

## Isolation of Guaianoid Pigments from the Gorgonian *Calicogorgia granulosa*

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Received May 23, 1996<sup>®</sup>

Three pigments of the guaiazulene class have been isolated from the gorgonian *Calicogorgia granulosa*. Structures of these compounds have been determined as guaiazulene, 2,2'-diguaiiazulenylmethane, and a new compound, 2,2'-biguaiiazulenyl, by combined chemical and spectroscopic methods. 2,2'-Biguaiiazulenyl exhibited moderate antimicrobial activity.

Guaiazulene and related sesquiterpenoids are widely distributed among marine octocorals of the order Scleractinia.<sup>1</sup> These compounds are well recognized for their distinctive blue and purple colors. Several compounds of this class have been reported to exhibit various bioactivities including antibacterial, antifungal, cytotoxic, antitumor, and immunoregulating activities as well as inhibitory activities against cell division of fertilized sea urchin and ascidian eggs.<sup>2–5</sup> In our search for novel secondary metabolites from Korean water organisms, we collected the gorgonian *Calicogorgia granulosa* Kukenthal (Paramuriceidae) off the shore of Jaeju Island.<sup>6</sup> Silica vacuum flash chromatography of the CH<sub>2</sub>Cl<sub>2</sub> extract followed by silica HPLC of nonpolar fractions (0–5% EtOAc in hexane) yielded three blue and bluish-green colored pigments of the guaiazulene class, including a new dimeric compound. In this note we report the structure elucidation and antimicrobial activity of these compounds.

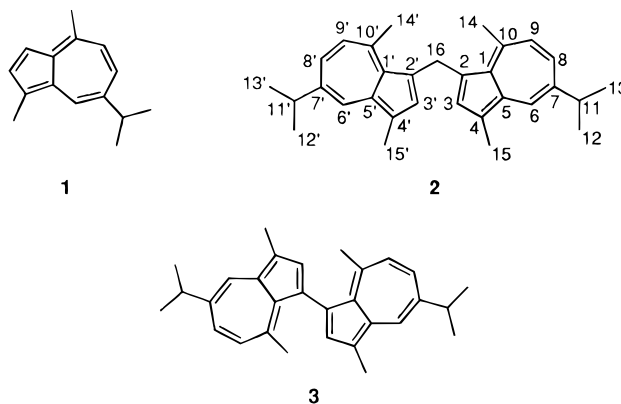
Compound **1** was isolated as a gum, which was analyzed for C<sub>15</sub>H<sub>18</sub> by HRMS and <sup>13</sup>C NMR. Compound **1** was readily identified as guaiazulene by comparison of spectral data with authentic material. Since originally being found in the essential oils of terrestrial plants, guaiazulene has been isolated from a marine red alga and various gorgonian corals.<sup>2–5,7–9</sup>

A closely related hydrocarbon **2** was isolated as a blue gum. Spectral data of **2** were highly compatible with those derived from **1**. A combination of <sup>1</sup>H- and <sup>13</sup>C-NMR, HMQC, and HMBC experiments determined the structure of this compound as 2,2'-diguaiiazulenylmethane. A literature survey revealed that **2** was originally reported as a synthetic product.<sup>10,11</sup> Later, the same compound was isolated from the deep-sea gorgonian *Pseudotesia* sp.<sup>3</sup> Spectral data of **2** were identical to those reported for this compound. The structure of **2** was further confirmed by chemical transformation of guaiazulene following the method developed by Treibs.<sup>10</sup>

Compound **3** was isolated as a bluish-green amorphous solid that was analyzed for C<sub>30</sub>H<sub>34</sub> by a combination of HRMS and <sup>13</sup>C-NMR methods. The appearance of only 15 signals in the <sup>13</sup>C-NMR spectrum revealed that **3** was a symmetric dimer, and comparison of its

