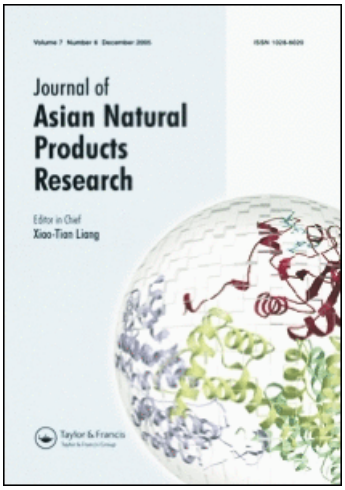

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Two new flavonoid glycosides from *Artemisia frigida* Willd.

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An investigation of the *n*-BuOH-soluble fraction from the aerial parts of *Artemisia frigida* has led to the isolation of two new flavonoid glycosides, named friginoside A and friginoside B. Their structures were characterized as 5,7-dihydroxy-3′,4′,5′-trimethoxy flavone 7-*O*-β-D-glucuronide (**1**) and 5,7-dihydroxy-3′,4′,5′-trimethoxy-flavone 7-*O*-β-D-glucuronyl-(1 → 2)*O*-β-D-glucuronide (**2**) on the basis of 1D and 2D NMR spectral analysis.

Keywords: *Artemisia frigida* Willd.; friginoside A; friginoside B; flavone

1. Introduction

Artemisia frigida Willd., Agi in Mongolian, is a commonly used medical material in Mongolian folk medicine [1–6], distributed throughout Inner Mongolia, occupying 10.38% of its steppe [7]. The aerial parts of *A. frigida* are used as a clinical medicine to treat many diseases, such as hemorrhage, arthrocele, nepropyrexia, rheumatoid, and menoxenia [8–10]. Sesquiterpenoids [11,12], coumarins [13], and flavonoids [14–16] have been reported from *A. frigida*. Recently, we carried out a systematic chemical study on the aerial parts of *A. frigida*, which resulted in the isolation of two new flavonoid glycosides together with two known compounds. Here, we report the structural characterization of the new compounds by spectral analysis.

2. Results and discussion

The 95% ethanol extract of *A. frigida* was suspended in water, and then partitioned with petroleum ether (PE), $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble fraction was separated by chromatography and afforded two new flavonoid glycosides, 5,7-dihydroxy-3′,4′,5′-trimethoxy flavone 7-*O*-β-D-glucuronide (**1**) and 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone 7-*O*-β-D-glucuronyl-(1 → 2)*O*-β-D-glucuronide (**2**), along with two known compounds, 5,7-dihydroxy-3′,4′-dimethoxy flavone-7-*O*-β-D-glucuronide (**3**) and chrysoeriol-4′-*O*-β-D-glucoside (**4**) (Figure 1). The structures of the known compounds were identified by comparing their spectroscopic data with those reported in the literature [17].

Compound **1** was obtained as a yellow powder. The molecular formula was determined to be $C_{24}H_{24}O_{13}$ by HR-ESI-MS at *m/z* 519.1146 $[M - H]^-$. The UV spectrum of **1** showed absorption maxima at 255, 268, and 336 nm. In the ^1H NMR spectrum, the signals of five aromatic protons at δ 6.44 (1H, d, $J = 2.0$ Hz, H-6),

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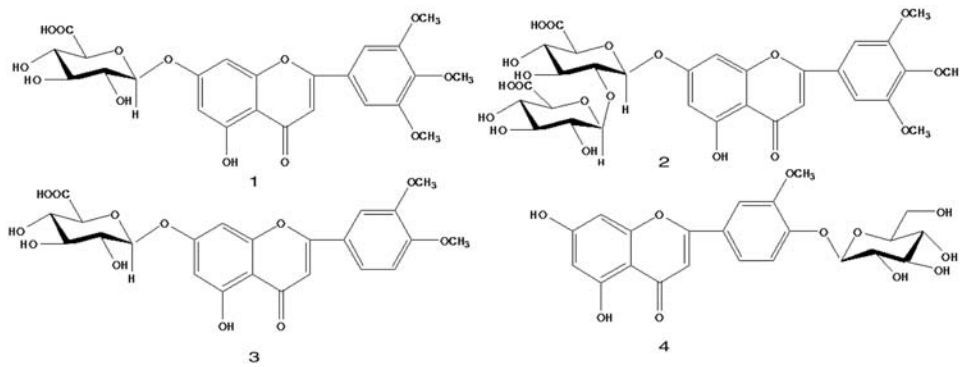

Figure 1. Structures of compounds **1**–**4**.

