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Lignans from *Saururus chinensis* inhibiting the transcription factor NF-κB

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Abstract

The sesquineolignans, saucerneol D and saucerneol E were isolated from the roots of *Saururus chinensis* together with four known lignans, manassantin A, manassantin B, (−)-saucerneol methyl ether, and (+)-saucernetin. Structure elucidation was based on the analysis of spectroscopic data and anti-inflammatory activity was studied using HeLa cells transfected with NF-κB reporter construct. All compounds except for (+)-saucernetin inhibited NF-κB dependent reporter gene expression with IC_{50} values of 2.5–16.9 μM.

Keywords: Saururus chinensis; Saururaceae; Sesquineolignan; Lignan; Saucerneol D; Saucerneol E; Nuclear factor κB

1. Introduction

Saururus chinensis (Saururaceae) is a perennial herb distributed in China and southern Korea and has been used in folk medicine to treat edema, gonorrhea, and also as an uretic in Korean traditional medicine (Kim, 1989). Previous chemical studies of the genus *Saururus* have shown the presence of more than 20 lignans (Rao and Alvarez, 1982, 1985; Rao and Rao, 1990; Chattopadhyay and Rao, 1987), some of which exhibited neuroleptic (Rao et al., 1987), hepatoprotective (Sung and Kim, 2000), and antifeedant activities (Kubanek et al., 2000). Recently, a triene, 12,13-dehydrogeranylgeraniol, has been demonstrated to inhibit intracellular reactive oxygen species (ROS)-catalyzed oxidation within HL-60 cells (Rajbhandari et al., 2001). Different types of compounds have also been isolated from *Saururus* species including aristolactams, flavonoids, anthraquinones, and furanoditerpenes (Rao and Reddy, 1990; Wang et al., 1996; Sung et al., 1997; Hwang et al., 2002).

In our search for anti-inflammatory substances from natural products, the methanol extract of the roots of *S. chinensis* (Saururaceae) potently inhibited the transcription factor NF-κB, which serves as a central regulator of human immune, inflammatory, and antiapoptotic responses (Ghosh et al., 1998). NF-κB regulates the transcription of various inflammatory cytokines, such as interleukin-1,-2,-6, and-8 and TNF-α, as well as genes encoding cyclooxygenase-II, inducible nitric oxide synthase, immunoreceptors, cell adhesion molecules, hematopoetic growth factors, and growth factor receptors (Tak and Firestein, 2001). NF-κB also controls transcription of genes encoding antiapoptotic proteins such as the caspase inhibitors c-IAP1, c-IAP2, and X-IAP, the TNF receptor-associated factors TRAF1 and TRAF2, the zinc finger protein A20, the immediate-early response gene IEX-1L, and the prosurvival Bcl-2 homolog Bfl-1/A1 (Yamamoto and Gaynor, 2001). Therefore, pharmacological inhibition of NF-κB could be a valuable strategy to modulate inflammatory processes as well as cell death. We have recently demonstrated that well-known anti-inflammatory medicinal plants contain structurally diverse NF-κB inhibitors such as sesquiterpene lactones, kaurane-type diterpenes, and triterpene (Koo et al., 2000; Hwang et al., 2001; Lee et al., 2002; Jin et al., 2002).

Bioactivity-guided fractionation of methanol extracts of *S. chinensis* using the NF-κB directed luciferase assay

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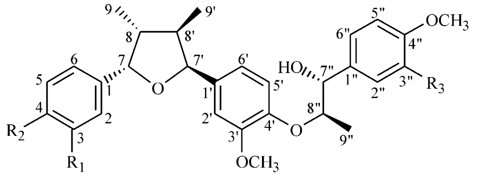
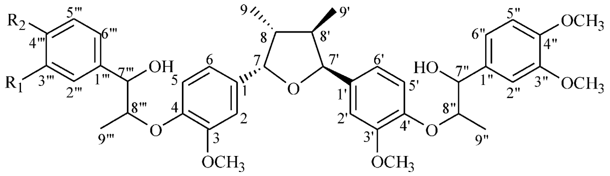
system has led to isolation of two new sesquineolignans named saucerneol D (**1**) and saucerneol E (**2**), and four known lignans, (−)-saucerneol methyl ether (**3**), manassantin A (**4**), manassantin B (**5**), and (+)-saucernetin (**6**) (Rao and Alvarez, 1982, 1983; Rao and Oruganty, 1997; Sung and Kim, 2000; Fig. 1). We report herein the isolation and structure elucidation of **1** and **2** using spectroscopic methods such as HMBC, HMQC and 2D NOESY NMR techniques as well as the inhibitory effects of compounds **1**–**5** on the NF-κB activation in HeLa cells transfected with NF-κB reporter construct.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, with a molecular formula of $C_{31}H_{36}O_8$ determined by HRFABMS (*m*/*z*; found 554.2751 $[M+NH_4]^+$; calcd 554.2754). The UV and IR spectra of **1** revealed the presence of hydroxyl (3490 cm^{-1}), and phenolic groups (235.6 and 286.2 nm, 1605 and 1595 cm^{-1}). Comparison of its ^{1}H and ^{13}C NMR spectral data with saucerneol, saucerneol methyl ether, saucerneol A, saucerneol B, and saucerneol C indicates that **1** is a tetrahydrofuran-type sesquineolignan (Rao and Alvarez, 1983; Rao and Oruganty, 1997; Sung et al., 2001).



