Triterpene Saponins from *Clematis mandshurica*

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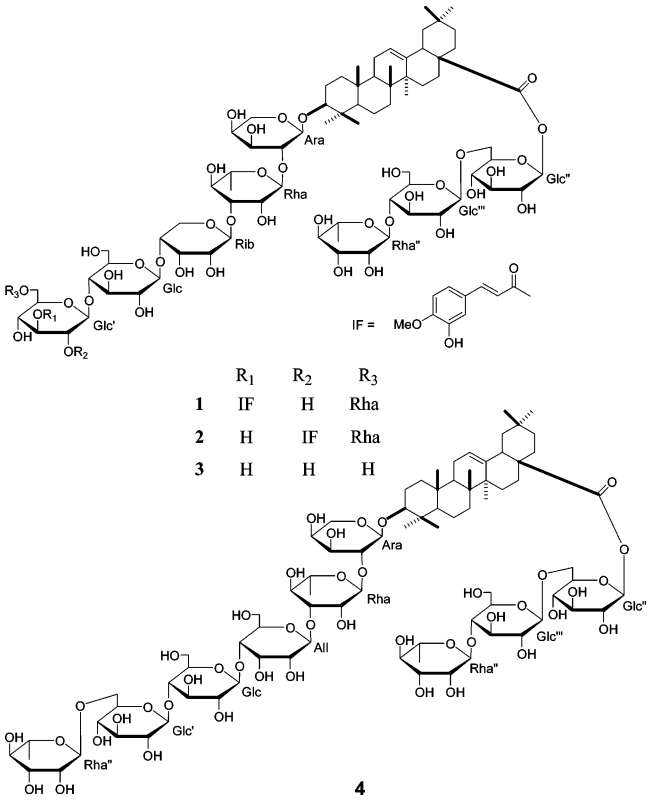
Four new triterpene saponins, clematomandshurica saponins A−D (**1**−**4**), together with three known saponins (**5**−**7**) have been isolated from the roots and rhizomes of *Clematis mandshurica*. Their structures were elucidated on the basis of their spectroscopic evidence and hydrolysis. Clematomandshurica saponins A and B showed significant inhibitory activity on cyclooxygenase-2 (IC_{50} = 2.66 and 2.58 μM, respectively).

The genus *Clematis* (Ranunculaceae) is a large genus in the Dicotyledoneae, with ca. 300 species being known worldwide. In the Chinese Pharmacopoeia, the roots and rhizomes of *C. chinensis* Osbeck, *C. mandshurica* Rupr., and *C. hexapetala* Pall. are all named *weilingxian*, which is commonly used as an anti-inflammatory, antitumor, and analgesic agent. Previous investigations were mainly directed toward *C. chinensis*. Up to date, more than 40 triterpene saponins and lignans have been isolated from the roots and rhizomes of *C. chinensis*.[1−11] In contrast, there are only a few phytochemical investigations concerning *C. mandshurica*. From this latter species, only four triterpene saponins, clematosides A−C and clematoside A′, were reported in 1967.[12] Our preliminary pharmacological studies suggested the total saponins prepared from the 50% EtOH extract of the roots and rhizomes of *C. mandshurica* showed significant inhibitory activity against cyclooxygenase-2 (COX-2). Chromatographic separation of the saponin fraction has resulted in the isolation of four new (**1**−**4**) and three known (**5**−**7**) triterpene saponins. Herein, we report the structural elucidation of the new compounds and also the inhibitory activities against COX-2 of the isolated saponins.

Results and Discussion

A 50% EtOH extract of the dried roots and rhizomes of *C. mandshurica* (5 kg) was suspended in H_2O and extracted with EtOAc and *n*-BuOH. The *n*-BuOH extract was subjected to column chromatography over porous polymer resin (D101), silica gel, and octadecylsilanized (ODS) silica gel to yield compounds **1**−**7**. Compounds **5**−**7** were identified as 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**5**),[11] huzhangoside B (**6**),[7] and clematichinenoside C (**7**),[7] respectively.

