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苏铁蕨的化学成分

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摘要：从蕨类植物苏铁蕨 (*Brainea insignis*) 中分离得到 1 个新的苯乙烯基吡喃酮苷，利用波谱技术鉴定了其结构。此外，还得到了 1 个已知的苯乙烯基吡喃酮苷和 4 个其它已知化合物。同时，还测定了分离得到的两个苯乙烯基吡喃酮苷化合物的 DPPH 自由基清除活性。

关键词：蕨类植物；苏铁蕨；苯乙烯基吡喃酮苷；DPPH 自由基清除活性
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Chemical Constituents from the Fern *Brainea insignis* (Blechnaceae)

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Abstract: A new styryl-2-pyrone glucoside (1) and a known styryl-2-pyrone glucoside (2), together with four other known compounds (3 - 6), were isolated from the ethanol extract of the fern *Brainea insignis*. The structures of these compounds were determined by means of spectroscopic analysis including 1D and 2D NMR, HR-MS. And the capacities to scavenge against DPPH radical of two styryl-2-pyrone glucosides were assayed.

Key words: Fern; *Brainea insignis*; Styryl-2-pyrone glycoside; DPPH radical scavenging activity

Braniea insignis (Hook.) J. Smith is a monotype genus plant belonging to Blechnaceae. As an ancient fern species, its early fossils belong to the Mesozoic era, near 400 million years ago. Now it is mainly distributed in the South of China, and used as a Chinese traditional medicinal herb for the treatment of common cold, suffer burn, trauma bleeding, ascarid disease etc. (Wu, 1990). A new flavonoid glycoside and four lignans have been isolated from *Brainea insignis* (Murakami *et al.*, 1986; Wada *et al.*, 1992). In the present paper, we report the isolation and structural elucidation of a new styryl-2-pyrone glucoside (1), a known styryl-2-pyrone glucoside (2), and four other known compounds (3 - 6), from *Brainea insignis* (Fig. 1). And the activities of two styryl-2-pyrone glucosides in scavenging DPPH radical were also reported.

Compound 1 was obtained as a yellow amorphous powder. The positive-ion HRESI-MS spectrum gave a $[M + H]^+$ ion peak at *m z* 393.1168 (calcd. *m z* 393.1186), indicating a molecular formula of $C_{19}H_{20}O_9$, with ten degree of unsaturation. In addition, the ESI-MS gave a $[M+H-162]^+$ ion at *m z* 231, which showed that compound 1 might be a glycoside. Its ^1H NMR and ^{13}C NMR spectra (Table 1) confirmed that it

