Compounds from the Aerial Part of *Saururus chinensis* and Their Cytotoxic Activity

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Abstract – Ten known compounds, 7-hydroxysauchinone (1), sauchinone (2), di-O-methyltetrahydrofuriguaiacin B (3), hencrine (4), saucerneol K (5), meso-dihydroguaiaretic acid (6), (−)-guaiacin (7), (3R,4S)-4-(4-hydroxy-3-methoxyphenyl)-7-methybut-8-en-9-one (9), and licarin A (10), were isolated from aerial part of *Saururus chinensis*. The chemical structures of these compounds were determined on the basis of spectroscopic analyses including 2D NMR. Compounds 1 - 10 were evaluated for their cytotoxic activity against the HL-60, MCF-7, and A549 cancer cell lines in *in vitro*.

Keywords – *Saururus chinensis*, Saururaceae, Lignan, Cytotoxic activity

Introduction

*Saururus chinensis* Baill., a perennial herb, belongs to the family Saururaceae, grows throughout East Asia such as China and Korea. It has been used as a medicinal herb to remedy various diseases such as edema, gonorrhea, jaundice, and inflammatory diseases for a long period of time in China and Korea (Chung et al., 1990). This plant has been investigated extensively, resulting in the isolation of various lignans (sauchinone, saucerneol, manassantin A, and manassantin B), flavonoids (rutin, hyperoside, quercitrin and quercetin) and alkaloid (Kim et al., 2009; Sung, 2006; Kim et al., 2004). Previous biological studies of this plant have shown antiasthmatic, antioxidant, and anti-inflammatory (Wang et al., 2008), cytotoxic (Park et al., 1997), vasorelaxant and inotropic (Ryu et al., 2008), hypolipidemic (Yu et al., 2008; Yun et al., 2007), hypoglycemic (He et al., 1992; Joo et al., 2006), analgesic (Park et al., 1998), neuroprotective (Wie, 2000), antihypertensive (Wang et al., 2008), and hepatoprotective effects (Sung and Kim, 2000; Sung et al., 1997, Sung et al., 2000). *S. chinensis* was also reported to contain compounds such as diterpenes, tannins, steroids, and lipids (Wang et al., 2008). Lignans (Sung and Kim, 2000; Sung et al., 2000) and flavonol glycosides (Sung et al., 1999) from *S. chinensis* were reported to have hepatoprotective effects in *in vitro*. Sauchinone, one of the active compounds from this plant, has been reported to inhibit bone destruction and to decrease mortality rate induced by LPS (Han et al., 2007; Seo et al., 2008). Furthermore, sauchinone attenuated LPS-induced TNF-α, inducible NO synthase and cyclooxygenase-2 gene expression in macrophages stimulated with LPS through the suppression of nuclear translocation of NF-κB (Lee et al., 2003; Hwang et al., 2003-1). In our continuing investigation to discover cytotoxic compounds, further fractionation of the EtOAc-soluble fraction resulted in the isolation of ten compounds (1 - 10). This study describes the isolation, structural elucidation of isolated compounds and evaluation their cytotoxic activity against various cancer cell lines.

Experimental

General experimental procedures – Optical rotations were measured with a JASCO DIP 370 digital polarimeter. UV spectra were taken in MeOH using a Thermo spectrometer, and IR spectra were obtained on a JASCO FT/IR – 4100 spectrometer. The nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Inova 400 MHz spectrometer. EI-MS spectrometric data were acquired with a JMS-700 MSTATION mass spectrometer (JEOL, Japan). Silica gel (Merck, 63 - 200
μm particle size) and RP-18 (Merck, 75 μm particle size) were used for column chromatography. TLC was carried out using Merck silica gel 60 F_{254} and RP-18 F_{344} plates. HPLC was performed using a Waters 600 Controller system with a UV 486 Tunable Absorbance Detector, and an YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size, YMC Co., Ltd., Japan) and HPLC solvents were from Burdick & Jackson, USA.

