New Structures and Bioactivity Patterns of Bengazole Alkaloids from a Choristid Marine Sponge

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JANUARY 1994

Impact Factor: 3.8 · DOI: 10.1021/np50102a002 · Source: PubMed

3 AUTHORS, INCLUDING:

Jaime Rodriguez
University of A Coruña
125 PUBLICATIONS **2,767**
CITATIONS

SEE PROFILE

Phil Crews
University of California, Sant…
179 PUBLICATIONS **6,167**
CITATIONS

SEE PROFILE

Journal of Natural Products
Vol. 56, No. 12, pp. 2034-2040, December 1993

NEW STRUCTURES AND BIOACTIVITY PATTERNS OF BENGAZOLE ALKALOIDS FROM A CHORISTID MARINE SPONGE[1]

JAIME RODRÍGUEZ, ROSA M. NIETO, and PHILLIP CREWS*

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

ABSTRACT.—New bengazoles **3–9** as inseparable mixtures are reported from the sponge *Jaspis* cf. *coricea* collected in Papua New Guinea. These compounds contain a new variation of the unusual bisoxazole core present in bengazole A [**1**]. The B-ring oxazole hexatetraol side chain is varyingly substituted with either myristic acid or 13-methylmyristic acid. Hydrolysis of three different bengazole mixtures each yielded an identical tetraol, bengazole Z [**11**].

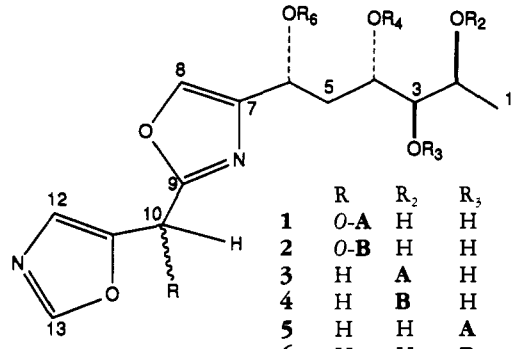
In 1989 (1) we reported on the diverse amino-acid-containing alkaloids of a thick, encrusting, orange Indo-Pacific sponge. Our interest in this specimen has been sustained because different collections, which can now be identified as *Jaspis* cf. *coriacea* (family Jaspidae, order Choristida=Astrophorida) have exhibited substantial variations among five different amino acid types. To date, these constituents include the bengamides (six compounds), isobengamide E, the bengazoles A [**1**] and B [**2**], a diketopiperazine, and the methyl ester of *N*-acetyl-L-phenylalanine. The bengazoles A and B (isolated from coll. no. 86009) proved to be only occasionally available but were of interest because of their potency in antiparasitic assays. Further biotesting was thwarted because on standing these compounds underwent a condensation reaction with oxygen followed by a fragmentation (2), and other Fijian specimens (coll. nos. 87009, 88062, 89139, and 91002) did not yield either **1** or **2**, even though they contained the bengamides (3). Recently, we were delighted to discover a Papua New Guinea (PNG) specimen (coll. no. 90187) containing inseparable mixtures of seven new bengazoles **3–9** accompanied by bengamides A and B and bengazole A [**1**] (2). The structures and cytotoxicity properties of these new bengazoles characterized by obtaining a hydrolysis product, bengazole Z [**11**], are described below.

RESULTS AND DISCUSSION

An nmr spectrum of the dark viscous oil obtained from the sponge MeOH extract contained low field resonances suggesting the presence of oxazole-containing metabolites. This oil was further processed by solvent partitioning of the MeOH solubles with hexanes (FH) and then CH_2Cl_2 (FD), followed by flash chromatography of the FD fraction. Repeated chromatography, as shown in Figure 1, of flash fraction seven from the FD, coded as FDF7, by successive reversed-phase hplc with MeOH-H_2O (85:15), then MeOH-H_2O (80:20) or MeCN-H_2O-MeOH (4:3:0.5), afforded three key fractions, FDF7H3H2, FDF7H2H1, and FDF7H2H2. The ^{1}H-nmr spectra shown in Figure 2 of these mixtures clearly indicated complex mixtures of new bengazoles which appeared to be missing the C-10 oxygen substituent present in bengazole A [**1**].

