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A Novel Bihomoflavanonol with an Unprecedented Skeleton from *Pteridium aquilinum*

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Abstract: **Objective** To seek the flavonoids with the unique structure and to investigate the chemical ingredients in the flavonoid-rich plant—*Pteridium aquilinum*. **Methods** The 80% EthOH extract from the degreased powder of *P. aquilinum* was partitioned by petroleum ether, $CHCl_3$, EtOAc, *n*-butanol, and water, respectively. The EtOAc fraction was sequentially subjected to silica gel column, repeated Sephadex LH-20 column, and preparative TLC to give a new compound. The antitumor activity of the novel flavonoid was primarily evaluated by MTT. **Results** Compound **1**, a biflavonoid with the unique structure named as pteridium III with an unprecedented bihomoflavanonol skeleton, was isolated from *P. aquilinum*. Compound **1** showed the *in vitro* antitumor activity against lung cancer cell NCI-H46, melanoma cell A375, and glioma cell U-7MG corresponding to the IC_{50} values of 22.9, 106.7, and 1540.5 μmol/L, respectively. No inhibition on gastric carcinoma SGC-7901 and prostatic carcinoma PC-3 was observed in the experiment. **Conclusion** A rare bihomoflavononol derivative, pteridium III, is obtained from the plant, which could enrich our knowedge on the chemical structures of flavonoids and bioactive constituents in *P. aquilinum*.

Key words: antitumor activity; bihomoflavononol; Pteridaceae; pteridium III; *Pteridium aquilinum*
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Introduction

Pteridium aquilinum (L.) Kuhn (Pteridaceae) is one of the most widely-distributed vascular plants in the world (Chen *et al*, 2008). The mature and tightly curled emerging fronds are regarded as a popular and delicious vegetable and processed to a series of food in fresh, canned, dried, or pickled forms. The species is traditionally used to heal jaundice with damp heat pathogen, rheumarthritis, and hypertension (Wu, 1990) by Chinese people. Previous phytochemical investigations on this plant have led to the isolation of a variety of proanthocyanidins (Markham, 1988) and flavonol glycosides (Cooper, 1976; Imperato, 1995; 1996; 1997; 1998; Imperato and Minutiell, 1997; Wang, Mahir, and Bryan, 1973).

Homoflavonoids, the family of flavonoids with an additional carbon designated as C-11 in their skeleton (Anders and Dana, 1982; Chang, Shen, and Chen, 2002; Zhao *et al*, 2008; Meng *et al*, 2010), are relatively rare secondary metabolites with novel and diverse chemical frameworks, and exhibit the biological activities, such as the prevention of cancer (Dixon, 2004; Holzbeierlein and Thrasher, 2005) and cardiovascular disease (Lee, 1992; Nonaka, 1992). The natural homoflavonoids with the unprecedented carbon frameworks have recently become of research object for seeking the lead compounds in medicinal chemistry.

In our seeking for homoflavonoids with the unique structure, we have isolated a biflavonoid named as pteridium III (compound **1**) with a bihomoflavanonol

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skeleton (Fig. 1) from this plant. In this paper, we describe the isolation and structure elucidation as well as the primary *in vitro* screening of the antitumor activities of compound **1** for six cancer cell lines.

