Conyzasaponins I−Q, Nine New Triterpenoid Saponins from *Conyza blinii*

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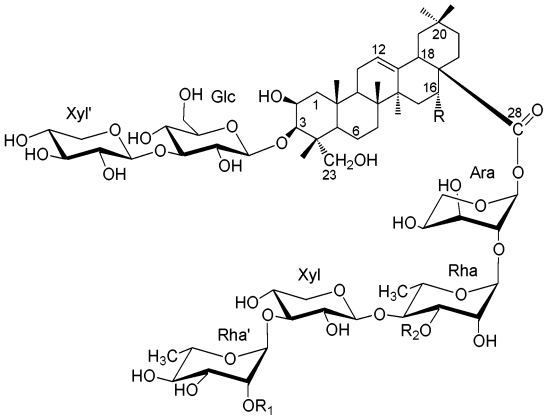
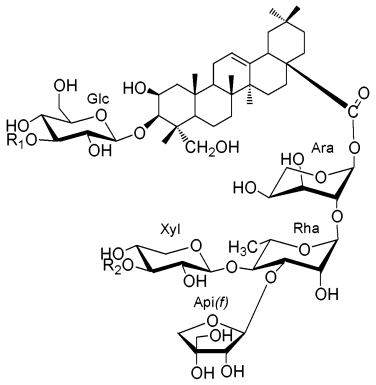
Nine new triterpenoid saponins, conyzasaponins I−Q (**1**−**9**), were isolated from the aerial parts of *Conyza blinii*. Their structures were established on the basis of extensive 1D and 2D NMR spectra as well as by chemical degradations. Among these compounds, conyzasaponins M−O (**5**−**7**) share a common pentasaccharide unit attached to C-28 of the aglycon, 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester, which contains two apiofuranosyl residues. To the best of our knowledge, they are among the few examples of natural products possessing two apiofuranose units in a single sugar chain.

The folk medicinal plant *Conyza blinii* Lévl. (Compositae) is a native herbaceous plant distributed mainly in the southwest region of the People's Republic of China. Its aerial parts and polar extracts made from this species have been used to treat inflammatory diseases, especially chronic bronchitis.[1] A detailed chemical investigation on this plant has led to the isolation and identification of eight new triterpenoid saponins, conyzasaponins A−H, which were reported earlier.[2,3] Further study on the polar fractions of *C. blinii* furnished nine new saponins named conyzasaponins I−Q (**1**−**9**). Among these saponins, conyzasaponins M−O (**5**−**7**) seem to be unusual by having a common pentasaccharide unit attached to C-28 of the aglycon, namely, 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester, containing two apiofuranosyl residues. Although most of the saponins isolated from *C. blinii* contain one or two apiose units, conyzazaponins I (**1**), J (**2**), and L (**4**) contain no apiose residue, which are three exceptions among the more polar saponins from *C. blinii*. This paper reports the isolation and structure elucidation of compounds **1**−**9** by extensive NMR studies and chemical degradations.



	R	R_1	R_2
1	H	H	H
2	OH	H	H
3	OH	H	Api(*f*)
4	OH	Gal	H



	R_1	R_2
5	Xyl'	Api(*f*)'
6	H	Api(*f*)'
7	Glc'	Api(*f*)'
8	Xyl'	H
9	Xyl'	Xyl"

Results and Discussion

Conyzasaponin I (**1**) was obtained as a white amorphous powder (MeOH). The molecular formula was determined to be $C_{63}H_{102}O_{30}$ according to its high-resolution MALDI FTMS (*m*/*z* 1361.6343, calcd for $C_{63}H_{102}NaO_{30}$ $[M + Na]^+$, 1361.6354). The six tertiary methyl groups (*δ* 0.90, 0.98, 1.12, 1.23, 1.33, and 1.54) and one trisubstituted olefinic proton (*δ* 5.45, br s) observed in the ^{1}H NMR spectrum coupled with information from its ^{13}C NMR spectrum (six sp^3 carbons at *δ* 15.0, 17.3, 17.5, 23.7, 26.1, and 33.1 and two sp^2 olefinic carbons at *δ* 123.1 and 144.1) indicated that the aglycon possesses an olean-12-ene skeleton. A 2D NMR study revealed that the aglycon was bayogenin (2*β*,3*β*,23-trihydroxyolean-12-en-28-oic acid).[2,4] The 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 63 carbon signals observed in the ^{13}C NMR spectrum of **1**, 30 were assigned to the aglycon part, and the remaining 33 were assigned to the oligosaccharide moiety. The ^{1}H and ^{13}C NMR spectra of **1** exhibited signals for six sugar anomeric protons at *δ* 5.06 (d, *J* = 7.8 Hz), 5.13 (d, *J* = 7.6 Hz), 5.20 (d, *J* = 7.6 Hz), 5.76 (br s), 6.20 (br s), and 6.46 (d, *J* = 2.6 Hz) and six anomeric carbons at *δ* 93.4, 101.0, 102.6, 105.4, 106.3, and 106.8 (Tables 2 and 3). The methyl carbon signals at

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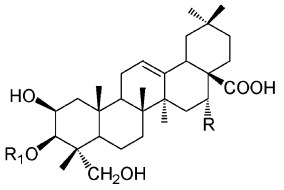
Table 1. ^{13}C NMR Spectral Data for the Aglycon Moieties of Compounds **1**−**9** (125 MHz in pyridine-d_5)

