Arch Pharm Res Vol 28, No 9, 1023-1026, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

A New Furofuran Lignan with Antioxidant and Antiseizure Activities from the Leaves of *Petasites japonicus*

Byung-Sun Min, Hui Song Cui[1], Hyeong-Kyu Lee, Dai-Eun Sok[1], and Mee Ree Kim[2]

College of Pharmacy, Catholic University of Daegu, Gyongbook 712-702, Korea, [1]College of Pharmacy and [2]Department of Food Science and Nutrition, Chungnam National University, Gung-Dong 220, Yuseong-Gu, Daejeon 305-764, Korea

(Received March 18, 2005)

A new furofuran lignan (**1**) was isolated from the *n*-butanol fraction of the methanolic extract of the leaves of *Petasites japonicus* (Sieb. et Zucc.) Maxim. (Compositae). The structure of compound **1** was determined to be 2α-(4'-hydroxy-3'-methoxyphenyl)-6α-(4"-hydroxy-3"-methoxyphenyl)-8α-hydroxy-3,7-dioxabicyclo[3.3.0]octane 4'-*O*-(β-D-glucopyranoside) by spectroscopic methods including 2D-NMR. In further studies, it was found that the compound **1** expressed an antioxidant activity in DPPH radical scavenging assay, and moreover, ameliorated the seizure in kainic acid-treated mice.

Key words: *Petasites japonicus*, Petaslignolide A, Furofuran lignan, DPPH Radical scavenging, Antiseizure

INTRODUCTION

Extracts from *Petasites* plants (Compositae) have been used for thousands of years for therapeutic purposes in folk medicine. The extract of *Petasites hybridus*, a native European perennial, has been found to contain spasmolytic (Ziolo and Samochowiec, 1988) and analgesic (Grossman and Schmidramsl, 2001) compounds such as petasin or isopetasin. *Petasites formosanus,* an indigenous species of *Petasites* in Taiwan, has been used for the treatment of hypertension; one of hypotensive constituents was identified to be *S*-petasin, a sesquiterpene (Wang *et al.,* 2001). Additionally, phenolic compounds such as phenylpropenoyl derivatives were isolated from the leaves of this specie (Lin *et al.,* 2001, 2004). Separately, the leaves of *Petasites japonicus* (Sieb. et Zucc.) Maxim. (Compositae), a perennial plant widely grown in Japan and Korea, is used as an edible vegetable. Recent studies showed that petasinophenol (Mizushina *et al.*, 2002) and flavonoid glycosides (Mizushina *et al.*, 2003), isolated from *P. japonicus*, inhibited eukaryotic DNA polymerase rhamda and DNA polymerase alpha, respectively. In the course of screening antioxidant compounds from the extract of leaves of *P. japonicus*, the butanol-soluble fraction was found to contain a remarkable antioxidative action in DPPH radical scavenging assay. In the present study, we report the isolation and structural elucidation of a new furofuran lignan, one of neuroprotective antioxidant constituents, from the butanol-soluble fraction of leaves of *P. japonicus*.

Correspondence to: Mee Ree Kim, Department of Food and Nutrition, Chungnam National University, 220 Gung-Dong, Yuseong-Gu, Daejeon 305-764, Korea
Tel: 82-42-821-6837, Fax: 82-42-822-8283
E-mail: mrkim@cnu.ac.kr

MATERIALS AND METHODS

General experimental procedures

Optical rotation was measured with a JASCO DIP-370 digital polarimeter in MeOH. UV spectrum was recorded on a UV-2450 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Unity Inova 400 spectrometer with the tetramethylsilane as an internal standard. Chemical shifts were presented as ppm. API-MS was measured with a Perkin-Elmer SCIEX API III Biomolecular Mass Analyzer.

Plant material

The leaves of *Petasites japonicus* were collected from agricultural fields in Kongju, Korea in May, 2004, and verified by Dr. Young Jin Choi. A voucher specimen was deposited in the Herbarium of Wild Vegetable Experiment Station, Kangwon-Do, Korea.

