Arch Pharm Res Vol 26, No 12, 1009-1013, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

A New Pterocarpan, (-)-Maackiain Sulfate, from the Roots of *Sophora subprostrata*

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(Received August 6, 2003)

A new pterocarpan, (-)-maackiain 3-sulfate (**1**) was isolated from the methanol extract of roots of *Sophora subprostarata* together with (-)-maackiain (**2**), trifolirhizin (**3**), lupeol (**4**), ononin (**5**), 7,4'-dihydroxyflavone (**6**), and (+)-syringaresinol (**7**). The structure of **1** was determined by analyses of 2D NMR and HRFABMS. Compounds **5-7** were isolated from this plant for the first time.

Key words: *Sophora subprostrata*, Pterocarpan, (-)-Maackiain 3-sulfate, Ononin, Dihydroxy-flavone, Syringaresinol

INTRODUCTION

The roots of *Sophora subprostrata* Chun et T. Chen (Leguminosae) have been used as a Korean traditional medicine for the treatment of fever, inflammation, peptic ulcer and cancer (Sakamoto *et al.*, 1992). Many chemical components have been reported from this plant including triterpenoid saponins; soyasaponins I, II, A_3, kaikasaponin, abrisaponin, (Sakamoto *et al.*, 1992; Kitagawa *et al.*, 1988), flavonoids; sophoradochromene, sophoranochromene (Komatsu *et al.*, 1970), alkaloids; matrine, oxymatrine, and pterocarpans; trifolirhizin, maackiain (Kojima *et al.*, 1970).

Pterocarpans are isoflavonoids found in many species of the Leguminosae. Since these compounds possess asymmetric carbons at 6a- and 11a-position, four diastereomeric structures are possible. However, the junction between C-6a and C-11a has been established to be a *cis*-fusion of the heterocyclic rings resulting in only one pair of naturally occurring enantiomers (Vanetten *et al.*, 1983). Pterocarpans are also well known for their antifungal activity as phytoalexins, and their occurrence in leguminous species is of taxonomic significance (Máximo *et al.*, 1998).

By means of chromatographic separation, we have isolated a new pterocarpan, (-)-maackiain 3-sulfate (**1**) along with the known compounds; (-)-maackiain (**2**), trifolirhizin (**3**), lupeol (**4**), ononin (**5**), 7,4'-dihydroxyflavone (**6**), and (+)-syringaresinol (**7**). Among the isolates, compounds **5-7** were isolated from this plant for the first time. In this paper, we describe the isolation of compounds and the structure of which was determined by analyses of spectroscopic method (^{1}H-^{1}H COSY, DEPT, HMQC and HMBC).

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MATERIALS AND METHODS

General experimental procedures

Optical rotations were determined on an Autopol III Automatic polarimeter (Rudolph Research Flanders, NJ). IR spectra were recorded on a Midac High Resolution FT-IR spectrometer in KBr disks. FABMS (positive ion mode) and CIMS were obtained on a JEOL JMS-700. Electrospray mass spectra were determined on an Api ES/MS (HP 59987A ES/5989A MS) mass spectrometer. NMR spectra were recorded on a Bruker 300 and 500 spectrometer. ^{1}H-^{1}H COSY, HMQC and HMBC NMR spectra were obtained with the usual pulse sequences. TLC and column chromatography were carried out on precoated silica gel F_{254} plates (Merck, art. 5715), RP-18 F_{254S} plates (Merck, art. 15423), silica gel 60 (230-400 mesh, Merck), Sephadex LH-20 (Bead size 25-100 μ, Sigma) and LiChroprep RP-18 (40-63 μm, Merck).

Plant material

The roots of *Sophora subprostrata* were purchased from a local Korean herbal drug market in July 2000. The plant was identified by Prof. Chang Su Yuk at Kyung Hee University in Korea. Voucher specimens (812-15) have been deposited in the laboratory of Korea Institute of Science & Technology (KIST).

