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Neolignans from *Piper kadsura* and their anti-neuroinflammatory activity

Ki Hyun Kim [a], Jung Wook Choi [a], Sang Keun Ha [b], Sun Yeou Kim [b], Kang Ro Lee [a,*]

[a] Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, 300 Chonchon-dong, Jangan-ku, Suwon 440-746, Republic of Korea
[b] East–West Medical Science Integrated Research Center, Graduate School of East–West Medical Science, Kyung Hee University, #1 Seocheon-dong, Kihung-ku, Yongin-City, Kyungki-Do 446-701, Republic of Korea

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ABSTRACT

Bioassay-guided column chromatographic separation of the methanolic extract of dried aerial parts of *Piper kadsura* (Piperaceae) led to the isolation of a new neolignan, piperkadsin C (**1**), together with eight known neolignans (**2**–**9**). The structures of the isolated compounds were elucidated by combined spectroscopic methods. The anti-neuroinflammatory activities of these compounds were evaluated by assessing nitric oxide (NO) production in LPS-activated BV-2 cells, a microglia cell line. Piperkadsin C (**1**) and futoquinol (**2**) potently inhibited NO production with an IC_{50} value of 14.6 and 16.8 μM in microglia cells, respectively. Compounds **3**, **4**, **5**, **8**, and **9** also exhibited moderate inhibition of NO production in BV-2 cells.

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Piper kadsura Ohwi (Piperaceae) is a medicinal vine-like plant that covers rocks and trees distributed in coastal forest regions of Korea, Japan, Taiwan, and China.[1] The stem part of this plant, known as haifengteng, is widely used in the Chinese herbal medicinal prescriptions for the treatment of asthma and arthritic conditions.[2] Moreover, its fruit is used for cooking, similar to pepper, and for improving digestive function in Japan.[3] Various compounds have been isolated from this plant, including amides, lignans, terpenes, and cyclohexanes.[2,4] In particular, a number of neolignans, such as kadsurenone, kazurenin M, piperenone, (−)-denudatin B, piperkadsin A, and futokadsurin A, have been isolated from *P. kadsura*.[2,5–7] Among the components, kadsurenone showed anti-platelet activating factor (PAF) activity[8] and piperenone blocks insect feeding.[9]

Much research has focused on drug discovery for neurodegenerative diseases, including cluster headaches, Parkinson's disease, Alzheimer's disease, trigeminal neuralgia, and epilepsy. Natural spicy plants, such as *Capsicum annum*, *Piper nigrum*, *Zingiber officinale*, and *Allium sativum*, can prevent or delay the onset and progression of neurodegenerative disorders,[10–14] with piperine isolated from *P. nigrum* (Piperaceae) exhibiting prominent nootropic activity.[14] Other spicy plants may help prevent neurodegenerative disorders via anti-neuroinflammatory activity. Therefore, in the course of our continuing search for biologically active compounds from natural Korean medicinal sources, we investigated the active principles of *P. kadsura* for anti-neuroinflammatory activity, since *P. kadsura* gave off strong spicy incense.

To identify the active ingredients responsible for anti-neuroinflammatory activity, the MeOH extract of *P. kadsura* was fractionated by solvent (*n*-hexane, $CHCl_3$, *n*-BuOH), and then each fraction was evaluated by assessing nitric oxide (NO) production in LPS-activated BV-2 cells, a microglia cell line. The *n*-hexane layer showed a potent inhibitory effect on NO production. The active *n*-hexane soluble fraction was separated on a silica gel and C-18 open-column chromatography, followed by semi-preparative HPLC to afford a new neolignan, piperkadsin C (**1**), together with eight known neolignans (**2**–**9**). The known isolated compounds were identified as futoquinol (**2**),[15] wallichinine (**3**),[16] kadsurenone (**4**),[5] denudatin A (**5**),[17] kadsurenin L (**6**),[18] isofutoquinol A (**7**),[7] futokadsurin C (**8**),[6] and 2-(3′-allyl-2′,6′-dimethoxy-phenyloxy)-1-acetoxy-(3,4-dimethoxy-phenyl)-propyl ester (**9**)[19] by comparisons with previously published data. This is the first report of compounds **5** and **9** being isolated from this plant. We elucidated the structure of the new neolignan, piperkadsin C (**1**), and evaluated the anti-neuroinflammatory activity of **1**–**9**.

