(2*E*,9*E*)-Pyronaamidine 9-(*N*-Methylimine), a New Imidazole Alkaloid from the Northern Mariana Islands Sponge *Leucetta* sp. cf. *chagosensis*

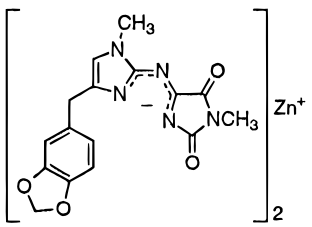
Anuchit Plubrukarn, David W. Smith, Roger E. Cramer, and Bradley S. Davidson*

Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822

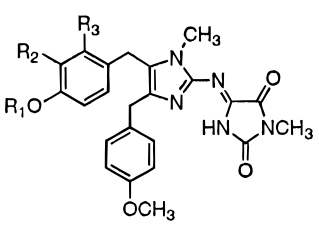
Received October 2, 1996[⊗]

A new imidazole alkaloid, (2*E*,9*E*)-pyronaamidine 9-(*N*-methylimine) (**4**), was isolated along with the known compounds pyronaamidine (**3**) and kealiiquinone (**5**) from the yellow sponge *Leucetta* sp. cf. *chagosensis* collected near the Island of Rota, Northern Mariana Islands. Single-crystal X-ray diffraction analysis allowed the unambiguous assignment of the structure of compound **4**, including the position of the exchangeable proton and the geometry of the two imino double bonds. Compound **4** exhibited mild cytotoxicity toward the A-549, MCF-7, and HT-29 human tumor cell lines.

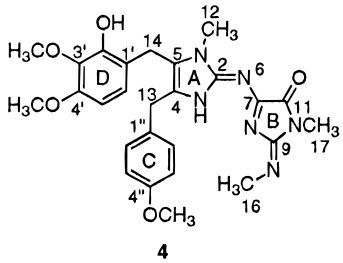
Sponges of the genera *Leucetta* and *Clathrina* have been sources of interesting imidazole alkaloids, including clathridine (**1**), which was isolated as a stable zinc complex,[1,2] naamidine A (**2**),[3,4] and pyronaamidine (**3**).[5] The alkaloids in this group are similar in that they each possess a central imidazole ring to which one or two functionalized benzyl groups are attached at the C-4, C-5, or N-3 positions, and an amino or imino moiety linked to a hydantoin or hydantoin-like ring is often connected to the N-6 position. From the antimicrobial extract of the round, yellow sponge *Leucetta* sp. cf. *chagosensis* (Leucettidae) collected near the Island of Rota in the Northern Mariana Islands, we have isolated a new imidazole alkaloid (2*E*,9*E*)-pyronaamidine 9-(*N*-methylimine) (**4**) along with the known alkaloids pyronaamidine (**3**) and kealiiquinone (**5**).[5]



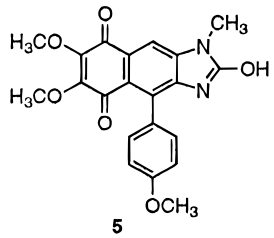
1



2 $R_1=R_2=R_3=H$
3 $R_1=CH_3$; $R_2=OCH_3$; $R_3=OH$



4



5

A methanolic extract of freeze-dried sponge tissue was subjected to a solvent-partition scheme resulting in fractions of hexane-, CCl_4-, and $CHCl_3$-soluble materials.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **4**

position	δ^1H (*J* in Hz)	δ^{13}C	HMBC
2		148.5	H-12
4		128.8	H-13, H-14
5		123.8	H-12, H-13, H-14
7		155.0	
9		151.9	H-16, H-17
11		163.9	H-17
12	3.50 s	29.6	
13	3.87 s	31.2	H-2"/H-6"
14	3.94 s	23.1	H-6'
16	3.06 s	35.3	
17	3.07 s	25.4	
1′		116.3	H-14
2′		147.6	H-14, H-5', H-6'
3′		135.9	OMe-3', H-14, H-6'
4′		151.8	OMe-4', H-14, H-5′, H-6'
5′	6.41 d (8.7)	104.0	H-6'
6′	6.57 d (8.7)	123.7	H-14, H-5'
1″		130.7	H-13
2″/6″	7.16 d (8.7)	130.2	H-13, H-3"/H-5"
3″/5″	6.82 d (8.7)	114.4	H-2"/H-6"
4″		158.8	H-3"/H-5", OMe-4"
OMe-3'	3.88 s	61.2	
OMe-4'	3.82 s	56.1	
OMe-4"	3.76 s	55.5	

