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Flavonoids of *Crotalaria sessiliflora*

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Phytochemical investigation of the whole plants of *Crotalaria sessiliflora* L. led to the isolation of four flavonoids. The structures of these compounds were identified as 2',4',5,7-tetrahydroxyisoflavone (**1**), 2',4',7-trihydroxyisoflavone (**2**), 4',7-dihydroxyflavone (**3**), and isovitexin (**4**) using spectroscopic analysis. Among these, compounds **2**, and **3** have not been reported from *Crotalaria* species, whereas compounds **1**, and **4** were reported from this plant for the first time.

Key words: *Crotalaria sessiliflora*, Leguminosae, Isoflavone, Flavone, Flavone glycoside

INTRODUCTION

Crotalaria sessiliflora L. (Leguminosae) is an annual plant, and is used as a diuretic, a cardiotonic, an analgesic, and a hemogogue in folk medicine (Kim, 1996). Röders group (1992) reported three pyrrolizidine alkaloids from the seeds of this plant, and monocrotaline showed cytotoxic effects against sarcoma 180, leukemia 615, and Walker carcinoma 256 (Huang *et al.*, 1980). In this paper, we describe the isolation of four flavonoids from *C. sessiliflora* as well as the elucidation of their structures using spectroscopic analysis.

MATERIAL AND METHODS

General experimental procedure

IR spectra were obtained with a Perkin Elmer 1710 spectrophotometer. The NMR spectra were taken on a JEOL LA 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz), a Bruker GPX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz), and a Bruker AMX 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. EIMS spectra were obtained on a VG Trio-2 spectrometer. FAB-MS spectra were obtained on a JMS AX505WA spectrometer. TLC was carried out on silica gel 60 F_{254} and RP-18 F_{254} plates (Merck, Germany). Column chromatography was performed over silica gel 60 (Merck, particle size 230-400 mesh) and Sephadex LH-20 (Pharmacia, Sweden).

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Plant Materials

The whole plants of *Crotalaria sessiliflora* L. (Leguminosae) was collected from Mt. Kwanak, Seoul, Korea in September 1998 and identified by Dr. Dae Suk Han, an emeritus professor from the College of Pharmacy at Seoul National University. A voucher specimen (SNUPH-0054) has been deposited in the College of Pharmacy herbarium at Seoul National University in Korea.

Extraction and Isolation

The dried whole plants (393 g) of *C. sessiliflora* were extracted five times with 80% MeOH in an ultrasonic apparatus for 3 h. This residue was evaporated *in vacuo* to yield the total extract (26.8 g). This extract was then suspended in distilled water and partitioned sequentially with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH.

The EtOAc fraction (1.2 g) was subjected to silica gel column chromatography using $CHCl_3$-EtOAc gradient system (90:1→1:1) to provide 11 fractions (fractions 1-11). From fraction 8, compound **2** (2.2 mg) was isolated using a silica gel column chromatography ($CHCl_3$-EtOAc, 20:1), and then purified by semipreparative RP-HPLC (YMC Jsphere-H80, 4 μm, 250×10 mm, MeOH-H_2O = 6:4). Compounds **1** (1.4 mg) and **3** (1.5 mg) were also separated from fraction 9 by the same procedure as described for the isolation of compound **2**. Fraction 11 was rechromatographed over silica gel and eluted with solvent mixtures ($CHCl_3$-MeOH-H_2O = 50:4:1→ $CHCl_3$-MeOH-H_2O = 8:4:1) to give six fractions (subfractions 1-6). Subfraction 6 was chromatographed with Sephadex LH-20 (MeOH) to provide

four fractions (subfractions 7-10). Compound **4** (5.0 mg) was further purified by RP-HPLC (Waters, μ-Bondapak, 5 μm, 300 x 3.9 mm) with solvent mixtures (MeOH-AcCN-H_2O = 7:13:80).

2',4',5,7-Tetrahydroxyisoflavone (1)

Pale yellow powder; mp: 271-273 °C; IR λ_{max} (KBr, cm^{-1}): 3410 (OH), 1619 (C=O), 1575, 1510, 1462 (C=C); MS (EI, *m/z*) : 286 (M^+, 100), 269 (25), 217 (8), 153 (RDA, 65), 134 (RDA, 43); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm) 13.03 (1H, brs, 5-OH), 8.14 (1H, s, H-2), 6.96 (1H, d, *J* = 8.3 Hz, H-6'). 6.35 (1H, d, *J* =1.6 Hz, H-8), 6.35 (1H, d, *J* = 2.3 Hz, H-3'), 6.26 (1H, dd, *J* = 8.3, 2.3 Hz, H-5'), 6.20 (1H, d, *J* = 1.6 Hz, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm) 180.8 (C-4), 164.7 (C-7), 162.3 (C-5), 159.0 (C-4'), 158.1 (C-9), 156.8 (C-2'), 155.7 (C-2), 132.6 (C-6'), 120.8 (C-3), 109.0 (C-1'), 106.6 (C-5'), 104.8 (C-10), 103.0 (C-3'), 99.3 (C-6), 94.0 (C-8).

