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TWO NEW TRITERPENOID SAPONINS FROM *Lysimachia davurica*

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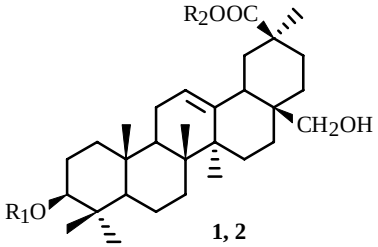
Two new saponins were isolated from an ethanol extract of the whole plants of Lysimachia davuria. *The new saponins were respectively characterized as 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl}-3*β*,28-dihydroxyolean-12-en-30-oic acid-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl]-ester (**1**) and 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl}-3*β*,28-dihydroxyolean-12-en-30-oic acid-*O*-*β*-D-glucopyranosyl-ester (**2**). Their structures were determined by 1D, 2D NMR and MS techniques.*

Key words: *Lysimachia davuria,* saponins.

Lysimachia davuria is a folklore medicinal plant that grows in northeastern China. The whole plant is used for treating hypertension [1]. Active saponins have been isolated from *Lysimachia* genus [2–4]. In previous paper we have isolated some flavones, chlorogenic acid ester, fatty acid, and eleven saponins from *Lysimachia davuria* [5–12]; now we continue to report the isolation and structural elucidation of two new saponins.

The whole plant of *L. davuria* was extracted with EtOH and partitioned between H_2O and petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH extract was chromatographed on an AB-8 resin column to afford a saponin-rich portion, which was separated by repeated Si-gel column chromatography on normal and HPLC, yielding the pure saponins **1** and **2**.

Saponin **1** gave positive result in the Liebermann-Burchard test. it showed quasimolecular ion peaks at *m/z* 1245 $[M+Na]^+$ in the positive ESIMS. The molecular formula was determined to be $C_{53}H_{94}O_{27}$ by HRFABMS. The six tertiary methyl groups δ 1.54, 1.24, 1.11, 1.01, 0.89, and 0.84 and one trisubstituted olefinic proton (δ 5.31, br.t) observed in the ^{1}H NMR spectrum (Table 1) as well as the information from the ^{13}C NMR spectrum (six sp^3 carbons at δ 28.1, 25.9, 19.1, 16.8, 16.7, and 15.6, two olefinic carbons at δ 123.0 and 144.2 analyzed with DEPT and HMQC) showed that the compound was a triterpenoid saponin. D-glucose, D-xylose, and L-arabinose were detected by GC analysis after acid hydrolysis and preparation of their thiazolidine derivatives. Assignment for all carbon signals was achieved by HMBC, HMQC, and DEPT (Table 2). Comparison of the ^{13}C NMR data with that of the known jacquinic acid (3*β*,16*α*,28-trihydroxy-olean-12-en-30-oic acid) [13] showed that they shared very similar ^{13}C NMR data except C-3, C-16, and C-30; C-16 and C-30 were upshifted by 50.8 and 3.7 ppm, respectively, suggesting that no hydroxyl was substituted at C-16 and carboxyl-30 was esterified by sugar; C-3 was downshifted for 10.5 ppm, suggesting that the glycoside linkage was at C-3. The above analysis revealed that the aglycone of saponin **1** was 3*β*,28-dihydroxy-olean-12-en-30-oic acid.



1, 2

	R_1	R_2
1:	*β*-D-Glc*p*-(1→2)-[*β*-D-Glc*p*-(1→4)]-*α*-L-Ara*p*-	*β*-D-Xyl*p*-(1→2)-*β*-D-Glc*p*-
2:	*β*-D-Glc*p*-(1→2)-[*β*-D-Glc*p*-(1→4)]-*α*-L-Ara*p*-	*β*-D-Glc*p*-

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TABLE 1. ^{1}H NMR Spectral Data for Compounds **1** and **2** in Pyridine-d_5

C atom	**1**	**2**	C atom	**1**	**2**
3	3.10 (dd, 12.0, 4.0)	3.11 (dd, 11.5, 4.5)	Sugar	Glc I at Ara C_2	Glc I at Ara C_2
5	0.85 (t, 7.0)	0.82 (t, 7.5)	1	5.16 (d, 8.0)	5.17 (d, 7.5)
12	5.31 (br.t)	5.17 (br.t)	2	3.97	3.98
15	1.63 (t, 7.0)	1.65 (t, 7.0)	3	3.78	3.80
16	1.71 (t, 7.0)	1.74 (t, 7.0)	4	4.17	4.20
18	3.04 (s)	3.12 (s)	5	3.90	3.92
23	1.11	1.16	6	4.44	4.45
24	1.01	1.02	Sugar	Glc II at Ara C_4	Glc II at Ara C_4
25	0.84	0.84	1	5.12 (d, 8.0)	5.13 (d, 8.0)
26	0.89	0.88	2	4.04	4.02
27	1.24	1.27	3	3.91	3.91
28	3.88 (d, 11.0)	3.86 (d, 11.0)	4	4.27	4.25
	3.63 (d, 11.0)	3.60 (d, 11.0)			
29	1.54	1.59	5	3.90	3.93
30			6	4.48	4.43
3-*O*-Sugar	Ara	Ara	30-*O*-Sugar	Glc III	Glc III
1	4.90 (d, 5.0)	4.91 (d, 5.5)	1	6.32 (d, 8.0)	5.81 (d, 8.0)
2	4.53	4.52	2	4.67	3.98
3	4.45	4.42	3	3.99	3.87
4	4.43	4.39	4	4.23	4.25
5	3.84	3.85	5	3.88	3.89
				4.45	
				Xyl at Glc III C_2	
				5.32 (d, 8.0)	
				3.27	
				3.58	
				3.53	
				4.00	

