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Note

Isolation and characterization of two new saponins from *Lysimachia capillipes*

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Abstract—Two new saponins, capilliposide K (**1**) and capilliposide L (**2**), were isolated from the whole plants of *Lysimachia capillipes*. Their structures were established by spectral and chemical techniques.

Keywords: *Lysimachia capillipes*; Capilliposide K; Capilliposide L

1. Introduction

Lysimachia capillipes Hemsl (Primulaceae) is a medicinal plant that grows in southeastern China and is well known in the folklore of the region. The whole plant is used for treating colds and rheumatoid arthritis.[1] In the course of our ongoing screening for active antitumor constituents, it was found that the ethanol extracts displayed cytotoxicities. We have isolated nine saponins from this plant, together with some flavones and lactones.[2–8] Two of the saponins have been shown to have the stronger antitumor activities among the compounds isolated.[6,8] Now, we continue to report the isolation and structural elucidation of two new saponins, capilliposides K (**1**) and L (**2**). They showed no cytotoxic activities against human A-2780 cells.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −14.33 (*c* 0.60, pyridine), and gave a positive Liebermann–Burchard reaction. The molecular formula was determined to be $C_{58}H_{96}O_{27}$ on the basis of HRFABMS: *m/z* 1247.5671 $[M+Na]^+$ (calcd for $C_{58}H_{96}O_{27}Na$ 1247.6037). The negative-ion ESIMS showed a signal for the quasi-molecular ion peak at *m/z* 1223.8 $[M-H]^-$. The presence of seven tertiary methyl groups (δ 1.84, 1.78, 1.19, 1.09, 0.97, 0.92, and 0.82) and one olefinic proton (δ 5.36, br t) observed in the ^{1}H NMR spectrum as well as in the ^{13}C NMR data (seven sp^3 carbons at δ 15.4, 16.5, 16.5, 25.1, 27.2, 27.7, 33.3 and two sp^2 olefinic carbons at δ 122.0 and 144.9 analyzed with DEPT and HMQC) indicated that the compound might be an olefinic triterpene saponin. D-Glucose, D-xylose, and L-arabinose were detected by GC analysis after acid hydrolysis and preparation of their thiazolidine derivatives.[11] Assignments of all carbon signals (Table 1) were achieved by HMQC and HMBC spectra. The ^{13}C NMR spectrum of **1** showed the signals of aglycone moiety identical to those of camelliagenin A (3β,16α,22α,28-tetrahydroxy-olean-12-ene)[9] except those for the C-3 and C-22 carbon atoms. The chemical shifts of C-3 and C-22 had shifted downfield for 9.9 and 15.3 ppm, respectively, which suggested that the sugar chains were linked at positions C-3 and C-22. The NOESY correlations between H-16, H-22, H-26, H-28β, and Me-30 were in agreement with

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Table 1. The ^{1}H NMR (500 MHz) spectral data of compounds **1** and **2**[a]

No.	Camelligenin in CD_3OD	**1**	**2**	No.		**1**	**2**
1	1.03, 1.64	1.03, 1.43	1.06, 1.50	3-O-Ara	1′	4.77	4.71
2	1.74, 2.03	1.80, 1.94	1.83, 1.97		2′	4.41	4.38
3	3.20	3.17	3.13		3′	4.33	4.30
4					4′	4.49	4.46
5	0.79	0.86	0.88		5′	3.81, 3.68	3.80, 3.63
6	1.41, 1.56	1.55, 1.60	1.59, 1.66	At C-2′ Glc	1″	5.49	5.50
7	1.35, 1.54	1.20, 1.63	1.19, 1.57		2″	4.07	4.03
8					3″	3.86	3.84
9	1.64	1.49	1.53		4″	4.24	4.20
10					5″	3.94	3.92
11	1.88	1.87, 1.95	1.87, 1.98		6″	4.50, 4.35	4.50, 4.34
12	5.26	5.36	5.38	At C-4′ Glc	1‴	4.96	4.88
13					2‴	4.01	3.99
14					3‴	3.69	3.66
15	1.40, 1.83		2.98		4‴	4.08	4.02
16	4.15	5.17	3.84		5‴	3.80	3.77
17					6‴	4.60, 4.46	4.59, 4.36
18	2.42	2.51	2.55	At C-2‴ Xyl	1⁗	4.89	4.81
19					2⁗	4.05	4.00
20					3⁗	4.17	4.15
21	1.46, 2.10	1.83, 2.55	1.86, 2.59		4⁗	4.13	4.11
22	4.03	4.67	4.78		5⁗	3.72, 4.20	3.65, 4.21
23	1.07	1.19	1.15	At C-22 Glc	1⁗′	5.18	5.20
24	0.86	1.09	1.02		2⁗′	4.33	4.35
25	0.98	0.82	0.68		3⁗′	3.85	3.91
26	0.96	0.92	0.80		4⁗′	4.22	4.27
27	1.44	1.84	1.43		5⁗′	3.95	4.05
28	3.32, 3.64	4.08, 3.79	4.20, 4.53		6⁗′	4.64, 4.43	4.60, 4.32
29	0.90	1.78	0.85				
30	0.97	0.97	1.07				

