New Antifeedant Bromopyrrole Alkaloid from the Caribbean Sponge *Stylissa caribica*

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In this first report on the chemistry of the sponge *Stylissa caribica*, two known bromopyrrole metabolites and a new compound, *N*-methyldibromoisophakellin (**1**), were isolated and identified. The structure of **1** was determined using spectroscopic methods and the computer program COCON. *N*-Methyldibromoisophakellin (**1**) was shown to be the only secondary metabolite in *Stylissa caribica* that, at its natural concentration, is active as a feeding deterrent against a common omnivorous reef fish.

In a recent survey of the chemical antipredatory defenses of 71 species of Caribbean sponges, it was discovered that all of the five species within the family Axinellidae yielded crude organic extracts that deterred the feeding of predatory reef fish in aquarium assays.[1] More recently it has been shown that one species, *Axinella corrugata* (previously *Teichaxinella morchella*), yielded high concentrations of stevensine as the single compound responsible for feeding deterrency.[2] The purpose of the study reported herein was to isolate and identify the metabolite(s) responsible for the chemical defense of *Stylissa caribica*, a sponge closely related to *A. corrugata*. To the best of our knowledge, this is the first report on the chemistry of *S. caribica*.

A series of brominated pyrrole alkaloids have been isolated from a specimen of the sponge *S. caribica* collected off the coast of Sweetings Cay, Bahamas. Bioassay-guided fractionation of the methanol extract of this sponge resulted in the isolation of two known bromopyrrole-derived alkaloids, dibromoisophakellin (**2**, 13 mg) and ageliferin (**3**, 3 mg), as well as the new compound *N*-methyldibromoisophakellin (**1**, 140 mg). We describe herein the isolation, structural elucidation, and antifeedant properties against the common predatory reef fish *Thalassoma bifasciatum* of the new bromopyrrole alkaloid **1**.

The compounds **1**−**3** could be isolated using previously reported methods (details see Experimental Section and Supporting Information). The brominated alkaloids dibromoisophakellin (**2**) and ageliferin (**3**) were identified by comparison of their spectroscopic data with those previously reported.[3] The FAB mass spectrum (positive ion mode) of the new compound *N*-methyldibromoisophakellin (**1**) showed an isotopic cluster at *m*/*z* 402, 404, and 406 [M + H]$^+$ in the ratio 1:2:1, suggesting the presence of two bromine atoms. The molecular formula of **1** was established as $C_{12}H_{14}Br_2N_5O$ by HRFABMS (*m*/*z* 405.9526, [M + H]$^+$, Δ +0.2 mmu), which is in accordance with the ^{1}H and ^{13}C NMR data (summarized in Table 1). By comparison of δ_C of **1** with all known intramolecular cyclized oroidin derivatives, the isophakellin skeleton was obtained.[4] Therefore, the structure of **1** was identified and confirmed by COCON[5] as the *N*-methyl derivative of dibromoisophakellin (**2**). The absolute configuration of **1** was determined by comparison of the optical rotation of **1** with that of **2**.[3a]

Scheme 1

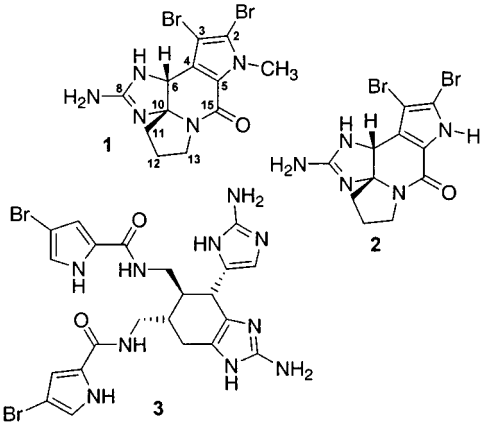

Table 1. ^{1}H and ^{13}C NMR Spectral Data of **1** in DMSO-d_6

position	δ(^{13}C)[a]	δ(^{1}H)[b]	COSY[c]	HMBC[d]
N-CH_3	35.0	3.92 (3H)		2,5
C-2	114.0			
C-3	95.9			
C-4	123.3			
C-5	121.1			
C-6	53.8	5.23 (1H)		3, 4, 5, 8, 10, 11
N-7[e]		9.01 (1H)		6, 8, 10
C-8	157.1			
N-9[e]		10.08 (1H)		6, 8, 10
C-10	83.4			
C-11	39.3	2.23 (2H)	12	10, 12
C-12	19.1	2.02 (2H)	11, 13	10, 11
C-13	44.4	3.57/3.47 (2H)	12	10, 12, 15
C-15	155.0			
N-16		8.17 (2H)		

