48. New Ecotoxicologically and Biogenetically Relevant Terpenes of the Tropical Green Seaweed *Caulerpa taxifolia* which Is Invading the Mediterranean

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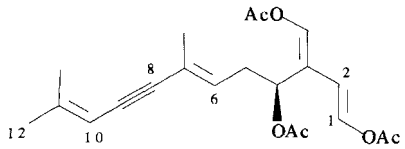
(13.XI.92)

The tropical green seaweed *Caulerpa taxifolia* (VAHL) C. AGARDH (Caulerpales) which is invading the Mediterranean is shown to contain trace amounts of two further novel terpenes, 7,7-*C*-didehydro-6-hydroxy-6,7-dihydrocaulerpenyne (= (4*S*,6*S*,1*E*)-3-[(*Z*)-acetoxymethylidene]-6-hydroxy-11-methyl-7-methylidenedodeca-1,10-dien-8-yne-1,4-diyl diacetate; **3a**) and taxifolione (= 6-methylhept-5-en-3-yn-2-one; **4**). The former is the most active of the toxins so far isolated from this seaweed, both as an *in vitro* inhibitor of the growth of marine bacteria and as a cytotoxic agent toward marine ciliate protists. This suggests a central ecotoxicological role for triacetate **3a** as an adjuvant factor in the invasion of the Mediterranean by this seaweed. Moreover, the almost equally toxic 10,11-epoxycaulerpenyne (**2**) which is scarcely available from Nature for bioassays can now be obtained by peroxy-acid epoxidation of caulerpenyne (**1**), along with the 6,7-epoxycaulerpenynes **6b** and **6a**. The latter are very labile, **6a** giving triacetate **3a**, suggesting epoxides to be late biogenetic intermediates in *C. taxifolia*.

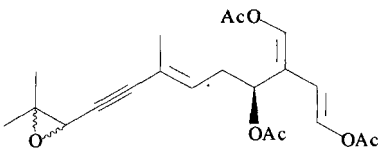
1. Introduction. – The recent, unprecedented phenomenon of massive invasion of the Mediterranean by a tropical seaweed, which started in front of Monaco and heavily spread to the neighboring areas of Cap Martin and Cap d'Ail and which is already touching distant localities both west up to the Balearic Islands and east up to Boccale/Livorno, is currently of much concern. The implied seaweed, *Caulerpa taxifolia* (VAHL) C. AGARDH, is one of the few toxic seaweeds[1]) and has stimulated studies from the ecological [3a], natural-product-chemistry [4], and ecotoxicological point of view [5]. These studies showed that in the Mediterranean, *C. taxifolia* competes with the endemic flora [3b] and contains the toxic [1] sesquiterpene caulerpenyne (**1**) in impressively large amounts, larger than in *Caulerpa* species in the tropics, accompanied by other sesqui- and monoterpenes [4]. Moreover, sesquiterpenes and raw juice of this seaweed are powerfully toxic; they inhibit the growth, *in vitro*, of marine bacteria and are cytotoxic agents toward marine ciliate protists [5]. Of particular concern is 10,11-epoxycaulerpenyne (**2**) which, in such tests, surpasses all other terpenes so far isolated from *C. taxifolia* [4] [5]; possibly it may also have mutagenic properties, owing to an oxirane functionality linked in an unusual manner to an enyne grouping.

The observation that, on standing, seawater/EtOH solutions of 10,11-epoxycaulerpenyne (**2**) become more active in inhibiting marine ciliates [5] suggests chemical transfor-

[1]) Along with other Caulerpales [1] and a few red seaweeds [2].

