Two New Lignans from the Roots of *Saururus chinensis*

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Saururus chinensis (Saururaceae) is a perennial herbaceous plant that has been used in the treatment of various diseases such as edema, jaundice, gonorrhea, fever, and inflammation in Korean folk medicine.[1] Chemical studies of the genus *Saururus* have shown the presence of lignans,[2-6] aristolactams, flavonoids, anthraquinones, and fruanoditerpenes,[7-10] some of which exhibited neuroleptic,[11] hepatoprotective,[12] antifeedant[13] and antioxidant activities.[14] Previously, we reported the isolation of protective agents against sepsis in the animal model from this plant.[15]

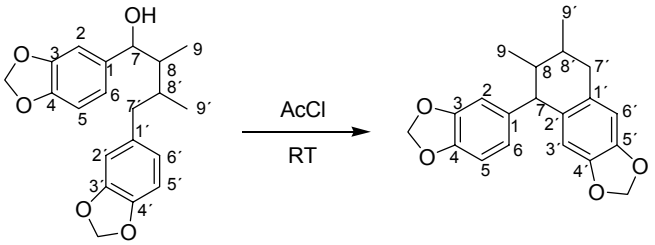
Two lignans (**1** and **2**) were isolated from the EtOAc fraction of the roots of *S. chinensis* by repetitive column chromatography. Compound **1** was obtained as yellow oil. It showed the value of $[a]_D^{20}$ -65.7° (*c* 1.806, $CHCl_3$). A molecular formula of **1** was found to be $C_{20}H_{22}O_5$ by HRFABMS (*m/z*; found 343.1538 $[M+H]^+$; calcd. 343.1545). The UV spectra of **1** revealed the presence of phenolic groups (234 and 287 nm). The ^{1}H-NMR spectrum showed the presence of two methyl doublets (H-9 and H-9′), two methine groups (H-8′ and H-8), one benzylic methylene (H-7′), one benzylic methine group substituted by oxygen (H-7) and two 3,4-methylenedioxyphenyl groups. The 2D ^{1}H-^{1}H COSY spectrum indicated coupling between the oxygenated methine at δ 4.25 (H-7) and the methine signal at δ 1.75 (H-8), which was also coupled to the methyl signal at δ 0.54 (H-9). On the other hand, the methine signal at δ 2.39 (H-8′) was coupled to the methyl signal at δ 0.81 (H-9′). These observations are consistent with a lignan-7-olic skeleton. The positions of each functional group were determined by a HMBC experiment. One set of correlations was observed between the oxygenated methine H-7 (δ 4.25) and C-1, C-2, C-6, C-8, C-8′ and C-9 and the other set was between H-7′ (δ 2.46) and C-1′, C-2′, C-6′, C-8, C-8′ and C-9′. Additionally, the correlations between two methylenedioxy signal at δ 5.88 and 5.89 and C-3′/C-3 and C-4′/C-4 supported the proposed link the 3,4,3′,4′-dimethylenedioxy phenyl moiety. The absolute stereochemistry at C-7 of **1** was established by modified Mosher's method.[16-18] The differences of chemical shift values obtained by subtracting (*R*)-MPTA ester from (*S*)-MTPA ester [$\Delta\delta_H$ (δ_S - δ_R)] are shown in Table 1, and the negative values of $\Delta\delta_H$ (δ_S - δ_R) at H-9, H-7′, H-8′ and H-9′ suggested a *S* configuration at C-7 in compound **1**. To determine the configurations at C-8 and C-8′, **1** was converted to an aryl-tetralin type compound (**1a**) with acetyl chloride by the previously reported reaction, in which inversion of the stereochemistry at C-7 of **3** to that of **3a** was shown.[19] The observed spin coupling constants, $J_{7,8}$ = 10.2 Hz, $J_{8,8'}$ = 10.5 Hz and $J_{8',7'ax}$ = 11.3 Hz for **1a** confirmed the all-*trans* stereochemistry with two methyl groups and the pendant phenyl substituent all *pseudo*-equatorial positions (Figure 2).[6] Based on this evidence, **1** was determined to be (7*S*,8*R*,8′*S*)-3,4,3′,4′-dimethylenedioxylignan-7-ol and named as saucerneol J.

Table 1. Characteristic ^{1}H-NMR data of Mosher esters of **1** for determination of stereochemistry

Position	7	9	7′	8′	9′
1$_S$ (δ_S)	5.58	0.53	2.32	1.77	0.68
1$_R$ (δ_S)	5.52	0.57	2.42	2.09	0.78
$\Delta\delta$ (δ_S - δ_R)	*S*	-0.04	-0.10	-0.32	-0.10

[a]These authors contributed equally to this work.

