Two New Glycosides from *Sanicula lamelligera*

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Two new glycosides, 21-*O*-β-D-glucopyranosyl-olean-12-ene-3β, 16α, 21β, 22α, 28-pentol-3-*O*-β-D-glucopyranosyl $(1 \rightarrow 2)$-[β-D-arabinopyranosyl $(1 \rightarrow 3)$]-β-D-glucopyranoside (**1**) and 4-*O*-β-D-glucopyranosyl rosmarinic acid (**3**), along with the known compound rosmarinic acid (**2**) were isolated from the whole plants of *Sanicula lamelligera* Hance. Their structures were elucidated by spectroscopic analysis and chemical means.

Key words: Sanicula lamelligera, Triterpenoid Saponin, 4-*O*-β-D-Glucopyranosyl, Rosmarinic Acid

Introduction

Sanicula lamelligera Hance (Umbelliferae) is a folk medicine used for treatment of wounds, mild catarrhs of the respiratory system and chronic chest pains [1]. It was recently reported that saponins from this genus were responsible for antimicrobial, hemolytic, and antioxidant activities, whereas hydroxycinnamic derivatives, especially rosmarinic acid, were responsible for antioxidant activity and acted as active constituents against HIV-1 [2, 3]. Up to now, the chemical constituents of this plant have not been reported. Our investigation on this plant led to the isolation of two new glucosides (**1** and **3**) (see Figs 1 and 2).

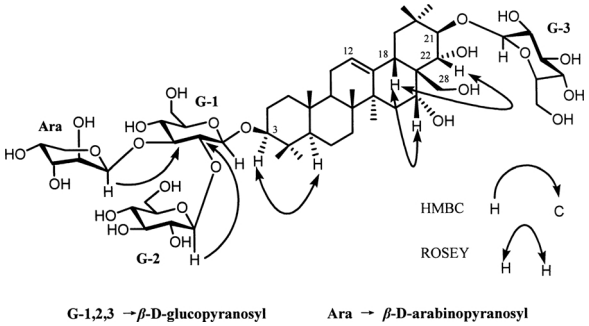

Fig. 1. Chemical structure and key correlations of compound **1**.

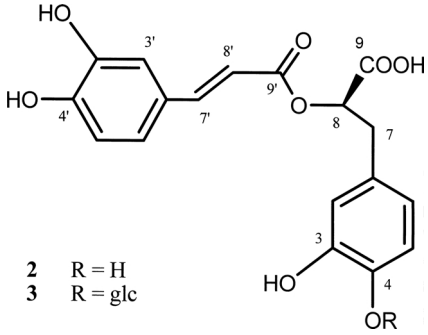

Fig. 2. Chemical structures of compounds **2** and **3**. **glc** → **β-D-glucopyranosyl**.

Results and Discussion

Compound **1** was isolated as a white amorphous powder and analyzed for $C_{53}H_{88}O_{24}$ by negative-ion HRESI-MS. The ^{1}H NMR and ^{13}C NMR spectrum of **1** displayed four anomeric proton signals at $\delta = 5.70$ (d, $J = 7.8$ Hz), 5.24 (d, $J = 7.8$ Hz), 4.98 (d, $J = 7.8$ Hz) and 4.86 (d, $J = 7.8$ Hz) and four anomeric carbon signals at $\delta = 103.8, 105.3, 106.6$ and 105.3. Consequently **1** was assumed to possess four sugar units. On acid hydrolysis, **1** gave an aglycone, identified as barringtogenol C (**1a**) by comparison of its spectral features with those previously reported [4], and sugar components which were identified as glucoside, arabinose by comparison with authentic samples on TLC. The sugar linkages were determined by 2D NMR experiments. In the HMBC spectrum, long-range correlations were observed between H-1 ($\delta = 4.98$, d, $J = 7.8$ Hz) of G-3 and C-21 ($\delta = 92.5$) of the agly-

