Lignan Glycosides from *Neoalsomitra integrifoliola*

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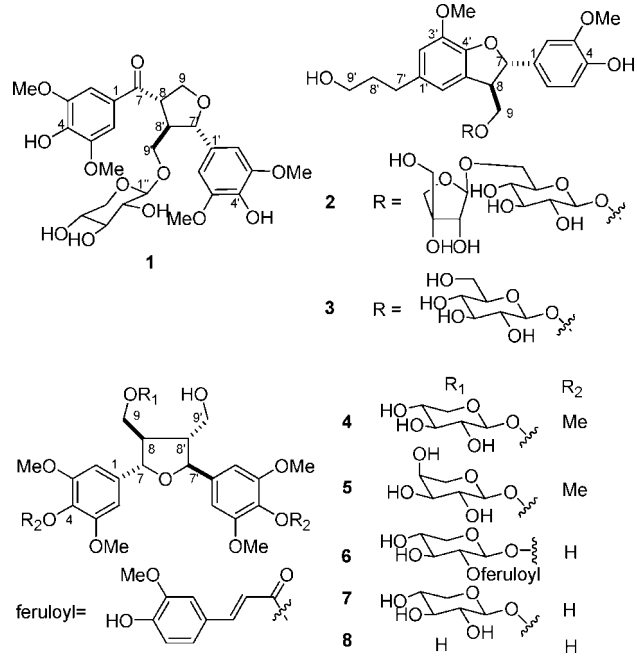
Five new lignan glycosides, (8*R**,7′*S**,8′*R**)-5,5′-dimethoxy-7-oxolariciresinol 9′-*O*-*β*-D-xylopyranoside (**1**), (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol 9-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside (**2**), (7*S**,8*R**,7′*S**,8′*R**)-4,4′-dimethoxyhuazhongilexin 9-*O*-*β*-D-xylopyranoside (**4**), (7*S**,8*R**,7′*S**,8′*R**)-4,4′-dimethoxyhuazhongilexin 9-*O*-α-L-arabinopyranoside (**5**), and (7*S**,8*R**,7′*S**,8′*R**)-huazhongilexin 9-*O*-(2-feruloyl)-*β*-D-xylopyranoside (**6**), together with four known compounds, (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 9-*O*-*β*-D-glucopyranoside (**3**), the 9-*O*-*β*-D-xylopyranoside of icariol A_2 (**7**), huazhongilexin (**8**), and nudiposide (**9**), were isolated from the leaves of *Neoalsomitra integrifoliola*. Their structures were determined by spectroscopic analysis and chemical methods. Compounds **2**, **3**, **8**, and **9** showed weak anti-inflammatory activities.

Neoalsomitra integrifoliola (Cogn.) Hutch (Cucurbitaceae) is a toxic shrub distributed in the south and southeast of China. It was reported that the stems, leaves, and fruits of *N. integrifoliola* were toxic and might cause severe diarrhea or tetany.[1] To date, 32 tetracyclic triterpenoids and their saponins were isolated from the bark of the plant.[2–5]

As part of an ongoing search for active compounds from toxic herbs, we found that the 95% alcohol extract, the *n*-BuOH-soluble fraction, and its subfraction Fr 3 of the leaves showed weak and moderate anti-inflammatory activities through evaluating mouse ear edema induced by croton oil *in vivo*, with inhibitory ratios of 30.3, 45.3, and 49.6%, respectively. Bioassay-guided fractionation of the active fraction led to the isolation of five new lignan glycosides (**1**, **2**, **4**−**6**) and four known compounds, (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 9-*O*-*β*-D-glucopyranoside (**3**), the 9-*O*-*β*-D-xylopyranoside of icariol A_2 (**7**), huazhongilexin (**8**), and nudiposide (**9**). Compounds **2**, **3**, **8**, and **9** showed marginal anti-inflammatory activities. Compounds **3**, **7**, **8**, and **9** were isolated from this genus for the first time.



