A New Norcembranoid Dimer from the Red Sea Soft Coral *Sinularia gardineri*

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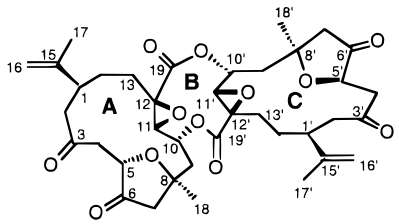
A study of *Sinularia gardineri* (Pratt) (Alcyoniidae), collected in the Red Sea, revealed a new heptacyclic norcembranoid dimer singardin (**1**). The structure of singardin was deduced by spectroscopic analysis. A known sesquiterpene, guaianediol (**2**), and the known cembranolides (1*R*,5*S*,8*R*,10*S*,11*R*)-11-hydroxy-1-isoprenyl-8-methyl-3,6-dioxo-5,8-epoxycyclotetradec-12-ene 10,12-carbolactone (5-*epi*-sinuleptolide) and sinuleptolide were also isolated. Compounds **1** and **2** show cytotoxicity to murine leukemia (P-388), human lung carcinoma (A-549), human colon carcinoma (HT-29), and human melanoma cells (MEL-28).

The marine environment, comprising approximately half of the total global biodiversity, offers an enormous source of novel and biologically active compounds. Soft corals are Coelenterates (class Anthozoa, subclass Octocorallia, order Alcyonacea, family Alcyoniidae) and are symbiotic associations of coral animals (polyps) with their algal partners (Zooxanthellae).[1] They are rich sources of terpenoids, notably cembranoid diterpenes. Their abundant production and accumulation of diterpenoids is intriguing, as it seems unlikely that these compounds act solely as repellents against predators.[2,3] Instead, the diterpenoids may play an as yet unknown physiological role in these benthic animals. These animals are characterized by a low concentration of such common lipids as glycerides or fatty acid esters, which are of vital importance to higher animals, where they function in connective tissues and as energy sources.[2,3] The genus *Sinularia* is reputed for its versatile chemical constituents and their biological activity. Terpenoids, including sesquiterpenes, cembrane, norcembrane, flexibilene, cladiellane, and lobane diterpenoids,[4−7] and steroids,[8−10] compose the main secondary metabolites isolated from the genus *Sinularia*. Many biological activities were reported for these metabolites, including cytotoxicity,[11] enhancement of glucose transport in rat adipocytes,[12] and histamine-release inhibition.[13]

Four reports have been published on norcembranoids from the genus *Sinularia*. (1*R*,5*S*,8*R*,10*S*,11*R*)-11-Hydroxy-1-isoprenyl-8-methyl-3,6-dioxo-5,8-epoxycyclotetradec-12-ene 10,12-carbolactone (5-*epi*-sinuleptolide) was the first norcembranoid lacking a methyl group at C-4,[1] and additional related compounds were reported later.[14] The structures of two of these were confirmed by single-crystal X-ray analysis. Another cytotoxic norcembrane was subsequently reported from *Sinularia polydactyla*.[15] Recently, the C-4 norcembranolide sinuleptolide was isolated from an unidentified Okinawan *Sinularia* species.[16] The present study illustrates the first report of a norcembranoid dimer from marine organisms. There are several reports of dimeric cembranoids from *Sarcophyton tortuosum*[17−19] and *Sarcophyton glaucum*.[20,21]

The CH_2Cl_2 extract of freshly collected *Sinularia gardineri* (Pratt) (family Alcyoniidae) was chromatographed on Si gel with a hexane−Me_2CO gradient.[22] The least polar fraction afforded the norcembranoid dimer singardin (**1**) after RP C18 chromatography, eluting with MeOH followed by $CHCl_3$ and crystallization from EtOH. The intermediate polar fraction was subjected to preparative TLC on polyamide, using MeOH−H_2O (4:6) to afford the guaianediol (**2**). The more polar fraction afforded 5-*epi*-sinuleptolide and sinuleptolide after fractional crystallization from ether and repeated HPLC on RP C18 using MeOH−H_2O (1:1).