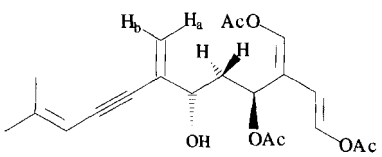


1

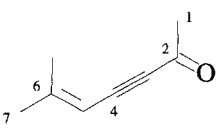


1:1 diastereoisomer mixture

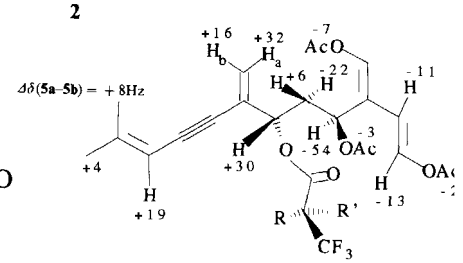
2



3a



4



5a R = MeO, R′ = Ph
b R = Ph, R′ = OMe

mations to a more active compound. Moreover, the C_{10} enyne-aldehyde taxifolial D, that appears as a truncated sesquiterpene [4], might be rationalized to derive biogenetically in *C. taxifolia* from caulerpenyne through oxiranes. This stimulated us to search for new natural metabolities of this seaweed and to study the epoxidation of caulerpenyne (**1**; available in large amounts from *C. taxifolia*) as a semisynthetic route to both the rare 10,11-epoxycaulerpenyne (**2**) and the isomeric, unknown 6,7-epoxycaulerpenyne (**6**) in adequate amounts for biological assays and reactivity studies. It was rewarding to find in *C. taxifolia* the new, strongly bioactive and biogenetically significant, hydroxylated terpene **3a** which was also obtained by epoxidation of caulerpenyne (**1**). Another, new truncated sesquiterpene of biogenetic significance, **4**, was also isolated from *C. taxifolia*.

2. Results and Discussion. – 2.1. *New Terpenes of* C. taxifolia. As indicated in the *Exper. Part*, the new hydroxylated sesquiterpene and the new truncated terpene isolated from *C. taxifolia* of Cap Martin have the structure of 7,7-*C*-didehydro-6-hydroxy-6,7-dihydrocaulerpenyne (**3a**) and of taxifolione (**4**), respectively.

The structure of the volatile taxifolione (**4**) is supported by the NMR resonances for the enyne portion (*Table 1* and *Exper. Part*) which are similar to those of the corresponding portion of both caulerpenyne (**1**) and the C_{10} terpene taxifolial D [4]. The position of the carbonyl group in **4** is supported by UV spectra and $^1H,^{13}C$-COSY showing a ^nJ between $CH_3(1)$ and both C(2) and C(3); $^nJ(^1H,^{13}C)$ correlations support also the remaining portion (*Exper. Part*), while $CH_3(7)$ is assigned by a positive NOE with *H*–C(5).

Structure **3a** is suggested by the ^{13}C-NMR spectra (*Table 1* and *Exper. Part*) which are similar to those of caulerpenyne [4] [6] (**1**), except for a shielding of C(6), typical for a CHOH group, and a deshielding of *C*–C(7), typical for a CH_2=C group. This is confirmed by the ^1H-NMR spectra, which show, besides the methylidene protons coupled (small *J*) with a methine proton at 4.27 ppm, all other coupling patterns requested for structure **3a** (*Exper. Part*).

The absolute configuration of triacetate **3a** at C(6) is determined from high-field ^1H-NMR examination ($CDCl_3$) of the diastereoisomeric 3,3,3-trifluoro-2-methoxy-2-phenylpropanoates $CF_3C(OMe)(Ph)COOR''$, **5a** and **5b**, prepared by reaction of **3a** with

Table 1. *^{13}C-NMR Data for 7,7-C-Didehydro-6-hydroxy-6,7-dihydrocaulerpenyne* (**3a**), *Its 6-Epimer* **3b**, *Taxifolione* (**4**), *and Compounds* ***6a,b*** *and* **8a,b**. In C_6D_6, unless otherwise stated.

