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Fubo Han & lk-Soo Lee

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A new flavonol glycoside from the aerial parts of *Epimedium koreanum* Nakai

Fubo Han 🕩 and Ik-Soo Lee 🕩

College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju, Republic of Korea

ABSTRACT

A new prenylated flavonol glycoside (1) was isolated from a 95% methanol extract of the dried and powdered aerial parts of *Epimedium koreanum* Nakai (Herba Epimedii), along with seven previously known flavonoids (**2–8**). The chemical structure of the new compound (1) was established to be 5-hydroxy-4'-methoxy-8-(2-hydroxy-3-methyl-3-butenyl)flavone 3-O- α -L-rhamnopyranosyl-7-O- β -D-gluco pyranoside on the basis of spectroscopic methods. The antioxidant activities of these compounds were determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and kaempferitrin (**8**) showed a high reactivity with DPPH.



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KEYWORDS

Epimedium koreanum Nakai; flavonol glycoside; antioxidant activity

1. Introduction

Herba Epimedii (Yinyanghuo in Chinese), the dried aerial parts of various *Epimedium* plants, has been safely used as an important traditional Chinese herbal medicine for thousands of years (Guo & Xiao 2003). Plants of the genus *Epimedium* are geographically distributed in China, Korea, Japan, North Italy and some countries in South-east Asia (Fan & Quan 2012). Herba Epimedii, mainly used as a kidney tonic, aphrodisiac and anti-rheumatic medicinal herb (Zhao et al. 2007), has also been used to treat many diseases such as cardiovascular diseases, osteoporosis, sexual dysfunction and tumours (Sze et al. 2010; Ma et al. 2011). Previous studies on the chemical constituents of the *Epimedium* species have led to the isolation of various flavones, lignans, diphydrophenanthrenes, ionones, terpene glycosides

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CONTACT Ik-Soo Lee 🖾 islee@chonnam.ac.kr



Figure 1. Structures of compounds 1-8.

and phenylethanoids (Matsushita et al. 1991; Miyase & Ueno 1991; Sun et al. 1998; Li et al. 2011; Zhou et al. 2015). These studies have shown that the *Epimedium* species are a good source of flavonol glycosides, which exhibit antioxidant, anti-osteoporosis, androgenic and antidepressant-like actions (Pan et al. 2007; Zhang et al. 2008; Li et al. 2012; Miura et al. 2015). In the course of our search for potent bioactive components from *Epimedium* species, the chemical investigation of the aerial parts of *E. koreanum* Nakai was carried out. This paper describes the isolation and structure elucidation of one new flavonol glycoside (1) along with seven known flavonoids (2–8). In addition, the antioxidant activities of these compounds were determined using DPPH radical scavenging assay.

2. Results and discussion

The 95% MeOH extract of the aerial parts of *E. koreanum* was partitioned with *n*-hexane, CHCl₃ and EtOAc. The EtOAc fraction was chromatographed on silica gel columns and semi-preparative HPLC system, which afforded eight compounds including one new flavonol glycoside (**1**, 12.2 mg) (Figure 1). The known compounds (**2–8**) were identified as icariin (**2**, 474.4 mg, Mizuno et al. 1987), epimedin B (**3**, 181.9 mg, Ito et al. 1988), epimedin A (**4**, 29.0 mg, Ito et al. 1988), epimedoside A (**5**, 30.4 mg, Mizuno et al. 1988), diphylloside A (**6**, 6.7 mg, Mizuno et al. 1988), epimedoside E (**7**, 5.4 mg, Mizuno et al. 1988) and kaempferitrin (**8**, 9.4 mg, Cardoso et al. 2013), respectively, by comparing their spectroscopic data with previously published data.

