Isolation and Characterization of Chemical Constituents from the Roots of *Silene viscidula* Franch.

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In the present study, eight compounds were isolated and characterized by alcohol extraction method from the roots of *Silene viscidula* Franch. These compounds were emodin (**1**), physcion (**2**), α -spinasterol (**3**), hexadecanoic acid (**4**), sinocrassuloside VI (**5**), sinocrassuloside VII (**6**), sinocrassuloside VIII (**7**) and sinocrassuloside IX (**8**). Among them, compounds **1**, **2**, **3** and **4** were isolated from the roots of *Silene viscidula* Franch for the first time and compounds **1**, **2** and **4** were first reported from the genus of silene. The chemical compositions show that *Silene viscidula* had distantly related with its confused specie of *Psammosilene tunicoides*.

Key Words: *Silene viscidula*, Chemical constituents, Sinocrassuloside VIII, Sinocrassuloside IX.

INTRODUCTION

Silene viscidula Franch, which is one species of genus *silene* (Caryophyllaceae), is an important medicinal herb in China. It is mainly distributed in southwest of China. Its roots have been used to treat hemorrhagic diseases for years. Modern studies have demonstrated its broad effects of antipyretic, analgesic, diuretic and antineoplastic^{1,2}.

Saponins, polysaccharides, sterols, triterpenes, cardiac glycosides, flavonoids and volatile oils of genus *silene* have been proved in previous work³. However, a small number of chemical constituents such as molting steroid ketones⁴, cyclotides⁵, sinocrassuloside VI, sinocrassuloside VII⁶ and sinocrassuloside VIII and sinocrassuloside IX⁷ were isolated from *Silene viscidula*.

In order to clarify its active constituents and compare with the chemical compositions of *Psammosilene tunicoides*, an investigation on the ethanol extract of the roots of *Silene viscidula* was carried out. Eight compounds were isolated and identified as emodin (**1**), physcion (**2**), α -spinasterol (**3**), hexadecanoic acid (**4**), sinocrassuloside VI (**5**), sinocrassuloside VII (**6**), sinocrassuloside VIII (**7**), sinocrassuloside IX (**8**) (Fig. 1). Among these compounds, compounds **1**, **2**, **3** and **4** were isolated from the roots of *Silene viscidula* Franch for the first time and compounds **1**, **2** and **4** were first reported from the genus of silene.

EXPERIMENTAL

The HPLC used in this study consisted of a Waters Empower system (Milford, MA, USA), a model 600 pump, a

model 600 system controller, a model 600 multisolvent delivery system, a model 996 photodiode array detector (PAD), a sample injector with a 10 μ L loop and an Empower workstation. The ¹H and ¹³C NMR spectra were obtained on a 600 MHz NMR spectrometer instrument with TMS as internal reference (Varian, INOVA USA). MS analyses were performed on an Agilent 5973N spectrometer.

Organic solvents including ethanol, petroleum ether, chloroform, ethyl acetate and methanol were all of analytical grade (Guangcheng Chemical Factory, Tianjin, China). Acetonitrile used for HPLC analysis was of chromatographic grade (Tedia Company Inc, Fairfield, USA). Reverse osmosis Milli-Q water (Millipore, Bille-rica, USA) was used for all solutions and dilutions. Silica gel (Qingdao Marine Chemical Factory, 200-300 mesh), Sephadex LH-20 (GE Healthcare).

The roots of *Silene viscidula* were collected from Yunnan China and identified by Yuting Chen, Beijing University of Chinese Medicine¹.

The roots of *Silene viscidula* were dried and ground into powder. The *Silene viscidula* powder (21kg) were extracted with 95 % ethanol three times under reflux. The ethanol extract was then suspended in distilled water and extracted with petroleum ether and chloroform. The petroleum ether and chloroform solutions were evaporated. The residues of petroleum ether and chloroform were consolidated and subjected to a silica gel column eluting repeatedly with petroleum ether and ethyl acetate. Then by a combination of a Sephadex LH-20 column we obtained compound **1** (80 mg), compound **2** (70 mg), compounds **3** (30 mg) and **4** (40 mg), respectively.

