11-Oxoaerothionin: A Cytotoxic Antitumor Bromotyrosine-Derived Alkaloid from the Caribbean Marine Sponge Aplysina lacunosa

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11-OXOAEROTHIONIN: A CYTOTOXIC ANTITUMOR BROMOTYROSINE-DERIVED ALKALOID FROM THE CARIBBEAN MARINE SPONGE *APLYSINA LACUNOSA*[1]

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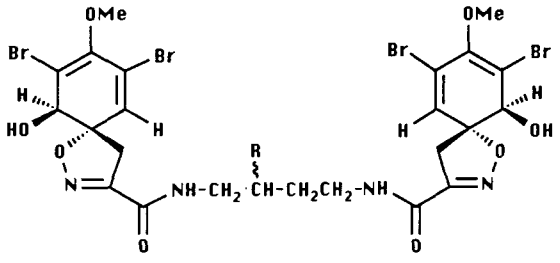
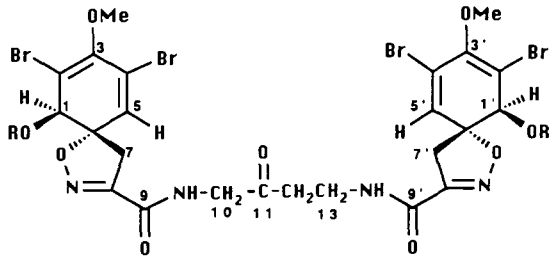
ABSTRACT.—A new cytotoxic bromotyrosine-derived secondary metabolite, 11-oxoaerothionin [**3**], was isolated from the Caribbean sea sponge *Aplysina lacunosa*. The structure of **3** was argued on the basis of detailed spectroscopic analysis and by chemical conversion to the known antibiotic compound 11-hydroxyaerothionin [**2**]. When screened against a panel of four human cell lines, 11-oxoaerothionin [**3**] showed pronounced as well as selective antitumor activity toward the human colon (HCT 116) cell line within the limited concentration range of 0.01–0.1 μg/ml.

The accumulation of biologically active substances in marine invertebrates has been observed as a general phenomenon and has been suggested to reflect the defensive strategy of these often sedentary, filter-feeding animals (1). Since marine invertebrates do not produce antibodies, their defense mechanisms are based primarily on phagocytosis by leucocytes, aided by the natural exudation of low mol wt, nonproteinaceous substances (2). These low mol wt compounds may have a high level of cytospecificity for malignant as opposed to normal cells, and they may therefore provide models for the synthesis of novel anticancer compounds (3).



1 R=H
2 R=OH



3 R=H
4 R=Ac

Marine sponges of the order Veron-

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gida have been praised for their ability to produce bromotyrosine-derived alkaloids, many of which have been described as powerful antibiotics or strongly cytotoxic antitumor substances (4). A range of unusual secondary metabolites containing up to four bromotyrosine residues have been isolated from sponges belonging to this order which includes the genera *Aplysina, Verongula, Pseudoceratina, Psammaplysilla,* and *Ianthella*. In such metabolites the tyrosine side chain has either been removed oxidatively or has been converted into a variety of nitrogenous groups while the aromatic ring has either been retained or has undergone rearrangement or partial reduction (5). More recently, remarkable examples of these unusual secondary metabolites containing disulfide linkages have been reported (6–9).

In a continuation of our systematic evaluation of extracts of marine invertebrates with anticancer activity, we now report the isolation of a new metabolite from *Aplysina lacunosa* Lamarck (family Aplysinellidae) collected off the west coast of Puerto Rico. A specimen of the sponge collected in July 1990 by SCUBA from a depth of 25 m near Desecheo Island was diced and blended in MeOH-$CHCl_3$ (1:1). Our interest in *A. lacunosa* stemmed from the potent in vitro antimicrobial activity and cytotoxic action (ED_{50} 7.3 μg/ml) shown by the crude MeOH/$CHCl_3$ extract against CHO-k1 cells. The solvent was evaporated in vacuo to an aqueous suspension which was partitioned between H_2O and $CHCl_3$. The bioactive $CHCl_3$ extract (21.2 g) was chromatographed on a Si gel column using mixtures of $CHCl_3$/MeOH (saturated with NH_3) of increasing polarity. Selected fractions were combined on the basis of tlc analyses and chromatographed on hplc on a C-18 reversed-phase column using mixtures of MeOH/H_2O as mobile phase.

