Isolation of γ-Lactam Alkaloids from the *Macrolepiota neomastoidea*

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Macrolepiota neomastoidea Hongo (Agaricaceae) 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.[1] To the best of our knowledge, the active principles of this mushroom are unknown except for two compounds, lepiotins A and lepiotins B.[1] 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 *n*-hexane, $CHCl_3$ and *n*-BuOH. Purification of the *n*-BuOH fraction by repeated column chromatography furnished four γ-lactam pyrrolidinone alkaloids, lepiotins A (**1**), lepiotins B (**2**), lepiotins C (**3**) and (*R*)-5-hydroxypyrrolidin-2-one (**4**). Although compound **3** has previously been reported as a synthetic compound,[2] 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 little different from those of synthetic compound.[2] Lepiotins A (**1**) and lepiotins B (**2**) were isolated from this mushroom by Tomihisa *et al.*,[1] but their absolute configurations of these compounds at C-5 were not clarified. The structures of the isolated metabolites, Lepiotins A (**1**),[1] lepiotins B (**2**)[1] and (*R*)-5-hydroxypyrrolidin-2-one (**4**)[3] 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 $C_{10}H_{11}NO_2$ from the $[M + H]^+$ peak at m/z 178.0866 ($C_{10}H_{12}NO_2$, 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 ^{1}H-NMR spectrum (Table 1) of **3** displayed signals for the presence of two methylene groups at δ_H: 1.89-1.98 (2H, m) and at δ_H: 2.42-2.45 (2H, t, J = 7.0 Hz), and one methylene group adjacent to the nitrogen function at δ_H: 3.21-3.23 (2H, t, J = 7.0 Hz). The ^{1}H- and ^{13}C-NMR spectra of **3** exhibited signals for two sets of methine groups (δ_H/δ_C; 6.67/114.9, 7.35/120.8) on a 1,4-disubstituted aromatic ring (Figure 1). The ^{13}C-NMR spectrum displayed ten carbon signals, composed of a carbonyl carbon of amide, one benzene ring, and three methylene carbons (Table 1). The ^{13}C-NMR resonances were similar to those of the related alkaloid, lepiotins A (**1**),[1] except for the replacement of the hydroxylated methine group at C-5 (δ_C: 87.3) in **1** with the methylene group at C-5

Table 1. ^{1}H- and ^{13}C-NMR data of **1** and **3** and key HMBC correlations of **3**

No	**1**		**3**		
	δ_H	δ_C	δ_H	δ_C	HMBC (H → C)
2		177.1		176.8	
3	2.69-2.74 (1H, m)	30.6	2.42-2.45 (2H, t, 7.0)	32.7	C-2, C-5
	2.42-2.50 (1H, m)				
4	2.42-2.50 (1H, m)	29.3	1.89-1.98 (2H, m)	27.1	C-2, C-5
	1.96-2.42 (1H, m)				
5	5.51 (1H, dd, 4.5, 2.0)	87.3	3.21-3.23 (2H, t, 7.0)	53.5	C-2, C-3
6		130.1		130.9	
7, 11	7.21 (2H, dd, 8.0, 2.0)	128.2	7.35 (2H, d, 8.0)	120.8	C-8, C-9,
8, 10	6.82 (2H, dd, 8.0, 2.0)	116.6	6.67 (2H, d, 8.0)	114.9	C-6, C-7
9		157.7		153.1	

NMR data were obtained in 500 MHz for ^{1}H and 125 MHz for ^{13}C in CD_3OD

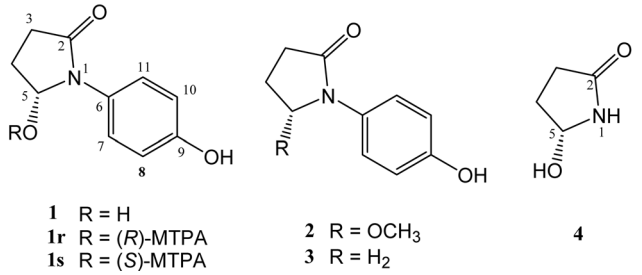

Figure 1. Structures of Compounds **1**-**4**.

