Mediterraneols: A Novel Biologically Active Class of Rearranged Diterpenoid Metabolites from *Cystoseira mediterranea* (Pheophyta)

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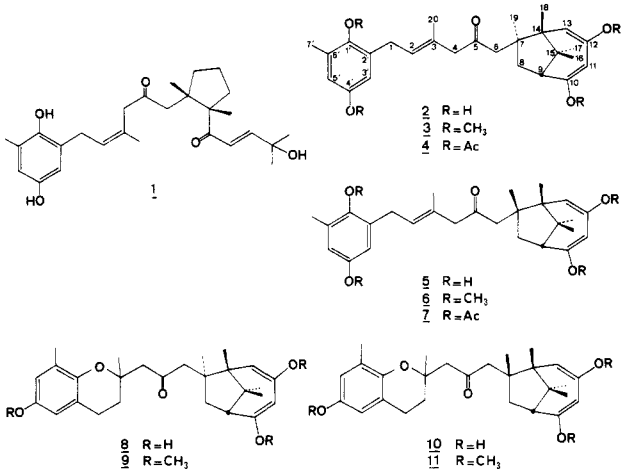
Isolation and structure of mediterraneols A, B, C, and D, a novel class of rearranged diterpenoids of mixed biogenesis, are reported from the brown alga *Cystoseira mediterranea*. These compounds inhibit mitotic cell division (ED_{50} = 2 μg/mL) and present an in vivo activity during P388 tests (T/C = 128%). The structures of these metabolites were determined by chemical and spectral methods including ^1H–^{13}C 2-D shift correlation. The relative stereochemistry was obtained by NOE difference spectroscopy.

During the past few years, many authors have reported new metabolites involved in the chemical defense of marine animals. By comparison, little data on the chemical defense of marine seaweeds have been published with the exception of Fenical's works on green[1] or brown[2] algae. Nevertheless, we began a systematic study of Cystoseiraceae (Phaeophyta) because in the Mediterranean sea these algae seem to offer more resistance than other brown seaweeds to their potent predators, sea urchins. Our first experiments on *Cystoseira elegans*[3] allowed us to isolate—mainly from fronds in agreement with Steinberg's works[4]—new diterpenoid hydroquinones, inhibitors of mitotic cell division (ED_{50} = 3.75 μg/mL) in the fertilized urchin (*Paracentrotus lividus*) egg essay. Unfortunately, this activity has not been observed during in vivo P388 leukemia tests.

In this paper, we report the isolation and structure determination of the main diterpenoids of mixed biogenesis from the related alga *Cystoseira mediterranea*. These metabolites inhibit the mobility of sea urchin sperm and the mitotic cell division (ED_{50} = 2 μg/mL) fertilized urchin eggs. Moreover, compounds **2** and **5** present in vivo activity during P388 leukemia tests (T/C = 128% at 32 mg/kg).[5] Meroditerpenoids have already been isolated from Cystoseiraceae,[6] but the novel metabolites presented in Chart I possess an unprecedented rearrangement in the diterpene skeleton that we had earlier based on chemical degradations.[7]

C. 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 purified by standard silica gel chromatography of the crude extract.

Chart I



Compound **1** was directly obtained by high-pressure liquid chromatography (HPLC) during purification of the main fraction, and the structure was rapidly assigned as bifurcarenone[8] by comparison of spectral results. Mediterraneol B (**2**) and A (**5**) were isolated as a mixture by HPLC in the same conditions. These two closely related metabolites were very difficult to purify in their natural form, but successful separation was achieved by using methylation (ICH_3/K_2CO_3) or acetylation (Ac_2O/py).

The tetramethoxy product **3**, isolated as a white foam, showed $[\alpha]_D$ −8.3° (*c* 2.9, $CHCl_3$) and analyzed for $C_{31}H_{44}O_5$ by mass spectrometry (peak matching) and ^{13}C NMR analysis. IR absorption established the presence of an unstrained ketone ($\nu_{C=O}$ = 1710 cm^{-1}), a conjugated double bond ($\nu_{C=C}$ = 1615 cm^{-1}), and an aromatic ring (ν = 1595 cm^{-1}). In the UV spectrum, absorptions at 215 and 289 nm (ϵ = 18 500, 2900) indicated a hydroquinone chromophore group and a conjugated diene was observed to produce a shoulder at 237 nm.

A formula of $C_{35}H_{44}O_9$ (14 unsaturation equivalents) for the tetracetate **4** was obtained by high-resolution mass spectrometry. The presence of important ions—$C_{15}H_{17}O_4$ (56%), $C_{13}H_{15}O_3$ (89%), $C_{12}H_{13}O_4$ (100%), $C_{12}H_{15}O_2$ (89%), $C_{11}H_{13}O_2$ (52%), $C_8H_9O_2$ (17%)—in conjunction with ^{13}C NMR (Table I) and ^1H NMR analysis allowed the structure **12** to be proposed for the phenol moiety and the first

(1) For some recent examples see: (a) Paul, V. J.; Sun, H. H.; Fenical, W. *Phytochemistry* **1982**, *21*, 468. (b) Paul, V. J.; Fenical, W. *Tetrahedron Lett.* **1982**, 5017. (c) Sun, H. H.; Paul, V. J.; Fenical, W. *Phytochemistry* **1983**, *22*, 743.

(2) (a) Gerwick, W. H.; Fenical, W. *J. Org. Chem.* **1981**, *46*, 22. (b) Gerwick, W. H.; Fenical, W.; Sultanbawa, M. U. S. *Ibid.* **1981**, *46*, 2233. (c) Gerwick, W. H.; Fenical, W. *Ibid.* **1983**, *48*, 3325.

(3) (a) Banaigs, B.; Francisco, C.; Gonzalez, E.; Fenical, W. *Tetrahedron Lett.* **1982**, 3271. (b) Banaigs, B.; Francisco, C.; Gonzalez, E.; Fenical, W. *Tetrahedron* **1983**, *39*, 629. (c) Banaigs, B.; Marcos, B.; Francisco, C.; Gonzalez, E.; Fenical, W. *Phytochemistry* **1983**, *21*, 2865.

(4) Steinberg, P. D. *Science (Washington, D.C.)* **1983**, *233*, 405.