Compound **1** was obtained as a viscose gum with optical rotation $[\alpha]_D^{25}$ −26.2 (*c* 0.1, $CHCl_3$). IR (ν_{max} 1740, 1636 cm^{-1}) and UV (λ_{max} 235, 280 nm) spectra showed phenyl and α,β-unsaturated carbonyl absorption systems. In the positive mode FABMS of **1**, a quasimolecular ion peak at 340 $[M]^+$ was observed, and the molecular formula of **1** was determined to be $C_{20}H_{20}O_5$ by HR-FABMS: observed, 340.1315 (calculated for $C_{20}H_{20}O_5$, 340.1311). The ^1H NMR spectrum of **1** (Table 1) indicated the presence of a set of three aromatic protons at δ 6.75 (1H, dd, *J* = 1.5, 7.5 Hz, H-6), 6.76 (1H, d, *J* = 7.5 Hz, H-5), and 6.92 (1H, d, *J* = 1.5 Hz, H-2), an olefinic proton at δ 7.00 (1H, s, H-2′), one methoxy group at δ 3.82 (3H,

* Corresponding author. Tel.: +82 31 290 7710; fax: +82 31 290 7730.
E-mail address: krlee@skku.ac.kr (K.R. Lee).

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Table 1
^{1}H and ^{13}C NMR data for compound **1** (δ in ppm, 500 MHz for ^{1}H and 125 MHz for ^{13}C, in $CDCl_3$)

Number	1		
	δ_H	δ_C	HMBC
1		133.9, C	
2	6.92 (d, 1.5)	108.8, CH	1, 3, 4, 6, 7
3		147.6, C	
4		146.8, C	
5	6.76 (d, 7.5)	108.1, CH	1, 3, 4, 6
6	6.75 (dd, 1.5, 7.5)	122.7, CH	1, 2, 4, 5, 7
7	3.64 (s)	65.3, CH	1, 2, 6, 8, 9, 3′, 5′, 6′
8		50.4, C	
9	0.96 (s)	16.8, CH_3	7, 8, 2′, 3′, 4′, 5′
1′		132.6, C	
2′	7.00 (s)	157.2, CH	8, 1′, 3′, 4′, 6′, 7′
3′		131.7, C	
4′		147.0, C	
5′	3.36 (s)	58.6, CH	7, 8, 9, 1′, 3′, 4′, 6′
6′		197.2, C	
7′	2.91 (dd, 1.5, 5.5)	33.0, CH_2	1′, 2′, 6′, 8′, 9′
8′	5.82 (m)	135.5, CH	1′, 7′, 9′
9′	5.06 (dd, 1.5, 5.5)	116.7, CH_2	1′, 7′, 8′
	5.08 (dd, 1.5, 12.0)		
O–CH_2–O	5.94 (s)	101.1, CH_2	3, 4
OCH_3-4′	3.82 (s)	59.2, CH_3	4′

Assignments were confirmed by ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra.

s), one methylenedioxy group at δ 5.94 (2H, s), one methine and one oxymethine protons at δ 3.36 (1H, s, H-5′), 3.64 (1H, s, H-7), and a methyl group at δ 0.96 (1H, s, H-9). The other methylene at δ 2.91 (2H, dd, J = 1.5, 5.5 Hz, H-7′) and olefinic protons at δ 5.06 (1H, dd, J = 1.5, 5.5 Hz, H-9′a), 5.08 (1H, dd, J = 1.5, 12.0 Hz, H-9′b), and 5.82 (1H, m, H-8′) were assigned to the allyl group. These data were similar to those of neolignans isolated from this plant.[2,18] As expected, the ^{13}C NMR spectra showed 20 signals, including one carbonyl carbon signal at δ 197.2 (C-6′), six carbons for an aromatic ring, six carbons for olefinic carbon, one quaternary carbon at δ 50.4 (C-8), two methine carbons at δ 58.6 (C-5′), 65.3 (C-7), one methylene carbon at δ 33.0 (C-7′), one methyl carbon at δ 16.8 (C-9), one methoxy carbon at δ 59.2 (OCH_3-4′), and one methylenedioxy carbon at δ 101.1 (O–CH_2–O). These spectroscopic data suggested that the structure of **1** may be a neolignan skeleton.[2,18]

The full NMR assignments and connectivites of **1** were determined by ^{1}H–^{1}H COSY, HMQC, and HMBC spectroscopic data analysis. The presence of the 3,4-methylenedioxy aromatic ring system was confirmed by HMBC data analysis (Fig. 2). The HMQC spectrum revealed that the proton at δ 3.64 (H-7) is attached to the carbon at δ

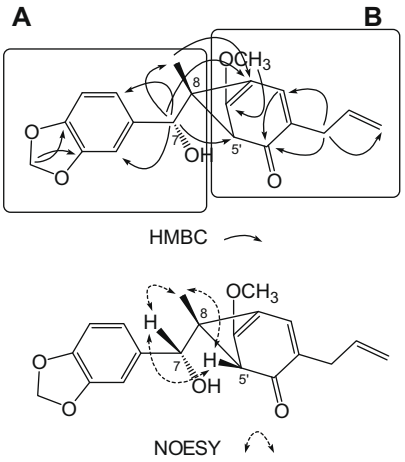

Figure 2. Key 2D-NMR (HMBC, NOESY) correlations of **1**.

