New Fish Feeding Deterrents, Including a Novel Sesquiterpenoid Heterogorgiolide, from the Brazilian Gorgonian *Heterogorgia uatumani* (Octocorallia, Gorgonacea)

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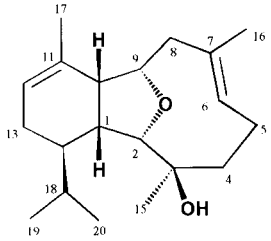
Studies of the Brazilian gorgonian octocoral *Heterogorgia uatumani* have resulted in the discovery of two metabolites that inhibit fish feeding under natural conditions. These are the previously reported eunicellane diterpenoid, (6*E*)-2α,9α-epoxyeunicella-6,11(12)-dien-3*β*-ol (**1**) and a new sesquiterpene lactone, heterogorgiolide (**2**). The structures of **1** and **2** were determined by spectroscopic methods and by comparison of spectral data with literature values. Field bioassays of the two compounds, at their natural concentrations, confirmed that they deter predation by a complex assemblage of reef fishes. This is an unusual observation showing that defenses are derived from both sesqui- and diterpenoid metabolites.

Secondary metabolites isolated from gorgonian octocorals (sea whips and sea fans) are structurally diverse[1] and many exhibit biological activities.[2] Since the polyps of the gorgonians lack physical defenses, the secondary metabolites from these animals have been generally hypothesized to serve as chemical defenses.[2,3] During the past decade, laboratory and field experiments have shown that numerous gorgonians possess metabolites which are highly deterrent to generalist carnivorous fishes.[4−13] Of the gorgonians studied in detail, several metabolites responsible for icthyodeterrent activity have been isolated.[6−12] Studies, mainly involving gorgonians from the Caribbean Sea[4−10] and from Guam,[11,12] show that diverse compounds, including acetogenins,[7] sesquiterpenes,[8,9] and diterpenes,[10−12] are the responsible metabolites. The Southern Atlantic Ocean along the Brazilian coast (more than 7500 km) is rich in octocorals, but investigations of their secondary metabolites, and their functions in defense, are few.[13]

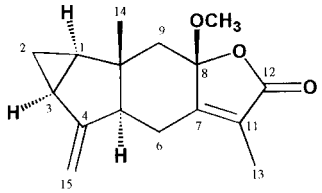
We report here the results of an investigation of the secondary metabolites and chemical defense of the gorgonian *Heterogorgia uatumani* Castro (Plexauridae, Gorgonacea, Octocorallia, Cnidaria) collected in Ilha Grande Bay, Angra dos Reis, a region southwest of Rio de Janeiro, Brazil. This represents the first chemical study of *H. uatumani*, a gorgonian distributed in the tropical Atlantic Ocean from the southeast Caribbean Sea to the south Brazilian littoral zone.[14]

Results and Discussion

H. uatumani was collected using SCUBA in March, 1994, at 10−12 m depth and immediately frozen. The freeze-dried animals were extracted with methanol and dichloromethane, and the combined extracts were fractionated by vacuum flash chromatography on Si gel to furnish a fraction (eluted with 30% ethyl acetate/isooctane) containing two major compounds. From this fraction, the major deterrent metabolites eunicellane diterpenoid **1** and heterogorgiolide (**2**) were subsequently purified by flash Si gel chromatography and by C_8 reversed-phase HPLC.



1



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The eunicellane ether **1** [(6*E*)-2α,9α-epoxyeunicella-6,-11(12)-dien-3*β*-ol] was isolated as 0.1% of the dry weight of the gorgonian. Compound **1** had the molecular formula $C_{20}H_{32}O_2$ by HRMS ($[M]^+$ *m/z* obsd 304.2370, calcd 304.2402) and by combined NMR methods. The ^{13}C and DEPT NMR spectra for **1** revealed the presence of five methyl groups, four methylene carbons, eight methines, and three quaternary carbons, all of which suggested that the molecule was of diterpenoid origin. An IR absorption at 3417 cm^{-1} suggested that one of the oxygen atoms present was part of an alcohol functionality. Comprehensive NMR data, involving $^1H-^1H$ COSY, HMQC, and HMBC experiments, allowed complete NMR assignments to be made. A literature survey revealed that this compound, (6*E*)-2α,9α-epoxyeunicella-6,11(12)-dien-3*β*-ol, had been previously isolated from an Indo-Pacific alcyonacean soft-coral of the genus *Cladiella*.[15] NMR experiments provided independent confirmation of the structure of **1**, and allowed all proton and carbon assignments to be confidently made.

