A New Flavonol Glycoside, Epimedin K, from *Epimedium koreanum*

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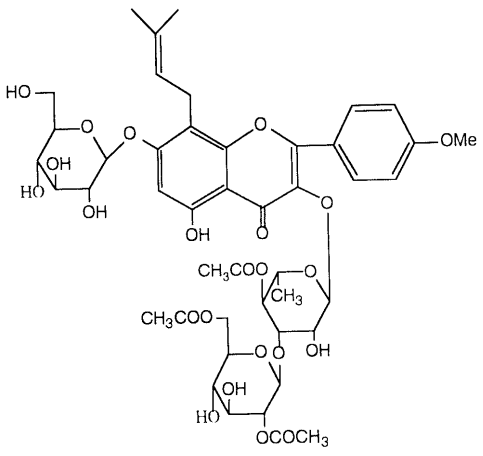
A new flavonol glycoside named epimedin K was isolated from the aerial parts of *Epimedium koreanum* NAKAI (Berberidaceae). Its chemical structure was found to be anhydroicaritin 3-*O*-β-D-(2,6-di-*O*-acetyl) glucopyranosyl (1→3)-α-L-(4-*O*-acetyl) rhamnopyranoside-7-*O*-β-D-glucopyranoside on the basis of spectroscopic and chemical evidence.

Key words *Epimedium koreanum*; Berberidaceae; anhydroicaritin; epimedin K

Constituent studies of *Epimedium* species have been carried out and many new flavonol glycosides have been isolated. As a general characteristic of the glycosides in *Epimedium* species, the aglycone moiety has a γ,γ-dimethylallyl group at the C-8 of kaempferol. As regards the sugar moiety, an α-L-rhamnose is always linked at C-3 as an *endo*-sugar. They can be classified into four groups A, B, C, and D, according to the presence or absence of a methyl group at C-4′ and a glucose residue at C-7.[1] The first group (A) contained glucosides both with a methyl group at C-4′ (anhydroicaritin)[2] and a glucose residue at C-7 as in the epimedins (A—C)[3] and hexandraside D.[4] The second group (B) consists of 7-hydroxylflavones with a methyl group at C-4′ as in the sagittatosides (A—C).[5] The third group (C) consists of flavonol glycosides which lack a methyl group at C-4′ in the aglycone moiety (8-isopentenylkaempferol), but the hydroxy group at C-7 is glucosylated as in the epimedosides (A, C, E)[6] and diphyllosides (A, B).[7] The fourth group (D) are 7-hydroxyflavones without a methyl group at C-4′ as in the ikarisosides (A, B, D, F).[8]

In our previous paper,[9] the isolation and structural determination of one new flavonol glycoside named epimedoside, together with three known compounds, icariside A_1, maltol and salidroside, were reported as constituents of the aerial parts of *Epimedium koreanum* NAKAI. Further investigation of *E. koreanum* revealed another new flavonol glycoside named epimedin K. The structure was determined on the basis of chemical evidence and spectral data.



