Cytotoxic Lavandulyl Flavanones from *Sophora flavescens*

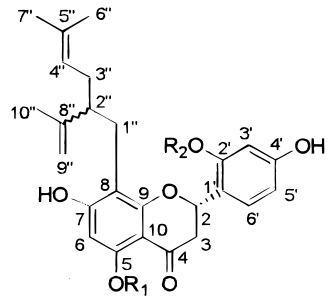
Tai-Hyun Kang,[†] Sei-Joon Jeong,[‡] Won-Gil Ko,[†] Na-Young Kim,[†] Byung-Hoon Lee,[†] Masanori Inagaki,[‡] Tomofumi Miyamoto,[‡] Ryuichi Higuchi,[‡] and Youn-Chul Kim*[,†]

College of Pharmacy, Wonkwang University, Iksan, 570-749, Korea, and Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Received November 8, 1999

Two new lavandulylated flavanones, (2*S*)-2′-methoxykurarinone (**1**) and (−)-kurarinone (**2**), were isolated from the root of *Sophora flavescens*, together with two known lavandulyl flavanones, sophoraflavanone G (**3**) and leachianone A (**4**), and two known isoflavonoids, formononetin and *l*-maakiain. The structures of **1** and **2** were determined on the basis of optical rotation and spectral evidence and by comparison with known compounds. Compounds **1**−**4** exhibited cytotoxic activity against human myeloid leukemia HL-60 cells.

The root of *Sophora flavescens* Aiton (Leguminosae) is a well-known Chinese herbal medicine used as a diuretic and for the treatment of diarrhea, gastrointestinal hemorrhage, and eczema.[1] Phytochemical studies of *S. flavescens* have reported the isolation of quinolizidine alkaloids, flavonoids, and triterpenoids.[2] In a study to find bioactive compounds from plants, the CH_2Cl_2 fraction of the MeOH extract of the root of *S. flavescens* showed significant cytotoxicity against human myeloid leukemia HL-60 cells. This active fraction was separated by column chromatography on Sephadex LH-20 and silica gel to afford two new lavandulyl flavanones, (2*S*)-2′-methoxykurarinone (**1**) and (−)-kurarinone (**2**). Two known lavandulyl flavanones, sophoraflavanone G (**3**)[3,4] and leachianone A (**4**),[3] and two known isoflavonoids, formononetin and *l*-maakiain, were also isolated. The structure characterization of **1** and **2** and the cytotoxic effects of **1**−**4** against HL-60 cells are reported in this paper.



1 $R_1 = R_2 = CH_3$
2 $R_1 = CH_3$ $R_2 = H$
3 $R_1 = R_2 = H$
4 $R_1 = H$ $R_2 = CH_3$

Compound **1** was obtained as a pale yellow powder, $[\alpha]^{23}_D$ −35.7° (*c* 1.3, MeOH). The HRFABMS of **1** indicated a molecular formula $C_{27}H_{32}O_6$. Its IR spectrum showed absorptions typical of hydroxyl and aromatic ring functionalities, and the UV spectrum was consistent with that of a flavanone.[5] The ^{1}H NMR spectrum of **1** showed the presence of lavandulyl group with signals at δ 1.46, 1.54, 1.63 (each 3H, s), 1.99 (2H, m), 2.53 (1H, m), 2.64 (2H, m), 4.53 (1H, br s), 4.57 (1H, br s), 4.96 (1H, br t, J = 6.7 Hz), and the protons at C-3 and C-2 in a flavanone skeleton as three one-proton double doublets at δ 2.58 (J = 2.5, 16.0 Hz), 2.81 (J = 13.2, 16.0 Hz), and 5.56 (J = 2.5, 13.2 Hz) together with two methoxyl groups at δ 3.72, 3.80. In the aromatic region of the ^{1}H NMR spectrum of **1**, a singlet appearing at δ 6.19 was assigned to the proton of a trisubstituted ring A, and one doublet proton at δ 7.42 (J = 8.0 Hz) and two overlapped protons at δ 6.51 (m) suggested that the B ring was oxygenated at C-2′ and C-4′.

The ^{13}C NMR assignments of **1** were confirmed by performing DEPT, ^{1}H−^{1}H, and ^{1}H−^{13}C correlation experiments. The chemical shift values of **1** were similar to those of kurarinone.[6] The point of attachment of the lavandulyl group and the positions of two methoxyl groups in the flavanone skeleton were established unambiguously as lavandulyl (C-8) and methoxyl (C-5 and C-2′) by the ^{1}H−^{13}C long-range coupling experiment (HMBC). The absolute configuration at C-2 was determined as *S* by CD analysis, which showed a positive Cotton effect at 336 and 291 nm.[7] Therefore, **1** was characterized as (2*S*)-2′-methoxykurarinone.

