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Ircinamine B, bioactive alkaloid from marine sponge *Dactylia* sp.

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Abstract—Ircinamine B was isolated from the marine sponge *Dactylia* sp., which was collected at Cape Sada in Japan. Based on extensive spectral analysis, the structures of the isolated metabolites were established. This novel compound showed moderate activity against the murine leukemia cell line P388 (IC_{50} 0.28 μg/mL).

During our search for bioactive compounds from marine organisms, we isolated ircinamine B from the marine sponge *Dactylia* sp.[1] Ircinamine B (**1**) is an analog of ircinamine (**2**),[2–4] which was reported by our group in 2002. In this study, we report the isolation and structural elucidation of ircinamine B, and the biological activities of the ircinamines (Fig. 1).

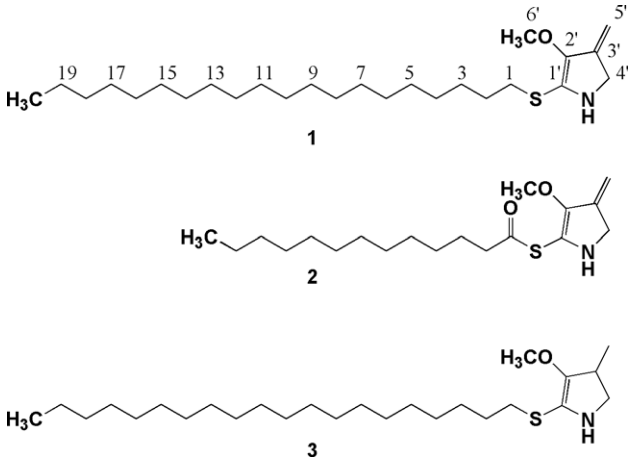

Figure 1. Structures of ircinamines.

Keywords: *Dactylia*; Marine natural products; Alkaloid; Sponge; Ircinamine.
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We collected the sponge *Dactylia* sp.[1] (3.7 kg) at Cape Sada in Ehime Prefecture (Japan) in 2001. The fresh sponges were broken into pieces and then extracted with MeOH for ca. one year at room temperature. The combined extracts were concentrated in vacuo, and the resulting aqueous suspension was partitioned between H_2O and EtOAc. The EtOAc layer was concentrated by vacuum evaporation, and the resulting residue was partitioned with 70% aq MeOH and hexane. Bioassay guided the fractionation of murine leukemia cell line P388 inhibiting hexane soluble material by sequential application of silica gel chromatography (eluent: $CHCl_3$ to MeOH) and silica gel chromatography (eluent: hexane to MeOH). The active fraction was purified by SiO_2 PTLC (eluent: 40% EtOAc/hexane) and SiO_2 PLC (eluent: 25% MeOH/$CHCl_3$). Finally, the mixture was purified with reversed-phase HPLC (eluent: 95% MeOH/H_2O) to give ircinamine B (**1**, 16.4 mg) as a colorless oil.

Ircinamine B (**1**) gave a $[M+H]^+$ ion at *m*/*z* 424.3607 in the positive ion HRFABMS spectrum that was appropriate for a molecular formula of $C_{26}H_{49}NOS$.[5] The ^1H, ^{13}C, ^1H–^1H COSY, ^{13}C–^1H COSY, and HMBC NMR spectral data of **1** readily identified two methyl groups, 21 methylene carbons, three quaternary carbons, and one exchangeable proton. The ^1H and ^{13}C NMR spectral data ($CDCl_3$) of **1** are shown in Table 1.[6,7]

HMBC experiments and EI-MS spectral data suggested the partial structures A and B shown in Figure 2. The proton connectivities H1/H2 and H20/H19 were

