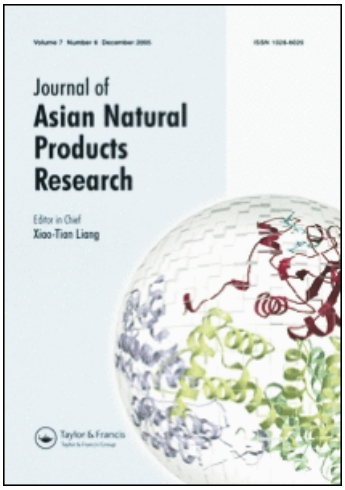

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Chen, Zhen-Hua , Zhang, Ru-Jun , Wu, Jian and Zhao, Wei-Min(2009) 'New dihydrochalcone glycosides from *Lithocarpus litseifolius* and the phenomenon of C-H→C-D exchange observed in NMR spectra of phenolic components', Journal of Asian Natural Products Research, 11: 6, 508 — 513
To link to this Article: DOI: 10.1080/10286020902920752
URL: http://dx.doi.org/10.1080/10286020902920752

Journal of Asian Natural Products Research
Vol. 11, No. 6, June 2009, 508–513

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New dihydrochalcone glycosides from *Lithocarpus litseifolius* and the phenomenon of C—H → C—D exchange observed in NMR spectra of phenolic components

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(*Received 24 November 2008; final version received 20 February 2009*)

Two new dihydrochalcone glycosides named 6″-*O*-acetyltrilobatin (**1**) and 3″-*O*-acetylphloridzin (**2**) as well as four known compounds were isolated from the leaves of *Lithocarpus litseifolius* (Hance) Chun (family Fagaceae). Their structures were elucidated on the basis of spectroscopic analyses. The phenomena of C—H → C—D exchange were observed in NMR spectra of the isolated phenolic components when measured in deuterated methanol.

Keywords: *Lithocarpus litseifolius*; Fagaceae; dihydrochalcone glycoside; C—H → C—D exchange

1. Introduction

Lithocarpus litseifolius (Hance) Chun (Fagaceae) is an evergreen arbor distributed widely throughout the mountainous regions in southern China. Its tender leaves have been used as sweet tea with anti-hypertension effect [1]. Previous phytochemical study on the genus *Lithocarpus* led to the identification of triterpenes [2,3] and dihydrochalcones, such as phloridzin and trilobatin [4]. Trilobatin was reported to be 300 times sweeter than that of sucrose [5]. In order to know more about the chemical and bioactive components of *L. litseifolius*, chemical investigation was undertaken on the leaves of *L. litseifolius* collected in Bama, Guangxi Zhuang Autonomous Region of China. This paper describes the isolation and structural elucidation of two new dihydrochalcones named 6″-*O*-acetyltrilobatin (**1**) and 3″-*O*-acetylphloridzin (**2**), and four known compounds phloridzin [4], trilobatin [4], 2″-*O*-acetylphloridzin [6], and phloretin (Figure 1) [7]. It was interesting to observe the phenomena of C—H → C—D exchange in the NMR spectra of phenolic compounds with phloroglucinol nucleus. A plausible mechanism of the phenomenon of C—H → C—D exchange was suggested.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with a molecular formula of $C_{23}H_{26}O_{11}$ determined by the analysis of HR-ESI-MS and NMR. The ^{13}C NMR spectrum of **1** showed 23 carbon signals separated by DEPT experiment into 1 methyl, 3 methylenes, 11 methines, and 8 quaternary carbons. The ^{1}H NMR spectrum of **1** revealed the presence of six aromatic proton signals at δ_H 7.04 (2H, d, $J = 8.1$ Hz), 6.68 (2H, d, $J = 8.1$ Hz), and

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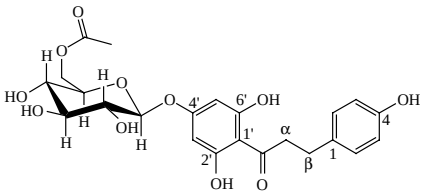
ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020902920752
http://www.informaworld.com



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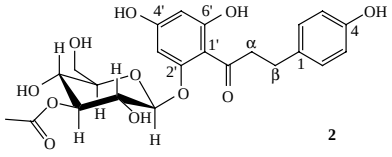

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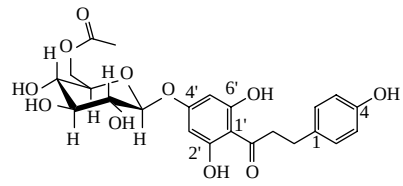
Figure 1. Structures of compounds **1** and **2**.