The ^{1}H and ^{13}C NMR data of **2** showed the presence of one lavandulyl and one methoxyl group in the flavanone skeleton. Comparison of the spectral data of **2** with those of kurarinone[6] revealed these constituents to be identical. The optical rotation of **2** was −60.5° (*c* 0.66, MeOH); however, that of kurarinone had been reported as +12.0° (*c* 0.1, MeOH)[6] and +25.5° (*c* 1.0, EtOH).[8] From the CD experiment, the absolute configuration at C-2 was determined to be *S*. There is no report for the absolute configuration at C-2″ of kurarinone, and the difference of optical rotation between **2** and reported values suggests **2** most probably to be (−)-kurarinone.

The cytotoxicities of the six isolated compounds were tested against human myeloid leukemia HL-60 cells. Compounds **1**−**4** showed significant cytotoxicities with IC_{50} values of 13.7, 18.5, 12.5, and 11.3 μM, respectively. Formononetin and *l*-maackiain were inactive. Cisplatin, as a positive control, showed an IC_{50} value of 2.3 μM.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO

* To whom correspondence should be addressed. Tel.: +82-653-850-6823, Fax: +82-653-852-8837. E-mail: yckim@wonnms.wonkwang.ac.kr.
[†] Wonkwang University, Korea.
[‡] Kyushu University, Japan.

10.1021/np990567x CCC: $19.00
Published on Web 03/29/2000

DIP-370 digital polarimeter. UV spectra were recorded on a JASCO U-best30 spectrophotometer. CD spectra were recorded on a JASCO J-720W spectropolarimeter. IR spectra were recorded on a JASCO FT/IR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 500 (500 MHz) spectrometer. FABMS and HRFABMS were recorded on a JEOL SX-102 spectrometer.

Plant Material. Roots of *S. flavescens* were collected at the Botanical Garden of Wonkwang University, Iksan, Korea, in September 1998. A voucher specimen (No. WP 030) is deposited in the Herbarium of College of Pharmacy, Wonkwang University, Korea.

Extraction and Isolation. Dried roots of *S. flavescens* (3 kg) were extracted with MeOH three times at room temperature. The MeOH extract (200 g) was partitioned between hexane and 60% aqueous MeOH and then between CH_2Cl_2 and 60% aqueous MeOH. The bioactive CH_2Cl_2 extract (112 g; IC_{50}, 57.0 μg/mL) was subjected to gel permeation chromatography on Sephadex LH-20 eluting with CH_2Cl_2/MeOH (10:1) and MeOH to give five fractions (A1−A5). Fraction A4 (32.5 g) was further subjected to Sephadex LH-20 column chromatography (CC) (eluent: CH_2Cl_2/MeOH, 20:1) to give three fractions (B1−B3). Fraction B2 (8.9 g) was chromatographed on Sephadex LH-20 column (eluent: CH_2Cl_2/MeOH, 50:1 and 20:1) to give three fractions (C1−C3). Fraction C1 (730 mg) was purified by silica gel CC and eluted with hexane/EtOAc (5:1) to afford (−)-kurarinone (**2**, 50.3 mg). Fraction C2 (4.1 g) was also purified by silica gel CC and eluted with hexane/EtOAc (4:1) to yield sophoraflavone G (**3**, 136.0 mg). Fraction B1 (4.8 g) was subjected to Sephadex LH-20 CC (eluent: CH_2Cl_2/MeOH, 25:1) to give four fractions (D1−D4). Fraction D2 (1.1 g) was purified by silica gel CC and eluted with hexane/EtOAc (5:1) to afford *l*-maakiain (127.9 mg) and formononetin (6.0 mg). Fraction D3 (980 mg) was purified by silica gel CC and eluted with hexane/EtOAc (4:1) to obtain (2*S*)-2′-methoxykurarinone (**1**, 140.3 mg) and leachianone A (**4**, 112.7 mg). The structures of sophoraflavone G (**3**),[3,4] leachianone A (**4**),[3] *l*-maakiain,[4] and formononetin[9] were identified by comparison of their spectral data (mp, UV, ^{1}H and ^{13}C NMR) with those reported in the literature.

