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Two new triterpenoid saponins from *Centella asiatica*

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ABSTRACT

Two undescribed ursane-type triterpene saponins, named asiaticoside H (**1**) and I (**2**), were isolated from the whole plants of *Centella asiatica*. The chemical structures of **1** and **2** were mainly characterized by extensive analysis of their 1D and 2D NMR and HRESIMS spectroscopic data and chemical derivation.

1. Introduction

Centella asiatica (L.) Urban, belonging to the family Apiaceae, is widely distributed throughout the world, especially in the moist and tropical regions of Asia, Africa and Oceania (James and Dubery, 2009). In some countries of the world, *C. asiatica* has been used for treating skin diseases and healing skin wounds for thousands of years (Brinkhaus et al., 2000). In this plant, the triterpene saponins are main characteristic constituents, which have been confirmed to be responsible for the above medicinal benefits (Shen et al., 2019; Kwon et al., 2014). *C. asiatica* has been traditionally used to improve brain function, boost memory and prevent cognitive deficits (Shinomol and Muralidhara, 2011), which was proved to associate with the triterpene saponins and caffeoylquinic acids in the plant that exhibit neuroprotective effects in several *in vitro* models (Wu et al., 2020; Viswanathan et al., 2019; Subaraja and Vanisree, 2019). Recently, *C. asiatica* extracts were confirmed to have antibacterial activity (Sieberi et al., 2020). Asiaticoside, a main component in *C. asiatica*, showed strong antibacterial activity against some antibiotic-resistant bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (Zhang et al., 2006). In order to fully understand the chemical compositions of *C. asiatica* and search for novel antibacterial ingredients from this plant, the present study was focused on the chemical investigation of the minor bioactive compounds. As a result, two new triterpene saponins (Fig. 1) were obtained and identified.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder. The molecular formula of **1** was determined to be $C_{54}H_{88}O_{24}$according to the $[M—H]^-$ ion peak at *m/z* 1119.5604 (calc. for 1119.5593) in its negative HRESIMS spectrum. The ^{1}H-NMR spectrum of **1** displayed several characteristic proton singals for six methyl proton signals at δ_H 0.90 – 1.15, an olefinic proton signal at δ_H 5.41 (brs) and four anomeric methines at δ_H 6.14 (1H, d, J =8.16 Hz), 4.97 (1H, d, J =7.86 Hz), 5.30 (1H, d, J =3.54 Hz) and 5.79 (1H, brs) (Table 1). In the ^{13}C-NMR spectra of **1**, four signals at δ_C 95.9, 105.0, 101.2 and 102.8 were readily assigned to the anomeric carbons for three glucoses and one L-rhamnose with the aid of HSQC spectrum (Table 1). The sugar residues were further identified as D-glucose and L-rhamnose based on the methods previously reported (Li et al., 2017). The ^{1}H-NMR and ^{13}C-NMR spectroscopic data were very similar to those of asiaticoside (Sung et al., 1992), a major component in *C. asiatica*, except for the presence of one more glucose moiety in **1**. The double methyl proton signal assigned to H-29 at δ_H 0.92 (3H, d, J =3.18 Hz) showed an HMBC correlation with C-18 (δ_C 53.5), while another double methyl proton signal assigned to H-30 at δ_H 0.90 (3H, d, J =2.94 Hz) exhibited HMBC correlations with C-19 (δ_C 39.5) and C-21 (δ_C 31.0), suggesting the ursane-type aglycone of **1** (Fig. 2). The double bond was located between C-12 and C-13 in view of the HMBC correlations of H-18 (δ_H 2.48) with C-12 (δ_C 126.1) and C-13 (δ_C 138.9). The chemical shifts of C-2 and C-3 at δ_C 69.8 and 78.2 suggested the equatorial position of the hydroxyl groups at C-2 and C-3 (Uddin Ahmad et al., 1986; Kojima et al., 1987). In the NOESY spetrum of **1**, the obvious cross signals of H-2 (δ_H 4.20)/H-25 (δ_H 1.06), H-2 (δ_H 4.20)/H-24 (δ_H 1.03), H-5 (δ_H 1.77)/H-23 (δ_H 3.33) and H-3 (δ_H 4.02)/H-5 (δ_H 1.77) further confirmed the stereochemistries of H-2, H-3 and the presence of an oxygen atom connected to C-23 (Fig. 2). A glucose residue was confirmed to be connected to C-23 of the aglycone due to the HMBC correlation between the anomeric proton at δ_H 5.30 (1H, d, J =3.54 Hz) and the methylene signal at δ_C 73.6 (C-23).