Extraction and isolation

The dried roots of *Sophora subprostrata* (3 kg) were cut into small pieces and extracted three times with MeOH at room temperature to afford 469 g of residue on removal of the solvent under reduced pressure. The methanol extract was suspended with water and then partitioned in turn with CH_2Cl_2, EtOAc, *n*-BuOH. The CH_2Cl_2 and EtOAc extracts were evaporated under reduced pressure to give 92.5 g and 23.6 g residues, respectively. The CH_2Cl_2 extract was divided by column chromatography on silica gel column eluting with CH_2Cl_2/EtOAc/MeOH (3:1:0.1) and CH_2Cl_2/MeOH/H_2O (7:1:0.1 → 4:1:0.1) gradient system to give six fractions (Fr.1~6). Fraction 5 (6.17 g) was divided by column chromatography on silica gel column eluting with CH_2Cl_2/EtOAc/MeOH (7:2:1→2:2:1) and CH_2Cl_2/MeOH/H_2O (7:1:0.1) to give eight fractions (Fr.5A~5H). Fraction 5F (1.1 g) was divided by column chromatography on silica gel column eluting with hexane/CH_2Cl_2/MeOH (3:3:0.7→3:3:1) to afford seven fractions (Fr.5FA~5FG). Fraction 5FF (193 mg) was purified by column chromatography on LiChroprep® RP-18 column eluting with MeOH/H_2O (3:7→8:2) gradient system to yield compound **1** (13.4 mg) in addition to compound **5** (4.8 mg) and compound **3** (11.9 mg). Fraction 5FE (131.9 mg) gave compound **3** (32.1 mg) by crystallization with methanol. Fraction 1 (54.3 g) was subjected to silica gel column chromatography using hexane/CH_2Cl_2/EtOAc (5:1:0.3→5:1:0.5) to give compound **4** (132.8 mg). Fraction 2 (9 g) was divided into three fractions (Fr.2A~2C) by gel filtration on Sephadex LH-20 using MeOH/CH_2Cl_2 (8:2). Fraction 2B (3.5 g) was partitioned with hexane/acetone, and this hexane layer was combined with fraction 2C (101.2 mg) and then subjected to silica gel column chromatography using hexane/EtOAc (1:2) and CH_2Cl_2/EtOAc/MeOH (3:3:0.1→3:3:1) to give seven sub-fractions (2BA~2BG). Sub-fraction 2BF (258.8 mg) was purified by LiChroprep® RP-18 TLC eluting with H_2O/CH_3CN (1:1) to afford compound **7** (5.9 mg).

The EtOAc extract (3.01 g) yielded compound **3** (232.2 mg) by crystallization, and then the rest of this extract was divided into two fractions (Fr.A~B) by gel filtration on Sephadex LH-20 eluting with MeOH. Fraction B (1.1 g) was subjected to silica gel column chromatography using with hexane/CH_2Cl_2/EtOAc (1:1:1→1:1:3) and CH_2Cl_2/MeOH (5:1) to afford six fractions (Fr.BA~BF). Fraction BB (201.1 mg) was purified by preparative silica gel TLC developed with hexane/CH_2Cl_2/EtOAc (3:3:1) to afford compound **2** (11.3 mg). Fraction BD (36.7 mg) and fraction BF (210.9 mg) gave compound **6** (25.4 mg) and an additional compound **3** (71.3 mg), respectively.

(-)-Maackiain 3-sulfate (1)

m.p. 210-220°C (dec); [α]: -161.8° (*c* 0.67, MeOH); UV (MeOH): λ_{max} (log ε) 276 (sh, 1.92), 284 (2.28), 309 (4.23) nm; IR (KBr): ν_{max} 1620, 1476, 1262, 1150, 1058 cm^{-1}; ^1H and ^{13}C-NMR data, see Table I; ESMS (negative-ion mode): *m/z* 363 $[M-H]^-$; HRFABMS $[M]^+$ *m/z* 364.0251, calcd for $C_{16}H_{12}O_8S$, 364.0253.

(-)-Maackiain (2)

m.p. 165°C; [α]: -242.8° (*c* 0.565, acetone); UV (MeOH): λ_{max} (log ε) 280 (2.25), 286 (2.34), 308 (2.91) nm; IR (KBr): ν_{max} 3400, 2910, 1620, 1472, 1150, 1034 cm^{-1}; CIMS: *m/z* 285 $[M+H]^+$; ^1H- and ^{13}C-NMR data, see Table I.