Both the CCl_4 and $CHCl_3$ fractions exhibited antimicrobial activity against *B. subtilis* and *C. albicans*. Chromatography of the active fractions over Si gel provided compound **4**, together with the known compounds **3** and **5**.

The combination of four downfield doublets and a large number of singlet signals assignable to both heterosubstituted methyls and methylenes in the ^{1}H-NMR spectra for compounds **3** and **5** suggested that they belonged to the naamidine class of compounds. In fact, direct comparison of spectral data to published values indicated that compounds **3** and **5** were the known alkaloids pyronaamidine and kealiiquinone, respectively, both isolated previously from a *Leucetta* sp. sponge collected in nearby Saipan and Guam.[3]

The IR spectrum of compound **4** suggested the presence of hydroxyl (3500 cm^{-1}) and amide carbonyl (1669 cm^{-1}) functionalities. The ^{1}H- and ^{13}C-NMR spectra (see Table 1) were almost identical to those of **3**, except for the presence of one additional methyl group (3.07/25.4 ppm). The HREIMS data, which indicated a molecular formula of $C_{26}H_{30}N_6O_5$, supported the pres-

* To whom correspondence should be addressed. Present address: Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322-0300. Phone: (801) 797-1628.

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1997.

S0163-3864(96)00673-8 CCC: $14.00 © 1997 American Chemical Society and American Society of Pharmacognosy

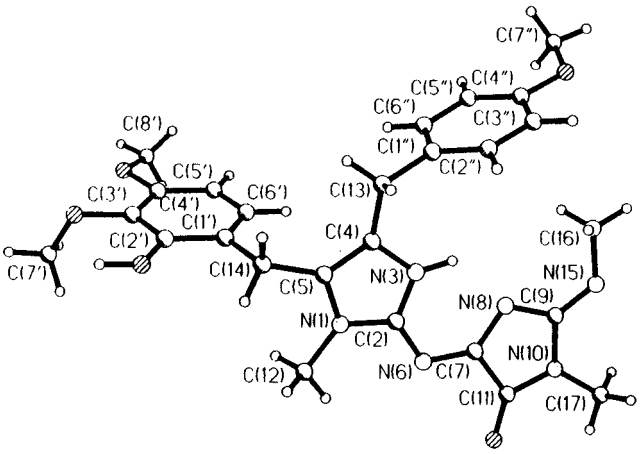
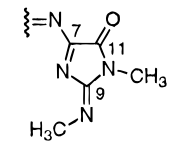


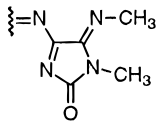
Figure 1. Crystal structure of compound **4**.

ence of an additional CH_3 group, while also revealing that an oxygen atom had been exchanged for nitrogen, when compared to pyronaamidine (**3**).

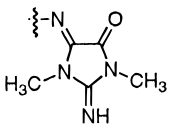
Although the NMR data for the functionalized benzylic C and D rings in **4** are virtually identical to those observed for pyronaamidine, there are small chemical-shift differences for the atoms assigned to rings A and B. The new CH_3 group (H-16, 3.07 ppm) exhibited only one correlation in the HMBC experiment (see Table 1) to a ring B carbon at δ 151.9 (C-9), indicating that the difference between compounds **3** and **4** was located in ring B. Correlations were also observed between the *N*-CH_3 group at δ 3.06 (H-17) and the ring B carbons at δ 151.9 (C-9) and 163.9 (C-11). The replacement of an oxygen with a nitrogen suggests the formation of an imine moiety as in partial structures **6**−**9**. These structures differ both in the position of the imine bond (C-9 in **6** and **8**; C-11 in **7** and **9**) and in whether the methyl group is bound to the imine nitrogen (**6** and **7**) or to N-8 (**8** and **9**). Structures **6** and **8** allow the formation of a more common guanidine functional group at C-9. Although incorporation of moiety **8** would yield a structure similar to that tentatively assigned to leucettamidine (**10**),[6] our observation of only a single HMBC correlation to the methyl group at δ 3.07 is more consistent with substructure **6**.



