Neolignans and Glycosides from the Stem Bark of *Illicium difengpi*

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Five new neolignans (**1**−**4** and **9**), two pairs of neolignan epimers (**5**−**8**), and two new aromatic glycosides (**10** and **11**) have been isolated from the stem bark of *Illicium difengpi*. Their structures were determined by spectroscopic methods, including 1D and 2D NMR, HRESIMS, CD experiments, and chemical methods. The absolute configurations of the 3,4-diol moiety in **1** and 1,3-diol moiety in **2** were confirmed by Snatzke's method, observing the induced circular dichroism after addition of dimolybdenum tetraacetate in DMSO. Compounds **3**, **4**, and **11** exhibited moderate anti-inflammatory activities with IC_{50} values ranging from 1.62 to 24.4 μM, while compound **3** displayed antioxidant activity with an IC_{50} value of 42.3 μM.

Illicium difengpi K. I. B et K. I. M. (Illiciaceae), indigenous to China, is a toxic shrub that grows in the mountainous areas of Guangxi Province. The stem bark is listed in *Chinese Pharmacopeia* and has been applied as a traditional Chinese medicine to treat rheumatic arthritis.[1] To date, only 20 compounds including neolignans and glycosides were isolated from the stem bark of this plant.[2−5]

As part of a program to search for bioactive compounds from toxic medicinal plants, a MeOH extract of the stem bark of *I. difengpi* was investigated. Five new neolignans, difengpiol A (**1**), difengpiol B (**2**), (7*R*,8*S*)-4,7,9-trihydroxy-3,5,3′,5′-tetramethoxy-8-*O*-4′-neolignan-8′-ene (**3**), (7*S*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan (**4**), and neodifengpin (**9**), two pairs of neolignan epimers, (7*R*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,5,3′-trimethoxy-8-*O*-4′-neolignan (**5**), (7*S*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,5,3′-trimethoxy-8-*O*-4′-neolignan (**6**), (7*R*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (**7**), and (7*R*,8*S*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (**8**), as well as two new aromatic glycosides, 2-hydroxy-4,5-methylenedioxyphenol-1-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**10**) and 3-hydroxy-4,5-dimethoxyphenol-1-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**11**), were isolated from the extract. Compounds **1** and **2** are rare dihydrobenzofuran neolignans with a partially hydrogenated aromatic ring, while **4**−**8** are unusual 8-*O*-4′-neolignans with a glycerol moiety at C-4. We describe herein the isolation and structural elucidation of the new compounds and determination of absolute configurations through spectroscopic analysis and chemical methods, especially the employment of Snatzke's method. The anti-inflammatory and antioxidant activities of **1**−**11** are also assayed.

Results and Discussion

Compound **1** was obtained as an amorphous, white powder. The molecular formula $C_{19}H_{26}O_6$ was established on the basis of positive HRESIMS (*m*/*z* 373.1632 $[M + Na]^+$, calcd for $C_{19}H_{26}O_6Na$, 373.1622). The IR spectrum of **1** showed the presence of hydroxy (3355 cm^{-1}) and aromatic (1607 and 1499 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) revealed signals of a 1,2,3,5-tetrasubstituted aromatic ring at δ_H 6.69 (1H, br s, H-2′) and 6.70 (1H, br s, H-6′) together with signals of an aromatic *O*-methyl group at δ_H 3.85 (3H, s). Three oxymethine protons [δ_H 3.97 (1H, m, H-3), 3.68 (1H, m, H-4), and 4.98 (1H, d, J = 6.0 Hz, H-7)], a pair of oxymethylene protons [δ_H 3.76 (1H, dd, J = 11.5, 5.5 Hz, H-9a) and 3.70 (1H, dd, J = 11.5, 7.5 Hz, H-9b)], an olefinic methine proton at δ_H 5.68 (1H, br s, H-2), two methylenes [δ_H 1.93 (1H, m, H-5a), 1.68 (1H, m, H-5b), 2.19 (1H, m, H-6a), and 2.06 (1H, m, H-6b)], and a methine proton at δ_H 3.35 (1H, m, H-8) were also observed in the ^1H NMR spectrum. The carbon signals in the ^{13}C NMR and DEPT spectra of **1** further confirmed the above units. Correlations in the $^1H-^1H$ COSY and HSQC spectra of **1** indicated the presence of CH(2)−CH(3)−CH(4)−CH_2(5)−CH_2(6), CH(7)−CH(8)−CH_2(9), and CH(7′)−CH(8′)−CH_2(9′) units (Figure 2). In the HMBC spectrum, the long-range correlations from H-7 to C-4′, from H-8 to C-4′, C-5′, and C-6′, and from H-7′ to C-1′, C-2′, and C-6′ confirmed the presence of a dihydrobenzofuran unit with a propanol moiety linked to C-1′. Furthermore, the HMBC correlations from H-5 and H-6 to C-1 and from H-7 and H-8 to C-1 indicated the presence of a 3,4-dihydroxy-1-cyclohexenyl unit located at C-7.

The absolute configurations of C-7 and C-8 of **1** were determined as 7*S*,8*R*, on the basis of the NOE correlations (Figure 3) between H-8 and H-2, indicating H-7 and H-8 to be *trans*, and the positive Cotton effects at 237 and 286 nm in the CD spectrum.[6,7]

The absolute configuration of the 3,4-diol moiety in **1** was determined using induced circular dichroism spectra by Snatzke's method.[8−11] Briefly, this simple, practical, and reliable method involves the in situ complexation of a 1,2-diol with $[Mo_2(OAc)_4]$, which leads to a significant induced circular dichroism spectrum (ICD). Only the sign of the Cotton effect around 310 nm in the ICD is related to the chirality of the diol moiety, expressed by the sign of the O−C−C−O torsion angle. Thus, the absolute configuration of the 1,2-diol moiety can be determined by means of the empirical helicity rule correlating the helicity expressed by the O−C−C−O subunit with the sign of the Cotton effect around 310 nm. However, it is necessary to first determine the relative configuration of the 1,2-diol unit. An *erythro* configuration of C-3 and C-4 was confirmed by the NOE experiments (Figure 3), which displayed correlations between H-3 and H-4 and between H-3, H-4, and H-5b. Then, the positive Cotton effect at 314 nm in the ICD (Figure 4) permitted assignment of a 3*S*,4*R* absolute configuration for **1** on the basis of the empirical rule (Figure 5). Accordingly, the structure of difengpiol A (**1**) was elucidated as shown in Figure 1.