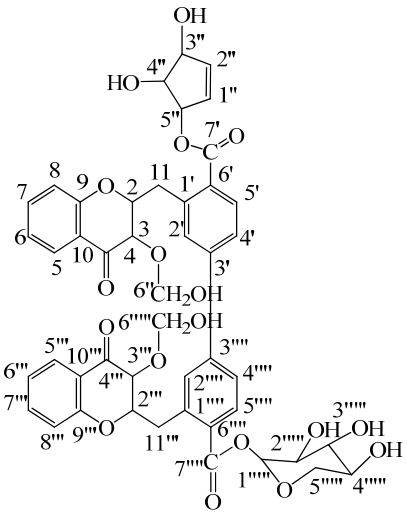

Fig. 1 Structure of compound 1

Materials and methods

General

Analytical TLC was performed on silica gel plates (Qingdao Marine Chemical Factory) with AcOEt-MeOH-H_2O (20:1:0.5) as eluant, and then was visualized under UV light by spraying with 5% $AlCl_3$ (in EtOH). Melting points were determined using an X4 Micro Melting Point Apparatus. Optical rotations were obtained with Jasco P—1020 Polarimeter. UV Spectra were determined by TU—1901 Spectrophotometer in anhydrous MeOH. IR Spectrum was recorded with Avatar 670 FTIR Spectrophotometer as KBr pellets in cm^{-1}. EI-MS was measured with Agilent 5973N Mass Spectrometer and the ^{1}H-NMR and ^{13}C-NMR spectra were obstained by Bruker AM—400 MHz and DRX—500 MHz Spectrometers

Plant material

The aerial parts of *Pteridium aquilinum* (L.) Kuhn were collected from Tiantangzhai, Anhui province, China in July 2010 and identified by Prof. ZHOU Shou-biao of Anhui Normal University (voucher No. 2010-07-3, in West Anhui Resource Plants Herbarium).

Extraction and isolation

The dried powder of *P. aquilinum* (16.5 kg) pretreated with ethyl ether (50 ℃) was extracted for three times with 80% EtOH for 5 h under reflux. EtOH was removed under reduced pressure to give a residue, which was partitioned between petroleum ether, $CHCl_3$, EtOAc, *n*-butanol, and water for three times for each solvent successively. After evaporation, the EtOAc fraction (174 g) was chromatographed on a silica gel column with gradient elution (EtOAc-MeOH 1:0, 40:1, 10:1, 2:1, and 0:1) and four fractions (Fr. 1—4) were obtained from the part eluted by EtOAc-MeOH (1:0). Fr. 3 (13.2 g) was submitted to Sephadex LH-20 column with an eluent of MeOH-water to afford six fractions (P1—P6). P1 (600 mg) was subjected to preparative TLC using EtOAc-MeOH-H_2O (20:1:0.5) as the eluting solvent system, and P1-b and P1-d were obtained. P1-b was then purified repeatedly by Sephadex LH-20 column ($CHCl_3$-MeOH 1:1→0:1) and compound **1** (50 mg) (Fig. 2) was yielded by repeated Sephadex LH-20 column chromatography with MeOH as eluent.

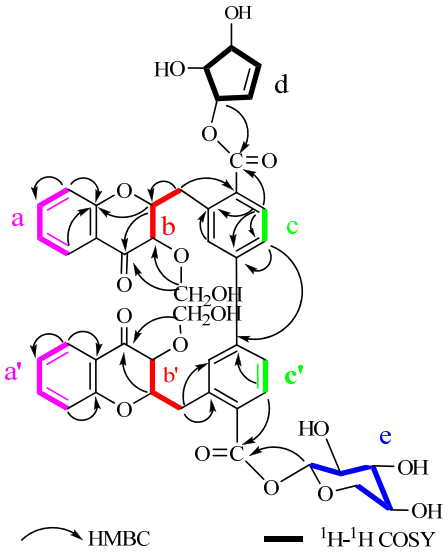

Fig. 2 Key HMBC and ^{1}H-^{1}H COSY of compound 1

Cytotoxicity for cancer cell lines

The six human cancer cell lines were maintained in RPMI-1640 medium. The cells were cultured in Nunc disposable 384-well plates containing 45 μL of growth medium per well and were incubated at 37 ℃ in a humidified incubator with 5% CO_2. Samples (5 μL) were added to the cultures at 24 h of incubation. After 72 h of incubation with the samples, 5 μL of presto blue (5 mg/mL) was added to each of the wells. The optical density was measured using a Freedom EVOlyzer (Tecan, Switzerland) at 560 nm with reference wave-length at 590 nm. In all experiments, three replicates were used. Adriamycin was used as the positive control.

