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# Cribrochalinamine oxides A and B, Antifungal β-Substituted Pyridines with an Azomethine *N*-Oxide from a Marine Sponge *Cribrochalina* sp.[1]

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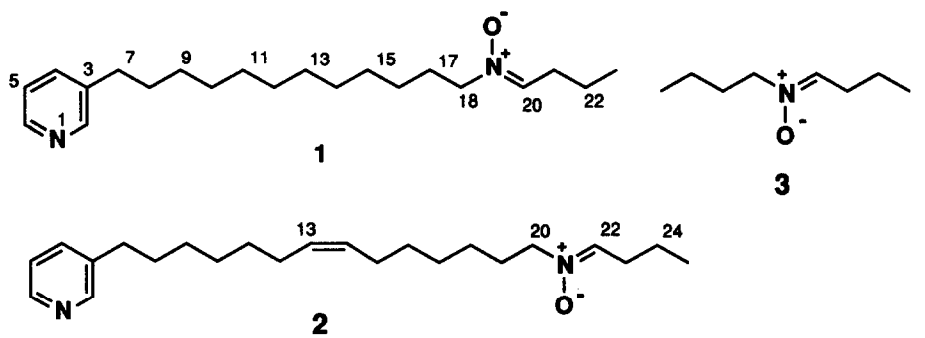
Abstract: Two β-substituted pyridine alkaloids, cribrochalinamine oxide A (**1**) and cribrochalinamine oxide B (**2**) were isolated from a marine sponge *Cribrochalina* sp. They bear a rare azomethine *N*-oxide function in the side chain.

Cytotoxic and/or antimicrobial monomeric β-substituted pyridines have been isolated from several marine sponges: niphatynes[2] and niphatesines[3] from *Niphates* sp.; xestamines[4] from *Xestospongia wiedenmayeri* and *Calyx podatypa*; theonelladines[5] from *Theonella swinhoei*; ikimines[6] from an unidentified Micronesian sponge. These compounds all have aliphatic sidechains with either terminal amino, *N*-methylamino, methoxyamino, or methoxyiminoether group. A marine sponge *Cribrochalina* sp. collected off Hachijo-jima Island exhibited antifungal activity against *Mortierella ramannianus*, and bioassay-guided fractionation of the sponge extract afforded two β-alkyl pyridine derivatives encompassing a rare azomethine *N*-oxide group.

The $Et_2O$ soluble portion of the EtOH extract of the frozen sponge (1 kg, wet weight) was partitioned between $H_2O$/MeOH (9:1) and *n*-hexane. The aqueous MeOH fraction was separated by a series of chromatographies, ODS flash ($H_2O$/MeOH), silica gel ($CHCl_3$/MeOH), and HPLC on ODS and dimethylamino-bonded phase to yield cribrochalinamine oxide A (**1**) ($8 \times 10^{-4}$ % yield based on wet weight) and cribrochalinamine oxide B (**2**) ($3 \times 10^{-4}$ % yield).[7]




Cribrochalinamine oxide A had a molecular formula of $C_{21}H_{36}N_2O$ as established by HRFABMS [*m/z* 333.2911 $(M+H)^+$, Δ 0.5 mmu]. Four mutually coupled $^1H$ NMR signals [δ 8.37 (brs), 8.34 (brd, *J*=5.0 Hz), 7.69 (brd, 7.6), and 7.35 (dd, 5.0, 7.6)] were reminiscent of β-substituted pyridine, which was corroborated by $^{13}C$ NMR data (δ 149.5, 146.5, 140.0, 137.2, and 124.7). The COSY spectrum showed the presence of a linear alkyl chain and a butylidene moiety [C20-C23; δ 7.18 (t, *J*=6.3 Hz), 2.42 (2H, dt, 6.3,

7.2), 1.57 (2H, tq, 7.2, 7.4), and 0.98 (3H, t, 7.4)]; an additional $sp^2$ signal at δ 146.0 d in the $^{13}C$ NMR spectrum implied an imino functionality. The $^1H$ and $^{13}C$ chemical shifts of the C18 methylene [$\delta_H$ 3.80 (2H, t, *J*=6.9 Hz); $\delta_C$ 65.4 t] suggested that this carbon was adjacent to either oxygen atom in an oxyimino group or a nitrogen atom. The HMBC crosspeak between H20 and C18 indicated that the two atoms were separated by three bonds, thus ruling out the possibility of an oxyiminoether. Therefore, C18 and C20 must be connected through a nitrogen atom. The deshielded chemical shift values for C20 methylene and the molecular formula strongly suggested that the nitrogen be oxidized to form a part of an azomethine *N*-oxide group. In fact, the FAB mass spectrum showed a prominent ion at *m/z* 317 $(M+H-16)^+$, demonstrating facile loss of an oxygen atom from the molecular ion, which supported the presence of an *N*-oxide.[8] Furthermore, the $^1H$ and $^{13}C$ NMR data for the C17 to C23 portion of **1** were superimposable on those of a synthetic model compound **3**.[9] A NOESY crosspeak between $H_2$18 and H20 secured the *Z* geometry of the N19,C20 double bond.

