# New Indole Derivatives with Free Radical Scavenging Activity from *Agrocybe cylindracea*

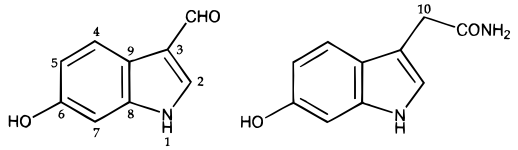
Won-Gon Kim, In-Kyoung Lee, Jong-Pyung Kim, In-Ja Ryoo, Hiroyuki Koshino, and Ick-Dong Yoo*,†

*Korea Research Institute of Bioscience and Biotechnology, KIST, P. O. Box 115, Yusong, Taejon 305-600, Korea, and The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama, 351-01, Japan*

*Received February 28, 1997*[⊗]

Two new indole derivatives were isolated as free radical scavengers from the MeOH extract of *Agrocybe cylindracea*. The structures of these compounds were determined to be 6-hydroxy-1*H*-indole-3-carboxaldehyde (**1**) and 6-hydroxy-1*H*-indole-3-acetamide (**2**) on the basis of spectroscopic studies. Compounds **1** and **2** inhibited lipid peroxidation in rat liver microsomes, with $IC_{50}$ values of 4.1 and 3.9 $\mu$g/mL, respectively.

It has been known that free radicals play an important role in the pathogenesis of diseases such as myocardial and cerebral ischemia, atherosclerosis, inflammation, and cancer-initiation and aging processes.[1,2] Lipid peroxidative disintegration of cells and organellar membranes induced by free radicals has been especially implicated in various pathological processes. Because free radical scavengers are expected to be useful as therapeutic agents for these diseases,[3] some free radical scavengers have been isolated from microbial metabolites.[4−6] In the course of screening for free radical scavenging substances from microorganisms, we isolated benzastatins A−G,[7−9] and betulinans A and B,[10] as reported previously. Further investigation has resulted in the discovery of two new indole derivatives, **1** and **2**, from the MeOH extract of an edible mushroom, *Agrocybe cylindracea* Bolbitiaceae. Previously agrocybenine,[11] polyacetylene compound agrocybin,[12] and illudine sesquiterpenes[13] have been isolated from *Agrocybe* sp. We report herein the isolation, structural determination, and lipid peroxidation inhibitory activity of **1** and **2**.




**1** **2**

A MeOH extract of *A. cylindracea* (10 kg) was partitioned between EtOAc and $H_2O$. The EtOAc solubles were subjected to Si gel column chromatography followed by reversed-phase HPLC to give compounds **1** (3.2 mg) and **2** (5.0 mg). The molecular formula of **1** was determined to be $C_9H_7NO_2$ on the basis of HREIMS (*m*/*z* found 161.0466 $M^+$, calcd 161.0475) in combination with $^{13}$C-NMR data. The UV absorption maxima ($\lambda_{max}$ 235, 276, 300) of **1** was characteristic of a 3-substituted-indole chromophore.[14] IR absorption of **1** at 1627 $cm^{-1}$ implied the presence of a carbonyl function in its structure. The $^1$H-NMR spectrum showed the following significant signals (Table 1): aromatic proton signals of a 1,2,4-trisubstituted benzene ($\delta$ 7.92, d, $J$ = 8.5 Hz; $\delta$ 6.77, dd, $J$ = 8.5, 2.0 Hz; $\delta$ 6.85, d, $J$ = 2.0 Hz), a singlet olefinic methine proton signal ($\delta$ 7.93), and a singlet signal ($\delta$ 9.77) attributable to an aldehyde proton. These spectral data of **1** in combination with $^{13}$C-NMR, DEPT, and HMQC spectral data suggested the presence of either a 3,5- or 3,6-disubstituted indole moiety. The $^1$H- and $^{13}$C-NMR spectral data also suggested the existence of a formyl group ($\delta_H$ 9.77, 1H, *s*; $\delta_C$ 187.4, *d*), and its upfield-shifted $^{13}$C chemical shift indicated that the formyl group could be attached at an aromatic ring. Taking into account the molecular formula ($C_9H_7NO_2$), the other substituent should be a hydroxyl group. The $^{13}$C chemical shift of $\delta$ 156.4 (C-6) indicated that this carbon was hydroxylated. These spectral data indicated that **1** was an indole compound having formyl and hydroxyl groups as substituents. The positions of these two substituents were determined by HMBC experiments (Table 1). Long-range couplings were observed from the *o*-coupled aromatic proton at $\delta$ 7.92 (H-4) to the hydroxylated carbon at $\delta$ 156.4 (C-6) and $sp^2$ quaternary carbons at $\delta$ 120.6 (C-3) and $\delta$ 140.5 (C-8). The formyl proton at $\delta$ 9.77 was, in turn, long-range coupled to $sp^2$ quaternary carbons at $\delta$ 120.6 (C-3) and $\delta$ 119.2 (C-9) and to the olefinic methine carbon at $\delta$ 139.4 (C-2). These long-range connectivities clearly revealed the formyl and hydroxyl groups to be at C-3 and C-6, respectively. Thus, **1** was determined to be 6-hydroxy-1*H*-indole-3-carboxaldehyde. Compound **1** is a new natural product related to 5-hydroxyindole-3-carboxaldehyde, which has been isolated from the marine sponge *Hyrtios erecta*.[15]

