THREE ANTIBACTERIAL COMPOUNDS FROM THE ROOTS OF *Pteris multifida*

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*A new eudesmane-type sesquiterpenoid, 3β-caffeoxyl-1β,8α-dihydroxyeudesm-4(15)-ene (**1**), together with two known compounds including ludongnin V (**2**) and isoneorautenol (**3**), were isolated from the roots of* Pteris multifida*. Their structures were determined by spectral and chemical methods, with their antibacterial activities being evaluated by the microdilution technique, respectively.*

Key words: *Pteris multifida*, Pteridaceae, eudesmane sesquiterpenoid, antibacterial activity.

*Pteris multifida (*Pteridaceae) is a perennial herb growing mainly in the south and southwest regions of China [1]. It is used in traditional Chinese medicine as an antimicrobial agent for the treatment of eczema, hematemesis, rheumatism, enterits, diarrhea, cold, and so on [2, 3], and its extracts also show high antimutagenic activity against picrolonic acid-induced mutation [4]. Previous chemical examination of the roots of *P. multifida* afforded saucerneol D, *β*-rosaterol, dehydrogoniothalamin, muxiangrine III, etc. [5–7]. In order to investigate the chemical composition and the antibacterial potential of *P. multifida* in more depth and define the chemodiversity of the plant and the consequent potential value of this natural resource, as well as to establish the best procedure to obtain extracts containing active principles, we have again isolated three active compounds, including a new eudesmane-type sesquiterpenoid and two known spirosecokaurene diterpenoid and furocoumarin from *P. multifida*. In this paper, we wish to report the isolation and structure elucidation as well as the antibacterial properties of these three compounds.

Compound **1** was obtained as a pale yellow amorphous powder from MeOH. The molecular formula was established as $C_{24}H_{32}O_6$ from the HREI-MS data at *m/z* 416.2204 $[M]^+$ (calcd for 416.2199), corresponding to nine degrees of unsaturation, ascribed to one carbonyl group, one benzene ring, two double bonds, and two alicyclic rings with the aid of spectral data. It exhibited UV maxima at 216, 220, 254, 298, and 325 nm, suggesting the presence of strong conjugation in the molecule. A bathochromic shift of 47 nm upon addition of NaOH indicated the presence of free phenolic hydroxy. A distinct bathochromic shift with $AlCl_3$ but again returning to the normal value upon addition of HCl indicated the presence of a catechol group. The IR spectrum exhibited absorption bands for hydroxyl groups (3408–3100 cm^{-1}, br), *α,β*-unsaturated ester moiety (1695 and 1270 cm^{-1}), isopropyl group (1358, 1160 cm^{-1}), exocyclic double bond (1650, 1475 cm^{-1}), and aromatic rings (1600 and 1510 cm^{-1}). Analysis of the NMR spectra with the aid of the DEPT technique demonstrated the presence of a caffeoyl group [including a *trans*-conjugated double bond signal at δ_H 6.38 (1H, d, J = 16.0 Hz) and 7.64 (1H, d, J = 16.0 Hz), which correlated with signals at δ_C 115.8 and 147.0, an ester carbonyl (δ_C 170.8), and a typical ABX aromatic ring spin system signal at δ_H 6.77 (1H, d, J = 8.1 Hz), 6.95 (1H, dd, J = 8.1, 2.0 Hz) and 7.04 (1H, d, J = 2.0 Hz), corresponding to δ_C 127.8, 115.1, 145.9, 149.2, 116.1 and 122.8] (Table 1). Besides the nine carbon signals for the caffeoyl group, 15 additional carbon and corresponding proton signals, including three methyls [one tertiary methyl (δ_C 12.3 and δ_H 0.72) and two secondary methyl groups (δ_C 14.8 and 20.6; δ_H 1.03 and 0.91)], three alicyclic methylenes [δ_C 31.8, 25.9, 37.2; δ_H 1.80, 1.90, 1.67], one exocyclic olefinic terminal methylene [δ_C 107.7; δ_H 4.82 and 4.96], one exocyclic olefinic quaternary carbon [δ_C 148.6], six methines [two alicyclic methines (δ_C 56.5, 39.4; δ_H 1.75, 2.35), one isopropyl methine (δ_C 31.4 and δ_H 1.71), three oxygenated methines [δ_C 78.0, 81.6, 80.7; δ_H 3.66, 5.12, 3.24], and one alicyclic quaternary carbon [δ_C 49.6] were recognized in the ^1H and ^{13}C NMR (DEPT) spectra.

