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BALEARONE, A METABOLITE OF THE BROWN ALGA CYSTOSEIRA BALEARICA

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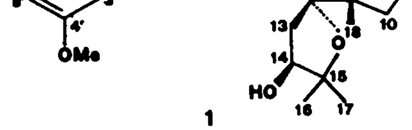
Abstract - The isolation of a new metabolite of mixed biogenesis, balearone 1, from the brown alga Cystoseira balearica is described. The structure was determined by an X-ray diffraction analysis.

In recent years a variety of tetraprenyl-hydroquinol derivatives have been isolated and characterized from marine algae of the families Cystoseiraceae and Sargassaceae.[1-12] While in several of these compounds the terpenoid component has an acyclic nature,[1-7] in others cyclization gives rise to a cyclopentane[8,12] or a bicyclo[4.3.0]-nonane ring system.[11,12] In this paper we wish to describe the structure of a new member of this family of metabolites of mixed biogenesis possessing a terpenoid moiety of an unprecedented skeletal type.

The chloroform extract of Cystoseira balearica Sauv. (collected at Portopalo, Sicily, Italy, April 1982) was chromatographed on a silica gel column to obtain several metabolites, one of which, balearone 1, crystallized from hexane, m.p. 94-96°, $[\alpha]_D^{20}$ +52°.

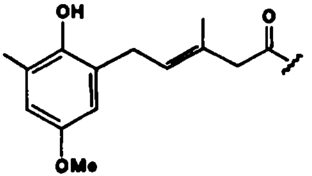


1

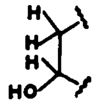
The IR spectrum showed absorptions attributable to hydroxyl (3430 cm^{-1}) and unconjugated carbonyl (1698 cm^{-1}) functions. The UV spectrum displayed absorptions at 289 (ε = 2620) and 218 nm (ε = 9500) indicative of a hydroquinol chromophore. High resolution mass spectral analysis of 1 indicated an elemental composition of $C_{28}H_{40}O_5$.

The ^{13}C NMR spectrum revealed a carbonyl absorption at δ 208.8s, a tetrasubstituted aromatic ring (153.2s, 146.5s, 127.9s, 126.0s, 114.1d, 113.1d), a trisubstituted double bond (130.8s, 127.8d) and four oxygen-bearing carbons (81.6, 80.2s, 78.3d, 55.6q). The remaining resonance lines in the spectrum were located at δ 59.8d, 56.0t, 52.1s, 46.1s, 40.8t, 35.7t, 35.6t, 30.7t, 24.1t, in addition to six methyl quartets at 27.4, 22.2, 19.1, 17.0, 16.9 and 16.5 accounting for all the 28 carbon atoms of the molecule.

The ^{1}H NMR spectrum and homonuclear decoupling studies indicated the presence of two <u>meta</u>-coupled aromatic protons at δ 6.58 and 6.54, an aromatic methoxyl at 3.75, a methyl group on an aromatic ring at 2.25 and a benzylic methylene at 3.36 (2H, m, AB part of an ABX, J=15, 7.5 and 6.5 Hz). The latter was coupled with a vinyl proton at δ 5.32 (1H, t, X part of an ABX) which was in turn allylically coupled with a vinyl methyl at 1.75. The spectrum also contained an isolated methylene resonance at δ 2.95 (s), a methine at 3.13 (s), an AMX pattern [AM part centered at 3.02 (1H, dd, J=13 and 6.5 Hz) and 1.91 (1H, dd, J=13 and 8.5 Hz), X part centered at 3.97, m], 4 methyl singlets at 1.19, 1.08, 0.94 and 0.91, and complex signals centered at 1.9, 1.7 and 1.4 integrating for a total 6 protons. According to the above data, balearone 1 was suggested to contain partial structures A and B.



A



B

Since further conclusions were not possible on the basis of spectral data alone, the structural elucidation (inclusive of stereochemistry) was completed by a single-crystal X-ray analysis.

A computer-generated perspective drawing of the final X-ray model is shown in Figure 1. Since the diffraction experiment did not distinguish enantiomers, the one depicted is an arbitrary choise.

