New Imidazole Alkaloids and Zinc Complexes from the Micronesian Sponge *Leucetta* cf. *chagosensis*

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Four new imidazole alkaloids, **2**−**5**, along with the known isonaamidine B (**1**) were isolated from a Pacific sponge, *Leucetta* cf. *chagosensis*, collected from Yap, Federated States of Micronesia. Among these, **4** and **5** are zinc complexes derived from isonaamidine B and isonaamidine D. The structures of the new compounds were elucidated from spectral data. Isonaamidine D (**3**) showed weakly antifungal activity against *Aspergillus niger* with MIC = 100 μg/mL.

Marine organisms have proven to be a source of antimicrobial metabolites.[1] Screening for antimicrobial metabolites drew our attention to *Leucetta* cf. *chagosensis* collected from Yap, Micronesia. Previous research has shown that sponges of this genus (order Clathrinida, family Leucettidae) are the source of a group of imidazole alkaloids.[2−11] The same type of alkaloid is also isolated from *Clathrina* spp.[12,13] and nudibranchs of the genus *Notodoris*[8−10,14] (family Aegiridae), which feed on these sponges. From the antifungal active extracts of *Leucetta* cf. *chagosensis* from Yap, we have isolated the known imidazole alkaloid isonaamidine B (**1**)[4] and four new related compounds, isonaamine B (**2**), isonaamidine D (**3**), isonaamidine B zinc complex (**4**), and the mixed zinc complex **5** containing both isonaamidines B and D. Several Zn^{2+} complexes of imidazole alkaloids have been isolated from calcareous sponges and *Notodoris* nudibranchs.[10−14] These include (naamidinato A) (naamidinato G) zinc (II), the first naturally occurring mixed-ligand metal complex, reported recently from a *Leucetta* sp. of sponge.[11] We now add two new members to the short list of Zn^{2+} complexes from marine sources. One of them, **5**, represents the second example of naturally occurring mixed-ligand metal complexes. In this paper, we report the isolation and structure elucidation of the new members of this imidazole alkaloid family and some antifungal activity of these imidazole alkaloids.

Samples of *Leucetta* cf. *chagosensis* were collected from Yap and extracted with MeOH and then MeOH−CH_2Cl_2 (1:1). The combined, concentrated extracts were successively partitioned using procedures described previously.[15] The CH_2Cl_2-soluble fraction inhibited the growth of *Aspergillus niger* and therefore was fractionated on Si gel open column and reversed-phase HPLC to yield the known isonaamidine B (**1**) as the major constituent, and four new minor compounds, **2**−**5**, which are related to isonaamidine B.

Isonaamine B (**2**) was obtained as yellow glass with the molecular formula $C_{19}H_{21}N_3O_2$ based on HRFABMS. The ^1H and ^{13}C NMR data, which were unambiguously assigned by COSY, HMQC, and HMBC experiments, were similar to those of isonaamine A[3], with the exception of the presence of one OMe (δ_H 3.76; δ_C 55.7), and one NMe (δ_H 3.31; δ_C 30.4). The proton singlets at δ 6.47 (1H, H-4) and 4.92 (2H, H-7) and the ^{13}C signals for the associated carbons [δ 113.8 (d) and 49.6 (t)] indicated that isonaamine B belonged to the isonaamine or the isonaamidine series but not the naamine or naamidine series.[3,4] The HMBC experiment defined not only the position of the two benzyl groups on the imidazole ring (long-range couplings H-7/C-2 and C-4; H-12/C-4 and C-5) but also the locations of the OMe and NMe. The OMe was located at C-16 instead of C-11 based on HMBC correlation between these methyl protons and C-16. The second methyl was placed at N-1 due to correlations of the NMe to C-2 and C-5. Thus, the structure of isonaamine B was assigned as **2**.

Isonaamidine D (**3**), obtained as yellow powder, showed virtually the same UV absorption as isonaamidine B (**1**). The ^1H NMR data for **3** were nearly identical to those of **1** except that the singlet for the NMe group in isonaamidine B (**1**)[4] was missing in the ^1H NMR spectrum of **3**. The ^{13}C signal for this NMe group was also absent in the ^{13}C NMR spectrum of **3**; however, the remaining protonated carbon signals were virtually the same as those in **1**.[4] (Due to the scarcity of sample, the quaternary carbon signals could not be unambiguously observed.) In agreement with this, a matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrum revealed an ion at *m*/*z* 406 $[M + H]^+$, 14 mass units less than that of isonaamidine B.[4] Therefore, structure **3** was assigned for isonaamidine D.

