New Flavonoids from *Lysimachia christinae* HANCE

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A chemical investigation of *Lysimachia christinae*, a traditional Chinese medicine used as an effective conservative treatment for gall stones, hepatolithiasis, and urinary calculi, resulted in the isolation of two new flavonoids, myricetin 3,3′-di-α-L-rhamnopyranoside (**1**) and quercetin 3,3′-di-α-L-rhamnopyranoside (**2**), along with the five known flavonoids quercetin 3-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranoside], amentoflavone, hyperin, quercetin 3-β-D-glucopyranoside, and kaempferol 3-α-L-rhamnopyranoside. Amentoflavone was reported for the first time from the genus *Lysimachia*, and quercetin 3-[*O*-α-L-rhamopyranosyl-(1 → 2)-β-D-galactopyranoside] was isolated from this plant for the first time. The structures of the new compounds were elucidated on the basis of their chemical reactions and extensive spectroscopic analyses, including UV, mass, and NMR spectra.

Introduction. – *Lysimachia christinae* HANCE, a herbaceous plant of the family Primulaceae, is widely distributed in southwestern China and the Yangzi River basin. The dry whole herb of *L. christinae*, a traditional Chinese medicine known as *Jinqiancao*, has long been used for an effective treatment of biliary calculus, hepatolithiasis, and urinary calculi [1]. Additionally, the title plant was used as diuretics [2], cholagogue [3], anti-inflammatory agent [4], antioxidant [5], and analgesic [4]. The aqueous extract of *L. christinae* was able to decrease blood uric acid in mice [6]. To search for its active constituents, phytochemical studies were carried out, and two new flavonoids, myricetin 3,3′-di-α-L-rhamnopyranoside (**1**) and quercetin 3,3′-di-α-L-rhamnopyranoside (**2**) (*Fig. 1*) were obtained (myricetin = 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4*H*-1-benzopyran-4-one; quercetin = 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one; α-L-rhamnopyranose = 6-deoxy-α-L-mannopyranose) along with five known flavonoids, quercetin 3-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranoside], amentoflavone (= 8-[5-(5,7-dihydroxyphenyl)-4-oxo-4*H*)-1-benzopyran-2-yl)-2-hydroxyphenyl]-5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one), hyperin (= 2-(3,4-dihydroxyphenyl)-3-(β-D-galactopyranosyloxy)-5,7-dihydroxy-4*H*-1-benzopyran-4-one), quercetin 3-β-D-glucopyranoside, and kaempferol 3-α-L-rhamnopyranoside (kaempferol = 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one). This article is about the isolation and structural elucidation of the new compounds.

Results and Discussion. – The dry whole plant (10 kg) of *Lysimachia christinae* was extracted with 95% EtOH. The concentrated residue was subsequently suspended in

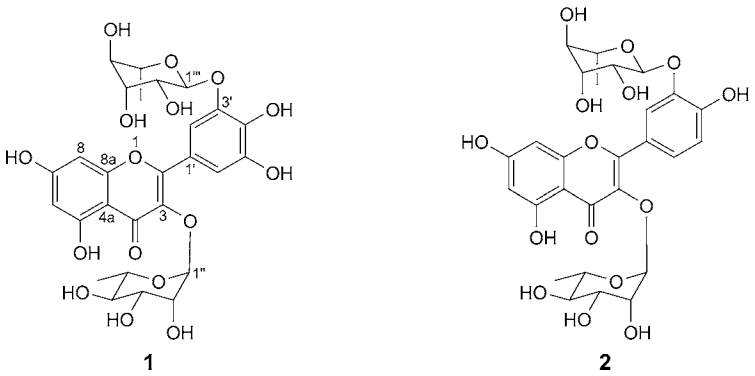

Fig. 1. *Compounds* **1** *and* **2**, *isolated from* Lysimachia christinae

H_2O and extracted in turn with petroleum ether, AcOEt, and BuOH. The AcOEt and BuOH extracts were subjected to column chromatography to yield compounds **1** and **2** and the five known flavonoids.