Compound **1** was obtained as a white, amorphous powder. The HRESIMS showed an accurate $[M + Na]^+$ ion at *m*/*z* 2005.8667, in accordance with an empirical molecular formula of $C_{92}H_{142}O_{46}$, which was supported by the ^{13}C NMR spectrum and DEPT data. The ^1H NMR spectrum showed seven tertiary methyl resonances at δ 0.84, 0.87, 0.87, 1.04, 1.12, 1.23, and 1.26 and an olefinic proton at δ 5.37, which was typical of the oleanolic acid skeleton. The resonances at δ 122.8 and 144.1 in the ^{13}C NMR spectrum also suggested that **1** possessed an oleanolic acid aglycone. In addition, the ^1H NMR spectrum indicated nine anomeric protons at δ 6.25 (1H, br s), 6.23 (1H, d, J = 7.5 Hz), 5.85 (1H, br s), 5.82 (1H, d, J = 5.5 Hz), 5.44 (1H, br s), 5.22 (1H, d, J = 8.0 Hz), 4.98 (1H, d, J = 7.5 Hz), 4.96 (1H, d, J = 7.0 Hz), and 4.83 (1H, d, J = 6.0 Hz). The three-proton doublet at δ 1.70 (3H, d, J = 6.0 Hz), 1.59 (3H, d, J = 6.0 Hz), and 1.52 (3H, d, J = 6.0 Hz) indicated the presence of three deoxyhexopyranosyl units in **1**. Acid hydrolysis of **1** with 2 N CF_3COOH gave oleanolic acid and isoferulic acid, together with L-arabinose, D-glucose, L-rhamnose, and D-ribose. In the ^{13}C NMR spectrum of **1**, the C-3 and C-28 carbon signals were observed at δ 88.7 and 176.5, respectively, implying that **1** must be a bisdesmosidic triterpene saponin. The exact sugar sequence and its linkage position to the aglycone were solved by detailed analysis of the 1D TOCSY and 2D NMR spectra. The ^1H NMR subspectra of the individual monosaccharide units were obtained by using selective irradiation of the easily identifiable proton resonances, as well as irradiation of other nonoverlapping proton resonances in a series of 1D TOCSY experiments. Subsequent analysis of the $^1H-^1H$ COSY spectrum resulted in the sequential assignments of all the proton resonances due to the nine monosaccharides, including identification of most of their multiplet



	R_1	R_2	R_3
1	IF	H	Rha
2	H	IF	Rha
3	H	H	H

4

Figure 1. Structures of compounds **1**−**4**.

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Table 1. ^{1}H NMR Data of Compounds **1** and **4** (500 MHz, in C_5D_5N, *J* in Hz)

no.	1	4	no.	1	4
Ara			Rha′		
1	4.83 (1H, d, *J* = 6.0)	4.79 (1H, d, *J* = 6.0)	1	5.44 (1H, br s)	5.41 (1H, br s)
2	4.54 (1H, d, *J* = 6.0, 7.0)	4.54 (1H, d, *J* = 6.0, 7.0)	2	4.72 (1H, br s)	4.70 (1H, br s)
3	4.22 (1H, m)	4.18 (1H, m)	3	4.55 (1H, br d, *J* = 9.5)	4.53 (1H, br d, *J* = 9.5)
4	4.21 (1H, m)	4.18 (1H, m)	4	4.24 (1H, t, *J* = 9.5)	4.22 (1H, t, *J* = 9.5)
5a	4.28 (1H, m)	4.24 (1H, m)	5	4.30 (1H, m)	4.28 (1H, m)
5b	3.78 (1H, br d, *J* = 11.0)	3.77 (1H, m)	6	1.59 (3H, d, *J* = 6.0)	1.57 (3H, d, *J* = 6.0)
Rha			Glc″		
1	6.25 (1H, br s)	6.24 (1H, br s)	1	6.23 (1H, d, *J* = 7.5)	6.21 (1H, d, *J* = 8.0)
2	4.86 (1H, br s)	4.93 (1H, br s)	2	4.14 (1H, dd, *J* = 9.0, 7.5)	4.13 (1H, dd, *J* = 9.0, 8.0)
3	4.69 (1H, br d, *J* = 9.5)	4.74 (1H, br d, *J* = 9.5)	3	4.21 (1H, t, *J* = 9.0)	4.20 (1H, t, *J* = 9.0)
4	4.43 (1H, t, *J* = 9.5)	4.45 (1H, t, *J* = 9.5)	4	4.29 (1H, m)	4.28 (1H, t, *J* = 9.0)
5	4.62 (1H, m)	4.60 (1H, m)	5	4.10 (1H, m)	4.06 (1H, m)
6	1.52 (3H, d, *J* = 6.0)	1.48 (3H, d, *J* = 6.0)	6a	4.67 (1H, br d, *J* = 10.5)	4.66 (1H, br d, *J* = 10.5)
			6b	4.32 (1H, m)	4.32 (1H, dd, *J* = 10.5, 5.4)
Rib		All	Glc‴		
1	5.82 (1H, d, *J* = 5.5)	5.85 (1H, d, *J* = 8.0)	1	4.98 (1H, d, *J* = 7.5)	4.98 (1H, d, *J* = 7.5)
2	4.13 (1H, m)	4.01 (1H, br d, *J* = 8.0)	2	3.94 (1H, dd, *J* = 9.0, 7.5)	3.94 (1H, dd, *J* = 9.0, 7.5)
3	4.61 (1H, m)	5.00 (1H, br s)	3	4.15 (1H, t, *J* = 9.0)	4.15 (1H, t, *J* = 9.0)
4	4.30 (1H, m)	4.12 (1H, m)	4	4.42 (1H, t, *J* = 9.0)	4.42 (1H, t, *J* = 9.0)
5	4.29 (2H, m)	4.54 (1H, m)	5	3.65 (1H, m)	3.65 (1H, m)
6a		4.46 (1H, m)	6a	4.20 (1H, br d, *J* = 11.5)	4.20 (1H, br d, *J* = 11.5)
6b		4.32 (1H, m)	6b	4.08 (1H, br d, *J* = 11.5)	4.08 (1H, br d, *J* = 11.5)
Glc			Rha‴		
1	4.96 (1H, d, *J* = 7.0)	5.20 (1H, d, *J* = 8.0)	1	5.85 (1H, br s)	5.84 (1H, br s)
2	3.89 (1H, dd, *J* = 9.0, 7.0)	3.92 (1H, dd, *J* = 9.0, 8.0)	2	4.66 (1H, br s)	4.66 (1H, br s)
3	4.21 (1H, m)	4.20 (1H, m)	3	4.55 (1H, dd, *J* = 9.0, 3.0)	4.55 (1H, dd, *J* = 9.5, 3.0)
4	4.21 (1H, m)	4.18 (1H, m)	4	4.34 (1H, t, *J* = 9.0)	4.33 (1H, t, *J* = 9.5)
5	3.84 (1H, m)	3.83 (1H, m)	5	4.98 (1H, m)	4.97 (1H, m)
6a	4.42 (1H, m)	4.43 (1H, br d, *J* = 12.0)	6	1.70 (3H, d, *J* = 6.0)	1.69 (3H, d, *J* = 6.0)
6b	4.38 (1H, m)	4.27 (1H, m)			
Glc′					
1	5.22 (1H, d, *J* = 8.0)	5.11 (1H, d, *J* = 8.0)			
2	4.16 (1H, dd, *J* = 9.0, 8.0)	4.00 (1H, dd, *J* = 9.0, 8.0)			
3	5.97 (1H, t, *J* = 9.0)	4.16 (1H, t, *J* = 9.0)			
4	4.10 (1H, m)	3.96 (1H, m)			
5	4.07 (1H, m)	3.92 (1H, m)			
6a	4.58 (1H, m)	4.61 (1H, br d, *J* = 10.0)			
6b	4.05 (1H, m)	4.02 (1H, m)			

