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Research Paper

Phenolic derivatives from the rhizomes of *Dioscorea nipponica* and their anti-neuroinflammatory and neuroprotective activities

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ABSTRACT

Ethnopharmacological relevance: *Dioscorea nipponica* (Dioscoreaceae) have been used as traditional medicines for diabetes, inflammatory and neurodegenerative diseases in Korea. The aim of the study was to isolate the bioactive components from the rhizomes of *Dioscorea nipponica* and to evaluate their anti-neuroinfalmmatory and neuroprotective activities.

Material and methods: The phytochemical investigation of 50% EtOH extract of *Dioscorea nipponica* using successive column chromatography over silica gel, Sephadex LH-20, and preparative high performance liquid chromatography (HPLC) resulted in the isolation and identification of 17 phenolic derivatives, including four new phenolic compounds (1–4). The structural elucidation of these compounds was based on spectroscopic methods, including 1D and 2D nuclear magnetic resonance (NMR) spectroscopy techniques, mass spectrometry, and optical rotation. All isolated compounds were evaluated for their effects on nerve growth factor (NGF) secretion in a C6 rat glioma cell line and nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV2 cells. The neurite outgrowth of compound 16 was further evaluated by using mouse neuroblastoma N2a cell lines.

Results: Three new stilbene derivatives, diosniponol C (1), D (2) and diosniposide A (3) and one new phenanthrene glycoside, diosniposide B (4), together with 13 known compounds were isolated from the rhizomes of *Dioscorea nipponica*. Of the tested compounds (1–17), phenanthrene, 3,7-dihydroxy-2,4,6-trimethoxy-phenanthrene (16) was the most potent NGF inducer, with 162.35 ± 16.18% stimulation, and strongly reduced NO levels with an IC_{50} value of 19.56 μM in BV2 microglial cells. Also, it significantly increased neurite outgrowth in N2a cells.

Conclusions: This study supports the ethnopharmacological use of *Dioscorea nipponica* rhizomes as traditional medicine.

1. Introduction

The onset of neurological disorders may incidentally begin with the onset of chronic inflammation and abnormal metabolism such as diabetes etc. The most common thesis is that cerebrovascular events such as diabetic polyneuropathy, peripheral neuropathies, infections, hyperlipidemia and neurological injuries are the onset causes. Specially, recent study reported that diabetic neuropathy may increase the risk of neurodegenerative disease (Abrams et al., 2009). Therefore, regulation of neuroinflammation and neurotrophic factor may prevent and cure neurodegenerative-related diseases. Particularly, induction and substitution of nerve growth

Abbreviations: NMR, nuclear magnetic resonance; NO, nitric oxide; LPS, lipopolysaccharide; UV, ultraviolet; IR, infrared; HR, high resolution; FAB, fast atom bombardment; MS, mass spectrometry; COZY, correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond correlation; HPLC, preparative high performance liquid chromatography; RP, reversed-phase; LPLC, low-pressure liquid chromatography; TLC, thin-layer chromatography; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; L-NMMA, N^G-monomethyl-L-arginine; NOS, nitric oxide synthase; PS, penicillin-streptomycin; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; CNS, central nervous system; JNK, c-Jun N-terminal kinases

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factor may show promising results in that. So, many researchers try to find materials with NGF mimetic activity and the induction of NGF secretion. Recently, Zhao et al. (2014) group demonstrated that neurotrophic factor might prevent LPS-induced neuroinflammation in microglia by inhibition of JNK signaling. These facts suggest that neurotrophic factor may be a potential target for the treatment of neuroinflammation in the central nervous system (CNS) disorders. The neurotrophic factor inducer or mimetics with anti-neuroinflammatory activity can synergistically recover neuronal damage, furthermore, regulate neurodegenerative disease. Therefore, it is very meaningful research that focused on searching novel materials that regulate the integration of neuroinflammation and neurotrophic factor in neurological disorders.

Dioscorea nipponica, known as 'Buchema' is a typical aura medicine which eliminates or improves giheo symptoms. Giheo symtoms means diarrhea, chronic fatigue, malnutrition, loss of appetite. The rhizomes of *Dioscorea* have been used for replenishing the spleen and stomach. Therefore, it can promote fluid secretion. And, it had regulated the frequency of urination or diabetes due to deficiency condition of the kidney and seminal emission, too. Traditionally, it has been known that increase of energy in the kidney may prevent neurodegenerative diseases. In recent pharmacological study, the extract of this plant showed neurotrophic activity (Kim et al., 2011; Lee et al., 2013), which has also been confirmed by our screening tests exhibiting that the *Dioscorea nipponica* EtOH extract had considerable NGF agonistic activity against glioma cell line in C6 rats. Based on this theory, we investigated to isolate active compound which may act on anti-neuroinflammation and neurotrophic factor from *Dioscorea nipponica*. Column chromatographic purification resulted in the isolation of three new stilbene derivatives, diosniponol C (1), D (2) and diosniposide A (3) and one new phenanthrene glycoside, diosniposide B (4), as well as 13 known compounds (5–17). Herein, we report the isolation of bioactive components from the rhizomes of *Dioscorea nipponica* and evaluate their anti-neuroinflammatory and neurotrophic activities.

