Variation in the Alkaloids among Indo-Pacific *Leucetta* Sponges

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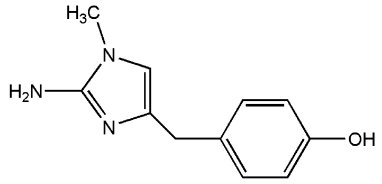
Nine different Indo-Pacific collections of calcareous *Leucetta* sp. sponges were investigated for variation in their alkaloid constituents. These alkaloids consisted of 2-amino imidazoles such as dorimidazole A (**1**) and a polyunsaturated fatty amino alcohol (PUFAA), leucettamol A (**3**). The nine *Leucetta* species were divided into five different groups based on taxonomy. Significantly, six specimens contained leucettamol A (**3**), while the other three contained imidazoles, and these two classes of alkaloids did not occur in the same sponge sample. We recently found a Fijian *Leucetta* sponge that was a source of spirocyclopentimidazolidins, including spirocalcaridine A (**4**). We now show that another Fijian collection affords three amino imidazoles consisting of the known alkaloid naamine A (**6**) plus two new structures, *N*,*N*-dimethyl naamine D (**5**) and leucettamine C (**7**).

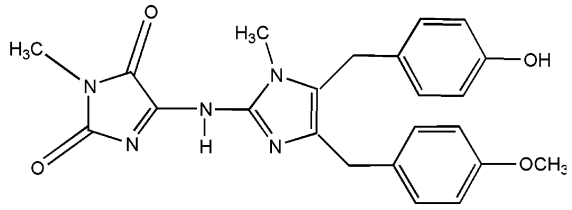
Introduction

Between 1989 and 1993 several research groups independently discovered that Calcarea Class sponges of the genus *Leucetta*[1] and their clathrivorous *Notodoris* nudibranchs[2] were rich in alkaloids. These natural products, headed by dorimidazole (**1**)[2c] or naamidinene A (**2**),[2a] consist of 2-amino imidazoles further functionalized with *p*-oxylbenzyl and imidazole dione (syn. dehydro hydantoin) residues. Such compounds are often isolable from a ubiquitous but undescribed *Leucetta* sp. A, which possesses a distinctive lemon yellow color and oval shape.[3] We have an abiding interest in both this class of natural products that also includes organometallic complexes (e.g., Zn-isonaamidine B)[2c,4] and in their *Leucetta* sources that are known throughout Oceania.[5] Recent developments indicate that it might be an oversimplification to consider *Leucetta* chemistry as being dominated by 2-amino imidazoles. The latest additions to *Leucetta*-derived compounds consist of polyunsaturated fatty amino alcohols (PUFAA), the leucettamols (such as **3**),[6] nonalkaloids including the polyene rhapsamine,[7] and an unnamed triene aldehyde.[8]

The intent of this study was to gain an overview of the chemistry of nine distinctly different *Leucetta* specimens collected from Fiji, Papua New Guinea, and Indonesia. All of the samples differed from the *Leucetta* sp. A[5] mentioned above and were distinct from one another by either overall color or morphology. Initially, 2-amino imidazoles were assumed to be a chemical signature of this assemblage. Our recent report of the unique 2-amino imidazoles headed by spirocalcaridine A (**4**) represents an initial outcome of preliminary studies.[9] Prior to this discovery, most *Leucetta* alkaloid metabolites could be divided into four categories, whose core frameworks are shown in Figure 1. These structures are distinguished by the content and position of a *p*-oxylbenzyl moiety, presumably derived from a tyrosine precursor, and by the presence or absence of an additional imidazoledione ring. The simplest, type **I**, has a single *p*-oxylbenzyl substituent at C4 and is exemplified by dorimidazole A (**1**),[4] preclathridine A,[10] or the clathridines.[1b,d] The type **II** and **III** categories have two *p*-oxybenzyl substituents at C4/C5 or C4/N1, respectively. Examples of type **II** include the naamines,[2a,3a] naamidines,[2a,c,d] and pyronaamidines.[1b,11,12] Those of type **III** are headed by the isonaamines[3,4] and isonaamidines,[2a,3a] and there are not any examples of the hypothetical type **IV** analogue. Reported below is our surprising find that *Leucetta* sponges appear to contain either 2-amino imidazoles or the amino polyene leucettamol A (**3**) but not both. In addition we outline the isolation and characterization of, *N*,*N*-dimethyl naamine D (**5**) and leucettamine C (**7**).



