

Sventrin, a New Bromopyrrole Alkaloid from the Caribbean Sponge *Agelas sventres*

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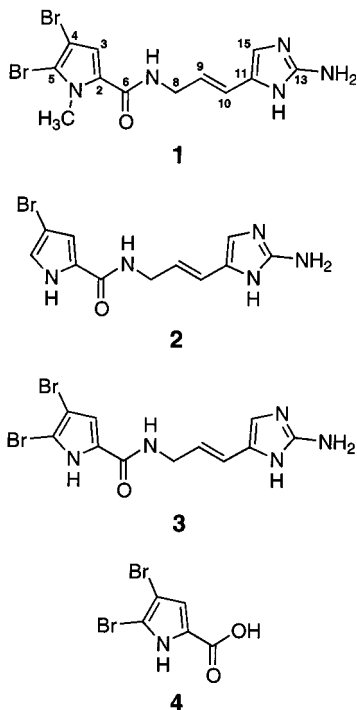
A detailed analysis of the secondary metabolites of a specimen of *Agelas sventres* was carried out here for the first time. The chemistry of *Agelas* sponges is dominated by bromopyrrole derivatives. Besides three known bromopyrrole metabolites, a new compound, sventrin (**1**), was isolated and its structure identified using spectroscopic methods. Sventrin (**1**) was shown to be a feeding deterrent compound against a common omnivorous reef fish.

A series of brominated pyrrole alkaloids have been isolated from a specimen of the sponge *Agelas sventres* collected off the coast of North Cat Cay, Bimini, Bahamas. *A. sventres* was first described by Lehnert and van Soest in 1996 and before known as *Agelas* sp. 3 for many years, but the chemistry was unknown.¹ This is the first report on the chemistry of this *Agelas* species from the Bahamas. The dichloromethane/methanol extract of this sponge (see Figure 1) resulted in the isolation of the known alkaloids hymenidin (**2**, 417 mg), oroidin (**3**, 328 mg), 4,5-dibromopyrrole-2-carboxylic acid (**4**, 35 mg), and a new bromopyrrole-derived alkaloid, sventrin (**1**, 75 mg). In this note, we describe the isolation and structural elucidation of this novel bromopyrrole alkaloid **1** and the feeding deterrent properties of **1** and **2** (data of **3** and **4** are already known) against the omnivorous reef fish *Thalassoma bifasciatum*.

The compounds **1**–**4** were isolated using previously reported methods.² The brominated alkaloids hymenidin (**2**), oroidin (**3**), and 4,5-dibromopyrrole-2-carboxylic acid (**4**) were identified by comparison of their spectroscopic data with those reported in the literature.³ The ESI mass spectrum (negative ion mode) of sventrin (**1**) showed an isotopic cluster at m/z 400, 402, and 404 $[M + H]^+$ 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 401.9565, $[M + H]^+$, $\Delta = 0.1$ mmu), which is in accordance with the ¹H and ¹³C NMR data. The NMR spectra of **1** are very similar to those of oroidin (**3**). The only differences are that **1** has one less NH signal and an additional methyl signal at 3.89 ppm. The signal at 3.89 ppm was assigned as an *N*-methyl group. The absence of the pyrrole NH and the presence of HMBC correlations from the *N*-methyl group to C-2 and C-5 established the structure of **1** as *N*(pyrrole)-methyl oroidin (= sventrin). 1D and 2D NMR data of **1** are summarized in the Experimental Section.

Deterrent metabolites appear to be the principal defensive strategy of Caribbean sponges against predatory reef fishes, because in a recent study no evidence was found for structural or nutritional defenses.⁴ Brominated pyrrole derivatives are known to function as feeding deterrents of sponges in the families Axinellidae and Agelasidae.⁵ To investigate the ecological role of the major metabolites of *A. sventres*, aquarium assays for the feeding deterrentcy were carried out against a common omnivorous reef fish using previously described methods.^{5c,6}

