Two Imidazole Alkaloids from a Sponge

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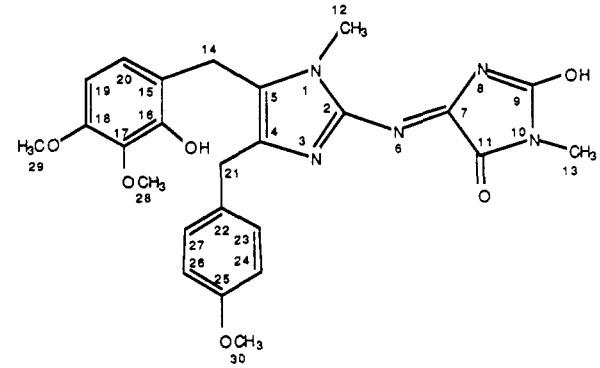
Two new imidazole alkaloids were isolated from a Micronesian sponge, *Leucetta* sp. Kealiiquinone (2) is a naphthoquinone which appears to be biogenetically derived from 1, which is a dibenzyl-substituted imidazole. Pyronaamidine (1) is cytotoxic against KB cells, MIC = 5 μg/mL.

Among marine invertebrates sponges are the leading producers of nitrogenous secondary metabolites according to a recent statistical analysis by Ireland et al.[1] Examples of new molecular entities are the naamines and naamidines, imidazole derivatives which Kashman and coworkers[2,3] isolated from a calcareous Red Sea sponge, *Leucetta chagosensis*. Our examination of a calcareous leuconoid sponge from Saipan and from Guam in Micronesia and tentatively identified as a *Leucetta* sp.[4] has resulted in two new imidazole alkaloids, which are related to Kashman's compounds and which we have named pyronaamidine (**1**)[5] and kealiiquinone (**2**).[6]

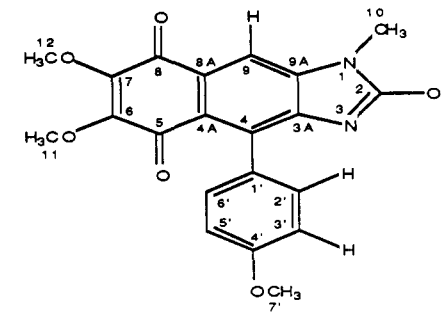
The yellow buttonlike sponges were collected in 1986 at depths of −13 to −30 m and frozen. The thawed animals were blended with ethanol. The concentrated extract was partitioned against hexane, carbon tetrachloride, chloroform, and 1-butanol. Successive chromatographies (flash, then HPLC) led to two crystalline compounds, the yellow pyronaamidine (**1**), mp 185–187 °C, and the red kealiiquinone (**2**), mp 300 °C (dec), in nearly equal amounts ((1.5 × 10^{-2})% yield).

The EI high-resolution mass spectrum of pyronaamidine (**1**) led to a formula of $C_{25}H_{27}N_5O_6$ (*m/z* 493.2043) with significant fragments at *m/z* 372 (C_8H_9O), 326 ($C_9H_{11}O_3$), 167 ($C_9H_{11}O_3$), and 121 (C_8H_9O). The ^1H NMR spectrum of **1** exhibited a broad singlet at 8.14 ppm, integrating for two exchangeable protons, two sets of ortho-coupled protons (δ 7.15, 6.78, *J* = 8.63 Hz, 2 H each, and δ 6.44, 6.34, *J* = 8.65 Hz, 1 H each), two 2 H singlets at 3.90 and 3.88 ppm, and five 3 H singlets, three OMe's at δ 3.87, 3.80, and 3.75, and two NMe's at δ 3.54 and 3.09, thus accounting for all 27 hydrogen atoms. A long-range COSY experiment[7] (Table I) suggested that each benzenoid moiety a and b was in fact benzylic.

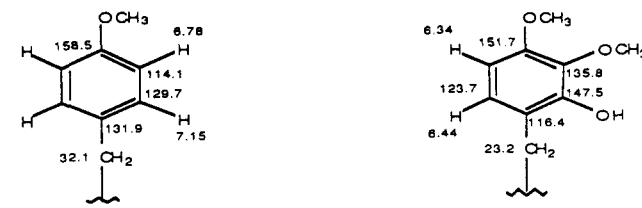
The large (9 ppm) chemical shift difference between the two benzylic carbons C14 (23.2 ppm) and C21 (32.1 ppm) finds an anology in the comparable (7.4 ppm) shift change of the benzylic carbons when going from 2-hydroxycumene (26.8 ppm) to cumene (34.2 ppm).[14]



(1)



(2)



(a) (b)

^{13}C NMR data, augmented by DEPT[8] and CSCM,[9-11] INAPT,[12,13] and NOE experiments (Tables II–IV) allowed assignment of all resonances in partial structures a, a *p*-

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(2) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003–3006.

