Chemistry of Verongida Sponges. 10.[1] Secondary Metabolite Composition of the Caribbean Sponge *Verongula gigantea*

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A detailed analysis of the secondary metabolites of the Caribbean sponge *Verongula gigantea* has been performed. A number of bromotyrosine derivatives, **1**, **2**, and **6**−**17**, were identified, one of which (**17**) is a novel compound. Its structure was determined on the basis of spectroscopic evidence. Additionally, aureol (**18**) and 5,6-dibromo-*N*,*N*-dimethyltryptamine (**19**) were isolated from one of the five analyzed specimens.

Marine horny sponges belonging to the order Verongida are of much current biological and chemical interest. They all show a peculiar biochemistry characterized by lack of terpenes, production of large amounts of sterols with the aplystane skeleton, and elaboration of typical bromo compounds biogenetically related to tyrosine. These last metabolites are considered distinct markers for Verongida sponges, helping to overcome difficulties inherent in the sponge's identification based on morphological grounds. To provide assistance in attaining correct identification and supporting taxonomic work, we have been studying for a long time the secondary metabolite content of several Verongida species from the Caribbean sea, where they are particularly abundant.[1−9] As a part of this chemotaxonomic study we wish to report herein the results obtained from the analysis of one of these species, identified as *Verongula gigantea*.

V. gigantea, which may attain a very large size (up to 70 cm), is generally bowl-shaped, but contorted and irregular forms are to be found. The external color in life is olive-green, whereas the choanosome is dark yellow. The sponge darkens as soon as is exposed to air, and the inner part becomes purplish.

The following data are reported in the literature on the secondary metabolite composition of this species. In 1981 Makarieva et al.,[10] by studying several Verongida sponges from Cuban coasts, analyzed two specimens of *V. gigantea*. The first one, collected at Barlavento, contained aeroplysinin 1 (**1**) and compound **2** (in its ketal and dienonic form) as the major compounds. The second one, collected at Punta del Este, contained aeroplysinin 2 (**3**) and compound **4** in addition to aeroplysinin 1. More recently, isolation of verongamine (**5**) from a specimen of *V. gigantea* collected at Little Stirrup Cay, Bahamas, was reported.[11]

During the Fenical expeditions along the coasts of the Bahama Islands in summers of 1990 and 1992, we collected several specimens of the same sponge species, which were observed and photographed in vivo and identified on the basis of their morphology as *V. gigantea*. We can reasonably suppose that all our specimens, which perfectly match with the literature descriptions, are cospecific with those studied by the authors cited before.

The first specimen to be studied (SS 1404) was collected in July 1990, along the coast of San Salvador Island, at 15 m depth, stored frozen at −20 °C until extraction, and incorporated, as a sub-sample, in the collection of the (Dipartimento per lo Studio del Territorio e delle sue Risorse), University of Genova, Italy, under the same reference number. It was extracted sequentially with MeOH−toluene (3:1) and $CHCl_3$. The methanol−toluene extracts were concentrated in vacuo, and the resulting residue, suspended in water, was extracted with EtOAc and then with *n*-BuOH. The combined lipophilic material and the *n*-BuOH extract were chromatographed on a Si gel column and on a RP_{18} column, respectively, using a linear gradient of solvents. Selected fractions from both separations were successively purified by direct and reversed-phase HPLC.

In agreement with the analysis performed by Makarieva et al.[10] our investigation confirmed the presence of aeroplysinin 1, **1**,[12] and **2**[13] as secondary metabolites of *V. gigantea*. In addition, our specimen yielded bromotyrosine-derived compounds **6**−**17**, a sesquiterpene hydroquinone ether (**18**),[14] and a bromotryptamine derivative (**19**).[14] Bromo-compounds **6**,[2] **7**,[3] **8**,[15] **9**,[4] **10**,[5] **11**,[16] **12**,[17] **13**,[18] **14**,[5] **15**,[6] and **16**,[6] previously isolated from other Verongida sponges, were identified by comparisons of their spectral data with literature values. Compound **17** is a novel compound, and its structure elucidation follows.