patterns and coupling constants as shown in Table 1. The HSQC spectrum correlated the proton resonances with those of the corresponding one-bond coupled carbons and the HSQC-TOCSY spectrum associated the anomeric protons with their respective carbon atoms, leading to unambiguous assignments of the carbons of all the individual monosaccharides. Comparison of the carbon chemical shifts thus assigned with those of the reference methyl glycosides,[13] taking into account the known effects of *O*-glycosylation, indicated that **1** contained one L-arabinopyranosyl unit, one D-ribopyranosyl unit, three L-rhamnopyranosyl units, and four D-glucopyranosyl units. The relatively large coupling constants (5.5−8.0 Hz) of the anomeric protons suggested the arabinopyranosyl moiety was α-configured and the glucopyranosyl and ribopyranosyl moieties were *β*-configured. The α-configuration of the rhamnopyranosyl moieties was identified by the correlation signals between H-1 and H-4 in the NOESY spectra. The linkage of the sugars and the sugars with the aglycone was established by the following HMBC correlations: Ara-H-1 (*δ* 4.83)/aglycone-C-3 (*δ* 88.7), Rha-H-1 (*δ* 6.25)/Ara-C-2 (*δ* 75.3), Rib-H-1 (*δ* 5.82)/Rha-C-3 (*δ* 82.0), Glc-H-1 (*δ* 4.96)/Rib-C-4 (*δ* 76.5), Glc′-H-1 (*δ* 5.22)/Glc-C-4 (*δ* 81.1), Rha′-H-1 (*δ* 5.44)/Glc′-C-6 (*δ* 68.0), Glc″-H-1 (*δ* 6.23)/aglycone-C-28 (*δ* 176.5), Glc‴-H-1 (*δ* 4.98)/Glc″-C-6 (*δ* 69.3), Rha‴-H-1 (*δ* 5.85)/Glc‴-C-4 (*δ* 78.2). All the linkages were also confirmed by the correlation signals observed in the NOESY spectrum; the anomeric protons at *δ* 4.83 (Ara-H-1), 6.25 (Rha-H-1), 5.82 (Rib-H-1), 4.96 (Glc-H-1), 5.22 (Glc′-H-1), 5.44 (Rha′-H-1), 4.98 (Glc‴-H-1), and 5.85 (Rha‴-H-1) showed correlations with H-3 of the aglycone at *δ* 3.26, H-2 of the Ara at *δ* 4.54, H-3 of the Rha at *δ* 4.69, H-4 of the Rib at *δ* 4.30, H-4 of the Glc at *δ* 4.21, H-6 of the Glc′ at *δ* 4.58 and 4.05, H-6 of the Glc″ at *δ* 4.67 and 4.32, and H-4 of the Glc‴at *δ* 4.42, respectively.