Extraction and isolation

The leaves of *P. japonicus* (1.5 kg) were extracted with MeOH at room temperature to obtain 220 g of the solid extract. The MeOH extract was suspended in H_2O and extracted successively with hexane (3 × 3 L), $CHCl_3$ (3 × 3 L), EtOAc (3 × 3 L), and BuOH (3 × 3 L) to give the hexane (8.1 g), $CHCl_3$ (7.6 g), EtOAc (8.9 g), and BuOH-soluble fractions (26.6 g), respectively. The BuOH-soluble fraction (26.6 g) was chromatographed on a silica gel column eluted with a stepwise gradient of $CHCl_3$ and MeOH to yield four fractions (Fr. A-D: 2.2 g; 5.3 g; 7.0 g; 3.3 g). Fr. B was chromatographed on a RP C-18 column eluted with MeOH-H_2O (1 : 4) to obtain four subfractions (Fr. B1-B4: 70.1 mg, 291.8 mg, 220.7 mg, 238.5 mg). Fr. B2 was precipitated from MeOH at 4°C to yield compound **1** (88 mg).

Petaslignolide A (1)

White amorphous powder (MeOH). $[\alpha]_D$: -10.0° (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.73), 229 (4.19), 279 (3.75) nm; API-MS *m/z*: 535 $[M-H]^-$, 373 $[M\text{-}Glc\text{-}H]^-$. ^1H- and ^{13}C-NMR (Table I).

Table I. ^1H-, ^{13}C-, and HMBC-NMR data of compound **1** (in DMSO-d_6)

	1		
	^1H (400 MHz)	^{13}C (100 MHz)	HMBC (H → C)
1	2.73 t (7.2)	62.0	C-2, 4, 5, 6, 8, 1'
2	4.76 d (7.2)	82.5	C-1, 4, 5, 8, 1', 2', 6'
4	3.91 dd (8.8, 2.6)	71.4	C-1, 2, 5, 6
	4.12 dd (8.8, 5.6)		
5	3.03 m	53.3	C-1, 2, 4, 6, 8, 1"
6	4.76 d (7.2)	86.1	C-1, 4, 5, 8, 1", 2", 6"
8	5.41 d (4.0)	100.9	C-1, 2, 5, 6
1'		135.9	
2'	6.95 d (2.0)	110.3	C-2, 3', 4', 5', 6'
3'		149.0	
4'		145.9	
5'	7.06 d (8.4)	115.3	C-1', 3', 4'
6'	6.84 d (8.4)	118.8	C-2, 2', 4', 5'
1"		134.0	
2"	7.10 d (2.0)	110.7	C-6, 4", 6"
3"		147.4	
4"		145.7	
5"	6.71 d (8.4)	114.9	C-1", 3", 6"
6"	6.84 d (8.4)	118.0	C-6, 2", 4", 5
OCH_3-3'	3.77 s	55.7	C-3'
OCH_3-3"	3.76 s	55.5	C-3"
Glc 1"'	4.89 d (7.2)	100.1	C-4'
2"'	3.26 m	73.2	
3"'	3.26 m	77.0	
4"'	3.16 m	69.6	
5"'	3.30 m	76.8	
6"'	3.44 dd (11.6, 5.6)	60.0	
	3.67 dd (11.6, 4.8)		

Enzymatic hydrolysis of 1

Naringinase (100 mg, from *Penicillium decumbens*) was added to a suspension of **1** (8 mg) in 50 mM acetate buffer (pH 5.5) and the mixture was stirred at 37°C for 5 h. The reaction mixture was extracted with EtOAc (10 mL × 3). The water layer was checked by silica gel TLC (EtOAc-MeOH-H_2O-AcOH, 65 : 20 : 15 : 15). The spot on the TLC plate was visualized by an anisaldehyde-H_2SO_4 reagent. The stereochemistry of glucose was determined by GC method described previously. The sugar derivative thus obtained showed a retention time of 21.30 min, identical to that of authentic D-glucose.

DPPH radical scavenging assay

Scavenging activity of petaslignolide A on DPPH radicals was measured as described previously (Blois, 1958; Sok *et al.*, 2003). 0.135 mL of DPPH radical solution (0.15 mM) in methanol and 0.015 mL of various concentrations of compound in DMSO were mixed in a microplate reader well. The mixture was kept at room temperature for 30 min, and the absorbance at 535 nm was measured. IC_{50} value was expressed as the concentration of compound to show 50% inhibition of DPPH radicals.

In vivo experiment

Mice were administered orally with petaslignolide A (50 mg/kg), suspended in saline, using an esophagus needle for 4 days consecutively before kainic acid injection. Then, the mice were injected intraperitoneally (50 mg/kg) with kainic acid, dissolved in saline, and control mice were administered with the same volume of saline. The behavioral change was expressed as the onset time of seizure as had been previously reported (Sok *et al.*, 2003).

