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Flavonoids from the leaves of *Pleioblastus argenteastriatus*

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A new flavonoid, 5,7,3'-trihydroxy-6-C- β -D-digitoxopyranosyl-4'-O- β -D-glucopyranosyl flavonoside (**1**), along with four known flavonoids 5,7,4'-trihydroxy-3',5'-dimethoxy flavone (**2**), 5,3',4'-trihydroxy-7-O- β -D-glucopyranosyl flavonoside (**3**), 5,4'-dihydroxy-3',5'-dimethoxy-7-O- β -D-glucopyranosyl flavonoside (**4**), 5,3',4'-trihydroxy-6-C-[β -D-glucopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranosyl flavonoside (**5**) were isolated from 95% EtOH extract of the leaves of *Pleioblastus argenteastriatus*. Their structures were determined on the basis of spectroscopic techniques and chemical methods.

Keywords: *Pleioblastus argenteastriatus*; flavonoids; 5,7,3'-trihydroxy-6-C- β -D-digitoxopyranosyl-4'-O- β -D-glucopyranosyl flavonoside

1. Introduction

Bamboo comprises over 1300 species, and more than 500 bamboo species have been found in China. *Pleioblastus argenteastriatus* is one of the bamboo species found in China. Chinese people realized the medical and health care effects of bamboo leaf long ago, and used it or its extract as a traditional Chinese medicine and food additive. The leaves of *Pleioblastus amarus*, the same genus bamboo species as *P. argenteastriatus*, had been used for the treatment of insomnia and diabetes and had the effects of improving eyesight and relieving alcoholism [1]. Previous phytochemical research on bamboo leaves showed the presence of orientin, homoorientin, vitexin, isovitexin, and flavonoids [2–4]. Flavonoids showed significant anti-inflammatory and antioxidant activities, and had the effects of treating cardiovascular diseases [5–7] and different types of cancer [8]. The leaves of *P. argenteastriatus* were rich in flavonoids, [9,10] which prompted us to further investigate

the flavonoids in this species. Extensive chromatography of the EtOH extract of the leaves of *P. argenteastriatus* had led to the isolation of a new flavonoid and four known flavonoids reported from this species for the first time. This paper deals with the isolation and structural elucidation of the new compound (**1**).

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder, mp 180.3–181.0°C, $[\alpha]_D^{20} + 20.3$ (c 0.15, MeOH). The molecular formula, C₂₇H₂₉O₁₄, was deduced from the negative HRESIMS at m/z 577.1513 [M-H]⁻. The ESIMS (negative) showed ion peaks at m/z 577.2 [M-H]⁻ and 1155.3 [2M-H]⁻. It was recognized as a flavonoid from a positive test with Mg–HCl and Molish reagents. The IR spectrum displayed characteristic absorption bands for hydroxyl (3413.6 cm⁻¹), carbonyl (1655.0 cm⁻¹), and aromatic rings (1627.0, 1508.3, 1491.3, 1439.3 cm⁻¹). The UV spectrum showed absorption maxima at 212.3,

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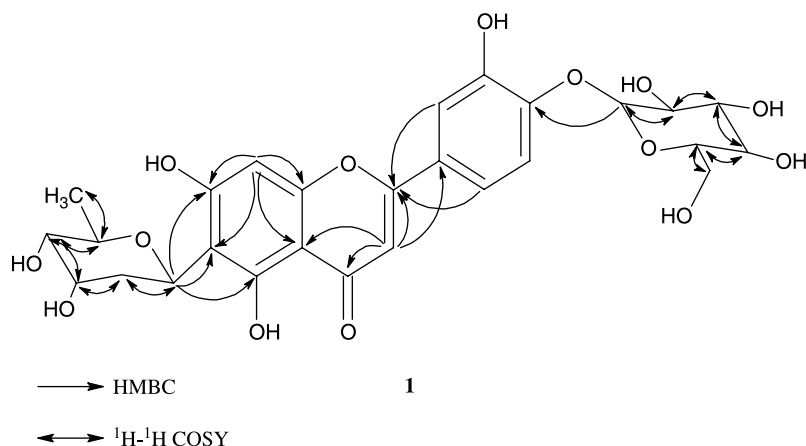


Figure 1. Significant HMBC and ¹H-¹H COSY correlations of **1**.