dorimidazole A (**1**)



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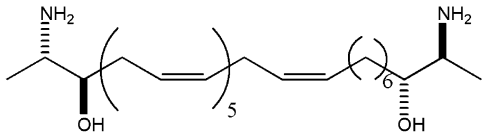
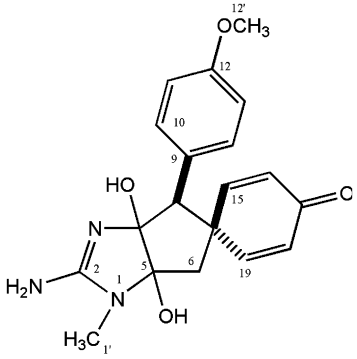


leucettamol A (**3**)



spirocalcaridine A (**4**)

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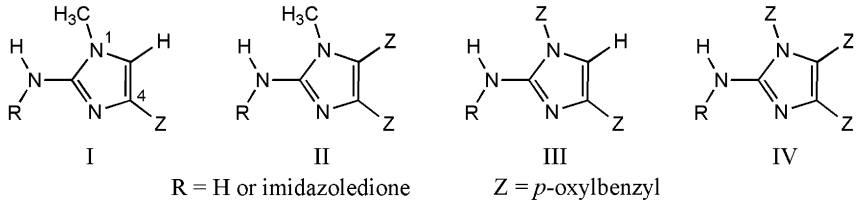


Figure 1. Structural categories of *Leucetta*-derived substituted 2-amino imidazoles.

Table 1. Summary of *Leucetta* Sponges

samp. no.	coll. no.	color	taxonomy	location[a]	2-amino imidazoles	leucettamol A
1	91136	brown	*L. avocado*[b]	PNG	yes	no
2	98156	green	*L. avocado*	PNG	no	yes
3	94649	yellow	*L. avocado*	IND	no	yes
4	95106	yellow	*L. avocado*	FIJ	yes	no
5	94566	purple	*L.primigenta*	IND	no	yes
6	98131	pink	*L. primigenta*	PNG	no	yes
7	95028	brown	*L.* sp. D	PNG	no	yes
8	95107	blue	*L.* sp. C	FIJ	no	yes
9	00111	yellow	*L.* sp. B	FIJ	yes	no

[a] PNG = Papua New Guinea; IND = Indonesia; FIJ = Fiji. [b] *L. avocado* (aka *Pericharax heterohaphis*).[5]

Table 2. ^{1}H and ^{13}C NMR[a] Data for *N,N*-Dimethyl Naamine D (**5**) in CD_3OD

no.	^{13}C NMR δ (mult)	^{1}H NMR δ (mult, *J* Hz, int)	^{1}H−^{1}H COSY	HMBC
2	146.4 (s)			
4/5	123.3 (s)			
6/6′	27.0 (t)	3.98 (s, 4H)		C4, C5, C8/8′, C12/12′
7/7′	128.4 (s)			
8/8′	128.7 (d)	7.08 (d, 8.5, 2H)	H9, H9′	C6/6′, C9/9′,C10/10′, C12/12′
9/9′	113.9 (d)	6.85 (d, 8.5, 2H)	H8/8′	C7/7′, C8/8′, C10/10′, C11/11′
10/10′	158.8 (s)			
11/11′	113.9 (d)	6.85 (d, 8.5, 2H)	H12/12′	C7/7′, C8/8′, C9/9′, C10/10′
12/12′	128.7 (d)	7.08 (d, 8.5, 2H)	H11/11′	C6/6′, C8/8′, C9/9′, C10/10′
13/13′	54.3 (q)	3.75 (s, 6H)		C10/10′
14/14′	29.0 (q)	3.26 (s, 6H)		C2, C4, C5

