Tetrahedron Vol. 39, No. 4, pp. 629 to 638, 1983
Printed in Great Britain.

0040-4020/83/040629-10$03.00/0

DITERPENOID METABOLITES FROM THE MARINE ALGA *CYSTOSEIRA ELEGANS*

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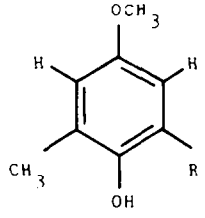
(*Received in France 27 July 1982*)

Abstract—The isolation and structure determination of three new diterpenoids from the brown alga *Cystoseira elegans* is reported. The structures of these compounds were determined by combined chemical and spectral (^{13}C and ^{1}H NMR with decoupling experiments, high resolution mass spectral) methods.

A number of marine metabolites have been found recently in *Phaeophyta* (brown algae), mainly in the families *Sargassaceae*[1] (order *Fucales*) and *Dictyotaceae*[2] (order *Dictyotales*). Continuing our research on the genus *Cystoseira*[3] (*Cystoseiraceae*), we describe here the isolation and structure determination of new diterpenoids of mixed biogenesis which were obtained from *Cystoseira elegans* collected along the Catalan coasts (June 1980).

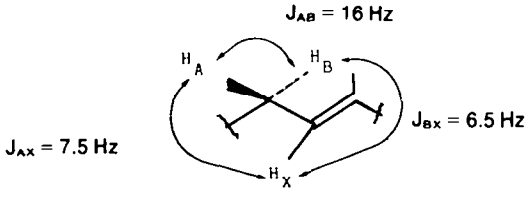
Compounds A–C were isolated from the ether soluble material of the aqueous methanol extract of the seaweed. The ether extract was fractionated on a silica gel open-column to yield various fractions, some of which were further purified by HPLC on Silicagel (μ-Porasil).

Compound A, obtained as a colorless oil, had a molecular formula $C_{28}H_{42}O_5$ (determined from high resolution mass spectrometry). IR absorption established the presence of an alcohol functionality (ν_{O-H} = 3450 cm^{-1}), an unstrained ketone ($\nu_{C=O}$ = 1705 cm^{-1}) and aromatic ring (ν_{max} = 3030, 1600 cm^{-1}). In the UV spectrum, absorptions at 214 nm and 288 nm (ϵ = 11500 and 3100), indicated a hydroquinol chromophore. Support for the assignment of a phenol moiety was obtained from the ^{13}C NMR (Table 1) with a –OMe group resonance (δ = 55.54 ppm), and from the ^{1}H NMR (δ_{OMe} = 3.73 ppm, δ_{CH_3} = 2.20 ppm, Table 2), in which the two meta-coupled (J = 2.9 Hz) proton resonances (δ = 6.52 and δ = 6.56 ppm for C-3', 5') were indicative of 1, 2, 3, 5-substitution on the phenol ring. Furthermore, the presence of important ions with *m/z* 150/151* in the mass spectrum,[4] established the following structure for the benzenoid moiety: (The exact position of the OMe group in 4' instead of 1' was further confirmed by the formation of the ion at *m/z* 189).



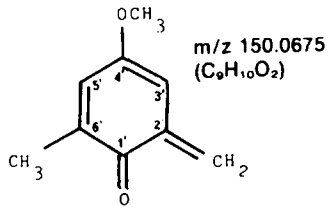
R($C_{20}H_{33}O_3$) = diterpenoid component with three unsaturation equivalents, a CO group and two alcohol functionalities.†

The acyclic nature of the diterpene component was determined by the presence of three olefinic protons and four olefinic Me groups, in combination producing three total unsaturations (all E).‡ The four first diterpenoid carbons were discerned by ^{1}H NMR; the C-1 benzylic methylene was observed as an ABX pattern at δ = 3.39 ppm (1 H, dd, J = 16 and 7.5 Hz) and 3.29 ppm (1 H, dd, J = 16 and 6.5 Hz) due to an adjacent olefinic proton at δ = 5.39 ppm (t, J = 7 Hz)



†Two OH groups were consistent with the ^{1}H NMR data obtained from the corresponding diacetate and from the high resolution mass spectrum.

‡The olefin geometries were assigned based upon the Me resonances observed at higher than 20 ppm in the ^{13}C NMR spectrum (γ shielding effect).



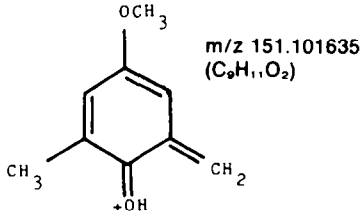


Table 1. ^{13}C chemical shift data in $CDCl_3$ (ppm/from TMS)

