Secondary Metabolites from Three Florida Sponges with Antidepressant Activity

Anna J. Kochanowska,[†] Karumanchi V. Rao,[†] Suzanne Childress,[‡] Abir El-Alfy,[‡] Rae R. Matsumoto,[‡] Michelle Kelly,[§] Gina S. Stewart,[⊥] Kenneth J. Sufka,[‡,⊥] and Mark T. Hamann*[,†,‡]

Departments of Pharmacognosy, Pharmacology, and Psychology and the National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, and the National Center for Aquatic Biodiversity and Biosecurity, National Institute of Water and Atmospheric Research, Auckland, New Zealand

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Brominated indole alkaloids are a common class of metabolites reported from sponges of the order Verongida. Herein we report the isolation, structure determination, and activity of metabolites from three Florida sponges, namely, *Verongula rigida* (order Verongida, family Aplysinidae), *Smenospongia aurea*, and *S. cerebriformis* (order Dictyoceratida, family Thorectidae). All three species were investigated chemically, revealing similarities in secondary metabolites. Brominated compounds, as well as sesquiterpene quinones and hydroquinones, were identified from both *V. rigida* and *S. aurea* despite their apparent taxonomic differences at the ordinal level. Similar metabolites found in these distinct sponge species of two different genera provide evidence for a microbial origin of the metabolites. Isolated compounds were evaluated in the Porsolt forced swim test (FST) and the chick anxiety−depression continuum model. Among the isolated compounds, 5,6-dibromo-*N,N*-dimethyltryptamine (**1**) exhibited significant antidepressant-like action in the rodent FST model, while 5-bromo-*N,N*-dimethyltryptamine (**2**) caused significant reduction of locomotor activity indicative of a potential sedative action. The current study provides ample evidence that marine natural products with the diversity of brominated marine alkaloids will provide potential leads for antidepressant and anxiolytic drugs.

Marine sponges have been a prolific resource of a huge diversity of secondary metabolites over the past 50 years of discovery. Sponges in the order Verongida have been reported to show unusual biochemical profiles characterized by the absence of terpenes and the production of sterols and brominated compounds biogenetically related to tyrosine.[1]

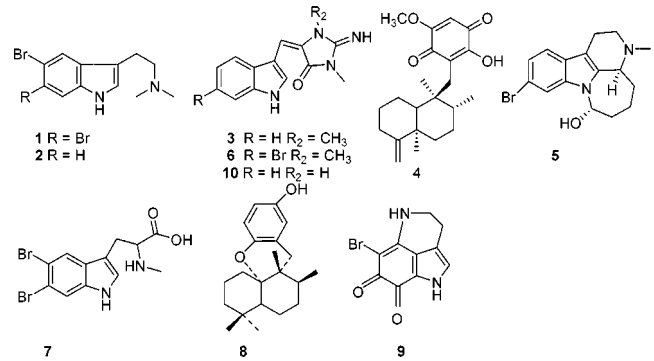
Many reported indole and pyrrole-imidazole alkaloids play a defensive role and exhibit interesting types of bioactivity, including calmodulin antagonism, cytotoxicity, antimicrobial, antiviral, antiparasitic, anti-inflammatory, and Ca^{2+}-releasing activity.[2,7,10,12,14] Bromination of members of this class of alkaloids occurs frequently when isolated from the marine environment and is catalyzed by haloperoxidases that oxidize halogen anions from seawater.[3] The utility of these metabolites in neuropsychiatric disorders remains to be characterized.

Central nervous system (CNS) disorders are common worldwide, with about one-fourth of adult Americans suffering each year from a diagnosable psychotic disorder.[4] Since the 1960s depression has been linked with decreased functional amine-dependent synaptic transmission.

All clinically used antidepressant drugs suffer from serious side effects, which may include metabolic, cardiovascular, and sleep disorders or increased suicidal thoughts and aberrant behavior. These drugs also require approximately 6 weeks before their full therapeutic effect occurs. Most known antidepressant drugs act as either monoamine oxidase inhibitors or reuptake inhibitors of noradrenaline and/or serotonin. These groups of drugs increase the concentration of monoamines in brain, facilitating their binding to receptors.[5] In addition, serotonergic neurotransmission systems have been implicated in the neuronal regulation of mood; thus enhancement of serotonin transmission is a recognized basis for the treatment of different types of depression.