	3a	**3b**	**4**[a])	**6a**	**6b**	**8a**[b])	**8b**[c])
C(1)	137.29 (*d*)	137.53 (*d*)	32.68 (*q*)	137.43 (*d*)	137.49 (*d*)	137.32 (*d*)	137.73 (*d*)
C(2)	109.86 (*d*)	109.67 (*d*)	184.71 (*s*)	109.52 (*d*)	109.56 (*d*)	110.12 (*d*)	109.93 (*d*)
C(3)	119.85 (*s*)	118.81 (*s*)	91.55 (*s*)	118.80 (*s*)	118.48 (*s*)	120.17 (*s*)	119.09 (*s*)
C(4)	67.08 (*d*)	67.60 (*d*)	89.70 (*s*)	67.16 (*d*)	67.48 (*d*)	67.35 (*d*)	68.14 (*d*)
C(5)	40.71 (*t*)	39.95 (*t*)	103.48 (*d*)	32.54 (*t*)	32.54 (*t*)	36.78 (*t*)	35.92 (*t*)
C(6)	70.88 (*d*)	72.02 (*d*)	158.19 (*s*)	61.48 (*d*)	61.67 (*d*)	72.95 (*d*)	73.25 (*d*)
Me–C(6)			21.77 (*q*)				
C(7)	135.36 (*s*)	135.37 (*s*)	25.46 (*q*)	51.45 (*s*)	51.35 (*s*)	80.32 (*s*)	80.38 (*s*)
C(8)	90.69 (*s*)	90.67 (*s*)		92.66 (*s*)	[d])	88.97 (*s*)[e])	88.76 (*s*)[e])
C(9)	89.87 (*s*)	89.51 (*s*)		80.39 (*s*)	80.39 (*s*)	86.69 (*s*)[e])	86.66 (*s*)[e])
C(10)	105.87 (*d*)	105.79 (*d*)		105.02 (*d*)	105.03 (*d*)	105.00 (*d*)	104.89 (*d*)
C(11)	149.04 (*s*)	149.06 (*s*)		149.69 (*s*)	149.58 (*s*)	150.61 (*s*)	150.65 (*s*)
C(12)	24.57 (*q*)	24.55 (*q*)		24.43 (*q*)	24.41 (*q*)	24.47 (*q*)	24.48 (*q*)
*C*H(Ac)=C(3)	134.39 (*d*)	135.15 (*d*)		134.89 (*d*)	135.24 (*d*)	134.69 (*d*)	136.20 (*d*)
CH_2=C(7) or *Me*–C(7)	119.12 (*t*)	119.50 (*t*)		19.20 (*q*)	19.26 (*q*)	22.16 (*q*)	22.78 (*q*)
Me–C(11)	21.04 (*q*)	21.04 (*q*)		20.91 (*q*)	20.91 (*q*)	21.12 (*q*)	21.11 (*q*)
MeCO_2C(1)	19.82 (*q*)[e])	19.88 (*q*)[e])		19.81 (*q*)[e])	19.82 (*q*)[e])	19.84 (*q*)[f])	19.92 (*q*)[f])
Me*C*O_2C(1)	166.62 (*s*)[f])	166.78 (*s*)[f])		166.34 (*s*)[f])	166.45 (*s*)[f])	167.23 (*s*)[g])	167.30 (*s*)[g])
MeCO_2C(4)	20.42 (*q*)	20.50 (*q*)		20.33 (*q*)	20.37 (*q*)	20.45 (*q*)	20.55 (*q*)
Me*C*O_2C(4)	169.91 (*s*)	169.25 (*s*)		169.06 (*s*)	169.00 (*s*)	169.93 (*s*)	169.26 (*s*)
MeCO_2CH=C(3)	19.95 (*q*)[e])	19.94 (*q*)[e])		19.92 (*q*)[e])	19.91 (*q*)[e])	19.94 (*q*)[f])	19.97 (*q*)[f])
Me*C*O_2CH=C(3)	167.15 (*s*)[f])	167.15 (*s*)[f])		167.19 (*s*)[f])	167.20 (*s*)[f])	166.66 (*s*)[g])	166.91 (*s*)[g])

[a]) In $CDCl_3$.