No	**1**		**1a**	No	**1**	
	^{13}C	^{1}H	^{13}C		^{13}C	^{1}H
1	39.6 (t)	1.38, 0.85 (m)	39.2	G1-1	105.3	4.86 (d, 7.8 Hz)
2	26.7 (t)	2.16 (br d, 10.5 Hz)	28.2	2	79.6	
3	89.3 (d)	3.27 (dd, 11.5, 4.0 Hz)	78.1	3	87.2	
4	38.9 (s)		39.5	4	69.7	
5	55.8 (d)	0.71 (d, 11.5 Hz)	55.9	5	77.8	
6	18.5 (t)	1.25, 1.47 (m)	18.8	6	62.5	
7	33.1 (t)	1.22, 1.58 (m)	33.3	G2-1	103.8	5.70 (d, 7.8 Hz)
8	40.6 (s)		40.1	2	76.6	
9	47.0 (d)	1.75 (m)	47.2	3	78.0	
10	37.3 (s)		37.3	4	72.5	
11	23.9 (t)	1.87 (m)	23.9	5	78.7	
12	123.1 (d)	5.39 (s)	123.1	6	63.3	
13	144.0 (s)		144.0	ara-1	105.3	5.24 (d, 7.8 Hz)
14	42.0 (s)		42.1	2	72.9	
15	34.4 (t)	1.61, 2.00 (m)	34.4	3	74.9	
16	68.0 (d)	4.0 (t, 9.4 Hz)	67.9	4	69.8	
17	48.0 (s)		47.4	5	68.0	
18	40.1 (d)	2.85 (dd, 13.0 Hz, 3.8 Hz)	41.3	G3-1	106.6	4.98 (d, 7.8 Hz)
19	48.0 (t)	1.38 (m)	48.3	2	75.7	
		3.14 (t, 13.0)		3	78.3	
20	36.8 (s)		36.5	4	71.5	
21	92.5 (d)	4.85 (d, 9.2 Hz)	78.7	5	78.6	
22	74.1 (d)	4.61 (d, 9.2 Hz)	77.3	6	62.6	
23	28.0 (q)	1.21 (s)	28.8			
24	16.8 (q)	1.06 (s)	16.6			
25	15.8 (q)	0.80 (s)	15.8			
26	16.9 (q)	0.86 (s)	17.0			
27	27.6 (q)	1.84 (s)	27.5			
28	67.3 (t)	3.65 (d, 10.0 Hz)	68.4			
		3.95 (d, 10.0 Hz)				
29	30.2 (q)	1.53 (s)	30.6			
30	20.6 (q)	1.36 (s)	19.5			

Table 1. ^{13}C NMR and ^{1}H NMR spectral data for **1**, ^{13}C NMR for **1a** (in pyridine-d_5, 125 MHz).

cone, H-1 ($\delta = 4.86$, d, $J = 7.8$ Hz) of G-1 and C-3 ($\delta = 89.3$) of the aglycone, H-1 ($\delta = 5.70$, d, $J = 8.0$ Hz) of G-2 and C-2 ($\delta = 79.6$) of G-1, H-1 ($\delta = 5.24$, d, $J = 7.8$ Hz) of terminal arabinosyl and C-3 ($\delta = 87.2$) of G-1. From the NMR data each sugar is a pyranosyl with β configuration for glucosyl and arabinosyl. Therefore, the structure of **1** is assigned as 21-*O*-β-D-glucopyranosyl-olean-12-ene-3β, 16α, 21β, 22α, 28-pentol-3-*O*-β-D-glucopyranosyl$(1 \rightarrow 2)$-[β-D-arabinopyranosyl $(1 \rightarrow 3)$]-β-D-glucopyranoside.

Compound **2** was obtained as gray amorphous powder. $\{[\alpha]_D^{25} + 91.9$ (*c* 0.42, MeOH).$\}$ Negative-ion FAB-MS giving an [M-1]$^-$ peak at m/z 359 indicated the molecular formula $C_{18}H_{16}O_8$. The ^{1}H NMR spectrum of **2** indicated the presence of two 1, 3, 4-trisubstituted phenyl proups [$\delta = 6.98$ (1H, *d*, $J = 8.0$ Hz), 6.78 (1H, *br d*, $J = 8.0$ Hz), 6.85 (1H, *br s*), 6.68 (1H, *br s*), 6.77 (1H, *d*, $J = 8.0$ Hz), 6.65 (1H, *br d*, $J = 8.0$ Hz)] and one *trans* double bond [$\delta = 6.04$, 7.24 (each 1H, *d*, $J = 16.0$ Hz)]. Finally, the structure of **2** was assigned as rosmarinic acid on comparison with the data reported [5], including absolute configuration (R) at C-8.

