· Letters ·

A New Flavan-4-ol Glycoside from *Pronephrium triphyllum*

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Abstract: **Objective** To study the chemical constituents of *Pronephrium triphyllum*. **Methods** The chemical constituents in the plant were isolated and purified with silica gel and Sephadex LH-20. Their structures were identified by analyses of spectral data and physicochemical properties. **Results** Six compounds were isolated and identified as shelincaoide A (**1**), *n*-butyl-β-*D*-fructopyranoside (**2**), triphyllin A (**3**), 6,7-di-hydroxycoumarin (**4**), daucosterol (**5**), and β-sitosterol (**6**), respectively. **Conclusion** Compound **1** is found to be a new compound. Compounds **2** and **4** are firstly isolated from the plants in *Pronephrium* Presl. and all compounds except **3** are obtained from the species for the first time.

Key words: 6,7-di-hydroxycoumarin; flavan-4-ol glycoside; *Pronephrium triphyllum*; shelincaoide A; triphyllin A

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Introduction

The genus *Pronephrium* Presl. (Thelypteridaceae) comprises 61 species that are found in the world and among them 18 are found in China (Flora of China Committee of Chinese Academy of Sciences, 1999). *P. triphyllum* (Sw.) Holtt. is widely distributed in the south of China and has been used as a folk medicine to treat ulcerative carbuncle, venomous snake bite, fracture, eczema, itch, and acute and chronic tracheitis. It is renowned to be effective in heat-clearing and detoxicating, detumescence and eliminating stasis, and resolving phlegm and relieving cough (Chinese Materia Medica Committee of State Administration of Traditional Chinese Medicine, 1999). Previous phytochemical investigations on Thelypteridaceae ferns showed the presence of some unusual flavan-4-ol glycosides (Zhao *et al*, 2006; 2007). So far, compounds triphyllin A, B, and C have been found from *P. triphyllum* (Tanaka *et al*, 1985). Since then there have been no further records on the chemical constituents. To seek the substantial basis of treatment for diseases, the whole plant of *P. triphyllum* has been studied. In this paper, we described the isolation and structure elucidation of one new flavan-4-ol glycoside, as well as five known compounds.

Materials and methods

Equipments

Melting points were determined on an X—4 Micro Melting Point Apparatus. UV spectra were measured with a Shimadzu UV—2550 Spectrometer. The IR spectra were recorded on a Nicolet Avatar 330 FT-IR Spectrometer, as KBr pellets. CD spectra were measured with a Chirascan Circular Dichroism Spectrometer. MS spectra were recorded on a Finnigan LCQ-DECA Spectrometer. HR-ESI-TOP-MS were obtained on a Shimadzu LC-MS-IT-TOF Spectrometer. NMR spectra were measured on a Bruker AV—400 Spectrometer. Silica gel for column chromatography and GF_{254} silica gel for TLC were produced by Qingdao Marine Chemistry Company and Sephadex LH-20 for column chromatography wzx produced by Fluka BioChemika.

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Solvents and chemicals were of analytical grade.

Plant material

The whole plants of *Pronephrium triphyllum* (Sw.) Holtt. were collected in Zhongshan (Guangdong, China), in 2007, and was taxonomically identified by Prof. PAN Chao-mei. A specimen (20070214) was deposited in the Pharmacy Department of Zhongshan Hospital of Traditional Chinese Medicine.

Extraction and isolation

The air-dried whole plants (8.5 kg) were extracted for three times with 95% EtOH under reflux for 9 h. After evaporation, the extract was suspended in water and partitioned with petroleum ether, EtOAc, and *n*-BuOH, sequentially. The *n*-BuOH extract (24 g) was chromatographed on silica gel column (200—300 mesh) eluted with $CHCl_3$-MeOH (30:1→5:1) to yield compounds **1** (9 mg), **2** (23 mg), and **3** (4.8 g). The EtOAc extract (80 g) was chromatographed on silica gel column (200—300 mesh) eluted with $CHCl_3$-MeOH (50:1→1:1), then subjected to silica gel and Sephadex LH-20 to yield compounds **4** (32 mg) and **5** (24 mg). The petroleum ether extract was chromatographed on silica gel column eluted with petroleum-acetone to yield compound **6** (34 mg).

Results and discussion

Compound **1**: White needles, mp 302—304 ℃. The molecular formula, $C_{30}H_{36}O_{14}$, was determined by HR-ESI-MS spectrum (*m*/*z*: 643.2008 $[M + Na]^+$ calcd for $C_{30}H_{36}O_{14}Na$, 643.2003). ^1H-NMR and ^{13}C-NMR data were shown in Table 1. The IR spectrum indicated the presence of hydroxyl (3422 cm^{-1}) and phenyl (1610, 1517, and 1461 cm^{-1}) groups (Fig. 1A).

The ^1H-NMR data of compound **1** showed characteristic signals for one methyl at δ 2.06, one methoxy at δ 3.78, two anomeric protons at δ 4.39 (d, J = 7.3 Hz) and 5.16 (d, J = 8.5 Hz), and the AA'BB' coupling system at δ 7.43 (2H, d, J = 8.7 Hz) and 7.02 (2H, d, J = 8.7 Hz), suggesting a *para*-substituted phenyl group (Table 1).

