Hydnellins A and B, Nitrogen-Containing Terphenyls from the Mushrooms *Hydnellum suaveolens* and *Hydnellum geogerirum*

Toshihiro HASHIMOTO,[a] Dang Ngoc QUANG,[a,b] Michio KURATSUNE,[a] and Yoshinori ASAKAWA*,[a]

[a] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University; Yamashiro-cho, Tokushima 770–8514, Japan: and* [b] *Faculty of Chemistry, Hanoi University of Education; 136 Xuan Thuy Road, Cau Giay, Hanoi, Vietnam.*

Received January 20, 2006; accepted March 7, 2006

Fractionation of methanol extracts of the fruit bodies of two inedible mushrooms *Hydnellum suaveolens* and *Hydnellum geogerirum* (Thelephoraceae) resulted in isolation of two new *p*-terphenyl derivatives named hydnellins A and B and sarcodonin δ possessing N-oxide. Their structures were determined by 2D NMR, IR, UV spectroscopy, and chemical reaction. These terphenyls showed moderate antioxidant activity.

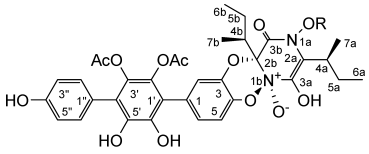
Key words *Hydnellum suaveolens*; *Hydnellum geogerirum*; antioxidant; Thelephoraceae

Mushrooms from *Hydnellum* genus have been chemically investigated since Khanna (1965) reported isolation of atrometin, a *p*-terphenyl, from *Hydnellum diabolus*.[1] One year later, aurantiacin was also purified from *Hydnellum caeruleum*.[2] Following this, several other *p*-terphenyls were obtained from this genus.[3] These *p*-terphenyls exhibit potent cytotoxic activity against a range of tumor and other hyperproliferative cell lines[4] and display neuroprotective[5] and antioxidant activity.[6] In the course of our investigation of antioxidant compounds from Thelephoraceae, we previously reported isolation of novel *p*-terphenyls thelephantins A—N from *Thelephora aurantiotincta*[7,8] and *H. caeruleum*.[9] Recently, we studied the chemical constituents of the fruit bodies of two fungi, *Hydnellum suaveolens* and *Hydnellum geogerirum*, and isolated two new *p*-terphenyls named hydnellins A and B (**1** and **2**) and a previously known *p*-terphenyl sarcodonin δ, which showed antioxidant activity. This paper describes their isolation, structural characterization, and antioxidative activity.

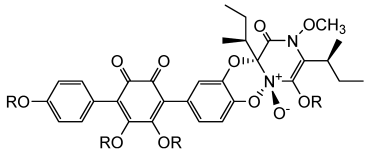
Methanolic extract of the fruit bodies of *H. suaveolens* was partitioned between EtOAc and water, then the EtOAc layer was concentrated and subjected to silica gel column chromatography to obtain three terphenyl compounds, namely hydnellins A and B (**1** and **2**) and sarcodonin δ (**3**), which latter was previously obtained from *Sarcodon scabrosus*.[10] In addition, **3** was also obtained from *H. geogerirum* as described in Experimental.

Hydnellin A (**1**) has a molecular ion peak at 717.2306 $[M+Na]^+$ in its FAB-MS, corresponding to the molecular formula $C_{35}H_{38}O_{13}N_2$ as determined by HR-FAB-MS. Interpretation of its 2D NMR revealed the presence of a terphenyl core with six *ortho*- and one *meta*-coupling aromatic protons, two aliphatic side chains, two acetyl groups, and a methoxyl group. Two aliphatic side chains were elucidated as *sec*-butyl partial structure by ^1H–^1H COSY and HMBC spectra, which were located at C-2a and C-2b by the HMBC correlations between H-4a and C-2a, H-4b and C-2b. These spectral data were identical with those of episarcodonin α (**5**)[11] except for the presence of the methoxyl group. Investigation of 2D NMR spectrum of **1**, especially ROESY spectrum (Fig. 2), located the methoxyl group at N-1a. It was also confirmed by a low field position of the methoxyl group at δ_H 3.99 in its ^1H-NMR spectrum. From the above evidence, **1** was determined as 1b-epimer of sarcodonin δ (**3**) as shown in Fig. 1.

