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Terpenoid constituents from the aerial parts of *Asplenium scolopendrium*

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Abstract – Phytochemical investigations on the aerial parts of *Asplenium scolopendrium* led to the isolation of four terpenoids, the structures of which were assigned as lutein (**1**), (6*S*,9*S*)-roseoside (**2**), icariside B_2 (**3**), and picrionoside A (**4**) using spectroscopic data.
Keywords – *Asplenium scolopendrium*, terpenoid, lutein, (6*S*,9*S*)-roseoside, icariside B_2, picrionoside A

Introduction

Asplenium scolopendrium L. (Aspleniaceae) is a bracken distributed in the southern areas and Ulleung Island in Korea. Previous phytochemical research on this plant afforded only a few kaempferol glycosides and amino acid derivatives (Mizuno *et al*., 1990). As a part of our ongoing search for biologically active materials with plant origins, *A. scolopendrium* was chosen. Chromatographic separation and purification of the aerial parts of *A. scolopendrium* resulted in the identification of four terpenoids, lutein (**1**), (6*S*,9*S*)-roseoside (**2**), icariside B_2 (**3**), and picrionoside A (**4**).

Experimental

General experimental procedures – Optical rotation was measured with a JASCO DIP-1000 digital polarimeter (Tokyo, Japan) and CD spectra were recorded on a JASCO J-715 spectrometer. ESI-MS spectra were obtained on an Agilent 1100 series LC/MSD. UV and IR spectra were recorded on a Shimadzu UV-2101 and a Perkin Elmer 1710 spectrophotometer, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker spectrometer at 400 MHz and at 100 MHz, respectively. Column chromatography was performed using a Sephadex LH-20 (Pharmacia) and a Kiesegel 60 (Art. 7734; Merck, Darmstadt, Germany). TLC was conducted on pre-coated Kiesegel 60 F_{254} plates (Art. 5715; Merck, Darmstadt, Germany). Spots on the TLC were detected under UV radiation.

Plant material – The aerial parts of *A. scolopendrium* were collected at Ulleung Island (Korea) in June 2002, and identified by one of authors. A voucher specimen (SNUPH-0032) has been deposited in the herbarium of our institute.

Extraction and isolation – The air-dried aerial parts of *A. scolopendrium* (1.7 kg) were cut into pieces and extracted with 100% MeOH. The methanolic extract was evaporated *in vacuo* to give a crude extract (150 g), which was successively extracted using CH_2Cl_2 and *n*-BuOH. The CH_2Cl_2 extract (11 g) was chromatographed over Sephadex LH-20 (*n*-hexane-CH_2Cl_2-MeOH = 10 : 10 : 1) giving three fractions. The second fraction was subjected to a silica gel using *n*-hexane-EtOAc (3 : 1 → 1 : 2) and resulted in compound **1** (34.0 mg). The *n*-BuOH fraction (6.8 g) was applied to a MCI-gel chromatography (100% H_2O → 100% MeOH) and then divided into four fractions. The first fraction (128.7 mg) was separated using HPLC (AcCN-H_2O = 30 : 70, 2 ml/min, YMC J'sphere ODS-H80) to afford compound **2** (10.2 mg). The second fraction (202.5 mg) was applied to HPLC (AcCN-H_2O = 17 : 83, 2 ml/min, YMC J'sphere ODS-H80) and yielded compound **3** (5.0 mg). The fourth fraction (94.4 mg) was applied to HPLC (AcCN-H_2O = 19 : 81, 2 ml/min, YMC J'sphere ODS-H80) and finally resulted in compound **4** (3.0 mg).