The ^{13}C-NMR data of compound **1** analyzed with the aid of the DEPT and HSQC spectra revealed there were one *para*-substituted and one fully-substituted phenyl groups, and one aromatic methyl group in the aglycon. The analysis of NMR data showed there were two sugar units in compound **1**. On acid hydrolysis of compound **1**, only *D*-glucose was detected by GC, suggesting that two sugars be all *D*-glucoses. The β-configuration of the glycosidic bonds was deduced from the coupling constants of the anomeric protons and the ^{13}C-NMR data of the sugar units (Table 1).

A substructure, -CH-CH_2-CH-, was confirmed in the aglycon through an ^1H-^1H COSY experiment of compound **1**, from the correlations of H-2 (δ 4.83) with H-3 (δ 2.24 and 2.12) and H-4 (δ 5.06) with H-3. In the HMBC spectrum of compound **1**, correlations of H-2 (δ 4.83) with C-2',6' (δ 127.8), H-2' (δ 7.43) with C-2 (δ 73.3), and H-4 (δ 5.06) with C-5 (δ 148.0), C-9 (δ 153.0), and C-10 (δ 108.0) (Fig. 1B) suggested that two ends of -CH-CH_2-CH- were linked to a *para*-substituted phenyl and a fully-substituted phenyl groups, respectively. The chemical shift of C-2 (δ 73.3) carbon indicated it was an oxygenated methine carbon. The above structural characteristics suggested the existence of a flavan moiety in compound **1**.

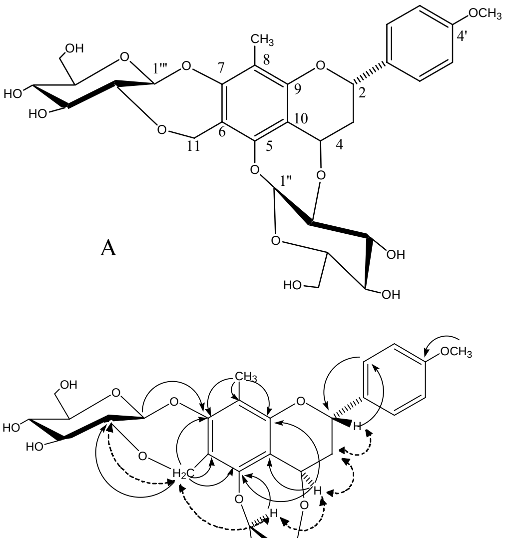

Fig. 1 Chemical structure (A) and key HMBC and NOESY correlations (B) of compound 1

As observed in the HMBC spectrum, correlations of H-1'' (δ 5.16) with C-5 (δ 148.0) and H-1''' (δ 4.39) with C-7 (δ 154.4) suggested that two *D*-glucoses were attached to C-5 and C-7, respectively. The correlation of H-11 (δ 5.16 and 4.24) with C-6 (δ 118.3), C-7 (δ 154.4), and C-8 (δ 113.6) suggested that C-11 (δ 64.1) was bonded to C-6, and the correlation of H-11 with

Table 1 ^{13}C-NMR (100 MHz) and ^{1}H-NMR (400 MHz) data of compound 1 and abacopterins D and I in DMSO-d_6