The FABMS (positive ion mode) of compound **17** showed two peaks of nearly equal intensity for the molecular ion peak at *m*/*z* 258−260, which indicated the presence of one bromine atom in the molecule and suggested the molecular formula $C_{11}H_{17}BrNO$ in agreement with ^{1}H and ^{13}C NMR data. The UV spectrum showed absorption maximum at λ 281 nm (ϵ 1485) at pH 7.0, which shifted at λ 301 nm (ϵ 2273) at pH 10.0, indicating for **17** a phenol chromophore. The presence of a trisubstituted benzene ring was indicated by the ^{13}C NMR spectrum, which exhibited, in the aromatic region, three doublets (δ 134.63, 130.33, and 117.68) and three singlets (δ 154.87, 111.18, and 129.20) and confirmed by the ^{1}H NMR spectrum, which showed three 1H signals in the downfield region of the spectrum (δ 6.90, 7.15, and 7.49). The coupling constant pattern of the above proton signals (Table 1) suggested that benzene substitution had to be of the 1,2,4 type. In addition the ^{1}H NMR spectrum contained a 9H-singlet at δ 3.21 and an AA′BB′ system at δ 3.53 and 3.05, identifying $-CH_2-CH_2-{}^+N(CH_3)_3$ as one of the three phenyl substituents; the remaining two were an OH group and a bromine atom, as suggested by the molecular formula. The location of these three substituents was deduced by an interproton contact detected through a NOE difference experiment upon irradiation at δ 3.05 (H_2-

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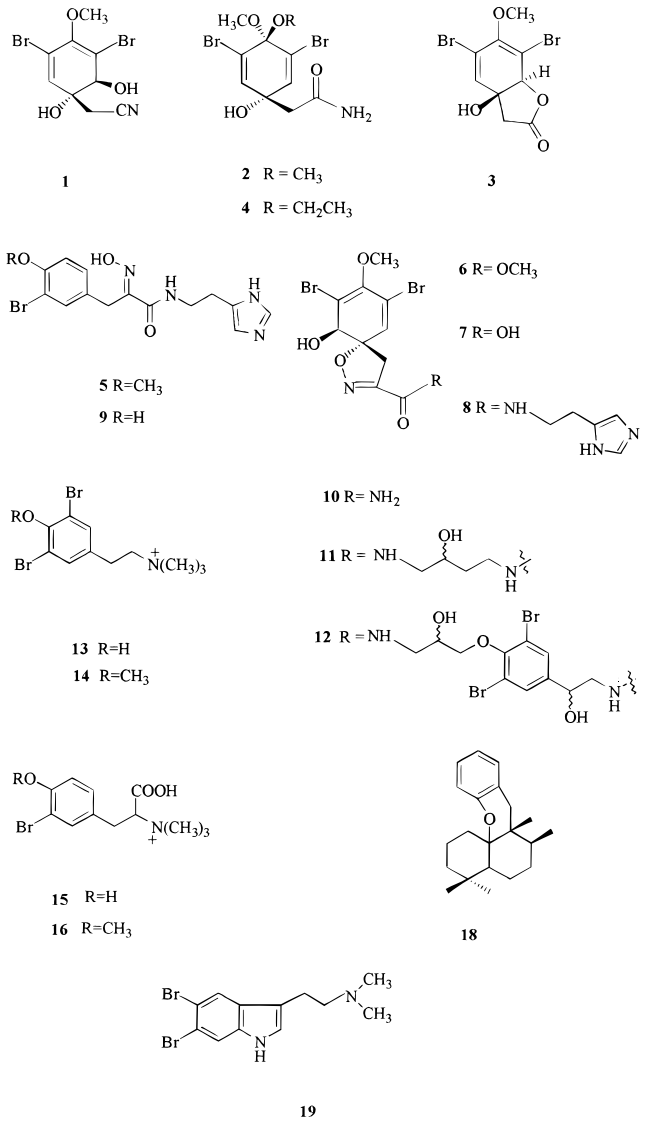
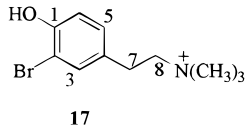
Table 1. ^{13}C and ^{1}H NMR Data for Compound **17** (CD_3OD)[a]

carbon	δ_C (mult.)	δ_H (mult., *J*/Hz)
1	154.87 (s)	
2	111.18 (s)	
3	134.63 (d)	7.49 (d, $J = 1.47$)
4	129.20 (s)	
5	130.33 (d)	7.15 (dd, $J = 8.08$; 1.47)
6	117.68 (d)	6.90 (d, $J = 8.08$)
7	29.07 (t)	3.05[b]
8	68.51 (t)	3.53[b]
$^{+}N(CH_3)_3$	53.73 (q)	3.22 (s)

