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Clathrynamides A, B, and C: Novel Amides from a Marine Sponge *Clathria* sp. That Inhibit Cell Division of Fertilized Starfish Eggs

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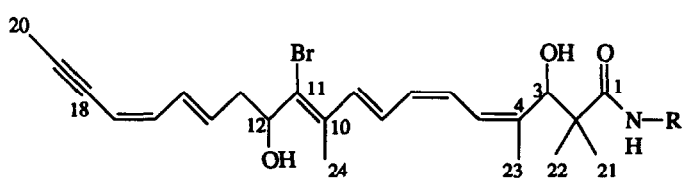
Abstract: Three novel bromine-containing amides, clathrynamides A (**1**), B (**2**), and C (**3**), were isolated from a marine sponge *Clathria* sp. Their structures were determined on the basis of spectroscopic data. Clathrynamide A (**1**) significantly inhibited cell division of fertilized starfish eggs and also showed inhibition of the growth of human myeloid K-562 cells *in vitro*.

In the course of our search for biologically active compounds from marine organisms,[1] we have found that the methanolic extract of a marine sponge *Clathria* sp. strongly inhibits cell division of starfish (*Asterina pectinifera*) embryos. Bioassay-guided purification of the crude extract resulted in the isolation of three novel bromine-containing amides which we have named clathrynamides A (**1**), B (**2**), and C (**3**). In this paper, we report the structures of **1**, **2**, and **3** which have been deduced from spectroscopic data.

The marine sponge *Clathria* sp. (10.0 g, wet weight) was collected off the coast of Sada-misaki, Ehime Prefecture, Japan. The methanolic extract (540 mg) was partitioned between benzene and water. The benzene-soluble material exhibited an inhibiting activity against cell division of fertilized starfish eggs. The active portion was chromatographed on silica gel with EtOAc-benzene (EtOAc: 20–80%) to afford three bioactive fractions. Each fraction was purified by HPLC (silica gel, 5% EtOH-hexane) to afford clathrynamides A (**1**) (0.84 mg; 0.0084% wet weight), B (**2**) (0.33 mg; 0.0033% wet weight), and C (**3**) (0.13 mg; 0.0013% wet weight) as colorless gummy substances.



1: R = H
2: R = $CH(CH_3)CH_2CH_2CH(OH)CH_3$
3: R = $CH(CH_3)CH_2CH_2COCH_3$

Table 1. NMR Spectral Data of Clathrynamides A (1), B (2), and C (3) (CD_3OD).[a]

Carbon No.	1		2	3
	δ_C[b]	δ_H	δ_H	δ_H
1	183.3 (s)			
2	46.7 (s)			
3	75.5 (d)	4.72 s	4.71 s	4.72 s
4	141.1 (s)			
5	125.8 (d)	6.56 d (12.8)	6.55 d (11.5)	6.56 d.(11.5)
6	127.0 (d)	6.44 t (11.5)	6.43 t (11.5)	6.43 t (11.5)
7	129.9 (d)	6.10 t (11.0)	6.10 t (10.5)	6.10 t (11.0)
8	128.8 (d)	6.97 dd (15.1, 11.5)	6.97 dd (14.9, 11.3)	6.97 dd (14.8, 11.3)
9	136.0 (d)	6.90 d (15.1)	6.90 d (15.1)	6.90 d (15.1)
10	133.7 (s)			
11	132.7 (s)			
12	71.4 (d)	4.77 t (7.3)	4.76 t (7.1)	4.77 t (6.9)
13	40.4 (t)	2.51 t (7.3)	2.51 t (6.9)	2.52 m[c]
14	132.5 (d)	5.73 dt (14.9, 7.3)	5.73 dt (15.1, 7.3)	5.73 dt (15.6, 7.2)
15	131.6 (d)	6.61 dd (14.7, 11.4)	6.61 dd (15.2, 10.7)	6.61 dd (15.4, 10.8)
16	139.5 (d)	6.23 t (10.8)	6.23 t (10.8)	6.23 t (10.8)
17	109.7 (d)	5.29 dd (10.5, 2.5)	5.28 dd (10.7, 2.7)	5.28 dd (10.8, 2.8)
18	77.6 (s)			
19	92.5 (s)			
20	4.0 (q)	1.98 d (2.3)	1.98 d (2.8)	1.98 d (2.8)
21(2-Me)	22.0 (q)	1.05 s	1.05 s	1.07 s
22(2-Me)	26.3 (q)	1.27 s	1.26 s	1.24 s
23(4-Me)	20.0 (q)	1.87 s	1.84 s	1.85 s
24(10-Me)	16.1 (q)	2.01 s	2.01 s	2.01 s
1'			3.85 m	3.84 m
2'			1.55 m	1.71 m
3'			1.46 m	2.52 m[c]
4'			3.70 m	
5'			1.15 d (6.4)	2.13 s
6'(1'-Me)			1.13 d (6.9)	1.13 d (6.9)

[a] ^1H and ^{13}C NMR spectra were taken at 500 MHz and at 125 MHz, respectively. Coupling constants, J_{H-H} (in Hz), are given in parentheses.

