Journal of Natural Products
Vol. 52, No. 4, pp. 753-756, Jul-Aug 1989

DIHYDROXYAEROTHIONIN AND AEROPHOBIN 1. TWO BROMINATED TYROSINE METABOLITES FROM THE DEEP WATER MARINE SPONGE *VERONGULA RIGIDA*

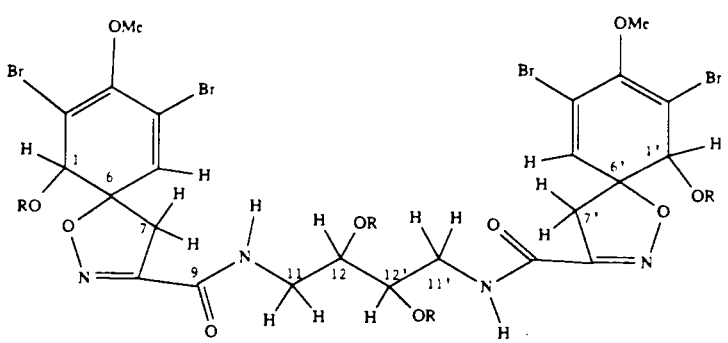
MALIKA GUNASEKERA and SARATH P. GUNASEKERA*

Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Fort Pierce, Florida 34946

ABSTRACT.—Two brominated tyrosine metabolites, dihydroxyaerothionin [**1**] and the known compound aerophobin 1 [**3**] have been isolated from the deep water marine sponge *Verongula rigida*. Their structures were determined on the basis of spectral data.

The sponge family Verongidae (1,2) has been a source of many bromotyrosine-derived metabolites; recent notable examples are the compounds containing disulfide linkages (3–5). During our investigation of the deepwater sponge *Verongula rigida* Esper, we encountered a novel addition to the aerothionin group that contains a 2,3-dihydroxybutyl-1,4-diamine moiety as the central unit. We also isolated aerophobin 1, a related metabolite previously reported from a *Verongia* sp. We report here details of the isolation and structure determination of the aerothionin metabolite, along with complete nmr spectral data for aerophobin 1, although these compounds did not show any biological activity.

The EtOAc-soluble fraction of the sponge *V. rigida* gave on repeated cc followed by hplc on both C-18 and amino reversed-phase columns metabolites **1** and **3**, respectively. Hrfabms suggested the molecular formula $C_{24}H_{26}Br_4N_4O_{10}$ for metabolite **1**, implying twelve degrees of unsaturation. Because only twelve carbon resonances (Table 1) were observed in the ^{13}C-nmr spectrum, it could be concluded that **1** was symmetrical. The uv spectrum (λ max 282, 225; ϵ 9400, 19000) indicated the presence of a heteroatom-substituted cyclohexadienyl moiety (6). The ir absorption bands showed the presence of NH and OH (3600–3000 cm^{-1}) and α-iminoamide (6) (1660, 1600, 1530 cm^{-1}) groups in the molecule. The ^{1}H-nmr spectrum contained signals [δ 3.19 and 3.61 (each 1H, d, J = 17.8 Hz), 3.92 (1H, s), 6.56 (1H, s)] corresponding to a spirocyclohexadienylisoxazole moiety (6). Further, a signal at δ 3.63 (3H, s) showed the presence of an OMe group. These data suggested that compound **1** has the same ring system as in the *Verongia* metabolite aerothonin reported earlier (7). The partial structure, -CONHCH$_2$CHOH-, was established from the following nmr data: N-*H*; δ 8.11 (t, J = 6.9 Hz, exchangeable); -*CH*$_2$-N, δ 3.14, 3.48 (2H, m), 39.02; *CH*OH, δ 3.43 (1H, m), 71.04. Decoupling experiments indicated that the CH$_2$ group is

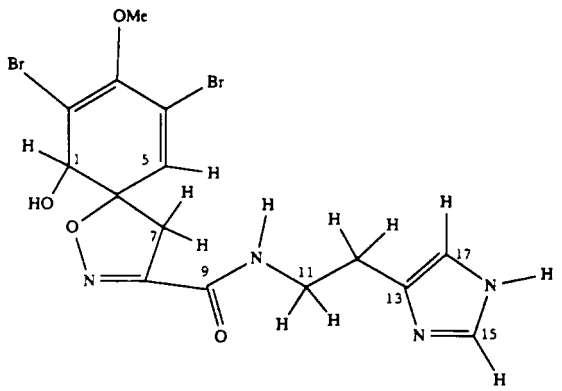


1 R=H
2 R=Ac

TABLE 1. ^{1}H- (360 MHz) and ^{13}C- (90.5 MHz) nmr Data.[a]