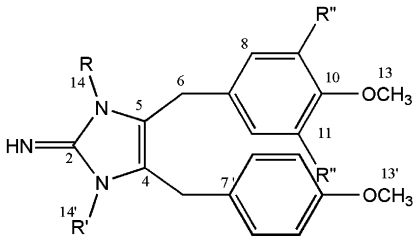
[a] Recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

Results and Discussion

The first step in this investigation was to obtain baseline data on the composition and levels of 2-amino imidazoles (types **I**−**III**) expected to be present in separate collections of the nine *Leucetta* sp. sponges. This ensemble, summarized in Table 1, was obtained from three Indo-Pacific regions, Fiji, Papua New Guinea, and Indonesia. There were five species represented in this group including *L.* sp. B, *L.* sp. C, *L.* sp. D, *L. avocado* (aka *Pericharax heterohaphis*),[5] and *L. primigenta*. The latter two species comprised the largest group and included several color morphologies with samples of *L. avocado* from each geographical zone. The individual crude extracts of all nine were examined by ^{1}H NMR, and on the basis of this preliminary data, the sponges were divided into two groups: those exhibiting aromatic and *N*CH_3 resonances characteristic of the oxylbenzyl and methyl substituents of the 2-amino imidazole and those with multiple vinyl peaks characteristic of leucettamol A (**3**), first isolated by Faulkner from *Leucetta microraphis* collected in Micronesia.[6] Six specimens contained **3** and were devoid of 2-amino imidazoles, whereas the other three contained 2-amino imidazoles. One of these latter specimens, coll. no. 00111, was the subject of our recent report describing three new 2-amino imidazoles.[9] Only two of the four specimens of *L. avocado* contained 2-amino imidazole, and one of these, coll. no. 95016, was chosen for detailed chemical investigation.

The *Leucetta avocado* (coll. no. 95106) obtained from Fiji afforded semipure extract fractions whose NMR properties indicate the presence of 2-amino imidazoles. The concentrated methanol crude extract of this sponge was fractionated according to our standard scheme.[13] Both the MeOH and CH_2Cl_2 solvent partition fractions exhibited such compounds, so their respective oils were further fractionated by size exclusion chromatography on LH-20 Sephadex (MeOH−CH_2Cl_2, 50:50). This was followed by flash chromatography on silica gel eluted with MeOH−CH_2Cl_2 of increasing polarity. Further purification of the MeOH solvent partition fraction (MeOH−H_2O, 1:1, labeled as "FM") by reversed-phase gradient HPLC (MeOH−H_2O, 60:40−100% MeOH) afforded the new derivative, *N,N*-dimethyl naamine D (**5**),[14] plus the known alkaloid naamine A (**6**).[2a] Additional purification of the CH_2Cl_2-soluble fraction by reversed-phase gradient HPLC (MeOH−H_2O, 70:30−100% MeOH) yielded the new 2-amino imidazole leucettamine C (**7**).

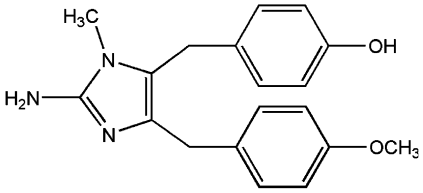
The first new compound characterized was *N,N*-dimethyl naamine D (**5**). Its molecular formula of $C_{21}H_{25}N_3O_2$ (unsaturation equivalence = 11) was established by HR-FABMS, $[M + H]^+$ m/z = 352.2022. A molecule with a C_{2v} symmetry was envisioned to account for the different formula of $C_{11}H_{12}NO$ derived by ^{13}C APT NMR. The NMR data shown in Table 2 (see also Figures S3 and S4) reinforced by HMQC NMR spectra revealed one OMe, one *N*Me, one CH_2, four sp^2 CH (as a disubstituted benzene), and four sp^2 C. Especially compatible with the features of a naamine analogue (i.e., **I** or **II** with R = H) were the combination of a downfield AA′XX′ spin system centered at δ 7.08 and 6.85, as well as three singlet peaks attributable to heterosubstituted methyls at δ 3.75 and 3.26 and a benzylic methylene at δ 3.98. On the basis of symmetry, 10 carbons, 12 hydrogens, and a single nitrogen and oxygen