The ^1H NMR spectra of compounds **4** and **5** contained features reminiscent of the spectra of the Zn^{2+} complexes isolated from marine sponges and nudibranchs,[10−14] and this facilitated the structure elucidations. Compound **4** was obtained as a yellow, amorphous solid. Its MALDI-TOF mass spectrum[16] showed ion clusters at around *m*/*z* 901 (most intense) $[M + H]^+$

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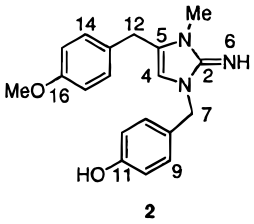
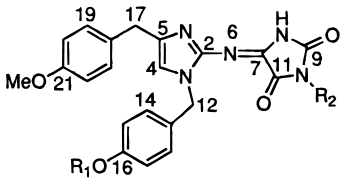
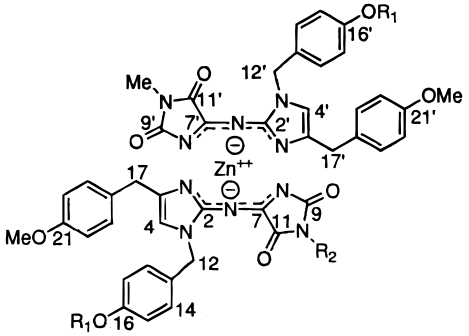
and at *m/z* 923 (most intense) $[M + Na]^+$ indicative of the presence of one zinc atom in the molecule.[10−13] The structure of **4** was determined by comparing its NMR data with those of the known Zn^{2+} complex (**6**).[10] The NMR data for compounds **4** and **6** were very similar, with slight differences due to the different NMR solvents; however, the ^1H (δ 3.80, s) and ^{13}C NMR signals (δ 55.1, q) for the OMe at C-16 and C-16′of **6** were missing in the spectra of **4**. Hence, compound **4** appeared to be a Zn^{2+} complex of isonaamidine B, and this was confirmed by HMBC data, which also permitted assignment of the quaternary ^{13}C NMR signals of **4**.



2



1 $R_1 = H$ $R_2 = Me$
3 $R_1 = H$ $R_2 = H$



4 $R_1 = H$ $R_2 = Me$
5 $R_1 = H$ $R_2 = H$
6 $R_1 = R_2 = Me$

The MALDI-TOF MS of the second Zn^{2+} complex, **5**, contained ion clusters at *m/z* 887 $[M + H]^+$ and 909 $[M + Na]^+$, different from those of compound **4** by 14 mass units. The ^1H NMR spectrum of **5** showed two sets of signals, one being identical to and another slightly different from those of **4**. This indicated that compound **5** was a Zn^{2+} complex composed of mixed ligands isonaamidine B (**1**) and isonaamidine D (**3**). Only one NMe signal was observed in the NMR spectra of compound **5**, consistent with its structure. The MALDI-TOF MS of **5** possessed two significant fragment ion peaks at *m/z* 420, and 406, ascribable to the ligands isonaamidine B and isonaamidine D, respectively, in agreement with the proposed structure. The fragment peak at *m/z* 420 was also observed in the MALDI-TOF MS of **4**.

It has been reported[17] that pyochelin-zinc complex, a siderophore from *Pseudomonas aeruginosa*, is probably an artifact of chromatography on Si gel. To determine whether the zinc complexes we isolated were artifacts, 10 mg of isonaamidine B (**1**) was passed through a Si gel column using the same eluent system that was used in the original isolation. ^1H NMR spectra of all three fractions collected matched the spectrum of **2** and did not contain any signals characteristic of **4**. This confirmed that **4** and **5** were not artifacts resulting from Si gel chromatography, a conclusion consistent with that reported for the zinc complexes isolated previously from sponges.[11,13]

The five imidazole alkaloids **1**−**5** were tested against *P. aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *A. niger* ATCC 9642, and *Saccharomyces cerevisiae* ATCC 18824 at concentrations of 200, 100, 10, and 1 μg/mL in a minimum inhibitory concentration (MIC) assay conducted in triplicate.[18] The only inhibitory activity observed was that of **3** against *A. niger* at 100 μg/mL.

Experimental Section

General Procedures. IR and UV spectra were recorded on Bio-Rad 3240-spc FT and Hewlett−Packard spectrophotometers, respectively. FABMS spectra were measured with a VG ZAB-E mass spectrometer. MALDI-TOF mass spectra were taken on a PerSeptive Biosystems Voyages Elite instrument. All NMR experiments were performed on a Varian VXR-500 spectrometer equipped with a 3-mm $^1H/^{13}C$ switchable gradient microprobe (MDG-500-3) and a pulsed field gradient driver, using standard Varian software, version 5.2. NMR signals are reported in parts per million (δ), referenced to the solvents used. HPLC was conducted using a UV detector (230 nm) and Phenomenex ODS-2 (300 × 10 mm) column.