Along with related metabolites aerothionin [**1**] (10), 11-hydroxyaerothionin [**2**] (11), 11,19-dideoxyfistularin-3 (11), and fistularin 3 (12), 11-oxoaerothionin [**3**] was obtained as an amorphous, optically active, white powder [mp 174.6–176.6° (dec)]. Although **3** failed to show a molecular ion in the eims, it produced a cluster of five ions centered at *m/z* 854 in the positive ion hr fabms that was attributed to $[M + Na]^+$, suggesting the molecular formula $C_{24}H_{24}Br_4N_4O_9$ and therefore implying thirteen degrees of unsaturation. Since most of the fourteen carbon resonances observed in the ^{13}C-nmr spectrum of 11-oxoaerothionin [**3**] (Table 1) appear as closely-spaced doublets, it could be concluded that **3** was made up of two nearly identical bromotyrosine residues. The ir spectrum contained bands characteristic of alcohol, amine, ketone, and α-iminoamide functionalities (3600, 3350, 1713, 1665 cm^{-1}), while the uv spectrum had absorptions at λ max 284 nm (ε 11,500) (cis-dienoid group) and 262 nm (ε11,600), indicative of the cyclohexadienyl moieties (11). The ^1H-nmr spectrum in DMSO-d_6 contained signals [δ 3.60 and 3.19 (each 2H, ABq, $J = 18.2$ Hz, H-7, H-7′), 3.94 and 3.88 (each 1H, overlapping doublets, $J = 8.5$ Hz, H-1, H-1′), 6.60 and 6.57 (each 1H, s, H-5, H-5′), and 6.41 and 6.35 (each 1H, overlapping doublets, $J = 8.5$ Hz, 1,1′-OH)] ascribable to two dibromospirocyclohexadienylisoxazole ring systems (10,12). The ^1H-nmr signals associated with these systems (Table 1) were assigned on the basis of ^1H-^1H COSY, homonuclear spin-decoupling experiments, and comparison of J values. The two-proton signal near δ 6.38 (pseudo triplet, 2H, exchangeable) due to overlapping doublet signals of two distinct -OH protons (1-OH, 1′-OH) is coupled to another two-proton signal near δ 3.91 (pseudo triplet, 2H) ascribable to overlapping doublet signals of two distinct oxygen-bearing-methine protons (H-1, H-1′). Further, signals at δ 3.63 and 3.60 (each 3H, s) were indicative of the presence of two nearly equivalent methoxy groups. These data

TABLE 1. ^{1}H- (300 MHz, DMSO-d_6) and ^{13}C- (75 MHz, DMSO-d_6) nmr Data of 11-Oxoaerothionin [3] and Comparison with ^{13}C-nmr Shifts of Aerothionin [1] and 11-Hydroxyaerothionin [2].

