Studies on the Constituents of *Epimedium koreanum*

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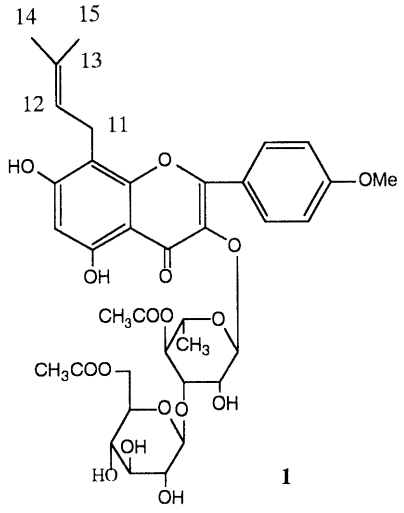
A new flavonol glycoside, epimedoside (1), was isolated together with three known compounds, identified as icariside A_1 (2), maltol (3) and salidroside (4), from the aerial parts of *Epimedium koreanum* NAKAI (Berberidaceae). Their structures were established by spectroscopic methods and chemical evidence.

Key words *Epimedium koreanum*; Berberidaceae; epimedoside; anhydroicaritin; icariside A_1; salidroside

The aerial parts of several plants of the genus *Epimedium* (Berberidaceae) have been used as a tonic in China and Japan. In chemical studies on the constituents of these plants a number of flavonoids,[1] alkaloids[2] and lignans[3] have been identified. We studied the aerial parts of *E. koreanum* NAKAI, with the aim of the isolating biologically active constituents. In this paper, we describe the isolation of one new flavonol glycoside, anhydroicaritin-3-*O*-β-D-(6-*O*-acetyl)glucopyranosyl(1→3)-α-L-(4-*O*-acetyl)rhamnopyranoside, named epimedoside (**1**), together with three known compounds, icariside A_1 (**2**),[4] maltol (**3**),[5] and salidroside (**4**).[6] The structures were determined on the basis of chemical evidence and spectral data.

Compound **1**, a yellow amorphous powder, mp 170—171 °C, was positive to the Molish and Mg–HCl tests. Its UV spectrum was characteristic of a flavone. Upon acidic hydrolysis, **1** gave a glucose and a rhamnose, so it should be a flavonol glycoside. In the UV spectrum of **1**, the bathochromic shift of band II by 9.2 nm caused by NaOAc indicated the presence of a hydroxyl group at C-7 in this compound. Its proton magnetic resonance (^{1}H-NMR) spectrum exhibited a characteristic singlet signal of 5-OH at δ 12.45, signals due to a methoxyl group at δ 3.88 (s), two methyl signals at δ 1.68 and 1.62 (each s) and a methylene signal at δ 3.38 (m), together with signal at δ 5.17 (br t, J=6.0 Hz), indicating the presence of a prenyl group. Furthermore, its ^{1}H-NMR spectrum showed a set of signals due to the 4′-substituted ring B at δ 7.86 (2H, d, J=8.5 Hz) and 7.15 (2H, d, J=8.5 Hz), along with a signal due to ring A at δ 6.32 (1H, s). Further, the heteronuclear multiple-bond correlation (HMBC) spectrum, which showed a ^3J correlation between 5-OH and C-6 (δ 98.4), and C–H correlation (C–H COSY) experiments with **1** allowed us to assign the proton signal at δ 6.32 to 6-H of the aglycone. The electron impact mass spectrum (EI-MS) of **1** showed characteristic ion peaks at m/z 368 (aglycone), 353 (aglycone−Me), 313 (aglycone $-C_4H_7$), 300, 165 and 135. The fragment at m/z 368, which corresponded to the aglycone of this compound, suggested 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. We concluded that the methoxyl group was at C-4′. The fragment of m/z 165 showed that the prenyl group was in ring A. It was attached at C-8 because the substituents at C-5, C-6 and C-7 had been assigned. Among the three hydroxyls, two were located above at C-5 and C-7, so the last one should be at C-3 due to other positions having been assigned in the aglycone. On the basis of the above data, the aglycone of **1** was identified as 8-prenyl-4′-methoxy-3,5,7-trihydroxyflavone (anhydroicaritin). In the ^{13}C-NMR spectrum, the signals due to a L-rhamnopyranosyl moiety and a D-glucopyranosyl moiety were observed. In the ^{1}H-NMR spectrum, the signal of the anomeric proton of the L-rhamnopyranose appeared at δ 5.35 (1H, br s) and the signal of the anomeric proton of the D-glucopyranose was observed at δ 4.25 (1H, d, J=7.9 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 anomeric proton of α-L-rhamnopyranose was correlated with C-3 (δ 133.5) of the aglycone, and the anomeric



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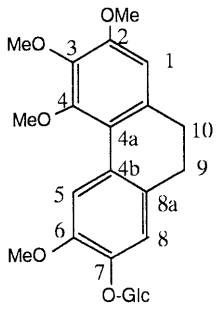

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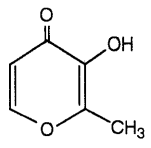

