A New Neolignan Glycoside from *Pteris multifida* Poir.

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Key Words : *Pteris multifida*, rel-(7*S*,8*S*)-$\Delta^{7'}$-2,9'-Dihydroxy-5'-methoxy-7,3'-dioxy-8,4'-neolignan-4-*O*-*β*-*D*-apiofuranosyl-(1→6)-*β*-*D*-glucopyranoside, Multifidoside A

Pteris multifida Poir. is widely distributed in the south and southwest district of China (Chinese name "fengweicao"),[1] which has been mainly used as a traditional Chinese folk drug for the treatment of eczema, haematemesis, enteritis, diarrhea, bacillary dysentery cold and are also known to have anticancer and antibacterial effects.[2] However, to the best of our knowledge, very little is known about its chemical constituents except for antimutagentic activity.[3] Our previous paper reported the isolation and characterization of six compounds from EtOAc fraction obtained by partition of the EtOH extract.[4] In a continuation of the phytochemical research on this plant, we now report the isolation and structural elucidation of a new neolignan glycosides, multifidoside A (**1**) from the *n*-BuOH fraction of the EtOH extract, along with four known compounds, scaphopetalone (**2**),[5] (−)-isolariciresinol 3*α*-*O*-*β*-apiofuranosyl-(1→2)-*O*-*β*-glucopyranoside (**3**),[6] 6,7-dihydroxy-3'-methoxy-4',5'-methylenedioxyisoflavone 6-*O*-*β*-*D*-xylopyranosyl-(1→6)-*β*-*D*-glucopyranoside (**4**),[7] polyporusterone I (**5**).[8]

Compound **1**, to which we assigned the name multifidoside A, was obtained as white amorphous powder and has a molecular formula of $C_{30}H_{38}O_{15}$ determined by HRFAB-MS which showed a quasi-molecular formula ion peak at *m/z*: 639.2289 $[M+H]^+$ (calcd. for $C_{30}H_{38}O_{15}$, 638.2211). This formula indicated 12 degrees of unsaturation. The ^{13}C-NMR and DEPT spectra of **1** clearly displayed 30 carbon signals (2 × CH_3, 4 × CH_2, 16 × CH, 8 × C), of which 11 carbons could be assigned to a glucose unit (δ_C 104.5, 74.8, 77.5, 71.1, 77.2, 67.8) and an apiose unit (δ_C 111.1, 77.8, 80.4, 75.0, 65.8), and the remaining 19 carbon signals were assigned to the aglycone. The UV spectrum showed the absorption bands at 208, 266 nm. Its IR spectrum (KBr) showed the absorption bands at 3328 (hydroxyl), 1630 (olefinic C=C), 1601, 1516 (phenyl). The ^{1}H and ^{13}C-NMR spectra of **1** showed the presence of two *meta*-coupling aromatic protons signals [δ_H 6.98 (1H, d, J = 1.7 Hz) and 6.83 (1H, d, J = 1.7 Hz), δ_C 110.8 and 116.8], three *asym*-coupling aromatic protons signals [δ_H 6.42 (1H, d, J = 2.4 Hz), 6.44 (1H, dd, J = 7.9, 2.4 Hz) and 6.96 (1H, d, J = 7.9 Hz), δ_C 103.9, 108.7 and 116.2], one methoxyl group [δ_H 3.76 (3H, s), δ_C 55.5], a (*E*)-coniferyl alcohol signal [δ_H 4.03 (2H, br d, J = 5.7 Hz), 6.39 (1H, d, J = 15.3 Hz) and 6.20 (1H, dt, J = 15.3, 5.7 Hz), δ_C 61.5, 128.8 and 126.7],[9] two methine signals [δ_H 4.79 (1H, d, J = 8.0 Hz) and 4.33 (1H, dq, J = 8.0, 6.4 Hz), δ_C 79.5 and 72.9], a methyl signal [δ_H 1.19 (3H, d, J = 6.6 Hz), δ_C 17.2], one hydroxyl signal [δ_H

