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Flavonoids from *Spatholobus suberectus*

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Two pterocarpans [(6a*R*,11a*R*)-maackiain, (6a*R*,11a*R*)-medicarpin], one flavanone [(2*S*)-7-hydroxy-6-methoxy-flavanone], one isoflavan (sativan) and two isoflavones (pseudobaptigenin, genistein) were isolated from the *Spatholobus suberectus* (Leguminosae). Their chemical structures were determined by comparison of their spectroscopic parameters of CD, EIMS, 1D-NMR and 2D-NMR with those reported in the literatures. All of these compounds are reported for the first time from this plant through the present study.

Key words: *Spatholobus suberectus*, Leguminosae, Pterocarpan, (6a*R*,11a*R*)-Maackiain, (6a*R*,11a*R*)-Medicarpin, (2*S*)-7-Hydroxy-6-methoxy-flavanone

INTRODUCTION

The vine stem of *Spatholobus suberectus* Dunn (Leguminosae) has been used for the improvement of blood circulation and treatment for dysmenorrhea, anemia, paralysis and arthralgia in Korean folk medicine (Kim and Cho, 1995). Even though several flavonoids, sterols and triterpenes have been reported from *S. suberectus* (Zhu 1988; Lin *et al.*, 1989; Cui *et al.*, 2002), no systematical studies on chemical constituents have been performed. Thus, we started to study the constitutents of *S. suberectus*. Two pterocarpans [(6a*R*,11a*R*)-maackiain, (6a*R*,11a*R*)-medicarpin], one flavanone [(2*S*)-7-hydroxy-6-methoxy-flavanone], one isoflavan (sativan) and two isoflavones (pseudobaptigenin, genistein) were isolated. Their chemical structures were determined by comparison of their spectroscopic parameters of CD, EIMS, 1D-NMR and 2D-NMR with those reported in the literatures. All of these compounds have not been previously found in the plant.

MATERIALS AND METHODS

Optical rotation was measured using a JASCO DIP-1000 polarimeter and CD data were recorded in MeOH on a JASCO-J715 spectrometer. FT-IR spectra were recorded on a Perkin Elmer 1710 spectrometer, and UV spectra were recorded on a Shimadzu UV-201 spectrometer. EIMS spectra were measured with a VG Trio II spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were run on a JEOL GSX 400 spectrometer at 400 MHz and 100 MHz, respectively, with TMS as an internal standard. TLC and column chromatography were carried out on precoated silica gel F_{254} plates (Merck, art. 5715), RP-18 F_{254} plates (Merck, art. 15423), silica gel 60 (230-400 mesh, Merck) and LiChroprep RP-18 (40-63 μm, Merck).

Plant materials

The stems of *S. suberectus* were purchased from Kyoungdong Oriental Herbal Market, Seoul, Korea in 2000 and identified by the late Dr. D.S. Han, an emeritus professor of the College of Pharmacy, Seoul National University. A voucher specimen (SNU-0031) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and isolation

The dried sterms of *S. suberectus* (12 kg) were extracted three times for 3 h with 80% MeOH in an ultrasonic apparatus. Removal of solvent *in vacuo* yielded a methanolic extract (1.7 kg). The methanolic extract was suspended in H_2O and partitioned with CH_2Cl_2. The CH_2Cl_2 fraction was

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then suspended in 90% MeOH and back-extracted with *n*-hexane. The residual 90% MeOH fraction (36.5 g) was fractionated by extensive column chromatography over silica gel using *n*-hexane : EtOAc : MeOH gradient and yielded thirteen major fractions (fr.1~fr.13). Following silica gel column chromatography of fr.5 (7 g) with a solvent gradient of MeOH in $CHCl_3$ yielded five subfractions (fr.5-1~ fr.5-5). Among them, fr.5-3 (2g) was eluted on C_{18} RP column chromatography with 100% H_2O to 100% MeOH as the eluent raising the ratio of MeOH and yielded seven subfractions (fr.5-3-1~fr.5-3-7). Among these subfractions, fr.5-3-3 (38 mg) yielded 1 (6.8 mg), 2 (21.3 mg), and 3 (5.1 mg) by additional purification step on C_{18} RP HPLC (AcCN : MeOH = 45 : 55). Compound 4 was isolated by C_{18} RP HPLC (AcCN : MeOH : H_2O = 45 : 10 : 45) from the fr. 5-3-4 (40 mg). Compounds 5 (4.5 mg) and 6 (13.5 mg) were purified by the additional purification step on C_{18} RP HPLC (AcCN : MeOH : H_2O = 35 : 10 : 55 (5) and 32 : 16 : 52 (6)) of fr. 6 and fr. 7, respectively.