The compound, a yellow powder, mp 200—201 °C, reacted positively to the Molish and Mg–HCl tests. Its UV spectrum was characteristic of a flavone. Upon acidic hydrolysis, the compound gave glucose and rhamnose, and so should be a flavonol glycoside. Its ^{1}H-NMR spectrum exhibited a characteristic singlet signal of 5-OH at δ 12.49 , signals due to a methoxyl group at δ 3.89 (s) , two methyl signals at δ 1.61 and 1.69 (each s) and a methylene signal at δ 3.57 and 3.43 (m), together with a signal at δ 5.18 (t, J=7.3 Hz) indicating the presence of a prenyl group. Furthermore, signals of five protons appeared in the aromatic region: A signal at δ 6.65 (1H, s) was due to the proton attached to C-6. A set of *ortho*-coupled doublet signals of four protons at δ 7.89 (2H, d, J=8.5 Hz) and 7.17 (2H, d, J=8.5 Hz) ppm corresponded to an AA′BB′ spin system assignable to the 4′-substituted ring B. In the ^{13}C-NMR spectrum, the carbon signals at δ 55.5 and 21.3, 122.0, 131.0, 25.4, 17.8 ppm further corroborated the presence of a methoxyl and a prenyl group. In the heteronuclear multiple bond correlation spectroscopy (HMBC) spectrum, correlations between the proton signal of a methoxyl group and the C-4′ signal, the methylene proton signal in the prenyl group and the C-8 signal were observed. So, we confirmed that the methoxyl group and prenyl group were attached to C-4′ and C-8 , respectively. The electron impact (EI)-MS showed characteristic ion peaks at m/z 368 (aglycone), 313 (aglycone−C_4H_7), 300, 165 and 135. The fragment at m/z 368, which corresponded to the aglycone of this compound, suggests the presence of three hydroxyls, one methoxyl and one prenyl group. Another fragment at m/z 135 (B2) indicated that the methoxyl group was in ring B. The m/z 165 fragment showed that two of the three hydroxyls were in ring A. In addition to 5-OH, the other should be at C-7 because the position of C-5, C-6, C-8 had been determined in ring A. The last one should be at C-3 due to the other positions having been assigned in the aglycone. On the basis of the above data, the aglycone was confirmed to be 8-γ,γ-dimethylallyl-4′-methoxy- 3,5,7-trihydroxyflavone (anhydroicaritin). The conclusion was further supported by acidic hydrolysis of this compound, when the aglycone obtained was shown to be identical to authentic anhydroicaritin. In the ^{1}H-NMR and ^{13}C-NMR

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spectra, signals due to an L-rhamnopyranosyl moiety and two D-glucopyranosyl moieties were observed. In the ^{1}H-NMR spectrum, the signal of the anomeric proton of the L-rhamnopyranose appeared at δ 5.34 (1H, br s), signals of the anomeric proton of the D-glucopyranose were observed at δ 4.52 (1H, d, J=7.9 Hz) and δ 5.01 (1H, d, J=7.3 Hz). The β-glucosidic and α-rhamnosidic linkages of these sugars were inferred from the coupling constants of the anomeric protons. In the HMBC spectrum, the signal of the anomeric proton at δ 5.01 (1H, d, J=7.3 Hz) was correlated with C-7 (δ 160.6) of the aglycone. The correlation indicated a β-D-glucopyranosyl moiety whose anomeric proton signal at δ 5.01 ppm was bonded to C-7. In addition, in the HMBC spectrum, a correlation between the anomeric proton of the α-L-rhamnopyranosyl moiety at δ 5.34 (1H, br s) and C-3 (δ 133.7) was shown. Thus, we determined that the α-L-rhamnopyranosyl moiety, with an anomeric proton signal at δ 5.34 ppm, was the substituent at C-3. Moreover, in the HMBC spectrum, the anomeric proton of β-D-glucopyranose at δ 4.52 (1H, d, J=7.9 Hz) was correlated with the signal at δ 76.2. The signal at δ 76.2 was assigned to C-3 of the α-L-rhamnopyranose. Therefore, it was deduced that the β-D-glucopyranosyl moiety with a signal of an anomeric proton at δ 4.52 ppm was substituted at C-3 of the α-L-rhamnopyranosyl moiety. The signals of protons at δ 1.98 (2×3H, s), 2.02 (3H, s) and the signals of the carbons at δ 20.6, 20.5, 20.4 ppm showed the presence of three acetyl methyls. The signals of carbons at δ 168.9, 169.4, 170.2 ppm showed existence of three carbonyls. Finally, as regards the positions of attachment of the three acetyl groups, confirmatory evidence was provided by HMBC and ^{1}H–^{1}H correlation spectroscopy (COSY) experiments as follows: one carbonyl signal (δ 169.4) was correlated with the proton of R4 of the rhamnosyl moiety at δ 4.78 ppm and the carbon of the carbonyl (δ 168.9) with the proton of G″2 at δ 4.57 and the other (δ 170.2) with the protons of G″6 of the glucosyl moiety at δ 4.34, 4.18 . Thus, it can be confirmed that the three acetyls were attached at R4-C, G″2-C and G″6-C, respectively. From the above data, we concluded that the compound was anhydroicaritin 3-*O*-β-D-(2,6-di-*O*-acetyl) glucopyranosyl-(1→3)-α-L-(4-*O*-acetyl) rhamnopyranoside-7-*O*-β-D-glucopyranoside. It was named epimedin K because it belonged to the A group.

