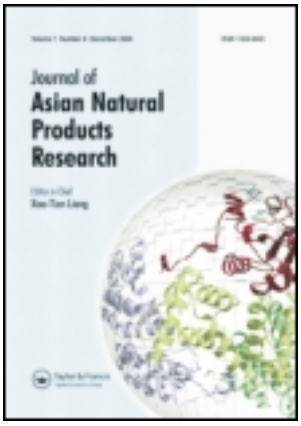

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Neuropective constituents from the rhizomes of *Abacopteris penangiana*

Han Wei [a], Guang-Hua Wu [a], Yong-Fang Lei [a], Chao-Mei Xiong [a b] & Jin-Lan Ruan [a]

[a] Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation of Hubei Province, Tongji Medical Center, College of Pharmacy, Huazhong University of Science and Technology, Wuhan, 430030, China

[b] Department of Pharmaceutical Analysis, Tongji Medical Center, College of Pharmacy, Huazhong University of Science and Technology, Wuhan, 430030, China

Available online: 13 Jul 2011

To cite this article: Han Wei, Guang-Hua Wu, Yong-Fang Lei, Chao-Mei Xiong & Jin-Lan Ruan (2011): Neuropective constituents from the rhizomes of *Abacopteris penangiana* , Journal of Asian Natural Products Research, 13:8, 707-713

To link to this article: http://dx.doi.org/10.1080/10286020.2011.586340

Journal of Asian Natural Products Research
Vol. 13, No. 8, August 2011, 707–713

Neuropective constituents from the rhizomes of *Abacopteris penangiana*

Han Wei[a], Guang-Hua Wu[a], Yong-Fang Lei[a], Chao-Mei Xiong[ab] and Jin-Lan Ruan[a]*

[a]*Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation of Hubei Province, Tongji Medical Center, College of Pharmacy, Huazhong University of Science and Technology, Wuhan 430030, China;* [b]*Department of Pharmaceutical Analysis, Tongji Medical Center, College of Pharmacy, Huazhong University of Science and Technology, Wuhan 430030, China*

(*Received 12 January 2011; final version received 4 May 2011*)

Two neuropective compounds were isolated from the rhizomes of *Abacopteris penangiana*, one was a new flavone and the other was a flavanone. Both compounds were firstly separated from natural plant. The isolation work was guided by the antioxidant activity. Both the compounds showed a significant antioxidant activity *in vitro* and a protective effect on dopamine-induced neurotoxicity in PC12 cells.

Keywords: *Abacopteris penangiana*; flavone; antioxidant activity; neuropective; PC12 cells

1. Introduction

Abacopteris penangiana (Hook.) Ching, a fern plant widely distributed in the south of China, has been used as a folk medicine to treat upper respiratory tract infections and dysentery [1]. Previous phytochemical investigation showed that rhizomes of *A. penangiana* were rich in antioxidant constituents [2]. In order to further investigate the antioxidant constituents of the title plant, macroporous resin HPD500 was employed to enrich and fractionate the extract of *A. penangiana*. The antioxidant activities were tested by various assays *in vitro*. A new flavone 5,2′,5′-trihydroxy-7-methoxyflavone (**1**, Figure 1), together with (2*S*)-5,2′,5′-trihydroxy-7-methoxyflavanone (**2**, Figure 1) firstly obtained from natural resource, was isolated from the active fraction D. Moreover, the 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity and protective effect against dopamine-induced neurotoxicity of the two compounds were further evaluated.