Compound **1** was isolated as a yellow amorphous powder, gave a positive reaction with Molisch reagent. HR-ESI-MS (*m/z*: 715.2214 [M + Na]⁺, calcd for C₃₃H₄₀O₁₆Na, 715.2214) indicated its molecular formula was C₃₃H₄₀O₁₆. In its thin-layer chromatography (TLC), **1** was detected under UV lamp at 254 nm and appeared as a yellow spot after spraying with anisaldehyde–H₂SO₄ spray reagent and heating.

Its UV spectrum was similar to that of icariin (**2**) at 268 and 350 nm. The ¹H NMR spectrum of **1** showed five aromatic proton signals. The four *ortho*-coupled protons at $\delta_{\rm H}$ 7.08 (2H, d, J = 8.2 Hz) and 7.90 (2H, d, J = 8.2 Hz) corresponded to an AA'BB' spin system assigned to the 4'-substituted ring B. The methoxyl group at $\delta_{\rm H}$ 3.90 (3H, s) was deduced to be attached to C-4' ($\delta_{\rm C}$ 162.1) based on the correlation between –OCH₃ and C-4' signals that was observed in the heteronuclear multiple bond correlation (HMBC) spectrum. A set of signals at $\delta_{\rm H}$ 3.00 (1H, dd, J = 10.3, 13.5 Hz, H-11a), 2.92 (1H, dd, J = 3.9, 13.5 Hz, H-11b), 4.30 (1H, m, H-12), 4.93 (1H, s, H-14a), 4.80 (1H, s, H-14b) and 1.79 (3H, s, H-15) in the ¹H NMR spectrum and the signals at $\delta_{\rm C}$ 28.7 (C-11), 75.0 (C-12), 147.9 (C-13), 109.6 (C-14) and 16.8 (C-15) in the ¹³C NMR spectrum were assigned to a 2-hydroxy-3-methyl-3-butenyl group. The location of the 2-hydroxy-3-methyl-3-butenyl group at C-8 position of **1** was supported by HMBC correlations of H-11 ($\delta_{\rm H}$ 3.00, 2.92) to C-7 ($\delta_{\rm C}$ 161.8) and C-9 ($\delta_{\rm C}$ 153.7). Therefore, the aglycone of **1** was deduced to be 5,7-dihydroxy-4'-methoxy-8-(2-hydroxy-3-methyl-3-butenyl)flavone (2"-hydroxy-3"-en-anhydroicaritin) (Luo et al. 2009).

In the ¹H and ¹³C NMR spectra of **1**, signals due to one D-glucopyranosyl moiety and L-rhamnopyranosyl moiety were observed. The anomeric proton signals were observed at $\delta_{\rm H}$ 4.98 (1H, d, J = 6.8 Hz, H-1") and $\delta_{\rm H}$ 5.37 (1H, s, H-1"), and the corresponding carbon signals appeared at $\delta_{\rm C}$ 101.5 (C-1") and $\delta_{\rm C}$ 102.2 (C-1"). The β -glucosidic and α -rhamnosidic linkages of these sugars were inferred from the coupling constants (*J*) of the anomeric protons.

The connectivities of 2"-hydroxy-3"-en-anhydroicaritin and glucose and rhamnose were elucidated by the HMBC correlation signals. The HMBC correlations between C-7 (δ_c 161.8) of 2"-hydroxy-3"-en-anhydroicaritin and H-1" (δ_H 4.98) of glucose confirmed the attachment of a glucose unit to the aglycone, and the position of rhamnose unit was confirmed in a similar manner by correlations between C-3 (δ_c 135.2) of 2"-hydroxy-3"-en-anhydroicaritin and H-1" (δ_H 5.36) of rhamnose. Based on these results, compound **1** was identified as 5 - h y d r o x y - 4' - m e t h o x y - 8 - (2 - h y d r o x y - 3 - m e t h y I - 3 - b u t e n y I) fl a v o n e 3-O- α -L-rhamnopyranosyl-7-O- β -D-glucopyranoside.