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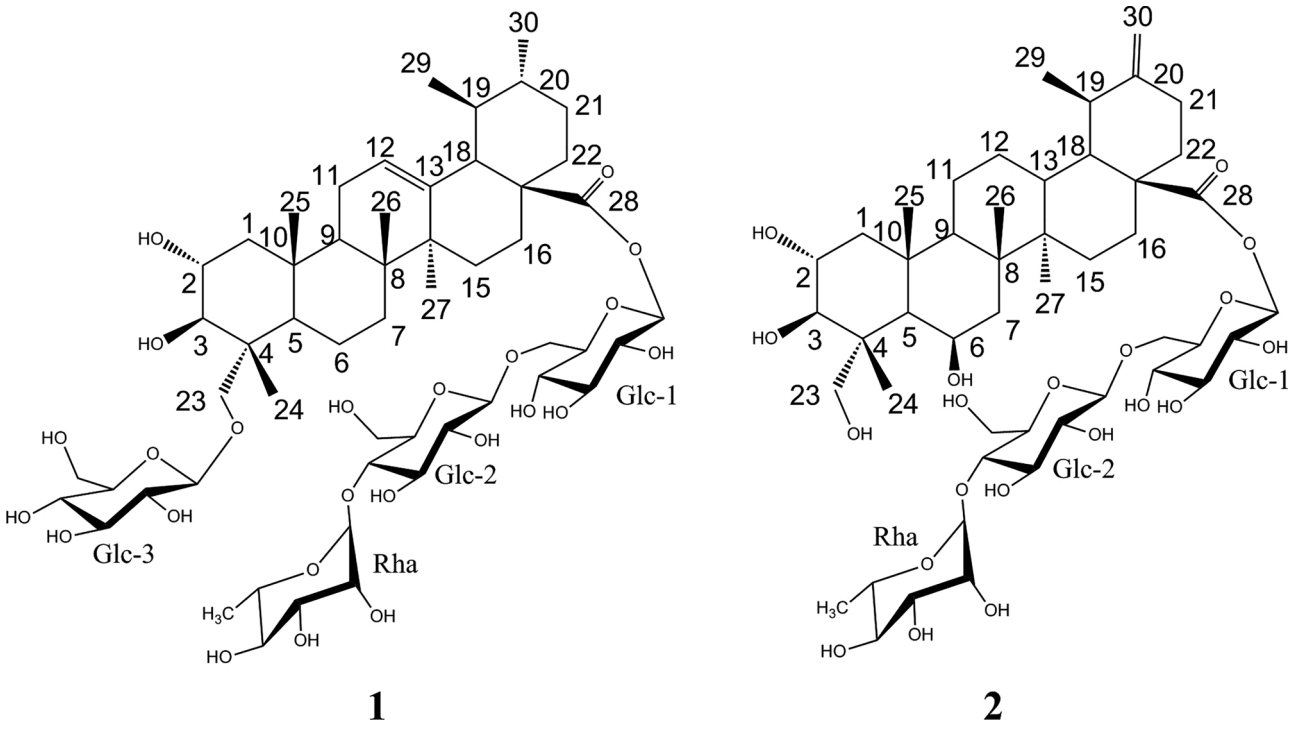

Fig. 1. Chemical structures of the compounds **1**-**2**.

Moreover, the chemical shift of the oxygenated methylene CH_2-23 in **1** shifted to lower field by around 7 ppm than that of CH_2-23 in asiaticoside, also suggesting a sugar residue was located at C-23 in **1**. The sugar chain at C-28 of the aglycone was verified by the following important HMBC correlations from H-1 (δ_H 6.14) of Glc-1 to C-28 (δ_C 176.7), H-1 (δ_H 4.97) of Glc-2 to C-6 (δ_C 69.5) of Glc-1, and H-1 (δ_H 5.79) of Rha to C-4 (δ_C 78.6) of Glc-2 (Fig. 2). Finally, compound **1** was etablished as 3-O-*β*-D-glucopyranosyl 2*α*,3*β*,23-trihydroxy-urs-12-ene-28-oic acid 28-O-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranoside by detailed analyses of HSQC, HMBC, NOESY and TOCSY spectra, and named asiaticoside H.

