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A New Apiofuranoside from the Rattan of *Piper flaviflorum*

Yu Wu[1a], Fei Long[1,2a], Chengjian Zheng[1*], Qianliang Ming[1] , Xuehong Deng[1,2], Jianyong Zhu[1] and Luping Qin[1,3*]

[1]*Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China,*

[2]*Department of Pharmacy, Fujian University of Traditional Chinese Medicine, 1 Huatuo Road, Fuzhou 350108, P. R. China,*

[3]*Shanghai Key Laboratory for Pharmaceutical Metabolite Research, Shanghai 200433, P. R. China*

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Abstract: A new apiofuranoside, named flavifloside A (**1**), together with two known hydroquinone diglycoside acyl ester, seguinoside E (**2**), seguinoside K (**3**) and a new natural product, 6'-*O*-(3, 4-dimethoxycinnamoyl)-arbutin (**4**), were isolated from the rattan of *Piper flaviflorum*. All these compounds were reported from this plant for the first time. Their structures were elucidated by detailed analyses of NMR, IR and MS data. In the mean time, all the compounds have been tested against A549, HCT116, MDA-MB-231 and CCRF-CEM cancer cell lines. Only flavifloside A showed moderate cytotoxicity against CCRF-CEM cancer cell line and none of them showed significant cytotoxicity against the other tested cell lines.

Keywords: *Piper flaviflorum*; apiofuranoside; flavifloside A.

1. Introduction

The *Piper* genus belongs to the Piperaceae family and contains more than 1000 species all over the world, widely distributed in the tropical and subtropical areas. *Piper* plants have been widely used for commercial, economical and medicinal applications, especially reputed for their versatile medicinal activities in Traditional Chinese Medicine, Indian Ayurvedic system of medicine and in folklore medicine of Latin America and West Indies [1]. *Piper* species possessed various pharmacological properties and has been applied mainly as anti-inflammatory [2], antifungal [3], antidepressant [4], anti-oxidative [5] and antinociceptive [6] agents.

The rattan of *Piper flaviflorum* was traditionally used in Dai ethnomedicine in Xishuangbanna, P. R. China, to treat some chronic and incurable diseases. Previous phytochemical investigations revealed that *P. flaviflorum* mainly contained alkaloids, lignans and sterols [7]. So far, there has been no report of the presence of glycosides from *P. flaviflorum* and the chemical constituents of this species have not yet been investigated thoroughly. Our study was therefore conducted to further elucidate the bioactive constituents from the rattan of *P. flaviflorum*, which was collected from

* Corresponding author: E-Mail: zheng_chengjian@hotmail.com, Phone:+86-21-81871309, *Fax*:+86-21-81871309; E-Mail: qinsmmu@126.com, Phone:+86-21-81871300, *Fax*:+86-21-81871300

[a] Both authors contributed equally to this work.

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Xishuangbanna in Yunnan province, China.

From the EtOAc extract, a new apiofuranoside (**1**), together with two known hydroquinone diglycoside acyl esters (**2** and **3**) and a new natural product (**4**), have been isolated and identified. Herein, details of the isolation and structure elucidation of the chemical constituents **1**-**4** (Figure 1) are described, and their cytotoxic activities were also tested against four cancer cell lines.

2. Materials and Methods

2.1. General

Optical rotations (ORD) were measured using a JASCO P-1020 spectropolarimeter. UV spectra were taken on a Shimadzu UV-260 spectrophotometer. IR Spectra were recorded on a Avatar 360-ESP spectrophotometer (Thermo Nicolet). NMR Spectra were performed on a DRX-600 spectrometer with tetramethylsilane (TMS) as an internal standard at 600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR. HR-MS spectra were obtained by electrospray ionization (ESI) in the positive mode on a Bruker APEX 7.0 TESLA FT-MS apparatus. TLC was carried out on silica-gel plates (Yan-tai Institute of Chemical Technology). Column chromatography (CC) was performed on silica gel (200 – 300, 300 – 400 mesh; Qingdao Marine Chemical Factory). All analytical grade solvents were produced by Sinopharm Chemical Reagent Co.,Ltd.

