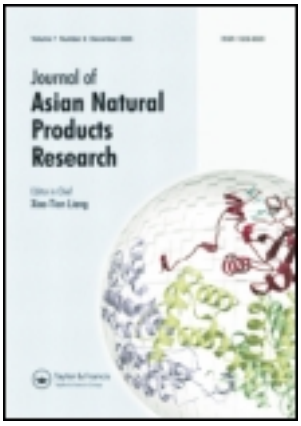

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Two new C-glycosylflavones from *Boea hygrometrica*

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Two new C-glycosylflavones, named 5,7,3′,4′-tetrahydroxy-6-methoxy-8-*C*-β-D-gluco-pyranosyl flavonoside (**1**), 5,3′,4′-trihydroxy-6,7-dimethoxy-8-*C*-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**2**), together with nine known compounds (**3**–**11**), were isolated from 50% acetone extract of *Boea hygrometrica* (Bunge.) R.Br. Their structures were established by spectroscopic techniques including MS, IR, UV, and 2D NMR.

Keywords: Gesneriaceae; *Boea hygrometrica*; C-glycosylflavones

1. Introduction

Boea hygrometrica (Bunge.) R.Br. belongs to Gesneriaceae, *Boea* Comm.ex Lam. *Boea* Comm.ex Lam consists of about 20 species in the world, and about 3 species of this genus is distributed in China. *B. hygrometrica*, a traditional Chinese herb, is widely distributed in China. It has been used in traditional Chinese medicine to activate blood circulation, dissipate stasis, and stop bleeding [1]. Studies on modern pharmacology demonstrated that it had functions such as antibiosis and anti-inflammatory [2]. However, few of the phytochemical studies of the plant have been reported previously. To obtain the active constituents, the plant was investigated. In this paper, two new C-glycosylflavones, named 5,7,3′,4′-tetrahydroxy-6-methoxy-8-*C*-β-D-glucopyra-nosyl flavonoside (**1**) and 5,3′,4′-trihydroxy-6,7-dimethoxy-8-*C*-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**2**) (Figure 1), along with nine known flavonoid glycosides 5,4′-dihydroxy-6,7-dimethoxy-8-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**3**) [3], 5,3′,4′-trihydroxy-6,7-dimethoxy-8-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**4**) [3], 5,3′,4′-trihydroxy-7,8-dimethoxy-6-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**5**) [4], 5,7,4′-trihydroxy-6-methoxy-8-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**6**) [4], 5,7,4′-trihydroxy-8-methoxy-6-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**7**) [4], 5,7,4′-trihydroxy-6-methoxy-8-*C*-β-D-glucopyranosyl flavonoside (**8**) [5], 5,4′-dihydroxy-6,7-dimethoxy-8-*C*-β-D-glucopyranosyl flavonoside (**9**) [3], 5,4′-dihydroxy-7-methoxy-6-*C*-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**10**) [6], and 5,4′-dihydroxy-7-methoxy-6-*C*-β-D-glucopyranosyl flavonoside (**11**) [7], among which, compounds **3**–**11** were

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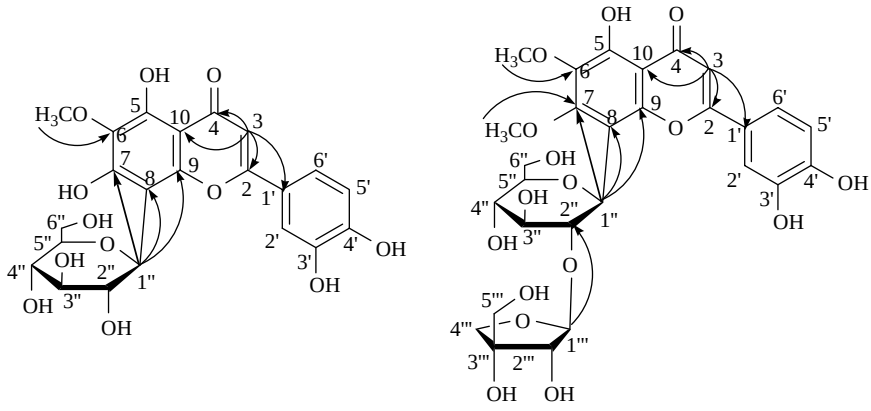


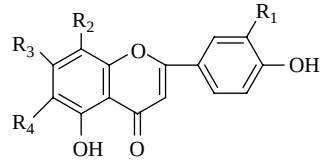
1 **2**

Figure 1. The chemical structures and selected HMBC correlations of compounds **1** and **2**.

obtained from this plant for the first time (Figure 2).

