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Extractives from the Bark of *Sophora japonica* L*1

Youngki Park*2†, Hak-Ju Lee*3, Don-Ha Choi*3,
Yeong-Han Kwon*4, and Jung-Soo Oh*2

ABSTRACT

This study was carried out to investigate the constituents of *Sophora japonica*(Leguminosae) bark. To isolate compounds, bark was extracted with ethanol and then partitioned with hexane, dichloromethane, ethylacetate and butanol successively. After partitioned, DCM fraction was subjected to column chromatography with various solvent system in silica gel and/or Sephadex LH-20. Structures were elucidated by spectroscopic methods including MS, ^{1}H, ^{13}C and 2D-NMR experiments.

Four compounds were isolated from the bark and identified as 3′,7-dihydroxy-4′-methoxyisoflavone (cyclosin), puerol A, maackiain-3-O-β-D-glucopyranoside (trifolirhizin), and 4′, 5, 7-trihydroxyisoflavone (genistein). Among these compounds, cyclosin and trifolirhzin were first isolated from *S. japonica*.

Keywords: *Sophora japonica*, Leguminosae, bark, cyclosin, puerol A, trifolirhizin, genistein

1. INTRODUCTION

Sophora japonica L.(Leguminosae) has common name as Chinese scholar tree or Japanese pagoda tree (Kim T. W., 1995) and is distributed throughout China, Korea, and Japan. It is a wide-branching, highly ornamental tree with a rounded crown (Michael, E. E., 1986). Its bark on old trunks is somewhat black locust-like except pale grayish brown in color. This tree is tolerant of pollution, heat, and drought and casts a light shade, allowing turf grasses to grow up to the trunk. For these properties, this tree uses for urban landscape and also used in the traditional medicine. The leaves are used for the treatment of hemorrhoids. It is also good at stanching and decreasing blood pressure. Sometime it has the effect of anti-inflammation. The flower is used for the prevention against paralysis to the patients who has high blood pressure. The flavonoids of the woods and roots (Park *et al.*, 2001; Takeda *et al.* 1977; Shirataki *et al.* 1987) have been studies but less is known of those in the bark.

This study was carried out to isolate and to identify the extractives from the bark of *S. japonica* L.

*1 Received on June 19, 2002; accepted on August 7, 2002.
*2 Dept. of Forest Resources, Dongguk University, Seoul 100-715, Korea
*3 Div. Wood Chemistry & Microbiology, Korea Forest Research Institute, Seoul 130-715, Korea
*4 Nambu Forest Experiment Station, KFRI, Chinju 660-300, Korea
† Corresponding author : Youngki Park (bay0601@hanmail.net)

2. MATERIALS and METHODS

2.1. Plant materials

The barks of *S. japonica* (A 20 years old, DBH : 9 cm) were collected from Jiri mountain, Kyungnam, Korea during June, 1996 and dried at room temperature. After drying, these samples were ground by Wiley mill.

2.2. Extraction and Fractionation

Dried and ground bark of *S. japonica* were extracted twice with ethanol (EtOH) and then evaporated to give the crude extractives. The crude extractives was successively partitioned with organic solvents, such as *n*-hexane, dichloromethane (DCM), ethylacetate (EtOAc) and *n*-butanol. For the isolation of compounds, silica gel and Sephadex LH-20 were used for column chromatography with various solvents. To verify the purity of compounds, silica gel 60 F_{254} (Merk) TLC plate was used with solvent A (toluene-ethyl formate-formic acid = 5:4:1, v/v/v) and solvent B (acetone-EtOAc-H_2O = 10:10:1, v/v/v). After developing, UV (254 nm) detector was used for monitoring.

From DCM soluble part, isolation was performed. This part of extractives (61.38 g) was chromatographed on Sehpadex LH-20 column eluted with MeOH-EtOH (1:1, v/v) to give 6 sets of fractions (SJBD 1~SJBD 6).

2.3. Instrumental Analysis

For the determination of molecular weights of the isolated compounds, EI-MS was performed at 70 eV ionization energy by direct inlet probe method, using JEOL JMS-600W mass spectrometer. About 10 mg of each sample was dissolved in 0.75 ml of methanol-d_4 (CD_3OD) or acetone-d_6 with TMS (tetramethylsilane) as an internal standard and NMR spectra were obtained using a Varian UI 500 spectrometer at the operating frequency of 500 MHz (^1H) and 125 MHz (^{13}C) equipped with a VXR computer system at Korea Basic Science Institute in Seoul. The operating conditions for one-dimensional (1D) spectrum was as followed : frequency; 500 MHz, sweep width; 6 KHz, flip angle; 90° (12.8 μs), sampling point; 48 K, accumulation; 256 pulses, temperature; 298 K.