Position	Dihydroxyaerothionin		Aerophobin 1		
	δ H[b]	δ C[b]	δ H[b]	δ C[b]	HMBC (^{1}H)[b]
1,1′	3.92 (s)	73.55 (d)[c]	3.90 (s)	73.57 (d, 147)[d]	5H, 7H
2,2′		120.81 (s)		120.87 (s)	5H
3,3′		147.15 (s)		147.18 (s)	1H, 5H, OMe
4,4′		113.09 (s)		113.08 (s)	1H, 5H
5,5′	6.56 (s)	131.25 (d)	6.55 (s)	131.21 (d, 176)	1H, 7H
6,6′		90.32 (s)		90.33 (s)	1H, 7H
7,7′	3.61, 3.19 (ABq, 17.8)	42.53 (t)	3.59, 3.16 (ABq, 18.2)	39.26 (t, 136)	5H
8,8′		154.47 (s)		154.37 (s)	7H
9,9′		158.98 (s)		159.10 (s)	10H, 11H
10,10′ (N)	8.10 (t, 6.9)		8.63 (t, 5.7)		
11,11′	3.48, 3.14 (m)	39.02 (t)	3.44 (ddd, 6.8, 6.8, 5.7)	37.66 (t, 137)	12H
12,12′	3.43 (m)	71.04 (d)	2.84 (dd, 6.8, 6.8)	24.12 (t, 126)	11H
13				130.79 (s)	11H, 12H, 15H, 17H
15			8.98 (br, s)	133.84 (d, 224)[e]	17H
16 (N)					
17			7.47 (br, s)	116.21 (d, 203)	12H, 15H
OMe	3.63 (s)	59.59 (q)	3.63 (s)	59.62 (q, 144)	

[a]Table entries are chemical shift, ppm from solvent (multiplicity, J in Hz).
[b]1% TFA-H in Me_2SO-d_6.
[c]Bruker's DEPT pulse sequence.
[d]Bruker's coupled DEPT pulse sequence.
[e]J_{CH} = 208 Hz in CD_3OD.



3

coupled to both the NH and CHOH groups. Acetylation of **1** with pyridine and Ac_2O yielded tetraacetate **2**, confirming the presence of four hydroxyl groups. Combination of the foregoing structural units yielded **1** for the symmetrical structure of dihydroxyaerothionin. X-ray studies as in aerothionin (7) are required to determine the absolute stereochemistry of dihydroxyaerothionin.

Analysis of metabolite **3** by hrfabms suggested the molecular formula $C_{15}H_{17}Br_2N_4O_4$, implying nine degrees of unsaturation. The presence of a cyclohexadienyl moiety was suggested by the uv absorptions (λ max 283, 235 sh, 225; ε 4300, 6200, 9100) in MeOH. The ir absorption bands showed the presence of NH and OH groups (3600–3000 cm^{-1}), and an α iminoamide group (1660, 1530 cm^{-1}) as in compound **1**.

The nmr data and the HMBC experiment (8) (see Table 1) indicated that compound **3** was identical to aerophobin 1.

Aerophobin 1 was isolated previously from a sponge *Verongia aerophoba* widespread along the Italian coast (9). *Aplysina* and *Verongula* are two genera described from the family Aplysinidae (10). To the best of our knowledge, this is the first report of bromotyrosine-derived metabolites from *Verongula*, although they are very common in the genus *Aplysina* (formerly *Verongia*). For a classification of the taxonomy, see Wiedenmayer (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectrum was obtained with a Perkin-Elmer Lambda 3 B uv/visible spectrophotometer. Ir spectra were obtained on a Perkin-Elmer 1310 spectrophotometer. Nmr spectra were obtained on a Bruker instrument operating at 360 MHz for ^1H and 90.5 MHz for ^{13}C. The high resolution mass spectra were obtained on a VG ZAB-2SE mass spectrometer. Melting point was measured by using Bristoline melting point apparatus, and optical rotation was measured with a Jasco DIP 360 digital polarimeter.

EXTRACTION AND ISOLATION.—The marine sponge *V. rigida* was collected from Sweetings Cay, Bahamas at a depth of 228 ft using an untethered manned submersible. A voucher specimen is deposited in the Indian River Coastal Museum of the Harbor Branch Oceanographic Institution. The freshly thawed sponge (377 g wet wt) was extracted three times with MeOH-toluene (3:1). The concentrated extract was then partitioned between EtOAc and H_2O. A portion of the EtOAc-soluble fraction (1 g) was chromatographed on Si gel (Kieselgel 60 H) using a CH_2Cl_2/MeOH step gradient. The fraction (140 mg) eluted with 10% MeOH/CH_2Cl_2 was rechromatographed on Si gel using CH_2Cl_2 and mixtures of CH_2Cl_2 and MeOH. Rechromatography of the fraction that eluted with 6% MeOH/CH_2Cl_2 (18 mg) on reversed-phase C-18 using MeCN/H_2O followed by reversed-phase hplc (5 μm, 250 × 10 mm, Alltech absorbosphere C-18) with 45% H_2O/MeOH gave dihydroxyaerothionin [**1**]. Similarly, rechromatography of the fraction that eluted with 8% MeOH/CH_2Cl_2 (23 mg) on a C-18 Sep-Pak with 20% H_2O/MeOH followed by hplc (7 μm, 250 × 10 mm, Phenomenex 1B-SIL 5 NH_2) with 8% MeOH/CH_2Cl_2 gave aerophobin 1 [**3**].

