New Agelasine Compound from the Marine Sponge *Agelas mauritiana* as an Antifouling Substance against Macroalgae

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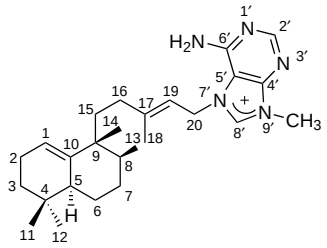
A new diterpene−alkaloid *epi*-agelasine C was isolated from the marine sponge *Agelas mauritiana* as an antifouling substance active against macroalgae. The structure of this substance was elucidated by spectral means.

Marine biofouling organisms such as blue mussels, barnacles, and macroalgae cause serious problems to ships' hulls, the cooling system of power plants, and fishing nets.[1] Organotin compounds such as TBTO [bis(*n*-tributyltin)oxide] have been used as antifouling agents against these organisms. However, stern warnings have been issued regarding the toxic effects of such heavy-metal compounds on the marine environment.[2,3] Therefore, antifouling substances with no or reduced toxicity must be discovered or developed. Our screening method[4] for antifouling activity against the macroalga *Ulva conglobata* enabled us to isolate an antifouling substance from *Agelas mauritiana* Carter (order Agelasida, family Agelasidae) collected from Pohnpei. We report here the structure of this compound.

A. mauritiana was soaked in ethanol for 24 h at room temperature, and the extract was evaporated and fractionated into water-soluble, ether-soluble, and water- and ether-insoluble fractions. Antifouling activity was recognized in the water- and ether-insoluble fraction. This fraction was chromatographed on silica gel and ODS columns. Further purification was made by 2-fold reversed-phase HPLC to afford the antifouling substance, *epi*-agelasine C (**1**).[5]



epi-agelasine C (**1**)

epi-Agelasine C (**1**) was obtained as a colorless powder, and its molecular formula was established as $C_{26}H_{39}N_5$ by HRFABMS. The ^{1}H- and ^{13}C-NMR spectra of this antifouling compound (see Table 1) were very similar to those of agelasine C.[5] The presence of a 9-methyladenium moiety was indicated by the observed signals for an *N*-methyl singlet, one olefinic proton singlet, and one amino proton (δ 3.96, 9.45, 7.85). Moreover, signals corresponding to a diterpene moiety were also observed as three methyl singlets (δ 1.06, 0.83, 0.84), two methyl doublets (δ 0.82, 1.75), and one vinyl proton (δ 5.33, J = 3.0 Hz). The *E* configuration of the linear olefin was determined by the vinyl methyl signal (δ 15.18) in the ^{13}C-NMR spectrum and by a NOESY experiment. The planar structure of **1** was confirmed by the correlation peaks observed in an HMBC experiment (Figure 1).

The stereostructure of *epi*-agelasine C was established from its NOE difference spectrum. NOEs were detected between H-2α/H-12, H-12/H-5α, H-5α/H-15, and H-6β/H-13 (see Figure 2). Thus, the structure of the new compound was assigned as a stereoisomer of agelasine C.

The antifouling activity of this compound and of $CuSO_4$ as a positive control against *Ulva* spores is shown in Figure 3. This result indicates that the activity of *epi*-agelasine C was not as high as that of $CuSO_4$. However, *epi*-agelasine C showed lethal activity against *Ulva* fronds at 50 ppm. This activity of *epi*-agelasine C was one-fifth that of $CuSO_4$. Antimicroalgal activity against *Oscillatoria amphibia* (Cyanophyceae), *Skeletonema costatum* (Diatomophyceae), *Brachiomonas submaria* (Chlorophyceae), and *Prorocentrum micans* (Dinophyceae) was observed at 1.0−2.5 ppm, so this compound seems to be useful as a measure to counter red tide. This compound did not exhibit antibacterial activity against *Staphylococcus aureus*, *Vibrio costicola*, *Escherichia coli*, and *Bacillus subtilis*.

Experimental Section

General Experimental Procedures. Optical rotation was determined with a HORIBA SEPA-300 high-sensitivity polarimeter. The IR spectrum was measured on a JASCO FT/IR-7000 spectrometer. The UV spectrum was recorded on a Shimazu UV-2200A spectrometer in MeOH. NMR experiments were carried out with a Varian Unity 500 NMR spectrometer using CD_3OD and DMSO-d_6 as the solvents for ^{1}H-NMR and CD_3OD for ^{13}C-NMR. HRFABMS was measured with a JEOL JMS-SX102 mass spectrometer.