6.06 (2H, s), one anomeric proton signal at δ_H 4.95 (1H, d, $J = 7.5$ Hz), two proton signals due to a methene at δ_H 2.82 (2H, t, $J = 7.3$ Hz), and one methyl signal at δ_H 2.08 (3H, s). The NMR spectral data of **1** were similar to those of the known compound trilobatin except for an additional acetyl group, which could be confirmed by the 42 Da difference between the molecular weight of **1** and trilobatin. The two characteristic proton signals of C-6 of glucosyl group at downfield δ_H 4.40 (1H, dd, $J = 11.8$, 1.8 Hz) and 4.26 (1H, dd, $J = 11.8$, 6.7 Hz) suggested the location of the acetyl group at C-6 of the glucose moiety. Further, analysis of $^1H-^1H$ COSY, HMQC, HMBC, and ROESY spectra of **1** enabled the assignment of all proton and carbon signals and the observation of $^{13}C-^1H$ long-range correlations between the carbon at δ_C 173.4 and δ_H 4.40 (1H, dd, $J = 11.8$, 1.8 Hz) and 4.26 (1H, dd, $J = 11.8$, 6.7 Hz) further revealed the location of the acetyl group at C-6 of glucosyl group. Therefore,



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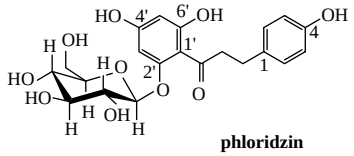


phloridzin

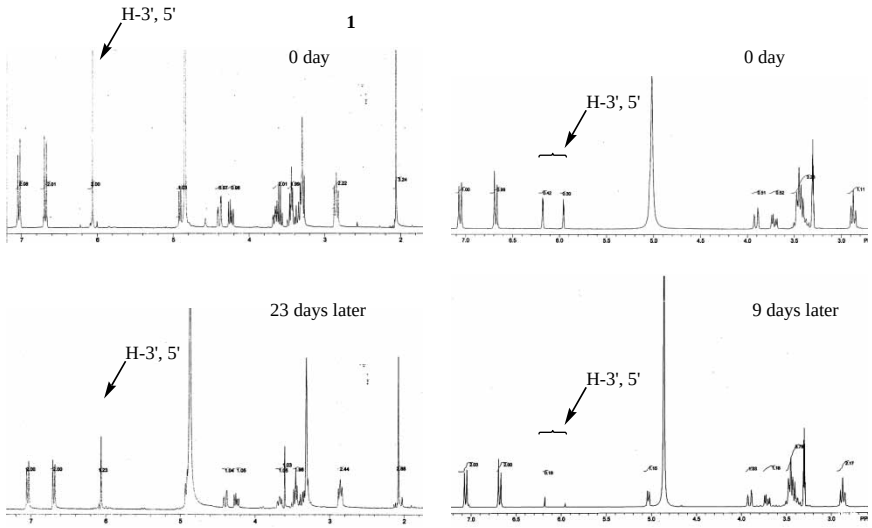
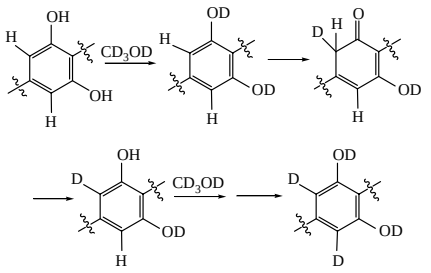


Figure 2. ^1H NMR spectra of 6″-*O*-acetyltrilobatin (**1**) and phloridzin in methanol-d_4.



Scheme 1. Plausible mechanism of C—H → C—D exchange in phloroglucinol nucleus.

the structure of **1** was finally identified to be 6″-*O*-acetyltrilobatin.

