# Neoilludins A and B, New Bioactive Components from *Lampteromyces japonicus*

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Neoilludins A and B were isolated from *Lampteromyces japonicus*, and their structures were elucidated by spectroscopic analysis. Their relative stereochemistries were also clarified by detailed analysis of proton-proton coupling constants and NOE experiments. These compounds are cytotoxic and exhibit antibacterial activity.

In the course of our continuing search for bioactive substances from natural resources, illudin S[1] (**1**) and two novel illudin congeners were isolated from *Lampteromyces japonicus*, and assigned the structures and stereochemistries shown in Figure 1. Illudin S and M show potent antitumor activity, but their selective toxicity toward tumor cells is poor. Illudin analogues that show greatly improved efficacy, compared with the parent compounds, have recently been prepared.[2-5] Especially, HMAF (hydroxymethylacylfulven) and an acetal derivative of illudin S have been shown to be highly effective.[6] Due to their antitumor activity, illudin congeners have been the focus of considerable attention. In this communication, we report the isolation and structural elucidation of new compounds, neoilludins A (**2**) and B (**3**), from the same toadstool. They showed weak activity against the murine leukemia cell line P388 [**2:** $IC_{50}$ 1.0 μg/ml, **3:** $IC_{50}$ 3.2 μg/ml, (**1**: $IC_{50}$ 3.7 ng/ml)].

These new compounds were isolated as follows. The toadstool *L. japonicus* (12.0 kg) was collected in Yamanashi Prefecture (Japan) in the middle of autumn. The ethanolic extract of *L. japonicus* was filtered and concentrated under reduced pressure, and extracted with ethyl acetate. The ethyl acetate extracts were concentrated, and the resulting residue was partitioned with 70% aqueous methanol and hexane. The aqueous methanol layer was then separated and concentrated. The oily material was purified by column chromatography on $SiO_2$ using a gradient elution of chloroform and methanol to give crude neoilludins as an oily mixture. Finally, this oily substance

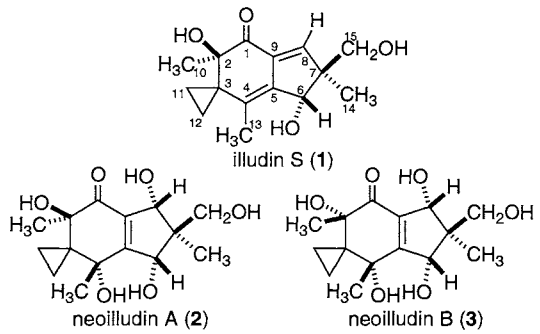


**Figure 1.** Structures of illudin S and neoilludins.

was purified by PTLC using ethyl acetate to give neoilludins A (38.4 mg, 3.20 x $10^{-6}$%) and B (31.1 mg, 2.59 x $10^{-6}$%) as a colorless glassy material.

Neoilludin A (**2**) shows an ion peak at *m/z* 321.1333 [$(M+Na)^+$] in HRFABMS,[7] indicating a molecular formula of $C_{15}H_{20}O_5$. Its $^1H$ and $^{13}C$ NMR spectral data ($CD_3OD$) are shown in Table 1.[8] Extensive NMR experiments ($^1H$ NMR, $^{13}C$ NMR, $^1H$-$^1H$ COSY, $^{13}C$-$^1H$ COSY and DEPT) and a detailed analysis of the results indicated that **2** has three methyl groups, three methylenes, two methines, and five exchangeable protons.

The proton-proton coupling network in the $^1H$ NMR spectrum of this compound was not readily assigned owing to the presence of numerous quaternary carbons. Therefore, complete assignment of the proton and carbon signals (Table 1) was achieved based on $^1H$-$^1H$ COSY, $^{13}C$-$^1H$ COSY and HMBC spectral data. Fortunately, HMBC experiments suggested that neoilludin A had a planar structure **2**. (Figure 2) As shown in Figure 2, HMBC correlation was observed from H6 ($\delta_H$ 4.77) to C5 ($\delta_C$ 166.0) and C7 ($\delta_C$ 53.4), and also from H8 ($\delta_H$ 5.05) to C1 ($\delta_C$ 203.1), C 9 ($\delta_C$ 136.0), and C7 ($\delta_C$ 166.0). These findings

