Pentacyclic Triterpenoid Saponins from *Silene viscidula*

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Three new pentacyclic triterpenoid saponins, viscidulosides A and B (**1** and **2**, resp.), and silenoviscoside D (**3**), were isolated from the roots of *Silene viscidula*, together with two known saponins, sinocrassulosides VIII and IX (**4** and **5**, resp.). Their structures were elucidated by spectroscopic data and chemical methods. Compounds **1/2** and **4/5** were two inseparable mixtures, which are glycosides of quillaic acid whose fucose residue is acylated by (*E*)- or (*Z*)-4-methoxycinnamic acid.

Introduction. – The roots of *Silene viscidula* are called 'Wacao' and are used in folk medicine for the treatment of rheumatism, cough, and bone pain in the southwestern region of China [1]. Many bioactive saponins of the genus *Silene* have been reported [2–5], but phytochemical and pharmacological studies on *S. viscidula* are limited. In the course of our research on bioactive constituents, three new saponins, named viscidulosides A and B (**1** and **2**, resp.), and silenoviscoside D (**3**), have been isolated from the roots of *S. viscidula*, along with two known saponins, sinocrassulosides VIII and IX (**4** and **5**, resp.) [6] (*Fig. 1*). Compounds **1/2** and **4/5** were two inseparable mixtures, which are glycosides of quillaic acid whose fucose residue is acylated by a (*E*)- or (*Z*)-4-methoxycinnamic acid. Here, we report the isolation and structural elucidation of the three new triterpenoid saponins **1**–**3**.

Results and Discussion. – The EtOH extract of the dried roots of the plant was suspended in H_2O and extracted successively with petroleum ether, AcOEt, and BuOH. The BuOH-soluble materials were repeatedly subjected to silica-gel and open *ODS* column chromatographies, followed by reversed-phase (RP) semipreparative HPLC to yield three new triterpenoid saponins **1**–**3**, and the two known saponins **4** and **5**. Saponins **1/2** (11:9) and **4/5** (8:10) were two inseparable mixtures, the composition of which were determined by the peak intensities of the corresponding ^{1}H-NMR spectra and HPLC chromatograms. Compounds **1** and **2** gave rise to two distinct peaks in the HPLC chromatogram, but each compound after separation returned to the original mixture of **1/2** when they were investigated by NMR spectra. All attempts to separate **1** and **2** were unsuccessful. Such a phenomenon could be explained by the configuration of the 4-methoxycinnamoyl group in **1** and **2**, which readily isomerize under the influence of light in aqueous MeOH solution [5]. Such isomerization of (*Z*)- and (*E*)-methoxycinnamoyl derivatives are common and have already been observed

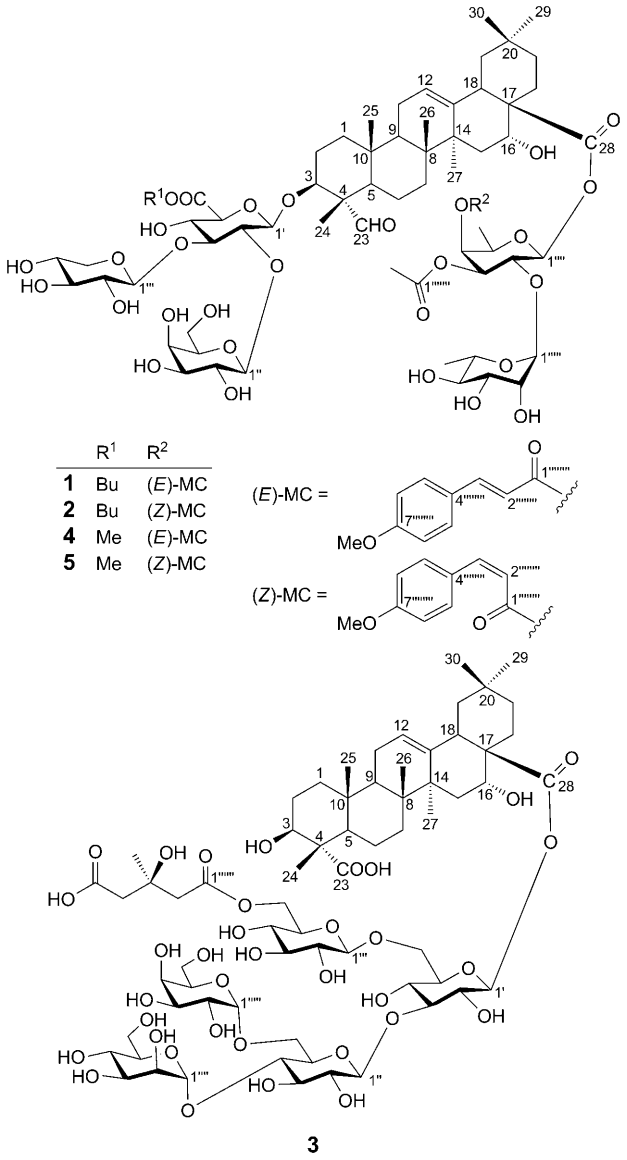


Fig. 1. *Structures of compounds* **1**–**5**

in saponins from *S. fortunei* [3] and *S. jenisseensis* [4][5], and *cis*- and *trans*-senegasaponins A from *Polygala senega* [7]. The structures of **1**–**3** were elucidated by NMR and MS data and sugar analysis. The known saponins **4** and **5** were identified by comparing their spectral data with those in the literature. They are reported here from the genus *Silene* for the first time.