Hymenidin (**2**) was first isolated in 1986,^{3c} but no reports of testing in the feeding deterrent assay against *Thalassoma bifasciatum* have yet appeared. Oroidin (**3**) and 4,5-dibromopyrrole-2-carboxylic acid (**4**) have been tested several times in this assay^{5a,c,7} and were therefore not tested again in this investigation. The minimum concentrations for a feeding deterrent activity of these two compounds are 0.7 mg/mL for **3** and 0.8 mg/mL for **4**. Sventrin (**1**) and hymenidin (**2**) were tested at concentrations of 1, 3, 5, and 10 mg/mL in the aquarium assay (for details see Experimental Section and Supporting Information). Hymenidin (**2**) is not active at 1 mg/mL (active at 3 mg/mL) and shows therefore a smaller feeding deterrentcy than oroidin (**3**). This was expected since it was shown for pyrrole-2-carboxylic acid derivatives that bromination is important for the feeding deterrentcy.^{5c} Also, *N*-methylation of the pyrrole nitrogen as in sventrin reduces the feeding



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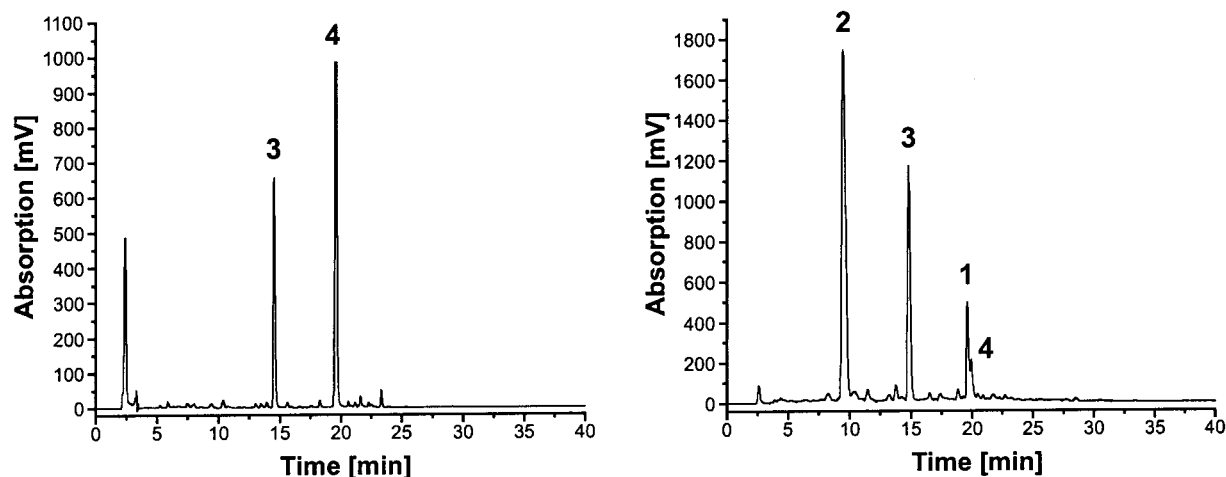


Figure 1. HPLC profiles of the *n*-butanol phase of *Agelas clathrodes* (left) and *Agelas sventres* (right). HPLC conditions, column: Kromasil RP18, 4.6 × 250 mm, 5 μm; gradient: 20–60% MeCN/H₂O + 0.1% TFA in 40 min; flow rate: 1 mL/min, UV detection at 280 nm. The retention times for *A. sventres* are as follows: hymenidin (**2**) t_R = 9.49 min, oroidin (**3**) t_R = 14.81 min, sventrin (**1**) t_R = 19.61 min, and 4,5-dibromopyrrole-2-carboxylic acid (**4**) t_R = 19.96 min.

deterrent activity (for details see Experimental Section). These results and previously obtained results showed that all four compounds deterred feeding of the Caribbean reef fish *T. bifasciatum* in aquarium assays. However, oroidin (**3**) is the only component in the tissue of *A. sventres* at sufficient concentration (0.9 mg/mL, the minimum concentration required for the feeding deterency is 0.7 mg/mL) to be most likely responsible for the feeding deterency. The other metabolites **1**, **2**, and **4** were found only in lower tissue concentrations (0.2, 1.1, and 0.1 mg/mL, respectively). The feeding deterency is probably effected by all four compounds; however, the mixture has not yet been tested.