272.4, 340.2 nm, characteristic for the flavonoid. Besides the characteristic signal for A,C-rings of the flavone skeleton at δ 6.57 (1H, s) and 6.85 (1H, s), the ¹H NMR spectrum of compound **1** displayed signals of a 1',3',4' tri-substituted phenyl moiety (B-ring) at δ 7.50 (1H, d, $J = 1.8$ Hz), 7.23 (1H, d, $J = 9.0$ Hz), and 7.52 (1H, dd, $J = 1.8, 9.0$ Hz), as well as nine oxygenated proton signals at δ 3.17–3.73, one methyl group at δ 1.17 (3H, d, $J = 6.6$ Hz), and one methylene group at δ 1.59 (1H, m), 2.03 (1H, dd, $J = 12.0, 2.4$ Hz). Two anomeric proton signals at δ 4.98 (1H, dd, $J = 12.0, 3.0$ Hz) and 4.80 (1H, d, $J = 7.8$ Hz) suggested the presence of two sugar units in compound **1**, which were identified as glucose and digitoxose by ¹³C NMR and ¹H NMR analyses. Acid hydrolysis of compound **1** affording only glucose indicated that the digitoxose was linked

to the aglycone with C–C bond. This assignment was also proved by the ¹³C NMR signals at δ 70.2, which was the characteristic signal of anomeric carbon of C-digitoxose. In the HMBC spectrum (see Figure 1), long-range correlations from H-1 of digitoxose to C-5, C-6, and C-7 of the flavone nucleus showed that the digitoxose was linked to C-6 of the flavone nucleus. The ¹³C NMR signal at δ 101.0 was assignable to the anomeric carbon of glucose. In the HMBC spectrum (see Figure 1), long-range correlations from H-1 of glucose to C-4' of the flavone nucleus and its δ_{c-1} 101.0 showed that glucose was linked to C-4' of the flavone nucleus by C–O–C bond. All the proton and carbon signals were assigned by HSQC, DEPT, ¹H-¹H COSY, and HMBC experiments. The coupling constants of anomeric protons indicated that glucosyl and digitosyl linkage were

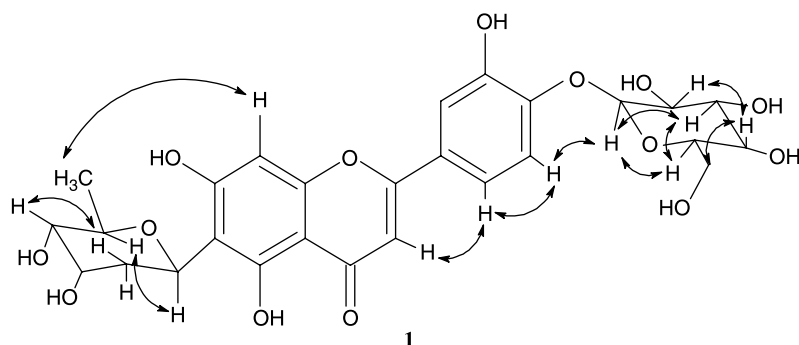


Figure 2. Significant NOESY correlations of **1**.

Table 1. NMR spectral data (δ , ppm) for compound **1** in DMSO- d_6 .^a

Aglycone moiety					Sugar moiety				
No.	δ_H	δ_{OH}	δ_C	DEPT	No.	δ_H	δ_{OH}	δ_C	DEPT
2			163.4	C	Dig				
3	6.85 (1H, s)		103.9	CH	1''	4.98 (1H, dd, 12.0, 3.0)		70.2	CH
4			182.0	C	2''	1.59 (1H, m), 2.03 (1H, q, 12.0, 2.4)		32.4	CH ₂
5		13.4 s	157.4	C	3''	3.45 (1H, m)	4.69 brs	69.6	CH
6			110.1	C	4''	3.71 (1H, d, 12.6)	4.76 brs	68.5	CH
7		9.1 brs	162.4	C	5''	3.64 (1H, dd, 6.6, 12.6)		74.5	CH
8	6.57 (1H, s)		94.8	CH	6''	1.17 (3H, d, 6.6)		17.4	CH ₃
9			156.2	C	Glu				
10			103.5	C	1''	4.80 (1H, d, 7.8)		101.0	CH
1'			124.6	C	2''	3.34 (1H, m)	5.39 brs	73.2	CH
2'	7.50 (1H, d, 1.8)		113.6	CH	3''	3.32 (1H, m)	5.12 brs	75.8	CH
3'		9.7 brs	146.9	C	4''	3.17 (1H, m)	5.07 brs	69.8	CH
4'			148.6	C	5''	3.40 (1H, m)		77.3	CH
5'	7.23 (1H, d, 9.0)		116.0	CH	6''	3.73 (1H, m), 3.48 (1H, m)	4.62 t (5.9)	60.7	CH ₂
6'	7.52 (1H, dd, 1.8, 9.0)		118.6	CH					