[b]) R part: 163.45 (*s*, C=O); 133.12 (*s*, C(1)); 129.96 (*d*, C(2)); 134.74 (*s*, C(3)); 132.91 (*d*, C(4)); 129.92 (*d*, C(5)); 127.96 (*d*, C(6)).

[c]) R part: 163.40 (*s*, C=O); 133.03 (*s*, C(1)); 129.98 (*d*, C(2)); 134.72 (*s*, C(3)); 132.89 (*d*, C(4)); 129.84 (*d*, C(5)); signal of C(6) hidden under the solvent signal.

[d]) Not detected.

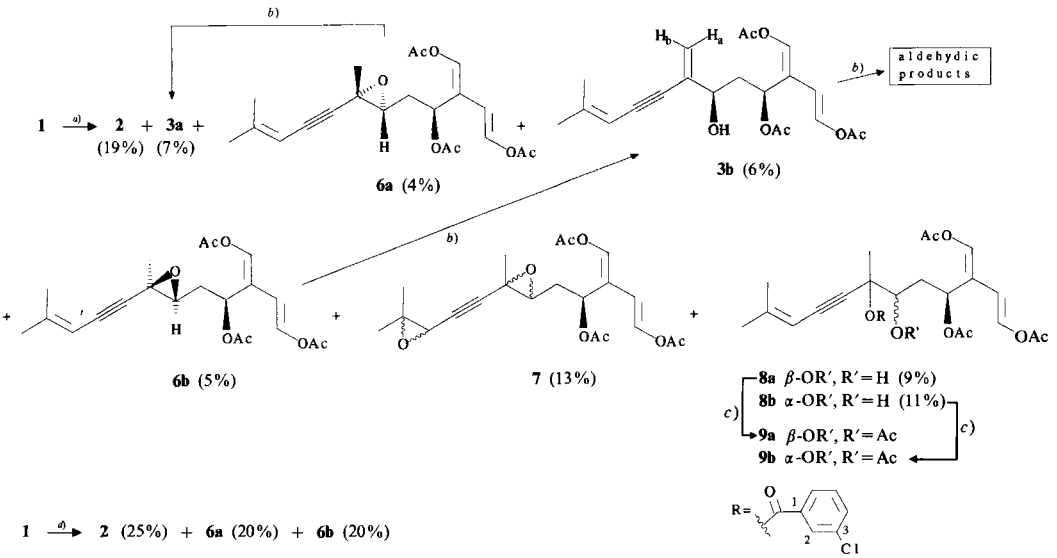
[e])[f])[g]) These signals can be interchanged.

(−)-(*R*)- or (+)-(*S*)-CF_3C(OMe)(Ph)COCl [7], respectively. However, the $\Delta\delta(\delta_S - \delta_R)$ value for H–C(6) (see *Formula* **5**) is far from ideal zero, as would be expected for coplanarity of H–C(6) with CF_3–C–COO (propanoate plane), and opposite $\Delta\delta$ values are measured for the diastereotopic 2 H–C(5). This suggests a conformational distortion from the ideal propanoate plane [8]. This notwithstanding, the $\Delta\delta$ values for all other protons of diastereoisomers **5** can only consistently be rationalized on the basis of the diamagnetic effect of the Ph ring for (6*S*)-configuration. Moreover, the allylic alcohol **3a** must have at C(4) the same configuration as in caulerpenyne [6] (**1**), as expected for a biogenetic descendant.

2.2. *Epoxidation of Caulerpenyne.* Caulerpenyne [4] (**1**) was subjected to peroxy-acid epoxidation under conditions suitable for acid-sensitive oxiranes. Thus, treatment of **1** with 3-chloroperoxybenzoic acid (3-$ClC_6H_4CO_3H$) under $NaHCO_3$ buffering yielded the desired diastereoisomer mixture **2**[2]), in a complex blend with other products, however

[2]) This synthetic sample of **2** was identical in every respect (including $[\alpha]_D$ and biological activities) to the 1:1 diastereoisomer mixture **2** previously isolated from *C. taxifolia* [4].