	R1	R2	R3
1	-OCH$_2$O-		-OCH$_3$
2	-OCH$_2$O-		-OH
3	-OCH$_3$	-OCH$_3$	-OCH$_3$



	R1	R2
4	-OCH$_3$	-OCH$_3$
5	-OCH$_2$O-	

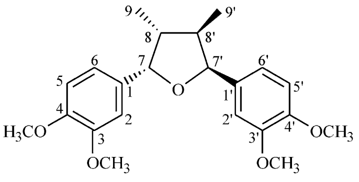


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Fig. 1. Chemical structures of 1–6 isolated from *Saururus chinensis*.

The ^{1}H NMR spectrum (Table 1) of **1** showed two methine groups at δ 2.26 (H-8) and δ 2.28 (H-8′) coupled with oxymethine groups at δ 5.42 (H-7) and δ 5.43 (H-7′) as well as with methyl groups at δ 0.71 (H-9) and δ 0.70 (H-9′), respectively. These signals were assigned to those protons in the asymmetric 8,8′-dimethyl-7,7′-disubstituted tetrahydrofuran moiety of **1**. Additionally, the proton of an oxygenated methine group at δ 4.13 (H-8″) was coupled with that of another oxygenated methine group at δ 4.65 (H-7″) and a methyl group at δ 1.17 (H-9″), indicating the presence of third phenylpropanoid moiety. The remaining nine aromatic protons exhibited characteristic signals belonging to three sets of 1,3,4-trisubstituted benzene rings. Two doublets at δ 6.81 (*J* = 1.7 Hz, H-2) and 6.79 (*J* = 8.0 Hz, H-5), one broad doublet at δ 6.76 (*J* = 8.0 Hz, H-6), and two proton singlets at δ 5.95 suggested that one substituent was a 3,4-methylenedioxyphenyl group. In addition, three methoxyl groups at δ 3.93 (3′-OCH$_3$), 3.89 (3″-OCH$_3$), and 3.88 (4″-OCH$_3$) and another two sets of 1,3,4-trisubstituted benzene protons at δ 6.90 (*d*, *J* = 1.6 Hz, H-2′), δ 6.99 (*d*, *J* = 8.0 Hz, H-5′), δ 6.82 (*dd*, *J* = 8.0, 1.6 Hz, H-6′), δ 6.95 (*d*, *J* = 1.7 Hz, H-2″), δ 6.84 (*d*, *J* = 8.2 Hz, H-5″), and δ 6.93 (*dd*, *J* = 8.2, 1.7 Hz, H-6″) indicated that the presence of an 8″-*O*-4′-neolignan containing a 3′-methoxyphenyl group and a 3″,4″-dimethoxylphenyl group (Sung et al., 2001). The ^{13}C NMR spectrum of **1**, including DEPT measurements, showed three methyl carbons, three methoxyl carbons, two methine carbons, four oxymethine carbons and one methylenedioxy carbon as well as carbon signals of three aromatic rings. The only differences of compound **1** from saucerneol B, which was recently isolated from the same plant (Sung et al., 2001), are the position of a methylenedioxy unit and the stereochemistry of teterahydrofuran moiety in the molecules.

The positions of each functional group were determined by an HMBC NMR experiment (Table 1). One set of correlations was observed between the carbinolic hydrogen H-7 (δ 5.42) and C-1 (δ 135.3), C-2 (δ 106.9), C-6 (δ 119.3), C-7′ (δ 83.4), C-8 (δ 43.9), C-8′ (δ 43.9), and C-9 (δ 14.7) and the other set was between H-7′ (δ 5.43) and C-1′ (δ 136.5), C-2′ (δ 110.2), C-6′ (δ 118.7), C-7 (δ 83.6), C-8 (δ 43.9), C-8′ (δ 43.9) and C-9′ (δ 14.7). Additionally, four-bond long-range correlations were observed between the methyl protons of H-9′ (δ 0.70) and H-9 (δ 0.71) and the quaternary carbons of C-1′ (δ 136.5) and C-1 (δ 135.3), respectively. Moreover, the correlations between the methylenedioxy signal at δ 5.95 and the signals for C-3 (δ 147.5) and C-4 (δ 146.4) strongly supported the proposed link between the 3,4-methylenedioxy phenyl group and the C-7 of the tetrahydrofuran ring of **1**. Furthermore, HMBC correlations between H-8″ (δ 4.13) and aromatic carbons resonance at δ 146.4 (C-4′) and δ 132.6 (C-1″) supported the proposition that **1** contains an 8″-*O*-4′ neolignan.