(3) Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193–2200.

(4) Professor Bergquist's identification was based on a slide, but is chemotaxonomically validated by Kashman's work (refs 2, 3).

(5) The prefix *pyro* indicates that the new imidazoles are substituted by pyrogallol derivatives rather than by phenol or catechol as is the case in the Red Sea compounds.

(6) *Kealii* (Hawaiian, meaning the chief or noble) is the middle name of the first author.

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(14) Johnson, L. F.; Jankowski, W. C. *Carbon-13 NMR Spectra, a Collection of Assigned, Coded, and Indexed Spectra*; Wiley: New York, 1972; pp 352–353.

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Table I. ^{1}H NMR and Long-Range COSY Data of 1 (CD_2Cl_2)

assignment	chem shift (δ)	mult	integration
H_3-13	3.09	s	3 H
H_3-12	3.54	s	3 H
H_3-30	3.75	s	3 H
H_3-29	3.80	s	3 H
H_3-28	3.87	s	3 H
H_2-21	3.88	s	2 H
H_2-14	3.90	s	2 H
H-19	6.34	d	1 H
H-20	6.44	d	1 H
H-24, H-26	6.78	d	2 H
H-23, H-27	7.15	d	2 H
C-9 OH	8.14	br s	1 H
C-16 OH	8.14	br s	1 H

Table II. ^{13}C NMR, DEPT, and CSCM Data of 1 (CD_2Cl_2)

assignment	mult	^{13}C chem shift (δ)	attached protons (δ)
14	t	23.16	3.90
13	q	24.68	3.09
12	q	29.93	3.54
21	t	32.09	3.88
30	q	55.48	3.75
29	q	56.04	3.80
28	q	61.11	3.87
19	d	103.97	6.34
24, 26	d	114.06	6.78
15		116.43	
20	d	123.65	6.44
5		126.92	
23, 27	d	129.69	7.15
22		131.87	
4		135.07	
17		135.84	
7		146.53	
2		146.66	
16		147.50	
18		151.65	
9		156.40	
25		158.47	
11		162.66	

Table III. CSCM and INAPT of 1 (CD_2Cl_2)

	CSCM		INAPT	
	proton (δ)	carbon (δ)	proton irradiated	carbon correlations[a]
CH	7.15	129.69	H-23, H-27	C-21, 23, **24**, 25, **26**, 27
CH	6.78	114.06	H-24, H-26	C-22, 24, **25**, 26
CH	6.44	123.65	H-20	C-14, 16, *17*, 18
CH	6.34	103.97	H-19	C-15, *16*, 17, 18, **20**
CH_3	3.54	29.93	H-12	2, 5
CH_3	3.09	24.68	H-13	9, 11

[a] Boldface numbers represent one bond carbon correlations (J_{CCH}) and italic numbers represent four bond carbon correlations (J_{CCCH}).

Table IV. NOE Data of 1

proton irradiated	proton enhanced
H_2-21	H_2-14, H-23, H-27
H_2-14	H_3-12, H-20
H_3-29	H-19
H_3-12	H_2-14

methoxybenzyl, and b, a 2-hydroxy-3,4-dimethoxybenzyl, moiety.

These data accounted for the two major mass spectral fragments representing a $C_{17}H_{20}O_4$ portion of 1. The nitrogenous moiety, $C_8H_7N_5O_2$, remained to be elucidated. Fortuitously, a seminar presented by Professor Y. Kashman in August 1988 suggested that 1 might be related to the imidazole alkaloids reported in 1987.[2] Comparison of the ^{13}C NMR data (Table V) left no doubt that pyronaamidine had structure 1.