DPPH radical scavenging activities of the flavonoids were determined by spectrophotometric assay. However, only the non-prenylated compound **8** showed high reactivity with DPPH (IC₅₀ 45.5 μ g/mL), which was a little weaker than the positive control Vitamin C (IC₅₀ 36.9 μ g/mL) (Table S1). Therefore, it can be indicated that C-8 prenylation could reduce the antioxidant activities of the flavonol glycosides isolated from *E. koreanum*, and this notion was the same as the result previously reported (Li et al. 2012).

3. Experimental

3.1. General experimental procedures

UV spectra were recorded on a JASCO V-530 spectrophotometer in MeOH, and IR spectra were obtained on a JASCO FT/IR-300E spectrometer in KBr discs. One-dimensional (1D) NMR experiments such as ¹H (500 MHz) and ¹³C (125 MHz) NMR experiments, 2D NMR experiments such as homonuclear correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC) and HMBC spectra were measured on a Varian Unity Inova-500 spectrometer with tetramethylsilane (TMS) as internal standard. The chemical shift values are reported in ppm units, and the coupling constants are in Hz. HR-ESI-MS data were recorded on a Synapt G2 mass spectrometer (Waters, UK) at the Korea Basic Science Institute. TLC

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analyses were carried out using pre-coated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) with CHCl₃/MeOH (8 : 2, volume ratio) and visualisation of the TLC plates was performed under UV radiation (254 nm) and spraying with anisaldehyde-H₂SO₄ followed by heating. Column chromatographic isolations were performed using silica gel 60 (70–230 mesh, Merck). The semi-preparative HPLC system consisted of a chromatographic pump (Waters 600E Multi Solvent Delivery System) and a UV detector (Waters 996 PDA). A Phenomenex Luna C₁₈ column (10 × 250 mm, particle size 5 µm) was used for separations.

3.2. Plant material

The aerial parts of *E. koreanum* Nakai were purchased from Kyungdong Oriental Herbal Market in Seoul, Korea, August 2014 and identified by one of the authors (ISL). A voucher specimen (CNU-HE-201408) has been deposited in the College of Pharmacy, Chonnam National University.

3.3. Extraction and isolation

Aerial parts of *E. koreanum* (600 g) were crushed and extracted with 95% MeOH (3L × 3) under room temperature. The combined MeOH extracts (40 g) were concentrated and redissolved in water (500 mL), and partitioned successively with *n*-hexane, CHCl₃ and EtOAc (each 500 mL × 2). The EtOAc fraction (5 g) was subjected to column chromatography on silica gel (3.6 × 30 cm), with a gradient of CHCl₃/MeOH mixtures (9 : 1 → 0 : 10) and six fractions (Fr. 1–6) were collected. A solid precipitate was separated from Frs. 3 and 4 and recrystallised from MeOH to give **2** (360.4 mg). The filtrate of Fr. 3 was subjected to semi-preparative HPLC (55% MeOH/H₂O, 2.5 mL/min) to give **1** (6.9 mg) and **2** (63.5 mg). The filtrate of Fr. 4 was subjected to semi-preparative HPLC (50% MeOH/H₂O, 3 mL/min) to give **1** (5.3 mg), **2** (50.5 mg), **3** (21.8 mg) and **4** (4.9 mg). Fr. 5 was directly subjected to a semi-preparative HPLC (55% MeOH/H₂O, 2.5 mL min⁻¹) to afford **3** (160.1 mg), **4** (24.1 mg), **5** (30.4 mg), **6** (6.7 mg), **7** (5.4 mg) and **8** (9.4 mg).