The relative stereochemistry of the tetrahydrofuran ring could be deduced by comparison with literature data on related lignans (Fonseca et al., 1979; Rimando et al., 1994; Rao and Oruganty, 1997; Tofen et al., 2000; Sung et al., 2001; Kraft et al., 2002). The proton and carbon chemical shifts of **1** are typical for a *cis*-configuration of one aryl and methyl substituent, respectively, whereas the two methyl groups are oriented *trans* to each other. This assignment was further supported by the ^{1}H NMR data and 2D NOESY experiment. In the ^{1}H NMR data, the upfield-shifted signals of H-9 and H-9′ at δ 0.71 and δ 0.70 are due to the shielding effect of the aromatic rings and the downfield shifting of the signals from H-7 and H-7′ at δ 5.42 and 5.43 are due to the deshielding effect of the aromatic rings (Yue et al., 1989; Prasad et al., 1995). Along with this, the coupling constant of 6.4 Hz for H-7 and H-7′ suggested that these two protons are in a *cis*-configuration relative to the adjacent protons H-8 and H-8′, respectively (Sarkanen and Wallis, 1973; Rao and Oruganty, 1997). Furthermore, the NOE correlation signals between H-9/H-8′ and H-2 and H-6; and H-9′/H-8 and H-2′ and H-6′ clearly indicated the 7,8-*cis*-8,8′-*trans*-7′,8′-*cis* configuration (Fig. 2). Also, the relative configuration of C-7″ and C-8″ was established as being *threo* by comparing the chemical shifts of C-9″ (δ 17.0), C-7″ (δ 78.3), and C-8″ (δ 84.0) and the coupling constants ($J = 8.3$ Hz) of H-7″ (δ 4.65) and H-8″ (δ 4.13), with data from previous reports (Zacchino, 1994; Tsai et al., 1996; Su et al., 1999; Tofen et al., 2000).

The absolute configuration of C-7″ in **1** was determined using the Mosher ester procedure (Dale and Mosher, 1973; Kasahara et al., 1995). Compound **1** was treated with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-trifluoromethyl-phenylacetyl chloride in anhydrous pyridine at room temperature for 4 h to afford the (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**, respectively). The differences of chemical shift values obtained by subtracting (*R*)-MTPA ester from (*S*)-MTPA ester [$\delta\Delta = \delta$ (*S*)-MTPA$-\delta$ (*R*)-MTPA] are shown in Fig. 3. The $\Delta\delta$ values for H-7, H-8, H-9, H-7′, H-8′, and H-9′

Table 1

^{1}H and ^{13}C NMR spectral data and HMBC correlations for compounds **1** and **2** (600/150 MHz, $CDCl_3$)

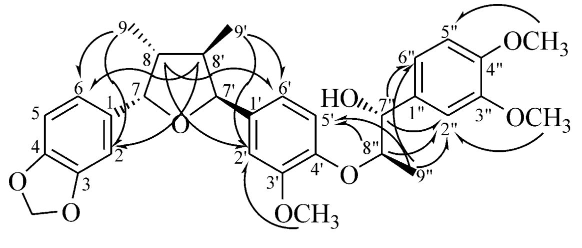
Carbon	**1**				**2**			
	δ_H	δ_C	Dept	HMBC correlations	δ_H	δ_C	Dept	HMBC correlations
1	–	135.3	C		–	135.4	C	
2	6.81 (*d*, 1.7)	106.9	CH	C-3, 4, 6, 7	6.82 (*d*, 1.5)	106.9	CH	C-3, 4, 6, 7
3	–	147.5	C		–	147.5	C	
4	–	146.4	C		–	146.4	C	
5	6.79 (*d*, 8.0)	107.8	CH	C-1, 3, 4	6.80 (*d*, 8.0)	107.8	CH	C-1, 3, 4
6	6.76 (*br d*, 8.0)	119.3	CH	C-2, 4, 7	6.76 (*br* d, 8.0)	119.3	CH	C-2, 4, 7
7	5.42 (*d*, 6.5)	83.6	CH	C-1, 2, 6, 7′, 8, 8′, 9	5.42 (*d*, 6.4)	83.7	CH	C-1, 2, 6, 7′, 8, 8′, 9
8	2.26 (*ddq*, 12.8, 6.5, 6.5)	43.9	CH	C-1, 7, 7′, 8′, 9, 9′	2.27 (*ddq*, 12.8, 6.4, 6.4)	44.0	CH	C-1, 7, 7′, 8′, 9, 9′
9	0.71 (*d*, 6.5)	14.7	CH_3	C-1, 7, 8, 8′	0.71 (*d*, 6.4)	14.7	CH_3	C-1, 7, 8, 8′
1′	–	136.5	C		–	136.6	C	
2′	6.90 (*d*, 1.6)	110.2	CH	C-3′, 4′, 6′, 7′	6.91 (*d*, 1.6)	110.2	CH	C-3′, 4′, 6′, 7′
3′	–	150.5	C		–	150.6	C	
4′	–	146.4	C		–	146.4	C	
5′	6.99 (*d*, 8.0)	118.7	CH	C-1′, 3′, 4′	6.99 (*d*, 7.9)	118.7	CH	C-1′, 3′, 4′
6′	6.82 (*dd*, 8.0, 1.6)	118.7	CH	C-2′, 4′, 7′	6.81 (*dd*, 7.9, 1.6)	118.8	CH	C-2′, 4′, 7′
7′	5.43 (*d*, 6.5)	83.4	CH	C-1′, 2′, 6′, 7, 8, 8′, 9′	5.43 (*d*, 6.4)	83.5	CH	C-1′, 2′, 6′, 7, 8, 8′, 9′
8′	2.28 (*ddq*, 12.8, 6.5, 6.5)	43.9	CH	C-1′, 7, 7′, 8, 9, 9′	2.29 (*ddq*, 12.8, 6.4, 6.4)	43.9	CH	C-1′, 7, 7′, 8, 9, 9′
9′	0.70 (*d*, 6.5)	14.7	CH_3	C-1′, 7′, 8′, 8	0.72 (*d*, 6.4)	14.7	CH_3	C-1′, 7′, 8′, 8
1″	–	132.6	C		–	132.0	C	
2″	6.95 (*d*, 1.7)	110.1	CH	C-3″, 4″, 6″, 7″	6.94 (*d*, 1.5)	109.4	CH	C-3″, 4″, 6″, 7″
3″	–	149.0	C		–	145.5	C	
4″	–	148.8	C		–	146.6	C	
5″	6.84 (*d*, 8.2)	110.9	CH	C-1″, 3″, 4″	6.88 (*d*, 8.6)	114.1	CH	C-1″, 3″, 4″
6″	6.93 (*dd*, 8.2, 1.7)	120.0	CH	C-2″, 4″, 7″	6.89 (*dd*, 8.6, 1.5)	120.7	CH	C-2″, 4″, 7″
7″	4.65 (*d*, 8.3)	78.3	CH	C-1″, 2″, 6″, 8″, 9″	4.63 (*d*, 8.3)	78.5	CH	C-1″, 2″, 6″, 8″, 9″
8″	4.13 (*dq*, 8.3, 6.2)	84.0	CH	C-1″, 4′, 7″, 9″	4.12 (*dq*, 8.3, 6.2)	84.1	CH	C-1″, 4′, 7″, 9″
9″	1.17 (*d*, 6.2)	17.0	CH_3	C-7″, 8″	1.17 (*d*, 6.2)	17.0	CH_3	C-7″, 8″
3-OCH_2O-4	5.95 (*s*)	100.8	CH_2	C-3, 4	5.96 (*s*)	100.9	CH_2	C-3, 4
3′-OCH_3	3.93 (*s*)	55.8	CH_3	C-3′	3.93 (*s*)	55.8	CH_3	C-3′
3″-OCH_3	3.89 (*s*)	55.9	CH_3	C-3″	–	–		
4″-OCH_3	3.88 (*s*)	55.9	CH_3	C-4″	3.90 (*s*)	55.9	CH_3	C-4″