Further analysis of the NMR spectra of **1** indicated the presence of an isoferuloyl moiety. The typical protons of the isoferuloyl moiety resonated at δ_H 7.43 (1H, d, *J* = 1.5 Hz), 7.00 (1H, dd, *J* = 8.0, 1.5 Hz), 6.89 (1H, d, *J* = 8.0 Hz), 7.87 (1H, d, *J* = 16.0 Hz), 6.57 (1H, d, *J* = 16.0 Hz), and 3.70 (3H, s) and δ_C 116.4, 121.3, 112.0, 145.3, 115.2, 150.0, 148.4, 128.3, 167.2, and 55.7. Comparison of the ^{1}H NMR data of **1** with those of the known compound **5** revealed that H-3 of the Glc′ resonated at a lower field (*δ* 5.97), which suggested that the isoferuloyl might be connected to C-3 (*δ* 78.9) of the Glc′. This was confirmed by the correlation between H-3 of the Glc′ and the carbonyl carbon (*δ* 167.2) of the isoferuloyl moiety in the HMBC spectrum. Hydrolysis of **1** with 0.1 N KOH afforded isoferulic acid and **5**. From the above evidence, the structure of **1** was established as 3-*O*-α-L-rhamnopyranosyl-(1→6)-[(3-*O*-isoferuloyl)-*β*-D-glucopyranosyl]-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, named clematomandshurica saponin A.

Compound **2** was obtained as an amorphous powder, with the molecular formula $C_{92}H_{142}O_{46}$, as deduced from the $[M + Na]^+$ peak at *m/z* 2005.8665 by HRESIMS and confirmed by ^{13}C NMR and DEPT data. The ^{1}H NMR spectrum of **2** showed nine anomeric proton resonances at *δ* 6.18 (1H, br s), 6.16 (1H, d, *J* = 8.0 Hz), 5.79 (1H, br s), 5.79 (1H, *J* = 5.5 Hz,), 5.39 (1H, br s), 5.29 (1H, d, *J* = 8.0 Hz), 4.95 (1H, d, *J* = 7.5 Hz), 4.85 (1H, d, *J* = 8.0 Hz), and 4.78 (1H, d, *J* = 6.5 Hz), three proton doublets at *δ* 1.64 (3H, d, *J* = 6.0 Hz), 1.55 (3H, d, *J* = 6.0 Hz), and 1.48 (3H, d, *J* = 6.0 Hz), and isoferuloyl resonances at *δ* 7.50 (1H, d, *J* = 2.0 Hz), 7.07 (1H, dd, *J* = 8.0, 2.0 Hz), 6.89 (1H, d, *J* = 8.0 Hz), 8.04 (1H, d, *J* = 16.0 Hz), 6.72 (1H, d, *J* = 16.0 Hz), and 3.70 (3H, s). Acid

hydrolysis of **2** with 2 N CF_3COOH gave oleanolic acid and isoferulic acid, together with L-arabinose, D-glucose, L-rhamnose, and D-ribose. Comparison of the NMR data of **2** with those of **1** revealed that **2** was also an oleane-type saponin with the same sugar moieties and sugar sequences as in **1**. The only difference was the NMR data of the Glc′. In the ^{1}H NMR spectrum of **2**, H-2 of the Glc′ resonated at a lower field, δ 5.67, which suggested the isoferuloyl might be connected to C-2 of the Glc′. This was confirmed by the long-range correlation between H-2 of the Glc′ and the carbonyl carbon (δ 166.5) of the isoferuloyl in the HMBC spectrum. Hydrolysis of **2** with 0.1 N KOH afforded isoferulic acid and **5**. Thus, the structure of **2** was identified as 3-*O*-α-L-rhamnopyranosyl-(1→6)-[(2-*O*-isoferuloyl)-*β*-D-glucopyranosyl]-(1→4)-*β*- D-glucopyranosyl-(1→4)-*β*-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, named clematomandshurica saponin B.

