# LEPIOTINS A AND B, NEW ALKALOIDS FROM THE MUSHROOMS, *MACROLEPIOTA NEOMASTOIDEA* AND *CHLOROPHYLLUM MOLYBDITES*

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Abstract--- Two new alkaloids, lepiotins A and B were isolated from the poisonous mushrooms *Macrolepiota neomastoidea* and *Chlorophyllum molybdites*, which were classified to family *Agaricaceae*. These structures were determined on the basis of a spectroscopic analysis and a synthetic study.

*Agaricaceous* mushroom *Chlorophyllum molybdites* closely related to the genus *Macrolepiota* has similar form to *M. neomastoidea*. Both mushrooms *C. molybdites* and *M. neomastoidea* have been known to cause similar poisoning with severe gastrointestinal symptoms including intestinal irritant, vomiting and profuse diarrhea.[1] Hypovolumetric shock from *C. molybdites* has also been reported.[2] In the course of our program to find a new biologically active compound from Japanese mushroom (Basidiomycetes),[3] we investigated the acute toxicity of the methanol extract on mice, and found temporary poisoning such as rising with both extracts from *C. molybdites* and *M. neomastoidea*. Attempts to find a toxic component led to the isolation of two new alkaloids, lepiotins A

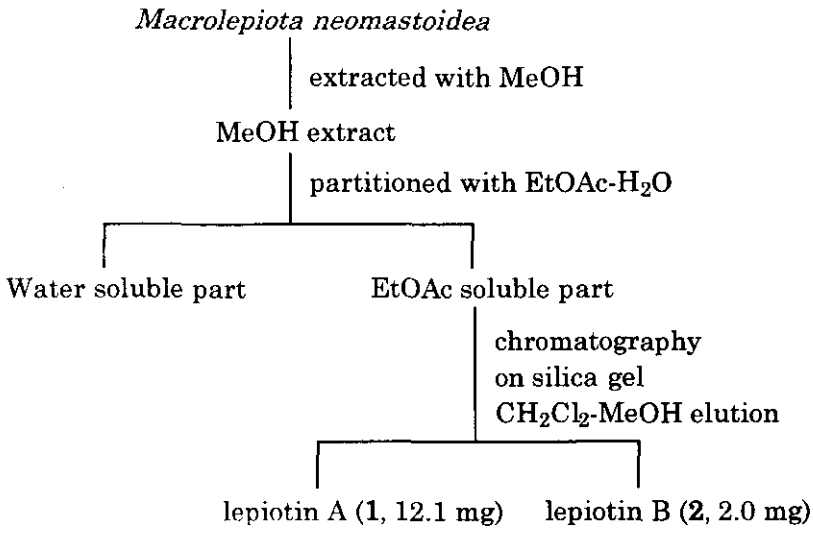



Chart 1. Isolation Procedure for Lepiotins A (**1**) and B (**2**) from *M. neomastoidea*

and B from the fruit bodies of *M. neomastoidea* and lepiotin B from *C. molybdites.* In this paper, we wish to report the isolation and the structural elucidation of lepiotins A (**1**) and B (**2**).