Chemical shifts are shown in the δ scale with *J* values (Hz) in parentheses.

were negative, while positive values were observed for 3″- and 4″-OCH_3, indicating a 7″*R*-configuration. Therefore, the structure of **1** was assigned as a new sesquineolignan, saucerneol D (Fig. 1). However, since the absolute stereochemistry is only known at C-7″ and C-8″, and these centers cannot be related to the other chiral centers the absolute configurations of the other chiral centers at C-7, C-8, C-7′, and C-8′ remain to be determined.

Since methanol was used for extraction and chromatographic purification, the possibility that the 3″-methoxyl group in compound **1** was from the solvent was tested by overnight incubation of **2** with methanol. However, not even a trace of compound **1** was detected. This is not unexpected since most of phenolic hydroxyl groups in phenylpropanoids could only be methylated by using diazomethane (Matsushita et al., 1991) or methyl iodide (Miyazawa et al., 1996).

Compound **2** was also obtained as a colorless powder. The molecular formula of **2** was determined to be $C_{30}H_{34}O_8$ from the positive HRFABMS spectrum (*m*/*z*; found 540.2593 $[M+NH_4]^+$; calcd 540.2597). The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**, except for the absence of a singlet signal of a phenyl methoxyl group, suggesting that **2** was the demethylated form of **1**. The HMQC and HMBC experiments supported the same connectivity in **2** as shown in **1**. The HMBC cross-peaks between 3′-methoxyl signal at δ 3.93 and C-3′ at δ 150.56 and between 4″-methoxyl signal at δ 3.90 and C-4″ at δ 146.57 indicated the positions of these methoxyl group as C-3′ and C-4″. These were further confirmed by a NOESY experiment, wherein NOE correlations were observed between H-2′ (δ 6.91) and 3′-OCH_3 (δ 3.93) and between H-5″ (δ 6.88) and 4″-OCH_3 (δ 3.90). Therefore, the 3″,4″-dimethoxylphenyl group in **1** is replaced by a 3″-hydroxy-4″-methoxylphenyl group in **2**.



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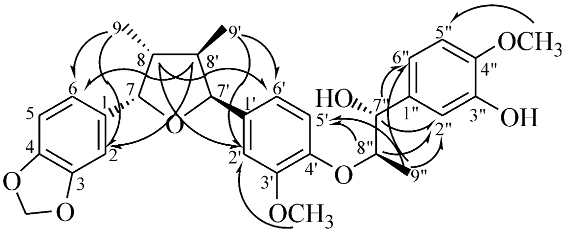

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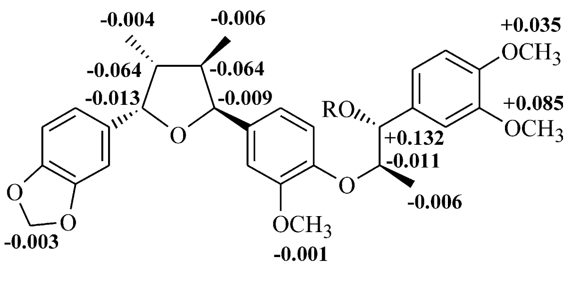
Fig. 2. Selected NOE correlations of **1** and **2**.