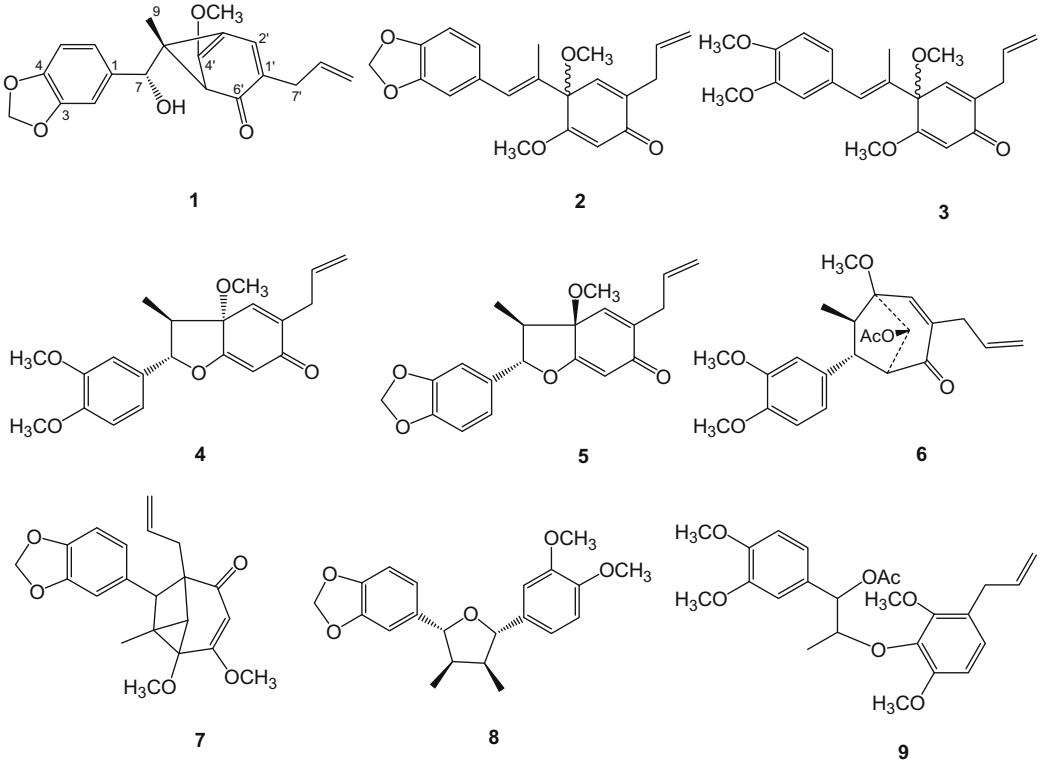

Figure 1. The structures of compounds **1**–**9** isolated from *P. kadsura*.

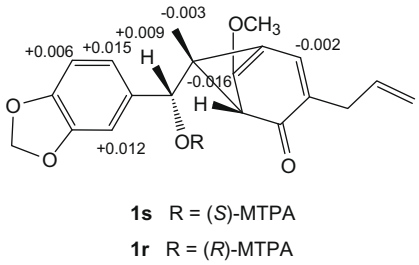

Figure 3. Difference in the Δ_{SR} ($\delta_S - \delta_R$) values of the MTPA esters of **1**.

