Four New Triterpenoid Saponins from *Conyza blinii*

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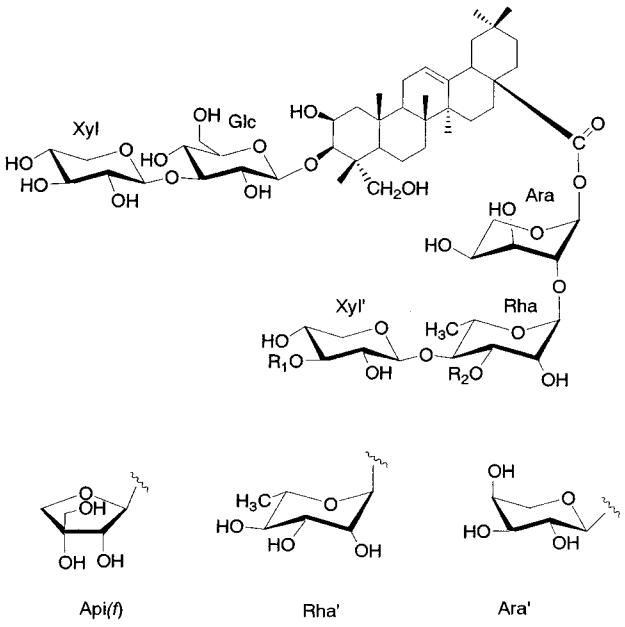
Three new bisdesmosidic saponins named conyzasaponins A, B, and C (**1**−**3**) and one new monodesmosidic saponin, conyzasaponin G (**4**), were isolated from the aerial parts of *Conyza blinii*. Their structures were elucidated on the basis of extensive NMR (DEPT, DQF-COSY, HOHAHA, HMQC, HMBC, and NOESY) and MS studies. Compounds **1**−**3** share a common prosapogenin, bayogenin 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranoside, which is identical with conyzasaponin G (**4**), and differ in the structures of the ester-linked sugar moieties at C-28. Conyzasaponin A (**1**) is the 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester, conyzasaponin B (**2**), the 28-*O*-*β*-D-apiofurano- syl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester, and conyzasaponin C (**3**), the 28-*O*-α-L-rhamnopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester of the prosapogenin, respectively.

Conyza blinii Lévl. (Asteraceae), commonly called "Jin Long Dan Cao", is distributed in the southwest region of the People's Republic of China. Its aerial parts are used in folk medicine for the treatment of chronic bronchitis and other inflammatory diseases. Preliminary pharmacological and clinical tests have shown that its polar extracts possess antibacterial, antiinflammatory, antitussive, antiulcer, and expectorant effects.[1] Phytochemical studies on *C. blinii* have led to the isolation of several types of compounds such as diterpenoids, flavonoids, and triterpenoids[2] which are regarded as characteristic constituents of the plants of the genus *Conyza*.[3] However, until now there has been no report on the saponins of *Conyza* species, although a number of triterpenoidal saponins were isolated from the plants of other genera in the Asteraceae. The medicinal uses and pharmacological activity of *C. blinii* prompted us to investigate its polar components, resulting in the isolation of four new triterpenoidal saponins named conyzasaponins A, B, C, and G (**1**−**4**). The present paper deals with the isolation and structural elucidation of these new saponins.