The mixture of compounds **1** and **2** was obtained as a white amorphous powder. ESI-MS exhibited *quasi*-molecular-ion peaks at *m/z* 1530 ($[M+Na+H]^+$; positive-ion mode) and 1541 ($[M+Cl]^-$; negative-ion mode), which yielded the molecular formula $C_{75}H_{110}O_{31}$ for **1** and **2**. The ^{1}H-NMR spectrum showed signals for six quaternary Me groups at δ(H) 0.81 (*s*, Me(26)), 0.89 (*s*, Me(29)), 0.98 (*s*, Me(30)), 0.99 (*s*, Me(25)), 1.10 (*s*, Me(24)), and 1.39 (*s*, Me(27)), one olefinic H-atom at δ(H) 5.35 (br. *s*, H−C(12)), two O-bearing CH goups at δ(H) 3.99 (*m*, H_β−C(3)) and 4.45 (*m*, H_α−C(16)), and one aldehyde H-atom at δ(H) 9.41 (*s*, H−C(23)). The structure of the triterpenoid moiety was obtained essentially from ^{1}H- and ^{13}C-NMR spectral data, supported by HMBC and HMQC spectral data, and confirmed to be quillaic acid (3β,16α-dihydroxy-23-oxoolean-12-en-28β-oic acid) [6].

The presence of a trisaccharide and of a disaccharide unit was deduced from the observation of anomeric signals at δ(H) 4.57 (*d*, *J* = 7.8, H−C(1′)), 4.78 (*d*, *J* = 6.6, H−C(1″)), 4.46 (*d*, *J* = 7.2, H−C(1‴)), 5.54 (*d*, *J* = 7.8, H−C(1⁗)), and 4.96 (br. *s*, H−C(1⁗)) in the ^{1}H-NMR spectrum, and δ(C) 103.9 (C(1′)), 104.7 (C(1″)), 105.1 (C(1‴)), 95.1 (C(1⁗)) and 102.7 (C(1⁗)) in the ^{13}C-NMR spectrum, respectively. A complete set of ^{13}C-NMR signals for each sugar residue was derived from HMQC and HMBC spectra (*Table*). Taken together with coupling-constant data for the anomeric H-atoms and other key H-atom resonances, the sugars present were identified as β-glucuronic acid, β-galactose, β-xylose, β-fucose, and α-rhamnose. This glycosidic profile was in agreement with the results of sugar analysis, by which the absolute configurations of the constituent monosaccharides of **1** and **2** were determined as D-glucuronic acid, D-galactose, D-xylose, D-fucose, and L-rhamnose [8]. Cross-peaks H−C(1″)/C(2′) (δ(C) 78.3), H−C(1‴)/C(3′) (δ(C) 86.5), and H−C(1⁗)/C(2‴) (δ(C) 72.1) in the HMBC spectrum confirmed two sugar chains as [β-D-galactopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranoside and [α-L-rhamnopyranosyl-(1 → 2)]-β-D-fucopyranoside.