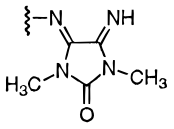
6



7

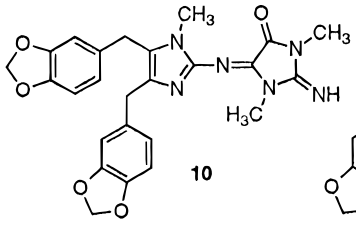


8

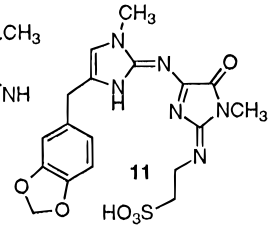


9

The ambiguity in the structure was resolved through the use of X-ray single-crystal diffraction analysis, allowing the unambiguous assignment of **4** as (2*E*,9*E*)-pyronaamidine 9-(*N*-methylimine). The computer-generated perspective drawing of compound **4** shown in Figure 1 clearly indicates that the molecule incorporates substructure **6**. Bond lengths of 1.356 and 1.322 Å for the C-2−N-6 and N-6−C-7 bonds, respectively, together with a 116.9° C-2−N-6−C-7 bond angle indicate a high degree of electron delocalization within the conjugated system, causing rings A and B to occupy a single plane. The exchangeable proton (NH-3), typically drawn in related compounds as associated with N-6,[1,7,8,9] N-8,[2] or as a C-9-OH,[3] resides on N-3 (bond length 0.9 Å) where it forms a hydrogen bond with N-8, as indicated by an NH-3 to N-8 distance of 2.06 Å. This dictates the geometry of the C-2−N-6 bond. The remaining two imino groups also can be assigned as *E*. The C-9−N-15 bond is presumably influenced primarily by potential steric repulsion between the two N-CH_3 groups. This structure is most similar to (9*E*)-clathridine 9-[*N*-(2-sulfoethyl)imine] (**11**),[10] which contains only a single benzyl group but has a taurine group attached at C-9 through an imine bond.



10



11

In order to rule out the possibility that (2*E*,9*E*)-pyronaamidine 9-(*N*-methylimine) (**4**) is simply an isolation artifact, being formed from pyronaamidine (**3**) and an adventitious supply of methylamine during extraction and chromatography, compound **3** was treated with methylamine hydrochloride in a mixture of CH_3OH−$CHCl_3$. Because compound **4** was clearly present after the initial extraction and solvent partition and before any chromatography was performed, we chose conditions that would simulate the extraction and solvent partition in the presence of methylamine. Although pyronaamidine did degrade under these conditions, no **4** was formed.

Although the initial chemical investigation was based on the observation of antimicrobial activity, only compound **3** exhibited antimicrobial activity against *Bacillus subtilis* and *Candida albicans* at a concentration of 100 μg/disk (zones of inhibition 10 and 7 mm, respectively). Compound **4**, however, did exhibit mild cytotoxicity toward the A-549 (lung), MCF-7 (breast), and HT-29 (colon) human tumor cell lines with GI_{50} values of 6, 3, and 6 μg/mL, respectively. These results are consistent with what has been observed for other *Leucetta* or *Clathrina* imidazole alkaloids. Pyronaamidine (**3**), for example, was reported to be mildly cytotoxic to KB cells with an MIC value of 5 μg/mL.[5]

Experimental Section

General Experimental Procedures. Unless otherwise noted, materials and solvents were obtained from commercial suppliers and used without further purification. Chromatography was carried out using Si gel, Merck 60 (60 Å), 230−400 mesh. Chromatography fractions were analyzed using TLC on 2 × 5 cm aluminum-backed plates covered with a 0.20-mm layer of Si gel 60 F_{254}, Art. 5554 (E. Merck, Darmstadt). UV light was used for visualization. Melting points were determined on a Laboratory Devices Mel-Temp II apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer. UV spec-

tra were obtained on a Milton Roy Spectronic 300 Array spectrophotometer. NMR spectra were recorded on a GE GN-Omega 500 spectrometer in CD_2Cl_2 (5.32 and 53.1 ppm). Mass spectra were recorded on a VG Analytical 70SE (EI) mass spectrometer.