Compound **2** was obtained as a white amorphous powder with a molecular formula of $C_{23}H_{26}O_{11}$ identical to that of **1** determined by the analysis of HR-ESI-MS and NMR. The ^{13}C NMR spectrum of **2** also exhibited 23 carbon signals, including 1 methyl, 3 methylenes, 11 methines, and 8 quaternary carbons. The ^{1}H NMR spectrum of **2** revealed the presence of six aromatic proton signals at δ_H 7.04 (2H, d, $J = 8.1$ Hz), 6.68 (2H, d, $J = 8.1$ Hz), 6.20 (1H, d, $J = 2.1$ Hz), and 5.96 (1H, d, $J = 2.1$ Hz), one anomeric proton signal at δ_H 5.15 (1H, d, $J = 7.8$ Hz), one oxygenated methine signal at δ_H 5.04 (1H, t, $J = 9.1$ Hz), two signals due to a methylene at δ_H 2.88 (2H, t, $J = 7.4$ Hz), and one acetyl methyl at δ_H 2.12 (3H, s). In contrast to the location of the glucosyl moiety at C-4′ in trilobatin, the splitting of the two aromatic proton signals at δ_H 6.20 (1H, d, $J = 2.1$ Hz) and 5.96 (1H, d, $J = 2.1$ Hz) indicated their location in *meta* position, and suggested the structure of **2** to be similar to phloridzin, another major component identified from the same plant species [4]. Analysis of $^{1}H-^{1}H$ COSY spectrum of **2** indicated that both the anomeric proton signal at δ_H 5.15 (1H, d, $J = 7.8$ Hz) and the oxygenated methine signal at downfield δ_H 5.04 (1H, t, $J = 9.1$ Hz) correlated with the proton signal at δ_H 3.57 (m, glc-2), which suggested the location of an acetyl group at C-3 of glucosyl group. The result was further confirmed by the $^{13}C-^{1}H$ long-range correlation between the proton at δ_H 5.04 (1H, t, $J = 9.1$ Hz) and the carbonyl carbon at δ_C 173.2. Thus, the structure of **2** was determined to be 3″-*O*-acetylphloridzin.

It was interesting to find that the integration data of the proton signal H-3′ (H-5′) of the new compound **1** shrunk with time severely compared with other proton signals in its ^{1}H NMR spectrum when measured in CD_3OD (Figure 2). C—H → C—D exchange was supposed to happen in the phloroglucinol nucleus, which could also be demonstrated by the shrink of C-3′ (C-5′) signal in its ^{13}C NMR spectrum, and by the 1 and 2 Da increase in the quasi-molecular weight of **1** stored in CD_3OD. Such phenomena could also be found when NMR spectra were measured with phloridzin (Figure 2). CF_3COOD may give the same result when it was used as an NMR solvent. Such a kind of deviation of integration data may influence the structural identification of chemical substances, and the deuteration may also lead to sample loss for further measurement of physico-chemical properties and evaluation of biological activity. A plausible mechanism of C—H → C—D exchange in phloresinol nucleus was suggested in Scheme 1.

Phloridzin, as a major component of the leaves of *L. litseifolius*, was reported to be a diuretic, and such an activity could contribute to the anti-hypertension effect of the plant [8].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with Perkin-Elmer 341 polarimeter. UV spectra were obtained on a Beckman DU-7 spectrometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. ESI-MS were measured using a Finnigan

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of **1**, **2**, and phloridzin (in methanol-d_4; J, Hz).

	1		**2**		Phloridzin	
No.	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1		134.3, s		134.3, s		134.4, s
2	7.04 (d, $J = 8.1$)	130.8, d	7.04 (d, $J = 8.7$)	130.8, d	7.06 (d, $J = 8.5$)	130.9, d
3	6.68 (d, $J = 8.1$)	116.6, d	6.68 (d, $J = 8.7$)	116.5, d	6.68 (d, $J = 8.5$)	116.6, d
4		157.0, s		157.0, s		156.8, s
5	6.68 (d, $J = 8.1$)	116.6, d	6.68 (d, $J = 8.7$)	116.5, d	6.68 (d, $J = 8.5$)	116.6, d
6	7.04 (d, $J = 8.1$)	130.8, d	7.04 (d, $J = 8.7$)	130.8, d	7.06 (d, $J = 8.5$)	130.9, d
1′		107.5, s		107.2, s		107.3, s
2′		166.0, s		162.5, s		162.8, s
3′	6.06 (s)	96.9, d	6.20 (d, $J = 2.1$)	96.0, d	6.16 (d, $J = 2.4$)	95.9, d
4′		165.6, s		166.4, s		166.4, s
5′	6.06 (s)	96.9, d	5.96 (d, $J = 2.1$)	98.9, d	5.94 (d, $J = 2.4$)	98.8, d
6′		166.0, s		168.1, s		168.0, s
α	3.30 (2H, m)	48.3, t	3.40 (2H, m)	47.4, t	3.40–3.48 (m)	47.4, t
β	2.82 (2H, t, $J = 7.3$)	31.7, t	2.88 (2H, t, $J = 7.4$)	31.3, t	2.84 (2H, t, $J = 7.7$)	31.3, t
C═O		207.6, s		207.0, s		207.1, s
Glc-1	4.95 (d, $J = 7.5$)	101.4, d	5.15 (d, $J = 7.8$)	102.3, d	5.05 (d, $J = 7.2$)	102.5, d
Glc-2	3.45 (m)	75.1, d	3.57 (m)	73.4, d	3.40–3.48 (m)	75.2, d
Glc-3	3.47 (m)	78.3, d	5.04 (t, $J = 9.1$)	79.8, d	3.40–3.48 (m)	78.9, d
Glc-4	3.37 (m)	72.1, d	3.54 (m)	69.7, d	3.40–3.48 (m)	71.6, d
Glc-5	3.68 (m)	76.0, d	3.55 (m)	78.6, d	3.40–3.48 (m)	79.0, d
Glc-6	4.40 (dd, $J = 11.8, 1.8$), 4.26 (dd, $J = 11.8, 6.7$)	65.1, t	3.90 (dd, $J = 12.0, 1.6$), 3.74 (dd, $J = 12.0, 6.5$)	62.5, t	3.90 (dd, $J = 12.0, 1.6$), 3.72 (dd, $J = 12.0, 6.5$)	62.9, t
CH_3CO—		173.4, s		173.2, s		
*CH_3*CO—	2.08 (s)	21.2, q	2.12 (s)	21.6, q		