Compound **2** was obtained as an colorless oil, with a molecular formula of $C_{20}H_{20}O_6$ determined by HREIMS (*m/z*; found 356.1258 $[M]^+$; calcd. 356.1260). The UV spectra of **2** revealed the presence of phenolic groups (244 and 291 nm). It showed the value of $[a]_D^{22}$ +21.9° (*c* 0.08, $CHCl_3$). The ^{1}H-NMR spectrum showed the presence of two methyl doublets (H-9 and H-9′), two methine groups (H-8′ and H-8), two benzylic methine groups substituted by oxygen (H-7′ and H-7), two dioxymethylene groups and five aromatic protons (H-2, H-5, H-6, H-3′ and H-6′). The positions of each



1 7*S*, 8*R*, 8′*S*
3 7*R*, 8*S*, 8′*R*

1a 7*R*, 8*R*, 8′*S*
3a 7*S*, 8*S*, 8′*R*

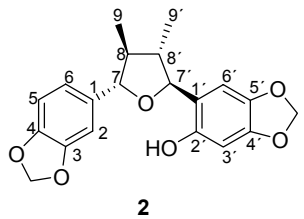


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Figure 1. Chemical structures of compounds **1-3**

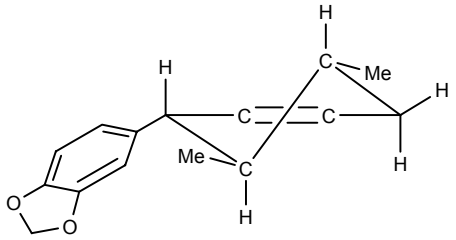

Figure 2. Partial structure of compound **1a**.

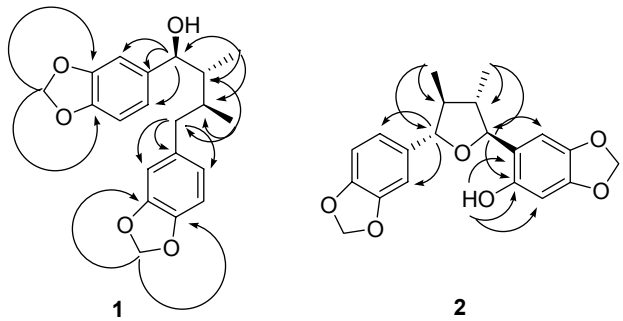

Figure 3. Key HMBC correlations of compounds **1**-**2**.

functional group were determined by a HMBC experiment. One set of correlations was observed between the oxygenated methine H-7 (δ 4.60) and C-2, C-6 and C-9 and the other set was between H-7′ (δ 4.70) and C-2′, C-6′ and C-9′. Additionally, the correlations of phenolic hydroxyl signal at δ 8.19 were observed among the C-1′, C-2′ and C-3′. The relative stereochemistry of the tetrahydrofuran ring could be deduced by comparison with literature data on related lignans.[2,20-26] In the ^{1}H-NMR data, upfield-shifted signals of H-9 and H-9′ at δ 1.07 and 1.06, respectively, indicated *trans* positions to those methyl groups because if those two protons were located on *cis* position to those of methyl group, chemical shift values of two protons should be about 0.7 ppm due to the shielding effect of the aromatic rings.[11,12] Along with this, coupling constants of 9.2 and 10.0 Hz for H-7 and H-7′, respectively, suggested *trans*-configurations both between H-7 and H-8 and between H-7′ and H-8′.[5,7] From the above evidence, **2** was confirmed as (7α,8β,7′β,8′α)-2′-hydroxy-3,4,4′,5′-dimethylenedioxy-7,7′-epoxy lignan and named as saucerneol K.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter. The NMR spectra were recorded on a Bruker 250 MHz (DMX 250, Germany) and Varian 400 MHz (INOVA-400, USA) spectrometer. Samples were dissolved in $CDCl_3$-*d* or CD_3OD, and chemical shifts were reported in ppm downfield from TMS. HIEIMS and HIFABMS were obtained on a JEOL JMS700 spectrometer (JEOL, Japan). The stationary phases used for column chromatography (Silica gel 60, 70-230 and 230-400 mesh and Lichroprep RP-18 gel, 40-63 μm, Merck) and TLC plates (Silica-gel 60 F_{254} and RP-18 F_{254s}, 0.25 mm, Merck) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under UV radiation and by spraying with 10% H_2SO_4, followed by heating. (*R*)-(-)-α-methoxy-α-(trifluoromethyl)-phenyacetyl chloride [(*R*)-MTPA-Cl] and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenyacetyl chloride [(*S*)-MTPA-Cl] were purchased from Aldrich (St. Louis, MO, USA; purity 99.0%). All other chemicals and solvents were of analytical grade, and used without further purification.

