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Neuroprotective Lignans from *Biota orientalis* leaves

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Abstract – We previously reported that 90% MeOH fraction of *Biota orientalis* leaves (L.) ENDL. had significant neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells. In the present study, (–)-savinin (**1**), (–)-hinokinin (**2**), dehydroheliobuphthalmin (**3**) were isolated by bioactivity-guided fractionation from the 90% MeOH fraction. All three lignans had significant neuroprotective activities against glutamate-induced neurotoxicity at the concentrations ranging from 0.1 to 10.0 μM.
Keywords – *Biota orientalis*, (–)-savinin, (–)-hinokinin, dehydroheliobuphthalmin, glutamate, primary cultures of rat cortical cells

Introduction

Glutamate is known to be involved with central excitatory neurotransmission as occurs in neuronal survival, synaptogenesis, neuronal plasticity, memory and in the brain (Aarts and Tymianski, 2003). However, high concentration of glutamate can evoke neuronal dysfunction and even damage or death (Choi, 1988). Glutamate-mediated neurotoxicity may be involved in several neuropathological disorders such as Alzhemer's disease, Parkinson's disease, ischemic stroke, and spinal cord trauma (Lipton and Rosenberg, 1994). Thus, neuroprotection against glutamate-induced neurotoxicity has been a therapeutic strategy to treat neurodegenerative disease (Rajendra *et al*., 2004).

During our search for neuroprotective compounds from natural products, we found that 90% MeOH fraction of *Biota orientalis* (L.) ENDL. (Cupressaceae) leaves had significant neuroprotective activity against glutamate-induced toxicity in primary cultures of rat cortical cells (Koo *et al*., 2002). The leaves of *B. orientalis* have been used in Oriental medicine for the treatment of gout, rheumatism, diarrhea, chronic tracheitis, leukotrichia and alopecia (Zhu *et al*., 2004). A number of flavonoids (Pelter *et al*., 1970), terpenes (Sung *et al*., 1998; Yang *et al*., 1995) and phenolics (Ohmoto and Yamaguchi, 1998) were reported to be isolated from this tree. By using activity-guided fractionation, we could isolate 15-MPA as an active constituent (Koo *et al*., 2002). In a continuation of our work, we attempted to isolate other neuroprotective compounds from 90% MeOH fraction of *B. orientalis* leaves.

In the present study, we isolated three lignans from 90% MeOH fraction of *B. orientalis* leaves and identified them as dibenzylbutyrolactone lignans, (–)-savinin (**1**), (–)-hinokinin (**2**), and dibenzylbutane lignan, dehydroheliobuphthalmin (**3**). These lignans exhibited significant neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells.

Experimental

General procedure – Optical rotation was measured using a JASCO DIP-1000 polarimeter. FT-IR spectra were recorded on a Perkin Elmer 1710 spectrometer, and UV spectra were recorded on a Shimadzu UV-201 spectrometer. EIMS spectra were measured with a VGTrio II spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were run on a JEOL GSX 400 spectrometer at 400 and 100 MHz, respectively, with TMS as an internal standard. The following reagents were used in the experiments : Heat-inactivated fetal calf serum (Logan, UT, U.S.A), Dulbecco's modified Eagle's medium (DMEM), glutamate, MK-801, penicillin, streptomycin, cytosine-β-D-arabinofuranoside, and MTT were all purchased from Sigma Chemical Co. (St, Louis, MO, U.S.A). All of the other reagents were of special grade.

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Plant material – The leaves of *B. orientalis* were purchased from Kyung-dong Market, Seoul, Korea, and identified by Dr. Jong Hee Park, a professor of the College of Pharmacy, Pusan National University. A voucher specimen (SNU-0008) has been deposited in Herbarium of the Medicinal Plant Garden, Seoul National University.