2. Material and methods

2.1. Plant material

The rhizomes of *Dioscorea nipponica* were imported from Heilongjiang, China, in January, 2009, and the plant was identified by one of the authors (K. R. Lee). A voucher specimen (SKKU NPL 0913) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

2.2. General

Thin-layer chromatography (TLC) was performed using Merck precoated Silica gel F_{254} plates and reversed-phase (RP)-18 F_{254}s plates. Spots were detected on TLC under ultraviolet (UV) light or by heating after spraying 10% H_2SO_4 in C_2H_5OH (v/v). Packing material of molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co. Ltd.). Low pressure liquid chromatography (LPLC) was carried out over a Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) and LiChroprep Lobar®-A RP-C_{18} (240 × 10 mm) column with an FMI QSY-0 pump (ISCO). Semi-preparative high performance liquid chromatography (HPLC) was performed using a Gilson 306 pump (Gilson, Middleton, WI) with a Shodex refractive index detector (Shodex, New York, NY) and Econosil® RP-C_{18} 10 u column (250 × 10 mm). Optical rotations were obtained on a JASCO P-1020 Polarimeter (Jasco, Easton, MD). Infrared (IR) spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra, including ^1H–^1H correlation spectroscopy (COZY), heteronuclear multiple quantum coherence (HMQC) and hetero nuclear multiple bond correlation (HMBC), were recorded on a Varian UNITY INOVA NMR spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C), with chemical shifts given in ppm (δ). High resolution (HR)-fast atom bombardment mass spectrometry (FABMS) and FABMS spectra were obtained on a JEOL JMS 700 mass spectrometer.

2.2.1. Extraction and isolation

The rhizomes of *Dioscorea nipponica* (10 kg) were extracted with 50% aqueous EtOH (3 × 4 L every 3 days) at room temperature and filtered. The filtrate was evaporated under reduced pressure to give an EtOH extract (1 kg), which was suspended in water (800 mL) and solvent-partitioned to give *n*-hexane (1 g), $CHCl_3$ (35 g), EtOAc (10 g), and BuOH (200 g) fractions. The $CHCl_3$ fraction (9.5 g) was separated over a silica gel column with a solvent system of $CHCl_3$/MeOH (60:1 to 1:1) to obtain 11 fractions (A–K). The B fraction (180 mg) was chromatographed on a Sephadex LH-20 column (CH_2Cl_2/MeOH, 1:1) to give two subfractions (B1–B2). Subfraction B1 (130 mg) was applied to LPLC on a LiChroprep Lobar-A Si column eluted with $CHCl_3$/MeOH (90:1) to give three subfractions (B11–B13). Subfraction B13 (23 mg) and B22 (39 mg) was purified with reverse phase-C_{18} silica gel semi-prep. HPLC (65% MeOH and 50% MeCN) to obtain compounds 5 (6 mg), 16 (6 mg), 1 (4 mg), and 2 (6 mg). The C fraction (55 mg) was chromatographed on Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) to yield three subfractions (C1–C3). Subfractions C2 (15 mg) and C3 (11 mg) were purified with RP-C_{18} silica gel semi-prep. HPLC (50% MeCN and 70% MeOH) to obtain compounds 11 (3 mg), 15 (3 mg) and 17 (2 mg). The H fraction (310 mg) was chromatographed on a Sephadex LH-20 column (CH_2Cl_2/MeOH, 1:1) and separated over an RP-18 open column with a solvent system of 60% MeOH, and then purified with RP-C_{18} silica gel semi-prep. HPLC (50% MeOH) to yield compound 6 (5 mg).

The EtOAc fraction (10.0 g) was separated over a silica gel column with a solvent system of $CHCl_3$/MeOH (20:1 to 1:1) to obtain 10 fractions (A–J). The B fraction was chromatographed on a Sephadex LH-20 column (CH_2Cl_2/MeOH, 1:1) and purified with RP-C_{18} silica gel semi-prep. HPLC (50% MeOH) to yield compound 8 (4 mg). The C fraction (560 mg) was separated over the RP-18 open column with a solvent system of 70% MeOH to yield six subfractions (C1–C6). Subfraction C1 (330 mg) was purified with RP-C_{18} silica gel semi-prep. HPLC (35% and 50% MeOH) to yield compounds 7 (22 mg) and 14 (3 mg). The D fraction (650 mg) was separated over the RP-18 open column with a solvent system of 50% MeOH to yield seventeen subfractions (D1–D17). Subfraction D2 (35 mg) was purified with RP-C_{18} silica gel semi-prep. HPLC (50% MeOH) to yield compound 13 (4 mg). Subfraction D11 (280 mg) was chromatographed on a Sephadex LH-20 column (80% MeOH) to yield three subfractions (D111–D113). Subfractions D113 (70 mg) and D111 (100 mg) were purified with RP-C_{18} silica gel semi-prep. HPLC (25 and 40% MeOH) to yield compounds 9 (70 mg) and 12 (9 mg). The E fraction (1.4 g) was separated over the RP-18 open column with a solvent system of 50% MeOH to yield six subfractions (E1–E6). Subfraction E5 (50 mg) was purified with RP-C_{18} silica gel semi-prep. HPLC (50% MeOH) to yield compound 10 (25 mg). The F fraction (1.3 g) was separated over the RP-18 open column with a solvent system of 50% MeOH to yield 11 subfractions (F1–F11). Subfraction F2 (160 mg) was purified with RP-C_{18} silica gel semi-prep. HPLC (40% and 50% MeOH) to yield compounds 4 (3 mg) and 3 (2 mg).

Diosniponol C (1): Colorless gum; $[\alpha]_D^{25}$ +11.0 (c 0.10 EtOH); IR (KBr) ν_{max} 3672, 2922, 2864, 1744, 1650, 1455, 1362, 1276, 1243, 1166, 1033, 796, 700 and 617 cm^{-1}; ^1H NMR (500 MHz, CD_3OD)