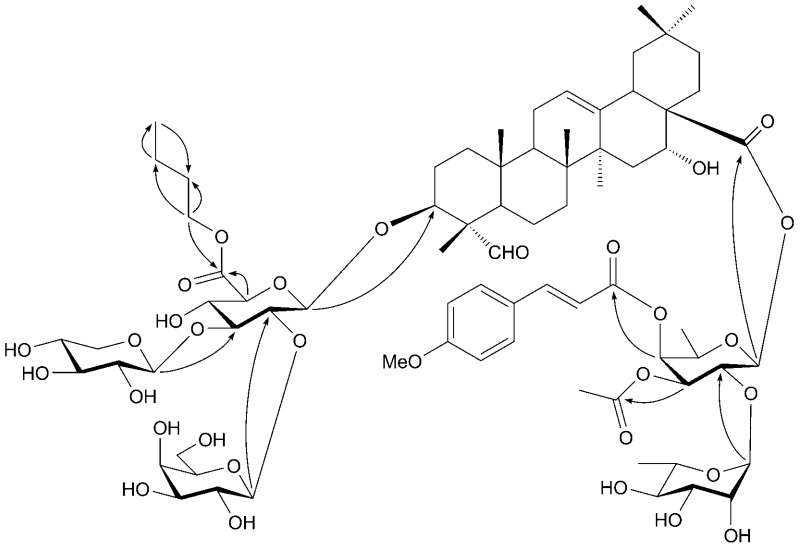
The characteristic signals displayed in the range of δ(H) 7.77 and 5.92 ppm in ^{1}H-NMR spectrum, together with ^{13}C-NMR, HMBC and HMQC data, indicated the presence of (*E*)- and (*Z*)-4-methoxycinnamoyl (MC) moieties, which was confirmed by two pairs of olefinic H-atom signals at δ(H) 6.48 (*d*, *J* = 16.2), 7.72 (*d*, *J* = 16.2), 5.93 (*d*, *J* = 12.6), and 6.99 (*d*, *J* = 12.6). The presence of an AcO group was suggested by the NMR data (δ(H) 2.03, *s* and δ(C) 21.4, 171.7), which was supported by HMBC and HMQC data. HMBCs H−C(3⁗) (δ(H) 3.76)/C(1⁗) and H−C(4⁗) (δ(H) 3.86)/C(1⁗) (δ(C) 168.6) confirmed that the AcO and the 4-methoxycinnates are at C(3) and C(4) of fucose, respectively. Further, the presence of a Bu group was implied by additional NMR signals, including those of a Me (δ(H) 0.94 (*m*, Me(4⁗)) and δ(C) 14.2 (C(4⁗))), three CH_2 groups (δ(H) 1.42 (CH_2(3⁗)) and δ(C) 20.2 (C(3⁗)), δ(H) 1.64 (CH_2(2⁗)) and δ(C) 31.9 (C(2⁗)), and δ(H) 4.19 (*t*, *J* = 6.0, CH_2(1⁗)) and δ(C) 66.4 (C(1⁗))), which was confirmed by the HMBC and HMQC experiments. The location of this group was assigned to be at C(6′) by the correlation H−C(1⁗)/C(6′) (δ(C) 170.4) (*Fig. 2*). The sugar chain ([β-D-galactopyranosyl-(1 → 2)][β-D-xylopyranosyl-(1 → 3)]-β-D-glucuronopyranoside) was shown to be connected at C(3). The other sugar unit ([α-L-rhamnopyranosyl-(1 → 2)]-β-D-fucopyranoside) was shown to be at C(28) by the correlations H−C(1′)/C(3) (δ(C) 86.5) and H−C(1‴)/C(28) (δ(C) 177.3) in the HMBC spectrum. Based on these findings, the structures of **1**

Table. *^{13}C-NMR Data* (150 MHz, δ in ppm, J in Hz) *of Compounds* **1**–**3** *in* CD_3OD[a])