[a] In pyridine-d_5.

β-configurations for H-16 and H-22. The above evidence revealed that the aglycone of compound **1** was camelliagenin A.

The ^{1}H and ^{13}C NMR spectra of compound **1** displayed signals of five sugar units. Their anomeric protons at δ 5.49 (1H, d, J 7.5 Hz), 5.18 (1H, d, J 8.0 Hz), 4.96 (1H, d, J 7.5 Hz), 4.89 (1H, d, J 7.5 Hz), and 4.77 (1H, d, J 5.5 Hz) in the HMQC spectrum were correlated with carbon signals at δ 104.2, 106.3, 104.0, 107.2, and 104.5, respectively. The spin systems associated with saccharides were identified by an HSQC–TOCSY experiment with the aid of a ^{1}H–^{1}HCOSY spectrum. All ^{1}H and ^{13}C signals of the sugar moieties were assigned by HMQC experiment. By combining these data with spin–spin couplings, the five sugar units were identified as three β-D-glucopyranosyl, one β-D-xylopyranosyl, and one α-L-arabinopyranosyl moieties.

The sugar sequences of the saccharide chains, as well as the glycoside sites were subsequently determined by the HMBC spectrum. In the HMBC spectrum of **1** (Fig. 1), the correlations between the anomeric proton of arabinose at δ 4.77 (1H, d, J 5.5 Hz) and C-3 of aglycone at δ 88.9, the anomeric proton of glucose-I at δ 5.49 (1H, d, J 7.5 Hz) and the C-2 of arabinose at δ 79.0, the anomeric proton of glucose-II at δ 4.96 (1H, d, J 7.5 Hz) and the C-4 of arabinose at δ 78.7, the anomeric proton of xylose at δ 4.89 (1H, d, J 7.5 Hz) and the C-2 of glucose-II at δ 84.9, and between the anomeric proton of glucose-III at δ 5.18 (1H, d, J 8.0 Hz) and the C-22 of aglycone at δ 85.6 were indicative of the sugar sequences of the saccharide chains as shown in Figure 1.

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

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −21.33 (c 0.75, pyridine). Its molecular formula, $C_{58}H_{94}O_{27}$, was deduced from HRFABMS: m/z 1245.4978 $[M+Na]^+$ (calcd for $C_{58}H_{94}O_{27}Na$ 1245.5880). Comparing the ^{13}C NMR data of **2** with those of **1**, **2** showed essentially the same carbon NMR data as **1** in the sugar portions, but different values in the aglycone part. The HMQC, HMBC, DEPT, ^{1}H–^{1}HCOSY, and NOESY spectra suggested the presence of an epoxy group between C-15 and C-16. In the NOESY spectrum of compound **2**, signals of H-15 [δ 2.98 (1H, d, J 3.5 Hz)], H-16 [δ 3.84 (1H, d, J 3.5 Hz)], and H-22 [δ 3.84 (1H, d, J 3.5 Hz)] were correlated to

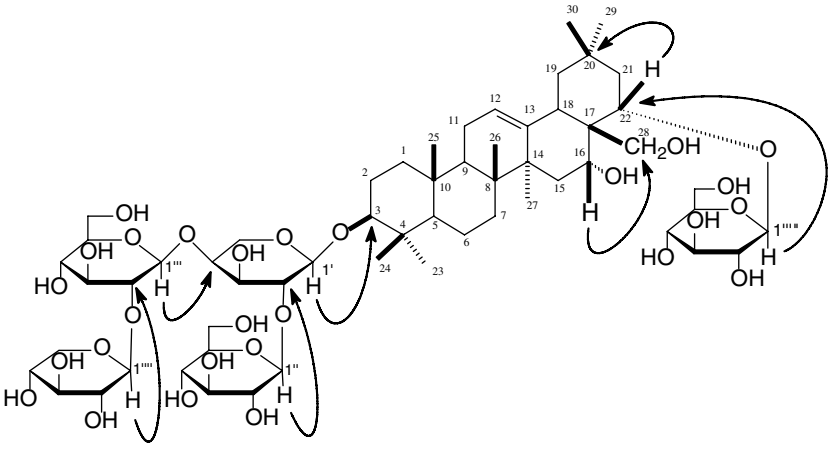

Figure 1. Structure and key HMBC correlations of compound **1**.