Extraction and isolation – The dried leaves of *B. orientalis* (12 kg) were extracted three times with MeOH in an ultrasonic apparatus. Removal of the solvent *in vacuo* yielded the MeOH extract (1.03 kg). MeOH extract was suspended in distilled water and partitioned with CH_2Cl_2. The CH_2Cl_2 layer was suspended in 90% MeOH and then partitioned with *n*-hexane. Column chromatography of the 90% MeOH fraction, showing neuroprotective activity (48.6% at 50 μg/ml), over silica gel using a mixture of *n*-hexane-EtOAc-MeOH with increasing polarity, yielded 14 fractions (fr. 1 - fr. 14). Consequently, Fr.3 was eluted on C_{18} column chromatography with 70% MeOH to 100% MeOH as an eluent raising the ratio of MeOH and yielded six frations (fr. 3 - 1 - fr. 3 - 6). Among these fractions, fr. 3 - 2 was subjected to column chromatography over Sephadex LH-20 using MeOH to yield 5 fractions (fr. 3 - 2 - 1 - fr. 3 - 2 - 5). Compound **1** (t_R: 25.1 min; 6.2 mg), **2** (t_R: 26.6 min; 4.0 mg), and **3** (t_R: 31.6 min; 8.7 mg) were obtained by an additional purification step on the C_{18} HPLC from fr. 3 - 2 - 5. The HPLC (Hitachi L-6200, Japan) system equipped with a UV visible detector and a YMC-Pack Pro C_{18} semi-preparative column (YMC Co.) was used for purification. The mobile phase for HPLC was a mixture of H_2O and AcCN (45 : 55) and, was detected at 254 nm.

Cell culture – Primary cultures of rat cortical cells containing both neurons and non-neuronal cells such as astrocytes and glia were prepared from 17-19-d-old fetal rats (Sprague-Dawley), a previously described (Kim *et al.*, 1998; Lee *et al.*, 2000; Kim and Kim, 2000). Cortical cells were seeded onto a collagen-coated 48-well-plate at a density of 1×10^6 cells/ml. The cultures were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal calf serum (Gibco) with 100 IU/mL penicillin and 10 μg/mL streptomycin at 37°C in a humidified atmosphere of 95% air-5% CO_2. cytosine-β-D-arabinofurnoside (1 μM) was added to the culture medium 3 d after plating to inhibit the proliferation of non-neuronal cells. Cultures were allowed to mature for 14, 15 d before being used for experiments.

Assessment of neuroprotective activity – All tested compounds were dissolved in DMSO (final culture concentration, 0.1%). To evaluate the neuroprotective activity of samples, cortical cell cultures were rinsed once with HBSS and then changed to a serum-free DMEM. Cortical cell cultures were pre-treated with test compounds for 1 h and then exposed to 100 μM glutamate. After incubation for an additional 24 h, neuronal viability of the cultures was assessed. In some experiment, cortical cell cultures were treated with test compounds for 24 h. Neuronal viability was assessed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, which reflects the mitochondrial enzyme function of cells. Cell viability was calculated as 100 × [(OD of test compound) + (glutamate-treated culture)] – [(OD of glutamate-treated culture) / (OD of control culture) – (OD of glutamate-treated culture)].

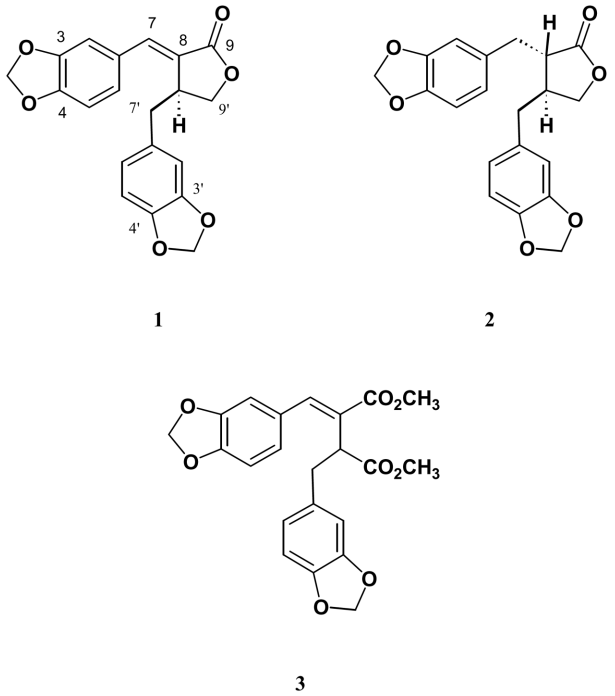

Fig. 1. Chemical structures of compounds **1** - **3**.

