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Anti-neuroinflammatory diarylheptanoids from the rhizomes of *Dioscorea nipponica*

Kyeong Wan Woo [a], Eunjung Moon [b,c], Oh Wook Kwon [d], Sung Ok Lee [d], Sun Yeou Kim [b,c], Sang Zin Choi [e], Mi Won Son [e], Kang Ro Lee [a,*]

[a] Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, 300 Chonchon-dong, Jangan-ku, Suwon, Gyeonggi-do 440-746, Republic of Korea
[b] Gachon Institute of Pharmaceutical Science, Gachon University, #191 Hambakmoe-ro, Yeonsu-gu, Incheon 406-799, Republic of Korea
[c] College of Pharmacy, Gachon University, #191 Hambakmoe-ro, Yeonsu-gu, Incheon 406-799, Republic of Korea
[d] Graduate School of East-West Medical Science, Kyung Hee University Global Campus, #1732 Deogyeong-daero, Giheung-gu, Yongin, Gyeonggi-do 446-701, Republic of Korea
[e] Dong-A Pharm Institute, Kiheung, Yongin, Gyeonggi-do 449-905, Republic of Korea

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ABSTRACT

In a continuing search for bioactive constituents from Dioscoreaceae medicinal plants, two new cyclic diarylheptanoids, diosniponol A (**1**) and B (**2**), together with 10 known compounds (**3**–**12**) were isolated from the rhizomes of *Dioscorea nipponica*. The structures of these new compounds were determined by spectroscopic analyses, including extensive two-dimensional nuclear magnetic resonance, high-resolution mass spectrometry, and optical rotation. All isolated compounds **1**–**12** were evaluated for their effects on nitric oxide (NO) production in murine microglia cell line BV-2. Compounds **8** and **11** showed potent inhibitory activities on NO production (IC_{50} 13.36 and 14.36 μM, respectively) without cell toxicity in lipopolysaccharide-activated BV-2 cells.

Microglia are resident immune cells of the central nervous system. Activated microglia induce production of neurotoxic factors including tumor necrosis factor alpha (TNF-α), nitric oxide (NO), and prostaglandin E_2 (PGE_2).[1,2] NO is a toxic molecule, and is related with neuronal death and central nervous system impairment.[3] In mammals, NO plays a pivotal role in pathophysiological processes including regulation of inflammatory response in neurodegenerative diseases.[4]

Dioscorea nipponica (Dioscoreaceae) is perennial lianas widely distributed in Southeast Asia, Korea, China, and Japan.[5] The rhizomes of *D. nipponica* have been used as Korean traditional medicine for the treatment rheumatism, asthma, and bronchitis.[6] Various compounds including steroidal saponins,[7,8] phenanthrenes,[9,10] and phenolic derivatives[11,12] have been isolated from this plant. Recently, we reported bioactive constituents from *Dioscorea japonica* that included cytotoxic withanolides and nerve growth factor inducing furostanol saponins.[13,14] In our continuing search for bioactive components from Dioscoreaceae medicinal plants, we investigated the rhizomes of *D. nipponica*. Presently, rhizomes (10 kg) were extracted with 50% aqueous ethanol (EtOH) at room temperature and filtered. The filtrate was evaporated under reduced pressure to give EtOH extract (1 kg), which was suspended in 800 mL water and solvent-partitioned to yield *n*-hexane (1 g), chloroform (35 g), ethyl acetate (10 g), and *n*-butanol (200 g) fractions. Repeated column chromatographic purification (Supplementary data) of the $CHCl_3$ and EtOAc fractions led to isolation of two new cyclic diarylheptanoids (**1**–**2**), named diosniponol A and B, together with ten known compounds (**3**–**12**) (Fig. 1).