1) Lepiotins A (**1**) and B (**2**) from *M. neomastoidea.*

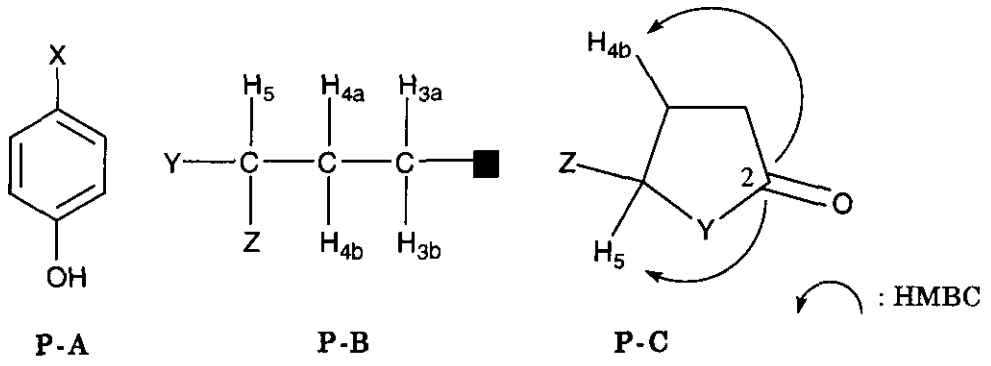
The fruit bodies of *M. neomastoidea* collected in Miyagi Prefecture were extracted with methanol. The methanol extract was partitioned between ethyl acetate and water. The ethyl acetate soluble part was concentrated *in vacuo* and the residue was repeatedly subjected to silica gel chromatography using dichloromethane-methanol mixed solvent system of increasing polarity to afford lepiotins A (**1**, 12.1 mg) and B (**2**, 2.0 mg). Lepiotin A (**1**) was obtained as a colorless oil. The molecular formula $C_{10}H_{11}NO_3$ was determined by the combination of HREI MS (m/z 193.0725, $M^+$), $^1H$ and $^{13}C$ NMR spectra. In the IR spectrum lepiotin A (**1**) showed the hydroxyl (3300) and carbonyl (1650 $cm^{-1}$) absorptions. Ferric chloride coloring test on TLC indicated the presence of phenol moiety. The $^{13}C$ NMR spectrum of lepiotin A (**1**) showed ten signals due to a carbonyl carbon of amide or ester moiety ($\delta_C$ 176.6), six $sp^2$ and three $sp^3$ carbons as shown in Table 1. The $^1H$ and $^{13}C$ NMR spectra of lepiotin A (**1**) exhibited signals for two sets of methine groups ($\delta_H/\delta_C$ 6.85/116.8, 7.22/127.7) on 1,4-disubstituted phenol ring (partial structure **P-A** in Figure 1) and a methine group ($\delta_H/\delta_C$ 5.48/86.8) attached to two hetero atoms. The H-H COSY

Table 1. $^{1}H$ and $^{13}C$ NMR Spectral Data for Lepiotin A (**1**)[a]

| position | $^{13}C$ | | $^{1}H$ |
|---|---|---|---|
| 2 | 176.6 | (s)[b] | |
| 3 | 30.5 | (t) | 2.75-2.77 (1H, m, $H_{3a}$) |
| | | | 2.45-2.48 (1H, m, $H_{3b}$) |
| 4 | 29.2 | (t) | 2.45-2.48 (1H, m, $H_{4a}$) |
| | | | 2.02-2.05 (1H, m, $H_{4b}$) |
| 5 | 86.8 | (d) | 5.48 (1H, dd, $J$=4.5, 2.0, $H_5$) |
| 6 | 129.7 | (s) | |
| 7, 11 | 127.7 | (d) | 7.22 (2H, dd, $J$=8.0, 2.0) |
| 8, 10 | 116.8 | (d) | 6.85 (2H, dd, $J$=8.0, 2.0) |
| 9 | 156.9 | (s) | |

[a] $CD_3OD$, 300 MHz for $^{1}H$, 75 MHz for $^{13}C$ NMR. Coupling constants ($J$) were given in Hz.

[b] q: methyl, t: methylene, d: methine, s: quaternary carbon.




**P-A** **P-B** **P-C**

Figure 1. Partial Structures for Lepiotin (**1**)

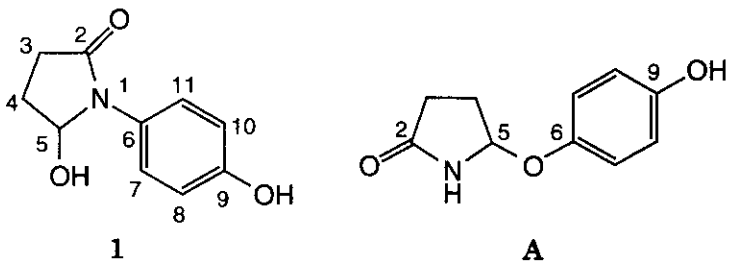
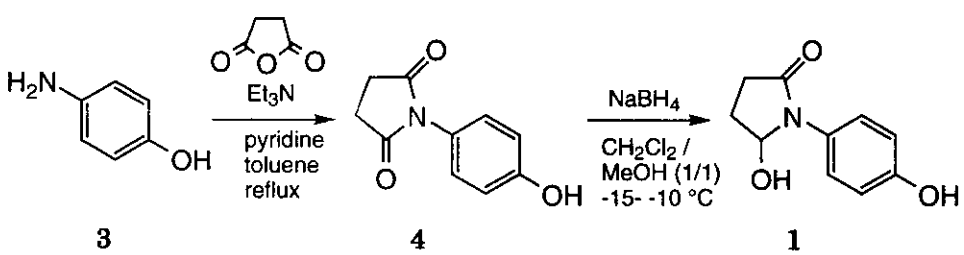



