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NEW ANTITUMOR TRITERPENE SAPONIN FROM *Lysimachia capillipes*

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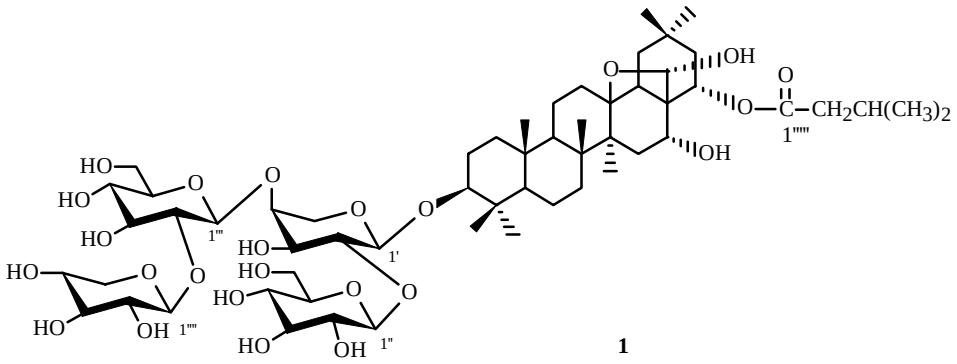
*One new saponin, named capilliposide D (**1**), and a known saponin, candidoside (**2**), were isolated from the whole plants of* Lysimachia capillipes*. Their structures were determined by 1D and 2D NMR, MS techniques, and chemical methods.*

Key words: *Lysimachia capillipes*, triterpene saponin, capilliposide D.

Lysimachia capillipes Hemsl (Primulaceae) is a folk medicinal plant that grows in southeastern China. The whole plant is used for treating colds and rheumatoid arthritis [1]. We have isolated some flavones from this plant [2]. Now we continue to report the isolation and structural elucidation of one new saponin, capilliposide D (**1**), and a known saponin, candidoside (**2**).

Compound **1** was obtained as an amorphous white powder and gave a positive result in the Liebermann- Burchard test. The molecular formula was determined to be $C_{57}H_{94}O_{24}$ by HRFABMS. In the negative ESIMS, it showed the signal of the quasi-molecular ion peak at *m/z* 1161 $[M–H]^-$. Seven tertiary methyl groups (δ 1.55, 1.28, 1.14, 1.09, 1.03, 1.02, and 0.77) were observed in the ^{1}H NMR spectrum, and the ^{13}C NMR spectrum (seven sp^3 carbons at δ 17.5, 17.7, 19.8, 20.9, 26.7, 29.1, and 34.4) showed that the compound was a triterpene saponin. Glucose, arabinose, and xylose were detected after acid hydrolysis. Assignment of all carbon signals (Table 1) was achieved by HMQC and HMBC. HMBC, DEPT, ^{1}H–^{1}H COSY, and HMQC-TOCSY showed that there is an isovaleryl group in the molecular structure.



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The ^{13}C NMR data was compared with that of the known anagalligenin A-22-acetate (3β, 16α, 22α, 28α-tetrahydroxy-22-acetate-13,28-epoxyoleanane) [3]. The aglycone of compound **1** and anagalligenin A-22-acetate showed very similar ^{13}C NMR data, except that the acetyl in anagalligenin A-22-acetate was replaced by isovaleryl in the aglycone of compound **1**. Correlations could also be achieved between the proton of H-22 [5.99 (1H, dd, J = 5.5, 6.0 Hz)] and the carbonyl of caproate at δ 173.9 by HMBC; the C-3 displayed down-shift for 12.2 ppm, suggesting that the glycoside linkage was at C-3; The NOESY correlations between H-16α, H-28α, H-22α, and Me-30 are in agreement with the β-configuration of OH-28.The above analysis revealed that the aglycone of compound **1** was anagalligenin A-22-isovalerat.

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TABLE 1. ^{13}C NMR Spectral Data for the Aglycone Moieties of **1** and **2** (125 MHz in pyridine-d_5)