To date, 14 types of serotonin receptors, grouped in seven families, have been reported to be expressed in mammalian CNS.[6] Research leading to selective serotonin receptor ligands is extremely important to further understand the role and function of these receptors. Considering the structural similarity of indole alkaloids and the endogenous monoamine serotonin, many efforts have been directed toward isolation and synthesis of serotonin-like molecules, which could possibly possess affinity to different 5HT receptors. Brominated marine indole alkaloids have been previously reported by Hu et al. to possess high affinity for human serotonin receptors.[7]

In order to further define the structure–activity relationship and search for possible leads to control depression, we have carefully examined the ethanol extract of three sponges collected from the Florida Keys. The sponges were collected from a variety of locations in the Florida Keys and separated based on morphology and color. The samples were identified as three species, two of which are well-known: *V. rigida* (Esper, 1794) (order Verongida, family Aplysinidae) and *S. aurea* (Hyatt, 1875) (order Dictyoceratida, family Thorectidae), and a third, *S. cerebriformis* (Duchassaing & Michelotti, 1864), is less common and separated based on subtle differences of morphology and coloration from the other two species. A table describing key field and histological characteristics that differentiate between the three species is available in the Supporting Information. Several known compounds were isolated, and those that bear structural similarity to serotonin were evaluated in two established animal models predictive of antidepressant drug action, namely, the rodent FST and the chick anxiety−depression model.



* To whom correspondence should be addressed. Tel: 662-915-5730. Fax: 662-915-6975. E-mail: mthamann@olemiss.edu.
[†] Department of Pharmacognosy.
[‡] Department of Pharmacology.
[§] National Center for Aquatic Biodiversity and Biosecurity, National Institute of Water and Atmospheric Research.
[⊥] Department of Psychology.

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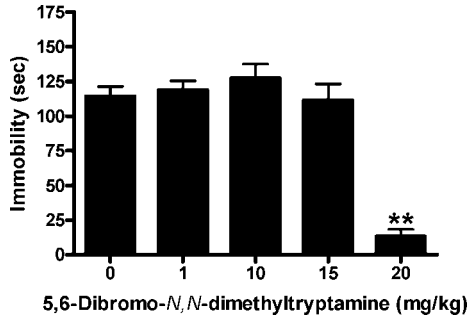

Figure 1. Reduction of immobility time in the forced swim test by 5,6-dibromo-*N,N*-dimethyltryptamine (**1**).

Results and Discussion

Exhaustive extraction of 3 kg of *V. rigida* yielded 211 g of crude extract. The fractionation and further purification (described in detail in the Experimental Section) of the crude extract yielded the following known metabolites: 5,6-dibromo-*N,N*-dimethyltryptamine (**1**),[8] 5-bromo-*N,N*-dimethyltryptamine (**2**),[8] aplysinopsin (**3**),[9,10] makaluvamine O (**9**),[7] arborescidine C (**5**),[11] 6-bromoaplysinopsin (**6**),[12,13] 5,6-dibromoabrine (**7**),[14] and small amounts of aureol (**8**)[8] and ilimaquinone (**4**).[15,16]

The ethanol extract of *S. aurea* was purified as described in the Experimental Section to yield aureol (**8**)[8] and four indole alkaloids, which were identified as 5,6-dibromo-*N-N*-dimethyltryptamine (**1**),[8] 2′-des-*N*-methylaplysinopsin (**10**),[17] 6-bromoaplysinopsin (**6**),[12,13] and makaluvamine O (**9**).[7]

S. cerebriformis (6 kg) yielded 260 g of extract, which exhibited significant activity against *Candida albicans* and MRS (IC_{50} of 15 μg/mL for both pathogens). The crude extract of this sponge was found to contain primarily ilimaquinone (**4**),[15,16] but polar fractions examined by HRMS showed also the characteristic peak of 5,6-dibromo-*N,N*-dimethyltryptamine.