Results and Discussion

Conyzasaponin A (**1**) was obtained as a white amorphous powder. Its molecular formula $C_{62}H_{100}O_{30}$ was determined from the $[M + Na]^+$ ion at *m*/*z* 1347 and $[M + K]^+$ ion at *m*/*z* 1363 in the MALDI-TOF MS (positive-ion mode) and from its ^{13}C, DEPT NMR spectral data. The IR spectrum exhibited absorptions at 3402 cm^{-1} (OH) and 1739 cm^{-1} (ester carbonyl). The six tertiary methyl groups (*δ* 0.90, 0.98, 1.13, 1.24, 1.33, and 1.53) and one trisubstituted olefinic proton (*δ* 5.46, br s) observed in the ^1H NMR spectrum coupled with the information from the ^{13}C NMR spectrum (six sp^3 carbons at *δ* 15.0, 17.3, 17.6, 23.7, 26.1, and 33.1 and two sp^2 olefinic carbons at *δ* 123.1 and 144.2) indicated that the aglycon possesses an olean-12-ene skeleton. After an extensive 2D NMR study, the aglycon was identified as bayogenin (2*β*,3*β*,23-trihydroxyolean-12-en-28-oic acid), a common aglycon that occurs in a number of plants in the family Asteraceae.[4,5] The downfield chemical shifts of C-3 (*δ* 83.0) and C-28 (*δ* 176.3) indicated that **1** is a bisdesmosidic glycoside (Table 1). Of the 62 carbons signals observed in the ^{13}C NMR spectrum of **1**, 30 were assigned to the aglycon part, and the remaining 32 to the oligosaccharide moiety, which consisted of two hexose and four pentose units. The ^1H and ^{13}C NMR spectra of **1** exhibited six sugar anomeric protons at *δ* 5.05 (d, *J* = 7.4 Hz), 5.15 (d, *J* = 7.3 Hz), 5.20 (d, *J* = 7.3 Hz), 5.78 (br s), 6.22 (d, *J* = 2.3 Hz), and 6.46 (d, *J* = 2.8 Hz) and carbons at *δ* 93.4, 101.0, 105.4, 106.3, 106.8, and 111.2 (Tables 2 and 3). The methyl carbon signal at *δ* 18.4 and the doublet methyl proton signal at *δ* 1.75 (3H, *J* = 6.0 Hz) indicated the presence of one 6-deoxy sugar. Acidic hydrolysis af-



1 R_1=Api(*f*), R_2=H

2 R_1=Api(*f*), R_2=Ara'

3 R_1=Rha', R_2= Api(*f*)

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Table 1. ^{13}C NMR Spectral Data for the Aglycon Moieties of **1**−**4** (125 MHz in pyridine-d_5)

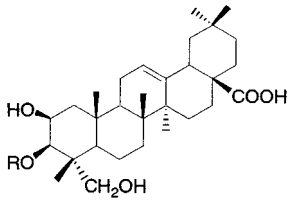
carbon	1	2	3	4	carbon	1	2	3	4
1	44.2	44.2	44.3	44.1	16	23.2	23.2	23.2	23.6
2	70.7	70.8	70.8	70.8	17	47.4	47.4	47.4	46.6
3	83.0	83.0	82.9	82.7	18	41.7	41.7	41.7	41.9
4	42.8	42.9	42.9	42.8	19	46.2	46.2	46.2	46.3
5	47.6	47.4	47.7	47.5	20	30.9	30.9	30.9	30.9
6	18.1	18.0	18.1	17.9	21	34.1	34.2	34.2	34.1
7	32.9	33.0	33.1	32.9	22	32.7	32.8	32.8	33.2
8	40.0	40.1	40.1	39.8	23	65.1	65.2	65.1	64.8
9	48.5	48.5	48.6	48.5	24	15.0	15.0	15.1	15.0
10	36.9	37.0	37.0	36.9	25	17.3	17.3	17.3	17.1
11	24.0	24.1	24.0	23.9	26	17.6	17.6	17.7	17.4
12	123.1	123.1	123.2	122.7	27	26.1	26.2	26.2	26.2
13	144.2	144.2	144.2	144.8	28	176.3	176.3	176.3	180.1
14	42.3	42.3	42.3	42.2	29	33.1	33.2	33.2	33.2
15	28.3	28.2	28.3	28.2	30	23.7	23.8	23.7	23.7

Table 2. ^{13}C NMR Spectral Data for the Sugar Moieties of **1**−**4** (125 MHz in pyridine-d_5)[a]

	1	2	3	4		1	2	3
Glc at C-3					Xyl′			
1	105.4	105.5	105.5	105.5	1	106.8	104.8	104.9
2	74.3	74.4	74.4	74.3	2	75.2[b]	75.1[b]	75.8[b]
3	87.7	87.7	87.7	87.6	3	84.8	85.4	83.5
4	69.4	69.4	69.4	69.3	4	69.4	69.7	69.6
5	77.9	78.0	78.0	77.9	5	66.9	66.5	67.1
6	62.2	62.3	62.3	62.1	Api(*f*)			
Xyl					1	111.2	111.3	112.0
1	106.3	106.4	106.3	106.3	2	77.7	77.7	77.5
2	75.3[b]	75.3[b]	75.3[b]	75.3	3	80.4	80.4	79.8
3	78.2	78.2	78.2	78.2	4	75.1	75.1	74.7
4	70.7	70.9	70.9	70.8	5	65.4	65.5	64.4
5	67.4	67.4	67.4	67.4			Ara′	Rha′
Ara at C-28					1		106.1	102.8
1	93.4	93.3	93.0		2		73.0	72.5
2	75.2	75.3	75.7		3		74.4	72.7
3	70.0	69.1	68.7		4		69.6	74.2
4	66.1	65.7	65.2		5		67.2	69.9
5	63.0	62.5	61.9		6			18.6
Rha								
1	101.0	100.9	101.0					
2	71.9	71.7	71.6					
3	72.7	82.3	82.5					
4	84.0	78.1	77.7					
5	68.5	68.7	68.8					
6	18.4	18.6	18.6					