Results and Discussion

Compound **1** was obtained as white, amorphous powder. The molecular formula $C_{27}H_{34}O_{13}$ was determined by HRESIMS indicating 11 degrees of unsaturation. The IR spectrum showed characteristic absorptions for a hydroxy group (3442 cm^{-1}), a carbonyl group (1662 cm^{-1}), and aromatic rings (1603, 1514 cm^{-1}). This observation was further supported by ^1H and ^{13}C NMR data. The ^1H NMR spectrum of **1** (Table 1) showed proton signals of two 1,3,4,5-tetrasubstituted aromatic rings (δ_H 7.70, s, 2H, H-2, 6; 7.27, s, 2H, H-2′, 6′), two oxymethylene groups (δ_H 4.66, dd, J = 8.5, 6.0 Hz, 1H, H-9a; 4.46, dd, J = 10.5, 3.5 Hz, 1H, H-9′a; 4.39, t-like, J = 8.5 Hz, 1H, H-9b; 3.91, dd, J = 10.5, 4.5 Hz, 1H, H-9′b), and three methines (δ_H 5.38, d, J = 8.5 Hz, 1H, H-7′; 4.73, m, 1H, H-8; 3.14, m, 1H, H-8′). In the ^{13}C NMR spectrum, carbon signals of two aromatic rings, a carbonyl, two methylenes, and three methines were observed. These data suggested that **1** was a tetrahydrofuranoid-type lignan.[6] The carbonyl was placed at C-7 according to the HMBC correlations (Figure 1). The methoxy groups at δ_H 3.80 (s, 6H) and 3.83 (s, 6H) in the ^1H NMR spectrum were similarly linked to C-3, 5 and 3′, 5′, respectively. From the HMBC correlations, compound **1** was revealed to be a 9-*O*-7′-tetrahydrofuran lignan. The coupling constant of H-7′ (J = 8.5 Hz) indicated a *trans* orientation between H-7′ and H-8′.[7] The NOE correlation between H-8 (δ_H 4.73, m) and H-7′ (δ_H 5.38, d, J = 8.5 Hz) revealed that H-8 and H-8′ were also *trans*. The existence of a sugar unit was indicated by the observation of an anomeric proton at δ_H 4.77 (1H, d, J = 8.0 Hz) and the corresponding anomeric carbon at δ_C 105.9 in the ^1H and ^{13}C NMR spectra, respectively. The ^{13}C NMR data of the sugar unit were consistent with those of xylose, which was confirmed by acid hydrolysis of **1** and co-TLC with an authentic sample.[8] The large coupling constant for the anomeric proton indicated a *β*-configuration for the xylopyranosyl. According to the literature,[9] the sugar unit was assigned as D-xylose by GC analysis. The long-range correlation from H-9′ to C-1″ suggested that the sugar unit was connected at C-9′. On the basis of this evidence, the structure of **1** was elucidated as (8*R**,7′*S**,8′*R**)-5,5′-dimethoxy-7-oxolariciresinol-9′-*O*-*β*-D-xylopyranoside.

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

	1 (C_5D_5N)		**2** (CD_3OD)		**3** (CD_3OD)	
no.	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	127.7		134.7		134.6	
2	107.5	7.70, br s	110.8	6.95, d (1.5)	110.7	6.93, d (1.5)
3	148.9		149.0		149.1	
4	143.6		147.4		147.7	
5	148.9		116.0	6.70, d (8.0)	116.1	6.71, d (8.0)
6	107.5	7.70, br s	119.7	6.80, dd (8.0, 1.5)	119.8	6.80, dd (8.0, 1.5)
7	198.4		89.2	5.55, d (6.5)	89.0	5.53, d (6.5)
8	49.1	4.73, m	53.0	3.56, m	53.2	3.56, m
9a	70.9	4.66, dd (11.0, 8.5)	72.5	4.00, m	72.5	4.15, dd (9.5, 5.5)
9b		4.39, t (8.5)		3.81, m		3.82, m
OMe	56.3	3.83, s	56.4	3.77, s	56.4	3.83, s
1′	131.7		136.9		136.9	
2′	105.1	7.27, br s	118.2	6.73, br s	118.2	6.74, br s
3′	149.2		145.2		145.2	
4′	137.2		147.4		147.5	
5′	149.2		129.5		129.7	
6′	105.1	7.27, br s	114.1	6.66, br s	114.2	6.66, br s
7′	84.3	5.38, d (8.5)	32.9	2.57, br t (8.0)	32.9	2.56, br t (7.5)
8′	53.2	3.14, m	35.8	1.76, m	35.8	1.76, m
9′a	67.8	4.46, dd (11.5, 3.0)	62.2	3.51, t (6.5)	62.3	3.51, t (6.5)
9′b		3.91, dd (11.5, 4.5)				
OMe	56.3	3.80, s	56.8	3.80, s	56.8	3.86, s
	Xyl		Glu		Glu	
1″	105.9	4.77, d (8.0)	1″ 104.2	4.28, d (7.5)	1″ 104.6	4.29, d (8.0)
2″	74.9	4.06, t (8.5)	2″ 75.1	3.16, m	2″ 75.2	3.17, m
3″	78.6	4.12, t (8.5)	3″ 76.9	3.30[a]), m	3″ 78.3	3.26[a]), m
4″	71.1	4.21, m	4″ 71.7	3.22, m	4″ 71.6	3.22, m
5″a	67.3	4.29, dd (11.0, 5.5)	5″ 78.1	3.35, m	5″ 78.1	3.31, m
5″b		3.66, t (11.0)	6″a 68.5	3.92, dd (11.0, 1.5)	6″a 62.8	3.68, t (10.0)
			6″b	3.56, m	6″b	3.59, m
			Api			
			1‴ 110.9	4.96, d (2.5)		
			2‴ 77.9	3.82, m		
			3‴ 80.5			
			4‴a 74.9	3.87, d (9.5)		
			4‴b	3.68, d (9.5)		
			5‴ 65.6	3.47, m		