Trifolirhizin (3)

m.p. 145-148°C; UV (MeOH): λ_{max} (log ε) 284 (1.29), 308 (1.62) nm; ESMS: *m/z* 447 $[M+H]^+$; ^1H-NMR (500 MHz, CD_3OD+pyridine-d_5): δ 7.20 (1H, d, *J*=8.4 Hz, H-1), 6.70 (1H, d, *J*=6.5 Hz, H-2), 6.63 (1H, s, H-7), 6.59 (1H, br s, H-4), 6.26 (1H, s, H-10), 5.68 (2H, d, *J*=14.9 Hz, $OC\underline{H_2}O$), 5.26 (1H, d, *J*=7.1 Hz, H-11a), 5.01 (1H, d, *J*=7.3 Hz, H-1'), 4.03 (1H, dd, *J*=4.3, 10.5 Hz, H-6), 3.94 (1H, d, *J*=11.5 Hz, H-6'), 3.77 (1H, dd, *J*=5.1, 11.9 Hz, H-6'), 3.67 (1H, m, H-3'), 3.63 (1H, m, H-2'), 3.59 (1H, m, H-4'), 3.54 (1H, m, H-5'), 3.38 (1H, m, H-6), 3.30 (1H, m, H-6a); ^{13}C-NMR (125 MHz, CD_3OD+pyridine-d_5): δ 160.1 (C-3), 157.7 (C-4a), 155.3 (C-10a), 149.2 (C-9), 142.8 (C-8), 133.0 (C-1), 119.3 (C-6b), 115.5 (C-11b), 111.6 (C-2), 106.0 (C-7), 105.6 (C-4), 102.3 ($O\underline{C}H_2O$), 102.1 (C-1'), 94.2 (C-10), 79.3 (C-11a), 78.6 (C-5'), 78.2 (C-3'), 74.9 (C-2'), 71.3 (C-4'), 67.3 (C-6), 62.4 (C-6'), 41.3 (C-6a).

Lupeol (4)

m.p. 190-193°C; [α] : +26.4° (*c* 0.7, $CHCl_3$); GCMS: *m/z* 426 $[M]^+$; ^1H-NMR (300 MHz, $CDCl_3$): δ 4.61 (1H, s, H-29), 4.49 (1H, s, H-29), 3.11 (1H, t, *J*=5.1 Hz, H-3), 2.30 (1H, dt, *J*=5.5, 11.0 Hz, H-19), 1.82 (1H, m, H-21); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 151.4 (C-20), 109.7 (C-29), 79.4 (C-3), 55.6 (C-5), 50.8 (C-9), 48.6 (C-18), 48.3 (C-19), 43.3 (C-17), 43.2 (C-14), 41.2 (C-8), 40.3 (C-22), 39.2 (C-4), 39.0 (C-1), 38.4 (C-13), 37.5 (C-10), 35.9 (C-16), 34.6 (C-7), 30.2 (C-21), 28.3 (C-23), 27.8 (C-2, 15), 25.5 (C-12), 21.3 (C-11), 19.7 (C-30), 18.7 (C-6), 18.4 (C-28), 16.5 (C-25), 16.3 (C-26), 15.7 (C-24), 14.9 (C-27).

Ononin (5)

m.p. 205-208°C; UV (MeOH): λ_{max} (log ε) 259 (4.80), 300

(sh, 1.62) nm; FABMS: *m/z* 431 $[M+H]^+$; ^1H-NMR (500 MHz, CD_3OD+pyridine-d_5): d 8.17 (1H, s, H-2), 8.13 (1H, d, *J*=8.8 Hz, H-5), 7.50 (2H, d, *J*=8.6 Hz, H-2', 6'), 7.24 (1H, d, *J*=2.0 Hz, H-8), 7.21 (1H, dd, *J*=2.2, 8.8 Hz, H-6), 6.99 (2H, d, *J*=8.7 Hz, H-3', 5'), 5.20 (1H, d, *J*=5.8 Hz, H-1"), 4.03 (1H, d, *J*=12.0 Hz, H-6"), 3.83 (1H, m, H-6"), 3.80 (3H, s, OMe) 3.65-3.68 (3H, m, H-2", 3", 5"), 3.56 (1H, m, H-4"); ^{13}C-NMR (75 MHz, CD_3OD): δ 177.1 (C-4), 163.1 (C-7), 160.8 (C-4'), 158.7 (C-9), 154.5 (C-2), 131.2 (C-2', 6'), 128.1 (C-5), 125.5 (C-1'), 125.3 (C-3), 120.0 (C-10), 116.7 (C-6), 114.7 (C-3', 5'), 104.7 (C-8), 101.8 (C-1"), 78.6 (C-5"), 78.0 (C-3"), 74.7 (C-2"), 71.2 (C-4'), 62.4 (C-6"), 55.6 (OMe).