[a] Assignments are based on DEPT, DQF-COSY, HMQC, HOHAHA, HMBC, and NOESY experiments. [b] Assignments may be interchangeable.

forded bayogenin (**1a**),[4,5] and the monosaccharide components were identified as glucose, xylose, arabinose, rhamnose, and apiose by GLC analysis. Alkaline hydrolysis also afforded a prosapogenin, which was identical with conyzasaponin G (**4**), namely, bayogenin 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranoside, as deduced from direct comparison of their ^1H NMR spectra and by co-TLC and co-HPLC analysis. From the above evidence, it was concluded that **1** is a bisdesmosidic triterpenoid glycoside with glucose and xylose linked to the C-3 position of the aglycon and the other four monosaccharides linked to the C-28 of the aglycon through an ester bond.



1a R= H

4 R= xyl(1→3)-glc

The identity of the monosaccharides and the sequence of the oligosaccharide chains in **1** were determined by a combination of DEPT, DQF-COSY, HOHAHA, HMQC, HMBC, and phase-sensitive NOESY experiments. The individual spin systems could be discerned from the spectra corresponding to the anomeric protons or methyl group (for the deoxy sugar) in the HOHAHA experiment. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were delineated using COSY with the aid of 2D-HOHAHA and phase-sensitive NOESY spectra. Information from COSY and 2D-HOHAHA furnished most of the assignments. On the basis of the assigned protons, a HMQC experiment then gave the corresponding carbon assignments and was further confirmed by HMBC (Tables 2 and 3). The apiofuranosyl group was easily identified by its C-3 being a quaternary carbon and C-4 and C-5 being two methylenes. After all of the proton and carbon signals were assigned, the six sugar units were identified as two xylose units, one glucose, one arabinose, one rhamnose, and one apiose and were confirmed by GLC and co-TLC analysis. The linkage of the sugar units at C-3 was established from the HMBC correlations of H-1 (δ 5.20) of Xyl with C-3 (δ 87.7) of Glc, and the long-range HMBC correlation between H-1 (δ 5.15) of Glc and C-3 (δ 83.0) of the aglycon, which confirmed the attachment of the disaccharide moiety to C-3 of the aglycon. The sequence of the sugar chain at C-28 was determined by the following HMBC correlations: H-1 (δ 5.78) of Rha

Table 3. ^{1}H NMR Spectral Data for the Sugar Moieties of **1**−**3** (500 MHz in pyridine-d_5)[a]

	1	2	3
Glc at C-3			
1	5.15 d (7.3)	5.15 d (7.6)	5.13 d (7.6)
2	4.02	4.03	4.04
3	4.06	4.08	4.05
4	4.08	4.11	4.12
5	3.85	3.84	3.83
6	4.27, 4.40	4.26, 4.42	4.28, 4.41
Xyl			
1	5.20 d (7.3)	5.20 d (7.8)	5.19 d (7.6)
2	4.00	4.01	4.02
3	4.16	4.16	4.13
4	4.18	4.15	4.14
5	3.70, 4.32	3.70, 4.31	3.70, 4.32
Ara at C-28			
1	6.46 d (2.8)	6.52 (br s)	6.55 (br s)
2	4.55	4.55	4.48
3	4.53	4.55	4.58
4	4.40	4.43	4.36
5	3.95, 4.51	3.92, 4.53	3.96, 4.56
Rha			
1	5.78 (br s)	5.70 d (0.9)	5.57 (br s)
2	4.57	4.82	4.72
3	4.56	4.57	4.37
4	4.35	4.50	4.48
5	4.36	4.41	4.30
6	1.75 d (6.0)	1.76 d (6.0)	1.73 d (6.2)
Xyl′			
1	5.05 d (7.4)	5.36 d (7.8)	5.27 d (7.8)
2	4.01	3.94	3.93
3	4.05	4.01	4.24
4	4.04	4.01	4.10
5	3.40, 4.18	3.18, 4.08	3.39, 4.09
Api(*f*)			
1	6.22 d (2.3)	6.18 d (2.3)	5.89 d (4.4)
2	4.80 d (2.3)	4.78	4.77
3			
4	4.32, 4.73	4.27, 4.71	4.17, 4.56
5	4.18	4.15	4.04
		Ara′	Rha′
1		5.06 d (7.6)	6.15 (br s)
2		4.44	4.73
3		4.00	4.55
4		4.15	4.27
5		3.52, 4.09	4.92
6			1.64 d (6.2)

