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Gorgiabisazulene and Gorgiagallylazulene, Two New Guaiazulenoid Pigments from a Gorgonian *Acalycigorgia* sp.

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Two new condensed guaiazulenoid pigments, gorgiabisazulene and gorgiagallylazulene, have been isolated from a gorgonian *Acalycigorgia* sp. and their structures have been established on the basis of spectroscopic and chemical evidence.

Guaiazulenes and related sesquiterpenes are widely distributed in gorgonian corals and some of them possess interesting biological activities such as antimicrobial, antitumor, and immunostimulatory activities as well as inhibitory activity against cell division of fertilized sea urchin eggs.[1-8] During the course of our investigation on the biologically active constituents of a gorgonian *Acalycigorgia* sp.,[9] we encountered two novel condensed guaiazulenoid pigments, designated gorgiabisazulene (**1**) and gorgiagallylazulene (**2**), together with the previously known pigments, guaiazulene (**3**),[2,3] linderazulene (**4**),[1,3] and 2,3-dihydrolinderazulene (**5**).[7] The present paper concerns with the structural elucidation of these new compounds. Gorgiabisazulene, guaiazulene, linderazulene, and 2,3-dihydrolinderazulene inhibited the cell division of fertilized ascidian (*Styela partita*) eggs with IC_{50} values of 8.0, 10, 6.5, and 3.0 μg/ml,[10] respectively, and gorgiagallylazulene and 2,3-dihydrolinderazulene displayed toxicity in the brine shrimp lethality bioassay (LC_{50}=30 and 7.5 μg/ml, respectively).[11]

Specimens of *Acalycigorgia* sp. (2.4 kg) were collected using SCUBA at Sukumo Bay in June 1990. The frozen materials were homogenized in methanol and left at room temperature for a few hours. After filtration, the methanol solution was evaporated to an aqueous suspension and extracted with dichloromethane. Fractionation of the dichloromethane extract (23 g) by sequential application of Sephadex LH-20 ($MeOH/CH_2Cl_2$), silica gel (hexane/AcOEt), and reverse phase liquid ($MeOH/H_2O$) chromatographies gave guaiazulene (662 mg), linderazulene (78 mg), 2,3-dihydrolinderazulene (478 mg), gorgiabisazulene (7 mg), and gorgiagallylazulene (74 mg).

The purple pigment, gorgiabisazulene (**1**), was isolated as an optically active amorphous solid, $[\alpha]_D^{20}$ -92° (*c* 0.05, $CHCl_3$); IR (CCl_4) 1605, 1555, 1455, 1335, 1215, 1115, 1080, 1025, 970, and 915 cm^{-1}; UV (EtOH) 572.8, 373.2, 357.6sh, 326.8, 310.4, 300.8sh, and 235.6 nm (ε 975, 5140, 4360, 20500, 28200, 25100, and 7450). The molecular formula, $C_{31}H_{32}O_2$, was established by high resolution EI mass spectrometry (*m/z* 436.2409, M^+, Δ +0.6 mmu). The close similarity between **1** and 2,3-dihydrolinderazulene (**5**) was revealed by the comparison of their spectral data. The ^{13}C NMR spectrum[12] (100 MHz, $CDCl_3$) of **1** assisted with INEPT experiments displayed fifteen signals compatible with the carbon frame work of **5**; including three methyl

groups, one oxygenated methylene group, one methine group, three one-protonated sp^2 carbon atoms and seven fully substituted sp^2 carbon atoms. The ^1H NMR spectrum[12] (400 MHz, $CDCl_3$) of **1** also showed signals due to one secondary methyl group at δ 1.42 (d, *J*=6.7 Hz), two methyl groups on aromatic ring(s) at δ 2.48 and 2.88, one methine group at δ 3.67 (ddq, *J*=8.8, 7.0, and 6.7 Hz), one oxygenated methylene group at δ 4.12 (dd, *J*=8.8 and 7.0 Hz) and 4.68 (dd, *J*=8.8 and 8.8 Hz), and three isolated aromatic protons at δ 6.51, 6.85, and 7.90, which correspond well with those of **5**. The difference between **1** and **5** resided merely in the presence of three aromatic protons in **1**, one less than those of **5**, and of a newly introduced methylene group [δ_H 5.11 (s); δ_C 35.52] with a half intensity in **1**. A combination of the ^1H-^1H and ^1H-^{13}C 2D-COSY spectra together with COLOC experiments established the 7-(2,3-dihydrolinderazulenyl)methyl system (**1a**), leading to a gross structure (**1**) for gorgiabisazulene. The location of the methylene substituent at C_7 position was revealed by the ^1H-^{13}C long-range correlation of H_{13} to C_6 and C_7 and an NOE between H_{13} and H_{12}. Finally, the structure was confirmed by the conversion of **5** into **1** according to the procedure of Matsubara *et al.*[13] Thus, the treatment of **5** with a solution of 0.15 wt% paraformaldehyde in glacial acetic acid afforded the condensed product, 7,7-methylenebis(2,3-dihydrolinderazulene), in 92% yield, which was found to be identical with gorgiabisazulene (**1**) in all respects.

