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HALOGENATED BLUE PIGMENTS OF A DEEP SEA GORGONIAN

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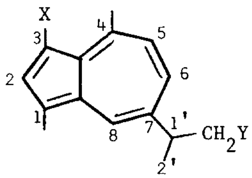
Three halogenated azulenes (**2**-**4**), one of them chiral, have been isolated from a deep sea gorgonian which was collected by minisubmersible at -350 m.

The strikingly brilliant colors of the tropical marine fauna include many shades of blue. The molecular structure of blue marine pigments was unknown before the recent isolation of guaiazulene (**1**) from the gorgonian _Euplexaura_ _erecta_ by Fusetani _et_ _al_.[1,2]

Dives aboard the minisubmersible _Makali'i_ have given us an opportunity to explore the rich deep water (-350 m) gorgonian fauna off O'ahu.[3] The intense blue color of some of these animals prompted us to study their chemistry. We have already reported[4] the presence of guaiazulene (**1**), of an aquamarine-colored bis (3,3-guaiazulenyl)methane, and of the purple linderazulene, which Thomson and coworkers[2] had earlier detected in a shallow water Sea of Marmara gorgonian. We now describe isolation and structure elucidation of three unprecedented halogenated guaiazulenes, one of them chiral, from a deep sea member of the family Paramuriceidae.

The animal (wet weight 191 g) was preserved on Dry Ice as soon as it was brought to the surface and steeped in hexane (800 mL, 24 h). The extract was dried (Na_2SO_4) and the solvent removed _in_ _vacuo_. The residue (624 mg) was chromatographed on BioBeads SX-8 (Bio-Rad Laboratories, Richmond, CA) with hexane/benzene (1:1) yielding seven fractions. Fraction 4 (20 mg) after HPLC on LiChrosorb RP-18 (Merck) and elution with methanol yielded fractions A and B. B was rechromatographed on the same column and eluted with methanol/H_2O (9:1). Two pigments were separated as blue oils in amounts of 1-2 mg and identified as 3-chloro- (**2**) and 3-bromo-7-isopropyl-1,4-dimethylazulene (**3**).[5] High resolution electron impact mass spectrometry (HREIMS) secured a formula of $C_{15}H_{17}Cl$ (_m/z_ 232.10188, calc'd. for $C_{15}H_{17}{}^{35}Cl$ 232.1020) for compound **2** and $C_{15}H_{17}Br$ (_m/z_ 276.05136; calc'd. for $C_{15}H_{17}{}^{79}Br$ 276.05141) for compound **3**. These formulas are compatible with halogenated sesquiterpenes possessing seven double bonds and/or rings. Comparison of the electronic spectra of **2** and **3** with the spectrum of authentic guaiazulene (**1**, Aldrich Chemical Co.) (Table 1) provided strong evidence that the pigments are haloazulenes.

Detailed analysis of the ^1H NMR spectra (Table 2), comparison with the guaiazulene spectrum, and with additional published spectral data[6] proved conclusively that both halogens are located at C-3 of guaiazulene. Moreover, a literature search revealed that 3-chloroguaiazulene (**2**) was a known synthetic compound, prepared from guaiazulene and _N_-chlorosuccinimide.[7] Comparison of the ^1H NMR spectra of natural and synthetic **2**, were superimposable. 3-Bromoguaiazulene (**3**) was not known previously, but both 3-haloazulenes had



1 X = Y = H guaiazulene

2 X = Cl, Y = H 3-chloro-7-isopropyl-1,4-dimethylazulene

3 X = Br, Y = H 3-bromo-7-isopropyl-1,4-dimethylazulene

4 X = H, Y = Br (7-(1'bromomethyl)ethyl-1,4-dimethylazulene ≡ ehuazulene

Table 1. Electronic Absorption Data of Guaiazulene (1), 3-Chloro- (2), 3-Bromoguaiazulene (3), and Ehuazulene (4) (Measured in chloroform on a Cary-14 spectrometer)

Compound No.	Maxima, nm
1	242, 285, 290, 305 sh, 350, 367, 600
2	246, ---, 293, 307, 352, 369, 620
3	246, ---, 294, 308, 353, 370, 612
4	244, 286, 292, 305 sh, 352, 369, 605

