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New apiose-containing triterpenoid saponins from *Conyza blinii*

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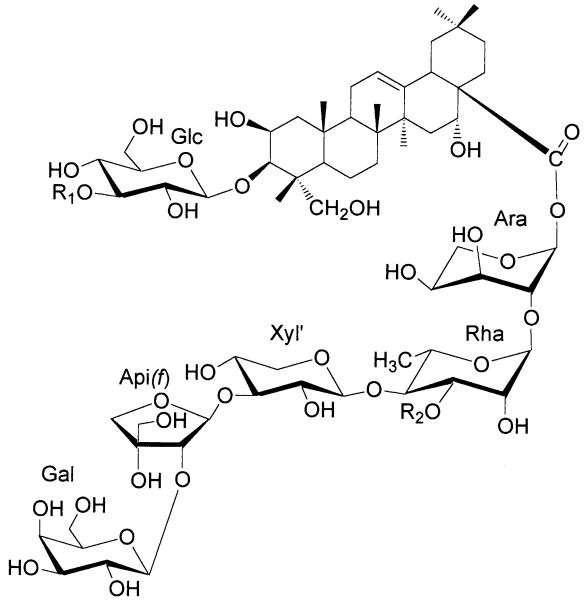
Abstract—Four new apiose-containing triterpenoid saponins, conyzasaponins D, E, F, and H, were isolated from the aerial parts of *Conyza blinii*. They are the first examples of triterpenoid saponins containing one apiose unit whose C-2 is substituted by a galactose. Conyzasaponins D and F have two apiose units, a sugar that has been rarely observed in natural products research. Their structures were established on the basis of extensive NMR studies and chemical degradation. Conyzasaponins D and F exhibited moderate cytotoxicity against HL-60 cell lines.

1. Introduction

Conyza blinii Lévl. (Asteraceae), commonly called 'Jin Long Dan Cao', is a native herbaceous plant distributed mainly in the southwest region in the People's Republic of China. In folk medicine, its aerial parts are used to treat some kinds of inflammatory diseases especially chronic bronchitis.[1] The isolation and identification of four saponins, conyzasaponins A, B, C, G and other compounds from it were reported earlier.[2,3] Further investigation of the saponin fraction of this plant resulted in four additional new saponins, named as conyzasaponins D, E, F, and H (**1**–**4**) respectively. Each of these saponins contains an apiose unit whose C-2 is substituted by galactose. Conyzasaponins D and F have two apiose units, a sugar that has been rarely observed in natural products research. In this paper, we report the isolation and structural elucidation of these saponins.

2. Results and discussion

Conyzasaponin D (**1**) showed pseudo-molecular peaks at *m/z* 1657 $[M+Na]^+$ and *m/z* 1673 $[M+K]^+$ in its MALDI-TOF MS spectrum (positive ion mode). Combined with its ^{13}C NMR data, its molecular formula was determined as $C_{73}H_{118}O_{40}$, which was confirmed by the $[M-H]^-$ ion at *m/z* 1633.7127 (calcd for $C_{73}H_{117}O_{40}$, 1633.7121) in its high resolution FAB MS. The six tertiary methyl groups (δ 1.01, 1.17, 1.17, 1.35, 1.61, and 1.78) and one trisubstituted olefinic proton (δ 5.64, br s) observed in the ^1H NMR spectrum coupled with the information from



	R_1	R_2
Conyzasaponin D (1)	Xyl	Api (*f*)'
Conyzasaponin E (2)	Xyl	Ara'
Conyzasaponin F (3)	H	Api (*f*)'
Conyzasaponin H (4)	Xyl	H

^{13}C NMR spectrum (six sp^3 carbons at δ 15.1, 17.4, 17.7, 24.9, 27.3, and 33.3 and two sp^2 olefinic carbons at δ 123.1 and 144.4) indicated that the aglycon possessed an olean-12-ene skeleton. After an extensive 2D NMR study, the aglycon was identified as polygalacic acid (2β, 3β, 16α, 23-tetrahydroxy-olean-12-en-28-oic acid).[4] The chemical shifts of