Sponge Taxonomy. The sponge was collected from depths of 6−12 m around Rota Island in the Northern Mariana Islands, Micronesia, on December 12, 1993, and was frozen until extraction. In life, the sponges are small, bright-lemon semispherical masses, and closely compare to *Leucetta chagosensis* Dendy 1913 (class Calcarea, order Clathrinida, family Leucettidae). A voucher specimen has been deposited at the Natural History Museum, London, UK (BMNH 1996:9.17.2).

Isolation and Purification. The freeze-dried sponge tissue (69 g) was exhaustively extracted with MeOH (3 x 200 mL). After evaporation to dryness, the crude organic-soluble material (795 mg) was subjected to a Kupchan solvent-partition scheme[11] yielding hexane-, CCl_4-, and $CHCl_3$-soluble material, weighing 260, 325, and 150 mg, respectively. The CCl_4 fraction, which showed antimicrobial activity against *B. subtilis* and *C. albicans* (12- and 11-mm zones of inhibition, respectively, at 100 μg/disk), was chromatographed over Si gel, using 2% MeOH in $CHCl_3$ as eluent. Pyronaamidine (**3**, 97 mg) was obtained as yellow crystals ($CHCl_3$−MeOH), and kealiiquinone (**5**, 5 mg) was obtained as orange crystals ($CHCl_3$−MeOH). The ^{1}H-NMR spectral data (300 MHz) of both compounds were identical to those reported previously.[3] The $CHCl_3$ fraction, which was also active against *B. subtilis* and *C. albicans* (10- and 8-mm zones of inhibition, respectively, at 100 μg/disk), was repeatedly chromatographed over Si gel, using 10% of MeOH in CH_2Cl_2 as the eluent, to yield compound **4** (26 mg), which was obtained as yellow crystals after recrystallization from a mixture of MeOH and CH_2Cl_2, together with a mixed fraction containing compounds **3** and **5** (37 mg).

(2*E*,9*E*)-Pyronaamidine 9-(*N*-methylimine) (4): obtained as yellow crystals (CH_2Cl_2−MeOH); mp 222−225 °C; UV (MeOH) λ_{max} (log ϵ) 211(5.14), 274 (4.78), 388 (4.96) nm; IR (dry film) ν_{max} 3500 (−OH), 1669 (C=O, amide), 1558 (imine) cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* [M$^+$] 506 (100), 502 (15), 486 (32), 340 (31), 167 (8), 121 (43), 69 (12); HREIMS obsd *m/z* [M$^+$] 506.2295; calcd for $C_{26}H_{30}N_6O_5$, 506.2278.

Single-Crystal X-ray Diffraction Analysis of (2*E*,9*E*)-Pyronaamidine 9-(*N*-Methylimine) (4). Recrystallization of pyronaamidine 9-(*N*-methylimine) (**3**) was accomplished in the solvent mixture of CH_2Cl_2 and MeOH to yield yellow crystals. A crystal measuring 0.16 × 0.37 × 0.9 mm was cut from a cluster of several crystals and mounted on a glass fiber. A Nicolet P1 bar diffractometer, using graphite monochromatized Mo Kα radiation, was used to determine unit-cell parameters and for data collection. During data collection, the intensities of the three check reflections, monitored every 97 reflections, showed no decreases. The data were corrected for Lorentz and polarization effects using the SHELXL system. An absorption correction was applied using three ψ-scans. The structure was solved by direct methods using SHELX-86. An initial attempt using default parameters gave a best CFOM of 0.33. Although this showed a promising fragment, it failed to lead to the correct structure. A second attempt, lowering the minimum E value from 1.2 to 1.0, and doubling the number of attempts, led to a solution with a CFOM of 0.07, from which the locations of all non-hydrogen atoms were found. The structure was refined on F^2 with SHELX-93 using the 4129 reflections with positive F_0 values among the 4638 independent reflections collected. In the final model, all non-hydrogen atoms were refined anisotropically. Hydrogen atoms were added to the methyl, benzyl, and benzene groups and were refined as riding on the atoms to which they were attached with common-group thermal parameters. Hydrogen atoms on N-3 and O-19 were found from difference Fourier maps and were allowed to refine independently. After the final cycle of refinement, the largest remaining peak was 0.23 eÅ^{-3} in the middle of C-7″, O-22, C-4″, C-5″ in a map with an ESD of 0.04. Final parameters are given in Table 2.[12]