Compound **2** was isolated as a white, amorphous powder, and its molecular formula $C_{20}H_{30}O_7$ was determined by positive

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** (500 and 125 MHz)

	1[a]		**2**[a]		**2**[b]	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	141.0		75.4		73.0	
2	125.3	5.68 br s	32.4	1.64−1.84 (overlapped)	31.3	1.47−1.69 (overlapped)
3	73.2	3.97 m	79.0	3.42 m	77.7	3.30 m
4	73.7	3.68 m	66.2	4.06 m	63.7	3.94 m
5a	29.5	1.93 m	27.0	1.64−1.84 (overlapped)	26.2	1.47−1.69 (overlapped)
5b		1.68 m		1.28 m		1.16 m
6a	23.4	2.19 m	26.7	1.64−1.84 (overlapped)	26.2	1.47−1.69 (overlapped)
6b		2.06 m		1.64−1.84 (overlapped)		1.47−1.69 (overlapped)
7	89.8	4.98 d (6.0)	93.5	4.27 d (5.5)	91.7	4.16 br s
8	51.9	3.35 m	47.0	3.49 m	45.0	3.38−3.45 (overlapped)
9a	65.7	3.76 dd (11.5, 5.5)	66.2	3.63 m	64.6	3.53 m
9b		3.70 dd (11.5, 7.5)				
1′	137.0		136.3		134.1	
2′	114.4	6.69 br s	114.1	6.59 br s	112.1	6.57 br s
3′	145.4		144.9		143.0	
4′	147.9		148.1		146.3	
5′	130.1		130.8		130.3	
6′	118.2	6.70 br s	118.0	6.70 br s	116.6	6.65 br s
7′	33.2	2.62 t (7.5)	32.9	2.54 t (7.5)	31.6	2.63 (overlapped)
8′	36.1	1.82 m	35.8	1.64−1.84 (overlapped)	34.8	1.47−1.69 (overlapped)
9′	62.5	3.58 t (6.5)	62.3	3.49 m	60.3	3.38−3.45 (overlapped)
3′-OCH_3	57.0	3.85 s	56.8	3.78 s	55.6	3.71 s
4-OCH_3			56.1	3.30 s	55.1	3.20 s
1-OH						4.33 s

[a] Recorded in MeOH-d_4. [b] Recorded in DMSO-d_6. Data assignments were based on HMQC and HMBC experiments.

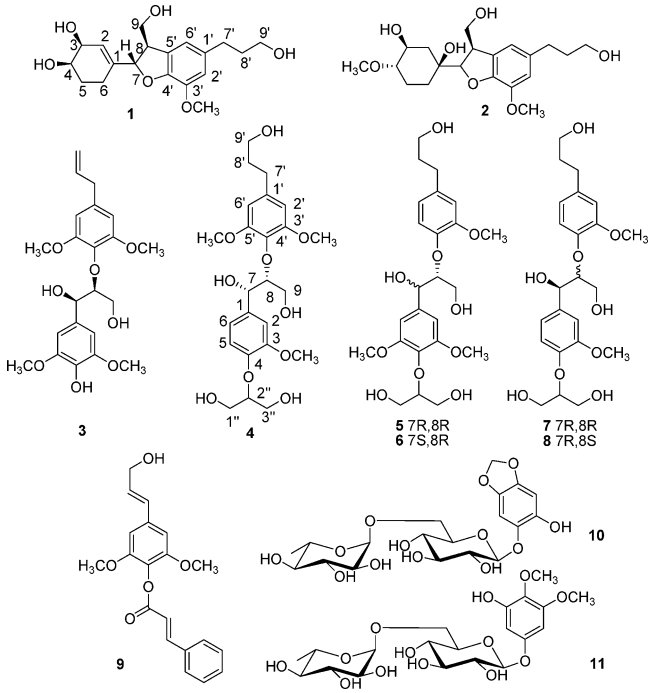

Figure 1. Structures of compounds **1**−**11**.

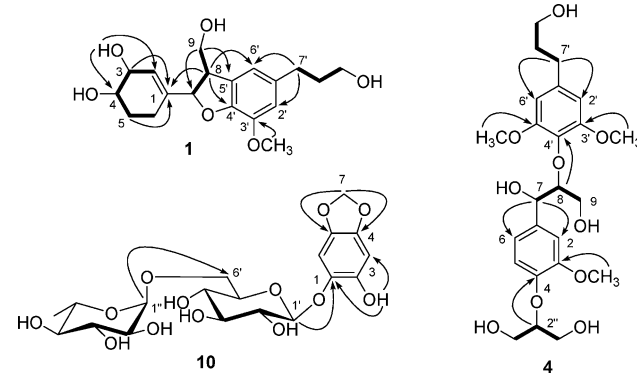

Figure 2. Selected COSY (bold bonds) and HMBC correlations of **1**, **4**, and **10**.

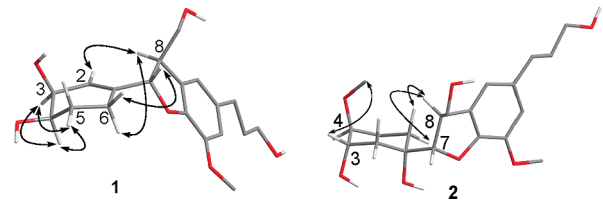

Figure 3. Key NOEs of **1** and **2**.

HRESIMS (*m*/*z* 405.1896 $[M + Na]^+$, calcd for 405.1884). The IR spectrum displayed absorption bands for hydroxy (3373 cm^{-1}) and aromatic (1606 and 1501 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those observed for **1** (Table 1), except for differences associated with the presence of a hydroxy group at C-1 (δ_C 75.4) and an *O*-methyl group at C-4 (δ_H 4.06, m; δ_C 66.2). The locations of the hydroxy and *O*-methyl groups were confirmed by long-range correlations in the HMBC spectrum between the OH proton (δ 4.33) and C-1 and between the OMe protons (δ 3.30) and C-4, respectively.