Results and discussion

Structure elucidation

Compound **1**: yellow powder; mp 163—165 ℃; $[\alpha]_D^{25}$ +11° (*c* 0.05, MeOH); UV λ_{max}^{MeOH} (nm): 224.6 (1.4), 250.1 (4.4), 263.4 (4.28), 294.5 (4.36); IR ν_{max}^{KBr} (cm^{-1}): 3348, 1652, 1427, 1121, 1041, 1216, 921; HR-ESI-MS$^+$ *m/z* 885.2597 $[M + H]^+$; ^{1}H-NMR, ^{13}C-NMR, and HMBC data were shown in Table 1; ^{1}H-^{1}H COSY and HMBC were reported in Fig. 2.

The ^{13}C-NMR spectra of compound **1** showed a total of 43 signals, three (δ_C 175.1, δ_C 146.1, and δ_C 61.5) of which were clearly twice the intensity of the others, giving 46 carbons in the molecule. The presence of paired signals in ^{13}C-NMR spectrum suggested a biflavonoid structure or a mixture of two closely related compounds. However, the HR-ESI-MS showed $[M + H]^+$ at *m/z* 885.2597 (calcd 885.2605) was consistent with a dimer and corresponding to molecular formula $C_{46}H_{44}O_{18}$. These observations hinted that compound **1** might be a biflavonoid.

Table 1 ^{1}H-NMR, ^{13}C-NMR, and HMBC data of compound 1 (DMSO-d_6)

No.	δ_C	δ_H	HMBC	No.	δ_C	δ_H	HMBC
2	67.0 d	3.83 (1H,m)	C-11, C-4, C-9	2‴	66.8 d	3.75 (1H, m)	C-3‴, C-11‴, C-4‴
3	75.1 d	4.75 (1H, d, *J* = 5.0 Hz)	−	3‴	72.0 d	3.42 (1H, d, *J* = 5.6 Hz)	C-2‴
4	175.1 s	−	−	4‴	175.1 s	−	−
5	115.8 d	6.84 (1H, d, *J* = 8.0 Hz)	C-10	5‴	125.7 d	6.29 (1H, d, *J* = 7.8 Hz)	C-10‴
6	114.9 d	7.07 (1H, m)	C-7	6‴	114.9 d	7.07 (1H, m)	C-7‴
7	121.1 d	6.96 (1H, dd, *J* = 7.7, 8.5 Hz)	C-6	7‴	121.0 d	6.96 (1H, dd, *J* = 7.5, 8.4 Hz)	C-6‴
8	116.1 d	6.78 (1H, d, *J* = 8.5 Hz)	C-7, C-9	8‴	116.1 d	6.78 (1H, d, *J* = 8.4 Hz)	C-7‴, C-9‴
9	166.5 s	−	−	9‴	166.2 s	−	−
10	125.0 s	−	−	10‴	124.7 s	−	−
11	33.4 t	2.64 (1H, dd, *J* = 1.5, 17.0 Hz); 1.99 (1H, dd, *J* = 2.0, 17.0 Hz)	C-6′, C-2	11‴	33.0 t	2.57 (1H, dd, *J* = 2.5, 18.0 Hz) 2.11 (1H, dd, *J* = 2.0, 18.0 Hz)	C-1‴′, C-2‴
1′	125.1 s	−	−	1‴′	144.4 s		
2′	130.0 d	d, 7.59 (1H, d, *J* = 1.6 Hz)	C-3′/C-3‴′, C-1′	2‴′	130.0 d	d, 7.56 (1H, d, *J* = 2.0 Hz)	C-3′/C-3‴′
3′	146.1 s	−	−	3‴′	146.1 s	−	−
4′	145.0 d	d, 7.52 (1H, d, *J* = 7.6 Hz)	C-3′/C-3‴′	4‴′	144.8 d	7.45 (1H, d, *J* = 7.8 Hz)	C-3′/C-3‴′
5′	114.1 d	6.25 (1H, b d, *J* = 9.0 Hz)	C-1′, C-3′, C-4′, C-7′	5‴′	113.8 d	6.22 (1H, b d, *J* = 9.5 Hz)	C-7‴′, C-6‴′
6′	149.3 s	−	−	6‴′	149.2 s	−	−
7′	170.6 s	−	−	7‴′	170.4 s	−	−
1″	130.1 d	6.42 (1H, m)	−	1‴″	100.0 d	5.05 (1H, m)	C-7‴′
2″	130.5 d	6.41 (1H, m)	−	2‴″	70.2 d	3.66 (1H, m)	−
3″	65.9 d	4.13 (1H, m)	−	3‴″	69.8 d	5.45 (1H, d, *J* = 7.5 Hz)	C-4‴″
4″	71.2 d	3.42 (1H, m)	−	4‴″	69.3 d	5.47 (1H, d, *J* = 4.0 Hz)	C-3‴″
5″	63.9 d	4.35 (1H, m)	C-7′	5‴″	64.0 t	3.98 (2H, m)	C-2‴″
6″	61.5 t	3.48 (2H, −)	C-4	6‴″	61.5 t	3.48 (2H, −)	C-4‴, C-3‴