**Plant material** – The dried aerial part of *S. chinensis* was purchased from a local folk medicine market named “Yak-yeong-si” in Daegu, Korea, in May 2010. Botanical identification was performed by Prof. Byung-Sun Min, and the voucher specimen CUD-1384 was deposited at the herbarium of the College of Pharmacy, Catholic University of Daegu, Korea.

**Extraction and isolation** – The dried aerial part of *S. chinensis* (12 kg) was extracted three times with MeOH at room temperature for seven days and then MeOH extract (1.5 kg) was suspended in hot-water (4 L) and partitioned with *n*-hexane (4 L x 3), ethyl acetate (4 L x 3), and *n*-butanol (4 L x 3), successively. The resulting fractions were concentrated in vacuo to give the hexane-soluble fraction (400.8 g), ethyl acetate-soluble fraction (584.93 g), and *n*-BuOH-soluble fraction (314.2 g), respectively. By the activity-guided fractionation, the ethyl acetate-soluble fraction was chromatographed on a silica gel column chromatography eluting with a gradient of CHCl₃-MeOH (50:1 → 5:1) to afford fifteen fractions (Fr. 1–15). Fraction 7 (80 g) was subjected on a silica gel column chromatography eluting with a gradient of *n*-hexane-acetone (5:1 → 0:1) to afford three subfractions (Fr. 7-1–7-3). Subfraction 7-1 (20 g) was subjected on a silica gel column chromatography eluting with a gradient of *n*-hexane-EtOAc (10:1 → 1:1) to afford compounds 2 (120.0 mg), 3 (32.8 mg), and 4 (25.0 mg). Subfraction 7-2 (25 g) was subjected to silica gel column chromatography with a gradient of *n*-hexane-EtOAc (5:1 → 1:1) to afford seven subfractions (Fr.7-2-1→7-2-7). Subfraction 7-2-5 (450 mg) was chromatographed over a RP-18 gel eluting with a gradient of MeOH-H₂O (3:1 → 6:1) to afford compounds 6 (34.4 mg), 7 (17.0 mg), 8 (12.3 mg), and 9 (8.0 mg). Subfraction 7-2-2 was chromatographed by MPLC on a ODS column using MeOH-H₂O (3:1) and purified by preparative HPLC on a RP-18 column using MeOH-H₂O (72:28 → 74:26) to yield compound 10 (8.0 mg). Subfraction 7-2-6 was chromatographed by MPLC on a ODS column using MeOH-H₂O (3:1) followed by recrystallization to yield compounds 1 (10.9 mg) and 5 (7.2 mg), respectively.

**7-HydroxySauchinone (1)** – White amorphous powder; [α]D²⁵ + 9.5 (c 0.37, CHCl₃); UV (CHCl₃) λₓₓₓₙ nm: 244, 297; IR (KBr) νₚₛₛ c⁻¹: 3695, 3019, 1214, 1054, 748; ¹H-NMR (400 MHz, CDCl₃) δ: 0.60 (3H, d, J = 7.6 Hz, H-9'), 1.25 (3H, d, J = 7.2 Hz, H-9), 1.74 (1H, m, H-7α), 1.84 (1H, m, H-7β), 2.06 (1H, m, H-8'), 2.50 (1H, m, H-8), 2.55 (1H, m, H-1'), 2.60 (1H, d, J = 13.2 Hz, H-6), 5.56 (1H, s, H-3'), 6.43 (1H, s, H-3), 7.0 (1H, s, H-6), 5.97 and 5.94 (each 1H, d, J = 1.2 Hz, 4',5'-OCH₃-O-), 5.68 and 5.67 (each 1H, s, 4,5-OCH₃-O-); ¹³C-NMR (100 MHz, CDCl₃) δ: 16.8 (C-9), 20.5 (C-9'), 24.4 (C-7), 34.9 (C-8), 40.2 (C-7), 44.7 (C-6), 68.9 (C-7), 98.3 (C-3), 100.7 (C-3'), 109.9 (C-4'), 110.6 (C-6), 119.1 (C-1), 143.8 (C-4), 144.4 (C-2), 148.7 (C-15), 168.2 (C-4'), 198.6 (C-2'). 101.8 (4',5-OCH₃-O-), 98.7 (4',5-OCH₃-O-); HR-EL-MS (rel. int.) m/z: 372.1209 [M⁺] (100), 373.1247 (22) (calcd. for C₂₉H₂₃O₇; 372.1209).

