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KALIHINENE AND ISOKALIHINOL B, CYTOTOXIC DITERPENE ISONITRILES FROM THE MARINE SPONGE *ACANTHELLA KLETHRA*[1]

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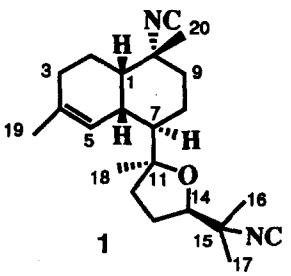
Abstract: Kalihinene and isokalihinol B, cytotoxic diterpene isonitriles have been isolated from the marine sponge *Acanthella klethra*. Structures of these compounds were elucidated by interpretation of NMR spectra as well as by single crystal X-ray diffraction.

Marine sponges of the order Halichondrida often contain sesquiterpene isonitriles and diterpene isonitriles which show a broad spectrum of biological activities, including antimicrobial and ichthyotoxic properties.[2] In our screening for antifungal and cytotoxic metabolites in Japanese marine invertebrates, the marine sponge *Acanthella klethra* collected during the cruise on the R/V Toyoshio-maru of Hiroshima University in Kuchinoerabu Island of the Satsunan Archipelago (-10 to -15 m) showed marked activity in both assays. Bioassay-guided fractionation of the ethanol extract of the sponge yielded two new diterpene isonitriles, named kalihinene (1) and isokalihinol B (2) along with the known diterpene kalihinol A (3). This paper deals with the isolation and structure elucidation of these substances.



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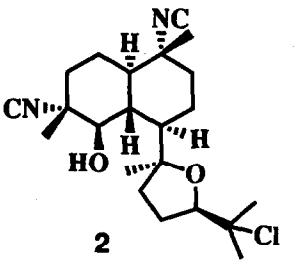

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The ether soluble portion of the ethanol extract of the frozen sponge (500 g) was partitioned between aqueous methanol and the solvent series of hexane, CCl_4, and CH_2Cl_2. The hexane layer was subjected to vacuum chromatography on Kieselgel 60H (E. Merck) with $CHCl_3$/MeOH. The active fractions eluted with $CHCl_3$ were gel-filtered on Toyopearl HW40 SF (Toso Co., Ltd.) with $CHCl_3$/MeOH (1 : 1) and successively purified by HPLC on YMC SH-043 Sil (Yamamura Chem. Lab. Co., Ltd.) with

hexane/ether (2 : 1) followed by on Develosil ODS-5 (Nomura Chem. Co., Ltd.) with 90% aq MeOH and Capcell Pak C_{18} (Shiseido Co., Ltd.) with 80% aq MeOH to obtain kalihinene (**1**) (31 mg). On the other hand, the CH_2Cl_2 and CCl_4 layers were fractionated by flash chromatography (Si/hexane - ether) and gel filtration followed by HPLC on YMC SH-043 Sil with $CHCl_3$ to afford isokalihinol B (**2**) (18 mg) and kalihinol A (**3**) (108 mg).

Kalihinene (**1**)[3] had a molecular formula of $C_{22}H_{32}N_2O$ which was established by FDMS (*m/z* 340 M^+) and ^{13}C NMR spectrum. The sharp IR absorption at 2160 cm^{-1} and two broad triplets (J = 6 Hz) at δ_C 60.4 and 60.9 in ^{13}C NMR spectrum indicated the presence of two isonitrile groups. The framework of **1** was straightforward by comparison of ^1H and ^{13}C NMR data (Table 1) with those reported for kalihinol F (**4**).[4,5] Instead of OH-4 and NC-5 in **4**, kalihinene had a trisubstituted double bond [δ_H 5.64 (1H, bd, J = 5.1Hz), 1.62 (3H, bs); δ_C 131.0 s, 126.3 d, 23.3 q], whose connectivity was confirmed by a ^1H-^1H COSY spectrum; a cross peak between H-5 (δ_H 5.64) and Me-19 (1.62) was observed. Similarly, connectivities of the remaining portion were secured by COSY spectra.