Keywords: *Conyza blinii*; triterpenoid saponins; apiose-containing.
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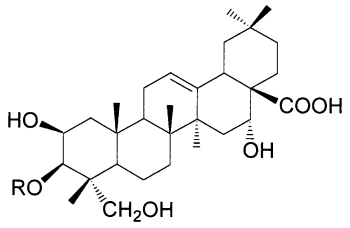
Table 1. ^{13}C NMR spectral data for the aglycon moieties of **1**–**4**, **1a**, and **1b** (125 MHz in pyridine-d_5)

	1	2	3	4	1a	1b
1	44.3	44.3	44.3	44.3	44.3	45.0
2	70.8	70.8	70.6	70.8	70.8	71.7
3	83.0	83.0	83.0	83.0	83.0	73.3
4	42.9	42.9	42.8	42.9	42.9	42.5
5	47.8	47.8	47.9	47.8	47.8	48.5
6	18.1	18.1	18.1	18.1	18.1	18.4
7	33.3	33.3	33.3	33.2	33.3	33.4
8	40.4	40.3	40.2	40.2	40.1	40.1
9	47.7	47.7	47.7	47.7	47.8	47.8
10	37.0	37.0	37.0	37.0	37.1	37.3
11	24.0	24.1	24.1	24.1	24.1	24.1
12	123.1	123.1	123.1	123.1	122.4	122.5
13	144.4	144.4	144.4	144.4	145.3	145.2
14	42.2	42.3	42.2	42.2	42.4	42.4
15	36.2	36.2	36.1	36.2	36.3	36.2
16	74.0	74.1	74.1	74.1	74.9	74.8
17	49.6	49.6	49.6	49.6	49.1	49.1
18	41.3	41.3	41.3	41.3	41.7	41.6
19	47.0	47.1	47.0	47.0	47.3	47.3
20	30.9	30.9	31.0	30.9	31.0	31.1
21	36.0	36.0	36.0	36.0	36.2	36.2
22	32.1	32.1	32.1	32.1	32.6	32.7
23	65.1	65.1	65.6	65.1	65.2	68.0
24	15.1	15.1	15.1	15.1	15.0	14.6
25	17.4	17.5	17.4	17.4	17.4	17.7
26	17.7	17.8	17.7	17.7	17.7	17.4
27	27.3	27.3	27.3	27.3	27.4	27.3
28	176.0	176.0	176.0	175.9	180.7	180.9
29	33.3	33.3	33.3	33.2	33.4	33.4
30	24.9	24.9	24.9	24.8	25.1	25.0

C-3 (δ 83.0) and C-28 (δ 176.0) indicated that **1** is a bisdesmosidic glycoside (Table 1). Of the 73 carbon signals observed in the ^{13}C NMR spectrum of **1**, 30 were assigned to the aglycon part, and the remaining 43 to the oligosaccharide moiety. The ^{1}H- and ^{13}C NMR spectra of **1** exhibited eight sugar anomeric protons at δ 5.15 (d, J=7.4 Hz), 5.20 (d, J=7.8 Hz), 5.26 (d, J=7.8 Hz), 5.28 (d, J=8.3 Hz), 5.58 (d, J=1.0 Hz), 5.96 (d, J=4.6 Hz), 6.47 (br s), and 6.59 (br s) and carbons at δ 93.1, 101.2, 104.8, 105.2, 105.5, 106.3, 109.4, and 111.9 (Tables 2 and 3). The methyl carbon signal at δ 18.5 and the doublet methyl proton signal at δ 1.70 (3H, J=6.0 Hz) indicated the presence of one 6-deoxy sugar. Acid hydrolysis afforded polygalacic acid (**1b**),[5] and the monosaccharide components were identified as glucose, xylose, arabinose, rhamnose, apiose and galactose (1:2:1:1:2:1) by TLC and GLC analysis. Alkaline hydrolysis afforded a new prosapogenin (**1a**) identified as polygalacic acid 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside on the basis of extensive NMR studies and MS studies. From the above evidence, it can be concluded that **1** is a bisdesmosidic triterpenoid glycoside with glucose and xylose linked to the C-3 position of the aglycon, and the other six monosaccharides linked to the C-28 of the aglycon through an ester bond.