carbon n°	Compound A		Compound B		Compound	
	off resonance	measured values	off resonance	measured values	off resonance	measured values
1	(t)	30.5	(t)	30.7	(t)	30.1
2	(d)	125.6	(d)	125.7	(d)	125.8
3	(s)	139.8	(s)	138.2	(s)	138.3
4	(t)	47.8	(t)	48.0	(t)	48.1
5	(d)	65.9	(d)	66.2	(d)	66.8
6	(d)	127.3	(d)	127.8	(d)	128.1
7	(s)	138.2	(s)	137.4	(s)	137.5
8	(t)	39.2	(t)	39.1	(t)	39.4
9	(t)	25.2	(t)	25.7	(t)	25.4
10	(t)	31.8	(d)	125.9	(t)	32.7
11	(d)	41.0	(s)	134.7	(d)	41.1
12	(s)	214.1	(d)	77.4	(s)	215.1
13	(d)	73.1	(t)	34.3	(t)	40.3.
14	(d)	121.2	(d)	120.4	(d)	118.2
15	(s)	134.0	(s)	134.4	(s)	133.6
16	(q)	25.9	(q)	25.8	(q)	25.3
17	(q)	17.7	(q)	18.0	(q)	17.8
18	(q)	18.6	(q)	16.5[a]	(q)	18.4
19	(q)	16.3[a]	(q)	16.5[a]	(q)	17.1[a]
20	(q)	16.3[a]	(q)	16.3[a]	(q)	16.3[a]
1'	(s)	146.4	(s)	146.7	(s)	147
2'	(s)	127.6[b]	(s)	128.0[b]	(s)	128.3[b]
3'	(d)	113.0[c]	(d)	113.3[c]	(d)	113.8[c]
4'	(s)	153.0	(s)	153.4	(s)	154.5
5'	(d)	113.8[c]	(d)	114.2[c]	(d)	114.3[c]
6'	(s)	125.4[b]	(s)	125.8[b]	(s)	125.3[b]
7'	(q)	16.3[a]	(q)	16.3[a]	(q)	16.3[a]
$-OCH_3$	(q)	55.5	(q)	55.7	(q)	55.6

Assignments are based on extrapolation from (7)

* These results were noted here only for attribution.

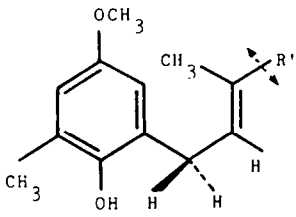
(a), (b) and (c) : assignments may be reversed.

Table 2. Chemical shifts and coupling constants in ^1H NMR spectra of compounds A, B and C (δ units/TMS as an internal standard)

Cdp.	A				B				C			
	δ(ppm)	δ(ppm)			δ(ppm)	δ(ppm)			δ(ppm)	δ(ppm)		
n°C	($CDCl_3$)	(C_6D_6)	mult.	J(Hz)	($CDCl_3$)	(C_6D_6)	mult.	J(Hz)	($CDCl_3$)	(C_6D_6)	mult.	J(Hz)
CH_3-O	3.73	3.43	s		3.74	3.43	s		3.74	3.44	s	
CH_3-Ø	2.20	2.15	s		2.21	2.19	s		2.21	2.19	s	
C_3'	6.52	6.63	d	2.9	6.53	6.62	d	2.9	6.52	6.63	d	2.9
C_5'	6.56	6.69	d	2.9	6.56	6.68	d	2.9	6.56	6.69	d	2.9
C_{1a}	3.39	3.36	dd	16, 7.5	3.38	3.41	dd	16, 7.5	3.39	3.38	dd	16, 7.5
C_{1b}	3.29	3.21	dd	16, 6.5	3.29	3.24	dd	16, 6.5	3.29	3.23	dd	16, 6.5
C_2	5.39	5.42	bt	7	5.39	5.45	bt	7	5.39	5.43	bt	7
C_5	4.50	4.42	ddd	8.5, 8.5, 5.5	4.51	4.45	ddd	8.5, 8.5, 5.5	4.51	4.44	ddd	8.5, 8.5, 5.5
C_6	5.13	5.16	bd	8.5	5.14	5.17	bd	8.5	5.14	5.19	bd	8.5
C_8	1.93	1.82	bt	7					1.97	1.86	bt	7
C_{10}					5.34	5.34	bt	7				
C_{11}	2.67	2.15	m		-	-			2.56	2.31	m	
C_{12}	-	-			3.98	4.02*	t	6.5	-	-		
C_{13a}	4.92	4.86	d	7		2.38	ddd	13, 6.5, 6.5	3.14	2.95	d	7
C_{13b}	s					2.27	ddd	13, 6 , 6				
C_{14}	4.92	4.99	d	7	5.09	5.21	bt	7	5.29	5.45	bt	7.5
C_{16}	1.85	1.52	bs		1.72	1.67	bs		1.74	1.62	bs	
C_{17}	1.79	1.51	bs		1.64	1.56	bs		1.62	1.47	bs	
C_{18}	1.09	0.84	d	7	1.60	1.63	bs		1.06	0.93	d	7
C_{19}	1.65	1.49	bs		1.70	1.54	bs		1.66	1.50	bs	
C_{20}	1.81	1.65	bs		1.82	1.67	bs		1.81	1.65	bs	

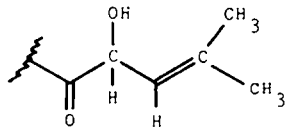
* other epimer = 3.96 ppm.

Acetylation of compound A (Ac_2O/pyridine/room temp.) produced a triacetate which showed simplified ^1H NMR signals from the C-1 methylene ($\delta = 3.26$ ppm, d), as illustrated in the related compound atomaric acid.[5] Finally, part structure **1** was strongly indicated by the base peak m/z 189* in the mass spectrum.



m/z 191.106909
($C_{12}H_{15}O_2$)

