The Structures of Aplysinamisines I, II, and III: New Bromotyrosine-Derived Alkaloids from the Caribbean Sponge Aplysina cauliformis

Abimael D. Rodríguez, and Ivette C. Piña

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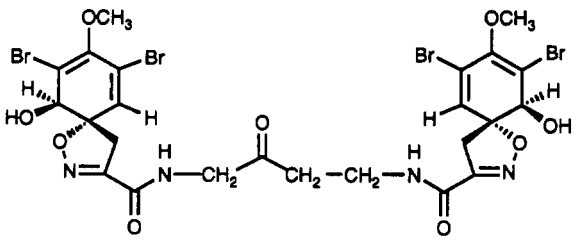
THE STRUCTURES OF APLYSINAMISINES I, II, AND III: NEW BROMOTYROSINE-DERIVED ALKALOIDS FROM THE CARIBBEAN SPONGE *APLYSINA CAULIFORMIS*[1]

ABIMAEL D. RODRÍGUEZ* and IVETTE C. PIÑA[2]

University of Puerto Rico, Department of Chemistry, PO Box 23346, U.P.R. Station, Río Piedras, Puerto Rico 00931-3346

ABSTRACT.—Three new bromotyrosine-derived alkaloids, aplysinamisines I [**4**], II [**5**], and III [**6**], were isolated from the sponge *Aplysina cauliformis* collected in Puerto Rico and their chemical structures elucidated on the basis of spectroscopic methods. Compounds **4–6** showed marginal antimicrobial activity, and aplysinamisines II [**5**] and III [**6**] displayed modest cytotoxicity.

An extraordinary number of marine natural products have been isolated from organisms belonging to the phylum Porifera, proving sponges to be a rich and varied source of new structural classes of secondary metabolites (1). All species of marine sponges belonging to the order Verongida that have been examined so far produce secondary metabolites derived from a brominated tyrosine (2). Many such compounds appear to be derived biosynthetically from the condensation of 3,5-dibromotyrosine with various amino acid residues arranged to form the same spirocyclohexadienylisoxazole system as in 11-oxoaerothionin [**1**] (3). Specifically, compounds containing a dibromotyrosine unit linked to a histamine residue, which may have undergone further modification, have been isolated from sponges collected in Italy, Okinawa, and the Caribbean. For instance, *Aplysina aerophoba* has been reported to produce two such polar metabolites, aerophobins 1 [**2**] and 2 [**3**] (4).



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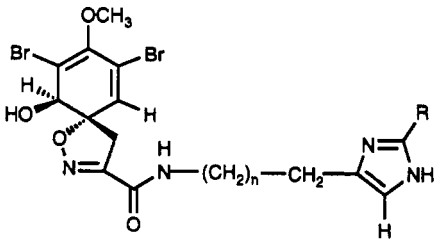

2 R=H, n=1
3 R= NH_2, n=2

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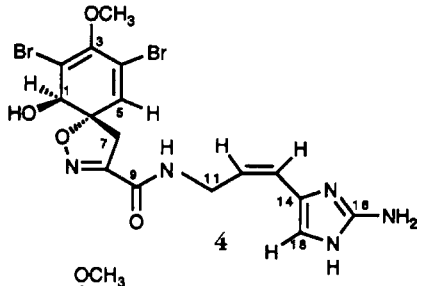
[2]Visiting graduate student from the Department of Chemistry, Universidad Central de Venezuela, Caracas, Venezuela, sponsored by the COPLAC and CONICIT Programs.

The majority of dibromotyrosine-derived natural products from Verongida have been reported to possess significant antimicrobial and cytotoxic activity (2). In the course of screening marine organisms collected from the coastal waters of Puerto Rico we have examined a number of *Aplysina* spp. Without exception, extracts of these specimens have exhibited antimicrobial activity and cytotoxic action against CHO-k1 cells. Prompted by the observation of antibiotic and cytotoxic activity in the MeOH/$CHCl_3$ extract of *Aplysina cauliformis* (Carter) (phylum Porifera, class Demospongea, family Aplysinidae), we have investigated this sponge and report here details of the isolation and structure determination of three previously unreported compounds **4**, **5**, and **6**. These compounds, which were named aplysinamisines I [**4**], II [**5**], and III [**6**], exhibited weak in vitro activity against Gram-positive bacteria and also against *Escherichia coli*. When tested against three human-tumor cell lines, aplysinamisine I [**4**] showed no activity, aplysinamisine II [**5**] was active against one cell line, and aplysinamisine III [**6**] showed cytotoxicity against all three cell lines.

