Pergamon

Tetrahedron Letters, Vol. 37, No. 19, pp. 3259-3262, 1996
Published by Elsevier Science Ltd
Printed in Great Britain.
0040-4039/96 $15.00 + 0.00

S0040-4039(96)00528-X

Suberosenone, a New Cytotoxin from *Subergorgia suberosa* [1a]

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Abstract: The organic extract of the gorgonian *Subergorgia suberosa* was found to contain a novel cytotoxic sesquiterpene, suberosenone (**1**), as well as two known piscicidal sesquiterpenes, buddledins C and D. The structure of **1** was defined by spectral methods, including 1D and 2D NMR experiments. Suberosenone, the first quadrone-class sesquiterpene from the marine biosphere, exhibited relatively potent cytotoxicity to solid tumor lines in comparison to leukemia lines in the NCI's 60 cell line *in vitro* screen.
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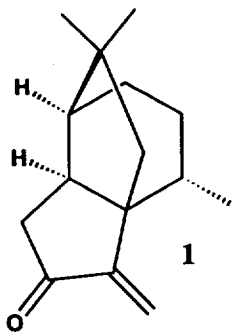
As part of our continuing investigation of natural products as leads to new antitumor agents,[2] we undertook an examination of the organic extracts of the gorgonian *Subergorgia suberosa*, which displayed an unusual profile of differential cytotoxicity in the NCI's human disease oriented 60-cell line *in vitro* screen[3]. Bioassay-guided fractionation of this extract led to the isolation of suberosenone (**1**), the first representative of the quadrone class of sesquiterpenes isolated from a marine organism or any nonfungal source.

The organic extract (5.1 g) of *S. suberosa* was partitioned between hexane and MeOH-H_2O (9:1). The cytotoxic, hexane-soluble material (3.8 g) was subjected to BioBeads S-X8 size exclusion chromatography (hexane-CH_2Cl_2-EtOAc, 2:4:1). A late eluting fraction (410 mg) was purified by HPLC (silica gel, hexane-EtOAc, 19:1) to yield suberosenone, **1** (110 mg), buddledin C, **2** (16 mg), and buddledin D, **3** (14 mg). HRFABMS analysis of the three compounds established a common molecular formula of $C_{15}H_{22}O$ (5 sites of unsaturation). Comparison of spectral and optical data reported in the literature identified the latter two metabolites.[4] Extensive ^1H and ^{13}C NMR analyses allowed us to define the structure of the remaining compound, which we have named suberosenone.[5]



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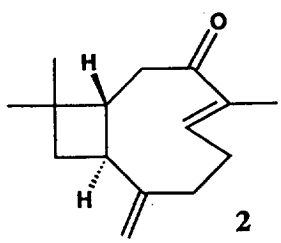

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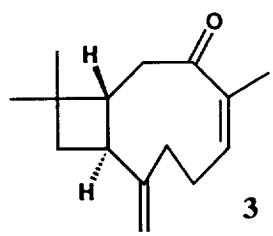

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A DEPT experiment showed the presence of three methyls, five methylenes, three methines, and four quaternary carbons. The ^{13}C resonances at δ208.6 (C-4), 151.8 (C-5) and 115.2 (C-6) indicated the presence of a ketone carbonyl

and an olefin, accounting for two of the five unsaturations required by the molecular formula. Therefore, suberosenone was tricyclic. DEPT and HMQC experiments identified the olefinic carbon at δ115.2 (C-6) as a methylene, consistent with an exocyclic double bond; UV (233 nm) and IR (1724, 1636 cm^{-1}) absorptions were consistent with an α-methylene cyclopentenone (**A**). The remaining structural fragments of suberosenone (**B-F**) consisted of small, isolated spin systems and their structures were deduced from analysis of COSY, DEPT and HMQC experiments. HMBC correlations (see Table 1) were used to assemble the fragments to give the gross structure **1** for suberosenone.

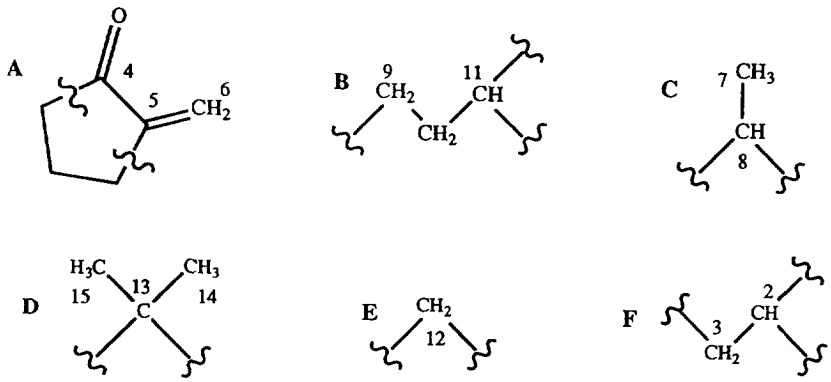


Table 1. ^{1}H and ^{13}C NMR Data for Suberosenone (**1**)[a]

