

Polyphenols from the Rhizomes of *Potentilla freyniana*

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Abstract

A new polyphenol, Potentillanin A (1), together with two known compounds (2–3), was isolated from the chloroform soluble fraction of *Potentilla freyniana*. The structure of Potentillanin A (1) was elucidated by spectroscopic methods.

Keywords: *Potentilla freyniana*; Potentillanin A; Polyphenols; Spectroscopic methods

Introduction

The rhizomes of *Potentilla freyniana* Borum in traditional Chinese medicine (TCM) are a source of Chinese folk drug “Wei-ling-Cai”, and are supposed to be effective as relieving pain and anti-inflammatory in folklore medicine [1]. It’s curative effect is well and adverse reaction is little. In the previous paper, only β -sitosterol and daucosterol were reported from this plant [2]. In the current research, a new polyphenol Potentillanin A (1), together with known compounds (2–3) was isolated from the rhizomes of *P. freyniana*. The structure of 1 was elucidated mainly by NMR techniques. Herein, we report the isolation and structure elucidation of these polyphenols.

Experimental

General experimental procedures

IR spectra were recorded with a Perkin-Elmer 577 spectrometer as KBr pellet. NMR spectra were recorded with a Bruker AM-400 spectrometer with TMS as an internal standard. EI-MS (70eV) was carried out with a Finnigan MAT 95 mass spectrometer. CD spectra were recorded on a JASCO J-810 spectropolarimeter. All solvents used were of analytical grade (Shanghai Chemical Plant). Silica gel (200 – 300 mesh) C₁₈ reversed-phase silica gel (150 – 200 mesh, Merck) etc. were used for column chromatography, and pre-coated silica gel GF₂₅₄ plate (QingDao Marine Chemical Plant) was used for TLC.

Plant material

The rhizomes of *Potentilla freyniana* were collected in July 2005 from Enshi County of Hubei Province, People’s Republic of China, and identified by Prof. Changgong Zhang, College of Pharmacy, Tongji Medical Center, Huangzhong University of Science and Technology. A specimen (DFZ0120) was deposited in the College of Pharmacy, Tongji Medical Center, Huangzhong University of Science and Technology.

Extraction and isolation

The air-dried rhizomes of *P. freyniana* (5.0 kg) were ground and extracted with 95% ethanol (10 × 5 L) at room temperature. The ethanol extract was concentrated under vacuum to leave a residue, which was suspended in H₂O (3 L) and extracted with Petroleum ether (3 × 3 L), CHCl₃ (3 × 3 L), EtOAc (3 × 3 L), and *n*-BuOH (3 × 3 L), sequentially. A part of the CHCl₃ extract (20 g) was subjected to a silica gel column chromatography eluting with gradient solvents of Petroleum ether – Acetone (15:1 – 1:1, and then pure Acetone) to afford four fractions (Fr. 1 – 4). Fraction 2 was chromatographed over silica gel eluting with CHCl₃-EtOAc (8:1) to yield 15 mg of 1 (0.0003 %, w/w) (*R*_f = 0.5, developed with CHCl₃-EtOAc (6:1)). Fraction 3 was chromatographed over silica gel eluting with CHCl₃-Acetone (5:1) to afford 2 (20 mg, 0.0004 %, w/w) and 3 (35 mg, 0.0007 %, w/w).

Potentillanin A (1) C₁₁H₁₄O₅, an amorphous powder, [α]_D²⁰: +79.0° (MeOH, *c* 0.20). CD (*c* 0.0038, MeOH), IR(KBr): ν_{\max} cm⁻¹: 3438, 2251, 1653, 1027. ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) (Table 1). EI-MS (70eV): *m/z*: 227 (2), 226 (40), 180 (15), 152 (10), 139 (100), 84 (50), 66 (65); positive HREIMS : *m/z* 226.0839 [M]⁺ (C₁₁H₁₄O₅, Calcd 226.0841).

Results and Discussion

Potentillanin A (1), an amorphous powder, showed molecular formula of C₁₁H₁₄O₅ determined on the basis of positive HREIMS at *m/z* 226.0839 [M]⁺ (Calcd 226.0841) with 5° of unsaturation. Eleven carbon signals including one methyl, two methylenes, four methines, four quaternary carbons were evident from the ¹³C-NMR spectra of 1 (Table 1). The IR spectrum showed absorptions at 3438, 1653, and 1027 cm⁻¹ representing the existence of hydroxyl, benzene ring and ether linkage group, respectively. In the ¹³C-NMR spectrum, one oxygenated methylene (δ 63.5, C-1’), one ketal carbon (δ 98.1, C-2), one oxygenated methine carbon (δ 63.1, C-3) and three oxygenated quaternary carbons

NO	δ_c	δ_H , Multi, J (Hz)
1	–	–
2	99.1	4.87 (d, 2.4)
3	63.2	3.74 (m)
4	23.9	2.42 (2H, m)
5	156.7	–
6	95.9	5.89 (d, 1.6)
7	157.0	–
8	94.9	5.68 (d, 1.6)
9	152.7	–
10	98.8	–
1’	63.6	3.59 (m)
		3.68 (m)
2’	15.6	1.08 (t, 6.8)

Table 1: ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectral data of 1 (in DMSO-*d*₆).