Position	Compound			
	3		1	2
	δ H[a]	δ C[b]	δ C[c]	δ C[d]
1,1′	3.94 (d, 1H, 8.5 Hz), 3.88 (d, 1H, 8.5 Hz)	73.55 (d, 2C)	75.1 (d)	73.8 (d, 2C)
2,2′	—	120.88 (s), 120.84 (s)	122.0 (s)	121.4 (s), 121.3 (s)
3,3′	—	147.13 (s), 147.11 (s)	148.7 (s)	147.7 (s, 2C)
4,4′	—	113.08 (s, 2C)	113.8 (s)	113.1 (s, 2C)
5,5′	6.60 (s, 1H), 6.57 (s, 1H)	131.20 (d, 2C)	132.3 (d)	130.6 (d, 2C)
6,6′	—	90.50 (s), 90.24 (s)	91.5 (s)	91.9 (s), 91.8 (s)
7,7′	3.60 (ABq, 2H, 18.2 Hz),[e] 3.19 (ABq, 2H, 18.2 Hz)	39.70 (t)[e], 39.45 (t)[e]	40.2 (t)	38.7 (t, 2C)
8,8′	—	154.37 (s), 154.05 (s)	155.3 (s)	153.9 (s, 2C)
9,9′	—	159.11 (s), 158.86 (s)	160.0 (s)	160.0 (s, 2C)
10	4.02 (d, 2H, 5.7 Hz)	48.52 (t)	39.4 (t)	36.2 (t)
11	—	204.43 (s)	26.7 (t)	68.0 (d)
12	2.70 (t, 2H, 6.8 Hz)	38.59 (t)[e]	—	45.0 (t)
13	3.34 (m, 2H)[e]	33.82 (t)	—	33.6 (t)
OMe	3.63 (s, 3H), 3.60 (s, 3H)	59.63 (q, 2C)	60.2 (q)	60.0 (q, 2C)
9-NH	8.66 (t, 1H, 5.7 Hz)	—	—	—
9′-NH	8.48 (t, 1H, 5.5 Hz)	—	—	—
1,1′-OH	6.41 (d, 1H, 8.5 Hz), 6.35 (d, 1H, 8.5 Hz)	—	—	—

[a]Assignments were aided by ^{1}H-^{1}H COSY, spin splitting patterns, selective decoupling experiments, and comparison of J values.

[b]Assignments were made on the basis of ^{1}H-^{13}C COSY and proton attachments via APT. The δ values are in ppm and are referenced to the residual DMSO signal (39.5 ppm).

[c]Recorded in Me_2CO-d_6 [see Kernan *et al.* (11)].

[d]Recorded in $CDCl_3$ [see Kernan *et al.* (11)].

[e]Signal partially obscured by the residual DMSO signal or by the H_2O peak.

suggested that 11-oxoaerothionin [**3**] has the same ring system as in the *Verongia* metabolite aerothionin [**1**] reported by Fattorusso *et al.* (10) and McMillan *et al.* (13).

The ^{1}H-nmr spectrum (Table 1) and the ^{1}H-^{1}H COSY spectrum revealed the partial structure -CO-NH-CH_2-CO-CH_2CH_2-NH-CO- : N-H; δ 8.66 (t, 1H, J = 5.7 Hz, exchangeable) and 8.48 (t, 1H, J = 5.5 Hz, exchangeable); -N-CH_2CO-, δ 4.02 (d, 2H, J = 5.5 Hz); -COCH_2CH_2N-, δ 2.70 (t, 2H, J = 6.8 Hz) and 3.34 (m, 2H). A ^{1}H-^{1}H COSY spectrum of **3** in DMSO-d_6 allowed assignment of the ^{1}H-nmr signals associated with this partial structure. Signals assigned to the C-12 methylene group (δ 2.70) showed a correlation in the COSY to a signal at δ 3.34 ascribable to the C-13 methylene group that in turn was coupled to a signal assigned to the upfield NH proton at δ 8.48. On the other hand, the NH proton resonating downfield at δ 8.66 showed coupling only to the signal absorbing at δ 4.02 assigned to the C-10 methylene, which in turn did not show additional correlations in the COSY. Hence, since the connectivity of the C-10 protons stopped there, C-11 must be a quaternary carbon.

Comparison of the ^{13}C-nmr spectrum of 11-oxoaerothionin [**3**] with the ^{13}C-nmr spectra of aerothionin [**1**] and 11-hydroxyaerothionin [**2**] (Table 1) re-

