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Callyspongamide A, a New Cytotoxic Polyacetylenic Amide from the Red Sea Sponge *Callyspongia fistularis*

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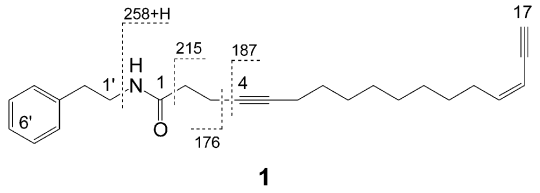
Callyspongamide A (**1**), a new cytotoxic polyacetylenic amide, has been isolated from the marine sponge *Callyspongia fistularis* collected in the Red Sea. Callyspongamide A is an amide derivative of a C_{17}-polyacetylenic acid and phenethylamine. It represents a new class of secondary metabolites within the family Callyspongiidae. Its structure was determined on the basis of 1D and 2D (COSY, HOHAHA, HMQC, and HMBC) NMR studies and high-resolution mass spectral measurement.

Marine sponges, particularly sponges of the order Haplosclerida, have been proven to be a rich source of straight-chain polyacetylenic compounds with different chain length and oxygenation pattern.[2] Many of the reported polyacetylenic compounds showed significant biological activity.[3−16]

In our continued search for pharmaceutically useful agents from marine invertebrates, we have isolated a new polyacetylenic amide, callyspongamide A (**1**), as a cytotoxic constituent from the Red Sea sponge *Callyspongia fistularis* (family Callyspongiidae). The structure determination of **1** was based on extensive NMR studies and high-resolution mass spectral determination.

Frozen specimens of the sponge (165 g) were extracted with $MeOH/CH_2Cl_2$ (1:1). The combined extracts were dried, and the residue was partitioned successively between 90% aqueous MeOH and hexane, and 60% MeOH and CH_2Cl_2. The organic extracts were combined and subjected successively to flash chromatography on reversed-phase and silica columns. The cytotoxic fractions were purified by reversed-phase HPLC to afford callyspongamide A (**1**) in a yield of 1.6×10^{-3}% (based on wet weight). It showed a moderate cytotoxicity against HeLa cells with an IC_{50} value of 4.1 μg/mL.

Callyspongamide A (**1**) was isolated as a light yellow oil. Its positive HRFABMS showed an $[M + H]^+$ peak at *m/z* 364.2649 for a molecular formula of $C_{25}H_{34}NO$, equivalent to 10 degrees of unsaturation. The structural determination of **1** was made possible from analysis of 1D (^1H and ^{13}C) and 2D (COSY, HOHAHA, HMQC, and HMBC) NMR data (Figures 1 and 2 and Table 1). The 1D NMR spectra of **1** were indicative of a polyacetylene-type metabolite. The presence of signals resonating at δ 3.00 (brs)/81.2 (d), 88.5 (s), 5.91 (td)/146.2 (d), and 5.36 (brd)/107.9 (d), attributed to a terminal enyne moiety, and δ 78.5 (s) and 81.6 (s) was indicative of a disubstituted acetylene moiety flanked by methylene groups. The phenyl moiety was assigned from five low-field protons resonating between δ 7.27 and 7.13 in the ^1H NMR spectrum. The assignment of this moiety was confirmed by ^{13}C NMR resonances at δ 138.8 (C-3′), 128.7 (C-4′/C-8′), 128.6 (C-5′/C-7′), and 126.5 (C-6′) (Table 1). The remaining six degrees of unsaturation were accounted for by an amide carbonyl [δ_C 171.5 (s), δ_{NH} 5.70 (brs)], a disubstituted olefin, and two acetylenic moieties. The phenethylamine moiety of **1** was assigned by interpretation of the COSY, HMQC, and HMBC data. In the COSY spectrum, the protons resonating at δ 3.46 (q, H_2-1′) showed only two correlations with N*H* (δ 5.70) and H_2-2′ (δ 2.75, t). HMBC cross-peaks of H_2-1′/C-3′, H_2-2′/C-3′, H_2-2′/C-4′, H_2-2′/C-8′, H-4′/C-2′, H-8′/C-2′, H-5′/C-3′, H-7′/C-3′, and H_2-1′/C-1 confirmed the assignment of this moiety and its placement at C-1. Moreover, HOHAHA cross-peaks of N*H*/H_2-1′, N*H*/H_2-2′, H_2-2′/H-4′, and H_2-2′/H-8′ secured this assignment.