**Sauchinone (2)** – White amorphous powder; [α]D²⁵ +85.0 (c 0.1, CHCl₃); UV (CHCl₃) λₓₓₓₙ nm: 244, 300; IR νₚₛₛ c⁻¹: 3020, 1214, 748; ¹H-NMR (400 MHz, CDCl₃) δ: 0.74 (3H, d, J = 7.6 Hz, H-9'), 1.21 (3H, d, J = 7.6 Hz, H-9), 1.67 (1H, m, H-7β), 1.94 (1H, m, H-7α), 1.91 (1H, m, H-8'), 2.45 (1H, m, H-8), 2.50 (1H, m, H-1'), 2.56 (1H, dd, J = 2.8, 12 Hz, H-6), 3.05 (1H, d, J = 4.8 Hz, H-7), 5.52 (1H, s, H-3'), 6.40 (1H, s, H-3), 6.85 (1H, s, H-6), 5.62 and 5.68 (each 1H, s, 4',5'-OCH₃-O-), 5.89 and 5.93 (each 1H, s, 4,5-OCH₃-O-); ¹³C-NMR (100 MHz, CDCl₃) δ: 20.9 (C-9), 21.4 (C-9'), 25.3 (C-7), 33.6 (C-8'), 34.9 (C-7), 35.1 (C-8), 35.2 (C-6), 37.6 (C-1'), 98.7 (4',5'-OCH₃-O-), 99.3 (C-3), 100.3 (C-3'), 100.5 (C-3'), 101.4 (4',5'-OCH₃-O-), 106.6 (C-6), 115.8 (C-1), 143.3 (C-4'), 145.1 (C-2'), 146.8 (C-5), 168.7 (C-4'), 199.7 (C-2'); EI-MS (rel. int.) m/z: 356 [M⁺] (100), 270 (13), 257 (15), 205 (20), 175 (25) (calcd. for C₂₉H₂₃O₇).

**Di-O-methyltetrahydrofuroguaianile B (3)** – White amorphous powder; [α]D²⁵ +28.0 (c 0.06, MeOH); UV (EtOH) λₓₓₓₙ nm: 241, 281; IR νₚₛₛ c⁻¹: 2980, 1214, 748; ¹H-NMR (400 MHz, CDCl₃) δ: 1.05 (6H, d, J = 5.6 Hz, H-7, H-7'), 2.33 (2H, m, H-8,8'), 4.52 (2H, dd, J = 5.6 Hz, H-7,7'), 6.87 (2H, d, J = 8.0 Hz, H-5'), 6.98 (2H, dd, J = 1.6, 8.0 Hz, H-6), 7.0 (2H, d, J = 1.6 Hz H-2,2'), 3.88-3.89 (12H, s, 3, 3', 4, 4'-OCH₃); ¹³C-NMR (100 MHz, CDCl₃) δ: 13.2 (C-9,9'), 44.6 (C-8,8'), 87.4 (C-7,7'), 109.9 (C-2,2'), 111.1 (C-5,5'), 118.8 (C-6,6), 135.0 (C-1'), 148.6 (C-4,4'), 149.1 (C-3,3'), 56.1 (3', 3',4,4'-OCH₃); EI-MS (rel. int.) m/z: 372 [M⁺] (25), 206 (100), 191 (75), 175 (70) (calcd. for C₃₉H₂₃O₇)

**Henricine (4)** – Yellow oil; [α]D²⁵ +6.0 (c 0.23, CHCl₃); UV (CHCl₃) λₓₓₓₙ nm: 242, 279; IR (KBr) νₚₛₛ c⁻¹: 3696, 3019, 1214, 1054, 748; ¹H-NMR (400 MHz, CDCl₃) δ: 1.03 (3H, d, J = 6.8 Hz, H-9'), 1.05 (3H, d, J = 7.6 Hz,