Compound **3** was obtained as a white, amorphous powder. The HRESIMS showed an accurate $[M + Na]^+$ ion peak at *m*/*z* 1683.7615, in accordance with an empirical molecular formula of $C_{76}H_{124}O_{39}$, which was supported by the ^{13}C NMR spectrum and DEPT data. The ^{1}H NMR spectrum of **3** showed eight anomeric proton resonances at δ 6.21 (1H, br s), 6.20 (1H, d, J = 8.0 Hz), 5.82 (1H, br s), 5.81 (1H, d, J = 5.5 Hz), 5.14 (1H, d, J = 8.0 Hz), 4.97 (1H, d, J = 7.5 Hz), 4.95 (1H, d, J = 8.0 Hz), and 4.81 (1H, d, J = 5.5 Hz), two three-proton doublet signals at δ 1.67 (3H, d, J = 6.0 Hz) and 1.50 (3H, d, J = 6.0 Hz), and seven tertiary methyls and one olefinic proton characteristic of oleanolic acid. Acid hydrolysis of **3** with 2 N CF_3COOH gave oleanolic acid, together with the monosaccharides L-arabinose, D-glucose, L-rhamnose, and D-ribose. Comparison of the NMR spectra (^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HSQC, TOCSY, NOESY, and HMBC) of **3** with the known compound **5** revealed that the terminal L-Rha (Rha′) moiety of the sugar chain linked to C-3 of the aglycone disappeared. Accordingly, the structure of **3** was determined as 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, named clematomandshurica saponin C.

Compound **4** was obtained as an amorphous powder, with the molecular formula $C_{83}H_{136}O_{44}$, as deduced from the $[M + Na]^+$ peak at *m*/*z* 1859.8311 by HRESIMS and confirmed by ^{13}C NMR and DEPT data. The ^{1}H NMR spectrum of **4** showed nine anomeric protons at δ 6.24 (1H, br s), 6.21 (1H, d, J = 8.0 Hz), 5.85 (1H, d, J = 8.0 Hz), 5.84 (1H, br s), 5.41 (1H, br s), 5.20 (1H, d, J = 8.0 Hz), 5.11 (1H, d, J = 8.0 Hz), 4.98 (1H, d, J = 7.5 Hz), and 4.79 (1H, d, J = 6.0 Hz), three proton doublets at δ 1.69 (3H, d, J = 6.0 Hz), 1.57 (3H, d, J = 6.0 Hz), and 1.48 (3H, d, J = 6.0 Hz), and seven tertiary methyls and one olefinic proton at δ 5.37 (1H, br s). Acid hydrolysis of **4** with 2 N CF_3COOH gave oleanolic acid, together with monosaccharides L-arabinose, D-glucose, L-rhamnose, and D-allose. The proton and carbon signals were unambiguously assigned by the ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, 1D TOCSY, HSQC, HSQC-TOCSY, NOESY, and HMBC experiments (Table 1, 3, and 4). Comparison of the NMR data of **4** with those of the known compound **5** revealed that the ribopyranosyl unit in **5** was substituted by an allopyranosyl unit. In the HMBC spectrum of **4**, H-1 of the allopyranosyl unit (δ 5.85) showed long-range correlation with C-3 of the Rha (δ 83.5), and H-1 of the Glc (δ 5.20) showed correlation with C-4 of the allopyranosyl unit (δ 78.2). Thus, the structure of **4** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-allopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, named clematomandshurica saponin D.

Saponins **1**−**7** were evaluated for inhibitory effects on COX-2 activity induced by lipopolysaccharide (LPS) in murine peritoneal macrophages. As a result, compounds **1** and **2** indicated inhibitory effects on COX-2 (IC_{50} = 2.66 and 2.58 μM, respectively). Celecoxib was used as a positive control (IC_{50} = 0.00051 μM).

Table 3. ^{13}C NMR Data for the Aglycone Moieties of **1**−**5** (125 MHz, in C_5D_5N)

no.	1	2	3	4	5
1	38.9	38.7	38.9	38.9	38.9
2	26.6	26.4	26.6	26.6	26.6
3	88.7	88.6	88.6	88.6	88.7
4	39.5	39.3	39.5	39.6	39.6
5	55.9	55.8	55.9	55.9	56.0
6	18.4	18.4	18.4	18.4	18.4
7	33.1	32.9	33.0	33.1	32.5
8	39.8	39.7	39.8	39.8	39.8
9	48.0	47.8	48.0	48.0	48.0
10	37.0	36.8	36.9	37.0	37.0
11	23.7	23.6	23.6	23.7	23.8
12	122.8	122.6	122.7	122.8	122.8
13	144.1	143.9	144.0	144.9	144.1
14	42.1	41.9	42.0	42.1	42.1
15	28.1	27.9	28.1	28.1	28.1
16	23.3	23.1	23.3	23.3	23.3
17	47.0	46.8	46.9	47.0	47.0
18	41.6	41.4	41.6	41.6	41.6
19	46.2	46.0	46.1	46.2	46.2
20	30.7	30.5	30.6	30.7	30.7
21	34.0	33.8	33.9	34.0	34.0
22	32.5	32.3	32.4	32.5	32.5
23	28.1	27.9	28.1	28.1	28.1
24	17.1	16.9	17.0	17.1	17.1
25	15.6	15.4	15.6	15.6	15.6
26	17.4	17.3	17.4	17.4	17.4
27	26.0	25.9	26.0	26.0	26.0
28	176.5	176.3	176.4	176.5	176.5
29	33.1	32.9	33.0	33.1	33.1
30	23.6	23.5	23.6	23.6	23.6