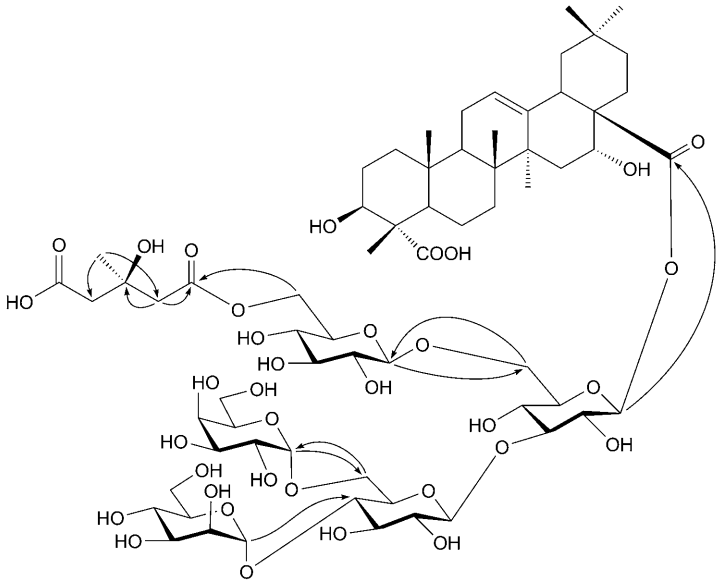
Position	**1**	**2**	**3**	Position	**1**	**2**	**3**
	The triterpenoid moiety				3′-*O*-*β*-D-Xyl		6′-*O*-*β*-D-Glc
1	39.4 (*t*)	39.4 (*t*)	40.1 (*t*)	1‴	105.1 (*d*)	105.1 (*d*)	105.1 (*d*)
2	25.9 (*t*)	25.9 (*t*)	27.7 (*t*)	2‴	75.3 (*d*)	75.3 (*d*)	75.3 (*d*)
3	86.5 (*d*)	86.5 (*d*)	76.6 (*d*)	3‴	78.4 (*d*)	78.4 (*d*)	77.9 (*d*)
4	56.0 (*s*)	56.1 (*s*)	55.7 (*s*)	4‴	70.9 (*d*)	70.9 (*d*)	71.9 (*d*)
5	48.7 (*d*)	48.7 (*d*)	52.9 (*d*)	5‴	67.3 (*t*)	67.3 (*t*)	75.4 (*d*)
6	21.0 (*t*)	21.0 (*t*)	22.4 (*t*)	6‴			64.9 (*t*)
7	31.9 (*t*)	31.9 (*t*)	33.9 (*t*)		28-*O*-*β*-D-Fuc		4″-*O*-*α*-D-Man
8	41.4 (*s*)	41.4 (*s*)	41.3 (*s*)	1⁗	95.1 (*d*)	95.1 (*d*)	103.6 (*d*)
9	48.2 (*d*)	48.2 (*d*)	48.5 (*d*)	2⁗	72.1 (*d*)	72.1 (*d*)	71.3 (*d*)
10	36.6 (*s*)	36.6 (*s*)	37.7 (*s*)	3⁗	75.1 (*d*)	75.1 (*d*)	71.6 (*d*)
11	24.7 (*t*)	24.7 (*t*)	24.7 (*t*)	4⁗	71.5 (*d*)	71.5 (*d*)	70.7 (*d*)
12	123.4 (*d*)	123.4 (*d*)	123.7 (*d*)	5⁗	71.2 (*d*)	71.2 (*d*)	73.9 (*d*)
13	144.9 (*s*)	144.9 (*s*)	144.9 (*s*)	6⁗	16.6 (*d*)	16.6 (*d*)	63.1 (*t*)
14	43.0 (*s*)	43.0 (*s*)	42.8 (*s*)		2‴-*O*-*α*-L-Rha		6″-*O*-*α*-D-Gal
15	36.6 (*t*)	36.6 (*t*)	36.2 (*s*)	1⁗′	102.7 (*d*)	102.7 (*d*)	100.2 (*d*)
16	73.9 (*d*)	73.9 (*d*)	75.2 (*d*)	2⁗′	71.9 (*d*)	71.9 (*d*)	70.6 (*d*)
17	50.5 (*s*)	50.5 (*s*)	50.1 (*s*)	3⁗′	72.1 (*d*)	72.1 (*d*)	71.8 (*d*)
18	42.7 (*d*)	42.7 (*d*)	42.1 (*d*)	4⁗′	73.9 (*d*)	73.7 (*d*)	71.2 (*d*)
19	48.2 (*t*)	48.2 (*t*)	47.9 (*t*)	5⁗′	71.2 (*d*)	71.2 (*d*)	72.4 (*d*)
20	30.9 (*s*)	30.9 (*s*)	31.5 (*s*)	6⁗′	18.5 (*q*)	18.5 (*q*)	62.9 (*t*)
21	37.3 (*t*)	37.3 (*t*)	36.6 (*t*)		6′-Butyl		HMG
22	31.5 (*t*)	31.5 (*t*)	32.2 (*t*)	1⁗″	66.4 (*t*)	66.4 (*t*)	172.8 (*s*)
23	210.8 (*d*)	210.8 (*d*)	182.2 (*s*)	2⁗″	31.9 (*t*)	31.9 (*t*)	47.0 (*t*)
24	11.0 (*q*)	11.0 (*q*)	11.9 (*q*)	3⁗″	20.2 (*t*)	20.2 (*t*)	71.3 (*s*)
25	16.5 (*q*)	16.5 (*q*)	16.7 (*q*)	4⁗″	14.2 (*q*)	14.2 (*q*)	47.4 (*t*)
26	18.2 (*q*)	18.2 (*q*)	17.9 (*q*)	5⁗″			175.3 (*s*)
27	27.3 (*q*)	27.3 (*q*)	27.4 (*q*)	6⁗″			28.1 (*q*)
28	177.3 (*s*)	177.3 (*s*)	177.1 (*s*)		3⁗′-*O*-Ac group		
29	33.6 (*q*)	33.6 (*q*)	33.5 (*q*)	1⁗‴	171.7 (*s*)	171.7 (*s*)	
30	25.4 (*q*)	25.4 (*q*)	25.2 (*q*)	2⁗‴	21.4 (*q*)	21.4 (*q*)	
	3-*O*-*β*-D-Glc-A		28-*O*-*β*-D-Glc		4⁗′-*O*-MC		
1′	103.9 (*d*)	103.9 (*d*)	95.3 (*d*)	1⁗⁗	168.6 (*s*)	167.6 (*s*)	
2′	78.3 (*d*)	78.3 (*d*)	73.0 (*d*)	2⁗⁗	115.7 (*d*)	116.6 (*d*)	
3′	86.5 (*d*)	86.5 (*d*)	89.8 (*d*)	3⁗⁗	147.4 (*d*)	146.1 (*d*)	
4′	71.2 (*d*)	71.2 (*d*)	69.5 (*d*)	4⁗⁗	128.4 (*s*)	128.8 (*s*)	
5′	76.7 (*d*)	76.7 (*d*)	77.7 (*d*)	5⁗⁗	131.4 (*d*)	133.8 (*d*)	
6′	170.4 (*s*)	170.4 (*s*)	70.0 (*t*)	6⁗⁗	115.7 (*d*)	114.7 (*d*)	
	2′-*O*-*β*-D-Gal		3′-*O*-*β*-D-Glc	7⁗⁗	163.6 (*s*)	162.5 (*s*)	
1″	104.7 (*d*)	104.7 (*d*)	105.4 (*d*)	8⁗⁗	115.7 (*d*)	114.7 (*d*)	
2″	74.2 (*d*)	74.2 (*d*)	74.9 (*d*)	9⁗⁗	131.4 (*d*)	133.8 (*d*)	
3″	75.5 (*d*)	75.5 (*d*)	77.9 (*d*)	4-MeO	56.3 (*q*)	56.1 (*q*)	
4″	70.9 (*d*)	70.9 (*d*)	82.6 (*d*)				
5″	76.7 (*d*)	76.7 (*d*)	74.9 (*d*)				
6″	62.5 (*d*)	62.5 (*d*)	68.7 (*t*)				