Among the compounds isolated, 6-bromoaplysinopsin (**6**) was previously reported to exhibit significant activity against *Plasmodium falciparum* and to display high affinity for human serotonin $5HT_2$ receptor subtypes.[7] Makaluvamine O (**9**), 5,6-dibromo-*N,N*-dimethyltryptamine (**1**), and aureol (**8**) were reported to display significant activity against the HCT-116 colon carcinoma cell line.[14] Both dimethyl bromotryptamine derivatives were reported as antimicrobial agents, and according to Tymiak and Rinehart, dibromotryptamine (**1**) had significantly greater antimicrobial activity than the monobrominated analogue (**2**).[12] Aplysinopsin (**3**) was previously isolated from different sponge species[9,10,12] and was found to possess antineoplastic activity.[10]

All the compounds previously reported from other Verongida species were identified by comparison of their spectral data with literature values. Similar patterns of secondary metabolite production were found in species belonging to two distinct orders (Verongida and Dictyoceratida), providing evidence for a common microbial source of these compounds. Recent isolation of both brominated tryptamine derivatives from algae *Bryopsis* sp. in our laboratory strengthens this hypothesis.

Due to limited amounts of isolated compounds, only four of them could be tested in the Porsolt forced swim test and chick anxiety−depression continuum models. The locomotor activity test was performed to demonstrate that reductions in immobility time showed by the isolated compounds were not a secondary consequence of their nonspecific stimulant actions.

5,6-Dibromo-*N,N*-dimethyltryptamine (**1**) was evaluated in the forced swim test and chick anxiety−depression model. The forced swim test showed that **1** possesses significant antidepressant-like activity ($F[4.44] = 31.56$, $p < 0.01$) (Figure 1). Post hoc comparisons of individual doses to the vehicle control showed that

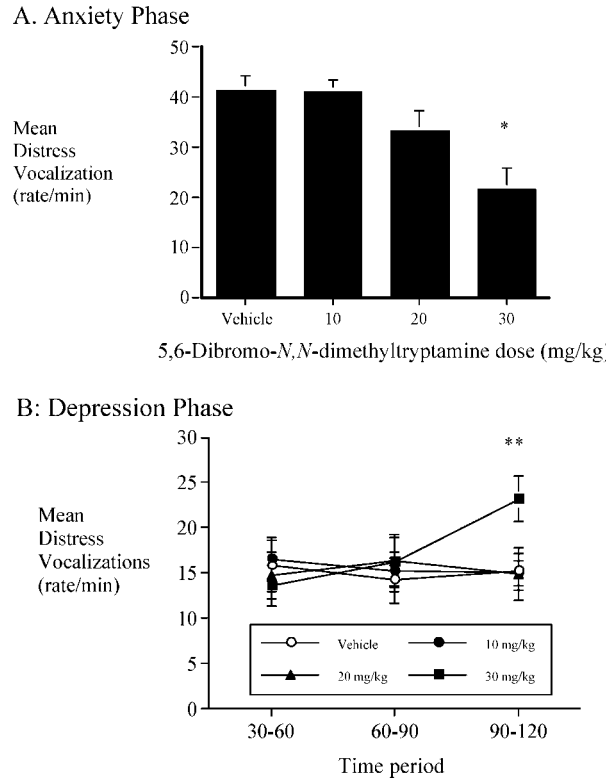

Figure 2. Effects of 5,6-dibromo-*N,N*-dimethyltryptamine (**1**) on separation distress vocalization rates during the anxiety phase (0 to 5 min, panel A) and the depression phase (30 to 120 min, panel B) of the test session. * indicates a significant decrease (i.e., anxiolytic effect) and ** indicates a significant increase (i.e., antidepressant effect) of vocalization rate compared to vehicle-treated chicks. All $p^s < 0.05$.

1 significantly reduced the immobility time only at the 20 mg/kg dose ($q = 8.28$, $p < 0.01$).[18]

In the chick anxiety−depression continuum model,[19] socially raised chicks are separated from conspecifics during a 2 h test session. Vehicle-treated chicks displayed high rates of vocalizations during the initial 5 min time block that declined over the next 20–25 min period to approximately 50% of the initial rate and remain stable throughout the remainder of the test session. Previous studies[19] have shown the first 5 min block to model the anxiety phase, whereby a diverse set of anxiolytic compounds reduce distress vocalizations, and that the last 90 min of the test session models the depressive phase of the model, whereby antidepressants increase distress vocalizations (i.e., block the onset of behavioral despair). In the present study, the 30 mg/kg dose of **1** possessed both anxiolytic and antidepressant properties by attenuating separation distress vocalizations in the anxiety phase and elevating separation distress vocalizations in the final 30 min of the depression phase of the model, respectively (Figure 2).