(6a*R*,11a*R*)-Maackiain (1)

Pale yellowish powder, $[\alpha]_D$: -261.1 (*c* 0.1, MeOH); CD (MeOH): $\Delta\varepsilon$ 288 (+2.59), 238 (-9.27) nm; UV λ_{max} (MeOH) (log ε) nm: 286 (3.22), 309 (3.31); IR ν_{max} (KBr) cm^{-1}: 3429, 1623, 1473, 932, 835; EIMS: *m/z* 284 $[M]^+$, 283, 267, 175, 162, 151, 147, 134; ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) see the Table I.

(6a*R*,11a*R*)-Medicarpin (2)

Yellowish powder, $[\alpha]_D$: -232.0 (*c* 0.1, MeOH); CD [MeOH, nm ($\Delta\varepsilon$)] : 3.04 (+3.19), 240 (-10.9); UV λ_{max} (MeOH) (log ε) nm: 285 (3.18); IR ν_{max} (KBr) cm^{-1} : 3424, 1621, 1496, 947, 837; EIMS: *m/z* 270 $[M]^+$, 269, 255, 148, 135; ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) see the Table I.

(2*S*)-7-Hydroxy-6-methoxy-flavanone (3)

Yellow powder, $[\alpha]_D$: -55.0 (*c* 0.1, MeOH); CD [MeOH, nm ($\Delta\varepsilon$)] : 348 (+2.29), 312 (-6.08), 242 (+7.06); UV λ_{max} (MeOH) (log ε) nm: 238 (4.23), 277 (4.04), 338 (*sh*) (2.38); IR ν_{max} (KBr) cm^{-1} : 3431, 1576, 1470; EIMS: *m/z* 270 $[M]^+$, 166, 151, 121, 69; ^{1}H-NMR (400 MHz, CD_3OD) : δ 7.52 (2H, br d, *J* = 7.2 Hz, H-2', H-6'), 7.43 (2H, t, *J* = 7.2 Hz, H-3', H-5'), 7.38 (1H, br d, *J* = 7.2 Hz, H-4'), 7.30 (1H, s, H-5), 6.45 (1H, s, H-8), 5.48 (1H, dd, *J* = 13.0, 3.0 Hz, H-2), 3.87 (3H, s, $OC\underline{H}_3$), 3.03 (1H, dd, *J* = 17.0, 13.0 Hz, H-3*ax*), 2.77 (1H, dd, *J* = 17.0, 3.0 Hz, H-3*eq*); ^{13}C-NMR (100 MHz, CD_3OD) : δ 193.7 (C-4), 161.0 (C-7), 158.5 (C-9), 146.3 (C-6), 141.6 (C-1'), 130.4 (C-3', C-5'), 130.2 (C-4'), 128.1 (C-2', C-6'), 114.2 (C-10), 108.7 (C-5), 105.5 (C-8), 81.9 (C-2), 57.3 ($O\underline{C}H_3$), 45.8 (C-3).

Sativan (4)

Yellow powder, $[\alpha]_D$: -22.0 (*c* 2, CD_3OD); EIMS: *m/z* 286 $[M]^+$, 164, 151, 97, 71, 57; ^{1}H-NMR (500 MHz, CD_3OD) : δ 7.00 (1H, d, *J* = 8.4 Hz, H-6'), 6.83 (1H, d, *J* = 8.2 Hz, H-5), 6.51 (1H, d, *J* = 2.3 Hz, H-3'), 6.44 (1H, dd, *J* = 8.4, 2.3 Hz, H-5'), 6.29 (1H, dd, *J* = 8.2, 2.4 Hz, H-6), 6.21 (1H, d,

Table I. ^{1}H- and ^{13}C-NMR data of **1** and **2** (CD_3OD)