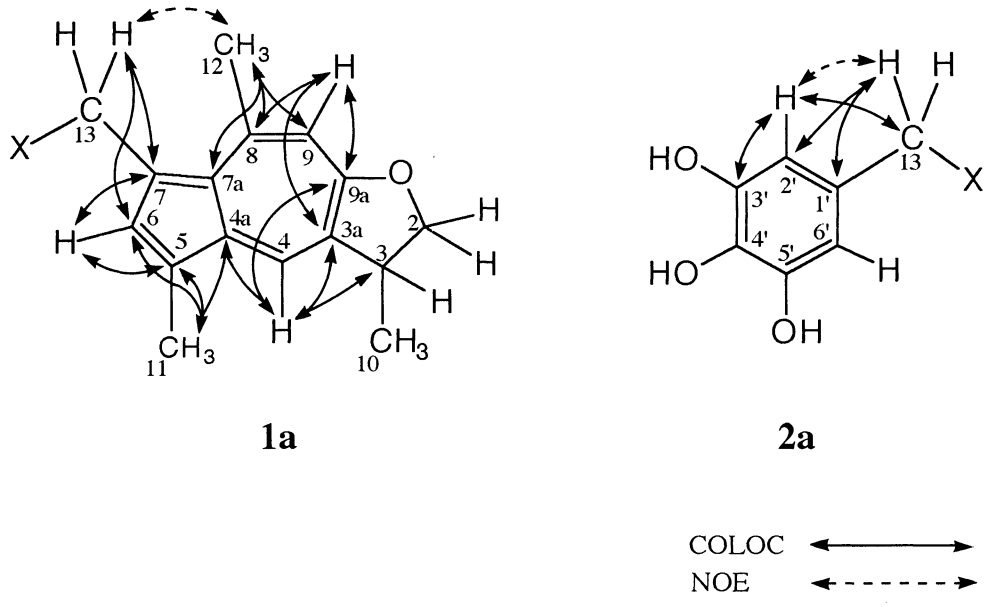
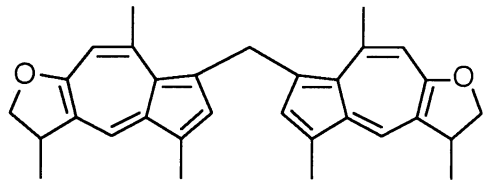


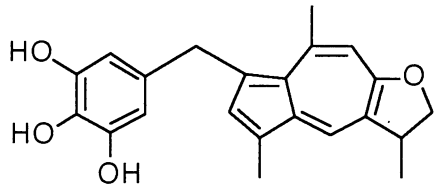
Fig. 1. Partial structures obtained from COLOC and NOESY experiments.

The second pigment, gorgiagallylzulene (**2**), was also obtained as an optically active purple amorphous solid, $C_{22}H_{22}O_4$, $[\alpha]_D^{21}$ -30° (*c* 0.11, $CHCl_3$), IR ($CHCl_3$) 3550, 3350, 1605, 1552, 1530, 1515, 1365, 1330, 1180, 1025, and 975 cm^{-1}; UV (EtOH) 564.0, 370.8, 353.2, 322.0sh, 306.8, and 206.8 nm (ε 260, 5480, 3970, 20300, 46400, and 37500), from a more polar fraction of the extract. The IR spectrum (3550 and 3350 cm^{-1}) and a positive $FeCl_3$-$K_3Fe(CN)_6$ test suggested the presence of phenolic hydroxyl group(s). The ^1H and ^{13}C NMR spectra[14] ($CDCl_3$) of **2** assisted with the ^1H-^1H COSY and COLOC experiments revealed the presence

of the 7-(2,3-dihydrolinderazulenyl)methyl moiety as found in **1** and another structural unit, gallyl group (**2a**). These findings led to the straightforward assignment of the gross structure (**2**) for gorgiagallylazulene.