Compound **1** was obtained as a yellow powder and gave a yellow fluorescence in the $AlCl_3$ test. The UV spectrum exhibited the characteristic absorption maxima for a flavonoid at 258, 306, and 353 nm [7]. A molecular formula of $C_{27}H_{30}O_{16}$ was assigned to **1** based on the quasimolecular-ion peak at *m*/*z* 633.1417 ($[M+Na]^+$) in its HR-ESI-MS. In the ^{1}H-NMR spectrum (*Table*), the two *meta*-coupled aromatic H-atoms at δ(H) 6.38 and 6.22 (2*d*, each $J=2.0$ Hz) demonstrated the characteristic substitution pattern of a 5,7-dihydroxy-substituted *A*-ring of a flavonoid. The other two *meta*-coupled aromatic H-atoms at δ(H) 7.13 and 7.12 (2*d*, each $J=1.9$ Hz) indicated a 3′,4′,5′-tri-*O*-substituted *B*-ring. The signal at δ(H) 12.64 was that of OH–C(5). There was no ^{1}H-NMR signal of H–C(3). The ^{13}C-NMR data of the aglycone moiety were very similar to those of myricetin 3-β-D-galactopyranoside 3′-β-D-xylopyranoside [8]. All of the above suggested that **1** was a derivative of myricetin. The signals at δ(H) 5.25 (*d*, $J=1.9$ Hz, 1 H) and 5.22 (*d*, $J=1.9$ Hz, 1 H) corresponding to anomeric H-atoms suggested the presence of two sugar residues, which was confirmed by the C-atom signals at δ(C) 102.3 and 101.1. Two Me signals at δ(H) 1.18 (*d*, $J=6.2$ Hz) and 0.84 (*d*, $J=6.1$ Hz), and δ(C) 18.4 and 17.9 as well as twelve glycosyl C-atom signals manifested that the sugars were methyl pentoses (=6-deoxyhexoses). The ^{1}H- and ^{13}C-NMR data of the sugar moieties were very similar to those of myricetin 3-α-L-rhamnopyranoside [9], which suggested that **1** was a rhamnopyranoside. Because the chemical shifts of C(2′) and C(3′) were different from those of C(6′) and C(5′), C(3′) must be link to a rhamnosyl unit. The other rhamnosyl residue was located at C(3) on the basis of the glycosidation shifts of C(2) (10.3 ppm), C(3) (−1.2 ppm), and C(4) (2.3 ppm) in comparison to the aglycone [9]. The HMBCs between δ(H) 5.25 (H–C(1″)) and δ(C) 134.7 (C(3)), and between δ(H) 5.22 (H–C(1‴)) and δ(C) 146.5 (C(3′)) (*Fig. 2*) confirmed the positions of the two rhamnosyl units. The two glycosidic bonds were α-oriented according to the small coupling constants of the anomeric H-atoms. Accordingly, the structure of **1** was established as myricetin 3,3′-di-α-L-rhamnopyranoside.

Table. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; (D_6)DMSO) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
Aglycone:				
C(2)		156.9		157.0
C(3)		134.7		134.7
C(4)		178.2		178.1
C(4a)		104.6		104.4
C(5)		161.8		161.8
H–C(6)	6.22 (*d*, J = 2.0)	99.2	6.19 (*d*, J = 1.8)	99.4
C(7)		164.7		164.8
H–C(8)	6.38 (*d*, J = 2.0)	94.0	6.36 (*d*, J = 1.8)	94.2
C(8a)		157.7		157.2
C(1′)		120.2		121.3
H–C(2′)	7.13 (*d*, J = 1.9)	111.6	7.56 (*d*, J = 1.8)	119.2
C(3′)		146.5		144.8
C(4′)		139.6		151.4
C(5′) or H–C(5′)	–	145.4	6.98 (*d*, J = 8.4)	116.7
H–C(6′)	7.12 (*d*, J = 1.9)	110.7	7.48 (*dd*, J = 8.4, 1.8)	125.3
3-Rha:				
H–C(1″)	5.25 (*d*, J = 1.9)	102.3	5.30 (*d*, J = 1.8)	102.2
H–C(2″)	3.97 (*t*, J = 1.8)	70.8	3.96 (*t*, J = 1.8)	70.8
H–C(3″)	3.76 (*dd*, J = 9.4, 2.3)	71.1	3.46 (*dd*, J = 8.6, 1.9)	71.1
H–C(4″)	3.31 (*t*, J = 9.4)	72.4	3.31 (*t*, J = 9.4)	72.4
H–C(5″)	3.70 (*m*)	70.4	3.68 (*m*)	70.5
Me(6″)	0.84 (*d*, J = 6.1)	17.9	0.81 (*d*, J = 5.8)	17.9
3′-Rha:				
H–C(1‴)	5.22 (*d*, J = 1.9)	101.1	5.26 (*d*, J = 1.8)	101.1
H–C(2‴)	3.97 (*t*, J = 1.8)	70.6	3.96 (*t*, J = 1.8)	70.6
H–C(3‴)	3.52 (*dd*, J = 9.2, 2.2)	70.9	3.73 (*dd*, J = 9.4, 2.2)	70.9
H–C(4‴)	3.17 (*t*, J = 9.4)	71.7	3.31 (*t*, J = 9.4)	71.6
H–C(5‴)	3.25 (*m*)	70.0	3.12 (*m*)	70.1
Me(6‴)	1.18 (*d*, J = 6.2)	18.4	1.18 (*d*, J = 6.2)	18.4

