AGELORINS A AND B, AND 11-*EPI*-FISTULARIN-3, THREE NEW ANTIBACTERIAL FISTULARIN-3 DERIVATIVES FROM THE TROPICAL MARINE SPONGE *AGELAS OROIDES*†

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Abstract-From the dichloromethane and methanol extracts of the tropical marine sponge *Agelas oroides* three fistularin-3 derivatives (**1-3**) were isolated. The structures of all isolates were determined by interpretation of their spectroscopic data. All isolates were found to show antibacterial activity.

In the course of our research program, aimed towards the isolation of marine derived biologically active compounds, the bright orange sponge *Agelas oroides* Schmidt (Agelasidae, Axinellida) was investigated. From this sample, collected at the Great Barrier Reef, Australia, three antibacterial compounds (**1-3**) were isolated. The three isolates were all identified as bromotyrosine-derived alkaloids. Interest in such brominated tyrosine derivatives was stimulated by their potent biological activities including; enzyme inhibition (1,2), cytotoxicity (3-6), antifungal (7), antibacterial (8) and antiviral (10) effects.

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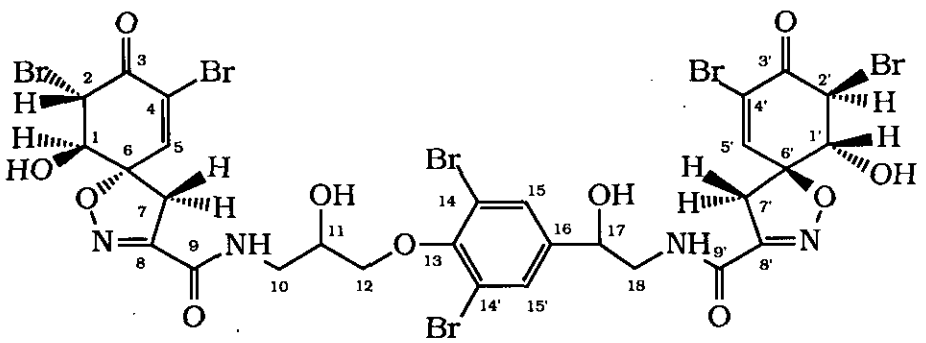
RESULTS AND DISCUSSION

Freeze dried sponge tissue was exhaustively extracted with CH_2Cl_2 and then MeOH. The combined extracts demonstrated strong antibacterial and molluscicidal activities. A 1:1 solution of CH_2Cl_2 and hexane was then added to the extract. The insoluble material was collected and fractionated by vlc. Further separation, using reversed phase hplc, yielded three compounds (**1-3**) with molecular weight in excess of 1000 D.

Compound (**1**) was found to have the molecular formula $C_{29}H_{26}N_4O_{11}Br_6$ by mass spectrometry. Of the sixteen degrees of unsaturation implied by the molecular formula of **1**, eleven were occupied with either carbon-carbon, carbon-oxygen or carbon-nitrogen double bonds; the molecule was thus pentacyclic. The ir and uv data for **1** indicated the presence of hydroxyl, iminoamide and conjugated ketone (3360, 1705, 1660, 1600 and 1540 cm^{-1}, 220 (ε 12,600), 250 (ε 7740) nm) functions. The ^1H and ^{13}C nmr spectra, on first appearance were complex due to many overlapping and broad resonances. After a detailed analysis of these spectra, Tables 1 and 2, it was evident that more than half of the ^{13}C and several of the ^1H nmr resonances were doubled, indicating the molecule to be composed of a number of identical/symmetrical units. A literature search for molecules having similar spectroscopic data and the structural elements there implied, yielded two compounds, fistularin-3 (3) and isofistularin-3 (4), that had many features in common with **1**. ^1H Nmr, ir and uv spectral comparisons made between **1** and fistularin-3 suggested the differences between the two molecules to be the absence of any methoxyl groups in **1**, together with the presence of a secondary bromo-function and a conjugated ketone.