2.4. Isolation of Compounds

Fraction SJBD 3 (7.02 g) was further subjected to repeated column chromatography on silica gel eluted with $CHCl_3$-MeOH (30:1, v/v) to give 4 sets of fractions (SJBD 3-1~SJBD 3-4). The first fraction (SJBD 3-1, 276 mg) of SJBD 3 was rechromatographed on silica gel column chromatography with benzene-MeOH (20:1, v/v) to give 6 sets of fractions (SJBD 3-1-1~SJBD 3-1-6). Among 6 fractions, second fraction (SJBD 3-1-2, 30 mg) was purified by column chromatography on Sephadex LH-20 and eluted with a solvent system of MeOH-H_2O (1:1, v/v) to give compound 1 (12 mg). The SJBD 3-4 fraction (5.16 g) was also chromatographed with a silica gel column (DCM-MeOH, 20:1, v/v) to give 6 subfractions (SJBD 3-4-1~SJBD 3-4-6). The SJBD 3-4-4 fraction (318 mg) was further subjected to column chromatography over Sephadex LH-20 (MeOH-H_2O, 7:3, v/v) to generate compound 2 (24 mg). Fraction 3-4-5 was recrystalized with MeOH to yield compound 3 (34 mg). Fraction SJBD 4 (6.95 g) was subjected to column chromatography on silica gel with DCM-MeOH (50:1, v/v) to give 7 fractions (SJBD 4-1~SJBD 4-7). SJBD 4-3 (1.20 g) was further chromatographed over Sephadex LH-20 with MeOH-H_2O (7:3, v/v) to give 5 fractions (SJBD 4-3-1~SJBD 4-3-5). Repeated column chromatography over Sepha-

dex LH-20 with MeOH-H_2O (1:1, v/v) of fraction SJBD 4-3-3 (170 mg) gave two fractions (SJBD 4-3-3-1～SJBD 4-3-3-2). The second fraction (SJBD 4-3-3-2, 80 mg) was further subjected to column chromatography over silica gel with benzene-MeOH (10:1, v/v) to give compound 4 (20 mg).

2.5. Spectral data of compounds

Compound 1. amorphous solid. EI-MS *m/z* : 284 (M^+, base ion), 241, 162, 137. ^{1}H NMR (500 MHz, methanol-d_4) : δ 3.88 (3H, *s*, OMe), 6.68 (1H, *d*, J = 2.2 Hz, H-8), 6.81(1H, *dd*, J = 2.2, 8.8 Hz, H-6), 6.83 (1H, *d*, J=8.1 Hz, H-5'), 6.90 (1H, *dd*, J = 2.0, 8.1 Hz, H-6'), 7.14 (1H, *d*, J=2.0 Hz, H-2'), 7.96 (1H, *d*, J = 8.8 Hz, H-5), 8.07(1H, *s*, H-2). ^{13}C NMR (125 MHz, methanol-d_4) : δ 56.47 (*s*, C4'-OMe), 103.66 (*d*, C-8), 114.25 (*d*, C-2'), 116.15 (*s*, C-10) 116.23 (*d*, C-5'), 118.76 (*d*, C-6), 122.90 (*d*, C-6'), 125.25 (*s*, C-3), 125.61 (*s*, C-1'), 127.96 (*d*, C-5), 147.71 (*s*, C-3'), 148.75 (*s*, C-4'), 154.36 (*d*, C-2), 160.46 (*s*, C-9), 169.65 (*s*, C-7), 178.15 (*s*, C-4). ^{1}H-^{1}H COSY (Correlation Spectroscopy) correlations : H-5↔H-6, H-2'/H-5'↔H-6', H-6↔H-8. HMBC (^{1}H Detected Multiple Bond Connectivity) correlations : H-2→C-3/C-9/C-4, H-2'→C-3, H-5→C-4/C-7/C-9, H-5'→C-1'/C-6', H-6'→C-2'/C-3, H-8→C-6, OMe→C-4'. NOESY correlations : H-5'↔H-OMe.