Specimens of *A. cauliformis* were collected at Mona Island off the west coast of Puerto Rico, freeze-dried, and extracted with MeOH-$CHCl_3$ (1:1). Fractionation of the $CHCl_3$ extract on a Si gel column, using CH_2Cl_2 with increasing amounts of MeOH previously saturated with NH_3, allowed the isolation of several bioactive fractions; further purifi-



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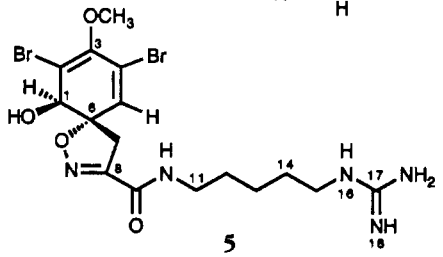

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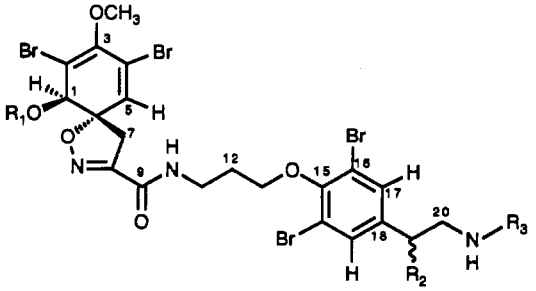
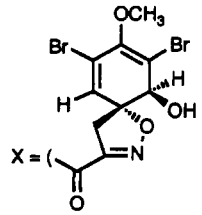


6 R_1=H, R_2=OH, R_3=Ac
7 R_1=R_3=Ac, R_2=OAc
8 R_1=R_2=R_3=H
9 R_1=R_3=Ac, R_2=H
10 R_1=H, R_2=OH, R_3=X



cation by chromatography (normal and reversed-phase cc and preparative tlc) led to the isolation of four pure biologically active dibromotyrosine derivatives. Of these, only **1** is known, and it was identified as 11-oxoaerothionin by comparison of its ^{1}H- and ^{13}C-nmr, ms, ir, and uv spectral properties with literature values (3). The structures of aplysinamisines **4–6** were readily established by spectral analysis and detailed comparison of their ^{1}H-nmr and ^{13}C-nmr data with well known Verongida metabolites.

Aplysinamisine I [**4**], an optically active, $[\alpha]_D = +121.9°$, colorless oil isolated in 0.0145% yield (based on the weight of freeze-dried sponge), showed intense $[M+H]^+$ ions in the ratio of about 1:2:1 at *m/z* 502, 504, and 506 in the positive fabms spectrum, which indicated the presence of two bromine atoms in the molecule. The molecular formula of aplysinamisine I [**4**] was determined to be $C_{16}H_{18}Br_2N_5O_4$ by hrfabms (*m/z* 503.97073, $[M+H]^+$, Δ 0.29 mmu). The uv spectrum of **4** showed one absorption at λ max 266 nm (ϵ 13,100) analogous to that of 11-oxoaerothionin [**1**], implying the presence of a common chromophore, and another absorption at λ max 226 nm (ϵ 13,700) not found in **1**. The ^{1}H nmr (Table 1) showed the following signals in DMSO-d_6: δ 6.56 (s, 1H, H-5), 3.92 (br s, 1H, H-1), 3.63 (s, 3H, -OMe) and an ABq system (δ 3.62 and 3.20, 1H each, J=18.0 Hz) characteristic of a spirocyclohexadienylisoxazole ring system commonly encountered in other Verongida constituents. The other signals: δ 9.01 (t, 1H, J=5.4 Hz, H-10, exchangeable), 6.06 (d, 1H, J=11.4 Hz, H-13), 5.24 (dt, 1H, H-12), and 4.16 (br t, 2H, J=5.1 Hz, H-11), correlated by a ^{1}H-^{1}H COSY experiment, suggested the partial structure -CO-NH-CH_2-CH=CH- (cis). A sharp signal for one aromatic proton at δ 6.52 (s, 1H, H-18) and an intense broad resonance at δ 5.28 (br s, 3H) for exchangeable protons indicated the existence of a monosubstituted aminoimidazole unit in aplysinamisine I [**4**]. The presence of the latter moiety was also suggested by the ^{13}C-nmr signals at δ 149.97 (C-16), 115.05 (C-14), and 131.23 (C-18) (Table 2). The connection of the aminoimidazole ring to olefinic carbon C-13 was argued on the basis of detection of a weak cross peak due to long range coupling between H-13 and H-18 in the ^{1}H-^{1}H COSY spectrum and on the basis of uv data considerations (λ max 226 nm) (5).