Compound **2** was isolated as a yellow powder and showed positive results in the $AlCl_3$ test. The UV spectrum of **2** exhibited absorption maxima at 256, 275, and 357 nm, characteristic absorption bands of a flavonol skeleton [7]. The HR-ESI-MS showed a quasimolecular-ion peak at m/z 617.1473 ($[M+Na]^+$), suggesting that its molecular formula was $C_{27}H_{30}O_{15}$. In the ^{1}H-NMR spectrum (*Table*), an *AMX* spin system at δ(H) 7.48 (*dd*, J = 8.4, 1.8 Hz, 1 H), 7.56 (*d*, J = 1.8 Hz, 1 H), and 6.98 (*d*, J = 8.4 Hz, 1 H) revealed the existence of a 1,3,4-trisubstituted aromatic moiety, which, together with two *meta*-coupled *d* at δ(H) 6.36 and 6.19 (2*d*, each J = 1.8 Hz), indicated that **2** was a derivative of quercetin. The signals at δ(H) 5.30 (*d*, J = 1.8 Hz, 1 H) and 5.26 (*d*, J = 1.8 Hz, 1 H) and the signals at δ(C) 102.2 and 101.1 demonstrated that **2** was a glycoside. The ^{1}H- and ^{13}C-NMR data of the sugar residues were similar to those of **1**, which suggested that **2** had two rhamnopyranosyl units. The latter were located at C(3) and C(3′) from the HMBCs between δ(H) 5.30 (H–C(1″)) and δ(C) 134.7 (C(3)), and

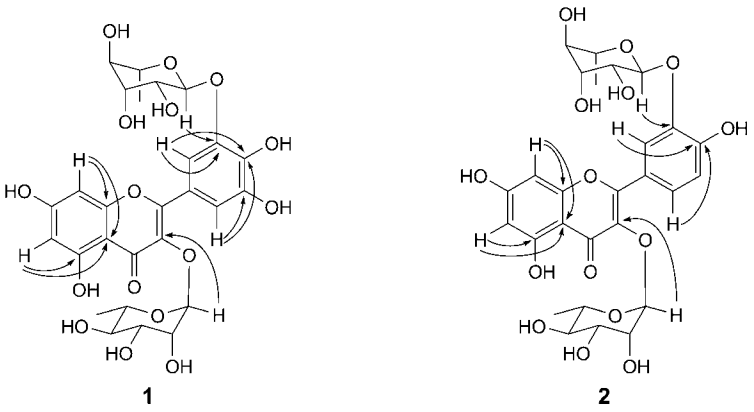

Fig. 2. *Key HMBCs of compounds* **1** *and* **2**

between δ(H) 5.26 (H–C(1‴)) and δ(C) 144.8 (C(3′)) (*Fig. 2*). Thus, compound **2** was identified as quercetin 3,3′-di-α-L-rhamnopyranoside.

The five other flavonoids were obtained and identified as quercetin 3-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranoside] [10], amentoflavone [11], hyperin [12], quercetin 3-β-D-glucopyranoside [13], and kaempferol 3-α-L-rhamnopyranoside [14], by comparing their spectroscopic data with those reported in the literature. Amentoflavone was reported for the first time from the genus *Lysimachia*, and quercetin 3-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranoside] was isolated from *L. christinae* for the first time.