RESULTS AND DISCUSSION

The BuOH-soluble fraction of the leaves of *P. japonicus* was subjected to repeated column chromatographies on normal and reverse phase silica gels and HPLC, and finally petaslignolide A (**1**) was obtained as an amorphous powder with a negative optical rotation, $[\alpha]_D$ -10.0°. The molecular formula of compound **1** was found to be $C_{26}H_{32}O_{12}$, based on a quasi-molecular ion at *m/z* 535 $[M-H]^-$ in the negative API-MS. The ^1H-NMR spectrum showed signals for two methine protons at δ 2.73 (t, *J* = 7.2 Hz) and 3.03 (m), two benzylic oxymethine protons at δ 4.76 (d, *J* = 7.2 Hz, 2H), an oxygenated methylene at δ 3.91 (dd, *J* = 8.8, 2.6 Hz) and 4.12 (dd, *J* = 8.8, 5.6 Hz), an oxymethine proton at δ 5.41 (d, *J* = 4.0 Hz), two 1,3,4-trisubstituted phenyl groups at δ 6.95 (d, *J* = 2.0 Hz), 7.06 (d, *J* = 8.4 Hz), and 6.84 (d, *J* = 8.4 Hz), 7.10 (d, J = 2.0 Hz), 6.71 (d, *J* = 8.4 Hz), and 6.84 (d, *J* = 8.4 Hz), which were assigned a lignan of the 3,7-dioxobicyclo[3,3,0]octane

type, compared with that of the 9-hydroxypinoresinol (Fig. 1) isolated from *Lonicera hypoleuca* (Khan and Shoeb, 1985). This observation was further supported by the ^{13}C-NMR spectrometric assignments; an oxygenated methine carbon at δ 100.9, four tri-substituted carbons at δ 86.1, 82.5, 62.0, and 53.3, an oxygenated methylene at δ 71.4, and two aromatic rings, coupled with DEPT and HMQC, and HMBC correlations between δ_H 4.76 (H-2) and δ_C 71.4 (C-4)/110.3 (C-2')/118.8 (C-6'), as well as δ_H 4.76 (H-6) and δ_C 71.4 (C-4)/110.7 (C-2")/118.0 (C-6") (Fig. 2). The C-8 position could be substituted by a hydroxy group due to an oxygenated methine carbon signal at δ 100.9. This hydroxyl group was further supported by the HMBC correlations between δ_H 4.76 (H-2,6) and δ_C 100.9 (C-8). In addition, the ^{1}H-NMR spectrum showed two methoxy groups at δ 3.77 and 3.76, which correlated with the quaternary carbons at δ 149.0 (C-3') and 147.4 (C-3"), respectively, in HMBC, establishing the positions of the methoxy groups. The large coupling constant of H-2 and H-6 (δ 4.76, 2H, d, J = 7.2 Hz) indicated that both were pseudoaxial protons (Hou *et al.*, 2003). This was further confirmed by the NOE effects of the NOESY spectrum between H-2 (δ 4.76) and H-4 (δ 3.91), as well as between H-4 and H-6 (δ 4.76). The α orientation of hydrogens at C-1 and -5 were deduced from the chemical shifts of ^{13}C-NMR, compared with that of the 9α-hydroxy-pinoresinol (Abe and Yamauchi, 1988). The orientation of the hydroxyl group at C-8 was assigned as a α-face, since

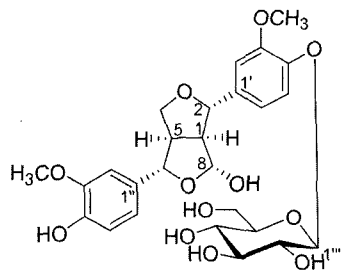

Fig. 1. Structure of compound **1** (petaslignolide A)