	1	2	3	4	5	6	7	8	9
1	44.2	44.2	44.3	44.3	44.2	44.1	44.3	44.2	44.3
2	70.7	70.7	70.8	70.8	70.7	70.5	70.9	70.8	70.8
3	83.0	82.9	82.9	83.0	83.0	83.0	82.8	82.9	82.9
4	42.8	42.8	42.9	42.9	42.8	42.8	42.9	42.9	42.9
5	47.6	47.6	47.8	47.8	47.7	47.7	47.6	47.6	47.4
6	18.1	18.0	18.0	18.1	18.0	18.1	18.0	18.0	18.0
7	33.0	33.2	33.3	33.2	33.0	33.0	33.0	33.0	33.0
8	40.0	40.1	40.2	40.2	40.1	40.0	40.1	40.0	40.0
9	48.5	47.7	47.7	47.7	48.5	48.5	48.5	48.5	48.5
10	36.9	36.9	37.0	37.0	37.0	37.0	37.0	36.9	37.0
11	24.0	24.0	24.0	24.1	24.0	24.0	24.0	24.0	24.0
12	123.1	123.0	123.1	123.1	123.1	123.1	123.2	123.2	123.2
13	144.1	144.3	144.4	144.4	144.2	144.2	144.2	144.2	144.2
14	42.3	42.2	42.2	42.2	42.3	42.3	42.3	42.3	42.3
15	28.3	36.1	36.1	36.2	28.2	28.2	28.2	28.2	28.2
16	23.2	73.9	74.1	74.1	23.2	23.2	23.2	23.2	23.2
17	47.3	49.5	49.6	49.6	47.4	47.4	47.4	47.4	47.4
18	41.7	41.2	41.3	41.3	41.7	41.7	41.7	41.7	41.7
19	46.2	47.0	47.0	47.0	46.2	46.2	46.2	46.2	46.2
20	30.9	30.8	30.9	30.9	30.9	30.9	31.0	30.9	30.9
21	34.1	35.9	36.0	36.0	34.2	34.1	34.2	34.1	34.2
22	32.7	32.0	32.1	32.1	32.8	32.8	32.8	32.8	32.8
23	65.2	65.1	65.1	65.2	65.1	65.6	65.0	65.0	65.0
24	15.0	15.0	15.1	15.1	15.0	15.1	15.1	15.0	15.0
25	17.3	17.3	17.4	17.4	17.3	17.3	17.3	17.3	17.3
26	17.5	17.6	17.7	17.7	17.6	17.6	17.6	17.6	17.6
27	26.1	27.1	27.3	27.3	26.2	26.2	26.2	26.2	26.2
28	176.3	175.9	176.0	176.0	176.3	176.3	176.3	176.3	176.3
29	33.1	33.2	33.3	33.2	33.2	33.2	33.2	33.2	33.2
30	23.7	24.7	24.9	24.9	23.7	23.7	23.8	23.7	23.7

δ 18.4 and 18.6 coupling with the doublet methyl proton signals at δ 1.64 (3H, d, J = 6.2 Hz) and 1.73 (3H, d, J = 5.7 Hz) indicated the presence of two 6-deoxy sugar units. Acid hydrolysis of **1** afforded bayogenin (**1a**),[2,4] and the monosaccharide components were identified as D-glucose, D-xylose, L-arabinose, and L-rhamnose, (1:2:1:2) by TLC, GLC, and HPLC analysis. Alkaline hydrolysis of **1** afforded a prosapogenin (**1b**), which was identified as bayogenin 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside based on a comparison with previously reported data and co-TLC.[2] These findings suggested 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.



	R	R_1
1a	H	H
2a	OH	H
1b	H	Xyl-(1→3)-Glc-
2b	OH	Xyl-(1→3)-Glc-

The identification of the monosaccharides and the full assignments of proton and carbon resonance signals of the sugar chains in **1** were accomplished by a combination of DEPT, DQF-COSY, HOHAHA, HETCOR, HMBC, and NOESY NMR experiments as previously described.[2,3,5] After all of the proton and carbon signals were assigned, combined with the $^3J_{H1,H2}$ coupling constants (Tables 2 and 3),[5] the six sugar units were identified as one β-D-glucopyranose unit, two β-D-xylopyranose units, one α-L-arabinopyranose unit predominantly in the $^1C_4$ form,[6,7] and two α-L-rhamnopyranose units. The disaccharide chain connected to the C-3 of the aglycon was identified on the basis of the following HMBC information: correlations between H-1 (δ 5.20) of Xyl′ and C-3 (δ 87.6) of Glc and between H-1 (δ 5.13) of Glc and C-3 (δ 83.0) of the aglycon. The sequence of the sugar chain at C-28 was established from the following HMBC correlations: H-1 (δ 6.46) of Ara with C-28 (δ 176.3) of the aglycon, H-1 (δ 5.76) of Rha with C-2 (δ 75.2) of Ara, H-1 (δ 5.06) of Xyl with C-4 (δ 83.9) of Rha, H-1 (δ 6.20) of Rha′ with C-3 (δ 83.3) of Xyl. In the NOESY experiments, the related peaks between H-1 (δ 5.13) of Glc and H-3 (δ 4.33) of the aglycon, H-1 (δ 5.20) of Xyl′ and H-3 (δ 4.06) of Glc, H-1 (δ 5.76) of Rha and H-2 (δ 4.53) of Ara, H-1 (δ 5.06) of Xyl and H-4 (δ 4.31) of Rha, and H-1 (δ 6.20) of Rha′ and H-3 (δ 4.18) of Xyl supported the above deduction. Therefore, the structure of conyzasaponin I (**1**) was established as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosylbayogenin 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Conyzasaponin J (**2**) showed quasi-molecular peaks at m/z 1377 $[M + Na]^+$ and m/z 1393 $[M + K]^+$ in its MALDI-TOFMS, which corresponded to 16 mass units higher than in **1**, indicating the addition of one oxygen atom. The signals for the sugar moiety in the ^1H and ^{13}C NMR spectra of compound **2** bore a strong resemblance to those of **1** (see Tables 2 and 3), and scrutiny of the NMR spectra led to the conclusion that compound **2** has the same sugar chains as **1**. Acid hydrolysis of **2** afforded polygalacic acid[3] as the aglycon and the sugars D-glucose, D-xylose, L-arabinose, and L-rhamnose in a ratio of 1:2:1:2, as determined by GLC and HPLC analysis, which supported the above deduction. Therefore, the structure of compound **2** was established as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosylpolygalacic acid 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Conyzasaponin K (**3**) had a molecular formula $C_{68}H_{110}O_{35}$ based on its high-resolution MALDI FTMS (m/z 1509.6716, calcd for $C_{68}H_{110}NaO_{35}$ $[M + Na]^+$, 1509.6725), 132 mass units higher than **2**. A comparison of the NMR spectra of **2** and **3** revealed that the signals of protons and carbons for the aglycon parts and the sugar chains at C-3 of the aglycon were superimposable, indicating that compound **3** possesses the same aglycon and the same oligosaccharide chain at C-3 as **2** (Tables 1−3). The composition of the sugar moieties of **3** (Glc-Xyl-Ara-Rha-Api, in a ratio of 1:2:1:2:1), as determined from acid hydrolysis and subsequent GLC and HPLC analysis, indicated that a pentasaccharide was attached to C-28 of the aglycon. Further study of the 1D and 2D NMR spectra of **3** revealed that the rhamnose at C-2 of arabinose was 1,3,4-substituted (Tables 2 and 3) instead of having a 1,4-substitution when compared with **2**. Using the same procedure as described for **1**, the overall structural assignments were achieved. Because all naturally occurring apiosides have been found in the D-furanosyl form,[8] the apiofuranosyl residue in **3** was determined to be in the β-D-configuration by the NMR spectral data (the β-D-apiofuranosyl C-1 signal resonates at about δ 110.0 ppm, which is about 7 ppm downfield when compared to the resonance of the corresponding α-D-anomer).[9] Accordingly, the structure of conyzasaponin K (**3**) was established as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosylpolygalacic acid 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. The molecular rotation difference ($\Delta[M]_D$ −279.0°) between **3** ($[M]_D$ −839.6°) and