Experimental Part

General. Column chromatoguaphy (CC): silica gel (SiO_2; 200–300 and 300–400 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Sweden), macroporous resin *AB-8* (*Qingdao Marine Chemical Factory*, Qingdao, P. R. China). TLC: silica gel GF_{254} plates (*Yantai Jiangyou Chemical Group Co.*, Yantai, P. R. China); spots visualized by UV light (254 and 365 nm), and by spraying with 1% $AlCl_3$ reagent. UV Spectra: *Shimadzu-UV-260* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Avatar-360-ESP* spectrophotometer (*Thermo Nicolet*); KBr tablets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR ((D_6)DMSO): *Bruker-Avance-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker-APEX-7.0-Tesla* FT-MS apparatus (*Bruker*, Germany); in *m*/*z*.

Plant Material. The whole plant of *Lysimachia christinae* was collected in Chongqing, China, in July 2010, and identified by *J. D.* A voucher specimen (No. 201007) was deposited with the Herbarium of Materia Medica, College of Pharmaceutical Sciences, Southwest University.

Extraction and Isolation. The whole plant of *Lysimachia christinae* was dried in the sun and ground. The dry powder (10 kg) was percolated with 95% EtOH at r.t. The combined extract was concentrated to yield 570 g of residue which was suspended in H_2O (2000 ml) and successively extracted with petroleum ether (4 × 2000 ml), AcOEt (4 × 2000 ml), and BuOH (4 × 2000 ml). The AcOEt extract (97 g) was subjected to CC (SiO_2 (200–300 mesh, 2.5 kg, 12 × 100 cm), $CHCl_3$/MeOH 100 : 1 → 1 : 1: *Fractions 1–7*. *Fr. 4* was subjected to repeated CC (SiO_2, $CHCl_3$/MeOH, then *Sephadex LH-20*, MeOH): *amentoflavone* (11 mg). *Fr. 5* was subjected to CC (SiO_2, $CHCl_3$/MeOH) and further purified by semi-prep. HPLC (*Phenomenex* C_{18} (21.2 × 100 mm, 10 μm), MeOH/H_2O 2 : 3): hyperine (6.5 mg), *quercetin 3-β-D-*

glucopyranoside (3.7 mg), and *kaempferol 3-α-L-rhannopyranoside* (7 mg). The BuOH extract (57 g) was subjected to CC (*AB-8*, EtOH/H_2O 20:80, 30:70, 50:50, 70:30, and 95:5): *Fractions I–V. Fr. II* was then applied on repeated CC (SiO_2, AcOEt/EtOH 90:10, 85:15, 80:20, and 0:100) and further purified by semi-prep. HPLC (*Phenomenex* C_{18} (21.2 × 100 mm, 10 μm), MeOH/H_2O 1:4): **1** (35 mg), **2** (25 mg), and quercetin *3-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranoside]* (28 mg).

Myricetin 3,3′-Di-α-L-rhamnopyranoside (= *3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-2-{3-[(6-deoxy-α-L-mannopyranosyl)oxy]-4,5-dihydroxyphenyl}-5,7-dihydroxy-4*H*-1-benzopyran-4-one*; **1**): Yellow powder. UV (MeOH): 258 (4.25), 306 (3.56), 353 (4.67). IR (KBr): 3400, 2930, 1656, 1606, 1454, 1074, 1058, 1040. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 633.1417 ($[M+Na]^+$, $C_{27}H_{30}NaO_{16}^+$; calc. 633.1426).

Quercetin 3,3′-Di-α-L-rhamnopyranoside (= *3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-2-{3-[(6-deoxy-α-L-mannopyranosyl)oxy]-4-hydroxyphenyl}-5,7-dihydroxy-4*H*-1-benzopyran-4-one*; **2**): Yellow powder. UV (MeOH): 256 (4.4), 275 (4.4), 357 (4.3). IR (KBr): 3410, 2935, 1658, 1560, 1502, 1260, 1075, 1035. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 617.1473 ($[M+Na]^+$, $C_{27}H_{30}NaO_{15}^+$; calc. 617.1477).

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