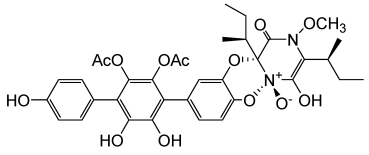
Hydnellin B (**2**) was not obtained in pure state. Thus acetylation of **2** resulted in formation of **4** with the molecular formula $C_{39}H_{40}O_{15}N_2$, containing four acetyl groups as detected by its 2D NMR spectra. ^{13}C-NMR data suggested the presence of *p*-terphenyl core of **4** with two *ortho*-quinones (179.0, 179.2 ppm) in the molecule, which were confirmed



1: R = Me
5: R = H



2. R = H
4. R = Ac



3

Fig. 1. Structures of **1**—**5**

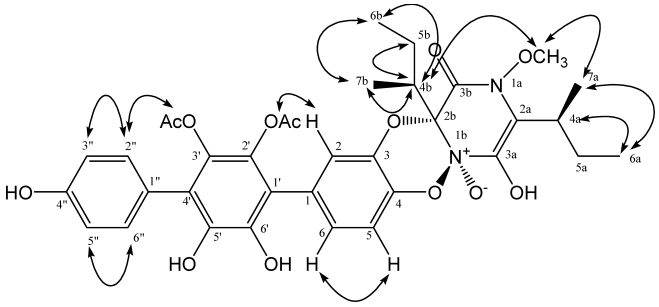


Fig. 2. ROESY Correlations of **1**

* To whom correspondence should be addressed. e-mail: asakawa@ph.bunri-u.ac.jp

Table 1. ^{1}H-NMR Data of Compounds **1** (CD_3OD) and **4** ($CDCl_3$)

Position	**1**	**4**
2	7.06 (d, 1.9)	7.02 (d, 1.9)
5	7.08 (d, 8.2)	7.15 (d, 6.5)
6	7.11 (dd, 1.6, 8.2)	7.04 (dd, 1.9, 6.5)
2″, 6″	7.16 (d, 8.8)	7.37 (d, 8.8)
3″, 5″	6.84 (d, 8.8)	7.23 (d, 8.8)
2′-OAc	1.92 (s)	
3′-OAc	1.93 (s)	
4a	3.00 (m)	3.04 (m)
5a	1.35 (m)	1.46 (m)
	1.61 (m)	1.61 (m)
6a	0.88 (t, 7.4)	0.89 (t, 7.4)
7a	1.07 (d, 7.1)	1.05 (d, 6.9)
4b	2.54 (m)	2.05 (m)
5b	1.31 (m)	1.58 (m)
	1.92 (m)	1.83 (m)
6b	1.04 (t, 7.4)	1.00 (t, 7.4)
7b	1.30 (d, 6.6)	1.18 (d, 6.9)
1a-OMe	3.99 (s)	4.03 (s)
3a-OAc		2.33 (s)
5′-OAc		2.27 (s)[a]
6′-OAc		2.25 (s)[a]
4″-OAc		2.18 (s)

a) Might be changed in each vertical column.

