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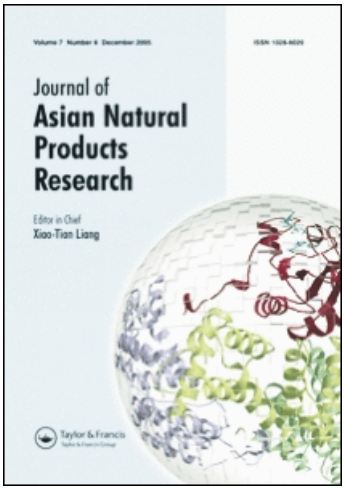

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Two new triterpenoid saponins from *Dianthus superbus* L.

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ORIGINAL ARTICLE

Two new triterpenoid saponins from *Dianthus superbus* L.

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Two new triterpenoid saponins (**1** and **2**) were isolated from the dried aerial parts of *Dianthus superbus* L. (Caryophyllaceae). Their structures were elucidated on the basis of spectral data to be 3-*O*-β-D-glucopyranosyl olean-9(11),12-diene-23,28-dioic acid 28-*O*-β-D-glucopyranoside (**1**) and 3-*O*-β-D-glucopyranosyl olean-11,13(18)-diene-23,28-dioic acid 28-*O*-β-D-glucopyranoside (**2**).

Keywords: *Dianthus superbus L.*; Caryophyllaceae; triterpenoid saponin

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1. Introduction

Dianthus superbus L. (Caryophyllaceae) is a small herb distributed in Shandong Province and elsewhere in the northeast of China. This plant, known as 'Qumai', is an important traditional Chinese medicine (TCM) used as a diuretic and an anti-inflammatory agent for the treatment of urinary infections, carbuncles, and carcinomas [1]. Previous chemical investigation on this species and its variety *D. superbus* L. var. *longicalycinus* Williams led to the isolation of saponins, flavones, and cyclopeptides [2–5]. As a part of our search for bioactive saponins from TCMs, we present in this report the isolation and structural elucidation of two new saponins (**1** and **2**; see Figure 1) from the dried aerial parts of *D. superbus*.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{42}H_{64}O_{15}$ determined from its pseudo-molecular ion peak at *m/z* 807.4167 $[M - H]^-$ in the HR-ESI-MS. Its UV spectrum with the absorption maxima at λ_{max} 203 and 282 nm indicated the presence of a homoannular conjugated diene system in the molecule [6]. The IR spectrum showed absorption bands at 3424 (OH), 1679 (C═O), and 1074, 1027 cm^{-1} (C—O—C).

The NMR spectra of the aglycone part showed six angular methyl groups at δ_H 0.84, 0.84, 1.19, 1.21, 1.38, 1.58 correlated with δ_C 23.6, 32.9, 20.5, 25.8, 20.7, 12.7 in the HSQC spectrum, respectively, two carboxyl carbons at δ_C 176.5, 180.5, two pairs of carbon signals at δ_C 116.2, 120.9, 145.9, 155.0, and two overlapped olefinic protons at δ_H 5.70 (s) correlated with δ_C 116.2 and 120.9 in the HSQC spectrum (see Table 1), which indicated the presence of an oleanane-type triterpene aglycone with a homoannular conjugated diene system [7].

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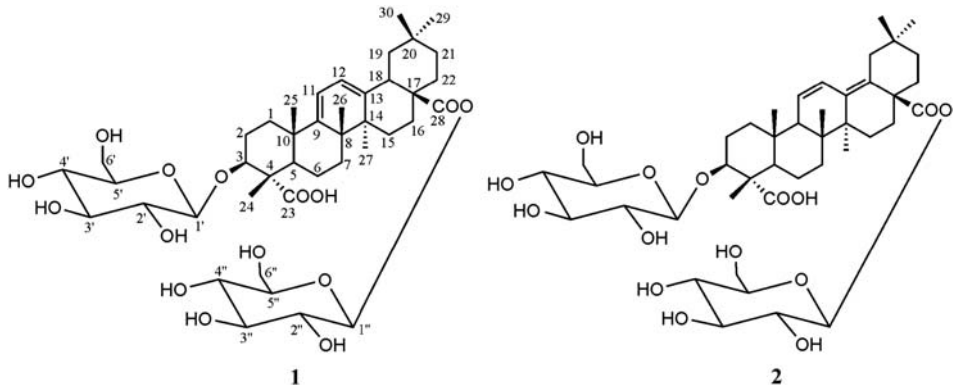

Figure 1. The structures of compounds **1** and **2**.