Compound **2** was isolated as a white amorphous powder. The molecular foumula of **2** was defined as $C_{48}H_{78}O_{20}$based on the $[M—H]^-$ ion peak at *m/z* 973.5018 (calc. for 973.5014) in the HRESIMS spectrum. Comparison of the ^{1}H-NMR and ^{13}C-NMR data of **2** with those of madecassoside, another major component in *C. asiatica*, suggested the chemical structures of these two compounds were only differed in the location of the double bonds. The configurations of the hydroxyl groups at C-2, C-3 and C-6 were further verified by the cross signals of H-2 (δ_H 4.49)/H-25 (δ_H 1.74), H-3 (δ_H 4.01)/H-5 (δ_H 1.82), H-5 (δ_H 1.82)/H-23 (δ_H 4.33) and H-5 (δ_H 1.82)/H-6 (δ_H 5.06) in the NOESY spetrum of **2** (Fig. 2). The appearance of a methylene carbon signal at δ_C 112.7 ppm in the ^{13}C-NMR spectrum of **2** indicated the presence of an exocyclic double bond. Detailed analysis of the ^{13}C-NMR data of **2** and 2α,3β-dihydroxy-urs-20(30)-en-28-oic acid (Qiao et al., 2014) demonstrated they might share the same E ring. This was further confirmed by the HMBC cross-peaks from H_3-29 (δ_H 1.13) to C-19, C-20 and H_2-30 (δ_H 5.00) to C-20, C-21. The sugar chain at C-28 of the aglycone was supported by the HMBC correlations from H-1 (δ_H 6.13) of Glc-1 to C-28 (δ_C 175.4), H-1 (δ_H 4.95) of Glc-2 to C-6 (δ_C 69.7) of Glc-1, and H-1 (δ_H 5.85) of Rha to C-4 (δ_C 78.6) of Glc-2 (Fig. 2). Thus, compound **2** was elucidated as 2*α*,3*β*,6*β*,23-tetrahydroxy-urs-20(30)-ene-28-oic acid 28-O-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranoside, and named asiaticoside I.

3. Experimental section

3.1. General experimental procedures

Silica gel (100–200 mesh) and TLC plates were obtained from Qingdao Haiyang Chemical Group Co. Ltd. (Qingdao, China). All solvents were analytical grade and bought from KeLong (Chengdu, China). Standard sugars were bought from Sigma-Aldrich (St. Louis, MO, USA). Optical rotations were recorded on a PerkinElmer 341 polarimeter. NMR spectra were performed on Bruker Avance-400 and Bruker Avance-600 spectrometers in C_5D_5N with TMS as an internal standard, respectively. HR-ESI-MS data were acquired on a LTQ Orbitrap XL mass spectrometer. The sugar derivatives were analyzed *via* a Shimadzu LC-20A series HPLC instrument equipped with a SinoChrom ODS-BP C18 column. Semi-preparative HPLC separation was carried out on a CXTH apparatus with an UV3000 detector equipped with a Unisil-10−120-C18 (250 × 30 mm, 10 μm) column.

3.2. Plant materials

The whole plant material of *C. asiatica* was collected in Sichuan Province, China, in July 2018. The plant was identified by Professor Yuntong Ma of Chengdu University of TCM. A voucher specimen (NO. ZZZYK20200803001−30) was deposited in the authors′ lab.

3.3. Extraction and isolation

The air-dried whole plants of *C. asiatica* (10.0 kg) were smashed and extracted with 80 % MeOH (20 L) for 3 times (24 h each time) at 60 °C. The combined extract was evaporated under reduced pressure to yield a dark brown residue, which was suspended in water and then partitioned successively with CH_2Cl_2 and *n*-BuOH. The resultant *n*-BuOH extract was dried under reduced pressure and then separated over a silica gel column (30 × 100 cm) eluted with a gradient solvent system of H_2O saturated CH_2Cl_2/MeOH (10:1, 8:1, 6:1, 4:1, 2:1, 1:1) to yield eleven fractions (Fr. 1 - Fr. 11) based on TLC analysis. Fr. 8 was subject to HPLC on a Unisil-10−120-C18 (10 μm, 250 × 30 mm, 20 mL/min) column

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **2** (δ, J in Hz, C_5D_5N).