Figure 2. Structures of Lepiotin A (**1**) and Structure **A**

correlations from $H_5$ to $H_{4a}$ and $H_{4b}$ as well as the one from $H_{4b}$ to $H_{3a}$ indicated a partial structure **P-B** as shown in Figure 1. The HMBC spectrum supported the 1,4-disubstituted phenol moiety (**P-A**) and the HMBC correlations from carbonyl carbon (2-C) to methine ($H_5$) and methylene ($H_{4a}$) hydrogens indicated a five-membered ring (partial structure **P-C**). Taking into account of hetero atoms in the molecular formula, a nitrogen and an oxygen atoms must be assigned to atoms X, Y and Z, two of those must be the same atom, in the partial structures **P-A** and **P-C**. Thus the partial structures **P-A** and **P-C** assembled to the structures (**1**) and (**A**) as shown in Figure 2. The $^{13}C$ chemical shift of C-6 at $\delta$ 129.7 indicated a nitrogen as a neighboring atom (structure **1**) instead of an oxygen (structure **A**).

For the structural confirmation and the estimate of the biological activity, lepiotin A (**1**) was synthesized from 4-aminophenol (**3**). 4-Aminophenol (**3**) and succinic anhydride were dissolved in pyridine-toluene (1:1). After adding triethylamine, the reaction mixture was heated under reflux for 48 h to give the imide (**4**) in 75% yield. The imide (**4**) in dichloromethane-methanol (1:1) was reduced with $NaBH_4$ at -15- -10 °C for 1 h to give




Scheme 1.

Table 2. $^{1}H$ and $^{13}C$ NMR Spectral Data for Lepiotin B (**2**)[a]

| position | $^{13}C$ | | $^{1}H$ | |
|---|---|---|---|---|
| 2 | 175.0 | (s)[b] | | |
| 3 | 29.7 | (t) | 2.68-2.82 | (1H, m) |
| | | | 2.44-2.55 | (1H, m) |
| 4 | 24.8 | (t) | 2.23-2.37 | (1H, m) |
| | | | 2.08-2.17 | (1H, m) |
| 5 | 92.9 | (d) | 5.20 | (1H, dd, J =6.1, 1.1) |
| 6 | 130.1 | (s) | | |
| 7, 11 | 126.1 | (d) | 7.22 | (2H, ddd, J =8.7, 3.1, 2.1) |
| 8, 10 | 116.1 | (d) | 6.75 | (2H, ddd, J =8.7, 3.1, 2.1) |
| 9 | 154.7 | (s) | | |
| *O*-$CH_3$ | 54.2 | (q) | 3.26 | (3H, s) |
| | | | 5.80 | (1H, br) |

[a] $CD_3OD$, 300 MHz for $^{1}H$, 75 MHz for $^{13}C$ NMR. Coupling constants (*J*) were given in Hz.

[b] q: methyl, t: methylene, d: methine, s: quaternary carbon.

aminal (**1**) in 62% yield, that was identical with lepiotin A (**1**).[4] Lepiotin B (**2**) was isolated as a colorless oil. The molecular formula $C_{11}H_{13}NO_3$ was determined by the combination of HREI MS (m/z 207.0912, $M^+$), $^{1}H$ and $^{13}C$ NMR spectra. The latter two spectra were similar to those of lepiotin A (**1**) indicating two sets of methine groups ($\delta_H/\delta_C$ 6.75/116.1, 7.22/126.1) on 1,4-disubstituted phenol ring and a methine group ($\delta_H/\delta_C$ 5.20/92.9) attached to two hetero atoms, except for a methyl ($\delta_H/\delta_C$ 3.26/54.2) in lepiotin B (**2**) as shown in the Figure 3. Analyses of the 2D NMR spectra (H-H COSY, HMQC and HMBC) of lepiotin B (**2**) indicated similar γ-lactam ring as that of lepiotin A (**1**). HMBC correlations from a carbonyl ($\delta_C$ 175.0) to a methine ($\delta_H$ 5.20), and a methine ($\delta_C$ 92.9) to a

methoxyl ($\delta_H$ 3.26) along with the molecular formula indicated that the hetero atom X must be a nitrogen. The gross structure of lepiotin B is thus represented by the structure (**2**) as shown in Figure 3.