Concerning the stereochemistry of the tetrahydrofuran ring of **2**, comparison of the proton and carbon chemical shifts with published data on related lignans (Fonseca et al., 1979; Rimando et al., 1994; Rao and Oruganty, 1997; Tofen et al., 2000; Sung et al., 2001; Kraft et al., 2002) and analysis of NOESY data suggested that compound **2** belonged to the same class as compound **1**, thus leading to the same stereochemistry of 7,8-*cis*-8,8′-*trans*-7′,8′-*cis* configuration (Fig. 2). The relative configuration of C-7″ and C-8″ was also established as being *threo* by comparing the chemical shifts of C-9″ (δ 17.0), C-7″ (δ 78.5), and C-8″ (δ 84.1), and the coupling constants ($J=8.3$ Hz) of H-7″ (δ 4.63) and H-8″ (δ 4.12) with data from previous reports (Zacchino, 1994; Tsai et al., 1996; Su et al., 1999; Tofen et al., 2000). Comparable optical rotation value $\{[\alpha]_D^{25}$ $-83.0°$(*c* 0.3, $CHCl_3$)$\}$ and physicochemical data of compound **2** with those of compound **1** suggested that compound **2** possessed an identical stereochemistry with that of compound **1**. Therefore, structure **2** was assigned as a new sesquineolignan, saucerneol E (Fig. 1). Additionally, a sesquineolignan, (−)-saucerneol methyl ether (**3**), two known dilignans, manassantin A (**4**) and manassantin B (**5**), and a lignan, (+)-saucernetin (**6**) were isolated and identified by comparison of the observed ^1H, ^{13}C NMR, HMQC, HMBC, and ESIMS data with literature values (Rao and Alvarez, 1982, 1983; Rao and Oruganty, 1997; Sung and Kim, 2000).

The inhibitory effects of these compounds on the NF-κB activation were measured in HeLa cells, which were transiently transfected with the plasmid of NF-κB directed luciferase expression system. The luciferase activity in the culture medium was measured after treatment with various concentrations of compounds in the presence or absence of TNF-α (1 ng/ml). Mana-



1r: R = (*S*)-MTPA
1s: R = (*R*)-MTPA

Fig. 3. Δδ values [Δδ (in ppm) = $\delta_S-\delta_R$] for (*R*)- and (*S*)-MTPA esters of compound **1**.

ssantin A (**4**) and B (**5**) showed the most potent inhibitory activity, with IC_{50} values of 2.5 and 2.7 μM, respectively. Saucerneol D (**1**), saucerneol E (**2**) and (−)-saucerneol methyl ether (**3**) also exhibited comparable inhibitory activities with IC_{50} values of 6.1, 12.7 and 16.9 μM, respectively. However, (+)-saucernetin (**6**) had a weak activity (IC_{50} >30 μM). The relative potency of these compounds on the inhibition of NF-κB activation was dilignan > sesquineolignan > lignan, suggesting that the phenylpropanoid moiety attached to C-4 and/or C-4′ was important for the inhibition of the stimuli induced NF-κB activation process.

3. Experimental

3.1. General

Mps were measured on an Electrothermal model 9100 without correction. Optical rotations were determined on JASCO DIP-370 polarimeter at 25 °C. UV and IR spectra were obtained on a Milton Roy 3000 spectrometer and JASCO IR Report-100, respectively. ^1H NMR, ^{13}C NMR, DEPT, HMQC, HMBC and NOESY spectra were obtained on Varian Unity 300 MHz and Bruker DMX 600 MHz NMR spectrometers using $CDCl_3$ as a solvent. HRFABMS and ESIMS were recorded on JEOL-HX 110 and Finnigan Navigator mass spectrometer, respectively. Kieselgel 60 (Merck No. 9385 and 7729) and LiChroprep RP-18 were used for cc.

3.2. Plant material

The dried roots of *S. chinensis* were collected at the Jeju island, Korea, in October 1999, and identified by Dr. Kyong Soon Lee, a plant taxonomist at Chungbuk National University. A voucher specimen (No. 99101) was deposited at our institute.