Table 1. NMR spectral data of ircinamine B measured in $CDCl_3$

Position	^{1}H NMR	^{13}C NMR
1	2.16 (2H, t, $J = 7.8$ Hz)	37.1
2	1.59 (2H, m)	25.7
3	[a]	29.3[c]
4–17	[a]	[b]
18	[a]	32.0[d]
19	1.22 (2H, m)	22.7[e]
20	0.88 (3H, t, $J = 8.4$ Hz)	14.1
1′	—	172.9
2′	—	166.9
3′	—	136.5
4′	4.09 (2H, br d)	40.6
5′a	5.83 (1H, s)	127.4
5′b	6.25 (1H, s)	—
6′	3.78 (3H, s)	52.0
NH	5.91 (1H, br s)	—

[a] These proton signals overlapped at 1.15–1.35 ppm (32H, m).
[b] The chemical shifts at 27.1, 27.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.7, 29.7, 29.8, 30.0, 30.1, and 32.8 ppm remain to be assigned.
[c] The chemical shift of C3 was indicated by an HMBC cross-peak between H1 and C3, and H2 and C3.
[d] The chemical shift of C18 was indicated by an HMBC cross-peak between H20 and C18.
[e] The chemical shift of C19 was indicated by an HMBC cross-peak between H20 and C19.

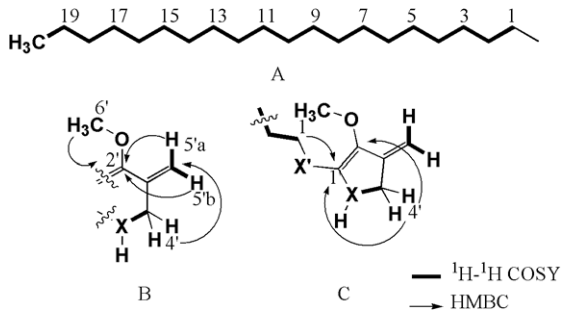

Figure 2. Partial structures of ircinamine B.

revealed by correlations in the ^{1}H–^{1}H COSY spectrum. On the other hand, the icosane group was indicated by a fragment peak (m/z 282: $C20H41^+$) of **1** in the EI-MS spectrum. These data suggest partial structure A. The location of quaternary carbon C3′ (δC 136.5) between C4′ and C5′ was verified by the HMBC correlations H4′/H3′, and H5′a, b/C3′. The ^{13}C–^{1}H COSY spectrum correlations also indicated the presence of this exomethylene group. Furthermore, the chemical shift of C4′ (δH 4.09, δC 40.6) and the ^{1}H–^{1}H correlation between H4′ and XH suggested that C4′ was adjacent to heteroatom, which is an imino proton. This assumption was confirmed by experiments with deuterium exchange. The chemical shift of C6′ (δH 3.78, δC 52.0) and the HMBC correlation between H6′ and C2′ (δC 166.9) indicated that C6′ was linked to an ether oxygen. These data were consistent with partial structure B. Furthermore, the HMBC correlations were observed between H4′/C1′ (δC 172.9) and H1 (δH 2.16)/C1′. Additionally, the only remaining olefinic carbon was C1′. Therefore partial structure B extends to partial structure C.

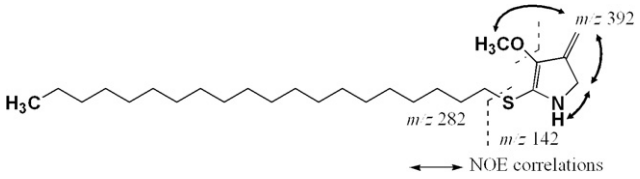

Figure 3. NOE correlations and MS fragmentation of ircinamine B.

