New Imidazole Alkaloids from the Sponge *Leucetta chagosensis*

Xiong Fu, John R. Barnes, Trang Do, and Francis J. Schmitz*

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019

Received October 22, 1996[⊗]

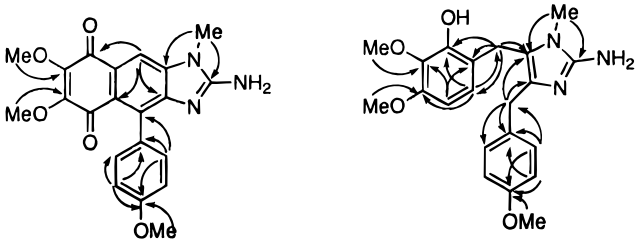
Chemical investigation of the sponge *Leucetta chagosensis* collected in Chuuk State, Federated States of Micronesia, has led to the isolation of two new alkaloids, 2-deoxy-2-aminokealiiquinone (**3**) and naamine C (**5**), along with the known alkaloid, pyronaamidine (**1**). The structures of **3** and **5** were assigned by spectroscopic and chemical methods.

A family of imidazole alkaloids has been isolated from calcareous sponges of the genera *Leucetta*[1−8] (order Clathrinida, family Leucettidae) and *Clathrina*[9,10] (order Clathrinida, family Clathrinidae) and nudibranchs of the genus *Notodoris*[5−7,11] (family Aegiridae). The *Notodoris* nudibranchs are known to feed on calcareous sponges of these two genera.[12,13] Some of these alkaloids show antimicrobial activity,[1,5,10] and pyronaamidine (**1**) is cytotoxic.[2] Several of these alkaloids have been isolated as zinc complexes.[7−10] As part of our ongoing study of marine organisms, we examined extracts of the sponge *Leucetta chagosensis* collected in Chuuk State, Federated States of Micronesia. In this paper, we report the isolation and structural elucidation of two new analogues of members of this imidazole alkaloid family.

The CH_2Cl_2 solubles from solvent partitioning of the MeOH and MeOH−CH_2Cl_2 (1:1) extracts of frozen specimens were fractionated by VLC on Si gel. Selected fractions were resolved further by C-18 reversed-phase HPLC to give the known alkaloid pyronaamidine (**1**)[2] and two new compounds, 2-deoxy-2-aminokealiiquinone (**3**) and naamine C (**5**), which is an oxygenated analogue of naamine A (**6**).[5]

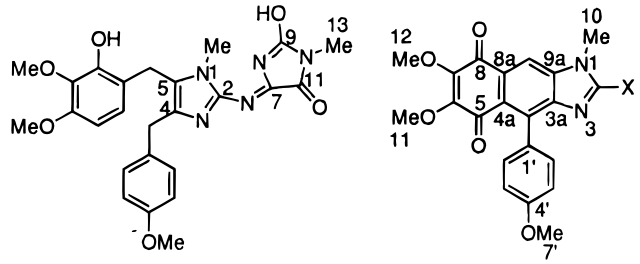
2-Deoxy-2-aminokealiiquinone (**3**) crystallized as red needles from a mixture of MeOH−CH_2Cl_2, mp >250 °C dec. The ^{1}H NMR and ^{13}C NMR data for compound **3** were similar to those of kealiiquinone (**2**)[2], especially the ^{13}C NMR data, which were nearly identical. However, the FABMS of **3** revealed a $[M + H]^+$ ion at *m/z* 394 and a $[M + Na]^+$ ion at *m/z* 416, both one mass unit less than expected for **2**, indicating replacement of O by NH in **3**. Assignment of an NH_2 group at C-2 was compatible with the mass data and was also consistent with the observation of a two-proton exchangeable signal at δ 7.10. In order to confirm the structure, **3** was acetylated (Ac_2O−pyridine) to afford a diacetate (**4**), whose FABMS showed peaks at *m/z* 478 $[M + H]^+$ and 391 $[M - 2Ac]^+$. The IR spectrum showed carbonyl absorption compatible with an imide, 1740. All spectral data of **3** and **4** were in good agreement with their structures. The ^{13}C NMR assignments for **3** were confirmed by HMQC and HMBC experiments (see Figure 1).

Naamine C (**5**) was isolated as an amorphous solid. Its molecular formula, $C_{21}H_{25}N_3O_4$, was deduced from FABMS (*m/z* 384 $[M + H]^+$) and NMR data. This formula differs from that of pyronaamidine (**1**) by the elements $C_4H_2N_2O_2$. Comparison of the ^{1}H- and ^{13}C-NMR spectral data of **5** with those of pyronaamidine (**1**) indicated that the ^{13}C signals for C-7, C-9, C-11, and C-13, and the three-proton singlet for Me-13 in the spectra of compound **1** were missing in the spectra of **5**, but that the rest of the signals for the two compounds were virtually identical. Hence, structure **5** was proposed, and this was confirmed by HMQC and HMBC data. HMBC correlations are outlined in Figure 1.



