Monobromoisophakellin, a New Bromopyrrole Alkaloid from the Caribbean Sponge *Agelas* sp.

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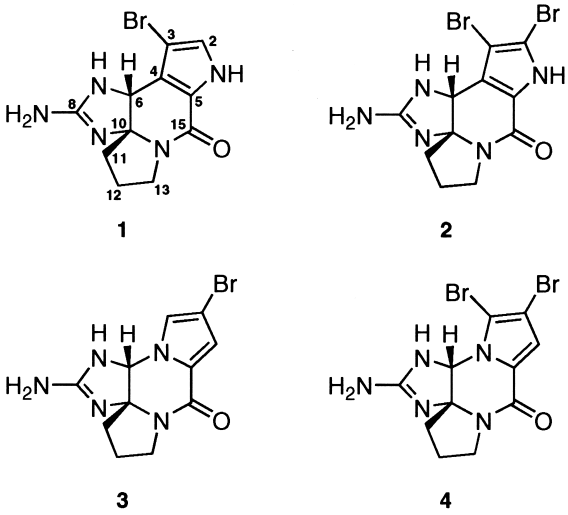
A detailed analysis of the chemical constituents of a Caribbean specimen of *Agelas* sp. was carried out. Four brominated compounds (**1**–**4**) were isolated and one of them was identified as a new bromopyrrole metabolite, monobromoisophakellin (**1**). The structure of **1** was determined using spectroscopic methods. All compounds were tested for their antifeedant activity against the Caribbean reef fish *Thalassoma bifasciatum* in an aquarium assay.

Introduction

Bromopyrrole alkaloids are well known in marine sponges of the genus *Agelas* (Braekman *et al.*, 1992). In our search for bioactive substances from marine organisms, a series of brominated pyrrole alkaloids have been isolated from a specimen of the Caribbean sponge *Agelas* sp. collected off the coast of Sweetings Cay (Bahamas). Examination of the dichloromethane/methanol extract of this sponge resulted in isolation of the known alkaloids dibromoisophakellin (**2**), which has been previously isolated from *Acanthella carteri* (Fedoreyev *et al.*, 1986), monobromophakellin (**3**), and dibromophakellin (**4**), both previously described from *Phakellia flabellata* (Sharma and Burkholder, 1971; Sharma and Magdoff-Fairchild, 1977) as well as of the new bromopyrrole alkaloid, monobromoisophakellin (**1**). In this communication we describe the isolation and structural elucidation of the new bromopyrrole alkaloid (**1**).



Scheme 1

Materials and Methods

The marine sponge *Agelas* sp. employed in this study was collected in September 1998 by SCUBA diving (15 m depth) off the coast of Sweetings Cay (Grand Bahama Island) during a scientific cruise of the *R/V Edwin Link* to the Bahamas. The specimen is an undescribed species of *Agelas* (order Agelasida, family Agelasidae), the colour in life is reddish-orange, consistency is tough, spongy, firm and almost incompressible. A voucher fragment has been deposited under registration no. ZMA POR. 13369 in the Zoölogisch Museum, Amsterdam, The Netherlands.

Samples of *Agelas* sp. were immediately frozen after collection and kept at –20 °C until extraction. For bulk extraction followed by isolation of brominated secondary metabolites, lyophilized sponge tissue (102 g) was ground and extracted exhaustively in a 1:1-mixture of dichloromethane/MeOH at room temperature. The orange-colored wet crude extract was partitioned between *n*-hex-

ane (3 × 500 ml) and MeOH (150 ml). The obtained MeOH extract was finally partitioned between *n*-BuOH (3 × 500 ml) and H_2O (300 ml). The resulting *n*-BuOH phase (5.9 g) 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 (details see figure caption 1) to afford **1** (86 mg, 0.08% of dry weight), **2** (28 mg, 0.03%), **3** (35 mg, 0.03%) and **4** (148 mg, 0.15%).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX600 NMR spectrometer. All NMR experiments were measured at 300 K. The 2D experiments (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, ^{1}H,^{15}N-HSQC and ^{1}H,^{15}N-HMBC) were carried out using 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. HPLC analysis was carried out as previously reported (Assmann *et al.*, 1999; Assmann *et al.*, 2000). 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.