2.2 Plant material

The aerial parts of *P. flaviflorum* were collected in Xishuangbanna, Yunnan Province, P. R. China, in May 2011, and were identified by Prof. Han-chen Zheng, Department of Pharmacognosy, Second Military Medical University. A voucher specimen (#201104) has been deposited at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

2.3 Extraction and Isolation

The air-dried and powdered aerial part (10 kg) of *P. flaviflorum* was exhaustively extracted with 80% EtOH under reflux. The EtOH extract was concentrated in vacuum to yield a semi-solid (700 g), which was later suspended in H_2O (700 mL) and then fractionated with EtOAc (3 × 700 mL). The gathered organic phase was concentrated to yield a residue (50 g) under reduced pressure at 60 °C, part of which (40 g) was subjected to column chromatography (1.2 kg Silica gel, 200-300 mesh), eluted with CH_2Cl_2/CH_3OH step gradients from 50:1, 20:1, 10:1, 5:1, 3:1, 1:1 to 0:1, to afford five fractions (*Fr. 1 – Fr. 5*). *Fr.1* (1.1 g), was applied to repeated CC (10 g Silica gel, 300-400 mesh, CH_2Cl_2/CH_3OH 10:1) to obtain compound **1** (9.1 mg). *Fr.3* (0.7 g), was subjected to repeated CC (15 g Silica gel, 300-400 mesh, CH_2Cl_2/CH_3OH 5:1) to give compound **4** (20 mg). *Fr.5* (1.5 g) was chromatographed over repeated CC (40 g Silica gel, 300-400 mesh, CH_2Cl_2/CH_3OH 5:1), followed by PTLC (CH_2Cl_2/CH_3OH 3:1) to afford compounds **2** (5.5 mg) and **3** (7.4 mg).

Compound **1**, 5-*O*-(4'-hydroxy-3'-methoxybenzoyl)-*β*-D-apiofuranoside, named flavifloside A, white amorphous powder, $[\alpha]^{25}_{D}$ = −8 (*c* 0.10, MeOH); ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data see Table 1; HR-ESI-MS: *m/z* 323.0720 $[M + Na]^{+}$ (calc. 323.0723).

Compound **4**, 6'-*O*-(3, 4-dimethoxycinnamoyl)-arbutin, white amorphous powder, $[\alpha]^{25}_{D}$ = −115 (c 0.05, MeOH); ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data see Table 2; HR-ESI-MS: *m/z* 485.1420 $[M + Na]^{+}$ (calc. 485.1426).

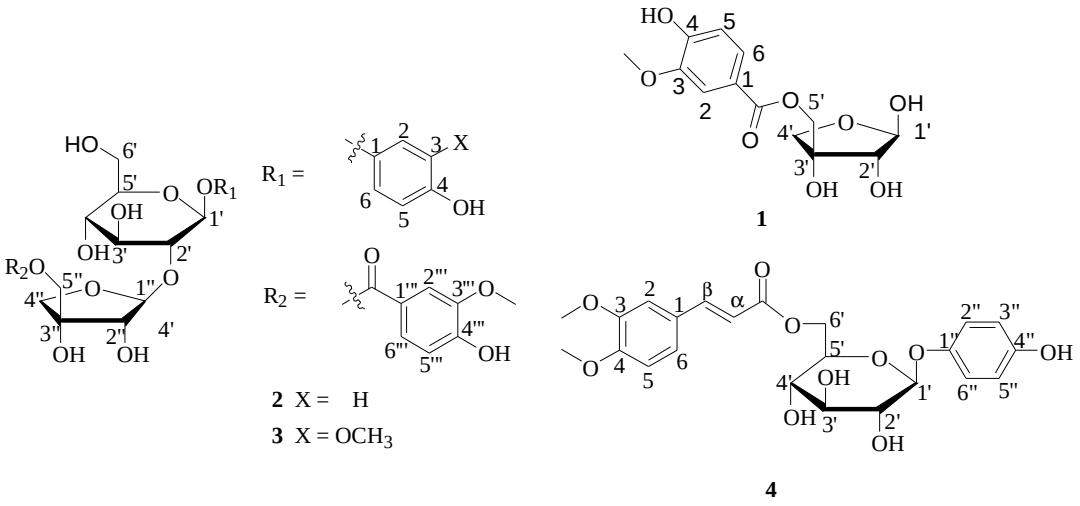

Figure 1. Structures of compounds **1**-**4**.