(5) These data are the results of screening performed under the auspices of the Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

(6) Oriente, G.; Piattelli, M.; Ruberto, G.; Tringali, C. *Phytochemistry* **1982**, *21*, 421. Amico, V.; Oriente, G.; Piattelli, M.; Ruberto, G.; Tringali, C. *J. Chem. Res.* **1982**, 262. Amico, V.; Cunsolo, F.; Piattelli, M.; Ruberto, G.; Fronczek, F. R. *Tetrahedron* **1984**, *40*, 1721. Amico, V.; Cunsolo, F.; Piattelli, M.; Ruberto, G. *Phytochemistry* **1984**, *23*, 2017.

(7) Francisco, C.; Banaigs, B.; Valls, R.; Codomier, L. *Tetrahedron Lett.* **1985**, 2629.

(8) Sun, H. H.; Ferrara, N. M.; McConnell, O. J.; Fenical, W. *Tetrahedron Lett.* **1980**, 3123.

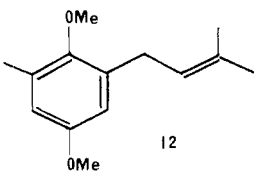
0022-3263/86/1951-1115$01.50/0

Table I. ^{13}C NMR Data for Compounds 3, 4, 6, and 7 in $CDCl_3$

	3		4		6		7	
C no.	off-res mult	measd values	off-res mult	measd values	off-res mult	measd values	off-res mult	measd values
1	t	28.6	t	28.8	t	28.5	t	28.8
2	d	127.1	d	127.0	d	127.6	d	127.3
3	s	130.4	s	131.5	s	130.8	s	131.9
4	t	39.4	t	39.6	t	39.3	t	39.6
5	s	210.3	s	209.5	s	210.8	s	210.3
6	t	48.3	t	48.9	t	49.3	t	49.1
7	s	47.8[a]	s	48.0[a]	s	47.8[a]	s	47.9[a]
8	t	35.6	t	36.0	t	35.1	t	35.4
9	d	38.4	d	38.3	d	38.8	d	38.9
10	s	155.8	s	145.2	s	156.9	s	147.2
11	d	90.7	d	88.6	d	90.8	d	89.1
12	s	160.1	s	149.9	s	158.9	s	148.0
	d	92.9	d	107.1	d	93.4	d	107.2
14	s	53.0[a]	s	52.7[a]	s	52.6[a]	s	52.2[a]
15	s	52.4[a]	s	52.6[a]	s	52.7[a]	s	53.0[a]
16	q	26.5	q	26.4	q	26.5	q	26.4
17	q	25.6	q	25.7	q	25.6	q	25.7
18	q	22.8	q	22.7	q	24.2	q	24.1
19	q	21.9	q	21.8	q	21.0	q	21.2
20	q	16.3	q	16.3	q	16.2	q	16.2
1′	s	154.0	s	153.9	s	154.1	s	153.9
2′	s	134.8[b]	s	134.2[b]	s	134.8[b]	s	134.3[b]
3′	d	114.0[c]	d	113.8[c]	d	114.1[c]	d	113.6[c]
4′	s	155.2	s	153.6	s	155.6	s	153.5
5′	d	113.2[c]	d	117.5[c]	d	113.3[c]	d	117.6[c]
6′	s	131.2[b]	s	130.5[b]	s	131.2[b]	s	130.7[b]
7′	q	16.0		16.0	q	16.0	q	16.0
OCH_3	q	55.3 55.4 55.5 56.4			q	55.3 55.5 × 2 56.2		
OAc								
CO			s s s	168.8 168.7 168.6			s s s s	169.4 168.8 168.7 168.6
CH_3			q q q q	20.05 19.7 20.8 21.0			q q q	20.0 × 2 20.9 21.1

[a–c] Assignments may be reversed. Assignments are based upon off-resonance multiplicities and comparison with suitable model products.

isoprene unit (C1 – C4 + C20) with E olefin geometry (δ_{CH_3} = 16.3 in ^{13}C NMR).



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Silver oxide oxidation was performed on **2**, giving the corresponding *p*-benzoquinone ($\nu_{C=C}$ = 1655 cm^{-1}; λ_{max} = 252 nm, ϵ = 10 000) without changes in the diterpenoid part, as indicated by ^1H NMR analysis.

In ^1H NMR of **3**, two deshielded methylenes [3.09 (2 H, s), 2.85 ppm (2 H, dd, J_{AB} = 16 Hz)] were consistent with the presence of an unstrained ketone by analogy with bifurcarenone. This was readily proved by reduction of **3** with $LiAlH_4$, giving a secondary alcohol [ν_{OH} = 3500 cm^{-1}; δ_{CH} = 4.1 (m)], which shifted the aforementioned methylene signals to 2.3 (d) and 1.93 ppm (d, AB system). Other ^1H NMR characteristics showed **3** to possess four quaternary methyl groups (1.04, 1.08, 1.23, 1.28 ppm), an ABM pattern with a methine proton [3.13 ppm (d, J = 4.5 Hz)] only coupled with the signal at 2.37 ppm (1 H, dd, J = 4.5, 12 Hz), methoxy signals [3.65 (s, 3 H), 3.74 (s, 6 H), 3.76 ppm (s, 3 H], and two deshielded olefinic protons (5.95, 5.99 ppm) weakly coupled (J = 1.8 Hz). A consistent downfield shift (6.48, 6.45 ppm) of these two protons in the ^1H NMR of **4** was in agreement with *W*-coupled protons in close relationship with the conjugated dienol displayed by ^{13}C NMR (see Table I).

In order to prove the bicyclic framework of **2**, compound **3** was treated with osmium tetroxide–H_2O_2 in *tert*-butyl alcohol, following by $Pb(OAc)_4$ treatment. After HPLC purification, the main product was submitted to CH_2N_2 to yield the corresponding methyl ester **13** (ν_{max} = 1735 cm^{-1}). The ^1H NMR spectrum of **13** showed that the four quaternary methyl groups were intact as well as an AB system. Signals of an ABM pattern were also discerned [3.37 (1 H, dd, J = 12.5, 6.5 Hz), 2.32 (1 H, bt, J = 12 Hz), 2.23 ppm (1 H, dd, J = 11.5, 6.5 Hz)] in agreement with a bridgehead position for C9. The lack of information concerning the various methyl groups led us to perform exhaustive ^1H NMR studies of **3** in order to accomplish the unequivocal characterization of all the protons included in the bicyclic system (see Figure 1), thus permitting us to propose structure **13** for the oxidative cleavage product in agreement with relative configurations defined subsequently.