The HPLC profile of the crude extract of *Agelas sventres* differs from that of *A. cervicornis*, *A. clathrodes*, *A. dispar*, and *A. wiedenmayeri*, where the major metabolites are only the “monomeric” building blocks **3** and **4** (see Figure 1). In none of these *Agelas* species was either sventrin (**1**) or hymenidin (**2**) isolated as a major metabolite. This is further evidence for the distinctiveness of *A. sventres* from *A. clathrodes*, which traditionally included the former within its variation (see Experimental Section and Supporting Information).

Experimental Section

General Procedures. ¹H NMR and ¹³C NMR spectra were recorded on Bruker AM250, AMX400, and DRX600 NMR spectrometers. All NMR experiments were measured at 300 K. The DQF-¹H, ¹H-COSY, ¹H, ¹³C-HSQC, ¹H, ¹³C-HMBC, ¹H, ¹⁵N-HSQC, and ¹H, ¹⁵N-HMBC experiments were carried out using standard parameters. Mass spectral analysis (HR-FABMS) was performed on a JEOL JMS-700 sector-field mass spectrometer with 3-nitrobenzyl alcohol as matrix or using a Fison VG Platform II for ESIMS. HPLC analysis was carried out as previously reported.² 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.

Animal Material. The marine sponge *Agelas sventres* (Lehnert and van Soest, 1996) investigated in this study was collected in August 2000 by scuba diving (18 m depth) at North Cat Cay, near Bimini, Bahamas. Voucher specimens are deposited at the Porifera collections of the Zoological Museum Amsterdam, The Netherlands (ZMA POR. 16400), and at the Instituto de Investigaciones Marinas y Costeras, INVEMAR, Santa Marta, Colombia (INV-POR 0584). A detailed taxonomic description is given in the Supporting Information. The studied

material from the Bahamas was kindly compared to the holotype from Jamaica (ZMA POR. 11322) by Dr. Rob W. M. van Soest (Zoological Museum Amsterdam), and the identity confirmed. The species in the Bahamas was originally described within the variation of *A. clathrodes* (Schmidt, 1870),⁸ but it was later discovered that it was a different, undescribed species.⁹ It was dubbed *Agelas* sp. 3 for many years by Caribbean spongologists until its formal naming and description.¹ However, its conspecificity with the original *A. schmidti* (Wilson, 1902) (non *A. schmidti* sensu Wiedenmayer, 1977⁸ and Zea, 1987,⁹ which is *A. wiedenmayeri* Alcolado, 1984¹⁰), remains to be determined.

Extraction and Isolation. The sample of *Agelas sventres* was immediately frozen after collection and kept at –20 °C until extraction. The freeze-dried sponge sample of *A. sventres* (93.5 g) was chopped into small pieces and extracted at room temperature exhaustively in a 1:1 mixture of CH₂Cl₂/MeOH. The orange-colored crude extract of *A. sventres* was partitioned between *n*-hexane (4 × 300 mL) and MeOH (150 mL). The MeOH extract was then partitioned between *n*-BuOH (5 × 300 mL) and H₂O (300 mL). The resulting *n*-BuOH (4.32 g) phase from the solvent partitioning scheme was purified by gel permeation chromatography on Sephadex LH-20 (Pharmacia) using MeOH as mobile phase. Final purification of the isolated compounds was achieved by preparative RP₁₈ HPLC (for details, see Supporting Information) to afford **1** (75 mg, 0.08% of dry weight), **2** (417 mg, 0.45% of dry weight), **3** (328 mg, 0.35% of dry weight), and **4** (35 mg, 0.04% of dry weight).

