Anti-neuroinflammatory diarylheptanoids from the rhizomes of Dioscorea nipponica

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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 (IC50 13.36 and 14.36 μM, respectively) without cell toxicity in lipopolysaccharide-activated BV-2 cells.

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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 E2 (PGE2). NO is a toxic molecule, and is related with neuronal death and central nervous system impairment. In mammals, NO plays a pivotal role in pathophysiological processes including regulation of inflammatory response in neurodegenerative diseases. Dioscorea nipponica (Dioscoreaceae) is perennial lianas widely distributed in Southeast Asia, Korea, China, and Japan. The rhizomes of D. nipponica have been used as Korean traditional medicine for the treatment rheumatism, asthma, and bronchitis. Various compounds including steroidal saponins, phanenanthrenes and phenolic derivatives 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. 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 CHCl3, 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 C19H22O6 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 [δH 7.08 (1H, d, J = 2.0 Hz, H-2′), 6.85 (1H, d, 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 [δH 4.28 (1H, dd, J = 11.0, 2.0 Hz, H-1′), 3.85 (1H, m, H-3′), and 3.44 (1H, ddd, J = 11.0, 8.0, 4.5, 2.0 Hz, H-5)], and four methylene groups [δH 2.69 (1H, m, H-7a), 2.63 (1H, m, H-7b), 2.10 (1H, ddd, J = 12.0, 4.0, 2.0, 2.0 Hz, H-2eq), 1.96 (1H, ddd, 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 [δH 3.84 (3H, s, 3′-OCH3) and 3.79 (3H, s, 3′-OCH3)]. The 13C NMR spectrum...
revealed resonances for 21 carbons attributable to six aromatic methine carbons (δC 121.6, 119.6, 115.6, 115.4, 112.9, and 110.6), six quaternary carbons (δC 148.2, 148.1, 146.6, 145.5, 135.7, and 134.5), three oxygenated carbons (δC 78.2, 75.6, and 68.5), four methylene carbons (δC 44.5, 42.2, 39.1, and 32.1), and two methoxy carbons (δC 56.3 and 56.2). The 1H and 13C NMR data of compound 1 were very similar to those of compound 3 isolated from Dioscorea villosa. 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-3α and C-3α0, respectively by heteronuclear multiple bond correlation spectroscopy (HMBC) cross-peaks of 3α-OCH3/C-3α and 3α0-OCH3/C-3α0, respectively (Fig. 2). The
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 15,3R,5S by comparison of its optical rotation with that of 3.15 Thus, the structure of 1 was determined as (1S,3R,5S)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,5-epoxy-3-hydroxyheptane and named dionsiponol A.

Compound 2, named dionsiponol 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 13C NMR spectra of 2 were almost similar to those of 1, except for the difference in the chemical shifts of the pyrene ring moiety in the 1H and 13C NMR spectrum (Table 1), implying that compounds 1 and 2 are stereoisomers at C-3.15,16 The relative stereochemistry of 2 was determined to be the same by comparing J values of 415 and the NOESY experiment (Fig. 3).16,19 The absolute configuration of 2 was determined to be 15,3S,5S by comparing the positive optical rotation value.15 From the foregoing, the structure of 2 was established to be (1S,3S,5S)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,5-epoxy-3-hydroxyheptane.

The isolated known compounds were identified as (1S,3R,5S)-1,7-bis-(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (3), (1S,3S,5R,6E)-1,7-bis-(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane (4), (1S,3S,5R,6E)-1,7-bis-(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptane-6-one (5),15 (3R,5R)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)heptanes (6),18 1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-penta-1,4-dien-3-one (7),21 tsaoaoxylone (8),22 1,7-bis-(4-hydroxyphenyl)hepta-4E,6E-dien-3-one (9),23 1,7-bis-(3,4-dihydroxyphenyl)hepta-4E,6E-dien-3-one (10),24 (4E,6E)-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 13C 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 IC50 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 IC50 of 13.36 and 14.36 μM, respectively, without evident cell toxicity. These compounds were more effective than N6-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 potent active compounds that have anti-neuroinflammatory properties via inhibition of NO production.

In conclusion, we isolated twelve diarylheptanoids (1–12) including two new cyclic diarylheptanoids, dionsiponol A (1) and B (2), from the rhizome of D. nipponica, and confirmed anti-inflammatory effects of tsaoaoxylone (8) and (4E,6E)-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.

References and notes