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THREE NEW LABDANE DITERPENOID GLYCOSIDES FROM *CONYZA BLINII*

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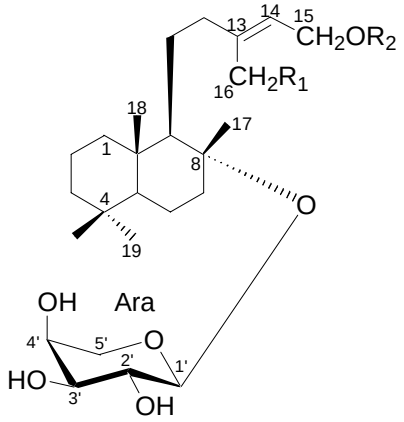
Abstract- Three new labdane-type diterpenoid glycosides, blinoside A, blinoside A-15-*O*-[3"*R*-hydroxy]octadecanoate and blinoside B, together with a known labdane diterpenoid (*E*)-8α,15,16-trihydroxy-13-labdene, were isolated and identified from *Conyza blinii*.

The genus *Conyza* (Asteraceae) is composed of more than 80 species, of which about 25 species have been studied chemically.[1] The most widespread compounds in the plants of this genus are clerodanes, meanwhile labdanes and phytanes have also been isolated.[2-8] *C. blinii* is a native herbaceous plant distributed in the southwest region of China and its aerial parts are used to treat inflammatory diseases in Chinese folk medicine.[1] Our investigation on the chemical constituents of *C. blinii* resulted in discovery of both types of diterpenoids. Two new clerodane-type diterpenoids from it, conyzalactone and 19-deacetyl conyzalactone, have been reported earlier.[9,10] In this paper, we report three new labdane-type diterpenoid arabinosides, together with a known diterpenoid from it.

RESULTS AND DISCUSSION

Blinoside A (**1**) was obtained as white solid. The HR-FABMS spectrum of **1** under the negative ion mode gave a quasi-molecular ion peak at *m/z* 455.3005 ([M-H]$^-$) corresponding to the molecular formula $C_{25}H_{43}O_7$ (*m/z* 455.3009). The IR spectrum of **1** showed the presence of hydroxyl group (3386 cm^{-1}) and olefin group (1657 cm^{-1}). The resonance signals of four tertiary methyl groups (δ 0.74, 0.77, 0.81, 1.39), one trisubstituted olefinic proton (δ 6.02, t, *J*=6.6 Hz), and four protons (δ 4.59, overlapped) attached to oxygen-bearing carbon atoms coupled with the information from its ^{13}C NMR spectrum (four sp^3 carbons at δ 15.9, 21.6, 21.7, 33.5, two sp^2 carbons at δ 127.3, 143.5, two secondary oxygen-bearing carbons

at δ 58.6, 59.9, and one quaternary oxygen-bearing carbon at δ 81.4 indicated that the presence of a labdane with one double bond, two primary hydroxyl groups, and one tertiary hydroxyl group. Further study of the spectral data of **1** revealed that the signals for the labdane aglycone part were similar as those of a known compound isolated from the same plant, (*E*)-8α,15,16-trihydroxy-13-labdene.[11] The stereochemistry of C-8 was determined from phase-sensitive NOESY spectrum. The methyl group at C-8 showed strong NOE interaction with the methyl group at C-10, which supported that the hydroxyl group on C-8 to be of α-equatorial orientation. The ^{1}H and ^{13}C NMR spectra of **1** indicated the presence of one α-L-arabinoside, which was confirmed by co-HPLC analysis with standard arabinose after hydrolysis. Comparison of spectral data of **1** with those of (*E*)-8α,15,16-trihydroxy-13-labdene showed that the arabinose moiety was attached to C-8 on the basis of the downfield chemical shifts of C-8 and H-17 of **1**. Cross peak between H-1' (δ 4.90) of arabinose and C-8 (δ 81.4) of the aglycone moiety in HMBC experiment confirmed the position of the glycoside linkage. The above analysis was confirmed by extensive study of HMQC and HMBC spectra. Finally, the structure of blinoside A (**1**) was established as (*E*)-8α,15,16-trihydroxy-13-labdene-8-*O*-α-L-arabinopyranoside.