^aThe ¹H and ¹³C NMR spectral data were measured at 600 MHz, and the *J* values (parentheses) are in hertz.

β -configurations [11,12]. This assignment was also proved by the significant NOESY correlations (see Figure 2). On the basis of the above evidence, the structure of compound **1** was determined as 5,7,3'-trihydroxy-6-C- β -D-diglycopyranosyl-4'-O- β -D-glucopyranosyl flavonoid.

3. Experimental

3.1 General experimental procedures

Melting points were determined with Shengguang WRX-1S thermal values analyzer with microscope and are uncorrected. The optical rotation was measured with a Perkin-Elmer 343 Polarimeter. UV spectra were obtained on Waters 2695 HPLC with photodiode array detector. IR spectra were taken on a Thermo Nicolet FT-IR NEXUS 670 spectrophotometer with KBr pellets. NMR spectra were recorded on Varian System-600. HRESIMS spectra were performed on AutoSpec Ultima-TOF mass spectrometer and ESIMS data were obtained with an Agilent 1100 Series mass spectrometer.

3.2 Plant material

The leaves of *P. argenteastratus* were collected from Anji County, Zhejiang Province, China in November 2005, and identified by Professor Yulong Ding, Bamboo Research Institute, Nanjing Forestry University, Nanjing, China. A voucher specimen (200511-04) is deposited at the International Centre for Bamboo and Rattan (ICBR), Beijing, China.

3.3 Extraction and isolation

The shade-dried leaves of *P. argenteastratus* (0.96 kg) were extracted with 95% EtOH by cold percolation for three times. A residue of 98.0 g was obtained after removal of the solvent by evaporation. The residue was suspended in H₂O and extracted with petroleum ether. The fraction after being extracted with petroleum ether was subjected to macroporous absorption resin (AB-8) and eluted with H₂O, 20% EtOH, 40% EtOH,

60% EtOH, 80% EtOH, and acetone. The 20% EtOH fraction (11.9 g) was then chromatographed over Sephadex LH-20 and eluted with MeOH repeatedly, to yield compound **5** (449.3 mg). The 40% EtOH fraction (33.2 g) was then chromatographed over Sephadex LH-20 and eluted with MeOH repeatedly, to yield compounds **1** (29.6 mg) and **4** (10.0 mg). The 60% EtOH fraction (7.3 g) was then chromatographed over Sephadex LH-20 and eluted with MeOH repeatedly, to yield compounds **2** (107.4 mg) and **3** (5.0 mg).

3.3.1 5,7,3'-Trihydroxy-6-C- β -D-diglycopyranosyl-4'-O- β -D-glucopyranosyl flavonoid (1)

Yellow amorphous powder (MeOH), mp 180.3–181.0 °C; $[\alpha]_D^{20} + 20.3$ (c 0.15, MeOH); UV λ_{max} (nm): 212.3, 272.4, 340.2; FT-IR (KBr) ν_{max} (cm⁻¹): 3413.6, 1655.0, 1627.0, 1508.3, 1491.3, 1439.3; ¹H and ¹³C NMR data (see Table 1); HRESIMS, *m/z* 577.1513 [M-H]⁻ (calcd for C₂₇H₂₉O₁₄, 577.1557); Negative ion ESIMS *m/z* 577.2 [M-H]⁻, 1155.3 [2M-H]⁻.

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