1



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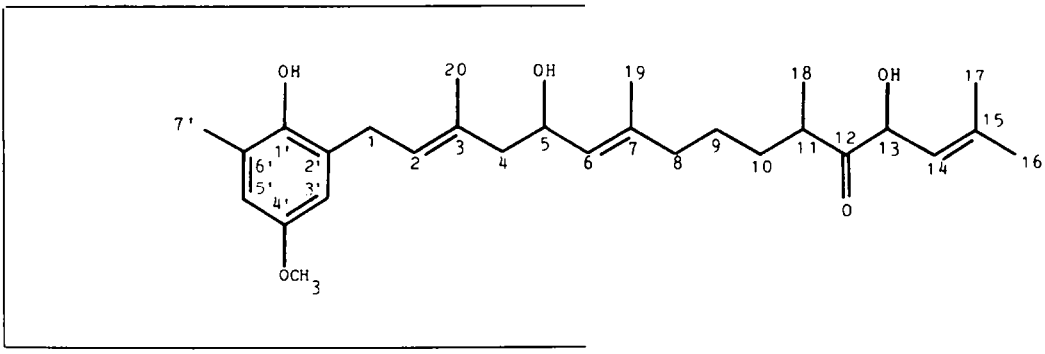
reaction smoothly afforded carboxylic acids which were methylated with CH_2N_2 to yield the corresponding methyl esters. The major product was investigated by ^1H NMR (in C_6D_6) and was consistent with structure **3** (Fig. 1). The methyl ester was observed at 3.42 ppm (ϕ–OMe, $\delta = 3.50$ ppm) and the doublet Me group at 1.06 ppm. Other bands in the ^1H NMR spectrum were identical to product A except that signals attributed to protons at C_{13}, C_{14}, C_{15}, C_{16} and C_{17} were lacking.

We conclude that compound A can be assigned the following structure:



The final structure assignment for compound A was accomplished by extensive 360 MHz ^1H NMR experiments involving double resonance techniques (Table 3). These data were consistent with the ^{13}C NMR spectrum, in particular with the off-resonance decoupling experiments. In the ^{13}C NMR spectrum, only three methylene carbons ($\delta = 31.84$, 30.48 and 25.18 ppm), and the unstrained ketone ($\delta = 214.07$ ppm, s) were not yet assigned. Considering the lack of quaternary carbons in the ^{13}C NMR spectrum, the multiplicity (AB pattern) and the chemical shift (4.86 ppm) of the α to alcohol proton in the ^1H NMR spectrum, it seemed reasonable to organize the ketone and alcohol to form an α-ketol (part structure 2).

This placement was confirmed by oxidative cleavage of the α ketol with alkaline hydrogen peroxide.[6] The

High resolution mass spectral fragmentations gave additional support for the structure of compound A (Fig. 2).

Compound B, obtained as a colorless oil, had a molecular formula $C_{28}H_{42}O_4$ (determined from high resolution mass spectrometry). IR absorptions suggested the presence of alcohol functionality ($\nu_{OH} = 3400\ cm^{-1}$), and the aromatic ring ($\nu_{C=C} = 1600\ cm^{-1}$). The UV spectrum was similar to that of A although the extinction coefficients of the absorptions (214 nm $\epsilon = 8900$ and 289 nm $\epsilon = 2400$) were lower. Compound B showed several spectral features in common with A, particularly the benzenoid chromophore and part of the diterpenoid moiety. The absence of the ketone functionalities and the presence of an additional double bond was established by ^{13}C and ^1H NMR analysis.

^1H NMR decoupling experiments allowed (Table 4) confident assignments of the additional unsaturation and OH groups at C-5 and C-12†. The high resolution mass and ^1H NMR data of the acetylated product confirmed

†In C_6D_6, the apparent presence of two epimers at C-12 was indicated ($\delta = 4.02$ and 3.96 ppm). The epimers appeared to be a 60/40 mixture, but the two metabolites were not separable.

m/z 189.091533
($C_{12}H_{13}O_2$)

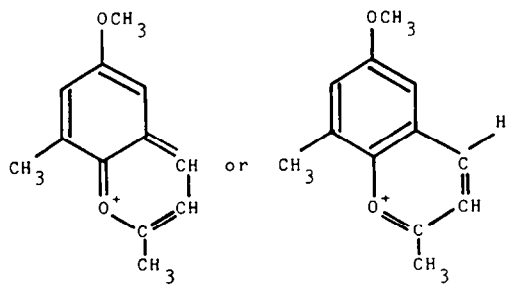
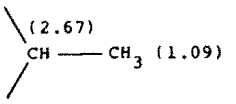
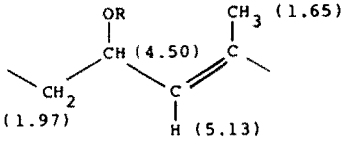
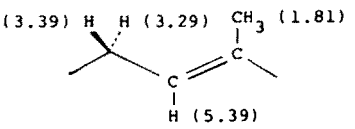
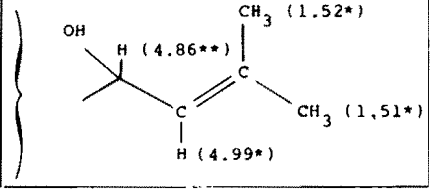
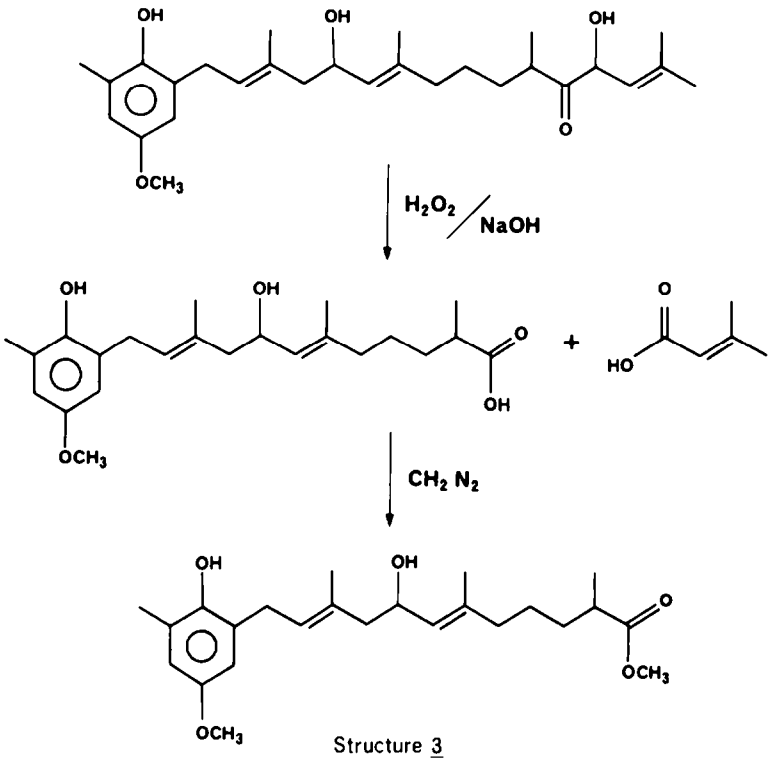