[a] Assignment based on DEPT, COSY, HMQC, and HMBC experiments. [b] AA′BB′ system ($J_{AB} = J_{A'B'} = -17.5$ Hz; $J_{A'B} = J_{AB'} = 5$ Hz; $J_{AA'} = J_{BB'} = 11.8$ Hz).



7); the diagnostic enhancement was observed for protons resonating at δ 7.49 (d, $J = 1.47$ Hz) and 7.15 (dd, $J = 1.47$ Hz; 8.08 Hz), which, on account of multiplicity of their signals, had to be located at C-3 and C-5, respectively. The upfield chemical shift value (δ 6.90) of H-6, consistent only with its location ortho to the phenolic group, fully defined structure **17** as reported in the figure.



It is to be noted that the AA′BB′ system resonated as a complex signal that was due to the preferred anti conformation along C-7−C-8 bond, as previously indicated for the dibromo derivative analogue **14**, isolated from *Verongula* sp. As for compound **14**,[5] coupling constant values of **17** ($J_{AB} = J_{A'B'} = -17.5$ Hz, $J_{AB'} = J_{A'B} = 5$ Hz, $J_{AA'} = J_{BB'} = 11.8$ Hz) were determined by visual fitting between experimental and calculated splitting patterns (Figure 1).

Compounds **1**, **2**, and **6**−**17**, typical Verongida metabolites, could be confidently considered endogenous. On the other hand, the origin of compounds **18** and **19** appeared more questionable. Both products were initially isolated by Djura et al.[14] from *Smenospongia echina* and *Smenospongia aurea*, species taxonomically far from Verongida sponges. Successively, they were found in some other species, and Tymiak et al.[19] hypothesized that aureol (**18**) was a dietary metabolite, while compound **19** and its analogues could be considered as peculiar metabolites of Dictyoceratida sponges.

The analysis we carried out on the metabolic content of four further specimens of *V. gigantea* present in our collection of Caribbean sponges, strongly indicated that both compounds were actually of exogenous origin. The four specimens (2003, 2310, 1609, and 1810) were extracted using the same experimental procedure performed on sample SS 1404, and none showed any trace of aureol (**18**) and 5,6-dibromo-*N*,*N*-dimethyltryptamine (**19**). On the contrary, bromotyrosine metabolites could play a role as chemotaxonomic markers, because the major bromocompounds found in the specimen SS 1404 (**2**, **6**, **8**, **10**, **11**, **12**, **13**, and **14**) were also contained in comparable amounts in the extracts of the other four specimens (Table 2). It is to be noted that we did not carry out the investigation of the minor compounds on specimens 2003, 2310, 1609, and 1810, because the occurrence of these compounds in very small amounts could not be considered significant in a chemotaxonomic perspective.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were determined on a Brüker AMX-500 spectrometer, and the solvent was used as an internal standard (CD_3OD: ^{1}H δ 3.34, ^{13}C δ 49.0). The nature of each carbon resonance was deduced from a DEPT experiment. Homonuclear ^{1}H connectivities were determined by using COSY experiments. One-bond heteronuclear $^{1}H-^{13}C$ connectivities were determined with the Bax−Subramanian[20] HMQC pulse sequence using a BIRD pulse 0.50 s before each scan, to suppress the signals originating from protons not directly bound to ^{13}C (interpulse delay set for $^{1}J_{CH} = 140$ Hz). During the acquisition time, ^{13}C broad-band decoupling was performed using the GARP sequence. Two- and three-bond $^{1}H-^{13}C$ connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 10 Hz. The FABMS was recorded in a glycerol matrix on a VC Prospec Fisons mass spectrometer. The FT−IR spectrum was recorded on a Bruker IFS-48 spectrophotometer using a KBr matrix. UV spectra were performed on a Beckman DU70 spectrometer in MeOH solution. MPLC was performed on a Büchi 861 apparatus using a SiO_2 (230−400 mesh) and a RP_{18} (40−63 μm) column. HPLC was performed on a Varian 2510 apparatus equipped with an RI-3 refractive index detector, using Hibar columns.