The ^{13}C nmr (DMSO-d_6) of **4** (Table 2) exhibited signals for two sp^3 methylenes, of which one constituted an isolated spin system (δ 39.33) and the other bore a nitrogen atom (δ 37.88), four sp^2 methines (δ 131.23, 131.23, 121.83 and 121.04), and seven fully substituted sp^2 carbons (δ 158.42, 154.46, 149.97, 147.04, 120.58, 115.05, and 112.95). The remaining ^{13}C-nmr signals corresponded to three sp^3 carbons, each bearing an oxygen atom: a methyl group (δ 59.47), a methine (δ 73.52), and a quaternary carbon (δ 90.08). The ^{1}H- and ^{13}C-nmr data described above are fully consistent with structure **4**. The detailed interpretation of the nmr data enabled complete assignment of the ^{1}H- and ^{13}C-nmr signals and thus established the structure of aplysinamisine I to be **4**.

The most polar compound, aplysinamisine II [**5**] (0.156% dry wt) was obtained as an optically active, $[\alpha]_D = +47.0$, colorless semisolid from the fraction eluting with 10% MeOH(NH_3) in CH_2Cl_2 and was purified further by chromatography on a column of Si gel (35–75 mesh), eluted with $CHCl_3$-MeOH(NH_3) (4:1) or reversed-phase cc on SiO_2-ODS [MeCN-H_2O (1:1)]. This major brominated metabolite displayed an isotopic cluster of $[M+H]^+$ ions in its fabms spectrum at *m/z* 508, 510, and 512 (ratio 1:2:1), consistent with the presence of two bromine atoms. Accurate mass measurement of these ions (hrfabms) confirmed a formula of $C_{16}H_{24}Br_2N_5O_4$ (*m/z* 510.01640, $[M+H]^+$, Δ −0.99 mmu). The ir spectrum contained bands characteristic of alcohol, amine, and secondary amide groups (3588, 3400, 1658 cm^{-1}), while the uv spectrum had absorptions at λ max 284 nm (ϵ 2500) and 218 nm (ϵ 4600), indicative of a cyclohexadienyl moiety. Like aplysinamisine I [**4**], aplysinamisine II [**5**] had a ^{1}H-nmr spectrum (Table 1) that indicated a dibromospirocyclohexadienylisoxazole ring system. The ^{1}H-^{1}H

TABLE 1. ^{1}H-nmr Data (300 MHz) of Aplysinamisine I [**4**], Aplysinamisine II [**5**], Aplysinamisine III [**6**], and Aplysinamisine III Diacetate [**7**].[a]

