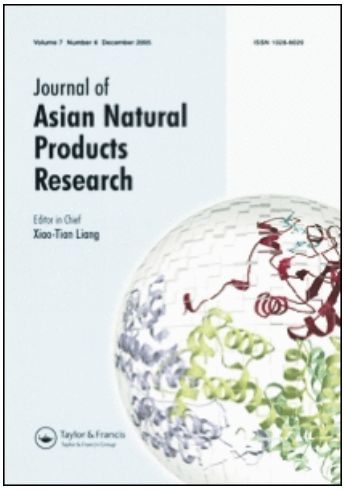

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 11 August 2010

To cite this Article Han, Wei-Li , Zhang, Xiao-Qi , Wang, Guo-Cai , Wang, Ying and Ye, Wen-Cai(2010) 'Three new flavane glucosides from the leaves of *Morus wittiorum*', Journal of Asian Natural Products Research, 12: 8, 680 — 684
To link to this Article: DOI: 10.1080/10286020.2010.490780
URL: http://dx.doi.org/10.1080/10286020.2010.490780

Journal of Asian Natural Products Research
Vol. 12, No. 8, August 2010, 680–684

ORIGINAL ARTICLE

Three new flavane glucosides from the leaves of *Morus wittiorum*

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(*Received 10 February 2010; final version received 29 April 2010*)

Three new flavane glucosides (**1**–**3**) were isolated from the leaves of *Morus wittiorum*. The structures with absolute configuration were determined on the basis of hydrolysis and spectroscopic methods including UV, IR, HR-ESI-MS, 1D, and 2D NMR.

Keywords: *Morus wittiorum*; mulberry; Moraceae; flavane glucosides

1. Introduction

The plants of the genus *Morus* (Moraceae) are widely spread in all temperate areas, and their leaves are commonly used as silkworm food. The leaves and root bark of mulberry have also been used as traditional Chinese medicines in the treatment of diabetes, rheumatism, arthritis, headache, and cough [1]. *Morus wittiorum* is widely cultivated in Guangdong, Guizhou, and Hunan Provinces and Guangxi Zhuang Autonomous Region of China. Previously, some phenolic compounds had been isolated from the stem bark of this plant [2]. During our investigation on structurally and pharmacologically interesting secondary metabolites from the plants of the genus *Morus* [3–5], three new flavane glucosides (**1**–**3**) were isolated from the leaves of *M. wittiorum* (Figure 1). This paper describes the isolation and structural elucidation of **1**–**3**.

2. Results and discussion

Compound **1** was obtained as a yellowish oil. The molecular formula of **1** was determined to be $C_{24}H_{30}O_{10}$ by HR-ESI-MS at *m/z* 501.1745 $[M + Na]^+$. The UV spectrum showed the absorption maxima at 227 and 278 nm. The IR spectrum of **1** implied the presence of hydroxyl group (3407 cm^{-1}) and aromatic ring (1615 and 1457 cm^{-1}). Acid hydrolysis of **1** afforded D-glucose, which was identified by gas chromatography of its aldononitrile peracetate derivative using an authentic sample as a reference. The ^1H NMR and ^1H–^1H COSY spectra indicated the presence of two sets of aromatic protons [δ_H 7.67 (d, $J = 8.4$ Hz), 6.65 (dd, $J = 8.4$, 2.2 Hz), and 7.33 (d, $J = 2.2$ Hz); δ_H 6.89 (d, $J = 8.4$ Hz) and 6.86 (d, $J = 8.4$ Hz)], a hydroxyethyl group [δ_H 3.59 (2H) and 4.32 (2H)], a methoxyl group [δ_H 3.70 (3H, s)], and an anomeric proton [δ_H 5.59 (d, $J = 6.2$ Hz)]. The ^{13}C NMR and DEPT spectra of **1** displayed 24 carbon signals including a methoxyl group (δ_C 55.3) and a hydroxyethyl group (δ_C 62.4, 28.3), as well as a β-D-glucopyranosyl unit. All the above data indicated that **1** was a flavane glucoside. With the aid of 1D and 2D

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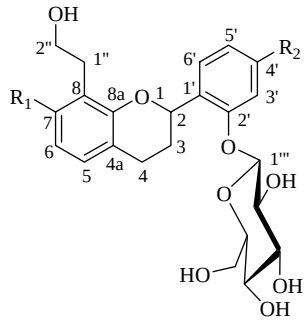
ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.490780
http://www.informaworld.com



1 (2*S*) R_1 = OH R_2 = OMe
2 (2*S*) R_1 = OMe R_2 = OH
3 (2*R*) R_1 = OMe R_2 = OH

Figure 1. Structures of compounds **1**–**3**.