**Table 1.** NMR spectral data of neoilludins A (**2**) and B (**3**)[a]

| Position | Neoilludin A (**2**) $^1H$ / ppm | $^{13}C$ / ppm (mult.)[b] | Neoilludin B (**3**) $^1H$ / ppm | $^{13}C$ / ppm (mult.)[b] |
|---|---|---|---|---|
| 1 | - | 203.1 (s) | - | 203.3 (s) |
| 2 | - | 77.2 (s) | - | 77.1 (s) |
| 3 | - | 34.6 (s) | - | 35.2 (s) |
| 4 | - | 73.4 (s) | - | 73.1 (s) |
| 5 | - | 166.0 (s) | - | 162.0 (s) |
| 6 | 5.05 (1H, d, $J$ = 1.1 Hz) | 80.1 (d) | 4.77 (1H, d, $J$ = 1.1 Hz) | 77.8 (d) |
| 7 | - | 53.4 (s) | - | 55.9 (s) |
| 8 | 4.47 (1H, d, $J$ = 1.1 Hz) | 80.0 (d) | 4.70 (1H, d, $J$ = 1.1 Hz) | 75.1 (d) |
| 9 | - | 136.0 (s) | - | 136.5 (s) |
| 10 | 1.47 (3H, s) | 28.1 (q) | 1.54 (3H, s) | 27.7 (q) |
| 11a | 0.63 (1H, ddd, $J$ = 10.6, 5.8, 4.4 Hz) | 5.09 (t) | 0.65 (1H, ddd, $J$ = 10.8, 6.0, 4.8 Hz) | 4.98 (t) |
| 11b | 0.72 (1H, ddd, $J$ = 10.6, 6.3, 4.8 Hz) | | 0.71 (1H, ddd, $J$ = 10.8, 6.0, 4.4 Hz) | |
| 12a | 0.88 (1H, ddd, $J$ = 9.9, 6.3, 4.8 Hz) | 8.31 (t) | 0.83 (1H, ddd, $J$ = 9.2, 6.0, 4.8 Hz) | 7.98 (t) |
| 12b | 0.53 (1H, ddd, $J$ = 9.9, 5.8, 4.8 Hz) | | 0.48 (1H, ddd, $J$ = 9.2, 6.0, 4.4 Hz) | |
| 13 | 1.31 (3H, s) | 25.1 (q) | 1.24 (3H, s) | 25.1 (q) |
| 14 | 0.95 (3H, s) | 18.0 (q) | 0.92 (3H, s) | 12.9 (q) |
| 15 | 3.80 (1H, dd, $J$ = 11.1 Hz) | 66.8 (t) | 3.44 (2H, s) | 67.4 (t) |
| | 3.67 (1H, dd, $J$ = 11.1 Hz) | | | |

[a]Spectra were recorded in $CD_3OD$ on a JEOL JNM-EX400 spectrometer. [b]Multiplicity was determined by DEPT experiments.

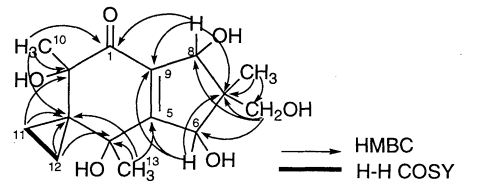


**Figure 2.** HMBC correlations of neoilludins A and B.

indicate the positions of two methine protons. The illudane framework and the positions of functional groups were thus clarified by HMBC correlations. Neoilludin A was treated with acetic anhydride and pyridine to give a triacetate.[9] This result also suggested the planar structure of **2**.[10] (Figure 2)

The molecular formula of neoilludin B (**3**) was determined to be $C_{15}H_{22}O_5$ by HRFABMS data [*m/z* 321.1301 $(M+Na)^+$].[7,8] The $^1H$ and $^{13}C$ NMR spectral data of **3** (Table 1) resembled those of **2**. All of the signals were assigned by a detailed comparison of the NMR spectral data with those of **2** and by 2D-NMR experiments. Interestingly, the HMBC correlation of **3** was the same as that of **2**. (Figure 2) Furthermore, **3** was also transformed to its triacetate.[9] As a result, neoilludin B was suggested to have a planar structure as shown in **3**.[10]

The relative stereochemistry of **2** (Figure 3) was clarified by NOE experiments. The NOE correlations between H6 and H15, and H8 and H15 suggested the stereochemistries of the two hydroxy groups at C6 and C8 and the hydroxymethyl group at C7. Furthermore, the NOE correlation for H10/H11a, H13/H11b, H13/H12b, and H13/H6, revealed the stereochemistries at C2 and C4. Therefore, the relative stereochemistry of neoilludin A is suggested to be **2**.

The relative stereochemistry of neoilludin B (**3**) was also deduced by NOE experiments. The relative stereochemistries at C6, C7, and C8 were suggested by the same NOE correlations as in **2**. The NOE correlations (H10/H11, H10/H12, H11/H13, H12/H13, and H6/H13) suggested the stereochemistries at C2 and C4 shown in Figure 3. Therefore, the relative stereochemistry of neoilludin B is suggested to be **3**. The relative high-field shift of the methyl protons (H10) of **2** is also reasonable.