1

Spectroscopically these differences were evident by the appearance of resonances for two conjugated ketone functions (183.7 (s) ppm) and two bromomethine functions (57.0 (d), 57.1 (d) ppm , δ 5.06 (d), 5.07 (d)), as well as the absence of resonances for methoxyl in the ^{1}H and ^{13}C nmr spectra of **1**.

Table 1. ^{1}H Nmr Data (300 MHz, acetone-d_6) for Agelorin A (**1**), Agelorin B (**2**) and 11-*epi*-Fistularin-3 (**3**).

Carbon	Agelorin A (**1**)	Agelorin B (**2**)	11-*epi*-Fistularin-3 (**3**)
1, 1'	4.39 (d, *J* 11.3 Hz)	4.56 (br m)	4.19 (m)
	4.40 (d, *J* 11.3 Hz)	4.56 (br m)	4.19 (m)
2, 2'	5.06 (d, *J* 11.3 Hz)	5.27 (br m)	
	5.07 (d, *J* 11.3 Hz)	5.27 (br m)	
5, 5'	7.61 (s)	7.46 (d, *J* 1.0 Hz)	6.49 (d, *J* 1.0 Hz)
	7.64 (s)	7.49 (d, *J* 0.9 Hz)	6.51 (d, *J* 0.9 Hz)
7, 7'	3.26 (d, *J* 18.2 Hz),	3.38 (d, *J* 18.2 Hz),	3.16 (d, *J* 18.5 Hz),
	3.88 (d, *J* 18.2 Hz)	3.87 (d, *J* 18.2 Hz)	3.85 (d, *J* 18.5 Hz)
	3.30 (d, *J* 18.2 Hz)	3.35 (d, *J* 18.2 Hz),	3.19 (d, *J* 18.5 Hz),
	3.86 (d, *J* 18.2 Hz)	3.90 (d, *J* 18.2 Hz)	3.83 (d, *J* 18.5 Hz)
10	3.53 (m), 3.81 (m)	3.53 (m), 3.80 (m)	3.55 (m), 3.76 (m)
11	4.26 (m)	4.25 (m)	4.25 (m)
12	4.04 (m), 4.08 (m)	4.04 (m), 4.08 (m)	4.05 (m)
15, 15'	7.67 (s)	7.67 (s)	7.65 (s)
	7.67 (s)	7.67 (s)	7.65 (s)
17	4.91 (dd, *J* 4.4, 7.5 Hz)	4.91 (ddd, *J* 4.2, 4.2, 7.5 Hz)	4.90 (dd, *J* 4.3, 7.5 Hz)
18	3.48 (m), 3.65 (m)	3.51 (m), 3.62 (m)	3.48 (m), 3.61 (m)
OCH_3			3.71 (s), 3.71 (s)
OH	5.97 (d, *J* 5.6 Hz)	4.45 (d, *J* 5.2 Hz)	5.41 (br s)
	5.99 (d, *J* 5.6 Hz)	5.00 (d, *J* 4.2 Hz)	5.43 (br s)
		5.92 (d, *J* 5.6 Hz)	
		5.92 (d, *J* 5.6 Hz)	
NH	7.65 (br), 7.67 (br)	7.66 (br), 7.68 (br)	7.66 (dd, *J* 5.9, 5.9 Hz)
			7.71 (dd, *J* 5.9, 5.9 Hz)

The stereostructure of the isoxazole moiety was deduced from a comparison of the ^{1}H nmr data of **1** with those of compounds showing a *cis* relationship between the hydroxyl group at C-1/C-1' and the oxygen atom in the isoxazoline unit. Further, the observation of two sharp doublets for H_2-7 in **1** versus the broad signals observed for the *cis-cis* compounds (9) indicates the C-1/C-1' hydroxyl groups and the corresponding isoxazole oxygen atoms to have a *trans* relationship. From the ^{1}H nmr data it was also evident that H-1 and H-2, as well as H-1' and H-2', were *trans*-diaxially disposed ($J_{1,2}$ and $J_{1',2'}$ 11.3 Hz). All of the above

deductions were supported by the results of nOe measurements made with **1**. For **1** the trivial name of agelorin A is proposed.