The correlations between H-6 and H-8 in the NOE experiments (Figure 3) indicated H-7 and H-8 to be *trans*. Positive Cotton effects at 233 and 286 nm in the CD spectrum confirmed the 7*S*,8*R* absolute configuration.[6,7]

The absolute configuration of the 1,3-diol moiety in **2** was also determined by observing the induced circular dichroism spectra after addition of dimolybdenum tetraacetate in DMSO.[8,12,13] However, for the 1,3-diol moiety, only the *syn*-parallel orientation of the hydroxy groups allows for the exchange of acetate groups from $Mo_2(OAc)_4$ and leads to an ICD spectrum. The observed sign of the Cotton effect around 400 nm in the ICD solely depends on the chirality of the 1,3-diol moiety. Thus, the absolute configuration of the 1,3-diol moiety can be determined by means of the empirical sector rule on the basis of the sign of the Cotton effect around 400 nm. Accordingly, the positive Cotton effect at 440 nm observed in the ICD confirmed the 1*S*,3*S* absolute configuration according to the sector rule (Figures 4 and 5). The correlation between H-3 (δ 4.06) and the OMe protons (δ 3.30) in the NOE experiment (Figure 2) indicated the C-3−C-4 *threo* configuration. Hence, the absolute

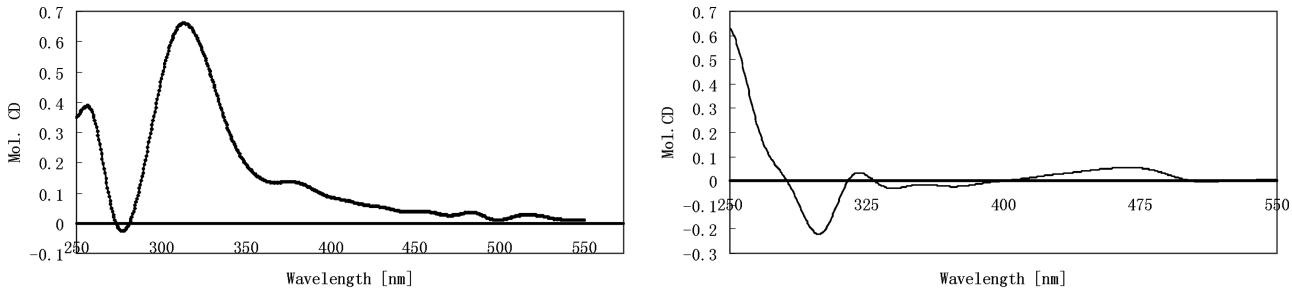

Figure 4. Circular dichroism spectra of **1** and **2** in a DMSO solution of dimolybdenum tetraacetate.

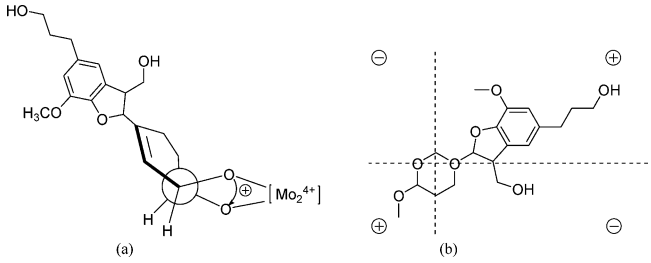

Figure 5. (a) Projection of the helicity rule for compound **1**. (b) Projection of the sector rule for compound **2**. Carbon atoms C-1 and C-3 are located behind the oxygen atoms.

configuration of C-4 was *R*. According to the above analysis, the structure of difengpiol B (**2**) was established as shown in Figure 1.

Compound **3** was obtained as a white powder. The molecular formula $C_{22}H_{28}O_8$ was established by HRESIMS (*m*/*z* 443.1688 [M + Na]$^+$). When its ^{1}H and ^{13}C NMR data (Tables 2 and 3) were compared with those of 7,9-dihydroxy-3,4,5,3′,5′-pentamethoxy-8-*O*-4′-neolignan-8′-ene,[14] the resonances of an *O*-methyl group were absent in **3**, indicating instead a hydroxy group at C-4. The assignments of the ^{1}H and ^{13}C NMR data were facilitated by comparison with those of the known compound and confirmed by the HMQC and HMBC data.

The configuration of **3** was elucidated by analysis of the NMR and CD data. The ^{1}H NMR spectrum of **3** showed a $J_{7,8}$ value of 3.0 Hz, suggesting that **3** possessed an *erythro* configuration.[15,16] The CD spectrum of **3** showed a positive Cotton effect at 228 nm, indicating that **3** had an 8*S* configuration.[17] Accordingly, compound **3** was characterized as (7*R*,8*S*)-4,7,9-trihydroxy-3,5,3′,5′-tetramethoxy-8-*O*-4′-neolignan-8′-ene.

Compound **4** showed an [M + Na]$^+$ ion at 505.2065 in the HRESIMS, indicative of a molecular formula of $C_{24}H_{34}O_{10}$. Its IR spectrum displayed absorption bands attributable to hydroxy (3390 cm^{-1}) and aromatic (1590 and 1508 cm^{-1}) groups. The ^{1}H NMR spectrum (Table 3) showed signals attributable to a 1,2,3,5-tetrasubstituted aromatic ring at δ_H 6.48 (2H, br s, H-2′, H-6′), a 1,2,4-trisubstituted aromatic ring at δ_H 7.02 (1H, br s, H-2), 6.80 (1H, d, J = 8.0 Hz, H-5), and 6.84 (1H, d, J = 8.0 Hz, H-6), and three *O*-methyl groups at δ_H 3.76 (3H, s) and 3.74 (6H, s). The ^{1}H NMR data also indicated the presence of two propane-triol moieties [δ_H 4.89 (1H, d, J = 5.0 Hz, H-7), 4.14 (1H, m, H-8), 3.83 (2H, dd, J = 12.0, 5.0 Hz, H-9), and 3.67 (4H, m, H-1″, H-3″), 4.16 (1H, m, H-2″)] and a propanol moiety [δ_H 2.58 (2H, t, J = 8.0 Hz, H-7′), 1.76 (2H, m, H-8′), and 3.49 (2H, t, J = 6.0 Hz, H-9′)]. These moieties were confirmed by ^{1}H−^{1}H COSY (Figure 2) and HSQC experiments. These data suggested that compound **4** was an 8-*O*-4′ neolignan. Three *O*-methyl groups were attached at C-3′, C-5′, and C-3 and were confirmed by long-range correlations between OCH$_3$ (δ 3.74) and C-3′, between OCH$_3$ (δ 3.74) and C-5′, and between OCH$_3$ (δ 3.76) and C-3 in the HMBC spectrum. HMBC correlations from H-2′ and H-6′ to C-7′, from H-2 and H-6 to C-7, from H-8 to C-4′, and from H-2″ to C-4 indicated that the gross structure of **4** was 4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan. An *erythro* configuration of **4** was confirmed by the $J_{7,8}$ value of 5.0 Hz in the ^{1}H NMR spectrum. A negative Cotton effect at 241 nm in the CD spectrum indicated an 8*R* configuration. Compound **4** was thus (7*S*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′- trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan.