Repeated efforts to obtain purified compounds from these mixtures were unsuccessful, so each fraction noted above was separately hydrolyzed, and all gave a single compound, bengazole Z [**11**]. Its molecular formula, $C_{13}H_{18}O_6N_2$, was consistent with the lrfabms peak at 299, assumed to be the $[M+H]^+$ ion. Side-by-side inspection of the $^{13}C/^1H$-nmr spectra of bengazole Z [**11**] and bengazole A [**1**] (Table 1) enabled

[1]Part 14 in the series Novel Sponge-Derived Amino Acids. For part 13 see C. Jiménez, and P. Crews, *Tetrahedron*, **47**, 2097 (1991).



	R	R_2	R_3	R_4	R_6
1	*O*-**A**	H	H	H	H
2	*O*-**B**	H	H	H	H
3	H	**A**	H	H	H
4	H	**B**	H	H	H
5	H	H	**A**	H	H
6	H	H	**B**	H	H
7	H	H	H	**A**	H
8	H	H	H	**B**	H
9	H	H	H	H	**A**
10	H	H	H	H	**B**
11	H	H	H	H	H

A=$CO(CH_2)_{12}Me$
B=$CO(CH_2)_{11}CHMe_2$

assignment of the resonances for the two oxazole rings [δ 152.2 (d)/8.18 (s) CH-13; 137.3 (d)/7.78 (bs) CH-8; 124.7 (d)/7.09 (bs) CH-12], and a ^1H-^1H COSY nmr spectrum proved the tetraol side chain [δ 78.7 (d)/3.21 (dd) CH-3; 71.3 (d)/3.70 (ddd) CH-4; 67.7 (d)/3.98 (dq) CH-2; 66.3 (d)/4.90 (dd) CH-6; 40.5 (t)/2.24 (ddd), and 1.92 (ddd) CH_2-5; 19.9 (q)/1.19 (q) Me-1]. A CH_2 moiety [δ 25.6 (t)/4.32 (bs)] joining the two oxazole rings was confirmed from a ^{13}C-^1H COSY (J=9 Hz) nmr spectrum, and diagnostic correlations were observed from the narrow doublet at δ 4.32, J=1 Hz, H_2-10, to the 161.4 (s) C-9 signal. That bengazole Z [**11**] possessed the same oxazole ring substitution pattern and the same relative stereochemistry within the side chain as present in bengazole A [**1**] (3) was demonstrated by their parallel nmr data summarized in Table 1.

A composition analysis of the three semi-pure fractions was addressed next. The goal was to first establish the regiochemistry of the ester functionality and then delineate its framework as **A** or **B** using mass spectral data, the areas of the highfield ^1H nmr region (Figure 2), and the ^1H-^1H COSY nmr correlations for the H's C-2 through C-6. Eight compounds **3–10** could be in these mixtures if all possible regioisomers with substituents **A** or **B** were present and assuming the bengazole A [**1**] side relative stereochemistry was conserved. The ^1H-nmr data for bengazole Z [**11**] provided handy reference shifts to establish clearly the side chain ester attachment points.

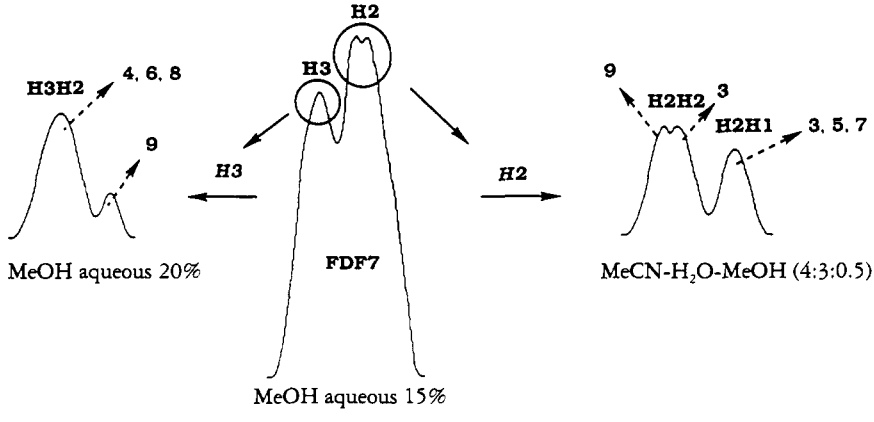


FIGURE 1. Overview of chromatography FDF7 hplc trace of FD flash chromatography fraction 7.

