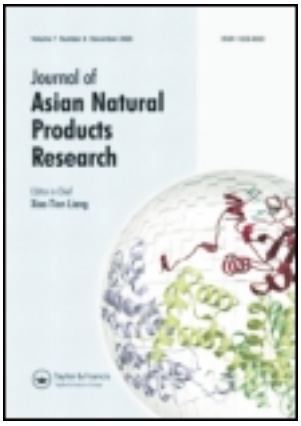

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[a] Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plants Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100193, China

[b] Musculoskeletal Research Laboratory, Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China

[c] Beijing Institute of Microchemistry, Beijing, 100091, China

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Triterpenoid saponins from *Dipsacus asper* and their activities *in vitro*

Jing-Jing Liu[a], Xin-Luan Wang[b], Bao-Lin Guo[a]*, Wen-Hua Huang[a], Pei-Gen Xiao[a], Chao-Qing Huang[a], Li-Zhen Zheng[b], Ge Zhang[b], Ling Qin[b] and Guang-Zhong Tu[c]

[a]*Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plants Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China;* [b]*Musculoskeletal Research Laboratory, Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China;* [c]*Beijing Institute of Microchemistry, Beijing 100091, China*

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Two new triterpenoid saponins **1** and **2**, along with six known saponins **3**–**8**, were isolated from the roots of *Dipsacus asper*. The structures of new compounds were established as 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranoside (dipsacus saponin J, **1**) and 3-*O*-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (dipsacus saponin K, **2**). The structures were determined by extensive analysis of their spectroscopic data. Compounds **6** and **7** could significantly stimulate UMR106 cell proliferation and increase alkaline phosphatase activities in UMR106 cell at the concentration of 4 μM.

Keywords: *Dipsacus asper*; triterpenoid saponin; anti-osteoporosis; UMR106 cell; cytotoxicity

1. Introduction

Dipsacus asper Wall. ex Henry[1] (Dipsacaceae) is widely distributed in southwest of China. The roots of *D. asper* have been used as a tonic and analgesic agent in traditional Chinese medicine for the treatment of low back pain, knee pain, and bone fractures [1]. Previous studies have been performed on the constituents of Radix Dipsaci (Xuduan), including those on triterpene glycosides, iridoids, caffeoylquinic acid, and so on. Radix Dipsaci extract (RDE) could prevent osteoporosis through increasing bone mass and changing bone histomorphology [2,3]. But few studies on osteoprotective effect have been carried out on monomeric compounds from RDE. Furthermore, some of the dipsacus saponins have been shown to have potent cytotoxicity [4]. In this paper, we describe the isolation and structural elucidation of eight triterpenoid saponins, including two new saponins, dipsacus saponins J (**1**) and K (**2**), as well as six known compounds (**3**–**8**) (Figure 1). The *in vitro* activities of the isolated compounds were screened using UMR106 cells for osteoprotective effect and A375, BGC823, MCF7, SW1990, and HEPG2 cells for cytotoxicity.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS exhibited an ion at *m/z* 1405.6615 $[M + Na]^+$, which was in accordance with the molecular formula of $C_{65}H_{106}$

*Corresponding author. Email: guobaolin010@163.com

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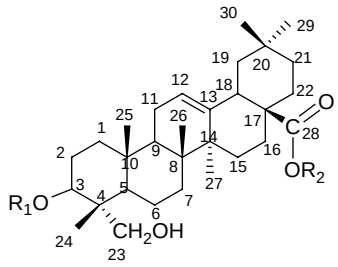

	R_1	R_2
1	glc$^{1\text{-}4}$(rha$^{1\text{-}3}$)glc$^{1\text{-}3}$rha$^{1\text{-}2}$ara-	glc-
2	ara-	glc$^{1\text{-}6}$glc$^{1\text{-}6}$glc-
3	ara-	glc-
4	ara-	glc$^{1\text{-}6}$glc-
5	glc$^{1\text{-}3}$rha$^{1\text{-}2}$ara-	glc$^{1\text{-}6}$glc-
6	glc$^{1\text{-}4}$(rha$^{1\text{-}3}$)glc$^{1\text{-}3}$rha$^{1\text{-}2}$ara-	glc$^{1\text{-}6}$glc-
7	xyl$^{1\text{-}4}$glc$^{1\text{-}4}$(rha$^{1\text{-}3}$)glc$^{1\text{-}3}$rha$^{1\text{-}2}$ara-	glc$^{1\text{-}6}$glc-
8	xyl$^{1\text{-}4}$glc$^{1\text{-}4}$(rha$^{1\text{-}3}$)glc$^{1\text{-}3}$rha$^{1\text{-}2}$ara-	glc-

Figure 1. Structures of compounds **1**–**8**.