The relative configuration of kalihinene was deduced by decoupling and NOE experiments. At first, we thought that **1** had a *trans*-decalin system as is the case in the kalihinols.[4-6] Therefore, it was unexpected that the H-6 proton at δ_H 2.19 became a doublet with a large coupling constant of 11 Hz upon irradiation of H-5 (δ_H 5.64), while it became a broad singlet upon irradiation of H-7 (δ_H 1.58), indicating that H-6 and H-7 were axially-oriented and H-1 was equatorial. Thus, kalihinene had a *cis*-octalin system. This assignment was supported by an NOE experiment, in which signals at δ_H 1.65 (H-1) and 1.53 (Me-20) were enhanced when the H-6 proton was irradiated. Similarly, NOEs were observed between Me-18 (δ_H 1.10) and both H-14 (3.79) and H-6.

The final confirmation of the stereochemistry was done by single crystal X-ray diffraction which confirmed the stereochemistry determined by the NMR analysis and clarified the stereochemistry of the tetrahydrofuran portion. Kalihinene (**1**) crystals from ethanol belonged to the orthorhombic space group $P2_12_12_1$ with cell constants of a = 12.345(3), b = 12.738(2), c = 13.315(5) Å and one molecule of composition $C_{22}H_{32}N_2O$ in the asymmetric unit. A total of 1629 independent diffraction maxima with $2\theta \leq 115°$ were measured using θ:2θ scans and graphite monochromated Cu Kα radiation. After correction for Lorentz, polarization and background effects, 1033 (63%) were judged observed ($|F_o| \geq 4\sigma|F_o|$). The structure was solved by direct methods and refined with full-matrix least-squares refinements with anisotropic heavy atoms and fixed isotropic riding hydrogens. The final crystallographic residual was 5.70 %.[7]

A computer generated perspective drawing is given in Figure 1. As anticipated from the NMR analysis, the ring fusion is *cis*; C-21, H-1, and H-6 are all mutually *cis*; and H-6 and H-7 are *trans* diaxial. The cyclohexane ring is in the chair conformation;

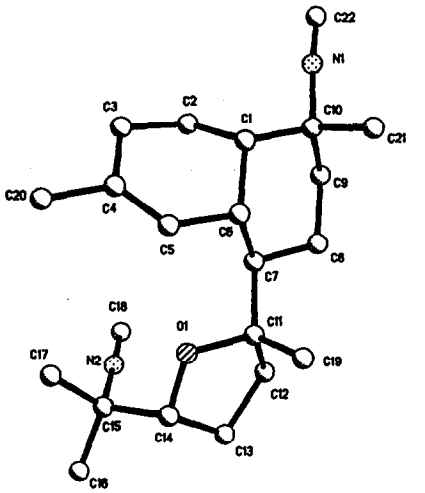
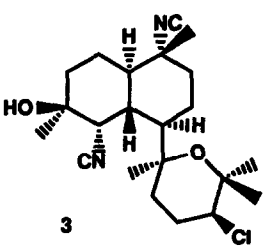


Figure 1. A computer generated perspective drawing of the final X-ray model of kalihinene (1). Hydrogens are omitted for clarity, and no absolute configuration is implied.



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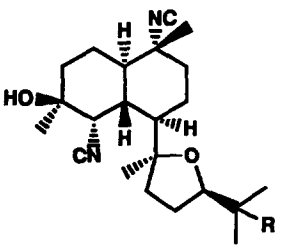

