Isolation of γ-Lactam Alkaloids from the Macrolepiota neomastoidea

Ki Hyun Kim, Il Kyun Lee, Ki Moon Park,† Wan Kyu Kim,‡ and Kang Ro Lee∗

Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
E-mail: krlee@skku.ac.kr
†Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 440-746, Korea
‡Division of Applied Microbiology, National Institute of Agricultural Science and Technology, RDA, Suwon 441-707, Korea
Received March 12, 2008

Key Words: Macrolepiota neomastoidea, Agaricaeae, γ-Lactam alkaloids, Lepiotins, Mosher’s method and circular dichroism

Macrolepiota neomastoidea Hongo (Agaricaeae) is a poisonous mushroom, which is widely distributed throughout Korea and other East Asian countries. M. neomastoidea has been known to cause severe gastrointestinal symptoms including intestinal irritation, vomiting and profuse diarrhea. To the best of our knowledge, the active principles of this mushroom are unknown except for two compounds, lepiotins A and lepiotins B.† Therefore, as part of our systematic study of Korean toxic mushrooms, we investigated the constituents of M. neomastoidea collected at Mt. Jiri, Namwon of Jeonbuk province, in Korea in November, 2005. Half dried aerial parts of M. neomastoidea were extracted with 80% aqueous MeOH at room temperature. The concentrated MeOH extract was partitioned with aqueous MeOH. Purification of the BuOH fraction by repeated column chromatography furnished four γ-lactam pyrrolidinone alkaloids, lepiotins A (1), lepiotins B (2), lepiotins C (3) and (R)-5-hydroxy-γ-pyrrolidin-2-one (4). Although compound 3 has previously been reported as a synthetic compound,‡ here we have isolated it for the first time from a natural source and named it lepiotins C. Moreover, the spectral data of isolated lepiotins C (3) were similar to those of the related alkaloid, lepiotins A (1), lepiotins B (2), and (R)-5-hydroxy-γ-pyrrolidin-2-one (4). The structures of the isolated metabolites, Lepiotins A (1), lepiotins B (2) and (R)-5-hydroxy-γ-pyrrolidin-2-one (4) were determined by comparison of spectral data with those reported previously. The compound 4 was for the first time isolated from this mushroom. This paper describes the isolation and structure elucidation of compound 3, as well as the determination of absolute configurations of 1 and 2 by the convenient Mosher’s method and Circular Dichroism (CD) study.

Compound 3 was obtained as a colorless gum, which tested positive against Dragendorff reagent. Its molecular formula was determined to be C10H11NO2 from the [M + H]+ peak at m/z 178.0866 (C10H12NO2, calcd. for 178.0868) in the positive-ion high resolution (HR)-FAB-MS spectrum. The IR spectrum indicated that 3 possessed hydroxyl (3443 cm⁻¹) and carbonyl (1662 cm⁻¹) groups. The 1H-NMR spectrum (Table 1) of 3 displayed signals for the presence of two methylene groups at δH: 2.42-2.45 (2H, t, J = 7.0 Hz), and one methylene group adjacent to the nitrogen function at δH: 3.21-3.23 (2H, t, J = 7.0 Hz). The 1H- and 13C-NMR spectra of 3 exhibited signals for two sets of methine groups (δH/δC: 6.67/114.9, 7.35/120.8) on a 1,4-disubstituted aromatic ring (Figure 1). The 13C-NMR spectrum displayed ten carbon signals, composed of a carbonyl carbon of amide, one benzene ring, and three methylene carbons (Table 1). The 13C-NMR resonances were similar to those of the related alkaloid, lepiotins A (1), except for the replacement of the hydroxylated methine group at C-5 (δC: 87.3) in 1 with the methylene group at C-5.

Table 1. 1H- and 13C-NMR data of 1 and 3 and key HMBC correlations of 3

<table>
<thead>
<tr>
<th>No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>HMBC (H → C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δH</td>
<td>δC</td>
<td>δH</td>
<td>δC</td>
</tr>
<tr>
<td>2</td>
<td>2.69-2.74 (1H, m)</td>
<td>30.6</td>
<td>2.42-2.45 (2H, t, 7.0)</td>
<td>32.7</td>
</tr>
<tr>
<td>3</td>
<td>2.42-2.50 (1H, m)</td>
<td>29.3</td>
<td>1.89-1.98 (2H, m)</td>
<td>27.1</td>
</tr>
<tr>
<td>4</td>
<td>1.96-2.42 (1H, m)</td>
<td>87.3</td>
<td>3.21-3.23 (2H, t, 7.0)</td>
<td>53.5</td>
</tr>
<tr>
<td>5</td>
<td>5.51 (1H, dd, 4.5, 2.0)</td>
<td>130.1</td>
<td>130.9</td>
<td>C-2, C-3</td>
</tr>
<tr>
<td>6</td>
<td>6.82 (2H, dd, 8.0, 2.0)</td>
<td>128.2</td>
<td>7.35 (2H, d, 8.0)</td>
<td>120.8</td>
</tr>
<tr>
<td>7, 11</td>
<td>116.6</td>
<td>114.9</td>
<td>6.67 (2H, d, 8.0)</td>
<td>153.1</td>
</tr>
</tbody>
</table>