2. Results and discussion

Compound **1** was isolated as a yellow amorphous powder with mp 118–121°C and $[\alpha]_D^{20}$ +9.20 (*c* 0.4, MeOH). The UV (MeOH) spectrum showed the absorption maxima at 274 and 350 nm. The molecular formula of **1** was determined as $C_{22}H_{22}O_{12}$ by the pseudo-molecular ion in the HR-ESI-MS at *m/z* 501.1006 $[M + Na]^+$. Its IR (KBr) spectrum showed absorption bands for hydroxyl (3393 cm^{-1}) and carbonyl (1653 cm^{-1}) groups, as well as aromatic rings (1593, 1561, 1496, 1460 cm^{-1}). The ^1H NMR spectrum showed the presence of three aromatic proton signals, resonated at δ_H 7.51 (d, 1H, $J = 2.1$ Hz), 7.46 (dd, 1H, $J = 8.1$, 2.1 Hz), 6.89 (d, 1H, $J = 8.1$ Hz) as one ABX system of B-ring, and a proton at δ_H 6.50 (s, 1H), a characteristic signal for



	R_1	R_2	R_3	R_4
3	H	glc^2-xyl	OCH_3	OCH_3
4	OH	glc^2-xyl	OCH_3	OCH_3
5	OH	OCH_3	OCH_3	glc^2-xyl
6	H	glc^2-xyl	OH	OCH_3
7	H	OCH_3	OH	glc^2-xyl
8	H	glc	OH	OCH_3
9	H	glc	OCH_3	OCH_3
10	H	H	OCH_3	glc^2-apiose
11	H	H	OCH_3	glc

Figure 2. The structures of compounds **3**–**11**.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **1** and **2** (CD_3OD, δ ppm).

	1		**2**	
No.	δ_H (J, Hz)	δc	δ_H (J, Hz)	δc
Aglycone				
2		166.7		168.8
3	6.50 (s, 1H)	103.3	6.50 (s, 1H)	101.4
4		184.4		184.2
5		153.3		154.6
6		132.3		137.3
7		153.7		159.1
8		105.3		111.8
9		158.3		153.0
10		105.6		108.7
1′		124.0		118.2
2′	7.51 (d, 1H, $J = 2.1$ Hz)	114.9	7.46 (d, 1H, $J = 2.0$ Hz)	112.5
3′		147.1		149.9
4′		151.0		151.2
5′	6.89 (d, 1H, $J = 8.1$ Hz)	116.8	6.73 (d, 1H, $J = 8.0$ Hz)	117.7
6′	7.46 (dd, 1H, $J = 8.1$, 2.1 Hz)	120.9	7.44 (dd, 1H, $J = 8.0$, 2.0 Hz)	121.9
6-OCH_3	3.88 (s, 3H)	60.9	3.90 (s, 3H)	61.2
7-OCH_3			4.00 (s, 3H)	62.8
Glc				
1″	5.01(d, 1H, $J = 9.6$ Hz)	75.7	4.86 (d, 1H, $J = 10.0$ Hz)	74.9
2″	4.11 (m, 1H)	72.9	4.28 (m, 1H)	77.5
3″	3.53 (m, 1H)	80.3	3.62 (m, 1H)	80.9
4″	3.68 (m, 1H)	72.3	3.70 (m, 1H)	72.5
5″	3.48 (m, 1H)	82.9	3.47 (m, 1H)	83.3
6″	3.97 (d, 1H, $J = 9.6$ Hz)	63.3	3.84 (d, 1H, $J = 10.0$ Hz)	63.6
	3.85 (m, 1H)		3.67 (m, 1H)	
Api				
1‴			5.11 (d, 1H, $J = 1.8$ Hz)	111.3
2‴			3.77 (d, 1H, $J = 1.8$ Hz)	78.5
3‴				80.6
4‴			3.11 (d, 1H, $J = 9.7$ Hz)	74.2
			2.71 (d, 1H, $J = 9.7$ Hz)	
5‴			3.26 (s, 2H)	64.7

C-ring of the flavone skeleton, as well as six protons linked to the oxygenated carbons at δ_H 3.30–4.11 and a methoxyl group at δ_H 3.88 (s, 3H). One anomeric signal at δ_H 5.01 (d, 1H, $J = 9.6$ Hz) suggested the existence of one sugar in compound **1**. The ^{13}C NMR signals at δ 75.7 (C-1″), 72.9 (C-2″), 80.3 (C-3″), 72.3 (C-4″), 82.9 (C-5″), and 63.3 (C-6″) indicated that the sugar was glucose and the C—C linkage between the aglycone and the sugar [3,5]. The coupling constant of the anomeric proton ($J = 9.6$ Hz) suggested that glucose should be β-configurated. The ^{13}C NMR spectrum (Table 1) clearly showed 22 carbon signals, 15 assigned to the aglycone, 6 to the glucose moiety, and 1 to the methoxyl. The ^{1}H NMR and ^{13}C NMR spectral data of compound **1** were very similar to those of 5,7,4′-trihydroxy-6-methoxy-8-*C*-β-D-glucopyranosyl flavonoside (**8**) [5], except for the presence of a hydroxyl group instead of H-3′ in compound **1**, i.e. the B-ring of compound **1** was 3′,4′-dihydroxyphenyl, different from 4′-hydroxyphenyl of compound **8**. In addition, the HMBC correlations of methoxyl group at δ_H 3.88