Cribrochalinamine oxide B (**2**) had a molecular formula of $C_{23}H_{38}N_2O$ as determined by HRFABMS [*m/z* 359.3016 $(M+H)^+$, Δ -4.6 mmu]. The NMR data of **2** were similar to those of **1** except for the presence of a disubstituted olefin [$\delta_H$ 5.35 (2H, m); $\delta_C$ 132.4 d, 130.1 d]. The position of the double bond in the sidechain was determined by the FAB-MS/MS data.[10] *Z*-Geometry of the C13,C14 double bond was deduced from $^{13}C$ NMR chemical shifts of 29.5 and 30.6 ppm for C12 and C15.[11]

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7. The crude antifungal fraction was a complex mixture of pyridine alkaloids, which did not give sharp peaks under various HPLC conditions, among which ODS HPLC with MeCN/50 mM $HCO_2NH_4$ (3:2) gave the best result.
   Cribrochalinamine oxide A (**1**). UV (MeOH) λmax 270 nm (ε 1900) and 255 (ε 2100); $^1H$ NMR ($CD_3OD$) δ 8.37 (brs), 8.34 (brd, *J*=5.0 Hz), 7.69 (brd, 7.6), 7.35 (dd, 5.0, 7.6), 7.18 (t, 6.3), 3.80 (2H, t, 6.3), 2.65 (2H, t, 7.6), 2.42 (2H, dt, 6.3, 7.2), 1.83 (2H, quint, 7.0), 1.65 (2H, quint, 7.0), 1.57 (2H, tq, 7.2, 7.4), 1.3-1.4 (m), 0.98 (3H, t, 7.4); $^{13}C$ NMR ($CD_3OD$) δ 149.5, 146.5, 146.0, 140.0, 138.2, 124.7, 65.4, 33.2, 31.2, 29.5-30.5, 28.5, 27.1, 18.9, 14.5.
   Cribrochalinamine oxide B (**2**). UV (MeOH) λmax 271 nm (ε 1500) and 255 (ε 1700); $^1H$ NMR ($CD_3OD$) δ 8.37 (brs), 8.33 (brd, *J*=4.9 Hz), 7.69 (brd, 7.5), 7.35 (dd, 4.9, 7.5), 7.18 (t, 5.9), 3.80 (2H, t, 6.5), 2.64 (2H, t, 7.6), 2.42 (2H, dt, 6.4, 7.2), 2.08 (2H, m), 2.03 (2H, m), 1.83 (2H, m), 1.65 (2H, quint, 7.0), 1.57 (2H, tq, 7.2, 7.4), 1.3-1.4 (m), 0.98 (3H, t, 7.4); $^{13}C$ NMR ($CD_3OD$) δ 150.0, 147.4, 146.0, 140.8, 138.2, 132.4, 130.1, 125.1, 65.4, 33.8, 32.3, 30-31, 29.6, 28.2, 27.1, 19.8, 15.5.
8. Loss of a hydroxyamino oxygen atom from peptide siderophores as well as loss of an oxygen atom from pyridine-*N*-oxides has been observed in mass spectrometry. (Persmark, M.; Pittman, JP.; Buyer, J. S.; Schwyn, B, Gill, P. R.; Neiland J. B. *J. Am. Chem. Soc.* **1993**, *115*, 3950; Richard J.; Ulrich, J. *Biomed. Environ. Mass Spectrom.* **1989**, *18*, 1)
9. Compound **3** was prepared by reduction of 1-nitro-*n*-butane with Zn (7 eq)/$NH_4Cl$ (2 eq) in THF/$H_2O$ (2:1) to *n*-butylhydroxylamine in the presence of *n*-butylaldehyde (10 eq). (Corey, E. J.; Estreicher H. *J. Am. Chem. Soc.* **1978**, *100*, 6294) **3**. $^1H$ NMR ($CD_3OD$) δ 7.18 (d, *J*=5.8 Hz), 3.81 (2H, t, 7.0), 2.43 (2H, q, 6.4), 1.83 (2H, quint, 7.3), 1.58 (2H, m), 1.35 (2H, m), 0.99 (3H, m), 0.96 (3H, m); $^{13}C$ NMR ($CD_3OD$) δ 145.8, 65.3, 30.3, 29.6, 20.5, 19.8, 14.4, 14.3.
10. Upon collisional activation of the $(M+H)^+$ ion at *m/z* 359, prominent ions were observed at *m/z* 341, 313, 299, 289, 275, 272, 258, 244, 230, 216, 202, 148, 134, 120, 106, and 93. (c.f. Tomer, K. B.; Crow, F. W.; Gross, M. L. *J. Am. Chem. Soc.* **1983**, *105*, 5487).
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