

MEDITERRANEOL A, A NOVEL REARRANGED DITERPENOID-HYDROQUINONE FROM THE
MARINE ALGA *CYSTOSEIRA MEDITERRANEA*

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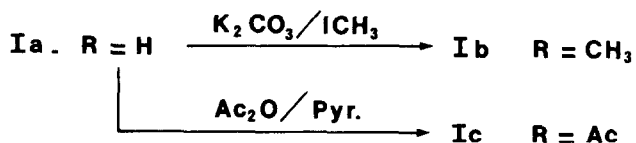
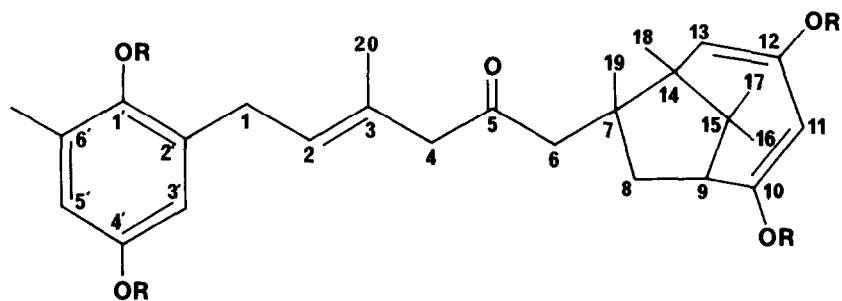
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Summary : The isolation and structure determination by combined chemical and spectral methods of a novel biologically active metabolite mediterraneol A (Ia) from *Cystoseira mediterranea* are reported. Mediterraneol A was found to be an inhibitor of mitotic cell division.

Along the French Mediterranean coastline, brown marine algae of the order Fucales (Phaeophyta) have been diminishing for the past fifty years. Those of the family Sargassaceae have essentially disappeared, while algae of the family Cystoseiraceae are abundant and appear to contain defensive chemicals which provide protection against potential predators such as sea urchins. We have focused our attention on the family Cystoseiraceae since this apparent adaptation has provided a useful rationale for the discovery of novel physiologically-active metabolites. In this report, we wish to provide the structure of an unprecedented bicyclic diterpenoid hydroquinone, mediterraneol A (Ia), from the Mediterranean alga *Cystoseira mediterranea*. Mediterraneol A was found to inhibit motility of the sperm and cell division of the fertilized eggs of the sea urchin *Paracentrotus lividus* with ED₅₀ values of 2 µg/ml.

Cystoseira mediterranea was collected near Banyuls-sur-Mer (France) during June and July (1981-1982). The alga was freeze-dried and subsequently extracted with chloroform/methanol (1/1). Diterpenoids were obtained by standard open-column silica gel chromatography of the crude extract (1.5 % of dry weight alga). The natural metabolite, mediterraneol A (Ia), was isolated (23 % from the ether extract) from a major chromatography fraction by HPLC on µ-Porasil (40 % EtOAc/isooctane). Since mediterraneol A was recognized as a tautomeric mixture, the compound was methylated (CH₃I/K₂CO₃) to yield the pure tetramethoxy product methyl mediterraneol A (Ib) as a white foam by repeated HPLC (8 % EtOAc/isooctane).

Ib showed $[\alpha]_D = 0$ (c 2.5, CHCl₃) and analyzed for C₃₁H₄₄O₅ by peak matching M⁺ m/z obs 496.3179 ; calc. 496.3186. The infrared spectrum (film) of this novel substance established the presence of an unstrained ketone ($\nu_{C=O}$ 1710 cm⁻¹), conjugated double bond



($\nu_{\text{C}=\text{C}}$ 1615 cm^{-1}) and an aromatic ring (ν 1595 cm^{-1}). In the UV spectrum, absorptions at 215 nm ($\epsilon = 19000$) and 289 nm (2800) indicated a hydroquinone chromophore and a shoulder at 237 nm the presence of conjugated double bonds.

The ^1H (Table 1) and ^{13}C NMR spectra including decoupling experiments, in conjunction with the other spectral characteristics, led us to conclude that Ib was a bicyclic diterpene coupled with a methyl-p-hydroquinone.

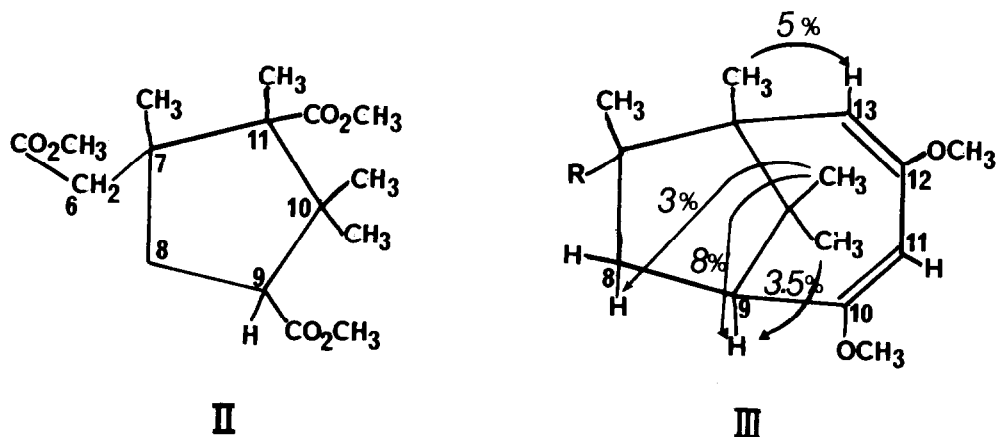
Table 1 : 360 MHz ^1H NMR data for compound Ib (6 ppm values relative to internal TMS)

