Ircinamine, a Novel Cytotoxic Alkaloid from *Ircinia* sp.

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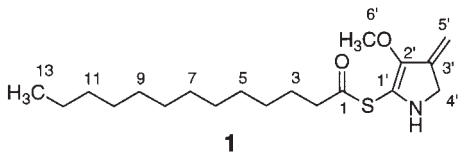
Ircinamine was isolated from the marine sponge *Ircinia* sp., and its structure was elucidated by detailed spectroscopic analysis. This novel compound showed moderate activity against the murine leukemia cell line P388.

In our continuing search for bioactive substances from marine organisms,[1,2] we isolated ircinamine (**1**) and kurospongin[3] (**2**) from the marine sponge *Ircinia* sp. Kurospongin (**2**), which was originally isolated by Higa's group in 1988, had been shown to be ichthyotoxic,[3] a feeding deterent[3] and to strongly inhibit DNA topoisomerase II.[4] Ircinamine has a unique structure with a tridecanoyl unit, a pyrroline ring, a thioester, and an enol ether, and shows moderate activity against the murine leukemia cell line P388 (IC_{50} 24.6 μg/ml). We report here the isolation and structural elucidation of ircinamine, a novel thioester compound.



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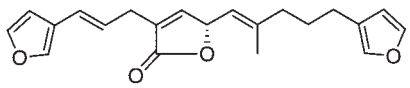

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The brown sponge *Ircinia* sp. (7.1 kg) was collected at Sada cape in Ehime Prefecture (Japan) in mid-spring. The methanolic extract was filtered and concentrated under reduced pressure, and extracted with ethyl acetate. The ethyl acetate layers were concentrated, and the resulting residue was partitioned with 70% aqueous methanol and hexane. The hexane layer was concentrated to give an oily material, which was separated by column chromatography on SiO_2 using a gradient elution with toluene and chloroform. The 50% toluene/chloroform eluate was concentrated under reduced pressure and the residue was purified by preparative TLC on silica gel with chloroform to give an oily mixture of ircinamine and kurospongin. Finally, this oily substance was purified by reversed-phase HPLC using acetonitrile to give ircinamine (2.3 mg, 3.2×10^{-7}%) (positive to Draggendorff test) and kurospongin (13.0 mg, 1.8×10^{-6}%) as a colorless glassy material, respectively.

Ircinamine (**1**) shows an ion peak at m/z 339.2304 (M^+) in HREI-MS, indicating a molecular formula of $C_{19}H_{33}NO_2S$.[5] Its ^{1}H and ^{13}C NMR spectral data ($CDCl_3$) are shown in Table 1.[6,7] Extensive NMR experiments (^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and DEPT) and a detailed analysis of the results indicated that **1** had two methyl groups, 13 methylene carbons, four quaternary carbons, and one exchangeable proton.

The proton-proton coupling network in the ^{1}H-NMR spectrum of this compound could not be readily assigned due to the presence of quaternary carbons and overlapping of the signals at δ 1.15–1.35 ppm. Therefore, complete assignment of the proton and carbon signals was achieved based on the analysis of ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and HMBC spectral data.

Table 1. NMR spectral data of ircinamine (**1**)

Position	^{1}H–NMR	^{13}C–NMR (mult.)
1	—	199.0 (s)
2	2.90 (2H, t, J = 7.3 Hz)	36.8 (t)
3	1.60 (2H, m)	23.2 (t)
4–10	[a]	[b]
11	[a]	31.9 (t)[c]
12	1.29 (2H, m)	22.7 (t)
13	0.88 (3H, t, J = 6.8 Hz)	14.1 (q)
1′	—	160.0 (s)
2′	—	166.2 (s)
3′	—	135.7 (s)
4′	4.14 (2H, br. d)	40.3 (t)
5′a	6.30 (1H, s)	127.7 (t)
5′b	5.81 (1H, s)	
6′	3.79 (3H, s)	52.2 (q)
NH	7.34 (1H, br. s)	

[a]These proton signals overlapped at 1.15–1.35 ppm (16H, m). [b]The chemical shifts at 29.1, 29.3, 29.3, 29.4, 29.5, 29.6, and 29.6 ppm remain to be assigned. [c]The chemical shift of C11 was indicated by an HMBC crosspeak between H13 and C11.

Fortunately, HMBC experiments and EI-MS spectral data suggested the partial structures a–c shown in Figure 1. The proton connectivities H2/H3 and H12/H13 were revealed by crosspeaks in the ^{1}H–^{1}H COSY spectrum. On the other hand, connectivity between C1 (δ_C 199.0) and C2 was deduced by HMBC crosspeaks (H2/C1 and H3/C1) and the chemical shift of H2 (δ_H 2.90). Furthermore, a tridecanoyl group was also indicated by a fragment peak (m/z 197: $C_{13}H_{25}O^+$) of **1** in the EI-MS spectrum. These data suggested partial structure a. The location of