1a R=xyl (1→3)-glc
1b R=H

The identity of the monosaccharides and the sequence of the oligosaccharide chains in **1** were determined by a

Table 2. ^{13}C NMR spectral data for the sugar moieties of **1**–**4**, and **1a** (125 MHz in pyridine-d_5)

	1	2	3	4	1a
C_3-Glc					
1	105.5	105.5	105.7	105.5	105.4
2	74.4	74.4	75.5	74.4	74.4
3	87.7	87.7	78.6	87.7	87.8
4	69.4	69.4	71.6	69.4	69.4
5	78.0	78.0	78.3	77.9	78.0
6	62.1	62.3	62.7	62.2	62.3
Xyl					
1	106.3	106.3		106.3	106.3
2	75.3[a]	75.2		75.3	75.2
3	78.2	78.2		78.2	78.2
4	70.9	70.9		70.9	70.9
5	67.4	67.4		67.4	67.4
C_{28}-Ara					
1	93.1	93.4	93.1	93.3	
2	76.0	75.5	76.0	75.6	
3	68.8	69.1	68.8	69.4	
4	65.2	65.6	65.2	65.7	
5	61.9	62.4	61.9	62.6	
Rha					
1	101.2	101.1	101.2	101.2	
2	71.6	71.7	71.6	71.9	
3	82.6[a]	82.4	82.6[a]	72.6	
4	77.7	78.0	77.7	83.5	
5	68.8	68.8	68.8	68.6	
6	18.5	18.6	18.5	18.3	

	1	2	3	4
Xyl′				
1	104.8	104.6	104.8	106.5
2	74.8	74.7	74.8	75.1
3	82.6[a]	84.0	82.5[a]	83.6
4	69.9	69.9	69.9	69.4
5	66.8	66.6	66.8	67.0
Api*f*				
1	109.4	109.5	109.4	109.6
2	84.5	84.3	84.5	84.4
3	81.3	81.2	81.3	81.2
4	75.8	75.6	75.8	75.6
5	65.9	65.9	65.9	65.8
Gal				
1	105.2	105.3	105.2	105.3
2	72.8	72.7	72.8	72.7
3	75.3[a]	75.3	75.3	75.2
4	70.2	70.3	70.2	70.2
5	77.1	77.0	77.1	77.2
6	62.3	62.3	62.1	62.2
	Api*f*′	Ara′	Api*f*′	
1	111.9	106.0	111.9	
2	77.5	72.9	77.5	
3	79.7	74.4	79.8	
4	74.5	69.7	74.5	
5	64.2	67.2	64.2	

The assignments are based upon DEPT, DQF-COSY, HMQC, HOHAHA, HMBC and PS-NOESY experiments.
[a] The data with the same labels in each column may be interchangeable.

Table 3. ^{1}H NMR spectral data for the sugar moieties of **1**–**4**, **1a** (500 MHz in pyridine-d_5)