Compound **5** was obtained as an amorphous powder, and the molecular formula $C_{24}H_{34}O_{10}$ was determined by HRESIMS. Its ^{1}H and ^{13}C NMR data (Tables 2 and 3) were in accordance with literature values for 4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,5,3′-trimethoxy-8-*O*-4′-neolignan.[3] Thus, the gross structure of **5** was established as shown. The $J_{7,8}$ value of 6.5 Hz in the ^{1}H NMR spectrum suggested that **5** had a *threo* configuration.[15,16] In the CD spectrum, a negative Cotton effect at 241 nm suggested that **5** possessed an 8*R* configuration.[17] Therefore, **5** was elucidated as (7*R*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,5,3′-trimethoxy-8-*O*-4′-neolignan.

Compound **6** was obtained as an amorphous powder with the molecular formula $C_{24}H_{34}O_{10}$ established by positive HRESIMS (*m*/*z* 505.2057 [M + Na]$^+$, calcd for 505.2044). When its ^{1}H and ^{13}C NMR data (Tables 2 and 3) were compared with those of **5**, small differences in the chemical shifts of C-7, C-8, and C-9 were observed. This indicated that **6** was a diastereisomer of **5**. The $J_{7,8}$ value of 4.0 Hz in the ^{1}H NMR spectrum indicated that **6** had an *erythro* configuration. The CD spectrum of **6** showed a negative Cotton effect at 245 nm, indicating an 8*R* configuration. Accordingly, compound **6** was elucidated as (7*S*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,5,3′-trimethoxy-8-*O*-4′-neolignan.

Compound **7** was isolated as a white, amorphous powder. Its molecular formula was determined to be $C_{23}H_{32}O_9$ by HRESIMS (*m*/*z* 475.1956 [M + Na]$^+$, calcd for 475.1939). The ^{1}H and ^{13}C NMR data (Tables 2 and 3) of **7** were in good agreement with those of 4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan.[3] The ^{1}H NMR spectrum showed a $J_{7,8}$ value of 6.5 Hz, suggesting that **7** possessed a *threo* configuration. In the CD spectrum, a negative Cotton effect at 240 nm indicated an 8*R* configuration. Therefore, **7** was characterized as (7*R*,8*R*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan.

Compound **8** was isolated as a white, amorphous powder, and its molecular formula $C_{23}H_{32}O_9$ was determined by HRESIMS at *m*/*z* 475.1959 [M + Na]$^+$. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) suggested **8** was a diastereisomer of **7**. An *erythro* configuration of **8** was confirmed by the $J_{7,8}$ value of 5.0 Hz in the ^{1}H NMR spectrum. In the CD spectrum, a positive Cotton effect at 237 nm indicated an 8*S* configuration. Consequently, **8** was determined to be (7*R*,8*S*)-4-*O*-(glycer-2-yl)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan.

Compound **9** was obtained as a white, amorphous powder, and the molecular formula was identified as $C_{20}H_{20}O_5$ by HRESIMS at *m*/*z* 363.1212 [M + Na]$^+$. The IR spectrum revealed the presence of hydroxy (3480 cm^{-1}), ester carbonyl (1712 cm^{-1}), and aromatic (1594 and 1507 cm^{-1}) groups. The ^{1}H NMR data (Table 3) indicated

Table 2. ^{13}C NMR Data of Compounds **3**−**9** (125 MHz in methanol-d_4)

position	**3**[a]	**4**	**5**	**6**	**7**	**8**	**9**[a]
1	130.4	135.9	136.1	136.1	136.2	137.3	134.3
2	102.4	111.2	105.7	105.3	111.5	112.8	128.9
3	147.0	150.5	154.2	154.3	150.6	152.0	128.3
4	133.8	146.8	139.2	139.0	146.9	148.5	130.5
5	147.0	117.3	154.2	154.3	117.3	118.8	128.3
6	102.4	119.5	105.7	105.3	119.8	120.9	128.9
7	72.6	76.9	74.2	73.8	72.7	74.0	146.6
8	87.1	86.1	86.0	86.7	85.2	87.5	116.8
9	60.6	61.7	62.4	61.9	60.8	62.1	164.7
1′	136.8	138.8	137.9	138.1	136.9	138.4	135.2
2′	105.4	105.7	113.9	113.9	112.8	114.2	103.2
3′	153.2	153.1	151.6	151.6	150.5	151.9	152.3
4′	134.2	133.5	147.2	147.5	145.9	147.8	131.0
5′	153.2	153.1	119.0	119.0	118.3	119.6	152.3
6′	105.4	105.7	121.7	121.9	120.6	122.2	103.2
7′	40.6	32.2	32.7	32.8	31.5	33.0	130.9
8′	135.2	34.3	35.5	35.5	34.4	35.8	129.0
9′	116.4	60.2	62.1	62.2	61.0	62.5	63.5
3′-OCH_3	56.1	55.4	56.6	56.6	55.3	56.7	56.2
5′-OCH_3	56.3	55.4	56.6	56.6			56.2
3-OCH_3		55.2	56.4	56.5	55.3	56.7	
1″,3″		60.8	62.2	62.1	60.8	62.3	
2″		81.9	84.7	84.7	81.8	83.3	

[a] Recorded in chloroform-d_1. Data assignments were based on HMQC and HMBC experiments.