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TABLE 1. ^{1}H and ^{13}C NMR Chemical Shifts of Compound **1** in $CDCl_3$, $SiMe_4$ as Internal Standard (δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	78.0	3.66 (1H, dd, J = 11.0, 4.5)	13	20.6	0.91 (3H, d, J = 6.9)
2	31.8	1.80 (1H, m)/1.51 (1H, m)	14	12.3	0.72 (3H, s)
3	81.6	5.12 (1H, dd, J = 6.0, 10.0)	15	107.7	4.82 (1H, d, J = 6.9)/4.96 (1H, d, J = 6.9)
4	148.6	-	1′	127.8	-
5	56.5	1.75 (1H, d, J = 10.1)	2′	115.1	7.04 (1H, d, J = 2.0)
6	25.9	1.90 (1H, m)/1.32 (1H, m)	3′	145.9	-
7	39.4	2.35 (1H, m)	4′	149.2	-
8	80.7	3.24 (1H, d, J = 9.8)	5′	116.1	6.77 (1H, d, J = 8.1)
9	37.2	1.67 (1H, m)/1.38 (1H, m)	6′	122.8	6.95 (1H, dd, J = 8.1, 2.0)
10	49.6	-	7′	147.0	7.64 (1H, d, J = 16.0)
11	31.4	1.71 (1H, m)	8′	115.8	6.38 (1H, d, J = 16.0)
12	14.8	1.03 (3H, d, J = 6.9)	9′	170.8	-

TABLE 2. MIC Values of Compounds **1-3** in μg/mL

Compound	Gram-positive bacteria			Gram-negative bacteria		
	B. sphaeriicus	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhimurium*
1	75	75	75	25	50	50
2	100	100	50	25	100	25
3	100	100	25	100	50	No activity
Ampicillin	12.5	6.25	6.25	12.5	3.13	12.5

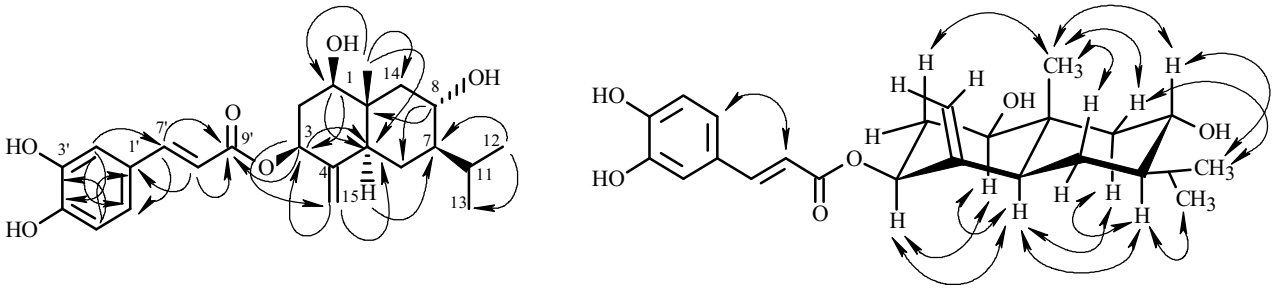

Fig. 1. Key HMBC and NOE correlations of compound **1**.

These spectral data suggested that compound **1** has a bicyclic sesquiterpene moiety possessing two hydroxyl groups and an exocyclic double bond in an eudesmane-type framework. The NMR spectra of the sesquiterpene moiety showed a very close similarity to those of eudesm-4 (15)-ene-1β,8α-diol [8], the only difference being an additional caffeoxyl group at C-3 from the HMBC correlations between H-3 with C-9′/C-2/C-4/C-15. This conclusion was further supported by the observed downfield shifts of C-3 (δ_C 81.6) and H-3 (δ_H 5.12) with respect to the corresponding signals in eudesm-4 (15)-ene-1β,8α-diol [8] and eudesm-4(15)-ene-1β,6α-diol [9]. The β-configuration of the 3-caffeoxyl group was determined from the chemical shifts and coupling constants of H-3 (5.12, dd, J = 6.0, 10.0 Hz), and further evidenced by the observed NOE interactions between H-3 and H-1/H-5 in the ROESY spectrum (Fig. 1). In turn, the HMBC correlations between H-1 and C-5/C-3, H-8 and C-6/C-7/C-9/C-10/C-11, H-6 and C-5/C-7/C-10/C-11, H-12 and C-7/C-13, H-15 and C-3/C-5, as well as H-14 and C-1/C-5/C-9, clearly indicated the attachment of the hydroxyl, isopropyl, exocyclic ethylenic bond, and angular methyl functional groups at C-1, C-8, C-7, C-4, and C-10, respectively. The observed NOE interactions between H-5 and H-1/H-3/H-7/H_{ax}-9, H_3-14 and H_{ax}-2/H_{ax}-6/H-8/H_{ex}-9, H-8 and H_{ax}-6/H_3-12, and H-7 and H-5/H_{ax}-9/H_3-13 distinctly indicated that the configurations of HO-1, CH_3-10, and C-7 isopropyl must be β-oriented. H-5 and HO-8 were α-oriented, suggesting that the A/B ring juncture was trans [10]. The

correlations between H-2′ and C-7′, H-7′ and C-6′/C-9′, H-8′ and C-1′/C-9′, H-2′ and H-7′, as well as H-6′ and H-8′, also were clearly observed in the HMBC and ROESY experiments.

On alkaline hydrolysis, compound **1** also yielded eudesm-4 (15)-ene-1β,8α-diol [8] and caffeic acid, which was compared with an authentic sample by melting point and co-TLC. Therefore, based on the above spectral features and physicochemical properties, the structure of compound **1** was characterized as 3β-caffeoxyl-1β,8α-dihydroxy eudesm-4(15)-ene, which has not been reported previously from any plant source.