^{1}H and ^{13}C NMR studies suggested the assignments listed in Tables 1 and 2, respectively, based on chemical shifts and correlation with related compounds.[11,12]

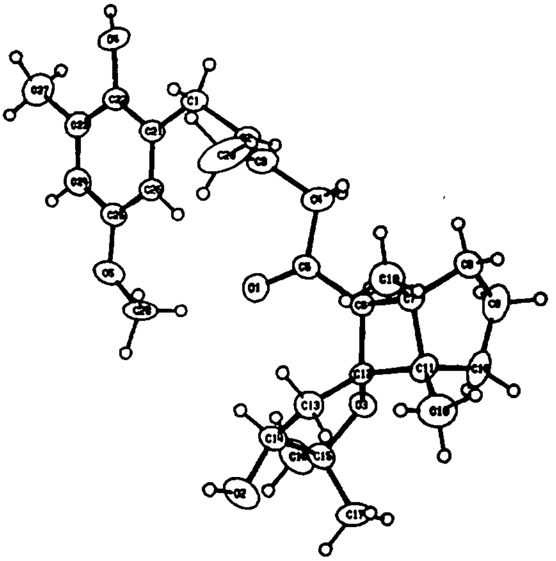
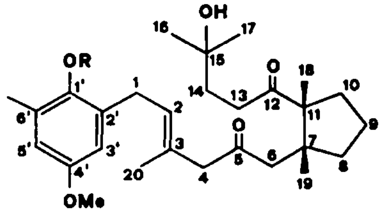


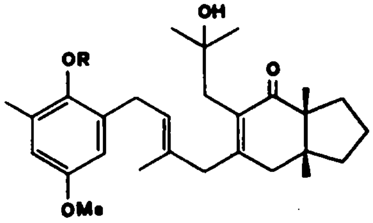
Fig. 1. A computer-generated perspective drawing of balearone 1 (no absolute configuration is implied).

The biogenesis of balearone 1 may proceed through a 5-membered ring precursor (2) <u>via</u> an internal aldol condensation involving C-6 to C-12 bond formation. The recent isolation of compound 3 from <u>C</u>. <u>algeriensis</u>[12] supports this biogenetic hypothesis. The alternative bonding of C-5 to C-13 would lead to the metabolite of the structure type 4 isolated from the same source[11,12] and possessing a bicyclo[4.3.0]-nonane ring system.



2 R=H

3 R=Me



4 R=H or OH

Table 1. ^{1}H NMR of balearone 1*

Position		
3'	6.54	AB (3)
5'	6.58	
1	3.45	dd (15, 7.5)
	3.38	dd (15, 6.5)
2	5.32	t (7.5)
4	2.95	s
6	3.31	s
8	complex multiplets at 1.4, 1.7 and 1.9	
9		
10		
13	3.03	dd (13, 6.5)
	1.91	dd (13, 8.5)
14	3.97	m
16	1.08	s
17	1.19	s
18	0.91	s
19	0.94	s
20	1.75	s
O-Me	3.75	s
6'-Me	2.25	s

* ^{1}H NMR spectrum was recorded in $CDCl_3$ at 250 MHz with TMS as internal standard; coupling constants (J in parentheses) are given in Hz; assignments were confirmed by decoupling.

Table 2. ^{13}C NMR of balearone 1*

(ppm)	Multiplicity	Assignment
208.8	s	C-5
153.2	s	C-4'
146.5	s	C-1'
130.8	s	C-3, 2' and 6'
127.9	s	
126.0	s	
127.8	d	C-2
114.1	d	C-3', and 5'
113.1	d	
81.6	s	C-12 and 15
80.2	s	
78.3	d	C-14
59.8	d	C-6
56.0	t	C-4
55.6	q	O-Me
52.8	s	C-7 and 11
46.1	s	
40.8	t	C-8, 10 and 13
35.7	t	
35.6	t	
30.7	t	C-1
24.1	t	C-9
27.4	q	C-16 and 17
22.2	q	
19.1	q	C-18 and 19
17.0	q	
16.9	q	C-20 and 6'-Me
16.5	q	

*^{13}C NMR spectrum was recorded in $CDCl_3$ at 75.5 MHz with TMS as internal standard; multiplicities were obtained by off-resonance decoupling experiments.