Neoilludins exhibited antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. Interestingly, neoilludin A showed selective activity against *S. aureus* at 10 ppm, compared

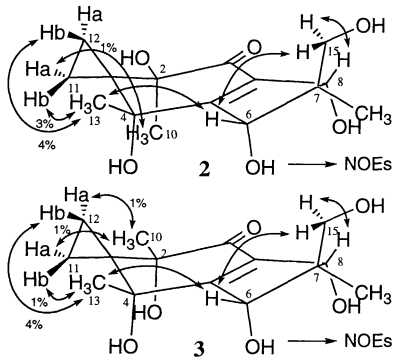


**Figure 3.** Relative stereochemistries of neoilludins.

**Table 2.** Antibacterial activity of neoilludins[a]

| | | *Bacillus subtilis* | *Staphylococcus aureus* |
|---|---|---|---|
| Illudin S | 10 ppm | 8.7±0.1[b] | 9.3±0.1 |
| | 100 ppm | 9.8±0.6 | 10.3±0.5 |
| Neoilludin A | 10 ppm | 0 | 9.8±0.4 |
| | 100 ppm | 9.9±0.6 | 10.1±0.1 |
| Neoilludin B | 10 ppm | 8.1±0.1 | 8.3±0.1 |
| | 100 ppm | 9.7±0.1 | 9.1±0.2 |

[a]A paper disc ( $\phi$ 8.0 mm) was used for the test. Cultures were grown for 3 days at 30 ℃ in the dark. [b]mean±standard error, n= 6.

with illudin S. (Table 2) Neoilludins were found to be less toxic than illudin S against murine leukemia cell line P388. Therefore, a further investigation of antibacterial activity will be necessary for these potential non-toxic antibacterial agents.

In summary, we isolated and performed a structural elucidation of neoilludins A (**2**) and B (**3**) from *L. japonicus*. Further experiments to determine their absolute stereochemistries and biosynthetic pathways are underway.

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7 Mass spectra were recorded on a JEOL JMS-LG2000 mass spectrometer.
8 Spectral data for neoilludins: **2**; IR($CHCl_3$) 3600-3100, 1690 $cm^{-1}$, $[\alpha]_D$= -2.2° (*c* 0.90, $CH_3OH$), HRFABMS *m/Z* 321.1333 [$(M+Na)^+$, Δ +1.9 mmu]: **3**; IR ($CHCl_3$) 3600-3100, 1685 $cm^{-1}$, $[\alpha]_D$= -0.5° (*c* 0.82, $CH_3OH$), HRFABMS *m/Z* 321.1301 [$(M+Na)^+$, Δ -1.3 mmu].
9 The triacetates of the neoilludins were obtained by treating the corresponding alcohols with acetic anhydride and pyridine at r.t. for 8 h. The resulting acetates were purified by PTLC on $SiO_2$ with AcOEt/hexane (1:1). The $^1H$ NMR spectrum was measured in $CD_3OD$. The $^1H$ NMR spectral data of the triacetates are as follows. 6,8,15-triacethyl neoilludin A; $^1H$ NMR, 0.55 (1H, m, H-12), 0.77 (1H, m, H-11), 0.83 (1H, m, H-11), 0.86 (1H, m, H-12), 1.12 (3H, s, H-14), 1.30 (3H, s, H-13), 1.37 (3H, s, H-10), 1.99 (3H, s, H-1'), 2.01 (3H, s, H-2'), 2.11 (3H, s, H-3'), 4.05 (1H, d, *J*= 11.7 Hz, H-15), 4.12 (1H, d, *J*= 11.7 Hz, H-15), 5.92 (1H, d, *J*= 1.1 Hz, H-8), 6.20 (1H, d, *J*= 1.1 Hz, H-6). 6,8,15-triacethyl neoilludin B; $^1H$ NMR, 0.51 (1H, m, H-12), 0.64 (1H, m, H-11), 0.76 (1H, m, H-11), 0.88 (1H, m, H-12), 0.93 (3H, s, H-14), 1.21 (3H, s, H-13), 1.46 (3H, s, H-10), 2.01 (3H, s, H-1'), 2.05 (3H, s, H-2'), 2.12 (3H, s, H-3'), 4.01 (1H, d, *J*= 11.0 Hz, H-15), 4.01 (1H, d, *J*= 11.0 Hz, H-15), 5.85 (1H, s, H-8), 5.895 (1H, s, H-6).
10 The significant difference in the $^{13}C$ NMR chemical shifts of only the carbinol carbon in $CD_3OD$ and $CD_3OH$ also suggested the planar structures. $^{13}C$ NMR ($CD_3OH$) **2**; 77.4 (C2), 73.4 (C4), 80.3 (C6), 80.2 (C8), 67.0 (C15). **3**; 77.2 (C2), 73.2 (C4), 77.9 (C6), 75.2 (C8), 67.6 (C15).