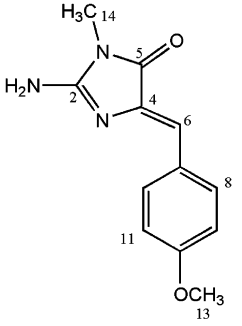
N,N-dimethyl naamine D (**5**)
R = R' = CH_3, R" = H

naamine D (**5a**)
R = R' = R" = H

naamine E (**5b**)
R = CH_3, R' = H, R" = OH



naamine A (**6**)



leucettamine C (**7**)

could be added to the NMR-computed molecular formula. The two missing pieces, an *N*H and one degree of unsaturation, were rationalized by proposing a guanidinium moiety in the form of a 2-amino imidazole ring. These data, together with the general similarity of ^{1}H and ^{13}C NMR resonances of **5** to that of symmetrical compounds naamine D (**5a**)[14a] and E (**5b**),[14b] indicated that the former was closely related to them as well as to the unsymmetrical congener naamine B.[2a] Further data in support of the proposed structure was derived from 2D NMR experiments that included ^{1}H−^{1}H COSY and HMBC.

The second new natural product, leucettamine C (**7**), had a molecular formula of $C_{12}H_{13}N_3O_2$ (unsaturation equivalence = 8) established by HRFABMS, $[M + H]^+$ *m/z* = 232.1082, and NMR data (CD_3OD) shown in Table 3. Multiple unsaturated rings were inferred from the high degree of unsaturation, but only a single aromatic ring was present. The APT ^{13}C and HMQC NMR spectra revealed carbon residues including one OMe, one *N*Me, four sp^2 CH (as a disubstituted benzene), one sp^2 CH, and four sp^2 C for an NMR-derived molecular formula of $C_{11}H_{11}NO$. Eventually a small signal was detected in the ^{13}C NMR for a carbonyl carbon at δ 171.0 (C5), further confirmed by key HMBC correlations between it and an olefinic proton singlet at δ 6.56 (H6) and the *N*CH_3 group at δ 3.14 (Me14).

Table 3. ^{1}H and ^{13}C NMR[a] Data for Leucettamine C (**7**) in CD_3OD

no.	^{13}C NMR δ (mult)	^{1}H NMR δ (mult, *J* Hz, int)	^{1}H−^{1}H COSY	HMBC
2	161.0 (s)			
4	138.6 (s)			
5	171.0 (s)			
6	117.4 (d)	6.56 (s, 1H)		C5, C8
7	129.6 (s)			
8	133.5 (d)	7.89 (d, 9, 1H)	H9	C6, C9, C10, C12
9	115.1 (d)	6.91 (d, 9, 1H)	H8	C7, C8, C10, C11
10	161.5 (s)			
11	115.1 (d)	6.91 (d, 9, 1H)	H12	C7, C9, C10, C12
12	133.5 (d)	7.89 (d, 9, 1H)	H11	C6, C8, C10, C11
13	55.9 (q)	3.82 (s, 3H)		C10
14	26.1 (q)	3.14 (s, 3H)		C2, C5

