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Triterpenoid saponins from *Clematis chinensis* and their inhibitory activities on NO production

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ABSTRACT

Four new triterpenoid saponins, clematochinenoside H–K (**1–4**), and five known structures (**5–9**), were isolated from the roots and rhizomes of *Clematis chinensis*. Their structures were elucidated on the basis of spectroscopic evidence and hydrolysis products. All isolates were evaluated for inhibitory effects against nitric oxide (NO) production in LPS-induced RAW 246.7 macrophages. Monodesmosidic saponins (**1–3**, **5**, and **6**) with a free carboxylic acid function at C-28 exhibited potent inhibitory activities with IC_{50} values in the range of 12.9–32.3 μM, where as bisdesmosidic saponin (**4**, and **7–9**) showed modest inhibitory effects with the inhibition ratios (%) from 39.9 to 59.0 at 50 μM. In addition, the hydroxyl group at C-21 showed negative effect on the NO production inhibitory activity.

1. Introduction

Clematis chinensis Osbeck. (Ranunculaceae) is distributed widely in the south of China. Its dried roots and rhizomes are a source of traditional Chinese medicine “Weilingxian”, with claims being made concerning its anti-inflammatory, anti-tumor, and analgesic properties (Mimaki et al., 2004). Chemical investigations on this genus afforded triterpene saponins, alkaloids, flavonoids, lignans, steroids, coumarins, macrocyclic compounds, and phenolic glycosides (Chawala et al., 2012). Several biological effects, such as anti-inflammatory, anti-tumor, analgesia, and cardioprotective activities, may be attributed to the characteristic markers of triterpene saponins based upon hederagenin and oleanolic acid (Chawala et al., 2012; Zhang et al., 2013; Zhao et al., 2016). Previously, we reported on the isolation and structural elucidation of triterpene saponins from *C. chinensis* (Fu et al., 2010, 2013) and alkaloids (Fu et al., 2016) from C. *mandshurica*. In our continuing effort to seek for the bioactive components from genus *Clematis*, a re-investigation on the roots and rhizomes of *C. chinensis* led to the discovery of four new and five known triterpene saponins. Herein, we report the isolation and structure elucidation, and anti-inflammatory activities of these compounds.

2. Results and discussion

In this paper, we report the isolation and structural elucidation of four new triterpene saponins clematochinenoside H–K (**1–4**) (Fig. 1), together with five known compounds saponin CP7 (**5**) (Shao et al., 1996), mandshunoside B (**6**) (He et al., 2011), 3-*O*-β-D-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranosyl oleanolic acid 28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**7**) (Zhao et al., 2014), 3-*O*-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**8**) (Zhang et al., 2013), clematichinenoside A (**9**) (Shao et al., 1995).

Their structures were elucidated on the basis of spectroscopic evidence and hydrolysis products. The inhibitory effects against nitric oxide production in LPS-induced RAW 246.7 macrophages of all compounds were also evaluated.

Compounds **1–4** were isolated as white amorphous powders. The monosaccharides obtained after aqueous acid hydrolysis of each compound were identified as glucose, rhamnose, arabinose, and ribose by TLC comparison with authentic samples. The absolute configuration of the monosaccharides was determined to be D for glucose and ribose and L for rhamnose and arabinose by GC analysis of chiral derivatives of the monosaccharides in the hydrolysate of each compound (see

