Two new neolignan glycosides from *Pteris multifida* Poir

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Two new neolignan glycosides, named as multifidoside A 1 and B 2, together with four known compounds have been isolated from the roots of *Pteris multifida* Poir. The structures of multifidoside A and B have been characterized by spectroscopic and chemical means as (7S, 8S)-\(\Delta^7\)-2,9-dihydroxy-3′-methoxy-7,3′-dioxo-8,4′-neolignan-4-\(\beta\)-D-apiofuranosyl-(1→6)-\(\beta\)-D-glucopyranoside and (7S, 8S)-\(\Delta^7\)-2,9,9′-trihydroxy-7,3′-dioxo-8,4′-neolignan-4-\(\beta\)-D-apiofuranosyl-(1→6)-\(\beta\)-D-glucopyranoside. The known compounds are identified by comparing their spectral data with those of authentic samples or data reported in the literature.

**Keywords:** *Pteris multifida* Poir, neolignan glycoside, (7S, 8S)-\(\Delta^7\)-2,9-dihydroxy-3′-methoxy-7,3′-dioxo-8,4′-neolignan-4-\(\beta\)-D-apiofuranosyl-(1→6)-\(\beta\)-D-glucopyranoside, (7S, 8S)-\(\Delta^7\)-2,9,9′-trihydroxy-7,3′-dioxo-8,4′-neolignan-4-\(\beta\)-D-apiofuranosyl-(1→6)-\(\beta\)-D-glucopyranoside, multifidoside A, multifidoside B

*Pteris multifida* Poir is widely distributed in the south and southwest districts of China (Chinese name “fengweicao”), which has been mainly used as a traditional Chinese folk drug for the treatment of eczema, haematemesis, enteritis, diarrhoea, bacillary dysentery and are also known to have anticancer and antibacterial effects. However, very little is known about its chemical constituents except for antimitogenic activity. A previous paper reported the isolation and characterization of six compounds from *EtOAc* fraction obtained by partition of the *EtOH* extract. In continuation of the phytochemical research on this plant, is now reported the isolation and structural elucidation of two new neolignan glycosides, multifidoside A, 1 and B, 2 from the *n*-BuOH fraction of the *EtOH* extract, along with the four known compounds (Figure 1), scaphopetalone 3 (Ref. 5), (-)-isolariciresinol 3\(\alpha\)-\(\beta\)-apiofuranosyl-(1→2)-\(\beta\)-D-glucopyranoside 4 (Ref. 6) 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone 6-O-\(\beta\)-D-xylopyranosyl-(1→6)-\(\beta\)-D-glucopyranoside 5 (Ref. 7) polyporustone 1, 6 (ref. 8).