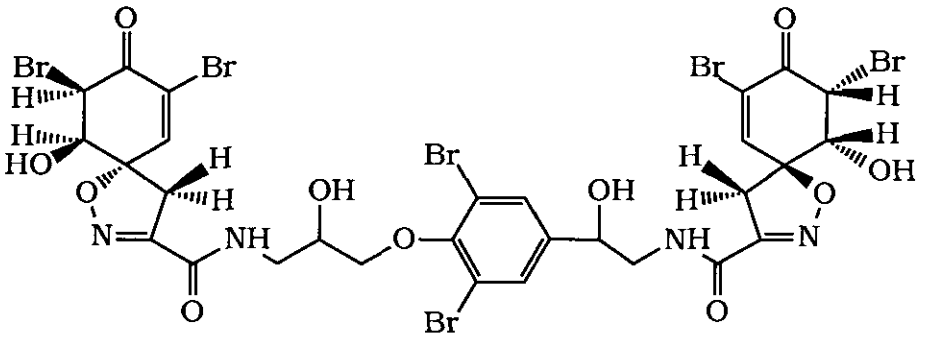
Compound (**2**), by mass spectrometry, was found to have the identical molecular formula as **1**, $C_{29}H_{26}N_4O_{11}Br_6$. All other spectroscopic data also indicated **2** to be similar to **1**, see Experimental and Tables 1 and 2. Significant differences between the two sets of spectroscopic data for **1** and **2** were; optical rotations, +50.0° for **2**, and -17.1° for **1**; ^{13}C nmr chemical shifts of the isoxazole unit (±0.1 to 3 ppm); as well as ^1H nmr chemical shifts and coupling constants for H-1, H-1', H-2 and, H-2' (see Table 1).

Table 2. ^{13}C Nmr Data (75.5 MHz, acetone-d_6) for Agelorin A (**1**), Agelorin B (**2**) and 11-*epi*-Fistularin-3 (**3**).

Carbon	Agelorin A (**1**)		Agelorin B (**2**)		11-*epi*-Fistularin-3 (**3**)	
1, 1'	74.6 d,	74.6 d	72.3 d,	72.3 d	75.0 d,	75.1 d
2, 2'	57.0 d,	57.1 d	54.8 d,	54.8 d	122.0 s,	122.1 s
3, 3'	183.7 s,	183.7 s	183.6 s,	183.6 s	148.6 s,	148.6 s
4, 4'	122.6 s,	122.6 s	124.9 s,	124.9 s	113.7 s,	113.7 s
5, 5'	149.2 d,	149.3 d	146.2 d,	146.4 d	132.0 d,	132.1 d
6, 6'	91.7 s,	91.7 s	90.8 s,	90.8 s	91.8 s,	91.8 s
7, 7'	38.4 t,	38.4 t	41.4 t,	41.4 t	39.8 t,	39.9 t
8, 8'	154.6 s,	154.7 s	155.4 s,	155.5 s	154.9 s,	155.0 s
9, 9'	160.2 s,	160.2 s	160.2 s,	160.2 s	160.4 s,	160.5 s
10	43.4 t		43.6 t		43.4 t	
11	69.7 d		69.9 d		69.7 d	
12	75.8 t		76.0 t		75.7 t	
13	152.6 s		152.7 s		152.5 s	
14, 14'	118.4 s,	118.4 s	118.4 s,	118.4 s	118.3 s,	118.3 s
15, 15'	131.4 d,	131.4 d	131.5 d,	131.5 d	131.3 d,	131.3 d
16	143.1 s		143.3 s		142.9 s	
17	71.0 d		71.4 d		71.3 d	
18	47.4 t		47.4 t		47.5 t	
OCH_3					60.2 q,	60.2 q

