Biosci. Biotechnol. Biochem., **74** (3), 652–654, 2010

Note

A New 2′-Oxygenated Flavone Glycoside from *Litsea glutinosa* (Lour.) C. B. Rob.

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Received September 28, 2009; Accepted December 11, 2009; Online Publication, March 7, 2010
[doi:10.1271/bbb.90701]

A new 2′-oxygenated flavone glycoside, named glutin, was isolated from *Litsea glutinosa* (Lour.) C. B. Rob. along with four known compounds. The structure was identified as 2′,5,7-trihydroxy-6-methoxyflavone 2′-O-β-D-glucopyranoside (1) on the basis of extensive spectroscopic analysis.

Key words: flavonoids; *Litsea glutinosa* (Lour.) C. B. Rob.; glutin

The genus *Litsea* (Lauraceae) comprises nearly 200 species, which are distributed widely throughout tropical and subtropical Asia, North America and subtropical South America. Previous phytochemical studies revealed the presence of alkaloids,[1–3] butanolides,[4,5] and sesquiterpenoid in the genus *Litsea*.[6–9] Few records are found in the literature with respect to the isolation of flavonoids from *Litsea* species.[10]

Litsea glutinosa (Lour.) C. B. Rob., locally known as "Chan Gao Shu" in China, is an evergreen medium-sized tree. Its bark and leaves are used as a demulcent and mild astringent for diarrhea and dysentery, and the roots are used to poultice sprains and bruises.[11] To date, there have been few phytochemical studies on the species *Litsea glutinosa* (Lour.) C. B. Rob.[12] Our previous investigation resulted in the isolation of two new aporphine alkaloids from this species.[13] As part of continuous research on plants of the *Litsea* genus, the chemical constituents of the leaves and twigs of *L. glutinosa* (Lour.) C. B. Rob. were reinvestigated.

Powdered leaves and twigs of *Litsea glutinosa* (Lour.) C. B. Rob. (12.0 kg) were repeatedly extracted with EtOH at room temperature. The extract was then concentrated under reduced pressure to give a brown syrup, which was partitioned in H_2O and extracted with solvents into a petroleum ether-fraction (80 g), an EtOAc-fraction (54 g), and an *n*-BuOH-fraction (108 g) fraction. The petroleum-ether fraction was subjected to silica gel column chromatography eluted with petroleum ether-EtOAc (10:1–1:1), by which five fractions (I–V) were obtained. Fraction II was resubmitted to silica gel column chromatography to yield compounds **2** (20 mg) and **3** (55 mg). The EtOAc-fraction was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (99:1–1:1) to afford eight fractions (I–VIII). Fraction III was resubmitted to silica gel column chromatography by gradient elution using petroleum ether-EtOAc, and crystals from Fraction III were purified by recrystallization ($CHCl_3$–MeOH 1:1) to give compounds **5** (38 mg). Fraction IV was resubmitted to Sephadex LH-20 (using MeOH as eluent) and RP C-18 (MeOH–H_2O 7:3, 0.7 liter; 6:4, 0.5 liter) to yield compounds **1** (8 mg). Fraction V was rechromatographed on a silica gel column, and eluted with $CHCl_3$ containing increasing amounts of MeOH, and then to Sephadex LH-20 column chromatography (using acetone as eluent) to give compounds **4** (12 mg).

Compound **1** was isolated as a yellow amorphous powder. A positive Shinoda and Fiegel's test indicated that compound **1** was a flavonoid glycoside.[14] Its formula, $C_{22}H_{22}O_{11}$, was derived from positive HR-ESI-MS measurements of the molecular-related ion at m/z 485.1053 (calcd. for 485.1059) $[M + Na]^+$, and from ^{13}C and DEPT NMR data. The IR spectrum (KBr) of **1** showed a hydroxyl absorption band at 3,435 cm^{-1} and a carbonyl absorption band at 1,658 cm^{-1}. The UV spectrum showed λ_{max} at 208, 267, and 326 nm (MeOH). A bathochromic shift (λ_{max} 326, 267, 215 nm) of 7 nm of band II with NaOAc indicated the presence of a free 7-OH group. Several strong, broad bands in the range of 1,650–1,051 cm^{-1} in the IR spectrum indicated a flavone skeleton. A prominent fragment at m/z 301 $[M + H - 162]^+$ and the ^1H and ^{13}C NMR spectra indicated the presence of a glucopyranosyl unit. Glycoside **1** (1 mg) was hydrolyzed with 2 N HCl at 100 °C for 40 min. The reaction mixture was diluted with water and extracted with EtOAc. The sugar in the aqueous layer was identified as D-glucose by co-PC using solvent system *n*-BuOH–HOAc–H_2O (4:1:5).

The ^1H NMR spectrum exhibited the presence of a sharp single hydroxyl signal at δ_H 13.0 and another hydroxyl signal, δ_H 10.75, that indicated that the A-ring had a 5,7-dihydroxy substituted pattern (Table 1). The aromatic region of the ^1H NMR spectrum of **1** displayed a sharp one-proton singlet signal at δ_H 7.01, correlated with C-3 (109.8) in its HMQC spectrum, which is characteristic the C-3 proton of 2′-oxygenated flavone.[15] Likewise, a second singlet resonance in the aromatic region at δ_H 6.58 (1H, δ_C 94.3 by HMQC) was assigned to H-8 on the basis of long-range connectivities to δ_C 157.5 (C-7), 152.8 (C-9), 131.4 (C-6), and 104.2 (C-10). A methoxyl signal at δ_H 3.76 was placed at C-6 based on ^3J correlation of these protons with C-6 at δ_C 131.4 in the HMBC spectrum. The presence of