[a] Recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

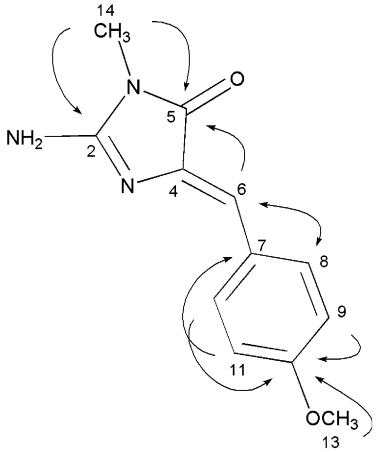
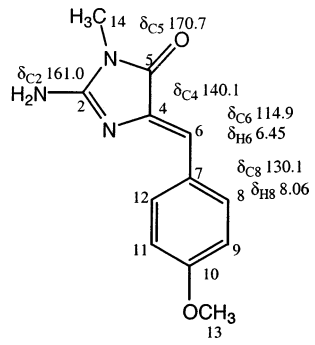


Figure 2. HMBC correlations for leucettamine C (**7**).

The remaining N_2H_2O and four degrees of unsaturation were accounted for by inserting a guanidinium moiety into a heterocyclic ring that was further functionalized by a carbonyl and a trisubstituted double bond exocyclic to the 2-amino imidazole ring and conjugated to a benzene ring. The HMBC correlations revealed a pattern of connectivities to link residues of the entire molecule from the imidazole through to the methoxy on the benzene ring, as shown in Figure 2. These data, together with the general similarity of ^{1}H and ^{13}C NMR resonances to that of other *Leucetta* alkaloids, suggested that leucettamine C (**7**) was a modified type **I** alkaloid with R = H, Z = *p*-methoxy aryl with two additional unsaturations at C4 and C5.

The substructures assembled above were used as seeds for dereplication. It was eventually concluded that leucettamine C (**7**) was comparable in overall structure to that of leucettamine B (**8**)[8] isolated from a *Leucetta* sponge, with the exception that a *p*-methoxy group replaces the trisubstituted benzene ring of the latter. The *Z*-double bond orientation in **8** was based on ^{13}C−^{1}H coupling constants[8] obtained via COLOC NMR data, but the small sample size of **7** remaining after bioactivity testing prevented us from obtaining these data. Alternatively, the *Z*-geometry proposed at the exocyclic double bond for **7** rests on the nearly identical shifts shown in Figure 3 for compounds **7** and **8** at positions 5, 6, and 8.

The two new compounds were tested for biological activity. Both were inactive in the soft agar diffusion assay, which explores for selective cytotoxicity.[15] Compounds **5** and **7** exhibited only mild activity in the antimicrobial panel consisting of *Eschericia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), and *Candida albicans* (ATCC 24433). For example,



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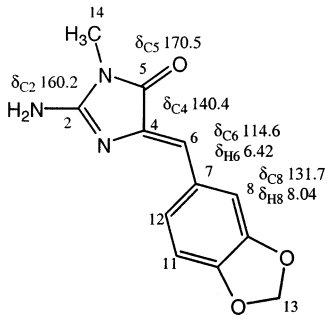

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Figure 3. Selective $^{13}C/^{1}H$ NMR shifts of leucettamines C (**7**) and B (**8**) in $(CH_3)_2CO$-d_6.

N,*N*-dimethyl naamine D (**5**) and leucettamine C (**7**) were weakly active against *C. albicans* in that their inhibition zone sizes relative to that of nystatin were 55% for **5** and 50% for **7**, with all compounds at 100 μg/disk.

Conclusions

There are two issues needing further discussion based on the results reported above. The first involves the variation in 2-amino imidazole constituents among the closely related sponges examined. The second pertains to biosynthetic relationships among compounds **5**−**7**.

The results in Table 1 contain information on multiple collections of two of the five *Leucetta* sponges regarding their content of amino imidizole[6] versus leucettamol unsaturated amino alcohol alkaloids. The four samples of *L. avocado* could be divided into two chemotypes[16] based on their nonoverlapping content of amino imidazoles and leucettamol A. Alternatively, both *L. primigenta* sponges were a source of leucettamol A. At this point in time the factors responsible for the chemotype differences are not explainable and are subjects of further ongoing investigations. Part of our approach involves a comparative chemical study of multiple collections of other calcareous sponges including the members of the *Clathrina*, *Leuconia*, and *Sycon* genera.