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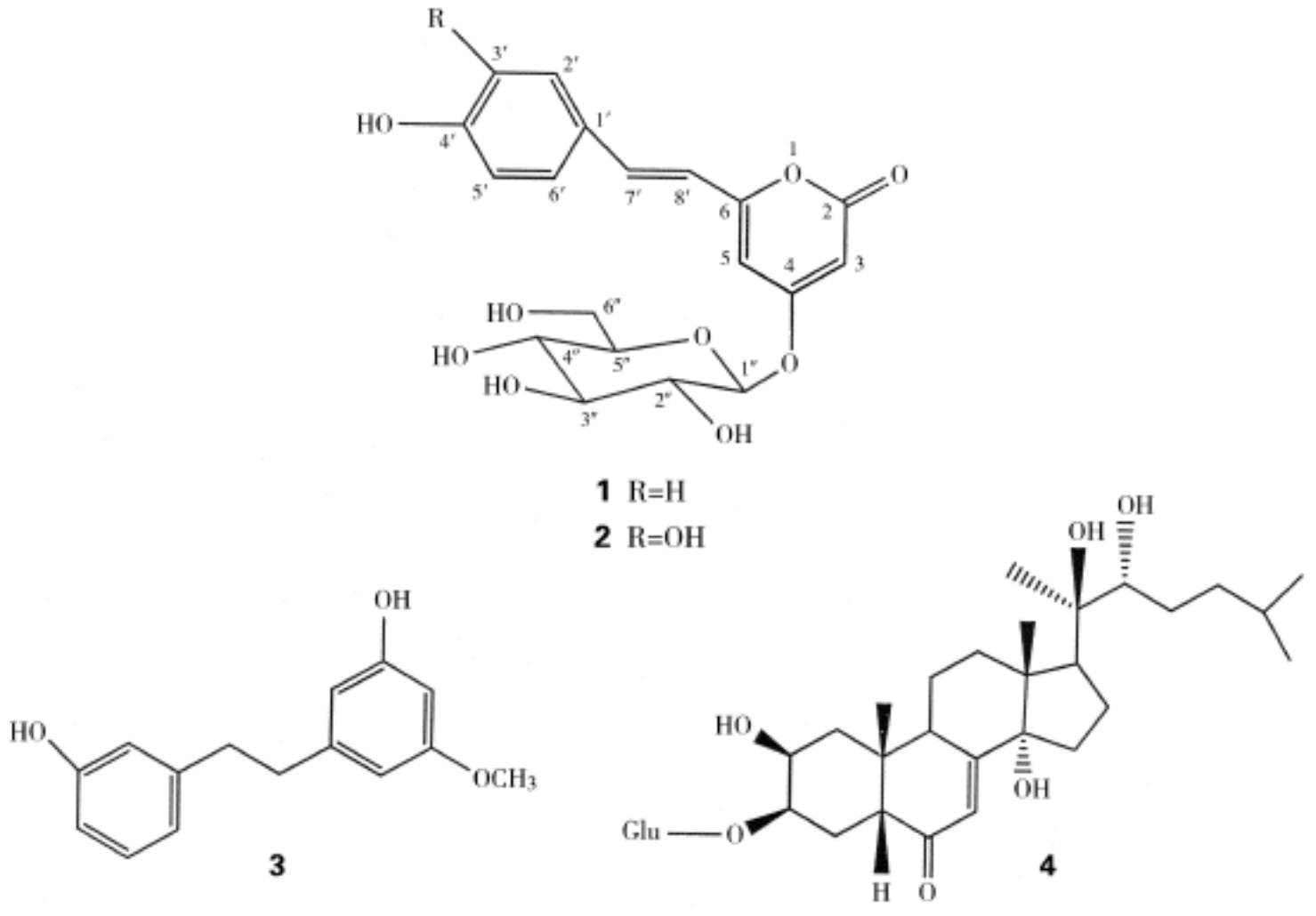

Fig . 1 Structures of compounds 1 - 4

Table 1 ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound 1 in C_5D_5N

No .	δ_C	δ_H
2	165.1	
3	94.0	6.21 (1H, s)
4	170.3	
5	101.8	6.22 (1H, s)
6	162.0	
1′	128.7	
2′	131.5	7.48 (1H, d, 7.8)
3′	118.4	7.14 (1H, d, 7.8)
4′	162.3	
5′	118.4	7.14 (1H, d, 7.8)
6′	131.5	7.48 (1H, d, 7.8)
7′	136.8	7.53 (1H, d, 16.4)
8′	118.1	6.67 (1H, d, 16.4)
1″	102.7	5.73 (1H, d, 7.4)
2″	76.1	4.27 (1H, br d, 6.6)
3″	80.0	4.30 (1H, m)
4″	72.5	4.32 (1H, m)
5″	80.9	4.11 (1H, m)
6″	63.7	4.34 (1H, br d, 11.2)
		4.29 (1H, br d, 12.2)