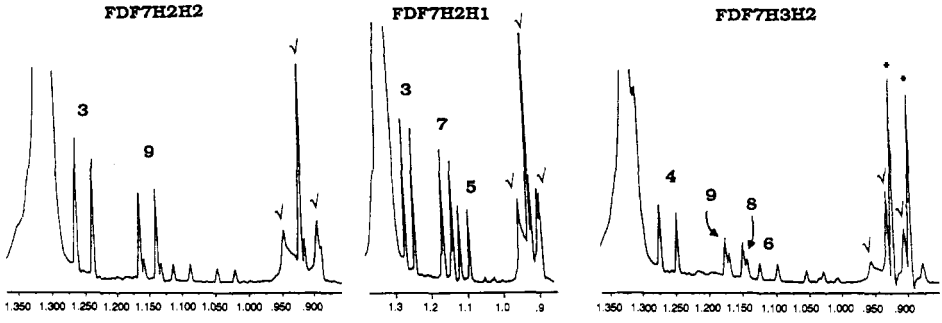


FIGURE 2. ^{1}H-nmr spectra showing the Me-1 region and myristate terminal Me's (√=type **A**, *=type **B**). **A**=$CO(CH_2)_{12}Me$; **B**=$CO(CH_2)_{11}CHMe_2$.

The hplc fraction FDF7H2H2 (Figures 1 and 2) was analyzed first, as it appeared to be the least complex mixture. Two compounds, bengazole C_2 [**3**] and bengazole C_6 [**9**], were identified in a ratio of 1.4:1.0. Clearly, only myristate ester **A** was present in these compounds, as evidenced by the Me-1 triplet at δ 0.925 (see √ marks of Figure 2). Also, the lrfabms of this mixture only showed an $[M+H]^+$ peak at *m/z* 509 in accord with the molecular formula $C_{27}H_{44}O_7N_2$. Key ^{1}H-^{1}H COSY nmr correlations to support the side chain regiochemistry assignment included cross peaks of **3** from δ 1.25 (Me-1) to the ester position δ 5.14 (H-2) and cross-peaks of **9** from the ester position δ 6.06 (H-6) to 2.45 (H_a-5) and 2.00 (H_b-5).

The adjacent hplc fraction FDF7H2H1 contained three components, bengazole C_2 [**3**], bengazole C_3 [**5**], and bengazole C_4 [**7**], in a ratio of 2.1:1.0:1.8. Indicative of the myristic ester **A** was the lrfabms $[M+H]^+$ peak at *m/z* 509, along with the Me-1 triplet at δ 0.92 (see √ marks of Figure 2). Diagnostic ^{1}H-^{1}H COSY nmr peaks were also

TABLE 1. Comparison of ^{13}C-nmr δ's (in ppm) and Coupling Constants (in Hz) of **1** and **11**.

Carbon	Compound	
	1	**11**
C-1	19.9	19.9
C-2	67.7	67.7
C-3	78.8	78.7
C-4	71.7	71.3
C-5	40.4	40.5
C-6	66.2	66.3
C-8	138.0	137.3
C-12	127.5	124.7
Coupling	***J* (Hz)[a]**	***J* (Hz)**
$J_{1\text{-}2}$	6.6	6.5
$J_{2\text{-}3}$	3.3	3.2
$J_{3\text{-}4}$	6.6	6.8
$J_{4\text{-}5a}$	9.0	9.5
$J_{4\text{-}5b}$	3.3	2.7
$J_{5a\text{-}5b}$	14.1	14.1
$J_{5a\text{-}6}$	5.7	7.0
$J_{5b\text{-}6}$	5.7	6.8

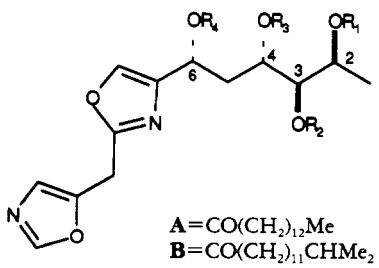
[a]Data for bengazole A hydrolysis product, **1**, R=OH.

identified, including those noted above of **3**, plus new ones of **5** from δ 1.10 (Me-1) to δ 4.07 (H-2) and to the ester position δ 4.74 (H-3), accompanied by those of **7** from the ester position δ 4.90 (H-4) to δ 2.42 (H_a-5) and δ 2.02 (H_b-5).