Animal Material. The sponge (36YA95) was collected from Yap, Federated States of Micronesia, in August 1995. It forms bright lemon yellow, semispherical masses. The sample is close to *Leucetta chagosensis* Dendy 1913 (class Calcarea, order Clathrinida, family Leucettidae), but differs from it in possessing an additional category of enormous triactinal spicules and has a much less compact skeleton. A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 1997.9.20.5) and the University of Oklahoma (36YA95).

Extraction and Isolation. Freshly thawed specimens of the sponge (201 g wet wt; 45 g dry wt after extraction) were cut into small pieces and soaked in MeOH (2 × 600 mL) followed by MeOH−CH_2Cl_2 (1:1) (2 × 600 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 (288 mg), CH_2Cl_2 (768 mg), *n*-BuOH (2.5 g), and H_2O (ca. 8 g) solubles. The CH_2Cl_2 extract showed antifungal activity, 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). Evaporation of the solvents of the 2% MeOH−CH_2Cl_2 eluate and the 20% MeOH−CH_2Cl_2 eluate, yielded isonaamidine B (**1**) (40 mg) and isonaamine B (**2**) (15 mg), respectively. The 5% MeOH−CH_2Cl_2 eluate contained a mixture of isonaamidine D (**3**) (0.6 mg) and Zn complexes **4** (0.9 mg) and **5** (0.6 mg), which was resolved by reversed-phase HPLC using 38% H_2O−MeOH as eluent. Isonaamidine B (**1**) was identified by comparison of its spectral data with literature values.[4]

Isonaamidine B (1): yellow amorphous solid; UV (MeOH) λ_{max} 226 (ϵ 19 400), 276 (4300), 374 (8000) nm; LRFABMS *m/z* 420 $[M + H]^+$.

Isonaamine B (2): yellow glass; UV (MeOH) λ_{max} 226 (ϵ 19 300), 276 (5800), 308 (3700) nm; IR (neat) ν_{max}

3000−3500 (br), 1654, 1625, 1605, 1506 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.14 (2H, d, J = 8.8 Hz, H-9), 7.12 (2H, d, J = 8.8 Hz, H-14), 6.87 (2H, d, J = 8.8 Hz, H-15), 6.79 (2H, d, J = 8.8 Hz, H-10), 6.47 (1H, s, H-4), 4.92 (2H, s, H-7), 3.82 (2H, s, H-12), 3.76 (3H, s, OMe), 3.31 (3H, s, NMe); ^{13}C NMR (125 MHz, CD_3OD) δ 160.3 (s, C-16), 159.1 (s, C-11), 147.5 (s, C-2), 130.6 (d, C-14), 130.5 (d, C-9), 129.6 (s, C-5), 128.9 (s, C-13), 126.1 (s, C-8), 116.8 (d, C-10), 115.3 (d, C-15), 113.8 (d, C-4), 55.7 (q, OMe), 49.6 (t, C-7), 30.4 (q, NMe), 29.8 (t, C-12); HRFABMS m/z 324.1689, calcd for $C_{19}H_{22}N_3O_2$ 324.1712 $[M + H]^+$.

Isonaamidine D (3): yellow amorphous solid; UV (MeOH) λ_{max} 226 (ϵ 18 900), 278 (4000), 370 (6600) nm; ^1H NMR (500 MHz, CD_3OD) δ 7.13 (2H, d, J = 8 Hz, H-14), 7.12 (2H, d, J = 8 Hz, H-19), 6.82 (2H, d, J = 8 Hz, H-15), 6.70 (2H, d, J = 8 Hz, H-20), 6.64 (1H, s, H-4), 5.15 (2H, s, H-12), 3.80 (2H, s, H-17), 3.74 (3H, s, 21-OMe); ^1H NMR (500 MHz, DMSO-d_6) δ 8.46 (1H, br, NH), 7.13 (2H, d, J = 8.5 Hz, H-14), 7.04 (2H, d, J = 8.5 Hz, H-19), 6.81 (2H, d, J = 8.5 Hz, H-15), 6.67 (2H, d, J = 8.5 Hz, H-20), 6.62 (1H, s, H-4), 6.44 (1H, s, NH), 5.02 (2H, s, H-12), 3.71 (2H, s, H-17), 3.69 (3H, s, 21-OMe); ^{13}C NMR (125 MHz, DMSO-d_6) δ 33.6 (t), 46.8 (t), 54.9 (q), 113.9 (d), 115.4 (d), 115.6 (d), 129.1 (d), 129.6 (d); ^{13}C NMR (125 MHz, CD_3OD) δ 34.3 (q), ca. 49 (t, overlapped with solvent peaks), 55.6 (q), 114.8 (d), 116.4 (d), 116.9 (d), 130.5 (d), 130.7 (d) (due to the scarcity of sample, the quaternary carbon signals are weak or could not be observed); MALDI-TOF MS m/z 406 $[M + H]^+$.