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 243B digital polarimeter. UV spectra were obtained on a TU-1901 spectrometer. IR spectra were recorded on an Avater-360 spectrometer. NMR spectra were recorded on an Inova 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer with TMS as internal standard. HRESIMS were measured on an Autospec-Ultima ETOF spectrometer in positive ion mode. HPLC were carried on an ODS column (Alltech 250 × 10 mm i.d., 5 μm) with an Alltech evaporative light scattering detector. GC were measured on an Agilent 6890N gas chromatograph, with an HP-5 capillary column (28 m × 0.32 mm) and an FID detector operated at 260 °C (column temp 180 °C), 1.0 mL/min N_2 as carrier gas. Column chromatography was performed with silica gel (200−300 mesh, Qingdao Mar. Chem. Ind. Co. Ltd.) and D101 porous polymer resin (Tianjin Chem. Ind. Co. Ltd.).

Plant Material. The roots and rhizomes of *C. mandshurica* were collected in August 2002 from Heilongjiang Province, in the Northeast of China. The identification of the plant was performed by Prof. Pengfei Tu, Peking University. A voucher specimen is kept in the herbarium of Peking University Modern Research Center for Traditional Chinese Medicine (CM200208).

Extraction and Isolation. The dried roots and rhizomes (5 kg) of *C. mandshurica* were extracted with 50% EtOH (× 3). After removal of the solvent under reduced pressure at 60 °C, the residue (600 g) was suspended in H_2O and defatted with EtOAc. The aqueous layer was further extracted with *n*-BuOH. The *n*-BuOH extract (200 g) was subjected to D101 porous polymer resin column chromatography and eluted with H_2O and 30% and 70% MeOH, successively. The fraction eluted with 70% MeOH (90 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$−MeOH−H_2O (7:3:0.1, 3:3:0.1, 3:3:1) to afford fractions 1−3. Fraction 2 was subjected to ODS C_{18} column chromatography and eluted with MeOH−H_2O in a gradient of MeOH to afford subfractions 2-1−2-4. Subfraction 2-1 was isolated by preparative HPLC (MeOH−0.05% TFA = 67:33, 2.5 mL/min) to yield compound **5** (1280 mg). Subfraction 2-2 was isolated by

Table 4. ^{13}C NMR Data for the Sugar Moieties of Compounds **1**−**5** (125 MHz, in C_5D_5N)

no.	1	2	3	4	5	no.	1	2	3	4	5
Ara						Rha′					
1	105.2	105.1	105.1	105.4	105.3	1	102.7	102.5		102.7	102.7
2	75.3	75.1	75.2	75.3	75.3	2	71.9	71.7		71.9	71.7
3	74.7	74.5	74.6	74.6	74.7	3	72.5	72.3		72.5	72.5
4	69.2	69.0	69.2	69.4	69.4	4	73.9	73.8		74.2	74.0
5	65.7	65.5	65.6	65.9	65.7	5	69.9	70.0		69.8	69.9
						6	18.5	18.3		18.6	18.6
Rha						Glc″					
1	101.4	101.2	101.3	101.3	101.4	1	95.6	95.4	95.6	95.6	95.6
2	71.9	71.7	71.9	71.9	71.9	2	72.9	72.8	73.8	73.9	73.8
3	82.0	81.7	81.9	83.5	81.9	3	78.7	78.5	78.6	78.7	78.7
4	72.7	72.5	72.6	72.5	72.7	4	70.8	70.6	70.7	70.8	70.8
5	69.7	69.5	69.7	69.6	69.9	5	78.0	77.8	78.0	78.0	78.0
6	18.4	18.2	18.4	18.4	18.5	6	69.3	69.2	69.0	69.2	69.2
Rib				All		Glc‴					
1	104.7	104.4	104.5	104.5	104.7	1	104.8	104.6	104.8	104.8	104.8
2	72.7	72.5	72.3	72.8	72.5	2	75.3	75.1	75.2	75.1	75.3
3	69.8	69.7	69.7	72.2	69.7	3	76.4	76.3	76.4	76.5	76.5
4	76.5	76.3	76.4	78.2	76.5	4	78.2	77.9	78.1	78.3	78.2
5	61.8	61.3	61.6	74.0	61.9	5	77.1	76.9	77.0	76.8	77.1
6				61.9		6	61.2	60.4	61.1	61.2	61.2
Glc						Rha″					
1	103.2	103.0	103.0	105.6	103.2	1	102.7	102.5	102.6	102.7	102.7
2	74.2	73.9	73.9	74.8	74.0	2	72.4	72.2	72.4	72.7	72.5
3	76.3	76.0	78.3	76.3	76.4	3	72.5	72.3	72.6	72.5	72.5
4	81.1	80.9	80.7	81.7	81.9	4	73.8	73.6	73.9	73.8	74.0
5	76.5	76.3	77.9	76.2	76.6	5	70.3	69.7	70.2	70.3	70.3
6	61.7	61.0	62.2	62.0	61.7	6	18.6	18.4	18.4	18.5	18.5
Glc′											
1	104.5	102.1	104.6	104.8	104.9						
2	74.1	74.8	74.6	74.8	74.8						
3	78.9	75.9	78.1	77.1	78.2						
4	69.8	70.1	71.8	71.6	71.9						
5	76.5	76.9	77.0	75.1	76.7						
6	68.0	68.0	61.6	68.5	68.5						