Compound 2. yellow powder. EI-MS *m/z* : 298 (M^+), 254, 239, 161, 107 (base ion). ; HR-EI-MS *m/z* : 298.0853 (calculated for $C_{17}H_{14}O_5$ 298.0841) ^{1}H NMR (500 MHz, methanol-d_4) : δ 2.79 (1H, *dd*, J = 6.0, 14.5 Hz, H-6ax), 3.24 (1H, *dd*, J = 4.0, 14.5 Hz, H-6eq), 5.92 (1H, *dd*, J = 4.0, 6.0 Hz, H-5), 6.11 (1H, *s*, H-3), 6.42 (1H, *d*, J = 2.0 Hz, H-10), 6.43 (1H, *dd*, J = 2.0, 9.0 Hz, H-8), 6.61 (2H, *dd*, J = 2.5, 11.0 Hz, H-3', 5'), 6.84 (2H, *dd*, J = 2.5, 11.0 Hz, H-2', 6') 7.29 (1H, *d*, J = 9.0 Hz, H-7). ^{13}C NMR (125 MHz, methanol-d_4) : δ 38.51 (*t*, C-6), 84.64 (*d*, C-5), 102.73 (*d*, C-10), 108.00 (*d*, C-8), 109.57 (*s*, C-12), 111.23 (*d*, C-3), 114.59 (*d*, C-3', 5'), 126.52 (*s*, C-1'), 130.62 (*d*, C-2', 6'), 131.01 (*d*, C-7), 156.05 (*s*, C-4'), 158.57 (*s*, C-11), 162.12 (*s*, C-9), 167.31 (*s*, C-4), 175.98 (*s*, C-2). ^{1}H-^{1}H COSY correlations : H-7↔H-8, H-2'/H-6'↔H-3'/H-5', H-5↔H-6eq/H-6ax, H-6eq↔H-6ax. HMBC correlations : H-3→C-2/C-4/C-5, H-5→C-1', H-8→C-10, H-10→C-9, H-2', 6'→C-4', H-3', 5'→C-1'/C-4', H-6→C-5/C-4. NOESY correlations : H-6eq/H-6ax/H-3/H-5↔H-7, H-2'/H-6'↔H-5/H-6eq/H-6ax.

Compound 3. yellow powder. EI-MS *m/z* : 284 (M^+-162, base ion), 284, 197, 162, 134, 69. ^{1}H NMR (500 MHz, acetone-d_6) : δ 3.42 (1H, *m*, H-4'), 3.43 (1H, *m*, H-2'), 3.52 (1H, *m*, H-3'), 3.54 (1H, *m*, H-5'), 3.60 (1H, *m*, H-6a), 3.69 (1H, *m*, H-6'), 3.70 (1H, *m*, H-6), 3.87 (1H, *dd*, J = 2.6, 11.9 Hz, H-6'), 4.30 (1H, *dd*, J = 4.5, 10.7 Hz, H-6), 4.95 (1H, *d*, J = 7.6 Hz, H-1'), 5.54 (1H, *d*, J = 7.2 Hz, H-11a), 5.92 (2H, *dd*, J = 0.9, 13.8 Hz, -OCH_2O-), 6.39 (1H, *s*, H-10), 6.58 (1H, *d*, J = 2.5 Hz, H-4), 6.74 (1H, *dd*, J = 2.4, 8.5 Hz, H-2), 6.90 (1H, *s*, H-7), 7.38 (1H, *d*, J = 8.5 Hz, H-1). ^{13}C NMR (125 MHz, acetone-d_6) : δ 41.07 (*d*, C-6a), 62.51 (*t*, C-6'), 67.02 (*t*, C-6) 71.18 (*d*, C-4'), 74.51 (*d*, C-2'), 77.76 (*d*, C-3', 5'), 79.05 (*d*, C-11a), 93.99 (*d*, C-10), 101.77 (*d*, C-1'), 102.15 (*t*, OCH_2O), 105.33 (*d*, C-4), 105.92 (*d*, C-7), 111.46 (*d*, C-2), 115.43 (*s*, C-11b), 119.35 (*s*, C-6b), 132.77 (*d*, C-1), 142.57 (*s*, C-8), 148.95 (*s*, C-9), 155.23 (*s*, C-10a), 157.5 (*s*, C-4a), 159.93 (*s*, C-3). ^{1}H-^{1}H COSY correlations : H-6a↔H-6/H-11a, H-2↔H-1/H-4, H-3'/H-1'↔H-2', H-4'↔H-3'/H-5', H-5'↔H-6'. HMBC correlations : H-1→C-3/C-4a, H-2→C-4/C-11b, H-4→C-2/C-3/C-4a/C-11b, H-6→C-11a, H-7→C-8/C-9/C-10a, H-10→C-8/C-9/C-6b/C-11a,

