A New Naphthopyrone from the Root of *Pleuropterus ciliinervis*

Byung-Sun MIN,[a] Jong-Pill LEE,[b] Min-Kyun NA,[c] Ren-Bo AN,[a] Sang-Myung LEE,[d] Hyeong-Kyu LEE,[a] KiHwan BAE,*[,c] and Sam-Sik KANG[e]

[a] *Laboratory of Immunomodulator, Korea Research Institute of Bioscience and Biotechnology; Daejeon, 305–333, Korea:* [b] *Korea Food and Drug Administration; 122–704, Seoul, Korea:* [c] *College of Pharmacy, Chungnam National University; Daejeon 305–764, Korea:* [d] *KT&G Central Research Institute; Deajeon 305–805, Korea: and* [e] *Natural Products Research Institute, Seoul National University; Seoul 110–460, Korea.* Received June 9, 2003; accepted July 28, 2003

A new naphthopyrone, pleuropyrone A (1), together with (−)-lyoniresinol 3a-*O*-*β*-D-glucopyranoside (2) and (+)-lyoniresinol 3a-*O*-*β*-D-glucopyranoside (3) was isolated from the roots of *Pleuropterus ciliinervis*. The structure of pleuropyrone A (1) was determined to be 2,5-dimethyl-8-hydroxynaphthopyrone 10-*O*-*β*-D-glucopyranoside by spectroscopic methods including 2D-NMR. Compounds 2 and 3 showed moderate antioxidant activity.

Key words pleuropyrone A; naphthopyrone; *Pleuropterus ciliinervis*; antioxidant activity

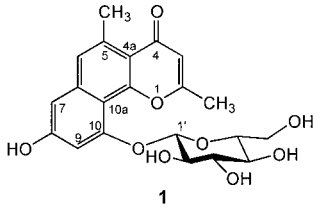
The root of *Pleuropterus ciliinervis* NAKAI (Polygonaceae) has been used in traditional Chinese folk medicine, which is known as "Hasuo," to treat inflammation, bacterial infections, suppurative dermatitis and gonorrhea in China as well as in Korea.[1,2] Stilbenes, anthraquinones and flavonoids have been isolated from the genus *Pleuropterus*.[3—5] Recently, we reported the isolation of resveratrol and stilbene glycosides from this plant, in addition to their antioxidant activity.[6] As a part of an ongoing study to identify novel antioxidant compounds from natural products, a new naphthopyrone, pleuropyrone A (**1**), together with (−)-lyoniresinol 3a-*O*-*β*-D-glucopyranoside (**2**) and (+)-lyoniresinol 3a-*O*-*β*-D-glucopyranoside (**3**) were isolated from a BuOH-soluble fraction of the root of *P. ciliinervis*, and their *in vitro* antioxidant activity, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals scavenging activities, as well as their inhibitory activity of lipid peroxidation, were examined. This paper reports the isolation and structure elucidation of a new naphthopyrone (**1**), as well as the antioxidant activity of the compounds (**1**—**3**).

Results and Discussion

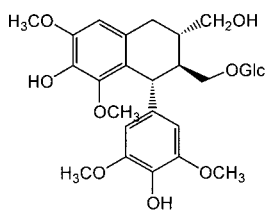
Silica gel column and preparative HPLC with a RP-18 column of a BuOH-soluble fraction of the root of *P. ciliinervis* resulted in the isolation of a naphthopyrone glucoside (**1**) and two lignan glycosides (**2**, **3**). The structures of the known compounds were identified as (−)-lyoniresinol 3a-*O*-*β*-D-glucopyranoside (**2**)[7] and (+)-lyoniresinol 3a-*O*-*β*-D-glucopyranoside (**3**),[8] when compared with the reported data (Chart).

