Bromosceptrin, an Alkaloid from the Marine Sponge *Agelas conifera*

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Six dimeric bromopyrrole alkaloids (**1**–**6**) were isolated from a Florida Keys specimen of *Agelas conifera*. One of the constituents was identified as a new bromopyrrole metabolite, bromosceptrin (**1**). The structure of **1** was established from MS spectrometry and 1D and 2D NMR spectrocopy.

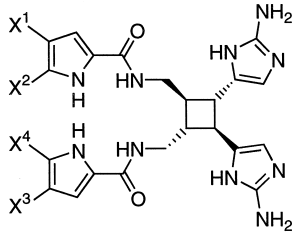
Introduction

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 dimeric pyrrole alkaloids have been isolated from a specimen of the sponge *Agelas conifera* collected off the coast of the Florida Keys, Florida, USA. Examination of the dichloromethane/methanol extract of this sponge resulted in isolation of the known alkaloids sceptrin (**2**), dibromosceptrin(**5**), ageliferin (**3**), bromoageliferin (**4**), and dibromoageliferin (**6**) which have been previously isolated from *Agelas* sponges (Walker *et al.*, 1981; Kobayashi *et al.*, 1990; Keifer *et al.*, 1991) as well as of the new bromopyrrole alkaloid, bromosceptrin (**1**). In this communication we describe the isolation and structure elucidation of **1**. Due to the isolation of bromosceptrin (**1**) the family of the sceptrins is now completed.

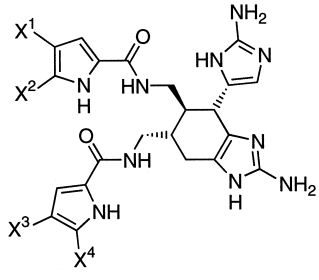
Materials and Methods

The marine sponge *Agelas conifera* (Schmidt, 1870) (order Agelasida, family Agelasidae) employed in this study was collected in May 1998 at Elbow Reef by SCUBA diving (19 m depth) off the coast of the Florida Keys, Florida, USA. The growth form of the specimen is repent-ramose with volcanoe-shaped oscules, colour in life is brownish, consistency is tough, spongy, firm and almost incompressible. A voucher fragment of the sponge has been deposited under registration no. ZMA POR. 16866 in the Zoölogisch Museum, Amsterdam, The Netherlands.

Samples of *Agelas conifera* were immediately frozen after collection and kept at –20 °C until



Sceptrin-Skeleton



Ageliferin-Skeleton

Sceptrin (2): X^1 = Br, X^2 = H, X^3 = Br, X^4 = H
Bromosceptrin (1): X^1 - X^3 = Br, X^4 = H
Dibromosceptrin (5): X^1 - X^4 = Br

Ageliferin (3): X^1 = Br, X^2 = H, X^3 = Br, X^4 = H
Bromoageliferin (4): X^1 - X^3 = Br, X^4 = H
Dibromoageliferin (6): X^1 - X^4 = Br

extraction. For bulk extraction followed by isolation of brominated secondary metabolites, freeze-dried sponge tissue (269 g ≈ 1360 ml) was extracted 3 times in MeOH, twice in a 1:1-mixture of dichloromethane/MeOH, and once in dichloromethane at room temperature each. The organic extracts were combined and evaporated to dryness. The obtained crude extract was partitioned between *n*-hexane (3 × 500 ml) and MeOH (150 ml). The remaining MeOH phase was concentrated and the residue (10.7 g) was purified by gel chromatography on Sephadex LH-20 (Pharmacia) using MeOH as mobile phase. A part of the fraction containing sceptrins and ageliferins (2.03 g, see Fig. 1) was finally purified by preparative RP_{18} HPLC (conditions: 5 min A, 45 min 45% B; A: 10% MeCN/H_2O + 0.1% TFA; B: MeCN + 0.1% TFA). The following compound proportions approximating those found in the sponge tissue by HPLC quantification can be given: bromosceptrin (**1**) (0.05 mg/ml corresponds to 0.03% of dry weight), sceptrin (**2**) (1.02 mg/ml ≈ 0.52%), dibromosceptrin (**5**) (0.13 mg/ml ≈ 0.07%), ageliferin (**3**) (0.07 mg/ml ≈ 0.04%), bromoageliferin (**4**) (0.12 mg/ml ≈ 0.06%), and dibromoageliferin (**6**) (0.09 mg/ml ≈ 0.05%).

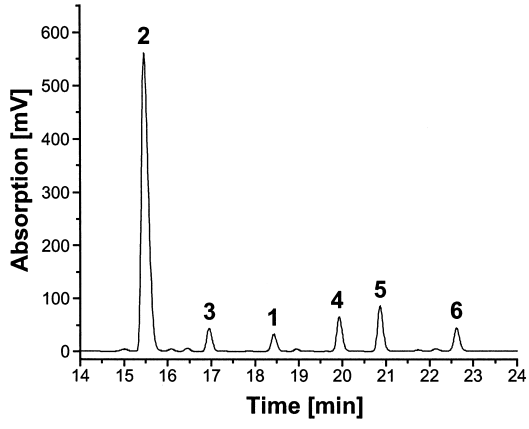
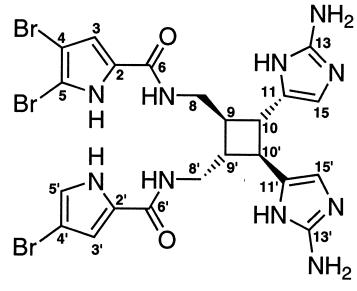


