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# Nostofungicidine, an Antifungal Lipopeptide from the Field-grown Terrestrial Blue-green Alga *Nostoc commune*

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***Abstract:*** In the course of our screening program for bioactive compounds, a novel lipopeptide, nostofungicidine (**1**), was isolated from the methanolic extract of a field-grown terrestrial blue-green alga, *Nostoc commune*. The structure of nostofungicidine was elucidated by chemical degradation and extensive NMR measurements including DQF-COSY, HOHAHA, HMBC, and ROESY techniques. Nostofungicidine contains a novel β-amino acid, 3-amino-6-hydroxy stearic acid (Ahs) in its structure.


*Keywords:* Natural products; Algae; Antifungals; Amino acid and derivatives

In recent years blue-green algae (cyanobacteria) have been recognized as a promising target in the search for new biologically active compounds such as antimicrobial compounds,[1-3] enzyme inhibitors,[4-6] cytotoxic compounds.[7-9] In a previous paper we have reported a novel antimitotic compound, nostodione A, from the field-grown terrestrial blue-green alga *Nostoc commune*.[10] The following experiment indicates that the methanolic extract of this alga contains an antifungal principle(s). This paper describes the isolation and structure of a novel antifungal lipopeptide named nostofungicidine (**1**).

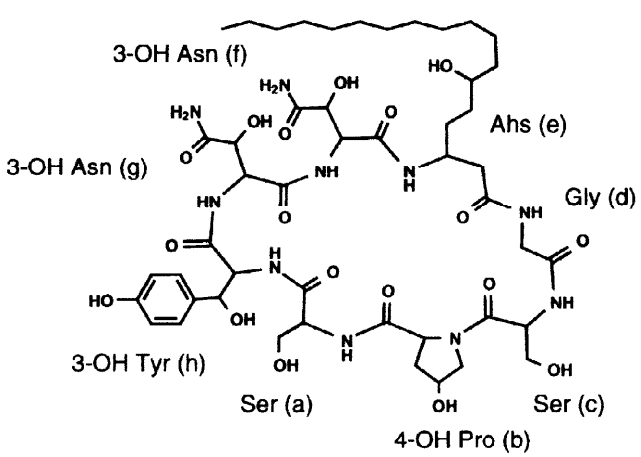


Fig. 1. Planar structure of nostofungicidine (**1**)

Field-grown *Nostoc commune* were collected at the campus of Okayama University in July 1995. The algal samples (6 kg dry weight) were washed with tap water and extracted with MeOH (18 L) for 10 days at room temperature. The resulting extract was concentrated to give an aqueous mixture which was then sequentially partitioned with *n*-hexane, ethyl acetate, and *n*-butanol. Antifungal activity was found in the *n*-butanol extract (2.4 g). The oily sample was chromatographed on ODS gel (Millipore Preparative C18 125 Å, 100 g) with a gradient elution from $H_2O$-MeOH (60:40) to 100% MeOH. Further purification was achieved by preparative HPLC (YMC AQ 313, MeOH-$H_2O$-$CHCl_3$, 80:15:5).

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Table 1. $^{1}H$ and $^{13}C$ NMR Data for Nostofungicidine (**1**) in DMSO-$d_6$