Table V. Comparison of ^{13}C NMR Data of the Imidazole Portion of 1 with the Chemical Shift (δ) of Kashman's Data[2]

carbon no.	1 (CD_2Cl_2)	Kashman ($CDCl_3$)
2	146.51	145.7
4	135.07	133.3
5	126.92	129.9
7	146.46	148.4
9	156.40	157.3
11	162.66	162.5
12	29.93	29.6
13	24.68	24.4

Table VI. ^{1}H NMR Data of 2 (DMSO-d_6)

assignment	mult	integration	chem shift (δ)
H_3-10	s	3 H	3.58
H_3-7′	s	3 H	3.78
H_3-12	s	3 H	3.83
H_3-11	s	3 H	3.92
H-3′, H-5′	d	2 H	6.88
H-2′, H-6′	d	3 H	7.12
H-9	s	1 H	7.69

Table VII. Coupled and Decoupled ^{13}C NMR Data (DMSO-d_6) Based on 1PDNA[15] and 1PDFA[16] Experiments

assignment	mult	chemical shift (δ)	J_{CH}, Hz	J_{CCCH}, Hz
10	q	29.18	140	
7′	q	55.43	144	
11, 12	q	61.04	146	
9	d	105.46	166	
3′, 5′	dd	113.27	160	4.63/4.93
4a	d	122.88		5.81
8a		124.07		
4	t	129.44		4.17/3.42
1′	t	130.64		8.72/7.75
2′, 6′	dd	131.06	160	7.73/7.56
9a	q	137.89		1.94/3.87/2.32
7	q	145.96		2.90/3.88/3.87
3a	d	147.82		6.15
6	q	148.20		2.90/3.88/3.87
2		158.28		
4′		158.97		2.90/2.91/2.72
8	d	181.83		4.85
5		182.39		

Based on a FAB mass spectrum, kealiiquinone (2) had a molecular formula of $C_{21}H_{18}N_2O_6$, thus differing from 1 by the elements $C_4H_9N_3$. However IR, UV, and NMR spectra revealed few structural similarities. In fact, only the *p*-anisyl moiety of partial structure a could be unambiguously identified (Table VI). Additional ^{13}C NMR experiments (Table VII) eventually allowed assignment of all carbon resonances, but only after the structure was solved by X-ray diffraction.

A computer generated perspective drawing of the final X-ray model of kealiiquinone is given in Figure 1. The aromatic tricyclic array and all of its attached atoms are planar within experimental error. The methyl group attached to O4, C15, is forced out of this plane. The attached phenyl ring, C1′–C6′, is also planar and roughly perpendicular to the plane of the tricyclic array. The phenyl ring is pushed out, away from the O3 substituent; the C12–C4–C1′ angle is 126° while the C11–C4–C1′ angle is 117°.

Once the structure of kealiiquinone (2) was established, its biogenetic relationship with pyronaamidine (1) was evident despite the absence of parallel spectral data. Kealiiquinone (2) is an oxidation product of (1) derived by ring closure (C-20 to C-21), aromatization, quinone formation in the pyrogallol dimethyl ether ring, plus oxidative loss of the aminoimidazoledione part of the mole-

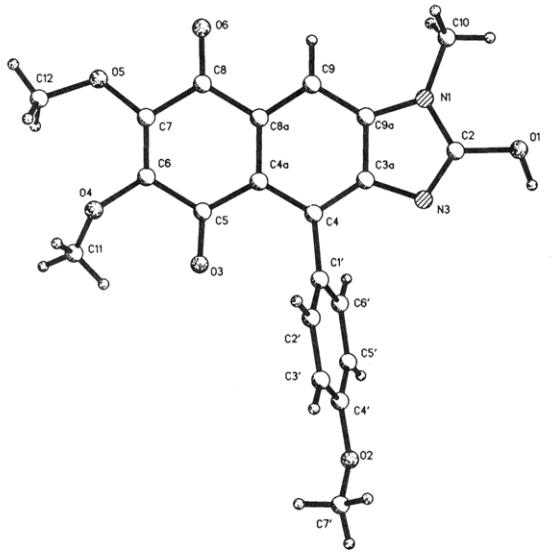

Figure 1. A computer-generated perspective drawing of kealiiquinone.

cule. We are showing the imino rather than the amino tautomer because of the low-field (δ 8.14) resonance of the exchangeable protons.[17]

Pyronaamidine (1) was cytotoxic against KB cells, MIC 5 μg/mL and inactive against HSV II virus. Kealiiquinone (2) showed no activity in our assay systems.

Experimental Section

General Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 1600 FTIR spectrophotometer and ultraviolet spectra on a Hewlett-Packard Model 8452A diode array spectrophotometer. Mass spectra were measured on a VG Analytical Model 70-SE mass spectrometer instrument and NMR spectra on a General Electric QE-300 instrument at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). Spectral grade solvents were used.

