Cytotoxic Sesquilignans from the Roots of *Saururus chinensis*

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*Saururus chinensis* Hort. ex Loudon (Saururaceae) is a perennial herb distributed in China and Korea, and has been used as a folk medicine for the treatment of edema, gonorrhea, jaundice, pneumonia, and several inflammatory diseases in Korea.\(^3\) Previous studies of *S. chinensis* reported the occurrence of lignans,\(^4\) aristolactams,\(^5\) flavonoids,\(^6\) and furanoditerpenes,\(^6\) and a wide range of biological activities including antioxidant activity,\(^1\) hepatoprotective activity,\(^2\) cytotoxic activity,\(^13\) anti-inflammatory activity,\(^13\) anti-atherogenic activity,\(^21\) and immunosuppressive activity.\(^25\) This paper reports the structures elucidation of the two new lignans and six known compounds, along with their cytotoxicity.

Compound 1 was obtained as a colorless powder and its molecular formula was determined to be C_{35}H_{33}O_{8}s, based on the [M-H] peak at m/z 537.2471 (calcd 537.2488) in the HRESIMS. The \(^1^H\)-NMR spectroscopic data of compound 1 indicated the presence of a tetrahydrofuran-type lignan unit, as judged from the signals for two methines at \(\delta_{HH} 2.34\) (H-8 and H-8'), for oxymethines at \(\delta_{HH} 5.65\) (H-7 and H-7'), and for methyl groups at \(\delta_{HH} 0.78\) (H-9) and 0.74 (H-9'), as well as the signals at \(\delta_{HH} 7.20\) (d, \(J = 1.7\) Hz, H-2), 7.30 (\(J = 8.2\) Hz, H-5), 7.36 (br d, \(J = 8.2\) Hz, H-6), 7.19 (d, \(J = 1.7\) Hz, H-2'), 7.34 (d, \(J = 8.2\) Hz, H-5'), and 7.09 (dd, \(J = 8.2, 1.7\) Hz, H-6') corresponding to two 1,3,4-trisubstituted benzene rings.\(^25\) An additional phenylpropanoid unit was observed in compound 1 from the proton signals for two oxymethines at \(\delta_{HH} 4.95\) (H-8''), and 5.42 (H-7''), for a methyl at \(\delta_{HH} 1.60\) (H-9'') and for a 1,3,4-trisubstituted benzene ring at \(\delta_{HH} 7.58\) (d, \(J = 1.6\) Hz, H-2''), 7.01 (d, \(J = 8.2\) Hz, H-5''), and 7.07 (dd, \(J = 8.2, 1.6\) Hz, H-6'').\(^4\) The location of the phenylpropanoid group was confirmed by the HMBC correlation between H-8'' (\(\delta_{HH} 4.95\)) and C-4'' (\(\delta_c 147.5\)), suggesting this phenylpropanoid is attached to C-4' on the tetrahydrofuran-type lignan moiety through an ether linkage. The relative stereochemistry of the tetrahydrofuran ring in compound 1 was established by the observed NOE correlations of H-9 with H-8', H-2, and H-6 as well as H-9' with H-8, H-2', and H-6', indicating the 7,8-cis-8',8'-trans-7',8'-cis configuration.\(^25\) In addition, the chemical shifts of C-9'' (\(\delta_c 15.3\)), C-7'' (\(\delta_c 175.7\)), and C-8'' (\(\delta_c 81.1\)), and the coupling constant (\(J = 3.4\) Hz) of H-7'' was supportive of the relative configuration of C-7'' and C-8'' as being erythro.\(^25\) Furthermore, the positive Cotton effect at 231 nm enabled to assign the configuration of C-7'' and 8'' as R and S, respectively.\(^27\) Thus, the structure of compound 1 was determined as 7''R,8''S-saucerone, a diastereomer of (-)-saucerone (3).

Saucerneol 2 had a molecular formula (C_{36}H_{34}O_{9}s) and exhibited a close resemblance to compound 1 in their \(^1^H\) and \(^1^C\) NMR spectroscopic data except for the presence of three methoxy groups. There were differences in the chemical shifts and coupling constants of H-7 in compounds 2 (\(\delta_{HH} 4.54, J = 9.3\) Hz) and 1 (\(\delta_{HH} 5.65, J = 6.4\) Hz), indicating the opposite configuration at C-7'' position in compound 2 compared to compound 1.

![Figure 1. Structures of compounds 1-2.](image1)

![Figure 2. Key \(^1^H\)-H COSY (——) and HMBC (H —— C) and NOESY (——) correlations of compound 1.](image2)
The CD spectroscopic data exhibited the positive Cotton effect at 232 nm in the same manner. Therefore, the structure of compound 2 was confirmed to be 7-epi-7'R,8'R,4-'s-demethylsaucerneol.