Heterogorgiolide (**2**) was isolated as an amorphous white solid (0.058% of dry wt gorgonian) with molecular formula $C_{16}H_{20}O_3$ by HRCI mass spectrometry ($[MH]^+$ *m/z* obsd 261.1486, calcd 261.1491) and combined NMR methods. The IR spectrum of **2** revealed absorptions assigned to lactone (1775 cm^{-1}) and double bond (1690 and 1658 cm^{-1}) functionalities. Carbon-13 NMR experiments, including DEPT analysis, indicated the presence of three methyl groups, four methylene carbons (one sp^2 and three sp^3), three methines and six quaternary carbons (four sp^2 and two sp^3), which suggested that **1** was a methoxy sesquiterpene skeleton containing four rings. The presence of one tetrasubstituted double bond and one exocyclic methylene was confirmed by ^{13}C NMR signals at δ 106.4 (CH_2), 128.1

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Table 1. NMR Data for Heterogorgiolide (**2**)

					HMBC correlations ($H^{\#}$)[a,c]	
$C^{\#}/H^{\#}$	δ ^{13}C (DEPT)[a]	δ ^{1}H (m, J in Hz)[a]	δ ^{1}H (m, J in Hz)[b]	$^{1}H-^{1}H$ COSY ($H^{\#}$)[a]	^{2}J	^{3}J
1	30.0 (CH)	1.38 (dt, 3.6, 7.5)	1.30 (ddd, 3.7, 7.3, 8.0)	2ab, 3	2ab	9b, 14
2a	17.1 (CH_2)	0.71 (dt, 3.6, 6.0)	0.64 (dt, 3.7, 5.4)	1, 2b, 3		
2b		0.83 (ddd, 6.0, 7.5, 8.0)	0.75 (ddd, 5.4, 8.0, 9.0)	1, 2a, 3		
3	24.6 (CH)	2.02 (m)	1.93 (m)	1, 2ab, 15a	1, 2ab	15ab
4	153.3 (C)				15ab	1, 2ab, 6a
5	56.3 (CH)	3.36 (m)	3.31 (m)	6ab, 15ab	6ab	1, 9a, 14, 15ab
6a	23.3 (CH_2)	2.35 (dd, 13.0, 18.0)	2.20 (ddq, 1.0, 12.1, 17.8)	5, 6b, 13		
6b		2.45 (m)	2.30 (ddq, 2.0, 7.0, 17.8)	5, 6a, 13		
7	159.4 (C)				6ab	13
8	109.8 (C)				9ab	6ab, 16
9a	51.4 (CH_2)	2.13 (d, 14.0)	2.15 (d, 14.0)	9b		1, 14
9b		2.48 (d, 14.0)	2.44 (d, 14.0)	9a		
10	38.6 (C)				1, 5, 9ab, 14	2ab
11	128.1 (C)				13	6ab
12	173.0 (C)					13
13	8.2 (CH_3)	1.82 (bs)	1.79 (dd, 1.0, 2.0)	6ab		
14	21.3 (CH_3)	0.52 (s)	0.45 (s)	9b		9ab
15a	106.4 (CH_2)	4.78 (bs)	4.65 (m)	3, 5, 15b		
15b		5.00 (bs)	4.94 (m)	5, 15a		
OCH_3	50.7 (CH_3)	3.08 (s)	3.06 (s)			

[a] ^{1}H and ^{13}C assignments made on the basis of HMQC experiments (CD_3OD, 400 and 100 MHz, respectively). [b] $CDCl_3$, 300 MHz. [c] HMBC J_{CH} = 8 Hz.

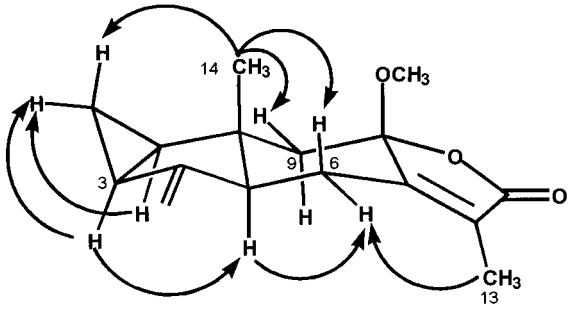

Figure 1. Selected nuclear Overhauser enhancements observed for heterogorgiolide (**2**).