O_{31}. The IR spectrum revealed absorption bands at 3412 (OH), 2939 (C—H), 1732 (C═O), 1637 (C═C), and 1074 (C—O—C) cm^{-1}. The resonances in the ^1H and ^{13}C NMR spectra for six methyls [δ_H/δ_C 0.86 (s)/23.7 (Me-30), δ_H/δ_C 0.87 (s)/33.1 (Me-29), δ_H/δ_C 0.96 (s)/16.2 (Me-25), δ_H/δ_C 1.08 (s)/17.5 (Me-26), δ_H/δ_C 1.09 (s)/14.1 (Me-24), and δ_H/δ_C 1.15 (s)/26.1 (Me-27)], an olefinic bond [δ_H/δ_C 5.40 (br s)/122.9 (C-12), 144.1 (C-13)], and an ester carbonyl [δ_C 176.4 (C-28)] suggested that **1** was an olean-12-en-28-oic acid derivative. By comparing the ^{13}C NMR spectral data with those reported [5], hederagenin was identified as the aglycone (Table 1). Six anomeric resonances at δ_H/δ_C 5.01 (d, $J = 6.5$ Hz)/104.7, δ_H/δ_C 5.35 (d, $J = 8.0$ Hz)/106.4, δ_H/δ_C 5.45 (d, $J = 7.5$ Hz)/104.4, δ_H/δ_C 6.22 (s)/101.4, δ_H/δ_C 6.31 (d, $J = 7.5$ Hz)/95.8, and δ_H/δ_C 6.36 (s)/101.9 indicated the presence of sugar moieties. The components of the above sugar moieties were three hexoses, two deoxyhexoses, and one pentose, which were inferred from the ESI-MS^n data at *m/z* 1405 $[M + Na]^+$, 1243 $[M + Na - 162]^+$, 1097 $[M + Na - 162 - 146]^+$, 773 $[M + Na - 162 - 146 - 2 \times 162]^+$, 495 $[M + Na - 162 - 146 - 2 \times 162 - 146 - 132]^+$, and confirmed by the calculation of relative molecular mass ($1382 = 472$ aglycone $+ 3 \times 162 + 2 \times 146 + 132$). Acid hydrolysis and GC-MS examination of **1** suggested the existence of D-glucose, L-rhamnose, and L-arabinose. The coupling constants of the anomeric protons from 6.5 to 8.0 Hz were deduced to be β-D-glucose or α-L-arabinose, and single signals to be α-L-rhamnose [6]. Furthermore, the α-anomeric configuration of L-rhamnose was confirmed by the chemical shift of C-5 (δ_C 68.8 or 69.6) [7]. By comparing the ^{13}C NMR spectral data of **1** with those of hederagenin [5], C-3 (δ_C 81.0) was observed to have a distinct downfield shift and C-28 (δ_C 176.4) an upfield shift, respectively, indicating that sugar moieties were attached to those positions [8]. The ^1H and ^{13}C NMR spectral data (Table 2) of the bisdesmosidic residues were assigned starting from the anomeric protons by means of the TOCSY, ^1H–^1H COSY, HSQC, HMBC, and NOESY spectra obtained for this compound. Linkage of the sugar units at C-3 of the aglycone was established from the following HMBC correlations: H-1 (δ_H 5.01) of arabinose with C-3 (δ_C 81.0) of the aglycone, H-1 (δ_H 6.22) of rhamnose with C-2 (δ_C 75.3) of arabinose, H-1 (δ_H 5.35) of glucose with C-3 (δ_C 82.8) of rhamnose, H-1 (δ_H 6.36) of rhamnose' with C-3 (δ_C 79.3) of glucose, H-1 (δ_H 5.45) of glucose' with C-4 (δ_C 75.3) of glucose (Figure 2). The sugar chain at C-28 was established from the HMBC correlations of H-1 (δ_H 6.31) of glucose'' with C-28 (δ_C 176.4) of the aglycone. Careful investigation of the ^1H and ^{13}C NMR spectral data of sugar chain at C-3 established that it was quite similar to those reported for dipsacus saponins XI and XII [9]. Evidence in ESI-MS^n data also

Table 1. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectral data for aglycone moieties of compounds **1** and **2** (C_5D_5N, δ in ppm and J in Hz).

