂₉H₃₂O₁₇Na, 675.1537).

2,5-Di-*O*- β -D-glucopyranosyloxy-1,3-dimethoxybenzene (3): amorphous powder; [α]_D²⁰ –52.1 (c 0.08, H₂O); UV (MeOH) $\lambda_{\max}$ (log ϵ) 226 (3.9), 280 (sh) nm; IR (KBr) $\nu_{\max}$ 3398, 2939, 2893, 1601, 1508, 1469, 1425, 1242, 1173, 1130, 1078, 816 cm^{–1}; ¹H NMR (DMSO-*d*₆, 500 MHz) and ¹³C 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 C₂₀H₃₀O₁₄Na, 517.1533).

1,4-Di-*O*- β -D-glucopyranosyloxy-2-methoxybenzene (4): amorphous powder; [α]_D²⁰ –25.7 (c 0.09, H₂O); UV (MeOH) $\lambda_{\max}$ (log ϵ) 230 (3.8), 280 (sh) nm; IR (KBr) $\nu_{\max}$ 3402, 2927, 1597, 1506, 1466, 1423, 1333, 1238, 1128, 1074 cm^{–1}; ¹H NMR (DMSO-*d*₆, 500 MHz) and ¹³C 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 C₁₉H₂₈O₁₃Na, 487.1428).

2-(4-Hydroxy-3-methoxyphenyl)ethanol 1-*O*-[β -D-apiofuranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside] (5): colorless gum; [α]_D²⁰ –61.8 (c 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 226 (3.8), 280 (3.4) nm; IR (KBr) $\nu_{\max}$ 3392, 2935, 2883, 1604, 1518, 1273, 1045, 822 cm^{–1}; ¹H NMR (DMSO-*d*₆, 500 MHz) and ¹³C 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 C₂₀H₃₀O₁₂Na, 485.1635).

2-(3,4-Dihydroxyphenyl)ethanol 1-O- β -D-apiofuranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (6): colorless gum; $[\alpha]_D^{20}$ -53.5 (c 0.54, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 228 (3.6), 278 (3.1) nm; IR (KBr) $\nu_{\max}$ 3388, 2933, 2885, 1606, 1527, 1282, 1049 cm^{-1} ; ^1H 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 $[\text{M} + \text{Na}]^+$ and 487 $[\text{M} + \text{K}]^+$; HRESIMS m/z 471.1498 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{28}\text{O}_{12}\text{Na}$, 471.1478).

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

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

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

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 $^\circ\text{C}$ 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); ^1H NMR (MeOH- d_4 , 400 MHz) and ^{13}C NMR (MeOH- d_4 , 100 MHz) data, see Table 1; ESIMS m/z 319 $[\text{M} - \text{H}]^-$, 343 $[\text{M} + \text{Na}]^+$.

Acidic Hydrolysis of 2. Compound 2 (6.6 mg) was refluxed in 2 N HCl (5.0 mL) at 80 $^\circ\text{C}$ for 3 h. The reaction mixture was extracted with CH_2Cl_2 (3 $\times$ 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 $^\circ\text{C}$ for 16 h. The reaction mixtures of 3 and 4 were extracted separately with CH_2Cl_2 (3 $\times$ 3 mL). The CH_2Cl_2 extracts were chromatographed over silica gel, eluting with CH_2Cl_2 –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_2Cl_2 –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 ^1H 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 C57BL/6J 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 $^\circ\text{C}$ 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 $\mu\text{g}/\text{mL}$), and 5% fetal calf serum was supplemented, the adhered macrophage cell line was incubated at 37 $^\circ\text{C}$ 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 $^\circ\text{C}$ in 5% CO_2 for 24 h, and the supernatant was removed. Then 100 μL of RPMI 1640 containing actinomycin D (0.5 $\mu\text{g}/\text{mL}$) was supplemented, and the supernatant was prepared as described above or RPMI 1640 was added. After incubation at 37 $^\circ\text{C}$ 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 Wellsan (LabSystem Drogen) 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 $^\circ\text{C}$ for 15 min, 50 μM FeSO_4 was added, and the reaction mixture was then incubated at 37 $^\circ\text{C}$ for 15 min again. An equal volume of 20% TCA was added to terminate the reaction, and the mixture was centrifuged at 3000g 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>.

References and Notes

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