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Triterpenoid saponins from the roots of *Cyathula officinalis* and their inhibitory effects on nitric oxide production

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[ABSTRACT] The present study was designed to investigate the chemical constituents of the roots of *Cyathula officinalis.* Compounds were isolated by silica gel, Sephadex LH-20, ODS column chromatography, and preparative HPLC. Their structures were determined on the basis of 1D and 2D NMR techniques, mass spectrometry, and chemical methods. One new oleanane-type triterpenoid saponin, 28-*O*-[*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-(1→3)-*β*-D-glucopyranosyl] hederagenin (**1**), was isolated from the roots of *Cyathula officinalis*. The anti-inflammatory activities of the isolates were evaluated for their inhibitory effects against LPS-induced nitric oxide (NO) production in RAW 264.7 macrophages cells. Compounds **2**, **4**, and **6** exhibited moderate anti-inflammatory activities.

[KEY WORDS] *Cyathula officinalis*; Amaranthaceae; Triterpenoid saponins; Nitric oxide inhibition

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Introduction

Cyathula officinalis Kuan belongs to Amaranthaceae family and grows in the southwest of China. The roots of *C. officinalis* as traditional Chinese medicine are widely used for removing blood stasis, restoring menstrual flow, easing joint movement, and inducing diuresis for treating stranguria, according to the theory of traditional Chinese medicine [1]. Nowadays, the aqueous extract of *C. officinalis* has been reported to possess anti-inflammation, antihypertensive and antifertility activities, and its clinical use is significant in China [2-4]. Previous studies on this species have resulted in the isolation of glycosides [5], phytoecdysteroids [6-7], and polysaccharide [8]. In our continued investigation on anti-inflammatory constituents from the roots of *C. officinalis*, a new triterpenoid saponin (**1**) and six known compounds (**2**–**7**) were isolated. All isolates were evaluated for their *in vitro* inhibitory effects on NO production in LPS-stimulated RAW 264.7 macrophages cells.

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Results and Discussion

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS of **1** showed a quasimolecular ion $[M + Na]^+$ at *m/z* 979.487 1 (Calcd. for $[C_{48}H_{76}O_{19} + Na]^+$ 979.487 1), consistent with a molecular formula $C_{48}H_{76}O_{19}$. The IR spectrum of **1** displayed absorption bands of hydroxyl group at 3 420 cm^{-1}, carbonyl group at 1 735 cm^{-1}, and double bond at 1 637 cm^{-1}. The ^1H NMR spectra showed six angular methyl proton signals at δ_H 0.87, 0.88, 0.91, 0.92, 1.05, and 1.21 (each 3H, s), and an olefinic proton at δ_H 5.35 (1H, br s, H-12). The ^{13}C NMR spectrum of **1** displayed 48 signals, including six tertiary methyl signals at δ_C14.1 (C-24), 16.6 (C-25), 18.0 (C-26), 24.1 (C-27), 33.6 (C-29), and 24.1 (C-30), two olefinic carbons at δ_C 122.4 (C-12) and 144.6 (C-13), one oxygenated methylene at δ_C 64.6 (C-23), one oxygenated methine at δ_C 73.2 (C-3), and one carbonyl group at δ_C 176.9 (C-28) (Table 1). The ^1H NMR data of **1** showed the presence of three anomeric protons at δ_H 6.30 (d, J = 8.2 Hz, H-1′of Glc), 5.14 (d, J = 7.2 Hz, H-1″of GluA), and 6.27 (br s, H-1‴of Rha), which were correlated with the carbon signals at δ_C 96.2 (C-1′), 106.5 (C-1″), as well as 103.2 (C-1‴) in the HSQC spectrum, respectively. The oleanane-type aglycone of **1** was revealed by comparison of the ^1H and ^{13}C NMR data with those of hederagenin-28-*O*-

Table 1 ^{1}H NMR and ^{13}C NMR data of compound 1 (500 MHz and 125 MHz, C_5D_5N)[a]