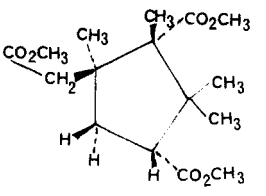

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Table II. NMR Data for Mediterraneol C (9): ^1H–^{13}C Shift Correlated 2-D NMR in $CDCl_3$ (Correlation via $^1J_{CH}$ and Long-Range J_{CH})

	J_{C-H}					J_{C-H}				
	^1H NMR		^{13}C NMR: δ (off-res			^1H NMR		^{13}C NMR: δ (off-res		
C no.	δ	J_{HH}, Hz	mult)	$^2J_{C-H}$	C no.	δ	J_{HH}, Hz	mult)	$^2J_{C-H}$	$^3J_{C-H}$
1	2.71 (bs)		22.6 (t)	H1–C2′	16	1.23 (s)		26.5 (q)		H16–C9
2	1.9 (m)		31.1 (t)		17	1.04 (s)		25.5 (q)		H17–C9
3			75.0 (s)		18	1.28 (s)		22.8 (a)	H18–C14	H18–C15
4	2.69 (bs)		53.8 (t)	H4–C3						H19–C8
					19	1.08 (s)		21.9 (q)	H19–C7	
5			210.8 (s)							H19–C14
6	2.90 (AB)	16	52.2 (t)	H6A/H6B–C7	20	1.37 (s)		24.7 (q)	H20–C3	H20–C4
7			47.2 (t)		1′			145.5 (s)		
8	2.47 (dd)	4.5, 12			2′			120.8 (s)		
			34.3 (t)							
	1.91 (d)	12			3′	6.38 (d)	3	111.0 (d)	H3′–C4′	H3′–C1′
9	3.15 (d)	4.5	38.4 (d)	H9–C10	4′			152.7 (s)		
10			156.8 (s)		5′	6.53 (d)	3	114.9 (d)	H5′–C4′	H5′–C1′
				H11–C10	6′			127.2 (s)		
11	6.00 (d)	1.8	90.6 (d)							
				H11–C12	7′	2.10 (s)		16.3 (q)	H7′–C6′	
12			159.9 (s)		4′-OMe	3.72 (s)		55.4 (q)		CH_3O–C4′
13	5.93 (d)	1.8	93.3 (d)	H13–C12	10-OMe	3.75 (s)		55.3 (q)		CH_3O–C10
14			53.2 (s)		12-OMe	3.70 (s)		55.6 (q)		CH_3O–C12
15			52.4 (s)							

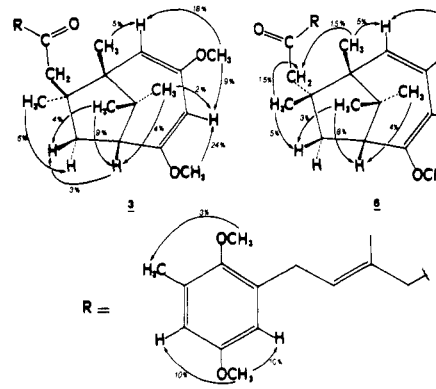

Figure 1. NOE for H(irradiated) → H(enhanced) for compounds **3** and **6**.

The row C10–C11–C12–C13 was verified by the observation of *W* coupling between H11 and H13 (J = 1.8 Hz), NOE on H11 (24%) upon irradiation of **10**–OCH_3 and NOE on H11 (9%) and H13 (18%) upon irradiation of **12**–OCH_3. The configuration of the protons attached to C8–C9 (2.37, 1.96, 3.13) were established as β, α, and β, respectively, from their coupling constants (90° dihedral angle confirmed by Drieding models) but also from NOE on H8β (3%) upon irradiation of H9.

Observations of NOE on H8β (3.5%) and H9 (9%) upon irradiation of **16**–CH_3 (1.23 ppm) suggested that this methyl group was oriented to the side of the five-membered ring, in agreement with high-field shift for **17**–CH_3 (1.04 ppm) due to the shielding effect of the cycloheptadiene ring. The α configuration of **19**–CH_3 was indicated by NOE (6%) of the 8α proton caused by irradiation at **19**–CH_3 (1.08 ppm). NOE irradiations at **18**–CH_3 (1.28 ppm) caused an enhancement (5%) on H13, proving that 18–CH_3 is located in the vicinity of H13. The downfield shift of **18**–CH_3 (1.28 ppm) can be explained by the proximity of the carbonyl group. Examination of a Dreiding model indicated an α configuration for this methyl group (in agreement with a cis junction) as the only acceptable possibility. From the evidence outlined above, relative stereochemistry can be assigned to C7 (*S**), C9 (*R**), and C14 (*R**).

At this point, such a rearranged structure as mediterraneol B with a conjugated dienol included in a bicyclo[4.2.1]nonane ring cannot be readily accepted without further proof.

In order to confirm the rearranged structure, several chemical degradations and various crystalline derivatizations were tried. Repeated unsuccessful attempts led us to lose the greater part of our sample of mediterraneol B as our only result. However, complete assignment of the proposed structure and supplementary spectroscopic evidence was obtained from a related substance—mediterraneol C—by ^1H–^{13}C shift correlation 2-D NMR spectroscopy including 2-D long-range ^1H–^{13}C chemical shift correlations.[9] The new compound **8** was isolated from the main fraction in a similar way than **2** and **5**. The oily trimethoxy product **9** showed $[\alpha]_D$ +5.7 (*c* 5.1, $CHCl_3$) and analyzed for $C_{30}H_{42}O_5$ by mass spectrometry (peak matching) and ^{13}C NMR analysis. IR absorptions and the UV spectrum were identical with those obtained for **3**. Three methoxy groups in ^1H NMR and ^{13}C NMR spectra supported the presence of a chromane moiety in **9**. Confident assignments of the chromane part were obtained from ^1H NMR spectra [2.71 (2 H, bs), 2.69 (2 H, bs), 1.37 ppm (3 H, s)] and the ^{13}C NMR spectrum (δ_{C3} = 75.0). Relative stereochemistry of mediterraneol C was assumed to be the same as mediterraneol B, on the basis of NOE results.