[a] Overlapped with solvent peak.

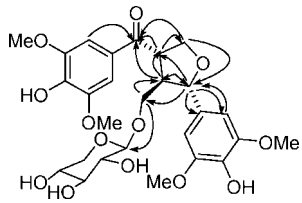

Figure 1. Key HMBC correlations of **1**.

Compound **2** showed a molecular formula of $C_{31}H_{42}O_{15}$ established by HRESIMS. The IR spectrum showed absorption bands for hydroxy groups (3356 cm^{-1}) and aromatic rings (1607, 1519 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **3**,[10,11] except for the additional signals for a sugar unit. Acid hydrolysis of **2** afforded glucose and apiose, which were identified by co-TLC with authentic samples. The large coupling constant of the anomeric proton revealed a *β*-configuration of the glucose, while the D-form was supported by the GC analysis.[9] The presence of *β*-D-apiose was in accord with the ^{1}H and ^{13}C NMR data.[12] Comparison of the ^{13}C NMR data of **2** with those of **3** exhibited that the signal of C-6″ of the glucose shifted downfield from δ_C 62.8 to 68.5, suggesting that the apiose was linked at C-6″, which was further confirmed by long-range correlations from H-1‴ to C-6″ and from H-6″ to C-1‴ in the HMBC spectrum. The absolute configuration of C-7 was determined by CD data. The positive Cotton effect at 286 nm indicated that the absolute configuration for C-7 was *S*.[13,14] Because of the large coupling constant of H-7, the configuration of C-8 was assigned as *R*. On the basis of the above evidence, the structure of **2** was elucidated as (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol 9-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside.

The molecular formulas of compounds **4** and **5** were both established as $C_{29}H_{40}O_{13}$ by HRESIMS. The IR and UV spectra of **4** and **5** showed very close resemblance to those of **7**.[15] In the ^{1}H and ^{13}C NMR spectra, two more O-methyl groups in their aglycones were clearly observed compared with **7**. These two O-methyl groups were linked at C-4 and C-4′ by HMBC data. Thus, the structure of **4** was elucidated as (7*S**,8*R**,7′*S**,8′*R**)-4,4′-dimethoxyhuazhongilexin 9-*O*-*β*-D-xylopyranoside. The presence of xylose and arabinose in **4** and **5**, respectively, was identified by the ^{13}C NMR data and the co-TLC behavior after acid hydrolysis. The arabinose was indicated as the α-anomer by the coupling constant of the anomeric proton (δ_H 4.14, d, J = 7.5 Hz). In the HMBC spectrum of **5**, correlation was observed from H-1″ to C-9, which suggested that the arabinosyl unit was attached to C-9 of the aglycone. Thus, the structure of **5** was elucidated as (7*S**,8*R**,7′*S**,8′*R**)-4,4′-dimethoxyhuazhongilexin 9-*O*-α-L-arabinopyranoside.