3.3. Extraction and isolation

The air-dried ground roots (3.2 kg) of *S. chinensis* were extracted with MeOH (3×10 l) at room temperature. After filtration and evaporation of the solvent, the resulting extract was diluted with H_2O and extracted with CH_2Cl_2 (3×5 l). The CH_2Cl_2 extract (95 g) was subjected to cc over silica gel (6.5×45 cm) eluted with *n*-hexane–acetone (hexane, 10:1, 5:1, 2:1, 1:1, acetone) to afford 12 fractions (F01–F12). F07 (6.5 g) was further applied to a silica gel cc (3.5×50 cm) eluting with CH_2Cl_2–MeOH (50:1, 30:1, 20:1, 10:1, 100% MeOH), affording five fractions (F07-I–F07-V). F07-I (2.5 g) was subjected to silica gel cc (3.5×45 cm) using hexane–EtOAc (3:1, 100% EtOAc) mixture, giving ten fractions (F07-I-1–F07-I-10). F07-I-6 (450 mg) was finally purified through a Sephadex LH-20 column eluting with MeOH to afford saucerneol D (**1**, 300 mg). F08 (1.5 g) was subjected to cc over RP-18 eluting with 40, 60, 80, and 100% MeOH–water in sequence to afford eight fractions (F08-I–F08-VIII). F08-V (150 mg) was further applied to a Sephadex LH-20 column eluting with MeOH to afford saucerneol E (**2**, 10.9 mg) and saucerneol methyl ether (**3**, 20.9 mg). F09 (6.5 g) was subjected to silica gel column cc (5×60 cm) with CH_2Cl_2–MeOH (50:1, 30:1, 15:1, 5:1, 100% MeOH) to give five fractions (F09-I–F09-V). F09-II (3.4 g) was further applied to a silica gel column (4.5×45 cm) eluting with CH_2Cl_2–acetone (20:1, 15:1, 10:1, 5:1, 100% acetone) to give five fractions (F09-II-1–F09-II-5). F09-II-4 (1.9 g) was purified through a Sephadex LH-20 column with MeOH to afford manassantin B (**5**, 1090 mg). F10 (16.9 g) was further separated by cc on silica gel (6.0×45 cm) eluting with a step gradient solvent of CH_2Cl_2–acetone (30:1, 20:1, 10:1, 7:1, 5:1, 100% acetone) to give six fractions (F10-I–F10-VI). F10-IV (1.0 g) was subjected to Sephadex LH-20 cc eluting with MeOH, resulting in manassantin A (**4**, 210 mg). F04 (5.8 g) was applied to a silica gel column (5.0×50 cm) eluting with *n*-hexane–EtOAc (10:1, 7:1, 5:1, 3:1, 2:1, 1:1, 100% EtOAc) to afford seven fractions (F04-I–F04-VII). F04-V (1.4 g) was purified further by silica gel cc (3.5×45 cm) eluting with CH_2Cl_2–EtOAc (40:1, 30:1, 20:1, 10:1, 5:1, 2:1, 100% MeOH) to give seven fractions (F04-V-1–F04-V-7). F04-V-5 (850 mg) was subjected to a Sephadex LH-20 cc eluting with MeOH, resulting in saucernetin (**6**, 300 mg).

3.4. Saucerneol D (**1**)

Colorless powder, mp 75–76 °C, $[\alpha]_D^{25}$ −88.1° (*c* 1.2, $CHCl_3$), UV λ_{max} MeOH nm (log ε): 208.5 (4.90), 235.6 (4.37), 286.2 (3.98). IR ν_{max} (KBr) cm^{-1}: 3490, 2960, 2920, 2900, 1605, 1595. HRFABMS *m*/*z*: 554.2751 ($C_{31}H_{36}O_8NH_4$, calc. 554.2754). For ^1H NMR (600 MHz, $CDCl_3$), ^{13}C NMR (150 MHz, $CDCl_3$) and HMBC data: see Table 1.

3.5. Saucerneol E (**2**)

Colorless powder, mp 76–78 °C, $[\alpha]_D^{25}$ −83.0°(*c* 0.3, $CHCl_3$), UV λ_{max} MeOH nm (log ϵ): 210.0 (4.90), 232.5 (4.35), 282.5 (3.87). IR ν_{max} (KBr) cm^{-1}: 3460, 2962, 2910, 2900, 1610, 1595. HRFABMS *m*/*z*: 540.2593 ($C_{30}H_{34}O_8NH_4$, calcd 540.2597). For ^1H NMR (600 MHz, $CDCl_3$), ^{13}C NMR (150 MHz, $CDCl_3$) and HMBC data: see Table 1.

3.6. Saucerneol methyl ether (**3**)

Colorless powder, mp 72–74 °C, $[\alpha]_D^{25}$ −63.0° (*c* 0.5, $CHCl_3$), UV λ_{max} MeOH nm (log ε): 207.5 (4.80), 235.0

(4.35), 285.2 (3.95). IR ν_{max} (KBr) cm^{-1}: 3450, 2950, 2900, 1600, 1590. For ^1H NMR, ^{13}C NMR and ESIMS data were in good agreement with literature values (Rao and Oruganty, 1997)

3.7. Manassantin A (4)

Colorless powder, mp 82–85 °C, $[\alpha]_D^{25}$ −102.1°(*c* 0.5, $CHCl_3$), UV λ_{max} MeOH nm (log ε): 210.1 (4.82), 235.2 (4.67), 280.5 (4.09), IR ν_{max} (KBr) cm^{-1}: 3500, 2962, 2915, 2900, 1610, 1590. The ^1H NMR, ^{13}C NMR and ESIMS data were in good agreement with literature values (Rao and Alvarez, 1983).

