

Calafianin, a Bromotyrosine Derivative from the Marine Sponge *Aplysina gerardogreeni*

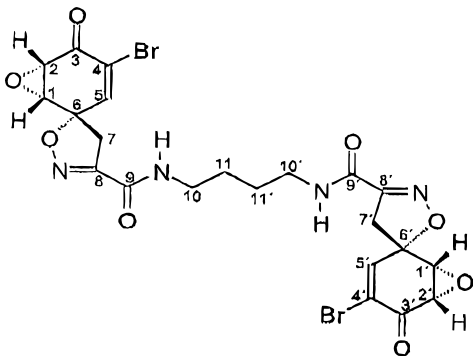
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Calafianin (**1**) and two known compounds, aerothionin and (3,5-dibromo-2-hydroxy-4-methoxyphenyl)-acetic acid, were isolated from the marine sponge *Aplysina gerardogreeni*. The structure of **1** was determined by NMR analysis and mass spectrometry.

During a search for bioactive compounds from Mexican marine organisms, the sponge *Aplysina gerardogreeni* n. sp. (Aplysinidae) was selected for further studies. The methylene chloride extract of this sponge was subjected to column chromatography. Separation of the extract gave rise to the isolation of compound **1**, which we named calafianin after the mythological Amazon queen Calafia, allegedly the ruler of the island California. Furthermore, aerothionin and (3,5-dibromo-2-hydroxy-4-methoxyphenyl)acetic acid were isolated. The structures of these two compounds were determined by comparison of their spectral data with those previously reported.^{1,2} Apparently the acetic acid derivative has only been characterized as a semisynthetic compound and has not been isolated from natural material before.



Compound **1** exhibited a positive ion FABMS pseudo-molecular ion at m/z 629.0 ($M + H$)⁺, and the isotopic pattern reveals the presence of two bromine atoms. This information is compatible with the molecular formula $C_{22}H_{20}Br_2N_4O_8$, implying 14 degrees of unsaturation. The proton NMR spectrum (Table 1) indicates the presence of one amide NH at δ 8.63, one olefinic proton at δ 7.49, and two methine and three methylene groups. In the ¹³C NMR spectrum 11 signals were observed and found by HETCOR experiments to correspond to two carbonyl, three quaternary, three methine, and three methylene groups. To account for these observations, the compound must be dimeric and composed of two identical units arranged symmetrically. The NMR signals are reminiscent of those reported for aerothionin, hinting at a close relationship with this compound. Taking into account these facts, in

Table 1. NMR Data for Calafianin (**1**)^a

no.	δ_C	δ_H	COSY	HMBC (C to H)
1, 1'	56.9	4.13(2.6 ^b / 3.7 dd)	H5, H5', H2, H2'	H5, H5', H7, H7'
2, 2'	53.0	3.93(3.5 d)	H1, H1'	
3, 3'	186.0			
4, 4'	122.8			H2, H2', H5, H5'
5, 5'	143.7	7.49(2.6 ^b d)	H1, H1'	H7, H7'
6, 6'	84.0			H7, H7'
7, 7'	43.6	3.68(17.8 d) 3.61(17.9 d)		
8, 8'	154.9			H7, H7'
9, 9'	158.3			H10, H10', NH, NH'
10, 10'	38.6	3.19(br)	H11, H11', NH, NH'	
11, 11'	26.4	1.50(br)	H10, H10'	H10, H10'
NH		8.63(5.7 t)	H10, H10'	

^a Solvent: DMSO-*d*₆. ^b ⁴ J_{HH} (W -coupling).

addition to the spectroscopic information, the remaining two unsaturation sites must belong to two epoxide rings. The positions of these rings reveal themselves by comparison with the aerothionin spectroscopic assignments; that is, they must occur bridging positions 1–2 and 1'–2'. This structural assignment is fully corroborated by the HMBC data shown in Table 1.