Table 3. ^1H NMR Data of Compounds **3**−**9** (500 MHz in methanol-d_4)

position	**3**[a]	**4**	**5**	**6**	**7**	**8**	**9**[a]
1							
2	6.60 br s	7.02 br s	6.69 br s	6.74 br s	7.02 br s	7.05 br s	7.59 dd (6.5, 3.0)
3							7.41 m
4							7.41 m
5		6.80 d (8.0)			6.96 d (8.0)	6.98 d (8.0)	7.41 m
6	6.60 br s	6.84 d (8.0)	6.69 br s	6.74 br s	6.87 d (8.0)	6.90 d (8.0)	7.59 dd (6.5, 3.0)
7	5.01 d (3.0)	4.89 d (5.0)	4.70 d (6.5)	4.87 d (4.0)	4.79 d (6.0)	4.86 d (5.5)	7.89 d (16.0)
8	4.12 m	4.14 m	4.26 m	4.24 m	4.15 m	4.15 m	6.72 d (16.0)
9	3.44 d (7.0)	3.83 dd (12.0, 5.0)	3.82 dd (11.5, 5.0)	3.74 m	3.80 dd (12.0, 6.5)	4.20 dd (11.5, 5.0)	
1′							
2′	6.50 br s	6.48 br s	6.70 br s	6.76 br s	6.72 br s	6.78 br s	6.67 br s
3′							
4′							
5′			6.71 d (8.0)	6.82 d (8.0)	6.72 d (8.0)	6.80 d (8.0)	
6′	6.50 br s	6.48 br s	6.57 d (8.0)	6.61 d (8.0)	6.58 d (8.0)	6.83 d (8.0)	6.67 br s
7′	3.38 d (6.5)	2.58 t (8.0)	2.53 t (7.5)	2.55 t (7.5)	2.53 t (7.5)	2.56 t (7.5)	6.59 d (16.0)
8′	5.99 m	1.76 m	1.73 m	1.74 m	1.73 m	1.74 m	6.34 m
9′	5.16 m	3.49 t (6.0)	3.48 t (6.5)	3.48 m	3.49 t (6.5)	3.49 t (6.0)	4.35 d (5.5)
3-OCH_3	3.90 s	3.76 s	3.75 s	3.76 s	3.74 s	3.78 s	
5-OCH_3	3.90 s		3.75 s	3.76 s			
3′-OCH_3	3.90 s	3.74 s	3.71 s	3.76 s	3.72 s	3.76 s	3.84 s
5′-OCH_3	3.90 s	3.74 s					3.84 s
1″,3″		3.67 m	3.66 d (4.5)	3.67 m	3.67 d (5.0)	3.69 m	
2″		4.16 m	3.90 t (4.5)	3.91 m	4.20 m	4.18 m	

[a] Recorded in chloroform-d_1. Chemical shifts are given in ppm. Figures in parentheses are coupling constants (J) in Hz. Data assignments were based HMQC and HMBC experiments.

the presence of a monsubstituted aromatic ring [δ_H 7.59 (2H, dd, J = 6.5, 3.0 Hz, H-2 and H-6), and 7.41 (3H, m, H-3, H-4, and H-5)], a 1,2,3,5-tetrasubstitued aromatic ring [δ_H 6.67 (2H, br s, H-2′, H-6′)], two *O*-methyl groups [δ_H 3.84 (6H, s)], and an allylic group [δ_H 4.35 (2H, d, J = 5.5 Hz, H-9′), 6.59 (1H, d, J = 16.0 Hz, H-7′), and 6.34 (1H, m, H-8′)]. A pair of *E*-olefinic methine protons at δ_H 7.89 (1H, d, J = 16.0 Hz, H-7) and 6.72 (1H, d, J = 16.0 Hz, H-8) were also observed in the ^1H NMR spectrum. The ^{13}C NMR data (Table 2) indicated the presence of an ester carbonyl group at δ_C 164.7. In the HMBC experiments, the long-range correlations from H-7 to C-1, C-2, and C-6 indicated the presence of a cinnamyl moiety. The long-range correlations from H-7′ to C-1′, C-2′, and C-6′ in the HMBC spectrum in combination with the HMQC and $^1H-^1H$ COSY spectra established the presence of an (*E*)-1-(3-hydroxyprop-1-enyl)-2,6-dimethoxyphenol moiety. Therefore, the structure of neodifengpin (**9**) was elucidated as shown in Figure 1.

Compound **10** was isolated as a white, amorphous powder. The molecular formula $C_{19}H_{26}O_{13}$ was established by HRESIMS (*m*/*z* 485.1273 $[M + Na]^+$, calcd for 485.1266). The ^1H NMR spectrum showed signals attributable to a 1,2,4,5-tetrasubstituted aromatic ring at δ_H 6.37 (1H, s, H-3), and 6.70 (1H, s, H-6) and a methylene dioxy group at δ_H 5.77 (2H, s, H-7). The observation of two anomeric protons at δ_H 4.68 (H-1″) and 4.49 (H-1′) and corresponding carbons at δ_C 102.4 (C-1″) and 105.9 (C-2″) in the NMR spectra (Table 4) suggested the existence of two sugar units. Acid hydrolysis of **10** afforded a glucose and a rhamnose. The large coupling constant (7.5 Hz) of the anomeric proton at δ_H 4.49 revealed that the glucose was in the β-configuration, while the small coupling constant (1.5 Hz) of the anomeric proton at δ_H 4.68 indicated that the rhamnose was in the α-configuration. GC analysis established the D- and L-configuration of the moieties, respectively.[18] Linkage information on the two sugar units was obtained on the basis of the ^{13}C NMR and HMBC spectra. The presence of a

Table 4. ^{1}H and ^{13}C NMR Data of Compounds **10** and **11**[a] (500 and 125 MHz in methanol-d_4)

	10		**11**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	140.0		152.1	
2	143.6		113.6	6.31 d (2.5)
3	99.0	6.37 s	155.1	
4	145.1		95.3	6.31 d (2.5)
5	141.4		99.1	
6	102.6	6.70 s	156.1	6.31 d (2.5)
7	102.3	5.77 s		
1′	105.9	4.49 d (7.5)	104.3	4.77 d (7.5)
2′	74.5	3.43 m	75.2	3.41−3.47 (overlapped)
3′	77.7	3.34−3.40 (overlapped)	78.1	3.41−3.47 (overlapped)
4′	71.5	3.34−3.40 (overlapped)	71.8	3.35−3.39 (overlapped)
5′	77.0	3.34−3.40 (overlapped)	77.3	3.54 m
6′	68.0	3.96 dd (11.0, 1.5)	68.1	4.03 d (11.0)
		3.45−3.64 (overlapped)		3.61−3.71 (overlapped)
1″	102.4	4.68 d (1.5)	102.5	4.74 s
2″	72.1	3.82 m	72.6	3.92 m
3″	72.4	3.45−3.64 (overlapped)	72.4	3.61−3.71 (overlapped)
4″	74.1	3.29−3.36 (overlapped)	74.4	3.35−3.39 (overlapped)
5″	69.8	3.45−3.64 (overlapped)	70.1	3.61−3.71 (overlapped)
6″	18.0	1.18 d (6.0)	18.2	1.23 d (6.0)
4-OCH_3			61.4	3.82 s
5-OCH_3			56.8	3.70 s