Statistical analysis – Data were evaluated for statistical significance using an analysis of variance (ANOVA) with a computerized statistical package. The data were considered to be statistically significant if the probability value was < 0.05.

Results and Discussion

In the present study, we isolated three lignans from the 90% MeOH fraction of *B. orientalis* and identified them as dibenzylbutyrolactone lignans, (–)-savinin (**1**) (Che *et al.*, 1984), (–)-hinokinin (**2**) (Rehnberg and Magnuson, 1988; Lopes *et al.*, 1983; Kato *et al.*, 1990), and dibenzylbutane

Table 1. Neuroprotective activities of lignans against glutamate-induced neurotoxicity in primary cultures of rat cortical cells

Compounds	Concentration (μM)	Protection (%)
Control		100.0 ± 0.0
Glutamate-treated[a)]		0.0 ± 0.0
	0.1	21.7 ± 5.8**
1	1.0	66.8 ± 7.9**
	10.0	32.2 ± 3.5**
	0.1	22.9 ± 5.0**
2	1.0	42.6 ± 2.4**
	10.0	56.9 ± 3.4**
	0.1	23.0 ± 5.7*
3	1.0	23.9 ± 3.6**
	10.0	43.4 ± 7.4*
	1.0	71.7 ± 1.2**
MK-801[b)]	10.0	77.4 ± 2.1**

Cells were pretreated with compounds 1 h before exposure to 100 μM glutamate. The values expressed as mean ± S.D of triplicate expriments. Optical density (OD) of control and glutamate-injured cultures were 0.95 ± 0.12 and 0.67 ± 0.09, respectively. *a)* Glutamate-injured cells differ from significantly from the control of $P < 0.01$. *b)* MK801: dizocipline maleate, a non-competitive antagonist of NMDA receptor. Astrisks indicate significance vs. glutamate-intoxicated cells (one-way ANOVA). *$P < 0.01$ and **$P < 0.001$.

lignan, dehydroheliobuphthalmin (**3**) (Jakupovic *et al*., 1986; Bohlmann *et al*., 1978) by the direct comparison of their physicochemical and spectroscopic data with those previously reported. All these three lignans were isolated for the first time from this tree.

Neuroprotective activities of these lignans against glutamate-induced neurotoxicity in primary cultures of rat cortical cells were evaluated using the MTT assay. All three lignans had significant neuroprotective activities against glutamate-induced neurotoxicity at the concentrations ranging from 0.1 to 10.0 μM (Table 1). Among these three neuroprotective lignans, (–)-savinin showed the most potent activity at a concentration of 1.0 μM (66.8 ± 7.9%, $P < 0.001$). As shown in Table 1, (–)-savinin and (–)-hinokinin, which have a butyrolactone ring, showed the more potent activity than dehydroheliobuphthalmin. At high concentration (over 10.0 μM), (–)-savinin caused a mild decrease in neuronal survival although the data did not showed statistical significance (0.1 μM: 100.6 ± 2.7%; 1.0 μM: 102.9 ± 0.5%; 10.0 μM; 94.3 ± 6.0% of untreated normal control, respectively).

We previously reported that arctigenin, a butyrolactone lignan isolated from the bark of *Torreya nucifera* (Jang *et al*., 2001; Jang *et al*., 2002) showed significant neuroprotective activity against glutamate-induced neurotoxicity. Both (–)-savinin and (–)-hinokinin are butyrolactone lignans possessing methylenedioxybenzyl unit at C–8' instead of veratryl unit which has been reported to be important for the neuroprotective activity of arctigenin (Jang *et al*., 2001).