Bromopyrrole alkaloids are known to be the principal defensive strategy of Caribbean sponges against predatory reef fishes (Pawlik *et al.*, 1995; Chanas *et al.*, 1996; Wilson *et al.*, 1999; Assmann *et al.*, 2000; Assmann *et al.*, 2001). To investigate the antifeedant activity of the four metabolites, aquarium assays were performed using previously described methods (Pawlik *et al.*, 1987; Pawlik *et al.*, 1995; Chanas *et al.*, 1996; Assmann *et al.*, 2000).

Results and Discussion

The compounds **1**–**4** could be isolated from the *n*-BuOH phase of *Agelas* species. The brominated alkaloids dibromoisophakellin (**2**), monobromophakellin (**3**), and dibromophakellin (**4**) were identified by comparison of their spectroscopic data with those previously reported (Sharma and Burkholder, 1971 → **4**; Sharma and Magdoff-Fairchild, 1977 → **3** + **4**; De Nanteuil *et al.*, 1985 → **4**; Fedoreyev *et al.*, 1986 → **2**; Jiménez and Crews, 1994 → **4**). The ESI mass spectrum (negative ion mode) of the new compound monobromoisophakellin (**1**) showed prominent pseudomolecular ion peaks at *m*/*z* 308 and 310 in the ratio 1:1, suggesting the presence of one bromine atom. The molecular formula of **1** was established as $C_{11}H_{13}BrN_4O$ by HRFABMS (*m*/*z* 310.0290, $[M + H]^+$, Δ −1.3 mmu), which is in accordance with the ^{1}H and ^{13}C NMR data (see Table I). The presence of a pyrrole ring conjugated with a carbonyl group part was supported by the UV absorption (MeOH) at λ_{max} 276 nm (lg ε 3.84 $mol^{-1}cm^{-1}$). The signal at δ_C 155.5 ppm was attributed to a carbonyl group which further supported by the IR (KBr) absorption band at ν_{max} 1697 cm^{-1}. The signal at δ_C 157.0 ppm is typical for the bromopyrrole alkaloids and is assigned to the carbon atom of the guanidine (C-8). From the ^{1}H,^{13}C-HMBC spectrum 28 correlations and from the ^{1}H,^{15}N-HMBC spectrum 7 correlations could be obtained which confirmed the proposed structure of **1**. Due to the ^{15}N data it was possible to distinguish between N-7 and N-9 (the aminoimidazol is protonated). The absolute configuration of **1** was obtained by comparison of the CD spectral data (c = 82 μol/l, MeOH, $[\theta]_{210}$ − 1220) with the values published in the literature (Fedoreyev *et al.*, 1986).

Table I. ^{1}H, ^{13}C and ^{15}N NMR chemical shifts (δ) of **1** in DMSO-d_6.

Position		δ(^{13}C)/δ(^{15}N)[a]	δ(^{1}H)[b]
1	NH	155	12.44 (1H, br)
2	CH	124.4	7.22 (1H, d), J = 3.0 Hz
3	C	93.3	–
4	C	121.6	–
5	C	121.4	–
6	CH	54.1	5.23 (1H, s)
7	NH	89	8.88 (1H, br)
8	C	157.0	–
9	NH	109	9.96 (1H, br)
10	C	84.2	–
11	CH_2	39.1	2.22 (2H, m)
12	CH_2	19.2	2.04 (2H, m)
13	CH_2	43.9	3.57/3.47 (2H, m)
14	N	123	–
15	C	155.5	–
16	NH_2	72	8.07 (2H, br)

[a] ^{13}C chemical shifts are given in [ppm] and are referenced to the DMSO-d_6 signal (39.5 ppm). ^{15}N chemical shifts are given in [ppm] and are calibrated according to the Bruker frequency, which is set to 0 ppm for NH_3, the accuracy is about 1 to 2 ppm.
[b] ^{1}H chemical shifts are given in [ppm] and are referenced to the DMSO-d_6 signal (2.50 ppm). The integration and the multiplicity of the proton signals are given in parenthesis.

