Mauritiamine, a New Antifouling Oroidin Dimer from the Marine Sponge *Agelas mauritiana*

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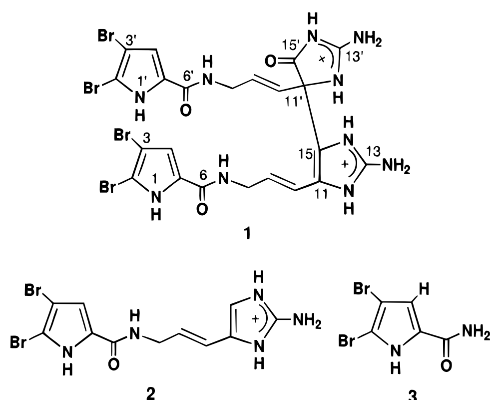
A new oroidin dimer, mauritiamine (**1**), has been isolated from the marine sponge *Agelas mauritiana* along with the known oroidin (**2**) and 4,5-dibromopyrrole-2-carbamide (**3**). Their structures were determined by spectral analysis. Compounds **1** and **2** inhibited larval metamorphosis of the barnacle *Balanus amphitrite* with ED_{50} values of 15 and 19 μg/mL, respectively, while **3** promoted larval metamorphosis of the ascidian *Ciona savignyi* at a concentration of 2.5 μg/mL.

Sessile marine organisms, such as barnacles, mussels, hydroids, bryozoans, and ascidians, cause serious problems by settling on fishing nets, hulls of ships, and cooling systems of power plants. Organotin compounds have been widely used for control of these organisms, but they are known to be toxic to marine biota.[1] Therefore, nontoxic antifouling substances are urgently needed. Sessile marine organisms possess various defense systems against predators, larvae of other sessile organisms, and pathogenic microorganisms. Hence, their secondary metabolites functioning as chemical defense substances might be potential nontoxic antifouling agents. During our search for antifouling compounds from Japanese marine invertebrates, we isolated three new diterpene formamides, kalihinenes X, Y, and Z, which inhibited larval settlement and metamorphosis of the barnacle *Balanus amphitrite*.[2] Subsequently, we found antifouling activity against *B. amphitrite* as well as metamorphosis-inducing activity on ascidian larvae[3−8] in the MeOH extract of the marine sponge *Agelas mauritiana* (Carter) (family Agelasidae, order Axinellida) collected off Hachijo-jima Island, Japan. Bioassay-guided isolation afforded a new oroidin dimer named mauritiamine (**1**) and the known oroidin (**2**)[9,10] as antifouling constituents along with 4,5-dibromopyrrole-2-carbamide (**3**)[9] as a larval metamorphosis-promoting compound.



The sponge (1.1 kg, wet wt) was extracted with MeOH. The concentrated aqueous residue was extracted with Et_2O and then *n*-BuOH. The *n*-BuOH layer, which showed both metamorphosis-inducing and antifouling activities, was fractionated by Sephadex LH-20 gel-filtration (EtOH−H_2O−*n*-BuOH) and ODS column chromatography (aqueous MeOH). Active fractions were purified by reversed-phase HPLC (CH_3CN−H_2O−TFA) to afford **1** (yield: 0.012%, wet wt), **2** (0.24%), and **3** (0.093%). Oroidin (**2**)[9,10] and 4,5-dibromopyrrole-2-carbamide (**3**)[9] were readily identified by comparison of spectral data with those in literature.

The positive FAB MS of mauritiamine (**1**) gave $(M + H)^+$ ion peaks at *m/z* 789/791/793/795/797 (intensity, 1:4:6:4:1), indicating that **1** contained four bromine atoms. The ion peak at *m/z* 793 corresponded to a formula of $C_{22}H_{21}{}^{79}Br_2{}^{81}Br_2N_{10}O_3$ (Δ −2.4 mmu). The ^{1}H-NMR spectrum (MeOH-d_4) exhibited four methylene protons at δ 3.92 (1H, dd, J = 16.5, 4.7 Hz, Ha-8′), 4.01 (1H, dd, J = 16.5, 4.7 Hz, Hb-8′), 4.01 (1H, dd, J = 16.5, 5.3 Hz, Ha-8), and 4.09 (1H, dd, J = 16.5, 5.3 Hz, Hb-8), four olefinic protons at δ 5.89 (1H, d, J = 15.5 Hz, H-10′), 5.99 (1H, dt, J = 15.5, 4.7 Hz, H-9′), 6.08 (1H, dt, J = 16.1, 5.3 Hz, H-9), and 6.47 (1H, d, J = 16.1 Hz, H-10), and two aromatic protons at δ 6.84 and 6.87 (each 1H, s, H-4 and H-4′). Interpretation of the ^{1}H−^{1}H COSY spectrum revealed two allyl groups, C(8)H_2C-(9)H=C(10)H and C(8′)H_2C(9′)H=C(10′)H. Ten D_2O-exchangeable protons were observed in the ^{1}H NMR spectrum in DMSO-d_6 (see Experimental Section). ^{13}C-NMR data were reminiscent of oxysceptrins,[11,12] except for C9−C11 and C9′−C11′. The absence of H-15 and the presence of two characteristic quarternary carbon signals at δ 66.8 (C11′) and 120.6 (C15) in **1** suggested that C15 in an aminoimidazole unit was linked to C11′ in an aminoimidazolinone unit. Connectivities of C10−C11−C15−C11′−C10′−C9′ were secured by HMBC cross peaks [δ 6.08 (H-9) and 6.47 (H-10)/δ 124.8 (C11); δ 5.99 (H-9′)/δ 66.8 (C11′); δ 5.89 (H-10′)/δ 66.8 and δ 120.6 (C15)]. Thus, the structure of **1** was determined; specific rotation, $[\alpha]^{25}_D$ 0.0° (*c* 0.14, MeOH), indicated that **1** was a racemate, which was confirmed by the CD spectrum; the CD curve was flat between 200 and 400 nm. Although ageliferins,[11,13] sceptrins,[11,14] and oxysceptrin[11,12] were considered oroidin dimers formed by Diels−Alder type cycloaddition, they are optically active. Mauritiamine (**1**) was optically inactive and is a new type of oroidin dimer with oxidation at C15′. A possible