Table 2. NMR Data for the Isoferuloyl Moieties of **1** and **2** (500 MHz, in C_5D_5N)

	1		2	
no.	^1H	^{13}C	^1H	^{13}C
1		128.3		128.2
2	7.43 (1H, d, J = 1.5 Hz)	115.2	7.50 (1H, d, J = 2.0 Hz)	115.2
3		150.0		150.8
4		148.4		148.3
5	6.89 (1H, d, J = 8.0 Hz)	112.0	6.89 (1H, d, J = 8.0 Hz)	111.9
6	7.00 (1H, dd, J = 8.0, 1.5 Hz)	121.3	7.07 (1H, dd, J = 8.0, 2.0 Hz)	121.3
7	7.87 (1H, d, J = 16.0 Hz)	145.3	8.04 (1H, d, J = 16.0 Hz)	145.8
8	6.57 (1H, d, J = 16.0 Hz)	116.4	6.72 (1H, d, J = 16.0 Hz)	115.9
9		167.2		166.5
OCH_3	3.70 (3H, s)	55.7	3.70 (3H, s)	55.6

preparative HPLC (CH_3CN−0.05% TFA = 30:70, 2.5 mL/min) to yield compounds **3** (52 mg) and **4** (16 mg). Subfraction 2-3 was isolated by preparative HPLC (CH_3CN−0.05% TFA = 31:69, 2.5 mL/min) to yield compounds **1** (25 mg), **2** (68 mg), and **6** (23 mg). Subfraction 2-4 was isolated by preparative HPLC (MeOH−0.05% TFA = 69:31, 2.5 mL/min) to yield compound **7** (48 mg).

Clematomandshurica saponin A (1): white, amorphous powder; $[\alpha]_D^{20}$ −96 (*c* 1.0, MeOH); UV(MeOH) λ_{max} (log ϵ) 241 (2.58), 296 (sh), 324 (4.28) nm; IR (KBr) ν_{max} 3458, 1718, 1636, 1520, 1058 cm^{-1}; ^1H NMR data, see Table 1 and 2; ^{13}C NMR data, see Tables 2, 3, and 4; HRESIMS *m/z* 2005.8667 $[M + Na]^+$ (calcd for $C_{92}H_{142}O_{46}Na$, 2005.8670).

Clematomandshurica saponin B (2): white, amorphous powder; $[\alpha]_D^{20}$ −92 (*c* 1.0, MeOH); UV(MeOH) λ_{max} (log ϵ) 249(2.38), 298 (sh), 327 (4.18) nm; IR (KBr) ν_{max} 3453, 1718, 1632, 1518, 1056 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz): δ 1.23 (3H, s, 23-CH_3), 1.19 (3H, s, 27-CH_3), 1.08 (3H, s, 24-CH_3), 1.00 (3H, s, 26-CH_3), 0.85 (3H, s, 29-CH_3), 0.84 (3H, s, 30-CH_3), 0.81 (3H, s, 25-CH_3), 3.23 (1H, br d, J = 11.0 Hz, H-3), 5.34 (1H, br s, H-12), 6.18 (1H, br s, Rha-H-1), 6.16 (1H, d, J = 8.0 Hz, Glc″-H-1), 5.79 (1H, br s, Rha″-H-1), 5.79 (1H, d, J = 5.5 Hz, Rib-H-1), 5.39 (1H, br s, Rha′-H-1), 5.29 (1H, d, J = 8.0 Hz, Glc′-H-1), 4.95 (1H, d, J = 7.5 Hz, Glc‴-H-1), 4.85 (1H, d, J = 8.0 Hz, Glc-H-1), 4.78 (1H, d, J = 6.5 Hz, Ara-H-1), 1.64 (3H, d, J = 6.0 Hz, Rha″-H-1), 1.55 (3H, d, J = 6.0 Hz, Rha′-H-6), 1.48 (3H, d, J = 6.0 Hz, Rha-H-6); ^1H NMR data of the isoferuloyl moiety, see Table 2; ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m/z* 2005.8665 ($[M + Na]^+$, calcd for $C_{92}H_{142}O_{46}$, 2005.8670).