Lutein (1) – $C_{40}H_{56}O_2$, yellow powder, $[\alpha]_D^{20}$: +54.3° (*c* 0.06, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 431 (3.95), 456 (4.04), 484 (3.94); ESI-MS (positive mode) *m/z*: 569 $[M+H]^+$; IR (KBr) ν_{max} (cm^{-1}): 3419, 1650, 971; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.92 (3H, s, H-16'), 1.07 (3H, s, H-17'), 1.15 (6H, s, H-16, H-17), 1.72 (3H, s, H-18'), 1.80 (3H, s, H-18), 1.94 (3H, s, H-19'), 2.02 (3H,s, H-19), 2.04 (6H, s, H-20, 20'), 2.48 (4H, m, H-2, 2', 4, 6'), 4.06 (1H, m, H-3), 4.30 (1H, m, H-3'), 5.51 (1H, dd, *J* = 5.1,

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15.0 Hz, H-7'), 5.60 (1H, br. s, H-4'), 6.13 (4H, m, H-8, 8', 10, 10'), 6.20 (1H, d, J = 16.0 Hz, H-7), 6.32 (2H, d, J = 10.0 Hz, H-14, 14'), 6.41 (2H, d, J = 16.0 Hz, H-12, 12'), 6.69 (2H, d, J = 10.0 Hz, H-15, 15'), 6.72 (2H, d, J = 16.0 Hz, H-11, 11'); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 12.7 (C-20'), 12.8 (C-19), 12.8 (C-20), 13.1 (C-19'), 21.6 (C-18) , 22.9 (C-18'), 24.2 (C-16'), 28.7 (C-16), 29.5 (C-17'), 30.2 (C-17), 34.0 (C-1'), 37.1 (C-1), 42.5 (C-4), 44.6 (C-2'), 48.4 (C-2), 54.9 (C-6'), 65.0 (C-3), 65.9 (C-3'), 124.5 (C-11'), 124.8 (C-4'), 124.9 (C-11), 125.6 (C-7), 126.2 (C-5), 128.7 (C-7'), 130.0 (C-15'), 130.1 (C-15), 130.8 (C-10'), 131.3 (C-10), 132.6 (C-14), 132.6 (C-14'), 135.0 (C-9'), 135.7 (C-9), 136.4 (C-13'), 136.5 (C-13), 137.5 (C-6), 137.6 (C-12), 137.7 (C-5'), 137.7 (C-12'), 137.8 (C-8'), 138.5 (C-8).

(6*S*, 9*S*)-Roseoside (2) – $C_{19}H_{30}O_8$, amorphous powder, $[\alpha]_D^{20}$: +62.0° (*c* 0.8, MeOH); UV (CD_3OD) λ_{max} nm (log ε): 230 (3.94), 310 (3.55); CD (*c* 0.03 mg/ml, MeOH): $[\theta]_{207.0}$ −8257, $[\theta]_{214.0}$ 0, $[\theta]_{243.5}$ +25352, $[\theta]_{295.0}$ 0; ESI-MS (positive mode) *m/z*: 409 $[M + Na]^+$; IR (KBr) ν_{max} (cm^{-1}): 3395, 2929, 1650; ^{1}H-NMR (400 MHz, CD_3OD): δ 1.01 (3H, s, H-12), 1.03 (3H, s, H-11), 1.27 (3H, d, J = 6.3 Hz, H-10), 1.91 (3H, s, H-13), 2.16 (1H, d, J = 15.6, H-2a), 2.60 (1H, d, J = 15.6 Hz, H-2b), 4.27 (1H, d, J = 7.6 Hz, H-1'), 4.53 (1H, m, H-9), 5.72 (1H, dd, J = 7.3, 15.6 Hz, H-8), 5.86 (1H, s, H-4), 5.96 (1H, d, J = 15.6 Hz, H-7); ^{13}C-NMR (100 MHz, CD_3OD): δ 18.0 (C-13), 20.4 (C-10), 23.0 (C-11), 24.3 (C-12), 51.0 (C-2), 43.2 (C-1), 63.1 (C-6'), 71.6 (C-4'), 75.0 (C-2'), 75.2 (C-9), 78.5 (C-5'), 78.7 (C-3'), 80.3 (C-6), 102.0 (C-1'), 127.3 (C-4), 131.3 (C-7), 134.5 (C-8), 167.9 (C-5), 202.1 (C-3).