[a]) Assignments were made on the basis of HMBC and HMQC experiments.

and **2** were determinated as 3-*O*-[β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-6-*O*-butyl-β-D-glucuronopyranosyl quillaic acid 28-*O*-[α-L-rhamnopyranosyl(1 → 2)]-3-*O*-acetyl-4-*O*-[(*E*)-4-methoxycinnamoyl]-β-D-fucopyranosyl ester and its (*Z*)-isomer. They were given the trivial names viscidulosides A and B.



1



3

Fig. 2. *Key HMBCs of* **1** *and* **3**

Compound **3** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{66}H_{104}O_{35}$ by the *quasi*-molecular-ion peaks at *m/z* 1480 ($[M+Na+H]^+$; positive-ion mode) and 1456 (M^-; negative-ion mode) in ESI-MS spectra. Complete assignments of all C-atom signals in the ^{13}C-NMR spectrum of **3** were achieved using HMQC and HMBC data (*Table*). The ^{1}H-NMR spectrum of **3** showed signals for six quaternary Me groups at δ(H) 0.78 (*s*, Me(26)), 0.88 (*s*, Me(29)), 0.96 (*s*, Me(30)), 0.97 (*s*, Me(25)), 1.08 (*s*, Me(24)), and 1.38 (*s*, Me(27)), of two O-bearing CH goups at δ(H) 3.98 (*m*, H_β–C(3)) and 4.52 (br. *s*, H_α–C(16)), and of one olefinic H-atom at δ(H) 5.31 (br. *s*, H–C(12)). The structure of the triterpenoid moiety was established as 3β,16α-dihydroxyolean-12-en-23α,28β-dioic acid by comparison with literature data [9].

In the HMQC spectrum of **3**, anomeric H-atom signals of sugars at δ(H) 5.42 (*d*, *J* = 7.8, H–C(1′)), 4.51 (*d*, *J* = 7.8, H–C(1″)), 4.37 (*d*, *J* = 7.8, H–C(1‴)), 5.15 (*d*, *J* = 3.6, H–C(1⁗)), and 4.94 (*d*, *J* = 4.2, H–C(1⁗′)) correlated with δ(C) 95.3 (C(1′)), 105.4 (C(1″)), 105.1 (C(1‴)), 103.6 (C(1⁗)), and 100.6 (C(1⁗′)), respectively, indicating the presence of a pentaglycoside. The sugars were identified as three β-glucoses (at C(28), C(3′), and C(6′)), one α-mannose, and one α-galactose by the coupling-constant data of the anomeric H-atoms and other key resonances. The absolute configurations of the monosaccharide were confirmed to be D-glucose, D-mannose, and D-galactose [8]. Cross-peaks H–C(1″)/C(3′) (δ(C) 89.8), H–C(1‴)/C(6′) (δ(C) 70.0), H–C(1⁗)/C(4″) (δ(C) 82.6), H–C(1⁗′)/C(6″) (δ(C) 68.7), CH_2(6′) (δ(H) 3.80 and 4.05)/C(1‴), and CH_2(6″) (δ(H) 3.66 and 3.82)/C(1⁗′) in the HMBC spectrum confirmed the sugar chain to be {[α-D-mannopyranosyl-(1 → 4)][α-D-galactopyranosyl-(1 → 6)]-β-D-glucopyranosyl-(1 → 3)}[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside. In addition, the presence of a 3-hydroxy-3-methylglutaryl (HMG) group was deduced by the remaining NMR signals, including those of a *tert*-Me group (δ(H) 1.36 (*s*, Me(6⁗′)) and δ(C) 28.1 (C(6⁗′))), two CH_2 groups (δ(H) 2.68 (CH_2(2⁗′)) and δ(C) 47.0 (C(2⁗′))), δ(H) 2.55 (CH_2(4⁗′)) and δ(C) 47.4 (C(4⁗′))), and three quaternary C-atoms (δ(C) 71.3 (C(3⁗′)), 172.8 (C(1⁗′)), and 175.3 (C(5⁗′))), which were confirmed by HMBC and HMQC experiments. The location of the HMG group was assigned to be at C(6‴) by the correlation CH_2(6‴) (δ(H) 4.19 and 4.45)/C(1⁗′) (*Fig. 2*), which was also supported by the presence of a deshielded signal of C(6‴) (2.1 ppm) in the ^{13}C-NMR spectrum. The sugar chain was connected to C(28) based on the correlation H–C(1′)/C(28) (δ(C) 177.1) in the HMBC spectrum.