DIHYDROXYAEROTHIONIN [**1**].—Compound **1** (2.8 mg): white powder, mp 162–164°; $[\alpha]^{25}D$ −64.2° (*c* = 0.1, MeOH); uv λ max (MeOH) 282 nm (ε 9400), 225 (19000), 202 (34000); ir (neat) 3600–3000, 1660, 1600, 1530 cm^{-1}; hrfabms *m/z* 850.8413 Δ 1.9 mmu for $C_{24}H_{27}{}^{79}Br_2{}^{81}Br_2N_4O_{10}$; lrfabms *m/z* (rel. int.) 853 (0.5%), 851 (0.8), 849 (0.5), 797 (0.2), 757 (0.3), 613 (0.1), 481 (3), 459 (3), 447 (3), 375 (4), 359 (6), 343 (5), 306 (4), 289 (12), 273 (13), 257 (14), 239 (20), 223 (18), 205 (72), 179 (50).

ACETYLATION OF **1** TO FORM **2**.—Compound **1** (1 mg) was treated with 0.5 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 tetraacetate **2**.

TETRAACETATE **2**.—Ir ($CHCl_3$) 3700, 1740, 1670, 1520, 1240, 935 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.09, 2.10, 2.12, 2.13 (3H each, s, 1-, 1′-, 12-, 12′-OAc), 3.03 (2H, d, *J* = 18.4 Hz, H-7, -7′), 3.43 (2H, d, *J* = 18.4 Hz, H-7, -7′), 3.46, 3.61 (4H, m, H-11, -11′), 5.06 (2H, m, H-12, -12′), 5.82, 5.84 (2H, 2s, H-1, -1′), 6.31, 6.32 (2H, 2s, H-5, -5′), 6.87 (2H, m, H-10, -10′); lrfabms *m/z* (rel. int.) $[M+H]^+$ 1019 (0.2%), 959 (0.5), 901 (0.3), 856 (0.2), 837 (0.1), 821 (0.2), 796 (0.3), 765 (0.3), 703 (22), 578 (10), 309 (42), 155 (100).

AEROPHOBIN 1 [**3**].—Compound **3** (9.0 mg): pale yellow gum; $[\alpha]^{25}D$ 107.8° (*c* = 0.6, MeOH) [lit. (9) $[\alpha]D$ 187° (MeOH)]; uv λ max (MeOH) 283 nm (ε 4300), 225 (9100), 200 (16300); ir (neat) 3600–3000, 1660, 1530 cm^{-1}; hrfabms *m/z* 476.9596 Δ 0.0 mmu for $C_{15}H_{17}{}^{79}Br^{81}BrN_4O_4$; lrfabms *m/z* (rel. int.) 479 (22%), 477 (43), 475 (22), 459 (15), 433 (10), 391 (12), 389 (25), 387 (13), 371 (52), 361 (15), 343 (18), 333 (80), 331 (80), 311 (65), 309 (90), 307 (75), 297 (60), 275 (35), 235 (50), 220 (20), 197 (75), 195 (100).

ACKNOWLEDGMENTS

We thank Dr. S. Pomponi for identification of the sponge and Dr. F.E. Koehn for assisting in the HMBC experiment. We also thank Professor K.L. Rinehart Jr., University of Illinois, Urbana, for fab mass spectra. This is Harbor Branch Oceanographic Institution Contribution No. 709.

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.* **3**, 1 (1986), and references cited therein.
2. P.J. Scheuer (Ed.), "Marine Natural Products: Chemical and Biological Perspectives," Academic Press, New York, Vols. I–V, 1978–1982.
3. L. Arabshahi and F.J. Schmitz, *J. Org. Chem.*, **52**, 3584 (1987).
4. A.D. Rodriguez, A.K. Rhone, and P.J. Scheuer, *Tetrahedron Lett.*, **28**, 4989 (1987).
5. E. Quinoa and P. Crews, *Tetrahedron Lett.*, **28**, 3229 (1987).
6. Y. Gopichand and F.J. Schmitz, *Tetrahedron Lett.*, 3921 (1979).
7. J.A. McMillan, I.C. Paul, Y.M. Goo, and K.L. Rinehart Jr., *Tetrahedron Lett.*, **22**, 39 (1981).
8. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
9. G. Cimino, S. DeRosa, S. DeStefano, R. Self, and G. Sodano, *Tetrahedron Lett.*, **24**, 3029 (1983).
10. A.A. Tymiak, K.L. Rinehart Jr., and G.J. Bakus, *Tetrahedron*, **41**, 1039 (1985).
11. F. Wiedenmayer, "Shallow Water Sponges of the Western Bahamas," Birkhauser Verlag, Basel, Switzerland, 1977, p. 64.

Received 20 January 1989