The molecular formula of **2** was determined to be $C_{10}H_{10}N_2O_2$ by HREIMS (*m*/*z* found 190.0746 $M^+$, calcd 190.0740) in combination with $^{13}$C-NMR data. The UV, $^1$H- and $^{13}$C-NMR spectral data (Table 1) of **2** were similar to those of **1**, suggesting that **2** also possessed an indole skeleton with two substituents. In the $^1$H- and $^{13}$C-NMR spectral data with HMQC data, the major differences between **1** and **2** are that a methylene signal ($\delta_H$ 3.39, 2H, s; $\delta_C$ 33.4, t) and a carbonyl carbon ($\delta_C$ 178.0, *s*) are newly observed in **2** instead of the signals corresponding of the formyl group of **1**. To account for the molecular formula ($C_{10}H_{10}N_2O_2$) of **2** together with its $^{13}$C-NMR data, the carbonyl carbon should be assigned to an amide, and this was supported by the observation of IR absorption data at 1654 $cm^{-1}$. From these spectral data, it was suggested that the formyl

* Author to whom correspondence should be addressed. FAX: 82-42-860-4595. E-mail: ybs@kribb4b80.kribb.re.kr.
† Korea Research Institute of Bioscience and Biotechnology.
‡ The Institute of Physical and Chemical Research.
⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1997.

S0163-3864(97)00150-X CCC: $14.00 © 1997 American Chemical Society and American Society of Pharmacognosy

**Table 1.** $^1$H- and $^{13}$C-NMR Spectral Data for **1** and **2** in $CD_3OD$

| | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ | $\delta_C$ | HMBC[b] | $\delta_H$ | $\delta_C$ | HMBC[b] |
| 2 | 7.93 (1H, s)[a] | 139.4 d | C-3, C-8, C-9, CHO | 6.91 (1H, s) | 123.2 d | C-3, C-8, C-9, C-10 |
| 3 | | 120.6 s | | | 109.5 s | |
| 4 | 7.92 (1H, d, $J$ = 8.5 Hz) | 123.2 d | C-3, C-6, C-8 | 7.24 (1H, d, $J$ = 8.9 Hz) | 119.8 d | C-3, C-6, C-8 |
| 5 | 6.77 (1H, dd, $J$ = 8.5, 2.0 Hz) | 113.8 d | C-7, C-9 | 6.52 (1H, dd, $J$ = 8.9, 1.9 Hz) | 110.0 d | C-7, C-9 |
| 6 | | 156.4 s | | | 154.1 s | |
| 7 | 6.85 (1H, d, $J$ = 2.0 Hz) | 98.8 d | C-5, C-9 | 6.67 (1H, d, $J$ = 1.9 Hz) | 97.5 d | C-5, C-9 |
| 8 | | 140.5 s | | | 139.0 s | |
| 9 | | 119.2 s | | | 122.2 s | |
| 10 | | | | 3.49 (2H, s) | 33.4 t | C-2, C-3, C-9, $CONH_2$ |
| CHO | 9.77 (1H, s) | 187.4 d | C-2, C-3, C-9 | | | |
| $CONH_2$ | | | | | 178.0 s | |

[a] Proton resonance multiplicity and coupling constant ($J$ = Hz) are in parentheses. [b] Carbons that correlate with proton resonance. The assignments were aided by DEPT, HMQC, and HMBC.