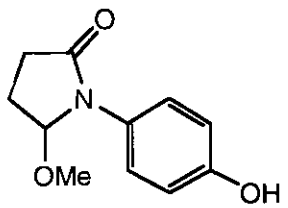



Figure 3. Gross Structure of Lepiotin B (**2**)

2) Isolation of lepiotin B (**2**) from *C. molybdites*

The ethyl acetate soluble part derived from the fruit bodies of *C. molybdites* as the similar procedure in Chart 1 was repeatedly subjected to silica gel chromatography using hexane-ethyl acetate solvent system and purified by preparative HPLC [ODS, methanol-water (3:7)] to afford lepiotin B (**2**, 2.3 mg) which was identical with the one isolated from *M. neomastoidea*.

Lepiotins A (**1**) and B (**2**) are new alkaloids having a γ-lactam and a phenol rings. Lepiotin A (**1**) is optically inactive implying an epimeric mixture related to the anomeric equilibrium at C-5. Although lepiotin B (**2**) showed optical activity, the absolute configuration at C-5 was not clarified.

Methanol extracts of *M. neomastoidea* and *C. molybdites* exhibited transient toxicity that included rising at the dose of 100 mg/kg on mice. Synthetic lepiotin A (**1**), however, did not show any activity at less than 400 mg/kg. We are now investigating assay system itself because of low toxicity of the methanol extracts of both mushrooms on mice.

## EXPERIMENT

UV spectra was recorded on a HITACHI U-3200 and IR spectra on JASCO A-100S and FT/IR 5300 spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were recorded on JEOL JNM GX500 (500 MHz for $^1H$ and 125 MHz for $^{13}C$) and VARIAN Gemini 2000 (300 MHz for $^1H$ and 75 MHz for $^{13}C$) spectrometers with TMS as an internal standard. Coupling

constants (*J*) were given in Hz. High resolution electron impact (HREI) MS spectra were recorded on a JEOL JMS DX303 spectrometer. Optical rotation was recorded on a JASCO DIP-370 spectrometer.

Isolation of lepiotins A (**1**) and B (**2**) from *M. neomastoidea*

The fruit bodies of *M. neomastoidea* (280 g) collected in Miyagi Prefecture were extracted three times with MeOH (3 x 400 mL) at rt for 30 h to give an extract (20 g) after being filtered and the solvent evaporated. This extract was partitioned between water and EtOAc. The organic layer was concentrated *in vacuo* and the residue (1.0 g) was repeatedly chromatographed on silica gel with mixtures of $CH_2Cl_2$ and MeOH of increasing polarity. Elution with $CH_2Cl_2$-MeOH (9:1) gave lepiotins A (**1**, 12.1 mg) and B (**2**, 2.0 mg).

Lepiotin A (**1**), colorless oil. UV λ (2-PrOH) nm ( log ε ): 281.8 (3.28), 230.4 (4.32), 203.0 (4.02). IR ν ($CHCl_3$): 3300, 2340, 1690, 1520, 760 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 500 MHz): δ 2.02-2.05 (1H, m), 2.45-2.48 (2H, m), 2.75-2.77 (1H, m), 5.48 (1H, dd, *J*=4.5, 2.0), 6.85 (2H, dd, *J*=8.0, 2.0), 7.22 (2H, dd, *J*=8.0, 2.0). $^{13}C$ NMR ($CD_3OD$, 125 MHz): δ 29.2 (t), 30.5 (t), 86.8 (d), 116.8 (d), 116.8 (s), 127.7 (d), 156.9 (s), 176.6 (s). HREI MS: *m/z* 193.0725 ($M^+$), Calcd 193.0739 for $C_{10}H_{11}NO_3$.

Lepiotin B (**2**), colorless oil. $[\alpha]_D^{22}$ +3.75° (*c* 0.160, MeOH). UV λ (MeOH) nm (log ε ): 279.0 (3.13), 232.2 (3.77), 202.4 (4.09). IR ν ($CHCl_3$): 3270, 2350, 1680, 1510, 1070 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 2.08-2.17 (1H, m), 2.23-2.37 (1H, m), 2.44-2.55 (1H, m), 2.68-2.82 (1H, m), 3.26 (3H, s), 5.20 (1H, dd, *J*=6.1, 1.1), 6.75 (2H, ddd, *J*=8.7, 3.1, 2.1), 7.22 (2H, ddd, *J*=8.7, 3.1, 2.1). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 24.8 (t), 29.7 (t), 54.2 (q), 92.9 (d), 116.1 (d), 126.1 (d), 130.1 (s), 154.7(s), 175.0 (s). HREI MS: *m/z* 207.0912($M^+$), Calcd 207.0895 for $C_{11}H_{13}NO_3$.

