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Novel triterpene saponins isolated from *Clematis mandshurica* and their inhibitory activities on NO production

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[ABSTRACT] Four new triterpene saponins, mandshunosides F–I (**1**–**4**), together with five known compounds (**5**–**9**), were isolated from the roots and rhizomes of *Clematis mandshurica*. Their structures were elucidated on the basis of spectroscopic evidences and hydrolysis products. Bisdesmosidic saponin (**3**–**9**) showed modest suppression of NO production with the inhibition ratios in the range of 51.3%– 64.6% at 50 μmol·L^{-1}, whereas monodesmosidic saponins with a free carboxyl group at C-28 (**1** and **2**) showed potent inhibitory activities with IC_{50} values being 12.7 and 8.3 μmol·L^{-1}, respectively.

[KEY WORDS] *Clematis mandshurica;* Triterpene saponins; Mandshunosides F–I; NO production

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Introduction

The roots and rhizomes of *Clematis mandshurica* Rupr. (Ranunculaceae) are a main source of traditional Chinese medicine "*Weilingxian*", which is widely used as an anti-inflammatory and anti-tumor agent [1]. Chemical investigations on genus *Clemaits* have afforded a series of triterpene saponins. The corresponding aglycones are oleanolic acid (Ole) and hederagenin (Hed). A trisaccharide and a complex long oligosaccharide chains can be linked to C-28 and C-3 in the aglycone, respectively [2-3]. Pharmacological studies have indicated that these saponins possess anti-inflammatory and anti-tumor effects, which are of particular interest as these are highly potent compounds probably responsible for most of activities shown by *Clematis* species [4-5]. However, detailed investigations are required to reveal the structure-activity relationship of these constituents. In our previous study, 27 triterpene saponins have been isolated from the roots and rhizomes of *C. chinensis*, and some of these compounds show inhibitory activities against COX-1 and COX-2 enzymes [2, 6]. As a continuing effort to seek for the anti-inflammatory components from genus *Clematis*, a re-investigation on the roots and rhizomes of *C. mandshurica* led to the discovery of four new and five known triterpene saponins (Fig. 1). Herein, we report the isolation, structure elucidation, and anti-inflammatory and cytotoxic activities of these compounds.

Results and Discussion

In the present study, we accomplished the isolation and structural elucidation of four new triterpene saponins mandshunosides F–I (**1**–**4**), along with five known compounds clematochinenoside E (**5**) [2], clematochinenoside F (**6**) [2], clematomandshurica saponin C (**7**) [7], clematernoside B (**8**) [8], and clematernoside D (**9**) [8], respectively, by comparing their NMR data with those reported in the literature.

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 in 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. The relatively large coupling constants (5.0–8.0 Hz) for the anomeric protons in the ^{1}H NMR spectra of compounds **1**–**4** suggested that the arabinopyranosyl moiety had an α-configuration and the glucopyranosyl and ribopyranosyl moieties has a β-configuration. The α-configuration of the rhamnopyranosyl moiety was determined from the broad singlet observed for the anomeric proton.

Compound **1** was isolated as white, amorphous powder. The HR-ESI/MS (negative-ion mode) experiment reveled a