Table 3. ^1H NMR decoupling experiments data for compound A (in $CDCl_3$)

Irradiated signal		Modified signals						Assigments
mult.	δ(ppm)	δ(ppm)	before	after	δ(ppm)	before	after	
d	1.09	2.67	m	bt				
m	2.67	1.09	d	s				
sext.	4.50	5.13	d	s	1.97	AB collapsed	d	
d	5.13	4.50	sext.	t	1.65	bs	s	
bt	5.39	1.81	bs	s	3.39/3.29	d.AB	AB pattern	
bs	4.92	1.79	bs	s	1.85	bs	s	
AB* pattern	4.86**	4.99	d	s				
	4.99	4.86	d	s	1.51	bs	s	
					1.52	bs	s	

* in C_6D_6

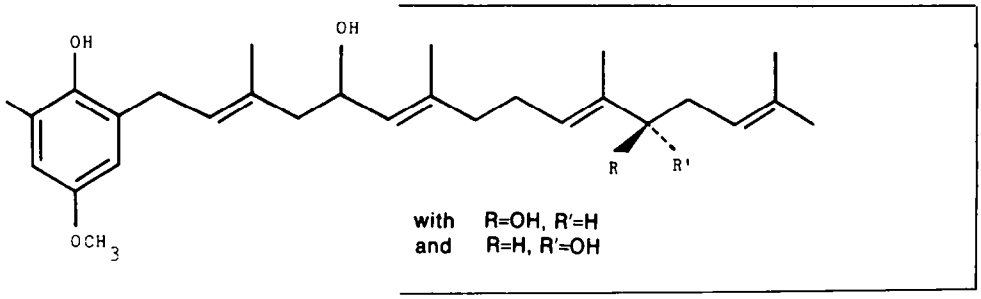
** by acetylation, this doublet downfield shifted from 4.86 to 5.95 ppm.



Structure 3

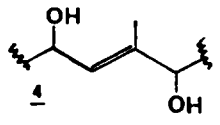
Fig. 1. Oxidative cleavage of compound A.

the presence of three hydroxylic groups. Thus structure **B** appeared to be:



Proof of the proposed structure for **B** was obtained by oxidation of the alcohol functionalities to the corresponding diketone (in particular to eliminate the possible part structure **4**).

Treatment of **B** first with methyl iodide in potassium carbonate, then with pyridinium chlorochromate at $-10°$ in CH_2Cl_2 yielded structure **5**



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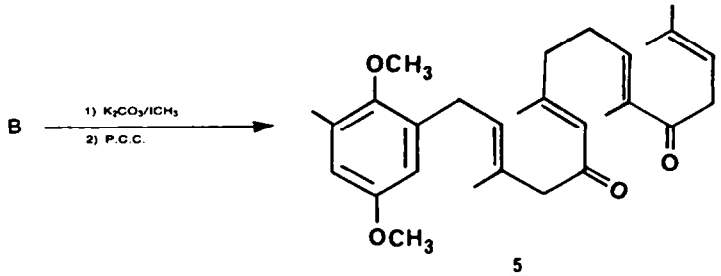
The lack of signals due to OH functionalities (IR and NMR), and in the ^1H NMR spectrum of **5**, the presence of bands due to methylene groups attached to CO functionalities (C_4 $\delta = 3.11$ ppm, bs, and C_{13} $\delta = 3.31$ ppm, d, $J = 7$ Hz) confirmed the presence of a diketone. The chemical shifts of C_6 ($\delta = 6.13$ ppm, s) and C_{10} ($\delta = 6.56$ ppm, t) provided considerable support in the subsequent assignment of structure **5**.