Position	Compound **1**		Abacopterin D		Abacopterin I	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	73.3	4.83 (br d *J* = 11.6 Hz)	72.4	5.25 (dd, *J* = 1.6, 12.0 Hz)	73.8	4.98 (br d, *J* = 12.0 Hz)
3	35.7	2.24 (br d, *J* = 14.6 Hz)	36.5	2.06 (m)	37.1	2.27 (br d, *J* = 14.0 Hz)
		2.12 (m)		1.86 (br t, *J* = 12.0 Hz)		1.96 (ddd, *J* = 14.0, 12.0, 3.0 Hz)
4	64.5	5.06 (br d, *J* = 3.3 Hz)	56.7	4.95 (s)	65.8	5.19 (d, *J* = 3.0 Hz)
5	148.0		150.3		151.3	
6	118.3		119.8		116.5	
7	154.4		153.9		156.1	
8	113.6		114.8		117.3	
9	153.0		152.7		152.0	
10	108.0		115.5		109.0	
11	63.7	5.16 (d, *J* = 13.1 Hz)	64.1	5.43 (br d, *J* = 13.0 Hz)		
		4.24 (d, *J* = 13.6 Hz)		4.34 (br d, *J* = 13.0 Hz)		
Me-6					11.1	2.87 (s)
Me-8	8.7	2.06 (s)	9.1	2.02 (s)	10.7	2.65 (s)
1'	132.0		133.3		133.4	
2',6'	127.8	7.43 (d, *J* = 8.7 Hz)	127.7	7.38 (d, *J* = 8.8 Hz)	128.2	7.25 (d, *J* = 8.7 Hz)
3',5'	113.9	7.02 (d, *J* = 8.7 Hz)	114.8	6.96 (d, *J* = 8.8 Hz)	114.2	6.97 (d, *J* = 8.7 Hz)
4'	159.1		159.0		159.9	
OMe-4	55.1	3.78 (s)	55.2	3.76 (s)	55.2	3.76 (s)
O-Glc-5						
1''	100.0	5.16 (d, *J* = 8.5 Hz)	105.3	4.44 (d, *J* = 7.6 Hz)	102.2	5.58 (d, *J* = 8.4 Hz)
2''	74.6	3.08 (t, *J* = 8.8 Hz)	73.9	3.22—3.26 (m)	79.6	4.02 (m)
3''	74.2	3.42 (m)	76.2	3.22 (m)	76.1	4.01 (m)
4''	70.1	2.94 (m)	70.1	3.11 (m)	71.7	3.96 (br t, *J* = 8.5 Hz)
5''	77.6	3.22—3.26 (m)	76.7	3.16 (m)	76.5	4.34 (m)
6''	60.9	3.66 (m)	61.0	3.43 (dd, *J* = 6.6, 11.5 Hz)	62.8	4.55 (br d, *J* = 11.1 Hz)
		3.41 (m)		3.64 (br d, *J* = 11.5 Hz)		4.36 (m)
O-Glc-7						
1'''	102.9	4.39 (d, *J* = 7.3 Hz)	102.2	4.33 (d, *J* = 7.2 Hz)	106.0	5.41 (d, *J* = 7.4 Hz)
2'''	85.7	3.20 (m)	85.8	3.18 (m)	75.8	4.32 (m)
3'''	73.9	3.22—3.29 (m)	73.9	3.22—3.26 (m)	78.3	4.30 (m)
4'''	69.4	3.22—3.26 (m)	69.6	3.20 (m)	71.4	4.28 (m)
5'''	77.9	3.22—3.26 m	78.0	3.21 (m)	78.5	3.87 (m)
6'''	60.5	3.72 (m)	60.5	3.50 (dd, *J* = 2.9, 11.7 Hz)	62.8	4.38 (m)
		3.53 (m)		3.68 (br d, *J* = 11.7 Hz)		4.35 (m)

C-2''' (δ 85.7) in the HMBC spectrum (Fig. 1B) confirmed that C-2''' of the sugar (located at C-7) formed an ether linkage to C-11. Thus, between the sugar and the aglycon, an unusual heptacyclic ring was formed. An HMBC correlation of MeO-4' (δ 3.78, s) to C-4' (δ 159.1) established the location of the methoxy at C-4'.

Comparison of the above NMR data of compound **1** with those of abacopterin D [(2*S*,4*R*)-4-hydroxy-4'-methoxy-8-methyl-11,2'''-oxidoflavan-5,7-di-*O*-β-*D*-glucopyranoside] (Zhao *et al*, 2006), which was a flavan-4-ol glycoside isolated from the rhizomes of *Abacopteris penangiana* (Hook.) Ching (Thelypteridaceae), showed that compound **1** had a similar structure, except for the carbon signals of C-4, C-10, and C-1''. In addition, the molecular weight of compound **1** was just 18 less than that of abacopterin D; Moreover, the chemical shift of C-4 (δ 64.5) was 7.8 downfield as observed for C-4 (δ 56.7) of abacopterin D, but similar with C-4 (δ 65.8) of abacopterin I (Zhao *et al*, 2007). Thus, it was postulated that C-2'' might be the binding site to the aglycon. The correlations of H-2'' (δ 3.08) with C-4 (δ 56.7) and H-4 (δ 5.06) with C-2'' (δ 74.6) in the HMBC spectrum (Fig. 1B) confirmed that C-2'' of the sugar (located at C-5) was attached to C-4. Thus, C-5, C-10, C-4, C-1'', and C-2'' formed a heptacyclic ring, including two oxygen atoms.

To determine the configurations at C-2 and C-4, an NOESY experiment was performed, and the correlation between H-4 and H-1'' was observed (Fig. 1B), which indicated that the configuration at C-4 was *R*. The coupling constants of H-2 (br d, J = 11.6 Hz) and H-4 (br d, J = 3.3 Hz) suggested that the stereochemical relationship between H-2 and H-4 was *trans* (Pouget *et al*, 2000). Therefore, the configuration at C-2 was deduced to be *S*. The CD spectrum and coupling constants of H-2 and H-4 were compared with those of abacopterin I to further confirm the configurations at C-2 and C-4. A positive Cotton effect at 282 nm ($\Delta\varepsilon$ + 1.91) was observed in the CD spectrum of compound **1**, which was quite comparable to that of abacopterin I, and the coupling constants of H-2 (br d, J = 11.6 Hz), and H-4 (br d, J = 3.3 Hz) of compound **1** were also consistent with those of abacopterin I. The results showed that compound **1** had the same configuration (2*S*,4*R*) as abacopterin I. On the basis of the above evidences, the structure of compound **1** was established as (2*S*,4*R*)-4'-methoxy-8-methyl-[(4,2''), (11,2''')]-dioxidoflavan-5,7-di-*O*-β-*D*-glucopyranoside named shelincaoide A.

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