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

Michael Assmann and Matthias Köck*

Alfred-Wegener-Institut für Polar- und Meeresforschung, Am Handelshafen 12, D-27570 Bremerhaven, Germany. Fax: 0049–471–4831–1425. E-mail: mkoeck@awi-bremerhaven.de

* Author for correspondence and reprint requests

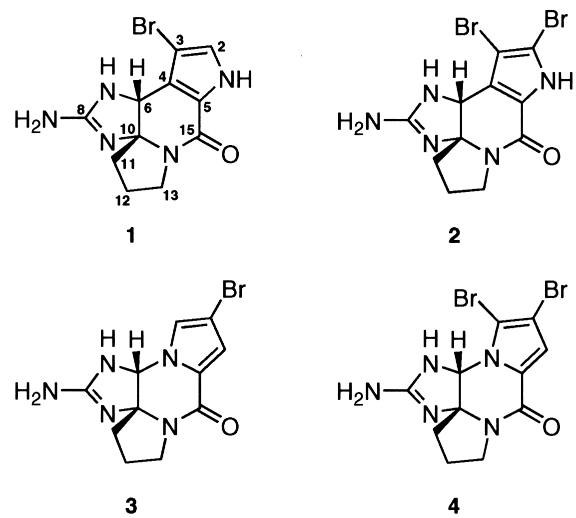
Z. Naturforsch. **57 c,** 153–156 (2002); received September 10/October 12, 2001

Sponges, *Agelas*, Bromopyrrole Alkaloids, Structure Elucidation

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*-hexane (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		$\delta(^{13}C)/\delta(^{15}N)^a$	$\delta(^{1}H)^b$
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.

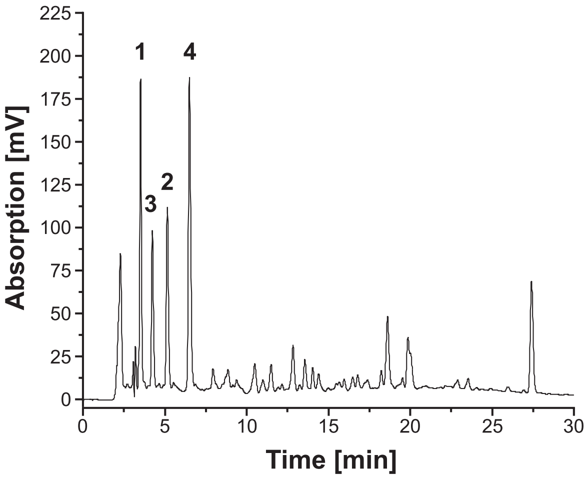


Fig. 1. HPLC profile of DCM/MeOH crude extract from *Agelas* sp. (column: Kromasil RP18, 4 × 250 mm, 5 µm; gradient: 20–50% $MeCN/H_2O$ + 0.1% TFA in 30 min; flow rate: 1 ml/min, UV detection at 280 nm). The retention times for *Agelas* sp. are: monobromoisophakellin (**1**) t_R = 3.52 min, monobromophakellin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

Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft (Ko 1314/3–1 to 3–4) is gratefully acknowledged. Furthermore, we are grateful to Professor Joseph R. Pawlik (Department of Biological Sciences, University of North Carolina Wilmington, USA) for giving M. A. the opportunity to participate in a scientific expedition to the Bahamas onboard the *R/V Edwin Link* in September 1998, during which the sponge *Agelas* sp. was collected. The use of the research vessel *Edwin Link* was made possible through financial support from the U. S. National Science Foundation (OCE-9711255 to J. R. Pawlik). We thank the captain and the crew of the *R/V Edwin Link* for their cooperation. We thank the government of the Bahamas for permission to perform research in their territorial waters. We are grateful to Dr. Rob W. M. van Soest (Instituut voor Biodiversiteit en Ecosysteemdynamica, Zoölogisch Museum, Universiteit van Amsterdam, The Netherlands) for his expert help with sponge identification. We thank Ellen Lichte for performing HPLC analyses.

Assmann M., Lichte E., Pawlik J. R. and Köck M. (2000), Chemical defenses of the Caribbean sponges *Agelas wiedenmayeri* and *Agelas conifera*. Mar. Ecol. Prog. Ser. **207**, 255–262.

Assmann M., Lichte E., van Soest R. W. M. and Köck M. (1999), New bromopyrrole alkaloid from the marine sponge *Agelas wiedenmayeri*. Org. Lett. **1**, 455–457.

Assmann M., van Soest R. W. M. and Köck M. (2001), New antifeedant bromopyrrole alkaloid from the Caribbean sponge *Stylissa caribica*. J. Nat. Prod. **64**, 1345–1347.

Braekman J. C, Daloze D., Stoller C. and van Soest R. W. M. (1992), Chemotaxonomy of *Agelas* (Porifera: Demospongiae). Biochem. Syst. Ecol. **20**, 417–431.

Chanas B., Pawlik J. R., Lindel T. and Fenical W. (1996), Chemical defense of the Caribbean sponge *Agelas clathrodes* (Schmidt). J. Exp. Mar. Biol. Ecol. **208**, 185–196.

De Nanteuil G., Ahond A., Guilhem J., Poupat C., Tran Huu Dau E., Potier P., Pusset M., Pusset J. and Laboute P. (1985), Invertebrates marins du lagon Neo-Caledonien. V. Isolement et identification des metabolites d'une nouvelle espece de spongiaire, *Pseudaxinyssa cantharella*. Tetrahedron **41**, 6019–6033.

Fedoreyev S. A., Utkina N. K., Ilyin S. G., Reshetnyak M. V. and Maximov O. B. (1986), The structure of dibromoisophakellin from the marine sponge *Acanthella carteri*. Tetrahedron Lett. **27**, 3177–3180.

Jiménez C. and Crews P. (1994), Novel sponge-derived amino acids. 15. Mauritamide A and accompanying oroidin alkaloids from the sponge *Agelas mauritiana*. Tetrahedron Lett. **35**, 1375–1378.

Pawlik J. R., Burch M. T. and Fenical W. (1987), Patterns of chemical defense among Caribbean gorgonian corals: a preliminary survey. J. Exp. Mar. Biol. Ecol. **108**, 55–66.

Pawlik J. R., Chanas B., Toonen R. J. and Fenical W. (1995), Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrency. Mar. Ecol. Prog. Ser. **127**, 183–194.

Sharma G. M. and Burkholder P. R. (1971), Structure of dibromophakellin, a new bromine-containing alkaloid from the marine sponge *Phakellia flabellata*. J. Chem. Soc. Chem. Comm. 151–152.

Sharma G. M. and Magdoff-Fairchild B. (1977), Natural products of marine sponges. 7. The constitution of weakly basic guanidine compounds, dibromophakellin and monobromophakellin. J. Org. Chem. **42**, 4118–4124.

Wilson D. M., Puyana M., Fenical W. and Pawlik J. R. (1999), Chemical defense of the Caribbean reef sponge *Axinella corrugata* against predatory fishes. J. Chem. Ecol. **25**, 2811–2823.

Zar J. H. (1999), *Biostatistical Analysis*, 4th ed.; Prentice-Hall: Upper Saddle River, NJ, USA.