was a mono-O-β-glucoside with the anomeric proton at δ 5.73 (1H, d, J = 7.4 Hz, H - 1″) and the anomeric carbon at δ102.7 (C - 1″) . The ^{1}H NMR spectrum exhibited an AA′BB′ system at δ 7.48 (2H, d, J = 7.8 Hz, H - 2′, 6′), 7.14 (2H, d, J = 7.8 Hz, H - 3′, 5′), indicating the presence of a *p*-disubstituted phenyl ring . And the ^{1}H NMR signals of a *trans* double bond was noted at δ 6.67 (1H, d, J = 16.4 Hz, H - 8′), 7.53 (1H, d, J = 16.4 Hz, H - 7′) . The HMBC spectrum showed the correlations (Fig . 2) between H - 8′ and C - 1′ (δ 128.7) suggesting the presence of an E-styryl moiety . The 2-pyrone unit was suggested by a detail comparison of ^{13}C NMR and ^{1}H NMR data with the literatures (Kraut *et al.*, 1996; Mcglacken and Fairlamb, 2005), and was identified as a 4, 6-disubstituted 2-pyrone with two proton signals at δ 6.22 (H

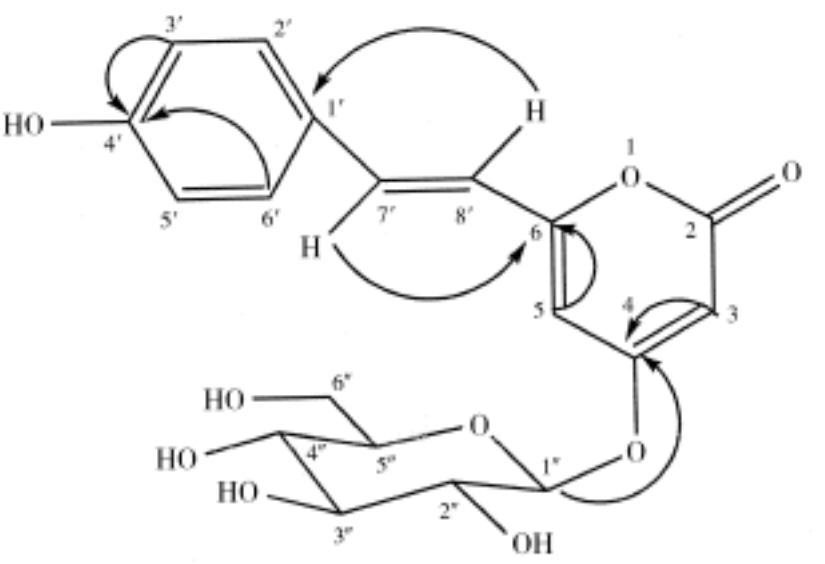

Fig . 2 Key HMBC correlations of compound 1

-5, s) and 6.21 (H-3, s) in ^{1}H NMR spectrum. The HMBC correlations clearly revealed that H-7′ in the E-styryl moiety was long-range coupled to the C-6 in 2-pyrone unit, and the anomeric proton (H-1″) of the glucose moiety was long-range coupled to the C-4 (δ 171.3). Therefore, compound 1 was determined to be bisnoryangonin-4-O-β-D- glucopyranoside.

Compound 2 was also obtained as a yellow amorphous powder. It displayed a $[M+H]^+$ peak at *m z* 409.1117 (calcd. *m z* 490.1135) in the positive ion HRESIMS, indicating a molecular formula of $C_{19}H_{20}O_{10}$. Compared with 1, all of the ^{13}C NMR data were very similar to that of the compound 1, except for the signals δ 149.2 (C-4′), δ 146.5 (C-3′). This indicated that there are two *o*-hydroxyls in E-styryl moiety of compound 2. In ^{1}H NMR spectrum, the signals of δ 6.96 (1H, d, *J* = 6.2 Hz, H-6′), 6.78 (1H, d, *J* = 6.2 Hz, H-5′), 7.05 (1H, s, H-2′) also proved this conclusion. Therefore, the structure of compound 2 was established as hispidin-4-O-β-D-glucopyranoside, which had been isolated from fern *Pteris ensiformis* (Chen *et al*., 2007).

Compound 3 was identified as batatasin (III) by analysis of its ^{1}H, ^{13}C NMR and MS spectra (Gao *et al*., 2006). Compound 4 was identified as ponasteroside A by comparison of its physicochemical and NMR data with the reported in the literature (Zhu *et al*., 2000; Hikino *et al*., 1969). And β-sitosterol (5), daucosterol (6) were identified by comparison with their authentic samples.

