Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Letter

A New Furan Flavonol Glycoside from *Epimedium koreanum*

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ARTICLE INFO

Article history

Received: May 7, 2013

Revised: August 28, 2013

Accepted: October 4, 2013

Available online:

March 24, 2014

DOI:

10.1016/S1674-6384(14)60027-7

ABSTRACT

Objective To study the chemical constituents of *Epimedium koreanum*. **Methods** Separation was carried out through silica gel and Sephadex LH-20 column chromatography and HPLC method. The chemical structures were elucidated by spectroscopic method including 1D-NMR and 2D-NMR. **Results** A new furanflavonol glycoside (**1**) was isolated and identified. **Conclusion** Compound **1** is a new furanflavonol glycoside, and its structure is corroborated as 5,4′-dihydroxyfurano [2″,3″:7,8] flavonol 3-*O*-α-*L*-rhamnoside.

Key words

Berberidaceae; *Epimedium koreanum*; furanflavonol glycoside; structural identification

1. Introduction

The aerial parts of *Epimedium koreanum* Nakai (Berberidaceae) have been used in China for over 2000 years for various medicinal treatments, in particular as tonic, antirheumatic, and aphrodisiac (Pharmacopoeia Committee of P. R. China, 2010). Up to now, many studies were focused on the flavonoids in plants of *Epimedium* Linn. for their various bioactivities *in vivo* or *in vitro* (Ma et al, 2011; Wang et al, 2010; Zhang et al, 2008; Sun et al, 1996; Li et al, 1995). In our previous study, the isolation and structural elucidation of two new prenylflavonols were reported from *Epimedium brevicornum* Maxim (Luo et al, 2009). As the continuation of our search for more new secondary metabolites, a new furanflavonol glycoside (**1**) was obtained from *E. koreanum*.

2. Materials and methods

2.1 General and plant materials

UV spectrum was run on a UV 210A Spectrophotometer. 1D NMR and 2D NMR spectra were recorded on Bruker DRX−400 Instrument with TMS as an internal standard. ESI-MS was taken on an API Qstar Pulsar Instrument, and HR-EI-MS was performed on a VG Autospec−3000 Spectrometer. Column chromatography (CC) was performed with silica gel (100−200 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

The leaves of *Epimedium koreanum* Nakai were purchased

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Fund: National Natural Science Foundation of China (21262021, 21062008)

from the market of Medical Building of Jilin province, China, in November 2010, and identified by Dr. Hai-zhou Li. A voucher specimen (KMUST 20101109) was deposited at the Laboratory of Phytochemistry, Faculty of Life Science and Technology, Kunming University of Science and Technology.

2.2 Extraction and isolation

The air-dried and powdered leaves of *E. koreanum* (1 kg) were extracted with 75% aqueous acetone (for three times). After removal of the solvent *in vacuo*, the residue was suspended in water and fractionated with $CHCl_3$, EtOAc, and *n*-BuOH, successively. The EtOAc extract (10.5 g) was purified by Sephadex LH-20, eluted with MeOH-H_2O (3:7→6:4→9:1), to give six fractions (Frs. A−F). Fr. C (600 mg) was subjected to silica gel column chromatography eluted with $CHCl_3$-MeOH gradients (30:1 and 20:1), to give eleven subfractions (Frs. C-1−C-11). Fr. C-7 (44.7 mg) was then purified on semipreparative HPLC (flow rate of 3 mL/min) with 40% MeOH in H_2O as mobile phase to give compound **1** (8 mg).

2.3 Acid hydrolysis for sugar analysis

A solution of compound **1** (1 mg) in 0.5 mL HCl (1 mol/L) was heated at 90−100 °C in a screw-capped vial for 5 h. The mixture was partitioned with $CHCl_3$ (0.5 mL), and the HCl layer was compared with the standard sample of rhamnose on TLC (EtOAc-MeOH-AcOH-H_2O 11:2:2:2) by visualizing the spots.

Compound **1**: yellow powder; ESI-MS: *m/z* 479 [M + Na]$^+$; HR-EI-MS: *m/z* 456.1049 ([M]$^+$, $C_{23}H_{20}O_{10}^+$, calcd. 456.1056); ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz) data are shown in Table 1.

3. Results and discussion

Compound **1** was obtained as yellow powder, and its molecular formula was established as $C_{23}H_{20}O_{10}$ by the ESI-MS ion peak at *m/z* 479 [M + Na]$^+$ as well as the HR-EI-MS data (*m/z* 456.1049 [M]$^+$, calcd. for $C_{23}H_{20}O_{10}$, 456.1056) (Figure 1). Compound **1** gave a positive reaction with Mg-HCl reagent, and its UV spectrum gave absorption maximum at 267 and 348 nm, which indicated the presence of flavonol skeleton in its structure (Tu et al, 2011).