(2*S*)-2′-Methoxykurarinone (1): pale yellow powder; mp 102−104 °C; $[\alpha]^{23}_D$ −35.7° (*c* 1.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 286 (4.36) nm; CD (*c* 7.4 × 10^{-5}, MeOH) $[\theta]_{336}$ +5.99, $[\theta]_{291}$ −15.36; IR (KBr) ν_{max} 3291, 2961, 2922, 1641, 1596, 1498, 1464, 1413, 1283 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.42 (1H, d, J = 8.0 Hz, H-6′), 6.51 (2H, m, H-3′, H-5′), 6.19 (1H, s, H-6), 5.56 (1H, dd, J = 2.5, 13.2 Hz, H-2), 4.96 (1H, br t, J = 6.7 Hz, H-4″), 4.57 (1H, br s, H-9″a), 4.53 (1H, br s, H-9″b), 3.80 (3H, s, 2′-OCH_3), 3.72 (3H, s, 5-OCH_3), 2.81 (1H, dd, J = 13.2, 16.0 Hz, H-3α), 2.64 (2H, m, H-1″), 2.58 (1H, dd, J = 2.5, 16.0 Hz, H-3β), 2.53 (1H, m, H-2″), 1.99 (2H, m, H-3″), 1.63 (3H, s, H-10″), 1.54 (3H, s, H-7″), 1.46 (3H, s, H-6″); ^{13}C NMR (acetone-d_6, 125 MHz) δ 189.5 (s, C-4), 163.8 (s, C-9), 162.6 (s, C-7), 161.2 (s, C-5), 159.6 (s, C-4′), 158.4 (s, C-2′), 149.2 (s, C-8″), 131.5 (s, C-5″), 128.1 (d, C-6′), 124.5 (d, C-4″), 119.7 (s, C-1′), 111.1 (t, C-9″), 108.5 (s, C-8), 107.8 (d, C-5′), 106.1 (s, C-10), 99.6 (d, C-3′), 93.5 (d, C-6), 74.7 (d, C-2), 55.7 (q, 2′, 5-OCH_3), 47.7 (d, C-2″), 45.6 (t, C-3), 31.9 (t, C-3″), 28.0 (t, C-1″), 25.8 (q, C-6″), 19.1 (q, C-10″), 17.8 (q, C-7″); FABMS *m*/z 453 $[M + H]^+$ (92), 423 (25), 329 (55), 307 (29), 289 (14), 233 (8), 179 (84), 154 (100); HRFABMS *m*/*z* 453.2307 ($[M + H]^+$, calcd for $C_{27}H_{33}O_6$, 453.2277).

(−)-Kurarinone (2): colorless powder; mp 115−117 °C; $[\alpha]^{23}_D$ −60.5° (*c* 0.66, MeOH); UV (MeOH) λ_{max} (log ϵ) 288 (4.58) nm; CD (*c* 3.8 × 10^{-5}, MeOH) $[\theta]_{337}$ +5.63, $[\theta]_{291}$ −15.78; IR (KBr) ν_{max} 3309, 2966, 1599, 1497, 1464, 1414, 1283 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.38 (1H, d, J = 8.0 Hz, H-6′), 6.46 (1H, d, J = 2.4 Hz, H-3′), 6.43 (1H, dd, J = 2.4, 8.0 Hz, H-5′), 6.19 (1H, s, H-6), 5.60 (1H, dd, J = 2.5, 13.2 Hz, H-2), 4.97 (1H, br t, J = 1.5 Hz, H-4″), 4.57 (1H, br s, H-9″a), 4.54 (1H, br s, H-9″b), 3.72 (3H, s, 5-OCH_3), 2.83 (1H, dd, J = 13.2, 16.5 Hz, H-3), 2.66 (2H, m, H-1″), 2.63 (1H, m, H-2″), 2.55 (1H, dd, J = 2.5, 16.5 Hz, H-3), 1.99 (2H, m, H-3″), 1.63 (3H, s, H-10″), 1.55 (3H, s, H-7″), 1.46 (3H, s, H-6″); ^{13}C NMR (acetone-d_6, 125 MHz) δ 189.6 (s, C-4), 163.8 (s, C-7), 162.7 (s, C-5), 161.2 (s, C-9), 159.1 (s, C-4′), 156.0 (s, C-2′), 149.2 (s, C-8″), 131.5 (s, C-5″), 128.4 (d, C-6′), 124.5 (d, C-4″), 118.3 (s, C-1′), 111.1 (t, C-9″), 108.5 (s, C-8), 107.7 (d, C-5′), 106.1 (s, C-10), 103.3 (d, C-3′), 93.5 (d, C-6), 75.0 (d, C-2), 55.7 (q, 5-OCH_3), 47.7 (d, C-2″), 45.7 (t, C-3), 31.9 (t, C-3″), 28.0 (t, C-1″), 25.8 (q, C-6″), 19.1 (q, C-10″), 17.8 (q, C-7″); FABMS *m*/*z* 439 $[M + H]^+$ (100), 391 (4), 315 (49), 297 (29), 179 (68), 154 (47), 136 (33); HRFABMS *m*/*z* 439.2160 ($[M + H]^+$, calcd for $C_{27}H_{33}O_6$, 439.2121).

Cytotoxicity Assay. Human myeloid leukemia HL-60 cells were seeded at 1 × 10^4 cells/well in a 96-well plate and incubated with test samples at doses of 0−50 μM for 96 h, and the viability was determined by MTT assay.[10]

Acknowledgment. This work was supported by the grants from the Medicinal Resources Research Center of Wonkwang University supported by the Chonbuk Principal Government and the Korea Science and Engineering Foundation (1999).

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NP990567X