The HRESIMS of compound **6** displayed an $[M + Na]^+$ ion peak at *m*/*z* 767.2482, consistent with a molecular formula of $C_{37}H_{44}O_{16}$. The IR, UV, and ^{1}H NMR spectra of **6** were also similar to those of **7**. Besides the characteristic signals attributed to a 2,5-diaryl tetrahydrofuranoid-type lignan glycoside, the ^{1}H NMR spectrum of **6** revealed the presence of a *trans*-feruloyl moiety (δ_H 7.12, d, J = 16.0 Hz, 1H, H-8‴; 6.06, d, J = 16.0 Hz, 1H, H-7‴; 6.76, br s, 1H, H-2‴; 6.69, br d, J = 8.8 Hz, 2H, H-5‴, 6‴; 3.80, s, 3H), which was further proved by the ^{13}C NMR spectrum.[16] By comparing the NMR data of **6** with those of **7**, it was found that the signal of H-2″ shifted downfield by +0.4 ppm, while the signals

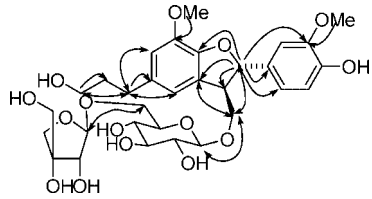

Figure 2. Key HMBC correlations of **2**.

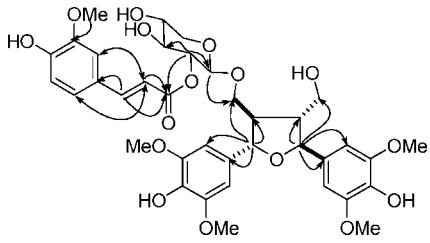

Figure 3. Key HMBC correlations of **6**.

of C-1″ and C-3″ shifted upfield by −1 and −2.2 ppm, respectively, which suggested that the *trans*-feruloyl moiety was located at C-2″. This linkage position was further confirmed by the correlation from H-2″ (δ_H 4.70) to C-9‴ (δ_C 165.9) in the HMBC spectrum (Figure 3). Thus, the structure of **6** was elucidated as (7*S**,8*R**,7′*S**,8′*R**)-huazhongilexin 9-*O*-(2-feruloyl)-*β*-D-xylopyranoside.

The known compounds were identified as (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 9-*O*-*β*-D-glucopyranoside (**3**),[10,14] the 9-*O*-*β*-D-xylopyranoside of icariol A_2 (**7**),[15] huazhongilexin (**8**),[17] and nudiposide (**9**)[18] by comparing with reported spectroscopic data.

The EtOH extract showed weak anti-inflammatory activity through evaluating mouse ear edema induced by croton oil *in vivo*, with an inhibitory ratio of 30.3%. The anti-inflammatory activities of the fractions were evaluated by the same procedure, and the *n*-BuOH-soluble fraction and its subfraction Fr 3 exhibited moderate activities with inhibitory ratios of 45.3 and 49.6%, respectively.

The anti-inflammatory activities of compounds **1**, **2**, **3**, **7**, **8**, and **9** isolated from subfraction Fr 3 were assessed by measuring the inhibitory ratios of *β*-glucuronidase release in rat PMNs induced by PAF *in vitro*, and the inhibitory ratios were − 3.89 ± 1.3, 29.2 ± 0.8, 31.1 ± 2.5, 4.67 ± 3.9, 18.1 ± 2.4, and 16.6 ± 2.7% at a concentration of 10^{-5} mol/L, respectively. Ginkgolide B was used as a positive control, with an inhibitory ratio of 78.4 ± 0.7%. These suggested that compounds **2**, **3**, **8**, and **9** showed marginal inhibitory activities of *β*-glucuronidase release from rat PMNs induced by PAF.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT_4-100x micromelting point apparatus and were uncorrected. Optical rotations data were determined on a PE model 343 digital polarimeter. UV spectra were obtained on a Hitachi UV-240 spectrophotometer. CD spectra were measured on a JASCO-712 polarimeter. IR spectra were recorded on a Nicolet IMPACT 400 FT-IR spectrometer. NMR spectra were obtained on INOVA-500 and Mercury-400 spectrometers. ESIMS were measured on an Agilent 1100 Series LC/MSD trap mass spectrometer. HRESIMS data were performed on an Autospec Ultima-TOF mass spectrometer. HPLC was carried out on a Shimadzu LC-6AD equipped with an SPD-10A detector. RP C_{18} columns (YMC-Pack ODS-A Φ 20 × 250 mm, 5 or 10 μm) were employed for preparative purpose. GC was carried out on a TSQ7000 (Finnigan, America) GC-MS instrument. Solvents were analytical or chromatographic grade and purchased from Beijing Chemical Company, Beijing, China. Macroporous resin D101 (26−60 mesh, Tianjin Haiguang Chemistry Company, Tianjin, China), Sephadex LH-20 (Pharmacia), and ODS (Merck, 20−45, 45−70 μm) were used for CC. Si gel 60 F-254 and HTLC (Qingdao Marine Chemical Factory) were used for TLC. Chromatograms were visualized under UV light or by spraying with 10% H_2SO_4/EtOH followed by heating.