Icariside B_2 (3) – $C_{19}H_{30}O_8$, amorphous powder, $[\alpha]_D^{20}$: −73.5° (*c* 1.0 MeOH); UV (MeOH) λ_{max} nm (log ε): 230 (4.08); CD (*c*, 0.04 mg/ml, MeOH): $[\theta]_{206.0}$ 0, $[\theta]_{233.5}$ −28526, $[\theta]_{272.0}$ 0; ESI-MS (positive mode) *m/z*: 409 $[M + Na]^+$; IR (KBr) ν_{max} (cm^{-1}): 3364, 2927, 1669; ^{1}H-NMR (400 MHz, CD_3OD): δ 0.95 (3H, s, H-12), 1.28 (3H, s, H-11), 1.29 (3H, s, H-13), 1.40 (1H, dd, J = 14.6, 10.1 Hz, H-2a), 1.74 (1H, dd, J = 14.6, 2.0 Hz, H-2b), 1.82 (1H, dd, J = 14.6, 11.5 Hz, H-4a), 2.28 (3H, s, H-10), 2.43 (1H, d, J = 14.6, 3.8 Hz, H-4b), 3.08-3.87 (sugar protons), 3.91 (1H, m, H-3), 4.33 (1H, d, J = 7.8 Hz, H-1'), 6.17 (1H, d, J = 17.0 Hz, H-8), 7.16 (1H, d, J = 17.0 Hz, H-7); ^{13}C-NMR (100 MHz, CD_3OD): δ 21.0 (C-13), 26.3 (C-12), 28.2 (C-10), 30.3 (C-11), 36.8 (C-1), 39.0 (C-4), 46.0 (C-2), 63.5 (C-6'), 69.2 (C-5), 71.9 (C-6), 72.4 (C-3), 73.5 (C-4'), 75.9 (C-2'), 78.7 (C-3'), 78.9 (C-5'), 103.7 (C-1'), 146.1 (C-7), 134.6 (C-8), 198.0 (C-9).

Picrionoside A (4) – $C_{19}H_{30}O_7$, amorphous powder; $[\alpha]_D^{20}$: +23.1° (*c* 1.2 MeOH); UV (MeOH) λ_{max} nm (log ε): 224 (3.89); CD (*c* 0.05 mg/ml, MeOH): $[\theta]_{199.5}$ 0, $[\theta]_{240.0}$ +30560, $[\theta]_{300.5}$ 0; ESI-MS (positive mode) *m/z*: 393 $[M + Na]^+$; IR (KBr) ν_{max} (cm^{-1}): 3392, 2925, 1667; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.82 (3H, s, H-11), 1.18 (3H, s, H-12), 1.43 (1H, dd, J = 13.4, 6.4 Hz, H-2a), 1.57 (3H, s, H-13), 1.77 (1H, dd, J = 13.4, 5.9 Hz, H-2b), 2.22 (3H, s, H-10), 2.52 (1H, d, J = 10.2 Hz, H-6), 4.32 (1H, m, H-3), 4.35 (1H, d, J = 7.8 Hz, H-1'), 5.64 (1H, brs, H-4), 6.21 (1H, d, J = 15.6 Hz, H-8), 6.55 (1H, dd, J = 15.6, 10.2 Hz, H-7); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 22.7 (C-13), 24.9 (C-11), 27.3 (C-10), 29.3 (C-12), 33.7 (C-2), 40.0 (C-6, overlapped with solvent peak), 54.1 (C-1), 61.6 (C-6'), 70.6 (C-3), 71.8 (C-4'), 73.9 (C-2'), 77.2 (C-5'), 77.3 (C-3'), 102.0 (C-1'), 125.3 (C-4), 133.9 (C-8), 135.0 (C-5), 147.7 (C-7), 198.3 (C-9).

