A New Sweet Dihydrochalcone-Glucoside from Leaves of *Lithocarpus pachyphyllus* (Kurz) Rehd. (Fagaceae)

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A new sweet dihydrochalcone-glucoside, trilobatin 2″-acetate (**1**), was isolated from the leaves of *Lithocarpus pachyphyllus*, together with phlorizin and trilobatin. The structures were established by spectroscopic methods, including one- and two-dimensional NMR (COSY, HMQC and HMBC).

Key words: Lithocarpus pachyphyllus, Sweet Dihydrochalcone-glucoside, Trilobatin 2″-Acetate

Introduction

The isolation and identification of several sweet principles from Chinese wild plants have been reported: rubusoside from Chinese *Rubus chigii* (Tanaka *et al.*, 1981), baiyunoside from *Salvia digitaloides* (Tanaka *et al.*, 1983), and phlorizin and trilobatin from *Lithocarpus litseiffolius* (Nie *et al.*, 1982). The leaves of *Lithocarpus pachyphyllus* (Kurz) Rehd. (Fagaceae) are known to taste sweet and used to be a kind of sweet tea in Yunnan province, south-west of China. The glycoside fraction of the leaves of *Lithocarpus pachyphyllus* collected in Yunnan was subjected to column chromatography, yielding a new sweet principle trilobatin 2″-acetate (**1**), together with two known sweet constituents phlorizin and trilobatin. This report describes the structure elucidation of trilobatin 2″-acetate.

Results and Discussion

Compound **1** was assigned the structure of trilobatin 2″-acetate based on the following facts. In the ^{1}H NMR spectrum, the expected peaks for the aromatic protons of a trilobatin unit were present, such as the AA′BB′ "quartet" for the disubstituted phenyl protons and a singlet (2H) for the *meta* protons, H-3′and H-5′, of the triloboglucinol ring, which must be a symmetrical structure. In addition, a three-proton singlet (CH_3) for an acetate was present at δ 1.9 ppm and a one-proton double doublet at δ 4.9 ppm was designated for the proton at the acetate-bearing carbon. The ^{13}C NMR data indicated the existence of a trilobatin unit plus an acetate unit. The partial structure of trilobatin unit can be identified by direct comparison with literature values (Nie *et al.*, 1982). The cross peak between carbonyl carbon of acetate and H-2″ was observed in HMBC spectrum. This strongly suggested that the acetate should be placed at C-2″. The correlation peaks between H-2″ and anomeric proton (H-1″) in the ^{1}H-^{1}H-COSY confirmed that the proton at the acetate-bearing carbon is coupled to the anomeric proton. All the above data supported the location of the acetate at C-2″. Combining the HMQC and HMBC data (Table I), the structure was determined to be trilobatin 2″-acetate.

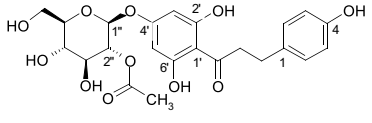

Fig. 1. The structure of compound **1**, trilobatin 2″-acetate.

Compound **2** was assigned the structure of phlorizin by comparison with literature values (Nie *et al.*, 1982).

Compound **3** was assigned the structure of trilobatin by comparison with literature values (Nie *et al.*, 1982).

Experimental

General

Melting points were obtained on an XRC-1 apparatus (Sichuan University, Sichuan, People's Republic of China). Optical rotations were taken on a Horiba SEPA-300 automatic polarimeter (Horiba, Tokyo, Japan). The nuclear magnetic resonance (NMR) spectra (^{1}H, ^{13}C, and two-dimensional NMR) were acquired on DRX-500 NMR instruments (Bruker, Karlsruhe, Germany) at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR; tetramethylsilane was used as an internal standard and coupling constants were represented in Hertz. Mass spectra were measured with a VG Autospec 3000 mass spectrometer (VG, Manchester, Eng-

Table I. ^{1}H- and ^{13}C-NMR spectral data of **1** (δ in ppm, *J* in Hz, CD_3OD).