The research into natural products as health protecting factors against oxidative damage is an interesting field. DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical that loses its purple color when accepts an electron from an antioxidant compound (Bao *et al*., 2004; Marino *et al*., 2007). In this paper, the radical scavenging capacities of the isolates (1 and 2) from *B. insignis* were evaluated by investigating them against the DPPH radical. The substances were assayed at three certain concentration of 0.01, 0.03, 0.06 mg ml, and their activities were compared with rutin, a known natural antioxidant compound. The results were reported in Table 2.

Table 2 Radical DPPH scavenging activity

Concentration (mg ml)	Rutin	Scavenging activity (SC%)	
		Compound 1	Compound 2
0.01	75.78	6.17	15.52
0.03	90.71	17.87	42.95
0.06	92.06	29.07	73.43

Our previous investigation showed that the extract of *B. insignis* exhibited high phenolic content and strong radical DPPH scavenging activity (Ding *et al*., 2008). In this work, all the substances showed activity against the radical DPPH, but rutin was more active than compound 1 and 2 at the tested contraction. Because compound 2 bears two phenolic hydroxyl groups on molecular skeleton, its observed activity was stronger than that of compound 1 which only has one phenolic hydroxyl group in its structure. This indicted that the phenolic hydroxyl group is the mainly active group for the activity against the radical DPPH.

Experimental

General Experimental Procedures Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^{1}H, ^{13}C NMR and 2D NMR spectra were measured on a Bruker DRX-500 NMR spectrometer (TMS as internal standard, δ in ppm, *J* in Hz). HRESIMS were obtained on an agilent G3250AA mass spectrometer. For column chromatography, silica gel (200-300 mesh, Qingdao Marine Chemical Inc., China). Lichroprep RP-18 (40-60 μm, Merck, Darmstadt, Germany) and Sephadex LH-20 (25-100 μm, Amersham Biosciences, Sweden) were used. A shimazu UV-VIS-PC 2410 spectrometer was used in DPPH radical scavenging activity test.

Plant Material The whole plant of *Brainea insignis* (Hook.) J. Smith was collected in February 2006, in Yunnan province, P. R. China. A voucher specimen (2006D011) was deposited in the school of Chemical Science and Technology of Yunnan University, and was identified by Prof. Shu-Gang Lu.

Extraction and Isolation The air-dried plant powder of *Brainea insignis* (1.4 kg) was extracted with EtOH for 24 h and filtered. The filtrate was evaporated in vacuo to give a residue. Then the residue (55 g) was subjected to column chromatography on silica gel, and was eluted with $CHCl_3$-MeOH (9∶1) to give four fractions. Fr. III was subjected to Sephadex LH-20 column chromatography eluting with MeOH to afford compound 1 (25 mg), 2 (12 mg), 4 (15 mg), 6 (30 mg), and were purified by RP-18 column chromatography eluting with CH_3OH-H_2O (9∶1).

Fr . II was subjected to silica gel column chromatography eluting with EtOAc-MeOH (96∶4) to afford compound 3 (10 mg). Compound 5 (50 mg) was from Fr . I .

Bisnoryangonin-4-O-β-D-glucopyranoside (1): yellow amorphous powder . $[\alpha]_D^{22.5}$ - 44.13 (*c* 0.64, MeOH) . ^{1}H- and ^{13}C-NMR data see table 1 . HR-ESI-MS: *m z* 393.1168 [M + H]$^+$ (calcd . for $C_{19}H_{21}O_9$ 393.1186) .

DPPH Radical Scavenging Assay DPPH radical scavenging activity was examined by the described method (Bao *et al.*, 2004; Marino *et al.*, 2007) . In brief, 3.0 ml each sample in ethanol was added to 2.0 ml DPPH (2.0×10^{-4} mol L) solution and incubated at room temperature for 30 min . Then the absorbance of the reaction mixture was determined at 517 nm on a shimazu UV-VIS-PC 2410 spectrometer .

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