	1		**2**	
	δ_C[a]	δ_H	δ_C	δ_H
1	39.1 (t)	1.05 (m), 1.54 (m)	38.8 (t)	1.03 (m), 1.56 (m)
2	26.4 (t)	1.99 (m), 2.19 (m)	26.1 (t)	2.20 (m), 2.26 (m)
3	81.0 (d)	4.26 (m)	81.9 (d)	4.24 (m)
4	43.6 (s)	–	43.5 (s)	–
5	47.6 (d)	1.70 (m)	47.6 (d)	1.65 (m)
6	18.1 (t)	1.26 (m), 1.64 (m)	18.3 (t)	1.33 (m), 1.65 (m)
7	32.7 (t)	1.30 (m), 1.51 (m)	32.8 (t)	1.58 (m), 1.74 (m)
8	39.9 (s)	–	40.0 (s)	–
9	48.2 (d)	1.72 (m)	48.2 (d)	1.74 (m)
10	36.9 (s)	–	37.0 (s)	–
11	23.9 (t)	1.91 (m), 2.01 (m)	23.7 (t)	1.92 (m), 2.01 (m)
12	122.9 (d)	5.40 (br s)	122.9 (d)	5.39 (br s)
13	144.1 (s)	–	144.2 (s)	–
14	42.1 (s)	–	42.1 (s)	–
15	28.3 (t)	1.05 (m), 2.27 (m)	28.3 (t)	1.07 (m), 2.27 (m)
16	23.4 (t)	1.91 (m), 2.01 (m)	23.4 (t)	1.87 (m), 1.91 (m)
17	47.0 (s)	–	47.0 (s)	–
18	41.7 (d)	3.16 (dd, $J = 14.0, 3.5$)	41.7 (d)	3.15 (dd, $J = 13.8, 3.0$)
19	46.2 (t)	1.21 (m), 1.70 (m)	46.2 (t)	1.19 (m), 1.69 (m)
20	30.8 (s)	–	30.7 (s)	–
21	34.0 (t)	1.06 (m), 1.31 (m)	34.0 (t)	1.09 (m), 1.29 (m)
22	32.6 (t)	1.73 (m), 1.80 (m)	32.6 (t)	1.72 (m), 1.87 (m)
23	64.0 (t)	3.84 (d, $J = 7.5$), 4.22 (m)	64.5 (t)	3.67 (d, $J = 10.2$), 4.26 (m)
24	14.1 (q)	1.09 (3H, s)	13.6 (q)	0.91 (3H, s)
25	16.2 (q)	0.96 (3H, s)	16.2 (q)	0.96 (3H, s)
26	17.5 (q)	1.08 (3H, s)	17.6 (q)	1.10 (3H, s)
27	26.1 (q)	1.15 (3H, s)	26.1 (q)	1.16 (3H, s)
28	176.4 (s)	–	176.5 (s)	–
29	33.1 (q)	0.87 (3H, s)	33.1 (q)	0.81 (3H, s)
30	23.7 (q)	0.86 (3H, s)	23.7 (q)	0.83 (3H, s)

Note: [a] Multiplicity determined by DEPT (135°) and HSQC.

supported this deduction. Finally, the structure of compound **1** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranoside, named dipsacus saponin J (Figure 1).