Plant Material. The leaves of *N. integrifoliola* were collected from Yunnan Province of China in July 2004 and identified by Prof. Lin Ma (Department of Natural Products Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences). A voucher specimen (No. 20040361) was deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and Isolation. The dried and crushed leaves (4.7 kg) of *N. integrifoliola* were extracted with 95% EtOH (3 × 30 L) under reflux for 2, 1, and 1 h, respectively. The extracts were combined and concentrated under reduced pressure to yield a dark brown syrup (699 g, 14.9% yield from dried leaves). The EtOH extract showed weak anti-inflammatory activity through evaluating mouse ear edema induced by croton oil *in vivo*, with an inhibitory ratio of 30.3%. The EtOH extract was dissolved in H_2O (5000 mL) and then partitioned with petroleum ether (3500, 3000, 2000 mL), EtOAc (3500, 3000, 2000 mL), and *n*-BuOH (3500, 3000, 2000 mL), successively. The *n*-BuOH-soluble fraction showed moderate anti-inflammatory activity with an inhibitory ratio of 45.3%. This active fraction (120 g) was passed over a D-101 macroporous resin column (2.5 kg) eluting with a gradient of aqueous EtOH (0, 20, 40, 60, 80, 100%, v/v) to yield six fractions (Fr 1−6). The anti-inflammatory activities of the fractions were evaluated in the same procedure, and Fr 3 exhibited moderate activity with an inhibitory ratio of 49.6%.

Thus, Fr 3 (27.8 g) was further fractionationed on Sephadex LH-20 (190 g) with 60% MeOH to give nine fractions (Frs 3.1−3.9). Fr 3.3 (3.3 g) was further purified by repeat ODS CC to give compounds **4** (22 mg) and **5** (9 mg). Fr 3.4 (4 g) was subjected to ODS CC (40−75 μm, 200 g) and eluted with 25−70% aqueous MeOH to provide nine fractions (Frs 3.4.1−3.4.9). Compound **7** (60 mg) was obtained from Fr 3.4.3 after repeated ODS CC. Fr 3.4.5 (370 mg) was further purified by repeated ODS CC and preparative HPLC (20% aqueous CH_3CN, 4 mL/min, t_R = 45.3 min) to give compound **9** (22 mg). Fr 3.4.7 (324 mg) was applied to repeated ODS, Sephadex LH-20 CC, and preparative HPLC (19% aqueous CH_3CN, 5 mL/min, t_R = 41.2 min) to give compound **2** (40 mg). Fr 3.5 (1.9 g) was subjected to ODS CC (40−75 μm, 200 g) and eluted with 20−70% aqueous MeOH to provide 14 fractions (Frs 3.5.1−3.5.14). Compound **1** (40 mg) precipitated from Fr 3.5.9. Fr 3.5.4 (56 mg) was purified by preparative HPLC (30% aqueous MeOH, 5 mL/min) to give compound **8** (23 mg, t_R = 49.5 min). Compound **3** (22 mg) was obtained from Fr 3.5.8 by preparative HPLC (19% aqueous CH_3CN, 5 mL/min, t_R = 35.5 min). Fr 3.6 (1.6 g) was subjected to ODS CC and preparative HPLC (19% aqueous CH_3CN, 5 mL/min) to give compound **6** (25 mg, t_R = 28.1 min).

(8*R,7′*S**,8′*R**)-5,5′-Dimethoxy-7-oxolariciresinol 9′-*O*-*β*-D-xylopyranoside (1):** white, amorphous powder; $[\alpha]^{20}_D$ −77.3 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 209 (2.20), 229 (1.02), 302 (0.59) nm; IR ν_{max} 3442, 2899, 1662, 1603, 1514, 1101, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 589.1884 for $[M + Na]^+$ (calcd for $C_{27}H_{34}O_{13}Na$, 589.1897).