Also, comparing (–)-savinin and (–)-hinokinin, (–)-savinin showed more potent activity than (–)-hinokinin. The only difference in the molecular structure of (–)-savinin compared to (–)-hinokinin, is the presence of a double bond at C-7 and C-8. Therefore, a butyrolactone and a double bond at C-7 and C-8 seem to be important to exert neuroprotective activity on cultured cortical neurons at least, in part. There are several reports on the biological activities, which include anti-estrogenic (Lee *et al*., 2005a), anti-bacterial (Nissanka *et al*., 2001) and anti-inflammatory activity (Lee *et al*., 2005b; Ban *et al*., 2002), of these dibenzylbutyrolactone lignans, which have a double bond at C-7 and C-8.

In addition, (–)-savinin and (–)-hinokinin have been reported as an antioxidant via scavenging free radicals or inhibiting the production of reactive oxygen species (Kim and Yang, 2004; Medola *et al*., 2007). Although this evidence was not produced from primary cultures of rat cortical cells, it could be suggested that one possible mechanism by which these lignans may exert a protective effect is by acting as antioxidants, and preventing the cells against glutamate-induced neurotoxicity.

Taken together, our results demonstrated that three lignans were isolated from *B. orientalis* leaves for the first time and these lignans exerted the neuroprotective activity against glutamate-induced toxicity in primary cultures of rat cortical cells. To assess a more relevant relationship between the structure and activity, more derivatives is necessary to be assessed for their neuroprotective activity, which will provide further insight into the design of new approaches to neurodegenerative disease.

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References

Aarts, M.M. and Tymianski, M., Novel treatment of excitotoxicity: targeted disruption of intracellular signaling from glutamate receptors. *Biochem. Pharmacol.* **66**, 877-886 (2003).

Ban, H.S., Lee, S.H., Kim, Y.P., Yamaki, K., Shin, K.H., and Ohuchi, K., Inhibition of prostaglandin E_2 production by taiwanin C isolated from the root of *Acanthopanax chiisanensis* and the mechanism of action. *Biochem. Pharmacol.* **64**, 1345-1354 (2002).

Bohlmann, F., Lonitz, T., and Knoll, K.H., Neue lignan-derivate aus der tribus heliantheae. *Phytochemistry* **17**, 330-331 (1978).

Che, C.T., Ahmed, M.S., Kang, A.S., Waller, D.P., Bingel, A.S., Martin, A., Rajamahendran, P., Bunyapraphatara, N., Lankin, D.C., Cordell, G.A., Soejarto, D.D., Wijesekera, W., and Fong, H.H.S., Studies on *Aristolochia III*. Isolation and biological evaluation of constituents of *Aristolochia indica* roots for fertility-regulating activity. *J. Nat. Prod.* **47**, 331-341 (1984).

Choi, D.W., Glutamate neurotoxicity and disease of the nervous system. *Neuron* **1**, 623-634 (1988).

Jakupovic, J., Huster, A., Bohlmann, F., King, R.M., and Robinson, H., New lignans and isobutylamides from *Heliopsis buphthalmoides*. *Planta Med.* **1**, 18-20 (1986).

Jang, Y.P., Kim, S.R., Choi, Y.H., Kim, J.W., Kim, S.G., Markelonis, G.J., Oh, T.H., and Kim, Y.C., Arctigenin protects cultured cortical neurons from glutamate-induced neurodegeneration by binding to kainate receptor. *J. Neurosci. Res.* **68**, 233-240 (2002).

Jang, Y.P., Kim, S.R., and Kim, Y.C., Neuroprotective dibenzylbutyrolactone lignans of *Torreya nucifera*. *Planta Med.* **67**, 470-472 (2001).

Kato, M.J., Yoshida, M., and Gottlieb, O.R., Lignoids and arylalkanones from fruits of *Virola elongata*. *Phytochemistry* **29**, 1799-1810 (1990).

Kim, J.Y. and Yang, K.S., Antioxidative activities of triterpenoids and lignans from *Acanthopanax divaricatus* var. *albeofructus*. *Yakhak Hoechi* **48**, 236-240 (2004).

Kim, S.R. and Kim, Y.C., Neuroprotective phenylpropanoid esters of rhamnose isolated from roots of *Scrophularia buergeriana*. *Phytochemistry* **54**, 503-509 (2000).