Sventrin (1): light yellow powder; UV (MeOH) λ_{max} (log ϵ) 274 (4.27) nm; IR (KBr) ν_{max} 3334, 2360, 1696, 1541, 1506, 1417, 1332, 1264, 1202, 1138, 956, 800, 722 cm⁻¹; ¹H NMR (DMSO-*d*₆, 250 MHz) δ 3.89 s, NCH₃; 3.93 t, J = 5.5, 5.7 Hz, H₂-8; 6.09, dt, J = 5.5, 16.1 Hz, H-9; 6.23, d, J = 16.1 Hz, H-10; 6.90, s, H-15; 7.06, s, H-3; 7.71, br, NH₂-16; 8.50, t, J = 5.7 Hz, NH-7; 12.19, br, NH-14; 12.86, br, NH-12; ¹³C NMR (DMSO-*d*₆, 62.9 MHz) δ 35.4, NCH₃; 39.9, C-8; 97.0, C-4; 110.7, C-5; 110.8, C-15; 114.1, C-3; 116.4, C-10; 124.9, C-11; 126.3, C-9; 127.6, C-2; 147.9, C-13; 159.6, C-6; ¹⁵N NMR (DMSO-*d*₆, 60.8 MHz) δ 59, N-16; 108, N-7; 132, N-12; 135, N-14; 156, N-1 (the chemical shifts were calibrated according to the Bruker frequency, which is set to 0 ppm for NH₃, the accuracy is about 1 to 2 ppm); COSY correlations H-7/H-8, H-8/H-9, H-9/H-10; ¹H, ¹³C-HMBC correlations H-1/C-2, H-1/C-5, H-3/C-2, H-3/C-5, H-3/C-6, H-7/C-6, H-7/C-8, H-8/C-6, H-8/C-9, H-8/C-10, H-9/C-8, H-9/C-11, H-10/C-8, H-10/C-9, H-10/C-11, H-10/C-15, H-15/C-10, H-15/C-11, H-15/C-13; ¹H, ¹⁵N-HMBC correlations H-3/N-1, H-8/N-7, H-9/N-7, H-10/N-12, H-15/N-12, H-15/N-14, H-16/N-12, H-16/N-14; HRFABMS m/z 401.9564 [M + H]⁺ (calcd for C₁₂H₁₄⁷⁹BrN₅O, 401.9565).

Hymenidin (2): light yellow powder; UV (MeOH) λ_{\max} (log ϵ) 268 (4.25) nm; ^1H NMR and ^{13}C NMR data same as reported in ref 3c; HRFABMS m/z 310.0315 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{13}\text{-}^{79}\text{BrN}_5\text{O}$, 310.0304.

Oroidin (3): light yellow powder; UV (MeOH) λ_{\max} (log ϵ) 274 (4.35) nm; ^1H NMR and ^{13}C NMR data same as reported in refs 3a,b,d; HRFABMS m/z 387.9404 $[\text{M} + \text{H}]^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{-}^{79}\text{Br}_2\text{N}_5\text{O}$, 387.9408.

4,5-Dibromopyrrole-2-carboxylic acid (4): light yellow powder; UV (MeOH) λ_{\max} (log ϵ) 271 (4.05) nm; ^1H NMR and ^{13}C NMR data same as reported in refs 3a,b; ESIMS (neg) m/z 266 (53), 268 (100), 270 (51).

Feeding Assays. A description of the feeding assay is given in the Supporting Information. Compounds **1** and **2** were tested at concentrations of 1, 3, 5, and 10 mg/mL. Three replicate assays were conducted at each concentration. 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).¹¹ The averaged values for the number of pellets eaten were (standard deviations in parentheses) 6.7 (0.6), 4.3 (0.6), 2.3 (0.6), and 0.3 (0.6) for **1** and 8.7 (0.6), 5.3 (0.6), 3.3 (0.6), and 0.7 (0.6) for **2**. Compounds **3** and **4** were not tested again in this investigation because we have carried out this before.^{5c}

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Supporting Information Available: A more detailed Experimental Section, including a detailed description of the Animal Material as well as some more information about the General Procedures and the Feeding Assays, is available free of charge via the Internet at <http://pubs.acs.org>.

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Supporting Information

Experimental Section

General Procedure. For extraction solvents were distilled prior to use, and gradient grade solvents were used for chromatographic applications. Fractions containing brominated metabolites were collected and monitored by TLC on pre-coated silica gel 60 F₂₅₄ plates (Merck) using a mixture of CHCl₃/MeOH/NH_{3aq} (40:20:1) as solvent system and detected by their UV absorbance at 254 or 366 nm. Thin-layer chromatography (TLC) was performed on pre-coated Si gel plates (Merck).