Except for the ketone group and the carbon-bearing oxygen of the chromane part, the assignment of the ^{13}C NMR spectrum was deduced from the ^1H NMR spectrum 2-D ^1H–^{13}C shift correlation. In this way, 21 out of the 30 carbons were easily identified (see Table II). Nine carbons remained to be identified, and for this, 2-D long-range heteronuclear ^1H–^{13}C shift correlations were used.[10] Table II shows the correlations obtained by this method. In the upfield region, H7′–C6′, H1–C2′, H3′/H5′–C4′, H11–C10,

(9) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.

(10) Long-range heteronuclear correlations were performed with maximum polarization for 12 Hz, leading to ^2J (geminal coupling) and ^3J (vicinal coupling) spots in the same spectrum. Generally, ^2J spots were distinctly greater than ^3J spots, and our experiment was in agreement with this rule. However, confident assignments of both couplings cannot be established on this sole statement. Recognition of most of these couplings was based on ^{13}C NMR values of the correlated carbons that were already identified unambiguously and on spectral evidences (for instance, CH_3–O–C was obviously a three-bond coupling). In doubtful cases (C7, C14, C15), correlations were first considered as ^2J and next as ^3J in order to investigate all structural possibilities.

Table III. NMR Data for Mediterraneol D (11): ^1H–^{13}C Shift Correlated 2-D NMR in $CDCl_3$ (Correlation via $^1J_{CH}$ and Long-Range J_{CH})

J_{C-H}					J_{C-H}					
	^1H NMR		^{13}C NMR: δ (off-res			^1H NMR		^{13}C NMR: δ (off-res		
C no.	δ	J_{HH}, Hz	mult)	$^2J_{C-H}$	C no.	δ	J_{HH}, Hz	mult)	$^2J_{C-H}$	$^3J_{C-H}$
1	2.71 (bs)		22.6 (t)	H1–C2′	16	1.23 (s)		26.4 (q)		H16–C9
2	1.9 (m)		31.0 (t)		17	1.04 (s)		25.5 (q)		H17–C9
3			74.9 (s)		18	1.10 (s)		24.4 (q)	H18–C14[a]	
4	2.69 (bs)		53.1 (t)	H4–C3						H19–C14[a] (?)
					19	1.12 (s)		20.0 (q)	H19–C7[a]	
5			210.0 (s)							H19–C8
6	2.92 (AB)	16	51.6 (t)	H6–C7	20	1.36 (s)		24.7 (q)	H20–C3	H20–C4
7			47.4 (s)		1′			145.4 (s)		
8	2.37 (dd)	4.5, 12	34.0 (t)		2′			120.7 (s)		
	1.87 (d)	12			3′	6.38 (d)	3	110.0 (d)	H3′–C4′	H3′–C1′
9	3.13 (d)	4.5	38.9 (d)	H9–C10	4′			152.4 (s)		
10			156.9 (s)		5′	6.53 (d)	3	114.9 (d)	H5′–C4′	H5′–C1′
				H11–C10	6′			127.2 (s)		
11	5.99 (d)	1.8	90.6 (d)							
				H11–C12	7′	2.10 (s)		16.3 (q)	H7′–C6′	
12			159.9 (s)		4′-OMe	3.72 (s)		55.4 (q)		CH_3O–C4′
13	5.95 (d)	1.8	93.2 (d)	H13–C12	10-OMe	3.75 (s)		55.3 (q)		CH_3O–C10
14			52.3 (s)		12-OMe	3.70 (s)		55.6 (q)		CH_3O–C12
15			52.8 (s)							

[a] Identified by comparison with 9.

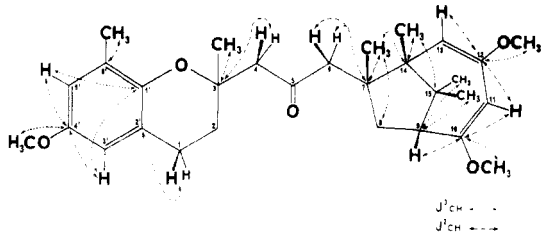

Figure 2. 2-D long-range heteronuclear ^1H–^{13}C shift correlations observed in mediterraneol C.

H11–C12, H13–C12, and H9–C10 two-bond couplings (geminal couplings) and H3′/H5′–C1′, CH_3O–C_4', CH_3O–C10, CH_3O–C12 three-bond couplings (vicinal couplings) identified the sp^2 quaternary carbons–C2′ (120.8 ppm), C6′ (127.2 ppm), C1′ (145.5 ppm), C4′ (152.7 ppm), C10 (156.8 ppm), C12 (159.9 ppm). A first element in the structural elucidation was given by the H9–C10 geminal coupling that, in connection with ^1H NMR decoupling experiments and NOE results, established the row C8–C9–C10–C11–C12–C13. In the downfield region H20–C3, H10–C4, and H4–C3, long-range couplings were easily observed, providing useful information to connect the row C4–C5–C6 to the chromane moiety. We noted also H18–C14 and H19–C7 geminal couplings that identified two of the last three quaternary carbons (respectively, 53.2 and 47.2 ppm).

Direct evidence for linkages of C6–C7, C7–C8, C7–C14, and C14–C15 were provided by H6A/H6B–C7, H19–C8, H19–C14, and H18–C15 couplings. All these results were resumed in Figure 2.

Due to the lack of coupling between H9–C15 and H13–C14, connections between C9–C15 and C13–C14 could not be established by this experiment. However, long-range couplings ($^3J_{CH}$) between H16–C9 and H17–C9 were observed, showing the close relationship between C9 and the function $(CH_3)_2C$, in agreement with NOE enhancements already described for H16/H17 and H9. As $^3J_{C-H}$ can be greater than $^2J_{C-H}$, the presence of this vinical coupling and the lack of geminal coupling (H16/H17–C15) were compatible with a structure in which both methyls were gauche relative to H9, leading to small coupling constants.[11]

(11) Wehrli, F. W.; Nishida, T. "Progress in the Chemistry of Organic Natural Products"; Springer-Verlag: New York, 1979; pp 2–229.