Proton	Compound			
	4 (DMSO-d_6)	**5** (DMSO-d_6)	**6** (CD_3OD)	**7** ($CDCl_3$)
H-1	3.92 (br s, 1H)	3.88 (br s, 1H)	4.08 (s, 1H)	5.75 (1H, s)
H-2	—	—	—	—
H-3	—	—	—	—
H-4	—	—	—	—
H-5	6.56 (s, 1H)	6.53 (s, 1H)	6.37 (s, 1H)	6.26 (s, 1H)
H-6	—	—	—	—
H-7	3.62 (ABq, 1H, 18.0 Hz) 3.20 (ABq, 1H, 18.0 Hz)	3.62 (ABq, 1H, 18.0 Hz) 3.20 (ABq, 1H, 18.0 Hz)	3.74 (ABq, 1H, 18.0 Hz) 3.03 (ABq, 1H, 18.0 Hz)	3.35 (ABq, 1H, 18.3 Hz) 3.00 (ABq, 1H, 18.3 Hz)
H-8	—	—	—	—
H-9	—	—	—	—
10-NH	9.01 (t, 1H, 5.4 Hz, exchangeable)	8.46 (br t, 1H, 5.4 Hz, exchangeable)	—	6.80 (t, 1H, 5.7 Hz, exchangeable)
H-11	4.16 (br t, 2H, 5.1 Hz)	3.10 (m, 2H)	3.48 (m, 2H)	3.74 (m, 2H)
H-12	5.24 (dt, 1H)[b]	1.42 (m, 2H)	2.02 (tt, 2H, 6.9, 13.8 Hz)	1.99 (m, 2H)
H-13	6.06 (d, 1H, 11.4 Hz)	1.24 (m, 2H)	4.01 (t, 2H, 6.0 Hz)	4.02 (t, 2H, 5.7 Hz)
H-14	—	1.42 (m, 2H)	—	—
H-15	—	3.10 (m, 2H)	—	—
16-NH	—	7.85 (br t, 1H, exchangeable)	—	—
H-17	5.28 (br s, 1H, exchangeable)	—	7.57 (s, 2H)	7.44 (s, 2H)
H-18	6.52 (s, 1H)	7.35 (br s, 1H, exchangeable)	—	—
H-19	—	—	4.75 (t, 1H)[b]	5.68 (dd, 1H, 3.6, 8.4 Hz)
H-20	—	—	3.42 (br t, 2H, 7.8 Hz)	3.50 (m, 2H)
21-NH	—	—	—	6.02 (br s, 1H, exchangeable)
H-23	—	—	1.93 (5, 3H)	1.92 (s, 3H)
NH_2	5.28 (br s, 2H, exchangeable)	7.35 (br s, 2H, exchangeable)	—	—
OMe	3.63 (s, 3H)	3.58 (s, 3H)	3.69 (s, 3H)	3.70 (s, 3H)
1-OH	6.56 (1H, exchangeable)[b]	6.56 (1H, exchangeable)	—	—
OAc	—	—	—	2.07 (br s, 6H)

[a]Assignments were aided by ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, spin splitting patterns, and comparison of *J* values.
[b]Signal partially obscured by the residual solvent signal or the H_2O peak.

TABLE 2. ^{13}C-nmr (75 MHz) Data for Aplysinamisines I [**4**], II [**5**], and III [**6**], and Aplysinamisine III Diacetate [**7**].[a]

Carbon	Compound				
	4 (DMSO-d_6)	**4** (CD_3OD)	**5** (DMSO-d_6)	**6** (CD_3OD)	**7** ($CDCl_3$)
C-1	73.52 (d)	75.45 (d)	73.44 (d)	75.40 (d)	72.49 (d)
C-2	120.58 (s)	122.68 (s)	120.64 (s)	122.72 (s)	122.09 (s)
C-3	147.04 (s)	149.23 (s)	147.07 (s)	149.23 (s)	149.94 (s)
C-4	112.95 (s)	114.12 (s)	113.00 (s)	114.10 (s)	107.79 (s)
C-5	131.23 (d)	132.21 (d)	131.22 (d)	132.14 (d)	130.35 (d)
C-6	90.08 (s)	92.39 (s)	90.12 (s)	92.55 (s)	90.21 (s)
C-7	39.33 (t)	40.19 (t)	39.50 (t)	40.06 (t)	39.72 (t)
C-8	154.46 (s)	155.32 (s)	154.44 (s)	155.11 (s)	153.48 (s)
C-9	158.42 (s)	161.33 (s)	158.82 (s)	161.61 (s)	158.94 (s)
C-11	37.88 (t)	39.49 (t)	38.53 (t)	37.86 (t)	37.51 (t)
C-12	121.04 (d)	123.47 (d)	28.23 (t)[b]	30.76 (t)	29.54 (t)
C-13	121.83 (d)	122.33 (d)	23.28 (t)	72.30 (t)	72.07 (t)
C-14	115.05 (s)	130.81 (s)	27.99 (t)[b]	—	—
C-15	—	—	40.65 (t)	153.61 (s)	153.21 (s)
C-16	149.97 (s)	151.45 (s)	—	118.94 (s, 2×C)	118.59 (s, 2×C)
C-17	—	—	157.20 (s)	131.66 (d, 2×C)	130.82 (d, 2×C)
C-18	131.23 (d)	118.11 (d)	—	142.82 (s)	136.30 (s)
C-19	—	—	—	71.64 (d)	73.33 (d)
C-20	—	—	—	47.62 (t)	44.01 (t)
C-22	—	—	—	173.29 (s)	170.03 (s)
C-23	—	—	—	22.68 (q)	23.35 (q)
OMe	59.47 (q)	60.42 (q)	59.56 (q)	60.42 (q)	60.21 (q)
1-*C*OMe					169.75 (s)[b]
19-*C*OMe					169.37 (s)[b]
1-COCH$_3$					20.91 (q)[c]
19-COCH$_3$					20.57 (q)[c]

