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 (1H–1H 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* HEMSLO (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 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 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 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 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 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 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 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 medicinal plant that grows in southeastern China. The whole plant is used for treating colds and rheumatoid arthritis.1)

[Image 48x50 to 290x329]
d, J = 7.5 Hz) and the C-2 of glucose-II (at C-4 of arabinose) at δ 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-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranoside, and called as capilliposide A.

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

The negative ESI-MS of compound 2 showed the 13C-NMR data were very similar, except that the caproate in compound 2 was replaced by a β-D-glucopyranoside in compound 3. Thus compound 3 was anagalligenin A-3-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranoside-(22-O-β-D-glucopyranoside, capilliposide.

Finally, the cytotoxic activities of compounds 1—3 were examined against human A-2780 cells; compound 2 showed significant cytotoxic activity, with an IC_{50} value of 0.1 μg/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. 1H- (500 MHz) and 13C-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 C18 (35—50 μm, Alltech) were used. TLC and HPTLC (silica gel GF 254 precoated plates, Qingdao Haiyang) detections were obtained by spraying 10% H_{2}SO_{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, then 95% EtOH extract and 50% EtOH extract were combined. After removal of the solvent by evaporation, the combined extracts were partitioned between H_{2}O and petroleum ether, CHCl_{3}, EtOAc and n-BuOH, successively. The n-BuOH extract was chromatographed over AB-8 resin column, eluting with H_{2}O 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_{2}O) in a gradient manner.
Fraction 25 was separated on an ODS C_{18} (35–50 μm) column, using MeOH/H₂O (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 μm) column and reverse-phase HPLC purification (MeOH/H₂O 41: 59), to afford 3 (38 mg).

**Compound 1:** White amorphous powder, [α]_{D}^{20} = -26.7° (c = 0.50, MeOH); IR (KBr) ν_{max} cm⁻¹: 3420 (OH), 2960, 2870, 1740, 1350, 1250, 1030, 940; ¹H-NMR (pyridine-d$_{5}$, 500 MHz): δ 5.19 (1H, s, H-28), 5.06 (1H, s, H-29), 4.30 (1H, dd, J = 5.5, 11.5 Hz, H-3), 1.72 (3H, s, Me-24), 1.61 (3H, s, Me-27), 1.18 (3H, s, Me-26), 0.74 (3H, t, 7.5 Hz, Me-30); ¹³C-NMR (pyridine-d$_{5}$, 125 MHz), see Table 2; ¹H-NMR data of others, see Table 1; ¹³C-NMR (pyridine-d$_{5}$, 125 MHz), see Table 1; negative ESI-MS m/z 1101 [M + Na]⁻, negative ESI-MS m/z 1077 [M⁺]; HR-FAB-MS m/z 1101.5447 [M + Na]⁺ (Calcd for C_{20}H_{25}O_{2}Na 1101.5458).

**Compound 2:** White amorphous powder, [α]_{D}^{20} = +23.4° (c = 0.55, MeOH); IR (KBr) ν_{max} cm⁻¹: 3410 (OH), 2960, 2870, 1720, 1470, 1340, 1200, 1050, 940; ¹H-NMR (pyridine-d$_{5}$, 500 MHz): δ 6.10 (1H, dd, J = 5.5, 6.5 Hz, H-22), 5.25 (1H, s, H-28), 4.80 (1H, brt, 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), ¹H-NMR data of others, see Table 2; ¹³C-NMR (pyridine-d$_{5}$, 125 MHz), see Table 1; positive ESI-MS m/z 1199 [M + Na]⁺, negative ESI-MS m/z 1175 [M⁻]; HR-FAB-MS m/z 1199.6208 [M + Na]⁺ (Calcd for C_{20}H_{25}O_{2}Na 1199.619). (Calcd for C_{20}H_{25}O_{2}Na 1199.619).

**Compound 3:** White amorphous powder, [α]_{D}^{20} = +5.0° (c = 0.50, MeOH); IR (KBr) ν_{max} cm⁻¹: 3410 (OH), 2960, 2870, 1470, 1330, 1200, 1050, 950; ¹H-NMR (pyridine-d$_{5}$, 500 MHz): δ 5.50 (1H, s, H-28), 5.14 (1H, brt, H-16), 5.11 (1H, brt, 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-25), 0.76 (3H, s, Me-30); ¹H-NMR data of others, see Table 2; ¹³C-NMR (pyridine-d$_{5}$, 125 MHz), see Table 1; positive ESI-MS m/z 1263 [M + Na]⁺, negative ESI-MS m/z 1239 [M⁻]; HR-FAB-MS m/z 1263.5975 [M + Na]⁺ (Calcd for C_{20}H_{25}O_{2}Na 1263.5986).

**Acid Hydrolysis of 1–3** Each saponin (5 mg) dissolved in water (100 mL) and 2 mL HCl (100 mL) was heated at 100 °C for 1 h. The mixture was added to 30 mL of 0.5% sodium hydroxide solution (pH 7) 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: N₂, retention time t (GLC) 16.4 min, t (GLC) 16.0 min, t-Xyl (19.9 min), t-Yl (9.6 min), t-Ara (9.4 min), t-Ara (10.0 min)]. From the new saponins n-glucose, n-xylene and l-arabino were detected.

**Cytotoxic Activity** 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, hydroxyxanthothecin (HCPT), as the positive controls. Among them, compound 2 exhibited cytotoxicity against human A-2780 with an IC₅₀ value of 0.1 μg/mL. On the other hand, compounds 1 and 3 displayed no cytotoxic effects against human A-2780 (>10 μg/mL).

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**References and Notes**