The last product, compound **C**, was rapidly assigned



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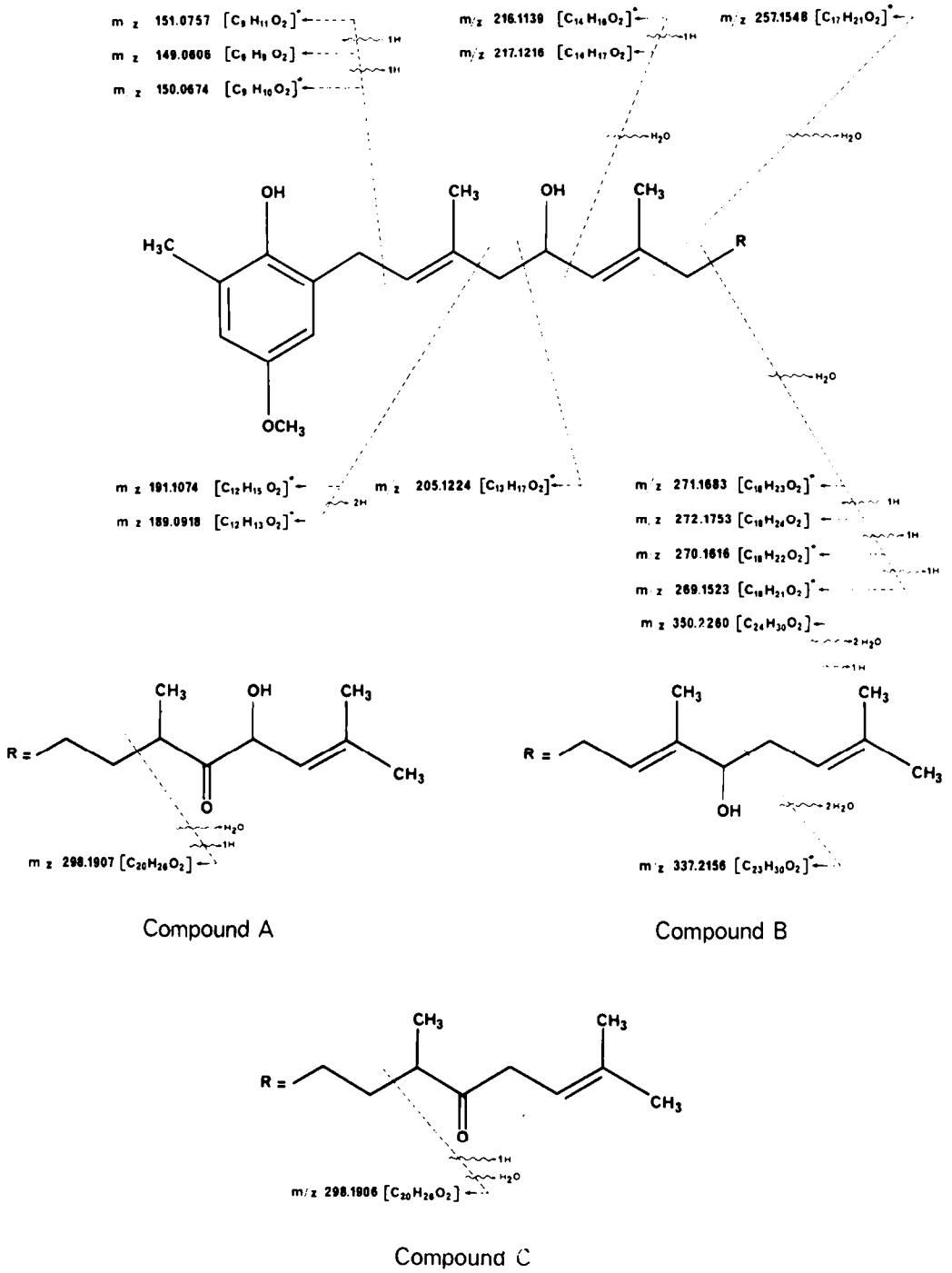
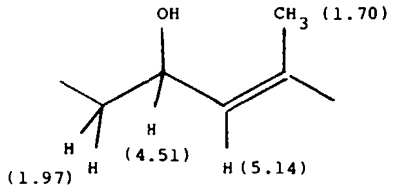
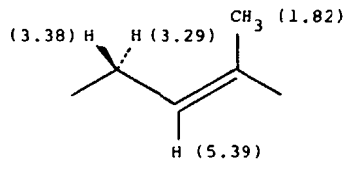
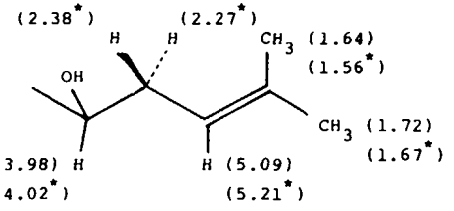


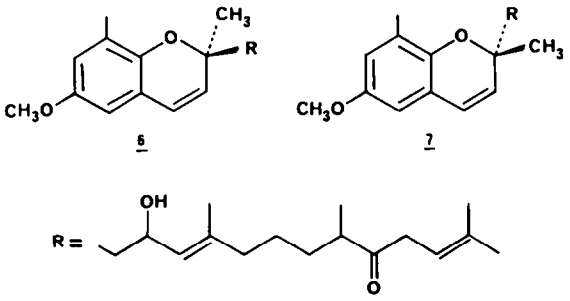
Fig. 2. Apparent mass spectral fragmentations for the diterpenoid-hydroquinones A–C. The ions indicated with the star exist at 42 amu higher values in the corresponding acetates.