The hplc fraction FDF7H3H2 was the most difficult mixture to analyze, because four bengazoles, D_2 [**4**], D_3 [**6**], D_4 [**8**], and C_6 [**9**], were eventually concluded to be present. The problem here was that the side chain of each compound had three OH's plus either a type **A** or **B** myristate. Consequently, each of the eight possible compounds **3**–**10** had to be considered. An lrfabms of FDF7H3H2 showed two strong $[M+H]^+$ peaks at *m/z* 509 ($C_{27}H_{44}O_7N_2$) and 523 ($C_{28}H_{46}O_7N_2$), which indicated the presence of both ester types **A** and **B** in the mixture. Equally informative were the Me resonances shown in Figure 2 for both the myristate group and the terminal Me-1 of the hexyl side chain. The relative areas of the Me-1 side chains intimated that four bengazoles were present in a ratio of 3.8:2.2:1.2:1.0 (Table 2). Likewise, the relative areas of the upfield triplet (√) and doublet (*) suggested the ratio of **B**:**A** was 2.8:1.0. This ratio of **B**:**A** was also in agreement with the analysis of a myristic methyl ester mixture obtained by saponification (KOH/MeOH) of fraction FDF7H3H2. A **B**:**A** ratio of 2.9:1.0 was obtained for this hydrolysate by measuring the relative ion current of the lrfabms $[M+H]^+$ peaks at *m/z*=243/257 corresponding to molecular formulae of $C_{15}H_{30}O_2$ and $C_{16}H_{32}O_2$, respectively. Alternatively, a **B**:**A** ratio of 2.8:1.0 was calculated by nmr from the relative areas of the myristate terminal methyl protons. The most intense Me-1 resonance at δ 1.26 in the FDF7H3H2 mixture was characteristic of the C-2 ester regiochemistry, and this assumption was verified by ^1H-^1H COSY nmr correlations observed between Me-1 and a relatively lowfield resonance at δ 5.16 (H-2). These signals were assigned to bengazole D_2 [**4**] rather than C_2 [**3**] because, as shown in entry 1 of Table 2, the highest ratio of **B**:**A**=1.15:1.0 that could be calculated was for an ester type **A** at C-2 and type **B** at the

TABLE 2. Analysis of Chain Types **A** and **B** in the Four-Component Mixture FDF7H3H2 Concluded to Contain **4**, **6**, **8**, and **9**.

Ester **A** or **B** at Carbon Position		C-2	C-3	C-4	C-6	
Me-1 Expt. Rel. Areas (Figure 2)		3.8	1.0	1.2	2.2	
Entry	Hypothetical mixture	Ester Type at Carbon Position				Calcd ratio **B**:**A**
		C-2	C-3	C-4	C-6	
1	**3, 6, 8, 10**	**A**	**B**	**B**	**B**	1.15
2	**4, 5, 7, 9**	**B**	**A**	**A**	**A**	0.86
3	**4, 5, 8, 9**	**B**	**A**	**B**	**A**	1.36
4	**4, 6, 7, 9**	**B**	**B**	**A**	**A**	1.71
5	**4, 5, 7, 10**	**B**	**A**	**A**	**B**	2.71
6	**4, 6, 8, 9**	**B**	**B**	**B**	**A**	2.73
7	**4, 6, 7, 10**	**B**	**B**	**A**	**B**	5.83
8	**4, 5, 8, 10**	**B**	**A**	**B**	**B**	7.20