(C), 153.3 (C) and 159.4 (C), and by two broad signals (1H each) at δ 4.78 and 5.00 in the ^{1}H NMR spectrum. The presence of a ^{13}C NMR carbonyl signal at δ 173.0, an infrared absorption at 1775 cm^{-1} and an UV absorbance at 207 nm illustrated that **2** possessed an α,β-unsaturated-γ-lactone. The presence of a methoxyl group was indicated by ^{13}C and ^{1}H NMR signals at δ 50.7 (CH_3) and δ 3.08 (3H, s), respectively. The downfield carbon chemical shift of the γ-lactone carbon [δ 109.8 (C)] indicated that the methoxyl group was part of a typical γ-methoxy-γ-lactone functionality. The presence of a cyclopropane ring was strongly suggested by the shielded signals at δ 0.71 (1H, dt, J = 3.6 and 6.0 Hz) and 0.83 (1H ddd, J = 6.0, 7.5 and 8.0 Hz). The remaining five- and six-membered rings were subsequently confidently assigned by $^{1}H-^{1}H$ COSY and HMBC correlation data (Table 1). These data allowed the complete planar structure of heterogorgiolide to be assigned.

The relative stereochemistry of heterogorgiolide (**2**) was assigned by interpretation of ^{1}H NMR coupling data ($CDCl_3$, 300 MHz) and nuclear Overhauser enhancement experiments (Table 1 and Figure 1). The rigid conformation of heterogorgiolide readily allowed the assignment of all substituents, as well as the three-dimensional arrangement of the three rings present. Homoallylic couplings were observed between the C-6 axial, [δ 2.20 (1H, ddq, J = 1.0, 12.1, 17.8 Hz)], and equatorial [(δ 2.30 (1H, ddq, J = 2.0, 7.0, 17.8 Hz)] protons and the H-13 methyl protons [(δ 1.79, 1H, dd, J = 1.0 and 2.0 Hz)]. The H-5 α-axial orientation was suggested by its axial−equatorial and axial−axial couplings with H-6ab (7.0 and 12.1 Hz, respectively). NOE enhancements observed for H-2β, H6β, and H-9β when the protons at δ 0.52 (3H, s) were irradiated, placed the bridghead methyl group in a β-axial position (Figure 1). The same correlations were observed for H-9α and H-2α, and for H-1 and H-2α when H-1 and H-3 were irradiated, respectively. These NOE enhancements indicated that the cyclopropane ring is in a cis relationship with H-14 and possesses a trans relationship to H-5. The methoxyl group was assigned to the β position on the basis of observed NOE correlations from the H-13 protons (C-13 methyl) to only the H-6α proton. Molecular modeling experiments showed that the observed NOE between the C-13 methyl group and H-6α proton requires that the methoxyl group be in the β or "up" axial position (Figure 1). The relative configurations of these groups are identical to those found in other linderane sesquiterpenes previously isolated from terrestrial plants.[15−22]

This is the first report of the isolation of a linderane sesquiterpenoid from a marine organism. Linderanes, on the other hand, are common metabolites of numerous plants. Two closely related linderane sesquiterpenoids, bearing α or β C-8/C-9 epoxides, have been isolated from *Chloranthus glaber*.[16,17] In addition, various linderanes have also been isolated from *Lindera strichnifolia*[18,19] *Chloranthus japonicus*,[20−25] *Chloranthus serratus*,[20,26,27] and *C. glaber*.[16,28]

The feeding deterrent properties of compounds **1** and **2** were determined using an in situ bioassay developed to assess feeding preferences of natural populations of carnivorous fish.[9,10] Palatable, carageenan-based food pellets were prepared, and consumption was measured by placing food arrays directly on coral reefs. Control foods, without extracts or purified metabolites, were readily consumed in all cases. Experiments which compared control to treated foods, in which compounds **1** and **2** were incorporated at the same concentrations as they occur in the gorgonian tissues, showed that extracts of *H. uatumani* and terpenoids **1** and **2** were highly deterrent. Diterpenoid **1** and heterogorgiolide (**2**), at natural volumetric concentrations (0.5 and 0.8 mg/cm^3), significantly inhibited feeding relative to controls with high statistical significance (Wilcoxon signed rank test, 1-tail, p = 0.0212 and p = 0.0068, respectively).[28]

Experimental Section

General Experimental Procedures. Reversed-phase (C_8) HPLC was carried out on a semipreparative column (9.4 mm i.d.) using a Waters model 510 pump and a model 410 differential refractometer detector. Corrected melting points were observed on a Thomas-Hoover capillary apparatus. IR spectra (film, $CHCl_3$) were recorded on a Perkin-Elmer model 1600 FTIR spectrometer. Mass measurements were obtained on a HP 5989A spectrometer. UV spectra were obtained on a Shimadzu model 1601 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 243B (D_{25} = 589 nm, c = 1.0, $CHCl_3$). NMR spectra were recorded on Varian Unity-500 and Bruker 200 spectrometers (CD_3OD solutions) and on a Varian Unity-300 spectrometer ($CDCl_3$ solutions).