EXPERIMENTAL

M.p. was taken on a Kofler block and is uncorr. MS analysis was performed with direct inject system at 70 eV. IR spectrum was determined on a Perkin-Elmer mod. 684 and UV spectrum on a Perkin-Elmer mod. 330 spectrophotometers. ^{1}H NMR spectra were measured at 250 MHz on a Bruker WP-250 instrument. ^{13}C NMR spectra were run at 75.5 MHz on a Bruker WM-300 instrument. Chemical shifts are quoted in ppm (δ) relative to TMS. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Preparative liquid chromatography (PLC) were carried out on a Jobin-Yvon Miniprep LC instrument.

Plant material. Cystoseira balearica Sauv. (voucher specimen deposited at the Erbarium of the Institute of Botany, Catania, Italy) was collected on rocks at about 1 m depth in April 1982 at Portopalo, Sicily.

Extraction and purification. Shade dried and ground alga (1.7 kg) was extracted x3 with $CHCl_3$ at room temp. with continuous stirring. The extracts were pooled and evaporated to give a dark green oil (35 g). The crude extract was applied to an open column (4 x 120 cm) of Si-gel. The column was eluted with increasing concentration of Et_2O in hexane. Fractions of 200 ml were collected and those exhibiting similar TLC profiles were combined. Fractions 90-112 were pooled and subjected to successive PLC (LiChroprep Si-60) using CH_2Cl_2-dioxane 97:3, CH_2Cl_2-Et_2O 90:10 and C_6H_{12}-dioxane 85:15 in that order. Crystallization from hexane gave pure 1 (954 mg, 0.056% dry weight) m.p. 94-96°; $[\alpha]_{20}$ (λ) +52° (589), +55° (578), +65° (546), +141° (436), +320° (365) (c 1.3, EtOH); UV λ_{max}^{EtOH} nm: 218 (ε=9500), 289 (ε=2612); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3430, 1698, 1605; HRMS: M^+ 456.2869 (calc. for $C_{28}H_{40}O_5$ 456.2875); MS m/z (%): 456 (0.6), 438 (7.8), 420 (44), 288 (27), 233 (27), 205 (5), 191 (15), 189 (28), 168 (15), 151 (21), 150 (100), 137 (29), 109 (27), 95 (15), 88 (78), 69 (13), 67 (26), 58 (56), 43 (32), 41 (22).

Crystal data. $C_{28}H_{40}O_5$, M = 456.6. Crystals were orthorhombic space group $P2_12_12_1$, a=10.048 (1), b=15.294 (3), c=17.251 (3) Å, V=2651 Å, Z=4, D_c= 1.144 g cm^{-3}, μ (MoKα)=0.72 cm^{-1}.

Crystallographic measurements. Data were collected on an Enraf-Nonius CAD4 diffractometer with graphite-monochromated Mo-Kα radiation (0.71073 Å). One octant of data having $1° < \theta < 25°$ was collected by ω-2θ scans of variable rate, designed to yield I≈25 (I) for all significant reflections. A maximum of 120 sec. was spent on any single scan. A total of 2644 unique data was collected, of which 1566 had $I > \sigma(I)$, and were used in the refinement.

Structure analysis. The structure was solved by direct methods and refined by full-matrix least squares, treating non-hydrogen atoms anisotropically. Hydrogen atoms were located from difference maps, but included as fixed contributions in calculated positions where possible, with B=7.0 $Å^2$. Convergence was achieved with R=0.052 for 299 variables, and maximum residual electron density 0.17eÅ^{-3}.

A computer generated perspective drawing of the final X-ray model is shown in Figure 1 and atomic coordinates are listed in Table 3.