The signals for H-1/H-1', H-2 and, H-2' appeared as broad unresolved multiplets in **2** as compared to four sharp doublets in **1**. These differences indicated H-1 and H-2, and thus H-1' and H-2', to have either an axial-equatorial or an equatorial-equatorial relationship in **2**. As the signals for H_2-7 were virtually identical to those found in **1** it was concluded that the relative stereochemistry of **2** at C-6 and C-1 was identical to that

in **1**. In contrast to **1**, however, in **2**, H-1 and H-2 as well as H-1' and H-2' are *cis*. These deductions were confirmed with the results from a 2D noesy measurement made with **2**. Compound (**2**) is thus a stereoisomer of **1**, for which the trivial name of agelorin B is proposed.



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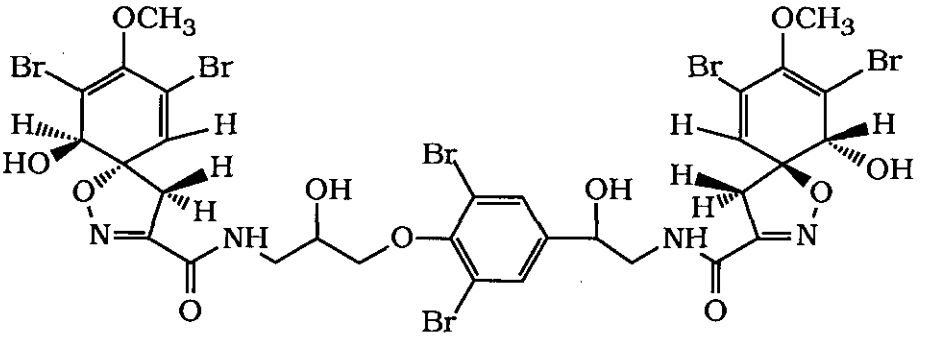

3

The third isolate, **3**, proved to be almost identical to the known metabolite fistularin-3 (3). Comparison of the ^{13}C and ^{1}H nmr of **3** with those published recently for fistularin-3 (10) revealed small but significant differences between the two, notably at C-11 (70.6 ppm in **3** compared to 69.5 ppm in fistularin-3). All other differences between the two sets of ^{13}C nmr data were in the range 0.1 to 0.2 ppm. Compound (**3**) also showed an optical rotation of +66° compared to +102° (10) and +104° (3) for fistularin-3. These observations suggested **3** to be the C-11 stereoisomer of fistularin-3. CD analysis of **3** permitted the absolute stereochemistry of the spiroisoxazole moiety to be deduced as 1*R*, 1'*R*, 6*S* and 6'*S* ($[\theta]^{max}_{284}$ 67,920, $[\theta]^{max}_{248}$ 78,260,

MeOH). The observed CD effects are virtually identical to those reported for aerothionin (11). Compound (**3**) is 11-*epi*-fistularin-3.

All three isolates showed antibacterial activity towards *Bacillus subtilis* and *Micrococcus luteus* in a bioautographic test system (12). No activity towards the gram negative bacterium, *Escherichia coli* and the fungus, *Penicillium oxalicum* was observed for **1-3**. Compound (**3**) was not cytotoxic towards KB-cells (IC_{50}>20 μg/ml) in contrast to fistularin-3 (3) but shows moderate cytotoxicity towards cultured breast cancer cells (BC1, IC_{50} 5.9 μg/ml; ZR-75-1, IC_{50} 4.5 μg/ml). In *in vitro* antimalarial assays compound **3** was also found to be inactive.

EXPERIMENTAL

General Experimental Procedures: As per reference 12.

CD spectra were recorded in MeOH using a JASCO J-500A spectropolarimeter.

Materials: Sponge material was collected by divers, using SCUBA, from Noggin Reef, The Great Barrier Reef, Queensland, Australia. The animals were all obtained from depths of 9-12 m during May of 1987, and then deep frozen. A voucher specimen is deposited at the Muséum d'Histoire Naturelle, Geneva, Switzerland (voucher number VV25).

