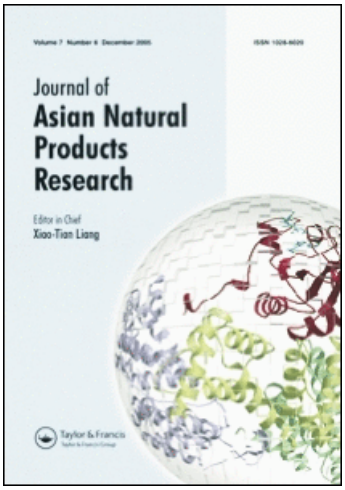

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Seven triterpene saponins were isolated from *Lysimachia christinae* and identified as lysichriside A (**1**), lysichriside B (**2**), primulanin (**3**), lysikokianoside 1 (**4**), anagallisin C (**5**), ardisiacrispin A (**6**), and ardisicrenoside B (**7**). Compounds **1** and **2** are new triterpene saponins, compounds **3**, **5**, and **7** were isolated from this genus, and compounds **4** and **6** were isolated from this species for the first time. Their structures were elucidated by means of 1D and 2D NMR experiments.

Keywords: *Lysimachia christinae*; lysichriside A; lysichriside B; triterpene saponin

1. Introduction

Lysimachia christinae Hance (Primulaceae) is a common medicinal plant, growing in southwestern China. The whole plant is used for diuretics and eliminating wetness.[1] Chemical studies on this plant were mostly limited to its flavonoids and essential oil.[1,2] In the activity screening experiments, its petroleum ether extract demonstrated remarkable efficacy of choleresis, its ethyl acetate extract demonstrated remarkable efficacy of diuresis, and its *n*-butanol extract demonstrated remarkable efficacy regarding anti-inflammation. In order to clarify its active constituents, we investigated its involatile constituents and obtained flavonoids[3] from its ethyl acetate extract, and we have also isolated seven triterpene saponins from its *n*-butanol extract. In this paper, we deal with the isolation and structural elucidation of seven triterpene saponins, lysichriside A (**1**), lysichriside B (**2**), primulanin (**3**), lysikokianoside 1 (**4**), anagallisin C (**5**), ardisiacrispin A (**6**), and ardisicrenoside B (**7**) (Figure 1). Amongst these, compounds **1** and **2** are new triterpene saponins. Compounds **3**, **5**, and **7** were isolated from this genus for the first time, and compounds **4** and **6** were isolated from this species for the first time. Their structures were elucidated by means of spectral and chemical methods.

2. Results and discussion

Compound **1** was an amorphous white powder, mp 280–282°C, $[\alpha]_D^{20}$ −2.0 (*c* 0.50, CH_3OH), and gave a positive result to the Liebermann–Burchard test. The molecular formula of **1** ($C_{54}H_{86}O_{23}$) was deduced from HRESI-MS (*m/z* 1103.5638 $[M + H]^+$). The negative ESI-MS showed a quasi-molecular ion peak at *m/z* 1101.5 $[M - H]^-$ and the fragment ion peaks at *m/z* 969 [M-132 (xylose)-H]$^-$, 897 [M-162(glucose)-42-H]$^-$, 807 [969-162 (glucose)]$^-$, 603 [807-162 (glucose)-42]$^-$, and 471 [603-132 (arabinose)]$^-$, indicating the presence of an inner arabinose. The six tertiary methyl groups, observed in the ^{1}H NMR (δ 1.39, 1.26, 1.14, 1.00, 0.99, and 0.85) and ^{13}C NMR (δ 16.3, 16.4, 18.4, 19.6, 24.0, 27.8) spectra, indicated that compound **1** was a triterpene saponin. Glucose, arabinose, and xylose were identified after acid hydrolysis of **1** by comparing with authentic samples (Sigma) on HPTLC. All the carbon signals were assigned by 2D NMR experiments including HMQC and HMBC spectra (Table 1). Comparison of the ^{13}C NMR spectral data of **1** with those of **6** showed that they were similar, except that the C-6 chemical shift of terminal glucose in **1** shifted towards downfield by 2.0 ppm, and an additional acetyl carbon signals at δ 20.8 and 170.9 appeared in the ^{13}C NMR spectrum of **1**, which indicated the presence of an acetoxy group at C-6 of terminal glucose. The presence of fragment ion peaks at *m/z* 897 [M-162(glucose)-42-H]$^-$ and the HMBC correlation from H-6 of terminal glucose to the carbon at δ 170.9 supported this conclusion.