NMR data with those of **1** showed that an aromatic methine carbon was replaced by a quaternary one. Compound **3** must, therefore, be a dimeric sesquiterpenoid formed by connection of two guaiazulenyl moieties at the aromatic ring. A combination of <sup>1</sup>H-COSY and HMQC experiments readily assigned the positions of the connection to C-2 and C-2' of guaiazulene, and this was confirmed by the observation of long-range correlations between the H-3, Me-14, and Me-15 protons and the C-1, C-2, and C-3 carbons in an HMBC experiment (see Experimental Section). The connection of the guaiazulenyl moieties at the C-2 and C-2' positions was further supported by NOESY experiments, in which the H-3 (H-3') and Me-14' (Me-14) protons were significantly enhanced by irradiations of each other. Study of a 3D model showed that connection at C-2 and C-2' made these protons close enough to each other to exhibit NOE correlations. Thus, the structure of **3** was unambiguously determined as 2,2'-biguaiiazulenyl, a novel sesquiterpene dimer of the guaiazulene class.

Guaiazulene and related sesquiterpenoids exhibit various bioactivities including antimicrobial, cytotoxic, and immunoregulating activities. In our measurement of antimicrobial activity using the paper disk method (disk diameter, 13 mm), compounds **2** and **3** were active against *Escherichia coli* (inhibition zone: 3 mm for both **2** and **3**) and *Bacillus subtilis* (3 and 8 mm for **2** and **3**, respectively) at a concentration of 25 μg/mL. In addition, **3** inhibited the growth of *Candida albicans* (5 mm) at the same concentration.



### Experimental Section

**General Experimental Procedures.** NMR spectra were recorded in C<sub>6</sub>D<sub>6</sub> solutions on a Varian Unity-500 spectrometer. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were measured

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<sup>®</sup> Abstract published in *Advance ACS Abstracts*, October 1, 1996.

at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me<sub>4</sub>Si. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV-vis spectra were obtained in MeOH using a Milton-Roy spectrophotometer. Mass measurements were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. Mps were measured on a Fisher-Johns apparatus and are reported uncorrected. All solvents used were spectral grade or were distilled from glass prior to use.

**Collection, Extraction, and Isolation.** *C. granulosa*<sup>6</sup> was collected by hand using Scuba at 20–25 m depth in January 1991, along the offshore of Jaeju Island, South Sea, Korea. The collected samples were briefly dried under shade and kept in the freezer until chemically investigated. The animals (2.5 kg) were defrosted, macerated, and repeatedly extracted with CH<sub>2</sub>Cl<sub>2</sub>. The crude extracts (12.2 g) were separated by silica vacuum flash chromatography by using sequential mixtures of *n*-hexane and EtOAc as eluents. Fractions eluted with nonpolar solvents (0–5% EtOAc in hexane) were combined and separated by semi-preparative C<sub>18</sub> reversed-phase HPLC (YMC ODS column, 1 cm × 25 cm, 100% CH<sub>3</sub>CN) to yield compounds **1–3** in the order of **1**, **3**, and **2**.

**Guaiazulene (1):** blue gum (400 mg, 3.3% of the crude extract).

**2,2'-Diguaiazulenylmethane (2):** blue solid (5 mg, 0.04% of the crude extract), mp 187–189 °C; UV-vis (MeOH) λ max (log ε) 629 (3.30), 372 (4.21), 355 (4.21), 309 (4.69), 294 (4.85), 248 (4.65) nm; IR (KBr) ν max 2960, 2920, 1540, 1445, 1385, 1200, 920, 810 cm<sup>-1</sup>; <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>) δ 8.13 (2H, d, *J* = 2.0 Hz, H-6, H-6'), 7.34 (2H, s, H-3, H-3'), 7.10 (2H, dd, *J* = 10.7, 2.0 Hz, H-8, H-8'), 6.66 (2H, d, *J* = 10.7 Hz, H-9, H-9'), 5.25 (2H, s, H-16), 2.82 (6H, s, Me-14, Me-14'), 2.79 (2H, m, H-11, H-11'), 2.46 (6H, s, Me-15, Me-15'), 1.22 (12H, d, *J* = 6.8 Hz, Me-12, Me-12', Me-13, Me-13'): <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>) δ 145.55 (C, C-10, C-10'), 141.38 (CH, C-3, C-3'), 138.57 (C, C-7, C-7'), 138.46 (C, C-4, C-4'), 134.69 (CH, C-8, C-8'), 133.52 (CH, C-6, C-6'), 133.39 (C, C-1, C-1'), 129.31 (C, C-2, C-2'), 126.40 (CH, C-9, C-9'), 124.46 (C, C-5, C-5'), 37.69 (CH, C-11, C-11'), 35.01 (CH<sub>2</sub>, C-16), 26.98 (CH<sub>3</sub>, C-14, C-14'), 24.69 (CH<sub>3</sub>, C-12, C-12', C-13, C-13'), 13.00 (CH<sub>3</sub>, C-15, C-15'); HREIMS [M]<sup>+</sup> *m/z* obsd 408.2825, C<sub>31</sub>H<sub>36</sub> requires 408.2817; LREIMS *m/z* 408 (39, rel int), 393 (13), 391 (13), 210 (38), 198 (100), 195 (20), 183 (60), 165 (16).

