BROMOPYRROLE ALKALOIDS FROM THE CARIBBEAN SPONGE *Agelas cerebrum*

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Bioguided fractionation of *Agelas cerebrum* crude extract resulted in isolation of four bromopyrrole and four bromopyrrole aminooimidazole alkaloids, identified as 5-bromopyrrole-2-carboxylic acid (1), 4-bromopyrrole-2-carboxylic acid (2), 3,4-bromopyrrole-2-carboxylic acid (3), 4,5-bromopyrrole-2-carboxylic acid (4), oroidin (5), bromoageliferin (6), dibromoageliferin (7) and dibromosceptrin (8) on the basis of spectroscopic data analyses (UV, IR, HRMS, 1D and 2D NMR) and comparison with literature data. This is the first report of compounds 2 and 3 in a marine sponge belonging to the *Agelas* genus and the first evidence of the presence of 1 from a natural source.

Keywords: *Agelas cerebrum*; bromopyrrole alkaloids; antitumoral and antiprotozoal activity.

**INTRODUCTION**

Interest in the biology and chemistry of sponges that belong to the genus *Agelas* continues despite many years of study devoted to these species. *Agelas* sponges are commonly found on the Caribbean and Indo-Pacific coral reefs, and new species are reported every year. For example, in 1992 it was noted that 12 species of *Agelas* were documented, while today the taxonomic record shows that there are 35 such species, from which more than 200 novel molecules have been isolated, some of them with high therapeutic potential.

Characteristic metabolites from this genus of sponges are bromopyrrole alkaloids which present a broad range of biological activities, including anticancer and antimalarial activity. Structurally, most of them are built up by a 4-bromo- or 4,5-dibromopyrrole-2-carboxylic acid moiety, which is often connected with a 2-aminooimidazole group through an aliphatic segment. Monomers of this chemotype can dimerize or trimerize to give a large variety of pyrrole 2-aminooimidazole alkaloids.

As part of our ongoing work on secondary metabolites produced by Caribbean marine sponges, we undertook the chemical study of the marine sponge *Agelas cerebrum* Assmann, van Soest & Köck, 2001 (phylum Porifera, class Demospongiae, order Agelasidae). This is the first report of a chemical study on the isolation and identification of metabolites from the marine sponge *A. cerebrum*.

**EXPERIMENTAL**

**General procedures**

UV measurements were performed on a Varian Cary 300 Scan UV-visible spectrophotometer. IR spectra were obtained with a PerkinElmer Paragon 1000 FT-IR spectrophotometer. NMR experiments were performed on a Bruker Avance 500 MHz spectrometer. Low resolution electrospray ionization (ESI) mass spectra were obtained with a Bruker Esquire 3000 Plus spectrometer in the positive or negative mode. High resolution mass spectra (HRESIMS) were conducted on a LTQ Orbitrap mass spectrometer (Thermo Finnigan). HPLC purification was carried out on a Waters 600 system equipped with a Waters 996 photodiode array detector, a Sedex 55 evaporative light-scattering detector (Sedere, France).

**Biological material**

An specimen of the marine sponge *A. cerebrum* was collected at a depth of about 20 m from “Boca de Calderas”, Havana, Cuba (23°05’55’’N 82°28’30’’W) in March 2008 and identified by Dr. P. M. Alcolado (Institute of Oceanology, Havana, Cuba). A voucher sample (ANC.02.010) has been deposited in the sponge collection of the Cuban National Aquarium. The sponge was kept frozen from collection until the extraction process.