A group of ^{1}H-NMR data at δ_H 5.47 (1H, brs, H-1‴), 4.29 (1H, brs, H-2‴), 3.78 (1H, dd, *J* = 9.0, 3.0 Hz, H-3‴), 3.76 (1H, m, H-4‴), 3.41 (1H, m, H-5‴) and 0.97 (3H, d, *J* = 6.0 Hz, H-6‴), as well as corresponding ^{13}C-NMR data at δ_C 103.6 (C-1‴, d), 72.2 (C-2‴, d), 73.1 (C-3‴, d), 72.1 (C-4‴, d), 71.9 (C-5‴, d), and 17.7 (C-6‴, q), indicated the presence of α-*L*-rhamnose (Tu et al, 2011), which was further confirmed by the acid hydrolysis of compound **1**. The correlation peak from the anomeric proton H-1‴ of rhamnose to C-3 (137.2, s) was observed in the HMBC spectrum, indicating that the rhamnose was located at C-3. Therefore, compound **1** was 5,4′-dihydroxyfurano [2″,3″:7,8] flavonol 3-*O*-α-*L*-rhamnoside.

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

No.	δ_C	δ_H (mult, *J*, Hz)
2	159.4	
3	137.2	
4	180.6	
5	159.5	12.46 (s, 5-OH)
6	95.6	6.91 (1H, s)
7	160.6	
8	109.9	
9	150.5	
10	108.7	
1′	122.3	
2′	132.1	7.90 (1H, d, 8.0)
3′	116.7	7.01 (1H, d, 8.0)
4′	161.9	
5′	116.7	7.01 (1H, d, 8.0)
6′	132.1	7.90 (1H, d, 8.0)
2″	146.1	7.77 (1H, d, 2.0)
3″	104.6	7.12 (1H, d, 2.0)
Rha		
1‴	103.6	5.47 (1H, brs)
2‴	72.2	4.29 (1H, brs)
3‴	73.1	3.78 (1H, dd, 9.0, 3.0)
4‴	72.1	3.76 (1H, m)
5‴	71.9	3.41 (1H, m)
6‴	17.7	0.97 (3H, d, 6.0)

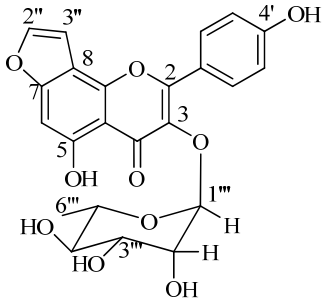

Figure 1 Structure of compound 1

A group of signals at δ_H 7.77 (d, *J* = 2.0 Hz, H-2″) / δ_C 146.1 (C-2″, d) and δ_H 7.12 (d, *J* = 2.0 Hz, H-3″) / δ_C 104.0 (C-3″), in combination with the HMBC correlations (Figure 2) of H-3″ with C-2″, C-7 (δ_C 160.6), and C-8 (δ_C 109.9), as well as H-2″ with C-3″, C-7, and C-8, indicated that a furan ring was fused at C-7 (oxygenated) and C-8 of ring A (Yadav, Ahmad, and Maurya, 2004). In addition, the signal at δ_H 12.46 in the ^{1}H-NMR spectrum was assigned as the chelated C_5-OH group. Therefore, the singlet signal at δ_H 6.91 (1H, s) was unambiguously assignable to H-6 of ring A, which was further proved by the HMBC correlations from H-6 to C-5 (δ_C 159.5), C-7, and C-10 (δ_C 108.7). Moreover, four low-field aromatic protons at δ_H 7.01 (2H, d, *J* = 8.0 Hz) and 7.90 (2H, d, *J* = 8.0 Hz) forming an AA′BB′ coupling system elucidated that only C-4′ was substituted in ring B. The low-field signal

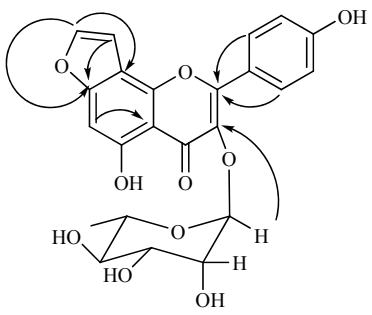

Figure 2 Key HMBC correlations of compound 1

of C-4′ at δ 161.9 combined with the molecular formula $C_{23}H_{20}O_{10}$ revealed that C-4′ was replaced by a hydroxyl group, which was further supported by the HMBC correlations from H-3′ and H-5′ to C-4′.

Therefore, based upon the above cumulative evidences, the structure of compound **1** could be explicitly determined as 5,4′-dihydroxyfurano [2″,3″:7,8] flavonol 3-*O*-α-*L*- rhamnoside.

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Acknowledgements

The reviewers in charge of manuscripts of this issue of CHM are as follows: Chun-fu Wu, Xiao-po Zhang, Ming-hua Qiu, Bin Wu, Jie Sun, Xiao-ping Qin, Hui-ye Zhang, Hai-hui Xie, Tie-jun Ling, Hui-ming Hua, Hai-feng Wu, Yan-jun Zhang, Shu-li Man, Mun-fei-Yam, Xi Huang, Yu-hong Bian, Zhi-min Wang.