Surprisingly, there is little definitive knowledge on the biosynthesis of simple 2-amino imidazoles[4] or the more complex compounds including oroidin-type alkaloids.[17] The current thinking can be summarized as follows. Hill[18] proposed that 2-amino imidazole terpenoids, which have been repeatedly isolated from tropical trees within the genus *Alchornea* (Euphorbiaceae), appear to be derived from the combination of a guanidine and isoprenoid units. Several years ago we suggested condensation of guanidine and a *p*-hydroxyphenyl pyruvate (phpp) could produce intermediate **i**, shown in Figure 4,[2b] which could then serve as a precursor for compounds such as dorimidazole A (**1**) and isonaamine A (**9**) as well as other related metabolites. This idea is consistent with the observation of marine phytoplankton being able to convert tyrosine to phpp.[19] Intermediate **i** is also a reasonable precursor to the other three compounds isolated here including the two new 2-amino imidazole alkaloids *N*,*N*-dimethyl naamine D (**5**) and leucettamine C (**7**), and naamine A (**6**). Also consistent with these ideas is that many of more than 50 2-amino imidazole-containing compounds known from marine sponges[20] can be dissected into a guanidine plus amino acid subunits. Examples include 2-methylaplysinopsin[21] from guanidine and tryptophan, corallistine[22] from guanidine and thiolhistidine, and keramadine,[23] possibly comprised of guanidine plus lysine and proline.

Experimental Section

General Experimental Procedures. NMR spectra were collected at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Multiplicities of ^{13}C NMR peaks were determined using DEPT and gHMQC data. High-resolution mass measurements were obtained on a benchtop ESI-TOF apparatus. Other procedures were as previously published.[24]

Biological Material, Collection, and Identification. Sponges of the species *Leucetta* sp. shown in Table 1 were collected by scuba from a variety of habitats at various depths. Voucher specimens and underwater photos (see Supporting Information Figure S2) are available (from P.C.). These sponges were identified by ourselves and Dr. M. C. Diaz (UCSC, IMS) in reference to previously published photographs[5] and ultrastructural properties.[25] The specimen (coll. no. 95106) was examined visually and by microscopy to reveal the absence of cyanobacteria on the ectosome. The details regarding collection locations are as follows. Four samples of *Leucetta avocado* (*Pericharax heterohaphis*): sample no. 1, coll. no. 91136, collected in Milne Bay, Papua New Guinea (S 01°19.5′, E 150°59.0′); sample no. 2, coll. no. 98156, collected at three sites near Wewak, Papua New Guinea (S 01°38.5′, E 143°59.8′; S 01°27.2′, E 144°02.3′; S 01°20.1′, E 144°10.0′); sample no. 3, coll. no. 94649, collected in the Sangihe Islands, Indonesia (N 02°40.1′, E 125°20.8′); and sample no. 4, coll. no. 95106, collected near Nayamoto, Fiji (S 18°24.0′, E 177°57.0′). Two samples of *Leucetta primigenta*: sample no. 5, coll. no. 94566, collected in the Sangihe Islands, Indonesia (N 02°10.7′, E 125°20.2′) and sample no. 6, coll. no. 98131, collected near Wewak, Papau New Guinea (S 03°25.0′, E 143°37.9′). *Leucetta* sp. D, sample no. 7, coll. no. 95028, collected in Milne Bay, Papau New Guinea (not available). *Leucetta* sp. C, sample no. 8, coll. no. 95107, collected near Nayamoto, Fiji (S 18°24.0′, E 177°57.0′). *Leucetta* sp. B, sample no. 9, coll. no. 00111, collected in Fiji at two sites (S 18°19.4′, E 178°01.4′; S 18°19.2′, E 178°02.0′).