[a] Data assignments were based on HMQC and HMBC experiment.

downfield methylene signal at δ_C 68.0 (C-6′) in the ^{13}C NMR spectrum indicated the attachment of the α-L-rhamnopyranosyl moiety at C-6′ of the β-D-glucopyranosyl moiety, and this was further confirmed by the HMBC correlation from H-1″ to C-6′. HMBC correlations between H-1′ and C-1 indicated the attachment of the β-D-glucopyranosyl moiety at C-1 of the aglycone. Thus, the structure of **10** was determined to be 2-hydroxy-4,5-methylenedioxyphenol-1-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **11** was obtained as a white, amorphous powder, and the molecular formula $C_{20}H_{30}O_{13}$ was deduced from HRESIMS (*m*/*z* 479.1758 $[M + H]^+$, calcd for 479.1759). The IR spectrum indicated the presence of hydroxy (3337 cm^{-1}) and aromatic ring (1601 and 1507 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **11** (Table 4) were similar to 3-hydroxy-4,5-dimethoxyphenol,[19] except for additional signals of two sugar units. A glucose and a rhamnose were obtained by acid hydrolysis of **11**. The large coupling constant (7.5 Hz) for the anomeric proton indicated the β-configuration for the glucose, while a broad singlet revealed the α-configuration for the rhamnose. GC data established the D- and L-configuration of the moieties, respectively.[18] In the HMBC spectrum, correlations from H-1″ and C-6′ and from H-1′ to C-1 suggested that the α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside unit was located at C-1 of the aglycone. Hence, the structure of **11** was elucidated as 3-hydroxy-4,5-dimethoxyphenol-1-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

The anti-inflammatory activities of **1**−**11** were evaluated by measuring the inhibitory ratios of β-glucuronidase release in rat polymorphonuclear leukocytes (PMNs) induced by platelet-activating factor (PAF) in vitro. Ginkgolide B was used as a positive control. As shown in Table 5, compounds **3**, **4**, and **11** exhibited moderate anti-inflammatory activities with IC_{50} values ranging from 1.62 to 24.4 μM, while the other compounds were inactive.

The antioxidant activities of **1**−**11** were assessed by measuring their inhibition activity on liver microsomal lipid peroxidation induced by the Fe^{2+}-Cys system in vitro. Vitamin E was selected as the positive control. Compound **3** displayed antioxidant activity with an IC_{50} value of 42.3 μM. The remaining compounds did not show antioxidant activity.

Table 5. Anti-inflammatory Activity of Compounds **3**, **4**, and **11**

compound[a]	IC_{50} values (μM)
3	24.4
4	1.62
11	7.66
ginkgolide B[b]	2.35

[a] Compounds **1**, **2**, and **5**−**10** were inactive (IC_{50} > 50 μM). [b] Positive control.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a P2000 automatic digital polarimeter. UV spectra were taken with a Hitachi UV-240 spectrophotometer. CD spectra were measured on a JASCO *J*-815 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer. NMR spectra were recorded on an Inova 500 MHz spectrometer. ESIMS data were obtained on a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS were measured on an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS. Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with a SPD-10A detector using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Analytical HPLC was measured on an Agilent 1100 Series instrument with a DAD detector using a YMC column (Rp-18 4.6 × 100 mm). GC data were recorded on an Agilent N6890 instrument. Column chromatography was performed with HP 20 macroporous resin (Mitsubishi Chemical Corporation), polyamide (30−60 mesh, Jiangsu Linjiang Chemical Reagents Factory, China), Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden), ODS (50 μm, Merck), and silica gel (200−300 mesh, Qingdao Marine Chemical Inc. China). TLC was carried out with glass precoated with silica gel GF_{254} (Qingdao Marine Chemical Inc. China). Dimolybdenum tetraacetate was purchased from Acros, USA.

Plant Material. The stem bark of *I. difengpi* (20 kg) was purchased from Tongrentang Pharmacy, Beijing, China, in September 2007, and identified by Prof. Lin Ma (Department of Natural Products Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences). A voucher specimen (No. 20070922) is deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences, People's Republic of China.

Extraction and Isolation. The air-dried stem bark of *I. difengpi* (20 kg) was ground, and the resultant powder was sequentially extracted with CH_2Cl_2 and MeOH to yield 1500 g of a MeOH extract. The crude MeOH extract was subjected to polyamide column chromatography and eluted with 40% EtOH and 60% EtOH to yield two corresponding fractions, A (640 g) and B (209 g). Fraction A was further applied to an HP 20 macroporous adsorbent resin (3500 g) column and eluted with H_2O, 30% EtOH, and 60% EtOH to yield three corresponding fractions (A_1−A_3, 40, 201, and 110 g, respectively). Fraction A_2 (201 g) was fractionated on a silica gel column (160−200 mesh, 850 g) with $CHCl_3$/MeOH/H_2O (100:1:0.1, 50:1:0.1, 30:1:0.1, 15:1:0.1, 10:1:0.1, 7:1:0.1, 5:1:0.1, 3:1:0.1, 1:1:0.1) to give 12 fractions (A_{2-1}−A_{2-12}). Fraction A_{2-1} (280 mg) was subjected to Sephadex LH-20 column chromatography and eluted with MeOH to yield six fractions (A_{2-1-1}−A_{2-1-6}). Fraction A_{2-1-3} (150 mg) was further fractionated by silica gel column chromatography with *n*-hexane/EtOAc (20:1, 10:1, 5:1, 2:1) to yield three subfractions ($A_{2-1-3-1}$−$A_{2-1-3-3}$). Subfraction $A_{2-1-3-3}$ was purified by preparative HPLC using 65% CH_3CN/H_2O to afford **9** (32 mg). Subfraction A_{2-1-5} (200 mg) was purified by preparative HPLC using 45% CH_3CN/H_2O to give **3** (26 mg). Fraction A_{2-3} (4.95 g) was subjected to Sephadex LH-20 column chromatography to afford five subfractions (A_{2-3-1}−A_{2-3-5}). Subfraction A_{2-3-2} (536 mg) was separated by preparative HPLC using 17% CH_3CN/H_2O to give **5** (100 mg) and **6** (27 mg). Fraction A_{2-4} (20.55 g) was chromatographed over a Sephadex LH-20 column to give eight fractions (A_{2-4-1}−A_{2-4-8}). Fraction A_{2-4-2} (7.80 g) was further chromatographed on an ODS column (50 μm, 300 g) with MeOH/H_2O (5:95, 10:90, 15:85, 20:80, 25:75, 30:70) to give 13 subfractions ($A_{2-4-2-1}$−$A_{2-4-2-13}$). Compound **2** (29 mg) was obtained from subfraction $A_{2-4-2-10}$ (231 mg) by preparative HPLC using 15% CH_3CN/H_2O. Subfraction $A_{2-4-2-9}$ (380 mg) was purified by preparative HPLC using 13% CH_3CN/H_2O to yield **4** (19 mg). Subfraction $A_{2-4-2-12}$ (700 mg) was separated by preparative HPLC using 15% CH_3CN/H_2O to give **7** (30 mg) and **8** (14 mg). The separation of fraction A_{2-5} (15.37 g) by Sephadex LH-20 chromatography eluted with MeOH/H_2O (60:40) and preparative HPLC using 14% CH_3CN/H_2O yielded **1** (32 mg). Fraction A_{2-8} (14.53 g) was subjected to a Sephadex LH-20 column and eluted with MeOH/H_2O (60:40) to give seven fractions (A_{2-8-1}−A_{2-8-7}).