The HMQC spectrum of compound **1** showed that it contained five sugar units, Their anomeric protons at δ 6.32 (1H, d, J = 8.0 Hz), 5.32 (1H, d, J = 8.0 Hz), 5.16 (1H, d, J = 8.0 Hz), 5.12 (1H, d, J = 8.0 Hz), and 4.90 (1H, d, J = 5.0 Hz) were correlated with carbons signals at δ 106.8, 105.8, 105.6, 104.2, and 93.8, respectively. The spin-systems associated with the monosaccharide were identified by a TOCSY experiment with the aid of the ^{1}H-^{1}HCOSY spectrum. All ^{13}C signals of the sugar moieties were assigned by HMQC experiment as shown in Table 2. Combining with spin-spin couplings, the four units were identified as three *β*-glucopyranosides (Glc), one *β*-xylopyranoside (Xyl), and one *α*-arabinopyranoside (Ara).

The sugar sequences of the disaccharide chains as well as the glycoside sites were subsequently determined by HMBC spectrum. In the HMBC spectrum of saponin **1**, correlations can be made between the anomeric proton of arabinose at 4.90 (1H, d, J = 5.0 Hz) and the C-3 of aglycone at δ 88.7, the anomeric proton of glucose-I at 5.16 (1H, d, J = 8.0 Hz) and the C-2 of arabinose at δ 80.9, the anomeric proton of glucose-II at 5.12 (1H, d, J = 8.0 Hz) and the C-4 of arabinose at δ 77.1, the anomeric proton of glucose III at 6.32 (1H, d, J = 8.0 Hz) and the C-30 of aglycone at δ 177.6, and the anomeric proton of xylose at 5.32 (1H, d, J = 8.0 Hz) and the C-2 of glucose III at δ 81.9, suggesting the sugar sequences of the disaccharide chains.

Thus, the structure of the saponin **1** was established as 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosy}-3*β*,28-dihydroxyolean-12-en-30-oic acid-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl]-ester.

Comparison of the NMR of saponin **2** with that of saponin **1** showed that they have very similar ^{13}C NMR data except that saponin **2** has one xylose less than saponin **1**, though saponin **2** was established as 3-*O*-{*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl}-3*β*,28-dihydroxyolean-12-en-30-oic acid-*O*-*β*-D-glucopyranosylester.

TABLE 2. ^{13}C NMR Spectral Data for Compounds **1** and **2** in Pyridine-d_5

C atom	**1**	**2**	C atom	**1**	**2**
1	38.6	38.7	3-*O*-Sugar	Ara	Ara
2	26.3	26.3	1	104.2	104.3
3	88.7	88.7	2	80.9	80.8
4	39.4	39.4	3	72.3	72.3
5	55.6	55.5	4	77.1	77.5
6	18.4	18.4	5	64.2	63.4
7	32.7	32.8	Sugar	Glc I at Ara C_2	Glc I at Ara C_2
8	41.8	41.8	1	105.8	105.8
9	47.7	47.6	2	76.1	76.1
10	36.8	36.7	3	79.4	78.7
11	23.8	23.8	4	71.5	71.3
12	123.0	123.0	5	78.2	78.2
13	144.2	144.2	6	62.5	62.5
14	40.0	40.0	Sugar	Glc II at Ara C_4	Glc II at Ara C_4
15	25.2	25.6	1	105.6	105.6
16	23.0	22.9	2	75.7	75.7
17	37.5	37.4	3	78.3	78.3
18	41.4	41.2	4	71.3	71.5
19	40.5	40.8	5	78.1	78.1
20	43.3	43.1	6	62.5	62.5
21	29.4	29.3	30-*O*-Sugar	Glc III	Glc III
22	30.5	30.4	1	93.8	94.1
23	28.1	28.1	2	81.9	72.7
24	16.8	16.7	3	78.7	78.3
25	15.6	15.6	4	70.5	70.9
26	16.7	16.7	5	79.4	79.2
27	25.9	25.9	6	61.8	61.9
28	68.2	68.0	Sugar	Xyl at Glc III C_2	
29	19.1	19.6	1	106.8	
30	177.6	178.2	2	76.2	
			3	78.7	
			4	71.1	
			5	67.6	

