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K. A. Alvi, Phillip Crews, and David G. Loughhead

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STRUCTURES AND TOTAL SYNTHESIS OF 2-AMINOIMIDAZOLES FROM A *NOTODORIS* NUDIBRANCH[1]

K.A. ALVI, PHILLIP CREWS,*

Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California, Santa Cruz, California 95064

and DAVID G. LOUGHHEAD

Syntex Research, Institute of Organic Chemistry, 3401 Hillview Ave., Palo Alto, California 94304

ABSTRACT.—The anthelmintic active crude extract of an Indo-Pacific nudibranch has yielded dorimidazole A [**1**], 1-methyl-2-amino-4(*p*-hydroxybenzyl)imidazole, accompanied by the known compound isonaamine A [**2**], 2-amino-1,4-di(*p*-hydroxybenzyl)imidazole. These overall structures were established with the aid of spectral data. The regiochemistry within the imidazole ring was deduced by employing ^{13}C-nmr chemical shift increment values. The structure of **1** was confirmed by a four-step total synthesis, in 21% overall yield. New insights on the biogenesis of sponge-derived amino imidazoles are provided by the structure of **1**.

Our discovery of novel nitrogen-containing secondary metabolites from soft-bodied coral reef invertebrate extracts continues to be guided by antiparasitic prescreens (1). Work was initiated on the crude extract of a small collection of *Notodoris gardineri* Eliot (Family Aegiridae), a common Indo-Pacific nudibranch, which registered potent in vitro activity at 50 μg/ml against the parasite *Nippostrongylus brasiliensis* (Tom Matthews, personal communication). We anticipated yellow-colored 2-aminoimidazoles as prominent constituents of *No. gardineri*. This bright yellow nudibranch accentuated with black markings (2) is often observed perched on the yellow calcarious sponge *Leucetta* sp. (3), and 2-aminoimidazoles have been reported from both of these organisms. Wilkinson (3) has observed *No. gardineri* eating the brown calcareous sponge, *Pericharax heteroraphis*, but no secondary metabolite chemistry has been reported for sponges of this genus. Carmely *et al.* (4) have reported 2-aminoimidazoles including naamidines, isonaamidines, naamines, and isomaamine from the Red Sea nudibranch-sponge pair *Notodoris citrina* and *Leucetta chagosensis* (4). Correspondingly, two 2-aminoimidazoles, kealiiquinone and pyronaamidine, have been recently reported by Akee *et al.* (5) from Indo-Pacific specimens of *Leucetta* sp., while an unusual ionophoric 2-aminoimidazole, clathridine, has been isolated by Ciminiello *et al.* (6) from the calcarious sponge *Clathrina clathrus*. Our bioassay-guided search for the active constituents of this nudibranch afforded a new 2-aminoimidazole, dorimidazole A [**1**], accompanied by the known metabolite isonaamine A [**2**] (4).

RESULTS AND DISCUSSION

The MeOH extract of three nudibranchs (1 g dry wt) yielded an *n*-BuOH concentrate (90 mg) which, after chromatography, afforded 6 mg of dorimidazole A [**1**] and 1 mg of isonaamine A [**2**]. Once the structure of **1** was established, **2** was deduced and verified by comparison of its ^{1}H-nmr and ms properties to those in the literature (4).

The high degree of unsaturation (seven double bond equivalents) in **1**, indicated by the formula of $C_{11}H_{13}N_3O$ (hreims $[M]^+$ 203.1068), suggested the presence of multi-

[1]Part 2 of the series Novel Marine Sponge Alkaloids. For part 1 see W.D. Inman, M. O'Neill-Johnson, and P. Crews, *J. Am. Chem. Soc.*, **112**, 1 (1990). The synthetic work represents contribution number 806 from the Syntex Institute of Organic Chemistry. This paper is dedicated to Professor William T. Doyle, Director, UCSC IMS, on the occasion of his retirement.