According to findings of Dukat et al., the two-atom chain that separates the indole from the terminal amine group is crucial to the binding of tryptamines to the $5HT_{1E}$ receptor, and any branching has been reported to reduce the affinity.[20] Research conducted by Glennon et al. revealed that replacing the primary amine moiety with a secondary or tertiary amine increases the lipophilicity of the molecule and makes it less prone to metabolism, hence improving drug-like properties.[21]

Interestingly, compound **2**, differing from **1** only by one bromine atom, did not exhibit antidepressant-like activity, but instead showed a significant sedative effect ($t = 3.55$; $p < 0.05$) (Figure 3b). Aplysinopsin (**3**) and ilimaquinone (**4**) did not show any significant antidepressant-like activity in the rodent swim test.

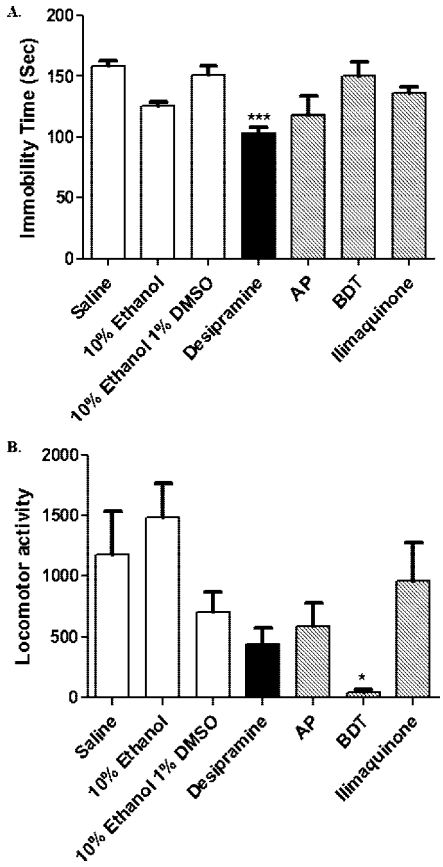

Figure 3. Effect of compounds **3** (AP), **2** (BDT), and **4** (ilimaquinone) in (A) forced swim test and (B) locomotor activity test in male Swiss Webster mice. *$p < 0.05$ and ***$p < 0.001$ versus corresponding vehicle.

In order to confirm that reduction of immobility in FST induced by the tested compounds is true and not a result of a nonspecific stimulant action, the effect on locomotor activity was determined, whereby a nonspecific stimulant action is reflected as a hyperlocomotive effect. Analysis of variance revealed an overall significant difference between the treatment groups ($F[6; 38] = 3.10$, $p < 0.05$). However, Bonferroni's multiple comparisons post hoc test revealed that there were no statistical differences between any of the tested compounds and their respective vehicle controls. Such results demonstrate that the observed antidepressant-like effect of 5,6-dibromo-*N,N*-dimethyltryptamine (**1**) is not associated with a stimulant action. In fact, **1** caused a nonsignificant trend toward decreasing locomotor activity, which would not account for its significant reduction of immobility time in the FST.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, MeOD, and DMSO-d_6 on an NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. The MS spectra were measured using a Bioapex FTESI-MS with electrospray ionization and on a Bruker microTOF instrument. TLC was carried out on precoated silica gel G_{254} or aluminum oxide ALOX-100 UV_{254} (500 μm) plates. HPLC was carried out on a Waters system with a Waters 2487 detector.

Animal Material. *S. aurea* was collected from the Florida Keys in August 2005. The sponges were collected from shallow coral reef habitat between 6 and 24 m depth at Key Largo, Florida, in July and August 2005. Voucher specimens have been deposited in the Natural History Museum, London (BMNH 2007.4.23.1 [University of Mississippi voucher 05FL-020(3)]; BMNH 2007.4.23.2 [University of Mississippi voucher 05FL-027]).

