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CHEMISTRY OF VERONGIDA SPONGES, II.[1] CONSTITUENTS OF THE CARIBBEAN SPONGE *APLYSINA FISTULARIS* FORMA *FULVA*

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ABSTRACT.—A detailed analysis of the secondary metabolites of the Verongida sponge *Aplysina fistularis* forma *fulva* has been performed. Twelve bromotyrosine derivatives have been identified, of which two, **14** and **15**, are novel compounds. Structure determination of compounds **14** and **15**, performed by spectroscopic means, resulted in revision of the ^{13}C-nmr assignments of aerothionine [**1**] and its derivatives. The sponge also elaborates an unusual imidazotetrahydropyrimidine derivative [**16**], probably derived from histidine, which has not yet been described as a naturally occurring compound.

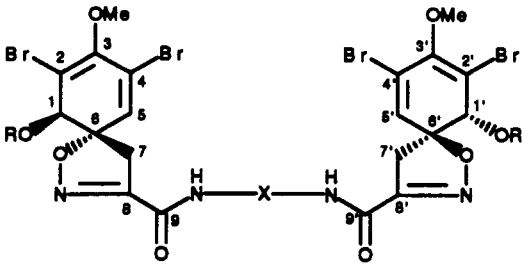
Verongida are marine horny sponges which are characterized by a unique biochemistry. They all lack terpenes, and possess a moderately high percentage of sterols, generally with the aplystane skeleton, and, above all, elaborate a series of brominated metabolites derived from tyrosine, which are considered to be peculiar to species belonging to this order. Another typical feature of Verongida sponges is their high phenotypic variability, which often results in ambiguous identifications based on diagnostic morphological criteria. In order to utilize their characteristic bromocompounds as chemical markers, which could support taxonomic work, we are studying in detail the secondary metabolites of a number of Verongida species collected during the Fenical expeditions (summers of 1990 and 1992) along the coasts of the Bahama Islands. In this paper we wish to report the results obtained from a detailed analysis of a specimen of a Verongida sponge from Grand Bahama Island, identified as *Aplysina fistularis* forma *fulva* (Pallas).

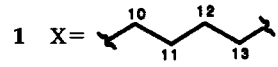
Some data on the secondary metabolites of this sponge have already been reported. In 1979, Gopichand and Schmitz (2) isolated aerothionine [**1**], the bis-2-oxazolidone derivative **2**, and fistularins-1 [**3**], -2 [**4**], and -3 [**5**] from a specimen collected along the coasts of the Virgin Islands, while in 1981 Makarieva *et al.* (3), by studying several Verongida from the Cuban coast, identified in the MeOH extract of *A. fistularis* aeroplysinin-2 [**6**] and 3,5-dibromo-1-hydroxy-4-oxo-2,5-cyclohexadien-1-acetamide [**7**], in addition to the ketal **8**, which was considered an artifact formed during the isolation. Moreover, in 1981 Tymiak and Rinehart (4) isolated the bromohydroquinone **9**, and in 1985 Walker *et al.* (5) identified aerothionine [**1**] and homoaerothionine [**10**] from specimens of *A. fistularis* collected along the coasts of California. However, none of the above papers has given a comprehensive view of the secondary metabolism of *A. fistularis*, which is the object of the present communication.

RESULTS AND DISCUSSION

Analysis of the BuOH-soluble material from the Me_2CO extracts indicated that our Caribbean specimen of *A. fistularis* elaborates all the bromo-compounds previously

[1]For Part 1 in this series, see Albrizio *et al.* (1).





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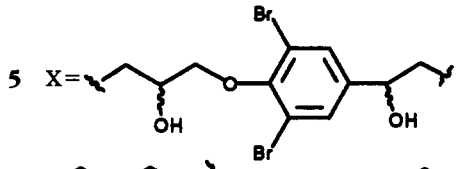

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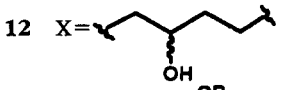

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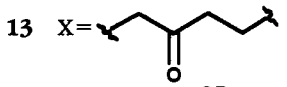

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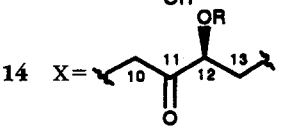