	R_1	R_2
1	OH	H
2	OH	—C(=O)(1")–CH$_2$(2")–CH(OH)(3", *R*)–(CH$_2$)$_{14}$(4"-17")–CH$_3$(18")
3	H	H

Blinoside A-15-*O*-[3"*R*-hydroxy]octadecanoate (**2**) was isolated as colorless oil. The HR-FABMS spectrum of **2** under the negative ion mode gave a quasi-molecular ion peak at *m/z* 737.5564 ([M-H]$^-$) corresponding to the molecular formula $C_{43}H_{77}O_9$ (*m/z* 737.5568). The ^{1}H and ^{13}C NMR spectra of **2** were

almost identical with those of **1**, and the observed differences were consistent with the existence in **2** of a hydroxyl fatty acid at C-15 (see Tables 1 and 2). The position of the hydroxyl fatty acid at C-15 was indicated by the downfield chemical shifts of C-15 and H-15 of **2** compared with those of **1** and confirmed by the cross peak between the C-1" (δ 172.3) of the fatty acid and the H-15 (δ 5.95) in HMBC experiments. Acidic hydrolysis of **2** gave L-arabinose. Alkaline hydrolysis of **2** afforded **1** and 3*R*-hydroxyoctadecanoic acid identified by its ^{1}H, ^{13}C NMR and MALDI-TOF MS spectra and comparison of its specific rotation with reported data.[12]

Table 1. ^{13}C NMR spectral data of compounds (1-3) (125 MHz in pyridine-d_5)

	1	**2**	**3**		**1**	**2**	**3**
1	40.1	39.9	40.1	Arabinose			
2	18.7	18.6	18.7	1'	97.6	97.5	97.6
3	42.2	42.2	42.3	2'	72.9	72.7	72.9
4	33.2	33.2	33.3	3'	74.6	74.3	74.6
5	55.9	55.9	56.0	4'	69.2	69.0	69.1
6	20.3	20.2	20.3	5'	65.9	65.8	65.9
7	40.2	40.2	40.3	Fatty acid			
8	81.4	81.2	81.2	1"		172.3	
9	59.8	59.8	59.7	2"		43.5	
10	39.3	39.2	39.4	3"		68.1	
11	25.4	25.1	24.8	4"		38.0	
12	38.8	38.6	43.3	5"		26.1	
13	143.5	147.4	138.7	6"-15"		29.5-29.9	
14	127.3	120.2	125.4	16"		32.1	
15	58.6	61.1	59.1	17"		22.9	
16	59.9	59.8	16.7	18"		14.2	
17	21.7	21.6	21.8				
18	33.5	33.4	33.5				
19	21.6	21.5	21.6				
20	15.9	15.8	15.9				

Blinoside B (**3**) was isolated as white solid. Its molecular formula, $C_{25}H_{44}O_6$, was found to be 16 mass units less than that of blinoside A (**1**), indicating the absence of one oxygen atom. Detailed comparison of the NMR data of **3** and **1** revealed that their resonance signals were very similar and only the signals of

the hydroxymethyl group (C-16: δ 59.9, H-16: δ 4.59 for **1**) were replaced by signals of an olefinic methyl group at δ 16.7 for carbon and δ 1.75 for proton (see Tables 1 and 2). Further study of 2D NMR spectra of **3** resulted in the establishment of the structure of **3** as (*E*)-8α,15-trihydroxy-13-labdene.

