# Three Novel Triterpenoid Saponins from *Lysimachia capillipes* and Their Cytotoxic Activities

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**Three new saponins, capilliposide A (1), capilliposide B (2) and capilliposide C (3) were isolated from an ethanol extract of *Lysimachia capillipes*. Their structures were determined by 1D and 2D NMR ($^1$H–$^1$H COSY, HMBC, HMQC, DEPT and TOCSY) techniques, MS, and hydrolysis. Capilliposide B showed significant cytotoxicity against human A-2780 cells.**

**Key words** *Lysimachia capillipes*; capilliposide A; capilliposide B; capilliposide C; triterpene; saponin

*Lysimachia capillipes* HEMSL (Primulaceae) is a folklore medicinal plant that grows in southeastern China. The whole plant is used for treating colds and rheumatoid arthritis.[1)] Active saponins have been isolated from the *Lysimachia* genus.[2,3)] In the course of our ongoing screening for active constituents on blood circulation promotion, we have isolated some flavones, lactones and two new saponins from *Lysimachia capillipes*.[4—6)] Now we continue to report the isolation and structural elucidation of three new saponins, capilliposide A (**1**), capilliposide B (**2**) and capilliposide C (**3**). Capilliposide B showed significant cytotoxic activity against human A-2780 cells.

Compound **1** is an amorphous white powder, and gave a positive result to the Liebermann–Burchard test. The positive and negative ESI-MS, showed *quasi*-molecular ion peaks at *m*/*z* 1101 $[M+Na]^+$ and 1077 $[M-H]^-$ respectively, the fragment ion peaks at *m*/*z* 945 $[M-132\ (xylose)-H]^-$, 783 $[945-162\ (glucose)]^-$, 621 $[783-162\ (glucose)]^-$ and 489 $[621-132\ (arabinose)]^-$, indicated the presence of an arabinose inner unit. The molecular formula of **1** ($C_{52}H_{86}O_{23}$) was deduced from HR-FAB-MS. Seven tertiary methyl groups ($\delta$ 1.61, 1.42, 1.27, 1.19, 1.18, 1.06, 0.84) were observed in the $^1$H- and $^{13}$C-NMR spectrum (seven *sp*$^3$ carbons at $\delta$ 15.9, 16.1, 18.4, 19.4, 25.7, 27.8 and 32.8 analyzed by DEPT and HMQC). The data showed that compound **1** was a triterpene saponin. D-Glucose, D-xylose and L-arabinose were detected by GC analysis after the acid hydrolysis and preparation of their thiazolidine derivatives.[7)] All the carbon signals were assigned by 2D NMR including $^1$H–$^1$H COSY, HMQC-TOCSY and HMBC experiments (Table 1). The $^{13}$C-NMR data were compared with those of a known compound anagalligenin A ($3\beta$,$16\alpha$,$22\alpha$,$28\alpha$-tetrahydroxy-13,28-epoxy-oleanane),[8)] indicating that the $^{13}$C-NMR data of the aglycone of **1** were very similar to those of anagalligenin A, except that the chemical shift of C-3 of **1** shifted downfield by 12.2 ppm, which indicated that the glycoside was linked at C-3. The NOESY correlations between H-16$\beta$, H-28$\beta$, H-22$\beta$ and Me-30 were in agreement with $\alpha$-configuration of OH-28. The above analysis revealed that the aglycone of compound **1** was anagalligenin A.

The HMQC spectrum of compound **1** showed that it contained four sugar units. Their anomeric protons at $\delta$ 5.69 (1H, d, $J$=8.0 Hz), 5.37 (1H, d, $J$=7.5 Hz), 4.90 (1H, d, $J$=7.5 Hz) and 4.87 (1H, d, $J$=5.5 Hz) were correlated with carbon signals at $\delta$ 104.6, 107.4, 103.9 and 104.4, respectively. The spin-systems associated with monosaccharides were identified by HMQC-TOCSY experiment with the aid of a $^1$H–$^1$H COSY spectrum. All carbon signals of the sugar moieties were assigned by HMQC experiment as shown in Table 1. Combining Combined with spin–spin couplings, the four units were identified as two $\beta$-glucopyranosides (Glc), one $\alpha$-arabinopyranoside (Ara) and one $\beta$-xylopyranoside (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 $\delta$ 4.87 (1H, d, $J$=5.5 Hz) and C-3 of aglycone at $\delta$ 88.7, the anomeric proton of glucose-I at $\delta$ 5.69 (1H, d, $J$=8.0 Hz) and the C-2 of arabinose at $\delta$ 79.4, the anomeric proton of glucose-II at $\delta$ 5.37 (1H, d, $J$=7.5 Hz) and the C-4 of arabinose at $\delta$ 78.3, the anomeric proton of xylose at $\delta$ 4.90 (1H,