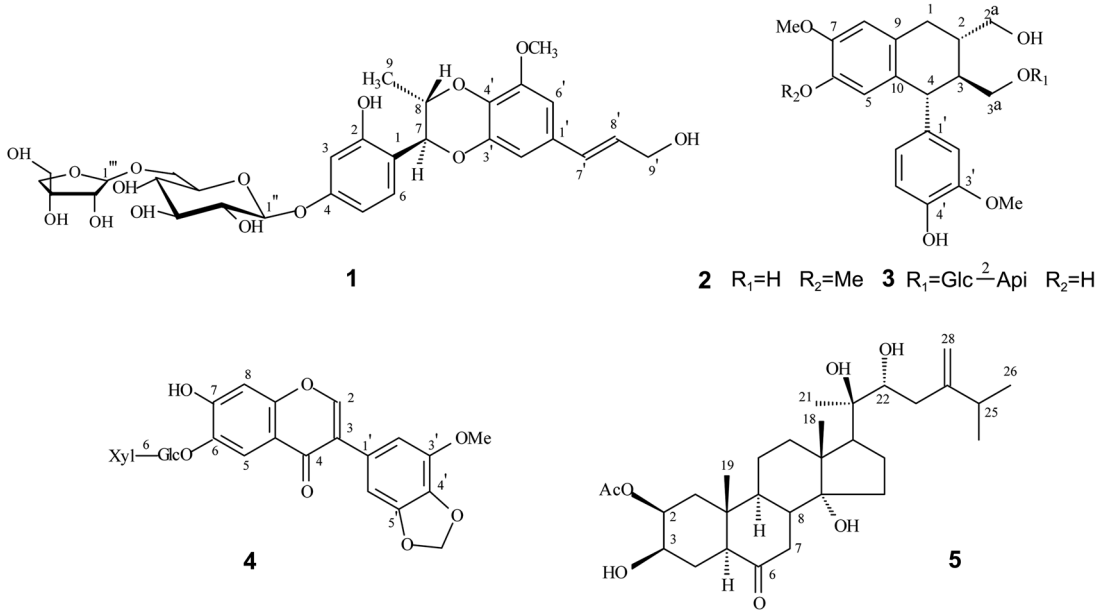

Figure 1. The structure of compounds **1-5**.

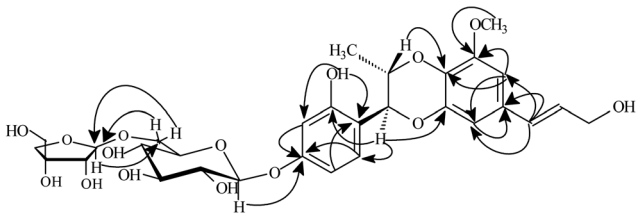

Figure 2. The key HMBC correlations of compound **1**.

9.68 (1H, s, HO-2), δ_C 154.8 (C-2)], and two anomeric protons of sugars [δ_H 4.81 (1H, d, J = 7.5 Hz, H-1") and 5.28 (1H, d, J = 2.2 Hz, H-1'"), the corresponding anomeric carbon signals at δ_C 104.5 (C-1") and 111.1 (C-1'")]. Comparison of the ^{1}H and ^{13}C-NMR data of **1** with those of eusiderin E[10] indicated that **1** is a 7,3'-dioxy-8,4'-neolignan glycoside. In HMBC experiment, the correlations of δ_C 145.8 (C-4) with δ_H 4.81 (H-1" of Glc)/6.42 (H-3)/6.44 (H-5)/6.96 (H-6); δ_C 131.2 (C-1') with δ_H 6.39 (H-7')/6.83 (H-6')/6.98 (H-2'); δ_C 149.0 (C-5') with δ_H 3.76 (-OMe)/6.83 (H-6'); and δ_C 154.8 (C-2) with δ_H 6.42 (H-3)/6.96 (H-6), suggested that the site of attachment of the disaccharide chain, (*E*)-coniferyl alcohol side-chain, the methoxyl and hydroxyl groups were at C-4, C-1', C-5' and C-2 of the aglycone, respectively.