[a] ^{13}C-nmr multiplicities were obtained by Attached Proton Test (APT) sequences. Assignments were made on the basis of homo- and heteronuclear chemical shift correlation methods and comparison to known models. The δ values are in ppm downfield from TMS.

[b,c] Values with identical superscripts in each column may be interchanged.

COSY spectrum allowed assignment of the ^{1}H-nmr signals associated with this system. In addition, the ^{1}H-nmr and ^{1}H-^{1}H COSY spectra contained signals that required an -CO-NH-CH_2-CH_2-CH_2-CH_2-CH_2-NH-C(=NH)-NH_2 group. A four-proton signal at δ 3.10 (br m, 4H), due to overlapping signals of two distinct methylenes bearing nitrogen (H-11 and H-15), was coupled to signals at δ 8.46 (br t, 1H, J=5.4 Hz) and 7.85 (br t, 1H) due, respectively, to 10-NH and 16-NH, and to a third signal at δ 1.42 (br m, 4H) assigned to two distinct overlapping sp^3 methylenes (H-12 and H-14). In turn, the signal at δ 1.42 was coupled to a multiplet at δ 1.24 (2H) that was assigned to the C-13 methylene protons. Aplysinamisine II [**5**] was shown to be a guanidine derivative by positive coloration with Sakaguchi reagent (6) and by its ^{13}C-nmr signal at δ 157.20 (s). This observation, however, could not be confirmed chemically by its conversion with 2,4-pentanedione to the corresponding 3,5-dimethylpyrimidine derivative because of concurrent thermal decomposition of **5** during the required reaction conditions (7). The complete structure of aplysinamisine II [**5**] was established from comparison of its ^{13}C-nmr spectrum with those of 11-oxoaerothionin [**1**] and aplysinamisine I [**4**] (Table 2). A 2D ^{13}C-,^{1}H-nmr chemical shift correlation experiment allowed the complete assignment of all protonated carbons of **5**.

From a less polar fraction eluted with CH_2Cl_2-MeOH(NH_3) (90:10), after successive cc on Si gel [$CHCl_3$-MeOH (NH_3) (95:5)] and preparative tlc [SiO_2; $CHCl_3$-MeOH(NH_3) (95:5)], we isolated aplysinamisine III [**6**] (0.024% dry wt), a colorless semisolid, $[\alpha]_D$= +69.0°; uv λ max 282 nm (5200) and 208 nm (145,000). The positive ion fabms indicated a cluster of five molecular ions: 794, 796, 798, 800, and 802 in the ratio 1:4:6:4:1 that was attributed to $[M+Na]^+$, indicating the presence of four bromine atoms. The molecular formula of aplysinamisine III [**6**] was determined to be $C_{23}H_{25}Br_4N_3O_7$ by hrfabms (*m/z* 797.82940 $[M+Na]^+$, Δ +1.23 mmu). The ^1H nmr (Table 1) showed the following resonances: δ 6.37 (ethylenic proton), 4.08 (*H*- C-OH), 3.69 (-OMe), and an ABq system (δ 3.74 and 3.03, 1H each, J=18.0 Hz), ascribable to the same spirocyclooxazoline ring system previously encountered in aplysinamisines I [**4**] and II [**5**]. The 2D ^1H-^1H COSY spectrum of **6** revealed the proton connectivities from NH-10 on C-9 [δ 6.80 (in $CDCl_3$), br t, 1H, exchangeable] to the first of three contiguous methylene units [H-11 (δ 3.48, m, 2H); H-12 (2.02, tt, 2H), and H-13 (4.01, t, 2H)]. The COSY spectrum also showed that the benzyl proton (δ 4.75, t, 1H, H-19) and the 21-NH [δ 6.02 (in $CDCl_3$), br s, 1H, exchangeable] were coupled to the same methylene unit (δ 3.42, br t, 2H, H-20). The remaining ^1H-nmr signals were two isolated aromatic protons (δ 7.57, s, 2H, H-17) and a sharp methyl signal (δ 1.93, s, 3H, H-23) ascribable to an acetamide unit.