A=$CO(CH_2)_{12}Me$
B=$CO(CH_2)_{11}CHMe_2$

other oxygen positions (e.g., C-3, C-4, and/or C-6) and this provided poor agreement to the experimental ratios summarized above. Also, as shown in Figure 1, it did not seem reasonable to propose **3** as a major component of both hplc fractions FDF7H2 and FDF7H3. Establishing that **4** was present in the FDF7H3H2 mixture narrowed the combinations of hypothetical mixtures to those shown in Table 2, and entries 3–6 had reasonable agreement between the observed and calculated **B**:**A** ratios. Tracing the various COSY nmr patterns from Me-1 of each of the three remaining compounds (shown in Figure 2) to the respective downfield proton shifts as compared to the corresponding shifts of **11** confirmed the assignment of esters at C-6 (Me-1=δ 1.16), at C-4 (Me-1=δ 1.14), and at C-3 (Me-1=δ 1.12). Comparing the observed (2.7–2.9:1.0) versus calculated ratios of **B**:**A** summarized in Table 2 indicated just two types of mixtures need to be considered further: entry 5, **4**, **5**, **7**, and **10** (calcd **B**:**A**=2.73) and entry 6, **4**, **6**, **8**, and **9** (calcd **B**:**A**=2.73). A choice in favor of the latter was made because compounds **5** and **7** were observed in fraction FDF7H2H1 (Figures 1 and 2) and not in FDF7H2H2 (Figure 2) and it would not be reasonable to expect these to be present in significant amounts in hplc peak FDF7H3 (Figure 1).

The bengazoles have been evaluated for their cytotoxicity in the NCI's 60 cell line screen. The parent compound of this family, bengazole A [**1**] [NSC 652603] has shown in vitro potency against two human tumor cell lines including colon, COLO-205, GI_{50}=0.181 μM (0.085 μg/ml), TGI=1.5 μM, LC_{50}=5.25 μM, and melanoma, SK-MEL-5, GI_{50}=1.13 μM, TGI=4.83 μM, LC_{50}=4.83 μM (4). The terms GI_{50} (same as IC_{50} or ED_{50}) and LC_{50} are further defined by Raub *et al.* (**4**). In contrast, the hydrolysis product bengamide Z [**11**] was inactive against these as well as 48 other solid tumor cell lines. It is relevant to note that oxazole-containing marine natural products are rather rare. They are currently known only from sponges (5–9) and one tunicate (10) and possess biopotency as antifungal, cytotoxic, antiparasitic, or tumor-promoting agents.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded in MeOH-d_4 at 250 MHz for ^{1}H and 62.5 MHz for ^{13}C. Multiplicities of ^{13}C nmr resonances were determined from DEPT data and COSY experiments. Both ^{1}H-^{1}H and ^{13}C-^{1}H COSY nmr data were used to assign resonances of compounds **3–9** and **11**. Lreims and lrfabms data were obtained on a double-focusing instrument. Hplc was done using a reversed-phase 10 μm ODS column.

COLLECTION AND IDENTIFICATION.—The sponge *J. cf. coricea* (coll. no. 90187) was collected at Boi-Boi Waga Island, Papua New Guinea in July 1990. It was preserved as described below. Voucher specimens and underwater photographs of coll. nos. 87009, 88062, 89139, 91002, and 90187 are available (from PC). This is a massive-encrusting sponge (0.5–2 cm thick), with an orange-yellowish color while alive and tan-brownish when dried. Its surface is smooth, and its consistency is compressible to soft. The skeleton is formed by oxeas, 400–520×6–8 μm, with strongylote or fusiform ends that are strewn or arranged in loose spongin-enforced tracts running sub-radially towards the sponge surface. Microspined oxyasters 12–26 μm in diameter, with 5–8 rays 6–12×1–2 μm in size, are spread in the choanosome and concentrated at the sponge surface. The species is found living on vertical walls or under ledges.

Our material is close in its external morphology and spicule composition to *Jaspis coriacea* (Carter) described extensively from Palau (11). However, our samples lack the large category of fusiform oxeas (730–1712×11–30 μm) from *J. coriacea*. Also, our samples possess asters with considerably fewer rays than the latter (8–15 μm). All specimens from Papua New Guinea, Fiji, and Indonesia (see coll. nos. above) conform to the description of 90187, with minor spicule size differences. The specimens from PNG lack the cirripedia (Crustacea) found abundantly on the underside of the body of Fijian samples.

A thorough comparison of *Jaspis* species is needed to determine if these differences should be considered as intraspecific variability or if they define a separate species. Furthermore, the genus *Jaspis* is badly in need of a revision due to the evident polyphyletic nature of the species currently assigned to it (12). Also, van Soest and Weinberg (13) have pointed out that many *Jaspis* species are in reality reduced species *Stelleta* or *Penares* (Stelletidae, Astrophorida).

