The Structure of Clathrodin, a Novel Alkaloid Isolated from the Caribbean Sea Sponge Agelas clathrodes

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THE STRUCTURE OF CLATHRODIN, A NOVEL ALKALOID ISOLATED FROM THE CARIBBEAN SEA SPONGE *AGELAS CLATHRODES*[1]

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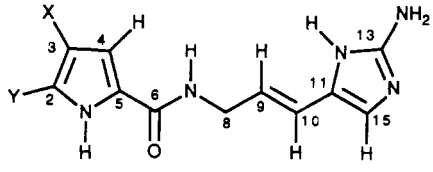
ABSTRACT.—A new alkaloid containing a nonbrominated pyrrole and a guanidine moiety, clathrodin, has been isolated from the MeOH extract of the Caribbean sea sponge *Agelas clathrodes*. The structure of clathrodin was determined to be **1** on the basis of its spectral data.

Several C_{11}-N_5 compounds containing a bromopyrrole and a guanidine moiety have been isolated from marine sponges belonging to the families Agelasidae and Axinellidae (1–11). However, not many related nonbrominated compounds are known from these families (12). During a recent expedition in search of physiologically active substances of marine organisms from Puerto Rico, we have examined the antimicrobial and cytotoxic actions of an MeOH extract of a sea sponge *Agelas clathrodes* (Schmitt) which was collected near Desecheo Island, Puerto Rico in March 1989. The material from the MeOH extract showed weak activity against *Proteus vulgaris, Staphylococcus aureus*, and *Shigella flexneri* (MIC = 1 mg/ml) using the standard disk assay. Similarly, the cytotoxicity assays inhibited the growth of SW-480 cells (ED_{50} = 53 μg/ml). We report here the isolation and the structure elucidation of the cytotoxic constituent clathrodin [**1**].



1 X=Y=H
2 X=Br, Y=H
3 X=Y=Br

The MeOH extract of the sponge was suspended in H_2O and extracted with $CHCl_3$ (3 × 250 ml) and *n*-BuOH (2 × 250 ml), successively. After concentration, the *n*-BuOH-soluble portion (3.30 g) was chromatographed on a reversed-phase (C_{18}, 20 g) column with H_2O followed by a Si gel column (48 g) with $CHCl_3$-MeOH (4:1) saturated with NH_3. Combination of like fractions on the basis of tlc analyses gave pure clathrodin [**1**] as a colorless semisolid (840 mg).

The hrfabms of **1** showed an intense $[M+H]^+$ peak at *m/z* 232.1198, indicating a molecular formula of $C_{11}H_{13}N_5O$ (Δ 0.3 mmu) for the compound. The spectral data recorded indicated that clathrodin [**1**] was the 3-debromo derivative of hymenidin [**2**], a potent antiserotonergic constituent of the Okinawan marine sponge *Hymeniacidon* sp. (7).

The ^{1}H-nmr spectrum (Table 1) and the ^{1}H-^{1}H COSY spectrum revealed the partial structure -CO-NH-CH2-CH= CH- (trans). The signals for two aromatic protons at δ 6.96 (H-2 and H-4, overlapping multiplets) and another at δ 6.12 (H-3, complex multiplet) indicated the existence of a monosubstituted pyrrole ring (9). The connection of the pyrrole ring to the amide carbonyl at C-5 was argued on the presence of an intense cross peak between H-4 and H-7 in the 2D nOe spectrum (13,14). An aminoimidazole unit was suggested by the ^{13}C-nmr signals at δ 127.35 (C-11), 151.79 (C-13), and 117.52 (C-15). Furthermore,

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TABLE 1. ^{1}H-nmr (300 MHz, DMF-d_7), ^{13}C-nmr (75 MHz, DMF-d_7), and nOe Spectral Data for Clathrodin [**1**].