65.3 (C-7), and the HMBC spectrum showed that H-7 was correlated to C-1, C-2, C-6, C-8, C-9, suggesting that the 3,4-methylenedioxyphenyl-7-hydroxy-8-substituted-propanol group (unit A) was present.[20] The HMBC spectrum indicated that H-7′ was correlated to C-1′, C-2′, C-6′, C-8′, C-9′, NMR data of which were similar to those of the partial structure of kadsurenin L.[18] This subunit was further connected by HMBC data, which revealed presence of 4-methoxy-3,5-disubstitued-cyclohexa-1,3-dienone group (unit B) from HMBC correlations between OCH_3-4′ and C-4′, between H-2′ and C-1′, C-3′, C-4′, C-6′, C-7′, and between H-5′ and C-1′, C-3′, C-4′, C-6′ (Fig. 2). Finally, correlations observed in the HMBC spectrum from H-7 to C-3′, C-5′, C-6′, from H-9 to C-2′, C-3′, C-4′, C-5′, from H-2′ to C-8, and from H-5′ to C-7, C-8, C-9 confirmed the proposed structure of **1** (Fig. 2). The NOESY spectrum displayed correlations between H-9 and H-7, between H-9 and H-5′, and between H-7 and H-5′, and vice versa, but correlations were not observed between H-9 and H-2 or H-6. This led to the assignment of a *trans* relation between the 3,4-methylenedioxyphenyl group at C-7 and the methyl at C-8, and a *cis* relation between the methyl at C-8 and H-5′ (Fig. 2). The absolute configuration of C-7 in **1** was established by a modified Mosher's method.[21] Compound **1** was subsequently esterified by (*S*)- and (*R*)-MTPA chlorides to yield the (*R*)- and (*S*)-MTPA esters, respectively. Analysis of ^{1}H NMR chemical shift differences between *S*- and *R*-MTPA ($\delta_S - \delta_R$) is shown in Figure 3, indicating that the absolute stereochemistry at C-7 of **1** was the *S* configuration. We therefore propose the structure of **1** in Figure 1, which is similar to those of macrophyllin.[22] To the

Table 2
Inhibitory activities of isolated compounds from *P. kadsura* on the NO production in LPS-activated BV-2 cells

Compounds	Inhibition[a] (%)	IC_{50} (μM)
n-Hexane layer	67.2	14.0[b]
1	60.8	14.6
2	55.3	16.8
3	18.5	45.6
4	21.3[c]	–
5	23.2[c]	–
6	na[d]	–
7	na	–
8	19.7	43.1
9	27.2	33.2
L-NMMA[e]	55.3	16.8

[a] Values mean the inhibition of NO production relative to the LPS control at 20 μM concentration of compounds ($n = 3$).
[b] Value in μg/ml.
[c] Cytotoxic effect was observed.
[d] na means not active.
[e] The L-NMMA was used as a positive control.