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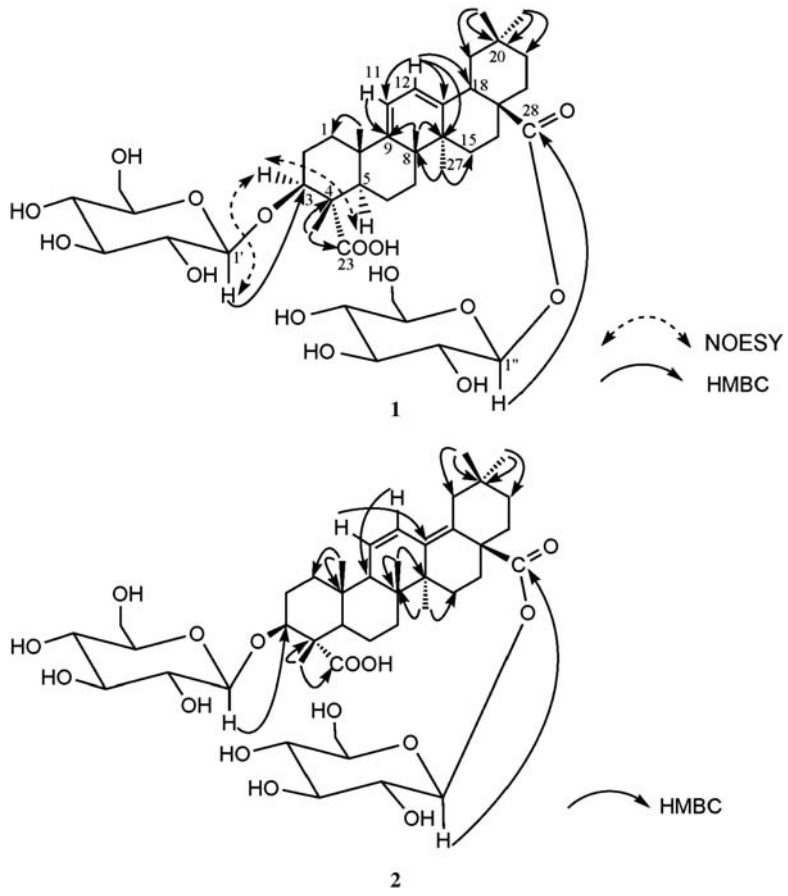

Figure 2. Selected HMBC correlations for compounds **1** and **2**.

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In the HMBC spectrum, the interactions of the protons at δ_H 5.70 (s) with C-8 (δ_C 43.4), C-9 (δ_C 155.0), C-10 (δ_C 38.5) and with C-11 (δ_C 116.2), C-13 (δ_C 145.9), C-18 (δ_C 40.2) (see Figure 2) confirmed the presence of a cisoid diene at C-9(11),12 in **1** [7,8]; accordingly, the two overlapped protons at δ_H 5.70 (s) were attributed to H-11 and H-12. In addition, the HMBC correlation of H-24 (δ_H 1.58) with C-23 (δ_C 180.5) showed that the carboxyl carbon at δ_C 180.5 was assigned to C-23, so the other carboxyl carbon at δ_C 176.5 was attributed to C-28 (see Figure 2). After extensive NMR (^{1}H NMR, ^{13}C NMR, HMBC, NOESY) spectral analysis, the aglycone was established to be an olean-9(11),12-diene-23,28-dioic acid, a new triterpenoid saponin, and its stereostructure was the same as that of an oleanane-type triterpene aglycone. Acid hydrolysis of **1** due to the paucity of the compound, the aglycone was not obtained from the hydrolysis. The sugar moieties from the hydrolysis were identified as D-glucose based on the GC-MS analysis of their chiral derivatives. Its ESI-MS exhibited fragment ion peaks at *m/z* 809 $[M + H]^+$, 647 $[M + H - 162]^+$, and 485 $[M + H - 162 - 162]^+$, which further confirmed the existence of two hexose residues. The chemical shifts of C-3 (δ_C 84.9) and C-28 (δ_C 176.5) indicated that **1** was a bisdesmosidic glycoside [9]. Inspection of its NMR spectral data of the sugar moiety (^{1}H and ^{13}C NMR, see Table 1) showed that **1** contained two anomeric carbons at δ_C 105.4 and 95.8, which correlated with the protons at δ_H 5.03 (d, $J = 7.8$ Hz) and 6.31 (d, $J = 8.1$ Hz), respectively, in the HSQC experiment, as well as the data from the NOESY experiment (see Figure 2), indicating the presence of two glucosyl units in the β-form. The positions of connectivity of the sugars were determined by the HMBC experiment. The long-range correlations between H-1′ (δ_H 5.03) and C-3 (δ_C 84.9), and between H-1″ (δ_H 6.31) and C-28 (δ_C 176.5) (see Figure 2), indicated that a D-glucose was connected at C-3 and another at C-28. On the basis of the above information, the structure of **1** was elucidated to be 3-*O*-β-D-glucopyranosyl olean-9(11),12-diene-23,28-dioic acid 28-*O*-β-D-glucopyranoside.