**2,2'-Biguaiazulenyl (3):** bluish green solid (15 mg, 0.12% of crude extract), mp 88–90 °C; UV-vis (MeOH) λ max (log ε) 626 (3.40), 374 (4.29), 354 (4.21), 309 (4.64), 283 (4.80), 250 (4.66) nm; IR (KBr) ν max 2960, 2925, 1540, 1460, 1365, 1200, 1160, 1050, 940, 920, 860, 810

cm<sup>-1</sup>; <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>) δ 8.23 (2H, d, *J* = 2.0 Hz, H-6, H-6'), 7.62 (2H, s, H-3, H-3'), 7.13 (2H, dd, *J* = 10.7, 2.0 Hz, H-8, H-8'), 6.64 (2H, d, *J* = 10.7 Hz, H-9, H-9'), 2.81 (2H, m, H-11, H-11'), 2.63 (6 H, s, Me-15, Me-15'), 2.28 (6 H, s, Me-14, Me-14'), 1.23 (12 H, d, *J* = 6.8 Hz, Me-12, Me-12', Me-13, Me-13'): <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>) δ 146.75 (C, C-10, C-10'), 141.75 (CH, C-3, C-3'), 139.08 (C, C-7, C-7'), 138.85 (C, C-4, C-4'), 134.80 (CH, C-8, C-8'), 134.11 (C, C-1, C-1'), 133.92 (CH, C-6, C-6'), 128.35 (C, C-2, C-2'), 126.54 (CH, C-9, C-9'), 123.80 (C, C-5, C-5'), 38.11 (CH, C-11, C-11'), 26.67 (CH<sub>3</sub>, C-14, C-14'), 24.73 (CH<sub>3</sub>, C-12, C-12'), 24.72 (CH<sub>3</sub>, C-13, C-13'), 13.05 (CH<sub>3</sub>, C-15, C-15'); HMBC correlations (optimized for 5 and 7 Hz of <sup>1</sup>H–<sup>13</sup>C couplings) H-3 – C-1, C-2, C-5: H-6 – C-1, C-4: H-8 – C-11: H-9 – C-1, C-14: H-12 (H-13) – C-7, C-11, C-13 (C-12): H-14 – C-1, C-9, C-10: H-15 – C-3, C-4, C-5; HREIMS [M]<sup>+</sup> *m/z* obsd 394.2638, C<sub>30</sub>H<sub>34</sub> requires 394.2660; LREIMS *m/z* 394 (100, rel int), 337 (29), 321 (9), 266 (7), 119 (12), 80 (18).

**Synthesis of 2.** Compound **2** was synthesized from guaiazulene via 2-methoxycarbonyl-guaiazulene followed by reductive coupling.<sup>10</sup> From 0.49 g (2.46 mmol) of guaiazulene, 0.13 g (0.32 mmol) of **2** was obtained; overall yield 13.0%.

**Acknowledgment.** The authors thank Mr. Hosung Jung, Polar Research Division, KORDI, for assistance of collecting gorgonian samples. We appreciate taxonomic assignments provided by Professor Jun-Im Song, Department of Biology, Ewha Womans University. MS data were kindly provided by Dr. Richard Kondrat, Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. This research was financially supported by Korean Ministry of Science and Technology Grant BSPN-00255 and BSPN-00283.

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NP960485Y