6.91 (1H, d, $J = 2.0$ Hz, H-8), and 7.35 (2H, s, H-2′, 6′) indicated the presence of an AB system and an A_2 system, and the proton singlet at δ 7.05 (1H, s) revealed the presence of H-3 of flavone. The three proton signals at δ 3.89 (9H, s, $-OCH_3$) corresponding to three methoxyls were attached to the positions 3′, 4′ and 5′, respectively. The remaining signals at δ 5.11 (1H, d, $J = 7.0$ Hz, H-1″) suggested the presence of the anomeric protons of glycoside.

The ^{13}C NMR signals (Table 1) also proved the presence of the methoxyl groups and aromatic rings. All protonated carbons were assigned by the analysis of the HMQC spectrum. The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of compound **3** [17], except for the B ring. Namely, the ABX system in compound **3** was substituted by the A_2 system in compound **1**, and this was confirmed by the proton signals at δ_H 7.35 (2H, s, H-2′, 6′) and δ_H 3.89 (9H, s, 3′,4′,5′-OCH_3).

The HMBC correlation from H-1″ to C-7 revealed that the sugar moiety was linked to the C-7 of the aglycone. Meanwhile, the correlation of NOESY between H-1″ and H-6, H-8 also confirmed the above link. The anomeric configuration in the sugar moiety was determined as β according to the coupling constant 7.0 Hz. So, the structure of **1** was elucidated as 5,7-dihydroxy-3′,4′,5′-trimethoxy flavone 7-*O*-β-D-glucuronide, and named as friginoside A. The key correlations of HMBC and NOESY are shown in Figure 2.

Compound **2** was obtained as a yellow powder. The molecular formula was determined to be $C_{30}H_{32}O_{19}$ by HR-ESI-MS at *m/z* 695.1449 $[M - H]^-$. ^{1}H and ^{13}C NMR spectra of compound **2** were similar to those of compound **1**, except for the sugar moiety. By means of HSQC, HMBC, HSQC-TOCOSY, and ^{1}H–^{1}H COSY spectra, compound **2** had two glucuronic acid, GluA1 (δ_C 98.3, 82.7, 75.3, 71.1, 74.4, and 170.6) and GluA2 (δ_C 104.7, 74.4, 75.8, 71.8, 75.8, and 170.6), respectively. The anomeric protons appearing at δ 5.29 (1H, d, $J = 6.0$ Hz) and 4.55 (1H, d, $J = 7.5$ Hz), and their corresponding carbons resonating at δ 98.3 (C-1″) and 104.7 (C-1‴) from the HSQC experiment suggested the presence of two β-D-glucuronyl groups. The HMBC cross-peaks between H-1″ and C-7 and between H-1‴ and C-2″ confirmed that the GluA1 was linked at C-7 and the GluA2 was linked at C-2″. Thus, the structure of **2** was elucidated as 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone 7-*O*-β-D-glucuronyl-(1 → 2)*O*-β-D-glucuronide, and named as friginoside B. The key correlations of HMBC are shown in Figure 2.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2** (300 MHz, in DMSO-d_6).