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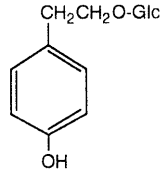

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proton of β-D-glucopyranose was correlated with the signal at δ 76.7. By C–H COSY and H–H COSY, the signal at δ 76.7 was assigned to C-3 of the α-L-rhamnopyranose. Therefore, the position of α-L-rhamnopyranosidation was decided to be at C-3 of the aglycone, and the location of β-D-glucopyranosylation was determined to be at C-3 of the α-L-rhamnopyranose. Finally, as regards the positions of attachment of the two acetyl groups, confirmatory evidence was provided by HMBC and ^{1}H–^{1}H COSY experiments as follows: one carbonyl signal (δ 169.6) was correlated with the proton of C-4 of the rhamnosyl moiety at δ 4.83 and the other (δ 170.3) with the protons of C-6 of the glucosyl moiety at δ 4.27 and 4.18, respectively. From the above data, we concluded that compound **1** was anhydroicaritin-3-*O*-β-D-(6-*O*-acetyl)glucopyranosyl-(1→3)-α-L-(4-*O*-acetyl)rhamnopyranoside. It was named epimedoside.

Compound **2**, was obtained as colorless needles, mp 219—220 °C. The UV spectrum showed absorption maxima at 216.8, 280.6, 302.2 and 312.8 nm, suggesting the presence of a 9,10-dihydrophenanthrene skeleton. This compound was identified as icariside A_1 (**2**) by further comparison of various data (^{1}H- and ^{13}C-NMR) with reported values.[4]

Compound **3**, was obtained as colorless needles, mp 159—160 °C. The ^{1}H-NMR spectrum exhibited a pair of olefinic proton signals at δ 6.37 (1H, d, J=5.5 Hz), 7.93 (1H, d, J=5.5 Hz) and one methyl signal at δ 2.36 (3H, s). Its ^{13}C-NMR spectrum showed one methyl signal at δ 14.3, one conjugated carbonyl signal at δ 175.3 and four olefinic carbon signals at δ 156.3, 152.2, 144.6 and 114.4. These data led us to conclude the structure of this compound to be 3-hydroxy-2-methylpyrone (maltol) (**3**), which was previously isolated by Wei.[5]

Compound **4** was obtained as colorless needles, mp 133—134 °C. It was identified as salidroside by the comparison of its data (UV, ^{1}H- and ^{13}C-NMR) with reported values.[6]

Experimental

General Procedures Melting points were determined on a Kofler apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GSX 400 and/or A-500 FT-NMR and chemical shifts are given in ppm with tetramethylsilane as an internal standard. EI-MS were measured on a JEOL JMS-SX 102. UV spectra were measured on UV-260. Silica gel was the product of the Qingdao Marine Chemical Factory, Shandong Province, China. High performance liquid chromatography (HPLC) was carried out on an LC-10 instrument.

Isolation The aerial parts of *E. koreanum* NAKAI (25 kg), purchased in October 1992 in Liao Ning Province, China, were extracted twice with 70% ethanol. After removal of the ethanol, the extract was absorbed on Amberlite D101 and the resin was eluted with water, 40% and 95% ethanol successively. A part of the 40% ethanol eluate (250 g) was chromatographed on silica gel with a chloroform–methanol gradient. The chloroform–methanol (20 : 1) eluate afforded crude crystals, which were recrystallized in methanol to give **3** (20 mg). The chloroform–methanol (10 : 1) eluate yielded a mixture, which was subjected to preparative HPLC on ODS-18 (Waters) with methanol–water (1 : 3), giving **4** (25 mg). A part of the 95% ethanol eluate (127 g) was also chromatographed on silica gel with a chloroform–methanol gradient. The chloroform–methanol (20 : 1) eluate yielded **2** (100 mg) and a mixture. The mixture was subjected to preparative HPLC on ODS-18 (Waters) with methanol–water (7.8 : 2.2), giving **1** (20 mg).