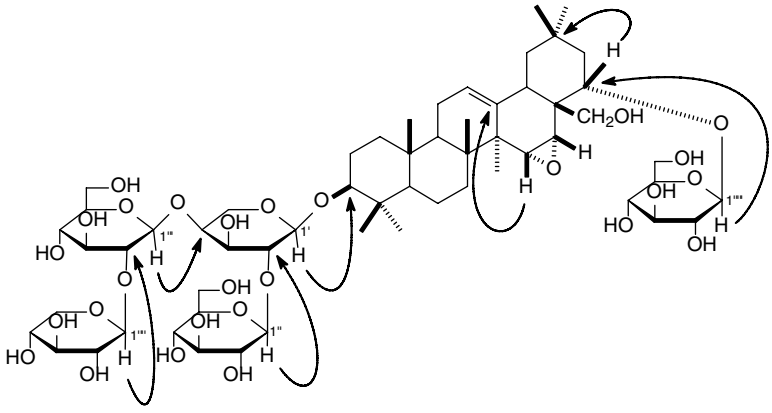

Figure 2. Structure and key HMBC correlations of compound **2**.

H-25 [δ 0.68 (3H, s)], H-26 [δ 0.80 (3H, s)], and H-28 [δ 3.96 (1H, d, J 10.5 Hz), 4.41 (1H, d, J 10.5 Hz)], respectively, from which it could be concluded that the conformations of H-15, H-16, and H-22 were all on the β side. The carbon signals of C-13, C-16, C-22, and C-28 were shifted upfield by 4.9, 13.1, 6.1, and 5.8 ppm, respectively, as a consequence of the epoxy group between C-15 and C-16. Based on the above findings, compound **2** was determined to be 22-*O*-β-D-glucopyranosyl-15α,16α-epoxy-28,3β,22α-trihydroxy-3-*O*-{β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl(1→2)]-α-L-arabinopyranosyl}-olean-12-ene, named as capilliposide L (Fig. 2).

3. Experimental

3.1. General methods

Optical rotations were obtained on a Perkin–Elmer 341 polarimeter in pyridine solution. IR spectra were recorded on a Perkin–Elmer 983G spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM-500 instrument. FABMS was carried out on a Zabspec E spectrometer, and ESIMS was obtained on an Esquire-LC00054 spectrometer. HPLC was performed using a Waters 510 pump with Alltech 500 ELSD (Evaporative Light Scattering Detector). GC–MS was performed using a Shimadzu QP5050A. For column chromatography, AB-8 resin (Tianjin Nankai), silica gel (200–300 mesh, Qingdao Haiyang), and ODS C_{18} (35–50 μm, Alltech) were used. TLC and HPTLC (silica gel GF_{254} precoated plates, Qingdao Haiyang) detections were obtained by spraying 10% H_2SO_4, followed by heating.

3.2. Materials

The dried whole plants of *Lysimachia capillipes* were collected in Guizhou province, the People's Republic of 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 is deposited in the Institute

of Medicinal Plants Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3. Extraction and isolation

The dried powdered plant materials (10 kg) were extracted with 95% EtOH and 50% EtOH under reflux, twice each, and then the 95% EtOH and 50% EtOH extracts were combined. After removal of the solvent by evaporation, the combined extracts were dispersed in H_2O and subsequently partitioned by petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, in sequence. The resultant *n*-BuOH extract was chromatographed over an AB-8 resin column, eluting successively with H_2O, 30%, 50%, 70%, and 95% EtOH. The 50% EtOH eluate was further chromatographed on a silica gel column, eluting with $CHCl_3$–MeOH (containing 5% H_2O) in a gradient manner. Fraction 19 was subjected to an ODS C_{18} (35–50 μm) column and reversed-phase HPLC purification (41:59 MeOH–H_2O), to afford **1** (37 mg) and **2** (12 mg).