Scheme



a) 3-$ClC_6H_4CO_3H$, $NaHCO_3$, CH_2Cl_2, 0°, 1 h. *b*) C_6H_6, −20°, over one week. *c*) Ac_2O/pyridine, r.t., overnight. *d*) 3-$ClC_6H_4CO_3H$, Na_2HPO_4, CH_2Cl_2, 0°, 1 h.

(*Scheme*). Two of these by-products were the diastereoisomeric 6,7-epoxycaulerpenynes (**6a** and **6b**), expected from competitive epoxidation at C(6)=C(7); they could be obtained by HPLC in pure form, besides the mixture **7** of bis-epoxides[3]). Unexpected products were 7,7-*C*-didehydro-6-hydroxy-6,7-dihydrocaulerpenyne (**3a**), the metabolite reported above as a constituent of *C. taxifolia*, and its 6-epimer **3b**. Esters **8b** and **8a**, which have entrapped the reagent, were also isolated from the reaction mixture.

Epoxidation of **1** could be improved in favor of the 10,11- and 6,7-epoxides by buffering with Na_2HPO_4, thus getting cleanly mixture **2** (25%) and mixture **6a**/**6b** (*ca.* 20% each). While mixture **2** resisted all attempts for separation [4], the diastereoisomeric epoxides **6b** and **6a** were obtained in pure form by HPLC and their structures established by their NMR spectra (*Table 1* and *Exper. Part*), after comparison with those of mixture **2** [4].

The absolute configuration of the labile epoxide **6a** could be assigned from its spontaneous chemical transformation in benzene at −20° into **3a** (*Scheme*). Similarly the diastereoisomeric epoxide **6b** gave rise to the allylic alcohol **3b** in benzene solution. However, this could only be ascertained by ^{1}H-NMR monitoring (C_6D_6) since **3b** decomposed into unidentified aldehydic products (typical ^{1}H-NMR signals at 8.85, 9.27, and 10.40 ppm).

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[3]) Evidence for a diastereoisomer mixture of bis-epoxides **7** rests on ^{1}H-NMR spectra in C_6D_6, which, though being of the same type as for epoxides **2** and **6**, are characterized by special features. Thus, signals for Me groups at olefinic bonds are absent, while *s*'s are observed at δ 0.88–1.42 for Me groups and both *s*'s and *dd*'s at δ 2.90–2.92 and 3.17–3.27, respectively, for epoxide protons.

Table 2. *Biological Assays of 7,7-C-Didehydro-6-hydroxy-6,7-dihydrocaulerpenyne* (**3a**) *with Marine Ciliates and Marine and Non-Marine Bacteria in Comparison with Data* [5] *of Caulerpenyne* (**1**) *and 10,11-Epoxycaulerpenyne* (**2**)

Bacteria			Growth inhibition zone diameter at 60 μg/disk		
Strain	Taxon	Origin	**1**[a])	**2**[a])	**3a**
I4b	*Xantomonas* sp.	*Funiculina quadrangularis*[b])	0	0	6
I6	*Planococcus* sp.	*Pteria hirundo*[c])	3	12	29
I9b	*Pseudomonas* sp. or *Alteromonas* sp.	demosponge SC3[d])	0	[h])	0
I11a	*Xantomonas* sp.	*Alcyonium palmatum*[e])	10	22	27
I17i	*Xantomonas* sp. or *Flavobacterium* sp.	*Cymbulia peroni*[f])	13	15	31
C21d	*Pseudomonas* sp.	*C. taxifolia*[g])	0	0	0
C24b	*Vibrio vulnificus*	*C. taxifolia*[g])	0	4	13
ATCC 25923	*Staphylococcus aureus*	non-marine reference strain	17	19	33