Singardin (**1**) Guaianediol (**2**)

Singardin (**1**), was obtained as colorless fine needles, mp 185−186 °C; LRFABMS *m/z* (fragment, %), 697 ($M^+ + 1$, 32) (calcd for $C_{38}H_{48}O_{12}$; 697.3); HRCIMS *m/z* (fragment, %), 349.1624, ($M^+ + 1$, 80.9) (calcd for the monomer $C_{19}H_{24}O_6$: 349.1651). The MS data suggested that **1** is a dimer. The IR spectrum revealed the lack of hydroxy groups, which was further confirmed by the absence of D_2O-exchangeable signals in the ^{1}H-NMR spectrum. Strong bands in the IR spectrum suggested lactone (1755 cm^{-1}) and ketone (1705−1710 cm^{-1}) functions. Analyses of 1D and 2D NMR spectra, including COSY, NOESY, HMQC, and HMBC, led to the conclusion that **1** was a dimer of 5-*epi*-sinuleptolide[1] and sinuleptolide,[16] but with an epoxide replacing the Δ^{12} olefin. Assignments of rings A and C were consistent with those previously reported in the literature. Two quaternary ^{13}C-NMR signals at δ 62.0 and 60.6 and two oxymethine carbons at δ 63.1 and 62.6, which correlated with proton singlets at δ 4.12 and 3.95, indicated two epoxy functions at C-11/C-12 and C-11′/C-12′. The long-range couplings of C-12 with H-11, H-10, and H-14, as well as those between C-12′ and H-11′ and H-10′ and H-14′ resonances, supported the structure of ring B. The unique HMBC ^2J off-resonance couplings between H-11/C-10 and H-11′/C-10′ confirmed the integrity of ring B (Figure 1). The ^3J HMBC couplings of C-19 and C-19′ carbonyl groups with H-11, H-13, H-10′ and H-11′,

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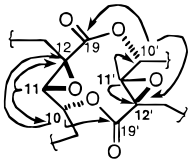

Figure 1. Important $^1H-^{13}C$-HMBC correlations of singardin (**1**).

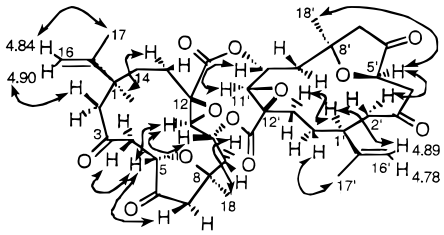

Figure 2. $^1H-^1H$-NOESY correlations of singardin (**1**).

H-13′, H-10 proton signals, respectively, provided further convincing evidence that the ten-membered bislactone ring B connects monomer rings A and C. The ^3J HMBC couplings of the C-8 and C-8′ quaternary carbons with H-10 and H-10′ proton signals further support linkage of ring B to rings A and C and thus suggest the dimeric nature of singardin.

The phase-sensitive NOESY data of singardin (Figure 2) confirmed the existence of two epimers. H-5 showed NOESY correlation with H-11, which suggested *S*-configuration of carbon 5. NOESY corrrelations of H-5 with H-7 and H-4 β-proton resonances further supported relative *cis* spatial arrangment. Similarly, *R* configuration of C-5′ was confirmed through NOESY correlations of H-5′ with α-Me18′ and H-2′ proton signals. The stereochemistry of C-5 and C-5′ induced large chemical shift differences in the ^{1}H-NMR and the ^{13}C-NMR signals of the neighboring carbons. The *R* configuration of C-5′ induced upfield shifts of carbons C-5′ (−2.2 ppm), C-3′ (−0.8 ppm), and C-18′ (−2.9 ppm) and a downfield shift of carbon C-6′ (+1.8 ppm) as compared to the *S* configuration of carbon C-5, which induced downfield shifts of carbons C-5, C-3, and C-18 and an upfield shift of carbon C-6. These shift values were comparable to those reported by Bowden *et al.*,[1] who confirmed the stereochemistry of the related monomer by X-ray crystallography, and with those reported for the other epimer by Shoji *et al.*[16] Attempts to grow appropriate singardin crystals for X-ray crystallography were unsuccessful.