Fig. 1. Structures of emodin (1), physcion (2), α -spinasterol (3), hexadecanoic acid (4), sinocrassuloside VI (5), sinocrassuloside VII (6), sinocrassuloside VIII (7) and sinocrassuloside IX (8)

The water phase was diluted and was subjected to D101 macroporous resin eluting with a solvent system composed of H₂O-CH₃CH₂OH (10:0-10:9). Then by a combination of Sephadex LH-20 column and MCI column we obtained compounds 5, 6, 7 and 8.

Eight compounds were isolated and identified as emodin (1)⁸, physcion (2)⁸, α -spinasterol (3)⁹, hexadecanoic acid (4)¹⁰, sinocrassuloside VI (5)¹¹, sinocrassuloside VII (6)¹¹, sinocrassuloside VIII (7)¹¹ and sinocrassuloside IX (8)¹¹ (Fig. 1). These compounds were confirmed with spectroscopic analysis including UV, HPLC, MS, ¹H and ¹³C NMR. Among these compounds, compounds 1, 2, 3 and 4 were isolated from *Silene viscidula* Franch for the first time and compounds 1, 2 and 4 were first reported from the genus *silene*.

RESULTS AND DISCUSSION

Compound 1 was isolated as orange-red granular crystal. The ESI-MS (m/z) of compound 1 showed a molecular ion at m/z 270 corresponding to a molecular formula C₁₅H₁₀O₅. ¹H NMR peaks at δ_H 12.29, 12.18, 10.64 could be ascribed to three phenolic hydroxyl group signals. ¹H NMR peak at δ_H 2.44 could be ascribed to methyl hydrogen signals. It was unambiguously identified as emodin (1,3,8-trihydroxy-6-methylantraquinone) on the basis of its ¹H and ¹³C NMR spectral data⁸.

Compound 2 was isolated as orange needle crystal. The ESI-MS (m/z) of compound 2 showed a molecular ion at m/z 284 corresponding to a molecular formula C₁₆H₁₂O₅. ¹H NMR data of δ_H 12.3, 12.1 could be ascribed to two phenolic hydroxyl group signals, methyl hydrogen signal δ_H 2.45 and methoxy hydrogen signal δ_H 3.94. It was unambiguously identified as physcion (1,8-dihydroxy-3-methoxy-6-methylantraquinone) on the basis of its ¹H and ¹³C NMR spectral data⁸.

Compound 3 was isolated as white needle crystal. The ESI-MS (m/z) of compound 3 showed a molecular ion at m/z 412 corresponding to a molecular formula C₂₉H₄₈O. It was unambiguously identified as α -Spinasterol on the basis of its ¹H and ¹³C NMR spectral data⁹.

Compound 4 was isolated as white powder. The ESI-MS (m/z) of compound 4 showed a molecular ion at m/z 256 corresponding to a molecular formula C₁₆H₃₂O₂. It was unambiguously identified as hexadecanoic acid on the basis of its ¹H and ¹³C NMR spectral data¹⁰.

Compounds 5 and 6 were obtained as white amorphous powder, ESI-MS (m/z): 1473.2 [M + Na]⁺, 1449.7 [M-H]⁻. ¹H and ¹³C NMR (Table-1). The ESI-MS (m/z) of compounds 5 and 6 showed a molecular ion at m/z 1450 corresponding to the same molecular formula C₇₁H₁₀₂O₃₁. The two kinds of groups were easily distinguished by coupling constant of two hydrogens on the double bond. It was unambiguously identified as sinocrassuloside VI and sinocrassuloside VII on the basis of its ¹H and ¹³C NMR spectral data¹¹. The structures of 5 and 6 were determined as 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)] [β -D-xylopyranosyl(1 $\rightarrow$ 3)]- β -D-glucuronopyranosyl quillaic acid 28-O-[α -L-rhamnopyranosyl(1 $\rightarrow$ 2)]-3-O-acetyl-4-O-(E/Z)-*para*-methoxycinnamoyl- β -D-fucopyranosyl ester.