Extraction and Isolation. Each sponge was preserved in the field according to our standard procedures and transported back to the laboratory at ambient temperature. The collection was extracted with MeOH (3×), after which the solvent was removed and the resulting oil was partitioned between hexanes and 10% aqueous MeOH. The MeOH layer was adjusted to 50% aqueous MeOH and extracted with CH_2Cl_2.

The specimen (coll. no. 95106, 2.0 kg wet weight) afforded, after extraction, a methanol-soluble oil that was solvent partitioned to obtain (0.2514 g) of the FM (methanol) fraction (see Supporting Information, Figure S1). Further fractionation by size exclusion chromatography using LH-20 Sephadex (MeOH−CH_2Cl_2, 50:50) was followed by flash chromatography on silica gel eluted with MeOH−CH_2Cl_2 of increasing polarity. The resulting third flash fraction was then subjected to reversed-phase HPLC (MeOH−H_2O, 60:40−100% MeOH) to afford 28.5 mg of *N*,*N*-dimethyl naamine D (**5**) as a dark amber solid. Additionally, 42.1 mg of naamine A (**6**) was isolated as an amorphous amber solid from this HPLC procedure. The solvent-partitioned methanol-soluble oil also produced 1.8743

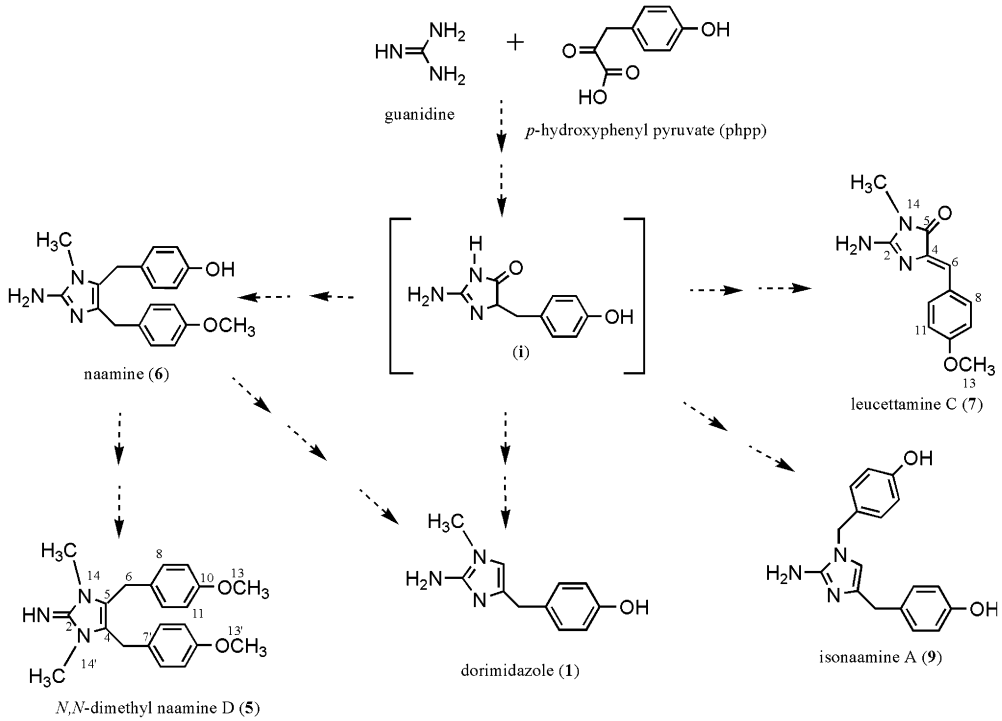

Figure 4. Biosynthetic relationships.

g of the FD, CH_2Cl_2 fraction (see Supporting Information, Figure S1). Further fractionation by size exclusion chromatography using LH-20 Sephadex (MeOH−CH_2Cl_2, 50:50) was followed by flash chromatography on silica gel eluted with MeOH−CH_2Cl_2 of increasing polarity. The resulting third flash fraction was then subjected to reversed-phase gradient HPLC (MeOH−H_2O, 70:30−100% MeOH) to afford 6.2 mg of leucettamine C (**7**) as an amorphous yellow powder.