Position	δ_H[a] (*J* in Hz)	δ_C[b]	nOe[c]
1	11.52 (1H, br s, exchangeable)	—	
2	6.96 (1H, m)	121.86 (d)	H-3
3	6.12 (1H, m)	109.04 (d)	
4	6.96 (1H, m)	110.34 (d)	H-7
5	—	130.93 (s)[d]	
6	—	161.45 (s)	
7	8.12 (1H, t, 5.6, exchangeable)	—	H-4
8	3.98 (2H, t, 5.5)	41.39 (t)	H-9, H-10
9	5.93 (1H, dt, 6.4, 15.6)	120.78 (d)	H-4, H-8
10	6.27 (1H, d, 15.5)	122.54 (d)	H-8, H-15
11	—	127.35 (s)[d]	
12	11.52 (1H, br s, exchangeable)	—	
13	—	151.79 (s)	
15	6.49 (1H, s)	117.52 (d)	H-10
-NH2	5.41 (2H, br s, exchangeable)	—	

[a]Assignments were aided by ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY, spin splitting patterns, and NOESY experiments. The chemical shifts are given in δ units (ppm downfield from TMS).

[b]Assignments were made on the basis of ^{1}H-^{13}C COSY and APT experiments. The δ values are in ppm and are referenced to the residual DMF signal (162.7 ppm).

[c]Obtained from the 2D nOe spectrum.

[d]Assignments may be interchanged.

the ^{13}C chemical shift values closely resembled those reported for hymenidin [**2**] (7) and oroidin [**3**] (1,2) and thus confirmed the structure **1** for clathrodin.

Clathrodin [**1**] is biogenetically related to bromine-containing antimicrobial alkaloids keramadine (5), oroidin [**3**] (2), sceptrin (3), and dibromoagelaspongin (9), which have been isolated from the same genus *Agelas*. Other related compounds reported are mono- and dibromophakelin from *Phakellia flabellata* (10), hymenialdisine from *Hymeniacidon aldis* (4,11) and hymenin and hymenidin [**2**] from *Hymeniacidon* sp (6–8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir and uv spectra were recorded on a Nicolet 600 FT-IR and a Hewlett-Packard Chem Station 8452A spectrophotometer, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on a General Electric Multinuclear QE-300; ^{1}H chemical shifts are recorded with respect to TMS (δ 0.0). Cc was performed on Analtech Si gel (35–75 mesh), and tlc analyses were carried out using Analtech glass packed precoated Si gel plates. All solvents used were either spectral grade or were distilled from glass prior to use.

EXTRACTION AND SEPARATION OF CLATHRODIN [**1**].—The sponge material was collected in March 1989, by the authors; a voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The collection was stored at 0° for several hours prior to freezing and lyophilization. The combined MeOH extracts of the freeze-dried specimen (60 g) upon filtration followed by concentration gave a residue (46.2 g) which was triturated with $CHCl_3$ and then with *n*-BuOH. The *n*-BuOH extract, after evaporation (3.3 g), was chromatographed over reversed-phase C_{18} column with H_2O followed by Si gel column with $CHCl_3$-MeOH (4:1) saturated with NH_3, to give several fractions which were then combined on the basis of tlc analyses. These were evaporated to give a pure semisolid residue (840 mg).

CLATHRODIN [**1**].—White semisolid: ir (KBr) 3330 (broad), 1683, 1617, 1563, 1522, 1407, 1328, 1127, 1042, 960, 746 cm^{-1}; uv (MeOH) 272 nm (ε 24,500); ^{1}H nmr (300 MHz, DMF-d_7) see Table 1; ^{13}C nmr (75 MHz, DMF-d_7) see Table 1; hrfabms *m/z* 232.1198 $[M+H]^+$ (55%) ($C_{11}H_{14}N_5O$ requires 232.1195), 154 (88), 136 (85), 122 (35), 107 (45), 102 (100). In vitro screening data for pure clathrodin show significant cytotoxicity against CHO-K1 cells (ED_{50} = 1.33 μg/ml). Clathrodin has also been found to possess potent blocking activity against cholinergic receptors on the isolated frog skeletal muscle at concentrations of 10^{-7}–10^{-3} M. The

pharmacological activities of clathrodin will be reported elsewhere in detail.

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