with C-6 at δ_C 132.3 indicated its linkage site. H-1″ at δ_H 5.01 correlated with the carbon signals at δ_C 153.7 (C-7), 105.3 (C-8), and 158.3 (C-9), which revealed that C-1″ of glucose was linked to C-8 of the aglycone. All the hydrogen and carbon signals were assigned by HSQC and HMBC experiments. On the basis of the above evidence, the structure of **1** was determined to be 5,7,3′,4′-tetrahydroxy-6-methoxy-8-*C*-β-D-glucopyranosyl flavonoside.

Compound **2** was isolated as a yellow amorphous powder with mp 151–153°C and $[\alpha]_D^{20}$ −85.33 (*c* 0.24, MeOH). The UV (MeOH) spectrum showed the absorption maxima at 268 and 348 nm. The molecular formula of **2** was determined as $C_{28}H_{32}O_{16}$ by the pseudo-molecular ion in HR-ESI-MS at *m/z* 647.1583 $[M + Na]^+$. Its IR (KBr) spectrum indicated the presence of hydroxyl (3377 cm^{-1}) and carbonyl (1652 cm^{-1}) groups, as well as aromatic rings (1602, 1570, 1523, 1457 cm^{-1}). The ^1H NMR spectrum showed the presence of three aromatic proton signals, resonated at δ_H 7.46 (d, 1H, $J = 2.0$ Hz), 7.44 (dd, 1H, $J = 8.0$, 2.0 Hz), and 6.73 (d, 1H, $J = 8.0$ Hz) as one ABX system, and a characteristic signal at δ_H 6.50 (s, 1H) for C-ring of the flavone skeleton. Two methoxyl groups at δ_H 3.90 (s, 3H) and 4.00 (s, 3H) were observed, as well as 11 protons linked to the oxygenated carbons at δ_H 2.71–4.28, suggested the existence of two sugars in compound **2**. Two signals at δ_H 4.86 (d, 1H, $J = 10.0$ Hz) and 5.11 (d, 1H, $J = 1.8$ Hz) were the anomeric protons of two sugars, respectively. The ^{13}C NMR spectrum (Table 1) clearly showed 28 carbon signals. Besides 15 carbon signals of aglycone and 2 methoxy1 groups, the rest 11 should be carbon signals of two sugars. According to the ^{13}C NMR analysis and acid hydrolysis, the sugars were identified as glucose and apiose. By comparison of its NMR spectral data with those reported in [6], the ^{13}C NMR signals of glucose are at δ 74.9 (C-1″), 77.5 (C-2″), 80.9 (C-3″), 72.4 (C-4″), 83.3 (C-5″), and 63.6 (C-6″) and those of apiose are at δ 111.3 (C-1‴), 78.5 (C-2‴), 80.6 (C-3‴), 74.2 (C-4‴), and 64.7 (C-5‴). Moreover, the β-configurations of glucose and apiose were determined by the coupling constants of the anomeric protons at δ_H 4.86 (d, 1H, $J = 10.0$ Hz) and 5.11 (d, 1H, $J = 1.8$ Hz). The ^1H NMR and ^{13}C NMR spectral data of compound **2** were very similar to those of 5,3′,4′-trihydroxy-6,7-dimethoxy-8-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (**4**) [3], except for the presence of apiose instead of xylose in compound **2**. In the HMBC spectrum (Figure 1), the following correlative signals were observed: the methoxyl at δ_H 3.90 with C-6 at δ_C 137.3 and the other methoxyl at δ_H 4.00 with C-7 at δ_C 159.1 indicated their linkage sites. H-1″ at δ_H 4.86 correlated with the carbon signals at δ_C 159.1 (C-7), 111.8 (C-8), and 153.0 (C-9), which revealed that C-1″ of glucose was linked to C-8 of the aglycone. The signal at δ_H 5.11 (H-1‴) correlated with the carbon signal at δ_C 77.5 (C-2″), indicating that the C-1‴ of the apiose was linked to C-2″ of the glucose. All the hydrogen and carbon signals were assigned by HSQC and HMBC experiments. On the basis of the above evidence, the structure of **2** was determined to be 5,3′,4′-trihydroxy-6,7-dimethoxy-8-*C*-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained using a Perkin–Elmer 341 polarimeter. UV spectra were measured with a Shimadzu UV-VIS 2201 spectrophotometer. IR spectra were measured with a Shimadzu FTIR-8201 PC spectrometer. The ^1H and ^{13}C NMR spectra were obtained on a Bruker DPX-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) with