The ^{13}C nmr of **6** (Table 2) exhibited signals for five sp^3 methylenes, among which one bore an oxygen atom (δ 72.30) and two bore nitrogens (δ 37.86 and 47.62), two sp^2 methines (δ 132.14 and 131.66), and nine fully substituted sp^2 carbons. The two signals at δ 118.94 (s) and 131.66 (d) were quite intense and were attributed to two carbons each, due to a symmetrically 1,2,4,6-tetrasubstituted benzene ring. The remaining ^{13}C-nmr signals consisted of five sp^3 carbon resonances: one singlet (δ 92.55), two doublets (δ 75.40, 71.64), and two quartets (δ 60.42, 22.68). The ^1H- and ^{13}C-nmr data described above were fully consistent with the structure proposed for aplysinamisine III [**6**]. Acetylation of **6** with pyridine and Ac_2O led to the diacetyl acetamide **7**, confirming the presence of two hydroxyl groups. The ^1H- and ^{13}C-nmr spectra of aplysinamisine III diacetate [**7**] (Tables 1 and 2) showed a close correspondence to those recorded for hexadellin A [**8**], isolated for the first time from a shallow-water specimen of a British Columbia species of *Hexadella* (8) and later reisolated under the name araplysillin I from the sponge *Psammaplysilla arabica* collected in the Red Sea (9), to its acetylacetamide derivative **9** (prepared from **8** upon acetylation with Ac_2O/pyridine) and to fistularin 3 [**10**], isolated from the Caribbean sponge *Aplysina fistularis* (10). Therefore, we propose structure **6**, with unassigned stereochemistry at C-19, for the new bromotyrosine-derived alkaloid aplysinamisine III. Curiously, structure **6** (when R_3=H) had been proposed earlier by Kashman's group for psammaplysin B, an antibiotic isolated from the sponge *Psammaplysilla purpurea* (11). The correct structure for psammaplysin B has since been revised by Scheuer's group (12).

The hydroxyl groups at C-1 in aplysinamisines I [**4**], II [**5**], and III [**6**] were determined to be cis to the methylene carbon at C-7 from comparison of the observed ^1H-nmr chemical shifts in DMSO-d_6 with those of 11-oxoaerothionin [**1**]. Furthermore, the spectra of **4**, **5**, and **6** all have two sharp doublets (AB quartet) attributed to the geminal C-7 protons because of the interaction of the hydroxyl group with one of the geminal protons. This is only possible in the trans, trans configuration found in **1** and aplysinamisines **4**–**6** (13). In the present work the absolute stereochemistry shown in structures **4**–**6** is inferred; the enantiomers drawn have been chosen arbitrarily to conform with that of 11-oxoaerothionin [**1**]. The relative stereochemistry of the 19-OH group in **6** could not be determined from the spectral data accumulated.

Albeit marginally, aplysinamisines I, II, and III all showed in vitro antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* within the concentration range of 50–100 μg/disk. However, aplysinamisines I [**4**] and II [**5**] did not exhibit significant in vitro antitumor activity against the human breast (MCF-7) and T cell leukemia (CCRF-CEM) cell lines (IC_{50}>50 μg/ml). Although aplysinamisine I [**4**] also proved inactive against the human colon (HCT 116) cell line, aplysinamisine II [**5**] showed selective activity against the same solid tumor cell line (IC_{50}=10 μg/ml). The cytotoxic activities of aplysinamisine III [**6**] were as follows: MCF-7 (IC_{50}=30 μg/ml), CCRF-CEM (IC_{50}=6 μg/ml) and HCT 116 (IC_{50}=10 μg/ml).