The sugar sequence of the oligosaccharide chain and the glycosidic site of **1** were determined by HMBC spectrum. In the HMBC spectrum (Figure 2), the anomeric proton of arabinose at δ 4.75 (d, 1H, $J = 6.0$ Hz) correlated with C-3 of the aglycone at δ 89.0, the anomeric proton of terminal glucose at δ 4.85 (d, 1H, $J = 8.0$ Hz) correlated with C-2 of the arabinose at δ 79.8, the anomeric proton of glucose-II at δ 4.80 (d,

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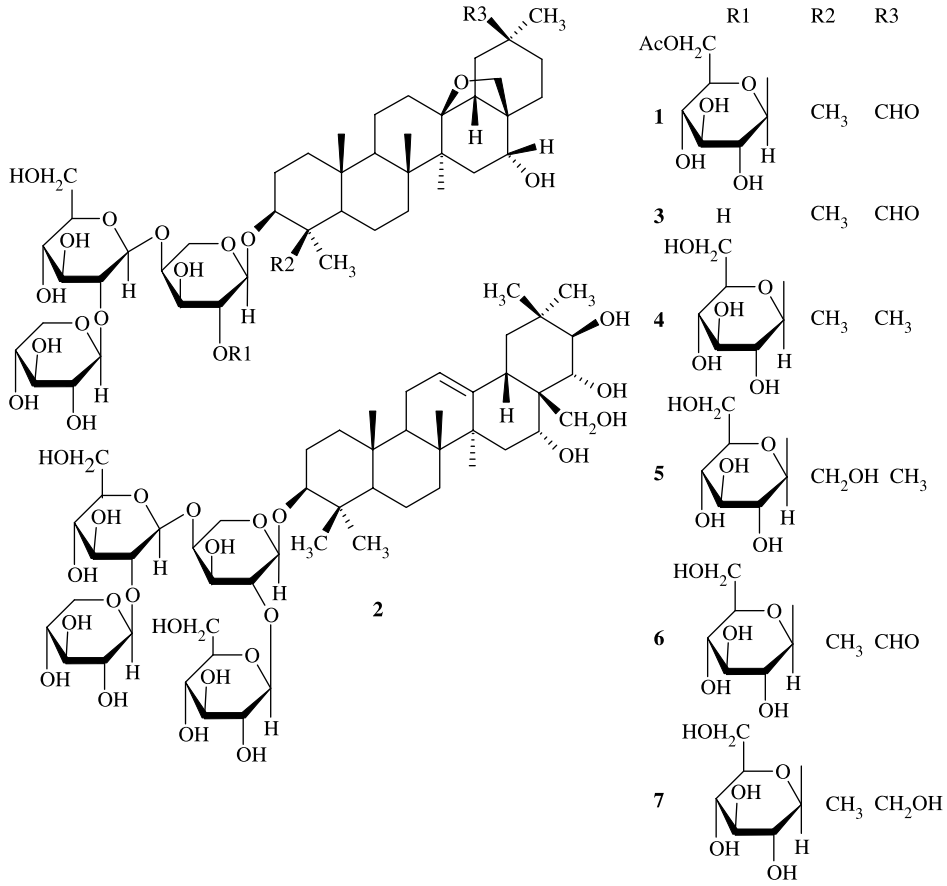

Figure 1. Structures of compounds **1**–**7**.

1H, $J = 8.0$ Hz) correlated with C-4 of the arabinose at δ 77.9, the anomeric proton of the xylose at δ 5.20 (d, 1H, $J = 7.5$ Hz) correlated with C-2 of the inner glucose at δ 85.4, confirmed the sugar sequence and configuration of **1**, identical to those of compound **6** as shown in Figure 1. Thus, the structure of compound **1** was established as 3β,16α-dihydroxy, oleanan-30-al, 13,28-epoxy-3-*O*-β-D-xylopyranosyl-(1–2)-β-D-glucopyranosyl-(1 → 4)-[β-D-6-acetyl-glucopyranosyl-(1 → 2)] -α-L-arabinopyranoside, 20 S, named lysichriside A.

