

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

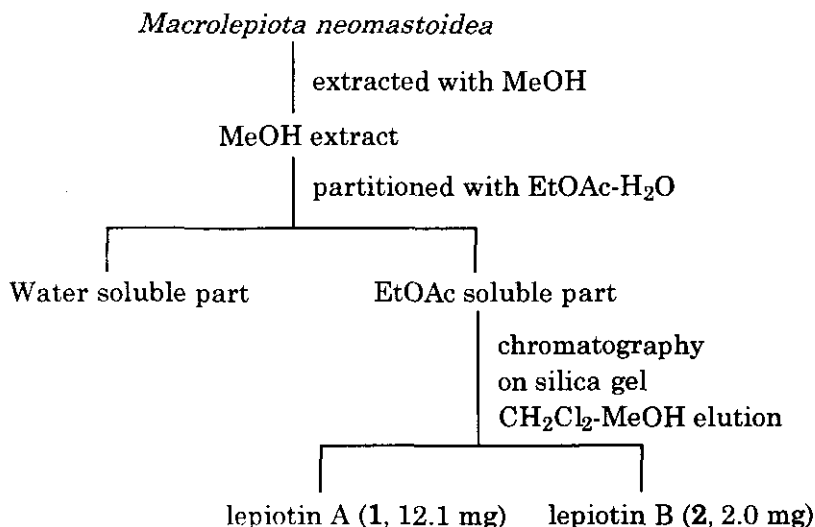


Chart 1. Isolation Procedure for Lepiotins A (1) and B (2) from *M. neomastoidea*

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 C₁₀H₁₁NO₃ was determined by the combination of HREI MS (*m/z* 193.0725, M⁺), ¹H and ¹³C 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 ¹³C NMR spectrum of lepiotin A (1) showed ten signals due to a carbonyl carbon of amide or ester moiety (δ_C 176.6), six sp² and three sp³ carbons as shown in Table 1. The ¹H and ¹³C NMR spectra of lepiotin A (1) exhibited signals for two sets of methine groups (δ_H/δ_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 (δ_H/δ_C 5.48/86.8) attached to two hetero atoms. The H-H COSY

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

position	^{13}C	^1H
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 CD₃OD, 300 MHz for ^1H , 75 MHz for ^{13}C NMR. Coupling constants (J) were given in Hz.

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

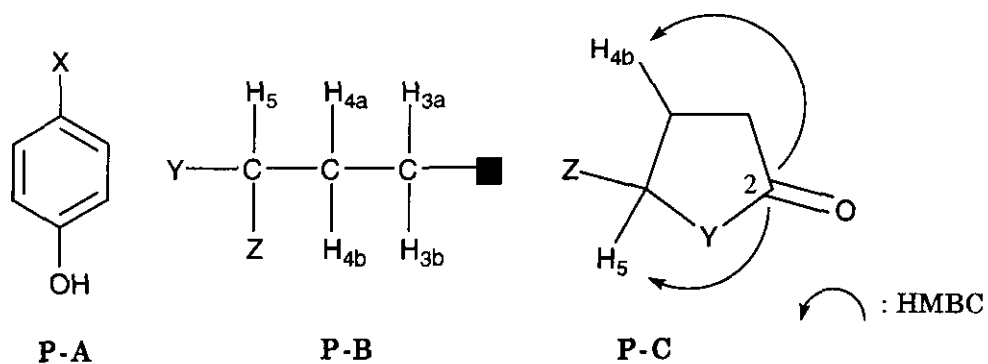


Figure 1. Partial Structures for Lepiotin (1)