Compounds 7 and 8 were obtained as white amorphous powder, ESI-MS (m/z): 1487.2 [M + Na]⁺, 1499.7 [M + Cl]⁻, 1463.8 [M-H]⁻. ¹H and ¹³C NMR (Table-1). The ESI-MS (m/z) of compounds 7 and 8 showed a molecular ion at m/z 1464 corresponding to the same molecular formula C₇₂H₁₀₄O₃₁. The two kinds of groups were easily distinguished by coupling constant of two hydrogens on the double bond. It was unambiguously identified as sinocrassuloside VIII and sinocrassuloside IV on the basis of its ¹H and ¹³C NMR spectral data¹¹. The structures of 7 and 8 were determined as 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)] [β -D-xylopyranosyl(1 $\rightarrow$ 3)] [6-O-methyl- β -D-glucuronopyranosyl quillaic acid 28-O-[α -L-rhamnopyranosyl(1 $\rightarrow$ 2)]-3-O-acetyl-4-O-(E/Z)-*para*-methoxycinnamoyl- β -D-fucopyranosyl ester.

Conclusion

The roots of *Silene viscidula* and *Psammosilene tunicoides* can be used medicinally in China. Both *Silene viscidula* and *Psammosilene tunicoides* are of the same family (Caryophyllaceae), but of different genera. *Silene viscidula* belongs to the genus *silene*. *Psammosilene tunicoides* which is an endangered endemic single species genus plant and has been listed as second degree national protective plant belongs to the genus *psammosilene*. The resource of *Psammosilene tunicoides* has rapidly declined in recent years. Consequently, the roots of *Silene viscidula* has been commonly used as substitutes of *Psammosilene tunicoides* in treatment because of their similar shapes, despite unclear chemical constituents