3.8. Manassantin B (5)

Colorless powder, mp 83–86 °C, $[\alpha]_D^{25}$ −99.8°(*c* 0.5, $CHCl_3$), UV λ_{max} MeOH nm (log ε): 209.5 (4.90), 236.0 (4.56), 282.5 (3.99), IR ν_{max} (KBr) cm^{-1}: 3460, 2950, 2900, 1610, 1590. The ^1H NMR, ^{13}C NMR and ESIMS data were in good agreement with literature values (Rao and Alvarez, 1983; Rao and Oruganty, 1997).

3.9. Saucernetin (6)

Colorless powder, mp 78–80 °C, $[\alpha]_D^{25}$ +48.1°(*c* 0.5, $CHCl_3$), UV λ_{max} MeOH nm (log ε): 209.2 (4.85), 235.0 (4.25), 283.5 (3.76), IR ν_{max} (KBr) cm^{-1}: 2965, 2900, 1600, 1590. The ^1H NMR, ^{13}C NMR and ESIMS data were in good agreement with literature values (Rao and Alvarez, 1982; Sung and Kim, 2000).

3.10. NF-κB activity assay

The HeLa cells transiently transfected with a plasmid pNFkB-Luc (Stratagene, La Jolla, CA, USA) for NF-κB luciferase reporter assay. Transfected cells were seeded in a 96-well plate at a density of 5×10^4 cells/well. After 24 h incubation at 37 °C, the cells were treated with various concentrations of compounds tested and TNF-α (1 ng/ml) for 24 h. Luciferase assay was performed according to the instructions of the manufacturer (Promega, Madison, WI, USA). Luciferase activity was determined in a Microlumat Plus luminometer (EG&G Berthold, Bad Wildbad, Germany) by injecting 100 μl of assay buffer containing luciferin and measuring light emission for 10 s. The results were normalized to the activity of β-galactosidase expressed by cotransfected lacZ gene under the control of a constitutive promoter.

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References

Chattopadhyay, S.K., Rao, K.V., 1987. Chemistry of *Saururus cernuus* IV: cyclooctadiene systems derived from austrobailignan-5. Tetrahedron 43, 669–678.

Dale, J.A., Mosher, H.S., 1973. Nuclear magnetic resonance enantiomer regents. Configurational correlations via nuclear magnetic resonance chemical shifts of diastereomeric mandelate, *O*-methylmandelate, and α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters. J. Am. Chem. Soc. 95, 512–519.

Fonseca, S.F., Barata, L.E.S., Ruveda, E.A., 1979. ^{13}C nuclear magnetic resonance spectral and conformational analysis of naturally occurring tetrahydrofuran lignans. Can. J. Chem. 57, 441–443.

Ghosh, S., May, M.J., Kopp, E.B., 1998. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. Ann. Rev. Immunol. 16, 225–260.

Hwang, B.Y., Lee, J.H., Koo, T.H., Kim, H.S., Hong, Y.S., Ro, J.S., Lee, K.S., Lee, J.J., 2001. Kaurane diterpenes from *Isodon japonicus* inhibit nitric oxide and prostaglandin E_2 production and NF-κB activation in LPS-stimulated macrophage RAW264.7 cells. Planta Medica 67, 406–410.

Hwang, B.Y., Lee, J.-H., Nam, J.B., Kim, H.S., Hong, Y.S., Lee, J.J., 2002. Two new furanoditerpenes from *Saururus chinenesis* and their effects on the activation of peroxisome proliferator-activated receptor γ. J. Nat. Prod. 65, 616–617.

Jin, H.Z., Hwang, B.Y., Kim, H.S., Lee, J.H., Kim, Y.H., Lee, J.J., 2002. Antiinflammatory constituents of *Celastrus orbiculatus* inhibit the NF-κB activation and NO production. J. Nat. Prod. 65, 89–91.

Kasahara, H., Miyazawa, M., Kameoka, H., 1995. Absolute configuration of 8-*O*-4′-neolignans from *Myristica fragrans*. Phytochemistry 40, 1515–1517.

Kim, J.G., 1989. Illustrated Natural Drugs Encyclopedia. Nam san dang, Seoul.

Koo, T.H., Lee, J.-H., Park, Y.J., Hong, Y.-S., Kim, H.S., Kim, K.-W., Lee, J.J., 2000. A sequiterpene lactone costunolide from *Magnolia grandiflora* inhibits NF-κB by targeting IκB phosphorylation. Planta Medica 67, 103–107.

Kraft, C., Jenett-Siems, K., Köhler, I., Tofen-Reblin, B., Siems, K., Bienzle, U., Eich, E., 2002. Antiplasmodial activity of sesquilignans and sesquineolignans from *Bonamia spectabilis*. Phytochemistry 60, 167–173.

Kubanek, J., Fenical, W., Hay, M.E., Brown, P.J., Lindquist, N., 2000. Two antifeedant lignans from the freshwater macrophyte *Saururus cernuus*. Phytochemistry 54, 281–287.

Lee, J.-H., Koo, T.H., Hwang, B.Y., Lee, J.J., 2002. Kaurane diterpene, kamebakaurin, inhibits NF-κB by directly targeting the DNA-binding activity of p50 and blocks the expression of antiapoptotic NF-κB target genes. J. Biol. Chem. 277, 18411–18420.