LCQ-DECA instrument, HR-ESI-MS data were obtained on a Mariner spectrometer. The NMR experiments were run on a Bruker AM 400 spectrometer with TMS as an internal standard. Preparative HPLC was carried out using a Varian SD-1 instrument equipped with a Merck NW25 C_{18} column (25 mm × 250 mm, 10 μm; Merck, Darmstadt, Germany) and ProStar 320 UV/VIS Detector. Column chromatographic separations were carried out using silica gel H60 (300–400 mesh; Yantai Chemical Industrial Institute, Yantai, China). HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC.

3.2 Plant material

The leaves of *L. litseifolius* were collected in Bama, Guangxi Zhuang Autonomous Region of China, and identified by Prof. Ding Fang of Guangxi Institute of Chinese Traditional Medicine. A voucher specimen (No. 20040108) is deposited in the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

Air-dried and powdered leaves (0.4 kg) of *L. litseifolius* were refluxed with 95% EtOH (1.5 l × 3), each for 1.5 h. The extract was concentrated to dryness *in vacuo* (105 g) and the chlorophyll was then precipitated with 30% EtOH. The filtrate was concentrated and the aqueous residue was partitioned with chloroform (500 ml × 3) and *n*-butanol (500 ml × 3), successively, to yield the chloroform extract (12 g) and *n*-butanol extract (55 g), respectively. The *n*-butanol extract (55 g) was subjected to a series of chromatography over silica gel H60, HW-40, and RP-18 preparative HPLC to afford **1** (12 mg), **2** (42 mg), phloridzin (12 g), trilobatin (15 g), 2″-*O*-acetylphloridzin (31 mg), and phloretin (120 mg).

*3.3.1 6″-O-acetyltrilobatin (**1**)*

A white amorphous powder; $[\alpha]_D^{22}$ −64 ($c = 0.075$, MeOH); UV λ_{max} (log ε): 281.0 (4.57), 224.0 (4.64) nm; IR (KBr) ν_{max} 3408, 2920, 1724, 1629, 1599, 1516, 1435, 1435, 1371, 1248, 1176, 1078, 829 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z*: 501.1 $[M+Na]^+$; HR-ESI-MS *m/z*: 501.1385 $[M+Na]^+$ (calcd for $C_{23}H_{26}O_{11}Na$, 501.1373).

*3.3.2 3″-O-acetylphloridzin (**2**)*

A white amorphous powder; $[\alpha]_D^{22}$ −43 ($c = 0.205$, MeOH); UV λ_{max} (log ε): 284.5 (4.58), 223.5 (4.65) nm; IR (KBr) ν_{max} 3443, 2920, 1722, 1629, 1604, 1515, 1464, 1385, 1263, 1211, 1080, 1043, 829 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z*: 501.2 $[M+Na]^+$; HR-ESI-MS *m/z*: 501.1369 $[M+Na]^+$ (calcd for $C_{23}H_{26}O_{11}Na$, 501.1373).

Acknowledgements

The authors are grateful to the financial support of Shanghai-Unilever Research and Development Fund (06SU07004). The authors also wish to thank Professors Ying Zhou and Ding Fang of Guangxi Institute of Chinese Traditional Medicine and Mr Jinchao Chen of Bama Longevity Insititute for supplying the plant material and valuable information and identification of the voucher specimen.

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