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), (6S,9S)-roseoside (2), icariside B$_2$ (3), and picrionoside A (4) using spectroscopic data.

Keywords − *Asplenium scolopendrium*, terpenoid, lutein, (6S,9S)-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), (6S,9S)-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 Kieselgel 60 (Art. 7734; Merck, Darmstadt, Germany). TLC was conducted on pre-coated Kieselgel 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$_2$Cl$_2$ and n-BuOH. The CH$_2$Cl$_2$ extract (11 g) was chromatographed over Sephadex LH-20 (n-hexane-CH$_2$Cl$_2$-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$_2$O → 100% MeOH) and then divided into four fractions. The first fraction (128.7 mg) was separated using HPLC (AcCN-H$_2$O = 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$_2$O = 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$_2$O = 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, [α]$_D^20$ = +54.3° (c 0.06, CHCl$_3$); UV (CHCl$_3$) $\lambda_{max}$ nm (log ε): 431 (3.95), 456 (4.04), 484 (3.94); ESI-MS (positive mode) m/z: 569 [M+H]$^+$; IR (KBr) $\nu_{max}$ (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), 1.15 (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, 10.2).
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'); 13C-NMR (100 MHz, CDCl3): 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, 9S)-Roseoside (2) = C19H18O8, amorphous powder, [α]20D = +62.0° (c 0.8, MeOH); UV (CD3OD) λmax nm (log ε): 230 (3.94), 310 (3.55); CD (c 0.03 mg/ml, MeOH): [θ]230D = −8257, [θ]222D = 25352, [θ]290D = 0; ESI-MS (positive mode) m/z: 0.05 [M + Na]+; IR (KBr) νmax (cm−1): 3399, 2929, 1667; 1H-NMR (400 MHz, CD3OD): δ 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); 13C-NMR (100 MHz, CD3OD): 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).

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, C40H40O15, was deduced from the quasi molecular ion peak at m/z 569 [M+H]+ and forty carbon signals in the 13C-NMR spectrum. These facts suggested that 1 was of a carotenoid (C40) derivative. The 1H-NMR spectrum of 1 displayed ten methyl protons at δ 0.9-2.04, two carbonyl protons at δ 4.06 and 4.30, and fifteen olefinic protons at δ 5.51-7.62. 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-17), and a γ-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...