C atom	**1**	**2**	C atom	**1**	**2**
1	40.3	38.6		*α*-L-Ara	*α*-L-Ara
2	27.3	26.4	1′	105.6	104.8
3	90.4	89.2	2′	80.5	79.3
4	40.9	39.6	3′	74.5	73.4
5	56.8	55.8	4′	80.1	78.3
6	19.1	18.4	5′	65.8	64.8
7	35.4	33.0		*β*-D-Glc I	*β*-D-Glc I
8	43.8	40.0	1″	105.9	104.4
9	51.4	47.1	2″	77.1	76.0
10	38.0	36.8	3″	78.6	77.8
11	20.4	23.8	4″	72.8	71.7
12	34.4	122.3	5″	79.2	78.1
13	88.9	145.2	6″	64.0	62.9
14	45.0	41.9		*β*-D-Glc II	*β*-D-Glc II
15	37.8	34.6	1‴	105.4	104.3
16	70.9	74.0	2‴	86.3	85.1
17	52.6	40.8	3‴	79.0	77.1
18	48.5	42.6	4‴	72.0	70.8
19	39.5	48.3	5‴	78.3	78.0
20	34.5	31.2	6‴	63.2	62.1
21	42.8	37.0		*β*-D-Xyl	*β*-D-Xyl
22	73.8	30.8	1⁗	108.65	107.4
23	29.1	28.0	2⁗	77.0	75.8
24	17.7	16.6	3⁗	79.2	77.4
25	17.5	15.7	4⁗	71.7	70.5
26	19.8	17.9	5⁗	68.5	67.3
27	20.9	27.3		Isovaleryl	
28	98.9	70.0	1′′′′′	173.9	
29	34.4	33.5	2′′′′′	27.7	
30	26.7	24.8	3′′′′′	45.3	
			4′′′′′	23.7	
			5′′′′′	23.6	
			6′′′′′	173.9	

The HMQC spectrum of compound **1** showed that it contained four sugar units, Their anomeric protons at δ 5.43 (1H, d, J = 8.0 Hz), 4.88 (1H, d, J = 8.0 Hz), 4.82 (1H, d, J = 7.5 Hz), and 4.72 (1H, d, J = 6.0 Hz) were correlated with the carbon signals at δ 105.9, 105.4, 108.6, and 105.6, respectively. The spin systems associated with monosaccharide were identified by HMQC-TOCSY experiment with the aid of the ^{1}H–^{1}H COSY spectrum. All ^{13}C signals of the sugar moieties were assigned by HMQC experiment as shown in Table 1. Combined with spin-spin couplings, the four units were identified as two *β*-glucopyranosides (Glc), one *α*-arabinpyranoside (Ara), and one *β*-xylpyranoside (Xyl).

The sugar sequences of the oligosaccharide chain as well as the glycoside sites were subsequently determined by HMBC spectrum. In the HMBC spectrum of **1**, correlations could be achieved between the anomeric proton of arabinose at 4.72 (1H, d, J = 6.0 Hz) and C-3 of aglycone at δ 90.4, the anomeric proton of glucose-I at 5.43 (1H, d, J = 8.0 Hz) and the C-2 of arabinose at δ 80.5, the anomeric proton of glucose-II at 4.88 (1H, d, J = 8.0 Hz) and the C-4 of arabinose at δ 80.1, and the anomeric proton of xylose at 4.82 (1H, d, J = 7.5 Hz) and the C-2 of glucose -II (at C-4 of arabinose) at δ 86.3, respectively, suggesting the sugar sequences of the oligosaccharide chain as shown in figure.

Thus, the structure of compound **1** was established as 22-isovalerate-anagalligenin A-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranoside, named capilliposide D.

TABLE 2. ^{1}H and ^{13}C NMR Spectral Data for the Sugar Moieties of **1** and **2** (125 MHz for ^{13}C NMR and 500 MHz for ^{1}H NMR in pyridine-d_5, δ, ppm, J/Hz)

H atom	**1**	**2**	H atom	**1**	**2**
	α-L-Ara	α-L-Ara		β-D-Xyl	β-D-Xyl
1′	4.72 (J = 6.0)	4.78 (J = 5.5)	1‴	4.82 (J = 7.5)	4.95 (J = 5.0)
2′	4.42	4.34	2‴	4.11	4.16
3′	4.33	4.32	3‴	4.19	4.23
4′	4.49	4.41	4‴	4.26	4.37
5′	3.83	3.99	5‴	3.70	3.82
5′	3.60	3.67	5‴	4.28	4.33
	β-D-Glc I	β-D-Glc I		Isovaleryl	
1″	5.83 (J = 8.0)	5.51 (J = 8.0)	2⁗	1.77 (1H, dd, J = 5.5, 12.0)	
2″	4.02	4.11	2⁗	1.75 (1H, dd, J = 5.5, 12.0)	
3″	3.88	3.90	3⁗	2.19 (1H, m)	
4″	4.24	4.22	4⁗	0.87 (3H, d, J = 6.0)	
5″	3.98	3.99	5⁗	0.85 (3H, d, J = 6.0)	
6″	4.51	4.48			
6″	4.36	4.36			
	β-D-Glc II	β-D-Glc II			
1‴	4.88 (J = 8.0)	4.90 (J = 8.0)			
2‴	4.26	4.20			
3‴	3.74	3.85			
4‴	4.23	4.25			
5‴	4.03	4.12			
6‴	4.60	4.57			
6‴	4.49	4.35			

EXPERIMENTAL

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. HPLC was performed using a Waters 510 pump with Alltech 500 ELSD (Evaporative Light Scattering Detector). 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 done by spraying 10% H_2SO_4 with following heating.