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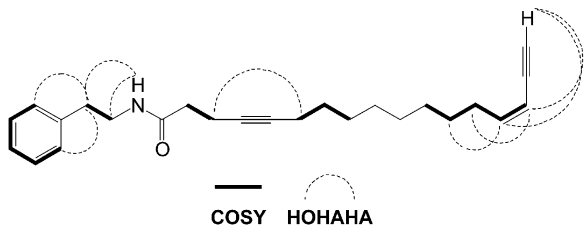

Figure 1. Observed COSY and HOHAHA correlations for **1**.

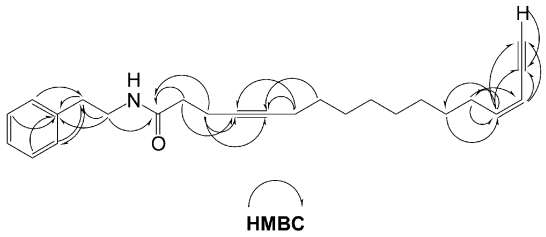

Figure 2. Observed HMBC correlations for **1**.

The assignment of the polyacetylenic part of **1** (C-1 to C-17) was inferred from interpretation of the COSY, HOHAHA, HMQC, and HMBC data. This assignment led to the assembly of C-2 to C-3, C-6 to C-8, and C-11 to C-17

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Table 1. ^{1}H and ^{13}C Chemical Shift Data of **1** ($CDCl_3$)

no.	δ_C (mult)	δ_H [mult, J(Hz)]	HMBC	HOHAHA
1	171.5 (s)			
2	36.1 (t)	2.24 (t, 7.3)	C-1, C-3, C-4	
3	15.3 (t)	2.38 (tt, 7.3, 2.3)	C-1, C-4, C-5	H-6
4	78.5 (s)			
5	81.6 (s)			
6	18.6 (t)	2.00 (tt, 7.3, 2.3)	C-4, C-5	H-3
7	28.6 (t)	1.36 (quint, 7.3)	C-5, C-6	
8	29.2 (t)[a]	1.22 (m)[b]		
9	28.9 (t)[a]	1.22 (m)[b]		
10	28.8 (t)[a]	1.22 (m)[b]		
11	29.0 (t)[a]	1.22 (m)[b]		
12	28.9 (t)	1.33 (quint, 7.3)	C-14	
13	30.2 (t)	2.25 (q, 7.3)	C-14, C-15	
14	146.2 (d)	5.91 (td, 10.7, 7.3)	C-12, C-13, C-16	
15	107.9 (d)	5.36 (br d, 10.7)	C-16, C-17	H-17
16	80.5 (s)			
17	81.2 (d)	3.00 (br s)	C-16	H-14, H-15
N*H*		5.70 (br s)		H-1′, H-2′
1′	40.6 (t)	3.46 (q, 6.9)	C-1, C-2′, C-3′	N*H*
2′	35.7 (t)	2.75 (t, 6.9)	C-1′, C-3′, C-4′, C-8′	N*H*, H-4′, H-8′
3′	138.8 (s)			
4′	128.7 (d)	7.13 (d, 7.6)	C-2′, C-6′	H-2′
5′	128.6 (d)	7.24 (t, 7.7)	C-3′	
6′	126.5 (d)	7.16 (t, 7.3)		
7′	128.6 (d)	7.24 (t, 7.7)	C-3′	
8′	128.7 (d)	7.13 (d, 7.6)	C-2′, C-6′	H-2′

[a] Signals may be interchangeable due to the proximity of signals. [b] Overlapped signals.

