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Two New Saponins from *Lysimachia davurica*

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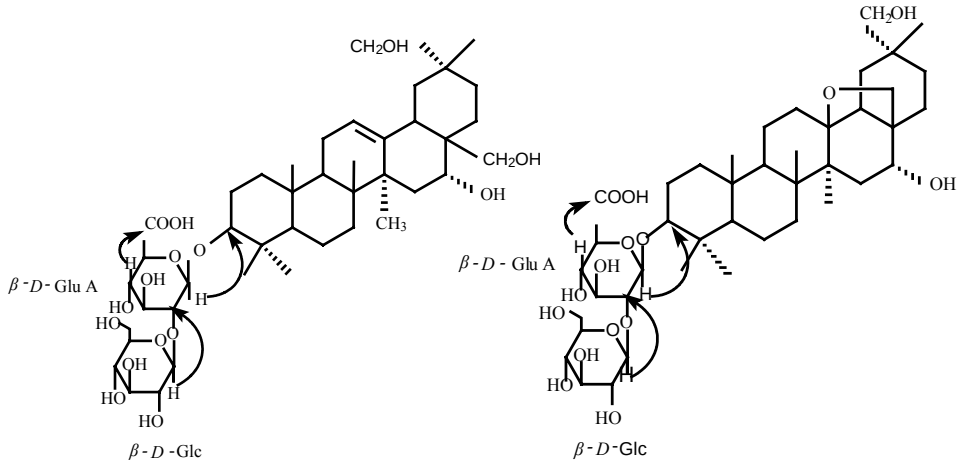
Abstract: Two new saponins named davuricoside I (**1**) and davuricoside E (**2**) were isolated from the whole plants of *Lysimachia davurica.* Their structures were determined by 1D and 2D NMR, FAB-MS techniques, and chemical methods.

Keywords: *Lysimachia davurica*, Primulaceae, triterpene saponin, davuricoside I, E.

Lysimachia davurica Ledeb. (Primulaceae) is a folk medicinal plant, growing in the Northeastern China. The whole plant is used for treating hypertension [1]. We have reported two new saponins (davuricoside L and O.) isolated from this plant[2], we now continue to report the isolation and structural elucidation of two new saponins, davuricoside I (**1**) and davuricoside E (**2**).

Figure 1 Structure and key HMBC correlations of compound **1** and **2**



1 **2**

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The whole plants of *Lysimachia davurica* was extracted with 95% and 50% EtOH and the EtOH extract was concentrated. The residue was dissolved in water and partitioned with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, successively. *n*-BuOH extract was chromatographed on AB-8 resin column to afford a saponin-rich portion, this portion was separated by repeated silica gel column chromatography and HPLC to afford compounds **1** and **2**.

Compound **1** was obtained as an amorphous white powder, mp 223-225℃, $[\alpha]_D^{20}$ -15.62 (c 0.10, pyridine), and gave positive result to Liebermann-Burchard test. In the positive and negative ESIMS, it showed quasi-molecular ion peak at *m/z* 835.6 $[M+Na]^+$ and 811.9 $[M-H]^-$, respectively. Its molecular formula $C_{42}H_{68}O_{15}$ was deduced from NMR and MS data. The six tertiary methyl groups (δ 1.72, 1.31, 1.15, 1.04, 0.88 and 0.79) and one olefinic proton (δ 5.33, br t) could be observed in the ^{1}HNMR spectrum. Correspondingly, the information of the ^{13}CNMR spectrum showed six sp^3 carbons at δ 15.5, 16.6, 16.9, 20.5, 27.1 and 27.8, and two sp^2 olefinic carbons at δ 122.0 and 144.9. Glucurose and glucose were detected after the acid hydrolysis. This result evidenced that the compound was a triterpene saponin. Assignment for all carbon signals was achieved by 2D NMR **(Table 1)**. Comparing with that of pridentigenin E (3β, 16α, 28, 30-tetrahydroxyolean-12-ene) [3], both compounds showed very similar ^{13}CNMR data except data of C-3, C-29 and C-30. In the ^{13}CNMR spectrum of **1**, the signal of C-3 down-shifted for 11.2 ppm, suggested the glycoside linkages at C-3. The methyl signal at C-20 up-shifted for 8.2 ppm, and the methylenehydroxyl signal at C-20 down-shifted for 7.0 ppm, suggested that C-29 and C-30 of compound **1** was CH_2OH and CH_3, respectively. In the NOESY spectrum, the proton at δ 4.26(H-16) had the correlation with methyl at δ 1.28 (26-CH_3), the methylenehydroxyl at δ 3.70 (29-CH_2OH) also correlated with methyl at δ 1.50 (27-CH_3). The above information revealed the aglycone of compound **1** to be 3β, 16α, 28, 29-tetrahydroxyolean-12-ene.