Compound **2** was obtained as a white amorphous powder. The HR-ESI-MS exhibited an ion at *m/z* 1113.5419 $[M + Na]^+$, which was in accordance with the molecular formula of $C_{53}H_{86}O_{23}$. The IR spectrum revealed absorption bands at 3399 (OH), 2927 (C—H), 1739 (C═O), 1653 (C═C), and 1076 (C—O—C) cm^{-1}. By comparing the ^{1}H and ^{13}C NMR spectral data of compound **1** with those reported in the literature [5], hederagenin was identified as the aglycone (Table 1). The sugar moieties were composed of three hexoses and one pentose, which was inferred from the calculation of relative molecular mass (1090 = 472 aglycone + 3 × 162 + 132). Acid hydrolysis and GC-MS examination of **2** suggested the presence of D-glucose and L-arabinose. The β-anomeric configuration for the glucopyranose and α-configuration for the arabinose were determined from their

Table 2. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectral data for sugar moieties of compounds **1** and **2** (C_5D_5N, δ in ppm and J in Hz).

	1			**2**	
	δ_C [a]	δ_H		δ_C [a,b]	δ_H
$^{3\text{-O-}}$ara-1	104.7 (d)	5.01 (d, $J = 6.5$)	$^{3\text{-O-}}$ara-1	106.7 (d)	4.96 (d, $J = 7.2$)
2	75.3 (d)	4.50 (m)	2	73.1 (d)	4.41 (t)
3	75.1 (d)	3.96 (br s)	3	74.7 (d)	4.05 (m)
4	69.8 (d)	4.08 (m)	4	69.6 (d)	4.27 (m)
5a, 5b	66.4 (t)	4.21 (m), 3.64 (d, $J = 11.5$)	5a, 5b	67.0 (t)	3.71 (d), 4.25 (m)
$^{\text{ara-2-}}$rha-1	101.4 (d)	6.22 (s)	$^{28\text{-O-}}$glc-1	95.6 (d)	6.23 (d, $J = 8.1$)
2	71.7 (d)	4.84 (m)	2	73.9 (d)	4.10 (t)
3	82.8 (d)	4.72 (m)	3	78.0 (d)	3.87 (m)
4	73.1 (d)	4.38 (m)	4	71.3 (d)	4.20 (m)
5	69.6 (d)	4.64 (m)	5	78.3 (d)[x]	4.20 (m)
6	18.6 (q)	1.49 (3H, d, $J = 5.0$)	6a, 6b	69.4 (t)	4.31 (m), 4.74 (m)
$^{\text{rha-3-}}$glc-1	106.4 (d)	5.35 (d, $J = 8.0$)	$^{\text{glc-6-}}$glc′-1	105.3 (d)	4.93 (d, $J = 7.8$)
2	76.8 (d)	4.09 (m)	2	75.0 (d)[y]	3.91 (m)
3	79.3 (d)	4.48 (m)	3	78.3 (d)[x]	4.20 (m)
4	75.3 (d)	4.65 (m)	4	70.9 (d)	4.31 (m)
5	78.0 (d)	3.78 (br s)	5	77.1 (d)	3.94 (m)
6a, 6b	60.6 (t)	4.81 (m), 4.41 (m)	6a, 6b	70.0 (t)	4.31 (m), 4.72 (m)
$^{\text{glc-3-}}$rha′-1	101.9 (d)	6.36 (s)	$^{\text{glc′-6-}}$glc″-1	105.7 (d)	5.01 (d, $J = 7.8$)
2	72.7 (d)	4.75 (m)	2	75.2 (d)[y]	4.02 (m)
3	72.4 (d)	4.75 (m)	3	78.7 (d)[x]	4.20 (m)
4	74.6 (d)	4.21 (m)	4	71.6 (d)	4.21 (m)
5	68.8 (d)	5.33 (m)	5	78.4 (d)[x]	4.20 (m)
6	18.4 (q)	1.50 (3H, d, $J = 5.0$)	6a, 6b	62.7 (t)	4.34 (d), 4.47 (dd)
$^{\text{glc-4-}}$glc′-1	104.4 (d)	5.45 (d, $J = 7.5$)			
2	75.4 (d)	4.07 (m)			
3	78.5 (d)	4.24 (m)			
4	70.4 (d)	4.54 (m)			
5	78.2 (d)	3.78 (m)			
6a, 6b	61.7 (t)	4.41 (m), 4.30 (m)			
$^{28\text{-O-}}$glc″-1	95.8 (d)	6.31 (d, $J = 7.5$)			