Table 4. ^1H NMR decoupling experiments for compound B in $CDCl_3$

Irradiated signals		Modified signals						Assignments
mult.	δ(ppm)	δ(ppm)	before	after	δ(ppm)	before	after	
m	4.51	5.14	d	s	1.97	m	d	
d	5.14	4.51	m	t	1.70	bs	s	
bt	5.39	1.82	bs	s	3.38/3.29	d.AB	AB pattern	
t*	4.02	2.38*/2.27*	dd.AB	d.AB pattern				
bt	5.09	1.72	bs	s	1.64	bs	s	
bt*	5 21*	1.67*	bs	s	2.38*/2.27*	ddd	d.AB pattern	
		1.56*	bs	s				

* in C_6D_6

by comparison of its ^1H and ^{13}C NMR data with those of a product obtained form *Halidrys siliquosa*:[8] 5'-hydroxy-12' oxohalidrol. Attempts to transform C to A were not successful. We noted however, that C spontaneously degraded to several products. One of these compounds (C_1) was isolated and its structure assigned. The IR spectrum of C_1 showed absorptions for ketone ($\nu_{C=O}$ 1710 cm^{-1}) and olefin ($\nu_{C=C}$ 1620 cm^{-1}) functionalities. The UV spectrum consisted mainly of absorption at 213 nm (ϵ = 10,000). A ^1H NMR (in C_6D_6) comparison between C and C_1 illustrated two sets of shifts for the meta-coupled aromatic protons (δ = 6.4 and 6.58 ppm), two Me's for C-20 (δ = 1.3 and 1.35 ppm double singlets indicative of two isomers) and the existence of an ethylenic AX pattern (δ = 6.1, d, J = 10 Hz for C-1 and δ = 5.42, d, J = 10 Hz for C-2). These ^1H NMR results, in comparison with model compounds[9] allowed the structure of C_1 to be assigned as the isomeric mixture of chromene structures **6** and **7**.



In an other part of our work, we determined the antibacterial properties of these new products (by the agar plates assay disc method: 0.4 mg/disc). Compound A showed *in vitro* activity against *Pseudomonas aeruginosa* (6 mm total zone of inhibition) but was not active against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae.* Compounds B and C showed no activity. Other biological activities were investigated and these results will be published in a subsequent paper.

Figure 2 illustrates the possible fragmentations of the major compounds as detemined by high resolution mass spectrometry.

EXPERIMENTAL

IR spectra were recorded on a Perkin Elmer Model 621 spectrophotometer and optical rotations were measured on a Roussel Jouan (T 71) polarimeter using a 0.5 cm microcell. ^1H NMR spectra were recorded at 360 MHz on an Oxford Magnetics/Nicolet Instruments "Home-Built" and at 250 MHz on CAMECA Proton Magnetic Resonance Spectrometers. ^{13}C NMR were recorded at 50 MHz on a Nicolet 1180 E Multinuclear (Wide-Bore) Spectrometer. All chemical shifts are reported relative to Me_4Si (δ = 0) and coupling constants are in Hertz. UV spectra were recorded on a Perkin Elmer 551 spectrophotometer and high-resolution mass spectra were obtained through the Biorganic and Biomedical Mass Spectrometry Resource Center, Space Sciences Laboratory University of California, Berkeley.

Extraction and isolation of constituents. The alga (0.530 kg) collected in June 1980 at Banyuls sur Mer, France, was frozen, ground to a fine powder with a blender in presence of $MeOH/H_2O$ (7/3 v/v) and extracted three times with $MeOH/H_2O$. The extracts were left one night at −30° in order to precipitate the lipids. After filtration, the MeOH was evaporated and the aqueous phase was extracted with ether. After solvent evaporation, the extraction yielded 1 g of extract that was applied to an open column of silica gel. The column was eluted with a solvent gradient from hexane to ether.

Fractions of 100 ml were collected: The more polar compounds (A and B) were eluted with 5/5 hexane/ether and the least polar fraction (6/4 hexane/ether) contained C. Each compound was subsequently purified by HPLC on a 10 mm × 100 cm preparative silica gel column using various proportions of EtOAc in TMP (trimethylpentane).

Compound A. (2E, 6E, 14E)-1-(1'-hydroxy-4'-methoxy-6'-methyl-phenyl)-5, 13-dihydroxy-12-one-3, 7, 11, 15-tetramethyl hexadeca-2, 6, 14-triene. The ketol was isolated as an oil (80 mg, 0.15% dry wt) and showed the following spectral features: $[\alpha]_D$ −96° (*c* = 9.96, MeOH); IR: ν_{max}^{film} = 3450, 1705, 1600, 1480 cm^{-1}; UV: λ_{max}^{MeOH} = 214 nm (ϵ = 11500), 288 nm (ϵ = 3100).

High resolution mass spectra: M^+-H_2O = 440.29499 $C_{28}H_{40}O_4$ (requires 440.29267) *m/z* (%): 440(6), 422(1.7), 298(1.45), 257(2.96), 255(2.19), 253(1.66), 217(7.49), 216(8.11), 215(2.25), 206(3.14), 205(4.02), 204(2.94), 203(5.02), 202(3.25), 192(13.89), 191(92.53), 190(15.17), 189(81.94), 188(9.96), 177(4.19), 175(5.66), 175(3.23), 165(2.77), 163(7.1), 152(13.16), 151(72.6), 150(13.37), 148(4), 147(11.8), 145(4.63), 141(3), 137(3.72), 135(4.33), 131(3.47), 125(4.04), 123(6.7), 121(7.9), 119(5.28), 117(3.5), 109(6.53), 107(16.7), 105(12.37), 95(13.4), 93(15.68), 91(14), 85(22.75), 84(7.56), 83(100), 81(18.29), 79(10.20), 77(5.72), 71(4.7).