2',4',7-Trihydroxyisoflavone (2)

Pale yellow powder; mp: 273-275 °C; IR λ_{max} (KBr, cm^{-1}): 3410 (OH), 1619 (C=O), 1575, 1510, 1462 (C=C); MS (EI, *m/z*): 270 (M^+, 100), 269 ([M-H]$^+$, 18), 253 (38), 137 (RDA, 48), 134 (RDA, 38); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 8.13 (1H, s, H-2), 7.92 (1H, d, *J* = 8.8 Hz, H-5), 6.97 (1H, d, *J* = 8.3 Hz, H-6'), 6.91 (1H, dd, *J* = 8.8, 2.2 Hz, H-6), 6.84 (1H, d, *J* = 2.2 Hz, H-8), 6.34 (1H, d, *J* = 2.4 Hz, H-3'), 6.26 (1H, dd, *J* = 8.3, 2.4 Hz, H-5').

4',7-Dihydroxyflavone (3)

Pale yellow powder; mp: 314-316 °C; MS (EI, *m/z*): 254 (M^+, 100), 226 (45), 137 (RDA, 98), 118 (RDA,52); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.91 (2H, d, *J* = 8.7 Hz, H-2', 6'), 7.84 (1H, d, *J* = 8.7 Hz, H-5), 6.93 (1H, s, H-8), 6.92 (2H, d, *J* =8.7 Hz, H-3', 5'), 6.89 (1H, d, *J* = 8.7 Hz, H-6), 6.71 (1H, s, H-3); ^{13}C-NMR (75 MHz, DMSO-d_6, δ ppm): 176.3 (C-4), 163.1 (C-7), 162.3 (C-2), 160.7 (C-4'), 157.5 (C-9), 128.1 (C-2', 6'), 126.4 (C-5), 121.7 (C-1'), 115.9 (C-10), 115.8 (C-3', 5'), 115.0 (C-6), 104.4 (C-3), 102.4 (C-8).

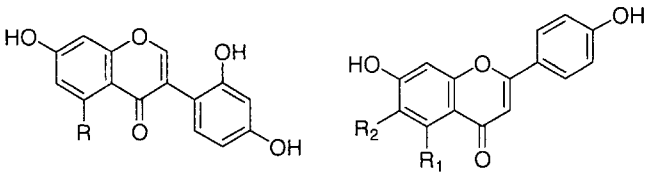
Isovitexin (4)

Yellow crystal (MeOH/EtOAc); mp: 237-239 °C; MS (FAB, glycerol) : 433 ([M+H]$^+$); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm) : 13.5 (1H, brs, 5-OH), 7.92 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.92 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.77 (1H, s, H-3), 6.49 (1H, s, H-8), 4.57 (1H, d, *J* = 10.0 Hz, H-1"), 4.02 (1H, t, *J* =10.0 Hz, H-2"), 3.9-3.0 (H-3", H-4", H-5", H-6"); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 182. 4 (C-4), 163.9 (C-2), 163.8 (C-7), 161.6 (C-9), 161.1 (C-4'), 156.7 (C-5), 128.9 (C-2', 6'), 121.5 (C-1'), 116.4 (C-3', 5'), 109.3 (C-6), 103.8 (C-10), 103.2 (C-3), 94.1 (C-8), 82.0 (C-5"), 79.4 (C-1"), 73.5 (C-2"), 71.0 (C-3"), 70.6 (C-4"), 61.9 (C-6").

