

Article

## An Acetylenic Alkaloid from the Calcareous Sponge *Leucetta* sp.

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**Abstract:** A new acetylenic alkaloid was isolated from the sponge *Leucetta* sp. The structure was established by analyzing spectroscopic data. The alkaloid showed cytotoxicity IC<sub>50</sub> 2.5 µg/mL against NBT-T2 cells.

**Keywords:** sponge; acetylene; alkaloid; cytotoxicity

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### 1. Introduction

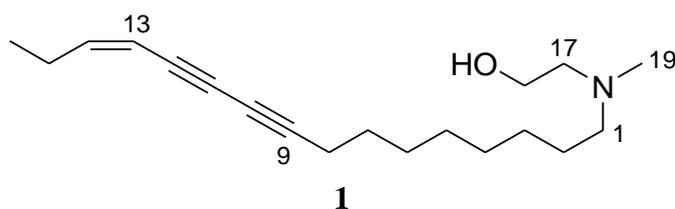
More than 8000 species of sponges have been recorded from intertidal to deep oceanic floors, from tropical to polar regions, and from marine to freshwater environments [1]. The majority of sponges prefer to grow in shallow warm waters such as coral reefs. Since coral reef sponges have been major subjects from an early stage of marine natural products study, they are recognized as the most prolific sources of diverse bioactive secondary metabolites among macrobenthos [2]. However, dereplication has become an increasingly major issue in natural product chemistry as it is common to encounter known molecules probably reflecting the fact that many overlapping specimens have been examined. To overcome this problem and to increase the diversity of source organisms, we collected sponges in a coral reef twilight zone (50–100 m), where the depth is too deep for conventional scuba diving and the fauna there have not been well examined. A few examples from the zone include seragamides [3] and a unique fatty acid [4]. In this short note, we describe a structure of a new cytotoxic acetylenic alkaloid from a sponge collected in the zone.

## 2. Results and Discussion

The small sponge was extracted with acetone and its lipophilic portion showing cytotoxicity at 5  $\mu\text{g/mL}$  was subjected to chromatographic separation to give compound **1** (0.088% from sponge). Compound **1** was found to have a molecular formula as  $\text{C}_{19}\text{H}_{31}\text{NO}$  indicating five degrees of unsaturation, which can be explained by the presence of two substituted acetylenes ( $\delta_{\text{C}}$  65.1 s, 71.9 s, 78.1 s, 84.8 s;  $2334\text{ cm}^{-1}$ ) and one *cis* double bond ( $\delta_{\text{H}}$  5.44 brd,  $J = 10.8\text{ Hz}$ , 6.04 dt,  $J = 10.8, 7.5\text{ Hz}$ ;  $\delta_{\text{C}}$  107.5 d, 149.2 d). Other structural features include an *N*-methyl group ( $\delta_{\text{H}}$  2.24 s (3H);  $\delta_{\text{C}}$  41.5 q), a terminal ethyl group ( $\delta_{\text{H}}$  1.01 t (3H), 2.36 m (2H)), a primary alcohol ( $\delta_{\text{H}}$  3.58 t (2H);  $\delta_{\text{C}}$  58.2 t;  $3407\text{ cm}^{-1}$ ), and nine methylenes ( $\delta_{\text{H}}$  1.30–2.32, 2.39 m (2H), 2.52 t (2H)). The primary alcohol group was coupled ( $J = 6.7\text{ Hz}$ ) to a methylene at  $\delta_{\text{H}}$  2.52, which showed HMBC correlation with the *N*-methyl group at  $\delta_{\text{H}}$  2.24 and also with another methylene at  $\delta_{\text{H}}$  2.39 indicating the presence of a tertiary amine with these substituents. The terminal ethyl group was connected to the double bond by observing COSY (H-13/H-14,15, H-14/H-15) and HMBC (H-13/C-15, H-15/C-13,14,16) cross peaks. This double bond was found to be conjugated to a diyne group connected to methylenes (H-13/C-11, H-14/C-12, H-8/C-9,10,11,12). By elucidating the remaining methylenes as a linear structural unit with HMBC (H-7/C-6,8,9, H-1/C-2,3, H-2/C-3), the whole structure was assigned as 2-(hexadec-13-ene-9,11-diynyl-methyl-amino)-ethanol (Figure 1).

A number of polyacetylenic molecules have been reported from marine sources [4–7], however, compound **1** is the first example of polyacetylene with an alkaloidal functionality from a marine sponge.