	1	**2**	**3**	**4**	**1a**
C_3-Glc					
1	5.15 d (7.4)	5.16 d (7.8)	5.17 d (7.8)	5.15 d (7.3)	5.14 d (7.4)
2	4.05	4.02	4.01	4.03	4.03
3	4.07	4.07	4.17	4.07	4.06
4	4.10	4.11	4.24	4.12	4.12
5	3.85 m	3.85 m	3.91 m	3.85 m	3.84
6	4.28	4.28	4.34	4.27	4.26
	4.43	4.42	4.47	4.41	4.40 br d (10.0)
Xyl					
1	5.20 d (7.8)	5.21 d (7.8)		5.20 d (7.5)	5.17 d (7.6)
2	4.02	4.01		4.00	4.00
3	4.15	4.14		4.15	4.16
4	4.17	4.15		4.16	4.16
5	3.69	3.70		3.70	3.69
	4.31	4.31		4.30	4.30
C_{28}-Ara					
1	6.59 (br s)	6.56 d (1.8)	6.60 (br s)	6.52 d (1.8)	
2	4.50	4.54	4.50	4.50	
3	4.59	4.57	4.59	4.54	
4	4.39	4.42	4.45	4.41	
5	3.99	3.94	3.99	3.96	
	4.60	4.56	4.61	4.55	
Rha					
1	5.58 d (1.0)	5.66 br s	5.59 d (1.2)	5.66 br s	
2	4.75 br s	4.81 br s	4.75 br s	4.51	
3	4.45	4.60	4.45	4.52	
4	4.49	4.46	4.48	4.32	
5	4.35	4.34	4.35	4.36	
6	1.70 d (6.0)	1.72 d (6.0)	1.71 d (6.2)	1.71 d (5.7)	
Xyl′					
1	5.28 d (8.3)	5.35 d (7.8)	5.29 d (7.8)	5.06 d (7.8)	
2	3.90 dd (8.2, 8.8)	3.87	3.90	3.96	
3	4.18	4.07	4.18	4.08	
4	4.01	3.97	4.00	3.99	
5	3.35 dd (11.0, 10.5)	3.20 dd (10.6, 10.0)	3.35 dd (11.0, 10.6)	3.37 dd (11.0, 10.8)	
	4.09	4.01	4.08	4.12	
Api*f*					
1	6.47 br s	6.42 d (0.9)	6.48 d (0.9)	6.40 br s	
2	5.09 d (1.0)	5.09 br s	5.10 br s	5.09 br s	
3	–	–	–	–	
4	4.13	4.12	4.14	4.17	
	4.58 d (11.4)	4.55 d (11.9)	4.59 d (8.9)	4.59 d (9.1)	
5	4.24	4.24	4.24	4.21	
Gal					
1	5.26 d (7.8)	5.27 d (8.2)	5.27 d (8.0)	5.21 d (7.8)	
2	4.53	4.54	4.52	4.54	
3	4.09	4.11	4.09	4.07	
4	4.49	4.47	4.50	4.48	
5	4.10	4.18	4.10	4.00	
6	4.26	4.28	4.38	4.36	
	4.38	4.42			
	Api*f*′	Ara′	Api*f*′		
1	5.96 d (4.6)	5.06 d (7.3)	5.97 d (4.8)		
2	4.83 d (4.6)	4.46	4.83 d (4.8)		
3	–	4.04	–		
4	4.17	4.15	4.16		
	4.55		4.55 d (9.1)		
5	4.07	3.50 d (11.5)	4.08		
		4.08			

The assignments are based upon DEPT, DQF-COSY, HMQC, HOHAHA, HMBC and PS-NOESY experiments. Overlapped signals are reported without designating multiplicity.