(7*S*,8*R*)-Dihydrodehydrodiconiferyl alcohol 9-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside (2): white, amorphous powder; $[\alpha]^{20}_D$ −25.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (1.84), 233 (0.48), 281 (0.18) nm; CD (MeOH) λ_{max} (Δε) 263 (0.01), 270 (1.09), 275 (2.89), 280 (4.62), 290 (6.61), 295 (4.00), 298 (0.97), 303 (−1.78) nm; IR ν_{max} 3356, 2934, 1607, 1519, 1039 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 693 $[M + K]^+$, 677 $[M + Na]^+$, 653 $[M - H]^-$; HRESIMS *m/z* 677.2371 for $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{15}Na$, 677.2421).

(7*S,8*R**,7′*S**,8′*R**)-4,4′-Dimethoxyhuazhongilexin 9-*O*-*β*-D-xylopyranoside (4):** white, amorphous powder; $[\alpha]^{20}_D$ −23.0 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 207 (1.57), 225 (0.16) nm; IR ν_{max} 3387, 2901, 1640, 1592, 1504, 1421, 1235, 1042 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 635 $[M + K]^+$, 619 $[M + Na]^+$, 595 $[M - H]^-$; HRESIMS *m/z* 619.2345 for $[M + Na]^+$ (calcd for $C_{29}H_{40}O_{13}Na$, 619.2367).

(7*S,8*R**,7′*S**,8′*R**)-4,4′-Dimethoxyhuazhongilexin 9-*O*-α-L-arabinopyranoside (5):** white, amorphous powder; $[\alpha]^{20}_D$ +1 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 204 (1.34), 223 (0.67) nm; IR ν_{max} 3381, 2903, 1644, 1592, 1504, 1421, 1235, 1042 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m/z* 635 $[M + K]^+$, 619 $[M + Na]^+$, 595 $[M - H]^-$; HRESIMS *m/z* 619.2328 for $[M + Na]^+$ (calcd for $C_{29}H_{40}O_{13}Na$, 619.2367).

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **4**−**6**

	4 (CD_3OD)[c]		**5** (CD_3OD)[c]		**6** (DMSO-d_6)[c]		**6** (CD_3OD)[d]	
no.	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	139.8		139.8		132.8		136.0	
2, 6	104.4	6.71, s	104.5	6.71, s	103.1	6.55, s	104.9	6.63, s
3, 5	154.6		154.6		147.7		149.2	
4	138.5		138.5		134.5		134.2	
OMe-3, 5	56.6	3.81, s	56.7	3.81, s	55.8	3.76, s	56.7	3.78, s
7	84.3	5.04, d (8.0)	84.3	5.07, d (8.0)	81.7	4.85, d (8.5)	84.1	5.04, d (8.0)
8	52.2	2.34, m ($W_{1/2}$ = 13.3)	52.2	2.36, m ($W_{1/2}$ = 12.7)	49.3	2.19, ddt (8.5, 9.5, 4.5)	50.7	2.23, m
9a	68.9	3.88, dd (11.5, 3.5)	68.7	3.99, dd (10.5, 4.0)	67.1	3.72[b]	67.3	3.90, br d (10.0)
9b		3.67, dd (11.5, 3.5)		3.67, dd (10.5, 4.0)		3.46, m		3.76[b]
1′	139.9		139.9		132.7		136.0	
2′, 6′	104.6	6.73, s	104.6	6.75, s	103.8	6.58, s	104.4	6.61, s
3′, 5′	154.6		154.6		147.8		149.3	
4′	138.4		138.4		134.7		134.0	
OMe-3′, 5′	56.7	3.81, s	56.7	3.81, s	55.8	3.76, s	56.7	3.83, s
OMe-4, 4′	61.1	3.70, s	61.1	3.70, s				
7′	84.0	4.99, d (8.0)	84.1	4.99, d (8.0)	81.4	4.76, d (8.5)	83.9	4.84, d (8.5)
8′	54.3	2.34, m ($W_{1/2}$= 13.3)	54.3	2.36, m ($W_{1/2}$= 12.7)	51.7	2.02, ddt (8.5, 9.5, 4.5)	52.2	2.23. m
9′a	61.0	3.73, m	61.1	3.73, dd (11.5, 3.0)	58.1	3.32, dd (11.5, 3.5)	58.7	3.81[b]
9′b		3.60, dd (11.5, 2.0)		3.60, dd (11.5, 3.0)		3.16, m		3.46, dd (12.4, 3.0)
Xyl								
1″	105.2	4.17, d (7.5)	105.1	4.14, d (7.5)	101.7	4.42, d (8.0)	104.3	4.36, d (7.6)
2″	75.0	3.15, t (8.0)	72.5	3.50, m	73.4	4.70, t (8.5)	75.0	3.50, t (8.5)
3″	78.0	3.24[a]	74.4	3.45, dd (9.5, 3.0)	74.0	3.37, t (8.5)	75.8	3.22, m
4″	71.2	3.43, m	69.7	3.75, m	69.8	3.42, m	71.4	3.58, m
5″a	67.1	3.79[b]	67.2	3.79[b]	65.9	3.79[b]	67.2	3.83[b]
5″b		3.12, dd (13.5, 2.50)		3.50, m		3.16, m		3.22, m
					feruyl			
					1‴	124.4		127.1	
					2‴	110.6	7.01, br s	111.6	6.76, br s
					3‴	147.8		149.3	
					4‴	148.1		151.2	
					5‴	115.5	6.66, br d (8.5)	116.5	6.69, br d (8.8)
					6‴	123.1	6.77, br d (8.5)	123.8	6.69, br d (8.8)
					7‴	113.5	6.23, d (16.0)	114.8	6.06, d (16.0)
					8‴	145.0	7.19, d (16.0)	147.2	7.12, d (16.0)
					9‴	165.9		168.1	
					OMe	55.5	3.75, s	56.4	3.80, s