[a] ^{13}C chemical shifts are given in ppm and are referenced to the DMSO-d_6 signal (39.5 ppm). [b] ^{1}H chemical shifts are given in ppm and are referenced to the DMSO-d_6 signal (2.50 ppm). The integration of the proton signals is given in parentheses. [c] The COSY correlations are given for both sides of the diagonal. [d] The HMBC correlations are given from protons to carbons. [e] The assignment may be interchanged.

Since recent studies[6] have found no evidence for structural or nutritional defenses, deterrent metabolites appear to be the principal defensive strategy of Caribbean sponges against predatory reef fishes. To investigate the feeding deterrency of the major metabolite of *S. caribica*, aquarium assays were performed using previously reported methods.[7] When incorporated into artificial foods at the same volumetric concentration as found in sponge tissue (0.9 mg/mL), *N*-methyldibromoisophakellin (**1**) deterred

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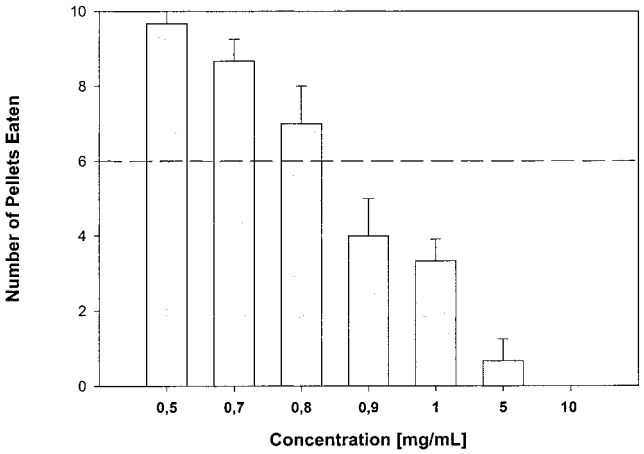

Figure 1. Aquarium assay results of feeding by *Thalassoma bifasciatum* on pellets treated with *N*-methyldibromoisophakellin (**1**). All control pellets were eaten in all assays. Three replicate assays have been performed at each concentration. 1 SD above the mean number of food pellets eaten is indicated. For any individual assay, a treatment was considered deterrent if the number of pellets eaten was less than or equal to 6 ($p < 0.043$ Fisher exact test, one-tailed), as indicated by the dotted line.[11]

feeding of the Caribbean reef fish *Thalassoma bifasciatum* in laboratory aquarium assays (Figure 1). Compound **1** is the only component in the sponge tissue of *S. caribica* at sufficient concentration likely to be responsible for the chemical defense: **2** (0.084 mg/mL) and **3** (0.02 mg/mL) were found only in low sponge tissue concentrations. Comparing the relative feeding deterrent activity with the major naturally occurring metabolites of *Agelas* sponges (1 mg/mL concentrations), a hierarchy of activity can be determined (expressed as mol/mL): sceptrin > *N*-methyldibromoisophakellin > oroidin > 4,5-dibromopyrrole-2-carboxylic acid.[7b] *N*-Methyldibromoisophakellin (**1**) represents another in the oroidin class of brominated pyrrole derivatives that function as chemical defenses of sponges in the families Axinellidae and Agelasidae.

