DOI 10.5012/bkcs.2010.31.7.2088

Cytotoxic Sesquilignans from the Roots of *Saururus chinensis*

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Received April 26, 2010, Accepted May 25, 2010

Key Words: *Saururus chinensis*, Sesquilignans, Cytotoxicity, Saururaceae

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.[1] Previous studies of *S. chinensis* reported the occurrence of lignans,[2-9] aristolactams,[10] flavonoids,[11] and furanoditerpenes,[12] and a wide range of biological activities including antioxidant activity,[5] hepatoprotective activity,[6] cytotoxic activity,[13-18] anti-inflammatory activity,[19-22] anti-atherogenic activity,[21,23] and immunosuppressive activity.[24] 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 $C_{31}H_{38}O_8$, 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 δ_H 2.34 (H-8 and H-8'), for oxymethines at δ_H 5.65 (H-7 and H-7'), and for methyl groups at δ_H 0.78 (H-9) and 0.74 (H-9'), as well as the signals at δ_H 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-trisubstitued benzene rings.[4,25] An additional phenylpropanoid unit was observed in compound **1** from the proton signals for two oxymethines at δ_H 4.95 (H-8"), and 5.42 (H-7"), for a methyl at δ_H 1.60 (H-9") and for a 1,3,4-trisubstituted benzene ring at δ_H 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" (δ_H 4.95) and C-4' (δ_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.[4,26] In addition, the chemical shifts of C-9" (δ_C 15.3), C-7" (δ_C 75.7), and C-8" (δ_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*.[4,26] Futhermore, 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*-saucerneol, a diastereomer of (-)-saucerneol (**3**).

Saucerneol J (**2**) had a molecular formula ($C_{30}H_{36}O_8$) and exhibited a close resemblance to compound **1** in their ^{1}H and ^{13}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** (δ_H 4.54, *J* = 9.3 Hz) and **1** (δ_H 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**.

Figure 2. Key ^{1}H-^{1}H COSY (▬) and HMBC (H ⟶ C) and NOESY (⟷) correlations of compound **1**.

Table 1. ^{1}H-and ^{13}C-NMR data (δ) of **1-2** (Pyridine-d_5)[a]

position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	-	136.5 (s)	-	135.6 (s)
2	7.20 d, 1.7	111.6 (d)	7.25 d, 1.7	112.1 (d)
3	-	151.5 (s)	-	151.5 (s)
4	-	147.6 (s)	-	147.8 (s)
5	7.30 d, 8.2	117.5 (d)	7.28 d, 8.1	116.5 (d)
6	7.36 br d, 8.2	120.1 (d)	7.31 br d, 8.1	120.6 (d)
7	5.65 d, 6.4	84.6 (d)	5.30 d, 8.6	82.7 (d)
8	2.34 ddq, 12.8, 6.4, 6.4	44.5 (d)	2.24 ddq, 8.6, 7.0, 6.5	45.7 (d)
9	0.78 d, 6.4	15.3 (q)	0.73 d, 7.0	14.9 (q)
1'	-	133.8 (s)	-	133.2 (s)
2'	7.19 d, 1.7	112.2 (d)	7.42 d, 1.7	111.2 (d)
3'	-	148.9 (s)	-	149.2 (s)
4'	-	147.5 (s)	-	148.5 (s)
5'	7.34 d, 8.2	116.6 (d)	7.30 d, 8.1	116.1 (d)
6'	7.09 dd, 8.2, 1.7	119.8 (d)	7.32 dd, 8.1, 1.7	120.5 (d)
7'	5.65 d, 6.4	84.3 (d)	4.54 d, 9.3	87.4 (d)
8'	2.34 ddq, 12.8, 6.4, 6.4	44.5 (d)	1.93 ddq, 9.3, 6.5, 6.5	47.9 (d)
9'	0.74 d, 6.4	15.3 (q)	1.05 d, 6.5	14.5 (q)
1"	-	136.8 (s)	-	135.1 (s)
2"	7.58 d, 1.6	112.2 (d)	7.59 d, 1.6	111.3 (d)
3"	-	150.2 (s)	-	148.9 (s)
4"	-	149.5 (s)	-	147.7 (s)
5"	7.01 d, 8.2	112.6 (d)	7.31 d, 8.1	115.8 (d)
6"	7.07 dd, 8.2, 1.6	120.3 (d)	7.29 dd, 8.1, 1.6	120.7 (d)
7"	5.42 d, 3.4	75.7 (d)	5.42 d, 3.9	74.8 (d)
8"	4.95 dq, 6.2, 3.4	81.1 (d)	4.90 dq, 6.2, 3.9	80.3 (d)
9"	1.60 d, 6.2	15.3 (q)	1.60 d, 6.2	14.3 (q)
3-OCH_3	3.84 s	56.5 (q)	3.82 s	56.4 (q)
3'-OCH_3	3.85 s	56.5 (q)	3.86 s	56.4 (q)
3"-OCH_3	3.82 s	56.3 (q)	3.80 s	56.3 (q)
4"-OCH_3	3.77 s	56.4 (q)	-	-

