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Two New Ecdysteroid Glycosides from the Rhizomes of *Silene tatarinowii* Regel

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Abstract: Two new ecdysteroid glycosides, sileneosides I and J (**1** and **2**), along with four known ecdysteroids (**3–6**) were isolated from the rhizomes of *Silene tatarinowii* Regel. Their structures were determined based on physicochemical properties and spectroscopic methods. In addition, the cytotoxicity of compounds **1–6** was evaluated *in vitro* in human SGC790, HCT116, A549, and BEL7404 tumor cell lines. The results showed that compounds **1** and **2** exhibited weak cytotoxicity against SGC790 (IC_{50} 82.58 ± 0.53 μM) and A549 cells (96.62 ± 0.58 μM), respectively.

Keywords: *Silene tatarinowii*; ecdysteroids; sileneoside I; sileneoside J; cytotoxic activity.

1. Introduction

The genus *Silene* L. (Caryophyllaceae family) consists of approximately 700 species extensively distributing in the temperate regions of the Northern Hemisphere [1]. *Silene tatarinowii* Regel, an annual herbaceous plant of genus *Silene*, was commonly used as folk medicines for the treatment of dropsy and rheumatism [2]. Previous studies had discovered the characteristic components such as ecdysteroids and triterpenoid saponins from 115 species of *Silene* genus [3-5]. Ecdysteroids have been considered as one of the most important chemotaxonomic markers for this genus [5], which mainly possess significant anti-inflammatory [6], antitumor [7], antibacterial or antioxidant activities [5]. As part of an ongoing search for bioactive constituents from the traditional folk herbal medicines [8], two new ecdysteroid glycosides, sileneosides I and J (**1** and **2**), and four known ones: stachysterone A (**3**) [9], 22-O-acetyl-20-hydroxyecdysone (**4**) [10], sidisterone (**5**) [11], 22-oxo-20-hydroxy-ecdysone (**6**) [12] (Figure 1), were isolated from the rhizomes of *S. tatarinowii*. This study aims to describe the structure identification and the cytotoxic activities of these compounds.

2. Materials and Methods

2.1. *General Experimental Procedures*

ESI-MS was performed on Waters Quattro Premier instrument. The HR-ESI-MS spectra were taken on an Agilent Technologies 6550 Q-TOF. 1D and 2D NMR spectra were recorded on a Bruker-AVANCE 400 instrument with TMS as an internal standard. Gas chromatography was taken on an Agilent Technologies 7890A. Semipreparative HPLC was performed on a system comprising an LC-

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6AD pump equipped with a SPD-20A UV detector and a Ultimate XB-C_{18} (10 mm × 250 mm, 5 μm particles). Silica gel was purchased from Qingdao Haiyang Chemical Group Corporation (Qingdao, China). MCI gel column were purchased from Mitsubishi Chemical Holdings. Sephadex LH-20 gel silica gel was purchased from GE Healthcare Bio-Sciences AB.