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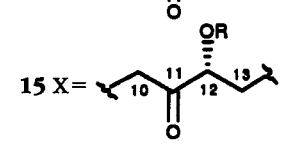

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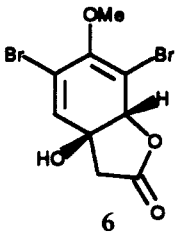
R=H **r** R=(*R*)-MTPA **s** R=(*S*)-MTPA



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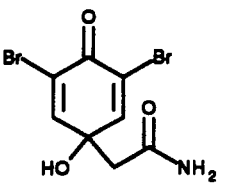

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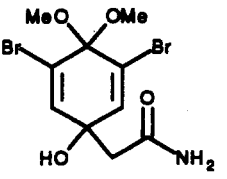

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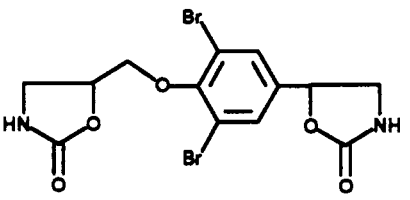

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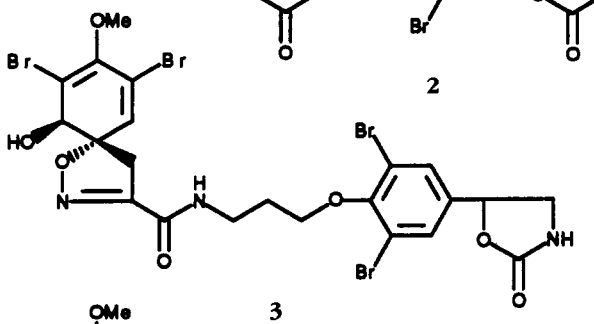

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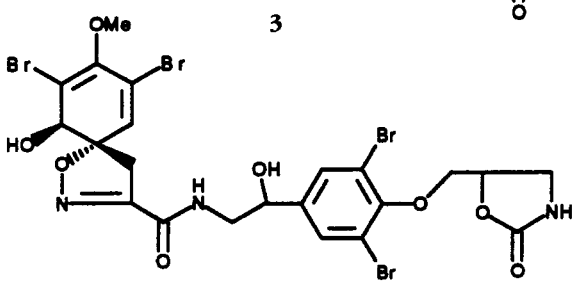

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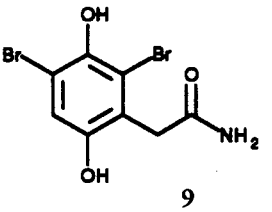

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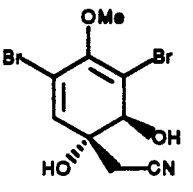

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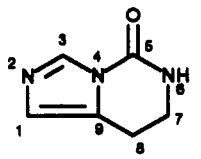

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isolated, apart from fistularin-2 and compound **2**, which were isolated only in trace amounts by Gopichand and Schimtz. Five further bromo-compounds [**11–15**] were also isolated. Aeroplysinin 1 [**11**] was first isolated from *Aplysina aerophoba* in 1972 (6), and since then has been found in several Verongida sponges. 11-Hydroxyaerothionin [**12**] has been isolated from *Pseudoceratina durissima* by Kernon *et al.* (7), and 11-oxoaerothionin [**13**] has recently been reported from the Caribbean sea sponge *Aplysina lacunosa* by Acosta and Rodriguez (8). The two epimeric 11-oxo-12-hydroxyaerothionins, **14** and **15**, are novel compounds.

Specimens of *A. fistularis* were collected by scuba at a depth of 1 m off Grand Bahama Island in July 1992. The BuOH-soluble material from the Me_2CO extracts was chromatographed on Si gel with solvents of increasing polarity. Further separation by repeated hplc on SiO_2 afforded compounds **1** (182 mg), **3** (14 mg), **5** (389 mg), **6** (155 mg), **7** (915 mg), **9** (37 mg), **10** (14.5 mg), **11** (140 mg), **12** (9.5 mg), **13** (13.5 mg), **14** (26.2 mg), and **15** (8.5 mg). Compounds **1**, **3**, **5–7**, and **9–13**, isolated as amorphous solids, were identified by comparison of their spectral data with those reported in the literature.