TABLE-1
¹H AND ¹³C NMR DATA FOR COMPOUNDS 5-8

	5		6		7		8	
	δ H mult (<i>J</i> in Hz) ^a δ C ^b		δ H mult (<i>J</i> in Hz) ^a δ C ^b		δ H mult (<i>J</i> in Hz) ^a δ C ^b		δ H mult (<i>J</i> in Hz) ^a δ C ^b	
3''	4.14 dd(7.8, 3.6)	74.0	4.14 dd(7.8, 3.6)	74.0	4.14dd	74.9	4.14dd	74.9
4''	4.55	68.9	4.55	68.9	4.57	68.5	4.57	68.5
5''	4.02	75.3	4.02	75.3	4.02	7.0	4.02	77.0
6''	4.43, 5.50	60.2	4.42, 4.50	60.2	4.44, 4.52	60.3	4.44, 4.51	60.3
3'-O- β -D-Xylopyranosyl								
1'''	5.31d (7.8)	103.5	5.31 d(7.8)	103.5	5.30 d(7.8)	103.6	5.30 d(7.8)	103.6
2'''	3.94 t(7.8)	73.8	3.94 t(7.8)	73.8	3.94 t(7.8)	74.1	3.93 t(8.4)	75.3
3'''	4.08	77.4	4.08	77.4	4.08	77.4	4.07	77.4
4'''	4.10	70.0	4.10	70.0	4.10	68.9	4.09	68.9
5'''	3.57, 4.21	66.2	3.57, 4.20	66.2	3.64, 4.21	66.2	3.62, 4.21	66.2
28-O- β -D-Fucopyranosyl								
1''''	6.17 d(8.4)	93.2	6.13d (8.4)	93.1	6.19d(7.8)	93.2	6.14 d(7.8)	93.2
2''''	4.71 t(8.4)	71.3	4.62 t(8.4)	71.3	4.72 t(9.6)	70.5	4.62 t(9.0)	70.6
3''''	5.68dd(9.3, 4.8)	73.1	5.68dd (9.3, 4.8)	73.1	5.70dd	73.8	5.69dd	73.8
4''''	5.75	70.1	5.75	70.1	5.75	69.9	5.77	69.8
5''''	4.20	69.9	4.21	68.5	4.19	68.5	4.20	68.5
6''''	1.22 d(6.6)	16.1	1.19 d(6.6)	16.1	1.24 d(6.0)	16.1	1.21 d(6.6)	16.1
2''''-O- α -L-Rhamnopyranosyl								
1'''''	5.76s	101.6	5.75s	101.6	5.77s	101.7	5.75s	101.7
2'''''	4.52	70.5	4.52	70.5	4.54	70.2	4.53	70.2
3'''''	4.36	70.6	4.36	68.5	4.36	71.9	4.35	71.9
4'''''	4.23	71.9	4.23	71.9	4.24	72.0	4.24	72.0
5'''''	4.40	70.0	4.40	70.0	4.44	68.9	4.44	68.9
6'''''	1.62 d(6.0)	18.4	1.62 d(6.0)	18.4	1.65 d(6.6)	18.4	1.64 d(6.6)	18.4
Acetyl group								
1''''''	–	169.9	–	169.8	–	169.9	–	169.9
2''''''	2.00 s	20.9	1.97 s	20.9	2.01 s	20.9	2.00 s	20.7
<i>para</i> -Methoxycinnamoyl group (MC)								
1'''''''	–	166.8	–	166.0	–	166.8	–	166.8
2'''''''	6.60 d(15.6)	114.7	5.91 d(12.9)	114.8	6.61d(16.2)	114.7	5.93 d(13.2)	114.8
3'''''''	7.95 d(15.6)	145.9	6.95 d(12.6)	145.9	7.96 d(16.2)	145.9	6.96 d(13.2)	145.9
4'''''''	–	126.8	–	127.1	–	126.9	–	127.1
5'''''''	–	–	–	–	–	–	–	–
and 8'''''''	7.53 d(12.6)	130.9	7.96d(9.0)	132.7	7.54 d(10.2)	130.9	7.99 d(8.4)	114.8
7'''''''	–	–	–	–	–	–	–	–
7'''''''	–	161.7	–	160.8	–	161.7	–	160.8
<i>p</i> -OCH ₃	33.67 s	55.7	3.64 s	55.7	3.68 s	55.8	3.65 s	55.7
6'-OCH ₃	–	–	–	–	3.72 s	52.4	3.72 s	52.4
Aglycon moiety								
1	0.81, 1.37	37.8	0.82, 1.37	37.8	0.85, 1.39	37.8	0.85, 1.39	37.8
2	1.82 2.09	24.8	1.82, 2.09	24.8	1.81, 2.01	24.7	1.81, 2.01	24.7
3	3.94 (7.8)	84.2	3.94	84.2	4.09	84.0	4.09	84.0
4	–	54.3	–	54.3	–	54.3	–	55.3
5	1.35	48.5	1.35	48.5	1.36	48.5	1.36	48.5
6	0.88, 1.37	20.2	0.88, 1.37	20.2	0.89, 1.36	20.2	0.89, 1.36	20.2
7	1.50	32.2	1.50	32.2	1.51	32.1	1.51	32.1
8	–	40.5	–	40.3	–	40.4	–	40.4
9	1.78	46.3	1.78	46.3	1.78	46.8	1.76	46.8
10	–	35.3	–	35.3	–	35.8	–	35.9
11	1.91	23.3	1.91	22.3	1.91	23.3	1.91	22.3
12	5.57 br s	121.8	5.57 br s	121.8	5.60 br s	121.8	5.60 br s	121.8
13	–	143.6	–	143.5	–	143.6	–	143.6
14	–	41.5	–	41.5	–	41.5	–	41.5
15	1.90, 2.18	35.8	1.90, 2.16	35.9	1.95, 2.18	35.8	1.91, 2.18	35.9
16	5.20 br s	73.0	5.17 br s	73.0	5.22 bre s	73.1	5.20 br s	73.1
17	–	49.0	–	49.0	–	48.5	–	48.5
18	3.39 d (13.8)	40.9	3.38	40.9	3.39 d(14.0)	40.9	3.39 d(14.0)	40.9
19	1.34, 2.74 t(14.0)	47.9	1.34, 2.47 t	47.9	1.34, 2.57 t(14.4)	47.9	1.36, 2.75 t(14.4)	47.9
20	–	30.6	–	30.5	–	30.5	–	30.6
21	1.30, 2.40	35.2	1.32, 2.41	35.2	1.30, 2.41	35.2	1.32, 2.41	35.3
22	2.22, 2.39	32.2	2.20, 2.38	32.2	2.22, 2.40	32.1	2.20, 2.40	32.1