7,4' -Dihydroxyflavone (6)

m.p. 290°C; UV (MeOH): λ_{max} (log ε) 255 (1.26), 323 (2.41) nm; CIMS: *m/z* 255 $[M+H]^+$; ^1H-NMR (500 MHz, pyridine-d_5): δ 8.45 (1H, d, *J*=8.6 Hz, H-5), 7.95 (2H, d, *J*=8.4 Hz, H-2', 6'), 7.30 (1H, d, *J*=1.4 Hz, H-8), 7.24 (3H, d, *J*=8.7 Hz, H-6, 3', 5'), 7.04 (1H, s, H-3); ^{13}C-NMR (125 MHz, pyridine-d_5): δ 177.4 (C-4), 164.1 (C-7), 163.4 (C-2), 162.1 (C-4'), 158.6 (C-9), 128.6 (C-2', 6'), 127.4 (C-5), 123.0 (C-1'), 117.4 (C-10), 116.7 (C-3', 5'), 110.4 (C-6), 105.7 (C-3), 103.4 (C-8).

(+)-Syringaresinol (7)

[α]: +2.4° (*c* 0.245, $CHCl_3$); ESMS: *m/z* 419 $[M+H]^+$; ^1H-NMR (300 MHz, $CDCl_3$): δ 6.58 (4H, s, H-2, 6, 2', 6'), 5.52 (2H, s, O<u>H</u>), 4.72 (2H, br s, H-7, 7'), 4.28 (2H, m, H-9, 9"), 3.90 (2H, m, H-9, 9'), 3.90 (12H, s, OMe), 3.09 (2H, br s, H-8, 8'); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 147.5 (C-3, 5, 3', 5'), 134.6 (C-4, 4'), 132.5 (C-1, 1'), 103.0 (C-2, 6, 2', 6'), 86.5 (C-7, 7'), 72.2 (C-9, 9'), 56.8 (OMe), 54.7 (C-8, 8').

RESULTS AND DISCUSSTION

Compound **1** was obtained as an amorphous powder and its molecular formula was established as $C_{16}H_{12}O_8S$ by HRFABMS (*m/z* 364.0251 $[M]^+$). The IR spectrum indicated the presence of double bond (1620 cm^{-1}) and $-SO_3$- (1262 and 1058 cm^{-1}) groups (Xiao *et al.* 2000). The ^1H and ^{13}C-NMR spectra of **1** were almost identical to those of (-)-maackiain (**2**) except for the shifts of peaks of the aromatic ring A. In the ^1H-NMR spectrum, compound **1** showed a characteristic set of peaks corresponding to a pterocarpan skeleton; the signals at δ 5.45 (d, *J*=6.7 Hz), 4.20 (dd, *J*=10.2, 2.8 Hz), 3.57 (m), and 3.50 (m) were assigned to H-11a, H-6, H-6, and H-6a, respectively (Chaudhuri *et al.*, 1995). The ^1H-NMR spectrum also displayed the doublet at δ 5.78 (*J*=7.4 Hz, -OC<u>H</u>$_2$O-) and two singlets at δ 6.30 (H-10) and 6.75 (H-7), which suggested compound **1** has a methylenedioxy moiety in the aromatic ring D. Furthermore, it showed a doublet at δ 6.77 (*J*=2.1 Hz, H-4), a doublet of doublets at δ 6.89 (1H, *J* = 8.4, 2.2 Hz, H-2) and a doublet at δ 7.35 (1H, *J*=8.4 Hz, H-1) assignable to the ABX system for the ring A. In their ^{13}C-NMR spectra, most of the signals of **1** and **2** (Table I) had very similar chemical shifts each other except for the signals at C-2, 3, 4 and -11b. Significant differences between the spectra of **1** and **2** were the large downfield shift (4-7 ppm) of the C-2, -4 and -11b signal and upfield shifts for C-3 (5 ppm) in **1** owing to an electron withdrawing group caused by sulfation of the 3-hydroxy group. *O*-Sulfate leads to a decreased electron density of *ortho* and *para* carbons and an increased electron density of the carbon carrying the sulfate group (Barron *et al.* 1988). The ^{13}C-NMR chemical shift of **1** was assigned based on further analyses of its HMQC and HMBC spectra. The cross-peaks between H-1/C-3, H-1/C-11a, H-2/C-3, H-2/C-11b, H-4/C-3, H-4/C-11b in the HMBC spectrum confirmed the planar structure of **1**. Moreover, the signal at δ 5.78 (OC<u>H</u>$_2$O) showed a long-range correlation with C-8 and -9 at δ 143.7 and 149.8, respectively. On the basis of the foregoing observations, compound **1** was elucidated to be a (-)-maackiain 3-sulfate.