[a] Assignments are based on DEPT, DQF-COSY, HMQC, HOHAHA, HMBC, and PS-NOESY experiments, with coupling constants (*J*) in parentheses.

with C-2 (δ 75.2) of Ara, H-1 (δ 5.05) of Xyl′ with C-4 (δ 84.0) of Rha, and H-1 (δ 6.22) of Api(*f*) with C-3 (δ 84.8) of Xyl′. The attachment of the tetrasaccharide moiety to C-28 of the aglycon was based on a HMBC correlation of H-1 (δ 6.46) of Ara with C-28 (δ 176.3) of the aglycon. All the monosaccharides except apiose were determined to be in the pyranose form from their ^{13}C NMR data. The two xyloses and the glucose were deduced to be in the β configuration, and the rhamnose and the arabinose in the α configuration, on the basis of their $^3J_{H-1,H-2}$ coupling constants and the NOE and HMBC information obtained.[6] The apiose was determined to be in the β configuration by comparing its spectroscopic data with literature value.[7] Analysis of all the above evidence led to the identification of conyzasaponin A (**1**) as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl bayogenin 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Conyzasaponin B(**2**), white needles (MeOH), was assigned a molecular formula of $C_{67}H_{108}O_{34}$, as determined from its $[M + Na]^+$ ion at *m*/z 1479 and $[M + K]^+$ ion at *m*/z 1495 in the MALDI-TOF MS (positive-ion mode) and

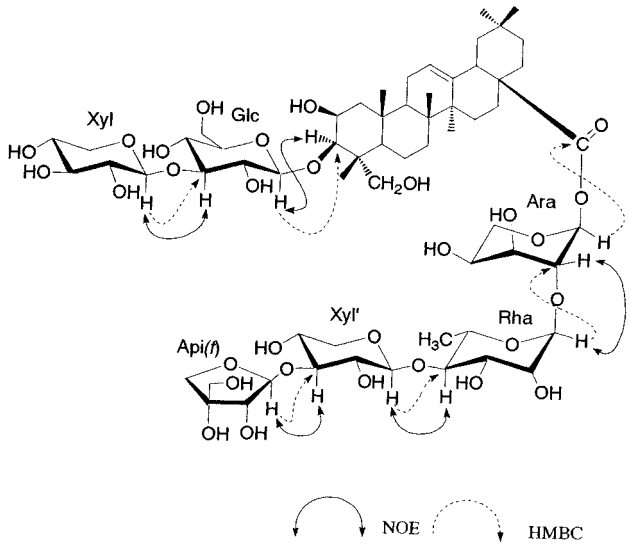


Figure 1. Sequence and linkage positions for the sugar moieties of compound **1** determined by HMBC and NOESY experiments.