ple aromatic rings. A *p*-hydroxy benzyl was apparent (^{1}H-nmr AB, δ 6.72, 7.04, J = 8.4 Hz, 4H and δ 3.68, s, 2H) as were an X-Me (δ 3.40, s, 3H), and -NH_2 (by difference of ^{13}C-nmr APT and hreims formulae). The remaining atoms consisting of C_3HN_2 were explained by a trisubstituted imidazole ring (^{13}C nmr δ 148.2, s; 115.0, d; 128.7, s; lreims *m/z* 107, [M − $C_4H_6N_3$]) bearing the -Me, -NH_2, and *p*-hydroxybenzyl appendages. The shift of the lowest field imidazole carbon was almost identical to C-2 of the 2-aminoimidazoles reported by Carmely *et al.* (4). However, technical difficulties in obtaining nOe measurements at UCSC prevented a straightforward assignment of the remaining vinyl C-H regiochemistry. A previous approach, successfully applied to complete assignments of the vinylic H's in oxazoles and thiazoles (7,8), was considered. The requisite reference ^{13}C-nmr chemical shift values, as shown in Figure 1, were assembled from the ^{13}C-nmr shifts of *N*-methyl imidazoles with further ring

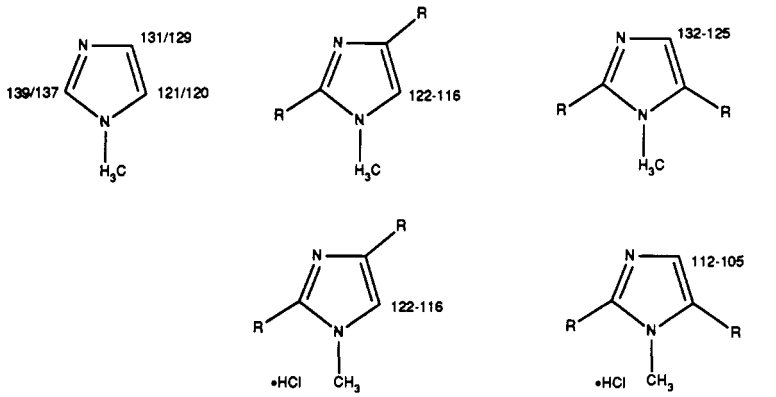


FIGURE 1. Predicted ^{13}C-nmr chemical shifts.

substituents of phenyl, methyl or amino. Substitution at C-2 imidazole ring position imparts a negligible effect on the shifts at C-4/5. Alternatively, chemical shift changes are observed at the C-H position of an *N*-Me imidazole carrying two additional substituents, and these range from a slight deshielding to shielding as summarized in Figure 1. Different ranges in δ's can be observed at the C-4 (neutral: δ125–132; HCl salt: δ 105–112) or C-5 (neutral or HCl salt: δ 116–122) positions for varyingly trisubstituted imidazole rings with 2-R and *N*-Me groups. The ^{13}C-nmr shift in **1** (neutral) of the protonated imidazole ring carbon at δ 115 ± 1 is much closer to that predicted for H-5 than for H-4. That the values of Figure 1 are widely applicable as a tool for assigning regiochemistry at C-4, C-5 of mono-substituted *N*-Me 2-aminoimidazoles is illustrated in Figure 2 by the data of compounds **3** and **4** (from the Sadtler ^{13}C-nmr catalog), keramadine [**5**] (9) (from an *Agelas* sponge), and isonaamine A [**2**] (from *Leucetta*) (4).