Fig. 1. HPLC profile of a fraction from the *n*-BuOH phase of *Agelas conifera* which has been purified by Sephadex LH-20 chromatography. This fraction contains only dimeric pyrrole alkaloids with the sceptrin and ageliferin skeleton. The retention times are: sceptrin (**2**) t_R = 15.46 min, ageliferin (**3**) t_R = 16.95 min, bromosceptrin (**1**) t_R = 18.43 min, bromoageliferin (**4**) t_R = 19.93 min, dibromosceptrin (**5**) t_R = 20.87 min, and dibromoageliferin (**6**) t_R = 22.62 min. HPLC conditions: column Kromasil RP_{18}, 4.6 × 250 mm, 5 µm; gradient 20 to 60% MeCN/H_2O + 0.1% TFA in 40 min; flow rate 1 ml/min.

^{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 and quantification 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.

Results and Discussion

The compounds **1–6** could be isolated from a Florida Keys specimen of the marine sponge *Agelas conifera*. The brominated alkaloids sceptrin (**2**), dibromosceptrin (**5**), ageliferin (**3**), bromoageliferin (**4**), and dibromoageliferin (**6**) were identified by comparison of mass spectrometry and NMR data with those previously reported (Walker *et al.*, 1981 → **2**; Kobayashi *et al.*, 1990 → **3**, **4**, **6**; Keifer *et al.*, 1991 → **2–6**). The ESI mass spectrum (negative ion mode) of **1** showed prominent pseudomolecular ion peaks at *m*/*z* 697, 699, 701, 703 in the ratio 1:2:2:1, suggesting the presence of three bromine atoms.



From this data it cannot be distinguished between the two different skeleton of the dimeric bromopyrrole alkaloids (ageliferin or sceptrin). Due to the cyclisation of oroidin type compounds to the dimeric forms new aliphatic protons are generated which can be used as fingerprint region.

The ageliferin skeleton has three methine and one methylene group in the region between 2.0 and 3.0 ppm whereas the sceptrin skeleton has four methine signals. Another criterion is the olefinic region since the sceptrin skeleton shows one signal more (H15′). The molecular formula of **1** was established as $C_{22}H_{24}{}^{79}Br_3N_{10}O_2$ by HRFABMS (*m/z* 696.9634, $[M+H]^+$, Δ +4.1 mmu), which is in accordance with the ^1H and ^{13}C NMR data of the new compound bromosceptrin (**1**) (see Table I). The absolute configuration of **1** was obtained by comparison of the CD spectral data (*c* = 43 µmol/l, MeOH, $[\theta]_{232}$ −1320) with the values published in the literature (Walker *et al.*, 1981; Kobayashi *et al.*, 1990; Keifer *et al.*, 1991; Shen *et al.*, 1998). Due to the isolation of bromosceptrin (**1**), which contains 3 bromines, the family of the sceptrins is completed. The members differ in the degree of bromination. Debromosceptrin (none bromine, Shen *et al.*, 1998), monobromosceptrin (one bromine, Keifer *et al.*, 1991), sceptrin (**2**, two bromines, Walker *et al.*, 1981) and dibromosceptrin (**5**, four bromines, Keifer *et al.*. 1991) were already described in the literature.

Bromopyrrole alkaloids are known to be feeding deterrent against predatory reef fishes (Chanas *et al.*, 1996; Wilson *et al.*, 1999; Lindel *et al.*, 2000; Assmann *et al.*, 2000; Assmann *et al.*, 2001). The dimeric compounds show a higher activity in comparison to the monomeric counterparts. Interestingly, the activity does not increase by the degree of bromination as known from the monomeric compounds (Assmann *et al.*, 2000). Only Sceptrin (**2**) appears in such concentration in the sponge tissue, which are above the required concentration of a single compound for feeding deterrency.

Acknowledgements

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

Position		δ(^{13}C)/δ(^{15}N)[b]	δ(^{1}H)[c]
1 (1′)	NH	166 (161)	12.65 (11.78)
2 (2′)	C	127.7 (126.4)	–
3 (3′)	CH	112.5 (111.4)	6.88 (6.80)
4 (4′)	C	97.7 (94.8)	–
5 (5′)	C/CH	104.5 (121.2)	(6.98)
6 (6′)	C	159.0 (159.7)	–
7 (7′)	NH	105 (105)	8.20 (8.16)
8 (8′)	CH_2	40.7 (40.7)	3.39 (3.39)
9 (9′)	CH	41.8 (41.8)	2.27 (2.27)
10 (10′)	CH	37.1 (37.1)	2.94 (2.94)
11 (11′)	C	126.8 (126.8)	–
12 (12′)	NH	137 (137)	12.20 (12.20)
13 (13′)	C	146.8 (146.8)	–
14 (14′)	NH	134 (134)	11.73 (11.73)
15 (15′)	CH	108.9 (108.9)	6.60 (6.60)
16 (16′)	NH_2	58 (58)	7.37 (7.37)

[a] The structure of bromosceptrin (**1**) was already published (Assmann *et al.*, 2000), but without any analytical data.
[b] ^{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.
[c] ^1H chemical shifts are given in [ppm] and are referenced to the DMSO-d_6 signal (2.50 ppm).

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