2.4 Cytotoxic Assay

Cytotoxicity assays against human cancer cell lines in vitro were performed by a modified 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium (MTT) method [8]. Cells were seeded in a 96 well plate at a concentration of 4-8 × 10^4 cells/well and each well was added to 100 μL on the average. Cells were then incubated in 5% CO_2 incubator at 37°C for 24 hours before treatment to allow attachment of cells to the wall of the plate. Subsequently, the cells were co-incubated with compounds **1-4** and doxorubicin (as a positive control) for 72 hours. Then each well was added 20 μL solvent of MTT at the concentration of 5 mg/mL and the cells were incu in a humidified atmosphere containing 5% CO_2. After that, the medium was removed and the violet crystals of formazan in viable cells were dissolved in DMSO. Optical density (OD) absorbance at 570 nm was measured by a full wavelength multi-function microplate reader.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** showed UV absorption maxima at 254 and 285 nm and IR absorptions at 1603 and 1515 cm^{-1}, revealing the presence of benzene ring. The ^{1}H-NMR spectrum indicated that compound **1** had one set of protons of aromatic system, at δ_H 7.60 (1H, *d*, *J*=1.8 Hz, H-2), 7.58 (1H, *dd*, *J*=7.8, 1.8 Hz, H-6) and 6.84 (1H, *d*, *J*=7.8 Hz, H-5), revealing three aromatic protons coupled in an ABX pattern. In addition, signals in the region of δ_H 3.50-5.50 mainly came from one sugar unit, characterized by an anomeric proton signal at δ_H 5.20 (1H, *d*, *J* = 1.8 Hz, H-1'). Combined with the ^{13}C spectrum and 2D NMR data, the sugar moiety was identified as an apioforanose [9]. According to previous reports, apiose unit with 1"-OH and 2"-OH in *trans* configuration presents constant coupling $J_{1,2}$ 0-2 Hz, whereas *cis* configuration is characterized by $J_{1,2}$ 3-4 Hz [10, 11]. The apiose unit was therefore identified as a β-D-apiofuranoside based on the anomeric proton singlet at δ_H 5.20 (1H, *d*, *J*=1.8 Hz, H-1') and the chemical shift of C-1' at δ_C 104.2 [12]. Furthermore, observed HMBC correlations (Figure 2) from H-5' (δ_H 4.32, *d*, *J* = 10.8 Hz; 4.30, *d*, *J* = 10.8 Hz) of the D-apiose to the ester carbonyl at δ_C 167.9 (C=O) suggested that the benzoyl group was attached to C-5' of the apiose unit. In addition, a methoxyl group was determined to be affixed at C-3 deduced from the HMBC correlation from the methoxyl protons (δ_H 3.89, *s*, 3H) to C-3 (δ_C 148.8) and the NOESY correlation between H-OCH_3 (δ_H 3.89, *s*, 3H) and H-2 (δ_H 7.60, *d*, *J* = 1.8 Hz). Consequently, the structure of **1** was elucidated as shown in Figure 1 and determined to be 5-*O*-(4'-hydroxy-3'-methoxybenzoyl)-*β*-D-apiofuranoside, trivially named flavifloside A.

Compounds **2**, **3** and **4** were determined to be seguinoside E (**2**) [13], seguinoside K (**3**) [14, 15] and 6'-*O*-(3, 4-dimethoxycinnamoyl)-arbutin (**4**) [16], respectively, by comparison with those

reported spectrographic data. Compound **4** was isolated as a new natural product in our study, which was recently synthesized by using immobilized lipase from *Penicillium expansum* [16]. However, in the reference, the proton and carbon signals of compound **4** were assigned just by comparing the NMR data with those of similar arbutin ester derivatives. Our present study assigned the ^{1}H and ^{13}C NMR signals of **4** (Table 2) totally on the basis of the 2D-NMR techniques (Figure 2 and supporting information), which therefore elucidated the accurate structure better than before.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** (recorded in CD_3OD, δ in ppm, J in Hz).

Position	δ_H	δ_C
1	—	122.3 (*s*)
2	7.60 (1H, *d*, *J* = 1.8)	125.3 (*d*)
3	—	148.8 (*s*)
4	—	153.1 (*s*)
5	6.84 (1H, *d*, *J* = 7.8)	115.9 (*d*)
6	7.58 (1H, *dd*, *J* = 7.8, 1.8)	113.8 (*d*)
Api		
1'	5.20 (1H, *d*, *J* = 1.8)	104.2 (*d*)
2'	3.86 (1H, *d*, *J* = 1.8)	78.6 (*d*)
3'	—	79.2 (*s*)
4'	4.14 (1H, *d*, *J* = 10.2) 3.83 (1H, *d*, *J* = 10.2)	74.3 (*t*)
5'	4.32 (1H, *d*, *J* = 10.8) 4.30 (1H, *d*, *J* = 10.8)	67.4 (*t*)
C=O	—	167.9 (*s*)
MeO-3	3.89 (3H, *s*)	56.5 (*q*)