Carbon	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H
1	133.8	7.28 (1H, d, 8.4)	133.6	7.30 (1H, d, 8.4)
2	111.5	6.50 (1H, dd, 8.4, 2.4)	111.2	6.51 (1H, dd, 8.4, 2.4)
3	160.9		160.6	
4	104.8	6.32 (1H, d, 2.4)	104.5	6.32 (1H, d, 2.4)
4a	158.8		158.5	
6	68.2	4.23 (1H, dd, 10.7, 4.7, H-6*eq*) 3.57 (1H, dd, 10.7, 10.6, H-6*ax*)	68.0	4.22 (1H, dd, 10.4, 3.0, H-6*eq*) 3.54 (1H, t, 10.4, H-6*ax*)
6a	42.3	3.48 (1H, m)	41.3	3.51 (1H, m)
6b	120.6		121.3	
7	106.7	6.28 (1H, s)	126.4	7.17 (1H,d, 8.2)
8	143.9		107.7	6.46 (1H, dd, 8.2, 2.2)
9	150.2		163.0	
10	95.0	6.39 (1H, s)	98.0	6.39 (1H, d, 2.2)
10a	156.3		162.5	
11a	80.8	5.46 (1H, d, 6.9)	80.5	5.47 (1H, d, 6.4)
11b	113.6		113.3	
$-OCH_2O-$	103.3	5.88 (2H, dd, 13.1, 1.1)		
$-OCH_3$			56.3	3.75 (3H, s)

J = 2.4 Hz, H-8), 4.16 (1H, br d, *J* = 10.1 Hz, H-2*ax*), 3.59 (1H, t, *J* = 10.1 Hz, H-2*eq*), 3.80 (3H, s, $OC\underline{H}_3$), 3.75 (3H, s, $OC\underline{H}_3$), 3.42 (1H, m, H-3), 2.88 (1H, dd, *J* = 15.5, 10.8 Hz, H-4*ax*), 2.74 (1H, dd, *J* = 15.5, 3.9 Hz, H-4*eq*); ^{13}C-NMR (100 MHz, CD_3OD) δ : 162.0 (C-4'), 160.3 (C-2'), 158.3 (C-7), 157.1 (C-9), 131.9 (C-5), 129.3 (C-6'), 123.7 (C-1'), 115.5 (C-10), 109.8 (C-6), 106.3 (C-5'), 104.5 (C-8), 100.2 (C-3'), 71.9 (C-4), 56.6 ($O\underline{C}H_3$), 56.5 ($O\underline{C}H_3$), 33.7 (C-3), 32.2 (C-2).

Pseudobaptigenin (5)

White amorphous powder, UV λ_{max} (MeOH) nm : 241 (*sh*), 249, 260 (*sh*), 296; EIMS: *m/z* 282 $[M]^+$, 146, 132, 84, 66; ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 8.32 (1H, s, H-2), 7.93 (1H, d, *J* = 8.8 Hz, H-5), 7.13 (1H, d, *J* = 1.6 Hz, H-2'), 7.04 (1H, dd, *J* = 8.0, 1.6 Hz, H-6'), 6.96 (1H, d, *J* = 8.0 Hz, H-5'), 6.90 (1H, dd, *J* = 8.8, 2.1 Hz, H-6), 6.81 (1H, d, *J* = 2.1 Hz, H-8), 6.04 (2H, s, $-OC\underline{H}_2O-$); ^{13}C-NMR (100 MHz, DMSO-d_6) : δ 174.8 (C-4), 160.9 (C-7), 157.9 (C-9), 153.7 (C-2), 147.3 (C-3'), 147.2 (C-4'), 127.5 (C-1'), 126.2 (C-5), 123.4 (C-3), 122.7 (C-6'), 116.0 (C-10), 113.9 (C-6), 109.8 (C-2'), 108.4 (C-5'), 102.4 (C-8), 101.4 ($-O\underline{C}H_2O-$).