Plant Material. The roots of *Saururus chinensis* was purchased in February 2003 from a folk medicine market, "Yak-ryong-si", in Daegu, Republic of Korea. These materials were confirmed taxonomically by Professor Ki-Hwan Bae, Chungnam National University, Daejeon, Republic of Korea. A voucher specimen (YNSC2004) has been deposited at the College of Pharmacy, Yeungnam University.

Extraction and Isolation. The dried roots of *Saururus chinensis* (9.7 kg) was extracted with 70% MeOH three times by refluxing for 24 hr, respectively and the MeOH solution was evaporated to dryness (1.0 kg). The MeOH extract was suspended in H_2O (1.4 L), and the resulting H_2O layer was successively partitioned with *n*-hexane, EtOAc and BuOH (each 1.4 L × 3). The EtOAc extract (130 g) was loaded on a silica gel column (60 × 12 cm, Silica-gel 70-230 mesh) and eluted by a stepwise gradient of *n*-hexane-EtOAc (100:0 → 0:100) and then EtOAc-MeOH (100:0 → 0:100). The eluates (500 mL in each flask) were combined into 39 fractions (SCE1-39) on the basis of silica gel TLC. Fraction SCE19 (970 mg) was chromatographed on a reverse-phase column (4 × 50 cm, LiChroprep RP-18) and eluted by a stepwise gradient of MeOH-H_2O (60:40 → 100:0) and then Fraction SCE19-10 (160 mg) was chromatographed on a Sephadex LH-20 column (3 × 90 cm, Sephadex LH-20) eluted with MeOH (5.0 L) to give **1** (90 mg). Fractions 26 (1.0 g) was chromatographed on a reverse-phase column (4 × 50 cm, LiChroprep RP-18) and eluted by a stepwise gradient of MeOH-H_2O (40:60 → 100:0) and then Fraction SCE26-13 (15 mg) was preparative TLC (10 × 10 cm, 0.25 mm coated silica gel, $CHCl_3$:MeOH = 95:5) to give **2** (5 mg).

(7*S*,8*R*,8′*S*)-3,4,3′,4′-Dimethylenedioxylignan-7-ol (1): Oil; $[a]_D^{20}$ -65.7° (*c* 1.806, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 234 (3.92), 287 (3.79) nm; ^{1}H-NMR (CD_3OD, 250 MHz) δ 6.77 (1H, d, *J* = 1.3 Hz, H-2), 6.69 (1H, dd, *J* = 8.0, 1.5 Hz, H-6), 6.69 (1H, d, *J* = 8.0 Hz, H-5), 6.69 (1H, d, *J* = 8.0 Hz, H-5′), 6.68 (1H, d, *J* = 1.5 Hz, H-2′), 6.60 (1H, dd, *J* = 7.9, 1.4 Hz, H-6′), 5.89 (2H, s, -OCH_2O-), 5.88 (2H, s, -OCH_2O-), 4.25 (1H, d, *J* = 9.6 Hz, H-7), 2.46 (2H, m, H-7′), 2.39 (1H, m, H-8′), 1.75 (1H, m, H-8), 0.81 (3H, d, *J* = 6.2 Hz, H-9′), 0.54 (3H, d, *J* = 7 Hz, H-9); ^{13}C-NMR (MeOD, 62.9 MHz) δ 149.1 (C-3), 148.9 (C-3′), 148.2 (C-4), 147.0 (C-4′), 140.1 (C-1), 136.7 (C-1′), 122.9 (C-6′), 121.6 (C-6), 110.3 (C-2′), 108.8 (C-5), 108.6 (C-5′), 107.9 (C-2), 102.2 (-OCH_2O-), 102.0 (-OCH_2O-), 77.8 (C-7), 44.1 (C-8), 43.0 (C-7′), 35.2 (C-8′), 13.1 (C-9′), 10.5 (C-9); HRFABMS *m/z* 343.1538 ($[M+H]^+$, calcd. for $C_{20}H_{23}O_5$, 343.1545).