**Note**

Compound 1 (Figure 1), to which is assigned the name multifidoside A, was obtained as white amorphous powder and has a molecular formula of C\(_{25}\)H\(_{32}\)O\(_{13}\), determined by HRFAB-MS which showed a quasi-molecular formula ion peak at \(m/z\) 659.2289 [M+H]\(^+\) (calcd. for C\(_{30}\)H\(_{30}\)O\(_{15}\), 658.2211). 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 could be assigned to a glucose unit (\(\delta\)_C 104.5, 74.8, 77.5, 71.1, 77.2, 67.8) and an apiose unit (\(\delta\)_C 111.1, 77.8, 80.4, 75.0, 65.8), and the remaining 19 carbon signals were assigned to the aglycone. The UV-Vis 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 and 1516 cm\(^{-1}\) (phenyl). The \(^1\)H and \(^{13}\)C NMR spectra of 1 showed the presence of two meta-coupling aromatic protons signals [\(\delta\)_H 6.98 (1H, d, \(J=1.7\) Hz) and 6.83 (1H, d, \(J=1.7\) Hz), \(\delta\)_C 110.8 and 116.8], three asymp-coupling aromatic protons signals [\(\delta\)_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), \(\delta\)_C 103.9, 108.7 and 116.2], one methoxyl group [\(\delta\)_H 3.76 (3H, s), \(\delta\)_C 55.5], a (E)-coniferyl alcohol signals [\(\delta\)_H 4.03 (2H, br d, \(J=5.7\) Hz), 6.39 (1H, d, \(J=15.3\) Hz) and 6.20 (1H, dd, \(J=15.3, 5.7\) Hz), \(\delta\)_C 61.5, 128.8 and 126.7], (ref 9), two methenyl signals [\(\delta\)_H 4.79 (1H, d, \(J=8.0\) Hz) and 4.33 (1H, dq, \(J=8.0, 6.4\) Hz), \(\delta\)_C 79.5 and 72.9], a methyl signal [\(\delta\)_H 1.19 (3H, d, \(J=6.6\) Hz), \(\delta\)_C 17.2], one hydroxyl signal [\(\delta\)_H 9.68 (1H, s, HO-2), \(\delta\)_C 154.8 (C-2)], and two anomic protons of sugars [\(\delta\)_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 \(\delta\)_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 (Ref. 10) indicated that 1 is a 7,3′-dioxo-8,4′-neolignan glycoside. In HMBC experiment, the correlations of \(\delta\)_C 145.8 (C-4) with \(\delta\)_H 4.81 (H-1″ of Glc)/6.42 (H-3)/6.44 (H-5)/6.96 (H-6); \(\delta\)_C 131.2 (C-1′) with \(\delta\)_H 6.39 (H-7′)/6.83 (H-6′)/6.98 (H-2′); \(\delta\)_C 149.0 (C-5′) with \(\delta\)_H 3.76 (-OMe)/6.83 (H-6′); and \(\delta\)_C 154.8 (C-2) with \(\delta\)_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 glucose and apiose respectively, which was compared with authentic sample by co-TLC, showing the presence of D-glucose and D-apiose. In addition, it was 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 sugars and the glucose was attached to the aglycone. Comparison of 13C NMR data of the sugar moieties with literature values11 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 (Figure 2) between the H-1″′ (δH 5.28) of apiose and the C-6″ (δC 67.8) of glucose as well as between the H-6″ (δH 4.05/3.96) of glucose and the C-1″′ (δC 111.1) of apiose, thus suggesting the linkage of apiose-(1→6)-glucose. The relative stereochemistry of 1 was determined based on the 13C NMR spectra data and the J values measured in the 1H NMR spectrum. The β-configuration on C-1″′ anomeric orientation of apiose was confirmed by comparing the 13C NMR spectra data of 1 with those of α-D-(δC 104.5) and β-D-apiofuranosides (δC 111.5), respectively12, 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 1H NMR spectrum appeared at slightly lower fields (δH 4.79 and 4.33, respectively) with a larger coupling constant (J=8.0 Hz) indicating a trans-orientation (axial-axial) of H-7 and H-8 pair in 1 (ref. 13). Comparison of the specific optical rotation of 1 with that of the known verticillatoside B (Ref. 14), suggested 1 to have the same absolute configurations of C-7 and C-8 as S and S, respectively. On these grounds, multifidoside A was elucidated as (7S, 8S)-Δ7′-2,9′-dihydroxy-5′-methoxy-7,3′-dioxo-8,4′-neolignan-4-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Compound 2 was obtained as white amorphous powder, possessing a molecular formula of C28H36O15 by HR FAB-MS data (m/z 625.2132 [M+H]+, calcd for 624.2054), 14 mass units lower than that of 1. Its UV-Vis, IR and MS spectra were very similar to those of 1. The 13C NMR and DEPT spectra clearly displayed 29 carbon signals (5 × CH2, 17 × CH, 7 × C). Comparing the NMR data with those of 1, the

