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Alertenone, a Dimer of Suberosenone from *Alertigorgia* sp.†

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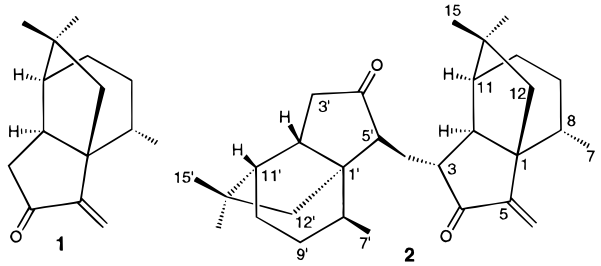
Bioassay-guided fractionation of organic extracts of the gorgonian *Alertigorgia* sp. has yielded the previously known suberosenone (**1**), a cytotoxic tricyclic sesquiterpene of the quadrone class, and alertenone (**2**), a dimer of suberosenone. The structure of **2** was determined by spectral analysis; the 1D TOCSY experiment was particularly useful in the structure elucidation. Comparison of the in vitro cytotoxicity of alertenone and suberosenone revealed that the dimeric alertenone was devoid of cytotoxicity below 35 μg/mL. In a hollow-fiber assay model of in vivo activity, suberosenone exhibited some growth inhibition of two of six tumor cell lines tested.

We recently reported the isolation, structure elucidation, and in vitro antitumor activity of suberosenone (**1**),[1] a novel sesquiterpene related to quadrone[2] and terrecyclic acid derivatives.[3,4] Motivated by the differential cytotoxicity[5] exhibited by **1**[1] in the NCI 60 cell line human tumor assay,[6] we undertook the acquisition of additional quantities of **1** for further biological evaluation. Using COMPARE analyses,[5] we identified extracts of several collections of the gorgonian *Alertigorgia* sp., whose cytotoxicity profile matched that of *Subergorgia suberosa*, the original source of **1**.[1] In the course of purifying quantities of suberosenone for additional bioassays, we isolated and identified smaller quantities of a related dimer, alertenone (**2**), the subject of this report.



The hexane-soluble portion of the organic extract of *Alertigorgia* sp. was permeated through Bio Beads S-X8; the resulting active fraction was subjected to vacuum−liquid chromatography on Si gel and, finally, normal-phase HPLC, to give **1** (1.5% yield) and the dimer **2** (0.6% yield). Suberosenone (**1**) was readily identified by comparison of its HPLC retention time and NMR spectra to previously established values.[1]

HREIMS of alertenone (**2**) yielded a molecular formula of $C_{30}H_{44}O_2$, twice the elemental composition of **1**. The NMR spectra were similar to, but more complex than, those of **1**. Other than a doubling of many highfield signals, the ^{1}H NMR spectrum was distinguished by the presence of only two olefinic proton signals and additional one-proton resonances at δ 3.06 (H-3) and 3.16 (H-5′), perhaps due to methines α to a carbonyl group. Each of these resonances was coupled to the same methylene pair (H-6′), at δ 1.34 and 1.47; the resonance at δ 3.06 was further coupled to H-2 (δ 1.84). This spin−spin system suggested that one molecule of suberosenone had added to another in a 1,4-Michael addition to give a dimer. Detailed analysis of COSY, HSQC, HMBC, and 1D TOCSY data confirmed the structure of alertenone (**2**) and permitted assignment of all proton and carbon resonances (Table 1). The 1D TOCSY experiment would seem to be particularly valuable for unraveling highly overlapped proton−proton spin systems such as those in such dissymmetric dimers. For example, selective irradiation of the doublet methyl proton signal for H-7 at δ 0.84 in a series of 1D TOCSY experiments, with mixing times ranging from 20 to 120 ms, revealed the progressive appearance of multiplets for H-8 through H-11, from which appropriate coupling constants could be extracted. A similar series of experiments, but with irradiation of the doublet methyl proton signal for H-7′ at δ 1.20, revealed the progressive appearance of multiplets for H-8′ through H-11′ in the other half of the dimer.

