Pyrinodemins B—D, Potent Cytotoxic bis-Pyridine Alkaloids from Marine Sponge *Amphimedon* sp.

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New bis-pyridine alkaloids, pyrinodemins B—D (1—3), have been isolated together with pyrinodemin A (4) and related 3-alkyl pyridine alkaloids 5—8 from the Okinawan marine sponge *Amphimedon* sp. and the structures were elucidated from spectroscopic data. Pyrinodemins B—D (1—3) showed potent cytotoxicity, while compounds 5—8 exhibited antimicrobial activity.

**Key words** sponge; *Amphimedon* sp.; bis-pyridine alkaloids; cytotoxicity; antimicrobial activity

A number of 3-alkyl pyridine alkaloids have been isolated from marine sponges of several genera.1) Almost of them possessed a long aliphatic chain with a variable nitrogen-containing terminus,2—7) and some had dimeric or polymeric structures of the 3-alkyl pyridine.8—11) During our search for bioactive metabolites from Okinawan marine sponges,12,13) we previously isolated cytotoxic pyridine alkaloids from sponges of the genera *Theonella*14) and *Nephytes*.14—16) More recently, potent cytotoxic bis-pyridine alkaloids with a unique cis-cyclopent[c]isoxazolidine moiety, pyrinodemins B—D (1—3), have been isolated together with pyrinodemin A17) (4) and its related 3-alkyl pyridine alkaloids 5—8 from the Okinawan marine sponge *Amphimedon* sp. Here we describe the isolation and structure elucidation of 1—3 and 5—8, and potent cytotoxicity of 1—3 against tumor cell lines as well as antimicrobial activity of 5—8.

The sponge *Amphimedon* sp. (SS-955) was collected off Nakijin, Okinawa, and extracted with MeOH. EtOAc-soluble materials of the MeOH extract were subjected to silica gel columns (CHCl3–MeOH and then hexane–EtOAc) to afford pyrinodemins B, C, and D (1—3) and some had dimeric or polymeric structures of the 3-alkyl pyridine rings. Proton and carbon chemical shifts of three methines at C-15 (δH 4.05; δC 77.2, d), C-6 (δH 2.83; δC 49.3, d), and C-20 (δH 3.46; δC 72.2, d) corresponded well to those of 4, suggesting the presence of an isoxazolidine ring. The presence of a cyclopent[c]isoxazolidine moiety was deduced from the intense fragment ion peak at *m/z* 270 ([C19H28N2O]+) in the electron impact mass spectrum (EI-MS), which might be generated from 1 through Hoffmann-like elimination of the isoxazolidine ring.19) Detailed analysis of the EI-MS fragmentation pattern (Fig. 1) suggested the presence of the two alkyl chains from C-7 to C-14 and from C-7' to C-19'. In the 1H-NMR spectrum of 4, two olefin proton signals (H-16 and H-17) were observed at δ 5.34 (2H), while such olefin signals were not observed for 1. The cis-ring junction of the bicyclic system was deduced from the nuclear Overhauser effect spectroscopy (NOESY) correlation for H-15/H-16. NOESY correlations of H-15/H-16 and H-15/H-20 indicated that the relative stereochemistry of H-15 and H-16 was cis. Therefore the structure of pyrinodemin B was concluded to be 1.

Pyrinodemins C (2) and D (3) were revealed to have the molecular formulae, C37H32N4O and C39H34N4O, respectively, by the HR-FAB-MS data. The structures of 2 and 3 were elucidated to be analogues lacking one of CH2 units from C-7' to C-16' in the alkyl side chain of pyrinodemin A (4) and from C-7' to C-19' in that of pyrinodemin B (1), respectively, by analyses of 1H-NMR and EI-MS data. The position of the dissubstituted olefin in 2 was assigned to C-15' on the basis of EI-MS fragment ions at *m/z* 190 ([C14H22N3]+) and 217 ([C15H32N3]+), and the Z-geometry of the olefin was implied by the chemical shifts of the allylic carbons (C-14' and C-17', δC ca. 27),20) which were deduced from HMBC cross-peaks.

Compound 5 was shown to have the molecular formula, C36H33N3O, by HREIMS (*m/z* 320.2352 [M]+, δ 0.6 mmu). The 1H- and 13C-NMR spectra suggested that 5 was a monomer 3-alkyl pyridine alkaloid with a dissubstituted double bond. In the 1H- and 13C-NMR spectra, a pair of sp3 carbon signals due to two pyridine rings, sp3 carbon signals due to three methines (C-15, δC 77.2; C-16, δC 49.3; C-20, δC 72.2), one methylene (C-19, δC 57.3) at relatively lower field, and methylenes in a long alkyl chain (δC 26—34). Aromatic proton signals [H-2 and H-2', δH 8.42 (2H); H-4 and H-4', δH 7.48 (2H); H-5 and H-5', δH 7.19 (2H); H-6 and H-6', δH 8.44 (2H)] in the 1H-NMR spectrum suggested the presence of two 3-alkyl-substituted pyridine rings. Proton and carbon chemical shifts of three methines at C-15 (δH 4.05; δC 77.2, d), C-16 (δH 2.83; δC 49.3, d), and C-20 (δH 3.46; δC 72.2, d) corresponded well to...
and EI-MS fragment ion peaks at \( m/z \) 106 and 132. The Z-geometry of the double bond was deduced from the \(^{13}\text{C}\) chemical shifts for the allylic methylene carbons (C-8, \( \delta^C \) 28.8; C-11, \( \delta^C \) 27.1).\(^{20}\) Thus compound 5 was assigned as a 3-alkyl (C14) pyridine with \( E \) and \( Z \)-forms (3:2) at the oxime terminus.