Matsushita, H., Miyase, T., Ueno, A., 1991. Lignan and terpene glycosides from *Epimedium sagittatum*. Phytochemistry 30, 2025–2027.

Miyazawa, M., Kasahara, H., Kameoka, H., 1996. O-demethylation of 7,7′-epoxylignans by *Aspergillus niger*. Phytochemistry 43, 111–113.

Prasad, A.K., Tyagi, O.D., Wengel, J., Boll, P.M., Olsen, C.E., Bisht, K.S., Singh, A., Sarangi, A., Kumar, R., Jain, S.C., Parmar, V.S., 1995. Neolignans and a lignan from *Piper clarkii*. Phytochemistry 39, 655–658.

Rajbhandari, I., Takamatsu, S., Nagle, D.G., 2001. A new dehydrogeranylgeraniol antioxidant from *Saururus cernuus* that inhibits intracellular reactive oxygen species (ROS)-catalyzed oxidation within HL-60 cells. J. Nat. Prod. 64, 693–695.

Rao, K.V., Alvarez, F.M., 1982. Chemistry of *Saururus cernuus*. I. Saucernetin, a new neolignan. J. Nat. Prod. 45, 393–397.

Rao, K.V., Alvarez, F.M., 1983. Manassantins A/B and saucerneol: novel biologically active lignoids from *Saururus cernuus*. Tetrahedron Lett. 24, 4947–4950.

Rao, K.V., Alvarez, F.M., 1985. Chemistry of *Saururus cernuus*. III. Some reactions of the diarylbutane-type neolignans. J. Nat. Prod. 48, 592597.

Rao, K.V., Rao, N.S.P., 1990. Chemistry of *Saururus cernuus*. VI. Three new neolignans. J. Nat. Prod. 53, 212–215.

Rao, K.V., Reddy, G.C.S., 1990. Chemistry of *Saururus cernuus*. V. sauristolactam and other nitrogenous constituents. J. Nat. Prod. 53, 309–312.

Rao, K.V., Oruganty, R.S., 1997. An improved method for the isolation of the lignan constituents of *Saururus cernuus* by reverse phase column chromatography. J. Liq. Chromatogr. Relat. Technol. 20, 3121–3134.

Rao, K.V., Puri, V.N., Diwan, P.K., Alvarez, F.M., 1987. Preliminary evaluation of manassantin A, a potential neuroleptic agent from *Saururus cernuus*. Pharmacol. Res. Commun. 19, 629–638.

Rimando, A.M., Pezzuto, J.M., Farnsworth, N.R., Santisuk, T., Reutrakul, V., Kawanishi, K., 1994. New lignans from *Anogeissus acuminata* with HIV-1 reverse transcriptase inhibitory activity. J. Nat. Prod. 57, 896–904.

Sarkanen, K.V., Wallis, A.F.A., 1973. PMR analysis and conformation of 2,5-bis-(3,4,5-trimethoxyphenyl)-3,4-dimethyltetrahydrofuran isomers (1). J. Heterocycl. Chem. 10, 1025–1027.

Su, B.-N., Takaishi, Y., Kusumi, T., 1999. Morinols A-L, twelve novel sesquineolignans and neolignans with a new carbon skeleton from *Morina chinensis*. Tetrahedron 55, 14571–14586.

Sung, S.H., Kim, Y.C., 2000. Hepatoprotective diastereomeric lignans from *Saururus chinensis* herbs. J. Nat. Prod. 63, 1019–1021.

Sung, S.H., Kwon, S.H., Cho, N.J., Kim, Y.C., 1997. Hepatoprotective flavonol glycosides of *Saururus chinensis* herbs. Phytother. Res. 11, 500–503.

Sung, S.H., Huh, M.S., Kim, Y.C., 2001. New tetrahydrofuran-type sesquilignans of *Saururus chinensis* root. Chem. Pharm. Bull. 49, 1192–1194.

Tak, P.P., Firestein, G.S., 2001. NF-κB: a key role in inflammatory diseases. J. Clin. Invest. 107, 7–11.

Tofen, B., Jenett-Siems, K., Siems, K., Jakupovic, J., Eich, E., 2000. Bonaspectins and neobonaspectins, first sesquilignans and sesquineolignans from a convolvulaceous species. Phytochemistry 53, 119–128.

Tsai, I.-L., Hsieh, C.-F., Duh, C.-Y., Chen, I.-S., 1996. Cytotoxic neolignans from *Persea obovatifolia*. Phytochemistry 43, 1261–1263.

Wang, E.-C., Shih, M.-H., Liu, M.-C., Chen, M.-T., Lee, G.-H., 1996. Studies on constituents of *Saururus chinensis*. Heterocycles 43, 969–975.

Yamamoto, Y., Gaynor, R.B., 2001. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. J. Clin. Invest. 107, 135–142.

Yue, J.-M., Chen, Y.-Z., Hua, S.-M., Cheng, J.-L., Cui, Y.-X., 1989. Ganschisandrine, A lignan from *Schisandra sphenanthera*. Phytochemistry 28, 1774–1776.

Zacchino, S.A., 1994. Enantioselective route to threo 8-O-4′-type neolignans: synthesis of (−)-virolin. J. Nat. Prod. 57, 446–451.