Extraction and Isolation: Deep frozen sponge tissue was freeze dried. Dry tissue (432.0 g) was extracted at room temperature overnight with dichloromethane (2.5 l) and then with methanol (3 l). From both extracts the dichloromethane solubles (67.2 g (15.6 %)) were taken and shaken with a 1:1 mixture (total volume 1 l) of CH_2Cl_2 and hexane. Undissolved material was collected and subjected to vacuum liquid chromatography (vlc) and reverse phase hplc separation (1:1 mixture of acetonitrile and water) to afford three alkaloids.

Agelorin A (1): (45.4 mg, 0.01 %); an amorphous off white powder, $[\alpha]_D^{25}$ -17.1° (c, 1.26, acetone); ir ν_{max} 3360, 2930, 1705, 1660, 1600, 1540, 1260, 1095 cm^{-1}; uv λ_{max} (EtOH) 220 (ε 12,600), 250 (ε 7740) nm; ^1H nmr see Table 1; ^{13}C nmr see Table 2; FABms, *m/z* (% rel. int.) 1092, 1090, 1088, 1086, 1084, 1082, (M^+, 1, 1.8, 1.8, 2, 1.8, 1.8, 1), 707 (0.8), 705 (1), 703 (0.8), 427 (10), 425 (18), 423 (10).

Agelorin B (2): (5.4 mg, 0.001 %); an amorphous white powder, $[\alpha]_D^{25}$ +50.0° (c, 0.27, acetone); ir ν_{max} 3350, 2920, 1700, 1665, 1605, 1540, 1255, 1095 910 cm^{-1}; uv λ_{max} (EtOH) 215 (ε 12,570), 250 (ε 7940) nm; ^1H nmr see Table 1; ^{13}C nmr see Table 2; FABms, *m/z* (% rel. int.) 1092, 1090, 1088, 1086, 1084, 1082, (M^+, <1), 707 (1), 705 (1), 703 (1), 427 (6), 425 (10), 423 (6).

11-*epi*-Fistularin-3 (3): (274.0 mg, 0.064 %); an amorphous white solid, $[\alpha]_D^{25}$ +65.2° (c, 1.04, acetone); ir ν_{max} 3350, 2920, 1655, 1545 cm^{-1}; uv λ_{max} (EtOH) 233 (ε 13,500), 283 (ε , 2,650) nm; ^1H nmr (300 MHz, Py-d_5) δ 3.22 (d, *J* 18.2 Hz, 1H), 3.28 (d, *J* 18.2 Hz, 1H), 3.43 (s, 6H), 3.61 (m, 2H), 3.78 (m, 2H), 4.01 (m, 2H), 4.14 (m, 1H), 4.24 (d, *J* 18.2 Hz, 1H), 4.25 (d, *J* 18.2 Hz, 1H), 4.42 (d, *J* 9.8 Hz, 1H), 4.45 (d, *J* 10.1 Hz, 1H), 4.58 (br m, 1H), 5.11 (br m, 1H), 6.39 (s, 1H), 6.45 (s, 1H), 7.11 (br m, 1H), 7.61 (br m, 1H), 7.72 (s, 2H), 9.21 (t, *J* 5.4 Hz, 1H), 9.60 (t, *J* 6.0 Hz, 1H); ^{13}C nmr (75.5 MHz, Py-d_5) 40.1 (tx2), 43.8 (t), 47.9 (t), 59.7 (qx2), 69.3 (d), 70.7 (d), 74.6 (dx2), 75.9 (t), 91.7 (sx2), 115.1 (sx2), 118.2 (sx2), 121.6(s), 121.7 (s), 130.9 (dx2), 132.1 (d), 132.2 (d), 143.3 (s), 147.9 (sx2), 152.1 (s), 155.1 (sx2), 160.4 (sx2) ppm, and Table 2; FABms, *m/z* (% rel. int.) 1141, 1139, 1137, 1135, 1133, ($[M+Na]^+$, 39, 64, 100, 80, 48).

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