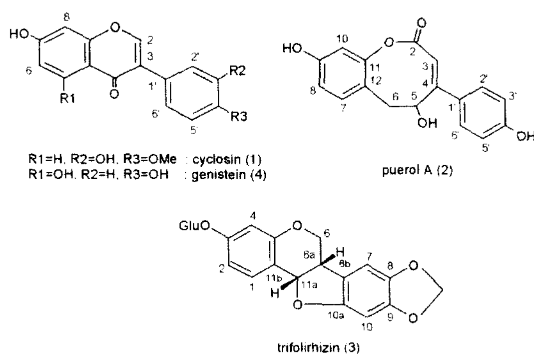

Fig. 1. Chemical structures of compounds isolated from the bark of *S. japonica.*

H-OCH_2O→C-8/C-9. H-1'→C-3, H-3'→C-2'/C-4', H-4'→C-3'/C-5'. NOESY correlations : H-1↔H-11a, H-1'↔H-2/H-4.

Compound 4. white amorphous powder. EI-MS *m/z* : 270(M^+, base ion), 243, 214, 154, 136, 118, 89. ^{1}H NMR (500 MHz, acetone-d_6) : δ 6.28 (1H, *d*, *J* = 2.1 Hz, H-6), 6.41 (1H, *d*, *J* = 2.1 Hz, H-8), 6.90 (2H, *dd*, *J* = 2.0, 8.8 Hz, H-3', 5'), 7.45 (2H, *dd*, *J* = 2.0, 8.8 Hz, H-2', 6'). 8.15 (1H, *s*, H-2), ^{13}C NMR (125 MHz, acetone-d_6) : δ 94.53 (*d*, C-8), 99.79 (*d*, C-6) 106.12 (*s*, C-10), 115.92 (*d*, C-3', 5'), 123.11 (*s*, C-1'), 124.08 (*s*, C-3), 131.19 (*d*, C-2', 6'), 154.25 (*d*, C-2), 158.36 (*s*, C-4') 159.08 (*s*, C-9), 163.65 (*s*, C-5), 165.04 (*d*, C-7), 181.57 (*s*, C-4). ^{1}H-^{1}H COSY correlations : H-8↔H-6, H-2'/6'↔H-3'/H-5. HMBC correlations : H-2→C-3/C-4/C-9, H-6→C-5/C-7/C-8/C-10, H-8→C-6/C-7/C-9/C-10, H-2'/6'→C-1'/C-2'/C-4'/C-6', H-3'/5'→C-1'/C-3'/C-4'/C-5'.

3. RESULTS and DISCUSSION

Compound 1 was isolated as amorphous solid. The EI-Mass afforded an [M^+] at *m/z* 284. The signals at δ 8.78 (1H, *s*, H-2) and at δ 154.36 (C-2) in its NMR spectra were suggestive of an isoflavonoid type skeleton (Yankep *et al.*, 2001). The ^{13}C NMR spectrum showed oxyolefinic methine carbon at δ 154.36 (C-2), olefinic quaternary carbon at δ 125.25 (C-3) and a carbonyl carbon at δ 178.15 (C-4). The ^{1}H NMR spectrum exhibited one methoxy group (δ 3.88, OMe), two *meta*-coupled aromatic protons (δ 6.68 and 6.83, H-8, H-6 and H-2'), two *ortho, meta*-coupled aromatic protons (δ 6.81 and 6.90, H-6 and H-6'), two *ortho*-coupled aromatic protons (δ 6.83 and 7.96, H-5 and H-5') and a non-coupled aromatic proton (δ 8.07, H-2). DEPT (45°, 90° and 135°) experiments showed the presence of one CH_3, seven CH and eight quaternary carbons. The NOESY spectrum showed cross peaks between OCH_3 δ_H 3.88 and H-2' δ_H 7.14, determined the position of methoxy group to be C-4' (Farag *et al.*, 2001). Consequently the structure of compound 1 was concluded to be 3', 7-dihydroxy-4'-methoxyisoflavone, cyclosin, the assignment of the ^{1}H, ^{13}C NMR spectrum was accomplished in this study. This compound did not found in the bark of *S. japonica* yet.