NMR data were obtained in 500 MHz for 1H and 125 MHz for 13C in CD3OD.
The chemical shift of C-6 at δ 130.9 indicated a nitrogen function as a neighboring atom (Figure 1). Thus, the structure of 3 was determined and it was named lepiotins C. The HMBC spectrum confirmed the connectivity of this structure. Compound 3 was previously described by Angela et al.\(^2\) as a synthetic compound, but here was isolated from a natural source for the first time. Although many alkaloids have been isolated from mushrooms,\(^4\)-\(^7\) N-aryl lactam derivatives have rarely been reported from natural sources. Some N-aryl lactam derivatives showed cooling activity,\(^2\) which were to be used to generate freshness in foods and beverages.

Compound 1 was obtained as a colorless gum, whose molecular formula was determined as C\(_{10}\)H\(_{11}\)NO\(_3\) from the [M + H\(^+\)] peak at m/z 194 in the positive-ion FAB-MS spectrum. Compound 1 was determined to be lepiotins A by comparison of their spectral data with values from the literature.\(^1\) However, the absolute configuration at C-5 of 1 has not been clarified previously. In the present study, we established the absolute configuration at C-5 in 1 and 2.\(^8\) The CD spectrum of (R)-5-hydroxy-2-pyrrolidin-2-one (4) showed negative absorption peaks at 217 and 224 nm, which were identical with those reported previously.\(^3\) The configuration of lactams can be determined by application of an octant rule using the Cotton effect of the \(\pi \rightarrow \pi^*\) band near 220 nm.\(^10\) The CD spectrum of 1 showed strong negative (\(\Delta \varepsilon \approx -9.1\) at 225 nm) Cotton effect (Figure 3). Similarly, 2 displayed strong negative (\(\Delta \varepsilon \approx -8.3\) at 212 nm) effect in the CD spectrum, indicating that the chirality of 1 and 2 was identical. The negative Cotton effect near 220 nm was reported to have (R)-enantiomer by an octant rule as (R)-5-hydroxy-2-pyrrolidin-2-one (4).\(^3\) Therefore, compounds 1 and 2 have (R)-configurations.

**Experimental Section**

**General procedures.** All melting points were determined on a Gallenkamp melting point apparatus and uncorrected. Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. CD spectra were measured on a JASCO J-715 spectropolarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Schimadzu UV-1601 UV-Visible spectrophotometer. FAB and HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including 1H-1H COSY and HMBC experiments, were recorded on a Varian INOVA 500 NMR spectrometer operating at 500 MHz (\(^1\)H) and 125 MHz (\(^13\)C), respectively. Optical rotations were measured on a Jasco P-1020 polarimeter.

**Plant materials.** Half dried aerial parts of *Macrolepiota neomastoidae* were collected at Mt. Jiri, Namwon of Jeonbuk province, Korea in November, 2005. A voucher

---

**Figure 1. Structures of Compounds 1-4.**

**Figure 2. Values of δ\(_S\)-δ\(_\text{MTPA}\) (data obtained in pyridine-\(d_5\)) of the MTPA esters of 1.**

**Figure 3. The CD spectra of 1 and 2 (in MeOH).**
specimen (SKSU-2005-11) of the mushroom was deposited at the College of Pharmacy at Sungkyunkwan University, Korea.

**Extraction and isolation.** Half dried aerial parts of *M. neomastoidea* (132 g) were extracted with 80% MeOH at room temperature. This extract was suspended in H2O, and partitioned and removed their solvent successively to give *n*-hexane (3.3 g), CHCl3 (283 mg), and n-BuOH fraction (10.4 g).

The n-BuOH soluble fraction (10.4 g) was chromatographed over a RP-C18 silica gel column with solvent system of MeOH-H2O (0:1→1:1) as the eluent to give nine fractions (B1-B9). Fraction B2 (2.5 g) was subjected to silica gel column chromatography using a step-wise gradient from CHCl3 to MeOH. The MeOH soluble fraction was further purified by recrystallization using a step-wise gradient from MeOH to H2O to afford 3 (120 mg). Fraction B6 (120 mg) was also subjected to a silica Lobar A®-column with CHCl3-MeOH-H2O (1:1:1) as the eluent to give three subfractions (B61-B63). Subfraction B61 was further purified by RP-C18 preparative HPLC (Econsil® RP-18 10 µm, 250 × 22 mm; 40% MeOH) to give two subfractions (B61-1-B61-2). Subfraction B61-1 (500 mg) was subjected to silica Lobar A®-column with CHCl3-MeOH-H2O (0:1:1) as the eluent to afford the desired compound 1 (5 mg).

**Lepiotins A (1).** Colorless gum, [α]D25 20.0° (c 0.200, MeOH); CD (c 5.31 × 10⁻³ M, MeOH) Δε (nm) +1.1 (248), −0.7 (238), −8.3 (212); UV λmax (MeOH) nm (ε): 203.0 (231), 231.2 (234), 329.4 (508); FAB-MS m/z: 208 [M+H]+.