| Assignment | δH (ppm),[a] mult., *J* (Hz) | δC (ppm),[a] mult. | Assignment | δH (ppm),[a] mult., *J* (Hz) | δC (ppm),[a] mult. |
|---|---|---|---|---|---|
| serine (**a**) | | | C-6 | 3.35 (1H, m) | 69.4 (d) |
| C-1 | - | 169.1 (s) | C-7[b] | 1.24 (2H, m)[c] | 36.9 (t) |
| C-2 | 4.18 (1H, m) | 55.3 (d) | C-8~C17 | 1.23 (20H, m)[c] | 21.7-31.1 (t)[c] |
| C-3 | 3.61 (2H, m)[c] | 61.3 (t) | C-18 | 0.98 (3H, t, *J*=6.8) | 14.0 (q) |
| NH | 8.36 (1H, d, *J*=7.6) | - | NH | 7.20 (1H, d, *J*=8.1) | - |
| OH | 4.77 (1H, t, *J*=5.9) | - | OH | 4.12 (1H, d, *J*=5.6) | - |
| 4-hydroxyproline (**b**) | | | 3-hydroxyasparagine (**f**) | | |
| C-1 | - | 171.7 (s) | C-1 | - | 167.7 (s) |
| C-2 | 4.49 (1H, t, *J*=8.0) | 59.3 (d) | C-2 | 4.65 (1H, dd, *J*=8.4, 4.8) | 55.5 (d) |
| C-3 | 1.88 (1H, m) | 37.3 (t) | C-3 | 4.07 (1H, dd, *J*=6.0, 4.8) | 71.4 (d) |
| | 2.05 (1H, m) | | C-4 | - | 173.2 (s) |
| C-4 | 4.38 (1H, m) | 68.8 (d) | NH | 7.78 (1H, d, *J*=8.4) | - |
| C-5 | 3.51 (1H, dd, *J*=10.8, 6.3) | 55.4 (t) | OH | 5.65 (1H, d, *J*=6.0) | - |
| | 3.72 (1H, dd, *J*=10.8, 3.6) | | $NH_2$ | 7.19 (2H, s) | - |
| OH | 5.07 (1H, d, *J*=3.6) | - | 3-hydroxyasparagine (**g**) | | |
| serine (**c**) | | | C-1 | - | 169.6 (s) |
| C-1 | - | 169.0 (s) | C-2 | 4.67 (1H, dd, *J*=8.8, 4.8) | 55.0 (d) |
| C-2 | 4.55 (1H, m) | 53.4 (d) | C-3 | 3.97 (1H, dd, *J*=5.6, 4.8) | 72.0 (d) |
| C-3 | 3.49 (1H, m) | 60.8 (t) | C-4 | - | 173.5 (s) |
| | 3.58 (1H, m)[c] | | NH | 7.66 (1H, d, *J*=8.8) | - |
| NH | 7.58 (1H, d, *J*=7.6) | - | OH | 5.50 (1H, d, *J*=5.6) | - |
| OH | 4.84 (1H, t, *J*=6.0) | - | $NH_2$ | 7.26 (2H, s) | - |
| glycine (**d**) | | | 3-hydroxytyrosine (**h**) | | |
| C-1 | - | 168.6 (s) | C-1 | - | 170.3 (s) |
| C-2 | 3.50 (1H, dd, *J*=16.8, 6.0) | 42.5 (t) | C-2 | 4.20 (1H, dd, *J*=7.6, 6.8) | 59.2 (d) |
| | 3.85 (1H, dd, *J*=16.8, 6.0) | | C-3 | 4.75 (1H, dd, *J*=6.8, 4.4) | 71.6 (d) |
| NH | 8.10 (1H, t, *J*=6.0) | - | C-1' | - | 131.4 (s) |
| 3-amino-6-hydroxystearic acid (**e**) | | | C-2', 6' | 7.11 (2H, d, *J*=8.4) | 127.7 (d)[c] |
| C-1 | - | 170.2 (s) | C-3', 5' | 6.67 (2H, d, *J*=8.4) | 114.5 (d)[c] |
| C-2 | 2.25 (2H, m) | 40.5 (t) | C-4' | - | 156.5 (s) |
| C-3 | 3.95 (1H, m) | 46.6 (d) | NH | 7.69 (1H, d, *J*=7.6) | - |
| C-4 | 1.38 (2H, m) | 34.0 (t) | OH | 5.53 (1H, d, *J*=4.4) | - |
| C-5[b] | 1.30 (2H, m) | 37.1 (t) | 4'-OH | 9.19 (1H, s) | - |

[a]Chemical shifts in δ ppm referenced to the solvent (DMSO) at 2.49 ppm for $^{1}H$ and 39.5 ppm for $^{13}C$.
[b]Interchangeable assignments. [c]Overlapping resonances.