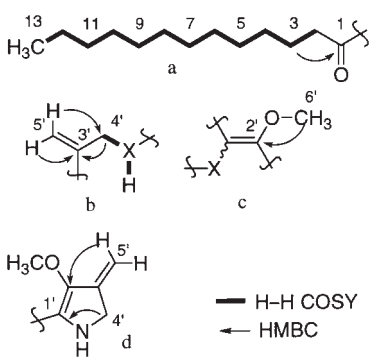

Figure 1. Partial structures of ircinamine.

quaternary carbon C3′ (δ_C 135.7) between C4′ and C5′ was verified by the HMBC crosspeaks H4′/C3′, and H5′a and H5′b/C3′. The IR absorption band (908 cm^{-1}, $CHCl_3$)[8] also indicated the presence of this exomethylene group. Furthermore, the chemical shift of C4′ (δ_H 4.14, δ_C 40.3) and the ^1H-^1H COSY crosspeak between H4′ and NH suggested that C4′ was adjacent to a heteroatom, which beared a proton (partial structure b). The chemical shift of C6′ (δ_H 3.79, δ_C 52.2) and the HMBC crosspeak between H6′ and C2′ (δ_C 166.2) indicated that C6′ was linked to an ether oxygen. Furthermore, C1′ (δ_C 160.0) was considered to link to C2′, since the only remaining olefinic carbon was C1′. These data were consistent with partial structure c.

Further extensive HMBC experiments revealed the connections between units a–c. Insertion of the quaternary carbon C3′ (δ_C 135.7) between C2′ and C4′ was supported by HMBC crosspeaks for H5′a,b/C2′ and H4′/C2′. Furthermore, the ^{13}C chemical shift of C1′ (δ_C 160.3) and the HMBC crosspeaks (H4′/C1′) corresponding to a three-bond correlation verified the presence of a pyrroline ring consisting of C1′–C4′, and a nitrogen atom[7] (partial structure d). Catalytic hydrogenation of **1** in MeOH using 10% Pd-C as a catalyst gave 3′,5′-dihydroircinamine (**3**).[9] As expected, a methine proton at 2.72 ppm and a methyl group (3H, 1.19 ppm) were observed in the ^1H NMR spectrum of 3′,5′-dihydroircinamine. This methine proton was correlated with methyl (1.19 ppm) and methylene protons (2H, 3.37 ppm), which bears the hetero atom (NH, 6.79 ppm). The existence of a pyrroline ring moiety was also supported by a detailed analysis of NMR data, as shown in Figure 2. Finally, the sulfur atom was necessarily connected to C1 (δ_C 199.0), which was an entirely reasonable ^{13}C NMR chemical shift.[10] Only one position remains in partial structure d in Figure 1 for a sulfur atom. Thus, the structure of ircinamine was deduced to be **1**.

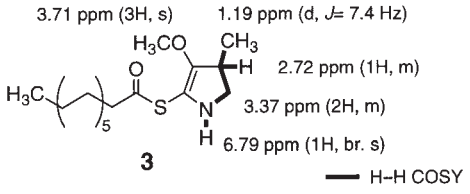

Figure 2. NMR assignment of dihydroircinamine (**3**).

The structure of **1** was also consistent with NOE data and EI-MS data, as shown in Figure 3. NOE crosspeaks among NH/H4′, H4′/H5′b, and H5′a/H6′ suggested carbon connectivities among C2′–C5′ and C6′. As noted above, a tridecanoyl group was indicated by an MS fragment peak (m/z 197). Furthermore, a methoxy group and pyrroline moiety were suggested by fragment peaks of 308 and 110, respectively.

In summary, we have elucidated the structure of ircinamine

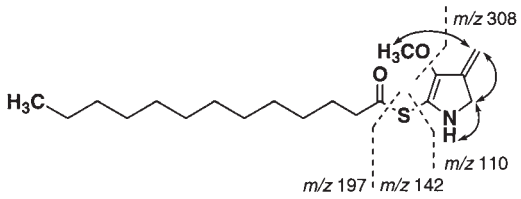

Figure 3. NOE correlations and MS fragmentation of **1**.

(**1**) isolated from *Ircinia* sp. Although ircinamine has only moderate activity toward P388, marked biological activity is expected based on the reactivity of the thioester moiety.[11] Further studies on biosynthetic pathways and its biological activity are in progress.

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References and Notes

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4 S. Fukuzawa and D. Uemura, the 61st Annual Meeting of the Chemical Society of Japan, Yokohama, March 1991, Abstr., No. 1C846.
5 Mass spectra were recorded on an M-80B mass spectrometer.
6 NMR spectral data were recorded in $CDCl_3$ on a JNM-EX 400 spectrometer.
7 Spectral data for ircinamine; IR ($CHCl_3$): 3411, 3000–2900, 2856, 1718, 1687, 1519, 1440, 908 cm^{-1}, UV (CH_3OH): λ_{max} 242 cm^{-1} (ε 1268), HREIMS m/z 339.2304 (M^+, Δ −7.4 mmu), EIMS m/z 339 (M^+), 308 (M^+-CH_3O), FABMS m/z 340 (M^++H).
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9 Ircinamine was nearly quantitatively converted into **3**. The ^1H NMR spectrum of **3** was also measured in $CDCl_3$. An upfield shift of H4′ was observed in the ^1H NMR spectrum of **3** [EIMS, m/z 341 (M^+)].
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