Isolation. The frozen sponge (0.66 kg wet weight) was thawed and extracted in a blender with ethanol. The ethanolic extract was reduced to $^1/_3$ of its original volume and partitioned against hexane, carbon tetrachloride, chloroform, and 1-butanol. The compounds of interest, as judged by ^{1}H NMR data, were found in the carbon tetrachloride extract (1.44 g). This extract was further purified by silica flash chromatography column (methylene chloride/methanol 95:5). Eight fractions were collected. Compounds 1 (101 mg) and 2 (98 mg) eluted in fractions 2 and 6. Further purification of 1 by HPLC on a Whatman Partisil semipreparative normal-phase silica column (ethyl acetate/hexane, 65:35), yielded 1, which was further purified by diffusion crystallization[17] using hexane and methylene chloride, yielding yellow feathery crystals, mp 185–187 °C (101 mg, (1.53×10^{-2})%). IR (thin film from $CHCl_3$): ν_{max} 3402.5 (br s), 1789.8 (sh, m), 1732.3 (sh, s), 1663.9 (sh, m), 1613.0 (sh, m), 1567.4 (sh, m), 1509.8 (sh, m), 1443.6, 1391.6, 1302.1, 1245.9, 1177.9, 1148.1, 1096.3, 1033.8, 967.9, 752.1, 605.9 cm^{-1}. UV (MeOH): λ_{max} 208 (log ϵ 4.69), 224 (4.54), 276 (3.78), 388 nm (4.22). HREIMS: m/z = 493.2043 ($C_{25}H_{27}N_5O_6$ requires m/z = 493.5182 Da). EIMS: 372 ($C_{17}H_{18}N_5O_5$), 327 ($C_{16}H_{17}N_5O_3$), 167 ($C_9H_{11}O_3$), 121 (C_8H_9O).

Fraction 6 was further purified by trituration with methylene chloride and diffusion crystallization using hexane and methanol/methylene chloride, yielding long red needles, mp 300 °C dec (98 mg, (1.485×10^{-2})%). IR (thin film from chloroform): ν_{max} 1654, 1647, 1618, 1597, 1541, 1511, 1353, 1309, 1286, 1235, 1176, 1033 cm^{-1}. UV (MeOH): λ_{max} 230 (log ϵ 4.18), 296 (4.35), 388 nm (3.14). FABMS: (M + 2) = 396.4 ($C_{21}H_{18}N_2O_6$).

Single-Crystal X-ray Diffraction Analysis. A rectangular (0.4 × 0.1 × 0.75 mm) crystal of kealiiquinone grown as described above was used in all X-ray experiments. Preliminary diffraction photographs showed only triclinic symmetry, and accurate lattice constants of a = 5.538 (7) Å, b = 13.32 (2) Å, c = 13.206 (15) Å, α = 106.50 (11)°, β = 92.21 (10)°, and γ = 91.91 (12)° were determined from a least-squares fit of 15 diffractometer measured 2θ values. Since density considerations suggested that Z = 2 for a molecular formula of $C_{21}H_{18}N_2O_6$ and there was no measurable optical rotation, we assumed space group $P1$, a choice that was validated by successful refinement. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer controlled four-circle diffractometer using graphite monochromated Cu Kα radiation (1.5418 Å) and variable speed 2θ–θ scans. After correction for Lorentz, polarization, and background effects, 1725 (70%) of the 2475 independent reflections were judged observed ($|F_o| \geq 4\sigma(F_o)$). A phasing model was found uneventfully using direct methods, and full-matrix least-squares refinements with anisotropic heavy atoms and fixed isotropic riding hydrogens have converged to a standard crystallographic residual of 0.099 for the observed data. Crystallographic parameters are available as supplementary material.

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Registry No. 1, 124535-77-9; 2, 124535-78-0.

Supplementary Material Available: Tables of crystal data, fractional coordinates, thermal parameters, bond distances, and bond angles for kealiiquinone (8 pages). Ordering information is given on any current masthead page.

(15) This experiment is based on the standard software of the QE-300 instrument. The pulse sequence is identical to the 1PULSE experiment except that the pulse programmer automatically turns the decoupling field off during data acquisition and on during the D5 delay period. This produces a coupled carbon spectrum with nuclear Overhauser enhancement (NOE) of the signals.

(16) This experiment is the complement of the 1PDFA experiment; the decoupler is turned on during data acquisition and off during the D5 delay period. It can be used to obtain a decoupled carbon spectrum without NOE.

(17) Kashman depicts the amino tautomer in ref 2, the imino in ref 3.