As NOE on H13 was found upon irradiation of H18, structures 3 and 8 were proposed for mediterraneol B and C in accordance with the whole study presented here.

Chemical degradations (see the Experimental Section for details) and part of the spectral characteristics have been previously published[7] to describe the gross structure of mediterraneol A (6, 7). We would like now to study the relative stereochemistry of 6 by comparison of its NOE data (Figure 1) with those of compound 3. The tetramethoxy product 6 afforded comparable NOE enhancements for C8, C9, C10, C11, C12, C13, C16, and C17. A normal NOE on H13 (5%) upon irradiation of 18–CH_3 was observed together with a little NOE on one of the C6 protons (1.5%). The same enhancement can be observed during NOE irradiation upon 19–CH_3 (1.12 ppm) on the same C6 proton (1.5%). This result compared with the lack of such an NOE in 3 indicated that the carbonyl group was not in the vicinity of the cyclopentane ring, as shown by differences in chemical shift for 18–CH_3 (1.10 ppm) and H8β (2.47 ppm). Observation of NOE on H8β (5%) upon irradiation of 19–CH_3 was more important and led us to conclude that methyl groups of 6 were all in β position on the cyclopentane ring. Thus, compound 6 was the C7 epimer (R^*) of mediterraneol B (4).

As for mediterraneol B, we applied 2-D NMR spectroscopy to a related substance, mediterraneol D (10), in order to obtain spectroscopic evidence for the proposed structure of mediterraneol A. After methylation, the new compound 11, obtained as an oil by repeated HPLC from the main fraction, showed $[\alpha_D]$ +14.1 (c 5, $CHCl_3$) and analyzed for $C_{30}H_{42}O_5$ by mass spectrometry and ^{13}C NMR analysis. A chromane moiety was suspected on the basis of only three methoxy groups in ^1H NMR and ^{13}C NMR spectra. The occurrence of a quaternary carbon bearing an oxygen atom (δ_{C3} = 74.9) in the ^{13}C NMR spectrum and of two methylene signals (2.71, 2.69 ppm) and a deshielded methyl group (1.36 ppm) in ^1H NMR spectrum reinforced the view that 11 contained a chromane part. NOE experiments confirmed that relative stereochemistry of 11 and 6 were identical.

The ^1H–^{13}C shift correlation 2-D NMR spectroscopy is summarized in Table III. The results are very similar to those obtained for 9. Because of the small chemical shift difference for 18 – CH_3 and 19 – CH_3 in the ^1H NMR

spectrum, vicinal couplings of these two methyl groups were not discerned and geminal couplings were identified by comparison with **9**. However, the same elements of structural elucidation have been found, providing considerable support for the proposed structures of the mediterraneol family.

To our knowledge, such structures are unique among the diterpenoids from natural sources. The presence of bifurcarenone—with the anti-Markovnikov cyclization between C7 and C11—in these novel metabolites rendered *C. mediterranea* one of the most original algae in regard to its very complex diterpenoid biosynthesis. Due to the isolation procedure we obtained structures with conjugated dienols included in the bicyclic system. However, the possibility of conjugated ketones representing the tautomeric equilibrium can be envisaged. Various observations during experimental workup led us to conclude that such structures would be bound only with quinone moieties. Unfortunately, we were unable to study these compounds, which were highly unstable during open-column chromatography. The instability of the conjugated ketonic form could also explain the poor yield obtained during silver oxide oxidation of **2** and **5** as the dienol form was the only one recovered. At least other meroditerpenoids,[12] from the same alga that seem not obviously related to bifurcarenone or to mediterraneols, are currently being investigated in this laboratory. We hope that these compounds will allow us a better understanding of the biosynthesis of such rearranged diterpenoids, particularly in the displacement of the methyl group at C11 of the regularly terpenoid precursor to C14 in the cyclic products.

Experimental Section

IR spectra were recorded on a Perkin-Elmer Model 621 spectrophotometer, and optical rotations were measured on a Roussel Jouan (T71) polarimeter using a 0.5-cm microcell. UV spectra were recorded on a Perkin-Elmer 551 spectrophotometer, and high-resolution mass spectra were obtained through the Bioorganic and Biomedical Mass Spectrometry Resource Center, Space Sciences Laboratory, University of California, Berkeley. ^{1}H NMR and ^{13}C NMR spectra (respectively, at 360 and 90.5 MHz) were recorded on a Bruker instrument. All chemical shifts are reported relative to Me_4Si (δ 0), and coupling constants are in Hertz.

All solvents used either were spectral grade or were distilled from glass prior to use. Purifications of all metabolites and reaction products were achieved by HPLC on preparative silica gel column using various proportions of EtOAc and isooctane.

Collection, Extraction, and Chromatography. The alga (150 g of dry weight by month) was collected in June and July (1981, 1982) at Banyuls-sur-Mer, France. The freeze-dried material was ground and extracted with $CHCl_3$/MeOH (1:1). After filtration and evaporation, the extract was partitioned between water and ether. The ether-soluble material was dried over $MgSO_4$ and filtered, and the filtrate was evaporated to yield 2.24 g of a crude organic extract. From various collections, compounds were eluted from a silica gel column with 10% hexane in ether or pure ether and further purified as oily substances by HPLC.