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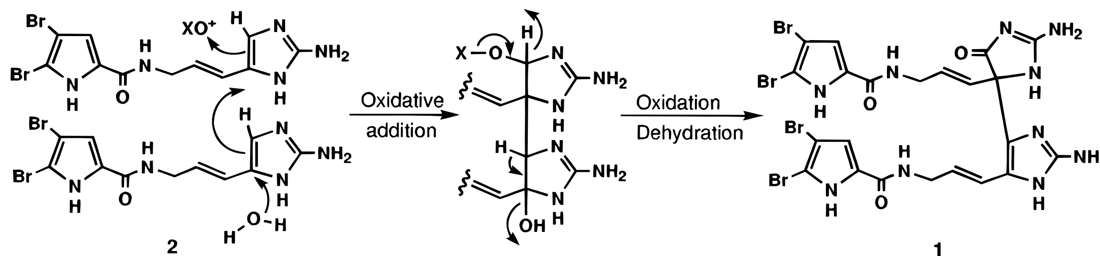

Figure 1. A possible biogenetic pathway of mauritiamine (**1**) from oroidin (**2**).

biogenetic pathway for **1** is shown in Figure 1, which includes two oxidation steps.

Mauritiamine (**1**) and oroidin (**2**) inhibited larval metamorphosis of the barnacle *Balanus amphitrite* at ED_{50} values of 15 and 19 μg/mL, respectively; they were not toxic at a concentration of 30 μg/mL. 4,5-Dibromopyrrole-2-carbamide (**3**) promoted larval metamorphosis of the ascidian *Ciona savignyi* at a concentration of 2.5 μg/mL. Compounds **1** and **2** exhibited moderate antibacterial activity against *Flavobacterium marinotypicum* with growth inhibitory zones of 10 and 15 mm at 10 μg/disk, respectively.

Experimental Section

General Experimental Procedures. Optical rotation was determined with a JASCO DIP-1000 digital polarimeter. CD spectrum was measured on a JASCO J-720W spectropolarimeter in MeOH. IR spectrum was measured on a JASCO IR-700 spectrometer. UV spectrum was recorded on a Hitachi U-2000 spectrometer in EtOH. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker ARX-500 NMR spectrometer at 27 °C in MeOH-d_4. Residual CHD_2OD (3.30 ppm) and CD_3OD (49.0 ppm) signals were used as internal standards. Multiplicities of ^{13}C signals were determined by HMQC experiments. Mass spectra were measured on a JEOL SX-102 mass spectrometer.

Collection and Isolation. The marine sponge was collected by SCUBA at a depth of 3 m off Hachijo-jima Island and identified as *Agelas mauritiana* by Dr. Rob van Soest. A voucher specimen (ZMA POR. 11017) was deposited at the Institute for Systematics and Population Biology, University of Amsterdam. The frozen sponge (1.1 kg, wet wt) was extracted with MeOH. The concentrated aqueous residue was successively extracted with Et_2O and *n*-BuOH. Part (2.00 g) of the *n*-BuOH layer (21.83 g) was gel-filtered through a Sephadex LH-20 column (2.2 × 70 cm, Pharmacia) with EtOH−H_2O−*n*-BuOH (3:2:1), and half of the active fractions (240−320 mL, 0.46 g) was fractionated by a reversed-phase column (2.2 × 30 cm; 30, 60, and 80% MeOH-H_2O, each 300 mL). The second fraction (60% MeOH−H_2O) afforded oroidin (**2**) and 4,5-dibromopyrrole-2-carbamide (**3**) after purification by HPLC (YMC-Pack ODS, 5 μm, 10 × 250 mm, YMC Co., Ltd.; 40% CH_3CN−H_2O containing 0.01% TFA; 2.0 mL/min). The third fraction (80% MeOH−H_2O) was purified by HPLC (YMC-Pack ODS, 5 μm, 10 × 250 mm; 40% CH_3CN−H_2O containing 0.01% TFA; 2.0 mL/min) to afford mauritiamine (**1**) (t_R 11 min; 12.0 mg; yield: 0.012%, wet wt).