**Lepiotins B (2).** Colorless gum, [α]D25 20.0° (c 0.200, MeOH); CD (c 5.31 × 10⁻³ M, MeOH) Δε (nm) +1.1 (248), −0.7 (238), −8.3 (212); UV λmax (MeOH) nm (ε): 203.0 (508), 231.2 (234), 329.4 (508); FAB-MS m/z: 208 [M+H]+.

**Lepiotins C (3).** Colorless gum, UV λmax (MeOH) nm (ε): 202.1 (5.55), 251.3 (5.27); IR (KBr) νmax cm⁻¹: 3443, 2253, 2127, 1662, 1056, 1029, 808, 761, 626 cm⁻¹; 1H- and 13C-NMR: see Table 1; FAB-MS m/z: 178 [M+H]+; HR-FAB-MS (positive-ion mode) m/z: 178.0866 (C₁₀H₁₂NO₂, calcd. for 178.0868).

(R)-5-Hydroxypropyridolin-2-one (4). Colorless gum, [α]D25 20.0° (c 0.050, MeOH); CD (c 4.95 × 10⁻³ M, MeOH) Δε (nm) −0.8 (224), −0.3 (220); UV λmax (MeOH) nm (ε): 203.1 (5.33), 229.5 (5.04); 1H-NMR (CD3OD, 500 MHz): δ 5.24 (1H, dd, J = 6.3, 1.7 Hz, H-5), 2.48 (1H, ddd, J = 16.7, 9.9, 7.5 Hz, H-3a), 2.37 (1H, ddd, J = 13.5, 9.9, 9.7, 6.3 Hz, H-4a), 2.19 (1H, ddd, J = 16.7, 9.7, 3.5 Hz, H-3b), 1.90 (1H, ddd, J = 13.5, 7.9, 3.5, 1.7 Hz, H-4b); 13C-NMR (CD3OD, 125 MHz): δ 182.3 (C-2), 80.9 (C-5), 31.3 (C-4), 29.5 (C-3); FAB-MS m/z: 102 [M+H]+.

Preparation of the (R)- and (S)-MTPA ester derivatives of 1 by a convenient meser ester procedure. Compound 1 (2.0 mg) in deuterated pyridine (1.0 mL) was transferred into clean NMR tube. (S)-(+)-α-Methoxy-α-(trifluoromethyl) phenylacetyl (MTPA) chloride (10 μL) was added into the NMR tube immediately under a N2 gas stream, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The NMR reaction tube was left at room temperature overnight. The reaction was then completed to afford the (R)-MTPA ester derivative (1r) of 1. The (S)-MTPA ester derivative of 1 (Is) was obtained as described for 1r. The 1H-NMR spectra of 1r and Is were measured directly in the NMR reaction tubes. Although strong proton signals of excess MTPA chlorides and MTPA acids (hydrolysis products from MTPA chloride, due to the trace amount of H2O in deuterated pyridine and moisture of the experimental environment) were present in the 1H-NMR spectra of 1r and Is, the undisturbed signals of 1r and Is were clearly different.

I: (500 MHz, pyridine-d₅): δ 7.806 (2H, dd, J = 8.0, 2.0 Hz, H-7, H-11), 7.146 (2H, dd, J = 8.0, 2.0 Hz, H-8, 10), 5.836 (1H, dt, J = 6.5, 1.5 Hz, H-5), 2.764 (1H, m, H-3a), 2.422 (1H, m, H-3b), 2.294 (1H, m, H-4a), 2.090 (1H, m, H-4b).

Ir: (500 MHz, pyridine-d₅): δ 8.503 (2H, dd, J = 8.0, 2.0 Hz, H-7, H-11), 7.584 (2H, dd, J = 8.0, 2.0 Hz, H-8, 10), 7.236 (1H, dd, J = 8.5, 2.5 Hz, H-5), 3.325 (1H, m, H-4a), 3.298 (1H, m, H-3a), 2.706 (1H, m, H-3b), 2.594 (1H, m, H-4b).

Is: (500 MHz, pyridine-d₅): δ 8.511 (2H, dd, J = 8.0, 2.0 Hz, H-7, H-11), 7.591 (2H, dd, J = 8.0, 2.0 Hz, H-8, 10), 7.245 (1H, dd, J = 8.5, 2.5 Hz, H-5), 3.335 (1H, m, H-4a), 3.309 (1H, m, H-3a), 2.716 (1H, m, H-3b), 2.606 (1H, m, H-4b).

**Acknowledgments.** The authors would like to thank Mr. Do Kyun Kim, Dr. Eun Jung Bang and Dr. Jung Ju Seo at Korea Basic Science Institute for the measurements of NMR and MS spectra. This work was supported by the grant “Classification of Poisonous Mushrooms and Study of Investigating Toxic Constituents” from the Rural Development Administration in Korea.

**References.**