Compound **14** showed in the fabms spectrum a 1:4:6:4:1 quintet at *m/z* 845, 847, 849, 851, and 853 for the pseudo-molecular ion peak, indicative of the presence of four bromine atoms. Combined analysis of the fabms and ^{13}C-nmr spectra suggested the molecular formula $C_{24}H_{24}N_4O_{10}Br_4$, implying thirteen degrees of unsaturation, which was confirmed by elemental analysis. The uv spectrum (λ max 262 nm) indicated the presence of a cyclohexadienyl chromophore. The ir absorption bands showed the presence of alcohol (3402 cm^{-1}), ketone (1728 cm^{-1}), and α-imino-amide (1665 cm^{-1}) groups in the molecule.

The ^{1}H- and ^{13}C-nmr spectra of **14** showed signals (see Table 1) corresponding to two 1-hydroxy-2,4-dibromo-3-methoxy-8-carbamoylspirocyclohexadienylisoxazole moieties. The splitting of some ^{1}H- and ^{13}C-nmr signals, which are slightly different for one ring as compared to the other, suggested that compound **14** has the same ring systems as aerothionin **1**, but unlike this latter bromo- compound it has an asymmetrical structure. The partial structure, which links the two spirocyclohexadienyl-8-carbamoyl moieties, must be a $C_4H_6O_2$ group from mass spectral data; the ^{13}C-nmr spectrum showed the four carbon atoms as being composed of one carbonyl (δ 207.84), one oxygen-bearing methine (δ 75.73), and two methylene (δ 49.86 and 43.43) groups. The cross-peaks between the methine proton at δ 4.402 (H-12) and the methylene protons at δ 3.662 and 3.628 (H_2-13) in the COSY spectrum, together with the low-field resonances of the same methine proton and the remaining methylene protons H_2-10 (δ 4.430 and 4.412), due to the deshielding effect of the carbonyl group, allowed establishment of this partial structure as reported in **14**. Additional support for structure **14** came from a COLOC experiment, which revealed coupling of C-9 with H-10, C-9′ with H-13a, and C-11 with H-12. The absolute stereochemistry of the chiral centers of the spirocyclohexadiene groups was suggested by the cd spectrum, which is analogous to the spectrum reported for aerothionin (9), whose absolute stereochemistry is known.

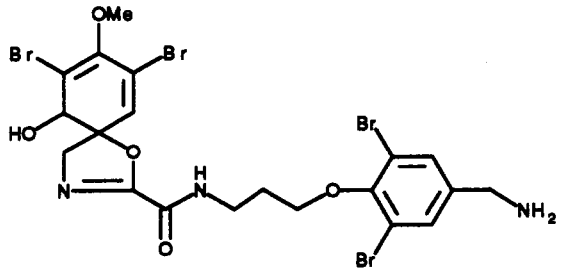
The COLOC nmr experiment performed on compound **14** also revealed a mistake in the ^{13}C-nmr resonance assignment reported in the literature (7, 8, 10) for the spirocyclohexadiene group frequently present in metabolites from Verongida sponges. It consists of the inversion of the chemical shifts of C-2 and C-4. Examination of literature data revealed that all the previous assignments of C-2 and C-4 in compounds possessing a similar spirocyclohexadiene group were based on a paper by Kashman *et al.* (10), in which the ^{13}C-nmr spectrum of aerothionin [**1**] was assigned by comparison with the spectrum of psammaplysin A. However, in a subsequent paper (11) Scheuer demonstrated that Kashman's structure of psammaplysin-A [**17**] was incorrect, and that the

TABLE 1. Nmr Spectral Data of Compounds **1**, **14**, and **15** (CD_3OD).[a]