$^{3\text{-O}}$ara-1	104.7 (d)	5.01 (d, $J = 6.5$)	$^{3\text{-O}}$ara-1	106.7 (d)	4.96 (d, $J = 7.2$)
2	74.2 (d)	4.17 (m)			
3	78.9 (d)	4.26 (m)			
4	71.2 (d)	4.34 (m)			
5	79.3 (d)	4.03 (m)			
6a, 6b	62.3 (t)	4.44 (m), 4.39 (m)			

Notes: [a] ^{13}C chemical shifts of substituted residues are underlined.
[b] ^{13}C NMR spectral data with the same marker (x or y) could be exchanged.

coupling constants ($^3J_{H1,H2}$: 7.2–8.1 Hz). The ^{13}C NMR chemical shifts of C-3 (δ_C 81.9) and C-28 (δ_C 176.5) indicated that **2** was a bidesmosidic saponin with glycosidic linkages at C-3 and C-28. The ^{1}H and ^{13}C NMR spectral data (Table 2) of the bisdesmosidic residues were assigned starting from the anomeric protons by means of the HSQC and HMBC spectra obtained for this compound. The anomeric proton signals were δ_H/δ_C 6.23 (d, $J =$ 8.1 Hz)/95.6, δ_H/δ_C 5.01 (d, $J = 7.8$ Hz)/105.7, δ_H/δ_C 4.96 (d, $J = 7.2$ Hz)/106.7, and δ_H/δ_C 4.93 (d, $J = 7.8$ Hz)/105.3, according to the HSQC spectrum. The long-range correlations between H-1 (δ_H 4.96) of arabinose and C-3 (δ_C 81.9) of the aglycone indicated that Ara moiety linked to the C-3 of hederagenin, and correlations between H-1 (δ_H 6.23) of glucose and C-28 (δ_C 176.5) of the aglycone, between H-1 (δ_H 4.93) of glc′ and C-6 (δ_C 69.4) of glc, and between H-1 (δ_H 5.01) of glc″ and C-6 (δ_C 70.0) of glc′ indicated that the trisaccharide moiety linked to C-28 of hederagenin was to be glc(1 → 6)glc(1 → 6)glc. By comparing the ^{13}C NMR spectral data of compound **2** with those of dipsacus saponin VI [5,10], the glycosylation shift of glc′-C-6 (δ_C 70.0) and one more group of glucopyranose signals (glc″) conformed this deduction (Figure 2). Then, the structure of **2** can be elucidated as 3-*O*-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside, named as dipsacus saponin K (Figure 1).

Known compounds were identified as HN saponin F (**3**) [10], dipsacus saponin VI (**4**) [5,10], macranthoidin A (**5**) [10,11], dipsacus saponin XII (**6**) [9], dipsacus saponin X (**7**) [12], and 3-*O*-β-D-xylopyranosyl(1 → 4)-β-D-glucopyranosyl-(1 → 4)]-[α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranoside (**8**) [13].