23	9.84 s	210.5	9.84 s	210.5	9.86 s	210.5	9.86 s	210.5
24	1.39 s	10.7	1.39 s	10.7	1.39 s	10.7	1.40 s	10.7
25	0.79 s	15.8	0.84 s	15.8	0.84 s	15.8	0.88 s	15.8
26	1.03 s	17.1	1.04 s	17.1	1.07 s	17.1	1.07 s	17.2
27	1.75 s	26.7	1.77 s	26.7	1.79 s	26.7	1.76 s	26.7
28	–	175.1	–	175.1	–	175.1	–	175.1
29	0.94s	33.2	0.94s	33.2	0.96s	33.2	0.98 s	33.2
30	0.98 s	24.7	0.99 s	24.6	1.02 s	24.6	1.02 s	24.6
3-O-β-D-Glucuronopyranosyl								
1'	4.88 d(7.2)	102.6	4.88 d(7.2)	102.6	4.86 d(7.8)	102.6	4.86 d(7.8)	102.6
2'	4.34 t(9.0)	7.4	4.34	77.4	4.36	77.4	4.36	77.4
3'	4.28 t(90.)	84.2	4.27	84.2	4.29	84.2	4.27	84.2
4'	4.44	70.2	4.44	70.2	4.24	69.9	4.24	69.8
5'	4.50	770.0	4.50	77.0	4.39	75.3	4.39	75.3
6'	–	170.4	–	170.4	–	169.5	–	169.5
2'-O-β-D-Galactopyranosyl								
1'	5.55 d(4.2)	102.7	5.55 d(4.2)	102.7	5.54 d(7.8)	102.7	5.54 d(7.8)	102.7
2'	4.46	72.0	4.46	72.0	4.47	73.0	4.46	73.0

in *Silene viscidula*. In this preliminary study we aimed to investigate the correlation between phylogeny, chemical constituents and therapeutic effects of *Silene viscidula* and *Psammosilene tunicoides*.

Eighteen saponins have been isolated from the roots of *Psammosilene tunicoides* so far¹². Both *Silene viscidula* and *Psammosilene tunicoides* contain pentacyclic triterpenoid saponins which may indicate there are some similar biosynthetic pathways and biogenetic associations. However, compounds **5**, **6**, **7** and **8** are primary components in the roots of *Silene viscidula* while *Psammosilene tunicoides* don't contain these compounds. We got the same result by HPLC detection. Furthermore, the number and types of glycosidic saponins between *Silene viscidula* and *Psammosilene tunicoides* were verified to be extremely dissimilar, which may suggest that they ate some differences in the metabolic pathways of glycosylation. These results indicate that the far relationship is determined between *Silene viscidula* and *Psammosilene tunicoides* in terms of chemical compounds and more importantly, that *Silene viscidula* should be avoided as *Psammosilene tunicoides* in homeopathic treatment.

The study of the chemical constituents of this genus has been infrequently reported, which allows to give an insight into plant phytochemistry classification and lay the foundation for biologically active ingredients screening.

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