EXTRACTION AND ISOLATION.—The freshly collected sponge (16.8 g, dry wt) was preserved by being

immersed in EtOH-H_2O (50:50). After approximately 24 h this solution was decanted and discarded. The damp organisms were placed in nalgene bottles and shipped back to the home lab at ambient temperature. Next, 100% MeOH was added and the organisms were soaked for 48 h. This procedure was repeated two more times. The combined organic extract yielded 3.2 g of crude oil, which was successively partitioned between equal volumes of aqueous MeOH, percent adjusted to produce a biphasic solution, and a solvent series of hexanes (FH), yield=0.8 g, and CH_2Cl_2 (FD), yield=0.66 g. The remaining H_2O solubles were extracted but did not contain any compounds of interest.

The CH_2Cl_2 (FD) fraction was chromatographed on a Si gel flash column (gradient of CH_2Cl_2/MeOH) affording ten fractions. Fraction seven (FDF7), as shown in Figure 1, was subjected to hplc [10 μm ODS, MeOH-H_2O (85:15)] and gave five fractions. The second and third fractions (FDF7H2 and FDF7H3) were repurified by hplc [10 μm, ODS, MeOH-H_2O (80:20) and MeCN-H_2O-MeOH (4:3:0.5), respectively] affording three key fractions coded as: FDF7H3H2 (33.6 mg) composed of compounds **4**, **6**, **8**, and **9** in a ratio 3.8:1.0:1.2:2.2; FDF7H2H1 (25.6 mg) composed of **3**, **5**, and **7** (2.1:1.0:1.8); and FDF7H2H2 (32.2 mg) containing **3** and **9** (1.4:1.0).

Myristate [**A**].—^{1}H nmr 2.38 (t, J=7.5 Hz, H-15), 1.60 (m, H-16); 1.30 (m, H-17–H-26), 0.90 (t, J=7.5 Hz, H-27); ^{13}C nmr 175.3 (C-14), 35.7 (C-15), 25.7 (C-16), 30.0 (C-17–C-24), 33.1 (C-25), 23.7 (C-26), 14.0 (C-27).

13-Methylmyristate [**B**].—^{1}H nmr 2.38 (t, J=7.5 Hz, H-15), 1.60 (m, H-16), 1.30 (m, H-17–H-25), 1.30 (m, H-26), 0.90 (d, J=6.6 Hz, H-27–H-28); ^{13}C nmr 175.4 (C-14), 35.8 (C-15), 25.7 (C-16), 30.0 (C-17–C-22), 28.5 (C-23), 40.2 (C-25), 29.1 (C-26), 23.0 (C-27–C-28).

Bengazole C_2 [**3**].—^{1}H nmr 1.25 (d, J=6.5 Hz, H-1), 5.14 (dq, J=6.5 and 3.2 Hz, H-2), 3.33 (under solvent, H-3), 3.54 (ddd, J=10.0, 7.9 and 2.5 Hz, H-4), 2.27 (m, H_a-5), 1.88 (m, H_b-5), 4.90 (under solvent, H-6), 7.76 (s, H-8), 4.31 (d, J=1.0 Hz, H-10), 7.09 (bs, H-12), 8.17 (s, H-13); ^{13}C nmr 16.9 (C-1), 71.0 (C-2), 77.3 (C-3), 70.5 (C-4), 40.7 (C-5), 66.2 (C-6), 145.1 (C-7), 137.2 (C-8), 161.4 (C-9), 25.7 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13).

Bengazole D_2 [**4**].—^{1}H nmr 1.26 (d, J=6.5 Hz, H-1), 5.16 (dq, J=6.5 and 2.7 Hz, H-2), 3.34 (under solvent, H-3), 3.56 (ddd, J=9.7, 7.2 and 2.7 Hz, H-4), 2.35 (m, H_a-5), 1.88 (m, H_b-5), 4.90 (under solvent, H-6), 7.77 (s, H-8), 4.32 (d, J=1.0 Hz, H-10), 7.10 (bs, H-12), 8.19 (s, H-13); ^{13}C nmr 16.9 (C-1), 71.0 (C-2), 77.3 (C-3), 70.4 (C-4), 40.7 (C-5), 66.2 (C-6), 145.1 (C-7), 137.2 (C-8), 161.4 (C-9), 25.6 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13).