NMR experiments, all the ^{1}H and ^{13}C NMR signals of **1** were assigned and are shown in Table 1.

Comparison of the NMR spectral data of **1** with those of the known acutifolin F [6] indicated that their NMR signals were very similar, except for a methoxyl group [δ_C 55.3 (q); δ_H 3.70 (3H, s)] in **1** instead of a hydroxyl group in acutifolin F at C-4′ position, and one more D-glucopyranosyl moiety was attached to the C-2′ position in **1**. The substituted positions were further confirmed by the HMBC correlations between a methoxyl group at δ_H 3.70 and C-4′ at δ_C 160.6, as well as between H-1‴ of glucose at δ_H 5.59 and C-2′ at δ_C 155.8 (Figure 2). The Cotton effect at λ_{max} 274 nm ($\Delta\varepsilon$ − 1.39) in the CD spectrum was similar to that of the known flavonoids (2*S*)-5-methoxyl-7-hydroxylflavane [λ_{max} 278 nm ($\Delta\varepsilon$ − 0.59)] [7] and (−)-(2*S*)-flavane [λ_{max} 276 nm ($\Delta\varepsilon$ − 0.43)] [8]. Therefore, the absolute configuration at C-2 of **1** was assigned to *S*. The structure of **1** was unambiguously elucidated as (2*S*)-7-hydroxyl-8-hydroxyethyl-4′-methoxylflavane-2′-*O*-β-D-glucopyranoside.

Compound **2** was isolated as a yellowish oil. It showed the same molecular formula ($C_{24}H_{30}O_{10}$) as that of **1** by its HR-ESI-MS at *m/z* 501.1730 $[M + Na]^+$. The presence of the hydroxyl group and aromatic ring was suggested by its IR spectrum at 3388, 1613, and 1511 cm^{-1}. Acid hydrolysis of **2** also afforded D-glucose, which was identified by gas chromatography. The ^{1}H NMR spectrum of **2** showed two sets of aromatic proton signals [δ_H 6.68 (1H, d, J = 2.2 Hz), 6.48 (1H, dd, J = 8.4, 2.2 Hz), and 7.23 (1H, d, J = 8.4 Hz); δ_H 6.87 (1H, d, J = 8.4 Hz) and 6.47 (1H, d, J = 8.4 Hz)], a methoxyl [δ_H 3.77 (3H, s)] and an anomeric proton [δ_H 4.86 (d, J = 6.8 Hz)]. The ^{13}C NMR and DEPT spectra of **2** displayed 24 carbons including a methoxyl group (δ_C 56.1) and a β-D-glucopyranosyl unit. All the above data indicated that **2** was a flavane glucoside. The assignments of the ^{1}H and ^{13}C NMR spectral data (Table 1) of **2** were completed with the aid of ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.

Comparison of the NMR spectral data of **2** with those of **1** indicated that their NMR signals were similar, except for the locations of the methoxyl and the hydroxyl groups. The position of the methoxyl group was indicated by its HMBC correlations between δ_H 3.77 (OMe) and δ_C 158.3 (C-7). The absolute configuration at C-2 of **2** was also determined as *S* by its Cotton effect at λ_{max} 275 nm ($\Delta\varepsilon$ − 1.40) in the CD spectrum, which was similar to that of the known flavonoid (2*S*)-5-methoxyl-7-hydroxylflavane [λ_{max} 278 nm ($\Delta\varepsilon$ −0.59)] [7]. Consequently, the structure of **2** was characterized as (2*S*)-7-methoxyl-8-hydroxyethyl-4′-hydroxylflavane-2′-*O*-β-D-glucopyranoside.

Compound **3** was also obtained as a yellowish oil. The molecular formula $C_{24}H_{30}O_{10}$ was established by HR-ESI-MS at *m/z* 501.1732 $[M + Na]^+$. The IR spectrum of **3** implied the presence of the hydroxyl group (3405 cm^{-1}) and aromatic ring (1612 and 1512 cm^{-1}). Acid hydrolysis of **3** afforded D-glucose. The ^{1}H and ^{13}C NMR spectral data of **3** were very similar to those of **2** (Table 1), indicating that **3** was the diastereomer of **2** with an asymmetric center at C-2. The Cotton effect at λ_{max}

Table 1. ^{1}H and ^{13}C NMR spectral data of **1**–**3** (J in Hz).