Compound **2** was obtained as a white amorphous powder. Its HR-ESI-MS showed the pseudo-molecular ion peak at *m/z* 807.4165 $[M - H]^-$, and thus compound **2** was also found to have the same molecular formula of $C_{42}H_{64}O_{15}$ as that of **1**. Compared to **1**, the UV spectrum of **2** showed the absorption maxima at λ_{max} 242, 250, and 259 nm, which revealed the presence of a heteroannular conjugated diene system in the molecule [10]. In ^{1}H and ^{13}C NMR spectra, the aglycone part of **2** showed six methyl proton signals (δ_H 0.83, 0.87, 0.88, 0.98, 0.98, 1.55), two olefinic proton signals at δ_H 5.68 (1H, d, $J = 10.5$ Hz) and 6.59 (1H, d, $J = 10.5$ Hz), and four olefinic carbons at δ_C 126.0, 126.9, 132.3, 137.0, respectively. Detailed analysis of the HSQC and HMBC spectral data indicated that heteroannular conjugated diene carbons were at C-11,13(18) in **2**. The aglycone moiety was further confirmed to be olean-11,13(18)-diene-23,28-dioic acid by comparison with the literature data [11]. Acid hydrolysis of **2** with 2M HCl also afforded D-glucose based on the GC/MS analysis of their chiral derivatives. The chemical shifts at δ_C 84.9 (C-3) and 175.7 (C-28) suggested that **2** was also a bidesmosidic saponin as **1**. In the HMBC spectrum, the long-range correlations between H-1′ (δ_H 5.09) and C-3 (δ_C 84.9) and between H-1″ (δ_H 6.31) and C-28 (δ_C 175.7) (see Figure 2) indicated that two D-glucose molecules were connected at C-3 and C-28, respectively. From the above information and detailed NMR spectral comparison of the sugar moiety for **2** with **1** (see Table 1), compounds **1** and **2** were demonstrated to possess an identical sugar sequence. Consequently, compound **2** was finally elucidated to be 3-*O*-β-D-glucopyranosyl

Table 1. ^{1}H and ^{13}C NMR spectral data for **1** and **2** (500 MHz for ^{1}H and 125 MHz for ^{13}C, C_5D_5N)[a].