Table 2. ^{13}C NMR Spectral Data for the Sugar Moieties of Compounds **1**−**9** (125 MHz in pyridine-d_5)[a]

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
C_3-Glc									
1	105.4	105.4	105.5	105.4	105.4	105.7	105.6	105.5	105.5
2	74.4	74.3	74.4	74.4	74.4	75.5	74.1	74.4	74.4
3	87.6	87.6	87.7	87.7	87.7	78.6	88.8	87.7	87.7
4	69.4	69.3	69.4	69.4	69.4	71.6	69.7	69.4	69.4
5	77.9	77.9	78.0	78.0	77.9	78.3	77.9	78.0	78.0
6	62.2	62.1	62.2	62.2	62.3	62.7	62.3	62.2	62.3
	Xyl′	Xyl′	Xyl′	Xyl′	Xyl′		Glc′	Xyl′	Xyl′
1	106.3	106.2	106.3	106.3	106.3		106.1	106.3	106.4
2	75.3	75.2	75.3	75.3	75.3		75.6	75.3	75.3
3	78.2	78.1	78.2	78.2	78.2		78.3	78.2	78.2
4	70.9	70.8	70.9	70.9	70.9		71.6	70.9	70.9
5	67.4	67.3	67.4	67.4	67.4		78.8	67.4	67.4
6							62.6		
C_{28}-Ara									
1	93.4	93.3	93.0	93.4	93.0	93.0	93.0	93.0	93.0
2	75.2	75.3	75.8	75.6	75.7	75.7	75.7	75.7	75.8
3	69.9	69.6	68.7	69.4	68.7	68.7	68.7	68.8	68.9
4	66.1	65.8	65.1	65.7	65.2	65.2	65.3	65.3	65.3
5	63.0	62.8	61.9	62.6	61.9	61.9	61.9	62.0	62.0
Rha									
1	101.0	101.0	101.1	101.2	100.9	100.9	101.0	101.0	101.0
2	71.9	71.8	71.5	71.9	71.6	71.5	71.6	71.6	71.6
3	72.7	72.6	82.6	72.6	82.4	82.5	82.6	82.5	82.5
4	83.9	83.4	77.6	83.3	77.8	77.8	77.8	78.1	77.8
5	68.6	68.6	68.7	68.6	68.7	68.7	68.7	68.8	68.7
6	18.4	18.3	18.5	18.4	18.6	18.6	18.6	18.6	18.6
Xyl									
1	106.8	106.5	104.9	106.5	105.0	105.0	105.1	105.3	104.9
2	76.2	76.1	75.8	75.8	75.0	75.0	75.1	75.6	74.6
3	83.3	83.2	83.2	83.5	84.4	84.2	84.2	78.3	86.6
4	69.3	69.2	69.6	69.3	69.7	69.7	69.7	71.3	69.4
5	67.3	67.2	67.1	67.2	66.7	66.7	66.7	67.2	66.6
			Api(*f*)		Api(*f*)	Api(*f*)	Api(*f*)	Api(*f*)	Api(*f*)
1			112.0		111.9	111.9	112.0	111.7	112.0
2			77.5		77.5	77.5	77.5	77.5	77.6
3			79.8		79.8	79.8	79.8	79.6	79.8
4			74.6		74.6	74.6	74.6	74.5	74.7
5			64.2		64.4	64.3	64.2	64.4	64.4
	Rha′	Rha′	Rha′	Rha′	Api(*f*)′	Api(*f*)′	Api(*f*)′		Xyl″
1	102.6	102.6	102.8	101.4	111.2	111.2	111.3		106.0
2	72.5	72.4	72.5	82.6	77.8	77.7	77.8		75.3
3	72.6	72.6	72.7	72.7	80.4	80.5	80.5		77.9
4	74.1	74.0	74.1	74.6	75.1	75.2	75.2		70.9
5	69.9	69.8	69.9	69.6	65.5	65.6	65.6		67.1
6	18.6	18.6	18.7	18.5					
				Gal					
1				107.9					
2				73.5					
3				75.4					
4				70.1					
5				77.0					
6				62.0					

[a] The assignments are based upon DEPT, DQF-COSY, HETCOR, HOHAHA, HMBC, and PS-NOESY experiments.