position	**1**		**2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	47.9	1.32 (m), 2.27 (m)	50.1	1.41 (m), 2.47 (m)
2	68.9	4.20 (m)	69.4	4.49 (m)
3	78.8	4.02 (m)	78.3	4.01 (m)
4	43.6		44.5	
5	48.4	1.77 (m)	49.5	1.82 (m)
6	19.1	1.37 (m), 1.53 (m)	67.9	5.06 (br s)
7	33.2	1.37 (m), 1.85 (m)	42.0	1.78 (m), 1.90 (m)
8	40.4		42.2	
9	48.5	1.75 (m)	51.9	1.70 (m)
10	38.4		40.9	
11	24.0	2.05 (m)	22.2	1.42 (m), 1.70 (m)
12	126.1	5.41 (br s)	26.1	2.39 (m), 2.45 (m)
13	138.9		38.5	3.01 (m)
14	42.8		42.0	
15	28.8	1.15 (m), 1.25 (m)	28.3	1.11 (m), 2.03 (m)
16	24.8	1.96 (m)	27.2	0.98 (m), 1.76 (m)
17	48.7		48.0	
18	53.5	2.48 (d, 11.16)	49.9	1.14 (m)
19	39.5	1.33 (m)	41.3	2.75 (m)
20	39.3	0.87 (m)	150.5	
21	31.0	1.25 (m), 1.37 (m)	34.4	1.42 (m), 2.10 (m)
22	37.0	1.78 (m), 1.92 (m)	35.3	1.35 (m), 2.53 (m)
23	73.6	3.33 (d, 9.48), 4.35 (m)	66.7	4.07 (m), 4.33 (m)
24	14.4	1.03 (s)	15.6	1.69 (s)
25	17.7	1.06 (s)	17.1	1.74 (s)
26	18.1	1.15 (s)	19.9	1.60 (s)
27	24.0	1.14 (s)	15.3	0.86 (s)
28	176.7		175.4	
29	17.6	0.92 (d, 2.94)	25.0	1.13 (d, 5.2)
30	21.6	0.90 (d, 3.18)	112.7	5.00 (br s)
Glc-1				
1	95.9	6.14 (d, 8.16)	95.5	6.13 (d, 7.48)
2	73.9	4.32 (m)	73.9	4.31 (m)
3	79.0	4.04 (m)	79.0	4.03 (m)
4	71.1	4.24 (m)	71.5	4.20 (m)
5	78.1	4.06 (m)	78.0	4.07 (m)
6	69.5	4.23 (m), 4.64 (m)	69.7	4.23 (m), 4.64 (m)
Glc-2				
1	105.0	4.97 (d, 7.86)	104.9	4.95 (d, 7.52)
2	75.5	3.93 (m)	75.5	3.92 (m)
3	76.7	4.12 (m)	76.6	4.12 (m)
4	78.6	4.36 (m)	78.8	4.36 (m)
5	77.3	3.65 (m)	77.2	3.65 (m)
6	61.5	4.08 (m), 4.20 (m)	61.5	4.08 (m), 4.22 (m)
Glc-3				
1	101.2	5.30 (d, 3.54)		
2	70.5	4.38 (m)		
3	72.9	4.65 (m)		
4	71.1	4.48 (m)		
5	72.0	4.38 (m)		
6	62.8	4.35 (m), 4.40 (m)		
Rha				
1	102.8	5.79 (br s)	102.3	5.85 (br s)
2	72.7	4.66 (m)	72.7	4.62 (m)
3	72.9	4.53 (m)	72.8	4.52 (m)
4	74.1	4.12 (m)	74.3	4.14 (m)
5	70.5	4.89 (m)	70.3	4.95 (m)
6	18.7	1.67 (d, 6.12)	18.6	1.67 (d, 5.4)

with MeOH-H_2O (57:43, v/v) as an eluent system to give six sub-fractions (Fr. 8.1 - Fr. 8.6). Fr. 8.5 was further purified by the same HPLC using CH_3CN-H_2O (25:75, v/v) as an eluant to afford compound **2** (61.6 mg). Fr. 10 was submitted to HPLC on a Unisil-10−120-C18 (10 μm, 250 × 30 mm, 20 mL/min) column with MeOH-H_2O (57:43, v/v) as an eluent system to give pure compound **1** (32.7 mg).

Asiaticoside H (**1**): White amorphous powder; $[\alpha]_D^{20}$ +11.7 (c 0.10, MeOH); ^{1}H NMR (600 MHz, C_5D_5N) and ^{13}C NMR (150 MHz, C_5D_5N) data see Table 1; HRESIMS: m/z 1119.5604 $[M-H]^-$ (calcd for $C_{54}H_{87}O_{24}^-$, 1119.5593).

Asiaticoside I (**2**): White amorphous powder; $[\alpha]_D^{20}$ -4.0 (c 0.15, MeOH); ^{1}H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (100 MHz, C_5D_5N) data see Table 1; HRESIMS: m/z 973.5018 $[M-H]^-$ (calcd for $C_{48}H_{77}O_{20}^-$, 973.5014).