Pleuropyrone A (**1**) was obtained as a white amorphous powder. Its positive FAB-MS spectrum gave a quasi-molecular ion peak at *m*/*z* 419 $[M+H]^+$. The corresponding high-resolution data suggested a molecular formula of $C_{21}H_{22}O_9$. The UV spectrum had maxima at 230, 269, 353 nm. The ^{1}H-NMR spectrum of **1** indicated the presence of an olefinic proton at δ 6.19 (s, H-3), an aromatic proton at δ 7.28 (s, H-6), a pair of *meta*-coupled aromatic protons at δ 6.76 (1H, d, J=1.8 Hz, H-7) and 6.79 (1H, d, J=1.8 Hz, H-9), two aromatic methyl groups at δ 2.42 (s) and 2.74 (s), and an anomeric proton at δ 5.07 (1H, d, J=7.8 Hz, H-1′). The ^{13}C-NMR including distortionless enhancement by polarization transfer (DEPT) and ^{1}H-detected multiple quantum coherence (HMQC) spectra of **1** revealed the presence of two methyl groups at δ 19.1 (C-2a) and 22.9 (C-5a), an olefinic methine at δ 111.8, three aromatic methines at δ 124.6 (C-6), 102.8 (C-7) and 102.4 (C-9), eight quaternary aromatic carbons, a lactonic carbonyl carbon at δ 178.6, and an anomeric carbon at δ 100.6. These ^{1}H- and ^{13}C-NMR spectral data indicated a naphthopyrone derivative, which is close to the structure of euplectin that was isolated from the lichen, *Flavoparmelia euplecta*,[9] except for the indenone moiety.

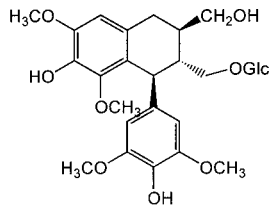
The presence of the chromone structure (partial structure A) in **1** was established by the spatial correlation observed between δ_H 6.19 (H-3)/δ_H 7.28 (H-6) and δ_C 116.9 (C-4a), and also between δ_H 7.28 (H-6) and δ_C 178.6 (C-4) in the heteronuclear multiple-bond correlations (HMBC) (Fig. 1).



1

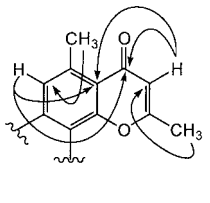


2

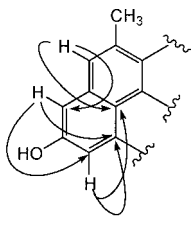


3

Chart



A



B

HMBC (⟶)

Fig. 1. HMBC Correlation for **1**

* To whom correspondence should be addressed. e-mail: baekh@cnu.ac.kr

Table 1. Antioxidant Activities of Compounds (**1**—**3**) from *P. ciliinervis*

	DPPH radical scavenging activity	Superoxide radical scavenging activity	Inhibitory activity of lipid peroxidation
1	>100[c]	>100	>100
2	45.7±4.0	>100	37.4±2.1
3	42.6±3.1	>100	39.1±3.1
α-Tocopherol[a]	25.4±0.9		6.6±1.0
Caffeic acid[a]		11.0±1.8	
BHT[a,b]	15.3±0.6	48.8±2.5	0.11±0.02

a) Positive control. *b*) Butylated hydroxytoluene (positive control). *c*) IC_{50} (μM) values were calculated from regression lines using six different concentrations in triplicate.

The ^{1}H–^{1}H correlation spectroscopy (COSY) correlations between the methyl signal at δ 2.42 and δ 6.19, and also between δ 2.74 and δ 7.28, indicated that the methyl groups were connected at C-2 and C-5. This finding further supported by the presence of long-range correlations between δ_H 2.42 (C-2a) and δ_C 111.8 (C-3) and also between δ_H 2.74 (C-5a) and δ_C 124.6 (C-6) in the HMBC. Further analysis of the NMR data indicated significant HMBC correlations between δ_H 7.28 (H-6)/δ_H 6.79 (H-9) and δ_C 107.9 (C-10a), δ_H 7.28 and δ_C 134.7 (C-5), and δ_H 6.79 and δ_C 159.0/δ 156.2 (C-10), which confirmed the substitution pattern of the naphthalene moiety (partial structure B). The ^{1}H- and ^{13}C-NMR assignments of this fragment were in good agreement with that of pannorin, which was obtained from *Chrysosporium pannorum*, containing the trisubstituted naphthalene moiety.[10]