The active compound nostofungicidine (**1**) was obtained as a white amorphous solid (15 mg). The molecular formula of **1** was established as $C_{48}H_{76}O_{18}N_{10}$ by high resolution FAB MS (+1.9 mmu error). The UV spectrum (in MeOH) displayed an absorption maxima at 274 nm (ε=2000) in neutral and acidic solutions, and at 292 nm (ε=2900) in alkaline solution. The IR spectrum indicated intense absorbances at 3400 $cm^{-1}$ (NH) and 1660 $cm^{-1}$ (amide CO). The $^{13}C$ NMR spectrum of **1**, assisted by a DEPT experiment, showed 45 carbon signals[11] attributable to one methyl, 19 methylenes, 16 methines, and 12 quaternary carbons including 10 carbonyl carbons. The $^1H$ NMR spectrum in DMSO-$d_6$ displays 26 protons in δ 1.23 ppm ~ δ 1.38 ppm and 11 exchangeable protons with a deuterium atom in δ 7.0 ppm ~ δ 8.5 ppm which are assignable to amide hydrogens. These data suggested that **1** was a lipopeptide. The amino acid analysis of the acid hydrolysate of **1** (6 N HCl, 12 h) indicated the existence of Ser, Gly (2:1) and one imino acid which was supposed to be hydroxy- proline. The detailed two-dimensional NMR studies (DQF-COSY, HOHAHA, and HMQC) indicated the presence of remaining 4 amino acid units (**e**)~(**h**) (Table 1, Fig. 2). The total sequence of amino acid units into gross structure **1** was achieved by HMBC and ROESY spectral data (Fig. 2). The planar structure of **1** was thus established as shown in Fig. 1.

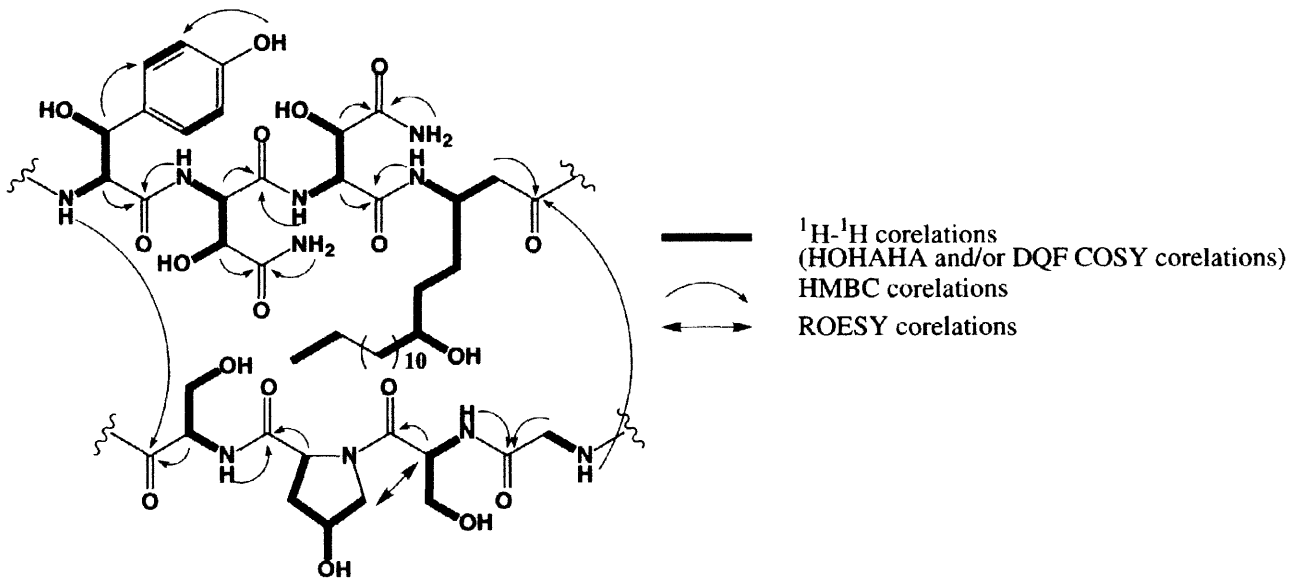


Fig. 2. DQF-COSY, HOHAHA, HMBC, and ROESY correlations of nostofungicidine (**1**)

β-Hydroxy-α-amino acids are often found in antifungal cyclic peptides of microbial origin such as pneumocandins[12] and xylocandins[13]. Long chain α-hydroxy-β-amino acids have also been found in puwainaphycins[14], calophycin[15], and microginin[16] from blue-green algae. To our knowledge, nostofungicidine is the first lipopeptide that includes 3-amino-6-hydroxy stearic acid (Ahs) in the structure. Determination of the absolute configuration of **1** is now in progress.

Nostofungicidine has shown potent antifungal activity[17] against *Aspergillus candidus* (MIC: 1.6 μg/mL) and

cytotoxicity (NSF-60 cell, $IC_{50}$=1.5 μM).[18] The stereochemistry and detailed biological activities of **1** will be reported elsewhere.

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