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
1	37.5	1.54 (m), 1.95 (m)	38.2	1.80 (m), 1.08 (m)
2	24.0	1.95 (m), 2.02 (m)	26.1	1.96 (m), 1.32 (m)
3	84.9	4.65 (dd, $J = 11.5$, 4.5 Hz)	84.9	4.71 (dd, $J = 12.0$, 4.5 Hz)
4	53.4		53.4	
5	48.1	2.06 (m)	51.6	1.92 (m)
6	21.1	1.61 (m), 1.74 (m)	21.3	1.51 (m), 1.64 (m)
7	32.3	1.78 (m), 1.89 (m)	32.8	2.21 (m), 1.70 (m)
8	43.4		41.4	
9	155.0		54.8	2.10 (br s)
10	38.5		36.4	
11	116.2	5.70 (s)[b]	126.9	5.68 (d, $J = 10.5$ Hz)
12	120.9	5.70 (s)[b]	126.0	6.59 (d, $J = 10.5$ Hz)
13	145.9		137.0	
14	41.3		42.3	
15	27.5	1.18 (m), 2.45 (m)	32.5	1.17 (m), 1.34 (m)
16	26.8	1.95 (m), 2.45 (m)	25.3	0.92 (m), 1.98 (m)
17	46.4		48.7	
18	40.2	3.32 (dd, $J = 14.0$, 4.0 Hz)	132.3	
19	46.2	1.27 (m), 1.68 (m)	40.8	2.18 (m), 2.65 (m)
20	30.7		32.7	
21	33.9	1.30 (m)	37.1	1.27 (m), 1.69 (m)
22	33.9	1.05 (m)	35.8	1.45 (m), 2.54 (m)
23	180.5		180.5	
24	12.7	1.58 (s)	12.1	1.55 (s)
25	25.8	1.21 (s)	18.7	0.88 (s)
26	20.7	1.38 (s)	16.7	0.98 (s)
27	20.5	1.19 (s)	20.0	0.98 (s)
28	176.5		175.7	
29	32.9	0.84 (s)	32.2	0.87 (s)
30	23.6	0.84 (s)	24.4	0.83 (s)
3-*O*-				
1′	105.4	5.03 (d, $J = 7.8$ Hz)	105.4	5.09 (d, $J = 7.5$ Hz)
2′	74.2	4.16 (dd, $J = 7.8$, 8.9 Hz)	75.6	3.97 (dd, $J = 7.5$, 8.5 Hz)
3′	78.9	4.24 (t, $J = 8.9$ Hz)	78.4	4.12 (t, $J = 8.5$ Hz)
4′	71.2	4.31 (d, $J = 8.9$ Hz)	71.7	4.19 (d, $J = 8.5$ Hz)
5′	78.3	3.86 (m)	79.4	4.02 (m)
6′	62.3	4.43 (dd, $J = 12.0$, 4.6 Hz) 4.37 (br d, $J = 12.0$ Hz)	62.9	4.36 (dd, $J = 12.0$, 5.5 Hz), 4.53 (br d, $J = 12.0$ Hz)
28-*O*-				
1″	95.8	6.31 (d, $J = 8.1$ Hz)	96.3	6.31 (d, $J = 8.0$ Hz)
2″	75.6	3.95 (dd, $J = 8.1$, 8.9 Hz)	74.1	4.10 (dd, $J = 8.0$, 9.0 Hz)
3″	78.3	4.11 (t, $J = 8.9$ Hz)	79.0	4.23 (t, $J = 9.0$ Hz)
4″	71.6	4.19 (d, $J = 8.9$ Hz)	71.3	4.26 (d, $J = 9.0$ Hz)
5″	79.3	3.99 (m)	78.3	3.91 (m)
6″	62.9	4.49 (dd, $J = 11.5$, 5.0 Hz) 4.35 (br d, $J = 11.5$ Hz)	62.4	4.32 (dd, $J = 12.0$, 5.0 Hz) 4.42 (br d, $J = 12.0$ Hz)

Notes:[a] The assignments were based upon ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, and NOESY spectra.
[b] The two protons were overlapped.

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olean-11,13(18)-diene-23,28-dioic acid 28-*O*-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter (cell length: 1.0 dm; Shimadzu Corporation, Tokyo, Japan). The IR (KBr disk) spectra were recorded on a Bruker Tensor 27 spectrometer (Bruker Company, Basel, Switzerland). 1D and 2D NMR spectra were measured in C_5D_5N at 300 K on a Bruker ACF-500 NMR (^{1}H: 500 MHz, ^{13}C: 125 MHz) spectrometer (Bruker Company). ESI-MS data were recorded on an MS Agilent 1100 series LC/MSD Trap Mass spectrometer (Agilent Technologies Inc., CA, USA), and HR-ESI-MS data were obtained on a G1969A TOF-MS instrument (Agilent Technologies Inc.). Gas chromatography was done on a Varian CP-3800 Gas Chromatograph (Agilent Technologies Inc.) equipped with a Saturn 2200 Mass detector (detection temperature: 220°C). Column: CP-sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 μm), column temperature: 150–260°C with a rate of 8°C/min, the carrier gas was He (0.8 ml/min), split ratio: 1/10, injection temperature: 250°C, and injection volume: 0.5 μl. Precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co., Qingdao, China) were employed for TLC. Spots were visualized by spraying 10% H_2SO_4–EtOH followed by heating. For column chromatography, silica gel (Qingdao Haiyang Chemical Co.), Sephadex LH-20 (20 × 100 mm; Amersham Pharmacia Biotech AB, Sweden), macroporous resin D101 (pore size B 13–14 nm, 26–60 mesh; Tianjin, China), and ODS-C_{18} (40–63 μm; Fuji, Japan) were used. Preparative HPLC was carried out using Agilent 1100 Series (Agilent Technologies Inc.) equipped with a Shim-park RP-C_{18} column (200 × 20 mm i.d.; Shimadzu Corporation) and a 1100 Series Multiple Wavelength detector.