The guaianediol (**2**) was isolated as an amorphous powder that decomposes at 143 °C. The physical and spectral data of **2** were consistent with the known but previously unnamed racemic sesquiterpene guaianediol isolated from the soft coral *Lemnalia africana*[23] and the gorgonian *Pacifigorgia eximia*.[4] Its structure was determined by X-ray analysis but apparently has not been published, because the crystal has two molecules per unit cell and only one of these molecules was clearly observed, while the second was extensively disordered.[4]

The final compounds isolated were the known cembranolides (1*R*,5*S*,8*R*,10*S*,11*R*)-11-hydroxy-1-isoprenyl-8-methyl-3,6-dioxo-5,8-epoxycyclotetradec-12-ene 10,12-carbolactone (5-*epi*-sinuleptolide) and sinuleptolide. Identity is based on comparison with HRCIMS, ^{1}H- and ^{13}C-NMR data previously reported.[1,16]

Singardin (**1**) and guaianediol (**2**) showed cytotoxic activity against the following: murine leukemia (P-388) cells, (**1**) 1 μg/mL, (**2**) 1 μg/mL; human lung carcinoma (A-549) cells, (**1**) 2.5 μg/mL, (**2**) 2.5 μg/mL; human colon carcinoma (HT-29) cells, (**1**) 5 μg/mL, (**2**) 5 μg/mL; and human melanoma cells (MEL-28): (**1**) 5 μg/mL, (**2**) 5 μg/mL. Singardin (**1**) also showed weak antifungal activity against *Candida albicans* B311 and *Cryptococcus neoformans*, giving 3-mm inhibition zones (50 μL, 1 mg/mL) as compared to amphotericin B, which gave 12- and 10-mm inhibition zones, respectively.

Experimental Section

Collection and Extraction. *S. gardineri* (Pratt) (class Anthozoa, subclass Octocorallia, order Alcyonacea, family Alcyoniidae) was collected in December 1994 in the Red Sea in shallow water (−10 m) near Hurghada, Egypt.[22] The sample was identified by Dr. Ahmed M. Helal, Department of Zoology, Faculty of Science, Al-Azaher University, Cairo, Egypt. A voucher specimen (94RS-9), is deposited at the Department of Pharmacognosy, University of Mississippi. The frozen sample (4.5 kg) was cut into small pieces and percolated twice with 4 L 95% EtOH for 12 h. The combined EtOH extract was evaporated under reduced pressure, yielding 22.0 g of dry extract. The dry extract was dissolved in 2 L CH_2Cl_2 and filtered. The residue (4.5 g) was flash chromatographed over 200 g of 40-μm Si gel with a hexane-Me_2CO gradient followed by an Me_2CO−MeOH gradient. The fraction eluted with Me_2CO−hexane (8:92, 490 mg) was dissolved in 2 mL of MeOH and passed over 4 g of RP C18, elution with 10 mL of MeOH and 10 mL of $CHCl_3$. The $CHCl_3$ fraction was evaporated and crystallized from EtOH to afford **1** (19.5 mg). The fraction eluted with Me_2CO−hexane (16:84) was subjected to repeated preparative TLC on polyamide using MeOH−H_2O (4:6) to afford **2** (16 mg). The fraction eluted with Me_2CO−hexane (22:78) was crystallized from Et_2O and subjected to repeated RP C18 HPLC, using MeOH−H_2O (1:1) to afford 5-*epi*-sinuleptolide (3.6 mg) and sinuleptolide (2.5 mg).

Singardin (1): [α]$_D$ −66° (*c* 0.25, $CHCl_3$); mp 185−186 °C λ_{max} (MeOH) 238 nm; LRFABMS *m/z* (fragment) 697 (M^+ +1, 32) (calcd for $C_{38}H_{48}O_{12}$: 697.3); HRCI *m/z* (fragment) 349.1624, (M^+ +1, 80.9) (calcd for the monomer $C_{19}H_{24}O_6$, 349.1651); ^{1}H- and ^{13}C-NMR (Table 1).