For HPLC analysis, samples were injected into a JASCO HPLC system equipped with a MD-910 photodiode-array detector. Routine detection was at 280 nm. HPLC separation columns (analytical: 4.6 × 250 mm, 5 μm; preparative: 16 × 250 mm, 7 μm) were pre-filled with Kromasil RP₁₈ (Knauer GmbH). The following gradient was used for preparative HPLC analysis: 5 min A, 35 min 35% B; A: 10% MeCN/H₂O + 0.1% TFA, B: MeCN + 0.1% TFA. Analytical HPLC was achieved by applying a linear gradient from 20-60% MeCN/H₂O + 0.1% TFA in 40 min. Fractions containing purified bromopyrrole alkaloids were combined on the basis of analytical HPLC.

The ¹H, ¹³C-HMBC experiment was acquired with 4096 data points in F₂, 256 increments and 4 acquisitions. The delay for evolution of the heteronuclear long range couplings was set to 80 ms and the relaxation delay to 2.0 s. The ¹H, ¹⁵N-HMBC experiment was acquired with 8192 data points in F₂, 64 increments and 16 acquisitions. The delay for evolution of the heteronuclear long range couplings was set to 120 ms and the relaxation delay to 2.0 s. The pulse programs were used from the Bruker library.

Animal Material. The specimen consists of a 15-22 cm in diameter cluster of bright orange, repent to erect, finger-shaped branches, each up to 2 cm in diameter, arising from a partly buried mass attached irregularly to corals and coral rubble; only the top few cm of the branches are seen by the diver. The surface is even, riddled with rounded to elongated holes, most of them roofed by a parchment-like membrane, and a few fully open, all 1-7 mm in diameter. On top of branches, the surface is a little rough, and there may be 1-2 roundish to elongated-contorted holes. The brighter color occurs near the top of branches; the non-exposed areas are cream; the interior tissue is orange; upon fixation in ethanol, it exudes an orange, opaque ink. The consistency is easily compressible, elastic, difficult to tear. The interior is extremely cavernous, with numerous roundish, interwoven tunnels, 3-6 mm in diameter, leaving relatively thin, 0.5-6 mm, walls of solid tissue. The skeleton consists of

primary fibers ascending sinuously and branching irregularly, 28-120 μm in diameter, separated 70-400 μm , cored with 1 to 5 spicules in an irregularly plumose fashion; their sinuous ascent make them look short in thick sections under the microscope; they have echinating spicules here and there. Ascending fibers are interconnected by relatively long, uncored secondary fibers, 24-57 μm in diameter, which run more or less parallel to each other and to the surface. In between ascending primary and interconnecting secondary fibers, there is an irregular tri-dimensional network of fibers, forming rounded meshes 60-190 μm in diameter. The dermis is supported by spicule brushes of the ascending primary fibers and by the tangentially placed, interconnecting secondary fibers, whose echinating spicules tend to pierce it. Spicules are acanthostyles, slender, relatively thin, showing a wide axial canal, with 9-18 whorls of 1-4 spines each; spines are slightly curved towards the head of the style and, although they are short ($<2.9 \mu\text{m}$), they look high due to the thinness of the spicules. When compared with those of other species, acanthostyles of *A. sventres* usually are distinguished by having the head with a few spines, and several whorls with just 1 or 2 spines; although the distance between whorls is usually regular, sometimes there is a single spine in between whorls that obscure the pattern. There are many developmental stages of spicules without spines, showing just an engrossed ring where the whorls of spines will appear. Dimensions (length by width, min-mean-max, n=25 spicules): 30-115.5-190 μm by 2.3-4.6-6.2 μm .

Feeding Assays. Purified compounds were dissolved in a minimal volume of MeOH and mixed with 1 mL of alginate-based food matrix¹ until all organic and water-soluble components were distributed uniformly throughout the paste. The alginate food matrix was then dispensed with a 1 mL syringe into a 0.25 M calcium chloride solution forming a strand that was allowed to harden for 2 min. The hardened strand was rinsed with filtered seawater and cut into 3 mm pellets with a scalpel. Control pellets were prepared identically but without the addition of natural compounds. Feeding assays were performed with fish (1 terminal phase and 2 females in each of 10 compartments) in aquaria onboard the *R/V Seward Johnson* using a common omnivorous reef fish, the bluehead wrasse *Thalassoma bifasciatum*. Rationale for the choice of this assay fish and an explanation of the methods for scoring the assay and the statistical analysis is well described in ref. 2. Each replicate assay was performed on a separate group of fish.

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