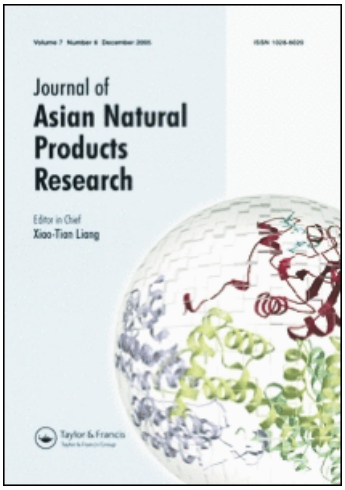

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Two new 9,10-*seco*-cycloartanes from the seeds of *Sphaerophysa salsula*

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Two new 9,10-*seco*-cycloartanes, named sphaerophyside SC (**1**) and sphaerophyside SD (**2**), together with four known compounds (**3**–**6**), were obtained from the ethanol extract of the seeds of *Sphaerophysa salsula*. The structures of these compounds were elucidated on the basis of spectral and chemical evidences. Compounds **3**–**6** were isolated from the plant for the first time.

Keywords: Leguminosae; *Sphaerophysa salsula*; Seeds; 9,10-Seco-cycloartane

1. Introduction

Sphaerophysa salsula (Pall.) DC (Leguminosae) is a plant widely distributed in Middle Asia and the northwest of China. It is used as a traditional medicine to treat hypertension in China. We previously [1–9] reported the chemical constituents from *S. salsula*. In this paper, two new cycloartanes and four known compounds were isolated from the plant and their structures were elucidated by spectral data, including 2D NMR spectra (DQFCOSY, HMQC and HMBC).

2. Results and discussion

By various means of separation, six compounds were obtained from the n-BuOH fraction of the ethanolic extract of the seeds of *Sphaerophysa salsula*. The known compounds, soyasaponin I (**3**), soyasaponin I 22-*O*-β-D-glucopyranoside (**4**), (2,5-dioxo-imidazolidin-4-yl)-urea (**5**) and tatarine C (**6**), were identified by comparing their NMR data with those of reported in the literature [10–12].

Compound **1** was obtained as a white powder. After hydrolysis with 0.5 mol/L HCl, **1** afforded a glucose, which was identified by co-TLC with authentic sample. The HRFAB-MS

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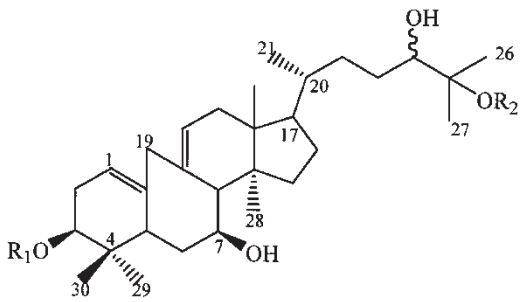
of **1** exhibited a pseudo-molecular ion peak at *m/z* 637.4320 $[M + H]^+$; together with the NMR data, the molecular formula $C_{36}H_{60}O_9$ was established. In the ^1H NMR spectrum of **1**, six methyl singlets [δ 0.73 (s, H-18), 1.24 (H-26), 1.21 (H-27), 0.92 (H-28), 1.02 (H-29), and δ 0.69 (H-30)] and one methyl doublet at δ 0.92 ($J = 7.5$ Hz, H-21) were observed. Two olefinic protons were presented at δ 5.27 (1H, br.s, H-1) and 5.36 (1H, br.s, H-11). An anomeric proton at δ 4.61 (1H, d, $J = 8.0$ Hz, H-1′) indicated the glucose should be the β anomer. In addition, three oxygen-bearing methine protons were observed at δ 3.39 (1H, dd, $J = 9.5, 3.0$ Hz, H-3), 3.80 (1H, m, H-7) and 3.36 (1H, t, $J = 9.0$ Hz, H-24). The ^1H NMR spectrum also revealed a pair of doublets at δ 2.75 and 3.02 (each 1H, d, $J = 14.0$ Hz, H-19). In the ^{13}C NMR and DEPT (90°, 135°) spectra of **1**, 36 carbon signals (7 × CH_3, 9 × CH_2, 14 × CH and 6 × C) were observed and among them, six were assigned to the signals of a glucose moiety. Four olefinic carbons at δ 117.6 (C-1), 140.6 (C-10), 139.3 (C-9) and 126.6 (C-11) and four oxygen-bearing carbons at δ 79.5 (C-3), 74.9 (C-7) and 81.9 (C-25) were also observed. From the above spectral data and comparison with those reported in the literature [13], compound **1** was inferred to be a 9,10-*seco* related derivative of sphaerophysone A. In the HMQC spectrum of **1**, the correlated peaks between H-1 (δ 5.27) and C-1 (δ 117.6), H-11 (δ 5.36) and C-11 (δ 126.6) were observed, and the protons at δ 2.75 and 3.02 (each 1H, d, $J = 14.0$ Hz, H-19) were correlated with the carbon at δ 45.8 (C-19). In the HMBC spectra of **1** (see figure 2), the protons at H-19 (δ 2.75 and 3.02) showed correlations with C-1 (δ 117.6), C-5 (δ 46.1), C-9 (δ 139.3), C-10 (δ 140.6), C-8 (δ 55.9) and C-11 (δ 126.6), respectively. H-1 (δ 5.27) showed HMBC correlations with C-10 (δ 140.6), C-19 (δ 45.8) and C-5 (δ 46.1). The HMBC spectrum also displayed that H-11 (δ 5.36) correlated with the C-19 (δ 45.8), C-8 (δ 55.9) and C-9 (δ 139.3); however, there were no HMBC correlations between H-1 (δ 5.27) and C-9 (δ 139.3) or H-11 (δ 5.36) and C-10 (δ 140.6), which confirmed that the structure of **1** is a *seco*-cycloartane type triterpene. In the HMBC of **1**, the anomeric proton at δ 4.61 (1H, d, $J = 8.0$ Hz, H-1′) showed correlation with C-25 (δ 81.9), so the C-25 was substituted by a glucosyl group. In the NOESY spectrum of **1**, the cross-peaks between H-3 (δ 3.39) and H-5 (δ 1.66), H-7 (δ 3.80) with H-28 (δ 0.92) indicated the β-orientations of 3,7-OH groups. The configuration of the hydroxyl group at C-24 was not determined. On the basis of the above evidence, the structure of **1** was determined and named sphaerophyside SC (figure 1).