vealed that the methylene group at C-11 in aerothionin was replaced by a carbonyl group in 11-oxoaerothionin. Acetylation of **3** with pyridine and Ac_2O yielded the expected diacetate **4**, confirming the presence of two hydroxyl groups. The hydroxyl groups at C-1 and C-1′ in 11-oxoaerothionin [**3**] were determined to be cis to the methylene carbons at C-7 and C-7′, respectively, from comparison of the observed ^{1}H-nmr chemical shifts in Me_2CO-d_6 of protons H-1, H-5, H-7, and 1-OH with those of aerothionin [**1**] and 11-hydroxyaerothionin [**2**] (11). Moreover, reduction of the 11-oxo group of 11-oxoaerothionin [**3**] under mild conditions ($NaBH_4$/EtOH, 25°) produced 11-hydroxyaerothionin [**2**] with apparent high stereoselectivity as determined by ^{1}H- and ^{13}C-nmr analyses of the product and by comparison of its tlc and hplc retention times with those of the natural product. These data suggested that 11-oxoaerothionin had the proposed structure **3**. The structure drawn implies relative stereochemistry only. The enantiomer shown has been chosen arbitrarily to conform with that of aerothionin [**1**]; the relative stereochemistry of the 11-OH group in **2** could not be established unambiguously from its synthesis or from the analysis of its spectral data.

In general, the cytotoxic effects of chemical agents on cells may include altered cellular morphology, failure of the cell to attach to surfaces, or changes in the rate of cell processes such as growth, death, and disintegration (14). Since the ultimate goal of a selection process is to find compounds that are selectively toxic to tumor cells with little toxicity to normal cells, a screening procedure using renegade cells is usually the best model for cytotoxicity studies, particularly those of slow growing tumors such as colon and mammary cancers which represent some of the tumors with the highest morbidity and mortality in man (15). Compound **3** was therefore screened for selective anticancer activity against a panel of four human tumor cell lines, including three solid tumors and one leukemic line. Five different concentrations of drug (0.01–100 μg/ml) were used in the basic screening studies. From these results, it was observed that within a limited concentration range (0.01–0.1 μg/ml), 11-oxoaerothionin showed pronounced selective cytotoxic activity toward the human colon (HCT 116) cell line. At concentrations equal to or greater than 20 μg/ml, compound **3** was cytotoxic to all human tumor cells tested. Because of its selective cytotoxic nature, compound **3** is a clear candidate for further testing as a potential antineoplastic agent. During in vitro antimicrobial assays 11-oxoaerothionin [**3**] inhibited the growth of *Escherichia coli* (MIC = 10 μg/ml), *Staphylococcus aureus* (MIC = 30 μg/ml), and *Pseudomonas aeruginosa* (MIC = 30 μg/ml).

EXPERIMENTAL

SPONGE COLLECTION AND TAXONOMY.—*A. lacunosa* was collected by the authors during July 1990 by SCUBA diving near Desecheo Island, at a depth of 25 m. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. *A. lacunosa* is a thick-walled, tubular species which usually occurs as single tubes, although occasionally multiple-tube individuals are observed. The outer surface is strongly convoluted or covered with circular pits or elongate grooves. The sponge appears in various colors (greenish yellow, pinkish lavender, red-brown) and reaches over 1 m in height and 10 cm in diameter with a large apical cloacal opening.

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra (neat and KBr) were recorded on a Nicolet 600 FT-IR spectrometer. Uv spectra of DMSO solutions were recorded on a Hewlett-Packard Chem Station 8452A spectrophotometer. Nmr spectra of DMSO-d_6 or $(CD_3)_2CO$ solutions, with TMS as internal standard, were recorded on a General Electric Multinuclear QE-300. High resolution mass measurements were supplied by Dr. Ronald L. Cerny from the Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska–Lincoln. All cc were carried out on Analtech Si gel (35–75 mesh) and tlc analyses using Analtech glass packed precoated Si gel plates. Final purifications were performed on a Beckman solvent delivery

system (Model 126) equipped with a Beckman uv detector (Model 167) and ri detector (Model 156), using a Beckman Ultrasil-ODS semipreparative column (10 mm × 25 cm; 40% H_2O in MeOH). Melting point was measured with a Büchi 535 melting point apparatus, and optical rotation was measured with a Perkin-Elmer 243B digital polarimeter. Antibiosis tests were done by the Petri-disc zonal inhibition technique at doses of 30, 10, and 1 μg of test compound per disc. All solvents used were either spectral grade or were distilled from glass prior to use.