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Table 1. $^{13}$C-NMR Spectral Data for Compounds of **1**—**3** (125 MHz in Pyridine-$d_5$)

| C | **1** | **2** | **3** | C | **1** | **2** | **3** |
|---|---|---|---|---|---|---|---|
| 1 | 38.9 | 38.9 | 38.9 | | $\alpha$-L-Ara | $\alpha$-L-Ara | $\alpha$-L-Ara |
| 2 | 26.3 | 26.3 | 26.3 | 1′ | 104.4 | 104.4 | 104.5 |
| 3 | 88.7 | 88.7 | 89.0 | 2′ | 79.4 | 79.4 | 79.1 |
| 4 | 39.4 | 39.4 | 39.5 | 3′ | 72.9 | 73.0 | 73.5 |
| 5 | 55.4 | 55.4 | 55.4 | 4′ | 78.3 | 78.1 | 78.7 |
| 6 | 17.4 | 17.7 | 17.6 | 5′ | 63.9 | 63.9 | 64.2 |
| 7 | 34.0 | 34.0 | 34.0 | | $\beta$-D-Glc I | $\beta$-D-Glc I | $\beta$-D-Glc I |
| 8 | 42.4 | 42.4 | 43.7 | 1″ | 104.6 | 104.6 | 104.2 |
| 9 | 50.1 | 50.0 | 50.0 | 2″ | 76.0 | 76.0 | 75.6 |
| 10 | 36.6 | 36.6 | 36.5 | 3″ | 77.7 | 77.6 | 77.2 |
| 11 | 19.1 | 19.0 | 19.4 | 4″ | 71.5 | 71.5 | 71.2 |
| 12 | 32.9 | 33.0 | 33.0 | 5″ | 78.1 | 78.0 | 77.7 |
| 13 | 87.1 | 87.3 | 87.2 | 6″ | 62.7 | 62.7 | 62.1 |
| 14 | 43.8 | 43.6 | 44.1 | | $\beta$-D-Glc II | $\beta$-D-Glc II | $\beta$-D-Glc II |
| 15 | 36.5 | 36.6 | 36.2 | 1‴ | 103.9 | 103.9 | 104.0 |
| 16 | 69.4 | 69.5 | 69.0 | 2‴ | 85.1 | 85.2 | 84.9 |
| 17 | 52.5 | 51.2 | 52.4 | 3‴ | 77.3 | 77.3 | 77.2 |
| 18 | 47.0 | 47.0 | 47.1 | 4‴ | 70.8 | 70.8 | 70.6 |
| 19 | 38.3 | 38.0 | 38.6 | 5‴ | 77.9 | 78.3 | 77.6 |
| 20 | 33.5 | 33.0 | 32.9 | 6‴ | 62.0 | 62.0 | 61.8 |
| 21 | 46.4 | 41.4 | 41.8 | | $\beta$-D-Xyl | $\beta$-D-Xyl | $\beta$-D-Xyl |
| 22 | 67.7 | 72.3 | 76.9 | 1⁗ | 107.4 | 107.5 | 107.2 |
| 23 | 27.8 | 27.8 | 27.7 | 2⁗ | 75.8 | 75.9 | 75.7 |
| 24 | 16.1 | 16.4 | 16.3 | 3⁗ | 77.5 | 77.3 | 77.2 |
| 25 | 15.9 | 16.0 | 18.1 | 4⁗ | 70.4 | 70.4 | 70.3 |
| 26 | 18.4 | 18.3 | 18.3 | 5⁗ | 67.2 | 67.2 | 67.1 |
| 27 | 19.4 | 19.3 | 19.8 | | | Caproyl | $\beta$-D-Glc III |
| 28 | 98.3 | 97.5 | 97.1 | 1⁗′ | | 172.7 | 103.6 |
| 29 | 32.8 | 33.1 | 33.3 | 2⁗′ | | 34.6 | 75.7 |
| 30 | 25.7 | 25.4 | 25.5 | 3⁗′ | | 24.8 | 77.6 |
| | | | | 4⁗′ | | 31.1 | 71.4 |
| | | | | 5⁗′ | | 22.2 | 78.1 |
| | | | | 6⁗′ | | 13.7 | 62.6 |