1. R_1=H, R_2=Glc
2. R_1=Glc, R_2=H

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

Compound **2** was obtained as a white powder. Based on the HRFAB-MS and NMR data, **2** has the same molecular formula as **1**. In the ^{1}H NMR spectrum of **2**, the typical AB type signals at δ 2.73 and 3.01 (each 1H, d, $J = 14.0$ Hz, H-19) indicated that **2** is also a 9,10-*seco*-cycloartane glucoside. The ^{13}C NMR and DEPT spectra of **2** revealed the presence of thirty-six carbons (7 × CH_3, 9 × CH_2, 14 × CH and 6 × C), six of them were in good accordance with the presence of a glucose. In fact, the difference of the NMR data between **2** and **1** were found mainly at C-3, C-25 and sugar moiety, the downfield C-3 at δ 85.6 and upfield C-25 at δ 73.9 indicated that the hydroxyl at C-3 was substituted by a glucosyl group. In the HMBC spectrum of **2** (see figure 2), the anomeric proton at δ 4.30 (1H, d, $J = 8.0$ Hz) showed the correlation with C-3 at δ 85.6, supporting the above conclusion. The configurations of C-3 and C-7 were determined by NOESY spectrum. The NMR data were also assigned by 2D NMR (figure 1).

3. Experimental

3.1 General experimental procedures

NMR spectra were recorded at 500 MHz for ^{1}H and 125.0 MHz for ^{13}C on a JNM-A-500 instrument, with TMS as internal standard. Optical rotations were measured in a Jasco P-1010 polarimeter in CH_3OH. UV spectra were performed on a Hitachi U-2000 spectrophotometer. EI–MS data were recorded on a Jeol JMS-SX 102A spectrometer. Silica gel (Wakogel C-200, Wako Pure Chemistry, Japan) was used for column chromatography and Sephadex LH-20 (Pharmacia) was used for molecular exclusion chromatography. TLC employed precoated Si gel 60F 254 plates (Merck) and RPTLC employed precoated RP-18F 254s plates (Merck). The preparative HPLC was performed on a Tosoh liquid chromatograph coupled to a Tosoh UV-8011 UV detector. The MPLC separations were performed on a system with a Toyosoda UV-8000 detector, a Toyosoda CCPM pump, and a Lichroprep precolumn (310 × 25 mm) with the stationary phase RP-18 (40–63 μm, Merck).

3.2 Plant material

The seeds of *Sphaerophysa salsula* (10.0 kg) were collected in August 1999 in Huhehaote City, Inner Mongolia, P.R. China. Authentication of the botanical material has been reported

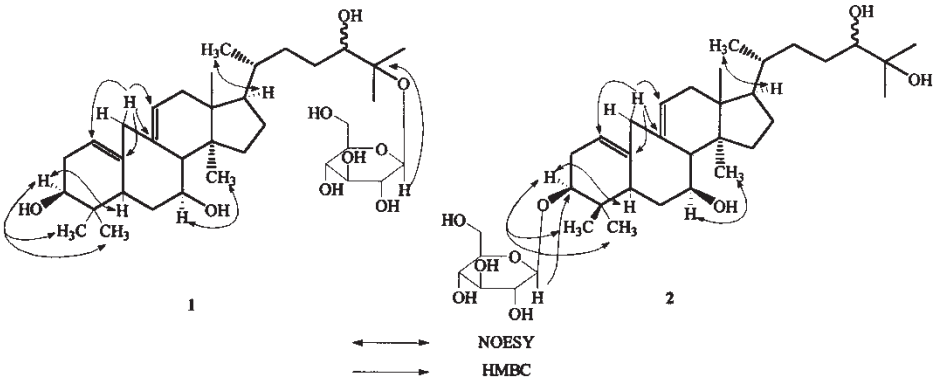


Figure 2. Significant NOESY and HMBC correlations for **1** and **2**.

previously. A voucher specimen (No. 990702) has been deposited in the Department of Natural Medicines, Shenyang Pharmaceutical University, Shenyang, China.