[a]Assignments were based on ^{1}H-^{1}H COSY, DEPT, HMQC and HMBC spectra.

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"*S*-4"-demethylsaucerneol.

The known compounds were in good agreement with previously reported NMR data and were consequently identified as (-)-saucerneol (**3**) with a $[\alpha]_D$ value of −71.1 (*c* 0.1 MeOH) [Lit. $[\alpha]_D$ −91.8],[25] *threo*,*erythro*-manassantin A (**4**),[16] 4-*O*-demehylmanassantin B (**5**),[18] *erythro*,*erythro*-manassantin A (**6**),[16] manassantin B (**7**)[28] and manassantin A (**8**).[17]

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 μM, respectively) against HL-60 cell lines (camptothecin, IC_{50} 0.8 μM).

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 μm, 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_2SO_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 kg of the solid extract. The MeOH extract was suspended in H_2O 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/H_2O, 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/H_2O, 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/H_2O, 2 : 1) to give compound **4** (17.8 mg). Fr. SC14 (0.88 g) was chromatographed on a RP C-18 column (MeOH/H_2O, 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/H_2O, 3 : 2) to give compound **2** (5.3 mg). Fr. SC14-6 (0.36 g) was chromatographed on a RP C-18 column (MeOH/H_2O, 3 : 2) to give compounds **5** (76.7 mg), **6** (5.3 mg), **7** (18.3 mg), and **8** (43.3 mg).

***erythro*-Saucerneol (1):** Colorless powder, mp 85 - 86 °C. $[\alpha]_D^{25}$ −18 (*c* 0.1, MeOH). UV λ_{max} (MeOH) nm (log ε): 206 (3.74), 284 (2.94). ^{1}H- and ^{13}C-NMR data see Tables 1. HRESIMS *m*/*z* 537.2471 $[M-H]^-$ (Calcd for $C_{31}H_{37}O_8$: 537.2488). CD (*c* 0.0004 MeOH): $[\theta]_{231}$ +12,075.

Saucerneol J (2): Colorless powder, mp 75 - 76 °C. $[\alpha]_D^{25}$ −10 (*c* 0.1, MeOH). UV λ_{max} (MeOH) nm (log ε): 206 (3.81), 282 (2.67). ^{1}H- and ^{13}C-NMR data see Tables 1 and 2. HRESIMS *m*/*z* 523.2310 $[M-H]^-$ (Calcd for $C_{30}H_{35}O_8$: 523.2332). CD (*c* 0.0006 MeOH): $[\theta]_{232}$ +18,121.

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

Acknowledgments. This research was supported by a grant of KRIBB Research Initiative Program (KGS2241012).

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