[b] Multiplicities were determined by DEPT experiments.

[c] These signals overlap with each other.

Clathrynamide A (**1**),[2] $[\alpha]_D^{25}$ +146° (*c* 0.0164, MeOH), has a molecular formula, $C_{24}H_{32}O_3NBr$, which was determined by high resolution fast-atom bombardment mass spectrometry (HRFABMS) [*m/z* 486.1448 and 484.1474 $(M+Na)^+$, Δ +0.2 mmu and Δ +1.1 mmu, respectively]. The IR spectrum of **1** indicated the presence of hydroxyl groups (3350 cm^{-1}), an acetylene bond (2200 cm^{-1}), and an amide group (1660 cm^{-1}). Further, ^1H NMR (CD_3OD) (Table 1), ^{13}C NMR (CD_3OD) (Table 1), and DEPT spectral data of **1** revealed the presence of four 1,2-disubstituted double bonds, a trisubstituted double bond, a tetrasubstituted double bond, two oxymethines, a methylene, a quaternary sp^3 carbon, and four methyl groups. Assignments of all of the protonated carbons were made by HMQC[3] spectrum. Detailed analysis of the ^1H-^1H COSY spectrum of **1** revealed the presence of two segments C-5–C-9 and C-12–C-17. The coupling constants between olefinic protons (H-6/H-7, H-8/H-9, H-14/H-15, and H-16/H-17) indicated that the geometries for Δ^6, Δ^8, Δ^{14}, and Δ^{16}-double bonds are *Z*, *E*, *E*, and *Z*, respectively.

The HMBC[4] spectrum showed two- or three-bond couplings for H-8 to C-10, H-9 to C-11 and C-24, H_3-24 to C-9, C-10 and C-11, and H-12 to C-11 and C-13, confirming the connection from C-9 to C-12 through the Δ^{10}-tetrasubstituted double bond. The HMBC spectrum showed two- or three-bond couplings for H-5 to C-23, H-6 to C-4, H_3-23 to C-3, C-4 and C-5, H-3 to C-1, C-2, C-4, C-5, C-21, C-22 and C-23, H_3-21 to C-1, C-2, C-3 and C-22, and H_3-22 to C-1, C-2, C-3 and C-21, confirming the partial structure C-1–C-5. Evidence of *Z* geometry assigned to the Δ^4-double bond arose from the NOESY experiment in which clear cross-peaks were observed for H-3/H-6 and H-5/H_3-23. The connection from C-17 to C-20 through the acetylene bond (C-18–C-19) was established by the cross peaks of H-20 to C-17 (the four-bond coupling), C-18 (the three-bond coupling), and C-19 (the two-bond coupling) in the HMBC spectrum. The ^1H NMR spectrum of **1** in DMSO-d_6 showed the signals of two amide protons at δ 6.86 (1H, brs) and δ 7.12 (1H, brs), and those of two hydroxyl protons at δ 5.35 (1H, d, J = 4.6 Hz, 12-OH) and δ 5.45 (1H, d, J = 5.0 Hz, 3-OH). The substitution of the bromine atom at C-11 was the only possible way to complete the structural assignment of **1**. This substitution was supported by the presence of the following prominent peaks in the EI-mass spectrum of **1**: *m/z* 358, 356 (M^+ - 105) and 105, corresponding to the fragments arising from cleavage of the C-12–C-13 bond, *m/z* 271 and 269 (M^+ - 87 - 105), corresponding to the fragments arising from cleavage of both the C-2–C-3 and C-12–C-13 bonds, and *m/z* 87 $[C(CH_3)_2CONH_2+H]^+$. Evidence of *Z* geometry assigned to the Δ^{10}-double bond arose from the NOESY experiment in which a clear cross-peak was observed for H-12/H_3-24. Thus, clathrynamide A was defined as (4*Z*,6*Z*,8*E*,10*Z*,14*E*,16*Z*)-11-bromo-3,12-dihydroxy-2,2,4,10-tetramethyl-4,6,8,10,14,16-icosahexaen-18-ynamide (**1**).