No.	δ_C	δ_H (*J*, in Hz)	No.	δ_C	δ_H (*J*, in Hz)
1	37.3	0.90 (d, 1.5),1.40 (d, 12.8)	28-Glc		
			1′	96.2	6.30 (d, 8.2)
2	26.6	1.24 (o), 2.28 (d, 12.8)	2′	74.7	4.36 (m)
3	73.2	4.60 (dd, 9.4,2.3)	3′	82.4	4.27 (m)
4	43.9	-	4′	76.3	4.10 (m)
5	47.5	1.65 (d, 12.0)	5′	79.8	4.03 (m)
6	19.1	1.72 (d, 6.1), 1.84 (d, 6.1)	6′	62.7	4.40 (m), 4.46 (m)
7	33.0	1.62 (m), 1.72 (d, 6.1)	GluA		
8	40.4	-	1″	106.5	5.14 (d, 7.2)
9	47.9	1.65 (d, 12.0)	2″	74.6	4.35 (m)
10	34.5	-	3″	82.6	4.36 (m)
11	24.3	1.89-1.90 (o)	4″	79.4	4.29 (m)
12	122.4	5.35 (br s)	5″	78.1	4.30 (m)
13	144.6	-	6″	173.7	
14	42.6	-	Rha		
15	28.8	1.13 (d, 12.0), 2.29 (m)	1‴	103.2	6.27 (br s)
16	23.9	1.89(m), 2.26 (d, 11.5)	2‴	73.0	4.76 (m)
17	48.6	-	3‴	71.6	4.34 (m)
18	42.2	3.17 (dd, 13.3,3.4)	4‴	72.2	4.43 (m)
19	46.6	1.22 (m), 1.71 (d, 6.1)	5‴	70.2	5.07 (m)
20	31.2	-	6‴	18.7	1.67 (d, 6.0)
21	33.0	1.62 (m), 1.72(d, 6.1)			
22	33.3	0.89(d, 6.9),1.33 (d, 7.0)			
23	64.6	3.64(d, 2.4), 4.13 (m)			
24	14.1	0.91 (s)			
25	16.6	0.88 (s)			
26	18.0	1.05 (s)			
27	24.1	1.21 (s)			
28	176.9	-			
29	33.6	0.92 (s)			
30	24.1	0.87 (s)			

[a] The assignments are based upon ^{1}H–^{1}H COSY, HSQC, HMBC and ROESY experiments. Coupling constants (*J*) in Hz are given in parentheses. o: the abbreviation foroverlapped

β-D-glucopyranosyl ester [9]. The upfield shift of δ_C 176.9 (C-28), compared with that of hederagenin [10], indicated that the sugar moieties were attached to the aglycone at C-28 position. Compound **1** had three hexoses according to the positive-ion ESI-MS data at *m/z* 828.43 $[M + NH_4 - 146]^+$, 652.30 $[M + NH_4 - 146 - 176]^+$, 471.43 $[M - H - 146 - 176 - 162]^+$. Acid hydrolysis of **1** gave L-rhamnose, D-glucose, and D-glucuronic acid, confirmed by HPLC-UV analysis of the respective isothiocyanate derivatives with authentic references. The linkages and sequence of sugar chain of **1** were corroborated by the following HMBC correlations: δ_H 6.30 (H-1′of Glc) / δ_C176.9 (C-28), δ_H 5.14 (H-1″of GluA) / δ_C 82.4 (C-3′of Glc), and δ_H 6.27 (H-1‴of Rha) /δ_C 82.6 (C-3″of GluA) (Fig. 2). The ^{1}H and ^{13}C NMR data were fully assigned by the ^{1}H–^{1}H COSY, HSQC, HMBC and ROESY spectra. Based on these evidences, compound **1** was identified as 28-*O*-[*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-(1→3)-*β*-D-glucopyranosyl]-hederagenin, as shown in Fig. 1.

The structures of known compounds were characterized as 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[*α*-L-rhamnopyanosyl-(1→3)]-(*β*-D-glucuronopyranosyl)}-28-*O*-*β*-D-glucopyranosy l oleanolic acid (**2**) [11], 3-*O*-[*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranosyl oeanolic acid (**3**) [12], 3-*O*-*β*-D-glucuronopy-ranosyl-28-*O*-*β*-D-glucopyranosyl oleanolic acid (**4**) [13], 3-[*α*-L-rhamnopyranosyl-(1→3)-*β*-D-glucuronopyranosyl]-28-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl] oeanolic acid (**5**) [13], cyasterone (**6**) [14-15], and 7- *O*-Methyl-loganin (**7**) [16], by comparisons of their ^{1}H and ^{13}C NMR data with those reported values. Compounds **2**,