TMS as an internal reference. HR-ESI-MS were recorded on an APEX II spectrometer. Column chromatography was performed on Diaion HP-20 (Mitsubishi Chemical Corp., Tokyo, Japan), silica gel (160–200 mesh, Qingdao Marine Chemical Industry, Qingdao, China), Toyopearl HW-40 and Sephadex LH-20 (TOSOH Corp., Tokyo, Japan). TLC was conducted on self-made silica gel G (Qingdao Marine Chemical Industry) plates. The chemical reagents were purchased from Beijing Chemical Plant (Beijing, China) and Tianjin No. 3 Reagent Plant (Tianjin, China).

3.2 Plant material

The fresh plants of *B. hygrometrica* were collected from Xixia County, Henan Province of China, in July 2009, and identified by Prof. Cheng-Ming Dong of Henan University of Traditional Chinese Medicine. Its voucher specimen (MED20090725) has been deposited in our laboratory.

3.3 Extraction and isolation

Air-dried whole plants of *B. hygrometrica* (5.0 kg) were extracted with 50% aq. Me_2CO two times at room temperature, and concentrated under reduced pressure below 45°C. The water-soluble part was chromatographed over Diaion HP-20 with H_2O containing increasing amounts of MeOH to afford H_2O eluate 42.1 g (A), 20% MeOH eluate 35.6 g (B), and 30% MeOH eluate 28.5 g (C). Fraction A was chromatographed on Toyopearl HW-40 (coarse grade) developing with 10% MeOH–50% MeOH. The 10% MeOH eluate (6.4 g) of A was rechromatographed on Toyopearl HW-40 (50% MeOH) and on silica gel ($CHCl_3$–MeOH–H_2O, 6:1:0.1) to yield compound **3** (30.1 mg). The 20% MeOH eluate (4.3 g) of A was rechromatographed on Toyopearl HW-40 (70% MeOH) and on silica gel ($CHCl_3$–MeOH–H_2O, 6:1:0.1) to yield compounds **4** (85.2 mg), **5** (85.2 mg), **6** (12.3 mg), and **7** (12.3 mg). Fraction B was chromatographed on Toyopearl HW-40 (coarse grade) developing with 10% MeOH–50% MeOH. The 10% MeOH eluate (7.1 g) of B was rechromatographed on Toyopearl HW-40 (70% MeOH) and on silica gel ($CHCl_3$–MeOH, 10:1) to yield compounds **1** (11 mg) and **8** (15 mg). The 20% MeOH eluate (5.4 g) of B was rechromatographed on Toyopearl HW-40 (MeOH) and on silica gel ($CHCl_3$–MeOH, 8:1:0.05) to yield compounds **9** (35 mg) and **10** (21 mg). Fraction C was chromatographed on Toyopearl HW-40 (coarse grade), developing with 10% MeOH–50% MeOH. The 10% MeOH eluate (6.8 g) of C was rechromatographed on Toyopearl HW-40 (MeOH) and on silica gel (EtOAc–EtOH–H_2O, 12:2:1) to yield compounds **2** (12 mg) and **11** (15 mg).

3.3.1 5,7,3′,4′-Tetrahydroxy-6-methoxy-8-C-β-D-glucopyranosyl flavonoside (1)

3.3.1.1 A yellow amorphous powder. $[\alpha]_D^{20}$ +9.20 (*c* 0.4, MeOH); IR (KBr) ν_{max}: 3393, 2925, 1653, 1593, 1561, 1496, 1460 cm^{-1}; UV (MeOH) λ_{max}: 274, 350 nm. ^1H and ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 501.1006 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_{12}Na$, 501.1009).

3.3.2 5,3′,4′-Trihydroxy-6,7-dimethoxy-8-C-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranosyl flavonoside (2)

3.3.2.1 A yellow amorphous powder. $[\alpha]_D^{20}$ −85.33 (*c* 0.24, MeOH); IR (KBr) ν_{max}: 3377, 2933, 1652, 1602, 1570, 1523, 1457 cm^{-1}; UV(MeOH) λ_{max}: 268, 348 nm. ^1H and ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 647.1583 $[M + Na]^+$ (calcd for $C_{28}H_{32}O_{16}Na$, 647.1588).

3.4 Acid hydrolysis of compound 2

Compound **2** (3 mg) was heated in 3 ml of $HCl/H_2O/EtOH$ (2:1:2) at 80°C for 4 h. The hydrolysate was partitioned between EtOAc and H_2O, and the aqueous layer was compared with authentic samples on TLC with silica gel [$CHCl_3/MeOH/H_2O$ (5:1:0.1)], which showed that the sugar was apiose and the glucose was linked to the aglycone with C—C bond.

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