2 ($[M]_D$ −560.6°) supported the notion that the apiose is in the β-configuration.[10]

Conyzasaponin L (**4**) was assigned a molecular formula of $C_{69}H_{112}O_{36}$, as deduced from its high-resolution MALDI FTMS (*m*/*z* 1539.6820, calcd for $C_{69}H_{112}NaO_{36}$ $[M + Na]^+$, 1539.6831). Its ^1H and ^{13}C NMR spectra showed seven anomeric proton and carbon signals (Tables 2 and 3). Acid hydrolysis of **4** gave polygalacic acid (**2a**), and the sugar units were determined by TLC, GLC, and HPLC analysis to be D-glucose, D-xylose, L-arabinose, L-rhamnose, and D-galactose (1:2:1:2:1). The chemical shifts of C-3 (δ 83.0) and C-28 (δ 175.9) of the aglycon in the ^{13}C NMR spectrum indicated that **4** is also a bisdesmosidic glycoside. Alkaline hydrolysis of **4** also afforded prosapogenin (**2b**), polygalacic acid 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside, indicating that the sugar chain at C-28 consisted of five monosaccharide units. Comparison of the NMR spectra of compounds **4** and **2** showed that the outer rhamnose in **4** was substituted by a galactose unit. The correlation peaks between H-1 (δ 5.16) of Gal and C-2 (δ 82.6) of Rha′ in the HMBC experiment and those between H-1 (δ 5.16) of Gal and H-2 (δ 4.75) of Rha′ in a phase-sensitive NOESY experiment proved that the galactose was attached to C-2 of the outer rhamnose. The full assignments of all the protons and carbons of **4**, using the same protocol as described for **1**, established that the structure of conyzasaponin L (**4**) was 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosylpolygalacic acid 28-*O*-β-D-galactopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Conyzasaponin M (**5**) was isolated as white needles. A molecular formula of $C_{67}H_{108}O_{34}$ was assigned on the basis of its high-resolution MALDI FTMS (*m*/*z* 1479.6619, calcd for $C_{67}H_{108}NaO_{34}$ $[M + Na]^+$, 1479.6620). Acid hydrolysis of **5** gave bayogenin (**1a**), and the monosaccharide units were identified as Glc-Xyl-Ara-Rha-Api in a ratio of 1:2:1:

Table 3. ^{1}H NMR Spectral Data for the Sugar Moieties of Compounds **1**−**4** (500 MHz in pyridine-d_5)[a,b]

	1	**2**	**3**	**4**
C_3-Glc				
1	5.13 d (7.6)	5.14 d (7.6)	5.15 d (7.6)	5.14 d (7.5)
2	4.00	4.03	4.04	4.02
3	4.06	4.07	4.08	4.08
4	4.09	4.13	4.13	4.12
5	3.84 m	3.84 m	3.84 m	3.84 m
6	4.26, 4.36	4.28, 4.40	4.28, 4.41	4.26, 4.40
Xyl′				
1	5.20 d (7.6)	5.20 d (7.6)	5.19 d (7.6)	5.19 d (7.5)
2	3.98	4.00	4.02	4.00
3	4.12	4.15	4.16	4.15
4	4.14	4.16	4.18	4.16
5	3.69, 4.29	3.70, 4.30	3.70, 4.32	3.70, 4.31
C_{28}-Ara				
1	6.46 d (2.6)	6.49 br s	6.56 br s	6.50 d (1.8)
2	4.53	4.52	4.48	4.51
3	4.50	4.53	4.59	4.54
4	4.36	4.39	4.44	4.41
5	3.92, 4.46	3.95, 4.55	3.97, 4.63	3.95, 4.54
Rha				
1	5.76 br s	5.70 br s	5.57 br s	5.66 br s
2	4.52	4.52	4.73	4.49
3	4.47	4.50	4.36	4.50
4	4.31	4.33	4.46	4.32
5	4.34	4.35	4.32	4.36
6	1.73 d (5.7)	1.72 d (5.3)	1.72 d (6.2)	1.72 d (5.7)
Xyl				
1	5.06 d (7.8)	5.09 d (7.8)	5.27 d (7.8)	5.05 d (7.8)
2	3.99	4.00	3.94	3.98
3	4.18	4.19	4.26	4.08
4	4.07	4.05	4.08	4.05
5	3.46 dd (10.9, 10.9), 4.16	3.44 dd (10.8, 10.8), 4.18	3.39 dd (10.8, 10.8), 4.14	3.39 dd (10.5, 10.5), 4.15
Rha′				
1	6.20 br s	6.20 d (1.2)	6.17 br s	6.31 br s
2	4.74	4.76 br s	4.74	4.75 br s
3	4.57	4.56	4.57	4.54
4	4.28	4.29	4.30	4.15
5	4.93 m	4.92 m	4.95 m	4.84 m
6	1.64 d (6.2)	1.66 d (6.2)	1.65 d (6.2)	1.59 d (6.2)
			Api (*f*)	Gal
1			5.90 d (4.3)	5.16 d (7.8)
2			4.78	4.53
3				4.12
4			4.18, 4.56	4.52
5			4.06	3.99
6				4.30, 4.40

[a] The assignments are based upon DEPT, DQF-COSY, HETCOR, HOHAHA, HMBC, and PS-NOESY experiments, with coupling constants (*J*) in parentheses. [b] Overlapped signals are reported without designating multiplicity.

