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Artigo

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 aminoimidazole 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,[1] while today the taxonomic record shows that there are 35 such species,[2] from which more than 200 novel molecules have been isolated,[3] some of them with high therapeutic potential.[4]

Characteristic metabolites from this genus of sponges are bromopyrrole alkaloids which present a broad range of biological activities,[5] including anticancer[6] and antimalarial activity.[7] 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-aminoimidazole group through an aliphatic segment. Monomers of this chemotype can dimerize or trimerize to give a large variety of pyrrole 2-aminoimidazole 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 Agelasida, family 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 717 plus autosampler, a Waters 996 photodiode array detector, and a Sedex 55 evaporative light-scattering detector (Sedere, France).

Biological material

A specimen of the marine sponge *A. cerebrum* was collected at a depth of about 20 m from "Boca de Calderas", Havana, Cuba (23º 05' 55" N 82º 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/CH_2Cl_2 (1:1) to give 2.3 g of a crude extract after concentration under reduced pressure. The crude extract was fractionated by RP-C_{18} flash chromatography (elution with a decreasing polarity gradient of H_2O/MeOH from 1:0 to 0:1, then MeOH/CH_2Cl_2 from 1:0 to 0:1). Fractions obtained were submitted to antitumoral assays by a colorimetric high-throughput screen.

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The bioactive H_2O/MeOH (1:3) fraction (180 mg) was chosen for a chemical investigation and was further fractionated by RP-C_{18} semi-preparative HPLC (Phenomenex, Luna C_{18}, 250 × 10 mm, 5 μm) with a gradient of H_2O/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-C_{18} analytical HPLC (Phenomenex Luna C_{18}, 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-C_{18} chromatography after stepwise elution with H_2O (which was discarded) and MeOH/CH_2Cl_2 (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, CD_3OD + $CDCl_3$) δ 6.26 (1H, d, *J* = 3.5 Hz, H-4), 6.59 (1H, d, *J* = 3.5 Hz, H-3); ^{13}C NMR (125 MHz, CD_3OD+$CDCl_3$) 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 $C_5H_3{}^{79}BrNO_2$, 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.[8] *In* V*itro* 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.[9] Briefly, erythrocytes infected with parasites of *P. berghei* ring forms/young 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 IC_{50}, which was determined according to reported methodology[10] using data of inhibition of schizont maturation measured as described by Schlichtherle *et al.*,[11] and adapting recommendations for *P. falciparum* isolates.[12] 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 $C_5H_4NO_2Br$ by HRESI-MS. The UV absorption [λ_{max} 273 nm (log ε 4.10)] was attributed to a substituted pyrrole chromophore.[13] The bands at 3356, 3122, and 1649 cm^{-1} in the IR spectrum suggested the presence of amine and carbonyl moieties. In the ^{13}C NMR spectrum, resonances due to a disubstituted pyrrole at δ 104.3 (s), 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 CO_2H moiety at C-2 and the bromine atom at C-5 were in agreement with other 2,5-disubstituted pyrroles.[14] 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, ^{13}C 1D and 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[15] and they are widely distributed in the species belonging to the genera *Agelas*, *Axinella*, *Acanthella*, *Pseudoaxinyssa*, and *Hymeniacidon*.[16] Oroidin (**5**), the first member of pyrrole-2-aminoimidazole alkaloids in this group, was isolated from the sponge *A. oroides*.[17] However, a revised structure was soon proposed.[18] 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.[19] 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*.[20] 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.[21] Chanas *et al*.[22] suggested that *A. conifera*, *A. dispar*, *A. inaequalis*, *A. sceptrum* and *A. wiedenmmeri* share both metabolites as a common chemical defense against fish predators.

Bromoageliferin (**6**) and dibromoageliferin (**7**), dimers of oroidin, were isolated from *A. conifera* and *A. cf. mauritiana*.[23] These metabolites are potent actomyosin ATPase activators[24] and other significant biological effects have been reported.[25] Since then, these compounds have been isolated from many *Agelas* species and from sponges of other genera such as *Astroscera willeyana*[26] and *Stylissa caribica*.[27] Dibromosceptrin (**8**), another dimeric bioactive metabolite, belonging to the sceptrin family, was discovered in *A. conifera*.[20] This compound, as well as **6** and **7**, were found to be potent feeding deterrents.[28]

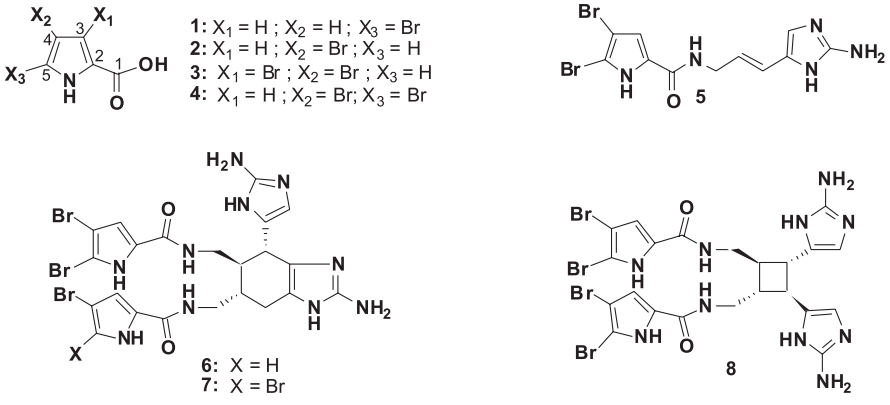

Figure 1. *Structures of compounds* ***1-8*** *isolated from A. cerebrum*

4-Bromopyrrole-2-carboxylic acid (**2**) and 3,4-bromopyrrole-2-carboxylic acid (**3**) were recently encountered in the Mediterranean sponge *Axinella verrucosa*[29] and the tropical sponge *Axinella damicornis*.[6] 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,[28] it is herein reported as a new natural bromopyrrole alkaloid isolated from this species. 5-bropyrrole 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*.[30] Then, in a sense, compound **1** may be useful as a chemotaxonomic marker for *Agelas cerebrum*.

Concerning bioactivity, the H_2O/MeOH (1:3) fraction from which compounds **1-8** were isolated showed strong cytotoxic activity in an *in vitro* antitumoral assay against three human tumor cell lines (A549 lung cancer cells, HT29 colonic cancer cells, and MDA-MB-231 breast cancer cells) at values equal and greater than 1 µg/mL; however, no antitumor activity against the same cell lines was detected below 10 µg/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 *A. cerebrum* exhibited a moderate antimalarial activity, which was evaluated according to recommended endpoint criteria for natural complex mixtures, with IC_{50} value equal to 60,35 ± 10,6 µg x mL^{-1} against *P. berghei*. Although this work revealed a different profile for bromopyrrole alkaloids isolated from *A. cerebrum* in comparison to that previously described for *A. oroides*, the presence of oroidin (**5**) and 4,5 dibromopyrrole-2-carboxilic acid (**4**) suggests that they are the main active principles in the antimalarial organic fraction. Both compounds were previously identified in *A. oroides* 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 antimalarial 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.[32]

In conclusion, this chemical study underscores the presence of bromopyrrole alkaloids as the representative secondary metabolites of *Agelas cerebrum*, and contributes to the chemotaxonomy within the Agelasidae family. Studies are in progress in order to advance the evaluation of the *in vivo* antimalarial activity of bromopyrrole alkaloids from *Agelas cerebrum.*

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