The HMQC spectrum of compound **1** showed that it contained two sugar units, their anomeric protons at δ 5.32 (d,1H, J 7.5Hz) and 4.82 (d,1H, J 8.0Hz) were correlated with carbon signals at δ 105.3 and 104.9, respectively. The spin-systems associated with two individual monosaccharides were identified by HMQC-TOCSY experiment with the aid of a ^{1}H-^{1}H COSY spectrum. All ^{13}C signals of the sugar moieties were assigned by HMQC experiment as shown in **Table 1**. Combining with spin-spin couplings of their anomeric protons, the two sugar units were identified as β-glucuronopyranoside (Glu A) and β-glucopyranosides (Glc). The sugar sequences of the oligosaccharide chains as well as the glycoside sites were subsequently determined by HMBC spectrum. In the HMBC spectrum of **1** (**Figure 1**), the correlations could be achieved between the anomeric proton of glucurose at δ 4.82 (d, 1H, J 7.50Hz) and C-3 of aglycone at δ 89.1, the anomeric proton of glucose at δ 5.32 (d, 1H, J 8.0Hz) and the C-2 of glucurose at δ 81.9, respectively, suggesting the sugar sequences of the oligosaccharide chains as shown in **Figure 1**.

Thus, the structure of the compound **1** was established as 3β, 6α, 28, 29-tetrahydroxyolean-12- en-3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucuronopyranoside.

Compound **2** was obtained as a white amorphous powder, mp 226-228℃, $[\alpha]_D^{20}$ -5.71 (c 0.50, pyridine), and gave positive result to Liebermann-Burchard test. In the positive and negative ESIMS, it showed quasi-molecular ion peak at *m/z* 835.5 $[M+Na]^+$ and 811.8

[M-H]$^-$, respectively. Its molecular formula $C_{42}H_{68}O_{15}$ was deduced from NMR and MS data. By comparison of NMR with that of compound **1**, compound **2** showed the same signals in the sugar portion with slightly difference in the aglycone. Compound **2** showed no olefinic signals between C-12 and C-13; instead, it showed a quaternary carbon signal at δ 86.5 ppm of C-13, which is the typical signal of epoxyoleanane of C-13 and C-18. Assignment of all the carbon signals of compound **2** was achieved by ^{1}H-^{1}HCOSY, HMQC, and HMBC experiments (**Figure 1** and **Table 1**). All the data assigned that compound **2** is 3β, 16α, 29-trihydroxy-13, 28-epoxyoleanane-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranoside.

We have also examined the cytotoxic activity of compound **1** and **2** against human A2780 cells. Compound **1** showed significant cytotoxic activity with IC_{50} values of 0.69 μg/mL, while compound **2** showed no cytotoxic activity.

Table 1 The ^{13}CNMR (125M Hz) spectral data of compound **1**and **2** (in pyridine-d_6, δ ppm)

position	**1** δ_c	**2** δ_c	position	**1** δ_c	**2** δ_c	position	**1** δ_c	**2** δ_c
1	38.7	39.0	16	73.6	76.8	3-O-glu A		
2	26.3	26.4	17	41.8	42.3	1'	104.9	104.7
3	89.1	89.4	18	41.3	50.6	2'	81.9	81.8
4	39.3	39.5	19	43.3	28.3	3'	77.7	75.7
5	55.6	55.5	20	36.6	50.6	4'	72.0	73.3
6	18.2	17.7	21	31.7	32.1	5'	77.8	77.8
7	33.1	34.3	22	29.0	31.3	6'	172.1	176.2
8	39.8	44.4	23	27.8	28.9	Glc(1→2)		
9	46.8	50.2	24	16.6	16.4	1"	105.3	105.3
10	36.6	36.6	25	15.5	16.3	2"	76.6	76.6
11	23.6	19.1	26	16.9	18.4	3"	77.5	77.5
12	122.0	36.7	27	27.1	19.5	4"	71.3	71.4
13	144.9	86.5	28	69.8	78.1	5"	78.2	78.3
14	41.8	45.0	29	74.1	74.7	6"	62.4	62.4
15	34.4	36.6	30	20.5	20.2			

Acknowledgments

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