RESULTS AND DISCUSSION

Phytochemical investigation of *C. sessiliflora* led to the isolation of four flavonoids from the EtOAc fraction using column chromatography and hplc. Compound **1** was a pale yellow powder, and showed characteristic phenolics in positive reaction with $FeCl_3$ reagents. The molecular formula of **1** was calculated as $C_{15}H_{10}O_6$ by EIMS and ^{13}C-NMR data. The EIMS spectrum of **1** showed the molecular ion peak at *m/z* 286. Peaks from *retro*-Diels Alder fragmentation at *m/z* 153 and 134 indicated that this flavonoid has two hydroxy functionalities in its A and B rings, respectively (Markham, 1982). The ^{1}H-NMR signals showed two *meta* coupled aromatic protons [δ 6.35 (1H, d, *J* =1.6 Hz, H-8), 6.20 (1H, d, *J* = 1.6 Hz, H-6)], and a hydroxy proton at 5-position [δ 13.03 (1H, brs)]. In addition, a singlet proton signal at d 8.13 indicated a characteristic proton at the C-2 position of the isoflavonoid (Markham and Geiger, 1994). The B-ring protons showed a characteristic 1,2,4-trisubstitued benzene ring [δ 6.96 (1H, d, *J* = 8.3 Hz), 6.35 (1H, d, *J* = 2.3 Hz), 6.26 (1H, dd, *J* = 8.3, 2.3 Hz)] and this result suggested that the compound **1** contained 2',4'-hydroxy aromatic system rather than 3',4'-dihydroxy aromatic system according to its chemical shift (Markham and Geiger, 1994). Therefore, the structure of **1** was determined to be 2',4',5,7-tetrahydroxyisoflavone (2'-hydroxygenistein) and was confirmed by comparison to previously reported data (Biggs, 1975; Lane and Newman, 1987; Agrawal and Bansal, 1989a).

The molecular formula of compound **2** was determined to be $C_{15}H_{10}O_5$ based on the ^{13}C-NMR spectral data and EIMS [*m/z* 270, M^+]. A positive reaction in $FeCl_3$ reagent also displayed the phenolic characteristic of this compound. Its ^{1}H-NMR spectrum and EIMS fragmentation were similar to those of compound **1**, except absence of the hydroxy group at the 5-position in the A ring. In addition, the molecular ion peak at *m/z* 270 and the fragment peaks from *retro*-Diels Alder fragmentation at *m/z* 137 and 134 confirmed the one hydroxy group in its A ring and the two hydroxy groups in its B ring. Therefore, the structure of compound **2** was determined as 2',4',7-trihydroxyisoflavone. The spectral data of **2** were in good agreement with the



1 R=OH
2 R=H
3 R_1=H, R_2=H
4 R_1=OH, R_2=Glc

Fig. 1. Chemical structures of compounds **1-4** isolated from *C. sessiliflora*

literature values (Woodward, 1980).

The ^{1}H-NMR signal of compound **3** displayed *para*-substituted benzene ring [δ 7.91 (2H, d, *J* = 8.7 Hz), 6.92 (2H, d, *J* = 8.7 Hz)], and a characteristic singlet at δ 6.71 (1H, brs) was assigned to the 3-position in flavone. In the EIMS spectrum, the molecular ion peak was shown at *m/z* 254, and the signals from *retro*-Diels Alder fragmentation at *m/z* 137 and 118 indicated the one hydorxy group in the A ring and in the B ring, respectively. These data indicated that compound **3** is 4',7-dihydroxyflavone, and this was further confirmed by comparison with the literature values (Shirataki *et al*, 1986).

Compound **4** was obtained as yellow crystal and showed a pseudomolecular ion peak at *m/z* 433. The molecular formula of compound **4** was established as $C_{21}H_{20}O_{10}$ by MS and ^{13}C-NMR data. The ^{1}H-NMR signals of compound **4** displayed a *para*-substituted benzene ring B [δ 7.92 (2H, d, *J* = 8.8 Hz), 6.92 (2H, d, *J* = 8.8 Hz)], two singlet peaks of H-3 and H-8 at δ 6.77 (1H, s) and 6.49 (1H, s), respectively, and one OH peak at δ 13.50 (1H, brs, 5-OH), which suggested that the aglycone of compound **4** was similar to apigenin. Interpretation of the chemical shift values in oxymethine and oxymethylene regions in the ^{13}C-NMR spectrum of **4** led to the identification of the sugar moiety as glucose and the chemical shift values were in good agreement with literature values (Agrawal and Bansal, 1989b). The *C*-glycosidic linkage was validated by the chemical shift of the 6-position at δ 109.3 that was shifted downfield (*ca.* + 10 ppm) due to the *C*-glycosylation and anomeric signals at δ 4.57 (^{1}H) and 79.4 (^{13}C). Therefore, the structure of **4** identified as isovitexin (Markham and Chari, 1982, Markham and Geiger, 1994, Agrawal and Bansal, 1989b).

The phytochemical study of the whole plants of *Crotalaria sessiliflora* led to the isolation of two isoflavones, one flavone, and one flavone glycoside. Of these, compounds **2** and **3** have not been reported from the *Crotalaria* genus, and compounds **1**, **4** are reported for the first time from this species.

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