Genistein (6)

Yellow amorphous powder, UV λ_{max} (MeOH) (log ε) nm: 264 (4.45); EIMS: *m/z* 270 $[M]^+$, 153; ^{1}H-NMR (300 MHz, DMSO-d_6) : δ 12.93 (1H, s, 5-OH), 8.29 (1H, s, H-2), 7.35 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.80 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.34 (1H, d, *J* = 2.1 Hz, H-8), 6.18 (1H, d, *J* = 2.1 Hz, H-6); ^{13}C-NMR (75 MHz, DMSO-d_6) : δ 180.0 (C-4), 164.8 (C-7), 161.9 (C-5), 157.6 (C-4'), 157.3 (C-9), 153.8 (C-2), 130.1 (C-2', 6'), 122.2 (C-3), 121.2 (C-1'), 115.0 (C-3', 5'), 104.2 (C-10), 99.0 (C-6), 93.7 (C-8).

RESULTS AND DISCUSSION

Compound **1** was isolated as yellowish powder, $[\alpha]_D$ -261.1°. Its molecular formula was deduced as $C_{16}H_{14}O_5$ from EIMS and carbon counts in ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of **1** showed a characteristic pattern corresponding to a pterocarpan skeleton; the signals at δ 5.46 (d, *J*=6.9 Hz), 4.23 (dd, *J*=10.7, 4.7 Hz), 3.57 (dd, *J*=10.7, 10.6 Hz) and 3.48 (m) were assigned to H-11a, H-6_{eq}, H-6_{ax} and H-6a, respectively (Kinoshita *et al.*, 1990). And the ^{1}H- and ^{13}C- NMR showed the presence of 1,2,4,5-tetrasubstituted and 1,3,4-trisubstituted aromatic rings and a methylenedioxy group. Complete assignments of these spectra were made with ^{1}H-^{1}H COSY, HMQC and HMBC techniques. The absolute stereochemistry of **1** was shown to be 6a*R* and 11a*R* (Garcez *et al.*, 1988; Tokes *et al.*, 1999), as these showed positive and negative cotton effects at 288 and 238 nm, respectively in its circular dichroism (CD) spectrum (Fig. 2). From these data, **1** was identified as (6a*R*,11a*R*)-maackiain (Ingham, 1981; Ingham, 1982; Maximo and Lourenco, 1998) (Fig. 1).

Compounds **2** was isolated as yellowish powder, ($[\alpha]_D$ -232°). Its molecular formula was deduced as $C_{16}H_{12}O_4$ from EIMS and carbon counts in ^{13}C-NMR. The ^{1}H- and ^{13}C-NMR spectra of **2** were almost identical to those of **1** except for signals of the some aromatic ring. Its spectra showed the presence of a methoxyl group (δ 3.75) and 1,4-disubstitued aromatic ring. Complete assignments of these spectra were made with ^{1}H-^{1}H COSY, HMQC and HMBC techniques. The absolute stereochemistry of **2** was shown to be 6a*R* and 11a*R* (Garcez *et al.*, 1988; Tokes *et al.*, 1999), as these showed positive and negative cotton effects at 304 and 240 nm, respectively in its circular dichroism (CD) spectrum (Fig. 2). From these data, **2** was elucidated as (6a*R*,11a*R*)-medicarpin (Ingham, 1981; Ingham, 1982; Hearth *et al.*, 1998) (Fig. 1).

Compound **3** was isolated as yellow powder with negative optical rotation ($[\alpha]_D$ -55°) and its molecular formula was deduced as $C_{16}H_{14}O_4$ from EIMS and carbon counts in ^{13}C-NMR. The ^{1}H-NMR spectrum showed a characteristic pattern of flavanone at δ 5.48 (1H, dd, *J*=13.0, 3.0 Hz), 3.03 (1H, dd, *J*=17.0, 13.0 Hz) and 2.77 (1H, dd, *J*=17.0, 3.0 Hz) (Reddy *et al.*, 2003). Complete assignments of these spectra were made with ^{1}H-^{1}H COSY and HMQC techniques. From all these data and the HMBC correlations, **3** was deduced as 7-hydroxy-6-methoxy-flavanone. The absolute configuration at C-2 was shown to be *S* (Matsuda *et al.*, 2002), as it showed positive and negative cotton effects at 348 and 312 nm, respectively in its circular dichroism (CD) spectrum (Fig. 2). Above all, **3** was concluded as (2*S*)-7-hydroxy-6-methoxy-flavanone (Fig. 1).

