FLAVONOIDS FROM THE LEAVES OF *BAMBUSA PERVARIABILIS* MCCLUREJIA SUN, YONG-DE YUE^{1*}, FENG TANG, XUE-FENG GUO

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ABSTRACT

A new flavonoid, 5,4'-dihydroxy-3',5'-dimethoxy-7-O[β -D-apiose-(1 $\rightarrow$ 2)]- β -D-glucopyranosyl flavonoside(**1**), along with four known flavonoids 5,7,3',4'-tetrahydroxy-6-C- β -L-arabinosyl flavonoside(**2**), 5,7,4'-trihydroxy-6-C- β -D-glucopyranosyl flavonoside(**3**), 5,7,3',4'-tetrahydroxy-6-C- β -D-glucopyranosyl flavonoside(**4**), 5,7,3',4'-tetrahydroxy-8-C- β -D-glucopyranosyl flavonoside(**5**) were isolated from the leaves of *Bambusa pervariabilis* McClure. Their structures were determined on the basis of spectroscopic techniques and chemical methods.

Keywords: *Bambusa pervariabilis* McClure; flavonoids; 5,4'-dihydroxy-3',5'-dimethoxy-7-O[β -D-apiose-(1 $\rightarrow$ 2)]- β -D-glucopyranosyl flavonoside

INTRODUCTION

Flavonoids, important active components of plant, showed significant anti-inflammatory and antioxidant activities, and had the effects of treating cardiovascular diseases¹⁻³ and different types of cancer-preventive activity^{4,5}. Phytochemical research on bamboo leaves showed that they were rich in flavonoids⁶⁻⁸. Consequently, flavonoids extracted from bamboo leaves have been used as health products and food additive. *B. pervariabilis* McClure is one of the bamboo species in China. Chinese people realized the medical and health care effects of *B. pervariabilis* McClure leaf long ago, and used it as a traditional medicine to treatment of febrile disorder, exogenous diseases, cooling blood and Hemostasis⁹. Our previous research exhibited that the presence of coumarins in the leaves of *B. pervariabilis* McClure¹⁰. Extensive chromatography of the EtOH extract of the leaves of *B. pervariabilis* McClure 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**.

EXPERIMENTAL

General experimental procedures

Melting points were determined with Shenguang 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.

Plant material

The leaves of *B. pervariabilis* McClure were collected from Nanning City, Guangxi Province, China in September 2008, and identified by Professor Dayong Huang, Bamboo Research Institute, Nanning Academy of Forestry,

Nanning, China. A voucher specimen (200810-01) is deposited at the International Centre for Bamboo and Rattan (ICBR), Beijing, China.

Extraction and isolation

The shade-dried leaves of *B. pervariabilis* McClure (8.24 kg) were extracted with 95% EtOH by cold percolation for three times. A residue of 765.3g 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 40% EtOH fraction (42.9 g) was then chromatographed over Sephadex LH-20 and eluted with MeOH repeatedly, to yield compound **1** (123.2 mg), compounds **2** (996.8 mg), compounds **3** (812.0mg), and compounds **4** (196.0mg) and **5** (137.6 mg).

5,4'-dihydroxy-3',5'-dimethoxy-7-O[β -D-apiose-(1 $\rightarrow$ 2)]- β -D-glucopyranosyl flavonoside(**1**)

Yellow amorphous powder (MeOH), mp 250.6-252.4 °C; $[\alpha]_D^{25} = -77^\circ$ ($c = 0.1$, DMSO); UV λ_{max} (nm): 251.0, 273.5 and 352.2, FT-IR (KBr) ν_{max} (cm⁻¹): 3266.4, 2917.2, 1651.2, 1596.9, 1571.5, 1493.1, 1455.4; ¹H and ¹³C NMR data (Table 1); HRESIMS, m/z 647.1557 [M+Na]⁺ C₂₈H₃₂NaO₁₆⁺ (calcd for 647.1588); Negative ion ESIMS m/z 659.2 [M+Cl]⁻.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow amorphous powder, mp 250.6-252.4°C, $[\alpha]_D^{25} = -77^\circ$ ($c = 0.1$, DMSO). The molecular formula, C₂₈H₃₂O₁₆, was deduced from the negative HRESIMS at m/z 647.1557 [M+Na]⁺ C₂₈H₃₂NaO₁₆⁺ (calcd for 647.1588). The ESIMS (negative) showed ion peaks at m/z 659.2 [M+Cl]⁻. 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 (3266.4cm⁻¹), methyl(2917.2), carbonyl (1651.2cm⁻¹), and aromatic rings (1596.9cm⁻¹, 1571.5cm⁻¹, 1493.1cm⁻¹, 1454.4cm⁻¹). The UV spectrum showed absorption maxima at 251.0, 273.5 and 352.2nm, characteristic for the Flavonoid.