Confirmation of the asignment of structure **1** was obtained via total synthesis. The overall strategy of this efficient effort is outlined in Scheme 1. Treatment of known diazoketone **6** (10) with HBr in Et_2O provided the unstable α-bromoketone **7**, which was converted to α-aminoketone **9** using a procedure described by Guzman *et al.* (11). Thus, **7** was treated first with methyl *N*-methylformimidate to provide the formamide **8**, which in turn was hydrolyzed with HBr/MeOH to the desired α-aminoketone **9**. Finally, condensation of **9** with aqueous cyanamide (12) provided the desired dorimidazole A [**1**] (as its HBr salt) in an overall yield of 21% from **6**. Synthetic **1** was found to be identical to natural dorimidazole A when examined by tlc. The ^{1}H- and ^{13}C-nmr were also consistent with the identity of natural and synthetic materials. However, the chemical shift of H-5 in the synthetic or natural dorimidazole A varied

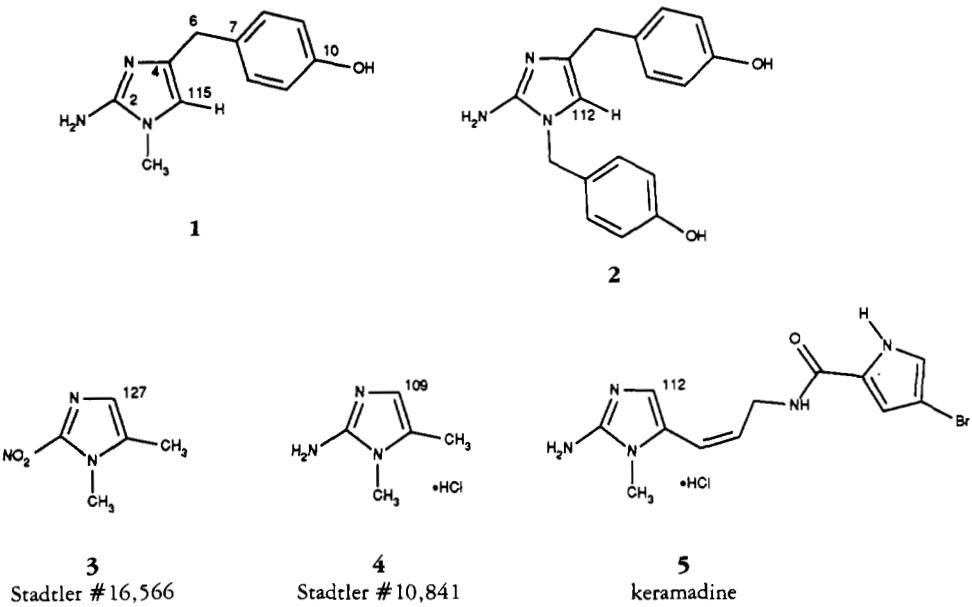
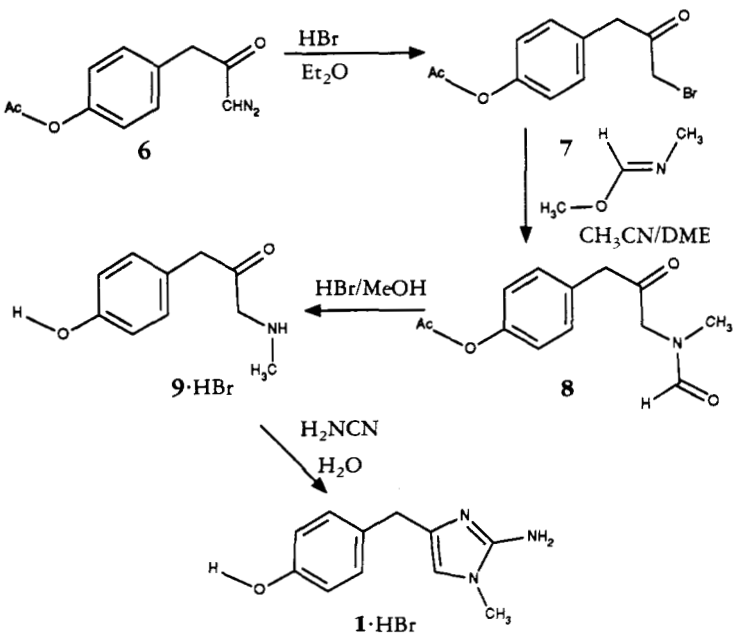


FIGURE 2. Observed chemical shifts.