2. Results and discussion

Compound **1** was obtained as a yellow needle. Its molecular formula was determined to be $C_{16}H_{12}O_6$ based on its HR-ESI-MS at *m*/*z* 323.0527 $[M + Na]^+$. The IR spectrum showed absorption bands at 3404 (OH), 1693 (conjugated CO), 1616, and 1574 (aromatic C=C) cm^{-1}. In the ^{13}C NMR spectrum of **1** (Table 1), the carbon signals of two aromatic rings (δ 92.9, 98.4, 105.1, 114.0, 117.5, 118.5, 121.0, 150.2, 150.5, 157.9, 161.3, and 165.7), together with a double band (δ 109.7 and 161.9) and a carbonyl (δ 182.6), confirmed the presence of a typical flavone skeleton. The ^1H NMR spectrum (Table 1) indicated the presence of a methoxyl at δ 3.88 (3H, s), three hydroxyls at δ 9.16 (1H, s), 10.18 (1H, s), and 12.91(1H, s), an AB system at δ 6.39 (1H, d, $J = 2.2$ Hz) and δ

*Corresponding author. Email: jinlan8152@163.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.586340
http://www.informaworld.com

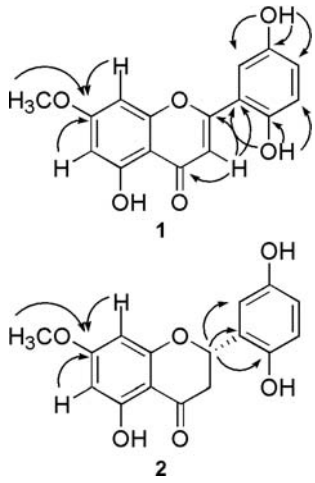

Figure 1. Structures and key HMBC correlations of **1** and **2**.

6.72 (1H, d, $J = 2.2$ Hz) and an ABX system at δ 6.87 (1H, dd, $J = 2.7$, 8.8 Hz), 6.90 (1H, d, $J = 8.8$ Hz), and 7.31 (1H, d, $J = 2.7$ Hz). The AB spin system and the correlation of 7-OCH_3 to C-7 in the HMBC spectrum (Figure 1) confirmed **1** as having 7-methoxy substitution in the A ring. The ABX spin system and the correlations of 2′-OH with C-1′, C-2′, C-3′, and 5′-OH with C-4′, C-5′, and C-6′ in the HMBC spectrum (Figure 1) supported **1** as having 2′,5′-dihydroxy substitution in the B ring. Furthermore, the ^{1}H and ^{13}C NMR spectra of **1** were very similar to those of 5,7,2′,5′-tetrahydroxyflavone [3]. Based on the above data, **1** was assigned as 5,2′,5′-trihydroxy-7-methoxyflavone.

(2*S*)-5,2′,5′-trihydroxy-7-methoxyflavanone (**2**) was firstly obtained through chemosynthesis method by Chan et al. [4]. Our study obtained **2** from the nature plant for the first time.

The antioxidant activities of fractions A–D obtained by macroporous resin column chromatography were investigated by assessing their roles in superoxide anion removal, hydrogen peroxide quenching ability, hydroxyl radicals trapping, potential lipid peroxidation inhibition, and

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for **1** and **2** (δ in ppm, J in Hz).

	1[a]			**2**[b]		
Position	δ_C	δ_H	DEPT[c]	δ_C	δ_H	DEPT
2	161.9	–	C	74.6	5.66 (dd, 12.7, 3.0)	CH
3	109.7	7.14 (s)	CH	41.7	2.74 (dd, 17.2, 3.0) 3.16 (dd, 17.2, 12.7)	CH_2
4	182.6	–	C	197.3	–	C
5	161.3	–	C	163.7	–	C
6	98.4	6.39 (d, 2.2)	CH	95.1	6.09 (d, 2.3)	CH
7	165.7	–	C	167.9	–	C
8	92.9	6.72 (d, 2.2)	CH	94.2	6.11 (d, 2.3)	CH
9	157.9	–	C	163.5	–	C
10	105.1	–	C	103.0	–	C
1′	117.5	–	C	125.6	–	C
2′	150.2	–	C	146.9	–	C
3′	118.5	6.90 (d, 8.8)	CH	116.7	6.67 (d, 8.6)	CH
4′	121.0	6.87 (dd, 8.8, 2.7)	CH	113.6	6.59 (dd, 8.6, 2.8)	CH
5′	150.5	–	C	150.4	–	C
6′	114.0	7.31 (d, 2.7)	CH	113.6	6.84 (d, 2.8)	CH
7-OCH_3	56.5	3.88 (s)	CH_3	56.4	3.79 (s)	CH_3
5-OH	–	12.91 (s)	–	–	12.07 (s)	–
2′-OH	–	10.18 (s)	–	–	–	–
5′-OH	–	9.16 (s)	–	–	–	–

Notes: [a]In DMSO-d_6.
[b] In CD_3OD.
[c] Distortionless enhancement by polarization transfer.