Table 1. NMR spectral data (d, ppm) for compound (**1**) in DMSO-d₆^a.

Aglycone moiety						Sugar moiety					
No.	δ_H	δ_{OH}	δ_C	DEPT	¹ H- ¹ H COSY	No.	δ_H	δ_{OH}	δ_C	DEPT	¹ H- ¹ H COSY
2			164.3	C		D-glucose					
3	7.06(1H,s)		104.0	CH		1''	5.07(1H,d,9.5)		98.4	CH	H-2''
4			182.2	C		2''	3.49(1H,m)		76.9	CH	H-1''
5			161.2	C		2''-OH		5.34brs			
5-OH		12.95s				3''	3.71(1H,m)		76.2	CH	H-2'',H-4''
6	6.43(1H,s)		99.6	CH		3''-OH		5.13brs			
7			162.8	C		4''	3.19(1H,m)		70.0	CH	H-3'',H-5''
8	6.91(1H,s)		95.4	CH		5''	3.53(1H,m)		77.3	CH	H-4'',H-6''
9			157.0	C		6''	3.71(1H,m) 3.59(1H,m)		60.7	CH ₂	H-5'',H-6''
10			105.5	C		6''-OH		4.69t(5.9)			
1'			120.3	C		D-apiose					
2'	7.34(1H,s)		104.6	CH		1'''	5.30(1H,d,2.1)		108.8	CH	H-2'''
3'			148.3	C		2'''	3.74(1H,m)		75.9	CH	H-1'''
3'-OCH ₃	3.87(3H,s)		56.5	CH ₃		2'''-OH		5.15brs			
4'			140.1	C		3'''			79.4	C	
4'-OH		9.37s				3'''-OH		4.63brs			
5'			148.3	C		4'''	3.64(1H,d,9.5) 3.90(1H,d,9.5)		74.1	CH ₂	H-4'''
5'-OCH ₃	3.87(3H,s)		56.5	CH ₃		5'''	3.28(1H,m) 3.34(1H,m)		64.3	CH ₂	H-5'''
6'	7.34(1H,s)		104.6	CH		5'''-OH		4.65brs			

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

Besides the characteristic signal for A,C-rings of the flavone skeleton at δ 6.43(1H,s) δ 6.91(1H,s), the ¹H NMR spectrum of compound **1** displayed signals of a 1',3',4',5' tetra-substituted phenyl moiety(B-ring) at 7.34(2H,s) and two methoxyl groups at δ 3.87(6H,s), as well as five oxygenated proton signals at δ 3.19-3.74, and three methylene group signals at δ 3.28-3.90. Acid hydrolysis of compound **1** affording glucose and apiose indicated that the glucose and apiose were linked to the aglycone with C-O bond. Two anomeric proton signals at δ 5.07 (1H,d,*J*=9.5Hz) and δ 5.30 (1H,d,*J*=2.1 Hz) suggested the presence of two sugar units in compound **1**, which were identified as glucose and apiose by ¹³C NMR and ¹H NMR. In the DEPT spectrum (Table1), two methylene group signals δ (C) 60.7 and δ (C) 64.3 were the signals of sugar-end. The anomeric proton signals at δ 5.07 (1H,d,*J*=9.5Hz) was identified as glucose. The anomeric proton signals at δ 5.30 (1H,d,*J*=2.1 Hz) was identified as apiose. This assignment was also proved by the ¹³C NMR signals at δ (C) 108.8, which was the characteristic signal of anomeric carbon of O-apiose, the signals of δ (C) 74.1 was the C-4' of apiose and the quaternary carbon signals δ (C) 79.4 was also confirmed the apiose was existed in the flavone. In the HMBC spectrum (Figure 2), long-range correlations from H-1 of glucose to C-7 of the flavone nucleus showed that the glucose was linked to C-O-C of the flavone nucleus and long-range correlations from δ 5.30 (1H,d,*J*=2.1 Hz) to C-2 δ (C) 76.9 of the glucose nucleus and δ 3.49(1H,m) to δ (C)108.8 of the apiose nucleus showed the apiose was linked to C-2 of the glucose nucleus by C-O-C bond., the chemical shift of the glucose C-2 δ (C) 76.9 moved to down-field about 4ppm, the assignment was also proved the apiose was linked to C-2 of the glucose. 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 apiosyl linkage were β -configuration^{11, 12}. This assignment was also proved by the significant NOESY correlations (Figure 3). On the basis of above evidence, the structure of compound **1** was

determined as 5,4'-dihydroxy-3',5'-dimethoxy-7-O[β -D-apiose-(1 $\rightarrow$ 2)]- β -D-glucopyranosyl flavonoid.