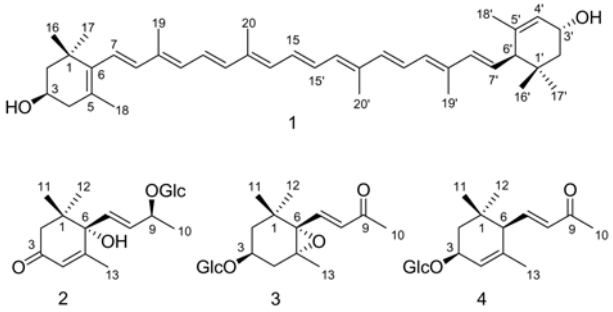

Fig. 1. Structures of compounds **1** - **4.**

Results and Discussion

Compound **1**, a yellow powder, exhibited the absorption maxima at 431, 456, and 484 nm that were characteristic in carotenoids. The molecular formula, $C_{40}H_{56}O_2$, was deduced from the quasimolecular ion peak at *m/z* 569 $[M+H]^+$ and forty carbon signals in the ^{13}C-NMR spectrum. These facts suggested that **1** was of a carotenoid (C_{40}) derivative. The ^{1}H-NMR spectrum of **1** displayed ten methyl protons at δ 0.9-2.04, two carbinol protons at δ 4.06 and 4.30, and fifteen olefinic protons at δ 5.51-6.72. The signals at δ 0.92 (H-16'), 1.07 (H-17'), and 1.15 (H-16 and 17) showed that there were cyclohexane groups at both ends of the nonaene side chain that consisted of four isoprenoid units (Bonnett *et al*., 1969). The types of the end groups turned out to be a β-ring with 3-OH demonstrated by the signals at δ 2.48 (H-4), 4.06 (H-3) and 6.20 (H-7), and a ε-ring with 3-OH with signals at δ 4.30 (H-3'), 5.51 (H-7') and 5.60 (H-4'), respectively. The chemical shifts of H-11 (δ 6.72) and H-15 (δ 6.69) appeared in the higher region than those of H-10 (δ 6.13), H-14 (δ 6.32), and H-12 (δ 6.41), which suggested that the stereochemistry of the polyene side

chain were all *trans* (Pfander *et al*., 1987). In the ^{13}C-NMR spectrum, the signals due to the double bonds in the cyclic alkene systems were observed at δ 126.2 (C-5) and 137.5 (C-6) as well as δ 124.8 (C-4') and 137.7 (C-5'), and two oxygenated carbon signals were identified at δ 65.0 (C-3) and 65.9 (C-3'). Information from ^{13}C-NMR, as well as ^{1}H-NMR and other spectral data led to the identification of the structure of compound **1** as lutein (Radics *et al*., 1981).