V. rigida was collected from shallow coral reef habitat between 3 and 21 m depth at Key Largo, Florida, in July and August 2005. Voucher specimens have been deposited in the Natural History Museum, London (BMNH 2007.4.23.3 [University of Mississippi voucher 05FL-020(2)]; BMNH 2007.4.23.4 [University of Mississippi voucher 05FL-089]).

S. cerebriformis was collected from shallow coral reef habitat between 3 and 21 m depth at Key Largo, Florida, on July 1 and August 7, 2005. Voucher specimens have been deposited in the Natural History Museum, London (BMNH 2007.4.23.5 [University of Mississippi voucher 05FL-020(1)]; BMNH 2007.4.23.6 [University of Mississippi voucher 0505FL-161]).

Extraction and Isolation. The sponge *S. aurea* was stored frozen until extracted. A sample of the sponge collected from Jamaica in November 2002 (35 g) was lyophilized, crushed, homogenized, and then extracted with ethanol at room temperature, yielding 1 g of extract. A second sample of sponge extract was obtained after grinding and exhaustive extraction with ethanol and yielded 21 g. TLC analysis indicated that the extracts contained various minor alkaloids. The extracts were subjected to silica gel vacuum liquid chromatography and eluted in order, with hexane (100%), hexane−acetone (9:1, 3:1, 1:1), acetone (100%), chloroform−methanol (1:1), and methanol (100%). Altogether seven major fractions were collected, and the elution of metabolites was monitored by TLC. Further workup (column chromatography on silica gel) of fraction 1 gave 80 mg (0.36% dry weight) of aureol (**8**); fraction 2 gave 45 mg (0.2% dry weight) of 5,6-dibromo-*N,N*-dimethyltryptamine. 2′-Des-*N*-methylaplysinopsin (**10**, 1.5 mg, 0.0068% dry weight), 6-bromoaplysinopsin (**6,** 1.2 mg, 0.0054%), and makaluvamine O (**9**, 1 mg, 0.0045% dry weight) were obtained from fractions 3 and 4. Purification of fraction 6 gave thymine (2 mg; 0.009% dry weight) and uracil (3.5 mg; 0.015% dry weight). The compounds were identified by comparison of their spectral data (^{1}H NMR, ^{13}C NMR, MS) with literature values.

Three kilograms of the frozen sponge *V. rigida* were extracted four times with 2000 mL of EtOH in a sonicator. The combined extracts were filtered and concentrated in vacuo until dried. The crude extract (211 g) was then subjected to vacuum-liquid chromatography using a gradient solvent system from hexanes through acetone to methanol, yielding 20 fractions. The acetone−methanol fraction (1:1) was further purified by flash column chromatography (C_{18} cartridge) with a water–methanol solvent system, yielding five fractions. Further purification of fraction 4 (H_2O−MeOH, 1:3) on a HPLC C_8 column (gradient from 100% H_2O to 100% MeOH) yielded 740 mg (0.35% dry weight) of 5,6-dibromo-*N,N*-dimethyltryptamine (**1**). The compound was isolated as a yellow, amorphous solid and could be purified by repeated recrystallization from methanol and identified on the basis of ^{1}H NMR, ^{13}C NMR, and HRMS spectra. Further workup of the residue of the same fraction by silica gel preparative thin-layer chromatography (chloroform−methanol, 8:2) and HPLC (C_8 columns, gradient from water to acetonitile) resulted in isolation of 0.1 mg (0.000047% dry weight) of makaluvamine O (**9**) and 0.3 mg (0.00014% dry weight) of arborescidine C (**5**), identified with high-resolution mass spectrometry and ^{1}H NMR analysis. Purification of fraction 3 on HPLC (C_8 column, water to acetonitrile solvent gradient system) yielded 3 mg (0.00142% dry weight) of 5-bromo-*N,N*-dimethyltryptamine (**2**). The presence of this compound was confirmed with ^{1}H NMR, ^{13}C NMR, and HRMS.