Clematomandshurica saponin C (3): white, amorphous powder; $[\alpha]_D^{20}$ −29 (*c* 1.0, MeOH); IR(KBr) ν_{max} 3416, 2928, 1730, 1061 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz): δ 1.24 (3H, s, 23-CH_3), 1.21 (3H, s, 27-CH_3), 1.10 (3H, s, 24-CH_3), 1.04 (3H, s, 26-CH_3), 0.86 (3H, s, 29-CH_3), 0.86 (3H, s, 30-CH_3), 0.83 (3H, s, 25-CH_3), 3.25 (1H, br d, J = 11.5 Hz, H-3), 5.36 (1H, br s, H-12), 6.21 (1H, br s, Rha-H-1), 6.20 (1H, d, J = 8.0 Hz, Glc″-H-1), 5.82 (1H, br s, Rha″-H-1), 5.81 (1H, d, J = 5.5 Hz, Rib-H-1), 5.14 (1H, d, J = 8.0 Hz, Glc′-H-1), 4.97 (1H, d, J = 7.5 Hz, Glc‴-H-1), 4.95 (1H, d, J = 8.0 Hz, Glc-H-1), 4.81 (1H, d, J = 5.5 Hz, Ara-H-1), 1.67 (3H, d, J = 6.0 Hz, Rha″-H-1), 1.50 (3H, d, J = 6.0 Hz, Rha-H-6); ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m/z* 1683.7615 $[M + Na]^+$ (calcd for $C_{76}H_{124}O_{39}$, 1683.7617).

Clematomandshurica saponin D (4): white, amorphous powder; $[\alpha]_D^{20}$ −68.6 (*c* 1.2, MeOH); IR (KBr) ν_{max} 3420, 2928, 1731, 1060 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m/z* 1859.8311 $[M + Na]^+$ (calcd for $C_{83}H_{136}O_{44}$, 1859.8302).

Acid Hydrolysis of Compounds 1−4. Each compound (5 mg) was hydrolyzed with 2 N aqueous CF_3COOH (10 mL) at 110 °C for 8 h in a sealed tube. The reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). The combined EtOAc extract was evaporated under reduced pressure and analyzed by TLC. Oleanolic acid was detected for compounds **1**−**4**, and isoferulic acid for compounds **1** and **2**. The aqueous layer was evaporated with MeOH repeatedly under vacuum to remove the solvent completely. The residue was dissolved in anhydrous pyridine (0.100 mL) and mixed with a pyridine solution of L-cysteine methyl ester hydrochloride (0.100 mL). After warming at 60 °C for 1 h, hexamethyldisilazine (0.100 mL) and trimethylsilyl chloride (0.040 mL) were added, the warming at 60 °C was continued for another 30 min, and then the mixture was filtered through a 0.45 μm membrane to remove the precipitate and analyzed by GC. L-Rha (t_R = 5.410 min), D-Glc (t_R = 11.628 min), D-Rib (t_R = 5.201 min), D-All (t_R = 11.368 min), L-Ara (t_R = 4.986 min).

Alkaline Hydrolysis of Compounds 1 and 2. Each compound (6 mg) was dissolved in 0.1 N KOH (2 mL) and reacted at room temperature for 1 h. The reaction mixture was neutralized with diluted HCl and analyzed by HPLC. Isoferulic acid and **5** were detected for compounds **1** and **2**.

Assay for Inhibition of COX-2 Activity. The assay for inhibition of COX-2 activity is based on the method previously reported.[14] Briefly, peritoneal macrophages were harvested from male C57BL-6J mice (Experimental Animal Center, Institute of Experimental Animal, Chinese Academy of Medical Sciences & Peking Union Medical College) 3 days after the injection (ip) of brewer thioglycollate medium, washed twice in D-Hanks' buffer, and resuspended in RPMI-1640 (GIBCO/BRL, Gaithersburg, MD). Macrophages were incubated with test compound at different concentrations for 1 h and were stimulated with LPS 1.0 mg/L for 9 h. The amount of PGE_2 in supernatants was measured by radioimmunoassay (RIA) using the PGE_2 RIA kit (PLA General Hospital, Beijing, China).

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