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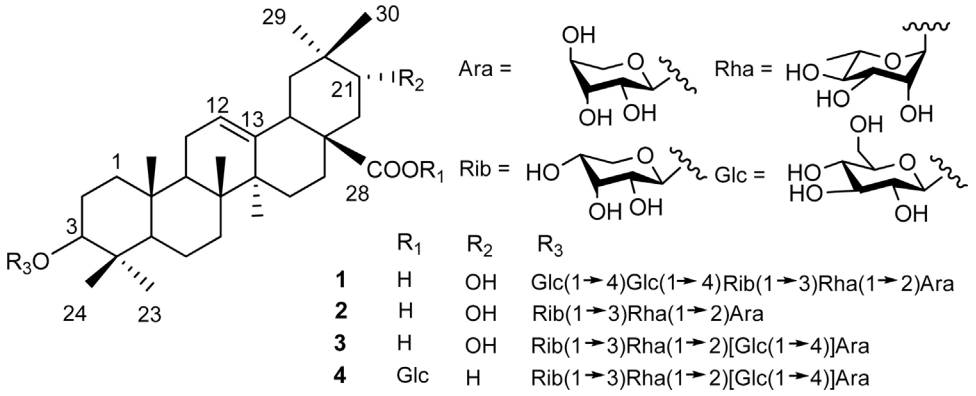

Fig. 1. The structure of compounds **1–4**.

Experimental Section). The relatively large coupling constants (5.0–8.0 Hz) for the anomeric protons in the ^{1}H NMR spectra of these compounds suggested that the arabinopyranosyl moieties have an α-configuration and the glucopyranosyl and ribopyranosyl moieties a β-configuration. The α-configurations of the rhamnopyranosyl moieties were determined from the broad singlets observed for the anomeric protons.

Compound **1** was isolated as white, amorphous powder. The HRESIMS (negative-ion mode) experiment revealed a pseudo-molecular ion peak $[M-H]^-$ at *m/z* 1205.5959, in agreement with the molecular formula $C_{58}H_{94}O_{26}$. The ^{1}H NMR spectrum (Table 1) showed seven tertiary methyl resonances at δ_H 1.42 (s, Me-27, 3H), 1.29 (s, Me-29, 3H), 1.23 (s, Me-23, 3H), 1.15 (s, Me-30, 3H),1.10 (s, Me-34, 3H), 1.06 (s, Me-26, 3H), and 0.87 (s, Me-25, 3H), and an olefinic proton atδ_H 5.51 (t, J = 3.0, H-12, 1H), which were typical signals of the oleanolic acid skeleton. However, the molecular formula of the aglycone moiety was higher by one oxygen atom than that of oleanolic acid, implying the presence of one more hydroxyl group in addition to the C-3 hydroxyl group. The hydroxymethine proton at δ_H 3.82 (t, J = 3.0 Hz) showed spin-couplings with the methylene protons at δ_H 2.36 (dd, J = 3.0, 14.0 Hz, H-22a) and 2.40 (dd, J = 3.0, 14.0 Hz, H-22b) in the ^{1}H-^{1}H COSY spectrum, and HMBC correlations with δ_C 47.4 (C-17), 41.4 (C-19), and 39.9 (C-22), indicating the presence of a hydroxyl group at C-21. This was confirmed by the downfield shift of C-21 at δ_C 73.5. The α-configuration of the C-21 hydroxyl group was evident from the small J value of H-21 (t, J = 3.0 Hz, 1H), characteristic of an equatorial proton, and confirmed by the ROESY correlation between H-21 and H-30. The ^{1}H NMR spectrum of **1** showed five anomeric protons at δ_H 6.26 (br s, 1H), 5.80 (d, J = 5.0 Hz, 1H), 5.14 (d, J = 8.0 Hz, 1H), 4.92 (d, J = 8.0 Hz, 1H), and 4.82 (d, J = 6.0 Hz, 1H) and one methyl group due to rhamnose unit at δ_H 1.51 (d, J = 6.0 Hz, 3H). Assignment for all ^{1}H and ^{13}C NMR signals (Tables 2 and 3) and determination of the structure were achieved by a combination of HMQC, HMBC and ^{1}H-^{1}HCOSY spectra. In the HMBC spectrum, the anomeric proton signals at δ_H 4.82 (Ara-H-1), 6.26 (Rha-H-1), 5.80 (Rib-H-1), 4.92 (Glc-H-1), and 5.14 (Glc′-H-1) showed cross-peaks with the carbon signals at δ_C 88.6 (Aglycone-C-3), 75.3 (Ara-C-2), 82.0 (Rha-C-3), 76.4 (Rib-C-4), and 81.0 (Glc-C-4), respectively. These signals provided ample evidence to determine the linkages between the sugars, and the sugar and the aglycone. These linkages were also confirmed by NOESY correlations between Aglycone-H-3/Ara-H-1, Ara-H-2/Rha-H-1, Rha-H-3/Rib-H-1, Rib-H-4/Glc-H-1, and Glc-H-4/Glc′-H-1. Thus, the structure of **1** was determined as 21α-hydroxyoleanolic acid 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside, which has been