(δ_C: 53.5) in **3.** The ^{13}C 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 to be $C_{10}H_{11}NO_3$ 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 determined the absolute configuration of **1** using the convenient Mosher ester procedure carried out in NMR tubes.[8] Two portions (each 2.0 mg) of **1** were treated with either (*S*)-(+)-α- or (*R*)-(−)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (10 μL) in deuterated pyridine (0.5 mL) directly in separate NMR tubes at room temperature, which afforded the (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**, respectively) of **1**. Analysis of ^{1}H-NMR chemical shift differences between *S*- and *R*-MTPA (δ_S-δ_R) is shown in Figure 2, indicating that the absolute stereochemistry at C-5 of lepiotins A was *R*-configuration. Although the positive values of ^{1}H-NMR chemical shift differences (δ_S-δ_R) at H-7 and H-8 were represented, the positive values of ^{1}H-NMR chemical shift differences (δ_S-δ_R) at H-3 and H-4 were significantly distinct. Accordingly, the structure of compound **1** was concluded to be 5*R*-lepiotins A. We have

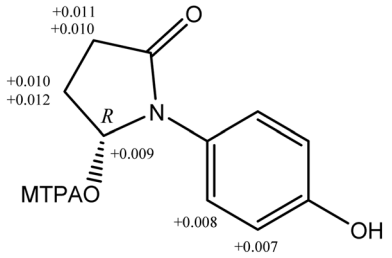

Figure 2. Values of δ_S-δ_R (data obtained in pyridine-d_5) of the MTPA esters of **1**.

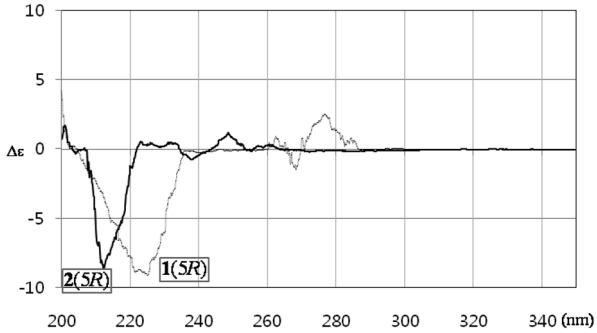

Figure 3. The CD spectra of **1** and **2** (in MeOH).

also studied the Circular Dichroism exciton chirality method to establish the absolute configuration at C-5 in **1** and **2**.[9] The CD spectrum of (*R*)-5-hydroxypyrrolidin-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 n $\rightarrow \pi^*$-band near 220 nm.[10] The CD spectrum of **1** showed strong negative ($\Delta\varepsilon$ −9.1 at 225 nm) Cotton effect (Figure 3). Similarly, **2** displayed strong negative ($\Delta\varepsilon$ −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-hydroxypyrrolidin-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 ^{1}H-^{1}H COSY and HMBC experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using TMS as an internal standard. Preparative HPLC used a Gilson 306 pump with Shodex refractive index detector. Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh) and RP-C_{18} silica gel (Merck, 230-400 mesh) was used for column chromatography. Merck pre-coated Silica gel F_{254} plates and RP-18 F_{254s} plates were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (v/v). Low pressure liquid chromatography was carried out over a Merck Lichroprep Lobar®-A Si 60 (240 × 10 mm) or a Lichroprep Lobar®-A RP-18 (240 × 10 mm) column with a FMI QSY-0 pump (ISCO).

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

speciemen (SKKU-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 H_2O, and partitioned and removed their solvent successively to give *n*-hexane (3.3 g), $CHCl_3$ (283 mg), and *n*-BuOH fraction (10.4 g).