Acid hydrolysis of **1** afforded the monosaccharide glucose, which was identified on TLC by a reference to an authentic sample. Furthermore, this sugar was determined to be D-glucose by the GLC of its pertrimethylsilylated L-cysteine methyl ester derivative.[11] The configuration of the glycosidic linkage of the glucopyranoside moiety in **1** was determined to be β based on the $J_{1',2'}$ value (7.8 Hz) of the anomeric proton. The connectivity of naphthopyrone and sugar in **1** was confirmed by HMBC, which showed a correlation between the signal at δ_H 5.07 (H-1′) and that at δ_C 156.2 (C-10), indicating glycosylation at the C-10 of the naphthopyrone structure. From these data, the structure of pleuropyrone A was established as 2,5-dimethyl-8-hydroxynaphthopyrone10-*O*-β-D-glucopyranoside.

Compounds (**1**—**3**) were tested for their antioxidant scavenging effects on DPPH and superoxide radicals, as well as their abilities to inhibit lipid peroxidation. Compounds **2** and **3** exhibited the scavenging activity against DPPH radicals with IC_{50} values of 45.7 and 42.6 μM (Table 1), respectively, whereas the two compounds were inactive against the superoxide radical scavenging activity. Furthermore, the two compounds (**2**, **3**) exhibited inhibitory activity against lipid peroxidation with IC_{50} 37.4 and 39.1 μM, respectively. However, pleuropyrone A (**1**) had no significant antioxidant activity.

Experimental

Optical rotations were measured on a JASCO DIP-370 digital polarimeter (JASCO Co., Tokyo, Japan). UV spectra were obtained on UV-2450 spectrophotometer (Shimadzu Co., Kyoto, Japan). ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC spectra were recorded on a Bruker DRX 600 NMR spectrometer (Bruker AXS Inc., Germany) with MeOH-d_4 as a solvent, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. FAB-MS was obtained on a JEOL HX 110 mass spectrometer (JEOL Co.). Prep. HPLC was carried out on a Shimadzu HPLC system; pump: LC10AD and detector: SPD-10AV. Column chromatography was carried out on silica gel (Kieselgel 60, 70—230 mesh, Merck Co., Darmstadt, Germany) and Amberlite MB-3 (Rohm and Haas Co., Philadelphia, U.S.A.). Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F_{254} plate (Merck) and RP-18 F_{254}S (Merck), and spots were detected under a UV light and by spraying 10% H_2SO_4 followed by heating.

Plant Material *P. ciliinervis* NAKAI was collected at Whachen, Korea, in August 1998. A voucher specimen (No. CNU477) is deposited at the Laboratory of Pharmacognosy, College of Pharmacy, Chungnam National University, Korea.

Isolation Procedure The air-dried roots of *P. ciliinervis* (3.0 kg) were extracted with MeOH, and the MeOH solution was then evaporated to dryness. The residue (315.6 g) was diluted with H_2O and partitioned against hexane, EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction (65.5 g) was subsequently fractionated on silica gel (1.5 kg) column chromatography and eluted using $CHCl_3$/MeOH/H_2O (70 : 30 : 4), to afford six fractions (Fr. 1—6; 5.9, 13.4, 7.8, 17.9, 10.3 and 3.7 g, respectively). Column chromatography of fr. 4 on silica gel ($CHCl_3$–MeOH, 6 : 1) and followed by prep. HPLC on RP-18 (7.8×300 mm, MeOH–H_2O, 48 : 52) furnished the pleuropyrone A (**1**, 20.8 mg, t_R 18.5 min). Fraction 5 (15.3 g) was further chromatographed on silica gel and eluted with EtOAc–MeOH (10 : 1→3 : 1) and then prep. HPLC (RP-18 column; MeOH–H_2O, 17 : 83) to yield two lignans, (−)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**2**, 22.5 mg, t_R 22.7 min) and (+)-lyoniresinol 3a-*O*-β-D-glucopyranoside (**3**, 22.2 mg, t_R 27.5 min).