1:2 by co-GLC analysis with standard sugars after being derivatized as trimethylsilyl ethers. Alkaline hydrolysis of **5** afforded a prosapogenin (**1b**). The NMR spectra of **5** showed the presence of seven sugar moieties (see Tables 2 and 4). The resonance signals of the sugar chain at C-3 and the arabinose, rhamnose, and xylose units of the sugar chain at C-28 were similar to those of **1**. Detailed examination of 1D and 2D NMR spectra of **5** revealed the presence of two terminal *β*-D-apiofuranosyl residues indicated by their characteristic resonance signals (with C-3 being quaternary and C-4 and C-5 being two secondary carbons). The correlation peaks between H-1 (*δ* 5.90) of Api(*f*) and C-3 (*δ* 82.4) of Rha and between H-1 (*δ* 6.17) of Api(*f*)′ and C-3 (*δ* 84.4) of Xyl in a HMBC experiment and the interactions through space between H-1 of Api(*f*) and H-3 (*δ* 4.38) of Rha and between H-1 of Api(*f*)′ and H-3 (*δ* 4.10) of Xyl in a NOESY experiment proved that one apiose was linked to C-3 of Rha, and another to C-3 of Xyl. On the basis of the foregoing evidence, the structure of **5** (conyzasaponin M) was established as 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosylbayogenin 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Conyzasaponin N (**6**), white needles from methanol, possessed a molecular formula of $C_{62}H_{100}O_{30}$ from its high-resolution MALDI FTMS (*m*/*z* 1347.6197, calcd for $C_{62}H_{100}NaO_{30}$ $[M + Na]^+$, 1347.6197), equivalent to 132 mass units fewer than **5**, implying the absence of a pentose. Extensive study of the NMR spectra of **6** indicated that the aglycon and the sugar chain at C-28 were the same as those of **5**, and the difference between these two compounds was in the sugar chain at C-3 of the aglycon. Compound **6** had a single terminal glucose linked to C-3 of the aglycon, as revealed by the upfield-shifted resonance signal (*δ* 78.6) assigned to C-3 of the glucose unit.[3] Hence, conyzasaponin N (**6**) was identified as 3-*O*-*β*-D-glucopyranosylbayogenin 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

The minor compound **7**, conyzasaponin O, was assigned a molecular formula of $C_{68}H_{110}O_{35}$, as deduced from its high-resolution MALDI FTMS (*m*/*z* 1509.6722, calcd for $C_{68}H_{110}NaO_{35}$ $[M + Na]^+$, 1509.6725). The ^{1}H and ^{13}C NMR data for **7** (Tables 1, 2, and 4) were very similar to those of conyzasaponin N (**6**), but included additional signals for one more glucopyranosyl residue in the sugar chain at C-3

Table 4. ^{1}H NMR Spectral Data for the Sugar Moieties of Compounds **5**−**9** (500 MHz in pyridine-d_5)[a,b]

	5	**6**	**7**	**8**	**9**
C_3-Glc					
1	5.13 d (7.6)	5.15 d (7.8)	5.14 d (7.3)	5.15 d (7.3)	5.14 d (7.4)
2	4.02	4.02	4.05	4.03	4.05
3	4.06	4.17	4.07	4.07	4.09
4	4.11	4.23	4.11	4.13	4.19
5	3.83 m	3.90 m	3.82 m	3.84 m	3.84 m
6	4.26, 4.39	4.33, 4.45	4.24, 4.39	4.27, 4.40	4.30, 4.43
	Xyl′		Glc′	Xyl′	Xyl′
1	5.18 d (7.6)		5.24 d (7.8)	5.20 d (7.6)	5.20 d (7.5)
2	4.01		4.07	4.01	4.03
3	4.13		4.27	4.15	4.16
4	4.17		4.21	4.16	4.17
5	3.69, 4.31		4.04	3.69, 4.32	3.70, 4.34
6			4.32, 4.55		
C_{28}-Ara					
1	6.53 br s	6.55 br s	6.57 br s	6.56 br s	6.57 d (0.9)
2	4.48	4.50	4.51	4.50	4.52
3	4.58	4.59	4.61	4.58	4.61
4	4.42	4.42	4.41	4.41	4.45
5	3.95, 4.56	3.97, 4.58	3.98, 4.60	3.97, 4.57	3.99, 4.59
Rha					
1	5.58 br s	5.60 br s	5.61 d (1.1)	5.62 br s	5.62 d (0.9)
2	4.71	4.74	4.75	4.74	4.76
3	4.38	4.39	4.39	4.45	4.47
4	4.48	4.50	4.51	4.53	4.53
5	4.32	4.33	4.34	4.37	4.38
6	1.73 d (5.9)	1.74 d (6.2)	1.75 d (6.0)	1.78 d (6.0)	1.73 d (6.2)
Xyl					
1	5.26 d (7.8)	5.28 d (7.8)	5.29 d (7.7)	5.36 d (7.8)	5.34 d (7.8)
2	3.96	3.97	3.97	3.97	3.99
3	4.10	4.13	4.14	4.15	4.17
4	4.02	4.04	4.06	4.16	4.05
5	3.35 dd (10.9, 10.9)	3.35 dd (11.0, 11.0)	3.35 dd (10.8, 10.8)	3.45, 4.19	3.41 dd (10.9, 10.9)
	4.13	4.15	4.14		4.20
Api(*f*)					
1	5.90 d (4.5)	5.91 d (4.5)	5.93 d (4.6)	6.01 d (4.6)	5.96 d (4.4)
2	4.79 d (4.5)	4.80 d (4.5)	4.82	4.77 d (4.6)	4.83 d (4.4)
3					
4	4.18 d (9.4)	4.18	4.19 d (9.4)	4.19 d (9.4)	4.21 d (9.2)
	4.56 d (9.4)	4.57 d (9.4)	4.58 d (9.4)	4.58 d (9.4)	4.60 d (9.2)
5	4.06	4.07	4.06	4.06	4.09
	Api(*f*)′	Api(*f*)′	Api(*f*)′		Xyl″
1	6.17 d (2.1)	6.20 d (2.0)	6.23 d (2.3)		5.26 d (7.6)
2	4.75 d (2.1)	4.77 d (2.0)	4.78		4.00
3					4.10
4	4.28 d (9.1)	4.29 d (9.4)	4.30 d (9.2)		4.17
	4.71 d (9.1)	4.73 d (9.4)	4.74 d (9.2)		
5	4.15	4.17	4.18		3.59 dd (11.1, 11.1)
					4.26

[a] The assignments are based upon DEPT, DQF-COSY, HETCOR, HOHAHA, HMBC, and PS-NOESY experiments, with coupling constants (*J*) in parentheses. [b] Overlapped signals are reported without designating multiplicity.