Isolation of lepiotin B (**2**) from *C. molybdites*

The fruit bodies of *C. molybdites* (186 g) collected in Shiga Prefecture were extracted three times with MeOH (3 x 300 mL) at rt for 30 h to give an extract (42 g) after being filtered and the solvent evaporated. This extract was partitioned between water and EtOAc. The organic layer was concentrated *in vacuo* and the residue (1.0 g) was chromatographed on silica gel (elution with hexane-EtOAc 1:1) and purified by preparative HPLC [ODS, MeOH-$H_2O$ (3:7)] to yield lepiotin B (**2**, 2.3 mg) as a colorless oil. $[\alpha]_D^{22}$ +3.75° (*c* 0.160, MeOH), UV λ (MeOH) nm (log ε ): 279.0 (3.13), 232.2 (3.77), 202.4 (4.09). IR ν ($CHCl_3$):

3270, 2350, 1680, 1510, 1070 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 2.08-2.17 (1H, m), 2.23-2.37 (1H, m), 2.44-2.55 (1H, m), 2.68-2.82 (1H, m), 3.26 (3H, s), 5.20 (1H, dd, *J*=6.1, 1.1), 6.75 (2H, ddd, *J*=8.7, 3.1, 2.1), 7.22 (2H, ddd, *J*=8.7, 3.1, 2.1). $^{13}C$ NMR ($CDCl_3$, 75 MHz): δ 24.8 (t), 29.7 (t), 54.2 (q), 92.9 (d), 116.1 (d), 126.1 (d), 130.1 (s), 154.7(s), 175.0 (s). HREI MS: *m/z* 207.0912 ($M^+$), Calcd 207.0895 for $C_{11}H_{13}NO_3$.

*N*-(4-Hydroxyphenyl)succimide (**3**)

4-Aminophenol (**3**, 1.1 g, 10 mmol) and succinic anhydride (1.5 g, 15 mmol) were dissolved in pyridine-toluene (1:1, 100 mL). After triethylamine (250 μL, 2.5 mmol) was added, the reaction mixture was heated under reflux for 48 h. The mixture was evaporated *in vacuo*, and the residue was chromatographed over silica gel (100 g). Elution with $CH_2Cl_2$-MeOH (9:1) afforded imide (**4**, 1.4 g, 75 %) as colorless columns, mp 260-265 °C (MeOH). IR ν ($CHCl_3$): 3690, 1712 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 500 MHz): δ 1.50 (4H, s), 6.80 (2H, d, *J*=8.3), 7.15 (2H, d, *J*=8.3). $^{13}C$ NMR ($CDCl_3$, 125 MHz): δ29.0 (t), 117.0 (d), 125.0 (s), 129.5 (d), 127.7 (d), 159.0 (s), 180.0 (s). HREI MS: m/z 191.0574 ($M^+$), Calcd 191.0583 for $C_{10}H_9NO_3$.

5-Hydroxy-*N*-(4-hydroxyphenyl)-2-pyrrolidinone (**1**)

To the imide (**4**, 1.5 g, 7.9 mmol) in $CH_2Cl_2$-MeOH (1:1, 150 mL) was added $NaBH_4$ (5 g, 132 mmol) at -15--10 °C and the mixture was stirred for 1 h. The reaction mixture was evaporated *in vacuo* and the residue was chromatographed on silica gel (300 g). Elution with EtOAc afforded aminal (**1**, 933.7 mg, 62 %) as an oil. IR ν ($CHCl_3$): 3300, 2340, 1690, 1520, 760 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz): δ 2.02-2.05 (1H, m), 2.40-2.50 (2H, m), 2.70-2.80 (1H, m), 5.50 (1H, dd, *J*=5.0, 1.8 ), 6.80 (2H, d, *J*=9.0), 7.22 (2H, d, *J*=9.0). HREI MS: *m/z* 193.0719 ($M^+$), Calcd 193.0721 for $C_{10}H_{11}NO_3$.

Acute toxicity test

Male ddy mice, 6 weeks old, were used in this study. The animals were housed in air conditioned room at rt (22 ± 1 °C) and with standard food and tap water available adlibitum. In this study five mice per group were used. The samples (100, 200, 400 mg/kg) were dissolved in 2 % gum Arabic solution and were injected intraperitoneally (ip) in mice. The appearance of mice was observed every an hour.

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