2,5-Dimethyl-8,10-dihydroxynaphthopyrone 10-*O*-β-D-Glucopyranoside (**1**, Pleuropyrone A): White amorphous powder (MeOH). $[\alpha]_D^{24}$ −51° (c=2.3, MeOH). UV λ_{max} nm: 230, 269, 353. Positive-ion FAB-MS *m/z*: 419 $[M+H]^+$, 441 $[M+Na]^+$. HR positive FAB-MS *m/z*: 419.1340 ($[M+H]^+$, Cald for $C_{21}H_{23}O_9$: 419.1342). ^{1}H-NMR (MeOH-d_4): δ 6.19 (1H, s, H-3), 7.28 (1H, s, H-6), 6.76 (1H, d, J=1.8 Hz, H-7), 6.79 (1H, d, J=1.8 Hz, H-9), 2.42 (3H, s, C-2-C$\underline{H}_3$), 2.74 (3H, s, C-5-C$\underline{H}_3$), 5.07 (1H, d, J=7.8 Hz, H-1′), 3.48 (1H, t, J=8.4 Hz, H-2′), 3.38 (1H, m, H-3′), 3.28 (1H, t, J=9.6 Hz, H-4′), 3.41 (1H, m, H-5′), 3.56 (1H, dd, J=10.8, 5.4 Hz, H-6′a), 3.74 (1H, br d, J=10.8 Hz, H-6′b). ^{13}C-NMR (MeOH-d_4): δ 164.0 (C-2), 111.8 (C-3), 178.6 (C-4), 134.7 (C-5), 124.6 (C-6), 102.8 (C-7), 159.0 (C-8), 102.4 (C-9), 156.2 (C-10), 156.0 (C-1b), 116.9 (C-4a), 138.1 (C-6a), 107.9 (C-10a), 19.1 (C-2-$\underline{C}H_3$), 22.9 (C-5-$\underline{C}H_3$), 100.6 (C-1′), 73.7 (C-2′), 77.0 (C-3′), 69.4 (C-4′), 77.1 (C-5′), 60.5 (C-6′).

(−)-Lyoniresinol 3a-*O*-β-D-Glucopyranoside (**2**): White amorphous powder (MeOH). $[\alpha]_D$ −23.4° (c=0.1, MeOH), lit. $[\alpha]_D$ −110° (c=0.7).[11] Positive FAB-MS *m/z*: 605 $[M+Na]^+$.

(+)-Lyoniresinol 3a-*O*-β-D-Glucopyranoside (**3**): White amorphous powder (MeOH), $[\alpha]_D$ +24.8° (c=0.1, MeOH), lit. $[\alpha]_D$ +35.8° (c=0.58, MeOH at 25 °C).[12] Positive FAB-MS *m/z*: 605 $[M+Na]^+$.

Determination of Sugar Compound **1** (2 mg) was refluxed with 4 N HCl–dioxane (1 : 1, 2 ml) for 2 h. The mixture was then extracted with EtOAc (5 ml×3). The residual water layer was neutralized with Amberlite MB-3 and dried to give a residue, which was dissolved in pyridine (1 ml), to which 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2 ml) was added. The mixture was kept at 60 °C for 1.5 h, dried *in vacuo*, and the residue was trimethylsilylated with hexamethyldisilazane-trimethylchlorosilane (0.1 ml) at 60 °C for 1 h. The mixture obtained was partitioned between hexane and H_2O (0.3 ml each) and the hexane extract was analyzed by GC-MS. In the acid hydrolysate of **1**, D-glucose was confirmed by comparing the retention times of the derivatives with those of D-glucose (21.30 min) and L-glucose (22.0 min), and derivatives prepared in a similar way.[13] The sugars obtained by the acid hydrolysis of **1** was identified by TLC on silica gel using of EtOAc–MeOH–H_2O–AcOH (65 : 20 : 15 : 15) as a solvent system. The spot on the plate was visualized by spraying with an anisaldehyde-H_2SO_4 solution.