EXPERIMENTAL

Material and Instrument. The *Lysimachia davuria* was collected from Tumen county of Jilin province in October 1998, China, and identified by Dr. Bao-Lin Guo, Institute of Medicinal Plants Development, Chinese Academy of Medical Sciences and Peking Union Medical College. The voucher specimen (No. 98023) is deposited in the Institute of Medicinal Plants Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Melting points were measured on a Fisher-Johns apparatus and were uncorrected. Optical rotations were obtained on a Perkin–Elmer 341 polarimeter. IR spectra were recorded on a Perkin–Elmer 983G spectrometer. NMR spectra were recorded on a Bruker AM-500 (500 MHz) instrument. FABMS were obtained on a Zabspec E spectrometer; ESIMS were obtained on an Esquire-LC00054 spectrometer. For column chromatography, AB-8 resin (Tianjin Nankai), silica gel (200–300 mesh, Qingdao Haiyang), and ODS C_{18} (35–50 *um*, Alltech) were used. TLC and HPTLC (silica gel GF_{254} precoated plates, Qingdao Haiyang) detection was by spraying with 10% H_2SO_4 following heating. GC analysis was carried out on a Shimadzu QP5050A gas chromatograph.

Extraction and Isolation. The dried powdered plant materials (10 kg) were refluxed with 95% EtOH twice and then with 50% EtOH twice; the 95% EtOH extract and 50% EtOH extract were combined. After removal of the solvent by evaporation, the combined extracts (2.8 kg) were partitioned between H_2O and petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH.

The *n*-BuOH extract (1.5 kg) was chromatographed over AB-8 resin column, eluting with H_2O and 30, 50, 70, and 95% EtOH. The 50% EtOH eluate (27 g) was chromatographed on Si-gel column, eluting with $CHCl_3$–MeOH (MeOH contains 5% H_2O) from 90:10 to 20:80 in a gradient manner. Fraction 20–24 ($CHCl_3$–MeOH 55:45) (4.8 g) was separated on an ODS C_{18} (35–50 μm) column (2.0×40.0 cm) using MeOH–H_2O (48:52) as eluent and reverse-phase HPLC purification (MeOH–H_2O 46.5:53.5) to afford **2** (28 mg); Fraction 27–31 ($CHCl_3$–MeOH 45:55) (2.3 g) was separated on the ODS C_{18} (35–50 μm) column (2.0×40.0 cm) using MeOH–H_2O (37:63) as eluent and reverse-phase HPLC purification (0.5×25.0 cm, MeOH–H_2O 34.5:65.5) to afford **1** (21 mg).

Saponin 1: white amorphous powder; $[\alpha]_D^{20}$ +7.78° (*c* 0.20, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3310 (OH), 2965, 2875, 1725, 1645, 1475, 1320, 1230, 1025; Positive and negative ESIMS *m/z* 1245 $[M+Na]^+$ and 1221 $[M-H]^-$; HRFABMS *m/z* 1245.5864 $[M+Na]^+$ (calcd for $C_{53}H_{94}O_{27}Na$, 1245.5880); ^1H and ^{13}C NMR see Tables 1 and 2.

Saponin 2: white amorphous powder; $[\alpha]_D^{20}$+6.60° (*c* 0.30, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3340 (OH), 2970, 2875, 1730, 1640, 1470, 1320, 1235, 1010; Positive and negative ESIMS *m/z* 1113 $[M+Na]^+$ and 1089 $[M-H]^-$; HRFABMS *m/z* 1113.5432 $[M+Na]^+$ (calcd for $C_{53}H_{86}O_{23}Na$, 1113.5458); ^1H and ^{13}C NMR see Tables 1 and 2.

Acid Hydrolysis. Each saponin (5 mg) dissolved in water (100 mL) and 2 M HCl (100 mL) was heated at 100°C for 1 h. The water was passed through an Amberlite IRA-60E column (6×50 mm), and the eluate was concentrated. The residue was dissolved in pyridine (25 mL) and stirred with D-cysteine methyl ester (4.0 mg) for 1.5 h at 60°C. To the reaction mixture, hexamethyldisilazane (10 mL) and trimethylsilyl chloride (10 mL) were added and the mixture was stirred for 30 min at 60°C. The supernatant was then analyzed by GC [Column: DB-50, 25 mm×30 m, column temperature 235°C; carrier gas N_2, retention time D-Glc (16.5 min), L-Glc (16.1 min), D-Xyl (10.0 min), L-Xyl (9.7 min), D-Ara (9.5 min), L-Ara (10.1 min). From the new saponins D-glucose, D-xylose, and L-arabinose were detected.

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