Figure 1. Structures of **1-5**

Figure 2. Significant HMBC correlations of compound (1)

Figure 3. Significant NOESY correlations of compound (1)

5,7,3',4'-tetrahydroxy-6-C- β -L-arabinosyl flavonoside(2). Yellow amorphous powder, mp 226.2-227.0°C. ^1H NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 6.806(s,1H), 6.405(s,1H), 7.276(s), 6.806,6.793(d,1H), 7.276,7.290(d,1H), 4.692,4.708(d,1H), 3.863,3.859(m,1H), 3.501,3.507,3.517,3.522(q,1H), 4.126,4.142,4.158(t,1H), 3.631,3.611(d,1H) (3.895,3.899,3.916,3.919(q,1H). ^{13}C NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 166.36(C), 103.91(CH), 184.04(C), 158.75(C), 109.30(C), 164.79(C), 95.59(CH), 158.75(C), 105.17(C), 123.75(C), 114.14(CH), 147.06(C), 151.06(C), 116.79(CH), 120.34(CH), 76.22(CH), 70.74(CH), 75.85(CH), 70.83(CH), 71.89(CH $_2$).

5,7,4'-trihydroxy-6-C- β -D-glucopyranosyl flavonoside(3). Yellow amorphous powder, mp 187.3--188.6°C. ^1H NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 6.532(s,1H), 6.435(s,1H), 6.854,6.868(d,2H), 7.779,7.765(d,2H), 3.414,3.404(d,1H), 4.834,4.851(d,1H), 4.124,4.110,4.096(t,1H), 3.429,3.420(d,1H), 3.364-3.351(m,1H), 3.828,3.810(d,1H) 3.698,3.689,3.678,3.669(q,1H). ^{13}C NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 166.18(C), 103.87(CH), 184.04(C), 158.75(C), 109.25(C), 165.14(C), 95.29(CH), 162.83(C), 105.15(C), 123.12(C), 129.45(CH), 117.06(CH), 162.06(C), 117.06(CH), 129.45(CH), 80.15(CH), 75.31(CH), 72.59(CH), 71.99(CH), 82.64(CH), 62.87(CH $_2$).

5,7,3',4'-tetrahydroxy-6-C- β -D-glucopyranosyl flavonoside(4). Yellow amorphous powder, mp 213.7-214.5°C. ^1H NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 6.663(s,1H), 6.474(s,1H), 7.391 (s,1H), 6.888,6.872(d,1H), 7.420,7.416,7.403,7.399(q,1H), 3.201,3.184(d,1H), 4.580,4.560(d,1H), 3.141,3.122,3.104,3.086(q,1H), 4.047,4.028,4.010(t,1H), 3.167,3.157(m,1H), 3.684,3.662(d,1H) 3.535,3.497,3.442,3.409,3.397,3.385,3.373(m,1H). ^{13}C NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 163.71(C), 102.88(CH), 181.94(C), 160.77(C), 108.95(C), 163.32(C), 93.54(CH), 156.25(C), 103.48(C), 121.50(C), 113.38(CH), 145.82(C), 149.76(C), 116.12(CH), 119.04(CH), 79.02(CH), 73.10(CH), 70.70(CH), 70.25(CH), 81.67(CH), 61.57(CH $_2$).

5,7,3',4'-tetrahydroxy-8-C- β -D-glucopyranosyl flavonoside(5). Yellow amorphous powder, mp 237.5-238.9°C. ^1H NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 6.537(s,1H), 6.156(s,1H), 7.369(d,1H), 6.763,6.737(d,1H), 7.434,7.408,7.281(q,1H), 3.135 (d,1H), 4.615,4.577(d,1H), 3.233(q,1H), 3.709 (d,1H), 3.055(m,1H), 3.576(d,1H); 3.436(m,1H). ^{13}C NMR (600 MHz, MeOD- d_4 , δ , ppm, J/Hz): 164.54(C), 102.87(CH), 182.47(C), 160.84(C), 98.57(CH), 162.99(C), 105.01(C), 156.45(C), 104.50(C), 122.49(C), 113.38(CH), 146.25(C), 150.05(C), 116.12(CH), 119.82 (CH), 79.23(CH), 73.85(CH), 71.50(CH), 71.24(CH), 82.45(CH), 62.10(CH $_2$).

Furthermore, four known compounds 2, 3, 4 and 5 were identified as 5,7,3',4'-tetrahydroxy-6-C- β -L-arabinosyl flavonoside(2), 5,7,4'-trihydroxy-6-C- β -D-glucopyranosyl flavonoside(3), 5,7,3',4'-tetrahydroxy-6-C- β -D-glucopyranosyl flavonoside(4) and 5,7,3',4'-tetrahydroxy-8-C- β -D-glucopyranosyl flavonoside(5), respectively, by comparison of their spectroscopic data with the literature^{13,14}.

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