Assay for Antifouling Activity against Spores of *U. conglobata* Kjellman. A test sample was dissolved in 0.1 mL of MeOH and dried in a polystyrene Petri dish (35 mm in diameter) at 25 °C before 5 mL PES medium was added. A total of 1500−3000 spores of *Ulva* were incubated in each Petri dish, triplicate dishes being prepared in each set of experiments. After a 5-day incubation at 20−25 °C under 3000 lx with a 14L:10D photoperiod, the number of germinated spores, ungerminated spores, and unattached spores on 1 cm^2 of the Petri dish were counted, and the germination rate and attachment rate were calculated.[4]

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Table 1. ^{1}H-[a] and ^{13}C-NMR[b] Data for **1** in CD_3OD and DMSO-d_6

no.	chemical shift (ppm) and multiplicity				HMBC
	^{1}H[c]	^{1}H[d]	^{13}C[c]	^{13}C[d]	
1	5.40 (dd, 3.0, 4.6)	5.33 (dd, 3.7, 3.7)	116.72 (d)	116.66 (d)	5
2	2.05 (m)	2.73 (m)	22.30 (t)	22.84 (t)	1, 3, 10
3	1.12, 1.41 (m)		30.45 (t)	30.92 (t)	2, 4, 11, 12
4			30.37 (s)	30.86 (s)	
5	1.62 (m)	1.43 (m)	43.21 (d)	43.11 (d)	6, 10
6	1.13, 1.84 (m)		29.40 (t)	29.63 (t)	
7	1.46, 1.55 (m)		30.55 (t)	31.36 (t)	
8	1.28 (m)		44.06 (d)	44.08 (d)	
9			41.74 (s)	42.15 (s)	
10			145.04 (s)	145.43 (s)	
11	0.86 (s)	0.83 (s)	26.37 (q)	27.56 (q)	3, 4, 5, 12
12	0.90 (s)	0.84 (s)	26.30 (q)	27.44 (q)	3, 4, 5, 11
13	0.87 (d, 6.9)	0.82 (d, 6.8)	14.80 (q)	16.25 (q)	7, 8, 9
14	1.06 (s)	1.06 (s)	21.70 (q)	22.70 (q)	8, 9, 10, 15
15	1.25, 1.86 (m)		28.65 (t)	28.89 (t)	9, 10, 14, 16
16	1.85, 1.95 (m)	3.25 (m)	33.27 (t)	33.58 (t)	15, 17, 19
17			148.22 (s)	146.66 (s)	
18	1.58 (d, 1.2)	1.75 (d, 1.2)	15.18 (q)	16.64 (q)	16, 17, 19
19	5.51 (ddq, 7.1, 7.1, 1.2)	5.43 (ddq, 7.2, 7.2, 1.2)	113.32 (d)	114.76 (d)	16, 18, 20
20	5.19 (d, 7.1)	5.17 (d, 7.0)	46.80 (t)	44.06 (t)	17, 19, 5′, 8′
2′	8.45 (s)	8.45 (s)	155.20 (d)	155.38 (d)	4′, 6′
4′			148.97 (s)	148.93 (s)	
5′			109.21 (s)	109.21 (s)	
6′		7.85 (brs)	152.19 (s)	152.32 (s)	
8′		9.45 (s)	119.45 (d)	119.48 (d)	
9′-CH	4.00 (s)	3.96 (s)	30.04 (q)	30.77 (q)	4′, 8′

[a] Data were recorded at 500 MHz. Multiplicity and coupling constants are given in Hz. [b] Data were recorded at 125 MHz. Multiplicity is given in DEPT. [c] Data were recorded in CD_3OD. [d] Date were recorded in DMSO-d_6.

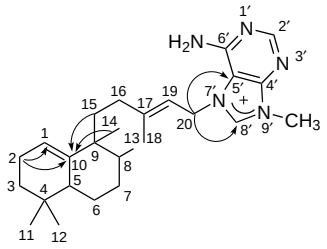

Figure 1. HMBC correlations for **1**.

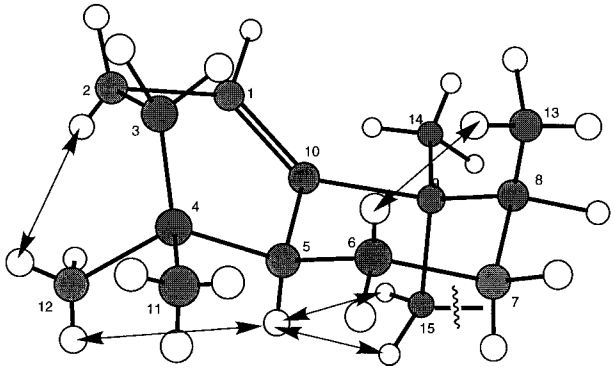

Figure 2. NOE correlations for **1**.