**Extraction and isolation**

A portion of *A. cerebrum* (250 g) was freeze-dried and ground to obtain a dry powder (15 g), which was exhaustively extracted with a mixture of MeOH/CHCl₃ (1:1) to give 2.3 g of a crude extract after concentration under reduced pressure. The crude extract was fractionated by RP-C₁₈ flash chromatography (elution with a decreasing polarity gradient of H₂O/MeOH from 1:0 to 0:1; then MeOH/CH₂Cl₂ from 1:0 to 0:1). Fractions obtained were submitted to antitumoral assays by a colorimetric high-throughput screen.
The bioactive H₂O/MeOH (1:3) fraction (180 mg) was chosen for a chemical investigation and was further fractionated by RP-C₁₈ semi-preparative HPLC (Phenomenex, Luna C₁₈, 250 × 10 mm, 5 μm) with a gradient of H₂O/MeOH/Formic acid from 60:40:0.1 to 20:80:0.1 in 35 min (flow 3.0 mL/min) and the subsequent mixtures were finally purified by RP-C₁₈ analytical HPLC (Phenomenex Luna C₁₈, 150 × 4.6 mm, 5 μm, flow 1.0 mL/min) to afford a new compound (1, 1.2 mg), together with seven known metabolites: 2 (5.2 mg), 3 (1.0 mg), 4 (2.5 mg), 5 (3.6 mg), 6 (2.7 mg), 7 (1.8 mg) and 8 (1.4 mg). Due to higher yield requirements for antimalarial assays, a preparative fraction was further obtained by RP-C₁₈ chromatography after stepwise elution with H₂O (which was discarded) and MeOH/CH₂Cl₂ (1:1).

Compound 1: Amorphous white solid; UV (MeOH) λ_max (log ε) 234 (3.80), 273 (4.10) nm; IR (thin film) ν_max 3356, 3122, 1649 cm⁻¹; ¹H NMR (500 MHz, CD₃OD + CDCl₃) δ 6.26 (1H, d, J = 3.5 Hz, H-4) and 6.59 (1H, d, J = 3.5 Hz, H-3); ¹³C NMR (125 MHz, CD₃OD + CDCl₃) δ 129.3 (C-2), 111.7 (C-3), 112.3 (C-4), 104.3 (C-5), 164.1 (C-6); HRESI-MS (−): m/z 190, 188 [M-H] (calcd for C₇H₁₃BrNO₂, 187.9396, Δ 0.3 ppm).

Antitumoral assay

A colorimetric assay using sulforhodamine B was adapted for a quantitative measurement of cell growth and viability following a technique described in the literature. In Vitro cytotoxicity was evaluated against three tumor cell lines: lung carcinoma A549, colon carcinoma HT29, and breast MDA-MB-231 and samples were tested at concentrations of 100, 10 and 1 μg/mL.

Antimalarial assay

In vitro drug susceptibility was determined in standard short-term cultures of Plasmodium berghei ANKA blood stages, as described before. Briefly, erythrocytes infected with parasites of P. berghei ring forms/trophozoites were incubated at 2% parasitemia at a final cell concentration of 1% in complete culture medium (RPMI 1640; 20% Fetal Calf Serum, Sigma) containing serial dilutions of samples from A. cerebrum, each in duplicate wells of 96-well culture plates. These plates were incubated for a period of 24 h at 37 °C under standardized in vitro culture conditions. The antimalarial activity was expressed as IC₅₀, which was determined according to reported methodology and using data of inhibition of schizont maturation measured as described by Schlichtherle et al., and adapting recommendations for P. falciparum isolates. Chloroquine phosphate and artemisinin (both from Sigma) were used as references.

RESULTS AND DISCUSSION

Compound 1 was isolated as amorphous white solid and its mass spectrum showed molecular ions at m/z 188 and 190 in a 1:1 ratio, indicative of the presence of one bromine atom. The molecular formula was determined as C₉H₇NOBr by HRESI-MS. The UV absorption [λ_max 273 nm (log ε 4.10)] was attributed to a substituted pyrrole chromophore. The bands at 3356, 3122, and 1649 cm⁻¹ in the IR spectrum suggested the presence of amine and carbonyl moieties. In the ¹³C NMR spectrum, resonances due to a disubstituted pyrrole at δ 104.3 (3), 112.3 (d), 111.7 (d), and 129.3 (s) were observed as well as a carbonyl (COOH) at δ 164.1 (s). The ¹H NMR spectrum indicated resonances due to 2,5-disubstituted pyrrole protons at δ 6.26 (1H, d, J = 3.5 Hz, H-4) and 6.59 (1H, d, J = 3.5 Hz, H-3). The positions of the CO₂H moiety at C-2 and the bromine atom at C-5 were in agreement with other 2,5-disubstituted pyroles. On this basis, compound 1 was concluded to be 5-bromopyrrole-2-carboxylic acid. On the other hand, compounds 2-8 were identified by a combination of spectroscopic methods (¹H, ¹³C NMR, 2D NMR, ESIMS) and comparison with the literature data.