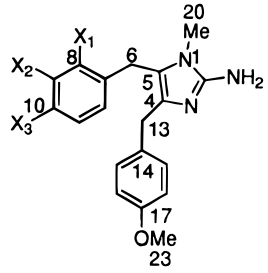
3 **5**

Figure 1. C/H long-range correlations obtained from HMBC spectra.



1

2 X=OH
3 X=NH_2
4 X=NAc_2



5 X_1 = OH; X_2 = OMe; X_3 = OMe

6 $X_1 = X_2$ = H; X_3 = OH

Experimental Section

General Experimental Procedures. IR and UV spectra were recorded on Bio-Rad 3240-spc FT and

* To whom correspondence should be addressed. Phone: 405-325-5581. FAX: 405-325-6111. E-mail: fjschmitz@chemdept.chem.uoknor.edu.

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

S0163-3864(96)00694-5 CCC: $14.00

Hewlett-Packard spectrophotometers, respectively. Mass spectra were measured with a VG ZAB mass spectrometer, and NMR spectra on a Varian VXR-500 instrument at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR). Preparative HPLC was performed using a Spherex 5 C-18 column (300 × 10 mm) with UV detection. Flash chromatography was carried out on Si gel 60-H (230−400 mesh).

Animal Material. The sponge (36T91) was collected from Northeast Pass, Chuuk Atoll, Micronesia, in 1991, and forms bright, lemon yellow semi-spherical masses.[14] The texture is quite firm, the surface smooth and undulating, the surface "catches" to the touch. This sponge is *L. chagosensis* Dendy 1913 (class Calcarea, order Clathrinida, family Leucettida). A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 1996:6:6:7).

Isolation. Freshly thawed specimens of the sponge (25 g dry wt after extraction) were cut into small pieces and soaked in MeOH (2 × 400 mL) followed by MeOH−CH_2Cl_2 (1:1) (2 × 400 mL). The extracts were concentrated, combined, and partitioned between aqueous MeOH and organic solvents as described previously,[15] to give, after evaporation of solvents under reduced pressure, hexane (0.16 g), CH_2Cl_2 (0.31 g), *n*-BuOH (1.23 g), and H_2O (0.64 g) solubles. Only the residue from the CH_2Cl_2 extract showed cytotoxicity against murine leukemia P-388 cell lines (IC_{50} 2 μg/mL), and hence, this was fractionated on an open column of SiO_2 using increasing amounts of MeOH in CH_2Cl_2 as eluent (CH_2Cl_2 to 50% MeOH−CH_2Cl_2). In all, 34 fractions (18 mL each) were collected. The major metabolite, **1**, was obtained from the seventh fraction (5% MeOH−CH_2Cl_2 eluate) by recrystallization from MeOH−CH_2Cl_2 (1:1). The mother liquor of fraction 7 was pooled with fraction 8 (5% MeOH−CH_2Cl_2) and 9 (10% MeOH−CH_2Cl_2) based on TLC and ^{1}H NMR, and the resulting mixture was subjected to C-18 reversed-phase HPLC using 30% H_2O in MeOH as eluent to afford **1** and **2**. Compound **3** was obtained from fraction 17 after removal of solvents. Pyronaamidine (**1**) was identified by comparison of its spectral data with literature values.[2]

2-Deoxy-2-aminokealiiquinone (3): obtained as red needles (15.7 mg) from MeOH−CH_2Cl_2, mp > 250 °C (dec.); UV (MeOH) λ_{max} 220 (log ϵ 4.49), 294 (4.57), 3.82 (3.73) nm; IR (dry film) ν_{max} 3400−2700 (br) 1645, 1620, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.75 (1H, s, H-9), 7.15 (2H, d, J = 8.7 Hz, H-2′ and H-6′), 7.10 (2H, br s, NH_2), 6.90 (2H, d, J = 8.7 Hz, H-3′ and H-5′), 3.94 (3H, s, Me-11), 3.85 (3H, s, Me-12), 3.80 (3H, s, Me-7′), 3.60 (3H, s, Me-10); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 182.0 (s, C-5), 181.4 (s, C-8), 158.5 (s, C-2), 157.8 (s, C-4′), 147.8 (s, C-7), 147.4 (s, C-3a), 145.5 (s, C-6), 137.5 (s, C-9a), 130.6 (d, C-2′ and C-6′), 130.2 (s, C-1′), 129.0 (s, C-4), 123.6 (s, C-8a), 122.5 (s, C-4a), 112.8 (d, C-3′ and C-5′), 105.1 (d, C-9), 60.60 (q, C-11 or C-12), 60.59 (q, C-12 or C-11), 54.98 (q, C-7′), 28.73 (q, C-10); FABMS *m/z* 394 [M + H]$^+$, 416 [M + Na]$^+$; HRFABMS *m/z* 394.1408 [M + H]$^+$ (calcd for $C_{21}H_{20}N_3O_5$ 394.1403); 416.1248 [M + Na]$^+$ (calcd for $C_{21}H_{19}N_3O_5Na$ 416.1222).