ABTS radicals scavenging activity. The results showed that fraction D had the most antioxidant activity among fractions A–D.

Additionally, **1** and **2** showed powerful antioxidant activity in ABTS scavenging activity assay (Figure 2). Previous research [5,6] demonstrated that **2** had a significant antioxidant activity. The results of ABTS radical scavenging activity assay of **2** in our study were in accordance with these researches.

Furthermore, the protective effect on dopamine-induced neurotoxicity of **1** and **2** was investigated in PC12 cells by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and confocal laser scanning fluorescence microscopy detection. As shown in Figure 3, 0.5 mM dopamine significantly reduced the cell viability and inversely **1**, **2**, or *N*-acetyl-L-cysteine (NAC, 5 μg/ml) protected dopamine-induced cell death. However, **1** and **2** had no effect by themselves on the cell viability. Additionally, nuclear staining with Hoechst 33258 demonstrated that normal PC12 cells had regular and round-shaped nuclei. In contrast, condensation and fragmentation of nuclei, characteristic of apoptotic cells, were demonstrated in cells treated with 0.5 mM dopamine. **1** and **2** attenuated the dopamine-induced nuclear damage. Mitochondria depolarization was considered as an irreversible step in the apoptosis process. In this respect, we detected the effect of **1** and **2** on the mitochondrial transmembrane potential. When PC12 cells were treated with dopamine, a decrease in the retention of rhodamine 123 was observed. **1** and **2** prevented the decrease in the retention of rhodamine 123 induced by dopamine. We further examined whether the inhibitory effect of **1** and **2** on dopamine-induced cell death was ascribed to the inhibited formation of reactive oxygen species (ROS) within cells by monitoring a conversion of 2′,7′-dichlorofluorescein diacetate (DCFH–DA) to 2′,7′-dichlorofluorescein (DCF). PC12 cells treated with 0.5 mM dopamine showed a significant increase in DCF fluorescence. Treatment with **1**, **2**, or NAC inhibited the dopamine-induced increase in DCF fluorescence (Figure 4). In conclusion, the results described above suggested that **1** and **2** had potential neuropective properties.

3. Experimental

3.1 General experimental procedures

All melting points were determined on an XT-4100Xb melting point apparatus and

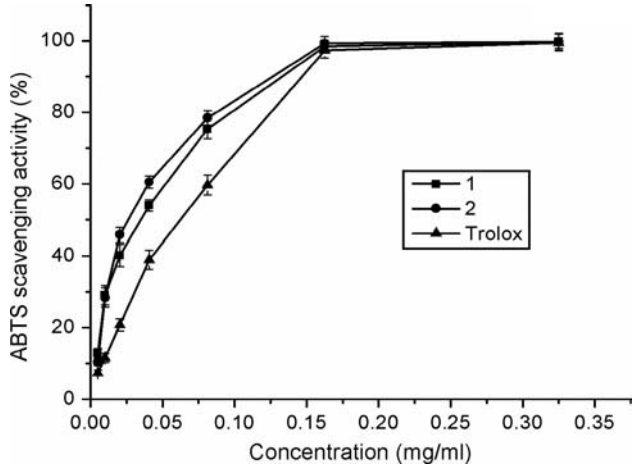

Figure 2. ABTS scavenging activity of **1** and **2**.