Guaianediol (2): amorphous powder; dec at 143 °C; [α]$_D$ −3.5° (*c* = 0.235, $CHCl_3$), LREIMS *m/z* (fragment) 238.0 (M^+, 50) (calcd for $C_{15}H_{26}O_2$, 238.2); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.86 (1H, ddd (11.4, 9.7, 6.0) H-1), 1.64 (1H, m), 1.74 (1H, m, H-2), 1.71 (1H, m), 1.61 (1H, m, H-3), 2.16 (1H, dd (9.2, 1.7) H-5), 5.49 (1H, br dd (1.9, 1.2) H-6), 2.18 (1H, ddd (15.8, 8.8, 1.7)), 1.92 (1H, ddd (16.2, 10.5, 1.4) H-8), 1.81 (1H, ddd (13.1, 10.5, 1.4)), 1.46 (1H, ddd (13.2, 10.3, 1.2) H-9), 2.24 (1H qq (6.9, 6.9) H-11), 0.97 (3H, d (6.9) H-12), 0.96 (3H, d (6.9) H-13), 1.26 (3H, s, H-14), 1.20 (3H, s, H-15); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 50.7 (d (C-1)), 21.5 (t (C-2)), 40.5 (t (C-3)), 80.2 (s (C-4)), 50.3 (d (C-5)), 121.3 (d (C-6)), 149.6 (s (C-7)), 25.1 (t (C-8)), 42.6 (t (C-9)), 75.3 (s (C-10)), 37.3 (d (C-11)), 21.4 (q (C-12)), 21.3 (q (C-13)), 21.1 (q (C-14)), 22.5 (q (C-15)).

(1*R*,5*S*,8*R*,10*S*,11*R*)-11-Hydroxy-1-isoprenyl-8-methyl-3,6-dioxo-5,8-epoxycyclotetradec-12-ene 10,12-carbolactone (5-*epi*-sinuleptolide): HRCIMS *m/z* (fragment) 349.1643, (M^+ +1, 10.2) (calcd for $C_{19}H_{24}O_6$, 349.1651); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.03

Table 1. ^{13}C- and ^{1}H-NMR Data of Singardin (**1**)[a]

C#	δ_C	δ_H	J (Hz)
1	39.8, d	2.77, dddd	7.6, 7.5, 4.9, 3.2
2	49.6, t	2.60, dd	14.2, 3.2
		2.26, dd	15.2, 7.9
3	208.3, s		
4	43.8, t	2.68, dd	14.2, 10.8
		2.59, dd	14.2, 3.5
5	78.0, d	4.44, dd	10.8, 2.2
6	211.8, s		
7	50.9, t	2.43, d	18.2
		2.48, d	18.1
8	79.4, s		
9	40.5, t	2.36, dd	15.8, 4.5
		2.13, dd	15.9, 2.8
10	78.1, d	4.70, dd	4.5, 2.8
11	63.1, d	4.12, s	
12	62.0, s		
13	21.3, t	2.30, ddd	15.7, 6.9, 2.2
		1.86, ddd	16.0, 7.9, 2.2
14	25.6, t	1.22, dddd	14.6, 7.8, 3.4, 2.4
		1.57, dddd	14.8, 7.0, 5.0, 2.8
15	145.3, s		
16	112.9, t	4.90, dq	1.4, 0.6
		4.84, dq	1.4, 0.9
17	18.6, q	1.67, s	
18	28.4, q	1.45, s	
19	172.2, s		
1′	40.7, d	2.61, m	
2′	48.2, t	2.20, dd	17.8, 9.2
		2.51, dd	17.4, 2.6
3′	207.5, s		
4′	44.6, t	2.72, dd	16.2, 5.9
		3.07, dd	16.3, 4.0
5′	75.8, d	4.18, dd	5.9, 4.1
6′	213.6, s		
7′	49.8, t	2.17, d	14.6
		2.61, d	14.2
8′	79.0, s		
9′	42.3, t	2.10, dd	12.4, 6.1
		2.31, dd	12.4, 6.9
10′	75.0, d	4.72, dd	6.7, 6.5
11′	62.6, d	3.95, s	
12′	60.6, s		
13′	21.2, t	2.18, ddd	15.4, 8.1, 2.8
		1.75, ddd	15.4, 7.7, 3.7
14′	26.8, t	1.33, dddd	10.8, 9.3, 7.5, 3.9
		1.66, dddd	10.7, 10.0, 8.8, 3.2
15′	145.8, s		
16′	112.5, t	4.89, dq	1.4, 0.6
		4.78, dq	1.4, 0.6
17′	18.7, q	1.67, s	
18′	25.5, q	1.45, s	
19′	172.3, s		