3.2 Plant material

The aerial parts of *D. superbus* were collected in Linyi City, Shandong Province, China, in June 2008, and identified by Prof. Mian Zhang of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 20080901) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3 Extraction and isolation

The air-dried aerial parts of *D. superbus* (5 kg) were powdered and refluxed three times with 95% EtOH. After concentrating *in vacuo*, the residue was suspended in 50% EtOH, cold preserved and allowed to stand, partitioned with supernatant, and precipitated (chlorophyll) successively. The solution was concentrated under reduced pressure to give a residue (84 g), which was further chromatographed over a macroporous resin D101 column eluted initially with water, and then with 50 and 70% EtOH to give fractions 1 and 2. Fraction 1 was subjected to MCI (MeOH–H_2O 5:5, v/v), repeated in ODS-C_{18} column (MeOH–H_2O 5:5, v/v), silica gel column using $CHCl_3$–CH_3OH–H_2O gradiently, followed by Sephadex LH-20 chromatographic purification (MeOH as the eluent) and prep-HPLC (MeCN–H_2O, 70:30, UV detection at 210 nm, $t_{R1} = 7.5$ min, $t_{R2} = 8.7$ min) affording **1** (8 mg) and **2** (7 mg), respectively.

3.3.1 3-O-β-D-Glucopyranosyl olean-9(11),12-diene-23,28-dioic acid 28-O-β-D-glucopyranoside (1)

White amorphous powder (MeOH); $[\alpha]_D^{23} + 78.2$ ($c = 0.10$, CH_3OH). UV λ_{max} (log ε): 203 (3.53), 282 (3.66) nm; IR ν_{max} (cm^{-1}): 3424, 2945, 1679, 1465, 1385, 1262, 1142, 1074, 1027. ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1. ESI-MS: *m/z* 807 $[M - H]^-$; HR-ESI-MS: *m/z* 807.4167 $[M - H]^-$ (calcd for $C_{42}H_{63}O_{15}$, 807.4172).

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*3.3.2 3-O-β-D-Glucopyranosyl olean-11,13(18)-diene-23,28-dioic acid 28-O-β-D-glucopyranoside (**2**)*

White amorphous powder; $[\alpha]_D^{23}$ −20.0 ($c = 0.10$, CH_3OH). UV λ_{max} (log ε): 200 (3.34), 242 (3.65), 250 (3.68), 259 (3.49) nm; IR ν_{max} (cm^{-1}): 3422, 2933, 1725, 1637, 1466, 1389, 1261, 1079, 1028. ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1. ESI-MS: *m/z* 807 $[M - H]^-$; HR-ESI-MS: *m/z* 807.4165 $[M - H]^-$ (calcd for $C_{42}H_{63}O_{15}$, 807.4172).

3.4 Acid hydrolysis of 1 and 2

Compound **1** (4 mg) was treated with 2 M HCl (4 ml) at 90°C for 2 h. The reaction mixture was extracted with $CHCl_3$ (3 × 5 ml). The remaining aqueous layer was neutralized with 0.5 M KOH and concentrated to dryness to give a residue, which was dissolved in pyridine (2 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution [12]. The mixture was heated at 60°C for 1 h, and trimethylchlorosilane (0.5 ml) was added, followed by heating at 60°C for 30 min. Then, the solution was concentrated to dryness and taken up in water (1 ml × 3), followed by extraction with *n*-hexane (1 ml × 3), and the supernatant was subjected to GC/MS analysis. The absolute configuration of the monosaccharides was confirmed to be D by comparison of the retention time (14.51 min) of monosaccharide derivatives with that of an authentic sample prepared in the same manner. Using the same method, the monosaccharides from **2** (4 mg) were also identified as D-glucose.

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