	1[a]		**2**[b]		**3**[b]	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.86 (1H, dd, J = 9.9, 1.8)	72.7	5.47 (1H, dd, J = 10.0, 2.0)	73.8	5.39 (1H, dd, J = 10.0, 2.0)	73.8
3	1.95 (1H, m)	29.7	1.84 (1H, m)	30.3	1.88 (1H, m)	29.9
	2.31 (1H, m)		2.18 (1H, m)		2.23 (1H, m)	
4	2.59 (1H, m)	25.8	2.65 (1H, m)	25.8	2.65 (1H, m)	25.9
	2.71 (1H, m)		2.93 (1H, m)		2.95 (1H, m)	
4a	–	113.4	–	116.2	–	116.2
5	6.89 (1H, d, J = 8.4)	127.8	6.87 (1H, d, J = 8.4)	128.2	6.88 (1H, d, J = 8.4)	128.2
6	6.86 (1H, d, J = 8.4)	108.6	6.47 (1H, d, J = 8.4)	110.4	6.48 (1H, d, J = 8.4)	110.3
7	–	156.2	–	158.3	–	158.2
8	–	114.3	–	114.6	–	114.7
8a	–	154.7	–	155.4	–	155.5
1′	–	124.6	–	124.2	–	123.9
2′	–	155.8	–	156.3	–	156.7
3′	7.33 (1H, d, J = 2.2)	102.6	6.68 (1H, d, J = 2.2)	104.1	6.71(1H, d, J = 2.2)	104.1
4′	–	160.6	–	159.0	–	159.0
5′	6.65 (1H, dd, J = 8.4, 2.2)	107.9	6.48 (1H, dd, J = 8.4, 2.2)	104.2	6.48 (1H, dd, J = 8.4, 2.2)	104.2
6′	7.67 (1H, d, J = 8.4)	127.3	7.23 (1H, d, J = 8.4)	128.4	7.23 (1H, d, J = 8.4)	128.3
1″	3.59 (2H, m)	28.3	2.89 (2H, m)	27.6	2.89 (2H, m)	27.6
2″	4.32 (2H)[c]	62.4	3.58 (2H, m)	62.2	3.57 (2H, m)	62.5
OMe	3.70 (3H, s)	55.3	3.77 (3H, s)	56.1	3.77 (3H, s)	56.1
Glc-1‴	5.59 (1H, d, J = 6.2)	102.9	4.86 (1H, d, J = 6.8)	102.9	4.88 (1H, d, J = 6.4)	102.7
2‴	4.30 (1H)[c]	74.9	3.43 (1H)[c]	75.0	3.44 (1H)[c]	74.9
3‴	4.09 (1H, m)	78.9	3.42 (1H)[c]	78.2	3.43 (1H)[c]	78.3
4‴	4.30 (1H)[c]	71.3	3.41 (1H)[c]	71.3	3.40 (1H)[c]	71.3
5‴	4.31 (1H)[c]	78.7	3.42 (1H)[c]	78.2	3.42 (1H)[c]	78.3
6‴	4.35 (1H)[c]	62.4	3.74 (1H, dd, J = 12.1, 2.7)	62.4	3.72 (1H, dd, J = 11.6, 2.1)	62.4
	4.56 (1H, dd, J = 11.4, 1.9)		3.91 (1H, d, J = 12.1)		3.90 (1H, d, J = 11.6)	

Notes: [a] 400 MHz for ^{1}H and 100 MHz for ^{13}C in C_5D_5N.
[b] 400 MHz for ^{1}H and 100 MHz for ^{13}C in CD_3OD.
[c] Overlapped signals were reported without designating multiplicity.

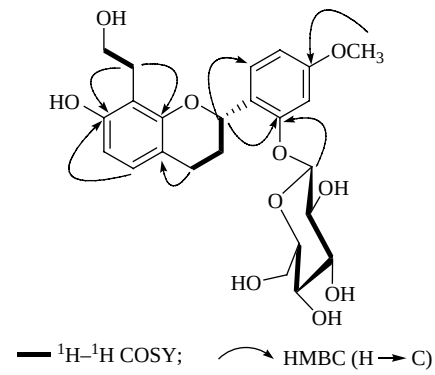


Figure 2. Key $^1H-^1H$ COSY and HMBC correlations of **1**.

274 nm ($\Delta\varepsilon + 1.15$) in the CD spectrum of **3** was contrary to that of **2**, which confirmed that the absolute configuration at C-2 was *R*. Thus, **3** was identified as (2*R*)-7-methoxyl-8-hydroxyethyl-4′-hydroxylflavane-2′-*O*-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were recorded on a JASCO UV-550 spectrometer. CD spectra were measured on a JASCO P-720 spectrometer. IR spectra (KBr pellets) were obtained on a JASCO FT/IR-480 infrared spectrometer. HR-ESI-MS data were obtained on an Agilent 6210 LC/MSD TOF mass spectrometer. NMR spectra were measured on a Bruker AV-400 spectrometer. For column chromatographies, silica gel (200–300 mesh; Qingdao Marine Chemical Co., Qingdao, China), Sephadex LH-20 (Pharmacia, Piscataway, NJ, USA), and ODS (YMC, Kyoto, Japan) were used. HPLC was performed on a COSMOSIL C_{18} preparative column (5 μm, 20 × 250 mm; Nacalai Tesque, Inc., Kyoto, Japan).