d, $J$=7.5 Hz) and the C-2 of glucose-II (at C-4 of arabinose) at $\delta$ 85.1, respectively. These suggested the sugar sequences of the oligosaccharide chain are as shown in figure. Thus, the structure of compound **1** was established as anagalligenin A-3-*O*-$\beta$-D-xylopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-(1→4)-[$\beta$-D-glucopyranosyl-(1→2)]-$\alpha$-L-arabinopyranoside, and called as capilliposide A.

The HR-FAB-MS of compound **2** indicated the molecular formula as $C_{58}H_{96}O_{24}$. Comparison of NMR data of **2** with those of compound **1** showed the data were very similar (Tables 1, 2), except that the OH of C-22 in **1** was replaced by those of a caproate in **2**, along with the HMBC cross-peak from H-22 [$\delta$ 6.08 (1H, dd, $J$=5.5, 6.5 Hz)] to the carbonyl of caproate at $\delta$ 172.7. All above analysis showed compound **2** to be 22-caproylanagalligenin A-3-*O*-$\beta$-D-xylopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-(1→4)-[$\beta$-D-glucopyranosyl-(1→2)]-$\alpha$-L-arabinopyranoside, named calledas capilliposide B.

The negative ESI-MS of compound **3** showed a signal of a *quasi*-molecular ion peak at *m/z* 1239 $[M-H]^-$ corresponding to a molecular formula $C_{58}H_{96}O_{28}$ obtained from HR-FAB-MS. Comparison of NMR data of **3** with those of compound **2** showed the $^{13}$C-NMR data were very similar, except that the caproate in compound **2** was replaced by a $\beta$-D-glucopyranoside in compound **3**. Thus compound **3** was anagalligenin A-3-*O*-{$\beta$-D-xylopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-(1→4)-[$\beta$-D-glucopyranosyl-(1→2)]-$\alpha$-L-arabinopyranosyl}-22-*O*-$\beta$-D-glucopyranoside, capilliposide C.

Finally, the cytotoxic activities of compounds **1**—**3** were examined against human A-2780 cells[9]; compound **2** showed significant cytotoxic activity, with an $IC_{50}$ value of 0.1 $\mu$g/ml while compound **1** and compound **3** showed no cytotoxic activity.

## Experimental

**General** Melting points were measured on a Fisher-Johns apparatus and uncorrected. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer 983G spectrometer. $^1$H- (500 MHz) and $^{13}$C-NMR (125 MHz) spectra were recorded on a Bruker AM-500 instrument. FAB-MS were obtained on a Zabspec E spectrometer; ESI-MS 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 $\mu$m, Alltech) were used. TLC and HPTLC (silica gel $GF_{254}$ precoated plates, Qingdao Haiyang) detections was were obtained by spraying 10% $H_2SO_4$ following heating.

**Extraction and Isolation** The *Lysimachia capillipes* was collected in Guizhou province, the People's Republic of China, and identified by Dr. Bao-lin Guo of the Institute of Medicinal Plants Development, Chinese Academy of Medical Sciences and Peking Union Medical College. The dried powdered plant materials (10 kg) were refluxed with 95% EtOH twice and then with 50% EtOH twice, then 95% EtOH extract and 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 AB-8 resin column, eluting with $H_2O$ and 30, 50, 70 and 95% EtOH. The 50% EtOH eluate was chromatographed on a silica gel column, eluting with $CHCl_3$/MeOH (containing 5% $H_2O$) in a gradient manner.

