A New Bromotyrosine Alkaloid from the Caribbean Sponge *Aiolochroia crassa***

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A detailed analysis of the chemical constituents of a Caribbean specimen of *Aiolochroia crassa* was performed. Five brominated products (**1–5**) were isolated and one of these was a new bromotyrosine metabolite. The structure of the new compound **1** has been established from spectral studies. Compounds **1** and **2**, which are the major brominated metabolites and have not been previously identified in any *Aiolochroia* species, could be usefully employed as chemotaxonomic markers.

Introduction

Bromotyrosine-derived alkaloids are well known in marine Verongida sponges (Minale, 1976; Minale *et al.*, 1976; Ciminiello *et al.*, 1994; Ciminiello *et al.*, 1994a; Ciminiello *et al.*, 1995). In our search for bioactive substances from marine organisms, a series of brominated isoxazoline alkaloids have been isolated from a specimen of the Caribbean sponge *Aiolochroia crassa* collected off the coast of Eleuthera Island (Bahamas). Examination of the methanolic extract of this sponge resulted in isolation of the known alkaloids aerophobin-1(**2**), aerophobin-2 (**3**) (Cimino *et al.*, 1983), purealidin L (**4**) (Kobayashi *et al.*, 1995), and isofistularin-3 (**5**) (Gopichand and Schmitz, 1979) as well as of the new bromotyrosine-derived alkaloid, N-methyl-aerophobin-2 (**1**) (Fig. 1). In this paper we describe the isolation and structural elucidation of the new bromotyrosine alkaloid (**1**).

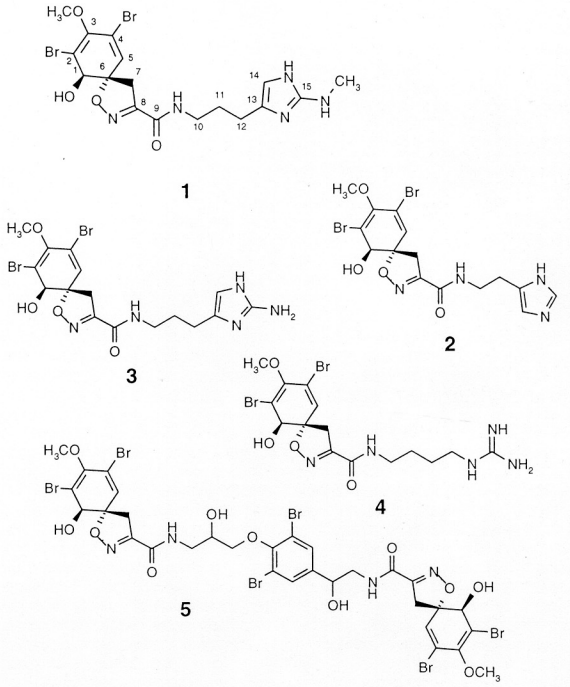

Fig. 1. Brominated secondary metabolites of *Aiolochroia crassa.*

Materials and Methods

The marine sponge *Aiolochroia crassa* employed in this study was collected in August 1995 by SCUBA diving (27 m depth) off the coast of

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Eleuthera Island (Bahamas) during a scientific cruise of the *R/V Seward Johnson* to the Caribbean Sea. The specimen is a massive sponge and yellow in color. It has a characteristic surface and a very characteristic dendritic system of thick pithed fibres, with a low proportion of fibre material compared to "flesh". A voucher specimen is deposited under registration no. ZMA POR. 11503 in the Zoölogisch Museum, Amsterdam, The Netherlands.

Samples were immediately frozen after collection and kept at −20 °C until extraction. For bulk extraction followed by isolation of brominated secondary compounds, lyophilized tissue was ground and successively extracted with MeOH at room temperature (3 x 500 ml). Following evaporation of the solvent the crude extract was partitioned between EtOAc and H_2O, and the aqueous layer was subsequently extracted with *n*-BuOH. The resulting EtOAc (1 g) and *n*-BuOH (4 g) fractions were taken to dryness, redissolved in a mixture of CH_2Cl_2/MeOH/TFA (75:25:0.1 v/v) and each fraction subjected to column chromatography on silica gel. Fractions were collected and monitored by TLC, which was performed on precoated TLC plates with Si gel 60 F_{254} (Merck, Darmstadt, Germany) using the same solvent system. Secondary compounds were detected by their UV absorbance at 254 or 366 nm. Final purification of the isolated compounds was usually achieved by column chromatography on Sephadex LH-20 with acetone or with mixtures of MeOH/CH_2Cl_2 (1:1 v/v) as eluents. Alternatively compounds were purified by column chromatography on reversed phase LiChroprep® RP-18 columns (Merck, Darmstadt, Germany) with a mixture of MeOH and H_2O (60:40 v/v) as eluents.

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX 400 NMR spectrometer locked to the major deuterium resonance of the solvent, CD_3OD. The nature of each carbon resonance was deduced from DEPT-135 experiments. FAB-MS spectra were recorded on a Finnigan MAT 8430 spectrometer with glycerol as matrix.

For HPLC analysis, samples were injected into a HPLC system equipped with a photodiode-array detector (Gynkotek, Germany). Routine detection was at 254 nm. The separation column (125 x 4 mm, i.d.) was prefilled with Eurospher C-18 (Knauer GmbH, Germany). Separation was achieved by applying a linear gradient from 100% H_2O (adjusted to pH 2 with phosphoric acid) to 100% MeOH in 40 min. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements.