(7*R*,8*R*,8′*S*)-3,4,4′,5′-Dimethylenedioxy-2′,7-cyclolignan (1a): $[a]_D^{22}$ +65.7° (*c* 0.036, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 6.72 (1H, d, *J* = 7.9 Hz, H-5), 6.60 (1H, dd, *J* = 7.9, 1.7 Hz, H-6), 6.50 (1H, s, H-6′), 6.50 (1H, d, *J* = 1.6 Hz, H-2), 6.14 (1H, s, H-3′), 5.91 (2H, s, -OCH_2O-), 5.80 and 5.79 (each

1H, d, J = 1.4 Hz, -OCH_2O-), 3.36 (1H, d, J = 10.2 Hz, H-7), 2.69 (1H, dd, J = 16.1, 4.5 Hz, H-7′a), 2.54 (1H, dd, J = 16.3, 11.3 Hz, H-7′b), 1.60 (1H, m, H-8′), 1.44 (1H, ddq, J = 10.5, 10.5, 6.2 Hz, H-8), 1.03 (3H, d, J = 6.3 Hz, H-9′), 0.84 (3H, d, J = 6.2 Hz, H-9); ^{13}C-NMR ($CDCl_3$, 62.9 MHz) δ 147.7 (C-3), 145.8 (C-4), 145.5 (C-5′), 145.4 (C-4′), 140.5 (C-1), 133.4 (C-2′), 130.1 (C-1′), 122.8 (C-6), 109.6 (C-3′), 109.1 (C-2), 107.7 (C-5), 107.6 (C-6′), 100.8 (-OCH_2O-), 100.5 (-OCH_2O-), 54.6 (C-7), 43.8 (C-8), 39.4 (C-7′), 35.4 (C-8′), 19.9 (C-9′), 17.1 (C-9).

(7*α*,8*β*,7′*β*,8′*α*)-2′-Hydroxy-3,4,4′,5′-dimethylenedioxy-7,7′-epoxy Lignan (2): Colorless oil; $[a]_D^{22}$ +21.9^o (*c* 0.08, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 244 (3.24), 291 (3.01) nm; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.19 (1H, s, OH), 6.87 (1H, d, J = 1.2 Hz, H-2), 6.81 (1H, dd, J = 6.5, 0.8 Hz, H-6), 6.79 (1H, d, J = 6.5 Hz, H-5), 6.45 (2H, s, H-3′ and H-5′), 5.96 (2H, s, -OCH_2O-), 5.89 (2H, s, -OCH_2O-), 4.70 (1H, d, J = 10.0 Hz, H-7′), 4.60 (1H, d, J = 9.2 Hz, H-7), 1.98 (1H, m, H-8′), 1.76 (1H, m, H-8), 1.07 (3H, d, J = 6.4 Hz, H-9), 1.06 (3H, d, J = 6.4 Hz, H-9′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 151.0 (C-2′), 148.2 (C-4), 147.9 (C-3), 147.5 (C-4′), 140.8 (C-1), 119.9 (C-6), 115.4 (C-1′), 108.4 (C-5), 106.9 (C-6′), 106.6 (C-2), 101.3 (-OCH_2O-), 101.2 (-OCH_2O-), 99.7 (C-3′), 89.5 (C-7′), 87.5 (C-7), 50.6 (C-8), 48.4 (C-8′), 14.1 (C-9′ and C-9); HREIMS *m/z* 356.1258 ([M]$^+$, calcd. for $C_{20}H_{20}O_6$, 356.1260).

Preparation of (*S*) and (*R*)-MTPA Esters of 1. Mosher's esters was prepared according to the reported method.[16-18] To compound **1** (3 mg) in 0.5 mL of CH_2Cl_2 were added sequentially 0.2 mL of anhydrous pyridine, 0.5 mg of 4-(dimethylamino)pyridine and 12.5 mg of (*R*)-(-)-*α*-methoxy-*α*-(trifluoromethyl)phenylacetyl chloride [(*R*)-MPTA-Cl]. The mixture was left at room temperature overnight and checked by TLC to determine if the reaction was finished. After addition of 1 mL of hexane, the reaction mixture was passed through a column (6 × 0.6 cm, silica gel, 230-400 mesh, 9385) with hexane-CH_2Cl_2 (1:2). The eluate was dried in *vacuo* to give the (*S*)-MTPA ester of **1**. Using (*S*)-MTPA-Cl, the (*R*)-MTPA ester of **1** was prepared.

Conversion of 1 to 1a. Compound **1** (10 mg) in 0.5 was dissolved in acetyl chloride (3 drops). The solution was kept at room temperature for 2 hr, and, after the addition of H_2O, neutralized with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), filtered and evaporated. The residue that dissolved in CH_2Cl_2 (1-2 mL) was passed through a column (6 × 0.6 cm, silica gel, 230-400 mesh, 9385) with CH_2Cl_2 mobile phase. The eluates were dried in *vacuo* to give compound **1a** (6 mg).

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