Table 2. $^1$H-NMR Spectral Data for Compounds of **1**—**3** (500 MHz for $^1$H-NMR in Pyridine-$d_5$)

| H | **1** | **2** | **3** | H | **1** | **2** | **3** |
|---|---|---|---|---|---|---|---|
| 1 | 1.03 1.43 | 1.01 1.37 | 1.06 1.47 | | *β*-D-Glc I | *β*-D-Glc I | *β*-D-Glc I |
| 2 | 1.80 1.94 | 1.78 1.95 | 1.82 1.95 | 1″ | 5.69 | 5.65 | 5.50 |
| 3 | 3.13 | 3.11 | 3.20 | 2″ | 4.06 | 4.10 | 4.03 |
| 5 | 0.86 | 0.82 | 0.85 | 3″ | 3.86 | 3.93 | 3.88 |
| 6 | 1.55 1.60 | 1.53 1.59 | 1.53 1.62 | 4″ | 4.22 | 4.23 | 4.17 |
| 7 | 1.20 1.63 | 1.21 1.62 | 1.18 1.60 | 5″ | 3.95 | 3.99 | 3.87 |
| 9 | 1.49 | 1.43 | 1.44 | 6″ | 4.51 4.37 | 4.50 4.36 | 4.47 4.34 |
| 11 | 1.87 1.95 | 1.86 1.91 | 1.86 1.93 | | *β*-D-Glc II | *β*-D-Glc II | *β*-D-Glc II |
| 12 | 2.40 2.79 | 2.37 2.82 | 2.36 2.80 | 1‴ | 5.37 | 5.29 | 5.38 |
| 15 | 1.91 2.33 | 1.88 2.30 | 1.92 2.32 | 2‴ | 4.27 | 4.19 | 4.31 |
| 16 | 5.06 | 4.80 | 5.14 | 3‴ | 3.73 | 3.85 | 3.88 |
| 18 | 2.23 | 2.20 | 2.27 | 4‴ | 4.19 | 4.25 | 4.20 |
| 19 | 1.35 2.90 | 1.31 2.88 | 1.37 2.95 | 5‴ | 4.01 | 4.12 | 3.97 |
| 21 | 2.57 3.02 | 2.64 2.99 | 2.60 3.06 | 6‴ | 4.63 4.49 | 4.59 4.32 | 4.63 4.48 |
| 22 | 5.00 | 6.08 | 5.11 | | *β*-D-Xyl | *β*-D-Xyl | *β*-D-Xyl |
| 23 | 1.27 | 1.18 | 1.20 | 1⁗ | 4.90 | 4.97 | 4.96 |
| 24 | 1.06 | 1.03 | 1.09 | 2⁗ | 4.02 | 4.08 | 4.01 |
| 25 | 0.84 | 0.82 | 0.76 | 3⁗ | 4.17 | 4.33 | 4.20 |
| 26 | 1.42 | 1.30 | 1.29 | 4⁗ | 4.16 | 4.30 | 4.24 |
| 27 | 1.61 | 1.58 | 1.59 | 5⁗ | 3.76 4.29 | 3.65 4.27 | 3.79 4.30 |
| 28 | 5.81 | 5.25 | 5.50 | | | Caproyl | *β*-D-Glc III |
| 29 | 1.18 | 1.12 | 1.08 | 1⁗′ | | | 5.33 |
| 30 | 1.19 | 1.01 | 1.01 | 2⁗′ | | 2.34 (2H, t, 7.5) | 4.15 |
| | *α*-L-Ara | *α*-L-Ara | *α*-L-Ara | 3⁗′ | | 1.54 (2H, m) | 3.91 |
| 1′ | 4.87 | 4.76 | 4.80 | 4⁗′ | | 1.16 (2H, m) | 4.19 |
| 2′ | 4.42 | 4.32 | 4.28 | 5⁗′ | | 1.15 (2H, m) | 4.03 |
| 3′ | 4.32 | 4.38 | 4.39 | 6⁗′ | | 0.72 (3H, t, 7.5) | 4.56 4.32 |
| 4′ | 4.47 | 4.46 | 4.41 | | | | |
| 5′ | 3.80 3.63 | 3.92 3.67 | 3.88 3.61 | | | | |

Fraction 25 was separated on an ODS $C_{18}$ (35—50 $\mu$m) column, using MeOH/$H_2O$ (44.5 : 55.5) as eluents to afford **1** (23 mg) and **2** (60 mg). Fraction 33 was subjected to an ODS $C_{18}$ (35—50 $\mu$m) column and reverse-phase HPLC purification (MeOH/$H_2O$ 41 : 59), to afford **3** (38 mg).