The gross structure of compound **1** was elucidated by analysis of ^{1}H-^{1}H COSY and HMBC spectra data. The ^{1}H-^{1}H COSY spectrum of compound **1** revealed connectivities of three pairs of partial structures: **a** [H_5 (6.84)-H_6 (7.07)-H_7 (6.96)-H_8 (6.78)] and **a′** [$H_{5‴}$ (6.29)-$H_{6‴}$ (7.07)-$H_{7‴}$ (6.96)-$H_{8‴}$ (6.78)], **b** [H_{11} (2.64,1.99)-H_2 (3.83)-H_3 (4.75)] and **b′** [$H_{11‴}$ (2.57, 2.11)-$H_{2‴}$ (3.75)-$H_{3‴}$ (3.42)], **c** [$H_{4′}$ (7.52)-$H_{5′}$ (6.25)] and **c′** [$H_{4‴′}$ (7.45)-$H_{5‴′}$ (6.22)], two azygos fragments **d** [$H_{1″}$ (6.42)-$H_{2″}$ (6.41)]-$H_{3″}$ (4.13)-$H_{4″}$ (3.42)-$H_{5″}$ (4.35)] and **e** [$H_{1‴″}$ (5.05)-$H_{2‴″}$ (3.66)-$H_{3‴″}$ (5.45)-$H_{4‴″}$ (5.47)-$H_{5‴″}$ (3.98)]. The HMBC cross peaks of δ_H 6.78 (H-8) to δ_C 121.1 (C-7) and δ_C 166.5 (C-9), δ_H 6.84 (H-5) to δ_C 125.0 (C-10), indicated that fragment **a** might be in the A-ring of the flavonoid. The HMBC correlations of δ_H 6.25 (H-5′) to δ_C 125.1 (C-1′), δ_C 146.1 (C-3′) and δ_C 149.3 (C-6′), and δ_H 7.59 (H-2′) to δ_C 125.1 (C-1′) were observed, disclosed fragment **c** was probably in the

B-ring of compound **1**. According to the presence of the HMBC cross peaks (Fig. 2) of δ_H 3.83 (H-2) to δ_C 166.5 (C-9) and δ_C 175.1 (C-4), δ_H 2.64/δ_H 1.99 (H-11) to δ_C 149.3 (C-6′), fragment **b** was confirmed in C-ring and the B-ring was linked with C-2 through C-11 (Fig. 2). Thus, a homoflavanonol skeleton was established in compound **1**. Similarly, **a′**, **c′** and **b′** were respectively in A′-, B′-, and C′-ring of the other homoflavanonol-structure moiety of compound **1**. The C-5, 6, 7, 8 and C-5″, 6″, 7‴, 8‴ were all tertiary carbons, and the quaternary carbons δ_C 149.3 (C-6′) and δ_C 149.2 (C-6⁗) were respectively linked with δ_C 170.6 (C-7′/C-7⁗), which suggested compound **1** might be a C-3′/3⁗-linked bihomoflavonoids. The HMBC correlations of δ_H 7.59 (H-2′) and δ_H 7.52 (H-4′) to δ_C 146.1 (C-3⁗/C-3′) further confirmed a C-3′/3⁗-linked bihomoflavanonol of compound **1**.