Table 2. ^{1}H NMR spectral data of compounds (1-3) (500 MHz in pyridine-d_5)[a]

	1	**2**	**3**
14	6.02 t (6.6)	5.70 t (6.6)	5.85 t (6.4)
15	4.59 (overlapped)	5.95 d (6.6)	4.44 d (6.4)
16	4.59 (overlapped)	4.49 br s	1.75 s
17	1.39 s	1.34 s	1.39 s
18	0.81 s	0.81 s	0.82 s
19	0.74 s	0.74 s	0.76 s
20	0.77 s	0.75 s	0.79 s
Arabinose			
1'	4.90 d (6.4)	4.82 d (6.4)	4.88 d (6.6)
2'	4.36 dd (6.7, 8.5)	4.24 (overlapped)	4.29 (overlapped)
3'	4.19 dd (8.5, 3.6)	4.10 dd (3.5, 8.5)	4.17 dd (8.5, 3.5)
4'	4.31 m	4.24 (overlapped)	4.29 (overlapped)
5'	3.75 dd (12.2, 1.9)	3.69 dd (12.0, 1.7)	3.74 dd (12.2, 1.9)
	4.23 dd (12.2, 3.5)	4.17 dd (12.0, 3.4)	4.23 dd (12.2, 3.5)
Fatty acid			
1"		--	
2"		2.64 dd (14.7, 4.5)	
		2.71 dd (14.7, 8.4)	
3"		4.38 m	
4"		1.58, 1.70	
5"		1.47, 1.60	
6"-15"		1.28	
16"		1.28	
17"		1.28	
18"		0.87 t (6.8)	

[a] Coupling constants *J* (Hz) in parentheses.

EXPERIMENTAL

Melting points were measured with a XT_{4A} micro-melting point apparatus and temperature was uncorrected. The IR spectra were recorded on a Perkin-Elmer IR spectrophotometer. 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) referring to TMS. MALDI-TOF MS and FAB MS were conducted using a PerSeptive Biosystems Voyager DESTR and KYKY-ZHP-5# mass spectrometers respectively. HR-FAB MS was measured using a KRATOS CONCEPT 32IH mass spectrometer. The resin D101 (Tianjin Chemical Co.) and silica gel (200-300 mesh and Type 60, Qingdao Marine Chemical Co.) were used for open column chromatography.

Extraction and Isolation 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 the School of Pharmaceutical Sciences, Peking University, Beijing, People's Republic of China. The air-dried powdered plant material (20 kg) was refluxed with 95% ethanol (20 L) twice and then with 60% ethanol (20 L) once. The 95% ethanol extract (689 g) was subjected to silica gel chromatography by eluting in turn with petroleum ether, chloroform, ethyl acetate, and methanol. The EtOAc eluate (80 g) was subjected to silica gel column and eluted with $CHCl_3$-MeOH to give compound (**2**) (200 mg). The methanol eluate (5 g) and the 60% ethanol extract (53 g) was 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 70% MeOH eluate (21 g) was subjected to silica gel chromatography twice with $CHCl_3$: MeOH: H_2O as the eluent to afford **1** (500 mg) and **3** (50 mg).

Blinoside A (1). White amorphous powder; mp 78-79 ^{o}C; $[\alpha]^{23}_{D}$ –5.09^{o} (*c* 1.49, methanol); IR ν_{max} (KBr) 3386, 2922, 2864, 1657, 1385, 1076, 995 cm^{-1}; ^{13}C NMR (pyridine-d_5, 125 MHz) data see Table 1; ^{1}H NMR (pyridine-d_5, 500 MHz) data see Table 2; negative FAB MS: *m/z* 455 $[M-H]^{-}$; HR-FAB MS (negative mode): calcd for $C_{25}H_{43}O_7$ $[M-H]^{-}$, 455.3009; found 455.3005.

Blinoside A-*O*-15-[(*R*)-3-hydroxy]octadecanoate (2). Colorless oil; $[\alpha]^{22}_{D}$ –3.00^{o} (*c* 3.53, methanol); IR ν_{max} (KBr) 3370, 2919, 2847, 1719, 1380, 1081, 1068, 991 cm^{-1}; ^{13}C NMR (pyridine-d_5, 125 MHz) data see Table 1; ^{1}H NMR (pyridine-d_5, 500 MHz) data see Table 2; MALDI TOF MS (positive ion mode): *m/z* 761 $[M+Na]^{+}$, 777 $[M+K]^{+}$; HR-FAB MS (negative mode): calcd for $C_{43}H_{77}O_9$ $[M-H]^{-}$, 737.5568; found 737.5564.