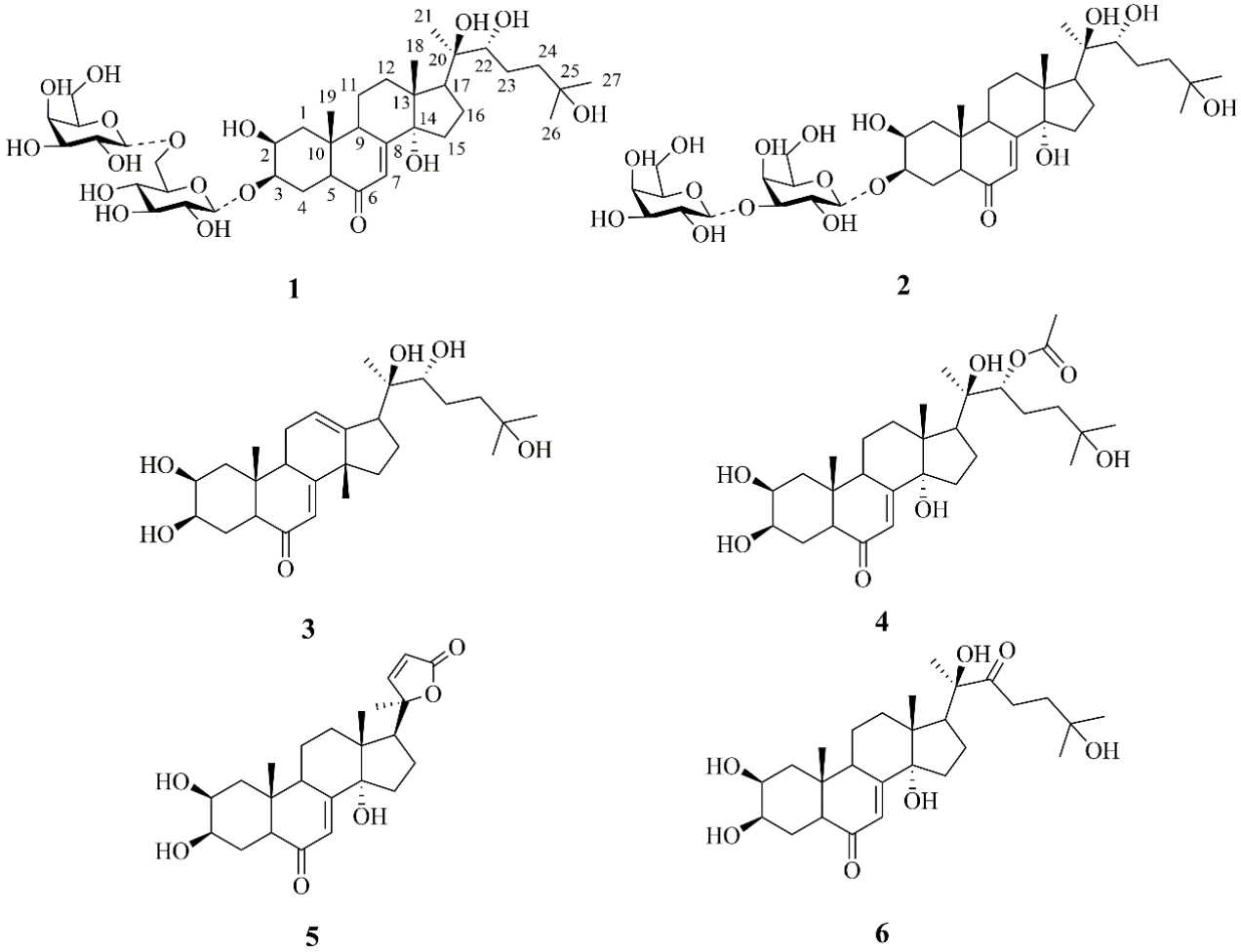

Figure 1. Structures of compounds **1**

2.2. *Plant Material*

The dried rhizomes of *S. tatarinowii* Regel were collected in October 2018 from north slope of Qinling mountains in Shaanxi Province of China, and was identified by Associate Professor Yuzhu Tan (Chengdu University of Traditional Chinese Medicine). A voucher specimen (No. 20181010) was deposited at the Medicinal Plants Herbarium (MPH), Chengdu University of Traditional Chinese Medicine, Chengdu, China.

2.3. *Extraction and Isolation*

The powdered rhizomes of *S. tatarinowii* Regel (3.8 kg) were extracted three times by 70% EtOH under the condition of 80 °C reflux to obtain a crude extract (160.3 g). The extraction was suspended in water, and then was sequentially extracted with *n*-BuOH. The *n*-BuOH layer residue (57.6 g) was subjected to silica gel column chromatography ($CHCl_3$:MeOH mixture 15:1 to 3:1, v/v), to yield four fractions (Fr. 1-Fr. 4). Fr. 2 (18.9 g) was decolorized on a MCI gel column (MeOH:H_2O mixture (8:1 to 1:1, v/v)), to yield three subfractions (Fr. 2-1-Fr. 2-3). Fr. 2-1 (3.3 g) was purified over Sephadex LH-20 column (MeOH), and then by semi-preparative HPLC eluted with MeOH:H_2O (21%, v/v, 2 mL/min) to get compound **1** (10 mg, t_R = 33 min) and compound **2** (9 mg, t_R = 36 min). Fr. 2-2 (3.9 g) was purified

by semi-preparative HPLC eluted with MeOH:H_2O (42%, v/v, 2 mL/min) to afford compound **3** (4 mg, t_R = 78 min), compound **4** (8 mg, t_R = 62 min), compound **5** (5 mg, t_R = 48 min), and compound **6** (6 mg, t_R = 30 min).

2.4. *Spectroscopic Data*

*Sileneoside I (**1**)*: A white amorphous powder, $[\alpha]_D^{25}$ 48.5 (*c* 0.2, CH_3OH); UV (CH_3OH) λmax: 232 nm, 275 nm; IR (KBr) ν_{max}: 3393, 2963, 2940, 1653, 1379 and 1057 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5) spectral data, see Table 1; HR-ESI-MS: *m/z* 827.4049 $[M+Na]^+$ (calcd for $C_{39}H_{64}O_{17}Na$, 827.4041).