from its ^{13}C, DEPT NMR spectral data. Its molecular weight was 132 mass units higher than that of **1**, which indicated the presence of one additional pentose unit. The occurrence of seven sugars in **2** was confirmed by the observation of seven anomeric protons [δ 5.06 d (J = 7.6 Hz), 5.15 d (J = 7.6 Hz), 5.20 d (J = 7.8 Hz), 5.36 d (J = 7.8 Hz), 5.70 d (J = 0.9 Hz), 6.18 d (J = 2.3 Hz), and 6.52 (br s)] and carbons [δ 93.3, 100.9, 104.8, 105.5, 106.1, 106.4, and 111.3]. The results of co-TLC analysis showed that the sugar components of **1** and **2** were identical. Comparison of the NMR spectra of **2** with those of **1** led to the conclusion that they share the common aglycon and prosapogenin (see Table 1) and also indicated that the remaining five sugars were connected to C-28. The overall structural identification and NMR assignments were accomplished using the same protocol as for **1**. Accordingly, the seven sugar units were determined to be two α-arabinopyranoses, two β-xylopyranoses, one β-glucopyranose, one α-rhamnopyranose, and one β-apiofuranose (see Tables 2 and 3). The exact linkage positions for the sugar units were established using HMBC and NOESY correlations, as described for **1**. Thus, conyzasaponin B was elucidated as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl bayogenin 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Conyzasaponin C (**3**), white needles (MeOH), displayed a $[M + Na]^+$ ion at *m*/z 1493 and a $[M + K]^+$ ion at *m*/z 1509 in the MALDI-TOF MS (positive-ion mode), consistent with the molecular formula $C_{68}H_{110}O_{34}$. The seven anomeric protons at δ 5.13 d (J = 7.6 Hz), 5.19 d (J = 7.6 Hz), 5.27 d (J = 7.8 Hz), 5.57 (br s), 5.89 d (J = 4.4 Hz), 6.15 (br s), and 6.55 (br s) in the ^{1}H NMR spectrum and the seven anomeric carbons at δ 93.0, 101.0, 102.8, 104.9, 105.5, 106.3, and 112.0 in the ^{13}C NMR spectrum showed that **3** contains seven sugar moieties. Two of those are 6-deoxy monosaccharides, as indicated by the two methyl carbons at δ 18.6 and two methyl proton doublets at δ 1.73 (J = 6.2 Hz) and 1.64 (J = 6.2 Hz), respectively. The results of co-TLC analysis indicated that compounds **1** and **3** possess the same sugar components. The NMR signals due to the aglycon and the oligosaccharide unit at the C-3 position of the aglycon were superimposable on those of **1** and **2**, demonstrating that the three compounds share the common aglycon and the same disaccharide chain at C-3 of the aglycon (see Tables 1−3). Thus, the sugar moiety at C-28

of the aglycon was a pentasaccharide chain in **3**. The overall structural assignment was accomplished using the same procedure as described in **1**. Extensive NMR (DQF-COSY, HOHAHA, HMQC, HMBC, NOESY) studies permitted the full assignments of the protons and carbons of **3**. The exact linkage positions for the sugar units were established using the HMBC and NOESY correlations as described for **1**. Hence, the structure of conyzasaponin C was established as 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl bayogenin 28-*O*-α-L-rhamnopyranosyl-(1→3)-*β*-D-xylopyra-nosyl-(1→4)-[*β*-D-apiofuranosyl (1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Conyzasaponin G (**4**), a white amorphous powder (MeOH), was assigned a molecular weight of 782 Da, as indicated by its $[M - H]^-$ ion at *m*/z 781 in the FABMS (negative-ion mode). The presence of two monosaccharides in **4** was demonstrated by the two anomeric proton signals at *δ* 5.17 d (J = 7.5 Hz) and 5.20 d (J = 8.0 Hz) in the ^{1}H NMR spectrum and two anomeric carbon signals at *δ* 105.5 and 106.3 in the ^{13}C NMR spectrum. Co-TLC analysis indicated that the two sugars were glucose and xylose. Comparison of the ^{1}H and ^{13}C NMR spectra of **4** and **1** revealed that the aglycon parts and the disaccharide chains attached to C-3 of the aglycons of the two compounds (see Tables 1 and 2) were the same, with **4** having a free carboxyl group indicated by the chemical shift (*δ* 180.1) of C-28 of the aglycon. The $[M - Xyl - H]^-$ ion at *m*/z 649 and $[M - Xyl - Glc - H]^-$ ion at *m*/z 487 in the FABMS of **4** confirmed the above deduction. Therefore, **4** was a monodesmosidic saponin and was determined structurally as bayogenin 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranoside.