Compound **2** was obtained as an amorphous powder and its ESI-MS spectrum exhibited the quasimolecular ion at *m/z* 409 $[M + Na]^+$ corresponding to the molecular formula of $C_{19}H_{30}O_8$. The ^{1}H-NMR spectrum of **2** revealed signals assignable to a vinyl proton at δ 5.86 (H-4), and two *trans* olefinic protons at δ 5.72 (1H, dd, *J* = 15.6, 7.3 Hz, H-8) and 5.96 (1H, d, *J* = 15.6 Hz, H-7). The three methyl singlets were assigned to a methyl proton at δ 1.91 (H-13) next to a double bond, and *gem*-dimethyl groups at δ 1.01 (H-12) and 1.03 (H-11) on a quaternary carbon. A doublet peak at δ 1.27 was designated to a secondary methyl proton (H-10) correlated with an oxygenated methine at δ 4.53 (1H, m, H-9). Geminally coupled signals at δ 2.16 (d, *J* = 15.6 Hz, H-2a) and 2.60 (d, *J* = 15.6 Hz, H-2b) suggested that a carbonyl group was linked to this methylene and *gem*-dimethyl were substituted to C-1. A doublet of the doublet peak at δ 5.72 (H-8) implied the presence of a vicinal proton which was assigned to the signal at δ 4.53 by virtue of ^{1}H-^{1}H COSY. Moreover, an anomeric proton with β configuration appeared at δ 4.27 (1H, d, *J* = 7.6 Hz) and the sugar moiety was identified as D-glucopyranoside when compared with the literature (Otsuka *et al*., 1995). The ^{13}C-NMR spectrum exhibited a carbonyl signal at δ 202.1 (C-3), two oxygenated carbon signals at δ 75.2 and 80.3 in addition to the signals of glucose. The absolute configuration of C-6 proved to be *S* by the positive molar ellipticity $([\theta]_{243.5}$ +25352) in the CD spectrum as described in the literature (Ito *et al*., 2001). The stereochemistry of C-9 were determined by comparing the chemical shift of C-9 in **2** with the previous data, which was reported as the ^{13}C-NMR chemical shifts for the C-9 in (9*R*)-or (9*S*)-3-oxo-α-ionol 9-β-D-glucopyranoside that appeared at δ 77.0 and 74.7, respectively (Pabst *et al*., 1992). Therefore, the absolute configuration of the C-9 (δ 75.2) in **2** was tentatively assigned as *S*. On the basis of this data, **2** turned out to be (6*S*, 9*S*)-roseoside (Murai *et al*., 2001).

The ^{1}H-NMR spectrum of **3** displayed two methylene groups, H-2 (δ 1.40 and 1.74) and H-4 (δ 1.82 and 2.43), with geminal couplings and vicinal couplings due to an adjacent chiral carbon (C-3). It was also observed that two mutually coupled olefinic protons appeared at δ 6.17 (H-8) and 7.16 (H-7) without additional correlations to other protons, and a methyl proton at δ 2.28 next to carbonyl group (C-9) in the ^{1}H-NMR spectrum. The absolute configuration of C-6 was determined as *R* by the negative CD value $([\theta]_{233.5}$ −28526) in the CD spectrum. Based on the above data, compound **3** was elucidated as icariside B_2, in good agreement with the literature (Miyase *et al*., 1987).

Compound **4** showed three independent spin coupling systems and four methyl singlets in the ^{1}H-NMR spectrum. On the basis of their chemical shifts, four singlets were assigned to H-12 (δ 0.82), H-11 (δ 1.18), H-13 (δ 1.57), and H-10 (δ 2.22), the last one due to a neighboring carbonyl group. One of the spin coupling systems exhibited two doublets of the doublets at δ 1.43 (*J* = 13.4, 6.4 Hz, H-2) and 1.77 (*J* = 13.4, 5.9 Hz, H-2), a multiplet at δ 4.32 (H-3), and a broad singlet at δ 5.64 (H-4). Another of the spin coupling systems was equipped with a methine proton signal at δ 2.52 (1H, d, *J* = 10.2 Hz, H-6), and a pair of *trans* olefinic proton signals at δ 6.21 (1H, d, *J* = 15.6 Hz, H-8) and 6.55 (1H, dd, *J* = 15.6, 10.2 Hz, H-7) that were conjugated to a carbonyl group. The remaining spin coupling system contained sugar protons including an anomeric proton at δ 4.35 (1H, d, *J* = 7.8 Hz). The ^{13}C-NMR spectrum of **4** exhibited 13 carbon signals besides the six signals due to a glucopyranosyl moiety. These results suggested that **4** was a glucoside of an α-ionone derivative and the *O*-glucoside linkage was at C-3. Further, the CD spectrum of **4** revealed a positive curve $([\theta]_{240.0}$ +30560), showing that the C-6 side-chain linkage is β. Thus, the structure of **4** was designated to picrionoside A (Uchiyama *et al*., 1990).

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