Acetates M^+-AcOH = 524.3130 $C_{32}H_{44}O_6$ (requires 524.3094) *m/z* (%): 524(1.5), 482(7.5), 464(9.3), 422(8.4), 404(1), 397(12.5), 379(2.4), 356(3.3), 342(1.4), 339(11.7), 299(8.4), 298(6.1), 271(12.0), 259(8.5), 257(6.1), 255(4.0), 248(5.8), 247(6.0), 245(3.2), 235(12.9), 234(3.7), 233(18.7), 229(13.9), 227(4.8), 218(9.7), 217(13.6), 206(6.9), 205(10.3), 204(7.4), 203(10.9), 199(2.7), 191(81.2), 189(59.1), 188(4.3), 177(5.4), 175(8.7), 165(5.9), 163(9.09), 152(17.5), 151(100), 150(13.5), 149(19.2), 147(24.5), 137(9.1), 135(7.7), 133(11.5), 127(12.9), 125(12.2), 123(8.6), 121(11.9), 119(12.7), 109(9.9), 107(17.7), 105(15.4), 95(16.0), 93(19.1), 91(14.3), 85(62.9), 83(29.6), 81(22.4), 79(11.1), 77(7.3).

^1H NMR acetylated product A ($CDCl_3$): δ = 6.59(bs, 1H, 6.74*), 6.56(bs, 1H, 6.54*), 5.80(bd, 1H, J = 7 Hz, 5.95*), 5.64(dd, 1H, J = 7 Hz, 5.95*), 5.26(bt, 1H, J = 7 Hz, 5.43*), 5.09(bd, 1H, J = 7.5 Hz, 5.20*), 5.06(d, 1H, J = 6.5 Hz, 5.20*), 3.75(s, 3H, 3.38*), 3.14(d, 2H, J = 6.5 Hz, 3.26*), 2.49(m, 1H), 2.30(s, 3H, 2.04*), 2.13(s, 3H, 1.89*), 2.12(s, 3H, 1.78*), 1.94(s, 3H, 1.72*), 1.85(s, 3H, 1.67*), 1.81(s, 3H, 1.67*), 1.70(s, 3H, 1.53*), 1.66(s, 3H, 1.49*), 1.13(d, 3H, J = 7 Hz, 1.16*). ^1H NMR (Table 2) and ^{13}C NMR (Table 1).

Compound B. (2E, 6E, 10E, 14E)-1-(1'-hydroxy-4'-methoxy-6'-methyl phenyl)-5, 12 dihydroxy-3, 7, 11, 15-tetramethyl hexadeca-2, 6, 10, 14-tetraène. The diol epimeric mixture was isolated as an oil (60/40 mixture) $[\alpha]_D$ − 1.9 (*c* = 9.3, MeOH); IR: ν_{max}^{film} = 3400, 1600, 1480 cm^{-1}; UV: λ_{max}^{MeOH} = 214 nm (ϵ = 8900), 289 nm (ϵ = 2400).

High resolution mass spectra: M^+-2H_2O = 406.28833 $C_{28}H_{38}O_2$ (requires 406.2862) *m/z* (%): 406(4.54), 404(3.6), 305(2.4), 337(2.22), 271(3.18), 207(2.11), 269(2.8), 257(1.45), 255(4.2), 217(2.9), 215(2.67), 205(2.3), 203(4.6), 201(7.8), 191(50.8), 190(14), 189(100), 188(22.8), 175(4.8), 173(10.1), 163(6), 159(5.76), 151(67.26), 150(9.72), 149(5.3), 147(7.34), 145(11), 143(4.25), 135(35.74), 133(11), 131(7.6), 121(12.9), 119(13.6), 117(4.5), 115(5.83), 109(11.4), 107(24.33), 105 (20.84), 97(7), 95(12.5), 93(40.4), 91(24.3), 81(31), 79(16.5), 77(14), 71(6), 70(6.58).

Acetate M^+-2 AcOH = 448.2976 (Calc for $C_{30}H_{40}O_3$ 448.2967). *m/z* (%): 448(10.8), 406(3.8), 392(8.6), 379(4.3), 313(17.6), 299(2.2), 284(1.8), 271(4.8), 257(2.0), 255(13.7), 233(5.8), 229(5.5), 217(3.7), 216(3.6), 215(2.6), 206(5.2), 205(6.3), 201(48.0), 200(8.8), 199(13.7), 192(6.7), 191(47.9), 190(6.8), 189(19.9), 185(7.7), 175(6.9), 173(9.4), 171(5.87), 165(3.6), 163(4.9), 161(4.7), 159(16.3), 157(11.2), 152(17.9), 151(100), 150(11.8), 149(18.7), 147(11.5), 145(32.4), 135(61.5), 134(13.07), 133(25.9), 131(13.1), 129(6.5), 123(5.9), 121(18.9), 119(26.7), 117(7.4), 109(20.5), 107(41.8). 105(30.8), 95(14.4), 93(71.7), 91(34.1), 81(22.3), 79(20.5), 77(18.4), ^1H NMR (Table 2) and ^{13}C NMR (Table 1).

^1H NMR (acetylated product B in $CDCl_3$) δ_{ppm} = 6.59 (bs, 1H), 6.55(bs, 1H), 5.66(dd, 1H, J = 7 Hz), 5.37(t, 1H, J = 7 Hz), 5.26(t, 1H, J = 7 Hz), 5.10(d, 1H, J = 6.5 Hz), 5.08(t, 1H, J = 7 Hz),

*Values in C_6D_6.

4.98(bt, 1H, J = 7.5 Hz), 3.75(s, 3H), 3.13(d, 2H, J = 7 Hz), 2.31(s, 3H), 2.12(s, 3H), 2.02(s, 3H), 1.95(s, 3H), 1.71(s, 3H), 1.70(s, 3H), 1.67(s, 3H), 1.60(s, 3H), 1.59(s, 3H).