Partial structure C and their NMR chemical shifts (^{1}H and ^{13}C) revealed the configuration of ircinamine. Therefore, the unidentified heteroatoms estimated were nitrogen and sulfur. Catalytic hydrogenation of **1** in MeOH using 10% Pd/C as a catalyst gave 3′,5′-dihydroircinamine B (**3**).[8] As expected, a methine proton (1H, 2.73 ppm) and methyl group (3H, 1.19 ppm) were observed in the ^{1}H NMR spectrum of 3′,5′-dihydroircinamine B. ^{1}H–^{1}H COSY correlations were observed between methine proton (H3′) and methyl proton (H5′), and between 3′H and methylene proton (2H, 3.30 ppm, H4′), which bears the heteroatom (NH, 5.92 ppm). The existence of a pyrroline ring moiety was also supported by detailed analysis of the NMR spectral data. Finally, the location of the sulfur atom was at one position in partial structure C. Thus, the structure of ircinamine B was deduced to be **1**.

The NOE correlations and EI-MS fragment peaks give support to the structure of **1** (Fig. 3). NOE correlations among NH/H4′, H4′/H5′b and H5′a/H6′ suggested carbon connectivity among C2′–C5′ and C6′. The EI-MS fragment peak at m/z 392 suggested elimination of the methoxy group in ircinamine B. Furthermore, m/z 142 and m/z 282 fragment peaks suggested fragmentation of sulfur and alkyl groups, respectively.

These NMR chemical shifts of ircinamine B and ircinamine suggested that the conformation of ircinamine B is not stabilized by intermolecular hydrogen bond (Fig. 4).

To summarize, we have elucidated the structure of ircinamine B (**1**) isolated from *Dactylia* sp. This compound is

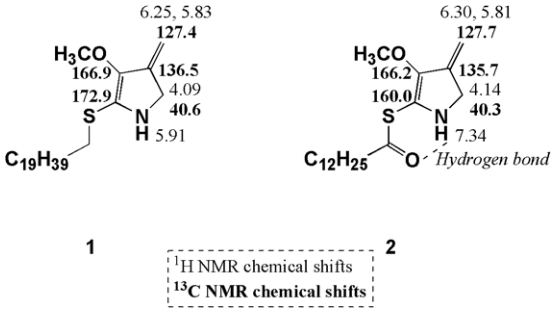

Figure 4. Characters of ircinamines.

Table 2. Biological activity of ircinamines

Compound	IC_{50} (murine leukemia cell line P388)
Ircinamine B (**1**)	0.28 μg/mL
Ircinamine (**2**)	24.9 μg/mL

an analog of ircinamine (**2**), which is a thioester compound. The biological activity of ircinamine B, which is a thioether compound, showed hundredfold more activity as compared to ircinamine against the murine leukemia cell line P388 (Table 2). This result may be related to the conformational stability of ircinamines. In addition, we isolated ircinamine B from a species other than *Ircinia* sp. Therefore, we surmise that the true producers may be the symbiotic microorganisms. Further studies on the biosynthetic pathway and biological activity of ircinamine B are in progress.

Acknowledgments

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References and notes

1. The sponge is an undescribed species and the genus to which it belongs is new to Japan.
2. Kuramoto, M.; Fujita, T.; Ono, N. *Chem. Lett.* **2002**, 464.
3. Kuramoto, M.; Chou, T.; Yamada, K.; Chiba, T.; Hayashi, Y.; Uemura, D. *Tetrahedron Lett.* **1996**, *37*, 3867.
4. Kuramoto, M.; Hayashi, K.; Yamaguchi, K.; Yada, M.; Tsuji, T.; Uemura, D. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 771.
5. Mass spectra were recorded on a JMS-700 mass spectrometer.
6. NMR spectral data were recorded in $CDCl_3$ on a JNM-EX400 spectrometer.
7. Spectral data for ircinamine B; IR ($CHCl_3$): 3451, 2927, 2854, 1716, 1670, 1512, 1457, 1442 cm^{-1}; HRFABMS *m/z* 424.3607 ($[M+H]^+$, $\Delta + 0.6$ mmu); EIMS *m/z* 423 (M^+), 392 ($M^+ - CH_3O$), 282, 142.
8. Ircinamine B was nearly quantitatively converted into 3′,5′-dihydroircinamine B (**3**). The ^1H NMR spectrum of **3** was also measured in $CDCl_3$.