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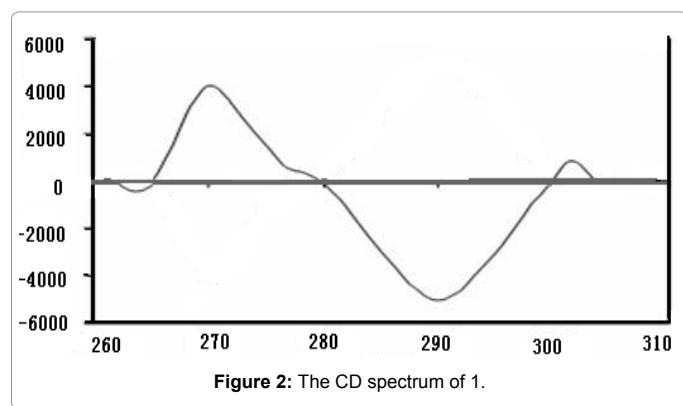
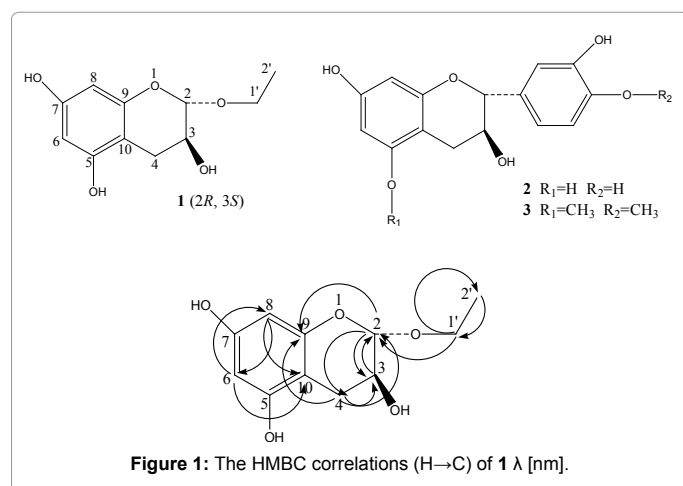
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(δ 156.7, C-5; δ 157.0, C-7; δ 152.7, C-9). A benzene ring and the presence of a cyclic system accounted for 5° of the unsaturation. The $^1\text{H-NMR}$ spectrum (in $\text{DMSO-}d_6$) of 1 (table 1) displayed one methine signal at 1.08 (t, $J=6.8$, $\text{CH}_3\text{-}2'$), two oxygenated methylene protons at δ 3.59 and δ 3.68 (m, $\text{CH}_2\text{-}1'$), one ketal carbon proton at δ 4.87 (d, $J=2.4$, H-2), one oxygenated methine proton at δ 3.74 (m, H-3), two methylene protons at δ 2.42 (m, $\text{CH}_2\text{-}4$), two methine protons at δ 5.89 (d, $J=1.6$, H-6) and δ 5.68 (d, $J = 1.6$, H-8). The coupling and oxygenated patterns revealed by $^1\text{H-NMR}$ were consistent with the functional groups indicated by the above $^{13}\text{C-NMR}$ analyses.

Analysis of the $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and HSQC spectra of 1 enabled the assignment of all the protons to the bonding carbons. The HMBC correlations and planar structure of 1 was outlined as Figure 1.

The structure is similar to character of a flavan-3-ol, and its CD spectrum (Figure 2) is consistent with that of a flavan-3-ol. It showed a strong positive effect near 270 nm, and a negative effect near 290 nm. Analysis of the CD spectrum, the stereochemical structure was fit to



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be (2R, 3S) referred to [3]. Thus, the structure of 1 was unambiguously elucidated as (2R, 3S) - 2 - ethoxy - 3,5,7 - trihydroxy - 3,4 - dihydro - Benzopyran (1).

The two known polyphenols were successively identified as 2 referred to [4], and 3 referred to [5] on the basis of EI-MS, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data. The compounds 2 and 3 were isolated from this plant for the first time.

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