Compound **2** was an amorphous white powder, mp 274–276°C, $[\alpha]_D^{20}$ + 16.00 (*c* 0.10, pyridine), and gave a positive result to the Liebermann–Burchard test. The molecular formula of **2** was established as $C_{52}H_{86}O_{23}$ by HRESI-MS (*m/z* 1079.5630 $[M + H]^+$). The negative ESI-MS showed a quasi-molecular ion peak at *m/z* 1077.5 $[M - H]^-$ and 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)]$^-$, indicating the presence of an inner arabinose. The seven tertiary methyl groups observed in the ^{1}H NMR (δ 1.69, 1.15, 1.05, 0.93, 0.92, 0.91, and 0.80) and ^{13}C NMR (δ 15.8, 16.8, 17.0, 27.4, 29.7, 19.2) spectra indicated that compound **2** was a triterpene saponin. Glucose, arabinose and xylose were identified after acid hydrolysis of **2** by comparing with authentic samples (Sigma) on HPTLC. All the carbon signals were assigned by 2D NMR including HMQC and HMBC experiments (Table 1). The ^{13}C NMR spectral data of the aglycone moiety of **2** were compared with those of barringtogenol C [4], indicating that the ^{13}C NMR spectral data of the aglycone of **2** were almost consistent with those of barringtogenol C, except that the chemical shift of C-3 of **2** was shifted downfield by 10.0 ppm, which indicated a glycosidic site at C-3 of **2**. The sugar sequence of the oligosaccharide chain and the glycosidic site of **2** were determined by HMBC spectrum. In the HMBC spectrum (Figure 2), the anomeric proton of arabinose at δ 4.77 (d, 1H, $J = 5.5$ Hz) correlated with C-3 of the aglycone at δ 89.0, the anomeric proton of terminal glucose at δ 4.90 (d, 1H, $J = 7.6$ Hz) with C-2 of the arabinose at δ 79.8, the anomeric proton of glucose-II at δ 4.98 (d, 1H, $J = 7.5$ Hz) with C-4 of the arabinose at δ 77.9, the anomeric proton of the xylose at δ 5.46 (d, 1H, $J = 7.4$ Hz) with C-2 of inner glucose at δ 85.3,

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (75 MHz in pyridine-d_5).

Position	1	2	Barringtogenol C	Sugar unit	1	2
1	39.0	38.9	39.1	Arabinose		
2	26.5	26.4	28.1	A-1	104.3	104.2
3	89.0	89.0	78.0	A-2	79.8	79.8
4	39.6	39.6	39.3	A-3	73.4	73.3
5	55.6	55.9	55.8	A-4	77.9	77.9
6	17.8	18.5	18.8	A-5	64.5	64.4
7	32.5	33.2	33.2	Glucose (terminal)		
8	42.4	40.1	40.1	G-1	104.9	104.9
9	50.3	47.1	47.1	G-2	76.3	76.2
10	36.7	36.9	37.2	G-3	78.4	78.3
11	19.0	23.8	23.9	G-4	71.2	71.2
12	32.2	122.6	122.9	G-5	78.3	78.2
13	86.2	145.1	143.8	G-6	64.9	63.0
14	44.4	40.5	42.0	CH_3CO	170.9	
15	34.2	34.9	34.2	CH_3CO	20.8	
16	76.7	71.8	67.8	Glucose (inner)		
17	43.9	47.0	47.2	G′-1	104.8	104.7
18	53.2	41.9	41.2	G′-2	85.4	85.3
19	36.7	47.1	48.2	G′-3	77.6	77.5
20	48.2	36.9	36.3	G′-4	71.0	71.0
21	30.3	78.6	78.6	G′-5	78.5	78.4
22	33.2	77.9	77.2	G′-6	62.4	62.4
23	27.8	28.1	28.7	Xylose (X)		
24	16.4	16.8	16.5	X-1	107.7	107.6
25	16.3	15.8	15.8	X-2	76.1	76.0
26	18.4	17.0	17.0	X-3	77.8	77.8
27	19.6	27.4	27.4	X-4	70.8	70.7
28	77.6	67.6	68.4	X-5	67.6	67.5
29	24.0	29.7	30.5			
30	207.4	19.2	19.4			

suggested the sugar sequence and configuration of **2** were identical to those of compounds **4**–**7**, as shown in Figure 1. Thus, the structure of compound **2** was established as 3β,16α,21β,22α,28-pentahydroxy,olean-12-en-3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside, named lysichriside B.

Five known triterpene saponins were identified as primulanin (**3**) [5], lysikokianoside 1 (**4**) [6], anagallisin C (**5**) [7], ardisiacrispin A (**6**) [8], and ardisicrenoside B (**7**) [8] by comparison of their spectral data with those reported in the literature.

3. Experimental

3.1 General experimental procedures

All melting points were determined by a XT-4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 polarimeter (Na filter, $\lambda = 589$ nm). IR spectra were obtained on a Perkin–Elmer 577 spectrometer with KBr disk. NMR spectra were recorded on a Bruker AV-300 spectrometer with TMS as internal standard. ESI-MS were measured on a Finnigan MAT 95 instrument.