*Sileneoside J (**2**)*: A white amorphous powder, $[\alpha]_D^{25}$ 47.8 (*c* 0.2, CH_3OH); UV (CH_3OH) λmax: 230 nm, 270 nm; IR (KBr) ν_{max}: 3393, 2963, 2940, 1653, 1379 and 1057 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5) spectral data, see Table 1; HR-ESI-MS: *m/z* 827.4034 $[M+Na]^+$ (calcd for $C_{39}H_{64}O_{17}Na$, 827.4041).

3. Results and Discussion

3.1. *Structure Elucidation*

Compound **1** was a white amorphous powder, showing maximum UV absorption at 232 nm. The IR spectrum of **1** showed typical absorption bands for OH (3393 cm^{-1}) and conjugated C=O (1653 cm^{-1}), corresponding to common characteristics of ecdysteroids [13]. It was appointed the molecular formula was $C_{39}H_{64}O_{17}$ via the HR-ESI-MS positive ion at *m/z* 827.4049 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{17}Na$, 827.4041). The ^{1}H-NMR spectrum (Table 1) of **1** showed five methyl protons at δ_H 1.22 (3H, s, Me-18), 0.99 (3H, s, Me-19), 1.62 (3H, s, Me-21), 1.40 (3H, s, Me-26) and 1.40 (3H, s, Me-27), an olefinic proton 6.20 (1H, s, H-7), as well as signals for two anomeric protons at δ_H 5.56 (d, J = 3.0 Hz) and δ_H 5.49 (d, J = 3.0 Hz). The ^{13}C-NMR spectrum (Table 1) displayed 39 carbon signals, 27 of which belonged to the aglycone carbons, while the remaining signals were assignable to one glucopyranosyl moiety (δ_C 104.4, 73.0, 80.5, 72.1, 79.8, 68.7) and one galactopyranosyl moiety (δ_C 101.0, 71.4, 71.8, 71.4, 72.4, 63.0). Among the carbon signals of the aglycone, δ_C 18.4, 22.0, 24.8, 30.5 and 30.6 were due to five methyl groups, δ_C 203.6, 167.1 and 122.0 were due to an α, β-unsaturated ketone group [14]. In comparison of the proton and carbon data, the aglycone resonances of **1** were almost consistent with those of 20-hydroxyecdysone [15], the difference was that the chemical shift of C-3 was transfered to downfield at δ_C 80.1 ($\triangle\delta_C$ + 11.6), indicating that the glycosidation positions was at C-3. In detail, in the HMBC spectrum (Figure 2), the correlation between the proton signal at δ_H 2.76 (H-5) with the carbon signal at δ_C 204.3, suggested the location of the keto group at C-6, which was further confirmed by the singlet proton at δ_H 6.20 (H-7). These data allowed us to recognize the aglycone of compound **1** as a cholest-7en-6-one derivative. HMBC (Figure 2) correlations of H-21/C-17 and C-20, H-22/C-20, C-21, C-23 and C-24, H-26/C-24, C-25 and C-27, indicated that the appearance of an isooctane group linked at C-17 position. Moreover, HMBC correlations of H-1/C-3 and C-5, H-7/C-5, C-9 and C-14, H-17/C-13, C-18 and C-21, H-18/C-12, C-13, C-14 and C-17, H-19/C-1, C-5, C-9 and C-10, were assigned. In addition, HMBC correlations of H-Gal-1′/C-Glc-6 and H-Glc-1/C-3 disclosed that the terminal galactopyranosyl unit was linked at C-6 of the inner glucopyranosyl unit, then the glucopyranosyl unit was linked at C-3 of the aglycone. The glucopyranosyl moiety and galactopyranosyl moiety were identified as D-glucopyranose and D-galactopyranose by acid hydrolysis of **1**, followed by TLC comparison with a reference compound and the optical rotation determination ($[\alpha]_D^{20}$+49.2 (*c* 0.16, H_2O), $[\alpha]_D^{20}$ +80.1 (*c* 0.14, H_2O)) [16], and judged to be in α-configuration from the coupling constants of two anomeric protons (3.0 Hz each). In the NOESY spectrum of **1** (Figure 2), the NOE correlations of H-4/H-2/H-9, H-1a/H-5 and Me-19, indicated β-orientations of 2-OH and 5-H, which supported the A/B *cis* ring junction pattern; the NOE correlations of H-12a/H-15a/H-16a and Me-18, H-12b/H-17, supported the C/D *trans* ring junction pattern; and the NOE correlations of H-12b/Me-21 and H-22/H-16a, suggested α-axial configurations of Me-21 and 22-OH, and β-orientations of 20-OH. Therefore, compound **1** was identified as 2β,3β,14α,20*R*,22*R*,25-hexahydroxy-5β-cholest-7-en-6-on-3-*O*-[α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl], which was named as sileneoside I.