Bis(isonaamidinato B)zinc (II) (4): yellow amorphous solid; UV (MeOH) λ_{max} 228 (ϵ 22 400), 278 (3900), 368 (8700), 380 (8500) nm; ^1H NMR (500 MHz, CD_3OD) δ 7.42 (4H, d, J = 8.9 Hz, H-14 and 14′), 7.23 (2H, s, H-4 and 4′), 6.83 (4H, d, J = 8.9 Hz, H-15 and 15′), 6.38 (4H, d, J = 8.9 Hz, H-20 and 20′), 6.32 (4H, d, J = 8.9 Hz, H-19 and 19′), 5.47 (2H, d, J = 14.2 Hz, H-12 and 12′), 5.20 (2H, d, J = 14.2 Hz, H-12 and 12′), 3.73 (2H, d, J = 16 Hz, H-17 and 17′), 3.16 (2H, d, J = 16 Hz, H-17 and 17′), 3.63 (6H, s, OMe), 2.89 (6H, s, NMe); ^{13}C NMR (125 MHz, CD_3OD) δ 166.4 (s, C-9 and 9′), 163.3 (s, C-11 and 11′), 159.3 (s, C-21 and 21′), 158.7 (s, C-16 and 16′), 155.1 (s, C-7 and 7′), 149.2 (s, C-2 and 2′), 137.8 (s, C-5 and 5′), 131.2 (d, C-14 and 14′), 131.1 (s, C-18 and 18′), 130.0 (d, C-19 and 19′), 129.1 (s, C-13 and 13′), 119.0 (d, C-4 and 4′), 116.5 (d, C-15 and 15′), 114.4 (d, C-20 and 20′), 55.4 (q, OMe), 49.6 (t, C-12 and 12′), 33.1 (t, C-17 and 17′), 24.6 (q, NMe); MALDI-TOF MS m/z (rel int) 901 (86), 903 (64), 905 (52), 907 (11) $[M + H]^+$, 923 (100), 925 (75), 927 (62), 929 (12) $[M + Na]^+$; 420 (100).

(Isonaamidinato B)(isonaamidinato D)zinc (II) (5): yellow amorphous solid; UV (MeOH) λ_{max} 228 (ϵ 24 800), 278 (4460), 364 (9900), 380 (9600) nm; ^1H NMR (500 MHz, CD_3OD) δ 7.42 (2H, d, J = 8.9 Hz, H-14′), 7.41 (2H, d, J = 8.9 Hz, H-14), 7.22 (1H, s, H-4′), 7.18 (1H, s, H-4), 6.82 (4H, d, J = 8.9 Hz, H-15 and 15′), 6.42 (2H, d, J = 8.9 Hz, H-20), 6.38 (2H, d, J = 8.9 Hz, H-20′), 6.36 (2H, d, J = 8.9 Hz, H-19), 6.31 (2H, d, J = 8.9 Hz, H-19′), 5.46 (1H), 5.45 (1H), 5.20 (2H), (each d, J = 14.2 Hz, H-12 and 12′), 3.72 and 3.23 (each 1H, d, J = 16 Hz, H-17′), 3.70 and 3.13 (each 1H, d, J = 16 Hz, H-17), 3.65 and 3.63 (each 3H, s, OMe), 2.89 (3H, s, NMe); ^{13}C NMR (125 MHz, CD_3OD) δ 166.5 (s, C-9′), 164.4 (s, C-9), 163.9 (s, C-11), 163.3 (s, C-11′), 159.3 (s, C-21 and 21′), 158.7 (s, C-16 and 16′), 155.9 (s, C-7), 155.2 (s, C-7′), 149.4 (s, C-2), 149.2 (s, C-2′), 137.8 (s, C-5′), 137.5 (s, C-5), 131.2 (d, C-14 and 14′), 130.9 (s, C-18′), 130.8 (s, C-18), 130.0 (d, C-19 and 19′), 129.2 (s, C-13 and 13′), 118.8 (d, C-4′), 118.7 (d, C-4), 116.5 (d, C-15 and 15′), 114.7 (d, C-20), 114.4 (d, C-20′), 55.44 (q, OMe), 55.39 (q, OMe), 49.6 (t, C-12 and 12′), 33.14 (t, C-17′), 33.07 (t, C-17), 24.6 (q, NMe); MALDI-TOF MS m/z (rel int) 887 (100), 889 (67), 891 (48), 893 (12) $[M + H]^+$, 909 (69), 911 (66), 913 (51), 915 (14) $[M + Na]^+$, 420 (100), 406 (100).

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