All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai). Silica gel (200–300 mesh) and C18 reversed-phase silica gel (250 mesh, Merck) were used for column chromatography, and precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, China) for TLC.

3.2 Plant material

The whole plant of *Lysimachia christinae* was collected from Sichuan Province of China in July 2004, and was identified by Professor Shi-hui Qian. A voucher specimen (No. Lys-2004-07) is deposited in the Herbarium of Jiangsu Academy of Traditional Chinese Medicine.

3.3 Extraction and isolation

The dried powder of the whole plant (10 kg) of *L. christinae* was percolated with 80% C_2H_5OH. After removal of the C_2H_5OH under reduced pressure, a dark green residue (1002 g) was obtained, which was

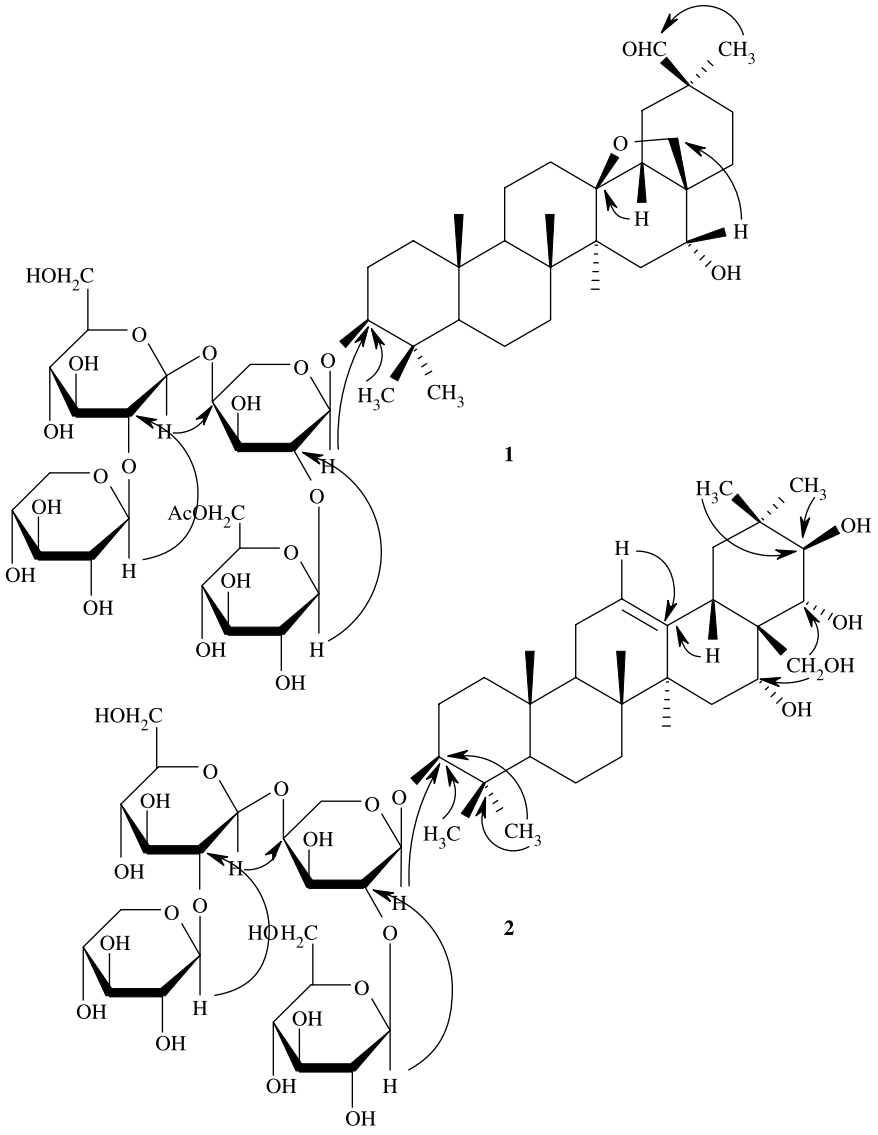

Figure 2. Key HMBC correlations of **1** and **2**.

