Two New Cytotoxic Linderazulenes from a Deep-Sea Gorgonian of the Genus *Paramuricea*

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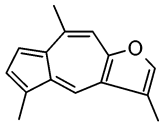
The known compound linderazulene (**1**) and two new linderazulenes (**2**, **3**) were isolated from a deep-sea gorgonian *Paramuricea* sp. The structures of **2** and **3** were determined through spectroscopic methods. Compounds **1**−**3** show moderate in vitro cytotoxicity against the P388 murine leukemia cell line with IC_{50}'s of 18.8, 2.7, and 15.6 μg/mL, respectively. Compound **2** showed moderate activity against the PANC-1 pancreatic cell line with an IC_{50} of 18.7 μg/mL.

Gorgonians have proven to be a rich source of guaiazulene-related pigments.[1] These compounds show a variety of activities including antifungal, antitumor, antibacterial, immunoregulatory, and antiproliferative effects on fertilized sea urchin and ascidian eggs.[2−7] As part of our continued research on biologically active secondary metabolites from marine organisms,[8] a deep-sea gorgonian of the genus *Paramuricea* (family Paramuriceidae) collected at Westpunt, Curaçao, was investigated. The EtOAc partition of the gorgonian was fractionated by regular-phase vacuum column chromatography followed by reverse-phase HPLC to yield three compounds (**1**, **2**, and **3**). Compound **1** was identified as the known compound linderazulene by comparison of its spectroscopic data to the published data. Linderazulene was first reported from the gorgonians *Acalycigorgia* sp.[4] and *Paramuricea chamaeleon*.[9] Detailed interpretation of the spectroscopic data observed for **2** and **3** identified them as new members of the linderazulene class of compounds. This paper describes the isolation, structure elucidation, and cytotoxic activity of compounds **2** and **3**.



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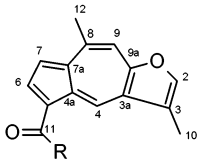

2 R = OMe

3 R = H

Compound **2** was obtained as a pink amorphous solid (mp 138−139 °C). HRFABMS suggested a formula of $C_{16}H_{14}O_3$ for **2** (*m/z* 255.1033 $[M + H]^+$ observed, 255.1021 calculated) requiring 10 degrees of unsaturation. This formula was further supported by the ^{13}C, DEPT, and HMQC spectra (see Table 1), which indicated the presence of 16 carbon resonances including seven quaternary olefinic carbons (δ 159.0, 141.1, 139.3, 136.3, 126.3, 120.3, 116.4), five olefinic methine carbons (δ 141.1, 137.0, 130.7, 116.1, 115.7) two methyl carbons (δ 25.2, 8.0), one methoxyl carbon (δ 50.9), and one ester carbon (δ 166.3). The ^1H NMR spectrum showed resonances corresponding to two aromatic methyls (δ 2.45 (s, 3H) and 2.93 (s, 3H)); one ester methyl (δ 3.94 (s, 3H)); two mutually coupled olefinic resonances (δ 8.24 (d, $J = 3.8$ Hz, 1H) and 7.28 (d, $J = 3.8$ Hz, 1H)); and three one-proton aromatic singlets (δ 9.99, 7.69, and 7.56). The IR spectrum of compound **2** showed bands corresponding to an ester carbonyl functionality (1678 cm^{-1}). The foregoing spectral data suggested that compound **2** is a new linderazulene metabolite in which the C-11 methyl has been oxidized to an ester. Analysis of the $^1H-^1H$ COSY and proton-detected HMQC and HMBC spectra allowed for full assignment of the NMR data (Table 1). An alternative structure in which the C-11 ester functionality is attached at C-7 could be ruled out on the basis of correlations observed in the NOESY spectrum between H-7 and H_3-12 as well as by chemical shift arguments. We propose the common name 11-carbomethoxylinderazulene for **2**.