C#[b]	δ ^{13}C	δ ^{1}H	^{1}H-^{1}H COSY	HMBC (C to H#)	nOe[c]
1	57.8	-		3α, 6a, 6b, 7, 8, 9α, 11, 12b	
2	45.1	2.30 (ddd, 11.5, 9.5, 0.5)	3α, 3β	3α, 3β, 8, 10α, 12b	7
3	41.8	α 2.44 (dd, 19.5, 9.5)	2, 3β		
		β 2.64 (dd, 19.5, 12.0)	2, 3α		
4	208.6	-		3α, 3β, 6b	
5	151.8	-		3α, 6b, 12a	
6	115.2	a 4.97 (d, 0.8)			
		b 5.95 (d, 0.8)			
7	17.2	0.88 (3H, d, 7.0)	8	8, 9α, 9β	2, 6a, 8
8	36.3	2.12 (m)	7	2, 7, 9α, 9β, 10β, 12a, 12b	
9	26.4	α 1.32 (ddd, 14.0, 6.5, 1.0)	9β, 10α	7, 8, 10α, 11	
		β 2.06 (m)	9α, 10α		
10	27.8	α 1.59 (m)	9α, 9β, 10β, 11	2, 8, 9α, 9β	2
		β 1.67 (m)	9β, 10α, 11		14
11	49.6	1.87 (ddd, 3.5, 3.0, 0.5)	10β	3β, 9α, 10α, 14, 15	
12	54.2	a 1.69 (d, 14.5)	12b	2, 11, 14, 15	15
		b 1.79 (d, 14.0)	12a		
13	40.2	-		2, 10α, 10β, 12a, 14, 15	
14	26.9	1.15 (3H, s)	15	12b, 15	9β, 12b
15	34.9	1.19 (3H, s)	14	11, 12a, 14	3β, 11

[a] recorded in $CDCl_3$ at 500 MHz

[b] the numbering system used is consistent with published data on the quadrone class of carbon skeleton

[c] from difference nOe and NOESY spectra

The inherently rigid nature of suberosenone is readily apparent. The relative stereochemistry of suberosenone was elucidated on the basis of difference nOe experiments. An enhancement of H-2 (δ2.30) and one of the protons on C-10 (δ1.59) upon irradiation of the H-7 methyl signal indicated that they were all in *cis* 1,3-diaxial positions relative to one another around the six-membered ring, and defined a chair conformation for the cyclohexane ring. H-6a was also enhanced, placing it *trans* to the ketone carbonyl on the exocyclic methylene. The large coupling constant (J=11.5 Hz) between H-2 and H-3ß identified these protons as *trans*. This was supported by nOe data showing enhancement of the

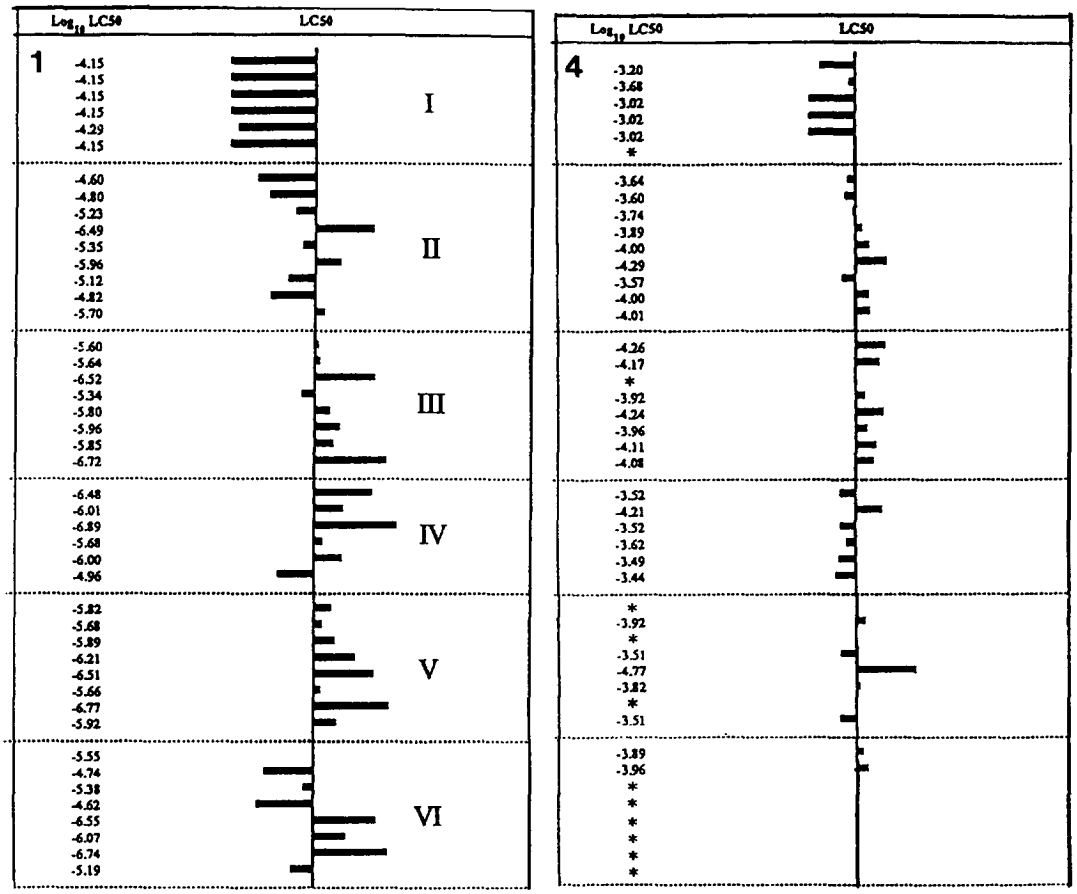
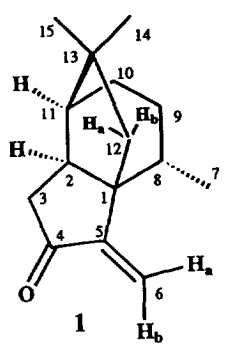