	1		**2**	
	^{1}H (J in Hz)	^{13}C	^{1}H (J in Hz)	^{13}C
Aglycone				
2		164.2		164.3
3	7.05 (1H, s)	103.8	7.09 (1H, s)	103.8
4		182.1		182.2
5		161.1		161.2
6	6.44 (1H, d, $J = 2.0$)	99.6	6.47 (1H, s)	99.5
7		163.1		162.7
8	6.91 (1H, d, $J = 2.0$)	95.1	6.93 (1H, s)	95.5
9		156.9		156.9
10		105.2		105.6
1′		120.2		120.3
2′	7.35 (1H, s)	104.6	7.39 (1H, s)	104.7
3′		148.3		148.3
4′		140.0		140.1
5′		148.3		148.3
6′	7.35 (1H, s)	104.6	7.39 (1H, s)	104.7
Gly				
1″	5.11 (1H, d, $J = 7.0$)	103.3	5.29 (1H, d, $J = 6.0$)	98.3
2″	3.28 (1H, m)	73.1	3.53 (1H, m)	82.7
3″	3.32 (1H, m)	73.9	3.56 (1H, m)	75.3
4″	3.18 (1H, m)	72.0	3.39 (1H, m)	71.1
5″	3.63 (1H, m)	76.6	3.90 (1H, m)	74.4
6″		172.1		170.6
1‴			4.55 (1H, d, $J = 7.5$)	104.7
2‴			3.17 (1H, m)	74.4
3‴			3.23 (1H, m)	75.8
4‴			3.25 (1H, m)	71.8
5‴			3.61 (1H, m)	75.8
6‴				170.6
OCH_3-3′, 5′	3.89 (9H, s)	56.5(2)	3.90 (9H, s)	56.5(2)
OCH_3-4′		59.6		59.7
OH-5	12.9 (1H, s)		12.9 (1H, s)	

3. Experimental

3.1 General experimental procedures

Optical rotations were measured in MeOH at 25°C on a Perkin-Elmer 241 polarimeter. The UV spectra were recorded on a Shimadzu UV-2201 spectrometer. The IR spectra were recorded in KBr disks on a Thermo Nicolet 200 double beam spectrophotometer. The HR-ESI-MS spectra were measured on Bruker Daltonics MicroTOFQ. NMR spectra were measured on a Bruker ARX-600 NMR spectrometer with tetramethylsilane as the internal reference, and chemical shifts are expressed in δ (ppm). Semi-preparative HPLC was performed using a Japanese liquid chromatograph equipped with a Zorbax SB-C18 column. Column chromatography was performed using silica gel (200–300 mesh; Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Fractions were monitored by TLC (silica gel GF_{254} 10–40 μm; Marine Chemical Factory), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

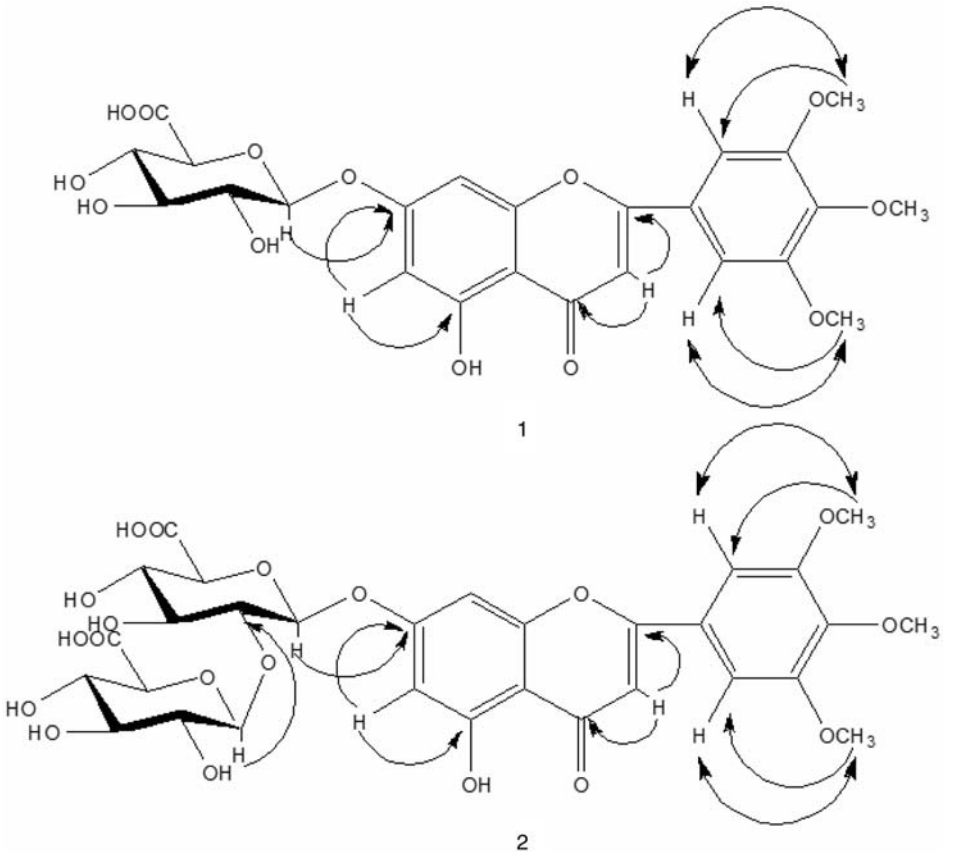

Figure 2. Selected HMBC (→) and NOESY (↔) correlations for **1** and **2**.