Bifurcarenone (1). Compound 1 was isolated as an oil (1.9% from ether extract) by HPLC (40 % AcOEt in isooctane): IR (film) 3500, 1710, 1680, 1620 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.89 (1 H, d, J = 16), 6.67 (1 H, d, J = 16), 6.57 (1 H, d, J = 2.8), 6.46 (1 H, d, J = 2.8), 5.43 (1 H, t, J = 8), 3.33 (2 H, d, J = 8), 3.05 (2 H, s), 2.48 (1 H, d, J = 15), 2.36 (1 H, d, J = 15), 2.24 (3 H, s), 1.63 (3 H, s), 1.35 (3 H, s), 1.33 (3 H, s), 1.22 (3 H, s), 1.21 (3 H, s); HRMS, M$^+$ m/z (relative intensity) m/z 442.2708 (7) (calcd for $C_{27}H_{38}O_5$ 442.2709), 424.2608 ($C_{27}H_{36}O_4$, 33), 406.2488 ($C_{27}H_{34}O_3$, 32), 251.1665 ($C_{15}H_{23}O_3$, 33), 233.1478 ($C_{15}H_{21}O_2$, 61.5), 205.1572 ($C_{14}H_{21}O$, 36), 177.0803 ($C_{11}H_{13}O_2$, 100), 150.1047 ($C_{10}H_{14}O$, 37), 137.0588 ($C_8H_9O_2$, 24), 123.0811 ($C_8H_{11}O$, 20), 121.0652 (C_8H_9O, 12), 95.0866 (C_7H_{11}, 29).

Mediterraneols A and B. Compounds **2** and **3** were isolated in mixture as white solid (23% from ether extract) by HPLC (40% AcOEt in isooctane). The final purification was obtained mainly by methylation.

Methylation. To a solution of the mixture **2** + **3** (200 mg) in dry acetone (20 mL) were added MeI (2 mL) and anhydrous K_2CO_3 (1 g). The reaction was refluxed for 3 h, diluted with water, and extracted with ether (3 × 200 mL). The ether layer was washed with water, dried over anhydrous $MgSO_4$, and evaporated to dryness. The resulting product (205 mg) was subjected to repeated HPLC (8% AcOEt in isooctane) to give pure tetramethoxymediterraneol A (**4**) (36 mg) and tetramethoxymediterraneol B (**6**) (25 mg).

Acetylation. The natural mixture (10 mg) was combined with excess pyridine (2 mL) and Ac_2O (2 mL) and stirred at room temperature overnight. Ice water was then added, and the mixture was extracted with Et_2O (3 × 20 mL). The Et_2O phase was dried over anhydrous $MgSO_4$ and reduced in vacuo to yield the acetylated products. Purification was performed with silica HPLC (10% AcOEt in isooctane).

Mediterraneol B (3). Compound 3, white foam, was isolated by repeated HPLC (8% EtOAc/isooctane) as 4.5% of the extract and showed the following spectral features: IR (film) 2970, 2870, 1710, 1615, 1595, 1500, 1460, 1380, 1325, 1220, 1200, 1140, 1120, 1060, 870, 815 cm^{-1}; UV (MeOH) λ_{max} 215 nm (ϵ 18500), 237 (sh), 289 (2900); ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.54 (2 H, s), 5.99 (1 H, d, J = 1.8), 5.95 (1 H, d, J = 1.8), 5.38 (1 H, t, J = 7.5), 3.75 (3 H, s), 3.74 (6 H, s), 3.65 (3 H, s), 3.34 (2 H, d, J = 7.2), 3.13 (1 H, d, J = 4), 3.09 (2 H, s), 2.85 (2 H, AB system, J = 16), 2.37 (1 H, dd, J = 4, 12), 2.26 (3 H, s), 1.87 (1 H, d, J = 12), 1.74 (3 H, s), 1.28 (3 H, s), 1.23 (3 H, s), 1.08 (3 H, s), 1.04 (3 H, s).

Acetate 4. The tetraacetate was isolated as an oil by HPLC (10% EtOAc/isooctane): IR (film) 2980, 2880, 1765, 1710, 1620, 1595, 1480, 1430, 1370, 1200, 1110, 1070, 1020, 900, 870 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.80 (1 H, d, J = 2.4), 6.78 (1 H, d, J = 2.4), 6.48 (1 H, bs), 6.45 (1 H, bs), 5.26 (1 H, t, J = 8), 3.21 (2 H, d, J = 8), 3.10 (2 H, s), 3.08 (1 H, bs), 2.81 (2 H, AB system, J = 14), 2.44 (1 H, dd, J = 4.8, 12), 2.33 (3 H, s), 2.29 (3 H, s), 2.26 (3 H, s), 2.24 (3 H, s), 2.13 (3 H, s), 2.10 (1 H, d, J = 12), 1.68 (3 H, s), 1.24 (3 H, s), 1.22 (3 H, s), 1.06 (3 H, s), 1.05 (3 H, s).

5: ^{1}H NMR (360 MHz, C_6D_6) δ 7.00 (1 H, bs), 6.80 (1 H, bs), 6.70 (1 H, bs), 6.64 (1 H, bs), 5.28 (1 H, bs), 3.43 (2 H, bs), 2.90 (2 H, d, J = 16), 2.88 (2 H, d, J = 7), 2.70 (1 H, bs), 2.27 (1 H, bd, J = 12), 2.14 (1 H, d, J = 12), 1.95 (3 H, s), 1.88 (3 H, s), 1.80 (3 H, s), 1.72 (3 H, s), 1.67 (3 H, s), 1.62 (3 H, s), 1.25 (3 H, s), 1.09 (3 H, s), 0.98 (3 H, s), 0.93 (3 H, s).

High-resolution mass spectra for 4: M$^+$ − 2 AcOH, m/z 488.2548 (calcd for $C_{31}H_{36}O_5$ (488.256), 488.2548 (1.5), 428.2340 ($C_{29}H_{32}O_3$, 1.2), 404.2329 ($C_{27}H_{32}O_3$, 2.6), 386.2038 ($C_{27}H_{30}O_2$, 3.0), 368.2118 ($C_{27}H_{28}O$, 1.0), 263.0841 ($C_{14}H_{15}O_5$, 70.9), 261.1084 ($C_{15}H_{17}O_4$, 56), 233.1431 ($C_{14}H_{17}O_3$, 18.4), 221.0805 ($C_{12}H_{13}O_4$, 100), 219.0877 ($C_{13}H_{15}O_3$, 88.9), 193.0856 ($C_{11}H_{13}O_3$, 16.3), 179.0708 ($C_{11}H_{15}O_2$, 89.1), 177.0817 ($C_{11}H_{13}O_2$, 52.3), 137.0605 ($C_8H_9O_2$, 17.4), 95.0858 (C_6H_7O, 62.9).