EXTRACTION AND ISOLATION OF BROMOTYROSINE COMPOUNDS.—A frozen sample of *A. lacunosa* was cut into small pieces, and the H_2O was removed by lyophilization. The resulting sponge residue (301 g) was blended (3 × 1500 ml) with MeOH-$CHCl_3$ (1:1) mixtures and filtered to remove solids. The combined extracts were concentrated in vacuo; the residue (48.3 g) was taken up with H_2O (1000 ml) and extracted with $CHCl_3$ (3 × 1000 ml). The organic layer was filtered and the solvent removed to give a dark green oil (21.2 g). A portion of the $CHCl_3$-soluble material (5.05 g) was chromatographed on a Si gel column (150 g) and eluted with $CHCl_3$ containing increasing proportions of MeOH previously saturated with NH_3, to afford 25–50 ml fractions. Fractions eluted with $CHCl_3$-MeOH (90:10) were combined (1.50 g) and rechromatographed on a glass column (2.25 cm × 144 cm) of Si gel (90 g) with $CHCl_3$-MeOH (95:5) saturated with NH_3. All the bromotyrosine compounds present eluted out as follows: 11,19-dideoxyfistularin 3 (40.7 mg), aerothionin [**1**] (109 mg), 11-oxoaerothionin [**3**] (151 mg), fistularin 3 (342 mg), and 11-hydroxyaerothionin [**2**] (122 mg). All the known compounds were identified by comparison of their ir, ^{1}H-nmr, ^{13}C-nmr, and fabms spectra with reported values. Analytically pure samples of 11-oxoaerothionin [**3**] were generated subsequently by reversed-phase hplc (5 μm, 10 × 250 mm, Beckman Ultrasil C-18) with 40% H_2O/MeOH.

11-Oxoaerothionin [**3**].—Compound **3**: white powder; mp 174.6–176.6° (dec); $[\alpha]^{25}D$ +181.15° (c = 2.17 g/100 ml, DMSO); uv λ max (DMSO) 284 nm (ε 11,500), 262 (ε 11,600); ir (neat) 3600–3000, 1713, 1665, 1558, 1552, 1435, 1293, 1271, 1131, 1100, 1025 cm^{-1}; hrfabms *m/z* 831.8235 (Δ 0.1 mmu) for $C_{24}H_{24}{}^{79}Br_2{}^{81}Br_2N_4O_9$; ^{1}H nmr (300 MHz, DMSO-d_6) see Table 1; ^{13}C nmr (75 MHz, DMSO-d_6) see Table 1. The cytotoxic activities of 11-oxoaerothionin [**3**] were determined as follows: on human breast (MCF-7) IC_{50} = 20 μg/ml; melanoma (SK5-MEL) IC_{50} = 10 μg/ml; colon (HCT 116) IC_{50} = 10 μg/ml; and T cell leukemia (CCRF-CEM) IC_{50} = 3.5 μg/ml. The assays were based on the ability of living tumor cells to reduce a tetrazolium dye (XTT) to a soluble purple formazan metabolite (16,17).

ACETYLATION OF **3** TO FORM DIACETATE **4**.—Compound **3** (20 mg) was treated with 3 ml of Ac_2O-pyridine (1:1) and left overnight at room temperature. The product was diluted with H_2O and freeze-dried. Purification of the product by hplc using C-18 (5 μ) with 15% H_2O/MeOH afforded diacetate **4**.

11-Oxoaerothionin diacetate [**4**].—Compound **4**: white powder; mp 111.9–112.6°; ir ($CHCl_3$) 3363, 3062, 3014, 2938, 2840, 1755, 1710, 1673, 1537, 1371, 1217, 1018, 912, 759 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 7.21 (1H, t, exchangeable), 7.06 (1H, t, exchangeable), 6.34 (1H, s), 6.30 (1H, s), 5.85 (1H, s), 5.78 (1H, s), 4.27 (2H, m), 3.83 (6H, s), 3.68 (2H, m), 3.47 (2H, m), 3.10 (2H, ABq), 2.84 (2H, m), 2.20 (6H, s); ^{13}C nmr (75 MHz, $CDCl_3$) δ 203.04 (s), 169.59 (s), 169.52 (s), 158.87 (s, 2C), 153.48 (s), 153.16 (s), 149.86 (s), 149.77 (s), 130.48 (d), 130.44 (d), 121.98 (s), 121.82 (s), 107.91 (s), 107.75 (s), 90.16 (s), 89.78 (s), 73.17 (d, 2C), 60.24 (q, 2C), 48.83 (t), 39.85 (t), 39.58 (t), 39.40 (t), 33.94 (t), 20.68 (q, 2C); fabms *m/z* (rel. int.) $[M + Li]^+$ 923 (18), 715 (5), 581 (17), 496 (7), 447 (40), 362 (2), 294 (22), 241 (3), 160 (100).