1



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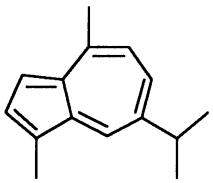

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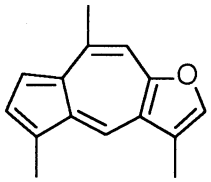

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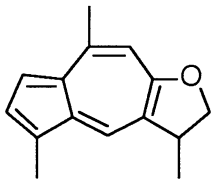
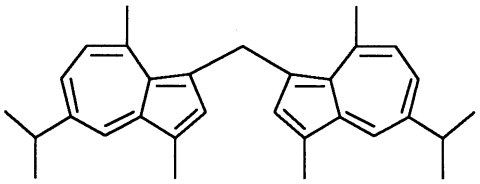


5



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Now, it is a question whether gorgiabisazulene (**1**) and gorgiagallylazulene (**2**) are the artifacts formed in isolation processes or not. Scheuer and his co-workers have previously reported the isolation of bis-guaiazulenyl compound (**6**)[3] along with 3-haloguaiazulenes[4] from a deep sea gorgonian *Pseudothesia* sp. and have pointed out the possibility that the former might be an artifact derived from the latter. In the present work, 3-haloguaiazulenes and related compounds could not be isolated from *Acalycigorgia* sp. On the other hand, **1** and **2** could be detected by HPLC analysis (ODS column, MeOH/H_2O) in the supernatant solution soon after soaking the specimens in ethanol. These facts suggest that gorgiabisazulene and gorgiagallylazulene are possibly the genuine natural products.

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12) **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (6H, d, *J*=6.7 Hz, 10- and 10′-H_3), 2.48 (6H, s, 11- and 11′-H_3), 2.88 (6H, s, 12- and 12′-H_3), 3.67 (2H, ddq, *J*=8.8, 7.0, and 6.7 Hz, 3- and 3′-H), 4.12 (2H, dd, *J*=8.8 and 7.0 Hz, 2- and 2′-Ha), 4.68 (2H, dd, *J*=8.8 and 8.8 Hz, 2- and 2′-Hb), 5.11 (2H, s, 13-H_2), 6.51 (2H, s, 9- and 9′-H), 6.85 (2H, s, 6- and 6′-H), and 7.90 (2H, s, 4- and 4′-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.86 (C_{11} and $C_{11'}$), 20.31 (C_{10} and $C_{10'}$), 27.55 (C_{12} and $C_{12'}$), 35.32 (C_{13}), 39.16 (C_3 and $C_{3'}$), 77.44 (C_2 and $C_{2'}$), 109.88 (C_9 and $C_{9'}$), 121.87 (C_{3a} and $C_{3a'}$), 125.37 (C_{4a} and $C_{4a'}$), 128.61 (C_4 and $C_{4'}$), 129.12 (C_8 and $C_{8'}$), 131.41 (C_7 and $C_{7'}$), 133.09 (C_5 and $C_{5'}$), 136.40 (C_6 and $C_{6'}$), 147.47 (C_{7a} and $C_{7a'}$), and 165.19 (C_{9a} and $C_{9a'}$); LREIMS *m/z* 436 (M^+), 421, 391, 224, 212 (base peak), 197, and 182.
13) Y. Matsubara, M. Morita, S. Takekuma, Z. Zhao, H. Yamamoto, and T. Nozoe, *Bull. Chem. Soc. Jpn.*, **64,** 2865 (1991).
14) **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (3H, d, *J*=6.7 Hz, 10-H_3), 2.54 (3H, s, 11-H_3), 2.74 (3H, s, 12-H_3), 3.66 (1H, ddq, *J*=8.8, 7.0, and 6.7 Hz, 3-H), 4.10 (1H, dd, *J*=8.8 and 7.0 Hz, 2-Ha), 4.36 (2H, s, 13-H_2), 4.65 (1H, dd, *J*=8.8 and 8.8 Hz, 2-Hb), 6.12 (2H, s, 2′- and 6'-H), 6.49 (1H, s, 9-H), 7.08 (1H, s, 6-H), and 7.90 (1H, s, 4-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.81 (C_{11}), 20.34 (C_{10}), 27.16 (C_{12}), 36.95 (C_{13}), 39.05 (C_3), 77.44 (C_2), 108.07 ($C_{2'}$ and $C_{6'}$), 110.31 (C_9), 122.26 (C_{3a}), 125.21 (C_{4a}), 127.89 ($C_{1'}$), 128.65 (C_4), 129.40 ($C_{7'}$), 129.54 (C_8), 133.12 (C_5), 135.97 ($C_{4'}$), 136.94 (C_6), 144.01 ($C_{3'}$ and $C_{5'}$), 147.58 (C_{7a}), and 165.26 (C_{9a}); LREIMS *m/z* 350 (M^+), 348, 212 (base peak), 197, 182, and 153; HREIMS *m/z* 350.1534 (M^+, $C_{22}H_{22}O_4$, Δ +1.5 mmu).

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