	δ (C)(DEPT)	δ (H)	^{1}H, ^{1}H COSY	HMBC (selected)
C(1)	133.8 (C)			
CH(2)	130.3 (CH)	6.8 (dd, *J* = 2.0, 6.5)	CH(3), CH(5), CH(6)	
CH(3)	116.1 (CH)	6.5 (m)	CH(2), CH(5), CH(6)	
C(4)	156.4 (C)			
CH(5)	116.1 (CH)	6.5 (m)	CH(2), CH(3), CH(6)	
CH(6)	130.3 (CH)	6.8 (dd, *J* = 2.0, 6.5)	CH(2), CH(3), CH(5)	
C(1′)	107.0 (C)			H-3′, H-5′
C(2′)	165.4 (C)			
CH(3′)	96.3 (CH)	5.8 (s)		
C(4′)	164.5 (C)			H-1″
CH(5′)	96.3 (CH)	5.8 (s)		
C(6′)	165.4 (C)			
CH(1″)	99.2 (CH)	4.9 (d, *J* = 8.0)	CH(2″)	H-3″, H-5″
CH(2″)	74.8 (CH)	4.7 (dd, *J* = 8.0, 9.5)	CH(1″),CH(3″)	H-4″
CH(3″)	75.9 (CH)	3.4 (t, *J* = 9.5)	CH(4″), CH(2″)	
CH(4″)	71.1 (CH)	3.3 (d, *J* = 7.1)	CH(3″)	
CH(5″)	78.4 (CH)	3.3 (d, *J* = 7.1)	CH^{α}(6″), CH^{β}(6″)	H-3″, H-1″
CH_2(6″)	62.2 (CH_2)	3.5 (dd, *J* = 4.9, 12.1)	CH(5″), CH^{β}(6″)	
		3.7 (dd, *J* = 12.1, 2.0)	CH^{α}(6″), CH(5″)	
CH_2(α)	47.5 (CH_2)	3.1 (m)	CH_2(b)	
CH_2(β)	31.1 (CH_2)	2.6 (t, *J* = 7.8)	CH_2(a)	
C(a)	207.1 (C)			CH_2(β)
C(b)	171.8 (C)			H-2″, CH_3(c)
CH_3(c)	20.9 (CH_3)	1.9 (s)		

Table II. ^{1}H- and ^{13}C-NMR spectral data of **2** and **3** (δ in ppm, *J* in Hz, CD_3OD).

	2		**3**	
	δ (C)(DEPT)	δ (H)	δ (C)(DEPT)	δ (H)
1	133.9 (C)		133.8 (C)	
2	130.4 (CH)	7.0 (dd, *J* = 2.3, 8.5)	130.3 (CH)	6.9 (d, *J* = 8.4)
3	116.1 (CH)	6.6 (dd, *J* = 2.5, 8.5)	116.1 (CH)	6.6 (d, *J* = 8.4)
4	156.4 (C)		156.2 (C)	
5	116.1 (CH)	6.6 (dd, *J* = 2.5, 8.5)	116.1 (CH)	6.6 (d, *J* = 8.4)
6	130.4(CH)	7.0 (dd, *J* = 2.3, 8.5)	130.3 (CH)	6.9 (d, *J* = 8.4)
1′	106.8 (C)		106.8 (C)	
2′	162.3 (C)		165.2 (C)	
3′	95.4 (CH)	6.1 (d, *J* = 2.3)	96.4 (CH)	6.0 (s)
4′	167.6 (C)		164.8 (C)	
5′	98.3 (CH)	5.9 (d, *J* = 2.3)	96.4 (CH)	6.0 (s)
6′	166.0 (C)		165.2 (C)	
α	47.0 (CH2)	3.4 (m)	47.4 (CH_2)	3.1 (2H, t, *J* = 7.8)
β	30.8 (CH2)	2.8 (t, *J* = 14.9)	31.0 (CH_2)	2.7 (2H, t, *J* = 7.8)
O=C	206.5 (C)		207.0 (C)	
1″	102.0 (CH)	5.0 (d, *J* = 7.3)	100.9 (CH)	4.9 (d, *J* = 7.3),
2″	74.7 (CH)	3.4 (m)	74.5 (CH)	3.4 (m)
3″	78.4 (CH)*	3.4 (m)	78.0 (CH)*	3.4 (m)
4″	71.1 (CH)	3.4 (m)	71.0 (CH)	3.4 (m)
5″	78.5 (CH)*	3.4 (m)	77.8 (CH)*	3.4 (m)
6″	62.4 (CH2)	3.9 (dd, *J* = 2.0, 12.0), 3.7 (dd, *J* = 5.3, 12.0)	62.3 (CH2)	3.8 (dd, *J* = 12.0, 1.7), 3.6 (d, *J* = 12.0, 5.2)