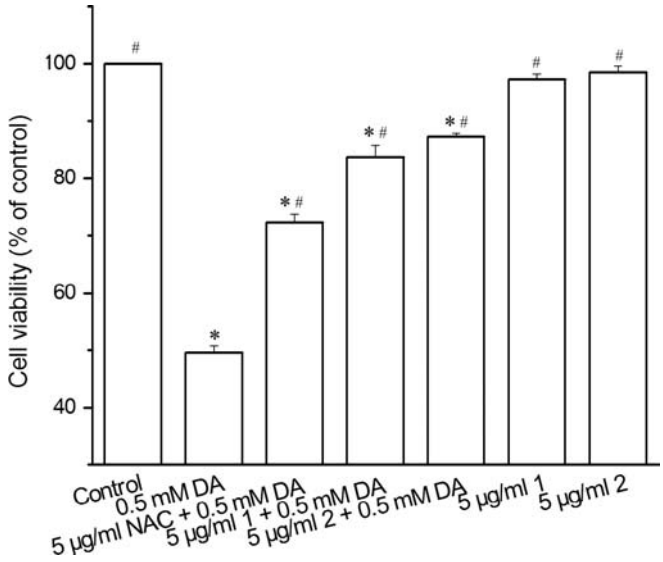

Figure 3. The cell viability of **1** and **2**.

are uncorrected. Optical rotation was determined on a PerkinElmer model 341 polarimeter. UV spectra were recorded on a Shimadzu UV-260 spectrophotometer. IR spectra were obtained on a PerkinElmer 577 spectrophotometer. NMR spectra were obtained on a Bruker AM-400 spectrometer using tetramethylsilane (TMS) as the internal standard. HR-ESI-MS and ESI-MS were obtained on a Marine Instrument. CD data were recorded on a JASCO J-810 Spectropolarimeter. The macroporous resin HPD500 was purchased from Bonherb Technology Company (Hebei, China). Silica gel (300–400 mesh, Qingdao Marine Chemical Company, Qingdao, China) and Sephadex LH-20 (Fluka BioChemika, Buchs, Switzerland) were used for column chromatography. All solvents used in this experiment were of analytical grade.

3.2 Plant material

The dried rhizomes of *A. penangiana* were collected from Jiujiang, Jiangxi Province, China, in June 2009 and authenticated by Prof. Ce-Ming Tan, Jiujiang Forest Plants Specimen Mansion. The voucher specimen (PZX0311) has been deposited in College of Pharmacy, Tongji Medical Center, Huazhong University of Science and Technology, Wuhan, China.

3.3 Extraction and isolation

Dried rhizomes (500 g) of *A. penangiana* were extracted with 80% ethanol. The solvent was evaporated under reduced pressure to yield the ethanolic extract (90 g). The extract (15 g) was fractionated by macroporous resin HPD500 (EtOH–H_2O, 20:80, 40:60, 60:40, and 80:20, each 3000 ml) to give fractions A (2.0 g), B (5.9 g), C (2.8 g), and D (1.5 g), respectively. Fraction D (300 mg) was repurified by silica gel column chromatography (300–400 mesh) using $CHCl_3$–MeOH (30:1) to yield compound **1** (24 mg) and fraction D1 (105 mg). Fraction D1 was subjected to Sephadex LH-20 using $CHCl_3$–MeOH (1:1) and then further purified on a silica gel (300–400 mesh) column, eluting with $CHCl_3$–MeOH (30:1) to give compound **2** (11 mg).

3.3.1 5,2′,5′-Trihydroxy-7-methoxyflavone (1)