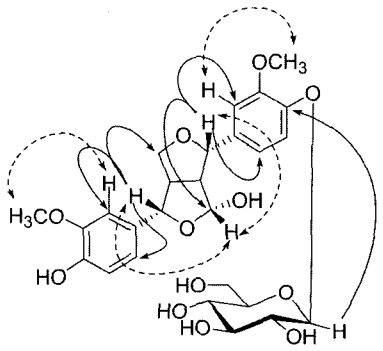

Fig. 2. Significant MHBC (→) correlations and NOE (←···→) in the NOESY spectrum for compound **1**

the coupling constant of H-8 (δ 5.41, d, J = 4.0 Hz) was large compared with that of 4β-hydroxy-2,6-di(4'-hydroxy-3'-methoxy)phenyl-3,7-dioxabicyclo[3,3,0]octane isolated from *Lonicera hypoleuca* (Khan and Shoeb, 1985), as well as the NOE effect of the NOESY spectrum between H-8 (δ 5.41) and H-2/H-6 (δ 4.76). On the other hand, the ^{1}H-NMR spectrum of compound **1** indicated the signals for an anomeric proton at δ 4.89, and six oxygenated protons due to a hexose unit. In support of this, the enzymatic hydrolysis of compound **1** with naringinase yielded a monosaccharide unit, which was identified by co-TLC with an authentic sample. Its absolute configuration was determined by gas chromatography to be D-glucose (Min *et al.*, 2000). The configuration of the glycosidic linkage for the glucopyranoside unit was determined to be β form, based on the $J_{1,2}$ value of the anomeric proton at 7.2 Hz (δ 4.89). The linkage of D-glucose moiety was determined, based on the HMBC correlation between at δ_H 4.89 (H-1''') and δ_C 145.9 (C-4'). Therefore, the structure of petaslignolide A (**1**) was determined to be 8-hydroxy-2α-(4'-hydroxy-3'-methoxyphenyl)-6α-(3",4"-methylenedioxyphenyl)-8α-hydroxy-3,7-dioxabicyclo[3.3.0]octane 4'-O-(β-D-glucopy-ranoside). Although previous data (Khan and Shoeb, 1985; Hou *et al.*, 2003; Yeo *et al.*, 2004) demonstrated that 9-hydroxypinoresinol 1- or 5-hydroxylated 2α-(4'-hydroxy-3'-methoxyphenyl)-6α-(3",4"-methylenedioxyphenyl)-8α-hydroxy-3,7-dioxabicyclo[3.3.0]octane 4'-O-(β-D-glucopy-ranoside) analogues were present in some plants, there has been no report on 8-hydroxylated derivative. Furthermore, this is the first report on the existence of a dioxabicyclo [3.3.0]octane lignan compound in the extract of *P. japonicus*.

Analysis of DPPH free radical-scavenging activity indicates that petaslignolide A (**1**) showed IC_{50} value of 113.04 μg/mL, while the IC_{50} value for alpha-tocopherol and quercetin was estimated to be 25.65 μg/mL and 6.83 μg/mL, respectively (Table II). Next, in an animal experiment (Table III), it was found that oral administration of petaslignolide A (50 mg/kg weight) to ICR mice for 4 days delayed the onset time of seizure from 12.5 ± 4.0 min for kainic acid-treated mice to 29 ± 5.7 min, suggestive of anti-seizure activity of petaslignolide A. In contrast, quercetin, despite its strong antioxidant action, showed no significant effect on the seizure caused by kainic acid

Table II. DPPH free radical scavenging activity of compounds

Compound	IC_{50} (μg/mL)[a]
Petaslignolide A	113.04 ± 4.21
Alpha-tocopherol	25.65 ± 0.49
Quercetin	6.83 ± 1.60

[a]DPPH radical scavenging activity was expressed as the mean ± SE of the 50% inhibitory concentration (IC_{50}) from three determinations, obtained by interpolation of the concentration-inhibition curve.

Table III. Effect of petaslignolide A on seizure in mice injected with kainic acid

Group	Onset time of seizure, min
Kainic acid only	12.5 ± 4.0
Kainic acid + PA	29.0 ± 5.7

Mice (8 animals) were administrated orally (50 mg/kg) with petaslignolide A (PA) for 4 days before kainic acid injection, and then the behavioral sign of seizure was monitored for 60 min.

under the same condition. Although the anti-seizure activity of petaslignolide A (50 mg/kg weight) is comparable to that of melatonin (20 mg/kg), a representative neuroprotective compound, intraperitoneally administered 30 min before kainic acid challenge (Mohanan and Yamamoto, 2002), the present result does not allude the similar potency between petaslignolide A and melatonin. Actually, the neuroprotective effect of melatonin differed greatly according to its administration period (Mohanan and Yamamoto, 2002). Meanwhile, the efficacy of petaslignolide A seemed to require multiple administrations; a single administration of petaslignolide A prior to kainic acid challenge on the same day failed to prevent the seizure (data not shown). Therefore, it is suggested that the prior metabolism of petaslignolide A *in vivo* may be important for the anti-seizure activity of petaslignolide A. Then, it is likely that the enzymatic deglucosylation of petaslignolide A may be implicated in the formation of a bioactive metabolite, membrane-permeable, which needs further study. Taken together, the present data indicate that the extract of *P. japonicus* leaves contains petaslignolide A, a new furofuran lignan compound, showing antioxidant and anti-seizure activities.