Compound 2 was isolated as a yellow powder and exhibited [M+] at *m/z* 298.0853 in the HR-EI-Mass spectrum, from which molecular formular could be postulated as $C_{17}H_{14}O_5$. The ^{1}H NMR spectrum showed signals for seven aromatic protons, one *ortho*-coupled proton (δ 7.29, H-7), one *meta*-coupled proton (δ 6.42, H-10) and two sets of *ortho-meta*-coupled aromatic protons (δ 6.84, H-2', 6' and δ 6.61, H-3', 5'). A total of 17 carbons appeared in the ^{13}C NMR spectrum which included one methylene, nine methine, and seven quaternary carbons. The ^{13}C NMR spectrum showed a carbonyl carbon at δ 175. 98 (C-2). A singlet at δ 6.11 (H-3) was coupled with C-3 (δ 111.23) in the HMQC (^{1}H Detected Single Qantum Coherence) spectrum and HMBC showed cross peaks between H-3 and C-2, C-4 and C-5, determined the assignment of H-3 and C-3. Consequently

the structure of compound 2 was concluded to be puerol A and the assignment of the ^{13}C NMR spectrum was accomplished in this study. The spectral data (^{1}H, ^{13}C NMR) of compound 2 were almost same as those of results of Shirataki *et al.* (1987) and Kinjo *et al.* (1985).

The compound 3 was isolated a yellow powder The EI-Mass afforded an [M^{+}] at *m/z* 284. The proton signal at δ 5.92 (2H, *dd*) and the carbon signal at δ 102.15 revealed the presence of a methylenedioxy group in compound 3. The position of this group on ring B was suggested to be between C-8 and C-9 owing to a signal in HMBC spectrum. The HMBC showed cross peaks between H-1' δ_H 4.95 and C-3 δ_C 159.93, determined the position of glucose to be C-3. According to Harbone (1989), the coupling constant of H-1'(anomeric) proton can indicate whether the glycosidic linkage of glucopyranose is α (7-8 Hz) or β (3-4 Hz). Since, the coupling constant of H-1' of this compound was 7.6 Hz, glycosidic linkage of compound 3 was β type. Consequently the structure of compound 3 was concluded to be maackiain-3-O-β-D-glucopyranoside, trifolirhizin. Compound 3 were found from *Cicer mongoltavicum* root (Harborne, 1994) and *S. flavescens*, but this is the first isolation from *S. japonica* bark.

Compound 4 was obtained as a white amorphous powder. The EI-MS presented a signal at *m/z* 270. The presence of a singlet at δ 8.15 (H-2) in the ^{1}H NMR spectrum and a carbon signal at δ 154.25 (C-2) suggested that it was an isoflavonoid (Talukdar *et al.*, 2000). The ^{1}H NMR spectrum exhibited one set of *meta*-coupled aromatic protons (δ 6.28, H-6 and 6.41, H-8), two sets of *ortho-meta*-coupled aromatic protons (δ 7.45, H-2', 6' and δ 6.90 H-3', 5'), and a non-coupled aromatic hydrogen (δ 8.15, H-2). These signals were assignable to H-6, H-8, H-2', 6', H-3', 5' and H-2 protons respectively of a 4', 5, 7-trihydroxyisoflavone skeleton. The corresponding carbons were identified by HMQC as seven methine carbon atoms at δ 99.79 (C-6), 94.53 (C-8), 131.19 (C-2', 6'), 115.92 (C-3', 5') and 154.25 (C-2), respectively. A carbon signal at δ 165.04 was assigned to C-7 based on its cross peaks ^{13}C-^{1}H correlations to both H-6 (δ 6.28) and H-8 (δ 6.41). In the ^{13}C NMR spectrum, C-3 at δ 124.08, C-4 at δ 181.57, C-5 at δ 163.65, C-9 at δ 159.08, C-10 at δ 106.12, C-1' at δ 123.11 and C-4' at δ 158.36 were assigned. These carbon chemical shifts were assigned by comparison with literature values (Lee *et al.*, 2001). From the above evidence, the structure of compound 4 was determined to be 4', 5, 7 -trihydroxyisoflavone, genistein.

4. CONCLUSIONS

From DCM fraction of the bark of *S. japonica*, four compounds were isolated by column chromatography using Sephadex LH-20 and/or silica gel and identified using EI-MS and ^{1}H and ^{13}C NMR spectroscopy as follows : 3', 7-dihydroxy-4'-methoxyisoflavone (cyclosin), puerol A, maackiain-3-O-β-D-glucopyranoside (trifolirhizin) and 4', 5, 7-trihydroxy isoflavone (genistein). This is the first report of the isolation and identification of cyclosin and trifolirhizin from *S. japonica*.

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