Table 3. Atomic coordinates for balearone 1

a) non-hydrogen atoms with estimated standard deviations in parentheses

Atom	x	y	z
O1	0.1941(5)	0.3989(3)	1.1727(2)
O2	-0.0998(4)	0.5536(3)	1.3128(3)
O3	-0.0512(4)	0.3238(2)	1.3153(2)
O4	0.4207(4)	0.3109(2)	0.7835(2)
O5	-0.0337(4)	0.4417(3)	0.9180(2)
C1	0.4079(6)	0.3021(4)	0.9443(4)
C2	0.3666(7)	0.2769(5)	1.0247(3)
C3	0.3973(7)	0.3123(5)	1.0900(4)
C4	0.3433(8)	0.2780(7)	1.1644(3)
C5	0.2382(6)	0.3349(5)	1.2038(3)
C6	0.1848(6)	0.2991(4)	1.2782(3)
C7	0.2767(5)	0.2817(4)	1.3489(2)
C8	0.3121(7)	0.1875(4)	1.3637(4)
C9	0.1822(10)	0.1438(4)	1.3839(4)
C10	0.0953(6)	0.2138(4)	1.4225(4)
C11	0.1534(6)	0.3039(4)	1.4009(3)
C12	0.0857(5)	0.3481(3)	1.3300(2)
C13	0.0839(6)	0.4476(4)	1.3285(3)
C14	-0.0437(6)	0.4728(4)	1.2895(3)
C15	-0.1389(5)	0.3992(4)	1.3123(3)
C16	-0.2428(7)	0.3816(4)	1.2499(6)
C17	-0.2010(8)	0.4117(4)	1.3915(4)
C18	0.1758(9)	0.3584(6)	1.4754(3)
C19	0.3950(7)	0.3430(4)	1.3559(4)
C20	0.4842(8)	0.3900(9)	1.0958(4)
C21	0.2934(6)	0.3397(4)	0.8966(3)
C22	0.3028(6)	0.3380(4)	0.8171(3)
C23	0.1978(6)	0.3707(3)	0.7705(3)
C24	0.0873(5)	0.4040(5)	0.8076(3)
C25	0.0797(6)	0.4057(4)	0.8872(3)
C26	0.1826(5)	0.3738(3)	0.9322(3)
C27	0.2105(8)	0.3706(5)	0.6843(4)
C28	-0.0406(7)	0.4551(5)	0.9989(4)

b) hydrogen atoms

Atom	x	y	z
H1a	0.4429	0.2490	0.9173
H1b	0.4800	0.3471	0.9479
H2	0.3064	0.2250	1.0282
H4a	0.4192	0.2710	1.2012
H4b	0.3023	0.2196	1.1539
H6	0.1516	0.2497	1.2455
H8a	0.3765	0.1827	1.4078
H8b	0.3520	0.1604	1.3163
H9a	0.1980	0.0941	1.4205
H9b	0.1380	0.1214	1.3359
H10a	0.0968	0.2060	1.4801
H10b	0.0018	0.2089	1.4034
H13a	0.1617	0.4704	1.2985
H13b	0.0867	0.4715	1.3824
H14	-0.0271	0.4806	1.2328
H16a	-0.1976	0.3739	1.1988
H16b	-0.2933	0.3273	1.2632
H16c	-0.3057	0.4322	1.2469
H17a	-0.1294	0.4231	1.4306
H17b	-0.2634	0.4626	1.3900
H17c	-0.2510	0.3577	1.4063
H18a	0.0885	0.3673	1.5023
H18b	0.2381	0.3265	1.5106
H18c	0.2146	0.4164	1.4614
H19a	0.4676	0.3228	1.3205
H19b	0.3673	0.4035	1.3412
H19c	0.4281	0.3428	1.4106
H20a	0.5273	0.3945	1.0332
H20b	0.5564	0.3808	1.1349
H20c	0.4321	0.4434	1.1096
H24	0.0117	0.4274	0.7761
H26	0.1765	0.3755	0.9901
H27a	0.3066	0.3965	0.6660
H27b	0.1383	0.4076	0.6617
H27c	0.2015	0.3093	0.6649
H28a	-0.1113	0.5000	1.0176
H28b	0.0487	0.4758	1.0169
H28c	-0.0612	0.3975	1.0238
H20H	0.0547	0.6035	1.3008
H40H	0.4180	0.2715	0.7324

Supplementary material available. Bond distances and angles (Tables 4 and 5), anisotropic thermal parameters (Table 6) and selected torsion angles (Table 7) are deposited in the Tetrahedron archives, for distribution on request.

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