Bengazole C_3 [**5**].—^{1}H nmr 1.10 (d, J=6.5 Hz, H-1), 4.07 (dq, J=6.5 and 3.5 Hz, H-2), 4.74 (dd, J=7.1 and 3.5 Hz, H-3), 3.80 (ddd, J=10.0, 7.1, and 3.4 Hz, H-4), 2.30 (m, H_a-5), 1.90 (m, H_b-5), 4.90 (under solvent, H-6), 7.76 (s, H-8), 4.31 (d, J=1.0 Hz, H-10), 7.09 (bs, H-12), 8.17 (s, H-13); ^{13}C nmr 19.9 (C-1), 66.6 (C-2), 80.0 (C-3), 69.3 (C-4), 40.7 (C-5), 66.2 (C-6), 145.1 (C-7), 137.2 (C-8), 161.4 (C-9), 25.6 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13).

Bengazole D_3 [**6**].—^{1}H nmr 1.12 (d, J=6.5 Hz, H-1), 4.08 (dq, J=6.5 and 3.1 Hz, H-2), 4.73 (dd, J=7.0 and 3.1 Hz, H-3), 3.80 (m, H-4), 2.20 (m, H_a-5), 1.90 (m, H_b-5), 4.90 (under solvent, H-6), 7.76 (s, H-8), 4.31 (d, J=1.0 Hz, H-10), 7.09 (bs, H-12), 8.17 (s, H-13); ^{13}C nmr 19.9 (C-1), 66.6 (C-2), 80.0 (C-3), 69.3 (C-4), 40.7 (C-5), 66.2 (C-6), 145.1 (C-7), 137.4 (C-8), 161.4 (C-9), 25.7 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13).

Bengazole C_4 [**7**].—^{1}H nmr 1.14 (d, J=6.4 Hz, H-1), 3.67 (dq, J=6.4 and 5.1 Hz, H-2), 3.42 (t, J=5.1 Hz, H-3), 4.90 (under solvent, H-4), 2.42 (m, H_a-5), 2.02 (m, H_b-5), 4.90 (under solvent, H-6), 7.72 (s, H-8), 4.31 (d, J=1.0 Hz, H-10), 7.09 (bs, H-12), 8.19 (s, H-13); ^{13}C nmr 16.9 (C-1), 71.0 (C-2), 77.3 (C-3), 70.4 (C-4), 40.7 (C-5), 66.2 (C-6), 145.1 (C-7), 137.2 (C-8), 161.4 (C-9), 25.6 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13).

Bengazole D_4 [**8**].—^{1}H nmr 1.16 (d, J=6.5 Hz, H-1), 3.70 (dq, J=6.5 and 3.4 Hz, H-2), 3.45 (m, H-3), 4.90 (under solvent, H-4), 2.40 (m, H_a-5), 2.05 (m, H_b-5), 4.90 (under solvent, H-6), 7.77 (s, H-8), 4.32 (d, J=1.0 Hz, H-10), 7.10 (bs, H-12), 8.19 (s, H-13); ^{13}C nmr 16.9 (C-1), 71.0 (C-2), 77.3 (C-3), 70.4 (C-4), 40.7 (C-5), 66.2 (C-6), 145.1 (C-7), 137.2 (C-8), 161.4 (C-9), 25.6 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13).

Bengazole C_6 [**9**].—^{1}H nmr 1.16 (d, J=6.5 Hz, H-1), 3.94 (dq, J=6.5 and 3.4 Hz, H-2), 3.17 (dd, J=6.8 and 3.4 Hz, H-3), 3.51 (m, H-4), 2.40 (m, H_a-5), 2.00 (m, H_b-5), 6.05 (dd, J=9.8 and 5.2 Hz, H-6), 7.89 (s, H-8), 4.33 (d, J=1.0 Hz, H-10), 7.10 (bs, H-12), 8.19 (s, H-13); ^{13}C nmr 19.9 (C-1), 67.7 (C-2), 79.0 (C-3), 69.6 (C-4), 37.5 (C-5), 67.5 (C-6), 149.0 (C-7), 139.4 (C-8), 161.5 (C-9), 25.6 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13).