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 6-deoxy sugar) in the HOHAHA experiment. Starting from the anomeric hydrogen of each sugar unit, all the protons 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 eight sugar units were identified as one β-D-glucose unit, two β-D-xylose units, one α-L-arabinose unit in the $^1C_4$ form,[4] one α-L-rhamnose unit, and two β-D-apiose units (in the furanose form), and one β-D-galactose unit. The disaccharide chain connected to the C-3 of the aglycon was confirmed by the following HMBC information: the correlations between H-1 (δ 5.20) of Xyl and C-3 (δ 87.7) of Glc, the H-1 (δ 5.15) 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 6.59) of Ara with C-28 (δ 176.0) of the aglycon, H-1 (δ 5.58) of Rha with C-2 (δ 76.0) of Ara, H-1 (δ 5.28) of Xyl′ with C-4 (δ 77.7) of Rha, H-1 (δ 6.47) of Api(*f*) with C-3 (δ 82.6) of Xyl′, H-1 (δ 5.26) of Gal with C-2 (δ 84.5) of Api(*f*), H-1 (δ 5.96) of Api(*f*)′ with C-3 (δ 82.6) of Rha. In the NOESY experiments, the cross peaks between H-1 (δ 5.15) of Glc and H-3 (δ 4.36) of the aglycon, H-1 (δ 5.20) of Xyl and H-3 (δ 4.07) of Glc; H-1 (δ 5.58) of Rha and H-2 (δ 4.50) of Ara, H-1 (δ 5.28) of Xyl′ and H-4 (δ 4.49) of Rha, H-1 (δ 6.47) of Api(*f*) and H-3 (δ 4.18) of Xyl′, H-1 (δ 5.26) of Gal and H-2 (δ 5.09) of Api(*f*), H-1 (δ 5.96) of Api(*f*)′ and H-3 (δ 4.45) of Rha confirmed the above deduction. Therefore, conyzasaponin D was identified as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl polygalacic acid 28-*O*-β-D-galactopyranosyl-(1→2)-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 E (**2**) had the same molecular formula as **1**, $C_{73}H_{118}O_{40}$, determined from the quasi-molecular peaks at *m/z* 1657 $[M+Na]^+$ and *m/z* 1673 $[M+K]^+$ in its MALDI-TOF MS spectrum and its NMR data, which was further proved by the $[M-H]^-$ ion at *m/z* 1633.7125 (calcd for $C_{73}H_{117}O_{40}$, 1633.7121) in its negative HR-FAB MS. Its ^1H and ^{13}C NMR spectra indicated that compound **2** also bore polygalacic acid as its aglycon (see Table 1) and had eight monosaccharide units proved by the eight anomeric protons [δ 5.06 (d, *J*=7.3 Hz), 5.16 (d, *J*=7.8 Hz), 5.21 (d, *J*=7.8 Hz), 5.27 (d, *J*=8.2 Hz), 5.35 (d, *J*=7.8 Hz), 5.66 (br s), 6.42 (d, *J*=0.9 Hz), and 6.56 (d, *J*=1.8 Hz)] and carbons (δ 93.4, 101.1, 104.6, 105.3, 105.5, 106.0, 106.3, and 109.5). The alkaline hydrolysis of compound **2** provided the same prosapogenin (**1a**) as that of **1**, and the acid hydrolysis of **2** afforded Glc, Xyl, Ara, Rha, Api and Gal in the ratio of 1:2:2:1:1:1, indicating that the sugar chain attached to C-28 was composed of two arabinose units, one rhamnose unit, one xylose unit, one apiose unit, and one galactose unit. Comparison of the NMR spectra of **1** and **2** indicated the difference between the two saponins was the monosaccharide unit at C-3 of rhamnose, compound **2** bearing an α-L-arabinopyranosyl unit instead of an apiose unit at this position. Further study of the 2D NMR spectra of compound **2** led to the establishment of its structure as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl polygalacic acid 28-*O*-β-D-galactopyranosyl-(1→2)-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester. Worthy of mention is that the conformation of the two α-L-arabinopyranosyl units in the structure of **2** are different, with Ara in the $^1C_4$ chair form and Ara′ in the $^4C_1$ chair form.[6]