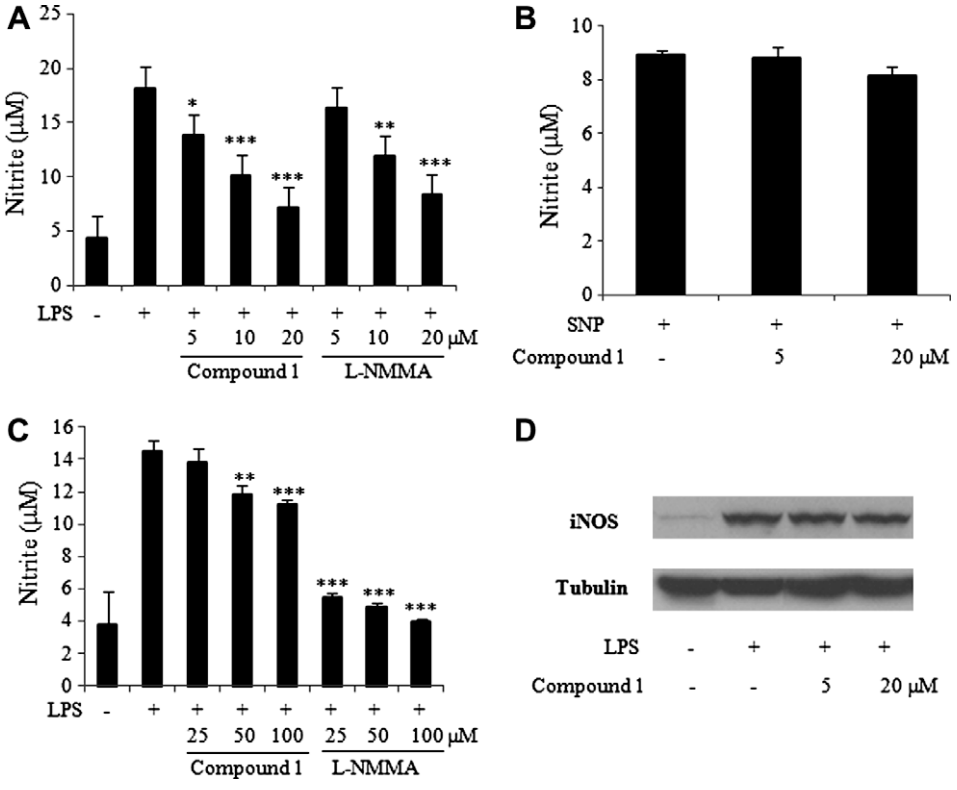

Figure 4. The inhibitory effect of compound **1** on NO production in LPS-activated BV-2 cells. (A) Effect of compound **1** on LPS-induced NO production in BV-2 cells. Nitrite was measured using the Griess reaction at 24 h after treatment with LPS (100 ng/ml) in the presence or absence of compound **1**. (B) Effect of compound **1** on NO scavenging activity after a mixture of SNP and compound **1** was incubated at 25 °C for 150 min. (C) Effect of compound **1** on iNOS enzyme activity in BV-2 cells. (D) Effect of compound **1** on iNOS expression in BV-2 cells. iNOS protein was detected using Western blot analysis at 6 h after treatment with LPS (100 ng/ml) in the presence or absence of compound **1** (5, 20 μM). L-NMMA was used as a positive control. All data are presented as the mean ± S.D. $^*p$ <0.05, $^{**}p$ <0.01 and $^{***}p$ <0.001 indicate statistically significant differences from treatment with LPS alone.

best of our knowledge, compound **1** represents an unprecedented rearranged neolignan of macrophyllin-type bearing C-8 bridged to C-3′ and C-5′. Thus, compound **1** was determined to be a new neolignan, (7*S*)-7-hydroxy-3,4-methylenedioxyphenyl-4′-methoxy-6′-oxo-Δ-$^{1',3',8'}$-8.3′.5′-lignan, namely, piperkadsin C.

Neuroinflammation can cause neuronal damage in neurodegenerative diseases.[23] Brain inflammation results from activation of microglia, the resident immune cells. The activated microglia cells produce excessive inflammatory substances such as NO, various cytokines, and prostaglandins, to cause brain injury and neurodegenerative diseases.[24] Therefore, modulation of activated microglia can protect neurons from exposure to inflammatory substances.

Here, we investigated the anti-neuroinflammatory effects of compounds (**1**–**9**) isolated from *P. kadsura* in LPS-activated BV-2 cells, a microglia cell line (Table 2).[25] Compounds **1** and **2** dose-dependently inhibited NO production, with IC_{50} values of 14.6 and 16.8 μM in LPS-activated BV-2 cells, respectively. Compound **1** was more potent than N^G-monomethyl-L-arginine (L-NMMA), an inducible NO synthase (iNOS) inhibitor, in inhibiting NO production (Fig. 4A). Compounds **3**, **4**, **5**, **8**, and **9** also mildly inhibited NO production.

To further understand the mechanism of the action of compound **1**, we next evaluated the effect of compound **1** on NO scavenging activity,[25] iNOS enzyme activity,[26] and iNOS protein expression.[25] Compound **1** did not affect NO scavenging activity or iNOS protein expression (Fig. 4), but inhibited iNOS enzyme activity. By the way, piperkadsin C (**1**) exhibited lower inhibitory effect on iNOS enzyme activity than that of L-NMMA, a classical iNOS inhibitor (Fig. 4C). These results suggest that piperkadsin C (**1**) may have other inhibitory mechanism on NO production. Further investigations are required to elucidate the inhibitory mechanism of **1** on neuroinflammation.

In the structure–activity relationship (SAR), it appears that the methylenedioxy function at C-3/C-4 in **1** and **2** improves anti-neuroinflammatory activity, compared to compound **3**. Compounds **5**, **7**, and **8** possess this moiety but were inactive, perhaps because of other substituents or skeletal differences. The skeleton of hydrobenzofuranoids (**4** and **5**) are cytotoxic but could also inhibit NO production. The veratryl moiety could also decrease inhibitory activity, as compounds (**3**, **8**, and **9**) were less active than **1** and **2**. Inhibition of NO production might be more positively influenced by linear-type than cyclic-type linkages of neolignan, since **6** and **7** were inactive and possess bicyclooctene or tricyclooctene rings. This structure–activity data could be valuable in future synthetic and pharmacological studies.

In summary, piperkadsin C (**1**) isolated from *P. kadsura* exhibited anti-neuroinflammatory activity by suppressing the release of NO in LPS-stimulated microglia cells. Therefore, compound **1** may have beneficial therapeutic potential for neuroinflammatory diseases through inhibition of microglial activation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.016.

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