Blinoside B (3). White amorphous powder; mp 78-80 ^{o}C; $[\alpha]^{22}_{D}$ –1.22^{o} (*c* 0.16, methanol); IR ν_{max} (KBr) 3411, 2922, 2864, 1660, 1385, 1076, 997 cm^{-1}; ^{13}C NMR (pyridine-d_5, 125 MHz) data see Table 1; ^{1}H NMR (pyridine-d_5, 500 MHz) data see Table 2; negative FAB MS: *m/z* 439 $[M-H]^-$; HR-FAB MS (negative mode): calcd for $C_{25}H_{43}O_6$ $[M-H]^-$, 439.3061; found 439.3060.

Acid Hydrolysis of 1. Compound (**1**) (20 mg) was heated in 3 mL of 0.4 M HCl (dioxane-H_2O, 2:1) at 95 ^{o}C for 2 h in a water bath. After dioxane was removed, the solution was washed with $CHCl_3$ (4 mL×4). The water layer was neutralized by passing through an Amberlite MB-3 resin column eluted with water, concentrated and then dissolved in 1 mL of water, to which an ethanol solution (1 mL) of L-(-)-α-methylbenzylamine (5 mg) and $NaBH_3CN$ (8 mg) were added. The mixture was heated and stirred at 40 ^{o}C for 4 h, acidified by 0.3 mL of glacial acetic acid, and evaporated to dryness to give a colorless oil which was acetylated with acetic anhydride (0.3 mL) and pyridine (0.3 mL) for 24 h at rt. 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 the 1-[(*S*)-*N*-acetyl-α-methylbenzyl-amino]-1-deoxyalditol acetate of the monosaccharide, which was identified by co-HPLC analysis with the derivative of standard arabinose prepared under the same conditions. HPLC conditions: Column, Shiseido Capcell Pak ODS, 4.6 mm i.d.×250 mm; mobile phase, 37% MeCN in H_2O; flow rate, 1 mL/min; detection, UV 230 nm. Retention times of the derivative of L-arabinose: 22.2 min.

Alkaline Hydrolysis of 2. 5 mL of 10% KOH in EtOH (w/v) was added to 2 mL of EtOH solution of **2** (20 mg), and the mixture was kept at rt for 18 h till TLC detection showed no presence of **2**. The product was diluted with 50 mL of H_2O, and then extracted with $CHCl_3$ (20 mL×4). The $CHCl_3$ soluble fraction was subjected to silica gel column and eluted with $CHCl_3$:MeOH (12:1) to afford compound (**1**) and 3*R*-hydroxyoctadecanoic acid.

3*R*-hydroxyoctadecanoic acid. White solid from $CHCl_3$; $[\alpha]^{22}_{D}$ –17.01^{o} (*c* 0.67, chloroform); ^{1}H NMR ($CDCl_3$, 500 MHz): 0.88 (t, *J* = 7.0 Hz, 3H, C18-CH_3), 1.25-1.56 (m, 28H, $(CH_2)_{14}$), 2.47 (dd, *J* = 16.5, 8.9 Hz, 1H, C2-CH_b), 2.57 (dd, *J* = 16.5, 3.1 Hz, 1H, C2-CH_a), 4.03 (m, 1H, C3-CH); ^{13}C NMR ($CDCl_3$, 125 MHz): 14.2 (C18), 22.8 (C17), 25.5 (C5), 29.4-29.8 (C6-C15), 32.0 (C16), 36.6 (C4), 40.9 (C2), 68.1 (C3), 176.8 (C1). MALDI-TOF MS: *m/z* 323 $[M+Na]^+$.

Acid Hydrolysis of 3. Compound (**3**) (10 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. The monosaccharide constituent in the sugar fraction was converted to the

corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative, which was then analyzed by HPLC. The derivative of L-arabinose was detected.

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