Table 1. ^{1}H-NMR (400 MHz, in pyridine-d_5) and ^{13}C-NMR (100 MHz, in pyridine-d_5) spectral data of compounds **1** and **2**

No.	**1**		**2**	
	δc	δ_H (J in Hz)	δc	δ_H (J in Hz)
1	39.9	2.12 (m, H-1a) 1.75 (m, H-1b)	39.0	2.13 (m, H-1a) 1.73 (m, H-1b)
2	68.5	4.06, m	68.6	4.06, m
3	80.1	4.02, m	81.5	4.72, m
4	32.2	2.33 (m, H-4a) 1.85 (m, H-4b)	32.2	2.13 (m, H-4a) 1.86 (m, H-4b)
5	53.0	2.76 (1H, dd, 3.0, 10.1)	52.9	2.67 (1H, dd, 2.4, 12.6)
6	204.3	-	203.4	-
7	122.1	6.20, s	122.0	6.21, s
8	167.8	-	167.1	-
9	34.8	3.50 (1H, t, 8.3)	34.7	3.47 (1H, t, 8.2)
10	39.1	-	39.8	-
11	21.5	1.82 (m, H-11a) 1.67 (m, H-11b)	21.5	1.76 (m, H-11a) 1.66 (m, H-11b)
12	32.4	2.60 (m, H-12a) 2.01 (m, H-12b)	32.4	2.55 (m, H-12a) 1.98 (m, H-12b)
13	48.6	-	48.5	-
14	84.6	-	84.6	-
15	32.2	2.10 (m, H-15a) 1.85 (m, H-15b)	32.4	2.13 (m, H-15a) 1.76 (m, H-15b)
16	22.2	2.45 (m, H-16a) 2.10 (m, H-16b)	22.2	2.44 (m, H-16a) 2.09 (m, H-16b)
17	50.6	3.02 (1H, t, 8.1)	50.6	2.97 (1H, t, 8.4)
18	18.4	1.22 (s, 3H)	18.4	1.20 (s, 3H)
19	24.8	0.99 (s, 3H)	24.7	0.93 (s, 3H)
20	77.5	-	77.4	-
21	22.0	1.62 (s, 3H)	22.0	1.59 (s, 3H)
22	78.1	3.90, m	78.1	3.86, m
23	28.0	2.18 (m, H-23a) 1.85 (m, H-23b)	28.0	2.13 (m, H-23a) 1.81 (m, H-23b)
24	43.2	2.33 (m, H-24a) 1.85 (m, H-24b)	43.2	2.29 (m, H-24a) 1.81 (m, H-24b)
25	70.2	-	70.1	-
26	30.7	1.40 (s, 3H)	30.6	1.36 (s, 3H)
27	30.5	1.40 (s, 3H)	30.5	1.36 (s, 3H)

Table 1 continued..

Glc-1	104.4	5.49 (1H, d, 3.0)		
2	73.0	4.73, m		
3	80.5	4.63, m		
4	72.1	4.51, m		
5	79.8	4.50, m		
6	68.7	4.59 (m, H-6a) 4.25 (m, H-6b)		
Gal-1'	101.0	5.56 (1H, d, 3.0)	104.2	5.77 (1H, d, 3.6)
2'	71.4	4.67, m	71.3	4.70, m
3'	71.8	4.79, m	79.9	4.06, m
4'	71.4	4.67, m	71.8	4.50, m
5'	72.4	4.63, m	73.1	4.70, m
6'	63.0	4.42 (m, H-6a) 4.42 (m, H-6b)	61.3	4.56 (m, H-6a) 4.28 (m, H-6b)
Gal-1"			103.7	5.53 (1H, d, 1.6)
2"			71.8	4.54, m
3"			71.6	4.70, m
4"			71.8	4.54, m
5"			73.9	4.90, m
6"			63.1	4.44 (m, H-6a) 4.31 (m, H-6b)