The known compounds were in good agreement with previously reported NMR data and were consequently identified as (-)-saucerneol (3) with a \( [\alpha]_0 \) value of -71.1 (c 0.1 MeOH) [Lit. \( [\alpha]_0 \) -91.8], 25 \( \alpha, \beta, \gamma, \delta \)-erythro-manassantin A (4), 26 4-O-demethylmanassantin B (5), 27 4'-erythro-4'-manassantin A (6), 28 manassantin B (7) 29 and manassantin A (8). 30

All the compounds isolated were evaluated against HL-60 (human promyelocytic leukemia) cells. Compounds 1-8 exhibited cytotoxicity (IC_{50}; 0.5, 7.1, 3.3, 5.2, 3.6, 2.3, 8.5 and 0.8 \( \mu \text{M} \), respectively) against HL-60 cell lines (camptothecin, IC_{50} 0.8 \( \mu \text{M} \)).

**Experimental Section**

**General experimental procedures.** Melting points were determined on a Kofler micro-hotstage (uncorrected). Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-1601 UV-visible. CD spectroscopic data were obtained from JASCO-720 CD spectrometer. The NMR spectra were recorded on a Varian Unity 400 FT-NMR spectrometers with the tetramethylsilane as an internal standard. Chemical shifts are presented in ppm. HRESIMS were measured on a Waters Q-Tof Premier mass spectrometer. Column chromatography (CC) was performed on silica gel (70 - 230 and 230 - 400 mesh, Merck), reverse-phase C18 gel (40 \( \mu \text{m} \), Nacalai Inc., Japan). Thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} (Merck) or RP-18 F_{254} (Merck) plates. Spots were visualized by spraying 10% aqueous H$_2$SO$_4$ solution on the plates and heating them for 5 min.

**Plant material.** The roots of *Saururus chinensis* was collected at Jeju (Korea) in July 2008 and dried at room temperature. A voucher specimen (00250) is deposited at the Plant Extract Bank, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea.

**Extraction and isolation.** The dried roots of *S. chinensis*
(6.5 kg) was extracted with MeOH at room temperature (3 × 20 L) to obtain 0.65 mg of the solid extract. The MeOH extract was suspended in H2O and extracted with EtOAc (3 × 3 L) to give the EtOAc-soluble fractions (85 g). The EtOAc-soluble fraction (84 g) was chromatographed on a silica gel column eluted with a stepwise gradient of hexane and EtOAc to yield 14 fractions (fr. SC1-SC14). Fr. SC12 (1.2 g) was chromatographed on a RP C-18 column (MeOH/H2O, 7:3) to yield 19 sub-fractions (fr. SC12-1-SC12-19). Fr. SC12-15 (0.14 g) was chromatographed on a RP C-18 column (MeOH/H2O, 2:1) to give compounds 1 (13.2 mg) and 3 (98.1 mg). Fr. SC12-19 (0.103 g) was chromatographed on a RP C-18 column (MeOH/H2O, 2:1) to give compound 4 (17.8 mg). Fr. SC14 (0.88 g) was chromatographed on a RPC-18 column (MeOH/H2O, 8:2) to yield seven sub-fractions (Fr. SC14-1-SC14-7). Fr. SC14-3 (0.12 g) was chromatographed on a RP C-18 column (MeOH/H2O, 3:2) to give compound 2 (5.3 mg). Fr. SC14-6 (0.36 g) was chromatographed on a RP C-18 column (MeOH/H2O, 3:2) to give compounds 5 (76.7 mg), 6 (5.3 mg), 7 (18.3 mg), and 8 (43.3 mg).

eythro-Saucerone (1): Colorless powder, mp 85 - 86 °C. [α]D
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18
(0.1, MeOH). UV λmax (MeOH) nm (log e): 206 (3.74), 284 (2.94). 1H- and 13C-NMR data see Tables 1, HRESIMS m/z 537.2471 [M-H]− (Calcd for C31H37O8: 537.2488). CD (c 0.0004 MeOH): [θ]211 +12.075.

Saucerone (2): Colorless powder, mp 75 - 76 °C. [α]D
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(0.1, MeOH). UV λmax (MeOH) nm (log e): 206 (3.81), 282 (2.67). 1H- and 13C-NMR data see Tables 1 and 2, HRESIMS m/z 523.2310 [M-H]− (Calcd for C29H35O8: 523.2332). CD (c 0.0006 MeOH): [θ]222 +18.121.

Cytotoxicity evaluation. All the isolates were assessed with the HL-60 (human promyelocytic leukemia) cells according to the established protocol.29

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References and Notes