Bromopyrrole alkaloids are known to be one of the most common metabolites contained in marine sponges and they are widely distributed in the species belonging to the genera Agelas, Axinella, Acanthella, Pseudaxinyssa, and Hymeniacidon. Oroidin (5), the first member of pyrrole-2-aminomidoazole alkaloids in this group, was isolated from the sponge A. oroides. However, a revised structure was soon proposed. It was not until 1981, during solidstate photodimerization studies of (−)-sceptrin, a related dimeric bromopyrrole alkaloid, that the final structure of 5 was confirmed by X-ray diffraction analysis. Oroidin has been isolated from many Agelas sponges and species of other genera such as: Axinella damicornis, Axinella verrucosa, Acanthella aurantiaca, Goreauiella sp and Pseudaxinyssa cantharella. Compound 4 (4,5-bromopyrrole-2-carboxylic acid) has been also isolated from many Agelas species and together with oroidin have been reported to exhibit significant biological properties. Chanas et al. suggested that A. conifera, A. dispar, A. inaequalis, A. sceptrum and A. wiedenmmeri share both metabolites as a common chemical defense against fish predators.

Bromoaegeliferin (6) and dibromoaegeliferin (7), dimers of oroidin, were isolated from A. conifera and A. cf. mauritiana. These metabolites are potent actomyosin ATPase activators and other significant biological effects have been reported. Since then, these compounds have been isolated from many Agelas species and from sponges of other genera such as Astronserca willeyana and Stylissa caribica. Another dimeric bioactive metabolite, belonging to the sceptrin family, was discovered in A. conifera. This compound, as well as 6 and 7, were found to be potent feeding deterrents.
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4-Bromopyrrole-2-carboxylic acid (2) and 3,4-bromopyrrole-2-carboxylic acid (3) were recently encountered in the Mediterranean sponge Axinella verrucosa\(^2\) and the tropical sponge Axinella damicornis.\(^4\) However, to our knowledge, this is the first report of the occurrence of both compounds in a sponge belonging to the Agelas genus. While compound 1 had been previously synthesized,\(^8\) it is herein reported as a new natural bromopyrrole alkaloid isolated from this species. 5-bromopyrrole alkaloids are not commonly isolated from marine sources and there are only two examples of other new 5-bromopyrrole derivatives identified in the genus Agelas.\(^9\) Then, in a sense, compound 1 may be useful as a chemotaxonomic marker for Agelas cerebrum.

Concerning bioactivity, the H\(_2\)O/Methanol (1:3) fraction from which compounds 1-8 were isolated showed strong cytotoxic activity in an \textit{in vitro} antitumoral assay against three human tumor cell lines (A549 lung cancer cells, HT29 colon cancer cells, and MDA-MB-231 breast cancer cells) at values equal and greater than 1 \(\mu\)g/mL; however, no antitumor activity against the same cell lines was detected below 10 \(\mu\)g/mL for each isolated compound. Probably, undetectable quantities of a very potent antineoplastic substance justify the activity of the crude fraction, or the synergism of natural product mixtures.

The organic extract of \textit{A. cerebrum} exhibited a moderate antimarial activity, which was evaluated according to recommended endpoint criteria for natural complex mixtures,\(^1\) with IC\(_{50}\) value equal to 60.35 \pm 10.6 \(\mu\)g x mL\(^{-1}\) against \textit{P. berghei}. Although this work revealed a different profile for bromopyrrole alkaloids isolated from \textit{A. cerebrum} in comparison to that previously described for \textit{A. ovoides}, the presence of ovoidin (5) and 4,5 dibromopyrrole-2-carboxylic acid (4) suggests that they are the main active principles in the antimarial organic fraction. Both compounds were previously identified in \textit{A. ovoides} as devoid of any cytotoxicity against L6 cells, while they exhibited an IC\(_{50}\) value of 3.9 mg/mL for 4 and a limited antimarial activity for 5, in the whole cell parasite assays.\(^7\) Sceptrin was evaluated against D6 and W2 strains by other authors and showed no activity.\(^15\)

In conclusion, this chemical study underscores the presence of bromopyrrole alkaloids as the representative secondary metabolites of \textit{Agelas cerebrum}, and contributes to the chemotaxonomy within the Agelasiidae family. Studies are in progress in order to advance the evaluation of the \textit{in vivo} antimarial activity of bromopyrrole alkaloids from \textit{Agelas cerebrum}.

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