3.2 Plant material

The aerial parts of *A. frigida*, used as the experimental material, were collected in Tongliao, Inner Mongolia of China, in July 2007, and identified by Prof. Buhebateer (Inner Mongolia University for Nationalities). A voucher specimen (No. 20070720) has been deposited in the School of Traditional Mongolian Medicine of Inner Mongolia University for Nationalities.

3.3 Extraction and isolation

The air-dried aerial parts of *A. frigida* (2 kg) were crushed and extracted twice under reflux with 95% EtOH. Evaporation of the solvent under reduced pressure yielded the 95% EtOH extract. The extract was partitioned with PE, $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble fraction (45.0 g) was isolated by column chromatography on silica gel and gradiently eluted with $CHCl_3$–MeOH (50:1–1:1) to give 10 fractions (fractions 1–10). Fraction 4 [300 mg, $CHCl_3$–MeOH (20:1) eluate] was loaded onto a column of silica gel and eluted with $CHCl_3$–acetone (50:1–1:1) to give 10 fractions (fractions 4-1 to 4-10). Fraction 4-6 (45 mg) was further purified by column chromatography on silica gel with $CHCl_3$–acetone (10:1) to give **4** (20 mg). Fraction 8 [5.2 g, $CHCl_3$–MeOH (3:1) eluate] was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (5:1–1:1) to give three fractions (fractions 8-1 to 8-3). Fraction 8-2 [700 mg, $CHCl_3$–MeOH

(3:1) eluate] was further chromatographed on a Sephadex LH-20 column eluted with MeOH, and then separated by semi-preparative HPLC (CH_3CN-H_2O, 20:80) yielding **2** (18 mg) and **3** (15 mg). Fraction 8-3 [200 mg, $CHCl_3$–MeOH (1:1) eluate] was further separated by semi-preparative HPLC (CH_3CN-H_2O, 18:85) to yield **1** (30 mg).

*3.3.1 5,7-Dihydroxy-3′,4′,5′-trimethoxy flavone 7-O-β-D-glucuronide (**1**)*

Yellow powder (MeOH); mp 263–265°C; $[\alpha]_D^{25}$ −38.7 ($c = 0.1$, MeOH); UV (MeOH) λ_{max} (nm) ($\log \varepsilon$): 255 (4.27), 268 (4.21), 336 (4.10); IR (KBr) ν_{max} (cm^{-1}): 3480–3300, 1663, 1652, 1607, 1495, 1457, 1341, 1258; ^1H NMR (300 MHz, in DMSO-d_6) and ^{13}C NMR (75 MHz, in DMSO-d_6) spectral data: see Table 1; HR-ESI-MS: *m/z* 519.1146 $[M - H]^-$ (calcd for $C_{24}H_{23}O_{13}$, 519.1139).

*3.3.2 5,7-Dihydroxy-3′,4′,5′-trimethoxyflavone 7-O-β-D-glucuronyl-(1 → 2)O-β-D-glucuronide (**2**)*

Yellow powder (MeOH); mp 271–273°C; $[\alpha]_D^{25}$ −40.2 ($c = 0.1$, MeOH); UV (MeOH) λ_{max} (nm) ($\log \varepsilon$): 269 (4.46), 351 (4.11); IR (KBr) ν_{max} (cm^{-1}): 3480–3300, 1665, 1652, 1611, 1494, 1457, 1337, 1258; ^1H NMR (300 MHz, in DMSO-d_6) and ^{13}C NMR (75 MHz, in DMSO-d_6) spectral data: see Table 1; HR-ESI-MS: *m/z* 695.1449 $[M - H]^-$ (calcd for $C_{30}H_{31}O_{19}$, 695.1460).

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