Compound **3** was obtained as yellow gum. Negative-ion HRESI-MS gave an [M-1]$^-$ peak at m/z 521.1287, corresponding to a molecular formular of $C_{24}H_{26}O_{13}$. The ^{1}H NMR and ^{13}C NMR spectra of **3** were similar to those of **2**, except that signals corresponding to the sugar moiety included an anomeric proton signal [$\delta = 4.67$ (1H, d, $J = 7.0$ Hz)] and anomeric carbon signal ($\delta = 102.5$). The absolute configuration of **3** deduced as follows. Enzymic hydrolysis of **3** yielded rosmarinic acid $\{[\alpha]_D^{25} + 118.0$ (*c* 0.5, MeOH)$\}$ and D-glucose. The glucosyl unit attached to C-4 was deduced from the heteronuclear multiple-bond correlation (HMBC) interactions between the anomeric proton signal ($\delta = 4.67$) and the carbon signal of C-4 ($\delta = 144.4$). Furthermore, the *J* value (> 7) of the anomeric proton of the sugar moieties indicated the β-orientation at the anomeric center of D-glucose. Thus, **3** was determined to be 4-*O*-β-D-glucopyranosyl rosmarinic acid.

Table 2. ^{13}C NMR and ^{1}H NMR spectral data for **2**, **3** (in D_2O, 125 MHz).

No	**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	131.3 (s)		134.5 (s)	
2	118.1 (d)	6.85 (br s)	118.0 (d)	6.78 (br s)
3	144.7 (s)		146.4 (s)	
4	143.5 (s)		144.4 (s)	
5	117.1 (d)	6.98 (d, 8.0 Hz)	117.8 (d)	6.90 (d, 8.0 Hz)
6	122.6 (d)	6.78 (br d, 8.0 Hz)	122.6 (d)	6.68 (br d, 8.0 Hz)
7	37.8 (d)	2.97 (dd, 14.0, 4.3 Hz) 3.08 (dd, 14.0, 8.6 Hz)	37.8 (d)	2.97 (dd, 14.0, 4.3 Hz) 3.12 (dd, 14.0, 8.6 Hz)
8	77.3 (d)	5.18 (dd, 4.3, 8.6 Hz)	77.1 (d)	5.18 (dd, 4.3, 8.6 Hz)
9	177.5 (s)		177.5 (s)	
1'	127.9 (s)		127.9 (s)	
2'	117.0 (d)	6.68 (br s)	117.0 (d)	6.64 (br s)
3'	145.2 (s)		145.1 (s)	
4'	148.0 (s)		148.0 (s)	
5'	116.0 (d)	6.77 (d, 8.0 Hz)	116.0 (d)	6.80 (d, 8.0 Hz)
6'	123.4 (d)	6.65 (br d, 8.0 Hz)	123.4 (d)	6.63 (br d, 8.0 Hz)
7'	146.8 (d)	7.24 (d, 16.0 Hz)	146.9 (d)	7.21 (d, 16.0 Hz)
8'	115.4 (d)	6.04 (d, 16.0 Hz)	115.2 (d)	6.02 (d, 16.0 Hz)
9'	169.7 (s)		169.7 (s)	
glc-1			102.5 (d)	4.67 (d, 7.8 Hz)
2			73.8 (d)	
3			76.3 (d)	
4			70.1 (d)	
5			76.9 (d)	
6			61.3 (d)	

The above three compounds showed no anti-tumor activity against K562, K549, while compound **2** (rosmarinic acid) showed a weak anti-epiphyte activity.

Experimental Section

General

Melting points were measured on a Kofler melting point apparatus produced by Sichuan University (China) and are uncorrected. UV spectra were obtained on a UV-210A spectrometer Company apparatus. IR spectra were performed on a Bio-Rad FTS-135 spectrometer. Optional rotations were measured on a Japanese Fasco DIP-370 digital polarimeter. The negative ion FABMS and HR-ESIMS were recorded on a VG Auto Spec-3000 spectrometer. All NMR experiments were recorded on a Bruker DRX-500 spectrometer with TMS as internal standard. The silica gel for TLC and column chromatography were obtained from Qingdao Marine Chemical Inc., China.