Experimental

General Procedures The melting point was determined on a Kofler apparatus and is uncorrected. ^{1}H- and ^{13}C-NMR spectra, with tetramethylsilane (TMS) as internal standard, were recorded on a JEOL GSX 400 and/or α-500 FT-NMR. EI-MS was measured on a JEOL JMS-SX 102. UV spectra were measured on a Shimadzu UV-260. Silica-gel was from the Qingdao Marine Chemical Factory, Shandong Province, China. High performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-10 instrument. The plant was purchased in October 1992 in Liaoning Province, China.

Isolation The aerial parts of *E. koreanum* NAKAI (25 kg) were extracted twice with 70% ethanol. After removal of the ethanol, the extract was absorbed on Amberlite D 101 and the resin was eluted successively with water, 40% and 95% ethanol . Part of the 95% ethanol eluate (127 g) was chromatographed on silica-gel with a chloroform–methanol gradient. The chloroform–methanol (10 : 1) eluate gave a mixture, which was subjected to preparative HPLC on an ODS-RP18 column (Waters) with methanol–water (6.4 : 3.6) to give the compound (28 mg).

Epimedin K A yellow powder, mp 200—201 °C, reacting positively to the Molish and Mg–HCl tests. UV λ_{max}^{MeOH} (nm): 270.2, 313.2, 349.2; 271.8, 354.4 (+NaOMe); 278.8, 306.0, 343.0, 408.6 (+$AlCl_3$); 279.8, 304.6, 337.8, 411.2 (+$AlCl_3$/HCl); 270.0, 313.2 (+NaOAc); 270.0, 313.0, 348.2 (+NaOAc/H_3BO_3). EI-MS (*m/z*): 368, 353, 313, 300, 165, 135. ^{1}H-NMR (DMSO-d_6) δ: 12.49 (1H, s, 5-OH), 7.89 (2H, d, J=8.5 Hz, H-2′, 6′), 7.17 (2H, d, J=8.5 Hz, H-3′, 5′), 6.65 (1H, s, H-6), 5.18 (1H, t, J=7.3 Hz, H-12), 3.57, 3.43 (2H, m, H-11), 1.69 (3H, s, H-15), 1.61 (3H, s, H-14); G′: 5.01 (1H, d, J=7.3 Hz, H-1), 3.32 (2H, m, H-2,3), 3.19 (1H, m, H-4), 3.45 (1H, m, H-5), 3.73 (1H, m, H-6a), 3.47 (1H, m, H-6b), R: 5.34 (1H, br s, H-1), 4.10 (1H, m, H-2), 3.82 (1H, dd, J=2.4, 9.7 Hz, H-3), 4.78 (1H, t, J=9.7 Hz, H-4), 3.26 (1H, m, H-5), 0.73 (3H, d, J=6.1 Hz, CH_3-6), G″: 4.57 (1H, m, H-2), 4.52 (1H, d, J=7.9 Hz, H-1), 3.41 (1H, m, H-3), 3.21 (1H, m, H-4), 3.61 (1H, m, H-5), 4.34 (1H, m, H-6a), 4.18 (1H, m, H-6b); 3.89 (3H, s, 4′-OCH_3), 2.02 (3H, s, R_4-Ac), 1.98 (2×3H, s, $G''_{2,6}$-2×Ac). ^{13}C-NMR (DMSO-d_6): 178.0 (C-4), 161.6 (C-4′), 160.6 (C-7), 159.0 (C-5), 157.3 (C-2), 153.0 (C-9), 133.7 (C-3), 131.0 (C-13), 130.4 (C-2′, 6′), 122.0[a] (C-12), 121.9[a] (C-1′), 114.1 (C-3′, 5′), 108.4 (C-8), 105.5 (C-10), 98.2 (C-6), 25.4 (C-14), 21.3 (C-11), 17.8 (C-15); G′: 100.5 (C-1), 73.2 (C-2), 76.5 (C-3), 69.6 (C-4), 77.1 (C-5), 60.5 (C-6); R: 100.9 (C-1), 69.1 (C-2), 76.2 (C-3), 71.2 (C-4), 68.0 (C-5) 16.9 (C-6) ; G″: 101.3 (C-1), 73.2 (C-2), 73.9 (C-3), 70.23(C-4), 73.7 (C-5), 63.5 (C-6); 55.5 (4′-OCH_3), 169.4, 20.6 (R_4-Ac), 168.9, 20.5 (G''_2-Ac), 170.2, 20.4 (G''_6-Ac) (note: *a*, assignment may be interchanged, G′, G″ and R are those of the glucose at C-7 and the *exo*-glucose and *endo*-rhamnose at C-3, respectively). *Anal.* Calcd for $C_{45}H_{56}O_{23}$: C, 56.01; H, 5.85. Found: C, 55.98; H, 5.87.