The secondary ion mass spectrum (SIMS) of clathrynamide B (**2**),[5] $[\alpha]_D^{25}$ +76° (*c* 0.0033, MeOH), exhibited the $[M+H]^+$ ion peaks at *m/z* 564 and 562, and the $[M-H_2O+H]^+$ ion peaks at *m/z* 546 and 544, indicating that the molecular weight of clathrynamide B (**2**) is larger than that of clathrynamide A (**1**) by 100 a.m.u. The ^1H NMR spectrum (Table 1) and ^1H-^1H COSY spectrum of **2** showed that the C-1–C-24 portion of the molecule is identical with that of clathrynamide A (**1**). The ^1H NMR spectrum of **2** in DMSO-d_6 exhibited a doublet signal of an amide proton at δ 7.22 (J = 7.8 Hz) assigned to 1-CONH. Analysis of the ^1H-^1H COSY spectrum of **2** in DMSO-d_6 revealed that the amide proton signal is coupled to the methine proton signal at δ 3.70 (m) assigned to H-1'. Further, the ^1H decoupling experiments of **2** in DMSO-d_6 indicated that a 4'-hydroxy-1'-methylpentyl group is attached to the nitrogen atom of the 1-amide group. The indication was confirmed by the presence of NOEs between H_3-21 and H_3-6' (1'-Me), and between H_3-22 and H_3-6'. Thus, the structure of clathrynamide B was determined to be **2**.

The SIMS of clathrynamide C (3)[6] exhibited the $[M+H]^+$ ion peaks at *m/z* 562 and 560, and the $[M-H_2O+H]^+$ ion peaks at *m/z* 544 and 542, indicating that the molecular weight of clathrynamide C (3) is smaller than that of clathrynamide B (2) by 2 a.m.u. The IR absorption band at 1711 cm^{-1} and the ^1H NMR signal at δ 2.13 indicated the presence of an acetyl function. The ^1H NMR spectrum (Table 1) and ^1H-^1H COSY spectrum of 3 showed that the C-1–C-24 portion of the molecule is identical with those of clathrynamides A (1) and B (2). The ^1H decoupling experiments of 3 in DMSO-d_6 indicated that a 1'-methyl-4'-oxopentyl group is attached to the nitrogen atom of the 1-amide group (CONH: δ_H 7.14, d, J = 8.3 Hz). This was supported by the presence of NOEs between H_3-21 and H_3-6', and between H_3-22 and H_3-6'. Thus, the structure of clathrynamide C was determined to be 3.

Clathrynamide A (1) inhibited mitotic cell division of starfish eggs at an extraordinarily low concentration (IC_{50} = 6 ng/ml). On the other hand, clathrynamides B (2) and C (3) were not very active, the IC_{50} values of 2 and 3 being 0.2 and 1 μg/ml, respectively. It is noteworthy that the difference of the structure of the amide moiety affects profoundly biological activities. Clathrynamide A (1) also inhibited the growth of human myeloid K-562 cells *in vitro* with an IC_{50} value of 0.2 μg/ml.

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2. 1: UV (MeOH) λ_{max} 267 (log ε 4.29), 308 (4.47), 321 (4.58), and 336 nm (4.46); IR (film) 3350, 2200, 1660, and 1590 cm^{-1}; CIMS *m/z* 486, 484 $[M+Na]^+$, 464, 462 $[M+H]^+$, and 446, 444 $[M-H_2O+H]^+$; EIMS *m/z* 358 (1%), 356 (2), 271 (32), 269 (32), 105 (70), 87 (24), 77 (100), and 44 (99); CD (MeOH) $\Delta\varepsilon_{335}$ +10, $\Delta\varepsilon_{319}$ +16, $\Delta\varepsilon_{306}$ +15, and $\Delta\varepsilon_{264}$ -21.
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5. 2: UV (MeOH) λ_{max} 267 (log ε 4.24), 307 (4.37), 321 (4.48), and 337 nm (4.37); IR (film) 3330, 2300, 1630, and 1540 cm^{-1}; EIMS *m/z* 458 (1%), 456 (1), 271 (5), 269 (5), 187 (8), 114 (23), 105 (28), and 44 (100); CD (MeOH) $\Delta\varepsilon_{333}$ +6, $\Delta\varepsilon_{318}$ +10, $\Delta\varepsilon_{304}$ +9, and $\Delta\varepsilon_{261}$ -11.
6. 3: UV (MeOH) λ_{max} 268 (log ε 4.41), 307 (4.50), 321 (4.58), and 337 nm (4.47); IR (film) 3340, 2300, 1711, 1630, and 1530 cm^{-1}; EIMS *m/z* 456 (2%), 454 (2), 271 (3), 269 (4), 185 (19), 142 (16), 105 (17), 99 (36), and 44 (100); CD (MeOH) $\Delta\varepsilon_{333}$ +6, $\Delta\varepsilon_{318}$ +11, $\Delta\varepsilon_{303}$ +11, and $\Delta\varepsilon_{261}$ -13.

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