Presupposing the absolute stereochemistry around position 6 to be identical to the one encountered in aerothionin, viz., *S*, NOE-difference spectroscopy studies resolve the remaining configuration around the epoxide ring at positions 1 and 2. Saturation with the frequency corresponding to the shift of each of the epoxide protons results in 5% enhancement of the signal of the other, confirming the *cis* relationship of these protons. Furthermore irradiation with the frequency corresponding to the signal from the proton at position 1 results in a 2% enhancement of the protons at position 7. Saturation of the signals originating from the latter group produces a 5% enhancement of the proton attached to position 2, attesting to the *cis* relationship of these groups. The same saturation experiment results in an 11% enhancement of the signal originating from the olefinic proton at position 5, supporting these assignments. Taking into account the strict spectroscopic identity of the two parts of calafianin, including the fact that the product is optically active, and the assumption mentioned above define the structure and absolute configuration of calafianin as 6,6'-*S*, 1,1'-*R*, 2,2'-*S*.

The presence of aerothionin has been reported to have no chemotaxonomical significance within the Aplysinidae family.³ We agree with this conclusion.

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Experimental Section

General Experimental Procedures. NMR spectra were recorded in DMSO- d_6 on a Varian 400 FT-NMR spectrometer at 400.0 MHz and 100.6 MHz for ^1H and ^{13}C NMR spectra, respectively. The UV spectrum was recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Circular dichroism (CD) spectra were measured on a JASCO J-710 spectropolarimeter. FABMS was recorded on a JEOL JMS-MX7HX 110 A spectrometer using a *m*-NBA matrix.

Animal Material. The sponge was collected by scuba diving at the Espíritu Santo-La Partida Island, La Paz B. C. S. (México), in June, 1996. It was kept frozen prior to freeze-drying and extraction. A voucher specimen (RED 9616) has been deposited at the Pharmacognosy Laboratory of the Agronomy Department of Universidad Autónoma de Baja California Sur (México). The identification was performed by Martín García and Carlos Sánchez at the Marine Biology Department of UABCS by comparison with material collected in 1985 (samples M101, M102) identified by Patricia Gómez at the Instituto de Limonología y Ciencias del Mar of UNAM as *Aplysina gerardogreeni*. This species was later reported as new by Gomez and Bakus.⁴

Extraction and Isolation. The sponge (600 g) was extracted exhaustively by maceration with hexane, CH_2Cl_2 , and EtOH (yield of extracts 20, 81, and 40 g, respectively). The CH_2Cl_2 extract was separated into MeOH-soluble (61 g) and -insoluble (18 g) parts. The soluble material (21 g) was subjected to chromatography on a Si gel column (70–230 mesh, ratio 1:60), eluted with a stepwise gradient from hexane, hexane–acetone (increasing polarity), to MeOH, giving 16 fractions. Repeated Si gel chromatography on fraction 5 led to the isolation of (3,5-dibromo-2-hydroxy-4-methoxyphenyl)-acetic acid (30 mg). Fraction 8 (6.2 g) gave crystals of aerothionin from cold acetone. Aerothionin showed activity

against *Mycobacterium tuberculosis*. The mother liquid of this fraction was further purified by Si gel chromatography (230–400 mesh, ratio 1:88) using hexane– Me_2CO (6:4), Me_2CO , and MeOH as eluents. One of the fractions (1020 mg) was chromatographed several times on a Si gel column (230–400 mesh, ratio 1:100), eluted with a stepwise gradient of hexane, Me_2CO , and CH_2Cl_2 –MeOH, yielding 30 mg of **1**.

Calafianin (1): colorless solid. The solubility in solvents useful for recording optical spectra was too low to allow meaningful concentration measurements; consequently the spectra were determined using saturated solutions in EtOH. Extinction coefficients can therefore not be given, and only characteristic wavelength and signs are recorded: UV (EtOH) λ_{max} (absorbance) 260 sh (0.63), 230 sh (0.78), 210 sh (0.90) nm; CD, λ , (rotation in millidegrees) 344.1 (2.5), 270.3 (13.8), 233.6 (24.1), 199.0 (–25.0); the optical rotation in EtOH was determined to be positive; NMR data, see Table 1; NOE experiments were performed using DMSO- d_6 solutions; FABMS in the positive ion mode m/z 629 ($\text{M} + \text{H}$).

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