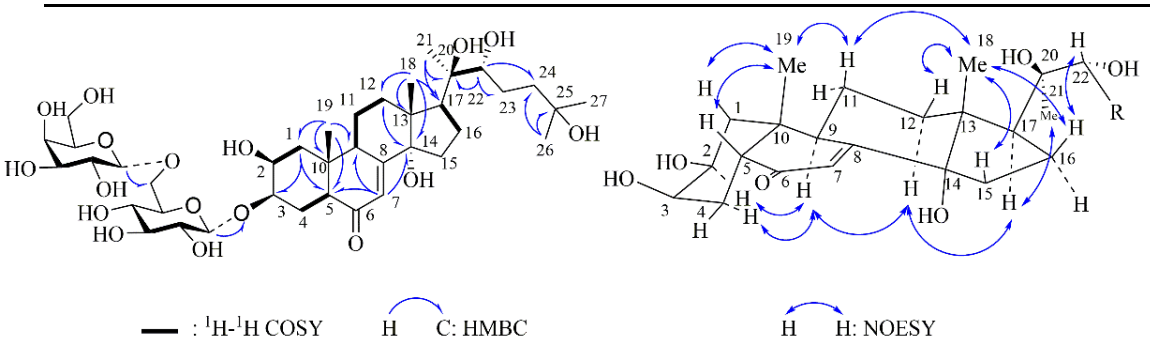

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **1**

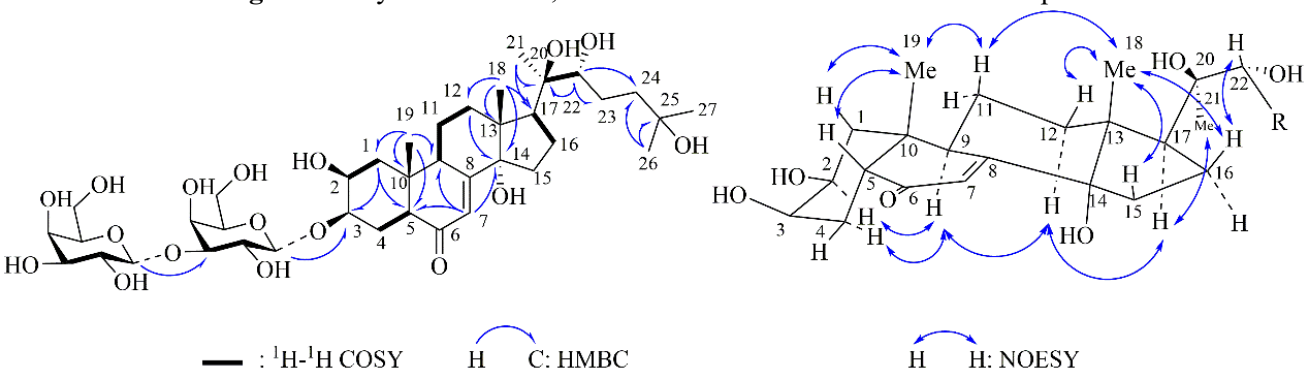

Figure 3. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **2**

3.2. *Cytotoxicity Assay*

The cytotoxic activity assay toward the SGC790, HCT116, A549, and BEL7404 tumor cell lines were measured by the MTT method *in vitro*, using 5-fluorouracil as positive control. Briefly, 1×10^4 mL^{-1} cells were seeded into 96-well plates and allowed to adhere for 24 h. Compounds **1-6** were dissolved in DMSO and diluted with complete medium to six degrees of concentration (from 0.001 $mmol/L^{-1}$ to 0.4 $mmol/L^{-1}$) for inhibition rate determination. After incubation at 37 °C for 4 h, the supernatant fraction was removed before adding DMSO (100 μL) to each well. The inhibition rate (IR) and IC_{50} were calculated (see Table 2). Values are mean ± SD, n = 3.

Table 2. Cytotoxicity of compounds **1-6** against four human cancer cell lines *in vitro* (IC_{50},μM)[a]

Compounds	Cell lines			
	SGC790	HCT116	A549	BEL7404
[b]5-Fu	8.4 ± 1.9	14.3 ± 2.1	8.0 ± 1.6	9.0 ± 2.5
1	82.58 ± 0.53	>100	>100	>100
2	>100	>100	96.62± 0.58	>100
3	>100	>100	>100	>100
4	>100	>100	>100	>100
5	>100	>100	>100	>100
6	>100	>100	>100	>100

[a]IC_{50} values are means from three independent experiments (average ± SD) in which each compound concentration was tested in three replicate wells; [b]5-fluorouracil (5-Fu) as positive control.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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