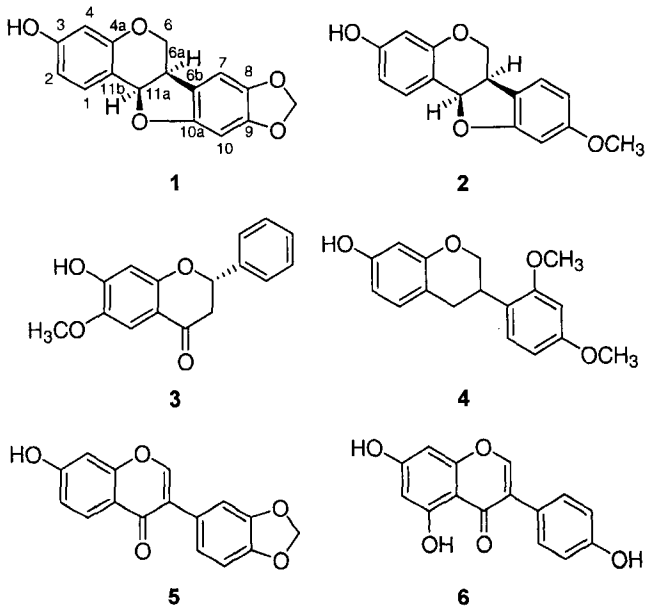

Fig. 1. Chemical structures of compounds **1-6**

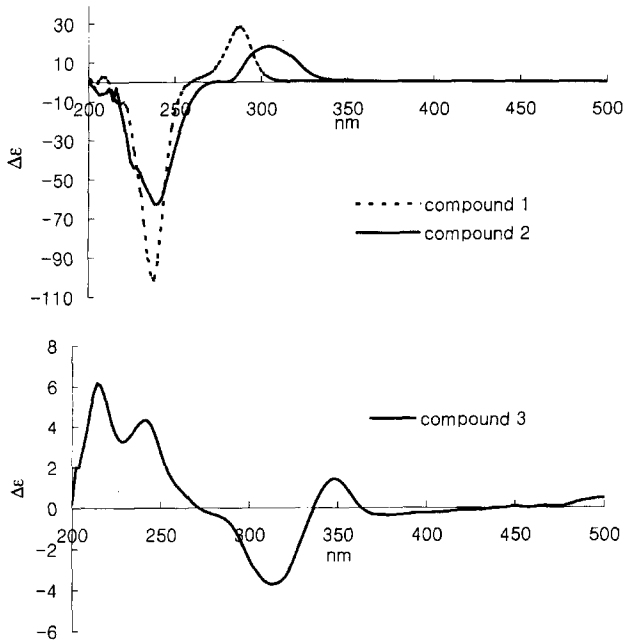

Fig. 2. Cricular dichroism spectra of **1**, **2**, and **3**

Compound **4** was isolated as a yellow power and its molecular formula were deduced as $C_{17}H_{18}O_4$ from EIMS and carbon counts in ^{13}C-NMR. The ^{1}H-NMR spectrum showed characteristic pattern of isoflavan at δ 4.16 (1H, br d, *J* = 10.1 Hz, H-2*ax*), 3.59 (1H, t, *J* = 10.1 Hz, H-2*eq*), 3.42 (1H, m, H-3), 2.88 (1H, dd, *J* = 15.5, 10.8 Hz, H-4*ax*), 2.74 (1H, dd, *J* = 15.5, 3.9 Hz, H-4*eq*) (Subarnas *et al.*, 1991). Compound **4** was identified as sativan by comparison with previously reported spectral data (Bonde *et al.*, 1973; Ingham, 1977) (Fig. 1).

Compound **5** and **6** were obtained as amorphous powders and their molecular formula were deduced as $C_{17}H_{18}O_4$ from EIMS and carbon counts in ^{13}C-NMR respectively. Their ^{1}H- and ^{13}C-NMR spectra showed characteristic pattern of isoflavone. (Harbone *et al.*, 1988). By comparison with previously reported spectral data, **5** and **6** were identified as psedobaptigenin and genistein, respectively (Ohashi *et al.*, 1976, Kinjo *et al.*, 1987) (Fig. 1).

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