Compound **1**: White amorphous powder, $[\alpha]_D^{20}$ −26.7° ($c$=0.50, MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3420 (OH), 2960, 2870, 1475, 1350, 1210, 1030, 940; $^1$H-NMR (pyridine-$d_5$, 500 MHz): $\delta$ 5.81 (1H, s, H-28), 5.06 (1H, br t, H-16), 5.00 (1H, br t, H-22), 3.13 (1H, dd , $J$=4.0, 11.5 Hz, H-3), 1.61 (3H, s, Me-27), 1.42 (3H, s, Me-26), 1.27 7 (3H, s, Me-23), 1.19 (3H, s, Me-30), 1.18 (3H, s, Me-29), 1.06 (3H, s, Me-24), 0.84 (3H, s, Me-25); $^1$H-NMR data of others, see Table 2; $^{13}$C-NMR (pyridine-$d_5$, 125 MHz), see Table 1; positive ESI-MS $m/z$ 1101 $[M+Na]^+$, negative ESI-MS $m/z$ 1077 $[M-H]^-$; HR-FAB-MS $m/z$ 1101.5447 $[M+Na]^+$ (Calcd for $C_{52}H_{86}O_{23}Na$ 1101.5458).

Compound **2**: White amorphous powder, $[\alpha]_D^{20}$ −23.4° ($c$=0.55, MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3410 (OH), 2960, 2870, 1720, 1470, 1340, 1200, 1050, 940; $^1$H-NMR (pyridine-$d_5$, 500 MHz): $\delta$ 6.08 (1H, dd, $J$=5.5, 6.5 Hz, H-22), 5.25 (1H, s, H-28), 4.80 (1H, br t, H-16), 3.11 (1H, dd, $J$=4.5, 12.0 Hz, H-3), 1.58 (3H, s, Me-27), 1.30 (3H, s, Me-26), 1.18 (3H, s, Me-23), 1.12 (3H, s, Me-29), 1.03 (3H, s, Me-24), 1.01 (3H, s, Me-30), 0.82 (3H, s, Me-25); $^1$H-NMR data of others, see Table 2; $^{13}$C-NMR (pyridine-$d_5$, 125 MHz), see Table 1; positive ESI-MS $m/z$ 1199 $[M+Na]^+$, negative ESI-MS $m/z$ 1175 $[M-H]^-$; HR-FAB-MS $m/z$ 1199.6208 $[M+Na]^+$ (Calcd for $C_{58}H_{96}O_{24}Na$ 1199.6191).

Compound **3**: White amorphous powder, $[\alpha]_D^{20}$ −5.0° ($c$=0.50, MeOH); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3410 (OH), 2960, 2870, 1470, 1335, 1200, 1050, 950; $^1$H-NMR (pyridine-$d_5$, 500 MHz): $\delta$ 5.50 (1H, s, H-28), 5.14 (1H, br t, H-16), 5.11 (1H, br t, H-22), 3.20 (1H, dd, $J$=4.0, 11.5 Hz, H-3), 1.59 (3H, s, Me-27), 1.29 (3H, s, Me-26), 1.20 (3H, s, Me-23), 1.09 (3H, s, Me-24), 1.08 (3H, s, Me-29), 1.01 (3H, s, Me-30), 0.76 (3H, s, Me-25); $^1$H-NMR data of others, see Table 2; $^{13}$C-NMR (pyridine-$d_5$, 125 MHz), see Table 1; positive ESI-MS $m/z$ 1263 $[M+Na]^+$, negative ESI-MS $m/z$ 1239 $[M-H]^-$; HR-FAB-MS $m/z$ 1263.5975 $[M+Na]^+$ (Calcd for $C_{58}H_{96}O_{28}Na$ 1263.5986).

**Acid Hydrolysis of 1—3** 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-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.

**Cytotoxic Activity**[9] Three isolated compounds (**1**—**3**) were evaluated for their cytotoxicities against human A-2780 cell lines by using methylene blue dye assay and the anti-cancer drug, hydroxycamptothecin (HCPT), as the positive controls. Among them, compound **2** exhibited cytotoxicity against human A-2780 with an $IC_{50}$ value of 0.1 $\mu$g/ml. On the other hand, compounds **1** and **3** displayed no cytotoxic effects against human A-2780 (>10 $\mu$g/ml).

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