[a] Overlapped with solvent peak. [b] Overlapped with −OMe peak. [c] 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. [d] 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR.

(7*S,8*R**,7′*S**,8′*R**)-Huazhongilexin 9-*O*-(2-feruloyl)-*β*-D-xylopyranoside (6):** pale yellow, amorphous powder; $[\alpha]^{20}_D$ −33.3 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (2.21), 238 (0.69), 325 (0.47) nm; IR ν_{max} 3405, 2936, 2844, 1701, 1602, 1515, 1325, 1113, 830 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 767 $[M + Na]^+$, 743 $[M - H]^-$; HRESIMS *m*/*z* 767.2482 for $[M + Na]^+$ (calcd for $C_{37}H_{44}O_{16}Na$, 767.2527).

Acid Hydrolysis of Compounds 1−5 and 7. Compounds **1** (8 mg) and **2** (11 mg) were dissolved in 5% HCl (50 mL) and heated at 90 °C for 8 h. The mixture was evaporated under vacuum to 20 mL and then extracted with EtOAc three times (25, 25, 20 mL). After addition of H_2O, the acidic solution was evaporated again and then dried *in vacuo* to furnish a monosaccharide residue. From the residue, xylose, glucose, and apiose were detected by co-TLC [$CHCl_3$−MeOH−HOAc−H_2O (25:12:2:2)] with authentic samples. The R_f values for the above sugars were 0.43, 0.20, and 0.66, respectively.

Compounds **3**−**5** and **7** were individually hydrolyzed with HCl vapor on an HPTLC precoated plate (80 °C water bath for 40 min) followed by developing with $CHCl_3$−MeOH−HOAc−H_2O (25:12:2:2).[9] The sugars were visualized by spraying with phenylamine-*ortho*-benzenedicarboxylic acid reagent followed by heating, and compared with authentic samples (R_f of D-glucose, D-xylose, L-arabinose: 0.21, 0.44, 0.39).

Absolute Configuration of the Sugars. The absolute configuration of the xylose and glucose was determined according to a reported procedure.[9] An authentic sugar sample (3 mg) was trimethylsilylated, and the derivative was analyzed by GC-MS. The monosaccharide residues obtained from the acid hydrolysis of compounds **1** and **2** were treated as the authentic sugar samples following the same procedures, and the retention times of sugar derivatives were compared with those of the authentic samples (D-xylose 17.67 min, D-glucose 19.57 min).

Anti-inflammatory Bioassays. The anti-inflammatory activities of the EtOH extract and the fractions were measured through evaluating mouse ear edema induced by croton oil *in vivo*.[19] According to the reported procedures,[20] the anti-inflammatory activities of compounds **2**, **3**, **7**, **8**, and **9** were assessed by measuring the inhibition of the PAF-induced release of *β*-glucuronidase from rat polymorphonuclear leukocytes (PMNs) *in vitro*. The absorbance was read at 550 nm, and the inhibitory ratio was calculated. Ginkgolide B was used as reference compound.

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