Experimental Section

General Experimental Procedures. Melting points were measured with a XT4A micro-melting point apparatus and are uncorrected. Optical rotations were measured using an AA-10R automatic polarimeter. The IR spectra were recorded on a Perkin-Elmer IR spectrometer. The ^{1}H and ^{13}C NMR spectra were measured on either a JEOL ECP-500 or INOVA 500 FT-NMR spectrometer in pyridine-d_5 solution, and chemical shifts are expressed in *δ* (ppm) referring to TMS. MALDI-TOF MS and FAB MS were conducted using a PerSeptive Biosystems Voyager DESTR and KYKY-ZHP-5# mass spectrometer, respectively. The resin D101 (Tianjin Chemical Co.), Si gel (200−300 mesh and Type 60, Qingdao Marine Chemical Co.), and ODS (Chromatorex DM1020T, 100−200 mesh) were used for open column chromatography. HPLC was performed using an ODS column (PEGASIL ODS-2, Senshu Pak, 20 mm i.d. × 150 mm; detector, UV 210 nm), respectively. GLC: Shimadzu GC-7A; column, Silicone OV-17 on Uniport HP (80−100 mesh), 3 mm i.d. × 2.1 m; column temperature, 160 °C; carrier gas, N_2; flow rate, 30 mL/min.

Plant Material. The aerial parts of *Conyza blinii* were collected from Sichuan Province, People's Republic of China in August 1996 and a voucher specimen (No. 960818) has been deposited at the Herbarium of School of Pharmaceutical Sciences, Peking University, Beijing, People's Republic of China.

Extraction and Isolation. The air-dried powdered plant material (20 kg) was refluxed with 95% ethanol twice and then with 60% ethanol once. The 95% ethanol extract was subjected to Si gel column chromatography and eluted in turn with petroleum ether, chloroform, ethyl acetate, and methanol. The methanol eluate and the 60% ethanol extract were treated with acetone supersonically, then the insoluble parts were combined and chromatographed over D101 resin column eluting with H_2O and 30, 50, 70, and 95% ethanol. The 50% ethanol eluate (150 g) was chromatographed on Si gel, eluting with $CHCl_3$−MeOH−H_2O in a gradient manner. Fraction 61, eluted with $CHCl_3$−MeOH−H_2O (70:30:10, lower layer), was chromatographed over an ODS column eluting with 30−70% MeOH in H_2O to afford **1** (500 mg). Fractions 83−85, eluted with $CHCl_3$−MeOH−H_2O (65:35:10, lower layer), were pooled and recrystallized with MeOH−H_2O to yield a white crystalline solid. The white crystalline solid was subjected to ODS column chromatography and reverse-phase HPLC purification (65% MeOH in H_2O) to furnish **2** (40 mg) and **3** (35 mg). The 70% ethanol eluate (25 g) was chromatographed on Si gel, eluting with EtOAc−EtOH−H_2O in a gradient manner, and fractions 64−65, eluted with EtOAc−EtOH−H_2O (70:30:3), gave **4** (15 mg) after further Si gel column chromatography with the same eluting system.

Conyzasaponin A (1): white amorphous powder from MeOH; mp 219−220 °C; $[\alpha]^{25}_D$ −13° (*c* 0.94, MeOH); IR ν_{max} (KBr) 3402, 2924, 1739, 1640, 1036 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon *δ* 5.46 (1H, br s, H-12), 4.80 (1H, br s, H-2), 4.32 (1H, d, J = 4.4 Hz, H-3), 3.71, 4.35 (each 1H, d, J = 10.3 Hz, H_2-23), 3.27 (1H, dd, J = 13.3, 3.7 Hz, H-18), 1.53, 1.33, 1.24, 1.13, 0.98, 0.90 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1−3; MALDI-TOF MS (positive-ion mode) *m*/*z* 1347 $[M + Na]^+$ and 1363 $[M + K]^+$.

Conyzasaponin B (2): white needles from MeOH; mp 233−234 °C; $[\alpha]^{25}_D$ +6° (*c* 0.63, MeOH); IR ν_{max} (KBr) 3404, 2936, 1746, 1637, 1037 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon *δ* 5.47 (1H, br s, H-12), 4.78 (1H, br s, H-2), 4.33 (1H, d, J = 3.5 Hz, H-3), 3.71, 4.35 (each 1H, d, J = 10.5 Hz, H_2-23), 3.29 (1H, dd, J = 13.8, 4.2 Hz, H-18), 1.57, 1.34, 1.24, 1.16, 1.00, 0.90 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data see Tables 1−3; MALDI-TOF MS (positive-ion mode) *m*/*z* 1479 $[M + Na]^+$ and 1495 $[M + K]^+$.