Table 2. ^{13}C-NMR Data of Compounds **1** (CD_3OD) and **4** ($CDCl_3$)

Pposition	**1**	**4**
1	124.6	140.4
2	119.7	118.6
3	142.6	148.0[b]
4	141.7	148.2[b]
5	117.6	116.9
6	128.6	125.2
1′	122.4	140.4
2′	134.6[a]	179.0[a]
3′	134.8[a]	179.2[a]
4′	124.7	124.9
5′	129.8	132.9[c]
6′	129.8	132.2[c]
1″	124.7	124.9
2″, 6″	132.5	131.3
3″, 5″	116.0	121.5
4″	142.6	151.8
2a	158.8	168.2
3a	158.1	164.5
4a	35.1	35.8
5a	27.2	27.2
6a	12.6	11.9
7a	16.8	15.6
2b	94.2	
3b	160.7	165.8
4b	43.8	42.8
5b	24.9	24.8
6b	12.7	12.6
7b	14.9	13.3
1a-OMe	67.7	67.5
3a-OAc		21.2[d]; 169.1[e]
2′-OAC	20.1[b]; 170.5[c]	
3′-OAc	20.2[b]; 170.5[c]	
5′-OAc		20.4[d]; 168.2[e]
6′-OAc		20.4[d]; 168.2[e]
4″-OAc		19.5[d]; 168.1[e]

a—*e*) Might be changed in each vertical column.

by the absorption band at 342 nm in its UV spectrum. Comparing the spectral data of **4** and sarcoviolin α[11] revealed that two *ortho*-quinones were located at C-3′ and C-4′. Detailed analysis of its ^{1}H–^{1}H COSY and HMBC spectra pointed out that **4** contained two *sec*-butyl partial structures at C-2a and C-2b. Other parts of **4** could be elucidated by comparing its spectral data with **3**, in which the presence of a methoxyl group at N-1a was clearly detected. The relative stereochemistries of two *sec*-butyls and N-1b of **4** were also established by comparing their ^{1}H- and ^{13}C-NMR spectral data with those of **3**. Thus the structure of **2** was elucidated as shown in Fig. 1.

Terphenyl compounds have been shown to have strong antioxidant activity.[12] Compounds **1** and **3** were evaluated for their ability to inhibit free radical by DPPH method. They showed moderate antioxidant activity with IC_{50} values of 29.1 and 25.0 μM, respectively. Meanwhile, the IC_{50} of α-tocopherol as standard was 22.8 μM.

Experimental

General Experimental Procedures Optical rotations were measured by JASCO DIP-1000 polarimeter. IR spectra were measured by Perkin Elmer Spectrum One FT-IR spectrometer. UV spectra were obtained by Shimadzu UV-1650PC in EtOH solution. TLC was performed on silica gel plates (Kieselgel 60 F254, Merck). Column chromatography was carried out on silica gel 60 (0.2—0.5 mm, and 0.04—0.063 mm, Merck). Preparative medium-pressure liquid chromatography (MPLC) was performed with Work-21 pump (Lab-Quatec Co., Ltd., Japan) and a Lobar column (Merck). NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR) using CD_3OD and $CDCl_3$ as solvent. Mass spectra including high-resolution FAB mass spectra were recorded on a JEOL JMS AX-500 spectrometer.

Fungal Material Fruit bodies of *H. suaveolens* (No. GY0401) and *H. geogerirum* (No. GY0402) were collected on Fuji mountain, Japan in August and September 2004, respectively, and identified by Mr. Yasuhiko Gotoh. Voucher specimens have been deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan.

Extraction and Isolation Fresh fruit bodies of *H. suaveolens* (480 g) were extracted with MeOH and the methanolic extract was concentrated to give a residue, which was partitioned between EtOAc and water. The EtOAc extract (6.6 g) was separated by Sephadex LH-20 column chromatography, using $CHCl_3$–MeOH (1 : 1) as solvent system, to yield 12 fractions. Fraction 2 (2.5 g) was purified by silica gel column, $CHCl_3$–MeOH gradient to afford **1** (143.6 mg), **3** (156.3 mg), and a mixture (151.7 mg), which was later subjected to MPLC with DIOL column, mobile phase $CHCl_3$–EtOAc (1 : 1), flow rate 1 ml/min to obtain a mixture of **2** (150.7 mg). A part of the mixture (75.6 mg) was acetylated with acetic anhydride (2.0 ml) in pyridine (2.0 ml) and worked up as usual to afford **4** (63.8 mg).