**Figure 1.** Structure of compound **1**.



## 3. Experimental Section

### 3.1. General Procedures

FTIR spectrum was taken on a Varian FTS-3000 instrument.  $^1\text{H}$ ,  $^{13}\text{C}$  and 2D (COSY, HSQC, HMBC) NMR spectra were obtained on a Bruker Avance III 500 spectrometer in  $\text{CDCl}_3$  with reference to an internal standard of TMS. Chemical shifts and coupling constants were given as  $\delta$  and Hz. ESIMS was measured on a Jeol JMS-T100LP instrument.

### 3.2. Animal Material

The sponge, an undescribed *Leucetta* sp. (Figure 2, Leucettidae, Clathrinida, Calcarea), was collected at 50 m depth off Kume Island in Okinawa on September, 2009 and kept frozen until extraction. The sponge was identified by one of us (NJdV) and deposited at NCB Naturalis under the code RMNH POR 3927. The sponge is small, pink, pyriform and has one prominent osculum. The

skeleton consists of regular triactines and tetractines densely and irregularly scattered throughout the ectosome and choanosome. The rays of the spicules range from 5–110  $\mu\text{m}$ , of which the smaller ones are juvenile forms.

**Figure 2.** The sponge *Leucetta* sp.



### 3.3. Isolation of Alkaloid 1

The sponge (wet, 3.54 g) was extracted two times with acetone (30 mL). The resulting residue (0.31 g) was partitioned between EtOAc and water, and the organic layer was concentrated to give 14.7 mg of the extract. It was then separated on a silica gel column with stepwise elution using *n*-hexane-EtOAc (2-1, 1-1, and 1-2), EtOAc-MeOH (1-5), and MeOH to give a total of 7 fractions. Fraction 7 contained 3.1 mg (0.087%) of the alkaloid 1.

### 3.4. Alkaloid 1

Yellow oil, FTIR 3407, 2931, 2857, 2334, 1458, 1043  $\text{cm}^{-1}$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR: see Table 1. ESIMS obsd  $m/z$  290.24798, calcd for  $\text{C}_{19}\text{H}_{32}\text{NO}^+$  290.24784.

### 3.5. Cytotoxicity Testing

NBT-T2 cells (BRC-1370, purchased from Riken BioResource Center) were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum and antimicrobials under standard protocol and seeded in 200  $\mu\text{L}$  wells. After preincubation (37  $^{\circ}\text{C}$ , 24 h), cells were exposed to graded concentrations of compound 1 in duplicate (37  $^{\circ}\text{C}$ , 48 h). Then, the cells were treated with MTT solution (15  $\mu\text{L}$ , 5 mg/mL in PBS) after removal of the medium and incubated for 3 h. The residual formazan was dissolved in DMSO (100  $\mu\text{L}$ ) and the absorbance was measured with a Tecan sunrise microplate reader at 560 nm. The  $\text{IC}_{50}$  values were found by plotting the absorbance values against concentrations. The alkaloid showed cytotoxicity  $\text{IC}_{50}$  2.5  $\mu\text{g}/\text{mL}$  against NBT-T2 cells.

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for compound **1** in  $\text{CDCl}_3$ .

C#	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}$ (mult., $J$ in Hz)	COSY	HMBC
1	57.7 t	2.39 m	H-2	C-2,3,17,19
2	27.2 t	1.46 m	H-1,3	C-3
3	27.2 t	1.30 m	H-2	C-1
4	29.3 t	1.30 m		
5	29.0 t	1.30 m		
6	28.8 t	1.39 m		C-5
7	28.2 t	1.53 m	H-6,8	C-6,8,9
8	19.6 t	2.32 m	H-7	C-9,10,11,12
9	84.8 s	-		
10	65.1 s	-		
11	78.1 s	-		
12	71.9 s	-		
13	107.5 d	5.44 brd, $J = 10.8$ Hz	H-14,15	C-11,15
14	149.2 d	6.04 dt, $J = 10.8, 7.5$ Hz	H-13,15	C-10
15	24.1 t	2.36 m	H-13,14,16	C-13,14,16
16	13.3 q	1.01 t, $J = 7.6$ Hz	H-15	C-14,15
17	58.7 t	2.52 t, $J = 6.7$ Hz	H-18	C-1
18	58.2 t	3.58 t, $J = 6.7$ Hz	H-17	C-17
19	41.5 q	2.24 s		C-17

<sup>a</sup> Multiplicities were determined by DEPT experiments.

#### 4. Conclusions

A new acetylenic alkaloid **1** was characterized with spectroscopic methods. Together with our previous work [4], Calcareous sponges are still promising sources of unique bioactive molecules.

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*Samples Availability:* As the original amount of compound **1** was small, we do not plan to distribute.

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