dissolved in hot water, and successively extracted with petroleum ether, EtOAc, and *n*-butanol. The *n*-butanol extract was evaporated *in vacuo* to give a residue (150 g), which was chromatographed over silica gel column eluting with $CHCl_3$–CH_3OH (in gradient, 10:1 to 0:100, v/v) to yield eight major fractions on the basis of TLC analysis. Fraction 4 ($CHCl_3$/CH_3OH, 4:1) was separated by reversed-phase C-18 silica gel column chromatography and eluted with a gradient of CH_3CN in H_2O (H_2O/CH_3CN, 3:2 to 1:1) to give compounds **1** (18 mg) and **3** (30 mg). Fraction 5 ($CHCl_3$/CH_3OH, 3:1) was separated by reversed-phase C-18 silica gel column chromatography and eluted with a gradient of CH_3CN in H_2O (H_2O/CH_3CN, 2:1 to 1:1) to afford compounds **7** (90 mg), **6** (1505 mg), **5** (75 mg), and **4** (400 mg). Fraction 6 ($CHCl_3$/CH_3OH, 2:1) was separated by reversed-phase C-18 silica gel column chromatography and eluted with a gradient of CH_3CN in H_2O (H_2O/CH_3CN, 3:1) to furnish compound **2** (35 mg).

3.3.1 *Compound **1***

An amorphous white powder, mp 280–282°C (CH_3OH), $[\alpha]_D^{20}$ −2.0 (*c* 0.50, CH_3OH). IR ν_{max} (KBr) cm^{-1}: 3409, 2930, 2874, 1711(C═O), 1630, 1542, 1451, 1384, 1360, 1248, 1161, 869; ^1H NMR (C_5D_5N-d_5, 300 MHz) δ: 0.85 (3H, s, H-25), 0.99 (3H, s, H-24), 1.00 (3H, s, H-29), 1.14 (3H, s, H-23), 1.26 (3H, s, H-26), 1.39 (3H, s, H-27), 3.11 (1H, br d, J = 11.4 Hz, H-3), 3.33 (1H, d, J = 7.4 Hz, Ha-28), 3.56 (1H, d, J = 7.4 Hz, Hb-28), 4.19 (1H, m, H-16), 9.40 (1H, s, H-30), 4.75 (1H, d, J = 6.0 Hz, H-1′),

4.85 (1H, d, $J = 8.0$ Hz, H-1″), 4.80 (1H, d, $J = 8.0$ Hz, H-1‴), 5.20 (1H, d, $J = 7.5$ Hz, H-1⁗); ^{13}C NMR (C_5D_5N-d_5, 75 MHz) spectral data: see Table 1; HRESI-MS: *m/z* 1103.5638 $[M + H]^+$ (calcd for $C_{54}H_{87}O_{23}$, 1103.5637); ESI-MS: *m/z* 1101.5 $[M - H]^-$ (100), 969 (60), 897 (35), 807 (16), 603 (9), 471(25).

3.3.2 *Compound* **2**

An amorphous white powder, mp 274–276°C (CH_3OH), $[\alpha]_D^{20}$ −16.00 (*c* 0.01, pyridine). IR ν_{max} (KBr) cm^{-1}: 3406, 2930, 2875, 1640, 1540, 1448, 1380, 1358, 1257, 1151, 870; ^{1}H NMR (C_5D_5N-d_5, 300 MHz) δ: 0.80 (3H, s, H-25), 0.91 (3H, s, H-26), 0.92 (3H, s, H-30), 0.93 (3H, s, H-29), 1.05 (3H, s, H-24), 1.15 (3H, s, H-23), 1.69 (3H, s, H-27), 3.23 (1H, br d, $J = 11.0$ Hz, H-3), 3.88 (1H, d, $J = 8.0$ Hz, H_a-28), 4.03 (1H, d, $J = 8.0$ Hz, H_b-28), 4.23 (1H, m, H-16), 4.63 (1H, d, $J = 9.0$ Hz, H-22β), 4.83 (1H, d, $J = 9.0$ Hz, H-21α), 5.27 (1H, br s, H-12), 4.77 (1H, d, $J = 5.5$ Hz, H-1′), 4.90 (1H, d, $J = 7.6$ Hz, H-1″), 4.98 (1H, d, $J = 7.5$ Hz, H-1‴), 5.46 (1H, d, $J = 7.4$ Hz, H-1⁗); ^{13}C NMR (C_5D_5N-d_5, 75 MHz) spectral data: see Table 1; HRESI-MS: *m/z* 1079.5630 $[M + H]^+$ (calcd for $C_{52}H_{87}O_{23}$, 1079.5637); ESI-MS: *m/z* 1077.5 $[M - H]^-$ (100), 945 (50), 783 (20), 621 (12), 489 (30).

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