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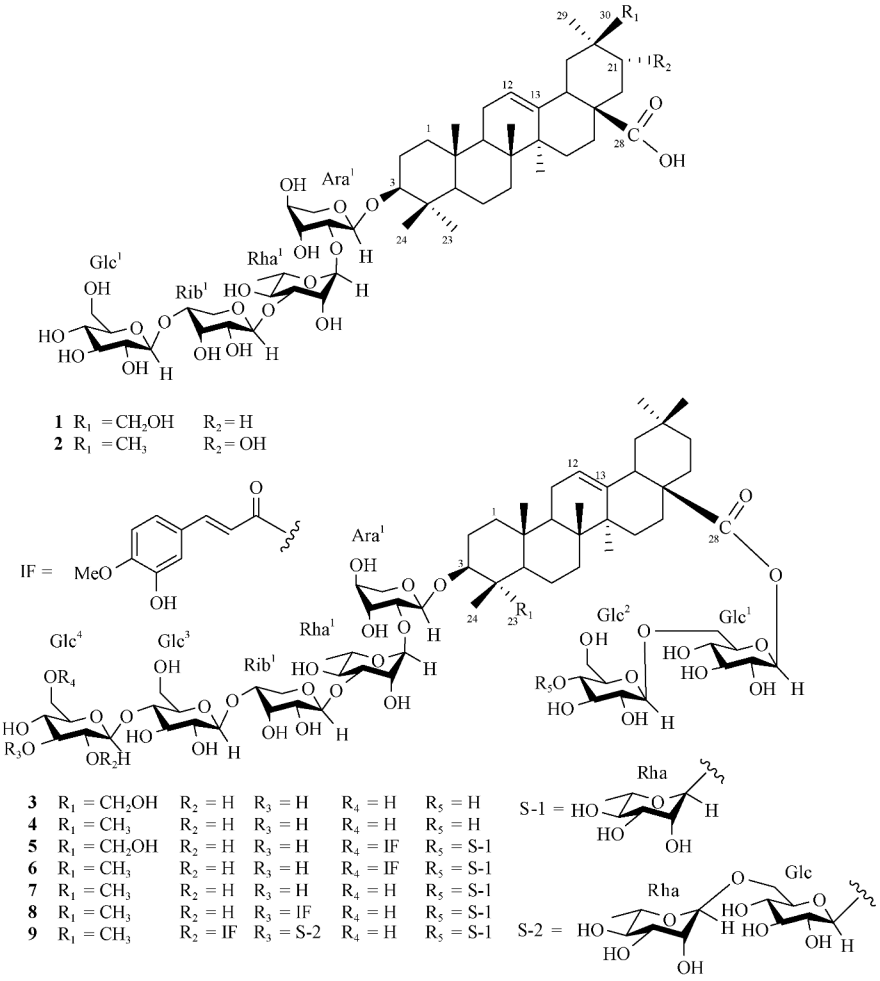

Fig. 1 Structure of compounds 1–9

pseudo-molecular-ion peak $[M-H]^-$ at *m/z* 1 043.542 4 (Calcd. 1 043.542 7), in agreement with the molecular formula $C_{52}H_{84}O_{21}$. The negative-ion mode ESI mass spectrum of **1** exhibited signals at *m/z* 1 043 $[M-H]^-$, 881 $[(M-H)-162]^-$, 749 $[(M-H)-162-132]^-$, 603 $[(M-H)-162-132-146]^-$, and 471 $[(M-H)-162-132\times2-146]^-$, which showed the presence of a linear sugar chain, and the sugar sequence appeared to be that of Ara-Rha-Rib-Glc [9]. The peak at *m/z* 471 was assigned to the aglycone moiety. The ^{1}H NMR spectrum showed six tertiary methyl resonances at δ_H 1.35 (3H, s, Me-27), 1.24 (3H, s, Me-23), 1.23 (3H, s, Me-29), 1.10 (3H, s, Me-24), 1.06 (3H, s, Me-26), and 0.84 (3H, s, Me-25), and an olefinic proton at δ_H 5.47 (1H, t, J = 3.0 Hz, H-12), 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 NMR signals of an additional hydroxymethyl group at δ_H 3.92 (d, J = 10.5 Hz) and 4.02 (d, J = 10.5 Hz) appeared in **1**. In the HMBC spectrum, the oxygenated methylene protons showed correlations with C-19 (δ_C 42.2), C-20 (δ_C 35.8), and C-21 (δ_C 29.6), indicating the presence of a hydroxymethyl group at C-20 (Fig. 2). These results indicated that either the C-29 or the C-30 methyl group was hydroxylated. C-29 hydroxylation gave mesembryanthemoidigenic acid [10], while C-30 hydroxylation gave queretaroic acid [11]. Further comparison of the carbon chemical shift for the hydroxymethyl signal (δ_C 65.5) of **1** with those for the known compound queretaroic acid revealed that the hydroxyl group was located at C-30 [11]. Thus, the aglycone of **1** was identified as queretaroic acid. The downfield chemical shift at δ_C 88.7 (Aglycone-3) in the ^{13}C NMR spectrum of **1** (Table 1) indicated that a sugar chain was linked to C-3 of aglycone.

The ^{1}H NMR spectrum of **1** showed four anomeric protons at δ_H 6.26 (br s, 1H), 5.80 (d, 1H, J = 5.0 Hz), 5.02 (d, 1H, J = 8.0 Hz), and 4.82 (d, 1H, J = 6.0 Hz) and one methyl group due to rhamnose unit at δ_H 1.51 (d, 3H, J = 6.0 Hz). Assignment for all ^{1}H and ^{13}C NMR signals 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^1-H-1), 6.26 (Rha^1-H-1), 5.80 (Rib^1-H-1), and 4.91 (Glc^1-H-1) showed