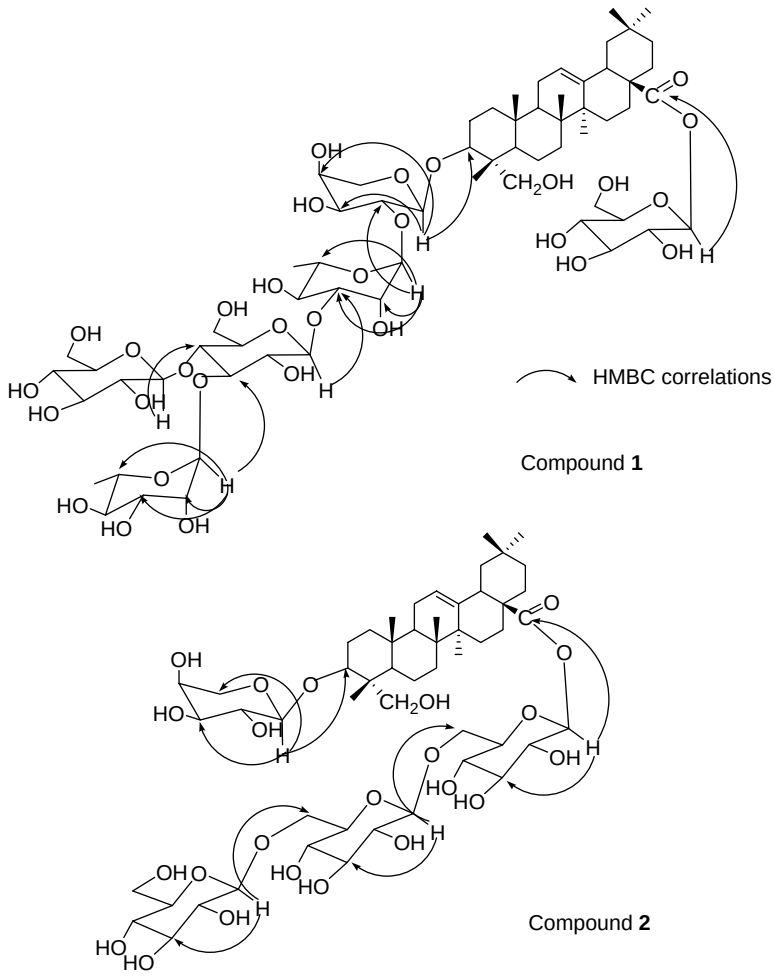

Figure 2. Selected HMBC correlations for compounds **1** and **2**.

As one of the best characterized clonal osteoblastic cell lines, UMR106 was used to study the mechanisms of drugs on osteoporosis [14,15]. All isolated compounds were tested on UMR106 cell lines for their anti-osteoporosis activities, except **1** and **3** due to their poor dissolvability. The results given in Table 3 are expressed as relative percent proliferation of UMR106 cells at concentrations of 0.4, 4, and 40 μM. The six compounds exerted a biphasic effect on cell proliferation, being slightly inhibitory at low concentration (0.4 μM, except compound **7**) and high concentration (40 μM, except compound **2**), and stimulation at middle concentration (4 μM, except compound **4**). Compounds **6** and **7** displayed significant promotion on UMR106 cells and revealed anti-osteoporotic potential. Furthermore, as one of the markers of osteoblastic differentiation, alkaline phosphatase (ALP) activity was measured in the UMR106 cells. It can be seen from Table 3 that all tested compounds showed significant increasing ALP activity at the concentration of 4 μM.

The cytotoxic activities of the isolated compounds were evaluated against A375, BGC823, MCF7, SW1990, and HEPG2

Table 3. Effect of isolates **2** and **4**–**8** on UMR106 cell proliferation and ALP activity.

	Proliferation rate (%)[ab]			
Sample	0.4 μM	4 μM	40 μM	Relative increased percentage of ALP activity (%)[c]
2	−0.6 ± 0.11	13.2 ± 0.04	8.9 ± 0.03	27.5*
4	−19.0 ± 0.01**	−5.4 ± 0.02	−26.0 ± 0.06**	90.8*
5	−5.8 ± 0.05	4.0 ± 0.07	−11.8 ± 0.05	69.5 (0.056)
6	−3.3 ± 0.06	24.8 ± 0.09*	−11.7 ± 0.06	29.6*
7	10.0 ± 0.04	29.7 ± 0.12**	−16.3 ± 0.02	39.0 (0.093)
8	−2.3 ± 0.04	12.6 ± 0.06	−26.2 ± 0.04**	32.2 (0.077)

Notes: [a]The proliferation rate (%) is calculated as 100 × (value of sample/value of control−1). Each value represents the mean ± SD of two individual experiments ($n = 3$/experiments).
[b]Significantly different from the control group by *t*-test (*$p < 0.05$, **$p < 0.01$).
[c]Tests were operated under the middle concentration of 4 μM. Relative increased percentage is calculated as 100 × (value of sample/value of control−1) and exact values of p between 0.05 and 0.1 are shown in parentheses.