of the aglycon. By comparison of the NMR data with those of **6**, it was found that C-3 of the inner glucose unit shifted downfield by 10.2 ppm and C-2 and C-4 shifted upfield by 1.4 and 1.9 ppm, respectively, indicating the outer glucose was linked to the C-3 of the inner glucose. HMBC NMR correlations between H-1 of the outer glucose unit (Glc′, δ 5.24) and the downfield-shifted C-3 (δ 88.8) of the inner glucose comfirmed the above deduction. Consequently, the structure of conyzasaponin O (**7**) was established as 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosylbayogenin 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

A molecular formula of $C_{62}H_{100}O_{30}$ was assigned to conyzasaponin P (**8**) on the basis of its high-resolution MALDI FTMS (*m*/*z* 1347.6186, calcd for $C_{62}H_{100}NaO_{30}$ [M + Na]$^+$, 1347.6197). The identification of compound **5** made the structure determination of **8** straightforward. Comparison of the NMR spectra of the two compounds revealed the lack of signals corresponding to an apiose moiety in the structure of **8**. The upfield-shifted resonance signal of C-3 (δ 78.3) of Xyl indicated that in compound **8** the xylose unit is a terminal sugar. Hence, conyzasaponin P (**8**) was identified as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosylbayogenin 28-*O*-β-D-apiofuranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

The high-resolution MALDI FTMS of another minor compound, conyzasaponin Q, displayed pseudomolecular ion peaks at *m*/*z* 1479.6618 (calcd for $C_{67}H_{108}NaO_{34}$ [M + Na]$^+$, 1479.6620), indicating the molecular formula of $C_{67}H_{108}O_{34}$, 132 mass units higher than that of **8**. Compound **9** was therefore assigned the same molecular weight as **5**. Comparison of the NMR data of **9** with those of **5** and **8** revealed the addition of a group of resonance signals assigned to a third xylopyranosyl unit (Xyl″), instead of an apiofuranosyl unit (see Tables 2 and 4). The HMBC correlation between H-1 (δ 5.26) of Xyl″ and C-3 (δ 86.6) of Xyl and NOESY space interaction between H-1 (δ 5.26) of Xyl″and H-3 (δ 4.17) of Xyl made it clear that Xyl″ was

attached to the C-3 of Xyl. Therefore, the structure of conyzasaponin Q (**9**) was established as 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosylbayogenin 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanaco microscope apparatus and were uncorrected. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The IR spectra were recorded on a JASCO 300E FTIR spectrometer. The ^{1}H and ^{13}C NMR spectra were measured on a JEOL ECP-500 spectrometer in pyridine-d_5 solution, and chemical shifts are expressed in δ (ppm) with reference to TMS. MALDI-TOFMS and high-resolution MALDI FTMS were conducted using a PerSeptive Biosystems Voyager DESTR spectrometer and IonSpec 4.7 T FT mass spectrometer, respectively. The resin D101 (Tianjin Chemical Co.), silica 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 ODS columns (PEGASIL ODS-II, Senshu Pak, 20 mm i.d. × 150 mm, and YMC ODS column; detector: UV 210 nm). GLC: Shimadzu GC-7A, column: Silicone OV-17 on Uniport HP (80−100 mesh), 3 mm i.d. × 2.1 m; column temperature, initial temperature 120 °C, programmed at 4 °C/min, and initial time 16 min; carrier gas N_2, flow rate 40 mL/min.

Plant Material. The aerial parts of *Conyza blinii* were collected from Chengdu, Sichuan Province, People's Republic of China, in August 1996, and a voucher specimen (No. 960818) has been deposited at the Herbarium of the School of Pharmaceutical Sciences, Peking University, Beijing 100083, People's Republic of China.

Extraction and Isolation. The extraction procedure was the same as described previously.[2] Fractions 68−69 (part I), fraction 73 (part II), fractions 83−85 (part III), fractions 103−108 (part IV), and fractions 117−119 (part V) obtained by Si gel column chromatography were subjected to open ODS column chromatography by eluting with 10%, 40%, 80%, and 100% MeOH. The 80% MeOH eluate of part I was subjected to HPLC purification with 60% MeOH to give **1** (500 mg). The 80% MeOH eluate of part III was chromatographed over a Lobar RP-C_{18} (Merck) column, and the 60% MeOH fraction gave **2** (90 mg) after HPLC purification (PEGASIL ODS-II column, 58% MeOH as the mobile phase). The 80% MeOH eluate of part IV was passed over a Lobar RP-C_{18} (Merck) column twice, and then HPLC (PEGASIL ODS-II column) was conducted with 52% MeOH as the eluent to afford **3** (80 mg). The 80% MeOH eluate of part V was purified with a Lobar RP-C_{18} (Merck) column twice with 52% MeOH as the eluent to obtain a white mixture, and the mixture was further subjected to HPLC purification (PEGASIL ODS-II column) to give **4** (131 mg) with 26% CH_3CN as the mobile phase. The 80% MeOH eluate of part II was further separated using a Lobar RP-C_{18} (Merck) column twice with MeOH−H_2O mixtures eluted in a gradient manner, and the fractions obtained were subjected to HPLC (PEGASIL ODS-II, 28% MeCN in H_2O) to give **5** (120 mg), **7** (6 mg), **8** (35 mg), and a mixture of **6** and **9**. This mixture was subjected to HPLC (YMC ODS column, 28.2% MeCN in H_2O) to afford **6** (36 mg) and **9** (24 mg).

Conyzasaponin I (1): amorphous white solid; mp 240−242 °C; $[\alpha]^{23}_D$ −26.7° (*c* 1.14, methanol); IR ν_{max} (KBr) 3411, 2914, 1734, 1644, 1052 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.45 (1H, br s, H-12), 4.77 (1H, H-2), 4.33 (1H, H-3), 3.71, 4.33 (1H each, d, J = 10.8 Hz, H-23), 3.27 (1H, dd, J = 14.0, 3.8 Hz, H-18), 1.54, 1.33, 1.23, 1.12, 0.98, 0.90 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1−3; MALDI-TOFMS (positive-ion mode) *m*/*z* 1361 [M + Na]$^+$, 1377 [M + K]$^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1361.6343 (calcd for $C_{63}H_{102}NaO_{30}$ [M + Na]$^+$, 1361.6354).