Extraction and Isolation. The gorgonian *H. uatumani* Castro (1.18 L volume) was collected using SCUBA in 1994 at a depth of 6−15 m, in Ilha Grande Bay, Angra dos Reis, south of Rio de Janeiro state. The specimens were stored frozen until extraction. The animals were freeze-dried and the tissues (1022 g) were extracted at room temperature once with MeOH/CH_2Cl_2 (3:7) and twice with 100% CH_2Cl_2. After removal of the solvents from the combined extracts under reduced pressure, 32.6 g of a brownish gum was obtained. The resulting crude extract was purified by vacuum flash chromatography using TLC grade Si gel and employing a gradient of 0−100% of EtOAc in isooctane. Part of the fraction eluted with 30% of EtOAc [0.2 of the 4.5 g obtained] was refractionated by Si gel flash chromatography [EtOAc:hexane (2:8)] to yield 20 fractions. Fractions 5 and 6 were combined (76.5 mg) and further purified by reversed-phase HPLC using MeOH/H_2O (4:1, flow rate of 2.5 mL/min) furnishing compound **1** (47.2 mg, 0.1% of dry wt gorgonian). Fraction 3 (42.4 mg) was purified by reversed-phase HPLC using MeOH/H_2O (6.5:3.5, flow rate of 3 mL/min) to give heterogorgiolide (**2**) (26.2 mg 0.058% of dry wt gorgonian).

(6*E*)-2α,9α-Epoxyeunicella-6,11(12)-dien-3*β*-ol (1):[15] white powder; IR (film, $CHCl_3$) ν_{max} 3417, 2953, 2911, 1712, 1459, 1371, 1258, 1072 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 5.48 (1H, m, H-6), 5.40 (1H, m, H-12), 4.11 (1H, bd, J = 6.0 Hz, H-9), 3.84 (1H, d, J = 7.2 Hz, H-2), 2.52 (1H, m, H-1), 2.44 (2H, m, H-5b and H-10), 2.42 (1H, m, H-8b), 2.18 (1H, m, H-13b), 2.10 (1H, m, H-4b), 2.06 (1H, m, H-5a), 2.02 (1H, m, H-8a), 1.95 (1H, m, H-13a), 1.81 (3H, bs, H-16), 1.68 (3H, bs, H-17), 1.60 (1H, m, H-14), 1.52 (1H, m, H-18), 1.50 (1H, m, H-4a), 1.39 (3H, s, H-15), 0.99 (3H, d, J = 6.3 Hz, H-20), 0.85 (3H, d, J = 6.3 Hz, H-19); ^{13}C NMR (CD_3OD, 50 MHz) δ 134.3 (C, C-11), 130.1 (CH, C-6), 127.8 (C, C-7), 122.2 (CH, C-12), 90.8 (CH, C-2), 81.9 (CH, C-9), 77.8 (C, C-3), 48.1 (CH, C-10), 45.5 (CH_2, C-8), 41.0 (CH, C-1), 39.4 (CH, C-14), 36.6 (CH_2, C-4), 30.4 (CH, C-18), 27.0 (CH_3, C-15), 23.6 (CH_2, C-13), 23.3 (CH_2, C-5), 22.0 (CH_3, C-17), 21.9 (CH_3, C-20), 21.8 (CH_3, C-19), 18.9 (CH_3, C-16; HREIMS (70 eV): $[M]^+$ m/z obsd. 304.2370, calcd. 304.2402 for $C_{20}H_{32}O_2$.

Heterogorgiolide (2): white amorphous solid; mp 149−151 °C; $[\alpha]^{25}_D$ +116° (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 nm (1.845); IR (film, $CDCl_3$) ν_{max} 3065, 2937, 2851, 1775, 1689, 1657, 1438, 1316, 1278, 1145 cm^{-1}; ^1H and ^{13}C NMR data (see Table 1); LREIMS (70 eV): m/z 260 $[M]^{+\cdot}$(2), 245 (3), 228 (45), 213 (12), 200 (15), 157 (14), 105 (100); HRCIMS (70 eV): $[M + H]^+$ m/z obsd 261.1486, calcd 261.1491 for $C_{16}H_{21}O_3$.

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