Mediterraneol A (6). Compound 6 white foam, was isolated by HPLC (8% EtOAc/isooctane) as 4% of the extract and showed the following spectral characteristics: $[\alpha]_D$ 0° (c 2.4, $CHCl_3$); IR (film) 2970, 2870, 1710, 1615, 1595, 1500, 1465, 1380, 1320, 1220, 1205, 1140, 1120, 1060, 870, 815 cm^{-1}; UV (MeOH) λ_{max} 215 nm (ϵ 19000), 237 (sh), 289 (2800); ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.55 (1 H, bs), 6.54 (1 H, bs), 6.00 (1 H, d, J = 1.8), 5.93 (1 H, d, J = 1.8), 5.38 (1 H, t, J = 7.5), 3.75 (3 H, s), 3.73 (3 H, s), 3.72 (3 H, s), 3.65 (3 H, s), 3.30 (2 H, d, J = 7.5), 3.15 (1 H, d, J = 4.5), 3.08 (2 H, s), 2.81 (2 H, AB system, J = 16), 2.47 (1 H, dd, J = 4.5, 12), 2.26 (3 H, s), 1.91 (1 H, d, J = 12), 1.73 (3 H, s), 1.23 (3 H, s), 1.12 (3 H, s), 1.10 (3 H, s), 1.04 (3 H, s).

Acetate 7. The tetraacetate was isolated as an oil by HPLC (10% EtOAc/isooctane): IR (film) 2970, 2870, 1765, 1710, 1620, 1595, 1480, 1430, 1370, 1200, 1170, 1110, 1070, 1020, 900, 870 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.80 (1 H, d, J = 2.5), 6.77 (1 H, d, J = 2.5), 6.47 (1 H, bs), 6.44 (1 H, bs), 5.26 (1 H, t, J = 8), 3.21 (2 H, d, J = 8), 3.10 (2 H, s), 3.08 (1 H, bs), 2.80 (2 H, AB system,

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J = 14), 2.61 (1 H, dd, J = 4.8, 12), 2.30 (3 H, s), 2.29 (3 H, s), 2.26 (3 H, s), 2.24 (3 H, s), 2.13 (3 H, s), 2.04 (1 H, d, J = 12), 1.68 (3 H, s), 1.22 (3 H, s), 1.12 (3 H, s), 1.10 (3 H, s), 1.06 (3 H, s).

7: ^{1}H NMR (360 MHz, C_6D_6) δ 7.03 (1 H, bs), 6.8 (1 H, bs), 6.71 (1 H, bs), 6.67 (1 H, bs), 5.29 (1 H, bs), 3.44 (2 H, bs), 2.93 (2 H, d, J = 16), 2.89 (2 H, d, J = 7), 2.71 (1 H, bs), 2.47 (1 H, bd, J = 12), 2.15 (1 H, d, J = 12), 1.95 (3 H, s), 1.89 (3 H, s), 1.80 (3 H, s), 1.73 (3 H, s), 1.69 (3 H, s), 1.62 (3 H, s), 1.47 (3 H, s), 1.16 (3 H, s), 1.11 (3 H, s), 0.98 (3 H, s).

High-resolution mass spectra for 7: $M^+ - 2$ AcOH = m/z 488.2535 (calcd for $C_{31}H_{36}O_5$ 488.256), 488.2535 (1.3), 446.2419 ($C_{29}H_{34}O_4$, 1.5), 428.2350 ($C_{29}H_{32}O_3$, 1.0), 404.2345 ($C_{27}H_{32}O_3$, 2.4), 386.2029 ($C_{27}H_{30}O_2$, 2.8), 368.2120 ($C_{27}H_{28}O$, 1.2), 263.0847 ($C_{14}H_{15}O_5$, 70.1), 261.1079 ($C_{15}H_{17}O_4$, 55), 233.1427 ($C_{14}H_{17}O_3$, 19.2), 221.0811 ($C_{12}H_{13}O_4$, 100), 219.0871 ($C_{13}H_{15}O_3$, 86.7), 193.0845 ($C_{11}H_{13}O_3$, 15.7), 179.0709 ($C_{11}H_{15}O_2$, 89.0), 177.0819 ($C_{11}H_{13}O_2$, 51.6), 137.0612 ($C_8H_9O_2$, 18.4), 95.0853 (C_6H_7O, 57.0).

Silver Oxide Oxidation of 2 and 5. Ag_2O (100 mg) and Na_2SO_4 (80 mg) were added to the mixture of **2** and **5** (40 mg) in Et_2O (5 mL), and the suspension was stirred for 1 h. After filtration, the solution was evaporated to give 33 mg of a material that was purified by HPLC (10% AcOEt in isooctane): 7 and 4 mg were obtained.

Data for the first quinone (7 mg): IR (film) 3400, 1700, 1655, 1625, 1615 cm^{-1}; UV (MeOH) 252 nm (ϵ 10 000) 237 (8900); ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.55 (1 H, d, J = 2.5), 6.47 (1 H, d, J = 2.5), 5.91 (1 H, d, J = 1.8), 5.88 (1 H, d, J = 1.8), 5.24 (1 H, t, J = 7), 3.13 (2 H, bd, J = 7), 3.07 (2 H, s), 3.05 (1 H, d, J = 4), 2.69 (1 H, d, J = 15), 2.40 (1 H, dd, J = 3.9, 12), 2.06 (3 H, s), 1.98 (1 H, d, J = 12), 1.64 (3 H, s), 1.25 (3 H, s), 1.21 (3 H, s), 1.10 (3 H, s), 1.06 (3 H, s).

Data for the second quinone (4 mg): IR (film) 3400, 1705, 1650, 1625, 1615 cm^{-1}; UV (MeOH) λ_{max} 252 nm (ϵ 12 000) 237 (10 500); ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.54 (1 H, d, J = 2.5), 6.48 (1 H, d, J = 2.5), 5.98 (1 H, d, J = 1.8), 5.91 (1 H, d, J = 1.8), 5.22 (1 H, t, J = 6.5), 3.11 (2 H, bd, J = 7), 3.07 (2 H, s), 3.04 (1 H, d, J = 4.5), 2.7 (1 H, d, J = 15), 2.56 (1 H, dd, J = 4, 12.5), 2.05 (3 H, s), 1.93 (1 H, d, J = 12.5), 1.63 (3 H, s), 1.21 (3 H, s), 1.10 (6 H, bs), 1.06 (3 H, s).