On acid hydrolysis, compound **1** gave *D*-glucose and *D*-apiose on the basis of *co*-TLC with authentic sample and rotational analysis according to Hudson rules,[11] showing the presence of *D*-glucose and *D*-apiose units. In addition, they were deduced from the FAB-MS spectral observation of *m/z* 507 $[M+H-132]^+$ and *m/z* 345 $[M+H-132-162]^+$ fragment ions, arising from the elimination of an apiose and a glucose unit, indicating the apiose was terminal sugar and the glucose was attached to the aglycone. Comparison of ^{13}C-NMR data of the sugar moieties with literature values[12] revealed that the glucose was present in pyranoside form and the apiose was in furanoside form. The HMBC experiment of **1** showed long-range correlations (Fig. 2) between the H-1'" (δ_H 5.28) of apiose and the C-6" (δ_C 67.8) of glucose as well as between the H-6" (δ_H 4.05/3.96) of glucose and the C-1'" (δ_C 111.1) of apiose, thus suggesting the linkage of apiose-(1→6)-glucose. The relative stereochemistry of **1** was determined based on the ^{13}C-NMR spectra data and the J values measured in the ^{1}H-NMR spectrum. The β-configuration on C-1'" anomeric orientation of apiose was confirmed by comparing the ^{13}C-NMR spectra data of **1** with those of α-*D*-(δ_C 104.5) and β-*D*-apiofuranosides (δ_C 111.5), respectively,[13] and the glucose had the β-configuration according to the coupling constant (J = 7.5 Hz) of H-1" of glucose. The coupling constants observed between H-7' and H-8' (J = 15.3 Hz) suggested that the (*E*)-coniferyl alcohol side-chain had a *trans*-configuration. The signals of H-7 and H-8 in the ^{1}H-NMR spectrum appeared at slightly lower fields (δ_H 4.79 and 4.33, respectively) than verticillatoside A,[14] and with a larger coupling constant (J = 8.0 Hz) indicating a *trans*-orientation (axial-axial) of H-7 and H-8 pair in **1**.[15] On these grounds, multifidoside **A** was elucidated as rel-(7*S*,8*S*)-$\Delta^{7'}$-2,9'-dihydroxy-5'-methoxy-7,3'-dioxy-8,4'-neolignan-4-*O*-β-*D*-apiofuranosyl-(1→6)-β-*D*-glucopyranoside.

The known compounds were identified by comparing their spectral data with reported values in the literature or their melting points and R_f values with authentic samples.

Experimental

General Procedures. Melting points were observed with a Chinese X-4 melting point apparatus (uncorrected). Optical rotations were measured with Perkin-Elmer 241 digital polarimeter. UV and IR (KBr disks) spectra were obtained on Shimadzu UV-300 (double beam) and Alpha-Centari FT-TR spectrophotometer. ^{1}H and ^{13}C-NMR (DEPT) spectra were recorded on Bruker AM-400 NMR spectrometer. Mass

Table 1. ^{1}H and ^{13}C-NMR spectral data of compound 1 (400 and 100 MHz, J_{Hz}, DMSO-d_6, TMS)*