Compound (**1**) was obtained as a colorless gum. The molecular formula of **1** was determined to be $C_{19}H_{22}O_4$ by the positive mode high resolution-fast atom bombardment mass spectrometry (HR-FABMS) data at *m/z* 375.1807 $[M+H]^+$ (calcd for 375.1805). The ^1H NMR spectrum (Table 1) of **1** indicated the presence of six aromatic ring protons [δ_H 7.08 (1H, d, *J* = 2.0 Hz, H-2′), 6.85 (1H, dd, *J* = 7.5, 2.0 Hz, H-6′), 6.79 (1H, d, *J* = 2.0 Hz, H-2″), 6.78 (1H, d, *J* = 8.5 Hz, H-5′), 6.71 (1H, d, *J* = 8.0 Hz, H-5″), and 6.64 (1H, d, *J* = 8.0, 2.0 Hz, H-6″)], three oxygenated methine protons [δ_H 4.28 (1H, dd, *J* = 11.0, 2.0 Hz, H-1), 3.85 (1H, m, H-3), and 3.44 (1H, dddd, *J* = 11.0, 8.0, 4.5, 2.0 Hz, H-5)], and four methylene groups [δ_H 2.69 (1H, m, H-7a), 2.63 (1H, m, H-7b), 2.10 (1H, dddd, *J* = 12.0, 4.0, 2.0, 2.0 Hz, H-2eq), 1.96 (1H, dddd, *J* = 12.0, 4.0, 2.0, 2.0 Hz, H-4eq), 1.84 (1H, m, H-6a), 1.76 (1H, m, H-6b), 1.38 (1H, ddd, *J* = 11.0, 11.0, 11.0 Hz, H-2ax), and 1.20 (1H, ddd, *J* = 12.0, 11.0, 11.0 Hz, H-4ax)], and two methoxy protons [δ_H 3.84 (3H, s, 3′-OCH_3) and 3.79 (3H, s, 3″-OCH_3)]. The ^{13}C NMR spectrum

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

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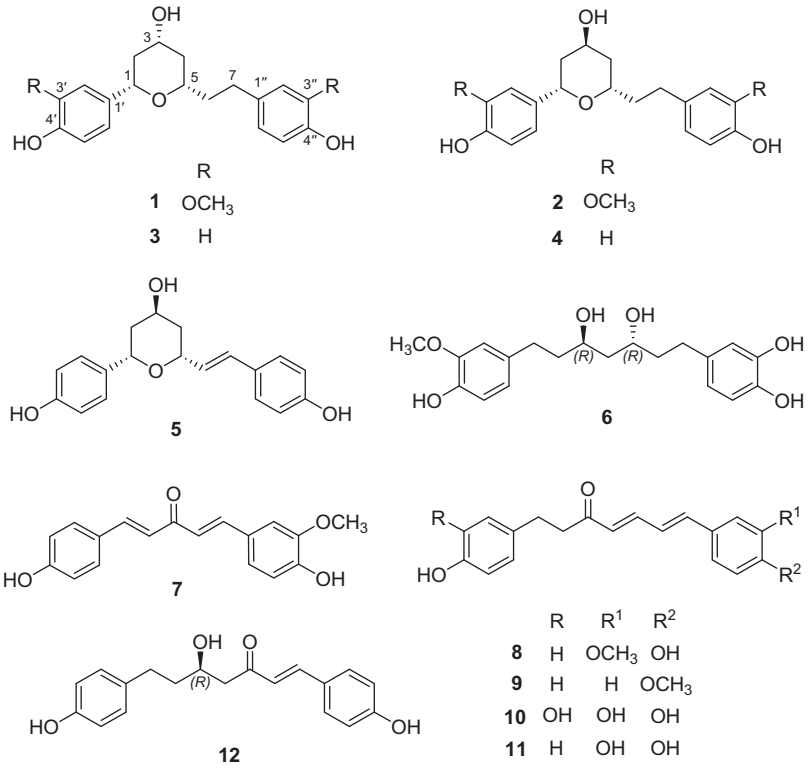

Figure 1. Chemical structures of compounds **1**–**12** from the rhizome of *D. nipponica*.

Table 1
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compounds **1** and **2**[a]