EXPERIMENTAL

SPONGE COLLECTION AND TAXONOMY.—*A. cauliformis* was collected by one of the authors by scuba diving during an underwater expedition conducted in August 1, 1992 near Mona Island (Carabinero Beach) from a depth of 20 m. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. *A. cauliformis* is ramose (branching) with the digitate-like branches only a few cm in diameter. The branches often reconnect and there is little tendency for the sponge to be massive. Color varies from gray to greenish to yellow. The sponge is abundant in the South West of Puerto Rico and its depth range is from 15 to 25 m.

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra (neat) were recorded on a Nicolet 600 FT-IR spectrometer. Uv spectra of MeOH solutions were recorded on a Hewlett-Packard Chem Station 8452A spectrophotometer. Nmr spectra, with TMS (δ 0.00 ppm) as internal standard, were recorded on a General Electric Multinuclear GN-300. All cc were carried out on Analtech Si gel (35–75 mesh) or Analtech Si gel-ODS. Optical rotations were measured with a Perkin-Elmer 243B digital polarimeter. All chromatographic separations described required distilled MeOH saturated with NH_3.

EXTRACTION AND ISOLATION OF BROMOTYROSINE COMPOUNDS.—A frozen sample of *A. cauliformis* was cut into small pieces, and the H_2O was removed by lyophilization. The resulting sponge residue (651.2 g) was blended (2×2000 ml) with MeOH-$CHCl_3$ (1:1) and filtered to remove solids. The combined extracts were concentrated in vacuo; the residue (142.2 g) was taken up with H_2O (1500 ml) and extracted successively with hexane (18.5 g) and $CHCl_3$ (3×1000 ml). The $CHCl_3$ extract was filtered with suction through two layers of sand and Celite, and the solvent was later removed to give a dark green oil (21.6 g). A portion of the $CHCl_3$-soluble material (15.1 g) was chromatographed on a Si gel column (550 g) and eluted with CH_2Cl_2 containing increasing proportions of MeOH previously saturated with NH_3, to afford 25-ml fractions. A total of 23 fractions, combined on the basis of tlc analyses, were eluted with CH_2Cl_2-MeOH(NH_3) (90:10). Of these, fractions 6 (1.79 g), 7 (1.38 g), 12 (200 mg), and 19 (1.26 g) showed modest antimicrobial activity and therefore were subsequently purified by chromatography. Fraction 6 (1.79 g, 0.4% based on the dry wt of the sponge) was identified as known 11-oxoaerothionin [**1**] by comparison of its ir, ^{1}H-nmr, ^{13}C-nmr, and lrfabms spectra with reported values (3). Fraction 7 (1.38 g) was rechromatographed on a glass column of Si gel (52 g) with $CHCl_3$-MeOH(NH_3) (95:5) to yield 8 new subfractions. One of these, subfraction 4 (431 mg), was subsequently purified by preparative tlc [$CHCl_3$-MeOH(NH_3) (95:5)] with multiple developments (14) to give pure aplysinamisine III [**6**] (159 mg). Fraction 12 (200 mg) was passed successively through two short columns of Si gel [$CHCl_3$-MeOH(NH_3) (90:10)] to give analytically pure aplysinamisine I [**4**] (31 mg). Fraction 19 (1.26 g) was rechromatographed on a glass column of Si gel (50 g) with $CHCl_3$-MeOH(NH_3) (80:20) to yield nearly pure aplysinamisine II [**5**] (702 mg). An analytically pure sample of **5** was generated subsequently by reversed-phase cc (Analtech SiO_2-ODS, 9 g) with 50% H_2O/MeCN (69.2 mg).