Fraction A_{2-8-3} (7.80 g) was further subjected to ODS column (50 μm, 300 g) chromatography eluted with MeOH/H_2O (5:95, 10:90, 15:85, 20:80) to yield eight subfractions ($A_{2-8-3-1}$−$A_{2-8-3-8}$). Subfraction $A_{2-8-3-4}$ (520 mg) was further purified by Sephadex LH-20 and preparative HPLC using 15% CH_3CN/H_2O to yield **10** (28 mg). Compound **11** (43 mg) was obtained from subfraction $A_{2-8-3-7}$ (1.25 g) by preparative HPLC using 15% CH_3CN/H_2O.

Difengpiol A (1): white, amorphous powder; $[\alpha]^{20}_D$ +45.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.5), 285 (3.7) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 237 (1.93), 286 (0.49) nm; IR (KBr) ν_{max} 3355, 2934, 1607, 1499, 1325, 951 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 373 [M + Na]$^+$; HRESIMS *m/z* 373.1632 [M + Na]$^+$ (calcd for $C_{19}H_{26}O_6Na$, 373.1622).

Difengpiol B (2): white, amorphous powder; $[\alpha]^{20}_D$ +27.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.4), 284 (3.6) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 233 (0.67), 286 (0.37) nm; IR (KBr) ν_{max} 3373, 2934, 1606, 1501, 1452, 1212, 1009 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 405 [M + Na]$^+$, 421 [M + K]$^+$, 787 [2 M + Na]$^+$; HRESIMS *m/z* 405.1896 [M + Na]$^+$ (calcd for $C_{20}H_{30}O_7Na$, 405.1884).

(7*R*,8*S*)-4,7,9-Trihydroxy-3,5,3′,5′-tetramethoxy-8-*O*-4′-neolignan-8′-ene (3): white, amorphous powder; $[\alpha]^{20}_D$ +2.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.7), 278 (3.2) nm; IR (KBr) ν_{max} 3422, 2937, 1665, 1590, 1503, 1459, 1121, 915, 827 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 443 [M + Na]$^+$, 459 [M + K]$^+$, and 419 [M − H]$^-$; HRESIMS *m/z* 443.1688 [M + Na]$^+$ (calcd for $C_{22}H_{28}O_8Na$, 443.1676).

(7*S*,8*R*)-4-*O*-(Glycer-2-yl)-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan (4): white, amorphous powder; $[\alpha]^{20}_D$ −12.0 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.5), 276 (3.0) nm; IR (KBr) ν_{max} 3390, 2939, 1590, 1508, 1460, 1125 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 505 [M + Na]$^+$, 481 [M − H]$^-$; HRESIMS *m/z* 505.2065 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_{10}Na$, 505.2044).

(7*R*,8*R*)-4-*O*-(Glycer-2-yl)-7,9,9′-trihydroxy-3,5,3′-trimethoxy-8-*O*-4′-neolignan (5): white, amorphous powder; $[\alpha]^{20}_D$ −12.5 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.4), 277 (3.2) nm; IR (KBr) ν_{max} 3395, 2940, 1593, 1509, 1461, 1226, 1125, 1032 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 505 [M + Na]$^+$, 521 [M + K]$^+$, 481 [M − H]$^-$; HRESIMS *m/z* 505.2075 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_{10}Na$, 505.2044).

(7*S*,8*R*)-4-*O*-(Glycer-2-yl)-7,9,9′-trihydroxy-3,5,3′-trimethoxy-8-*O*-4′-neolignan (6): white, amorphous powder; $[\alpha]^{20}_D$ −3.0 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.4), 278 (3.3) nm; IR (KBr) ν_{max} 3397, 2940, 1593, 1509, 1461, 1228, 1125, 1032 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 505 [M + Na]$^+$, 521 [M + K]$^+$, 481 [M − H]$^-$; HRESIMS *m/z* 505.2057 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_{10}Na$, 505.2044).

(7*R*,8*R*)-4-*O*-(Glycer-2-yl)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (7): white, amorphous powder; $[\alpha]^{20}_D$ −9.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.5), 279 (3.3) nm; IR (KBr) ν_{max} 3376, 2939, 1590, 1510, 1463, 1264, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 475 [M + Na]$^+$, 491 [M + K]$^+$, and 451 [M − H]$^-$; HRESIMS *m/z* 475.1956 [M + Na]$^+$ (calcd for $C_{23}H_{32}O_9Na$, 475.1939).

(7*R*,8*S*)-4-*O*-(Glycer-2-yl)-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (8): white, amorphous powder; $[\alpha]^{20}_D$ +4.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.5), 279 (3.2) nm; IR (KBr) ν_{max} 3384, 2938, 1590, 1511, 1463, 1264, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 475 [M + Na]$^+$, 451 [M − H]$^-$; HRESIMS *m/z* 475.1959 [M + Na]$^+$ (calcd for $C_{23}H_{32}O_9Na$, 475.1939).