The *n*-BuOH soluble fraction (10.4 g) was chromatographed over a RP-C_{18} silica gel column with solvent system of MeOH-H_2O (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 $CHCl_3$ to MeOH. The MeOH soluble fraction was further purified by recrystalization using a step-wise gradient from MeOH to H_2O to afford **3** (120 mg). Fraction B6 (120 mg) was also subjected to a silica Lobar A®-column with $CHCl_3$-MeOH-H_2O = 7:3:0.5 as the eluent to give three subfractions (B61-B63). Subfraction B61 was further purified by RP-C_{18} preparative HPLC (Econosil® RP-18 10 μ column, 250 × 22 mm; 25% MeOH) to obtain **1** (10 mg). Fraction B8 (30 mg) was directly purified by RP-C_{18} preparative HPLC (Econosil® RP-18 10 μ column, 250 × 22 mm; 40% MeOH) to give **2** (5 mg). Fraction B9 (250 mg) was also subjected to a RP-C_{18} silica Lobar A®-column with 50% MeOH as the eluent to give two subfractions (B91-B92). Subfraction B91 was further purified by RP-C_{18} preparative HPLC (Econosil® RP-18 10 μ column, 250 × 22 mm; 40% MeOH) to yield **4** (5 mg).

Lepiotins A (1). Colorless gum, $[\alpha]_D^{25}$: +7.73° (c 0.075, MeOH); CD (c 3.11 × 10^{-3} M, MeOH) $\Delta\varepsilon$ (nm) +2.5 (276), −1.4 (268), −9.1 (225); UV λ_{max} (MeOH) nm (ε): 201.0 (4.66), 220.8 (4.45), 274.0 (4.51); ^{1}H- and ^{13}C-NMR: see Table 1; FAB-MS m/z: 194 $[M+H]^+$.

Lepiotins B (2). Colorless gum, $[\alpha]_D^{25}$: +8.30° (c 0.200, MeOH); CD (c 5.31 × 10^{-3} M, MeOH) $\Delta\varepsilon$ (nm) +1.1 (248), −0.7 (238), −8.3 (212); UV λ_{max} (MeOH) nm (ε): 203.0 (5.08), 231.2 (3.74), 329.4 (5.08); FAB-MS m/z: 208 $[M+H]^+$.

Lepiotins C (3). Colorless gum, UV λ_{max} (MeOH) nm (ε): 202.1 (5.55), 251.3 (5.27); IR (KBr) ν_{max} cm^{-1}: 3443, 2253, 2127, 1662, 1056, 1029, 1008, 825, 761, 626 cm^{-1}; ^{1}H- and ^{13}C-NMR: see Table 1; FAB-MS m/z: 178 $[M+H]^+$; HR-FAB-MS (positive-ion mode) m/z: 178.0866 ($C_{10}H_{12}NO_2$, calcd. for 178.0868).

(*R*)-5-Hydroxypyrrolidin-2-one (4). Colorless gum, $[\alpha]_D^{25}$: +5.0° (c 0.050, MeOH); CD (c 4.95 × 10^{-3} M, MeOH) $\Delta\varepsilon$ (nm) −0.8 (224), −0.3 (220); UV λ_{max} (MeOH) nm (ε): 203.1 (5.33), 229.5 (5.04); ^{1}H-NMR (CD_3OD, 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, dddd, *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, dddd, *J* = 13.5, 7.9, 3.5, 1.7 Hz, H-4b); ^{13}C-NMR (CD_3OD, 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 mosher 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 N_2 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** (**1s**) was obtained as described for **1r**. The ^{1}H-NMR spectra of **1r** and **1s** 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 H_2O in deuterated pyridine and moisture of the experimental environment) were present in the ^{1}H-NMR spectra of **1r** and **1s**, the undisturbed signals of **1r** and **1s** were clearly different.

1: (500 MHz, pyridine-d_5): δ 7.806 (2H, dd, *J* = 8.0, 2.0 Hz, H-7, 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).

1r: (500 MHz, pyridine-d_5): δ 8.503 (2H, dd, *J* = 8.0, 2.0 Hz, H-7, 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).

1s: (500 MHz, pyridine-d_5): δ 8.511 (2H, dd, *J* = 8.0, 2.0 Hz, H-7, 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).

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