DPPH Radical Scavenging Activity DPPH radical scavenging activity was measured according to the procedure of Takao *et al.*[14] The reaction mixture was incubated at room temperature for 30 min. The purities of compounds used for the assay were above 95% checked by HPLC.

Superoxide Radical Scavenging Activity Superoxide was generated by xanthine/xanthine oxidase and measured by the nitroblue tetrazolium (NBT) reduction method.[15]

Preparation of Rat Brain Homogenate The rat brain homogenate was prepared as previously described, with some modification.[16] Rat brains were removed, washed with ice-cold saline, homogenized in 9 volumes of ice-cold 5 mM phosphate buffer (pH 7.4) using a glass homogenizer and then centrifuged at 1000 rpm for 10 min. The supernatant was stored at −70 °C until required for the lipid peroxidation determination.

Lipid Peroxidation Inhibitory Activity Lipid peroxidation inhibitory activity in rat brain homogenate was evaluated by the thiobarbituric acid (TBA) method with some modification.[16]

Acknowledgments This research was supported by a grant of the Korea Health 21 R & D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6-01NA01-0002). We are grateful to KBSI for ^{1}H- and ^{13}C-NMR spectral measurements.

References and Notes

1) Namba T., "The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures," Vol. 1, Hoikusha Publishing Co. Ltd., Osaka, 1993, p. 77.
2) Xiao K., Xuan L., Xu Y., Bai D., Zhong D., *Chem. Pharm. Bull.*, **50**, 605—608 (2002).
3) Han D. S., Cho H. J., *Kor. J. Pharmacogn.*, **12**, 221—226 (1981).
4) Yoshizaki M., Fujino H., Arise A., Ohmura K., Arisawa M., Morita N., *Planta Med.*, **53**, 273—275 (1987).
5) Tang W., Eisenbrand G., "Chinese Drugs of Plant Origin," Springer-Verlag, Berlin, 1992, pp. 787—789.
6) Lee J. P., Min B. S., An R. B., Na M. K., Lee S. M., Lee H. K., Kim J. G., Bae K. H., Kang S. S., *Phytochemistry*, **64**, 759—763 (2003).
7) Ohashi K., Watanabe H., Okumura Y., Uji T., Kitagawa I., *Chem. Pharm. Bull.*, **42**, 1924—1926 (1994).
8) Inoshiri S., Sasaki M., Kohda H., Otsuka H., Yamasaki K., *Phytochemistry*, **26**, 2811—2814 (1987).
9) Ernst-Russell M. A., Chai C. L. L., Wardlaw J. H., Elix J. A., *J. Nat. Prod.*, **63**, 129—131 (2000).
10) Ogawa H., Hasumi K., Sakai K., Murakawa S., Endo A., *J. Antibiot.*, **44**, 762—767 (1991).
11) Achenbach H., Löwel M., Waibel R., Gupta M., Solis P., *Planta Med.*, **58**, 270—272 (1992).
12) Shibuya H., Takeda Y., Zhang R. S., Tanitame A., Tsai Y. L., Kitagawa I., *Chem. Pharm. Bull.*, **40**, 2639—2646 (1992).
13) Min B. S., Nakamura N., Miyashiro H., Kim Y. H., Hattori M., *Chem. Pharm. Bull.*, **48**, 194—200 (2000).
14) Takao T., Kitatani F., Watanabe N., Yagi A., Sakata K., *Biosci. Biotech. Biochem.*, **58**, 1780—1783 (1994).
15) Cheng Z. J., Kuo S. C., Chan S. C., Ko F. N., Teng C. M., *Biochim. Biophys. Acta*, **1392**, 291—299 (1998).
16) Huong N. T. T., Matsumoto K., Kasai R., Yamasaki K., Watanabe H., *Biol. Pharm. Bull.*, **21**, 978—981 (1998).