Compound 1 (Epimedoside) A yellow amorphous powder, mp 170—171 °C, positive to the Molish and Mg–HCl tests. UV λ_{max}^{MeOH} (nm): 271.0 (+MeOH), 281.6, 376.8 (+NaOMe), 278.6, 305.2 (sh), 342.6, 400.2 (+$AlCl_3$), 280.4, 303.0 (sh), 339.8, 407.0 (+$AlCl_3$/HCl), 280.2, 365.6 (+NaOAc), 270.4 (+NaOAc/H_3BO_3). EI-MS *m/z*: 368, 353, 313, 300, 165, 135. ^{1}H-NMR (DMSO-d_6) δ: 0.71 (3H, d, J=6.8 Hz, H-6″), 1.62 (3H, s, H-14), 1.68 (3H, s, H-15), 1.94 (3H, s , R_4-Ac), 1.97 (3H, s, G_6-Ac), 3.38 (2H, m, H-11), 3.88 (3H, s, OMe), 4.18 (1H, m, H-6‴b), 4.25 (1H, d, J=7.9 Hz, H-1‴), 4.27 (1H, m, H-6″a), 4.83 (1H, t, H-4″), 5.17 (1H, t, J=6.0 Hz, H-12), 5.35 (1H, br s, H-1″), 6.32 (1H, s, H-6), 7.15 (2H, d, J=8.5 Hz, H-3′, 5′), 7.86 (2H, d, J=8.5 Hz, H-2′, 6′), 12.45 (1H, s, 5-OH) (the hydrogens shown with two and three primes are those of the *endo*-sugar and the *exo*-sugar at C-3, respectively). ^{13}C-NMR (DMSO-d_6) δ: 156.6 (C-2), 133.5 (C-3), 177.7 (C-4), 158.7 (C-5), 98.4 (C-6), 161.4 (C-7), 105.9 (C-8), 153.7 (C-9), 104.8 (C-10), 122.1 (C-1′), 130.3 (C-2′), 114.0 (C-3′), 161.4 (C-4′), 114.0 (C-5′), 130.3 (C-6′), 21.1 (C-11), 122.1 (C-12), 130.9 (C-13), 25.3 (C-14), 17.7 (C-15), 100.9 (C-1″), 69.5 (C-2″), 76.7 (C-3″), 71.0 (C-4″), 68.2 (C-5″), 16.9 (C-6″), 104.8 (C-1‴), 72.8 (C-2‴), 76.5 (C-3‴), 70.2 (C-4‴), 73.5 (C-5‴), 63.8 (C-6‴), 55.5 (OMe), 169.6, 20.4 (R_4-Ac), 170.3, 20.6 (G_6-Ac) (the carbons shown with two and three primes are those of the *endo*-sugar and *exo*-sugar at C-3, respectively). *Anal.* Calcd for $C_{37}H_{44}O_{17}$: C, 58.42; H, 5.83. Found: C, 58.27; H, 5.88.

Compound 2 (Icariside A_1) Colorless needles, mp 219—220 °C. UV λ_{max}^{MeOH} (nm): 216.8, 280.6, 302.2, 312.8. ^{1}H-NMR (pyridine-d_5) δ: 2.68 (4H, m, H_2-9/H_2-10), 3.82, 3.87, 3.89, 4.09 (each 3H, s, 2-OCH_3/3-OCH_3/4-OCH_3/6-OCH_3), 5.75 (1H, d, J=7.3 Hz, H-1′), 6.89 (1H, s, H-1), 7.42 (1H, s, H-8), 8.30 (1H, s, H-5). ^{13}C-NMR (pyridine-d_5) δ: 29.5, 30.8 (C-9/C-10), 55.9, 56.2 (2-OCH_3/6-OCH_3), 60.7, 61.4 (3-OCH_3/4-OCH_3), 62.5 (C-6′), 71.4 (C-4′), 75.0 (C-2′), 78.8, 79.1 (C-3′/C-5′), 102.6 (C-1′), 112.2, 112.5, 112.7 (C-1/C-5/C-8), 122.3, 125.6 (C-8a/C-10a), 131.4 (C-4a′), 134.6 (C-3), 142.9 (C-4b), 148.3, 148.7 (C-6/C-7), 150.9, 152.0 (C-2/C-4).

Compound 3 (Maltol) Colorless needles, mp 159—160 °C. UV λ_{max}^{MeOH} (nm): 213.8, 275.6. EI-MS *m/z*: 126, 97, 71, 55. ^{1}H-NMR (CD_3OD) δ: 2.36 (3H, s, 2-CH_3), 6.37 (1H, d, J=5.5 Hz, H-5), 7.93 (1H, d, J=5.5 Hz, H-6). ^{13}C-NMR (CD_3OD) δ: 175.3 (C-4), 156.3 (C-6), 152.2 (C-2), 144.6 (C-3), 114.4 (C-5), 14.3 (2-CH_3).

Compound 4 (Salidroside) Colorless needles, mp 133—134 °C. UV λ_{max}^{MeOH} (nm): 218.4, 271.2, 277.8 (sh). ^{1}H-NMR (CD_3OD) δ: 3.22 (2H, t, J=7.3 Hz, H-1′, β-CH_2), 4.66 (2H, t, J=7.3 Hz, α-CH_2), 4.87 (1H, d, J=7.3 Hz, H-1′), 7.05 (2H, d, J=8.6 Hz, H-3, 5), 7.18 (2H, d, J=8.6 Hz, H-2, 6). ^{13}C-NMR (CD_3OD) δ: 33.6 (C-β), 62.6 (C-6′), 71.4 (C-4′), 74.9 (C-2′), 77.6 (C-α), 78.0, 78.2 (C-3′/C-5′), 102.4 (C-1′), 118.1 (C-3/C-5), 130.8 (C-2, C-6), 131.7 (C-1), 158.3 (C-4).

Acid Hydrolysis Compound **1** (0.5 mg) was dissolved in 0.1 ml of methanol and concentrated HCl (4—5 drops). The solution was collected in capillaries. The solution was heated for 3 h at 60 °C, then subjected to silica gel TLC (developing solvent: $CHCl_3$–CH_3OH=2 : 1), together with authentic samples (glucose and rhamnose). Glucose and rhamnose were detected.

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References and Notes

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