Compound **C**. (2E, 6E, 14E)-1-(1'-hydroxy-4'-methoxy-6'-methylphenyl)-5-hydroxy-12-one-3, 7, 11, 15-tetramethyl hexadeca-2, 6, 14-triene. This compound was obtained as an oil and presented the spectral features described in 5'-hydroxy-12' oxohalidrol. High resolution mass spectra: $M^{+}-H_2 = 440.2940$ $C_{28}H_{40}O_4$ (requires 440.2926). *m/z*(%): 440(0.9), 424(2.7), 422(1.8), 298(1.06), 257(1.24), 217(4), 215(1.7), 205(3.3), 203(2.65), 192(5.76), 191(47.56), 190(14.4), 189(100), 188(21.65), 173(8.8), 152(6.2), 151(39), 150(6), 149(7.22), 147(11), 145(6.3), 137(10.8), 135(8.9), 133(4.1), 123(6.09), 121(6.12), 119(9.2), 117(10.4), 115(6.44), 113(5.18), 107(13.1), 105(12.3), 95(12.56), 93(18.55), 91(15), 85(10.5), 83(11.11), 81(19.45), 79(16.49), 77(10.9), 71(8.9), 70(2.48). ^{1}H NMR (Table 2) and ^{13}C NMR (Table 1).

Compound C_1 (*yellow oil*). IR: $\nu_{max}^{film} = 1710\,cm^{-1}$, $1620\,cm^{-1}$; UV: $\lambda_{max}^{MeOH} = 213$ nm ($\epsilon = 10.000$). ^{1}H NMR (C_6D_6) $\delta = 6.58$(bs, 1H), 6.4(bs, 1H), 6.1(d, 1H, J = 10 Hz), 5.47(bt, 1H, J = 7.5 Hz), 5.42(bd, 1H, J = 10 Hz), 4.92(bd, 1H, J = 8.5 Hz), 4.41(ddd, 1H, J = 8.5, 8.5 and 6 Hz), 3.34(s, 3H), 2.96(d, 2H, J = 7 Hz), 2.13(s, 3H), 1.85(bt, 1H, J = 7 Hz), 1.63(s, 3H), 1.55(s, 3H), 1.48(s, 3H), 1.35(s) + 1.30(s) = 3H, 0.93(d, 3H, J = 7Hz).

Acetylations. Excess Ac_2O and dry pyridine were added to milligram quantities of natural products and the resultant soln was stirred at room temp. overnight.

Oxidative cleavage with alkaline hydrogen peroxide. To 0.22 ml of methanolic soln (0.022 mM) of **A** (10 mg) was added 0.05 ml 2M NaOH, 0.044 m mole EDTA and 0.06 ml H_2O_2 (0.5 M soln). After 1 hr, the soln was extracted with diethyl ether. After solvent evaporation, the acidic products were treated with CH_2N_2 in order to obtain the corresponding methyl esters which were purified on PLC (thickness 1 mm) in hexane/AcOEt 50/50. The main product **3** ($R_f = 0.38$) gave the following spectral data. IR: $\nu_{max}^{film} = 3440\,cm^{-1}$, $1730\,cm^{-1}$, $1600\,cm^{-1}$; ^{1}H NMR (C_6D_6) δ = 6.84(d, 1H, J = 2.9 Hz), 6.8(d, 1H, J = 2.9 Hz), 5.50(bt, 1H, J = 7 Hz), 5.25(bd, 1H, J = 8.5 Hz), 4.46(ddd, 1H, J = 8.5, 8.5 and 5.5 Hz), 3.50(s, 3H), 3.42(s, 3H), 3.36(dd, 1H, J = 16 and 7.5 Hz), 3.21(dd, 1H, J = 16 and 6.5 Hz), 2.30(m, 1H), 2.20(s, 3H), 1.82(bt, 1H, J = 7 Hz), 1.64(s, 3H), 1.52(s, 3H), 1.06(d, 3H, J = 7 Hz).

Methylation of compound **B**. To a soln of **B** (22 mg) in dry acetone (2 ml) were added MeI (0.1 ml) and anhyd K_2CO_3 (300 mg). The mixture was refluxed for 5 hr, diluted with water and extracted with ether. The ether layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. The resulting product was subjected to HPLC to give the pure dimethoxyphenyl product.

Oxidation of methylated compound **B** *with PCC.* The methylated **B** (60 mg) was treated for 5 hr at $-10°$ with excess (100 mg) pyridinium chlorochromate (PCC) in CH_2Cl_2 (2 ml) after which the reaction was quenched with the addition of 50 ml diethyl ether. After filtration through a thin layer of silica gel, the oxidation product **5** was purified using a "lobar" column (24 × 1 cm) (20% AcOEt/80% isooctane). IR: $\nu_{max}^{film} = 1680\,cm^{-1}$, $1600\,cm^{-1}$. ^{1}H NMR ($CDCl_3$): $\delta = 6.57$(bs, 2H), 6.56(t, 1H, J indeterminable), 6.13(bs, 1H), 5.43(t, 1H, J = 7 Hz), 5.28(t, 1H, J = 7 Hz), 3.76(s, 3H), 3.68(s, 3H), 3.37(d, 2H, J = 7 Hz), 3.31(d, 2H, J = 7 Hz), 3.11(bs, 2H), 2.30(s, 3H), 2.28(s, 3H), 1.88(s, 3H), 1.74(bs, 6H),(s, 3H).

Acknowledgements—We wish to thank M. M. Bandurraga, S. Look, V. Paul (La Jolla, SIO) and M. Bon (Toulouse) for the measurements of high resolution ^{1}H NMR spectra. Research at SIO was sponsored by NOAA, Office of Sea Grant under grant #NA80AA-0-00120, Project R/WP-22.

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