Animal Material. The specimens of *Verongula gigantea* were collected at 15 m depth in the summer of 1990, along the coast of San Salvador Island (SS 1404) and in the summer of 1992, along the coasts of Little San Salvador Island (2003, 2310), Grand Bahama Island (1609), and Abaco Island (1810). They were stored frozen at −20 °C until used and identified by Prof. M. Pansini (Dip.Te.Ris., University of Genova, Italy).

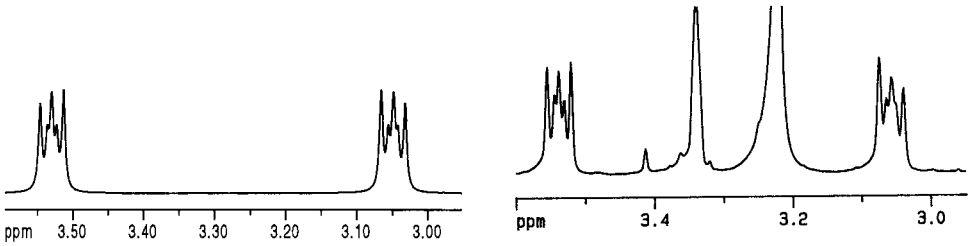


Figure 1. Observed (right) and calculated (left) ^{1}H NMR spectra (500 MHz) of the AA′BB′ system protons of compound **17**. The system was simulated on an IBM PS/2 70 with the aid of an unpublished program, NMR simulation by Prof. A. Mangoni.

Table 2. Percentages[a] of the Major Metabolites in Specimens of *Verongula gigantea*

	compound %									
specimen	**2**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**18**	**19**
SS 1404	0.198	0.321	0.622	0.176	0.361	0.164	0.139	0.487	0.138	0.110
2003	0.208	0.246	0.980	0.194	0.322	0.133	0.123	0.399	0	0
2310	0.162	0.274	0.677	0.185	0.284	0.141	0.098	0.535	0	0
1810	0.174	0.220	0.858	0.123	0.295	0.203	0.110	0.562	0	0
1609	0.208	0.330	0.954	0.174	0.401	0.207	0.182	0.451	0	0

[a] Percentage on dry weight of the specimens after extraction.

Reference specimens are deposited as sub-samples in the collection of the Dip.Te.Ris. under the same voucher numbers.

Extraction and Isolation. The extraction and isolation procedures were initially performed on the largest sample, SS 1404, as follows. The freshly thawed material (254 g dry wt after extraction) was chopped, homogenized, and then extracted with MeOH−toluene (3:1) (1 L × 3) and subsequently with $CHCl_3$ (1 L × 3) at room temperature. The combined MeOH−toluene solutions, after filtration, were concentrated in vacuo to give an aqueous suspension that was subsequently extracted initially with EtOAc and then with *n*-BuOH. The combined EtOAc and $CHCl_3$ extracts (22.0 g of a dark brown oil) were chromatographed by MPLC on a SiO_2 column using a solvent gradient system from *n*-hexane to EtOAc and then to MeOH. Selected fractions were combined on the basis of TLC analyses.