$n^\circ\text{C}$	$\delta(\text{CDCl}_3)$	$J(\text{Hz})$	$n^\circ\text{C}$	$\delta(\text{CDCl}_3)$	$J(\text{Hz})$
C4'-OCH ₃	3.73	s	C8 _a	2.47	dd 4.5, 12
C11-OCH ₃	3.72	s	C8 _b	1.91	d 12
C1'-OCH ₃	3.68	s	C9	3.15	d 4.5
C13-OCH ₃	3.75	s	C11	5.93	d 1.8
\emptyset -CH ₃	2.26	s	C13	6.00	d 1.8
C3'	6.55	bs	C16	1.23	s
C5'	6.54	bs	C17	1.04	s
C1	3.30	d 7.5	C18	1.10	s
C2	5.38	t 7.5	C19	1.12	s
C4	3.08	bs	C20	1.73	s
C6 _a	2.90	d 16			
C6 _b	2.72	d 16			

The following considerations provided considerable support in the assignment of structure Ib.

The first isoprene unit (C1-C4+C20) was arranged in agreement with spectral analysis and known compounds¹⁻³ with an E olefin geometry based upon the C20- methyl resonance observed at higher than 20 ppm in the ¹³C NMR spectrum (γ shielding effect).⁴⁻⁵ Carbons C4, C5 and C6 were organized by comparison with bifurcarenone,⁶ and this arrangement was confirmed by reduction with LiAlH₄ at room temperature to yield the corresponding C5 alcohol (ν_{OH} 3450 cm⁻¹ and $\delta_{\text{C}2}$ 2.35 ppm, 2 H, d, $J = 7$ Hz, $\delta_{\text{C}3} = 4.1$ ppm, 1 H, bm, $\delta_{\text{C}4}$ 2.0 ppm, 1 H, dd, $J = 9, 16$ Hz and 1.85 ppm, 1 H, dd, $J = 5, 16$ Hz).

Acetylation of mediterraneol A (Ac₂O/py/RT) gave, after HPLC purification (10 % EtOAc/isooctane), the oily tetraacetate Ic, which analyzed for C₃₁H₃₆O₅ by HRMS (M⁺ - 2 HOAc m/z 488.2535, calc. 488.2560). Infrared and NMR spectra of Ic showed that all hydroxyl functional groups had been acetylated. Unlike the ¹H NMR spectrum of Ia, that of Ic very clearly showed the meta positions and couplings of the 2 protons on the hydroquinone ring [δ 6.80 (1 H, d, $J = 2.5$) and δ 6.77 (1 H, d, $J = 2.5$)]. Acetylation of the C12 and C10 enol also induced significant low-field shifts of the C11 and C13 protons which themselves were coupled by a "W" coupling of 1.8 Hz. These protons were shifted from ca. 5.95 in Ib to 6.45 in Ic. Consideration of the methylation behavior, the presence of a conjugated diene system and proton decoupling information led to the overall formulation of the structure of Ib.



Support for the assignment of the bicyclic portion of mediterraneol A came from an oxidative cleavage reaction of Ib. Treatment of Ib with OsO₄/H₂O₂ in t-butanol, followed by treatment with Pb(OAc)₄ and CH₂N yielded the cleavage product II after purification by HPLC. Compound II analyzed for C₁₆H₂₆O₆ by HMRS (M⁺ m/z = 314.722, calc. 314.1731) and showed IR bands characteristic of an ester carbonyl (ν 1735 cm⁻¹). The ¹H NMR spectrum of II showed singlet methyl resonances at δ 1.07, 1.10, 1.17 and 1.49 for the methyl groups at C7, C10 and C11. Three methyl ester methyl resonances were observed at δ 3.61, 3.65 and 3.72 and the C6 protons were observed as an AB pattern at δ 2.75 and 2.27 ($J = 15.7$ Hz). The C8-C9 protons were readily distinguished in this spectrum as the expected ABM pattern [C8 : δ 2.55 (1 H, bt, $J = 11.5$), δ 2.24 (1 H, dd, $J = 12.5, 6.5$) ; C9 δ 3.37 (1 H, dd, $J = 6.5, 12.5$ Hz)].

Although the oxidation product II strongly supported the overall assignment of the structure of mediterraneol A, the relative stereochemistry of the methyl substituent at C7 was not clear. Nuclear Overhauser enhancement experiments in the ^1H NMR spectrum of Ib showed several additional features (shown in part structure III) which supported the formulation of the bicyclic portion of the molecule. Enhancements of the C13 olefin proton were observed upon irradiation of the C11 methyl. In addition, irradiation of the C10 methyl groups produced enhancements of the bridgehead proton at C9 and the exo proton at C8.

Mediterraneol A (Ia) possesses an unprecedented bicyclo [4.2.1] nonane skeleton which is probably produced by rearrangement of a regularly terpenoid precursor. *Cystoseira mediterranea* contains several related metabolites and a more comprehensive investigation, including assignment of the full stereochemistries of these compounds, will be provided in a full paper.

Acknowledgements

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4. ^{13}C NMR (50.32 MHz) δ TMS (CDCl_3): 210.8 (s), 158.9 (s), 156.9 (s), 155.6 (s), 154.1 (s), 134.8 (s), 131.2 (s), 130.8 (s), 127.6 (d), 114.1 (d), 113.3 (d), 93.4 (d), 90.8 (d), 56.2 (q), 55.5 (q), 55.5 (q), 55.3 (q), 52.7 (s), 52.6 (s), 49.3 (t), 47.5 (s), 39.4 (t), 38.8 (d), 35.1 (t), 28.5 (t), 26.5 (q), 25.6 (q), 24.2 (q), 21.0 (q), 16.2 (q), 16.0 (q).
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