1D NOESY experiments established that the two suberosenone subunits had identical relative configurations about the tricyclic ring system and also revealed the relative stereochemistry at the two new chiral centers (C-3 and C-5′) and the orientation of the two suberosenone units in the dimer (Figure 1).

In comparative testing against 10 human tumor cell lines (A-549, HOP-92, SF-295, SF-539, SNB-19, LOX, M14, MALME-3M, OVCAR-3, and MCF7), suberosenone (**1**) exhibited the same potency and differential cytotoxicity observed earlier,[1] with IC_{50} values of 0.002−1.6 μg/mL. However, alertenone (**2**) was surprisingly nontoxic; nine of the cell lines gave IC_{50} values of 35−45 μg/mL, while one was not responsive at 100 μg/mL.

Alertenone (**2**) does not appear to be an artifact of the isolation procedure. It was isolated as a single diastereomer of the four possible dimers. Further, it is relatively unstable when purified, gradually decomposing on standing or prolonged exposure to Si gel or $CDCl_3$. Considering the lack

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† Dedicated to the memory of Michael A. Westergaard, who lost his battle with prostate cancer on September 6, 1997.

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Table 1. ^{1}H and ^{13}C NMR Data for Alertenone (**2**)[a]

position	δ ^{13}C		δ ^{1}H (mult., *J* in Hz)	NOE	HMBC (C to H#)
1	55.1		1.84 (d, 12.0)	6′*RS*, 7, 10α, 11	3, 6′, 8, 10, 12, 13
2	53.4		3.06 (ddd, 4.2, 9.2, 11.7)	5′, 6′*S*, 11, 12*R*, 15	1, 4
3	48.2	a	4.96 (d,1)	6*β*	1
4	210.3	b	5.92 (d,1)	6α, 7, 8	1, 8, 9
5	151.9		0.84 (3H, d, 7.5)	2, 6α, 8, 9α	1, 9, 12
6	115.5		2.09 m	3, 6′*S*, 10α, 10*β*, 14, 15	11, 12, 13, 15
7	17.4	α	1.31 (dd, 5.9, 12.6)	8*β*, 11, 12*S*, 10*β*	11, 12, 13, 14
8	36.7	*β*	2.10 m	3′*β*, 5′, 7′, 10′α, 11′	1′, 2′, 4′, 5′, 11′
9	26.5	α	1.55 m	3′*β*, 5′, 7′, 10′α, 11′	1′, 4′, 6′, 8′, 12′
10	27.9	*β*	1.70 m	3′α, 11′, 12′*R*, 15′	1′, 8′, 9′
11	48.5		1.96 (t, 3.2)	2′, 3, 6′*R*, 7′	1′, 2′, 7′, 9′, 10′
12	54.4	pro*R*	1.74 (d, 14.2)	2′, 5′, 8′, 9′α	7′, 8′, 10′
13	39.9	pro*S*	1.80 (d, 14.2)	9′*β*, 10′*β*, 11′, 12′*RS*	2′, 9′, 11′, 13′
14	27.1		1.17 (3H, s)	3′*β*, 11′, 12′*RS*	11′, 12′, 13′, 15′
15	34.6		1.35 (3H, s)		11′, 12′, 13′, 14′
1′	56.9		2.44 (t, 9.0, 10.0)		
2′	43.6	α	2.46 (ddd, 1.5, 10.0, −22.0)		
3′	40.9	*β*	2.26 (dd, 9.0, −22.0)		
4′	220.7		3.16 (dt, 10.0,1.5,1.5)		
5′	52.1	pro*R*	1.34 m		
6′	25.4	pro*S*	1.47 (ddd, 4.3,10.3,13.6)		
7′	16.8		1.20 (3H, d, 7.2)		
8′	35.6		1.91 (pent, 7.2)		
9′	26.9	α	1.31 (dd, 5.3,14.6)		
10′	28.1	*β*	2.01 (dddd, 7.0,7.2,13.0,14.6)		
11′	49.6	α	1.59 m		
12′	47.5	*β*	1.62 m		
13′	39.3		1.70 (t, 2.9,2.9)		
14′	26.8	pro*S*	1.34 (d, 15.0)		
15′	34.1	pro*R*	1.38 (d, 15.0)		
			1.11 (3H, s)		
			1.05 (3H, s)		