REDUCTION OF **3** WITH $NaBH_4$ TO FORM 11-HYDROXYAEROTHIONIN [**2**].—A solution of 11-oxoaerothionin [**3**] (9.0 mg) in EtOH (5 ml) was treated with $NaBH_4$ (2.5 mg) and left stirring for 25 min at room temperature. After removal of the solvent at 35° in vacuo the product was purified by hplc using C-18 (5 μ) with 40% H_2O/MeOH. The identification of product **2** was established by comparison of its retention time (by tlc and hplc) and its ^{1}H- and ^{13}C-nmr spectra with those of authentic 11-hydroxyaerothionin [**2**].

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LITERATURE CITED

1. Y. Hashimoto, "Marine Toxins and Other Bioactive Marine Metabolites," Japan Scientific Societies Press, Tokyo, 1976, p. 245.
2. J.C. Harshbarger and C.J. Dawe, in: "Unifying Concepts of Leukemia." Ed. by R.M. Dutcher and L. Chieco-Bianchi, S. Karger, Basel, 1973, pp. 1–25.
3. A.F. Hegyeli and R.J. Hegyeli, in: "Antitumor Compounds of Natural Origin: Chemistry and Biochemistry." Ed. by A. Aszalos, CRC Press, Boca Raton, Florida, 1981, pp. 1–25.
4. M.H.G. Munro, R.T. Luibrand, and J.W. Blunt, in: "Bioorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, New York, 1987, Vol. 1, pp. 93–176.
5. D.J. Faulkner, *Nat. Prod. Rep.*, **7**, 269 (1990) and previous papers in the series, and references cited therein.
6. A.D. Rodríguez, A.K. Rhone, and P.J. Scheuer, *Tetrahedron Lett.*, **28**, 4989 (1987).
7. E. Quiñoá and P. Crews, *Tetrahedron Lett.*, **28**, 3229 (1987).
8. L. Arabshahi and F.J. Schmitz, *J. Org. Chem.*, **52**, 3584 (1987).
9. C. Jiménez and P. Crews, *Tetrahedron*, **47**, 2097 (1991).
10. E. Fattorusso, L. Minale, G. Sodano, K. Moody, and R.H. Thomson, *J. Chem. Soc., Chem. Commun.*, 752 (1970).
11. M.R. Kernan, R.C. Cambie, and P.R. Bergquist, *J. Nat. Prod.*, **53**, 615 (1990).
12. Y. Gopichand and F.J. Schmitz, *Tetrahedron Lett.*, **41**, 3921 (1979).
13. J.A. McMillan, I.C. Paul, Y.M. Goo, K.L. Rinehart Jr., W.C. Krueger, and L.M. Pschigoda, *Tetrahedron Lett.*, **22**, 39 (1981).
14. S. Horvath, *Toxicology*, **16**, 59 (1980).
15. M. Suffness, D.J. Newman, and K. Snader, in: "Bioorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, New York, 1989, Vol. 3, pp. 131–168.
16. M.C. Alley, D.A. Scudiero, A. Monks, M.L. Hursey, M.J. Czerwins, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, and M.R. Boyd, *Cancer Res.*, **48**, 589 (1988).
17. D.A. Scudiero, R.H. Shoemaker, K.D. Paull, A. Monks, S. Tierney, T.H. Nofziger, M.J. Currens, D. Seniff, and M.R. Boyd, *Cancer Res.*, **48**, 4827 (1988).

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