Reduction of 3 with $LiAlH_4$. A cold solution of **3** (10 mg) in dry ether (1 mL) containing $LiAlH_4$ (15 mg) was stirred at 0 °C for 1.30 h. Excess reagent was destroyed by slow addition of EtOAc. Addition of a saturated $MgSO_4$ solution and extraction with ether yielded 8 mg of an oil that was chromatographed on HPLC (10% AcOEt in isooctane) to give 3 mg of the corresponding reduction product: IR (film) 3450, 1615, 1595 cm^{-1}; selected values, ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.1 (1 H, m), 2.3 (2 H, d, J = 7), 2.04 (1 H, dd, J = 16, 6), 1.82 (1 H, dd, J = 16, 8.5), 1.27 (3 H, s), 1.17 (3 H, s), 1.08 (3 H, s), 1.03 (3 H, s).

Reduction of 6 with $LiAlH_4$. Reduction of the ketone of **6** (5 mg) was performed as described for **3**, and 1 mg of the reductive product was obtained after HPLC: IR (film) 3450, 1620, 1595 cm^{-1}; selected values, ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.05 (1 H, m), 2.35 (2 H, d, J = 7), 2.0 (1 H, dd, J = 16, 9), 1.85 (1 H, dd, J = 16, 5), 1.23 (3 H, s), 1.12 (3 H, s), 1.09 (3 H, s), 1.04 (3 H, s).

Oxidative Cleavage of 3. To a solution of **3** (20 mg) in *tert*-butyl alcohol and hydrogen peroxide was added a catalytic amount of OsO_4 in *tert*-butyl alcohol (2.5 wt %) and the resultant mixture stirred at room temperature overnight. The reaction mixture was extracted with EtOAc. After removal of the solvent in vacuo, the resulting oil was treated with $PbO(Ac)_4$ in EtOAc during 2 h and after filtration, purified by rapid open-column chromatography. The main fraction (60% EtOAc/isooctane) was further purified by HPLC (30% EtOAc/isooctane), and the main product was submitted to CH_2N_2 in order to obtain the corresponding methyl ester **11**: 6 mg; IR (film) 1735 cm^{-1}; HRMS, M^+ m/z 314.726 (calcd for $C_{16}H_{16}O_6$ 314.1731); ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.72 (3 H, s), 3.65 (3 H, s), 3.61 (3 H, s), 3.37 (1 H, dd, J = 12.5, 6.5), 2.75 (1 H, d, J = 15.7), 2.32 (1 H, bt, J = 12), 2.27 (1 H, d, J = 15.7), 2.23 (1 H, dd, J = 11.5, 6.5), 1.49 (3 H, s), 1.12 (3 H, s), 1.09 (3 H, s), 1.06 (3 H, s).

Oxidative Cleavage of 6. Oxidative cleavage of **6** (15 mg) with OsO_4 was performed as described above: IR (film) 1735 cm^{-1}; HRMS, M^+ m/z 314.722 (calcd for $C_{16}H_{16}O_6$ 314.1731); ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.72 (3 H, s), 3.65 (3 H, s), 3.61 (3 H, s), 3.37 (1 H, dd, J = 12.5, 6.5), 2.75 (1 H, d, J = 15.7), 2.55 (1 H, bt, J = 12), 2.27 (1 H, d, J = 15.7), 2.24 (1 H, dd, J = 11.5, 6.5), 1.49 (3 H, s), 1.17 (3 H, s), 1.10 (3 H, s), 1.07 (3 H, s).

Mediterraneol C (8/9). The oily substance **9** was obtained from the main fraction after methylation of **8** by HPLC purification: 100 mg, 0.11% from dry weight alga; HRMS M^+ 482.3032 (calcd for $C_{30}H_{42}O_5$ 482.3029); IR (film) and UV (MeOH), similar to those obtained for **3**.

NMR ^{1}H–^{13}C shift correlation: the applied pulse sequence was (π/2, ^{1}H) – ($t_{1/2}$) – (π, ^{13}C) – ($t_{1/2}$) – τ_1 – (π/2, ^{1}H; π/2, ^{13}C) – τ_2 – (BB, ^{1}H; FID, t_2) with τ_1 = 3.3 ms and τ_2 = 1.67 ms. Spectral width in F_1 was W_1 = ±500 Hz, and in F_2, W_2 = 6024 Hz.

NMR ^{1}H–^{13}C long-range shift correlation: Pulse sequence identical with ^{1}H–^{13}C shift correlation above except τ_1 = τ_2 = 41.7 ms. The pulse sequence was optimized to give maximum polarization for J = 12 Hz and at the same time suppress ^1J = 144 Hz interactions.

Mediterraneol D (10/11). Repeated HPLC (8% EtOAc/isooctane) gave the pure oily substance: 125 mg, 0.14% from dry weight alga; HRMS, M^+ m/z 482.3033 (calcd for $C_{30}H_{42}O_5$ 482.3029); IR (film) and UV (MeOH), similar to those obtained for **6**.

^{1}H–^{13}C shift correlation: See above.

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Synthesis of Substituted 1,2-Dihydroisoquinolines by the Intramolecular 1,3-Dipolar Alkyl Azide–Olefin Cycloaddition

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A general synthesis of substituted 1,2-dihydroisoquinolines based on intramolecular 1,3-dipolar cycloaddition of alkyl azides and olefins is described. Reaction of bromide **4** with sodium azide afforded azide **5**, which underwent 1,3-dipolar cycloaddition intramolecularly to give triazoline **6**. Rearrangement of triazoline **6** on silica gel gave diazo compound **7**. Treatment of **7** with rhodium acetate afforded substituted 1,2-dihydroisoquinoline **9** in good overall yield.

1,2-Dihydroisoquinolines are important heterocyclic systems.[1-3] Their use as the building blocks in the synthesis of alkaloids and medicinal compounds are indispensable to many preparations.[4] However, 1,2-dihydro-

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