4: R = NC

5: R = Cl

Table 1: ^{13}C and ^{1}H[a] NMR data for kalihinene and isokalihinol B

	kalihinene (1)		isokalihinol B (2)			kalihinene (1)		isokalihinol B (2)	
	^{13}C[b]	^{1}H	^{13}C[c]	^{1}H		^{13}C[b]	^{1}H	^{13}C[c]	^{1}H
1	43.8	1.65	48.0	1.53	13	25.7	1.89, 2.03	25.8	2.01, 2.13
2	20.4	1.71, 2.03	21.5	1.28, 1.98	14	82.9	3.79	84.6	4.08
3	30.7	2.03	37.1	1.87, 2.11	15	60.4[d]		70.9	
4	131.0		61.7[f]		16	26.3	1.30	29.4	1.53
5	126.3	5.64	76.8	3.59	17	25.1	1.34	28.1	1.56
6	34.7	2.19	43.0	1.39	18	19.7	1.10	18.5	1.26
7	48.1	1.55	54.3	1.53	19	23.3	1.62	19.8	1.43
8	24.7	1.15, 1.58	26.4	1.22, 1.69	20	26.8	1.53	20.6	1.35
9	34.0	1.75, 1.89	40.8	1.94, 2.06	5-OH				6.65
10	60.9[d]		60.2[f]		4-NC			154.1[g]	
11	86.5		87.8		10-NC	154.2[e]		152.3[g]	
12	37.2	1.71, 1.75	39.4	1.75, 1.82	15-NC	153.6[e]			

a. Recorded at 500 MHz in $CDCl_3$
b. Recorded at 125 MHz in $CDCl_3$
c. Recorded at 25 MHz in $CDCl_3$
d - g. Assignment may be interchanged.

the cyclohexene ring, the half-chair conformation; and the tetrahydrofuran, in the C-2 conformation with C-14 on the two-fold axis.

The molecular formula of isokalihinol B (**2**)[8] was determined to be $C_{22}H_{33}N_2O_2Cl$ by FDMS [*m/z* 366 and 368 (M - NC)$^+$, 356 (M - Cl)$^+$, and 330 (M - Cl - NC)$^+$] and ^{13}C NMR data (Table 1). The 13C NMR spectrum of **2** was in good accordance with that of kalihinol B (**5**) except that signals of C-1, C-3, C-6, and C-7 were shifted lowfield in isokalihinol B. The kalihinol B-type framework was also readily established by COSY experiments; the hydroxyl group was placed at C-5 and the NC group at C-4. The relative stereochemistry was deduced by coupling constants and chemical shift value as well as by NOE experiments. Large coupling constants (J = 10 Hz) were observed between H-6 (δ_H 1.39) and a series of signals at δ 1.53 (H-1), 3.59 (H-5), and 1.53 (H-7) indicated their axial-orientation, which was further secured by NOEs observed between H-5 and H-1, H-3$_{ax}$ (δ 1.87) and H-7. This provided evidence for the *trans*-fused decalin system. Similarly, the relative stereochemistry of the tetrahydrofuran ring was elucidated; NOEs were observed between Me-18 (δ 1.26) and H-14 (4.04). The axial-orientation of both Me-19 and Me-20 was based on the chemical shift values[5] of δ_C 19.8 and 20.6, respectively.

Kalihinol A (**3**) was identified by comparison of spectral data with those reported in the literature.[5]

Kalihinene and isokalihinol B were not only antifungal against *Mortierella ramannianus* and *Penicillium chrysogenum*, but also cytotoxic against P388 murine leukemia cells with IC_{50}s of 1.2 and 0.8 μg/mL, respectively.

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References and Notes

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2. D. J. Faulkner, *Nat. Prod. Rep.* **1**, 551 (1984); **3**, 1(1986); **4**, 539 (1987); **5**, 613 (1988).
3. Kalihinene (**1**): $[\alpha]_D$ 0° ($CHCl_3$); UV (MeOH) end absorption; IR (film) 2950, 2890, 2160, 1460,1380, 1185, 1130, 1070, 1040, 990, 820 cm^{-1}; FDMS *m/z* 340 (M)$^+$; ^1H and ^{13}C NMR data in Table 1.
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6. S. Omar, C. Albert, T. Fanni, P. Crews, *J. Org. Chem.* **53**, 5971 (1988).
7. Archival crystallographic data have been deposited with the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK. Please give a complete literature citation when ordering.
8. Isokalihinol B (**2**): $[\alpha]_D$ 0° ($CHCl_3$); UV (MeOH) end absorption; IR (film) 3300, 3000, 2950, 2900, 2875, 2150, 1460, 1380, 1180, 1150, 1100, 1090, 760 cm^{-1}; FDMS *m/z* 366 and 368 (M - NC)$^+$, 356 (M - Cl)$^+$, 330 (M - Cl - NC)$^+$; ^1H and ^{13}C NMR data in Table 1.

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