Position	**1**	**14**		**15**[b]
	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)
1,1′	75.5 (CH)	4.144 (d, 0.9), 4.126 (d, 0.9)	75.47 (CH), 75.47 (CH)	4.146 (d, 0.9), 4.129 (d, 0.9)
2,2′	114.2 (C)		114.14 (C), 114.14 (C)	
3,3′	149.3 (C)		149.28 (C), 149.28 (C)	
4,4′	122.7 (C)		122.78 (C), 122.78 (C)	
5,5′	133.2 (CH)	6.469 (d, 0.9), 6.450 (d, 0.9)	132.23 (CH), 133.21 (CH)	6.464 (d, 0.9), 6.473 (d, 0.9)
6,6′	92.6 (C)		92.64 (C), 92.54 (C)	
7a,7′a.....	40.1 (CH_2)	3.150 (d, 18.3), 3.124 (d, 18.3)	40.09 (CH_2), 40.03 (CH_2)	3.144 (d, 18.3), 3.130 (d, 18.3)
7b,7′b		3.822 (d, 18.3), 3.811 (d, 18.3)		3.822 (d, 18.3), 3.800 (d, 18.3)
8,8′	155.5 (C)		155.12 (C), 154.92 (C)	
9,9′	161.6 (C)		161.93 (C), 161.80 (C)	
10a.....	38.4 (CH_2)	4.430 (d, 18.8),	49.87 (CH_2)	4.430 (d, 18.8),
10b.....		4.416 (d, 18.8)		4.416 (d, 18.8)
11	26.1 (CH_2)		207.84 (C)	
12	26.1 (CH_2)	4.402 (dd, 6.3, 4.9)	75.73 (CH)	4.406 (dd, 6.3, 4.9)
13a.....	38.4 (CH_2)	3.662 (dd, 13.9, 4.9),	43.43 (CH_2)	3.662 (dd, 13.9, 4.9),
13b		3.628 (dd, 13.9, 6.3)		3.628 (dd, 13.9, 6.3)
-OMe.....	60.4 (CH_3)	3.761 (s) 3.757 (s)	60.39 (CH_3), 60.39 (CH_3)	3.761 (s) 3.757 (s)

[a]Assignment based on DEPT, COSY, HETCOR, and COLOC experiments.
[b]The ^{13}C-nmr spectrum of **15** is superimposable with that of **14**.

actual structure [**18**] possessed a oxacycloheptadiene rather than a cyclohexadiene ring. Consequently, the grounds for Kashman's assignment of the aerothionin ^{13}C-nmr spectrum also vanished; nevertheless, Kashman's assignment has continued to be used for reference purposes. Our reassignment rests on the presence of correlation peaks between H-1/H-1′ with C-2/C-2′ and between H-5/H-5′ with C-4/C-4′ in the COLOC spectrum of compound **14**, which clearly showed that the carbon signals at δ 114.14 and 122.7 are to be assigned to C-2/C-2′ and C-4/C-4′, respectively. The ^{13}C-nmr spectra of aerothionine (see Table 1) and its derivatives are reassigned accordingly.



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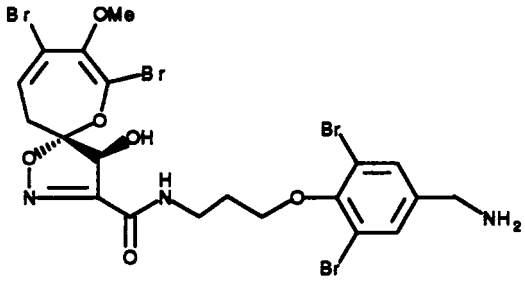

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Compound **15**, isolated in smaller amounts, exhibited ^{13}C-nmr, uv, ir, and fabms spectra identical to that of **14**. Comparison of the ^{1}H-nmr data of **15**, reported in Table 1, and assigned on the basis of a COSY experiment, with those of **14** confirmed the strong similarity between the two compounds, suggesting that they differ only in some stereochemical details. Because their cd spectra, which are sensitive to the configuration of the spirocyclohexadiene chromophores (9), are superimposable, **14** and **15** were concluded as being epimers at C-12.

In order to confirm this hypothesis, the stereochemistries at C-12 of compounds **14** and **15** were investigated by the modified Mosher's method suggested by Kakisawa (12). Two aliquots of each of the two epimers were separately treated with *R*-(+)- and *S*-(−)-methoxy(trifluoromethyl)phenylacetyl (MTPA) chloride in pyridine solution at room temperature for 2 h, thus obtaining the four triesters **14r**, **14s**, **15r**, and **15s**. The stereochemical determinations were based on the chemical shift differences of methylene protons at C-10 and C-13, which were assumed to be mainly affected by the MTPA group at C-12, as they are located relatively far away from the MTPA groups at C-1 and C-1′. The observed chemical shift differences (see Table 2) were in rather good agreement with the Mosher's model, except for the anomalous behavior of H-13b in compound **15r**–**15s**, which could be explained by the influence of the MTPA groups linked at C-1 and C-1′. Both H-10a and H-10b are deshielded in **14s** with respect to **14r**, while H-13a is shielded. Conversely, H-10a and H-10b are shifted upfield and H-13a is shifted downfield in compound **15s** compared to **15r**. The obtained results, taken together, suggested a 12*S* stereochemistry for compound **14** and a 12*R* stereochemistry for compound **15**.