3.2 Plant material

The leaves of *M. wittiorum* were collected in Zhenjiang City, Jiangsu Province of China in August 2006, and authenticated by Dr Li Liu of the Sericultural Research Institute, Chinese Academy of Agricultural Sciences. A voucher specimen (No. 2006081206) has been deposited at the Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University.

3.3 Extraction and isolation

The dried and powdered leaves of *M. wittiorum* (5.0 kg) were percolated with 70% EtOH (20 liters × 3), and the solution was concentrated under reduced pressure to afford a brownish residue (800 g), which was then dissolved in water and chromatographed over D101 macroporous resin using ethanol–water as the eluent. The 30% ethanol–water soluble fraction (140 g) was further subjected to silica gel column chromatography (CC; $CHCl_3$–CH_3OH, 100:0 → 0:100) to afford 14 subfractions (1–14). Subfraction 3 (3.8 g) was subjected to Sephadex LH-20 ($CHCl_3$–CH_3OH, 1:1) and ODS (CH_3OH–H_2O, 20:80 → 90:0) CC, followed by HPLC (CH_3OH–H_2O, 40:60) to afford **1** (11.0 mg), **2** (10.2 mg), and **3** (3.8 mg), respectively.

3.3.1 Compound **1**

A yellowish oil, $[\alpha]_D^{27}$ −20.2 ($c = 0.17$, MeOH); UV (λ_{max}): 227, 278 nm; CD (MeOH): $\Delta\varepsilon_{215\,nm}$ −1.42, $\Delta\varepsilon_{274\,nm}$ −1.39; IR (KBr) ν_{max}: 3407, 2924, 1615, 1457, 1384, 1076 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 501.1745 $[M + Na]^+$ (calcd for $C_{24}H_{30}O_{10}Na$, 501.1731).

3.3.2 Compound **2**

A yellowish oil, $[\alpha]_D^{27}$ −32.2 ($c = 0.20$, MeOH); UV (λ_{max}): 227, 278 nm; CD (MeOH): $\Delta\varepsilon_{213\,nm}$ −2.12, $\Delta\varepsilon_{275\,nm}$ −1.40; IR (KBr) ν_{max}: 3388, 2928, 1613, 1511, 1384, 1106 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS:

m/z 501.1730 $[M + Na]^+$ (calcd for $C_{24}H_{30}O_{10}Na$, 501.1731).

3.3.3 Compound **3**

A yellowish oil, $[\alpha]_D^{27}$ −5.2 ($c = 0.30$, MeOH); UV (λ_{max}): 227, 278 nm; CD (MeOH): $\Delta\varepsilon_{214\,nm} + 1.72$, $\Delta\varepsilon_{274\,nm} + 1.15$; IR (KBr) ν_{max}: 3405, 2927, 1612, 1512, 1442, 1106 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 501.1732 $[M + Na]^+$ (calcd for $C_{24}H_{30}O_{10}Na$, 501.1731).

3.4 Acid hydrolysis and gas chromatographic analysis

Each (1.5 mg) of the compounds (**1**–**3**) was heated in an ampoule with 1.5 ml of 2 N HCl (MeOH–H_2O, 1:1) at 100°C for 2 h. The aglycone was extracted with CH_2Cl_2 three times, and the aqueous residue was evaporated under reduced pressure. Pyridine (1 ml) and 2 mg of $NH_2OH{\cdot}HCl$ were added to the residue, and then the mixture was heated at 100°C for 1 h. Followed by the addition of Ac_2O (1.5 ml), the mixture was incubated in a water bath at 100°C for 1 h and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated for the GC analysis (front inlet 250°C, column temperature 230°C) using standard aldononitrile peracetates as reference samples. The monosaccharides of each compound were identified as D-glucose (t_R (min): 36.415 (D-glucose), 36.683 (reference D-glucose), 38.659 (reference L-glucose)).

Acknowledgements

This work was supported by grants from the National Natural Science Foundation for Outstanding Young Scholar (No. 30625039), the Program for Changjiang Scholars (to Dr Ye), the National Natural Science Foundation of China (No. 30472146), the Science Foundation of Ministry of Education of China (No. 707048), and the Team Project of Natural Science Foundation of Guangdong Province (No. 8351063201000003).

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