ACKNOWLEDGEMENT

This research was financially supported by Public Institute of Health Project (03-PJ1-PG3-22000-0016).

REFERENCES

Abe, F. and Yamauchi, T., 9α-Hydroxypinoresinol, 9α-hydroxymedioresinol and related lignans from *Allamanda neriifolia*. *Phytochemistry*, 27, 575-577 (1988).

Blois, M. S., Antioxidant determination by the use of a stable free radical. *Nature*, 181, 1199-1201 (1958).

Grossman, W. and Schmidramsl, H., An extract of Petasites hybridus is effective in the prophylaxis of migraine. *Altern. Med. Rev.*, 6, 303-310 (2001).

Hou, C. C., Lin, S. J., Cheng, J. T., and Hsu, F. L., Antidiabetic dimeric guianolides and a lignan glycoside from *Lactuca indica*. *J. Nat. Prod.*, 66, 625-629 (2003).

Khan, K. A. and Shoeb, A., A lignan from *Lonicera hypoleuca*. *Phytochemistry*, 24, 628-630 (1985).

Lin, C. H., R. Li, C. Y., Kuoh, C. S., and Wu, T. S., Constituents from the leaves of *Petasites formosanus* and their antioxidant activity. *Heterocycles*, 60, 1881-1889 (2003).

Lin, C. H., Li, C. Y., and Wu, T. S., A novel phenylpropenoyl sulfonic acid and a new chlorophyll from the leaves of *Petasites formosanus* Kitamura. *Chem. Pharm. Bull.*, 52, 1151-1152 (2004).

Min, B. S., Nakamura, N., Miyashiro, H., Kim, Y. H., and Hattori, M., Inhibition of Human Immunodeficiency Virus Type 1 Reverse Transcriptase and Ribonuclease H Activities by Constituents of *Juglans mandshurica*. *Chem. Pharm. Bull.*, 48, 194-200 (2000).

Mizushina, Y., Kamisuki, S., Kasai, N., Ishidoh, T., Shimazaki, N., Takemura, M., Asahara, H., Linn, S., Yoshida, S., Koiwai, O., Sugawara, F., Yoshida, H., and Sakaguchi, K., Petasiphenol: A DNA polymerase I inhibitor. *Biochemistry*, 41, 14463-14471 (2002).

Mizushina, Y., Ishidoh, T., Kamisuki, S., Nakazawa, S., Takemura, M., Sugawara, F., Yoshida, H., and Sakaguchi, K., Flavonoid glycoside: A new inhibitor of eukaryotic DNA polymerase A and a new carrier for inhibitor-affinity chromatography. *Biochem. Biophys. Res. Commun.*, 301, 480-487 (2003).

Mohanan, P. V. and Yamamoto, H. A., Preventive effect of melatonin against brain mitochondria DNA damage, lipid peroxidation and seizures induced by kainic acid. *Toxicol. Lett.*, 129, 99-105 (2002).

Sok, D.-E., Oh, S. H., Kim, Y. B., and Kim, M. R., Neuroprotective effect of rough aster butanol fraction against oxidative stress in the brain of mice challenged with kainic acid. *J. Agric. Food Chem.*, 51, 4570-4575 (2003).

Wang, G. J., Shum, A. Y., Lin, Y. L., Liao, J. F., Wu, X. C., Ren, J., and Chen, C. F., Calcium channel blockade in vascular smooth muscle cells: major hypotensive mechanism of S-petasin, a hypotensive sesquiterpene from *Petasites formosanus*. *J. Pharmacol. Exp. Therap.*, 297, 240-246 (2001).

Yeo, H., Chin, Y. W., Park, S. Y., and Kim, J., Lignans of Rosa multiflora roots. *Arch. Pharm. Res.*, 27, 287-290 (2004).

Ziolo, G. and Samochowiec, L., Study on clinical properties and mechanisms of action of *Petasites* in bronchial asthma and chronic obstructive bronchitis. *Pharm. Acta Helv.*, 72, 378-380 (1998).