Plant Material. The *Lysimachia capillipes* was collected in Guizhou province, China, and identified by Dr. Bao-Lin Guo, Institute of Medicinal Plants Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

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 the 50% EtOH extract were combined. After removal of the solvent by evaporation, the combined extracts were partitioned between H_2O and petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The *n*-BuOH extract was chromatographed over an AB-8 resin column, eluting with H_2O and 30, 50, 70, and 95% EtOH. The 50% EtOH eluate was chromatographed on Si gel column, eluting with $CHCl_3$/MeOH (contain 5% H_2O) in a gradient manner. Fraction 30 was separated on an ODS C_{18} (35–50 *um*) column using MeOH–H_2O (41.5:58.5) as eluent to afford **1** (120 mg) and **2** (15 mg).

Compound 1, white amorphous powder (MeOH–H_2O, 9:1), mp 239–241°C, $[\alpha]_D^{20}$ –10.00° (*c* 0.90, MeOH); IR (ν_{max}, KBr, cm^{-1}): 3320 (OH), 2960, 2870, 1730, 1720, 1460, 1340, 1200, 1060, 950.

^{1}H NMR (500 MHz, Py-d_5, δ, ppm, J/Hz): 5.99(1H, dd, J = 5.5, 6.0, H-22), 5.20 (1H, s, H-28), 4.78 (1H, br.t, H-16), 3.13 (1H, dd, J = 4.5, 12.0, H-3), 1.55 (3H, s, Me-27), 1.28 (3H, s, Me-26), 1.14 (3H, s, Me-23), 1.09 (3H, s, Me-29), 1.03 (3H, s, Me-24), 1.02 (3H, s, Me-30), 0.77 (3H, s, Me-25).

For the ^{1}H NMR data of the sugar moieties, see Table 2; for ^{13}C NMR (Py-d_5, 125 MHz), see Table 1; negative ESIMS *m/z* 1161 $[M–H]^-$; HRFABMS *m/z* 1185.6126 $[M+Na]^+$ (calcd. for $C_{60}H_{96}O_{29}Na$ 1185.6143).

Compound 2, white amorphous powder (MeOH–H_2O, 9:1), mp 227–229°C, $[\alpha]_D^{20}$ +9.47° (*c* 0.75, MeOH).

IR (ν_{max}, KBr, cm^{-1}): 3320 (OH), 2960, 2870, 1640, 1470, 1050, 950.

^{1}H NMR (500 MHz, Py-d_5, δ, ppm, J/Hz): 5.45 (1H, br.t, H-12), 4.68 (1H, br.t, H-16), 3.81 (1H, d, J = 11.5, H-28), 3.62 (1H, d, J = 11.5, H-28′), 3.19 (1H, dd, J = 4.5, 11.5, H-23), 1.82 (3H, s, Me-27), 1.23 (3H, s, Me-29), 1.10 (3H, s, Me-24), 1.09 (3H, s, Me-30), 1.04 (3H, s, Me-23), 0.97 (3H, s, Me-26), 0.84 (3H, s, Me-25).

For the ^{1}H NMR data of the sugar moieties, see Table 2; for ^{13}C NMR (Py-d_5, 125 MHz), see Table 1; negative ESIMS *m/z* 1045 $[M–H]^-$; HRFABMS *m/z* 1069.5541 $[M+Na]^+$ (calcd for $C_{61}H_{98}O_{29}Na$ 1069.5559). According with 3*β*,16*α*, 28-trihydroxy-olean-12-ene-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranoside (candidoside) [4].

Acid Hydrolysis of 1 and 2. Compounds **1** and **2** (each 5 mg) were refluxed with 5% HCl in MeOH (10 mL) for 5 h, and each mixture was diluted with H_2O and neutralized with $NaCO_3$. The neutral hydrolysate showed the presence of D-xylose, D-glucose, and L-arabinose by HPTLC [CH_3Cl–MeOH–H_2O (65:35:10) lower layer] when compared with authentic samples (Sigma).

Cytotoxic Activity. Compounds **1** and **2** were examined against human A2780 cells. Compound **1** showed significant cytotoxic activity with an IC_{50} value of 0.2 μg/mL. Compound **2** showed no cytotoxic activity.

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