Position	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	4.28 (dd, 11.0, 2.0)	78.2	4.75 (dd, 11.5, 2.0)	73.3
2ax	1.38 (ddd, 11.0 11.0, 11.0)	44.5	1.71 (dddd, 11.0, 3.0, 3.0, 2.0)	40.9
2eq	2.10 (dddd, 12.0, 4.0, 2.0, 2.0)		1.86 (ddd, 11.5, 3.0, 2.5)	
3	3.85 (m)	68.5	4.23 (br s)	64.0
4ax	1.20 (ddd, 12.0, 11.0, 11.0)	42.2	1.50 (ddd, 14.0, 11.5, 2.5)	38.5
4eq	1.96 (dddd, 12.0, 4.0, 2.0, 2.0)		1.69 (dddd, 13.0, 2.5, 2.5, 2.5)	
5	3.44 (dddd, 11.0, 8.0, 4.5, 2.0)	75.6	3.93 (dddd, 12.0, 8.5, 4.5, 2.5)	70.7
6a	1.84 (m)	39.1	1.80 (m)	38.4
6b	1.76 (m)		1.66 (m)	
7a	2.69 (m)	32.1	2.64 (m)	31.1
7b	2.63 (m)		2.61 (m)	
1′	—	135.7	—	135.6
2′	7.08 (d, 2.0)	110.6	6.99 (d, 2.0)	109.6
3′	—	148.1	—	147.0
4′	—	146.6	—	145.7
5′	6.78 (d, 8.5)	115.4	6.78 (d, 8.0)	114.5
6′	6.85 (dd, 7.5, 2.0)	119.5	6.83 (dd, 8.0, 2.0)	118.4
1″	—	134.5	—	133.7
2″	6.79 (d, 2.0)	112.9	6.82 (d, 2.0)	112.0
3″	—	148.2	—	147.0
4″	—	145.5	—	144.6
5″	6.71 (d, 8.0)	115.6	6.71 (d, 8.0)	114.8
6″	6.64 (dd, 8.0, 2.0)	121.6	6.64 (dd, 8.0, 2.0)	120.6
3′-OCH_3	3.84 (s)	56.3	3.84 (s)	55.3
3″-OCH_3	3.79 (s)	56.2	3.78 (s)	55.2

[a] ^{1}H and ^{13}C NMR data of **1** and **2** in $(CD_3)_2CO$ (δ values; *J* values in Hz are in parentheses).

revealed resonances for 21 carbons attributable to six aromatic methine carbons (δ_C 121.6, 119.6, 115.6, 115.4, 112.9, and 110.6), six quaternary carbons (δ_C 148.2, 148.1, 146.6, 145.5, 135.7, and 134.5), three oxygenated carbons (δ_C 78.2, 75.6, and 68.5), four methylene carbons (δ_C 44.5, 42.2, 39.1, and 32.1), and two methoxy carbons (δ_C 56.3 and 56.2). The ^{1}H and ^{13}C NMR data of compound **1** were very similar to those of compound **3** isolated from *Dioscorea villosa*.[15] A major difference between them was the substitution pattern of the two aromatic rings. The location of two methoxy groups were confirmed to be at C-3′ and C-3″, respectively by heteronuclear multiple bond correlation spectroscopy (HMBC) cross-peaks of 3′-OCH_3/C-3′ and 3″-OCH_3/C-3″, respectively (Fig. 2). The

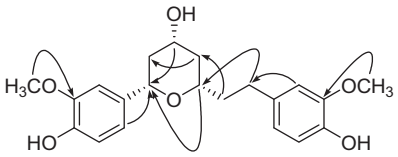

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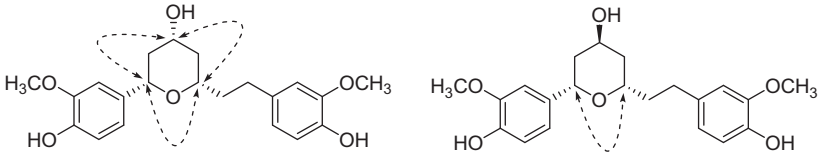
Figure 2. Key HMBC correlations of compound **1**.



1 2

Figure 3. Key NOE correlations of compounds **1** and **2**.

relative stereochemistry was assumed to be the same as that of **3**, based on the *J* values of **3**.[15,16] Nuclear Overhauser effect spectroscopy (NOESY) correlations reconfirmed the stereochemistry of **3** (Fig. 3).[16–18] The absolute configuration of **1** was established as 1*S*,3*R*,5*S* by comparison of its optical rotation value with that of **3**.[15] Thus, the structure of **1** was determined as (1*S*,3*R*,5*S*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,5-epoxy-3-hydroxyheptane and named diosniponol A.

Compound **2**, named diosniponol B, was isolated as a yellowish gum. It showed the same molecular formula as **1** (HR-FABMS *m/z* 375.1810 $[M+H]^+$) and ^1H and ^{13}C NMR spectra of **2** were almost similar to those of **1**, except for the difference in the chemical shifts of the pyrane ring moiety in the ^1H and ^{13}C NMR spectrum (Table 1), implying that compounds **1** and **2** are to be stereoisomers at C-3.[15,16] The relative stereochemistry of **2** was determined to be same by comparing *J* values of **4**[15] and the NOESY experiment (Fig. 3).[16,19] The absolute configuration of **2** was determined to be 1*S*,3*S*,5*S* by comparing the positive optical rotation value.[15] From the foregoing, the structure of **2** was established to be (1*S*,3*S*,5*S*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,5-epoxy-3-hydroxyheptane.