Conyzasaponin F (**3**) displayed a $[M+Na]^+$ ion at *m/z* 1525 and a $[M+K]^+$ ion at *m/z* 1541 in the MALDI-TOF MS (positive ion mode), with 132 mass units less than **1**. The negative HR-FAB MS showed a $[M-H]^-$ peak at *m/z* 1501.6699 (calcd for $C_{68}H_{109}O_{36}$, 1501.6699), this led to the determination of its molecular formula as $C_{68}H_{110}O_{36}$. Comparison of the NMR spectra of **1** and **3** revealed that the signals of protons and carbons for the aglycon parts and the sugar chains of C-28 were superimposable, indicating compound **3** possessed the same aglycon and the same oligosaccharide chain at C-28 (Tables 1–3). The composition of the sugar moieties of **3**, Glc/Ara/Rha/Xyl/Api/Gal in the ratio of 1:1:1:1:2:1, resulted from the acid hydrolysis and GLC analysis, indicated that there was only one glucose attached to C-3 of the aglycon. This terminal glucose was confirmed by its ^1H and ^{13}C NMR data. Hence, the structure of conyzasaponin F (**3**) was established as 3-*O*-β-D-glucopyranosyl polygalacic acid 28-*O*-β-D-galactopyranosyl-(1→2)-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 H (**4**) had the molecular formula $C_{68}H_{110}O_{36}$ deduced from a $[M-H]^-$ molecular peak at *m/z* 1501.6672 (calcd for $C_{68}H_{109}O_{36}$, 1501.6699) in its negative HR-FAB MS and from its ^{13}C NMR data. 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, and the sugar units were determined by GLC and TLC to be glucose, xylose, arabinose, rhamnose, apiose, and galactose (1:2:1:1:1:1). 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** was also a bisdesmosidic glycoside. Alkaline hydrolysis of **4** also afforded prosapogenin (**1a**), polygalacic acid 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside, indicating a pentasaccharide was attached to C-28. Extensive NMR study showed that the rhamnose in the pentasaccharide was 1,4-substituted (Tables 2 and 3) instead of the 1,3,4 substitution seen in **1**–**3**. The full assignment of all the protons and carbons of **4** using the same protocol described for **1** resulted the establishment of the structure of conyzasaponin H as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl polygalacic acid 28-*O*-β-D-galactopyranosyl-(1→2)-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

The saponins obtained in this investigation were evaluated for their bioactivity against HL-60 cells. Conyzasaponins D and F exhibited moderate cytotoxicity with IC_{50} value of 3.8 and 3.9 μM, respectively, compared with the positive control drug etoposide (IC_{50} 0.28 μM) and CPDD (IC_{50} 0.20 μM). Conyzasaponins E and H, however, did not show cytotoxicity (IC_{50}s are higher than 1.0×10^{-5} M). The presence of two apiose units in these two compounds is probably the key point related to their activity. Further studies on their bioactivity are under investigation.

3. Experimental

3.1. General procedures

Melting points were measured with a XT4A micro-melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The IR spectra were recorded on a Perkin–Elmer IR spectrometer. The ^{1}H and ^{13}C NMR spectra were measured on a JEOL α-500 spectrometer in pyridine-d_5 solution and chemical shifts are expressed in δ (ppm) referenced to TMS. MALDI-TOF and HR-FAB MS were conducted using a PerSeptive Biosystems Voyager DESTR and KRATOS CONCEPT 32IH mass spectrometers 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 an ODS column (PEGASIL ODS-2, Senshu Pak, 20 mm i.d.×150 mm, 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, rising rate 4°C min^{-1}, and initial time 16 min; carrier gas, N_2, flow rate 40 mL min^{-1}.

3.2. Plant material

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

3.3. Extraction and isolation

The extraction and isolation procedure was same as described in the previous paper.[2] Fractions 117–119 from Si gel column of the 50% ethanol eluate (150 g) was chromatographed over an ODS column eluting with 30% MeOH, 70% MeOH, and MeOH. The 70% MeOH eluate was subjected to medium pressure ODS column chromatography twice eluting with 52% MeOH to obtain a white mixture of saponins. This mixture was further exposed to reverse-phase HPLC purification to furnish **1** (120 mg), **2** (80 mg), **3** (20 mg) with 50% MeOH as the mobile phase and **4** (50 mg) with 26% CH_3CN as the mobile phase.