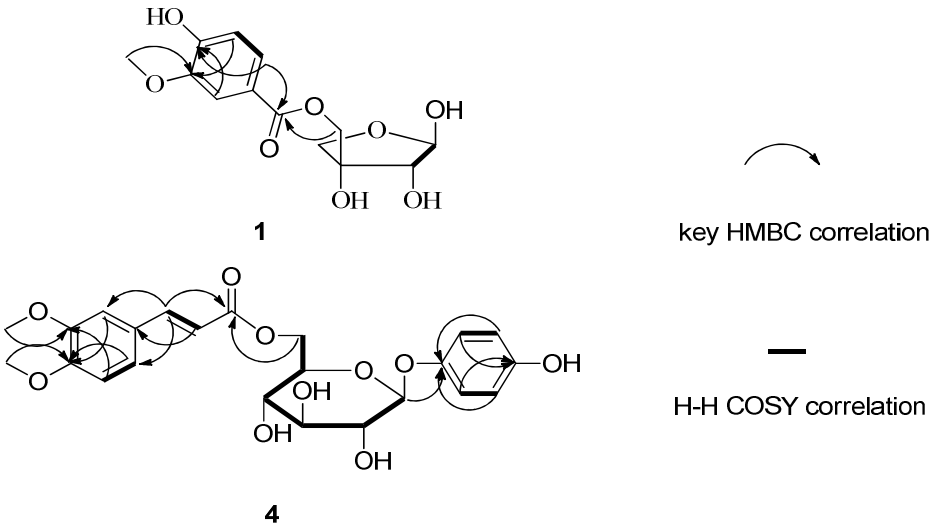

Figure 2. ^{1}H-^{1}H COSY and the key HMBC correlations of compounds **1** and **4**.

3.2 Cytotoxicity activity

Compounds **1-4** were evaluated for their cytotoxicity against human lung carcinoma (A549), colon carcinoma (HCT116), breast carcinoma (MDA-MB-231) and human promyelocytic leukaemia (CCRF-CEM) cell lines. Flavifloside A showed selective cytotoxicity against CCRF-CEM cancer cell line with IC_{50} value of 15.26 μg/mL. However, none of them showed significant cytotoxicity against the other tested cell lines, with all IC_{50} values > 100 μg/mL (Table 3).

Table 2. ^{1}H and ^{13}C NMR data for compound **4** (at 600 MHz in CD_3OD, δ in ppm, J in Hz).

Position	δ_H	δ_C
1	—	128.8 (*s*)
2	7.21 (1H, *d*, *J* = 1.8)	111.5 (*d*)
3	—	150.8 (*s*)
4	—	152.9 (*s*)
5	6.99 (1H, *d*, *J* = 8.4)	112.7 (*d*)
6	7.17 (1H, *dd*, *J* = 8.4, 1.8)	124.1 (*d*)
α	6.43 (2H, *d*, *J* = 15.6)	116.3 (*d*)
β	7.64 (2H, *d*, *J* = 15.6)	146.7 (*d*)
C=O	—	168.8 (*s*)
MeO-3	3.87 (3H, *s*)	56.5 (*q*)
MeO-4	3.86 (3H, *s*)	56.5 (*q*)
1'	4.72 (1H, *d*, *J* = 7.2)	103.7 (*d*)
2'	3.43 (1H, *m*)	74.9 (*d*)
3'	3.45 (1H, *m*)	77.9 (*d*)
4'	3.41 (1H, *m*)	71.8 (*d*)
5'	3.64 (1H, *m*)	75.5 (*d*)
6'	4.53 (1H, *dd*, *J* = 12.0, 2.4) 4.35 (1H, *dd*, *J* = 12.0, 6.6)	64.7 (*t*)
1"	—	152.3 (*s*)
2", 6"	6.94 (2H, *d*, *J* = 9.0)	119.6 (*d*)
3", 5"	6.64 (2H, *d*, *J* = 9.0)	116.4 (*d*)
4"	—	153.9 (*s*)

Table 3. Cytotoxic activity of compounds **1-3** (IC_{50}, μg/mL).

Compounds	Cell line [a]			
	A549	HCT116	MDA-MB-231	CCRF-CEM
1	>100	>100	>100	15.26
2	>100	>100	>100	>100
3	>100	>100	>100	>100
4	>100	>100	>100	>100
Doxorubicin	0.100	0.024	0.449	0.001

[a] Key to cell lines applied: human lung carcinoma (A549); colon carcinoma cell lines (HCT116); breast carcinoma cell lines (MDA-MB-231); human promyelocytic leukaemia cells (CCRF-CEM).

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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