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Table 1. ^{13}C- (125 MHz) and ^{1}H- (500 MHz) NMR Data for **1** (in DMSO-d_6)

Position	δ_C	δ_H (integral, mult, J in Hz)	Position	δ_C	δ_H (integral, mult, J in Hz)
2	160.9	/	4′	132.9	7.56 (1H, ddd, 1.6, 7.6, 7.8 Hz)
3	109.8	7.01(1H, s)	5′	122.0	7.23 (1H, ddd, 1.6, 7.6, 7.6)
4	182.3	/	6′	129.1	7.89 (1H, dd, 1.6, 7.6)
5	152.7	13.0 (1H, s, –OH)	2′-Glu:1″	100.2	5.12 (1H, d, 7.1)
6	131.4	/	2″	73.4	3.36 (1H, m)
7	157.5	10.75 (1H, s, –OH)	3″	76.8	3.37 (1H, m)
8	94.3	6.58 (1H, s)	4″	69.6	3.29 (1H, m)
9	152.8	/	5″	77.2	3.40 (1H, m)
10	104.2	/	6″a	60.6	3.47 (1H, dd, 14.6, 4.8)
1′	120.3	/	6″b		3.70 (1H, dd, 14.6, 5.5)
2′	155.4	/	6-OMe	60.0	3.76 (3H, s)
3′	115.5	7.36 (1H, dd, 1.6, 7.8)			

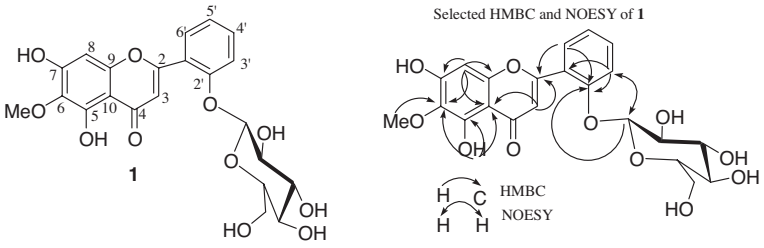

Fig. 1. The Structure, Key HMBC Correlations, and Selected NOESY of **1**.

7-hydroxy and 6-methoxyl groups was also confirmed by comparison of C-6 (δ_C 131.4) and C-8 (δ_C 94.3) ^{13}C NMR signals of **1** with those (δ_C 131.5, 94.5) of 5,7-dihydroxy-6-methoxyflavone.[16)]

The four spin-spin coupled multiplets at δ_H 7.89 (1H, dd, $J = 7.6$, 1.6 Hz), 7.56 (1H, ddd, $J = 1.6$, 7.6, 7.8 Hz), 7.36 (1H, dd, $J = 1.6$, 7.8 Hz), and 7.23 (1H, ddd, $J = 7.6$, 7.6, 1.6 Hz) comprised the remaining aromatic resonances and are characteristic ABCD signal pattern of a 2′-oxygenated B-ring,[15)] and were assigned to the C-6′, C-4′, C-3′, and C-5′ proton, respectively. The HMBC spectrum was utilized to identify the position of the sugar. A long-range correlation between the anomeric proton signal (δ_H 5.12) of glucose and C-2′ (δ_C 155.4) confirmed the attachment of the sugar at C-2′. Further useful correlations from H-3′ to 120.3s (C-1′), 155.4s (C-2′), H-4′ to 129.1d (C-6′), 155.4s (C-2′), and H-6′ to 132.9d (C-4′), 155.4s (C-2′), and 160.9s (C-2) confirmed this. The NOESY spectrum of **1** gave a cross peak between the H-3′ signal and the anomeric proton signal (δ_H 5.12) of glucose, indicating further that the glucose moiety in **1** is linked to the C-2′ hydroxy group. The coupling constant ($J = 7.1$ Hz) of the anomeric proton signal indicated the β-configuration of the glucopyranoside moiety. Thus, the new flavone glycoside was identified as 2′,5,7-trihydroxy-6-methoxyflavone 2′-O-β-D-glucopyranoside, and was named glutin (Fig. 1).

The structures of known compounds **2**–**5** were identified as sitosterol (**2**),[17)] stigmasterol (**3**),[18)] epicatechin (**4**),[19)] and sitosterol β-D-glucopyranoside (**5**)[20)] by a combination of spectroscopic analysis and comparison with the literature data. Compound **1** was characterized as a 2′-oxygenated flavone glycoside; this is the first time that a C-2′ oxygenated flavonoid has been isolated from the genus *Litsea*.

Our preliminary pharmacological study of this plant showed the EtOAc extract to have cytotoxic activity against human Hela (human carcinoma of the cervix) cell lines at a concentration of 10 μg/ml. But compound **1** did not show potential *in vitro* cytotoxicity screening against human Hela cell lines. Besides, **1** was screened for anti-oxidant activities using DPPH radicals by the described method.[21)] In brief, 5 μl of test sample (1 mM in DMSO) was seeded to 95 μl of 300 μM ethanolic DPPH and incubated at 37 °C. After 30 min, the absorbance of the mixture was measured at 517 nm in a microplate reader. Percent radical scavenging activity was determined by comparison with a DMSO-containing control. IC_{50} values represent concentrations of compounds scavenging 50% of DPPH radicals. BHA (3-*t*-butyl-4-hydroxyanisole) was used as a positive control. The results displayed that **1** scavenged DPPH radicals (53.5%), while standard anti-oxidant BHA as a positive control was active at a level of 92.1% at 1 mM.

Acknowledgments

This work was supported by the National Nature Science Foundation of China (no. 20862018), the Science Foundation of Yunnan University (Grant no. 2009B10Q and 2005Z001A), and the Science Foundation of Yunnan Province (Grants nos. 2006B0003Q, 2007B0006Z, and 2007PY01-23).

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