In addition to the above compounds, all biogenetically derived from bromotyrosine, fractionation of the extract of *A. fistularis* led to the isolation of a new metabolite (**16**, 220 mg), which is closely related to histidine. Compound **16**, isolated as a white solid, showed a pseudo-molecular ion peak at *m/z* 150 in the fabms spectrum, appropriate for a molecular formula of $C_6H_8N_3O$. Its very simple ^{1}H-nmr spectrum had two 1H singlets in the olefinic region and two mutually coupled 2H triplets at δ 2.30 and δ 1.71, while in the ^{13}C-nmr spectrum only six resonances were present (see Experimental). These data, together with proton-proton and proton-carbon chemical shift correlation 2D-nmr

TABLE 2. Selected ^{1}H-Nmr Chemical Shifts of Compounds **14r**, **14s**, **15r**, and **15s**.

Position	14r	14s	Δδ	15r	15s	Δδ
10a	4.35	4.49	+0.14	4.42	4.27	−0.15
10b	4.22	4.30	+0.08	4.39	4.27	−0.12
13a	3.99	3.88	−0.11	3.84	3.92	−0.06
13b	3.73	3.73	0.00	3.84	3.77	+0.07

experiments, showed **16** to be imidazo-[1,5-*c*]-tetrahydropyrimidine-5-one, a product previously prepared by Mechoulam and Hirshfeld (13) as an intermediate in the synthesis of zapotidine. To the best of our knowledge, compound **16** has not been found as a naturally occurring compound.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Fabms were obtained in a glycerol matrix on a VG ZAB mass spectrometer (Xe atoms of energy of 2-6 kV). Ft-ir spectra were recorded on a Bruker IFS-48 spectrophotometer with $CHCl_3$. Uv spectra were performed on a Beckman DU70 spectrometer in MeOH solution. Circular dichroism spectra were performed on a Jasco J-710 spectrophotometer in MeOH solution. ^{1}H- and ^{13}C-nmr spectra were determined on a Bruker AMX-500 spectrometer. Chemical shifts are referenced to the residual solvent signal (CD_3OD: δ_H 3.34, δ_C 49.0; CF_3COOD: δ_H 10.50, δ_C 163.7). Methyl, methylene, and methine carbons were distinguished by DEPT experiments. Homonuclear ^{1}H connectivities were determined by using the COSY experiment. One-bond heteronuclear ^{1}H-^{13}C connectivities were determined with a HETCOR pulse sequence optimized for $^1J_{CH}$ of 135 Hz. Two- and three-bond ^{1}H-^{13}C connectivities were determined by COLOC experiments, optimized for $^{2,3}J_{CH}$ of 8 Hz. Medium-pressure liquid chromatography (mplc) was performed on a Büchi 861 apparatus using a SiO_2 (230–400 mesh) column. Hplc separations were performed on a Varian 2510 apparatus equipped with an RI-3 refractive index detector, using Hibar columns.

EXTRACTION AND ISOLATION.—The sponge *A. fistularis* was collected (depth 1m) in the summer of 1992, along the coast of Grand Bahama Island and was stored frozen at −20°. Reference specimens are deposited at the Dipartimento di Chimica delle Sostanze Naturali dell'Università di Napoli.

The sponge (167 g dry wt after extraction) was homogenized and extracted with Me_2CO (4×1 liter). The combined solutions, after filtration, were concentrated *in vacuo* to an aqueous suspension which was subsequently extracted with *n*-hexane (4×500 ml) and BuOH (6×500 ml). The BuOH extract, after evaporation of the solvent, afforded 12 g of a dark brown oil, which was chromatographed by mplc on a Si gel column using a solvent gradient system from *n*-hexane to EtOAc and then to MeOH. Fractions eluted with *n*-hexane-EtOAc (6:4) (fraction A), *n*-hexane-EtOAc (3:7) (fraction B), and EtOAc (fractions C and D) containing bromotyrosines, and a fraction eluted with EtOAc-MeOH (9:1) containing compound **16**, were further separated.