Fresh fruit bodies of *H. geogerium* (50 g) were treated in the same manner as described above to give EtOAc extract (635.7 mg), which was purified by silica gel column using hexane–EtOAc gradient to give 14 fractions. Fraction 10 (54.9 mg) was rechromatographed on silica gel using $CHCl_3$–MeOH gradient as eluent to yield **3** (10.5 mg).

Hydnellin A (**1**): $[\alpha]_D^{20}$ −125.1° (c=1.0, $CHCl_3$). HR-FAB-MS: m/z $[M+Na]^+$: 717.2306 (Calcd for $C_{35}H_{38}O_{13}N_2Na$: 717.2272). IR (KBr) cm^{-1}: 3417, 1771, 1673. UV λ_{max} (EtOH) nm (log ε): 257 (4.2). ^{1}H- and ^{13}C-NMR (CD_3OD) (Tables 1, 2).

Acetylation of Hydnellin B (**4**): $[\alpha]_D^{20}$ +12.6° (c=0.68, $CHCl_3$). HR-FAB-MS: m/z $[M+Na]^+$: 799.2326 (Calcd for $C_{39}H_{40}O_{15}N_2Na$: 799.2326). IR (KBr) cm^{-1}: 1779, 1675, 1604. UV λ_{max} (EtOH) nm (log ε): 342 (3.4), 241 (4.2). ^{1}H- and ^{13}C-NMR ($CDCl_3$) (Tables 1, 2).

Antioxidant Activity Antioxidant activity of hydnellin A (**1**) and sarcodonin δ (**3**) was examined by DPPH radical scavenging method as reported previously.[12]

Acknowledgments We thank Dr. M. Tanaka and Miss Y. Okamoto (TBU, Japan) for recording NMR and mass spectra, respectively. Our special acknowledgments are also due to Mr. Y. Gotoh for his collection and identification of the mushrooms.

References

1) Khanna J. M., Malone M. H., Euler K. L., Brady L. R., *J. Pharm. Sci.*, **54**, 1016—1020 (1965).
2) Montfort M. L., Tyler V. J., Brady L. R., *J. Pharm. Sci.*, **55**, 1300—1302 (1966).
3) Sullivan G., Brady L. R., Tyler V. J., *Lloydia*, **30**, 84—90 (1967).
4) Stead P., Affleck K., Sidebottom P. J., Taylor N. L., Drake C. S., Todd M., Jowett A., Webb G., *J. Antibiot.*, **52**, 89—95 (1999).
5) Lee I. K., Yun B. S., Kim J. P., Ryoo I. J., Kim Y. H., Yoo I. D., *Biosci. Biotechnol. Biochem.*, **67**, 1813—1816 (2003).
6) Liu J. K., Hu L., Dong Z. J., Hu Q., *Chem. Biodiv.*, **1**, 601—605 (2004).
7) Quang D. N., Hashimoto T., Nukada M., Yamamoto I., Hitaka Y., Tanaka M., Asakawa Y., *Phytochemistry*, **62**, 109—113 (2003).
8) Quang D. N., Hashimoto T., Hitaka Y., Tanaka M., Nukada M., Yamamoto I., Asakawa Y., *Phytochemistry*, **63**, 919—924 (2003).
9) Quang D. N., Hashimoto T., Hitaka Y., Tanaka M., Nukada M., Yamamoto I., Asakawa Y., *Phytochemistry*, **65**, 1179—1184 (2004).
10) Ma B. J., Liu K. J., *Z. Naturforsch*, **60b**, 565—568 (2005).
11) Calı V., Spatafora C., Tringali C., *Eur. J. Org. Chem.*, **2004**, 592—599 (2004).
12) Quang D. N., Hashimoto T., Nukada M., Yamamoto I., Asakawa Y., *Planta Med.*, **69**, 1063—1066 (2003).