![Figure 1 — The structure of compounds 1-6](image-url)
NMR signals of the sugar moiety were almost the same as those of 1, except that an extra hydroxyl proton signal at $\delta_H 5.18$ (HO-9) and an aromatic proton singlet at $\delta_H 6.82$ (1H, d, $J=8.2$ Hz, H-5') was present in $^1H$ NMR spectrum of 2, and a methyl carbon signal ($\delta_C 17.2$) disappeared and a methylene carbon signal ($\delta_C 60.8$) appeared in the $^{13}C$ NMR and DEPT spectra of 2. All these data indicated that a hydroxyl group is linked to C-9 and a methoxyl group disappeared from C-5' of 1 (Table I). It was further supported by the upfield shift signal of C-5' (from $\delta_C 149.0$ to 117.3) and downfield shift signal of C-9 (from $\delta_C 17.2$ to 60.8) in $^{13}C$ NMR spectra of 2 (Table I). The absolute configurations of C-7 and C-8 were determined as S and S, respectively, by comparison of the specific optical rotation of 2 with that of 1. These data suggested 2 to be the analogue of 1. Therefore, the structure of 2 was characterized as $(7S, 8S)$-$\Delta^7$-2,9,9'-trihydroxy-7,3'-dioxy-8,4'-neolignan-5-O-$\beta$-D-apiofuranosyl-(1$\rightarrow$6)$-\beta$-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 Section

General Procedures

Melting points were observed with a Chinese X-4 melting point apparatus and are uncorrected. Optical rotations were measured with Perkin-Elmer 241 digital polarimeter. UV-Vis and IR (KBr disks) spectra were obtained on Shimadzu UV-300 (double beam) and Alpha-Centauri FT-TR spectrometer. $^1H$ and $^{13}C$ NMR (DEPT) spectra were recorded on Bruker AM-400 NMR spectrometer. Mass spectra were obtained on ZAB-HS and MAT-112 mass spectrometer, respectively. Separation and purification were performed by column chromatography over 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$_2$SO$_4$.

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. Lian Yunshan (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$\times$3) at RT. After removing the solvent, the crude extract (250 g) was suspended in warm water and partitioned successively with petroleum ether (60-90°C), CHCl$_3$, EtOAc and $n$-BuOH, concentrated under reduced pressure. The $n$-BuOH-soluble fraction was concentrated under reduced pressure to give 78.5
Table 1 — $^1$H and $^{13}$C NMR spectral data of compounds 1 and 2 (400 and 100 MHz, J\textsubscript{H,H}, DMSO-d$_6$, TMS)*

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** g of residues, which was isolated on a silica gel column eluting with CHCl$_3$-MeOH (8:0→1:5) in increasing polarity and 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 6 (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) and 2 (12 mg). Fraction C (3.1 g) was rechromatographed over a silica gel column eluting with EtOAc-MeOH (3:1→2:1) to yield 3 (9 mg) and subfraction. Subfraction was further purified by preparative TLC (silica gel) and developed with CHCl$_3$-MeOH (1:1) as development to provide compound 4 (13 mg) and 5 (11 mg).

** Compound 1: White amorphous powder (MeOH), m.p. 216-18°C; $[\alpha]_D^{20}$-11.2° ($c=0.45$, MeOH); HRFAB-MS: $m/z$ 639.2289 [M+H]$^+$ (calcd. for C$_{30}$H$_{38}$O$_{15}$, 638.2211); UV-Vis $\lambda_{max}^{MeOH}$ (nm): 208, 266; IR (KBr): 3328 (OH), 1630 (olefinic C=C), 1601, 1516 cm$^{-1}$ (phenyl); FAB-MS: $m/z$ 639 [M+H], 507
[M+H-132]⁺ and 345 [M+H-162 -132]⁺; for ¹H and ¹³C NMR data see Table I.

Compound 2: White amorphous powder (MeOH), m.p. 212-15°C; [α]_D⁰ -10.8° (c=0.45, MeOH); HRFAB-MS: m/z 625.2048 [M+H]⁺ (calcd. for C₂₉H₃₆O₁₅, 624.2054); UV-Vis λ_max (nm): 209, 266; IR (KBr): 3327(OH), 1628 (olefinic C=C), 1602, 1515 cm⁻¹ (phenyl); FAB-MS: m/z 625 [M+H]⁺, 493 [M+H-132]⁺ and 331 [M+H-162-132]⁺; for ¹H and ¹³C NMR data see Table I.

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