due to differences in solvent, state (salt or neutral), or concentration. For synthetic material, the signal for this proton was observed in the range from δ 6.06 to 6.24; for natural material this signal was observed in the range from δ 6.09 to 6.43. Also, the shifts of carbons C-4 and C-5 differed by as much as 1.8 ppm when the spectra of the natural product (CD_3OD) and the synthetic product ($CD_3OD/CDCl_3$) were compared (see Experimental). Alternatively, side-by-side comparison of nOe data for both samples (Figure 3) shows that they have identical arrangements in the vicinity of the C-4, C-5 dou-



SCHEME 1

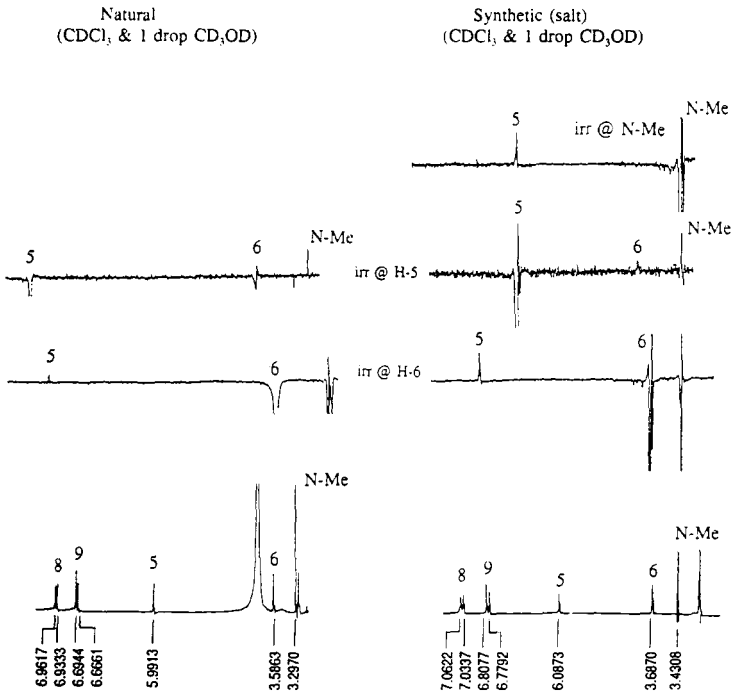
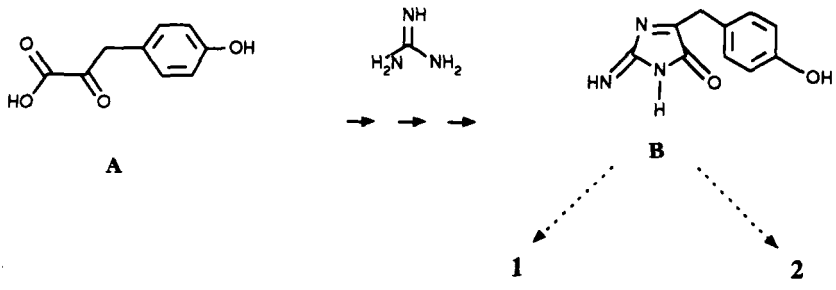


FIGURE 3. ^{1}H-nmr and difference nOe spectra of doramidazole A [**1**] at 300 MHz. Expansions are not the same for the spectra of natural and synthetic compounds (shifts are in ppm).

ble bond. The parallel nOe difference study of synthetic and natural **1**, conducted at Syntex, included irradiation at H-5, which revealed enhancements at δ 3.40 (Me) and 3.68 (H-6) in both samples.