The absolute configuration at C(3) of HMG of **3** was suggested as (*R*) by comparing the ^{13}C-NMR data of C(1) to C(6) of HMG (δ(C) 172.8 (C(1)), 47.0 (C(2)), 71.3 (C(3)), 47.4 (C(4)), 175.3 (C(5)), and 28.1 (C(6))) with those of sinocrassuloside II (δ(C) 171.7 (C(1)), 46.5 (C(2)), 70.7 (C(3)), 46.4 (C(4)), 174.6 (C(5)), and 28.2 (C(6))) [6], while the (*S*)-configuration at C(3) of HMG in dianversicoside A was based on the signals at δ(C) 172.6 (C(1)), 41.2 (C(2)), 71.0 (C(3)), 41.2 (C(4)), 172.6 (C(5)), and 28.4 (C(6)) [9]. The configuration at C(3) of HMG in sinocrassuloside II and dianversicoside A were established by *Fujimoto*'s method [10]. On the basis of the above evidence, the structure of **3** was elucidated as 3β,16α-dihydroxyolean-12-en-23α,28β-dioic acid 28-*O*-{[α-D-mannopyranosyl-(1 → 4)][α-D-galactopyranosyl-(1 → 6)]-β-D-glucopyranosyl-(1 → 3)}[β-D-6-*O*-((3*R*)-3-hydroxy-3-methylglutaryl)glucopyranosyl-(1 → 6)]-β-D-glucopyranoside, named silenoviscoside D.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China), *ODS* (50 μm; *Nacalai Tesque Inc.*, Japan). TLC: SiO_2 (10–40 μm; *Qingdao Marine Chemical Inc.*, P. R. China); visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Semiprep. HPLC: *Shimadzu LC-10AT* pump, *RID-10A* detector, *YMC-*C_{18}, 10 mm × 30 cm, flow rate: 1.5 and 1.2 ml/min. GC/MS: *Shimadzu GC-MS-QP5050*, *DB-1*, 0.25 mm × 30 m × 0.25 μm. Optical rotations: *Perkin-Elmer 241 MC* digital polarimeter. NMR Spectra: *Bruker AV-600* spectrometer, (D_4)MeOH (δ(H) 3.31 and δ(C) 49.15) as internal standard, δ in ppm, *J* in. ESI-MS: *Quattro Micro* mass spectrometer, in *m*/*z*.

Plant Material. The roots of *S. viscidula* were collected on July 10, 2006, from Kunming, Yunnan Province, P. R. China. The botanical identification was conducted by *Y.-S. L.* (Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, P. R. China). A voucher specimen (QGT 20060710) is deposited with the Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, P. R. China.

Extraction and Isolation. The dried roots of *S. viscidula* (3.5 kg) were ground and extracted with 70% EtOH (3 × 5 l) at 80°. The EtOH extract (700 g) was suspended in H_2O (3 l) and extracted with petroleum ether (PE; 3 × 3 l), AcOEt (3 × 3 l), and BuOH (3 × 3 l), successively. The BuOH-soluble extract (100 g) was subjected to CC (SiO_2 (900 g); AcOEt/MeOH gradient 100 : 0 → 0 : 100) to yield 14 fractions (*Frs. A–N*). *Fr. G* (4.5 g; AcOEt/MeOH 4 : 1) was subjected CC (open *ODS* (100 g); with MeOH/H_2O 0 : 100, 10 : 90, 30 : 70, 50 : 50, 70 : 30, 100 : 0) to yield six fractions (*Frs. GI–GVI*). *Fr. GVI* (350 mg; MeOH/H_2O 100 : 0), which was purified by semiprep. HPLC (MeCN/H_2O 55 : 45, containing 0.1% TFA, flow rate of 1.5 ml/min), gave **1** (11.5 mg; t_R 125.2 min), **2** (10.0 mg; t_R 141.2 min), **4** (28.0 mg; t_R 48.7 min), and **5** (40.0 mg; t_R 52.4 min). *Fr. J* (3.5 g, AcOEt/MeOH 2 : 1) was subjected to CC (open *ODS* (80 g); MeOH/H_2O 0 : 100, 10 : 90, 30 : 70, 50 : 50, 70 : 30, 100 : 0) to yield seven fractions (*Frs. JI–JVII*). *Fr. JII* (90.0 mg; MeOH/H_2O 30 : 70) was purified by semiprep. HPLC (MeOH/H_2O 58 : 42, containing 0.2% AcOH, flow rate of 1.2 ml/min) to afford **3** (10.0 mg, t_R 180.1 min).