Table I. ^1H- and ^{13}C-NMR data of compounds **1**[a] and **2**[b]

Position	^1H		^{13}C	
	1	2	1	2
1	7.35 d (8.4)[c]	7.14 d (8.4)	133.2	133.5
2	6.89 dd (8.4, 2.2)	6.37 dd (8.4, 2.4)	116.5	111.2
3			155.2	160.7
4	6.77 d (2.1)	6.19 d (2.3)	111.2	104.5
4a			157.8	158.4
6	4.20 dd (10.2, 3.8) 3.57 m	4.10 dd (10.5, 4.3) 3.44 t (10.4)	68.0	67.8
6a	3.50 m	3.34 ddd (11.0, 6.8, 4.3)	41.9	42.0
6b			120.0	120.2
7	6.75 s	6.68 s	106.5	106.4
8			143.7	143.5
9			149.8	149.8
10	6.30 s	6.26 s	94.8	94.6
10a			155.6	156.0
11a	5.45 d (6.7)	5.32 d (6.7)	80.0	80.5
11b			118.9	113.2
OCH_2O	5.78 d (7.4)	5.76 d (1.0), 5.74 d (1.0)	103.1	102.9

[a]Spectra were measured in CD_3OD+D_2O. [b]Spectra were measured in CD_3OD. [c]Values in parenthesis are coupling constants (Hz).

Six known compounds, (-)-maackiain (**2**) (Chaudhuri *et al.*, 1995; Máximo *et al.*, 1998; Mizuno *et al.*, 1990), trifolirhizin (**3**) (Yamamoto *et al.*, 1991), lupeol (**4**) (Sholichin *et al.*, 1980), ononin (**5**) (Barrero *et al.*, 1989), 7,4'-dihydroxyflavone (**6**) (Agrawal, 1989), and (+)-syringaresinol

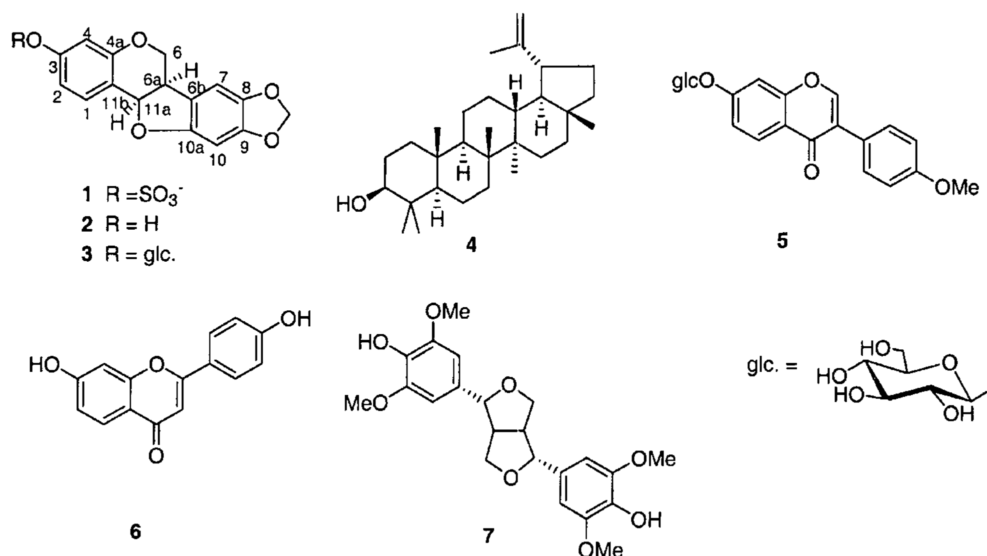

Fig. 1. Structure of compounds **1**-**7** isolated from *S. subprostrata*

(**7**) (Badawi *et al.*, 1983; Kwon *et al.*, 1999) were identified by comparing the physical and spectroscopic data with the literature values. Among the isolated compounds, **5**-**7** were isolated from this plant for the first time.

ACKNOWLEDGMENTS

This research was supported by a grant (PF002103-01) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government. The authors are grateful to Prof. Chang Su Yuk at Kyung Hee University in Korea for identification of the specimen and Dr. Dong-Hyun Kim at Korea Institute of Science and Technology for ESMS, and Dr. Jongki Hong at Korea Basic Science Institute for HRFABMS analysis.

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