Acid Hydrolysis Compound (0.5 mg) was dissolved in 0.1 ml methanol and concentrated HCl (3—4 drops). The solution was taken up collected in capillaries . The solution was heated for 3 h at 60 °C, then subjected to silica-gel TLC for analysis together with authentic samples (glucose, rhamnose and anhydroicaritin). 1) developing solvent: $CHCl_3$–CH_3OH 2 : 1. Glucose and rhamnose were detected. 2) developing solvent : cyclohexane : ethyl acetate, 1 : 1. The aglycone of the compound had the same *Rf* as anhydroicaritin.

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References

1) Mizuno M., Iinuma M., Tanaka T., Iwashima S., Sakakibara N., Liu X., Shi D., Mussel H., *Asian J. Plant Sci.*, **1**, 1—6 (1989).
2) Akai S., Imaida M., Matsukawa T., *Yakugaku Zasshi*, **55**, 1139—1152 (1935).
3) Oshima Y., Okamoto M., Hikino H., *Heterocycles*, **16**, 935—942 (1987).
4) Mizuno M., Kanie Y., Iinuma M., Tanaka T., Lang F. A., *Phytochemistry*, **30**, 2765—2768 (1991).
5) Mizuno M., Sakakibara N., Hanioka S., Iinuma M., Tanaka T., Liu X., Shi D., *Phytochemistry*, **27**, 3641—3643 (1988).
6) Takemoto T., Daigo K., Tokuoka Y., *Yakugaku Zasshi*, **95**, 312—320 (1975); Tokuoka Y., Daigo K., Takemoto T., *ibid.*, **95**, 321—325 (1975); *idem*, *ibid.*, **95**, 698—705, 825—829 (1975).
7) Mizuno M., Iinuma M., Tanaka T., Sakakibara N., Fujikawa T., Hanioka S., Ishida Y., Liu X., Murata H., *Phytochemistry*, **27**, 3645—3647 (1988).
8) Fukai T., Nomura T., *Phytochemistry*, **27**, 259—266 (1988).
9) Sun P., Ye W., Zhao J., Pei Y., Wang Z., Chen Y., Ogihara Y., Takeda T., *Chem. Pharm. Bull.*, **43**, 703—704 (1995).