3.3.1. 5-Hydroxy-4'-methoxy-8-(2-hydroxy-3-methyl-3-butenyl)flavone 3-O- α -L-rhamnopyranosyl-7-O- β -D-glucopyranoside (1)

Yellow amorphous powder. IR (KBr) v_{max} cm⁻¹: 3366, 1973, 1931, 1653, 1594, 1508, 1259. UV (MeOH) λ_{max} (log ε): 214 sh. (2.39), 222 (2.43), 269 (2.48), 314 (1.58), 350 (1.34) nm. ¹H NMR (500 MHz, CD₃OD): δ 7.88 (2H, d, J = 8.7 Hz, H-2', H-6'), 7.06 (2H, d, J = 8.7 Hz, H-3', H-5'), 6.64 (1H, s, H-6), 5.36 (1H, s, H-1''), 4.98 (1H, d, J = 6.8 Hz, H-1''), 4.93 (1H, s, H-14), 4.80 (1H, s, H-14'), 4.30 (1H, m, H-12), 4.25 (1H, s, H-2''), 3.97 (1H, m, H-6''), 3.89 (3H, s, -OCH₃), 3.79 (1H, m, H-6''), 3.74 (1H, m, H-3'''), 3.58 (1H, m, H-2''), 3.53 (1H, m, H-3''), 3.47 (1H, m, H-4''), 3.37 (1H, m, H-6''), 3.35 (1H, m, H-4'''), 3.00 (1H, dd, J = 10.3, 13.5 Hz, H-11), 2.92 (1H, dd, J = 3.9, 13.5 Hz, H-11), 1.79 (3H, s, H-15), 0.94 (3H, d, J = 5.6 Hz, H-6'''). ¹³C NMR (125 MHz, CD₃OD): δ 178.6 (C-4), 162.1 (C-4'), 161.8 (C-7), 160.1 (C-5), 157.5 (C-2), 153.7 (C-9), 147.9 (C-13), 135.2 (C-3), 130.4 (C-2', 6'), 122.4 (C-1'), 113.8 (C-3', 5'), 109.6 (C-14), 106.6 (C-8), 106.2 (C-10), 102.2 (C-1'''), 101.5 (C-1''), 98.0 (C-6), 77.0 (C-3''), 76.4 (C-5''), 75.0 (C-12), 73.7 (C-2''), 71.8 (C-4'''), 70.7 (C-3''', 5'''), 70.5 (C-2'''), 69.7 (C-4''), 61.1 (C-6''), 54.7 (-OCH₃), 28.7 (C-11), 16.8 (C-15), 16.3 (C-6'''). HR-ESI-MS m/z: 715.2214 [M+Na]⁺ (calcd C₃₃H₄₀O₁₆Na, 715.2214).

3.4. Acid hydrolysis of compound 1

Compound **1** (2 mg) was heated in 10% HCl (3 mL) at 80 °C for 2 h. The reaction mixture was then extracted with EtOAc (5 mL × 3). The water layer was evaporated to dryness and then dissolved in MeOH. The solution was then subjected to silica gel TLC together with authentic D-glucose ($R_{\rm fr}$ 0.12) and L-rhamnose ($R_{\rm fr}$ 0.30). The plate was developed with CHCl₃/MeOH/H₂O (13 : 7 : 2) lower phase, and visualised by anisaldehyde–H₂SO₄ solution (10 min at 105 °C).

3.5. DPPH radical scavenging activity

Each sample was dissolved in absolute MeOH and added to a DPPH solution (0.056 mg/mL, in absolute MeOH). After mixing gently for 30 min, optical densities were measured at 517 nm under 27 °C using a microplate reader (SpectraMax Plus 384, Molecular Devices, USA). Vitamin C was used as the positive control.

4. Conclusions

In conclusion, eight flavonol glycosides (1–8) including a new prenylated flavonol glycoside (1) were isolated from the aerial parts of *E. koreanum* Nakai. Among the isolates, the non-prenylated kaempferol glycoside 8 showed good antioxidant activity. The results indicated that C-8 prenylation of the flavonol glycosides isolated from *E. koreanum* could reduce their antioxidant activities.

Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCiD

Fubo Han (D) http://orcid.org/0000-0002-4283-5028 *Ik-Soo Lee* (D) http://orcid.org/0000-0003-4903-7106

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