Experimental Section

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AM 250, AMX 400, and DRX 600 spectrometers. A 50 mg sample of **1** in 0.5 mL of DMSO-d_6 was used for the NMR measurements. All NMR experiments were measured at 300 K. The DQF-^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, and ^{1}H,^{13}C-HMBC experiments were carried out with standard parameters. Mass spectral analysis (HRFABMS) was performed on a JEOL JMS-700 sector-field mass spectrometer with 3-nitrobenzyl alcohol (NBA) as matrix or using a Fison VG Platform II for ESIMS. IR (KBr) spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer. UV/vis spectra were obtained using a Perkin-Elmer UV/vis spectrometer Lambda 16. The CD spectra were obtained using the JASCO spectropolarimeter J-710.

Animal Material. The marine sponge *Stylissa caribica* investigated in this study was collected in September 1998 by scuba diving (15 m depth) at Sweetings Cay in the Bahamas. The sponge forms erect wedged-shaped, thick-bladed columns with irregularly corrugated lengthwise grooves and ridges, subdivided in places to form honeycomb-like depressions. The size of the specimen was 8 × 18 cm with orange-brown or dark reddish orange color in life, turning rather dark red-brown in alcohol. The surface in the depressions is shiny smooth, looking fleshy. The skeleton is composed of an irregular plumoreticulate arrangement of spongin-enforced spicule tracts, predominantly oriented longitudinally, with irregular interconnecting tracts. Peripherally, spicules are detached and in confusion. The ectosome is a thick organic layer which comes off easily as flakes and is devoid of spicules. The spicules are styles with occasional strongylote modifications of rather uniform dimensions, 240−350 × 6−10 μm. The specimen was compared with the type species of *Stylissa caribica* (Lehnert & van Soest, 1998)[8] from Jamaica and was found to match closely. There is a superficial resemblance with *Axinella corrugata* (George & Wilson, 1919), but in that species the surface is not fleshy-flaky; there is more definitely an axial and extra-axial arrangement of the skeleton, and the styles are significantly longer.[9] In addition, in contrast to *S. caribica*, the spicule complement includes oxeas. A voucher fragment of *S. caribica* has been deposited in the collections of the Zoological Museum of Amsterdam under reg. no. ZMA POR. 15607.

Extraction and Isolation. The sample of *S. caribica* was immediately frozen after collection and kept at −20 °C until extraction. For bulk extraction followed by isolation of brominated secondary compounds, frozen sponge tissue (155 mL) of *S. caribica* was chopped into small pieces and extracted at room temperature exhaustively in MeOH. The resulting *n*-BuOH (2.8 g) phase from the solvent partitioning scheme was purified by gel permeation chromatography on LH-20 Sephadex (Pharmacia) using MeOH as mobile phase. Final purification of the isolated compounds was achieved by preparative RP_{18} HPLC to afford compounds **1** (140 mg), **2** (13 mg), and **3** (3 mg). HPLC analysis was carried according to ref 10.

***N*-Methyldibromoisophakellin (1):** light yellow powder; UV (H_2O) λ_{max} (log ϵ) 288 (3.92) nm; CD (MeOH) λ ($\Delta\epsilon$) 207 (−1.56) nm; IR (KBr) ν_{max} 3383, 1697, 1661, 1560, 1425, 1347, 1202, 1134, 801, 722 cm^{-1}; ^{1}H and ^{13}C NMR data are shown in Table 1; HRFABMS *m/z* 405.9526 $[M + H]^+$, calcd for $C_{12}H_{14}{}^{81}Br_2N_5O$, 405.9524.

Dibromoisophakellin (2): light yellow powder; CD (MeOH) λ ($\Delta\epsilon$) 211 (−0.68) nm; ^{1}H and ^{13}C NMR data same as reported in ref 3a; HRFABMS *m/z* 387.9399 $[M + H]^+$, calcd for $C_{11}H_{12}{}^{79}Br_2N_5O$, 387.9408.

Ageliferin (3): light yellow powder; CD (MeOH) λ ($\Delta\epsilon$) 232 (−0.25) nm; ^{1}H and ^{13}C NMR data same as reported in refs 3b and 3c; ESIMS (neg) *m/z* 617 (52), 619 (100), 621 (52).

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Supporting Information Available: The contents of Supporting Information include the following topics: (1) more detailed comments on the structure elucidation and description of the COCON calculations (including a figure of structural proposals generated by COCON); (2) description of the feedings assays; (3) a more detailed Experimental Section.

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