Conyzasaponin J (2): amorphous white solid; mp 236−238 °C; $[\alpha]^{20}_D$ −41.4° (*c* 0.86, methanol); IR ν_{max} (KBr) 3405, 2931, 1735, 1637, 1063 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.63 (1H, br s, H-12), 5.24 (1H, H-16), 4.80 (1H, br s, H-2), 4.35 (1H, H-3), 3.69, 4.34 (1H each, d, J = 9.9 Hz, H-23), 3.58 (1H, dd, J = 14.2, 3.9 Hz, H-18), 1.77, 1.58, 1.34, 1.16, 1.15, 0.99 (3H each, s, H_3-27, 25, 24, 26, 30, 29); other NMR spectral data, see Tables 1−3; MALDI-TOFMS (positive-ion mode) *m*/*z* 1377 [M + Na]$^+$, 1393 [M + K]$^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1377.6288 (calcd for $C_{63}H_{102}NaO_{31}$ [M + Na]$^+$, 1377.6303).

Conyzasaponin K (3): amorphous white solid; mp 237−239 °C; $[\alpha]^{20}_D$ −56.5° (*c* 0.76, methanol); IR ν_{max} (KBr) 3415, 2933, 1728, 1645, 1045 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.64 (1H, br s, H-12), 5.25 (1H, H-16), 4.80 (1H, H-2), 4.35 (1H, H-3), 3.68, 4.31 (1H each, d, J = 9.4 Hz, H-23), 3.61 (1H, H-18), 1.77, 1.60, 1.35, 1.18, 1.17, 1.01 (3H each, s, H_3-27, 25, 24, 30, 26, 29); other NMR spectral data, see Tables 1−3; MALDI-TOFMS (positive-ion mode) *m*/*z* 1509 [M + Na]$^+$, 1525 [M + K]$^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1509.6716 (calcd for $C_{68}H_{110}NaO_{35}$ [M + Na]$^+$, 1509.6725).

Conyzasaponin L (4): amorphous white solid; mp 236−238 °C; $[\alpha]^{20}_D$ −33.6° (*c* 1.08, methanol); IR ν_{max} (KBr) 3390, 2933, 1732, 1653, 1055 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.63 (1H, br s, H-12), 5.23 (1H, H-16), 4.79 (1H, H-2), 4.34 (1H, H-3), 3.69, 4.33 (1H each, d, J = 10.1 Hz, H-23), 3.58 (1H, H-18), 1.77, 1.59, 1.35, 1.16, 1.15, 1.00 (3H each, s, H_3-27, 25, 24, 26, 30, 29); other NMR spectral data, see Tables 1−3; MALDI-TOFMS (positive-ion mode) *m*/*z* 1539 [M + Na]$^+$, 1555 [M + K]$^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1539.6820 (calcd for $C_{69}H_{112}NaO_{36}$ [M + Na]$^+$, 1539.6831).

Conyzasaponin M (5): white needles from MeOH; mp 236−238 °C; $[\alpha]^{20}_D$ −42.5° (*c* 0.36, methanol); IR ν_{max} (KBr) 3419, 2931, 1726, 1645, 1043 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.46 (1H, br s, H-12), 4.78 (1H, H-2), 4.32 (1H, H-3), 3.70, 4.33 (1H each, H-23), 3.30 (1H, dd, J = 13.7, 3.6 Hz, H-18), 1.55, 1.33, 1.24, 1.14, 1.01, 0.91 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1, 2, and 4; MALDI-TOFMS (positive-ion mode) *m*/*z* 1479 [M + Na]$^+$, 1495 [M + K]$^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1479.6619 (calcd for $C_{67}H_{108}NaO_{34}$ [M + Na]$^+$, 1479.6620).

Conyzasaponin N (6): white needles from MeOH; mp 231−233 °C; $[\alpha]^{20}_D$ −42.3° (*c* 0.36, methanol); IR ν_{max} (KBr) 3340, 2935, 1734, 1653, 1053 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.46 (1H, br s, H-12), 4.81 (1H, H-2), 4.33 (1H, H-3), 3.69, 4.36 (1H each, d, J = 10.3 Hz, H-23), 3.32 (1H, dd, J = 14.0, 4.1 Hz, H-18), 1.56, 1.35, 1.23, 1.14, 1.02, 0.91 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1, 2, and 4; MALDI-TOFMS (positive-ion mode) *m*/*z* 1347 [M + Na]$^+$, 1363 [M + K]$^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1347.6197 (calcd for $C_{62}H_{100}NaO_{30}$ [M + Na]$^+$, 1347.6197).

Conyzasaponin O (7): white needles from MeOH; mp 245−247 °C; $[\alpha]^{20}_D$ −38.6° (*c* 0.17, methanol); IR ν_{max} (KBr) 3404, 2935, 1734, 1653, 1053 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.46 (1H, br s, H-12), 4.79 (1H, H-2), 4.34 (1H, H-3), 3.70, 4.35 (1H each, H-23), 3.32 (1H, overlapped, H-18), 1.57, 1.35, 1.24, 1.15, 1.02, 0.91 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1, 2, and 4; MALDI-TOFMS (positive-ion mode) *m*/*z* 1509 [M + Na]$^+$, 1525 [M + K]$^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1509.6722 (calcd for $C_{68}H_{110}NaO_{35}$ [M + Na]$^+$, 1509.6725).