Dorimidazole A is an interesting compound as it and 2-aminoimidazole, isolated from *Reniera cratera* (13), are the simplest examples containing this moiety to be isolated from a nudibranch or sponge. There is little definitive knowledge on the biosyntheses of 2-aminoimidazoles. Hill (14) has suggested that the 2-aminoimidazole terpenoids, which are common from tropical trees within the genus *Alchornea* (Euphorbiaceae), appear to be derived from a fusion of guanidine and isoprenoid units. A parallel biogenesis mode might be represented (Scheme 2). We suggest that a guanidine and a *p*-hydroxyphenyl pyruvate **A** unite to generate intermediate **B** which could then serve as a precursor to both **1** and **2** or other related compounds. Consistent with this proposal is that marine phytoplankton have been shown to degrade tyrosine to **A** (15). To date, marine sponges are known as a source of more than forty 2-aminoimidazole-con-



SCHEME 2

taining compounds, and many of these structures can be dissected into guanidine plus amino acid subunits (e.g., keramadine [**5**] appears to be comprised of guanidine + lysine + proline moieties).

Conflicting follow-up antiparasite assay results were observed. The natural sample was active at 50 μg/ml, while, surprisingly, the synthetic material, administered as the HBr salt, was inactive. Trace amounts of unknown compounds appear to be present in the natural sample of **1** as shown by its ^{1}H-nmr spectra, and this could explain this difference in bioactivity behavior.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr experiments were carried out at 90 MHz (Syntex), 300 MHz (UCSC and Syntex), or 500 MHz (Syntex). Hreims data were obtained at the University of California, Berkeley mass spectrometry lab, and lreims data were obtained at Syntex. Ir spectra were measured as dispersions in KBr, and hplc was done using a 10 μ-ODS or 10μ Si gel column (25 × 1.0 cm). Flash chromatography was performed using 230–400 mesh Si gel. All solvents were distilled and dried for hplc and were spectral grade for spectroscopy. Standard pulse sequences were used for the two dimensional ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, and long range ^{1}H-^{13}C COSY nmr experiments (16,17).

ISOLATION PROCEDURES.—Nudibranchs (collection #88117: voucher and underwater photo available from PC) were collected for us in the Philippines during the winter of 1988 by Quality Marine, Los Angeles, California. Identification was provided by Dr. Terrence Gosliner, Curator of the Molluscs, California Academy of Sciences, and their appearance was similar to that shown in a color photo (2). A total of three animals were subjected to extraction (MeOH × 3). Evaporation of solvent afforded a greenish yellow oil (90 mg). The oil was purified by Sephadex (LH-20) cc using MeOH as solvent followed by reversed-phase hplc (10μ-ODS, solvent = 50% aqueous MeOH) to afford pure **1** (6 mg) and **2** (1 mg).

DORIMIDAZOLE A [**1**].—A yellow powder (6 mg): uv (MeOH) 228, 269, 297, 300 nm; lreims *m/z* (rel. int.) $[M]^+$ 203 (100), 188 (30), 160 (10), 147 (30), 110 (20), 107 (5), 96 (5); hreims $[M]^+$ *m/z* 203.1068 ($C_{11}H_{13}N_3O$, Δ 0.9 mmu of calcd); ^{1}H nmr (MeOD, 300 MHz) δ 3.40 (s, 3H, *N*-Me), 6.43 (s, H-5), 3.69 (s, H_2-6), 7.04 (d, J = 8.4, H-8, 8′), 6.72 (d, J = 8.4 Hz, 9, 9′) (see Figure 3 for an expansion of low field resonances); ^{13}C nmr (MeOD, 75 MHz) δ 148.2 (C-2), 128.7 (C-4), 115.0 (C-5), 30.8 (C-6), 128.3 (C-7), 130.7 (C-8, -8′), 116.5 (C-9, -9′), 157.6 (C-10), 32.3 (*N*-Me); ^{1}H nmr ($CDCl_3$ + 1 drop $MeOD_3$) see Figure 3. At the conclusion of the total synthesis work, difference nOe data were obtained at Syntex on both the natural product and synthetic sample, and these results are shown in Figure 3.

ISONAAMINE A [**2**].—A yellow powder (1 mg): uv (MeOH) 227, 265, 275, 294, 300 nm; ^{1}H nmr (MeOD, 300 MHz) δ 6.78 (s, 1H, H-5), 3.74 (s, 2H, H-6), 7.17 (d, 2H, J = 8.4 Hz, H-8, -12), 6.63 (d, 2H, J = 8.4 Hz, H-9, -11), 5.09 (s, 2H, H-13), 7.22 (d, 2H, J = 8.7 Hz, H-18, -15), 6.86 (d, 2H, J = 8.7 Hz, H-17, -16).