group of **1** was replaced in **2** by an acetamide group. The structure of **2** was determined by HMBC experiments (Table 1). Long-range couplings were observed from the *o*-coupled aromatic proton at $\delta$ 7.24 (H-4) to the hydroxylated carbon at $\delta$ 154.1 (C-6) and to $sp^2$ quaternary carbons at $\delta$ 109.5 (C-3) and $\delta$ 139.0 (C-8). The long-range correlations from methylene protons [$\delta$ 3.39 (H-10)] to carbons at $\delta$ 123.2 (C-2), $\delta$ 109.5 (C-3), and $\delta$ 122.2 (C-9), and the amide carbonyl carbon at $\delta$ 178.0 were also observed. The singlet olefinic methine proton at $\delta$ 6.91 (H-2) was also long-range coupled to carbons at $\delta$ 109.5 (C-3), $\delta$ 139.0 (C-8), $\delta$ 122.2 (C-9), and $\delta$ 33.4 (C-10). These HMBC data indicated that the hydroxyl and acetamide groups should be attached at C-3 and C-6, respectively. The structure of **2** was also supported by observation of the base peak at *m/z* 146 corresponding to the fragment ion generated by loss of the amide group. Thus, the structure of **2** was determined to be 6-hydroxy-1*H*-indole-3-acetamide. Compound **2** is a new amide derivative of 6-hydroxyindole-3-acetic acid, which has been reported as a product of the microbiological transformation of indole-3-acetic acid by *Aspergillus niger*.[16,17]

Compounds **1** and **2** both inhibited lipid peroxidation in rat liver microsomes in a dose-dependent manner. The $IC_{50}$ values of **1** and **2** were 4.1 and 3.9 $\mu$g/mL, respectively, which were weaker than that of vitamin E (1.6 $\mu$g/mL), a well known antioxidant. Indole-3-acetic acid, however, did not show lipid peroxidation inhibitory activity even at 50 $\mu$g/mL. Thus, the phenolic hydroxyl group of **1** and **2** appears to be important for their free radical scavenging activity.

## Experimental Section

**General Experimental Procedures.** UV−vis spectra were recorded on a Shimazu UV-260 spectrophotometer in MeOH. IR spectra were obtained on a Laser Precision Analect RFX-65 FT-IR spectrometer, respectively. EIMS and HREIMS spectra were measured on a Hewlett-Packard Model HP 5989A EIMS system and a JEOL JMS-HX 110/100A spectrometer, respectively, operating at 70 eV. NMR spectra were recorded on a JEOL Alpha 400 spectrometer in $CD_3OD$. Chemical shifts are given in parts per million using TMS as an internal standard. Si gel (Merck Kieselgel 60, 70−230 mesh) and silica TLC plates (Si gel 60 $F_{254}$) were purchased from Merck Company.

**Fungal Material.** The mushroom *A. cylindracea* was obtained from Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Korea, and deposited in Biomolecule Research Division, Korea Research Institute of Bioscience and Biotechnology. The sample was dried in the dark at room temperature and finely milled for solvent extraction.

**Extraction and Isolation.** *A. cylindracea* (10 kg) was extracted with MeOH (10 L) three times, and the MeOH extract was concentrated *in vacuo*. The resultant residue was extracted again with EtOAc (1 L) three times, and the EtOAc layer was concentrated *in vacuo*. The crude residue was then subjected to Si gel (Merck art no. 7734.9025) column chromatography followed by stepwise elution with $CHCl_3$−MeOH. The column was eluted with $CHCl_3$−MeOH (20:1) to give the first active fraction (fraction I) and subsequently eluted with $CHCl_3$−MeOH (10:1) to give the second active fraction (fraction II). Faction I was placed on a Si gel column, and the column was eluted with $CHCl_3$−MeOH (30:1). The active fraction was finally purified on an ODS HPLC column (Senshu Pak, 10 i.d. × 300 mm) eluted with MeOH−$H_2O$ (27:73) to afford **1** (3.2 mg) with a retention time of 16.4 min. Fraction II was further purified by Si gel column chromatography using elution of $CHCl_3$−MeOH (10:1) to give **2** (5.0 mg).

**Compound 1:** white powder; UV $\lambda_{max}$ (MeOH) ($\epsilon$) 220 (3950), 235 (3500), 276 (2730), 300 (1820) nm; IR (KBr) 3338, 2923, 1733, 1627 cm$^{-1}$; EIMS *m/z* 161 ($M^+$, 100), 132 ($M^+$ − CHO, 49.6); HREIMS *m/z* ($M^+$) 161.0466 ($C_9H_7NO_2$ requires 161.0475); $^1$H- and $^{13}$C-NMR data, see Table 1.