Conyzasaponin P (8): amorphous white solid; mp 243−245 °C; $[\alpha]^{20}_D$ −30.4° (*c* 0.51, methanol); IR ν_{max} (KBr) 3413, 2931, 1734, 1645, 1049 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.46 (1H, br s, H-12), 4.79 (1H, H-2), 4.34 (1H, H-3), 3.70, 4.35 (1H each, H-23), 3.31 (1H, dd, J = 14.0, 4.4 Hz, H-18), 1.56, 1.34, 1.24, 1.15, 1.02, 0.91 (3H each, s, H-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1, 2, and 4; MALDI-TOFMS (positive-ion mode) *m*/*z* 1347 [M + Na]$^+$,

1363 $[M + K]^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1347.6186 (calcd for $C_{62}H_{100}NaO_{30}$ $[M + Na]^+$, 1347.6197).

Conyzasaponin Q (9): white needles from MeOH; mp 242−244 °C; $[\alpha]^{20}_D$ −34.2° (*c* 1.26, methanol); IR ν_{max} (KBr) 3415, 2933, 1738, 1641, 1054 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) aglycon δ 5.46 (1H, br s, H-12), 4.79 (1H, br s, H-2), 4.36 (1H, H-3), 3.70, 4.36 (1H each, H-23), 3.32 (1H, dd, J = 13.8, 4.2 Hz, H-18), 1.56, 1.34, 1.24, 1.14, 1.01, 0.91 (3H each, s, H_3-25, 24, 27, 26, 30, 29); other NMR spectral data, see Tables 1, 2, and 4; MALDI-TOFMS (positive-ion mode) *m*/*z* 1479 $[M + Na]^+$, 1495 $[M + K]^+$; high-resolution MALDI FTMS (positive-ion mode) *m*/*z* 1479.6618 (calcd for $C_{67}H_{108}NaO_{34}$ $[M + Na]^+$, 1479.6620).

Acid Hydrolysis of Conyzasaponins I (1), J (2), K (3), L (4), and M (5). Compound **1** (42 mg) was heated in 1 mL of 1 M HCl (dioxane−H_2O, 1:1) at 95 °C for 2 h in a water bath. After dioxane was removed, the solution was extracted with EtOAc (1 mL × 3). The extractant was washed with H_2O, concentrated, and then subjected to silica gel column chromatography with $CHCl_3$−MeOH (21:1) as the eluent to give bayogenin (**1a**, 5 mg). The monosaccharide portion was neutralized by passing through an Amberlite MB-3 resin column eluted with water, concentrated, dried overnight [TLC detection, developing system $CHCl_3$−MeOH−H_2O−gHOAc (16:9:2:3), visualization by H_2SO_4 spray and then heating], and then treated with 1-(trimethylsilyl)imidazole at ambient 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 glucose, xylose, arabinose, and rhamnose (1:2:1:2) by co-GLC analysis with standard monosaccharides. By the same method, compounds **2** (6 mg), **3** (8 mg), **4** (11 mg), and **5** (20 mg) gave the sapogenin polygalacic acid (**2a**) (purified by HPLC, column Senshu Pak ODS-II; mobile phase 72% MeOH in H_2O; detection UV 210 nm) and bayogenin, and the monosaccharides were identified as Glc-Xyl-Ara-Rha (1:2:1:2) for **2**, Glc-Xyl-Ara-Rha-Api (1:2:1:2:1) for **3**, Glc-Xyl-Ara-Rha-Gal (1:2:1:2:1) for **4**, and Glc-Xyl-Ara-Rha-Api (1:2:1:1:2) for **5**. The retention times (t_R in parentheses, min) were Glc (28.9, 32.2); Xyl (22.4, 24.8); Rha (17.4, 20.4); Ara (16.1, 19.6); Api (13.5, 14.7), and Gal (27.6, 28.9).

Determination of the D,L-Configuration of Monosaccharide Subunits of Compounds 1−4. The monosaccharide subunits were obtained by hydrochloric acid hydrolysis as described above. The sugar residue was then dissolved in 1 mL water, and an ethanol solution (1 mL) of l-(−)-α-methylbenzylamine (5 mg) and $NaBH_3CN$ (8 mg) was added. The mixture was heated and stirred at 40 °C in a water bath for 4 h, acidified by 0.3 mL of glacial HOAc, and finally evaporated to dryness to give a colorless oil, which was further acetylated with acetic anhydride (0.3 mL) in 0.3 mL of pyridine for 24 h at ambient temperature. After co-distillation with toluene, the resulting products were suspended in 3 mL of water and then passed through a Sep-pak C_{18} cartridge eluted with H_2O and H_2O−MeCN (4:1, 1:1). The 50% MeCN eluate was passed through a Toyopak IC-SP M cartridge eluted by ethanol to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which were identified by co-HPLC analysis with the derivatives of standard sugars prepared under the same conditions. HPLC conditions: column, Senshu Pak Pegasil ODS, 4.6 × 150 mm; mobile phase, 33% MeCN in H_2O; flow rate, 0.8 mL/min; detection, UV 230 nm. Retention times of the derivatives of monosaccharides: L-arabinose, 24.5 min; D-xylose, 25.6 min; d-apiose, 25.6 min; D-galactose, 31.6 min; D-glucose, 38.3 min; L-rhamnose, 43.7 min.

Alkaline Hydrolysis of Conyzasaponins I (1), J (2), K (3), L (4), and M (5). Compound **1** (20 mg) was refluxed with 3 mL of 1 M KOH for 2 h. After cooling, the reaction mixture was neutralized with 1 M HCl to pH 6 and then extracted with *n*-BuOH (5, 4, 4, 3 mL). The organic layers were combined and then evaporated to dryness under reduced pressure. The residue was subjected to HPLC purification (Senshu Pak ODS-II column, UV detector 210 nm) with 73% MeOH as the eluent to give the prosapogenin **1b (**6 mg). Using the same method, compounds **2** (11 mg), **3** (5 mg), and **4** (5 mg) gave the prosapogenin **2b**; compound **5** also gave **1b**.

The physical and spectral data of bayogenin (**1a**), polygalacic acid (**2a**), and prosapogenins (**1b**, **2b**) have been reported previously.[2,3]

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