1-[4-(ACETOXY)PHENYL]-3-BROMO-2-PROPANONE [**7**].—A solution of 3.91 g (17.9 mmol) of 1-[4-(acetoxy)phenyl]-3-diazo-2-propanone [**6**] (12) in 100 ml of Et_2O was chilled in an ice-H_2O bath. This solution was then treated dropwise with HBr in Et_2O until further treatment did not induce additional gas evolution and tlc [Me_2CO-hexane (1:4)] indicated clean conversion to product. The Et_2O was then decanted from the precipitated solid product and washed with aqueous $NaHCO_3$. The solids were dissolved in CH_2Cl_2 and also washed with aqueous $NaHCO_3$. The Et_2O and CH_2Cl_2 solutions were combined, dried over $MgSO_4$, and concentrated on the rotary evaporator. The residue was flash chromatographed eluting with Me_2CO-hexane (1:4) to give a solid which was recrystallized from hexane to give 3.24 g (67%) of the desired product: mp 86–88°; ir (KBr) 1754, 1225, 1196 cm^{-1}; lreims *m/z* $[M]^+$ 270/272. *Anal.* calcd for $C_{11}H_{11}BrO_3$: C 48.73, H 4.09; found C 49.23, H 4.16. ^{1}H nmr ($CDCl_3$, 90 MHz) δ 2.26 (s, 3H), 3.85 (s, 2H), 3.89 (s, 2H), 6.99 (d, 2H, J = 9 Hz), 7.32 (d, 2H, J = 9 Hz).

N-[3-[4-(ACETOXY)PHENYL]-2-OXOPROPYL]-*N*-METHYLFORMAMIDE [**8**].—A solution of 1.10 g (4.06 mmol) of bromide **7** in 25 ml of MeCN was stirred in a flask equipped with a dry ice condenser. Then 0.80 ml of a 55% (as estimated by gc and nmr) solution of methyl *N*-methylformimidate (13) in dimethoxyethane was added, and the reaction mixture was heated in an 80° oil bath for 2.5 h. Since tlc then indicated incomplete conversion to product, an additional 0.50 ml of the same solution of methyl *N*-methylformimidate was added, and the reaction mixture was heated in the oil bath for an additional 2 h. The reaction mixture was then partitioned between CH_2Cl_2 and ice-H_2O. The H_2O was washed with additional CH_2Cl_2, and the combined organic phase was dried ($MgSO_4$) and concentrated on the rotary evaporator. The residue was flash-chromatographed eluting with Me_2CO-CH_2Cl_2 (1:9). The product, ob-

served by ^{1}H nmr to exist as a 4:1 mixture of geometrical isomers, was recrystallized from *t*-butyl methyl ether to give 0.511 g (50%) of **8**: mp 73–74°; ir (KBr) 1762, 1719, 1674, 1508, 1408, 1391, 1372, 1221 cm^{-1}; lreims *m/z* [M]$^+$ 249. *Anal.* calcd for $C_{13}H_{15}NO_4$: C 62.64, H 6.07, N 5.61; found C 62.81, H 6.20, N 5.57. ^{1}H nmr ($CDCl_3$, 500 MHz) δ 2.29 (s, 2.4H), 2.30 (s, 0.6H), 2.82 (s, 0.6H), 2.93 (s, 2.4H), 3.72 (s, 0.4H), 3.74 (s, 1.6H), 4.05 (s, 0.4H), 4.17 (s, 1.6H), 7.07 (d, 1.6H, J = 8.6 Hz), 7.09 (d, 0.4H, J = 8.4 Hz), 7.23 (d, 0.4H, J = 8.4 Hz), 7.24 (d, 1.6H, J = 8.6 Hz), 7.90 (s, 0.2H), 8.11 (s, 0.8H).