HYDROLYSIS OF PARTITION FRACTIONS.—Each mixture (10 mg), which included fractions FDF7H2H2,

FDF7H2H1, and FDF7H3H2, was separately stirred 48 h at room temperature in 1% KOH/MeOH (1.1 ml). After neutralization with a 1% solution of HCl, the mixture was partitioned between H_2O (4 ml) and CH_2Cl_2 (3×4 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness to afford the methyl esters of **A** and **B**. The aqueous extract was concentrated in vacuo, and the resulting crude extract was dissolved in MeCN-MeOH (1:1) and filtered. The dry residue gave a crude extract which was purified by hplc [10 μm ODS, MeCN-H_2O (15:85)], affording in each case bengazole Z [**11**].

Bengazole Z [**11**].—An oil as prepared above: uv (MeOH) λ max 224, 278 nm; $[\alpha]_D$ −2.5 (c=0.008 g/100 ml, MeOH); ^{1}H nmr 1.19 (d, J=6.5 Hz, H-1), 3.98 (dq, J=6.5 and 3.1 Hz, H-2), 3.21 (dd, J=6.8 and 3.1 Hz, H-3), 3.70 (ddd, J=9.5, 6.8, and 5.7 Hz, H-4), 2.24 (ddd, J=14.1, 6.8, and 5.7 Hz, H_a-5), 1.92 (ddd, J=14.1, 9.5, and 7.0 Hz, H_b-5), 4.90 (dd, J=7.0 and 6.8 Hz, H-6), 7.78 (bs, H-8), 4.32 (d, J=1 Hz, H-10), 7.09 (s, H-12), 8.18 (s, H-13); ^{13}C nmr C-1–C-6 see Table 1, 145.1 (C-7), 137.3 (C-8), 161.4 (C-9), 25.6 (C-10), 148.2 (C-11), 124.7 (C-12), 153.3 (C-13); lrfabms ($C_{13}H_{18}O_6N_2$, matrix glycerol) $[M+H]^+$ 299, $[M+H-H_2O]^+$ 281.

ACKNOWLEDGMENTS

Funding was from NIH Grants CA47135 and CA52955. We thank Ms. M.C. Diaz and Prof. Rob van Soest for taxonomy analyses, Ms. L.M. Hunter and Ms. Alexander for assisting in the collection of the sponges, the NCI-DTP program for bioassay data, and Dr. D. Niles of the Institute of Papua New Guinea Studies for his cooperation.

LITERATURE CITED

1. M. Adamczeski, E. Quiñoá, and P. Crews, *J. Am. Chem. Soc.*, **111**, 647 (1989).
2. M. Adamczeski, E. Quiñoá, and P. Crews, *J. Am. Chem. Soc.*, **110**, 1598 (1988).
3. M. Adamczeski, E. Quiñoá, and P. Crews, *J. Org. Chem.*, **55**, 240 (1990).
4. M.F. Raub, J.H. Cardellina, M.I. Choudhary, C.-Z. Ni, J. Clardy, and M.C. Alley, *J. Am. Chem. Soc.*, **113**, 3178 (1991).
5. T. Ichiba, W.Y. Yoshida, P.J. Scheuer, T. Higa, and D.G. Gravalos, *J. Am. Chem. Soc.*, **113**, 3171 (1991).
6. N. Fusetani, K. Yasumuro, S. Mastsunaga, and K. Hashimoto, *Tetrahedron Lett.*, **30**, 2809 (1989).
7. N. Fusetani, T. Sugawara, S. Mastsunaga, and H. Hirota, *J. Am. Chem. Soc.*, **113**, 7811 (1991).
8. J. Kobayashi, F. Itagaki, H. Shigemori, M. Ishibashi, K. Takahashi, M. Ogura, S. Nagasawa, T. Nakamura, H. Hirota, T. Ohta, and S. Nozoe, *J. Am. Chem. Soc.*, **113**, 7812 (1991).
9. H. Tada, T. Tozyo, Y. Tervi, and F. Hayashi, *Chem. Lett.*, **1**, 431 (1992).
10. M.P. Foster, G.P. Concepcion, G.B. Caraan, and C.M. Ireland, *J. Org. Chem.*, **57**, 6671 (1992).
11. P.R. Bergquist, *Pac. Sci.*, **19**, 123 (1965).
12. E. Hajdu and R.W.M. van Soest, *Bijdragen tot de Dierkunde*, **62**, 3 (1992).
13. R.W.M. van Soest and W. Weinberg, *Stud. Fauna Cur. Caribb. Isl.*, **70**, 1 (1988).

Received 1 April 1993