No.	δ_H	δ_C	DEPT	HMBC (H→C)	No.	δ_H	δ_C	DEPT	HMBC (H→C)
1		131.2	C	3,6,5,7, HO-2	9'	4.03 (2H, brd, 5.7)	61.5	CH_2	8', HO-9'
2		154.8	C	3,6,7, HO-2	HO-2	9.68 (1H, s)			
3	6.42 (1H, d, 2.4)	103.9	CH	HO-2,5	HO-9				
4		145.8	C	1",3,5,6	MeO-5'	3.76 (3H, s)	55.5	CH_3	
5	6.44 (1H, dd, 7.9, 2.4)	108.7	CH	3,6	Glc-1"	4.81 (1H, d, 7.5)	104.5	CH	
6	6.96 (1H, d, 7.9)	116.2	CH	5,7	2"	3.82 (1H, dd, 9.1, 7.4)	74.8	CH	
7	4.79 (1H, d, 8.0)	79.5	CH	8,6	3"	3.77 (1H, dd, 9.1, 8.5)	77.5	CH	
8	4.33 (1H, dq, 8.0, 6.4)	72.9	CH	7,9	4"	3.94 (1H, dd, 9.9, 8.5)	71.1	CH	
9	1.19 (3H, d, 6.6)	17.2	CH_3	8	5"	3.82 (1H, ddd, 9.9, 6.0, 1.6)	77.2	CH	
1'		131.2	C	2',6',7'	6"	4.05 (1H, dd, 11.3, 1.6) 3.96 (1H, dd, 11.3, 6.0)	67.8	CH_2	1'"
2'	6.98 (1H, d, 1.7)	110.8	CH	7',6'					
3'		143.6	C	2',7	Api-1'"	5.28 (1H, d, 2.2)	111.1	CH	6"
4'		135.5	C	6',8	2'"	4.29 (1H, d, 2.2)	77.8	CH	
5'		149.0	C	CH_3O-,6'	3'"		80.4	C	
6'	6.83 (1H, d, 1.7)	116.8	CH	2',7'	4'"	3.75 (1H, d, 9.4)	75.0	CH_2	
7'	6.39 (1H, d, 15.3)	128.8	CH	2',6'		3.96 (1H, d, 9.4)			
8'	6.20 (1H, dt, 15.3, 5.7)	126.7	CH	9'	5'"	3.69 (2H, s)	65.8	CH_2	

spectra analyses were carried out with ZAB-HS and MAT-112 mass spectrometer, respectively. Separation and purification were performed by column chromatography on silica gel (100-200, 200-300 mesh). TLC was performed on silica gel GF_{254} plates. The spots were visualized by UV (254 nm) and EtOH-H_2SO_4.

Plant Material. The roots of *P. multifida* Poir. were collected in August 2002, from Pingjiang district of Hunan Province, China. It was identified by Prof. Yun-Shan Lian (Department of Biology, Northwest Normal University). A voucher specimen (No. 107083) of the plant is deposited in the Herbarium of the Botany Department, Northwest Normal University, Lanzhou, 730070, China.

Extraction and Isolation. The air-dried and powered roots of *P. multifida* Poir. (5.0 kg) were soaked in 95% EtOH (15 L, 7 d × 3) at room temperature. After removing the solvent, the extract (250 g) was suspended in warm water and partitioned with petroleum ether (60-90 ^oC), $CHCl_3$, EtOAc, and *n*-BuOH, successively. The *n*-BuOH-soluble fraction was evaporated to give 78.5 g of residues, which was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (8:0→1:5) in increasing polarity and the eluates combined by monitoring with TLC to give three fractions (A, B and C). Fraction A (3.9 g) was further fractionated over silica gel column and eluted with $CHCl_3$-MeOH (4:1) to obtain **5** (21 mg). Fraction B (2.6 g) was purified by a silica gel column using $CHCl_3$-MeOH (3:1→1:1) as elution gradient to afford **1** (15 mg). Fraction C (3.1 g) was rechromatographed over a silica gel column eluting with EtOAc-MeOH (3:1→2:1) to yield **2** (9 mg) and subfraction. Subfraction was further purified by preparative TLC (silica gel) developed with $CHCl_3$-MeOH (1:1) to provide compound **3** (13 mg) and **4** (11 mg).

Compound **1**: White amorphous powder (MeOH), mp. 216-218 ^oC; $[\alpha]_D^{20}$ −11.2^o (c = 0.45, MeOH); HRFAB-MS: *m/z* 639.2289 $[M+H]^+$ (calcd. for $C_{30}H_{38}O_{15}$, 638.2211); UV λ_{max}^{MeOH} (nm): 208, 266; IR ν_{max}^{KBr} (cm^{-1}): 3328 (OH), 1630 (olefinic C=C), 1601, 1516 (phenyl); FAB-MS: *m/z* 639 [M+H], 507 $[M+H-132]^+$ and 345 $[M+H-162-132]^+$; ^1H and ^{13}C-NMR data see Table 1.

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