1-[4-(HYDROXY)PHENYL]-3-METHYLAMINO-2-PROPANONE HYDROBROMIDE [**9**·HBr].—A solution of 520 mg (2.09 mmol) of formamide **8** was dissolved in 21 ml of 1 N HBr in MeOH, and this solution was refluxed for 6.5 h. The reaction mixture was concentrated on the rotary evaporator to give a semi-solid which was crystallized from MeCN/Et_2O to give 327 mg of the desired product, mp 143–144°. Reworking the mother liquor gave an additional 119 mg, mp 144–145°, raising the yield to 82%: ir (KBr) 3351, 1721, 1518, 1219 cm^{-1}. *Anal.* calcd for $C_{10}H_{14}BrNO_2$: C 46.17, H 5.42, N 5.38; found C 46.24, H 5.46, N 5.43. ^{1}H nmr (DMSO-d_6, 300 MHz) δ 2.54 (s, 3H), 3.73 (s, 2H), 4.12 (s, 2H), 6.73 (d, 2H, J = 8.5 Hz), 7.02 (d, 2H, J = 8.5 Hz), 8.68 (bs, 2H), 9.33 (bs, 1H).

2-AMINO-4-(4-HYDROXY)PHENYLMETHYL-1-METHYLIMIDAZOLE HYDROBROMIDE [**1**·HBr].—A solution of 345 mg (1.32 mmol) of aminoketone **9** and 347 mg (8.25 mmol) of cyanamide in 5.5 ml of H_2O was heated in a 100° oil bath for 2 h and then concentrated on the rotary evaporator. The residue was flash chromatographed eluting with MeOH-CH_2Cl_2 (1:9). Product-containing fractions were concentrated and redissolved in MeOH/CH_2Cl_2. This solution was made acidic with MBr/MeOH and reconcentrated. The semi-solid residue was crystallized from MeCN to give 222 mg of the desired product, mp 175–176°. Reworking the mother liquor provided an additional 68 mg, mp 172–173°, raising the yield to 77%. Ir (KBr) 3287, 3141, 1674, 1516 cm^{-1}; lreims *m/z* [M]$^+$ 203. *Anal.* calcd for $C_{11}H_{14}BrN_3O$: C 46.50, H 4.97, N 14.79; found C 46.34, H 4.97, N 14.85. ^{1}H nmr ($CDCl_3$ + 1 drop CD_3OD, 500 MHz) δ 3.43 (s, 3H), 3.68 (s, 2H), 6.09 (s, 1H), 6.79 (d, 2H, J = 8.5 Hz), 7.04 (d, 2H, J = 8.5 Hz); ^{13}C nmr ($CDCl_3$ + 1 drop CD_3OD, 125 MHz) δ 30.3 (C-6), 32.3 (*N*-Me), 113.2 (C-5), 115.9 (C-9, -9′), 126.9, 127.4 (C-4, -7), 129.9 (C-8, -8′), 146.7 (C-2), 156.2 (C-10).

^{13}C-NMR COMPARISONS OF DORIMIDAZOLE A [**1**].—After the synthetic and natural samples of **1** were entirely consumed in the bioassay experiments, we realized that their ^{13}C-nmr data had not been measured in the same solvent. The following compilation summarizes the shift (in ppm) at each carbon for the natural sample in CD_3OD minus that of the synthetic sample in $CD_3OD/CDCl_3$ or $CD_3OD/CDCl_3$/pyridine-d_5: C-1 = 1.5, 0.9; C-4 = 1.8, 1.7; C-5 = 1.8, 1.7; C-6 = 0.5, 0.3; C-7 = 0.9, 0.6; C-8 = 0.8, 0.6; C-9 = 0.6, 0.3: C-10 = 1.4, 0.4; *N*-Me = 0.0, −0.2. Others (6) observed solvent effects on the shift at C-5 of 2-aminoimidazole such as clatharidine (Δδ in $CDCl_3$ vs. TFA = 3.0 ppm).

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