Figure 1. Averaged LC_{50}-centered mean graph derived from quadruplicate testing of suberosenone (**1**), and quadrone (**4**) in the NCI screen. The individual averaged $\log_{10} LC_{50}$ values for each cell line are provided in the vertical column to the left of the mean graph. The subpanel and individual cell line identifiers are presented top-to-bottom as follows: I (leukemia) CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226, SR; II (non-small cell lung) A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522; III (melanoma) LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62; IV (ovarian) IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3: V (renal) 786-O, A498, ACHN, CAKI-1, RXF-393, SN12C, TK-10, UO-31; VI (breast) MCF7, MCF/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N, BT-549, T-47D. An * indicates cell lines that were not tested with quadrone.

proton resonance at δ2.64 (H-3ß) upon irradiation of the Me-15 protons. Irradiation of Me-14 caused enhancement of the signal at δ2.06 (H-9), suggesting its projection upwards towards the methyl group. H-12b (δ1.79) was also enhanced by irradiation of Me-14, placing H-12b in a position *cis* to the C-14 methyl group. The absolute configuration of suberosenone has not been determined.

Only three naturally occurring compounds having the same carbon skeleton as suberosenone have been reported. Quadrone (**4**)[6] reportedly has antitumor properties, while terrecyclic acid A (**5**)[7] and terrecyclol (**6**)[8] have antibiotic properties but no reported antitumor activity. All three of the latter compounds were originally isolated from the fungus *Aspergillus terreus*; thus, suberosenone is the first reported occurrence of this carbon skeleton from a nonfungal source. Suberosenone demonstrated potent, differential cytotoxicity in the NCI human tumor-based primary screen; ovarian, renal and melanoma lines were particularly sensitive to **1**, while leukemia lines were relatively insensitive (Fig. 1). In contrast, quadrone (**4**) showed only weak differential cytotoxicity in the NCI screen (Fig. 1).[9]



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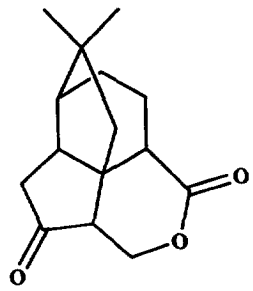

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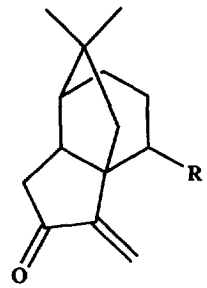

5 R=COOH
6 R=CH_2OH

Acknowledgements: The organism was collected under contract for the National Cancer Institute by P. Murphy (AIMS). Taxonomic identification was provided by P. Alderslade; a voucher specimen has been deposited at the Smithsonian Institute. We thank K. Snader and D. Newman for coordinating collections and taxonomic work, T. McCloud for extractions, L. Pannell for mass spectral analyses, and A. Monks and D. Scudiero for antitumor screening.

References and Notes

1. a) Taken in part from the M.S. Thesis of H.R. Bokesch, Hood College, Frederick, MD, 1996;
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5. **1**: colorless oil; $[\alpha]_D = +55.7^\circ$ (c 0.78, $CHCl_3$); UV(MeOH): λ_{max} 233 nm (ε=11100); IR (film): ν_{max} 2932, 1724, 1636, 1455, 1388, 1160, 1115, 928 cm^{-1}; HRFABMS: *m/z* 219.1748 (calcd for $C_{15}H_{23}O$, *m/z* 219.1750); for ^1H NMR and ^{13}C NMR data, see Table 1.
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9. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

(*Received in USA* 1 *December* 1995; *revised* 4 *March* 1996; *accepted* 12 *March* 1996)