Kim, Y.C., Kim, S.R., Markelonis, G.J., and Oh, T.H., Ginsenosides Rb_1 and Rg_3 protect cultured rat cortical cells from glutamate-induced neurodegeneration. *J. Neurosci. Res.* **53**, 426-432 (1998).

Koo, K.A., Sung, S.H., and Kim, Y.C., A new neuroprotective pinusolide derivative from the leaves of *Biota orientalis*. *Chem. Pharm. Bull.* **50**, 834-836 (2002).

Lee, M.K., Yeo, H.S., Kim, J.W., Markelonis, G.J., Oh, T.H., and Kim, Y.C., Cynandione A from *Cynanchum wilfordii* protects cultured cortical neurons from toxicity induced by H_2O_2, L-glutamate, and kainite. *J. Nurosci. Res.* **59**, 259-264 (2000).

Lee, S.H., Yoo, H.H., Piao, X.L., Kim, J.S., Kang, S.S., and Shin, K.H., Anti-estrogenic activity of lignans from *Acanthopanax chiisanensis* root. *Arch. Pharm. Res.* **28**, 186-189 (2005a).

Lee, S.H., Ban, H.S., Kim, Y.P., Kim, B.K., Cho, S.H., Ohuchi, K., and Shin, K.H., Lignans from *Acanthopanax chiisanensis* having an inhibitory activity on prostaglandin E_2 prodution. *Phytother. Res.* **19**, 103-106 (2005b).

Lipton, S.A. and Rosenberg, P.A., Excitatory amino acids as a final common pathway for neurologic disorders. *New Engl. J. Med.* **330**, 613-622 (1994).

Lopes, L.M.X., Yoshida, M., and Gottlieb, O.R., Dibenzylbutyrolactone lignans from *Virola sebifera*. *Phytochemistry* **22**, 1516-1518 (1983).

Medola, J.F., Cintra, V.P, Pesqueira E Silva, E.P., de Andrade Royo, V., da Silva, R., Saraiva, J., Albuquerque, S., and Bastos, J.K., (–)-Hinokinin causes antigenotoxicity but genotoxicity in peripheral blood of Wistar rats. *Food Chem. Toxicol.* **45**, 638-642 (2007).

Nissanka, A.P., Karunaratne, V., Bandara, B.M., Kumar, V., Nakanishi, T., Nishi, M., Inada, A., Tillekeratne, L.M., Wijesundara, D.S., and Gunatilaka, A.A., Antimicrobial alkaloids from *zanthoxylum tetraspermum* and *caudatum*. *Phytochemistry* **56**, 857-861 (2001).

Ohmoto, T. and Yamaguchi, K., Constituents of Pollen. XV. Constituents of *Biota orientalis* (L.) ENDL. (1). *Chem. Pharm. Bull.* **36**, 807-809 (1998).

Pelter, A., Warren, R., Hameed, N., Khan, N.U., Ilyas, M., and Rahman, W., Biflavonyl pigments from *Thuja orientalis* (cupressaceae). *Phytochemistry* **9**, 1897-1898 (1970).

Rehnberg, N. and Magnuson, G., Total synthesis of the lignans (–)- and (+)-burseran, (–)-cubebin, and (–)-hinokinin by diastereoselective conjugate addition of benzyl anions to 2-(R) and (s)-benzyloxy-2,5-dihydro-4-(3,4-methylenedioxybenzoyz-furan. *Tetrahedron Lett.* **29**, 3599-3602 (1988).

Sung, S.H., Koo, K.A., Lim, H.K., Lee, H.S., Cho, J.H., Kim, H.S., and Kim, Y.C., Diterpenes of *Biota orientalis* leaves. *Kor. J. Pharmacogn.* **29**, 347-352 (1998).

Yang, H.O., Suh, D.Y., and Han, B.H., Isolation and characterization of platelet-activating factor receptor binding antagonists from *Biota orientalis*. *Planta Med.* **61**, 37-40 (1995).

Zhu, J.X., Wang, Y., Kong, L.D., Yang, C., and Zhang, X., Effects of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J. Ethnopharmacol.* **93**, 133-140 (2004).

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