Visciduloside A (= *3*-O-*Acetyl-6-deoxy-2*-O-*(6-deoxy-α*-L-*mannopyranosyl)-1*-O-*[(3β,16α)-3-{[β*-D-*galactopyranosyl-(1 → 2)-[β*-D-*xylopyranosyl-(1 → 3)]-6-butyl-β*-D-*glucopyranuronosyl]oxy}-16-hydroxy-23,28-dioxoolean-12-en-28-yl]-4*-O-*[(2*E*)-3-(4-methoxyphenyl)prop-2-enoyl]-β*-D-*galactopyranose*; **1**). White amorphous powder. ^{1}H-NMR (600 MHz, CD_3OD): 0.81 (*s*, Me(26)); 0.89 (*s*, Me(29)); 0.94 (*t*, *J* = 7.2, Me(4‴‴)); 0.98 (*s*, Me(30)); 0.99 (*s*, Me(25)); 1.10 (*s*, Me(24)); 1.14 (*d*, *J* = 8.4, Me(6‴)); 1.24 (*d*, *J* = 6.0, Me(6⁗)); 1.39 (*s*, Me(27)); 1.41–1.43 (CH_2(3⁗)); 1.63–1.65 (CH_2(2⁗)); 2.03 (*s*, Me(2⁗)); 2.99 (*dd*, *J* = 14.2, 2.5, H–C(18)); 3.84 (*s*, MeO–C(7⁗)); 3.91–4.09 (*m*, H_β–C(3)); 4.19 (*t*, *J* = 6.0, CH_2(1⁗)); 4.41–4.49 (*m*, H_α–C(16)); 4.46 (*d*, *J* = 7.2, H–C(1″)); 4.57 (*d*, *J* = 7.8, H–C(1′)); 4.78 (*d*, *J* = 6.6, H–C(1″)); 4.96 (br. *s*, H–C(1‴)); 5.39 (br. *s*, H–C(12)); 5.54 (*d*, *J* = 7.8, H–C(1‴)); 6.48 (*d*, *J* = 16.2, H–C(2⁗)); 6.97 (*d*, *J* = 8.4, H–C(6⁗), H–C(8⁗)); 7.60 (*d*, *J* = 9.0, H–C(5⁗), H–C(9⁗)); 7.72 (*d*, *J* = 16.2, H–C(3⁗)); 9.42 (*s*, H–C(23)). ^{13}C-NMR (150 MHz, CD_3OD): *Table*. ESI-MS (neg.): 1541 ($[M+Cl]^-$). ESI-MS (pos.): 1530 ($[M+Na+H]^+$), 776, 281.

Visciduloside B (= *3*-O-*Acetyl-6-deoxy-2*-O-*(6-deoxy-α*-L-*mannopyranosyl)-1*-O-*[(3β,16α)-3-{[β*-D-*galactopyranosyl-(1 → 2)-[β*-D-*xylopyranosyl-(1 → 3)]-6-butyl-β*-D-*glucopyranuronosyl]oxy}-16-hydroxy-23,28-dioxoolean-12-en-28-yl]-4*-O-*[(2*Z*)-3-(4-methoxyphenyl)prop-2-enoyl]-β*-D-*galactopyranose*; **2**). White amorphous powder. ^{1}H-NMR (600 MHz, CD_3OD): 0.80 (*s*, Me(26)); 0.89 (*s*, Me(29)); 0.98 (*s*, Me(30)); 0.93 (*t*, *J* = 7.2, Me(4‴‴)); 0.99 (*s*, Me(25)); 1.11 (*s*, Me(24)); 1.12 (*d*, *J* = 8.4, Me(6‴)); 1.22 (*d*, *J* = 6.0, Me(6⁗)); 1.39 (*s*, Me(27)); 1.41–1.43 (CH_2(3⁗)); 1.63–1.65 (CH_2(2⁗)); 1.99 (*s*, Me(2⁗)); 2.96 (*dd*, *J* = 14.2, 2.5, H–C(18)); 3.82 (*s*, MeO–C(7⁗)); 3.90–4.06 (*m*, H_β–C(3)); 4.19 (*t*, *J* = 6.0, CH_2(1⁗)); 4.41–4.49 (*m*, H_α–C(16)); 4.46 (*d*, *J* = 7.2, H–C(1″)); 4.57 (*d*, *J* = 7.8, H–C(1′)); 4.78 (*d*, *J* = 6.6, H–C(1″)); 4.93 (br. *s*, H–C(1‴)); 5.32 (br. *s*, H–C(12)); 5.50 (*d*, *J* = 7.8,