cell lines according to the MTT assay. The result was that none of those compounds expressed inhibition more than 50% at the concentration from 0.1 to 80 μM. A study by Tran Manh Hung showed that cytotoxic saponins from *D. asper* had similar free carboxyl at C-28, but the saponins with sugar moiety linked at C-28 through an ester bond exhibited IC_{50} values more than 30 μg/ml [4]. It suggested that bidesmosidic triterpenoid saponins isolated from *D. asper* had non-cytotoxic activities.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer polarimeter. IR (KBr-disks) spectra were recorded by a Shimadzu FTIR-8400S spectrometer. Mass spectra were obtained on a JEOL JMS-T100 CS (HR-ESI-MS) and a Thermo Scientific LTQ Orbitrap XL (ESI-MS). NMR experiments were recorded in C_5D_5N on Bruker AV-600 spectrometers. Solvents used were of analytical grade and obtained from Beijing Chemical Works (Beijing, China) or chromatographic grade obtained from Burdick & Jackson Company (Swedesboro, NJ, USA). Silica gel for column chromatography and silica gel GF_{254} for TLC were obtained from Qingdao Marine Chemical Company (Qingdao, China). Sephadex LH-20 from Mitsubishi Chemical Corporation (Tokyo, Japan) and macroporous resin D101 (20–40 mesh) from Tianjin Nankai Hecheng S&T Co. Ltd (Tianjin, China) were also used for column chromatography. Preparative HPLC was carried out using Waters 600 pump with YMC-C_{18} column (250 × 10 mm i.d.) and 2487 detector. Gas chromatographic analysis was carried out with an Agilent 6890 system equipped with a Mass spectrograph 5973 detector (Agilent Technologies, Santa Clara, CA, USA). The column was a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies).

3.2 Plant material

The roots of *D. asper* were collected from Chengdu, Sichuan Province of China, in November 2007. It was identified by Prof. Bao-Lin Guo, Insititute of Medicinal Plant Development (IMPLAD), China Academy of Medical Sciences, Beijing, China. The voucher specimens had been deposited in the Herbarium of IMPLAD (No. 2007101).

3.3 Extraction and isolation

Dried roots (4.2 kg) of *D. asper* were powdered and extracted with 70% aqueous ethanol (33 liters × 3) under reflux. After evaporation, the residue was separated by column chromatography on macroporous resin D101 with an EtOH/H_2O (0:100, 30:70, 50:50, 95:5) gradient system to give four fractions (XUD-1 to -4). Fraction XUD-3 (453 g) was applied to a silica gel column using a $CHCl_3$/MeOH (v:v = 9:1, 8:2, 7.5:2.5, 7:3, 5:5, 0:10) gradient system to give six fractions (XUD-3-1 to -6). After recrystallization from MeOH, 2 g of compound **3** was obtained from fraction XUD-3-2 (16.2 g). Fraction XUD-3-3 (10.1 g) was subjected to Sephadex LH-20 column with MeOH as eluent and compound **4** (100 mg) was obtained. Fraction XUD-3-4 (18.7 g) was separated by silica gel column (100 mesh) with $CHCl_3$/MeOH/H_2O (7:3:0.2, 6.5:3.5:0.2, 6:4:0.2, 5.5:4.5:0.2) gradient system to give four fractions (XUD-3-4-1 to -4). After fraction XUD-3-4-2 was further fractionated by silica gel (200–300 mesh) column with *n*-BuOH/EtOAc/H_2O (4:1:2, underlayer) as eluent and YMC-C_{18} column (MeOH/H_2O, 57:43) through HPLC purification, compounds **5** (93 mg) and **2** (6 mg) were obtained. Compound **1** (49 mg) was obtained from fraction XUD-3-4-3 by silica gel (H) chromatography with $CHCl_3$/MeOH/H_2O (6.8:3.2:1, underlayer) as eluent. Compound **8** (46 mg) was gained from Fraction XUD-3-4-4 from silica gel (H) column chromatography with *n*-BuOH/EtOAc/H_2O (4:1:2, underlayer) as eluent and Sephadex LH-20 column eluted with Me_2CO/MeOH (3:7). Fraction XUD-3-5 (17.0 g) was subjected to silica gel (H) column eluted with *n*-BuOH/EtOAc/H_2O (4:1:2, underlayer), to yield compounds **6** (65 mg) and **7** (110 mg), respectively.