3.3.1. Conyzasaponin D (1). An amorphous white solid, mp 220–221°C, $[\alpha]^{20}_D=-47°$ (*c* 0.35, methanol); IR ν_{max} (KBr): 3402, 2925, 1727, 1642, 1039 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz): aglycon δ 5.64 (1H, br s, H-12), 5.25 (1H, H-16), 4.81 (1H, br s, H-2), 4.36 (1H, H-3), 3.69, 4.31 (each 1H, d, *J*=11.9 Hz, H-23), 3.59 (1H, dd, *J*=13.5, 4.2 Hz, H-18), 1.78, 1.61, 1.35, 1.17, 1.17, 1.01 (each 3H, s, H-27, -25, -24, -30, -26, -29); other NMR spectral data see Tables 1–3; MALDI-TOF MS (positive ion mode): *m/z* 1657 $[M+Na]^+$, 1673 $[M+K]^+$. HR-FAB MS (negative mode): calcd for $C_{73}H_{117}O_{40}$ $[M-H]^-$, 1633.7121; found 1633.7127.

3.3.2. Conyzasaponin E (2). An amorphous white solid, mp 238–239°C, $[\alpha]^{20}_D=-33°$ (*c* 0.34, methanol); IR ν_{max} (KBr): 3399, 2924, 1727, 1668, 1040 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz): aglycon δ 5.63 (1H, br s, H-12), 5.24 (1H, br s, H-16), 4.81 (1H, H-2), 4.40 (1H, H-3), 3.69, 4.25 (each 1H, d, *J*=10.5 Hz, H-23), 3.61 (1H, dd, *J*=14.2, 3.7 Hz, H-18), 1.78, 1.61, 1.35, 1.19, 1.16, 1.00 (each 3H, s, H-27, -25, -24, -26, -30, -29); other NMR spectral data see Tables 1–3; MALDI-TOF MS (positive ion mode): *m/z* 1657 $[M+Na]^+$, 1673 $[M+K]^+$. HR-FAB MS (negative mode): calcd for $C_{73}H_{117}O_{40}$ $[M-H]^-$, 1633.7121; found 1633.7125.

3.3.3. Conyzasaponin F (3). An amorphous white solid, mp 215–216°C, $[\alpha]^{20}_D=-44°$ (*c* 0.52, methanol); IR ν_{max} (KBr): 3369, 2924, 1725, 1635, 1039 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz): aglycon δ 5.64 (1H, br s, H-12), 5.25 (1H, H-16), 4.83 (1H, H-2), 4.35 (1H, H-3), 3.68, 4.34 (each 1H, d, *J*=10.5 Hz, H-23), 3.61 (1H, dd, *J*=14.0, 3.7 Hz, H-18), 1.77, 1.61, 1.37, 1.18, 1.17, 1.01 (each 3H, s, H-27, -25, -24, -30, -26, -29); other NMR spectral data see Tables 1–3; MALDI-TOF MS (positive ion mode): *m/z* 1525 $[M+Na]^+$, 1541 $[M+K]^+$. HR-FAB MS (negative mode): calcd for $C_{68}H_{109}O_{36}$ $[M-H]^-$, 1501.6699; found 1501.6699.

3.3.4. Conyzasaponin H (4). An amorphous white solid, mp 230–232°C, $[\alpha]^{20}_D=-36°$ (*c* 0.37, methanol); IR ν_{max} (KBr): 3395, 2927, 1731, 1662, 1082 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz): aglycon δ 5.63 (1H, br s, H-12), 5.24 (1H, H-16), 4.81 (1H, H-2), 4.35 (1H, H-3), 3.69, 4.33 (each 1H, d, *J*=10.1 Hz, H-23), 3.59 (1H, dd, *J*=14.0, 3.7 Hz, H-18), 1.77, 1.59, 1.35, 1.16, 1.16, 1.00 (each 3H, s, H-27, -25, -24, -30, -26, -29); other NMR spectral data see Tables 1–3; MALDI-TOF MS (positive ion mode): *m/z* 1525 $[M+Na]^+$, 1541 $[M+K]^+$. HR-FAB MS (negative mode): calcd for $C_{68}H_{109}O_{36}$ $[M-H]^-$, 1501.6699; found 1501.6672.