[a] Recorded in $CDCl_3$ at 500 MHz.

Table 2. Comparison of in Vitro Cytotoxicities of Suberosenone (**1**) and Alertenone (**2**)

	IC_{50} (μg/mL)	
cell line	**1**	**2**
nonsmall cell lung		
A-549	1.63	40
HOP-92	0.11	45
CNS		
SF-295	0.03	35
SF-539	0.002	35
SNB-19	0.006	45
melanoma		
LOX	0.006	>100
M14	0.010	40
MALME−3M	0.008	40
ovarian		
OVCAR-3	0.02	35
breast		
MCF7	0.43	40

of cytotoxicity in **2**, we speculate that alertenone may serve as a nontoxic, nonvolatile storage form of suberosenone, which, in turn, may be a chemical defensive agent of the gorgonian.

The suberosenone isolated in this study was utilized in a hollow-fiber assay for in vivo antitumor activity.[7] The quantity of **1** available precluded our identifying either a toxic or maximum-tolerated dose. Using a high dose of 40 mg/kg against six human tumor cell lines implanted subcutaneously and intraperitoneally in mice, we observed some activity against two of the cell lines. In the case of the H-522 lung tumor and U-251 CNS tumor lines, 10−30% net cell growth versus controls was observed at multiple doses in both the ip and sc fibers. These data require confirmation but are suggestive of in vivo antitumor activity.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin−Elmer 241 polarimeter in $CHCl_3$. UV spectra were determined on a Beckman DU-64 spectrophotometer; FT−IR spectra were obtained on a Perkin−Elmer 267 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-500 spectrometer using $CDCl_3$ as solvent and internal standard. The number of attached protons was determined from DEPT experiments. MS were determined on a JEOL SX102 spectrometer.

Animal Material. Samples of *Alertigorgia* sp. were collected along the east side of Joseph Bonaparte Gulf, Northern Territories, Australia, in August 1991, by the Australian Institute of Marine Sciences under contract to the National Cancer Institute. A voucher specimen (Q66C5606) is maintained at the Smithsonian Institution Sample Sorting Center, Suitland, Maryland.

Extraction and Isolation. Frozen coelenterate samples were processed as described[8] to give aqueous and organic extracts. The cytotoxic organic extract (3.46 g) was partitioned between MeOH−H_2O (9:1, 200 mL) and hexane (4 × 200 mL). The hexane-soluble extract (1.96 g) was permeated through BioBeads S-X8 (3 × 92 cm) with hexane−CH_2Cl_2−EtOAc (2:4:1); four fractions were obtained. Fraction 3 was subjected to vacuum−liquid chromatography on Si gel with a hexane−EtOAc gradient. The second of six fractions was purified by HPLC on Rainin Dynamax silica (2.1 × 25 cm) with hexane−EtOAc (19:1) to give suberosenone (**1**), 57 mg, and alertenone (**2**), 21 mg, $[\alpha]_D$ +15.4° (*c* 0.23, $CHCl_3$); UV λ_{max} (hexane) 231 nm (ϵ 3500); IR ν_{max} (film) 2926, 1733, 1636, 1453, 1389, 1240, 1179, 1084, 1044, 936, 757 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 436.3323 (M^+), calcd for $C_{30}H_{44}O_2$, 436.3341; 379.2635, calcd for $C_{26}H_{35}O_2$, 379.2640; LREIMS *m*/*z* 436 (39), 421 (8), 379 (100), 361 (8), 231 (10), 218 (15), 149 (38).