***N,N*-Diacetyl 2-Deoxy-2-aminokealiiquinone (4).** A solution of **3** (3.0 mg), Ac_2O (200 μL) and pyridine (200 μL) was kept overnight at room temperature, and then iced H_2O was added to destroy excess Ac_2O. The reaction solution was evaporated by a stream of N_2 to afford **4** (3.5 mg, 96.2%), as a powder: UV (MeOH) λ_{max} 286 (log ϵ 4.38), 378 (3.42) nm; IR (dry film) ν_{max} 2970, 2930, 1740, 1715, 1660, 1615, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.21 (1H, s, H-9), 7.22 (2H, d, J = 8.7 Hz, H-2′ and H-6′), 6.97 (2H, d, J = 8.7 Hz, H-3′ and H-5′), 4.08 (3H, s, Me-11), 4.00 (3H, s, Me-12), 3.83 (3H, s, Me-7′), 3.70 (3H, s, Me-10), 2.30 (6H, s, Ac) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.9 (s), 181.7 (s), 171.4 (s, 2C), 159.2 (s), 148.8 (s), 148.7 (s), 146.2 (s), 144.3 (s), 137.2 (s), 136.9 (s), 130.2 (d), 128.7 (s), 128.0 (s), 123.5 (s), 113.7 (d), 109.1 (d), 61.3 (q, 2C), 55.1 (q), 30.2 (q), 26.0 (q, 2C) ppm; FABMS *m/z* 478 [M + H]$^+$, 391 [M − 2Ac]$^+$.

Naamine C (5): obtained as a yellow powder (~2.9 mg); UV (MeOH) λ_{max} 228, 276, 368 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.06 (2H, d, J = 8.7 Hz, H-15 and 19), 6.76 (2H, d, J = 8.7 Hz, H-16 and 18), 6.46 (1H, d, J = 8.7 Hz, H-12), 6.34 (1H, d, J = 8.7 Hz, H-11), 3.87 (3H, s, Me-22), 3.81 (3H, s, Me-21), 3.73 (4H, br s, H-6 and 13), 3.72 (3H, s, Me-23), 3.26 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.5 (s, C-17), 151.5 (s, C-10), 147.1 (s, C-8), 146.4 (s, C-2), 135.5 (s, C-9), 129.5 (d, C-15 and 19), 129.0 (s, C-14), 123.3 (d, C-12), 122.7 (s, C-4), 121.1 (s, C-5), 115.1 (s, C-7), 114.2 (d, C-16 and 18), 103.8 (d, C-11), 61.0 (q, C-22), 55.8 (q, C-21), 55.3 (q, C-23), 29.6 (q, C-20), 29.3 (t, C-13), 22.6 (t, C-6); FABMS *m/z* 384 [M + H]$^+$.

Acknowledgment. The work was supported by NIH grant CA 52955. We gratefully acknowledge Dr. M. Kelly-Borges, The Natural History Museum, London, for sponge identification, the Coral Reef Research Foundation for use of its facilities, and the Government of Chuuk, Federated States of Micronesia, for permission to collect specimens. We also thank NSF Grant CHE 8113507 and the University of Oklahoma Research Associates Fund for funds to purchase a high-field NMR spectrometer.

References and Notes

(1) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003−3006.
(2) Akee, R. K.; Carroll, T. R.; Yoshida, W. Y.; Scheuer, P. J.; Stout, T. J.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 1944−1946.
(3) He, H. Y.; Faulkner, D. J.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1992**, *57*, 2176−2178.
(4) 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.
(5) Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193−2200.
(6) Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 1229−1234.
(7) Alvi, K. A.; Peters, B. M.; Hunter, L. M.; Crews, P. *Tetrahedron* **1993**, *49*, 329−336.
(8) Mancini, I.; Guella, G.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1995**, *78*, 1178−1184.
(9) Ciminiello, P.; Fattorusso, E.; Mangoni, A.; Di Blasio, B.; Pavone, V. *Tetrahedron* **1990**, *46*, 4387−4392.
(10) Ciminiello, P.; Fattorusso, E.; Magno, S.; Mangoni, A. *Tetrahedron* **1989**, *45*, 3873−3878.
(11) Alvi, K. A.; Crews, P.; Loughhead, D. G. *J. Nat. Prod.* **1991**, *54*, 1509−1515.
(12) Wilkinson, C. R. *Marine Biol.* **1978**, *49*, 161−167.
(13) Thompson, T. E. *Veliger* **1981**, *23*, 315−316.
(14) A colored plate of this sponge appears in Colin, P. L.; Arneson, C. "*Tropical Pacific Invertebrates*;" Coral Reef Press: Beverly Hills, CA, 1995; p 60, #230.
(15) Fu, X.; Schmitz, F. J.; Lee, R. H.; Papkoff, J. S.; Slate, D. L. *J. Nat. Prod.* **1994**, *57*, 1591−1594.

NP960694I