Table 2. ^{1}H NMR Data of Guaiazulene (1), 3-Chloro- (2), 3-Bromoguaiazulene (3), and Ehuazulene (4) (TMS = 0, *J* (Hz), m = multiplet measured on a Nicolet 300 MHz spectrometer)

Position No. / Compound No.	1^a	2^a	3^b	$4^{a(b)}$
1-Me	2.64	2.57	2.35	2.62 (2.55)
2-H	7.64, *J* = 3.6	7.40	7.51	7.62, *J* = 3.5
3-H	7.24, *J* = 3.6	--	--	7.27, *J* = 3.5
4-Me	2.82	3.09	3.09	2.82 (2.56)
5-H	7.04, *J* = 10.6	6.85, *J* = 10.5	6.58, *J* = 10.6	7.03, *J* = 10.6
6-H	7.45, *J* = 10.6, 1.8	7.31, *J* = 10.5, 2.1	6.98, *J* = 10.6, 2.1	7.38, *J* = 10.6, 1.95
8-H	8.24, *J* = 1.8	8.05, *J* = 2.1	7.92, J = 2.1	8.12, *J* = 1.95
1'-H	3.1, *J* = 7.0	3.0, *J* = 7.0	2.65 m	3.30 m (2.90 m)
2'-Me	1.4, *J* = 7.0	1.36, *J* = 7.0	1.12, *J* = 7.0	1.50, *J* = 7.0 (1.21, *J* = 7.0)
1'-CH_2Y	1.4, *J* = 7.0	1.36, *J* = 7.0	1.12, *J* = 7.0	3.63 m (3.25, *J* = 9.8, 6.17) (3.13, *J* = 9.8, 8.18)

[a] CD_2Cl_2 [b] C_6D_6

been synthesized by Anderson et al.,[8] who observed that the 3-bromo compound was less stable than the 3-chloro analog. This also holds true for our 3-haloguaiazulenes. Haloguaiazulenes may well be biosynthetic precursors of bis(3,3'-guaiazulenyl)methane (vide supra) and of 3-formylguaiazulene,[9] both of which we have encountered in the same gorgonian.

HPLC fraction A after further purification on a Bio-Sil A column (hexane) and HPLC (LiChrosorb RP-18, methanol/water, 9:1) furnished a third haloguaiazulene, isomeric with 3, but brominated unexpectedly in the isopropyl side chain. The structure of the new compound, 7-(1'-bromomethyl)ethyl)-1,4-dimethylazulene or ehuazulene (4),[10] was secured as follows.

HREIMS measurements provided the composition of $C_{15}H_{17}Br$ (*m/z* 276.05136, calc'd. for $C_{15}H_{17}{}^{79}Br$ 276.05141). The initial fragmentation to *m/z* 183 (M^+-CH_2Br instead of M^+-CH_3 as for 1-3, plus the typical electronic spectrum (Table 1) immediately suggested a novel haloguaiazulene. ^{1}H NMR measurements (Table 2) unambiguously supported structure 4 for ehuazulene. Signals for five aromatic protons and two methyls placed the bromine in the isopropyl group, a result already adumbrated by the tell-tale mass spectral fragmentation. The methine carbon of the isopropyl group thus is chiral and renders the adjacent methylene hydrogens non-equivalent (δ 3.25, 3.13), and coupled to each other by about 10 Hz. When we measured the optical rotation, we further discovered that ehuazulene displays a Cotton effect associated wth its 370 nm chromophore $[\alpha]_{578}^{\text{hexane}}$ +2°, $[\alpha]_{546}$ +5°, $[\alpha]_{436}$ +55°, $[\alpha]_{365}$ 0° (c 0.065).[11] This appears to be the first instance of a chiral azulene.

Biosynthetic capability by a gorgonian is noteworthy in view of the recent demonstration by Papastephanou and Anderson[12] that a terpenoid secondary metabolite is biosynthesized by a cell-free homogenate of the symbiotic algae without participation by the animal. In this case, no symbiotic algae can exist at -350 m. Nor is it likely that these compounds are of dietary origin, since sessile animals have few food choices.

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References and Notes

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9. Unpublished results from our laboratory.
10. In view of the cumbersome systematic name we propose the trivial name ehuazulene. *Ehu* is the Hawaiian word for reddish brown, the color of bromine.
11. Rotations were measured on a Perkin-Elmer Model 141 polarimeter.
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