Fraction A, containing aeroplysinins 1 and 2, which was chromatographed by hplc using a Hibar Lichrospher Si 60 10 μm (10×250 mm) column with a mobile phase of EtOAc-$CHCl_3$ (1:1), afforded 140 mg of aeroplysinin-1 [**11**] and 155 mg of aeroplysinin-2 [**6**].

Fraction B was purified by hplc on SiO_2 with a mobile phase of EtOAc-$CHCl_3$ (9:1) to obtain fistularin-3 (**5**, 208 mg) as a pure compound.

Fraction C, which was further purified using an SiO_2 column with a mobile phase of EtOAc-$CHCl_3$ (1:1), also contained fistularin-3 (**5**, 181 mg) in addition to aerothionine (**1**, 182 mg) and homoaerothionine (**10**, 14.5 mg).

Fraction D was chromatographed by hplc on SiO_2 with a mobile phase of EtOAc-$CHCl_3$ (9:1), giving 915 mg of 3,5-dibromo-1-hydroxy-4-oxo-2,5-cyclohexadien-1-acetamide [**7**], 37 mg of 3,5-dibromohydroquinone-2-acetamide [**9**], 9.5 mg of 11-hydroxyaerothionine [**12**], 13.5 mg of 11-oxoaerothionine [**13**], 14 mg of fistularin-1 [**3**], and 38 mg of a mixture of **14** and **15**. Final separation of this mixture was achieved by hplc on a Hibar Lichrospher Si 60 column (4×250 mm) using $CHCl_3$-MeOH (97:3) as eluent, to obtain 26.2 mg of **14** (relative R_t=1.00) and 8.5 mg of **15** (relative R_t=1.07) as pure compounds.

12S-Hydroxy-11-oxoaereothionine [**14**].—$[\alpha]^{25}D$ +152.5°; fabms *m/z* $[M+H]^+$ 845, 847, 849, 851, 853; *anal.* (Galbraith Labs, Inc.), found: C, 34.02; H, 2.80; N, 6.51; calcd for $C_{24}H_{24}N_4O_{10}Br_4$ C 33.96; H, 2.83; N, 6.60; uv λ max (MeOH) 280.5 (ε 10600), 231 (ε 17400) nm; cd (MeOH) $\Delta\epsilon_{288}$ +15.2, $\Delta\epsilon_{250}$ +18.2; ir (neat) ν max 3402, 1728, 1665 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1.

12R-Hydroxy-11-oxoaereothionine [**15**].—$[\alpha]^{25}D$ +160.7°; fabms *m/z* $[M+H]^+$ 845, 847, 849, 851, 853; uv λ max (MeOH) 281 (ε 10500), 230.5 (ε 17500) nm; cd (MeOH) $\Delta\epsilon_{288}$ +15.0, $\Delta\epsilon_{251}$ +18.3; ir (neat) ν max 3350, 1732, 1662 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1.

Fraction E afforded a mixture containing compound **16**. It was rechromatographed by hplc on an SiO_2 column with a mobile phase of EtOAc-MeOH (95:5) to obtain pure **16** (220 mg).

Imidazo-[1,5-c]-tetrahydropyrimidin-5-one [**16**].—White solid, mp 220°; fabms *m/z* $[M+H]^+$ 150; ^{1}H nmr (CF_3COOD) δ 7.87 (1H, s, H_1-3), 7.69 (1H, s, H_1-1), 2.30 (2H, t, J=6.5 Hz, H_2-7), 1.73 (2H, t, J=6.5 Hz, H_2-8); ^{13}C nmr (CF_3COOD) δ 140.1 (C-5), 134.7 (C-3), 131.9 (C-9), 117.0 (C-1), 39.9 (C-7), 18.8 (C-8).

Aereothionine [**1**] (14), fistularin-1 [**3**] (2), fistularin-3 [**5**] (2), aeroplysinin-2 [**6**] (15), 3,5-dibromo-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetamide [**7**] (16), 3,5-dibromohydroquinone-2-acetamide [**9**] (17), homoaereothionine [**10**] (14), aeroplysinin-1 [**11**] (6), 11-hydroxyaerothionine [**12**] (7), and 11-oxoaerothionine [**13**] (8) were identified by comparison of their spectral properties with those reported in the literature. A revised assignment of the ^{13}C-nmr spectrum of aerothionine [**1**] is reported in Table 1.