Yellow needles; mp 172–173°C; UV (CH_3OH) λ_{max} log (ε): 249 (4.13), 270

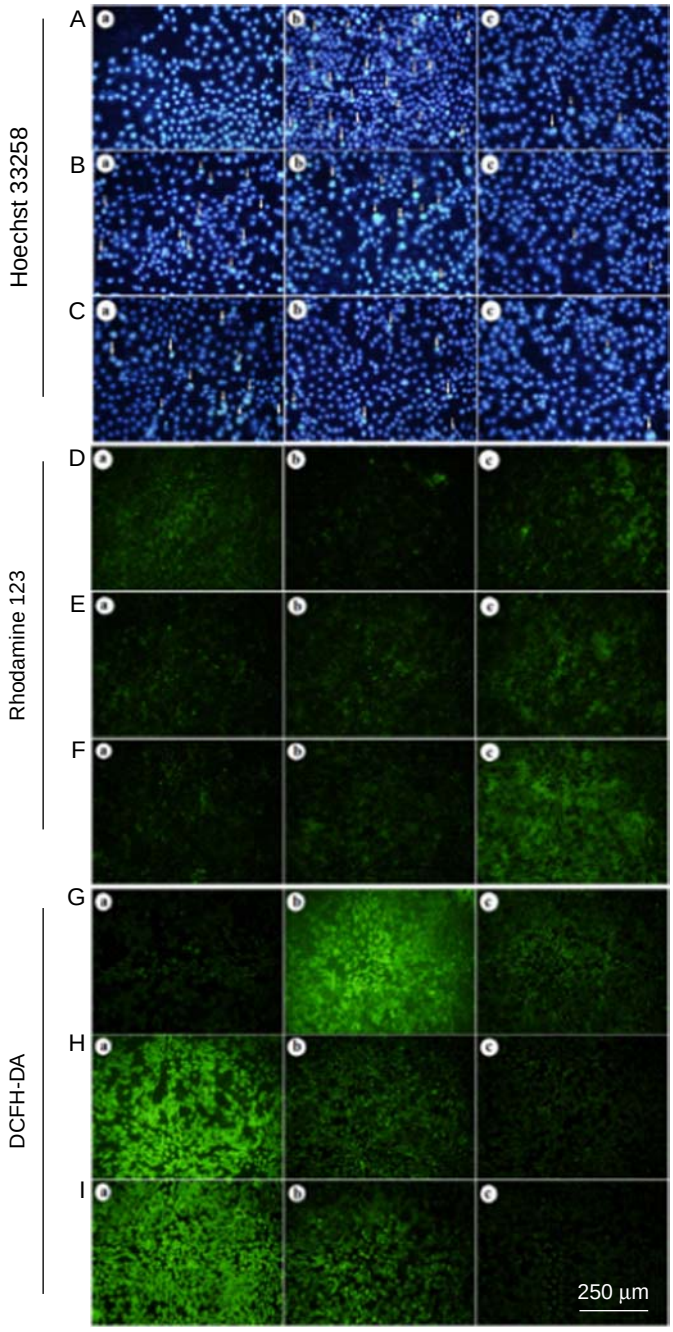

Figure 4. Protective effect of **1** and **2** on dopamine-induced nuclear damage, mitochondrial transmembrane potential ($\Delta\Psi_m$) dysfunction, and intracellular accumulation of ROS in PC12 cells. (A, D, G) PC12 cells exposed to vehicle alone (a), 0.5 mM dopamine for 12 h after 24 h preincubation with 0 μM (b), 300 μM (c) NAC. (B, E, H) PC12 cells exposed to 0.5 mM dopamine for 12 h after 24 h preincubation with 3 μM (a), 10 μM (b), and 20 μM (c) **1**. (C, F, I) PC12 cells exposed to 0.5 mM dopamine for 12 h after 24 h preincubation with 3 μM (a), 10 μM (b), and 20 μM (c) **2**. Arrows in A–C indicate the apoptosis nuclei of PC12 cells.

(3.87), and 346 (4.10) nm; IR (KBr, cm^{-1}): ν_{max} 3404 (OH), 1693 (conjugated CO), 1616, and 1574 (aromatic C=C); ^1H and ^{13}C NMR spectral data see Table 1; HR–ESI–MS: *m/z* 323.0527 $[M + Na]^+$ (calcd for $C_{16}H_{12}O_6$, 323.0522).

*3.3.2 (2S)-5,2′,5′-trihydroxy-7-methoxy flavanone (**2**)*

Colorless needle; mp 266–267°C; [α]25 D-9.5 ($c = 0.15$, CH_3OH); UV (CH_3OH) λ_{max} log (ε): 219 (4.38), 287 (4.30), and 335 (3.76) nm; ^1H and ^{13}C NMR spectral data, see Table 1; The configuration at C-2 was defined as *S* from the CD spectrum, which showed a positive Cotton effect at 326 nm and a negative Cotton effect at 287 nm [7]; ESI–MS: *m/z* 325 $[M + Na]^+$.