3.4. Acid hydrolysis of conyzasaponin D (1), E (2), F (3), H (4)

Compound **1** (30 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 applied to HPLC purification eluting with 75% MeOH to give polygalacic acid (**1b**, 5 mg). The monosaccharide portion was neutralized by passing through an Amberlite MB-3 resin column eluted with water, concentrated and dried overnight (TLC detection, developing system $CHCl_3$/MeOH/H_2O/glac. AcOH 16:9:2:3, visualization by H_2SO_4 spray and then heated), and then treated with 1-(trimethylsilyl)-imidozole 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, rhamnose, apiose, galactose (1:2:1:1:2:1) by co-GLC analysis with standard monosaccharides. By the same method, compound **2** (5 mg), **3** (5 mg), **4** (5 mg) gave the same sapogenin, and the monosaccharides were identified as Glc/Xyl/Ara/Rha/Api/Gal (1:2:2:1:1:1) for **2**, Glc/Ara/Rha/Xyl/Api/Gal (1:1:1:1:2:1) for **3**, and Glc/Xyl/Ara/Rha/Api/Gal (1:2:1:1:1:1) for **4**.

3.5. Alkaline hydrolysis of conyzasaponin D (1), E (2), H (4)

Compound **1** (46 mg) was refluxed with 3 mL 1 M KOH for 2 h. After cooling, the reaction mixture was neutralized with 1 M HCl to pH value 6 and then extracted with *n*-BuOH (5, 4, 4, 3 mL). The organic layers were combined and then evaporated to dryness under a reduced pressure. The residue was subjected to HPLC purification eluting with 72% MeOH affording a new prosapogenin (**1a**, 18 mg). Using the same method, compound **2** and **4** gave the same prosapogenin.

3.5.1. Prosapogenin (1a). White needles; mp 243–245°C; $[\alpha]^{20}_{D}$=17.1° (*c* 1.92, methanol); IR ν_{max} (KBr): 3419, 2931, 1695, 1554, 1052 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz): aglycon δ 5.66 (1H, br s, H-12), 5.20 (1H, br s, H-16), 4.80 (1H, br s, H-2), 4.34 (1H, H-3), 3.68, 4.33 (each 1H, d, *J*=10.6 Hz, H-23), 3.64 (1H, br d, *J*=14.2 Hz, H-18), 1.80, 1.59, 1.34, 1.18, 1.11, 1.03 (each 3H, s, H-27, -25, -24, -29, -26, -30); other NMR spectral data see Tables 1–3; MALDI-TOF MS (positive ion mode): *m*/*z* 821 $[M+Na]^+$, 837 $[M+K]^+$. HR-FAB MS (negative mode): calcd for $C_{41}H_{65}O_{15}$ $[M-H]^-$, 797.4324; found 797.4302.

3.5.2. Polygalacic acid (1b). White needles; mp 290–292°C; $[\alpha]^{20}_{D}$=49.5° (*c* 0.30, methanol); IR ν_{max} (KBr): 3467, 2937, 1687, 1560, 1039 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz): δ 5.68 (1H, br s, H-12), 5.23 (1H, br s, H-16), 4.54 (1H, br s, H-2), 4.27 (1H, d, *J*=4.1 Hz, H-3), 3.71, 4.15 (each 1H, d, *J*=10.6 Hz, H-23), 3.67 (1H, br d, *J*=13.5 Hz, H-18), 1.81, 1.65, 1.38, 1.19, 1.13, 1.04 (each 3H, s, H-27, -25, -24, -29, -26, -30); other NMR spectral data see Table 1; MALDI-TOF MS (positive ion mode): *m*/*z* 527 $[M+Na]^+$.

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