As for the fragment **d** (δ_H 4.35-δ_H 3.42-δ_H 4.13-δ_H 6.41-δ_H 6.42), the direct ^{1}H-^{1}H cosy correlations of δ_H 6.41 (1H, m) to δ_H 6.42 (1H, m), manifested **d** is a cyclopentenol analogue (named D-ring) (Barltrop *et al*, 1973; Steyn and Sable, 1971). The HMBC correlations of δ_H 4.35 (H-5″) to δ_C 170.6 (C-7′) demonstrated D-ring was probably linked with C-6′ through an esteratic linkage (C-7′).

With the respect to fragment **e**, the HSQC correlations of δ_H 5.05, δ_H 3.66, δ_H 5.45, δ_H 5.47, and δ_H 3.83 to δ_C 100.0 (C-1⁗), δ_C 70.2 (C-2⁗), δ_C 69.8 (C-3⁗), δ_C 69.3 (C-4⁗), and δ_C 64.0 (C-5⁗) (Table 1), revealed the existence of a β-*D* arabinopyranose ligand (Fraisse *et al*, 2000; Du *et al*, 2010). The sugar moiety was elucidated to link to C-6⁗ through a carbonyl ester bond from the HMBC correlation of δ_H 5.05 (H-1⁗) and δ_H 6.22 (H-5⁗) to δ_C 170.4 (C-7⁗) (Fig. 2). Therefore, the structure of compound **1** was shown in Fig. 1.

The relative stereochemistry of compound **1** was deduced from NOESY correlations as shown in Fig. 3. The NOESY correlation of H-4″ to H-11, H-1″ to H-5′, provided further evidences that the D-ring of compound **1** was linked to the B-ring. The NOESY cross-peaks of H-1⁗ to H-5⁗ and H-5⁗ to H-11‴ confirmed the D′-ring of compound **1** was linked to the B′-ring. Thus, the structure of compound **1**, a novel homoflavonoid derivative with an unprecedented bihomoflavanonol skeleton, was thus elucidated.

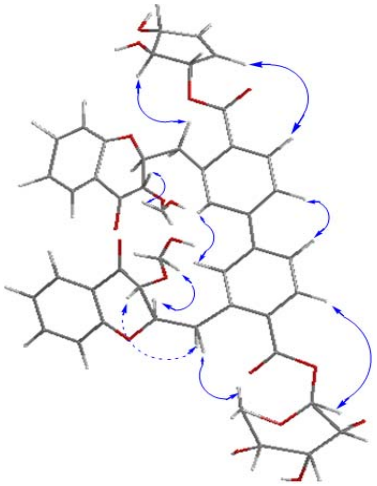

Fig. 3 Key NOESY correlations of compound 1

Cytotoxic activity

The primary antitumor activities of compound **1** against six types of cancer cells were evaluated (Table 2). Compound **1** showed moderate activity to lung cancer cell NCI-H46, and indistinctive activity to melanoma cell A375 and glioma U-7MG (IC_{50} = 22.6, 106.7, and 1540.5 μmol/L, respectively) by comparing with positive control, while no cytotoxic activities to hepatoma carcinoma cell BEL-7402, gastric carcinoma SGC-7901, and prostatic carcinoma PC-3 were observed in our experiments. The further investigations are undertaking.

Table 2 Cytotoxic activities of compound 1

Compound	IC_{50} / (μmol·L^{-1})					
	NCI-H46	A375	U-7MG	BEL-7402	SGC-7901	PC-3
1	22.9	106.7	1540.5	−	−	−
Adriamycin	1.1	6.43	5.3	5.5	3.56	5.6

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