3.4. Acid hydrolysis and determination of absolute configurations of monosaccharides

Compounds **1**-**2** (each 2.0 mg) were mixed and dissolved in 2% H_2SO_4 solution (2 mL) and heated at 100 °C for 12 h. The resultant solution was cooled to room temperature and then extracted with EtOAc three times (each 3 mL) to remove the aglycones. The remaining H_2O layer was neutralized to pH = 7 with aqueous solution of $Ba(OH)_2$,

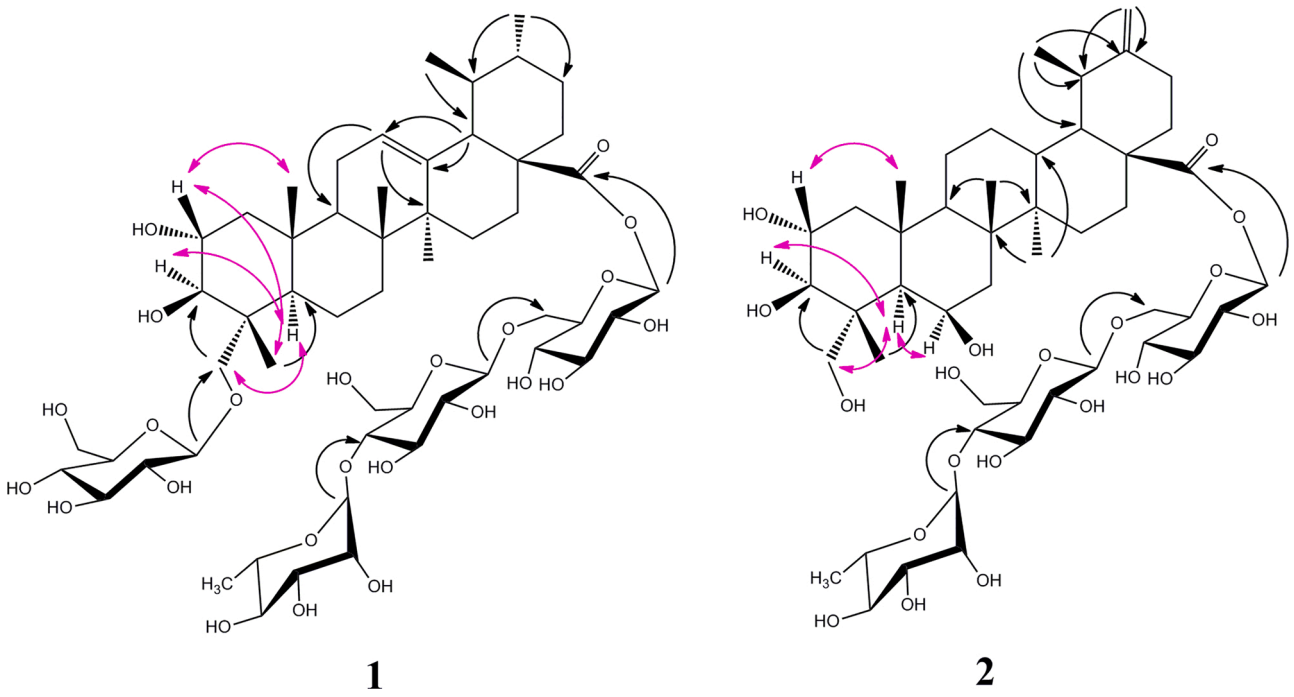

Fig. 2. Key HMBC and NOE correlations of compounds 1-**2**.

filtered, and identified using TLC with standard sugars. The filtered water solution of compounds **1** and **2** was dried under reduced pressure to give a residue (2.2 mg), which was dissolved in pyridine (0.3 mL) containing L-cysteine methyl ester hydrochloride (2.5 mg) and heated at 60 °C for 1 h. Then, phenylisothiocyanate (2.5 μL) was added and the mixture was heated at 60 °C for another 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC performed on a Shimadzu LC-20A series HPLC instrument equipped with a SinoChrom ODS-BP C18 column and CH_3CN-H_2O (25:75, v/v) containing 50 mM H_3PO_4 as the mobile phase. From the hydrolysate of compounds **1** and **2**, D-glucose and L-rhamnose were identified by comparison of the retention times of their derivatives with those of the authentic sugars derivatized in the same way, which showed retention times of 19.88 and 24.17 min, respectively.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2021.06.012.

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