[a] In $CDCl_3$, 500 MHz for ^{1}H and 125.75 MHz for ^{13}C. Carbon multiplicities: DEPT and APT; s = quaternary, d = methine, t = methylene, q = methyl carbon. In case of aliphatic methylenes, β-protons mentioned first.

(1H m, H-1) 2.48 (1H m), 2.85 (1H dd (16.5, 2.5), H-2), 2.69 (1H m), 2.99 (dd (16.3, 4.3) H-4), 4.28, (1H dd (11.5, 2.9) H-5), 2.50 (1H d (16.0)), 2.56 (1H d (16.1), H-7), 2.14 (1H dd (15.5, 2.3)), 2.51 (1H dd (15.8, 8.2), H-9), 4.63 (1H d (8.7), H-10), 4.81 (1H brs, H-11), 6.46 (1H ddd (11.1, 4.5, 0.8)), H-13), 2.17 (1H ddd (15.1, 3.8, 3.7)), 3.75 1H ddd (15.1, 10.9, 6.3), H-14), 4.60 (1H br s), 4.87 (1H dq (1.2, 0.7), H-16), 1.82 (3H s, H-17), 1.45 (3H s, H-18); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 42.0 (d (C-1)), 48.3 (t (C-2)), 208.0 (s (C-3)), 44.4 (t (C-4)), 77.2 (d (C-5)), 213.6 (s (C-6)), 51.9 (t (C-7)), 79.2 (s (C-8)), 42.3 (t (C-9)), 83.8 (d (C-10)), 75.7 (d (C-11)), 132.1 (s (C-12)), 145.2 (d (C-13)), 29.5 (t (C-14)), 147.9 (s (C-15)), 110.3 (t (C-16)), 20.9 (q (C-17)), 31.8 (q (C-18)), 169.1 (s (C-19)).

Sinuleptolide: HRCIMS *m/z* (fragment) 349.1658, (M^+ +1, 4.1) (calcd for $C_{19}H_{24}O_6$, 349.1651); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.01 (1H m, H-1), 2.37 (1H m), 2.88 (1H dd (16.6, 2.9), H-2), 2.59 (1H m), 3.03 (dd (16.1, 4.7) H-4), 4.40 (1H dd (11.1, 2.9) H-5), 2.46 (1H d (16.5)), 2.59 (1H d (16.4)), H-7, 2.12 (1H dd (16.0, 2.2)), 2.39 (1H dd (15.7, 7.8), H-9), 4.65 (1H d (8.3), H-10), 4.79 (1H d (0.9)), H-11), 6.53 (1H ddd (11.2, 4.8, 0.9), H-13), 2.19 (1H ddd (14.9, 4.1, 4.0)), 3.68 (1H ddd (15.0, 11.0, 6.1), H-14), 4.62 (1H br s), 4.85 (1H dq (1.1, 0.8), H-16), 1.55 (3H s, H-17), 1.49 (3H s, H-18); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 40.0 (d (C-1)), 46.3 (t (C-2)), 205.3 (s (C-3)), 43.3 (t (C-4)), 75.2 (d (C-5)), 215.2 (s (C-6)), 51.4 (t (C-7)), 79.2 (s (C-8)), 42.4 (t (C-9)), 83.2 (d (C-10)), 75.1 (d (C-11)), 132.5 (s (C-12)), 145.0 (d (C-13)), 28.8 (t (C-14)), 147.3 (s (C-15)), 110.4 (t (C-16)), 21.5 (q (C-17)), 26.5 (q (C-18)), 168.3 (s (C-19)).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, HMBC, and expanded HMBC and NOESY spectra of singardin (**1**) (5 pages). Ordering information is given on any current masthead page.

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