Table 1
^{1}H NMR data for the aglycone moieties of compounds **1** and **2** (500 MHz, in C_5D_5N).

Position	1	2
1	0.92 (m), 1.47 (m)	0.93 (m), 1.48 (m)
2	1.80 (m), 1.98 (m)	1.82 (m), 2.02 (m)
3	3.29 (dd, 12.0, 4.0)	3.30 (dd, 12.0, 4.0)
5	0.74 (dd, 6.5, 3.5)	0.75 (dd, 6.5, 3.5)
6	1.21 (m), 1.38 (m)	1.21 (m), 1.38 (m)
7	1.26 (m), 1.39 (m)	1.24 (m), 1.38 (m)
9	1.62 (t, 9.0)	1.63 (t, 9.0)
11	1.85 (m), 1.98 (m)	1.85 (m), 2.00 (m)
12	5.51 (t, 3.0)	5.52 (t, 3.0)
15	1.20 (m), 2.14 (m)	1.22 (m), 2.14 (m)
16	2.35 (m), 3.14 (m)	2.33 (m), 3.15 (m)
18	3.50 (dd, 3.5, 11.0)	3.51 (dd, 3.5, 11.0)
19	1.26 (m), 2.59 (m)	1.25 (m), 2.59 (m)
21	3.82 (t, 3.0)	3.80 (t, 3.0)
22	2.36 (dd, 3.0, 14.0)	2.37 (dd, 3.0, 14.0)
	2.40 (dd, 3.0, 14.0)	2.40 (dd, 3.0, 14.0)
23	1.23 (s, 3H)	1.24 (s, 3H)
24	1.10 (s, 3H)	1.11 (s, 3H)
25	0.87 (s, 3H)	0.86 (s, 3H)
26	1.06 (s, 3H)	1.05 (s, 3H)
27	1.42 (s, 3H)	1.42 (s, 3H)
29	1.29 (s, 3H)	1.29 (s, 3H)
30	1.15 (s, 3H)	1.14 (s, 3H)

Table 2
^{13}C NMR data for the aglycone moieties of compounds **1–4** (125 MHz, in C_5D_5N).

Position	1	2	3	4
1	38.9	39.0	39.0	38.9
2	26.6	26.6	26.7	26.7
3	88.6	88.7	88.7	88.7
4	39.3	39.4	39.3	39.5
5	55.6	55.6	55.6	55.7
6	18.6	18.6	18.5	18.5
7	33.1	33.1	33.1	33.1
8	39.8	39.9	39.9	39.9
9	48.0	48.0	48.0	48.0
10	36.9	36.9	36.9	37.0
11	23.8	23.7	23.7	23.8
12	122.6	122.6	122.6	122.7
13	144.8	144.8	144.8	144.1
14	42.3	42.3	42.2	42.1
15	28.5	28.4	28.5	28.2
16	27.0	27.0	27.0	23.4
17	47.4	47.5	47.5	47.3
18	41.8	41.8	41.8	41.6
19	41.4	41.4	41.5	46.1
20	35.6	35.6	35.6	30.6
21	73.5	73.5	73.5	33.9
22	39.9	39.8	39.9	32.3
23	28.1	28.0	28.0	28.0
24	16.8	17.2	17.3	17.1
25	15.4	15.4	15.4	15.4
26	17.4	17.4	17.4	17.5
27	25.7	25.6	25.6	26.0
28	180.1	180.0	180.1	176.5
29	28.4	28.3	28.3	33.1
30	24.8	24.7	24.8	23.6