The isolated known compounds were identified as (1*S*,3*R*,5*S*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (**3**), (1*S*,3*S*,5*S*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (**4**), (1*S*,3*S*,5*R*,6*E*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxy-hept-6-one (**5**),[15] (3*R*,5*R*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)heptanes (**6**),[20] 1-(4-hydroxy-3methoxyphenyl)-5-(4-hydroxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3one (**7**),[21] tsaokoarylone (**8**),[22] 1,7-bis(4-hydroxyphenyl)hepta-4*E*, 6*E*-dien-3-one (**9**),[23] 1,7-bis(3,4-dihydroxyphenyl)hepta-4*E*, 6*E*-dien-3-one (**10**),[24] (4*E*,6*E*)-1-(3′,4′-dihydroxyphenyl)-7-(4″-hydroxyphenyl)-hepta-4,6-dien-3-one (**11**),[25] and 5-hydroxy-1-(4′-hydroxyphenyl)-7-(4″-hydroxyphenyl)-hepta-1-en-3-one (**12**)[26] were identified by comparing the ^1H and ^{13}C NMR, and MS spectra with reported data.

The anti-inflammatory effects of diarylheptanoids have been investigated.[27–29] Therefore, we tested the anti-neuroinflammatory effects of diarylheptanoids derivatives (**1**–**12**) isolated from *D. nipponica,* based on the evaluation of the inhibitory activity on NO production in lipopolysaccharide (LPS)-activated murine microglia BV-2 cells. Among the tested compounds, compounds **5**, **7**, **9**, and **10** inhibited NO levels in the medium with IC_{50} of 18.58, 5.99, 8.44, and 7.84 μM, respectively (Table 2). However, these compounds induced significant cytotoxicity at a concentration of 20 μM. Compounds **8** and **11** significantly decreased the production of NO, with an IC_{50} of 13.36 and 14.36 μM, respectively, without evident cell toxicity. These compounds were more effective than N^G-monomethyl-L-arginine (L-NMMA), an inducible NO synthase inhibitor.[30] Therefore, we suggest that compounds **8** and **11** isolated from the rhizomes of *D. nipponica* may be potently active compounds that have anti-neuroinflammatory properties via inhibition of NO production.

Table 2
Inhibitory effect of compounds **1–12** on NO production in LPS-activated BV-2 cells

Compounds	IC_{50}[a] (μM)	Cell viability[b] (%, 20 μM)
1	95.07	109.2 ± 4.4
2	45.92	115.6 ± 9.4
3	39.67	96.83 ± 2.0
4	38.11	95.6 ± 4.2
5	18.58	91.4 ± 2.1*
6	89.66	99.2 ± 2.5
7	5.99	25.5 ± 1.9*
8	13.36	114.8 ± 5.8
9	8.44	18.5 ± 2.0*
10	7.84	24.62 ± 1.9*
11	14.36	103.61 ± 2.4
12	57.05	99.37 ± 3.1
NMMA[c]	18.26	103.1 ± 5.2

[a] IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.
[b] Cell viability after treatment with 20 μM of each compound was expressed as a percentage (%) of the LPS only treatment group. The data are expressed as mean ± SD. (Student's *t*-test, *p-value <0.05).
[c] NG-monomethyl-L-arginine (NMMA) was used as a positive control.

In conclusion, we isolated twelve diarylheptanoids (**1**–**12**) including two new cyclic diarylheptanoids, diosniponol A (**1**) and B (**2**), from the rhizome of *D. nipponica*, and confirmed anti-inflammatory effects of tsaokoarylone (**8**) and (4*E*,6*E*)-1-(3′,4′-dihydroxyphenyl)-7-(4″-hydroxyphenyl)-hepta-4,6-dien-3-one (**11**) in LPS-activated BV-2 cells. The results indicate that diarylheptanoid derivatives from *D. nipponica* may be potential candidates for the treatment of various neurodegenerative diseases associated with neuroinflammation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.073.

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