Verongamine, a Novel Bromotyrosine-Derived Histamine H-Antagonist from the Marine Sponge Verongula gigantea

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VERONGAMINE, A NOVEL BROMOTYROSINE-DERIVED HISTAMINE H_3-ANTAGONIST FROM THE MARINE SPONGE *VERONGULA GIGANTEA*

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ABSTRACT.—The novel bromotyrosine derivative verongamine [**1**] has been isolated from the marine sponge *Verongula gigantea*. The isolation of verongamine was facilitated by employing histamine-H_3 bioassay-guided fractionation. Verongamine is a specific histamine-H_3 antagonist at concentrations as low as 1 μg/ml.

In an effort to explore the pharmacology of histamine-H_3 agonists and antagonists, we have sought to discover novel H_3-active compounds that could be used as pharmacological probes, or as structure leads toward the development of therapeutically useful agents. Our interest is based on speculation that a selective histamine-H_3 agonist might hold considerable promise as a novel treatment for promoting sedation (1). Alternatively, a selective H_3-antagonist might find utility as a decongestant in allergic reactions (2). In the course of screening extracts from a variety of organisms for histamine-H_3 activity, a number of marine extracts were encountered which displayed activity in an H_3-receptor binding assay (3). Further investigation of one of these, an extract from the sponge *Verongula gigantea* Hyatt (Porifera: Demospongiae: Verongida), employing H_3 bioassay-guided fractionation, led to the identification of verongamine [**1**], a novel bromotyrosine derivative. Although bromotyrosine-derived sponge metabolites have been reported to possess a large variety of biological activities (4), the detection of histamine-H_3 activity is unprecedented among sponges and marine natural products in general. Herein we report the isolation, structure elucidation, and histamine-H_3 activity of verongamine.

The sponge, which had been kept frozen until workup, was homogenized with EtOH in a blender and filtered to give a crude extract. The extract was concentrated and then partitioned between EtOAc and H_2O. The EtOAc fraction was extracted with 1N HCl, and then the aqueous fraction was adjusted to pH 9 and back-extracted with EtOAc. The EtOAc was removed under vacuum and the resulting gum chromatographed on Si gel employing gradient elution of 2–20% MeOH in CH_2Cl_2. A group of fractions that eluted with 10–15% CH_3OH/CH_2Cl_2 and showed H_3 activity was pooled and concentrated. This material was subjected to further partitioning on Sephadex LH-20 with elution by CH_3CN-CH_2Cl_2 (1:1) to give pure verongamine.

The structure of verongamine [**1**] was elucidated in the following manner. The low-resolution fab mass spectrum showed dual $[M+H]^+$ molecular ions of nearly equal intensity at *m/z* 381 and 383, indicative of a monobrominated compound. Peak matching using high-resolution fab mass spectrometry established the molecular formula $C_{15}H_{18}N_4O_3{}^{79}Br$ (observed 381.0573, calcd 381.0562) for the $[M+H]^+$ ion. The ^{1}H-nmr spectrum (Table 1) revealed the presence of a 1,3,4-trisubstituted

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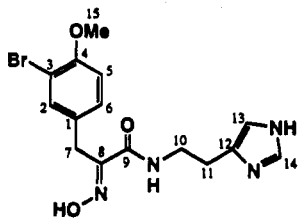

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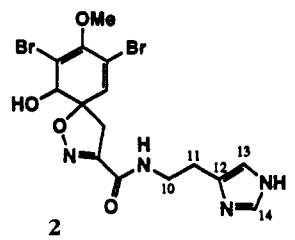

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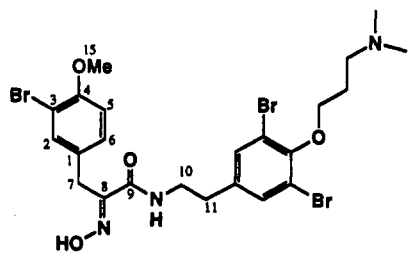

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phenyl ring, a methoxy, an isolated and deshielded methylene, two mutually coupled methylenes, and two slightly broadened heteroaromatic-like proton singlets reminiscent of the imidazole protons of aerophobin-1 [**2**] (5,6). Comparison of the spectral data of verongamine to the histamine substructure moiety of aerophobin-1 (5,6), and to the bromotyrosine substructure moiety of aplysamine-2 [**3**] (7), provided excellent correlation for structure **1** of verongamine. These data are shown in Table 1. The geometry of the oxime was determined to be *E* as shown in **1** based on the δ 28.7 ^{13}C-nmr chemical shift of C-7. Literature precedent (8) indicates the methylene α to the oxime in related compounds resonates near δ 28 for *E* oximes and near δ 35 for *Z* oximes.

Verongamine binds with an IC_{50} of 0.5 μM to the H_3-receptor isolated from guinea pig brain membranes (3). Verongamine also demonstrated H_3-antagonist activity in the electrical field stimulated (EFS) contracted guinea pig ileum (9). In

TABLE 1. Nmr Spectral Data for **1**, and Relevant Nmr Data for **2** (5) and **3** (7).