3.3 Extraction and isolation

The seeds were air-dried and de-fatted with petroleum ether, and extracted with 95% EtOH to give a black crude material (436.0 g), which was partitioned with petroleum ether and n-BuOH successively. The n-BuOH extract (212.0 g) was subjected to silica gel chromatography using a gradient mixture of $CHCl_3/CH_3OH$ as eluent to give eight fractions. Fraction 5 was applied to a silica gel column using a gradient mixture of $CHCl_3/CH_3OH$ as eluent to give six subfractions. Subfraction 3 was chromatographed on a Lobar column, and eluted with 60% and 100% CH_3OH/H_2O successively; the 60% eluate was subjected to preparative HPLC and eluted with 75% CH_3OH/H_2O to yield **1** (6 mg,

Table 1. NMR data for **1** and **2**[a].

	1			***2***	
No.	δ_H	δ_C	*No.*	δ_H	δ_C
1	5.27 br. s	117.6	1	5.27 br. s	118.1
2	1.70, 2.19 m	32.6	2	2.45, 2.06 m	32.3
3	3.39 dd (9.5, 3.0)	79.5	3	3.43 dd (9.5, 6.0)	85.6
4	–	38.8	4	–	38.9
5	1.66 m	46.1	5	1.76 m	46.4
6	1.90 m	36.8	6	1.94, 1.81 m	37.0
7	3.80 m	74.9	7	3.80 m	74.8
8	2.14 m	55.9	8	2.15 m	55.8
9	–	139.3	9	–	137.5
10	–	140.6	10	–	140.0
11	5.36 br. s	126.6	11	5.35 br. s	126.3
12	2.08, 1.95 m	38.5	12	2.03, 1.97 m	38.5
13	–	46.7	13	–	46.7
14	–	49.3	14	–	49.4
15	1.52 br. t (10.0)	36.2	15	1.74 m	36.2
16	1.56 m	29.3	16	1.91 m	29.3
17	1.62 m	52.0	17	1.62 m	52.0
18	0.73 s	15.3	18	0.73 s	15.3
19	3.02, 2.75 d (14.0)	45.8	19	3.01, 2.73 d (14.0)	45.8
20	1.40 m	35.0	20	1.38 m	35.0
21	0.92 d (7.5)	19.2	21	0.93 d (7.5)	19.2
22	1.89 m	37.9	22	1.89 m	37.9
23	1.95 m	29.3	23	1.51, 1.21 m	29.1
24	3.36 t (9.0)	75.5	24	3.15[b]	80.6
25	–	81.9	25	–	73.9
26	1.24 s	21.3	26	1.12 s	24.7
27	1.21 s	23.8	27	1.16 s	25.8
28	0.92 s	19.5	28	0.92 s	19.4
29	1.02 s	24.9	29	1.11 s	25.0
30	0.69 (3H, s, H-30)	13.4	30	0.79 s	14.8
1′	4.61 d (8.0′)	98.6	1′	4.30 d (8.0′)	106.2
2′	3.16 d (8.0′)	75.2	2′	3.17[b]	75.6
3′	3.34 m	78.1	3′	3.35 m	78.2
4′	3.30 m	71.6	4′	3.27 m	71.7
5′	3.31 m	77.7	5′	3.29 m	77.7
6′	3.81 m, 3.64 dd (12.5, 6.0)	62.6	6′	3.82 m, 3.64 dd (11.5, 5.5)	62.8

[a] The ^1H NMR and ^{13}C NMR spectra were measured in CD_3OD at 500 and 125 MHz, respectively, and the *J* values (parentheses) are in Hertz.
[b] Overlapped.

65 min) and **2** (2 mg, 60 min). Subfraction 5 was chromatographed on a Lobar column, and eluted with 10% and 20% CH_3OH/H_2O successively; the 10% eluate was subjected to preparative HPLC and eluted with 10% CH_3OH/H_2O to yield **6** (12 mg, 23 min). Subfraction 6 was to give **5** (14 mg, 100:14). Fraction 8 was chromatographed on a silica gel column to produce four sub subfractions. Subfraction 4 was chromatographed on a Lobar column, and eluted with 60% and 100%; the 100% eluate was subjected to preparative HPLC and eluted with 65% CH_3OH/H_2O to yield **3** (15 mg, 55 min) and **4** (24 mg, 70 min).

3.3.1 Sphaerophyside SC (1)

$C_{36}H_{60}O_9$, white powder. ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz): see table 1. HRFAB-MS *m/z* 637.4320 $[M + H]^+$ (calcd for $C_{36}H_{61}O_9$, 637.4326).

3.3.2 Sphaerophyside SD (2)

$C_{36}H_{60}O_9$, white powder. ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz): see table 1. HRFAB-MS *m/z* 637.4323 $[M + H]^+$ (calcd for $C_{36}H_{61}O_9$, 637.4326).

Acknowledgements

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