Conyzasaponin C (3): white needles from MeOH; mp 225−226 °C; $[\alpha]^{25}_D$ −20° (*c* 0.59, MeOH); IR ν_{max} (KBr) 3404, 2936, 1746, 1637, 1037 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon *δ* 5.46 (1H, br s, H-12), 4.77 (1H, br s, H-2), 4.32 (1H, d, H-3), 3.69, 4.32 (1H each, d, J = 10.1 Hz, H_2-23), 3.30 (1H, dd, J = 13.5, 3.9 Hz, H-18), 1.55, 1.33, 1.23, 1.13, 1.02, 0.91 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1−3; MALDI-TOF MS (positive-ion mode) *m*/*z* 1493 $[M + Na]^+$ and 1509 $[M + K]^+$.

Conyzasaponin G (4): a white amorphous powder from MeOH; mp 214−216 °C; IR ν_{max} (KBr) 3410, 2923, 1692, 1039 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon *δ* 5.48 (1H, br s, H-12), 4.80 (1H, br s, H-2), 3.69, 4.31 (1H each, d, J = 10.5 Hz, H_2-23), 3.28 (1H, dd, J = 14.0, 3.5 Hz, 18-H), 1.54, 1.33, 1.25, 1.06, 0.98, 0.91 (3H each, s, H_3-25, 24, 27, 26, 30, 29); sugar moiety *δ* 5.20 (1H, d, J = 8.0 Hz, xyl H-1), 5.17 (1H, d, J = 7.5 Hz, GLC H-1), 3.84 (1H, m, GLC H-5); other NMR spectral data see Tables 1 and 2; FABMS (negative-ion mode) *m*/*z* 781 $[M - H]^-$, 649 $[M - xyl - H]^-$, 487 $[M - xyl - GLC - H]^-$.

Acid Hydrolysis of Conyzasaponin A (1). Compound **1** (20 mg) was heated in 1 mL of 1 M HCl (dioxane−H_2O, 1:1) at 80 °C for 2 h in a waterbath. After dioxane was removed, the solution was extracted with EtOAc (1 mL × 3). The extractant was washed with H_2O and then concentrated to give bayogenin (**1a**, 4 mg). The monosaccharide portion was neutralized with Ag_2CO_3 and centrifuged. The supernatant was concentrated and dried overnight and then treated with 1-(trimethylsilyl)-imidozole at room temperature for 2 h. After the excess reagent was decomposed with H_2O, the reaction product was extracted with *n*-hexane (2 mL × 2). The TMSi derivatives of the monosaccharides were identified as D-glucose (GLC R_t, 17.89), D-apiose (GLC R_t, 10.29), D-xylose (GLC R_t, 8.97), L-arabinose (GLC R_t, 7.13), L-rhamnose (GLC R_t, 7.29) by co-GLC analysis with standard monosaccharides.

Alkaline Hydrolysis of Conyzasaponin A (1). Compound **1** (20 mg) was refluxed with 2 mL of 1 M KOH at 80 °C for 12 h. After cooling, the reaction mixture was neutralized with 1 M HCl and then extracted with *n*-BuOH (2 mL × 3). The organic layers were combined and then evaporated to dryness under a vacuum. The residue was subjected to HPLC purification eluting with 80% MeOH in H_2O, affording prosapogenin (**4**, 2 mg).

TLC Analysis of Sugars of Conyzasaponins A, B, C, and G (1−4). Compounds **1**−**4** were applied to the TLC plate respectively and then hydrolyzed under HCl vapor at 60 °C for 40 min. After the excess HCl was removed, the authentic sugars were applied to the same plate. The TLC plate was developed by $CHCl_3$−MeOH−H_2O−gHAc (16:9:2:2), sprayed with α-naphthol−H_2SO_4, and heated. Hexoses gave purple spots, 6-deoxy sugars orange spots, and pentoses blue spots. The R_f values of each sugar are as follows: apiose, 0.51; arabinose, 0.49; glucose, 0.42; rhamnose, 0.57; and xylose, 0.52.

Bayogenin (1a): amorphous solid; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.52 (1H, br s, H-12), 4.53 (1H, m, H-2), 4.27 (1H, d, J = 4.10 Hz, H-3), 3.72, 4.17 (1H each, d, J = 10.3 Hz, H_2-23), 3.31 (1H, dd, J = 13.8, 4.1 Hz, H-18), 1.61, 1.37, 1.27, 1.11, 1.01, 0.93 (3H each, s, H_3-25, 24, 27, 26, 30, 29).

References and Notes

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