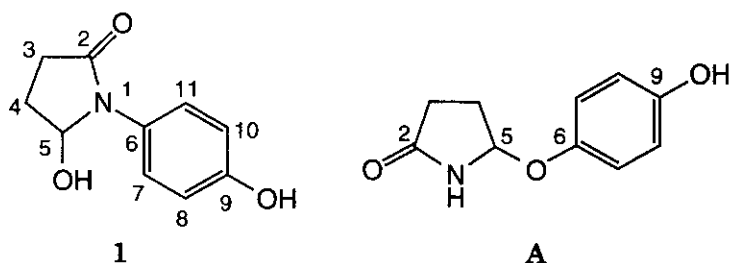
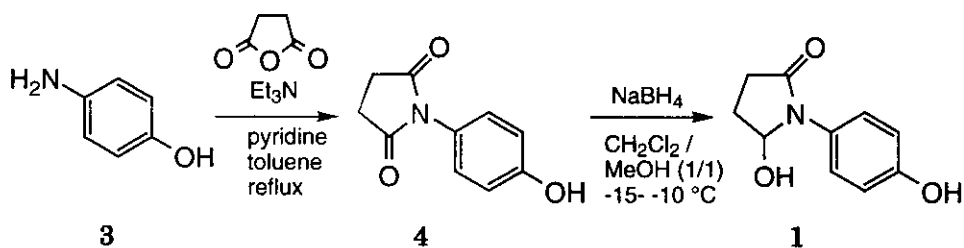


Figure 2. Structures of Lepiotin A (1) and Structure A

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 δ 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. ^1H and ^{13}C NMR Spectral Data for Lepiotin B (2)^a

position	^{13}C		^1H	
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 CD₃OD, 300 MHz for ^1H , 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 C₁₁H₁₃NO₃ was determined by the combination of HREI MS (m/z 207.0912, M⁺), ^1H and ^{13}C NMR spectra. The latter two spectra were similar to those of lepiotin A (1) indicating two sets of methine groups ($\delta_{\text{H}}/\delta_{\text{C}}$ 6.75/116.1, 7.22/126.1) on 1,4-disubstituted phenol ring and a methine group ($\delta_{\text{H}}/\delta_{\text{C}}$ 5.20/92.9) attached to two hetero atoms, except for a methyl ($\delta_{\text{H}}/\delta_{\text{C}}$ 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 (δ_{C} 175.0) to a methine (δ_{H} 5.20), and a methine (δ_{C} 92.9) to a

methoxyl (δ_{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.

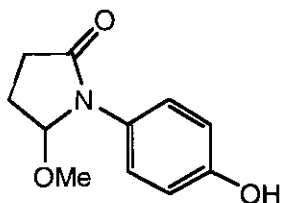


Figure 3. Gross Structure of Lepiotin B (2)

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 CH₂Cl₂ and MeOH of increasing polarity. Elution with CH₂Cl₂-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 (CHCl₃): 3300, 2340, 1690, 1520, 760 cm⁻¹. ¹H NMR (CD₃OD, 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, $J=8.0, 2.0$). ¹³C NMR (CD₃OD, 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 C₁₀H₁₁NO₃.

Lepiotin B (2), colorless oil. $[\alpha]_D^{22} +3.75^\circ$ (c 0.160, MeOH). UV λ (MeOH) nm (log ϵ): 279.0 (3.13), 232.2 (3.77), 202.4 (4.09). IR ν (CHCl₃): 3270, 2350, 1680, 1510, 1070 cm⁻¹. ¹H NMR (CDCl₃, 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, $J=8.7, 3.1, 2.1$). ¹³C NMR (CDCl₃, 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 C₁₁H₁₃NO₃.

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-H₂O (3:7)] to yield lepiotin B (2, 2.3 mg) as a colorless oil. $[\alpha]_D^{22} +3.75^\circ$ (c 0.160, MeOH), UV λ (MeOH) nm (log ϵ): 279.0 (3.13), 232.2 (3.77), 202.4 (4.09). IR ν (CHCl₃):

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 $\text{C}_{11}\text{H}_{13}\text{NO}_3$.

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 $^\circ\text{C}$ (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): δ 29.0 (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 $\text{C}_{10}\text{H}_9\text{NO}_3$.

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 $^\circ\text{C}$ 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 $\text{C}_{10}\text{H}_{11}\text{NO}_3$.

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 $^\circ\text{C}$) 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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