Neodifengpin (9): amorphous powder; UV (MeOH) λ_{max} (log ε) 206 (4.3), 280 (3.7) nm; IR (KBr) ν_{max} 3480, 3007, 2936, 1712, 1633, 1594, 1507, 1461, 1417, 1148 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 363 [M + Na]$^+$, 379 [M + K]$^+$; HRESIMS *m/z* 363.1212 [M + Na]$^+$ (calcd for $C_{20}H_{20}O_5Na$, 363.1203).

2-Hydroxy-4,5-methylenedioxyphenol-1-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (10): amorphous powder; UV (MeOH) λ_{max} (log ε) nm 203 (4.5), 228 (2.9), 304 (3.3); IR (KBr) ν_{max} 3366, 1637, 1502, 1484, 1660, 1064, 1038, 932, 859 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; ESIMS *m/z* 485 [M + Na]$^+$, 947 [2 M + Na]$^+$; HRESIMS *m/z* 485.1273 [M + Na]$^+$ (calcd for $C_{19}H_{26}O_{13}Na$, 485.1266).

3-Hydroxy-4,5-dimethoxyphenol-1-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (11): amorphous powder; UV (MeOH) λ_{max} (log ε) nm 205 (4.3), 225 (3.1); IR (KBr) ν_{max} 3337, 2934, 1601, 1507, 1453, 1228, 1064, 1045, 834, 810 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 4; ESIMS *m/z* 501 [M + Na]$^+$, 507 [M + K]$^+$, and 477 [M − H]$^-$; HRESIMS *m/z* 479.1758 [M + H]$^+$ (calcd for $C_{20}H_{31}O_{13}$, 479.1759).

Determination of Absolute Configuration of the Diol Moieties in Compounds 1 and 2 by Snatzke's Method. Following the reported procedure,[8] a solution of **1** (0.75 mg) in dry DMSO (1 mL) was mixed with dimolybdenum tetraacetate (1.2 mg). The first CD of the mixture (ca. 1:1.2 diol/dimolybdenum tetraacetate) was recorded immediately after mixing, and its time evolution was monitored until stationary. The observed sign of the diagnostic band at 314 nm in the ICD was correlated to the absolute configuration of the 3,4-diol moiety in **1**. To analyze **2**, ca. 1:2 diol/dimolybdenum tetraacetate mixtures were subjected to CD measurements as above. The observed sign of the diagnostic band at 440 nm in the ICD was correlated to the absolute configuration of the 1,3-diol moiety in **2**.[12]

HPLC Analysis of Compounds 4−8. HPLC analyses of compounds **4**−**8** were performed on a YMC column (Rp-18 4.6 × 100 mm) using a mobile phase of CH_3CN/H_2O (flow rate, 0.5 mL/min; temperature, 25 °C). The mobile phase gradient program was 10:90 (t = 0 min) and 23.5:67.5 (t = 40 min). The retention times of compounds **4**−**8** were t_{R4} = 25.90 min, t_{R5} = 19.20 min, t_{R6} = 20.94 min, t_{R7} = 22.26 min, and t_{R8} = 24.11 min, respectively.

Acid Hydrolysis of Compounds 10 and 11. Compounds **10** and **11** (10 mg) were dissolved in 5% HCl/H_2O (25 mL) and heated at 90 °C for 10 h. Each reaction mixture was diluted with water and extracted with EtOAc three times (15, 15, 15 mL). The aqueous layer was evaporated to dryness under pressure to give a monosaccharide residue. From the residue, glucose and rhamnose were detected by TLC with authentic samples.

Absolute Configuration of the Monosaccharides. The absolute configurations of the glucose and the rhamnose were determined according to a reported procedure.[18] Authentic sugar samples were trimethylsilylated, and the derivatives were analyzed by GC. The monosaccharides obtained from the acid hydrolysis of compounds **10** and **11** were treated similarly to the authentic sugar samples. The retention times of sugar derivatives were compared with those of the authentic samples (L-rhamnose 18.5 min, D-glucose 19.6 min).

Anti-inflammatory Activity Assays.[20] The anti-inflammatory activities of compounds **1**−**11** were assayed by measuring the inhibitory ratio of β-glucuronidase release in rat PMNs induced by PAF in vitro. Ginkgolide B was used as a positive control. Briefly, the samples were dissolved in DMSO at a concentration of 0.1 M and diluted to 10^{-3} M. The samples (5.0 μL) were incubated with a suspension of rat PMNs (250 μL) at a density of 2.5×10^6 cells mL^{-1} at 37 °C for 15 min, and 1×10^{-3} M cytochalasin B (2.5 μL) was added for another 5 min. After the addition of PAF (2.5 μL), the mixture was incubated for another 5 min. Subsequently, the mixture was put in an ice-bath to terminate the reaction. After centrifugation at 4000 rpm for 5 min, the supernatant was obtained. The supernatant (25 μL) and 2.5 mM phenolphthaleinglucuronic acid (25 μL) were incubated with 0.1 M HOAc buffer (100 μL) at 37 °C for 18 h. The reaction was terminated by the addition of 0.3 M NaOH (150 μL). The absorbance was read at 550 nm, and the inhibitory ratio (*IR*) was calculated as $IR\ (\%) = (A_{PAF} - A_t)/(A_{PAF} - A_C) \times 100\%$, where A_{PAF}, A_t, and A_c refer to the cell level of PAF, test compounds, and control groups, respectively.

Antioxidant Activity Assays.[21] The antioxidant activities of compounds **1**−**11** were evaluated by measuring their inhibition activities on liver microsomal lipid peroxidation induced by the Fe^{2+}-Cys system in vitro. Vitamin E was used as a positive control. Briefly, the test samples, liver microsomes suspension (1 mL), and 0.2 μM cysteine in 0.1 M PBS (pH 7.4) were incubated at 37 °C for 15 min, and 50 μM $FeSO_4$ was added to initiate lipid peroxidation. The reaction was terminated by addition of 20% TFA (1 mL). The mixture was centrifuged at 3000 rpm for 10 min. The supernatant (1 mL) was removed and reacted with 0.67% (w/v) thiobarbituric acid in a boiling H_2O bath for 10 min. After cooling, the absorbance was read at 532 nm, and the lipid peroxidation inhibitory ratio was calculated as $IR\ [\%] = 100\% - A_t/(A_p - A_c) \times 100\%$, where A_t, A_p, and A_c refer to the absorbance values of Fe^{2+}-cysteine, test compounds, and the control, respectively.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**11**, 2D NMR spectra of compounds **1**−**6** and **9**−**11**, CD spectra of compounds **1**−**8**, IR and MS spectra of compounds **1**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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