subunits (Figures 1 and 2 and Table 1). The placement of the acetylenic moiety at C-4/C-5 and the connectivities of subunits were confirmed by HMBC correlations. HMBC cross-peaks of H_2-2/C-1, H_2-3/C-1, H_2-2/C-4, H_2-3/C-4, H_2-3/C-5, H_2-6/C-4, H_2-6/C-5, and H_2-7/C-5 secured the position of the acetylenic moiety as well as the assignment of this fragment. Furthermore, HOHAHA long-range correlation between H_2-3 and H_2-6 ($J_{3,6}$ = 2.3 Hz) supported the assignment. Similarly, the structure assignment of the terminal enyne moiety was supported by HMBC cross-peaks of H-14/C-16, and H-15/C-16, H-15/C-17, and H-17/C-16. The *Z*-geometry at C-14/C-15 was deduced from the ^{1}H−^{1}H coupling constant of 10.7 Hz.[16−19] Finally, FABMS fragment ion peaks at *m/z* 259 (258+H), 215, 187, and 176 supported the substructural units of **1**.

Callyspongamide A represents a novel polyacetylenic amide within the family Callyspongiidae. It showed structural similarity with hermitamide A, which was isolated recently from a marine cyanobacterium *Lyngbya majuscula*.[20] Such structural similarity supported the microbial origin of callyspongamide A.

Experimental Section

General Experimental Procedures. The UV spectrum was recorded on a Hitachi 300 spectrometer. NMR spectra were recorded on a JEOL α-600 spectrometer. NMR chemical shifts were referenced to $CDCl_3$ solvent signals (δ_H 7.24; δ_C 77.0 ppm). Positive FAB mass spectral data were obtained with a JEOL JMS-700T mass spectrometer using NBA/NaCl as a matrix.

Animal Materials. The sponge was collected on October 20, 2001, by hand using scuba at depths between 15 and 20 m off Hurghada in the Red Sea. The sponge materials were frozen immediately and kept frozen at − 20 °C until processed. The sponge forms thick cushions with 2−3 cm high, thick-walled, volcano-shaped oscular elevations. The texture is elastic and the surface is microhispid, somewhat bumpy, and irregular in places. Color in live is reddish-pink, while it turns to light brown on preservation in alcohol. The ectosomal skeleton is a tangential reticulum of primary and secondary spongin fibers cored by thin oxeas making larger and smaller polygonal meshes of 120−250 μm diameter, rather irregular in shape. The choanosomal skeleton is primary spongin fibers measuring 55−60 μm in diameter, cored by 6 or more oxeas, and secondary fibers with 15−20 μm in diameter cored by a 1−3 oxeas, forming basically rectangular, but rather irregular meshes. Spongin was dominating the skeleton. Oxeas measure about 60−120 × 1−3 μm. The sponge conforms closely to the description of *Callyspongia fistularis* (Topsent, 1892 as Sclerochalina). The voucher is registered in the Zoological Museum of Amsterdam under No. ZMA POR. 16616.

Extraction and Isolation. Frozen specimens (165 g) of the sponge were extracted with MeOH/CH_2Cl_2 (1:1) (3 × 500 mL) at room temperature. The combined extracts were evaporated in vacuo. The concentrated brown residue was dissolved in 200 mL of MeOH/H_2O (9:1) and extracted with hexane (3 × 200 mL) to give 620 mg of hexane residue. The methanolic layer was diluted with H_2O to MeOH/H_2O (3:2) and then extracted with CH_2Cl_2 (3 × 300 mL) to afford 310 mg of CH_2Cl_2 extract. Both hexane and CH_2Cl_2 extracts were combined and subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 230/70 mesh), eluting with 50% to 0% H_2O in MeOH to obtain seven fractions. Fraction 5 (120 mg) was flash chromatographed on a silica column eluted with hexane/CH_2Cl_2 (1:1) through CH_2Cl_2/MeOH (1:1). The fraction eluted with hexane/CH_2Cl_2 (95:5) was concentrated, and the resulting residue (13 mg) was purified on a C18-reversed-phase HPLC column using 85% MeOH to give **1** (2.7 mg).

Callyspongamide A (1): pale yellow oil; UV (MeOH) λ_{max} 216 nm (log ϵ 4.12); NMR data, see Table 1; positive HR-FABMS *m/z* 364.2649 $(M + H)^+$ ($C_{25}H_{34}NO$, Δ +0.9 mmu).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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