3.3.1. Capilliposide K (1). White amorphous powder: $[\alpha]_D^{20}$ −14.33 (*c* 0.60, pyridine); IR (KBr): ν_{max} 3320 (OH), 2960, 2870, 1475, 1330, 1200, 1040, 950 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; positive-ion ESIMS: *m/z* 1247 $[M+Na]^+$; negative-ion ESIMS: *m/z* 1123 $[M-H]^-$; HRFABMS: *m/z* 1247.5671 $[M+Na]^+$ (calcd for $C_{58}H_{96}O_{27}Na$, 1247.6037).

3.3.2. Capilliposide L (2). White amorphous powder: $[\alpha]_D^{20}$ −21.33 (*c* 0.75, pyridine); IR (KBr): ν_{max} 3320 (OH), 2960, 2870, 1640, 1475, 1440, 1340, 1040, 950 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; positive-ion ESIMS: *m/z* 1245 $[M+Na]^+$; negative-ion ESIMS: *m/z* 1221 $[M-H]^-$; HRFABMS: *m/z* 1245.4978 $[M+Na]^+$ (calcd for $C_{58}H_{94}O_{27}Na$, 1245.5880).

3.4. Acid hydrolysis of 1 and 2

Each saponin (5 mg), which was 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,

Table 2. The ^{13}C NMR (125 MHz) spectral data of compounds **1** and **2**[a]

No.	Camelligenin in CD_3OD	1	2	No.		1	2
1	40.5	38.8	38.2	3-O-Ara	1′	104.5	104.6
2	26.9	26.1	26.1		2′	79.0	79.0
3	79.0	88.9	88.8		3′	73.2	73.2
4	40.0	39.3	39.3		4′	78.7	78.7
5	57.0	55.5	55.3		5′	64.5	64.5
6	20.4	18.2	18.1	At C-2′ Glc	1″	104.2	104.2
7	34.0	32.7	32.1		2″	75.7	75.4
8	40.9	39.8	41.8		3″	77.2	77.7
9	48.0	46.7	47.4		4″	71.4	71.4
10	37.7	36.5	36.7		5″	77.9	77.7
11	24.6	23.6	25.3		6″	62.6	62.1
12	124.6	122.7	123.0	At C-4′ Glc	1‴	104.0	104.0
13	143.8	144.0	139.1		2‴	84.9	84.9
14	42.8	41.9	39.3		3‴	76.9	76.9
15	35.5	36.2	57.4		4‴	70.6	71.0
16	70.1	68.4	55.3		5‴	77.8	77.8
17	45.0	44.9	43.0		6‴	61.8	61.8
18	42.3	41.7	43.2	At C-2‴ Xyl	1⁗	107.2	107.2
19	48.0	47.1	44.2		2⁗	75.6	75.6
20	32.4	31.6	31.4		3⁗	77.7	77.2
21	46.2	44.4	42.6		4⁗	70.3	70.3
22	70.3	85.6	79.5		5⁗	67.1	67.1
23	28.3	27.7	27.7	At C-22 Glc	1⁗′	106.3	105.0
24	16.9	16.5	16.4		2⁗′	75.2	75.4
25	16.2	15.4	15.1		3⁗′	77.6	77.8
26	17.5	16.5	18.5		4⁗′	71.1	70.6
27	27.6	27.0	23.3		5⁗′	78.1	78.7
28	67.0	68.4	62.6		6⁗′	62.1	61.8
29	33.7	33.3	33.4				
30	25.4	25.1	24.1				

[a] In pyridine-d_5.

and the mixture was stirred for 30 min at 60 °C. The supernatant was then analyzed by GC [column: DB-5, 0.25 mm × 30 m, column temperature; 230 °C; carrier gas: N_2, retention time D-Glc (16.4 min), L-Glc (16.0 min), D-Xyl (19.9 min), L-Xyl (9.6 min), D-Ara (9.4 min), L-Ara (10.0 min). From the new saponins D-glucose, D-xylose, and L-arabinose were detected.

3.5. Cytotoxic activity[10]

Two new saponins (**1** and **2**) were evaluated for their cytotoxicities against the human A-2780 cell line by using methylene blue dye assay and the anticancer drug, hydroxycamptothecin (HCPT), as the positive controls. They displayed no cytotoxic effects against human A-2780 (>10 μmol/mL).

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