3.4 Antioxidant activity of fractions A–D

The effect of scavenging superoxide radical was determined by the nitroblue tetrazolium reduction method [8]. Hydrogen peroxide scavenging activity was measured by using the modified method of peroxidase-dependent oxidation of phenol red [9]. The effect of fractions on hydroxyl radical was assessed by using the deoxyribose method [10]. The lipid peroxidation inhibition activity was determined using the ferric thiocyanate method [11]. ABTS radical scavenging activity was measured using an improved ABTS method as described by Re et al. [12]. Trolox was used as a positive control in all tests. All tests were run in triplicate and were averaged.

3.5 ABTS radical scavenging activity of 1 and 2

ABTS radical scavenging activity was measured using an improved ABTS method as described by Re et al. [12]. Trolox was used as a positive control in the test. All tests were run in triplicate and were averaged. Data were presented as mean ± SEM.

3.6 Protective effect of 1 and 2 on dopamine-induced neurotoxicity in PC12 cells

Differentiated PC12 cells were plated at a density of 1×10^5/100 μl in 96-well plates and grown for 24 h before treatment. Consequently, PC12 cells were preincubated for 24 h with **1**, **2**, or NAC, the positive control, respectively. PC12 cells were then exposed for 12 h with 0.5 mM dopamine. The cell viability was determined by the conventional MTT reduction assay. All tests were run in triplicate and were averaged. Data were presented as mean ± SEM. $^*p < 0.01$ versus control group and $^{\#}p < 0.01$ versus DA group.

3.7 Confocal laser scanning fluorescence microscopy detection

The treated PC12 cells (1×10^5/500 μl in 24-well plates) were dyed with the Hoechst 33258, rhodamine 123, and DCFH–DA. The fluorescence microscopy imaging was examined under an Olympus 1X71 fluorescence confocal microscope (Hoechst 33258: excitation, 488 nm; emission, 510 nm; rhodamine 123: excitation, 488 nm; emission, 510 nm; and DCFH–DA: excitation, 488 nm; emission, 525 nm).

Acknowledgements

This work was financially supported by grants from the National Natural Science Foundation of China (No. 30973864).

References

[1] Administration Bureau of National Chinese Traditional Medicine, *Chinese Herbal* (Shanghai Scientific and Technical Publisher, Shanghai, 1998), p. 164.
[2] Z.X. Zhao, J.L. Ruan, J. Jin, J. Zou, D.N. Zhou, W. Fang, and F. Zeng, *J. Nat. Prod.* **69**, 265 (2006).

[3] Y. Miyaichi, E. Hanamitsu, H. Kizu, and T. Tomomori, *Chem. Pharm. Bull.* **54**, 435 (2006).
[4] W.L. Chan, Y.C. Lin, W.H. Zhang, P.L. Tang, and Y.S. Szeto, *Heterocycles* **43**, 551 (1996).
[5] J.H. Zhao and S.B. Xu, *Chin. Pharmacol. Bull.* **13**, 438 (1997).
[6] H.L. Pu, J.H. Zhao, and S.B. Xu, *Chin. Tradit. Herb. Drugs* **31**, 113 (2000).
[7] W. Gaffield, *Tetrahedron* **26**, 4093 (1970).
[8] M. Nishikimi, N.A. Rao, and K. Yagi, *Biochem. Biophys. Res. Commun.* **46**, 849 (1972).
[9] R.J. Ruch, S.J. Cheng, and J.E. Klaunig, *Carcinogenesis* **10**, 1003 (1989).
[10] O.I. Aruoma and B. Halliwell, *Biochem. J.* **248**, 973 (1987).
[11] L.W. Chang, W.J. Yen, S.C. Huang, and P.D. Duh, *Food Chem.* **78**, 347 (2002).
[12] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, *Free Rad. Biol. Med.* **26**, 1231 (1999).