Aplysinamisine I [**4**].—Compound **4**: colorless oil; $[\alpha]^{26}D$ +121.9° (*c*=5.7,MeOH); uv λ max (MeOH) 266 nm (ε 13,100), 226 nm (ε 13,700); ir (neat) 3654–3000, 2937, 1660, 1595, 1543, 1435, 1273, 1219, 1047, 1025, 997, 824, 765 cm^{-1}; hrfabms *m/z* 503.97073 ($C_{16}H_{18}{}^{79}Br^{81}BrN_5O_4$ requires 503.97044); ^{1}H nmr (300 MHz, DMSO-d_6) see Table 1; ^{13}C nmr (75 MHz, DMSO-d_6 and CD_3OD) see Table 2; ^{1}H nmr (300 MHz, CD_3OD) δ 6.55 (s, 1H, H-18), 6.40 (s, 1H, H-5), 6.19 (d, 1H, *J*=11.4 Hz, H-13), 5.39 (dt, 1H, *J*=6.6, 11.4 Hz, H-12), 4.15 (dd, 1H, *J*=1.5, 6.6 Hz, H-11), 4.09 (s, 1H, H-1), 3.78 and 3.09 (ABq, 1H each, *J*=18.3 Hz, H-7), 3.71 (s, 3H, -OMe); fabms *m/z* (rel. int.) $[M+H]^+$ 506 (53), 504 (100), 502 (55), 307 (5), 154 (50).

Aplysinamisine II [**5**].—Compound **5**: colorless semisolid; $[\alpha]^{26}D$ +47.0° (*c*=7.9, MeOH); uv λ max (MeOH) 284 nm (ε 2500), 218 nm (4600); ir (neat) 3588–3050, 3024, 2782, 1658, 1402, 1024, 992 cm^{-1}; hrfabms *m/z* 510.01640 ($C_{16}H_{24}{}^{79}Br^{81}BrN_5O_4$ requires 510.01739); ^{1}H nmr (300 MHz, DMSO-d_6) see Table

1; ^{13}C nmr (75 MHz, DMSO-d_6) see Table 2; fabms *m/z* (rel. int.) $[M+H]^+$ 512 (25), 510 (50), 508 (25), 451 (7), 273 (22), 219 (12), 154 (100).

Aplysinamisine III [**6**].—Compound **6**: colorless semisolid; $[\alpha]^{26}D$ +69.0° (*c*=6.4, MeOH); uv λ max (MeOH) 282 nm (ε 5200), 208 nm (ε 145,000); ir (KBr) 3692–3026, 2933, 2884, 2853, 1649, 1643, 1545, 1400, 1384, 1281, 1095, 1044, 988, 918, 868, 765, 739, 704 cm^{-1}; hrfabms *m/z* 797.82940 ($C_{23}H_{25}{}^{79}Br_2{}^{81}Br_2N_3O_7Na$ requires 797.82817); ^{1}H nmr (300 MHz, CD_3OD) see Table 1; ^{13}C-nmr (75 MHz, CD_3OD) see Table 2; fabms *m/z* (rel. int.) $[M+Na]^+$ 802 (19), 800 (67), 798 (95), 796 (67), 794 (21), 776 (28), 700 (9), 681 (14), 661 (4), 612 (33), 532 (38), 510 (100), 482 (85), 464 (42), 437 (65), 413 (48).

ACETYLATION OF **6** TO FORM APLYSINAMISINE III DIACETATE [**7**].—Aplysinamisine III [**6**] (74.9 mg) was treated with 4 ml of Ac_2O-pyridine (1:1) and left overnight at room temperature with continuous stirring. After removal of the solvents in vacuo the crude oily residue isolated was loaded into a short Si gel column (40 g) and eluted with 1% MeOH(NH_3) in $CHCl_3$. Diacetate acetamide **7** was obtained pure (53 mg) as a colorless homogeneous oil.

Aplysinamisine III diacetate [**7**].–Compound **7**: colorless oil; $[\alpha]^{27}D$ +77.9° (*c*=2.99, $CHCl_3$); ir (neat) 3480–3130, 2936, 1753, 1745, 1672, 1665, 1547, 1538, 1371, 1223, 1045, 985 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) see Table 1; ^{13}C nmr (75 MHz, $CDCl_3$) see Table 2; fabms *m/z* (rel. int.) $[M+H]^+$ 860 (15) ($C_{27}H_{30}{}^{79}Br_2{}^{81}Br_2N_3O_9$ requires 860), 816 (43), 800 (55), 758 (15), 740 (23), 659 (5), 479 (30), 279 (100), 241 (18).

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