Compound **1** showed moderate activity against both gram-positive and gram-negative bacteria (Table 2).

Compound **2** was active against *S. typhimurium*, *E. coli*, and *S. aureus*, but less so against the remaining organisms.

Compound **3** had a minimum inhibitory concentration (MIC) of less than 50 μg/mL against *S. aureus* and *P. aeruginosa*, while it had no activity against *S. typhimurium*. In comparison with standard antibiotics, the activities of the compounds were not as promising, but the presence of these active constituents may contribute to the antibacterial and antiinflammation effects.

EXPERIMENTAL

General Experimemtal Procedures. Melting points were measured on a Chinese X-4 melting point apparatus (uncorrected), UV spectra were recorded on a Shimadzu UV-240 spectrometer, IR spectra (KBr disks) were obtained on an Alpha-Centari FT-IR spectrometer, NMR spectra were scanned on a Bruker AM-500 spectrometer (chemical shifts in δ downfield from TMS internal standard) operating at 500 and 125 MHz for ^{1}H and ^{13}C NMR respectively, and EI-MS spectra on JMS-02SB mass spectrometer.

Plant Material. *P. multifida* roots were collected at the flowering stage, in July to August 2002, from the Pingjiang District of Hunan Province (China), and authenticated by Prof. Yun-Shan Lian in the Department of Biology, Northwest Normal University. A voucher specimen (No.107083) of the plant was deposited in the Herbarium of the Faculty of Biology, Northwest Normal University, Lanzhou 730070, China.

Extraction and Isolation. The air-dried roots of *P. multifida* (1 kg) were ground and extracted with MeOH. The concentrated extract was partitioned with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble part was chromatographed over silica gel with the $CHCl_3$–Me_2CO (25:1→1:20, V/V) gradient system repeatedly, recrystallized, and purified on Sephadex LH-20 with $CHCl_3$-MeOH (5:1) to get **1** (8 mg), **2** (16 mg), and **3** (13 mg).

3β-Caffeoxyl-1β,8α-dihydroxyeudesm-4(15)-ene (1): $C_{24}H_{32}O_6$, mp 204–206°C (MeOH). UV (λ_{max}, nm), +MeOH: 216, 220, 254, 298, 325; +NaOH: 256, 372; +$AlCl_3$: 248, 304sh, 387; +$AlCl_3$+HCl: 228, 262sh, 296, 324. IR (KBr, ν, cm^{-1}): 3408-3100, 1695, 1650, 1600, 1510, 1475, 1358, 1270, 1160, 820; EI-MS: *m/z* 416 $[M]^+$ (5.2), 236 (1.8), 218 (12.4), 175 (8.9), 163 (100), 109 (23.1); ^{1}H and ^{13}C NMR are listed in Table 1.

Ludongnin V (**2**): colorless flaky crystals ($CHCl_3$–Me_2CO), mp 258–260°C, HREI-MS *m/z* 360.1567 $[M]^+$ (calcd for 360.1573, $C_{20}H_{24}O_6$). Comparison of the spectral (IR and NMR) data with the literature identified it as ludongnin V [11].

Isoneorautenol (**3**): white plate crystals (Me_2CO), mp 153–155°C, HREI-MS *m/z* 322.1211 $[M]^+$ (calcd for 322.1205, $C_{20}H_{18}O_4$). The physicochemical constants and spectral (IR, NMR, and MS) data of **3** indicate that it is identical to isoneorautenol [12].

Alkaline Hydrolysis of Compound 1. Compound **1** (5 mg) was refluxed with 5% KOH–MeOH solution (5mL) under heating at 90°C for 3 h. After cooling, the reaction mixture was diluted with H_2O (10 mL), then extracted with $CHCl_3$. The organic layers was dried with Na_2SO_4 and evaporated to afford colorless needle crystals of eudesm-4 (15)-ene-1β,8α-diol: mp 106–108° (lit. 105–106°C), which exhibited comparable NMR spectral data to literature values [8]. The aqueous layer was neutralized with 5% HCl and then extracted with Et_2O (twice); the ether layer was concentrated under pressure and recrystallized to give light-yellow plate crystals of caffeic acid, which was identified by comparison of the mixed melting point and R_f value [R_f 0.43, by co-TLC using $CHCl_3$–MeOH–HCOOH (9:1: 0.5, V/V) as developing solvent] with authentic samples.

Antimicrobial Screening. Antimicrobial screening of the compounds was performed by using the microdilution titer technique [13] to determine MICs of the metabolites against three gram-positive bacteria: *Bacillus sphaericus* (ATCC 14577), *Bacillus subtilis* (ATCC 6051), and *Staphylococcus aureus* (ATCC 6538), and three gram-negative bacteria: *Escherichia coli* (ATCC 10538), *Pseudomonas aeruginosa* (ATCC 25619), and *Salmonella typhimurium* (ATCC 23564). In the test, ampicillin is used as a positive control and resazurin as a color indicator.

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