Table 2. Crystal Data and Structure Refinement for **4**

	(2*E*,9*E*)-pyronaamidine 9-(*N*-methylimine)
empirical formula	$C_{26}H_{30}N_6O_5$
formula wt	506.56
temp	293(2) K
wavelength	0.71073 Å
cryst syst	triclinic
space grp	P_1 bar
unit cell dimens	a = 8.478(4) Å α = 110.73(3)°
	b = 12.033(5) Å β = 90.53(4)°
	c = 13.970(7) Å γ = 97.650(3)°
volume	1318.6(11) Å^3
Z	2
density (calculated)	1.276 mg/m^3
absorption coeff	0.091 mm^{-1}
F(000)	536
cryst size	0.16 × 0.37 × 0.9 mm
θ range for data collectn	1.83 to 25.00°
index ranges	$-10 \le h \le 10$, $-14 \le k \le 13$, $0 \le l \le 16$
reflns collected	4845
independent reflns	4638 (R_{int} = 0.0126)
refinement method	full-matrix least-squares on F^2
data/restraints/params	4129/0/345
goodness-of-fit on F^2	0.830
final R indices [I > 2r(I)]	$R1$ = 0.0491, wR2 = 0.1299
R indices (all data)	$R1$ = 0.0945, wR2 = 0.1497
largest diff peak and hole	0.225 and −0.177 eÅ^{-3}

Acknowledgment. This work was supported by the National Institutes of Health and the American Cancer Society, in the form of a Junior Faculty Research Award to B. S. D. We thank Dr. M. Kelly-Borges, The Natural History Museum, London, for identification of the sponge, Dr. C.-J. Chang, Purdue University, for providing cytotoxicity assays, Melani Puglisi and James Parham for their help in sponge collections, Wesley Yoshida for recording many of the NMR spectra, and Geeta Davidson for editorial comments. We would also like to thank Mark Michaels and the employees of Dive Rota for their assistance.

References and Notes

(1) Ciminiello, P.; Fattorusso, E.; Magno, S.; Mangoni, A. *Tetrahedron* **1989**, *45*, 3873−3878.
(2) Ciminiello, P.; Fattorusso, E.; Mangoni, A. *Tetrahedron* **1990**, *46*, 4387−4392.
(3) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003−3006.

(4) Carmely, S.; Ilan, M; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193−2200.
(5) Akee, R. K.; Carroll, T. R.; Yoshida, W. Y.; Scheuer, P. J.; Stout, T. J.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 1944−1946.
(6) Chan, G. W.; Mong, S.; Hemling, M. E.; Freyer, A. J.; Offen, P. H.; DeBrosse, C. W.; Sarau, H. M.; Westley, J. W. *J. Nat. Prod.* **1993**, *56*, 116−121.
(7) Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 1229−1234.
(8) Alvi, K. A.; Peters, B. M.; Hunter, L. M.; Crews, P. *Tetrahedron* **1993**, *49*, 329−336.
(9) Mancini, I.; Guella, G.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1995**, *78*, 1178−1184.
(10) He, H.; Faulkner, D. J.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1993**, *57*, 2176−2178.
(11) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178.
(12) Atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates and isotropic displacement parameters have been deposited in the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

NP960673O