Position	^{13}C Nmr				^{1}H Nmr		
	1[a]	**1**[b]	**2**[b]	**3**[b]	**1**[b]	**2**[b]	**3**[b]
1	112.1	112.1 s		113.1 s			
2	134.7	134.8 d		134.7 d	7.42 1H d 2.1		7.43 1H d 2.2
3	131.7	131.8 s		130.3 s			
4	155.9	155.9 s		155.8 s			
5	113.1	113.1 d		112.1 d	6.89 1H d 8.4		6.88 1H d 8.5
6	130.4	130.5 d		131.7 d	7.17 1H dd 8.4, 2.1		7.17 1H dd 8.5, 2.2
7	28.7	28.7 t		28.7 t	3.79 2H s		3.78 2H s
8	152.9	153.0 s		152.9 s			
9	165.9	165.7 s		165.8 s			
10	39.2	40.3 t	40.3 t	41.3 t	3.47 2H t 7.1	3.67 2H t 7.0	3.41 2H t 7.0
11	26.1	27.8 t	27.4 t		2.77 2H t 7.1	2.99 2H t 7.0	2.73 2H t 7.0
12	133.4	n.o.[d]	134.6 s				
13	117.6	n.o.[d]	117.7 d		6.80 1H br s	6.85 1H s	
14	135.0	136.1 d	135.9 d		7.55 1H br s	7.59 1H s	
15	56.7	56.7 q		56.7 q	3.80 3H s		3.81 3H s

[a]Run in 5% CD_3COOD in CD_3OD.
[b]Run in CD_3OD.
[c]Run in $CDCl_3/CD_3OD$.
[d]Not observed.

this assay, EFS-induced contractions are inhibited by the H_3-receptor agonist (*R*)-α-methyl histamine (RAM), and the effect of RAM can be blocked by H_3-antagonists. Verongamine at 1 μg/ml blocked the effect of RAM. For comparison, aerophobin-1 gave an H_3-receptor binding IC_{50} of 9.0 μM, and showed no activity in the guinea pig ileum assay. Additionally, verongamine at 1 μg/ml had no effect against the inhibition of EFS-induced ileum contractions by the α_2-adrenoceptor agonist clonidine. These results indicate verongamine is a specific H_3-receptor antagonist.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded at 300 MHz for proton and 75.5 MHz for carbon on a Varian XL-300 spectrometer. The low-resolution fabms was recorded on a Finnigan MAT-312 spectrometer. The high-resolution fabms was recorded on a VG-ZAB-SE double-focusing reverse-geometry mass spectrometer. The Si gel used was Merck Silica Gel 60, 230–400 mesh.

ANIMAL MATERIAL.—The specimen of *Verongula gigantea* (Porifera; Demospongiae; Verongida) was collected in March 1987, by the Johnson-SeaLink manned submersible at a depth of 210 m, north of Little Stirrup Cay, Bahamas. *Verongula gigantea* is not generally found at this depth, and the sponge collected appears to have been dislodged from the substrate and tumbled into deeper water. This specimen, however, was still alive at the time of collection. A taxonomic voucher specimen has been deposited in the Harbor Branch Oceanographic Institution Museum (catalog number 003:00406).

EXTRACTION AND PURIFICATION.—A 140 g portion of the frozen sponge was homogenized with EtOH in a blender and allowed to steep for 1 h before being filtered. This was repeated two additional times, and the resulting three extracts combined and concentrated under vacuum to give a brown gum. The gum was partitioned between EtOAc and H_2O, and the EtOAc fraction subsequently extracted 3 times with 1N HCl. The aqueous HCl fraction was treated with concentrated NaOH to adjust the pH to 9. The resulting alkaline solution was extracted 3 times with EtOAc, and the EtOAc extract concentrated under vacuum to give an oily residue (256 mg). A portion of the residue (45 mg) was chromatographed on Si gel employing a gradient elution consisting of 2–20% MeOH in CH_2Cl_2. A group of fractions which eluted with approximately 10–15% MeOH/CH_2Cl_2, and which displayed H_3 activity, were pooled. The residue from these combined fractions was subjected to gel filtration on Sephadex LH-20 with elution by MeCN/CH_2Cl_2 (1:1) to give pure verongamine (**1**; 1.4 mg, 0.006% of wet sponge).

Verongamine [**1**].—3-Bromo-α-(hydroxyimino)-N-[2-(1H-imidazol-4-yl)ethyl]-4-methoxy benzene propanamide (SCH-49483), yellow semisolid, oil; uv (MeOH) λ max 388 (630), 289 sh (2660), 280 (3040), 206 (33600) nm; ir (KBr) 3390 br, 3231 br, 2936, 2837, 1655, 1495, 1254, 1054 cm^{-1}; ^1H nmr and ^{13}C nmr, see Table 1; hrfabms, observed $[M+H]^+$ peak 381.0573, calcd for $C_{15}H_{18}N_4O_3{}^{79}Br$ 381.0562; fabms *m/z* for $[M+H]^+$; 381 (92) and 383 (100), for $[M+Na]^+$; 403 (36) and 405 (38).

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