H–C(1‴‴)); 5.93 (*d*, *J* = 12.6, H–C(2‴‴‴‴)); 6.92 (*d*, *J* = 9.0, H–C(6‴‴‴‴), H–C(8‴‴‴‴)); 6.99 (*d*, *J* = 12.6, H–C(3‴‴‴‴)); 7.77 (*d*, *J* = 9.0, H–C(5‴‴‴‴), H–C(9‴‴‴‴)); 9.41 (*s*, H–C(23)). ^{13}C-NMR (150 MHz, CD_3OD): *Table*. ESI-MS (neg.): 1541 ($[M+Cl]^-$). ESI-MS (pos.): 1530 ($[M+Na+H]^+$), 776, 281.

Silenoviscoside D (=*6-*O*-[(3*R*)-4-Carboxy-3-hydroxy-3-methylbutanoyl]-β-*D*-glucopyranosyl-(1 → 6)-[α-*D*-galactopyranosyl-(1 → 6)-[α-*D*-mannopyranosyl-(1 → 4)]-β-*D*-glucopyranosyl-(1 → 3)]-1-*O*-[(3β,16α)-3,16,23-trihydroxy-23,28-dioxoolean-12-en-28-yl]-β-*D*-glucopyranose*; **3**). Amorphous white powder. $[\alpha]_D^{20} = +13.3$ ($c = 0.025$, MeOH). ^{1}H-NMR (600 MHz, CD_3OD): 0.78 (*s*, Me(26)); 0.88 (*s*, Me(29)); 0.96 (*s*, Me(30)); 0.97 (*s*, Me(25)); 1.08 (*s*, Me(24)); 1.36 (*s*, Me(6‴‴‴)); 1.38 (*s*, Me(27)); 2.48–2.62 (CH_2(4‴‴‴)); 2.64–2.72 (CH_2(2‴‴‴)); 3.00 (*dd*, *J* = 14.2, 3.8, H–C(18)); 3.97–3.99 (*m*, H_β–C(3)); 4.37 (*d*, *J* = 7.8, H–C(1‴)); 4.51 (*d*, *J* = 7.8, H–C(1′)); 4.52 (br. *s*, H_α–C(16)); 4.94 (*d*, *J* = 4.2, H–C(1‴‴)); 5.15 (*d*, *J* = 3.6, H–C(1‴‴)); 5.31 (br. *s*, H–C(12)); 5.42 (*d*, *J* = 7.8, H–C(1′)). ^{13}C-NMR (150 MHz, CD_3OD): *Table*. ESI-MS (neg.): 1456 (M^-). ESI-MS (pos.): 1480 ($[M+Na+H]^+$), 954, 844, 619, 497, 414, 301, 275.

Sugar Analysis. A soln. of each saponin (3.0 mg each) in 1.0M HCl (dioxane/H_2O 1:1; 1 ml) was heated at 100° for 2 h in a H_2O bath. After removal of dioxane under vacuum, the soln. was extracted with AcOEt (3 × 1 ml). The H_2O-soluble part containing monosaccharides was neutralized by passing through an ion-exchange resin (*Amberlite MB-3*) column. The neutralized soln. was concentrated to dryness, and the residue was dissolved in pyridine (each 2 ml). L-Cysteine methyl ester hydrochloride (2 mg each) was added to the pyridine soln. subsequently. The mixture was kept at 60° for 1.5 h, dried under vacuum, and trimethylsilylated with 1-(trimethylsilyl)-1*H*-imidazole (0.1 ml) at 60° for 1 h. The mixture was suspended in H_2O (1 ml), then extracted with hexane (3 × 1 ml) [8]. The supernatant was analyzed by GC/MS under the following conditions: cap. column, *DB-1* (30 m × 0.25 mm × 0.25 μm); column temp., 230°; carrier gas, He at 0.8 ml/min, split ratio 1/10; injection temp., 250°; detection temp., 220°; injection volume, 0.5 μl. Compounds **1**, **2**, **4**, and **5** gave D-Glc A, D-Gal, D-Xyl, D-Fuc, and L-Rha at t_R 11.08, 13.88, 9.72, 10.75, and 10.21 min (identical to authentic standards), resp. Under the same conditions, compound **3** gave D-Glc, D-Gal, and D-Man at t_R 14.10 min, 13.88 min and 13.65 min, resp.

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