Compound **3** was also obtained as an amorphous pink solid (mp 136 °C). HRFABMS suggested a formula of $C_{15}H_{12}O_2$ for **3** (*m/z* 225.0917 $[M + H]^+$ observed, 225.0915 calculated) requiring 10 degrees of unsaturation. The NMR spectra of **3** (Table 1) were nearly identical to those observed for **2** with the primary difference being the observation of resonances attributable to an aldehyde functionality [^{13}C NMR: δ 187.1 (CH); ^1H NMR: δ 10.3 (s, 1H)] rather than the ester functionality observed for **2**. The IR spectrum of compound **3** confirmed the presence of an aromatic aldehyde functionality (ν 2746, 1641 cm^{-1}). A detailed analysis of the 2D spectra (COSY, NOESY, HMQC, and HMBC) confirmed the assignment of **3** as 11-formyllinderazulene.

Compounds **1**−**3** show moderate in vitro cytotoxicity against the P388 murine leukemia cell line with IC_{50}'s of 18.8, 2.7, and 15.6 μg/mL, respectively. Compound **2** also showed moderate activity against the PANC-1 pancreatic tumor cell line with an IC_{50} of 18.7 μg/mL. When tested for their ability to inhibit the growth of the fungal pathogen *Candida albicans,* no activity was detected at a concentration of 50 μg/mL under the conditions tested.[8]

Experimental Section

General Experimental Procedures. Melting points were recorded on a Gallenhamp melting point apparatus; IR spectra were collected on a MIDAC M-1200 with Galactic GRAMS/

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Table 1. NMR Spectral Data for Compounds **2** and **3** in $CDCl_3$

	compound **2**				compound **3**		
atom no.	δ_C, DEPT	δ_H, mult. (*J* in Hz)	HMBC[a]	NOESY	δ_C, DEPT	δ_H, mult. (*J* in Hz)	HMBC[a]
2	141.1 CH	7.56 s	C-3, C-3a, C-9a	H-10	141.8 CH	7.56 s	C-3, C-3a, C-9a
3	120.3 C				120.4 C		
3a	126.3 C				126.6 C		
4	130.7 CH	9.99 s	C-3a, C-4a, C-7a, C-9a	H-10	131.5 CH	9.99 s	C-3a, C-4a, C-7a, C-9a
4a	116.4 C				117.4 C		
5	136.3 C				135.1 C		
6	137.0 CH	8.24 d (3.8)	C-4a, C-5, C-7a	H-7	141.3 CH	8.09 d (3.8)	C-4a, C-5, C-7a
7	116.1 CH	7.28 d (3.8)	C-4a, C-5, C-6, C-7a	H-6 H-12	117.5 CH	7.32 d (3.8)	C-4a, C-5, C-6, C-7a
7a	139.3 C				141.7 C		
8	141.1 C				141.5 C		
9	115.7 CH	7.69 s	C-3a, C-7a, C-8, C-9a, C-12	H-12	117.4 CH	7.80 s	C-3a, C-7a, C-8, C-9a, C-12
9a	159.0 C				159.4 C		
10	8.0 CH_3	2.45 s 3H	C-2, C-3, C-3a	H-4, H-2	8.0 CH_3	2.46 s 3H	C-2, C-3, C-3a
11	166.3 C				187.1 CH	10.29 s	
12	25.2 CH_3	2.93 s 3H	C-7a, C-8, C-9	H-7, H-9	25.2 CH_3	2.94 s 3H	C-7a, C-8, C-9
$-OCH_3$	50.9 CH_3	3.94 s 3H	C-11				

[a] HMBC correlation from H number to carbon atoms listed.

386 software. The ^1H, COSY, NOESY, ^{13}C, DEPT 135, DEPT 90, HMQC, and HMBC (optimized for 10 Hz) spectra were recorded on a Bruker AMX-500 operating at 500 MHz (^1H) and 125 MHz (^{13}C). ^1H chemical shifts are referenced to $CDCl_3$ observed at 7.24 ppm, while ^{13}C chemical shifts are referenced to $CDCl_3$ observed at 77.0 ppm. The HRFABMS and EIMS were obtained on a Kratos MS50TC mass spectrometer at Oregon State University.