SYNTHESIS OF THE (*R*)- AND (*S*)-MTPA ESTERS OF **14** AND **15**.—To compound **14** or **15** (2.5 mg) in 200 μl of anhydrous pyridine, 5 μl of (*R*)-MTPA chloride [MTPA=α-methoxy-α-(trifluoromethyl)phenylacetyl] were added, and the mixture was allowed to react at room temperature. After 2 h, 5 ml of H_2O and solid K_2CO_3 were added, and the solution was extracted with $CHCl_3$ (5 ml). The organic phase, after evaporation of the solvent, yielded the (*R*)-MTPA triesters **14r** (2.2 mg) or **15r** (1.9 mg). The use of (*S*)-MTPA chloride in the same procedure led to the (*S*)-MTPA triesters **14s** (1.7 mg) or **15s** (1.6 mg).

12S-Hydroxy-11-oxoaereothionine (R)-*MTPA triester* [**14r**].—^{1}H nmr (CD_3OD) δ 6.54 and 6.51 (1H each, s, H-5 and H-5′), 6.00 and 5.93 (1H, each, s, H-1 and H-1′), 5.65 (1H, dd, *J*=7.1 and 4.4 Hz, H-12), 4.35 (1H, d, *J*=18.5 Hz, H-10a), 4.22 (1H, d, *J*=18.5 Hz, H-10b), 3.99 (1H, dd, *J*=14.5 and 4.4 Hz, H-12a), 3.73 (submerged), 3.19 and 3.13 (1H each, d, *J*=18.5 Hz, H-7a and H-7′a), 3.05 and 3.03 (1H each, d, *J*=18.5 Hz, H-7b and H-7′b).

12S-Hydroxy-11-oxoaereothionine (S)-*MTPA triester* [**14s**].—^{1}H nmr (CD_3OD) δ 6.65 and 6.61 (1H each, s, H-5 and H-5′), 6.08 and 6.01 (1H each, s, H-1 and H-1′), 5.64 (1H, dd, *J*=7.1 and 4.4 Hz, H-12), 4.49 (1H, d, *J*=18.5 Hz, H-10a), 4.30 (1H, d, *J*=18.5 Hz, H-10b), 3.88 (1H, dd, *J*=14.5 and 4.4 Hz, H-12a), 3.73 (submerged), 3.48 and 3.37 (1H each, d, *J*=18.5 Hz, H-7a and H-7′a), 3.27 and 3.22 (1H each, d, *J*=18.5 Hz, H-7b and H-7′b).

12R-Hydroxy-11-oxoaereothionine (R)-*MTPA triester* [**15r**].—^{1}H nmr (CD_3OD) δ 6.49 and 6.46 (1H each, s, H-5 and H-5′), 6.01 and 5.90 (1H each, s, H-1 and H-1′), 5.68 (1H, dd, *J*=6.2 and 4.5 Hz, H-12), 4.42 and 4.39 (2H, AB system, *J*=18.5 Hz, H-10a and H-10b), 3.84 (2H, m, H-12a and H-12b), 3.18 and 3.09 (1H each, d, *J*=18.5 Hz, H-7a and H-7′a), 3.07 and 2.96 (1H each, d, *J*=18.5 Hz, H-7b and H-7′b).

12R-Hydroxy-11-oxoaereothionin (S)-*MTPA triester* [**15s**].—^{1}H nmr (CD_3OD) δ 6.67 and 6.60 (1H each, s, H-5 and H-5′), 6.09 and 6.00 (1H each, s, H-1 and H-1′), 5.66 (1H, dd, *J*=6.8 and 3.8 Hz, H-12), 4.27 (2H, s, H-10a and H-10b), 3.92 (1H, dd, *J*=14.5 and 3.8 Hz, H-12a), 3.77 (submerged), 3.48 and 3.43 (1H each, d, *J*=18.5 Hz, H-7a and H-7′a), 3.28 and 3.25 (1H each, d, *J*=18.5 Hz, H-7b and H-7′b).

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