In Vitro Cytotoxicity Assay. The in vitro 10 cell-line bioassay was a 2-day bioassay. Cells were grown in RPMI-1640 without L-glutamine, supplemented with with 10% fetal

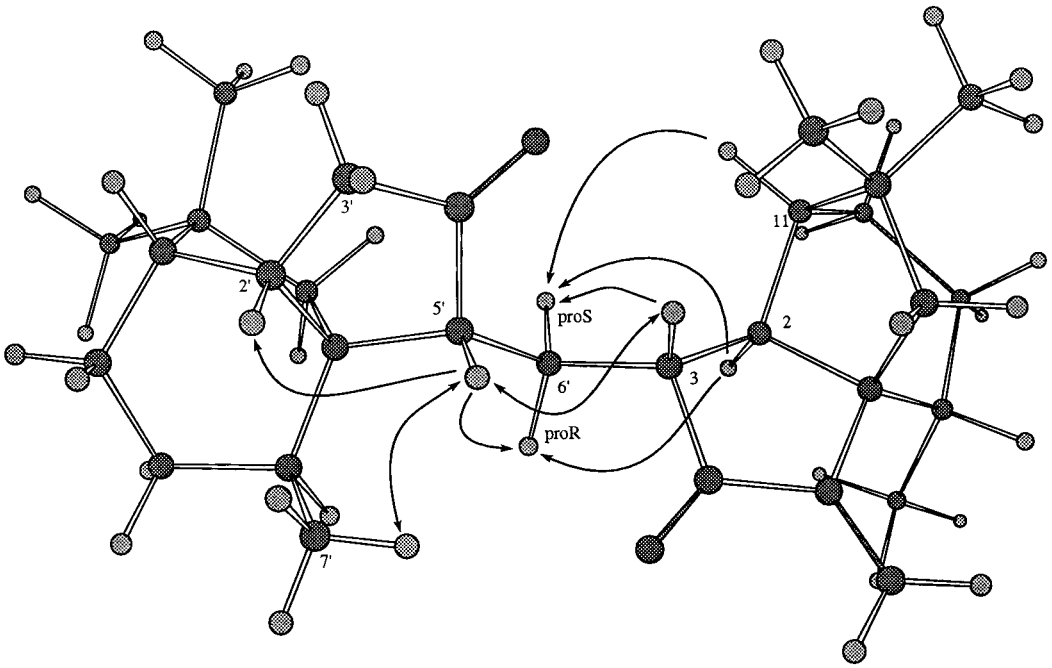

Figure 1. A computer-generated drawing of **2** showing key NOE correlations revealing the relative stereochemistry of the molecule.

bovine serum, 5.0 mL of a 200-mM glutamine stock, and 0.5 mL of gentamicin and plated out in T-162 cm^2 flasks. Once the cells were confluent, they were harvested and plated in 96-well microtiter flat-bottom plates at a seeding density of 50−100 000 cells per well, to yield optical density readings in the range of 1−2.0, and incubated for 1 h in a 37 °C, 5%, CO_2 incubator. After the 1-h incubation, the cells were then introduced to the test sample, via Beckman Biomek Workstation-1000. The Biomek-1000 performed eight serial dilutions in a 96-well round-bottom plate and then transferred aliquots of 100 μL to the assay plate. The plate was then returned to the incubator for 24 h. After the 2-day incubation, the cells were exposed to a tetrazolium salt (2,3-*bis*[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide, XTT) for a 4-h incubation in a 37 °C incubator, where viable cells reduced the tetrazolium salt to a colored formazan product. Once the incubation was completed, the plates were then read in a dual wavelength mode at 450 nm, with a 650 nm reference, using a SpectraMAX 250 (Molecular Devices) plate reader.

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