3.3.1 Dipsacus saponin J (1)

A white amorphous powder; mp 238–240°C; $[\alpha]_D^{20}$ −0.57 (c = 0.10, MeOH); UV λ_{max} (MeOH): 204 nm; IR ν_{max} (KBr): 3412, 2939, 1732, 1637, 1074 cm^{-1}; ^1H and ^{13}C NMR spectral data see Tables 1 and 2; HR-ESI-MS: *m/z* 1405.6615 $[M + Na]^+$ (calcd for $C_{65}H_{106}O_{31}Na$, 1405.6616).

3.3.2 Dipsacus saponin K (2)

A white amorphous powder; mp 246–250°C; $[\alpha]_D^{20}$ + 5.2 (c = 0.22, MeOH); IR ν_{max} (KBr): 3399, 2927, 1739, 1653, 1076 cm^{-1}; ^1H and ^{13}C NMR spectral data see Tables 1 and 2; HR-ESI-MS: *m/z* 1113.5419 $[M + Na]^+$ (calcd for $C_{53}H_{86}O_{23}Na$, 1113.5458).

3.4 Acid hydrolysis of compounds 1 and 2

Compounds **1** (6 mg) and **2** (3 mg) were hydrolyzed with 2N TFA (5 ml) for 6 h at boiling water bath. After extraction by CH_2Cl_2 for three times, the remaining aqueous layer was concentrated and identified by TLC ($CHCl_3$/MeOH/H_2O = 8:5:1) with authentic samples. Spots were detected by spraying with 1% anisaldehyde (in EtOH) followed by heating.

3.5 Determination of sugar components

The absolute configuration of monosaccharides from each aqueous layer was determined by GC-MS of their trimethylsilylated derivatives. Column temperature: 180–250°C, programmed increase: 15°C/min, carrier gas: N_2 (1 ml/min); injection temperature: 250°C, injection volume: 1 μl, split ratio: 1/50. The derivatives of L-arabinose, D-glucose, and L-rhamnose were detected at t_R: 13.02, 17.91, 16.26 min, respectively. By comparing with authentic samples, D-glucose, L-rhamnose, and L-arabinose were

detected from **1**, and D-glucose and L-arabinose from **2**.

3.6 Colorimetric MTT (tetrazolium) assay for cell proliferation

The UMR106 cell line was purchased from the American Type Culture Collection (Manassas, VA, USA), No. CRL-1661. Cells were cultured in basal medium constituted with Dulbecco's modified Eagle's medium containing 10% (v/v) fetal bovine serum and antibiotics for incubation at 37°C in a 5% CO_2/95% air humidified atmosphere [16]. UMR106 cells were placed into 96-well plate and maintained for 24 h and then treated with six saponins at the concentrations of 0.4, 4, and 40 μM, and the medium with equivalent vehicle as control. After 24 h, 50 μl/well of MTT solution (1 mg/ml) at 37°C was added. The cells were incubated for 4 h. The solution was then removed and 200 μl of DMSO was added into all wells. The plates were then read using a microplate reader system with a test wavelength of 570 nm against a reference wavelength of 650 nm [17].

3.7 Analysis of ALP activity

After 500 μl of 104 cells/well of UMR 106 cells were seeded into 24-well plates and incubated for 48 h, the culture medium with six saponins at concentrations of 0.4, 4, and 40 μM was added (the medium with equivalent vehicle as control). After culturing for 2 days, the ALP activity of UMR 106 cells was determined [18]. ALP activity was expressed as ALP activity normalized by the content of protein.

3.8 Cytotoxicity assay

An MTT assay was employed to evaluate the cytotoxic potential of the compounds. A375 (human malignant melanoma), HepG2 (human hepatocellular carcinoma), BGC823 (human gastric carcinoma), MCF-7 (human breast cancer), and SW1990 (human pancreatic carcinoma) were used for the MTT assay. Cells (2.5×10^3 cells/well) were incubated for 96 h with the test sample at final concentrations of 0.1, 1, 10, 40, and 80 μM/ml. Then, the MTT solution (0.5 mg/ml) was added, followed by further incubation for 4 h. The medium was then removed and the resulting intracellular purple formazan were dissolved in 180 μl of DMSO and the absorbance was measured at 570 and 450 nm using a microplate reader. The inhibition ratio = 1 − [OD (sample) 570 − OD (sample) 450]/[OD (control) 570 − OD (control) 450].

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Note

1. *D. asper* was sometimes named as *D. asperoides* in China.

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