Mauritiamine (1): colorless solid; $[\alpha]^{25}_D$ 0.0° (*c* 0.14, MeOH); IR (film) ν_{max} 3300, 1680, 1640, 1570, 1420, 1210, 1140, 840, 800, and 720 cm^{-1}; UV (EtOH) λ_{max} 222 (sh, ϵ 14 500) and 278 nm (18 400); FABMS (positive, glycerol) *m/z* 789/791/793/795/797 (M$^+$, intensity, 1:4:6:4:1); HRFABMS (positive, glycerol) *m/z* 792.8466 (calcd for $C_{22}H_{21}{}^{79}Br_2{}^{81}Br_2N_{10}O_3$, 792.8490); ^{1}H and ^{13}C NMR data in CD_3OD, see Table 1. ^{1}H NMR (DMSO-d_6) δ 3.88 (2H, s), 3.96 (2H, m), 5.81 (1H, d, J = 15.1 Hz), 5.84 (1H, d, J = 15.1 Hz), 6.05 (1H, dt, J = 16.1, 6.0 Hz), 6.45 (1H, d J = 16.1 Hz), 6.94 (1H, s), 6.95 (1H, s), 7.35 (2H, br s, 2 × NH), 8.40 (1H, t, J = 6.0 Hz, NH), 8.41 (1H, t, J = 6.0 Hz, NH), 9.36 (1H, br s, NH), 12.10 (1H, br s, NH), 12.58 (2H, br s, 2 × NH), and 12.67 (2H, s, 2 × NH).

Table 1. ^{1}H- and ^{13}C-NMR Data for **1**[a]

no.	^{1}H[b]	^{13}C[c]	HMBC
2′		106.4[e] s	
2′		106.5[e] s	
3′		100.13[f] s	
3′		100.09[f] s	
4	6.87[d] s	114.6 d	2, 5
4′	6.84[d] s	114.6 d	2′, 5′
5		128.6[g] s	
5′		128.5[g] s	
6		161.7 s	
6′		161.9 s	
8a	4.01 dd, 16.5, 5.3	41.7 t	9, 10
8b	4.09 dd, 16.5, 5.3		9, 10
8′a	3.92 dd, 16.5, 4.7	41.1 t	9′, 10′
8′b	4.01 dd, 16.5, 4.7		9′, 10′
9	6.08 dt, 16.1, 5.3	130.5 d	8, 11
9′	5.99 dt, 15.5, 4.7	132.1 d	8′, 10′, 11′
10	6.47 d, 16.1	117.0 d	8, 9, 11
10′	5.89 d, 15.5	127.2 d	15, 8′, 9′, 11′
11		124.8 s	
11′		66.8 s	
13		149.0 s	
13′		149.0 s	
15		120.6 s	
15′		194.7 s	

[a] Data are recorded in MeOH-d_4 at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 27 °C. [b] Multiplicities and coupling constants are given in Hz. [c] Multiplicities were determined by an HMQC experiment. [d−g] These resonances may be interchangeable.

Antifouling Assays against Barnacle Larvae. Cyprid larvae were obtained by the method previously reported.[15] Samples dissolved in EtOH were applied into each well of 24-well polystyrene plates. After removal of the solvent, 2 mL of 80% seawater and six cyprid larvae were added to each well, and the plates were incubated for 48 h at 25 °C in the dark. The number of settled and metamorphosed larvae was counted under a microscope.

Metamorphosis-Promoting Assays on Ascidian Larvae. Fifteen newly hatched larvae and 4 mL of artificial seawater (460 mM NaCl 10.1 mM KCl, 9.2 mM $CaCl_2$, 35.9 mM $MgCl_2$·H_2O, 17.5 mM $MgSO_4$·$7H_2O$, 10 mM Tris−HCl, pH 8.2) were added to each well of 12-well polystyrene plates. The sample to be assayed was added to the larvae in DMSO solution (10 μL), and to

the control group 10 μL of DMSO was added. The plates were incubated for 10 h at 18 °C in the dark. The number of settled and metamorphosed larvae was counted under a microscope.

Antibacterial Assay. Antibacterial activity was determined by the paper disk method. A paper disk (thick, 8 mm, Toyo Roshi Kaisha, Ltd., Tokyo) impregnated with 10 μg of sample was placed on agar plate containing *F. marinotypicum* ATCC 19260, and the plate was incubated for 24 h at 28 °C.

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