The fractions eluted with *n*-hexane−EtOAc (1:1) were purified by HPLC using a Hibar LiChroprep Si 60 column (10 × 250 mm) with a mobile phase of EtOAc−$CHCl_3$ (1:1) and afforded 219.0 mg of pure aeroplysinin 1 (**1**) and 815.3 mg of compound **6**, identified by comparison of their spectral properties with literature values.[12,2] The fractions eluted with EtOAc−MeOH (7:3) were purified by HPLC on a Hibar LiChroprep Si 60 column (10 × 250 mm) with EtOAc 100% as mobile phase to obtain 504.2 mg of compound **2**, identified by comparison of its spectral properties with literature values.[13] The fractions eluted with EtOAc−MeOH (3:7) were rechromatographed by MPLC using an RP_{18} column (40−63 μm) and a linear gradient solvent system from H_2O to MeOH to $CHCl_3$. The fractions eluted with H_2O−MeOH (4:6) were purified by HPLC using a Hibar LiChrospher RP_{18} column (4 × 250 mm) with H_2O−CH_3CN (1:1) and TFA 0.1% as mobile phase and afforded 5.32 mg of compound **7**, 1.58 g of pure aerophobine 1, (**8**), and 4.6 mg of compound **9**, identified by comparison of their spectral properties with literature values.[3,15,9] The fractions eluted with *n*-hexane−EtOAc (1:9) were purified by HPLC using a Hibar LiChroprep Si 60 column (10 × 250 mm) with a mobile phase of EtOAc−$CHCl_3$ (9:1) to obtain 448.3 mg of compound **10**, identified by comparison of its spectral properties with literature values.[5] The fractions eluted with EtOAc−MeOH (9:1) were rechromatographed by HPLC using a Hibar LiChroprep Si 60 column (10 × 250 mm) with a mobile phase of EtOAc−$CHCl_3$ (9:1) to obtain 918.0 mg of 11-hydroxyaerothionin (**11**), identified by comparison of its spectral properties with literature values.[16] The fractions eluted with EtOAc 100% were purified by HPLC using a Hibar LiChroprep Si 60 column (10 × 250 mm) with a mobile phase of EtOAc−$CHCl_3$ (9:1) and afforded 417.3 mg of pure fistularin 3 (**12**), identified by comparison of its spectral properties with literature values.[17] The fractions eluted with *n*-hexane−EtOAc (7:3) were purified by HPLC using a Hibar LiChroprep Si 60 column (10 × 250 mm) eluted initially with *n*-hexane−EtOAc (7:3) and successively with *n*-hexane−EtOAc−$CHCl_3$ (8:1:1) to obtain 350.2 mg of aureol (**18**), as a pure compound. It was identified by comparison of its spectral properties with literature values.[14] The fractions eluted with MeOH 100% were rechromatographed by MPLC using a RP_{18} column (40−63 μm) eluted with a linear gradient solvent system from H_2O to MeOH and afforded compound **19** as a pure compound (275.6 mg), identified by comparison of its spectral properties with literature values.[14]

The BuOH-soluble material (14 g) was subjected to a MPLC on a RP_{18} column using solvents of decreasing polarity from H_2O to MeOH to $CHCl_3$. The fractions eluted with H_2O 100% were rechromatographed by HPLC on a Hibar LiChrospher RP_{18} column (4 × 250 mm) with H_2O−MeOH (8:2) and TFA 0.1% as mobile phase to obtain 354 mg of compound **13**, identified by comparison of its spectral properties with literature values,[18] and 20.1 mg of compound **17**. The fractions eluted with H_2O−MeOH (8:2) were rechromatographed by MPLC using a RP_{18} column (40−63 μm) and a linear gradient solvent system from H_2O to MeOH to $CHCl_3$. It afforded 1.24 g of compound **14**, identified by comparison of its spectral properties with literature values,[5] and a mixture of compounds **15** and **16**, separated by HPLC using a Hibar LiChrospher RP_{18} column (4 × 250 mm) with H_2O−MeOH (9:1) and TFA 0.1% as mobile phase. Compound **15** (8.0 mg) and compound **16** (1.1 mg) were obtained, and both were identified by comparison of their spectral properties with literature values.[6]

The same extraction and isolation procedures were performed on specimens 2003 (137 g dry wt after extraction), 2310 (147 g dry wt after extraction), 1609 (86 g dry wt after extraction), and 1810 (28 g dry wt after extraction), and the obtained results, with respect to the major metabolites, are reported in Table 2 in percentages.

Compound 17: UV (MeOH) λ_{max} 281 nm (pH 7.0, ϵ 1485), 301 nm (pH 10.0, ϵ 2273), 275 nm (pH 4.0, ϵ 1475); IR (KBr) ν_{max} 2575 cm^{-1} (NC−H stretch), 1635 cm^{-1} (aromatic); FABMS [M]$^+$ 258−260; ^{1}H NMR and ^{13}C NMR data (CD_3OD, 500 MHz) are reported in Table 1.

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