Plant material

The whole plants of *Sanicula lamelligera* Hance were collected from Linglang County of Yunnan Province, P.R. China in April 2003 and identified by Prof. Y. M. Shui. A voucher specimen is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried plants (5.0 kg) were exhaustively extracted with 75% MeOH $(4\ l \times 3)$ at r. t., and the combined extracts were concertrated under reduced pressure. The crude extraction (200 g) was subjected to DM 101 resin column chromatography, eluting with water and 95% EtOH, successively. Then, the 95% EtOH faction was evaporated (Under red. press), giving a residue (140 g) that was purified on column chromatography (silica gel H-60, 15×110 cm) with a $CHCl_3$-MeOH gradient elution. **1** (70 mg) was obtained from fraction 4 ($CHCl_3$: MeOH: H_2O, 7: 3: 0.5 v/v) by repeated Rp-18 column chromatography. **2** (200 mg) was obtained from fraction 3 ($CHCl_3$: MeOH, 3: 1 v/v) by repeated column chromatography **3** (30 mg) was obtained from fraction 5 ($CHCl_3$: MeOH: H_2O, 7: 4: 0.5 v/v) by repeated Rp-18 column chromatography.

21-*O*-β-D-glucopyranosyl-olean-12-ene-3β, 16α, 21β, 22α, 28-pentol-3-*O*-β-D-glucopyranosyl $(1 \rightarrow 2)$-[β-D-arabinopyranosyl $(1 \rightarrow 3)$]-β-D-glucopyranoside **(1)**, white powder. – M. p. 292 – 294 °C. – $[\alpha]_D^{25} - 8.3$ (c 0.60, H_2O). – UV/vis$_{max}$ (pyridine): $\lambda_{max}(\lg \varepsilon) = 204$ nm (0.57). – IR (KBr) = 3424 (br, OH), 2884, 1632, 1078, 1041 cm^{-1}. – ^{1}H NMR and ^{13}C NMR see Table 1. – MS (FAB$^-$) m/z (%) = 1107 $[M-H]^-$ (100), 946 $[M-\text{glucose}]^-$ (9). – MS (HRESI): m/z = 1107.5597 ($C_{53}H_{88}O_{24}Na$. calcd. 1107.5587).

4-*O*-β-D-glucopyranosyl rosmarinic acid (**3**), yellow gum. – $[\alpha]_D^{25} - 16.7$ (c 0.60, H_2O). – UV/vis$_{max}$ (H_2O): $\lambda_{max}(\lg \varepsilon) = 283$ nm (3.17). – IR (KBr) ν = 3421 (br, OH), 1692, 1598, 1510, 1406, 1279 cm^{-1}. – ^{1}H NMR and ^{13}C NMR see Table 2. – MS (FAB$^-$) m/z (%) = 521 $[M-H]^-$ (100), 359 $[M-\text{glucose}-H]^-$ (8). – MS (HRESI): m/z = 521.1287 ($C_{24}H_{26}O_{13}Na$. calcd. 521.1295).

Acid hydrolysis of compound **1**: A solution of **1** (50 mg) in 5% H_2SO_4 was heated at 100 °C for 2 h. The reaction mixture was extracted with EtOAc and purified by MPLC ($CHCl_3$: MeOH = 30: 1, v/v) to provide compound **1a** (15 mg). ^{13}C NMR see Table 1.

Enzymic hydrolysis of Compound **3**: A mixture of compound **3** (20 mg) and complex cellulosic enzyme (1 g) in AcOH-AcONa buffer (pH 5.5, 10 ml) was incubated with gentle stirring at 28 °C for 72 h. The reaction mixture was extracted with EtOAc $(30\ ml \times 3)$. The extraction was chromatographed on silica gel ($CHCl_3$:MeOH, 7:3 v/v) to give rosmarinic acid {4 mg, $[\alpha]_D^{25} + 118.0$ (c 0.5, MeOH)}.

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