A fraction eluted with 100% MeOH from the VLC silica column after further purification on a C_{18} column yielded five fractions; further workup on the water and MeOH fraction yielded 32.5 mg (0.0154% dry weight) of aplysinopsin (**3**), identified by comparison of the spectral data (^{1}H NMR, ^{13}C NMR, HRMS) with literature values. Purification of the same fraction resulted in isolation of 1 mg (0.00047% dry weight) of 5,6-dibromoabrine (**7**) and 6-bromoaplysinopsin (**6**, 2.0 mg, 0.00094% dry weight). The fraction eluted with hexane−acetone (8:2) from VLC yielded small amounts of ilimaquinone (**4**, 5 mg, 0.00236% dry weight) and 2 mg (0.00094% dry weight) of aureol (**8**). The presence of these compounds was confirmed by TLC, MS, and NMR analysis, comparing with standards.

Six kilograms (wet weight) of the frozen sponge *S. cerebriformis* was extracted exhaustively with EtOH in a sonicator. The combined extracts were filtered and concentrated in vacuo until dried. The crude extract (260 g) was then subjected to vacuum-liquid chromatography using a gradient solvent system from hexanes through acetone to methanol, yielding 20 fractions. Nonpolar fractions after purification yielded 2.5 g (0.9615% dry weight) of ilimaquinone (**4**), which was identified by comparison of ^{1}H NMR and ^{13}C NMR data with a standard. Fractions eluted with methanol showed a characteristic

pattern of a dibrominated compound, and the HRMS comparison with a standard revealed the presence of 5,6-dibromo-*N,N*-dimethyltryptamine (**1**).

Locomotor Activity and the Forced Swim Test. To evaluate the isolated compounds for antidepressant-like activity, male Swiss Webster mice (Harlan, Indianapolis, IN) (25–30 g weight) were used. Animals were housed in groups of five with a 12 h light/12 h dark cycle. Food and water were provided ad libitum. All procedures involving animals were performed as approved by the Institutional Animal Care and Use Committee of The University of Mississippi. Animals were randomly divided into groups ($n = 6-10$/group). Each group was injected ip with either the compound (1–20 mg/kg), desipramine (20 mg/kg), or vehicle (saline, 10% ethanol, or 10% ethanol/1% DMSO). Following injection, locomotor activity was monitored using an automated activity monitoring system (San Diego Instruments, San Diego, CA). Each mouse was placed in a Plexiglas enclosure, and locomotor activity was recorded as the number of photobeam interruptions for 30 min after drug injection. The activity for the last 10 min was quantified and analyzed. Immediately at the end of the locomotor session, individual mice were subjected to the forced swim test. The mice were individually placed in a clear plastic cylinder (23 cm high, 10 cm internal diameter) filled with deionized water (8 cm high) at 25 °C. The mice were recorded with a video camera (positioned at about 30 cm above the cylinder) for a total of 6 min. The total period of immobility during the last 4 min was timed by three independent observers. The mean immobility time was then calculated. A mouse was judged to be immobile when it remained afloat, making only minimal movements to keep its head above water.[22]

Chick Anxiety−Depression Continuum Test. Group-housed white-leghorn cockerels (Cal-Maine W36) were tested at ages 5–6 days posthatch. Chicks were placed in isolation into a six-unit sound-attenuating apparatus containing video cameras and microphones 15 min after receiving ip injections of vehicle or 10, 20, or 30 mg/kg 5,6-dibromo-*N,N*-dimethyltryptamine. Vocalizations were recorded in 5 min blocks over a 120 min test period. The anxiety phase of the model is characterized by high rates of distress vocalizations during the first 5 min of the test period. The depression phase of the model is characterized by a reduced (approximately 50% of the initial rate) and stable rate of distress vocalizations during the 30–120 min period of the test session. All animal procedures were performed by the guidelines approved by the Institutional Animal Care and Use Committee.

Statistical Analysis. For the forced swim test, immobility times of the three independent raters were averaged for each mouse, and data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison post hoc tests to determine statistical differences from the corresponding vehicle control. Chick distress vocalization data were analyzed by two-way repeated measures ANOVA, one-way ANOVA, and simple effects analyses with post hoc comparisons conducted using Fisher's LSD test. *p*-Values less than 0.05 were considered statistically significant.

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Supporting Information Available: Table showing key field and histological characteristics of the three sponge species. This information is available free of charge via the Internet at http://pubs.acs.org.

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