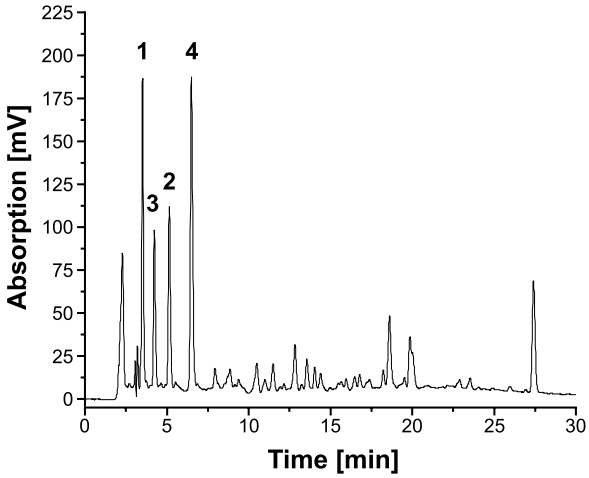


Fig. 1. HPLC profile of DCM/MeOH crude extract from *Agelas* sp. (column: Kromasil RP18, 4 × 250 mm, 5 μm; gradient: 20–50% MeCN/H_2O + 0.1% TFA in 30 min; flow rate: 1 ml/min, UV detection at 280 nm). The retention times for *Agelas* sp. are: monobromoisophakellin (**1**) t_R = 3.52 min, monobromophakellin (**3**) t_R = 4.23 min, dibromoisophakellin (**2**) t_R = 5.15 min, and dibromophakellin (**4**) t_R = 6.50 min.

Table II. Results of the aquarium assays for compounds **1** to **4** at different concentrations[a].

Compound	1 mg/ml[b]	5 mg/ml[b]	10 mg/ml[b]	Nat. conc.[c]
1	6.0 ± 1.0	3.3 ± 0.6	0.7 ± 0.6	0.15 mg/ml
2	4.3 ± 0.6	1.7 ± 0.6	0	0.05 mg/ml
3	8.7 ± 0.6	6.7 ± 0.6	2.7 ± 0.6	0.06 mg/ml
4	7.7 ± 0.6	5.0 ± 1.0	1.3 ± 0.6	0.26 mg/ml

[a] Aquarium assay results of feeding by *Thalassoma bifasciatum* on pellets treated with purified bromopyrrole alkaloids (**1** to **4**) isolated from *Agelas* species. All control pellets were eaten in all assays. Three replicate assays were performed at each concentration (mean ± SD are indicated in columns 2 to 4). 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, 1-tailed; Zar, 1999).
[b] Concentration of the pure compound (**1** to **4**) in the pellet. The molar concentrations are 3.22 μM (**1** and **3**) and 2.57 μM (**2** and **4**) for 1 mg/ml, 16.1 μM (**1** and **3**) and 12.9 μM (**2** and **4**) for 5 mg/ml, 32.2 μM (**1** and **3**) and 25.7 μM (**2** and **4**) for 10 mg/ml.
[c] Natural concentration of **1** to **4** in *Agelas* species. Sponge volume was determined by displacement of water with frozen material according to Pawlik *et al.* (1995).

The results of the antifeedant activity of the four phakellin derivatives against *Thalassoma bifasciatum* in the aquarium assay are given in Table II. This shows a higher activity for the isophakelline skeleton in comparison to the phakelline skeleton. The isophakellins which are active at 1 mg/ml are in the same range of the antifeedant activity as oroidin (Chanas *et al.*, 1996; Assmann *et al.*, 2000). It is further known from the literature that bromination increases the antifeedant activity which is confirmed by presented results (Assmann *et al.*, 2000). In contrast to other brominated alkaloids the natural concentration of the four compounds in *Agelas* sp. is relatively low (see Table II). All concentrations (0.05–0.26 mg/ml) are below the required concentration for feeding deterrency (1 mg/ml for **1** and **2** and even higher for **3** and **4**).

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