**Compound 2**: white powder; UV $\lambda_{max}$ (MeOH) ($\epsilon$) 224 (9870), 274 (1690), 295 (2360) nm; IR (KBr) 3336, 2923, 1654 cm$^{-1}$; EIMS *m/z* 190 ($M^+$, 32.7), 146 ($M^+$ − $CONH_2$, 100.0); HREIMS *m/z* ($M^+$) 190.0746 ($C_{10}H_{10}N_2O_2$ requires 190.0740); $^1$H- and $^{13}$C-NMR data, see Table 1.

**Inhibitory Activity against Lipid Peroxidation in Rat Liver Microsomes.** According to the method of Ohkawa *et al.*,[18] rat liver microsomes were prepared and suspended in 100 mM Tris−HCl buffer (pH 7.4). Lipid peroxidation was initiated by adding 500 $\mu$M $FeSO_4 \cdot H_2O$. After 30 min at 37 °C, the reaction was stopped by adding 3 M trichloroacetic acid in 2.5 N HCl. Lipid peroxidation was assessed by measuring thiobarbituric acid reactive products. Percent inhibition was calculated as follows: $[1 - (T - B)/(C - B)]100$, in which $T$, $C$, and $B$ are absorbance values at 530 nm of the drug treatment, the control (peroxidation without a drug), and the 0 time control (no peroxidation), respectively.

**Acknowledgment.** This research was supported by the Ministry of Science and Technology, Korea.

## References and Notes

(1) Hammond, B.; Kontos, H. A.; Hess, M. L. *Can. J. Physiol. Pharmacol.* **1985**, *63*, 173−187.
(2) Halliwell, B.; Gutterridge, J. M. C. *Free Radicals in Biology and Medicine*, 2nd ed.; Clarendon Press: Oxford, 1989; pp 416−508.
(3) Kinouchi, H.; Epstein, C. J.; Mizui, T.; Carlson, E.; Chen, S. F.; Chan, P. H. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11 158−11 162.
(4) Shin-ya, K.; Hayakawa, Y.; Heto, H. *J. Nat. Prod.* **1993**, *56*, 1255−1258.
(5) Shin-ya, K.; Imai, S.; Furihata, K.; Kato, Y.; Vanduyne, G. D.: Clardy, J.; Hayakawa, Y.; Seto, H. *J. Antibiot.* **1990**, *43*, 444−447.
(6) Kato, S.; Kawai, H.; Kawasaki, T.; Toda, Y.; Urata, T.; Hayakawa, Y. *J. Antibiot.* **1989**, *42*, 1879−1881.
(7) Kim, W. G.; Kim, J. P.; Kim, C. J.; Lee, K. H.; Yoo, I. D. *J. Antibiot.* **1996**, *49*, 20−25.
(8) Kim, W. G.; Kim, J. P.; Yoo, I. D. *J. Antibiot.* **1996**, *49*, 26−30.
(9) Kim, W. G.; Kim, J. P.; Koshino, H.; Shin-ya, K.; Seto, H.; Yoo, I. D. *Tetrahedron*, in press.
(10) Lee, I. K.; Yun, B. S.; Cho, S. M.; Kim, W. G.; Kim, J. P.; Ryoo, I. J.; Koshino, H. ; Yoo, I. D. *J. Nat. Prod.* **1996**, *59*, 1090−1092.
(11) Koshino, H.; Lee, I. K.; Kim, J. P.; Kim, W. G.; Uzawa, J.; Yoo, I. D. *Tetrahedron Lett.* **1996**, 37, 4549−4550.
(12) Jones, E. R. H.; Bu'Lock, J. D. *J. Chem. Soc.* **1953**, 3719−3720.
(13) Stransky, K.; Semerdzieva, M.; Otmar, M.; Prochazka, Z.; Budesinsky, M.; Ubik, K.; Kohoutova, J.; Streinz, L. *Collect. Czech. Chem. Commun.* **1992**, 57, 590−603.
(14) Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Academic Press: Oxford, 1964; p 176.
(15) Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamatsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. *Tetrahedron* **1990**, *46*, 7699−7702.
(16) Arinbasarov, M. U.; Baklashova, T. G.; Kozlovskii, A. G. *Prikl. Biokhim. Mikrobiol.* **1977**, *13*, 427−430.
(17) Koshcheenko, K. A.; Baklashova, T. G.; Kozlovskii, A. G.; Arinbasarov, M. U.; Skryabin, G. K. *Prikl. Biokhim. Mikrobiol.* **1977**, *13*, 248−254.
(18) Ohkawa, H.; Ohishi, N.; Yagi, K. *Anal. Biochem.* **1979**, *95*, 351−358.

NP970150W