* These signals may be reversed.

land). Infrared (IR) spectra were obtained in KBr pellets on a Bio-Rad FTS-135 IR spectrophotometer (Bio-Rad, Richmond, CA).

Material

The leaves of *Lithocarpus pachyphyllus* were collected in Kunming, Yunnan, P. R. China, in July 2000. The voucher specimen was deposited at the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

Dried and powdered leaves of *Lithocarpus pachyphyllus* (4 kg) were extracted three times with CH_3OH (5 l for each time). The combined organic phase was evaporated to small volume *in vacuo* and then suspended with water. The mixture was extracted four times with diethyl ether (2 l for each time) and then with ethyl acetate four times (in a total volume of 8 l). The EtOAC extract was washed with some water, then dried with anhydrous $NaSO_4$ and then evaporated to afford a deep brown gum (320 g). The crude extract was submitted to silica gel chromatography eluting with $CHCl_3$/MeOH. The fraction eluted with $CHCl_3$/MeOH (90:10, v/v) was evaporated, yielding compound **1** (2.6 g), compound **2** (5.7 g) and compound **3** (31 g).

Trilobatin 2″-acetate (**1**) ($C_{23}H_{26}O_{11}$) was obtained as white needles: $[\alpha]_D^{24}$ – 26.23° (MeOH, *c* 0.95), m.p. 196 ~ 197.5 °C; UV (MeOH) λ_{max}202, 225 and 284 nm; IR: cm^{-1} 3508, 1732, 1633, 1599; FAB-MS: *m/z* 477[M-H]$^-$. ^{1}H and ^{13}C NMR spectral data are given in Table I.

Compound **2** ($C_{21}H_{24}O_{10}$) was obtained as pale yellow needles: m.p. 126 ~ 127 °C; UV (MeOH) λ_{max}224 and 285 nm; IR: cm^{-1} 1620; FAB-MS: *m/z* 435 [M-H]$^-$; EI-MS: *m/z* 274, 255, 168, 153, 120, 107, 91, 69, 60; for ^{1}H and ^{13}C NMR data see Table II. Comparison of the physicochemical properties with the reported data allowed to identify compound **2** as phlorizin, previously isolated from leaves of *Lithocarpus litseifolius* (Nie *et al.*, 1982).

Compound **3** ($C_{21}H_{24}O_{10}$) was obtained as yellow needles: m.p. 161 ~ 162 °C; UV: λ_{max}288 nm; IR: cm^{-1} 1623; FAB-MS: *m/z* 435 [M-H]$^-$; for ^{1}H and ^{13}C NMR data see Table II. Comparison of the physicochemical properties with the reported data allowed to identified compound **3** as trilobatin, previously isolated from leaves of *Lithocarpus litseifolius* (Nie *et al.*, 1982).

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Nie R. L., Takashi T., Zhou J., and Tanaka O. (1982), Phlorizin and trilobatin, weet dihydrochalcone-glucosides from leaves of *Lithocarpus litseifolius* (Hance) Rehd. (Fagaceae). Agric. Biol. Chem. **46**, 1933–1934.

Tanaka T., Kohda H., Tanaka O., Chen F. H., Chou W. H., and Leu J. J. (1981), Rubusoside, a sweet principle of *Rubus chigii* Hu (Rosaceae). Agric. Biol. Chem. **45**, 2165–2166.

Tanaka T., Tanaka O., Lin Z. W., Zhou J., and Ageta H. (1983), Sweet and bitter glycosides of the Chinese plant drug, Bai-Yun-Shen (roots of *Salvia digitaloides*). Chem. Pharm. Bull. **31**, 780–783.

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