The molecular formula, \( \text{C}_{19}\text{H}_{30}\text{N}_2\text{O} \), of compound 6 was established by HR-EI-MS (\( m/z \) 302.2362 [M], \( \Delta \) +0.4 mmu). \(^1\text{H}\)-NMR data revealed a 3-alkyl pyridine moiety, a disubstituted olefin, and an oxime terminus consisting of a 3:2 mixture of \( E \)- and \( Z \)-forms. The position of the olefin was inferred as C-15–C-16 by EI-MS fragment ion peaks at \( m/z \) 190 and 216 (Fig. 2). This was also supported by EI-MS fragment ions at \( m/z \) 190, 205, and 220 observed for the reduction product (9) of 6 with \( D_2 \). The carbon chemical shifts of C-14 and C-17 (\( \delta^C \) 29.2 and 30.5, respectively) of 6 were indicative of 15Z-geometry.\(^{20}\) Thus compound 6 was elucidated to be a \( \Delta^{15(16)} \) analogue of 5.

Compounds 7 and 8 were revealed to possess the molecular formulae, \( \text{C}_{18}\text{H}_{30}\text{N}_2\text{O} \) and \( \text{C}_{17}\text{H}_{28}\text{N}_2\text{O} \), respectively, by
Table 1. Antimicrobial Activity of Pyrinodemin A (4) and Compounds 5—8

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Candida albicans ATCC 90028</td>
<td>&gt;33</td>
</tr>
<tr>
<td>Cryptococcus neoformans ATCC 900112</td>
<td>33</td>
</tr>
<tr>
<td>Aspergillus niger ATCC 40406</td>
<td>&gt;33</td>
</tr>
<tr>
<td>Paecllyomes varioti YM-1</td>
<td>33</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes ATCC 40769</td>
<td>&gt;33</td>
</tr>
<tr>
<td>Stephyllocus aureus 209P</td>
<td>&gt;33</td>
</tr>
<tr>
<td>Micrococcus luteus IFM 2066</td>
<td>&gt;33</td>
</tr>
<tr>
<td>Bacillus subtilis PCI 189</td>
<td>&gt;33</td>
</tr>
<tr>
<td>Corynebacterium xerosis IFM 2057</td>
<td>&gt;33</td>
</tr>
<tr>
<td>Escherichia coli NIH JC2</td>
<td>&gt;33</td>
</tr>
</tbody>
</table>

Mueller Hinton broth and Sabouraud dextrose broth were used for bacteria and fungi, respectively.
Compound 7: UV \( \lambda_{\text{max}} \) (MeOH) nm (\( \epsilon \)): 264 (3100). IR (neat) cm\(^{-1}\): 3200, 2925, 1575. 1H-NMR (CDCl\(_3\)) \( \delta \): 1.2—1.3 (16H), 1.45 (2H, m), 1.55 (2H, m), 2.19 (1.2H, m), 2.38 (0.8H, m), 2.65 (2H, \( J=7.6 \text{ Hz} \)), 6.71 (0.4H, \( J=5.1 \text{ Hz} \)), 7.21 (1H, \( J=5.6 \text{ Hz} \)), 7.43 (0.6H, \( J=6.0 \text{ Hz} \)), 7.49 (1H, d, \( J=5.6 \text{ Hz} \)), 8.47 (2H, m). EI-MS \( m/z \) (rel. int. %): 93 (100), 106 (93), 120 (28), 134 (12), 148 (18), 162 (29), 176 (33), 190 (16), 204 (22), 218 (24), 232 (78), 273 (9), 290 ([M]\(^{-}\), 4). HR-EI-MS \( m/z \): 290.2337 (Calcd for C\(_{18}\)H\(_{30}\)N\(_2\)O [M]\(^{-}\): 290.2358).

Compound 8: UV \( \lambda_{\text{max}} \) (MeOH) nm (\( \epsilon \)): 264 (3200). IR (neat) cm\(^{-1}\): 3200, 2925, 1575. 1H-NMR (CDCl\(_3\)) \( \delta \): 1.2—1.3 (14H), 1.45 (2H, m), 1.55 (2H, m), 2.19 (1.2H, m, H\(_2\)-17), 2.38 (0.8H, m), 2.65 (2H, \( J=7.6 \text{ Hz} \)), 6.71 (0.4H, \( J=5.1 \text{ Hz} \)), 7.21 (1H, \( J=5.6 \text{ Hz} \)), 7.43 (0.6H, \( J=6.0 \text{ Hz} \)), 7.49 (1H, d, \( J=5.6 \text{ Hz} \)), 8.47 (2H, m). EI-MS \( m/z \) (rel. int. %): 93 (100), 106 (86), 120 (20), 134 (9), 148 (14), 162 (13), 176 (16), 190 (10), 204 (18), 218 (74), 259 (4), 276 ([M]\(^{-}\), 2). HR-EI-MS \( m/z \): 276.2185 (Calcd for C\(_{17}\)H\(_{28}\)N\(_2\)O [M]\(^{-}\): 276.2202).

Reduction of Compound 6
To a solution of compound 6 (0.1 mg) in MeOH-\( d_4 \) (70 \( \mu l \)) was added 5% palladium on activated carbon (10 \( \mu g \)), and the mixture was stirred at room temperature for 1 h under a deuterium atmosphere. After filtration of the catalyst, the filtrate was evaporated in vacuo to afford compound 9 (0.08 mg): HR-EI-MS \( m/z \): 306.2630 (Calcd for C\(_{19}\)H\(_{30}\)D\(_2\)N\(_2\)O [M]\(^{-}\): 306.2638).

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References and Notes
18) The small amounts of 1—3 obtained from this sponge prevented measurement of their specific rotations.