Results and Discussion

Specimens of *Aiolochroia crassa* were frozen after collection and kept at −20 °C until extracted with methanol. The extract was concentrated and sequentially extracted with ethyl acetate and *n*-butanol. Fractionation of the *n*-butanol soluble fraction of the methanol extract employing successive application of silica gel and Sephadex LH-20 chromatographies gave pure compounds **1–4**. The ethyl acetate soluble fraction was first chromatographed on a silica gel column. Further separation by Sephadex LH-20 and column chromatography on reversed phase columns afforded compounds **1**

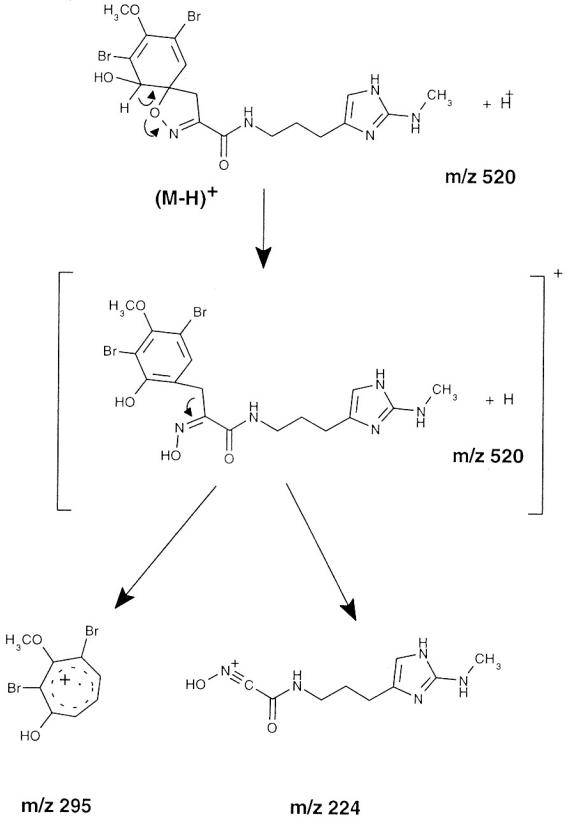

Fig. 2. Fragmentation pattern of **1**.

and **5**. Compounds **2–5** were identified by comparison of their spectroscopic data with those previously reported.

The new compound N-methyl-aerophobin-2 (**1**) showed an isotopic cluster of MH^+ ions in the ratio 1:2:1, consistent with two bromine atoms, in the FABMS at *m/z* 518, 520 and 522. The molecular formula $C_{17}H_{21}Br_2N_5O_4$ was established from the FABMS spectrum, which shows a characteristic fragmentation pattern (Figs 2 and 3) similar to that of aerophobin-2 reported previously (Cimino *et al.*, 1984). Complete structural elucidation of **1** was achieved by comparison of the ^1H and ^{13}C NMR spectra of the new compound (Tables I and II) with those of the previously reported aerophobin-2 (Cimino *et al.*, 1983). Like aerophobin-2 the ^1H NMR spectrum of **1** exhibited characteristic signals of a spirocyclohexdiene-isoxazole ring at δ 4.13 (1 H, d, H-1, J = 0.8 Hz), 6.46 (1 H, d, H-5, J = 0.9 Hz) and 3.14, 3.82 (two d, 1 H each, J = 18.5 Hz). This partial structure was further supported by the ^{13}C resonances for carbons 1–9 (Table II).

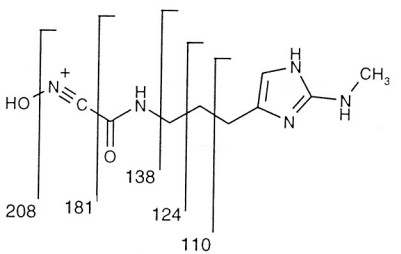

Fig. 3. Decomposition of the ion m/z 224.

Table I. ^1H NMR spectral data of **1** in CD_3OD.

Position	Chemical shift (δ)
H-1	4.13 (1H, d, J = 0.8)
H-5	6.45 (1H, d, J = 0.9)
H-7_A	3.82 (1H, d, J = 18.3)
H-7_B	3.12 (1H, d, J = 18.3)
H_2-10	3.38 (2H, t, J = 6.8)
H_2-11	1.90 (2H, quint; J = 7.1)
H_2-12	2.60 (2H, t, J = 7.1)
H-14	6.62 (1H, s)
3-OCH_3	3.77 (1H, s)
15-NCH_3	2.98 (3H, s)

Table II. ^{13}C NMR spectral data of **1** in CD_3OD.

Position	Chemical shift (δ)
C-1	75.5
C-2	114.1
C-3	149.3
C-4	122.8
C-5	132.2
C-6	92.4
C-7	40.1
C-8	155.2
C-9	161.7
C-10	39.5
C-11	22.9
C-12	28.9
C-13	128.5
C-14	110.3
C-15	149.5
3-OCH_3	60.4
15-NCH_3	29.5

The signal at δ 6.62 in the ^1H NMR spectrum was assigned to the heterocyclic proton of a 2-aminoimidazole. Whereas the ^1H NMR spectrum of **1** showed the same signals in the aliphatic region as those reported for aerophobin-2 it differed from aerophobin-2 by the presence of an additional singlet at δ 2.98 (3 H, s, 15-NCH_3). Thus, N-methyl-aerophobin-2 was assigned structure **1**, which was further supported by characteristic ^{13}C signals (Table II), which corresponded to the carbons of an spirocyclohexadiene-isoxazole unit and to a N-methyl-2-aminoimidazole ring, respectively (Table II).

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