***N,N*-Dimethyl naamine D (5):** amber amorphous solid; UV (MeOH) λ_{max} (log ϵ) 205 (4.25), 281 (3.75), 312 (3.58) nm; IR (neat) 3500, 1670, 1605, 1500 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125.7 MHz, CD_3OD) (see Supporting Information, Figures S3, S4). Additional diagnostic 2D NMR data include: (a) $^1H-^1H$ NMR COSY correlations between the aromatic protons H δ 7.08 (H8/12) and H δ 6.85 (H9/11); (b) HMQC correlations (see Supporting Information, Figure S5); (c) HMBC correlations (see Supporting Information, Figures S6−S8) beginning from the guanidinium carbon δ 146.4 (C2) to the *N*-methyl groups at δ 3.26 (Me14/14′), with correlation peaks to the double bond carbons C4 and C5 (δ 123.3); (d) HMBC correlation peaks from protons H6/6′ (δ 3.98) of the benzyl methylenes to the adjacent double bond carbons C4/C5 (δ 123.3), and from the H6/6′ (δ 3.98) methylene protons to carbons C8/8′ and C12/12′ (δ 128.7) of the benzene rings; (e) HMBC correlations linking all of the benzene ring atoms including CH δ 7.08 (H8/12) with carbons C9/11 (δ 113.9) and to the quaternary carbon C10 (δ 158.8), as well as CH δ 6.85 (H9/11) with carbons C8/12 (δ 128.7) and both quaternary carbons C7 (δ 128.4) and C10 (δ 158.8); and (f) HMBC correlations between the terminal methoxy fragments δ 3.75 (Me13/13′) and the aromatic quaternary carbons C10/10′ (δ 158.8); HRFABMS 352.2022 $[M + H]^+ = C_{21}H_{26}N_3O_2$ (Δ 0.3 mmu of calcd).

Naamine A (6): amber amorphous solid; UV (MeOH) λ_{max} (log ϵ) 206 (4.26), 288 (3.77), 307 (3.56) nm; IR (neat) 3515, 1675, 1610, 1503 cm^{-1}; ^1H and ^{13}C NMR data in accordance with literature values;[2a] HRFABMS 324.1711 $[M + H]^+ = C_{19}H_{22}N_3O_2$ (Δ 0.1 mmu of calcd).

Leucettamine C (7): yellow amorphous powder; UV (MeOH) λ_{max} (log ϵ) 207 (4.26), 230 (4.10), 368 (4.40) nm; IR (neat) 3525, 1710, 1605, 1570, 1480, 1260, 1150 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125.7 MHz, CD_3OD), see Table 3; ^1H NMR (500 MHz, Me_2CO-d_6) and ^{13}C NMR (125.7 MHz, Me_2CO-d_6) (see Figure 4). HMBC correlations revealed a pattern of connectivities to link residues of the entire molecule from the imidazole ring through to the methoxy on the benzene ring, as shown in Figure 2. These included correlations from the NCH_3 group at δ 3.14 (Me14) to the guanidinium carbon δ 161.0 (C2) and to δ 171.0 (C5); from the vinyl proton H6 (δ 6.56) to δ 171.0 (C5) and to C8/12 (δ 133.4); and from the methoxy δ 3.82 (Me13) to C10 (δ 161.5); HRFABMS 232.1082 $[M + H]^+ = C_{12}H_{14}N_3O_2$ (Δ 0.4 mmu of calcd).

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Supporting Information Available: The isolation scheme, NMR spectra of naamine D and leucettamine C (^1H, ^{13}C NMR and complete 2D spectra), and a photo gallery of *Leucetta* sponges. This material is available free of charge via the Internet at http://pubs.acs.org.

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