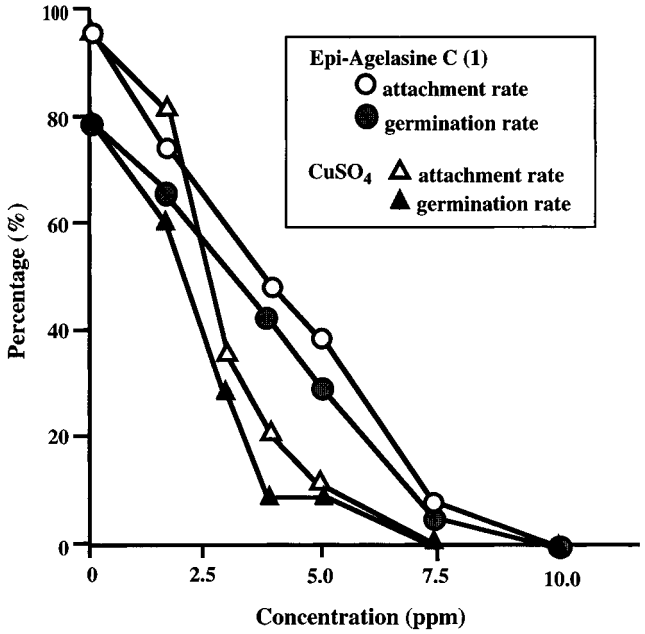

Figure 3. Effect of **1** and $CuSO_4$ on the attachment rate and germination rate of spores of *Ulva conglobate* at various concentrations.

Assay for Lethal Activity against *Ulva* Fronds. A test sample was dissolved in 0.1 mL of MeOH and dried in a polystyrene Petri dish (35 mm in diameter) before 5 mL of a PES medium was added. An *Ulva* frond (about 2.0 × 2.0 cm) was incubated under the same conditions as those already described, triplicate dishes being prepared in each set of experiments. The lethal activity was evaluated by direct visual observation.

Assay for Antimicroalgal Activity. A test sample was dissolved in 10 μL of MeOH and dried in 96-well polystyrene microplates. F/2 medium[6] (200 μL) and *Oscillatoria amphibia, Skeletonema costatum, Brachiomonas submaria*, or *Prorocentrum micans* were incubated in each well at 25 °C under 2000 lx with a 12L:12D photoperiod. Samples of 10 and 500 μg/mL of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea were used as positive controls, and duplicate dishes were prepared in each set of experiments. The activity against *P. micans* and *B. submaria* was evaluated with an optical microscope, while that against *O. amphibia* and *S. costatum* was evaluated by direct visual observation.[7]

Sponge Material. A specimen of *A. mauritiana* was collected at Pohnpei during a voyage on the Sohgen Maru and identified by Prof. P. Bergquist (University of Auckland). It was kept frozen until used.

Extraction and Isolation. *A. mauritiana* was soaked in ethanol for 24 h at room temperature and the extract evaporated and fractionated into water-soluble, ether-soluble, and water- and ether-insoluble fractions. Antifouling activity was recognized in the water- and ether-insoluble fraction. This fraction was chromatographed on silica gel 60 (230−400 mesh, Merck, Germany) with $CHCl_3$ and MeOH and on ODS (Cosmosil 140 C18-OPN, Nacalai Tesque) with MeOH (10−50%). Further purification of the active fraction was performed twice by HPLC using an ODS column (TSK gel ODS-80Ts, 20 mm × 25 cm, Tosoh) with CH_3CN-H_2O−TFA (80:20:0.1) and MeOH$-H_2O$ (75:25) to afford *epi*-agelasine C (**1**) (7 mg), 0.000 47% from fresh *A. mauritiana*.

***epi*-Agelasine C (1):** colorless powder; $[\alpha]^{25}_D$ +33.9° (*c* 0.056, MeOH); IR (KBr) ν_{max} 3440, 2926, 2860, 1649, 1615, 1460, 1383, 1301, 1205, 1180, 1131, 833, 803, 721 cm^{-1}; UV (MeOH) λ_{max} 273 nm (ϵ 8140); HRFABMS *m/z* $[M + H]^+$ 422.3288, calcd for $C_{26}H_{40}N_5$ 422.3284; ^1H- and ^{13}C-NMR data are shown in Table 1.

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