Animal Material. The gorgonian specimen (HBOI ID 19-V-00-3-003) was collected May 19, 2000, using Harbor Branch Oceanographic Institution's *Johnson-Sea-Link* research submersible off the northwest coast of Curaçao, 12°22.78′ N latitude and 69°10.15′ W longitude, on a rocky slope and at a depth of 342 m. The specimen was frozen immediately after collection and kept frozen until workup. This specimen most closely fits the genus *Paramuricea* Kölliker, 1865 [Cnidaria, Anthozoa, Gorgonacea, Plexauridae (Paramuriceidae)], but cannot be ascribed to a known species at this time. It differs from other specimens that were collected in the same region, in both morphology and spiculation. This holaxonian gorgonian specimen was 40 cm tall, 30 cm wide, and branching mostly in one plane. The base of the main stem is 6 mm diameter, which has several secondary branches and numerous short (1−2 cm) terminal branchlets, 0.5−0.75 mm diameter, which do not anastomose. The polyps are distributed over the entire surface, tend to alternate about 1−2 mm apart, and are denser on the branchlets. The calyces (anthostele) are tubular to conical, ~0.5 mm high, and consist of relatively few thorn scale spicules with very blunt, slightly spinous, projecting spine and sparse basal root. The large anthocodia are exert (1−2 mm), with long tentacles and pinnules. The anthocodial collaret is relatively strong with a crown of 3 or 4 rows of thin, curved, slightly spinous spindles and some spinose rods. The coenenchyme consists of a thin layer of spicules, dominated by knee-shaped rods that are warty on the convex side and smooth on the concave side, and a few crosses. The axis is soft and fibrous with a cross-chambered central core. The coenenchymal spicules range from ~0.07 to 0.20 mm in length; the thorn scales are ~0.25 mm; the anthocodial spindles are ~0.15 to 0.45 mm; and the anthocodial rods are ~0.23 to 0.30 mm. The color of the specimen was described in situ from the submersible as pale lavender. While still fresh in the lab, the main stalk and branches were pale lavender, and the branchlets were tan. In alcohol the specimen is dark brown. A museum voucher specimen is deposited at the Harbor Branch Oceanographic Museum, catalog number 012:00804.

Extraction and Isolation. The frozen specimen of the gorgonian (100 g wet wt) was chopped into small slices and extracted exhaustively with EtOH (3 × 400 mL). The combined EtOH extracts were concentrated by distillation under reduced pressure. The resulting residue was partitioned between water and EtOAc (3 × 100 mL). The combined EtOAc partition was concentrated to yield 1.2 g of a reddish brown solid. The EtOAc partition was fractionated by vacuum column chromatography on a Kieselgel 60H stationary phase using a step gradient of EtOAc in heptane. The fractions that eluted with heptane and heptane−EtOAc (95:5) (v/v) were combined and chromatographed by reversed-phase HPLC [(Vydac C-18 Protein and Peptide column, 10 × 250 mm, H_2O-CH_3CN, 2:8, flow rate = 3 mL/min, detected by UV absorbance at 254 nm] to yield compounds **1** (1.4 mg, 0.0014%), **2** (21 mg, 0.021% of wet weight), and **3** (1.2 mg, 0.0012%).

11-Carbomethoxylinderazulene (2): amorphous pink solid, mp 138−139 °C; UV (MeOH) λ_{max} (log ϵ) 389 (3.92), 376 (3.80), 325 (4.07), 308 (4.3), 247 (4.00), 228 (4.05) nm; IR (neat) ν_{max} 2946, 2918, 2853, 1678, 1447, 1408, 1387, 1305, 1217, 1188, 1133, 1079, 1056, 939 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 1; HRFABMS *m/z* 255.1033 (calcd for $C_{16}H_{15}O_3$ $[M + H]^+$, 255.1021).

11-Formyllinderazulene (3): amorphous pink solid, mp 136 °C; UV (MeOH) λ_{max} (ϵ) 395 (3.86), 332 (3.97), 316 (4.12), 293 (4.03), 260 (4.15), 215 (4.08) nm; IR (neat) ν_{max} 2956, 2923, 2853, 2746, 1641, 1634, 1398, 1374, 1297, 1286, 1233, 1148, 1081, 1044, 943 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 1; HRFABMS *m/z* 225.0917 (calcd for $C_{15}H_{13}O_2$ $[M + H]^+$, 225.0915).

Biological Assays. P388 murine leukemia cells and PANC-1 pancreatic cancer cells were obtained from the American Type Culture Collection (Rockville, MD). Cytotoxicity assays were run as previously described according to the methods of Alley et al.[10] Antifungal assays were run as previously described.[8]

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