Ciliates			LD_{100}[l]), ED_{100}[k]) [μg/ml]		
Strain	Taxon	Origin	**1**[a])	**2**[a])	**3a**
TB6	*Euplotes vannus*	Tanabe (Japan), 7/1983	> 20[l]), 20	20, 10	20, 5
CM1	*Euplotes* sp.	Cap Martin (France), 7/1992	> 20[l]), 10	10, 5	10, 5
CM2[m])	*Euplotes* sp.	Cap Martin (France), 7/1992	> 20[l]), 10	20, 10	10, 5
G-Lb5[n])	*Euplotes crassus*	Gesira (Somalia), 8/1974	20, 10	5, 1.2	1.2, 0.5
11Rec[n])	*Euplotes crassus*	Sciacca-Strazzone (Italy), 12/1974	20, > 10	10, 5	10, 0.5
SR2	*Euplotes minuta*	San Rossore (Italy), 3/1991	> 20[l]), 10	> 20[l]), 10	20, 10
MP1	*Euplotes minuta*	Marina di Pisa (Italy), 3/1991	> 20[l]), 10	20, 10	20, 5
SicAA	*Euplotes rariseta*	Milazzo (Italy), 9/1980	10, 5	5, 1.2	2.5, 0.5
PR5	*Diophrys oligothrix*	Porto Recanati (Italy), 6/1979	2, 1	1.2, [h])	0.5, [h])

[a]) Already reported in preliminary form [5].

[b]) Pennatulacea; dredging C2, May 20, 1991, from 43° 25.23′ N, 9° 58.15′ E to 43° 19.70′ N, 10° 00.90′, E, mean depth 137 m.

[c]) Ctenidobranchia; dredging D3, May 23, 1991, from 43° 09.66′ N, 9° 36.18′ E, to 43° 04.78′ N, 9° 38.31′ E, mean depth 357 m.

[d]) Dredging C3, May 23 1991, from 43° 16.34′ N, 10° 09.72′ E to 43° 10.36′ N, 10° 08.20′ E, mean depth 126 m.

[e]) Alcyonacea; dredging C6, May 23, 1991, from 43° 16.23′ N, 10° 19.90′ E to 43° 10.09′ N, 10° 25.45′ E, mean depth 64 m.

[f]) Acochlidiomorpha; dredging E1, May 6, 1991, from 43° 34.61′ N, 9° 33.36′ E, to 43° 31.79′ N, 9° 38.70′ E, mean depth 584 m.

[g]) Cap Martin (France).

[h]) Not checked.

[i]) Lowest concentration for 100% kills.

[k]) Lowest dose eliciting a fission rate delay in 100% of tested cells.

[l]) Less than 100% kills observed at the highest attainable concentration (20 μg/ml) of the terpene in the medium used for bioassays.

[m]) Their membership to *E. vannus* or *E. crassus* has not been determined as yet.

[n]) F1 strains established in the laboratory from F1 hybrid lines involving wild stocks.

These observations suggest that epoxides **6a** and **6b**, though elusive, are also metabolites of *C. taxifolia*, from which allylic alcohols **3a** and **3b** may descend, enzymatically or not. Epoxides **6a** and/or **6b**, can also be seen as precursors of taxifolione (**4**), and it may also be envisaged that the monoterpene taxifolial D present in *C. taxifolia* [4] arises along an epoxidation route from an elusive $\Delta^{5,6}$-sesquiterpene analog of caulerpenyne. Anyway, late biogenetic events in *C. taxifolia* of Cap Martin seem to be centered around sesquiterpene epoxides. This was not noticed during previous extensive examinations of the Caulerpales [1][4]).

2.3. *Biological Activities.* The antibacterial and cytotoxic activities of 7,7-*C*-didehydro-6-hydroxy-6,7-dihydrocaulerpenyne (**3a**), isolated from *C. taxifolia* or obtained from epoxide **6a**, were evaluated toward marine bacteria, as prokaryotes, and ciliate protists, as unicellular eukaryotes (*Table 2*), whose feeding relationships are an important factor in food-web models [9], and compared with the corresponding activities of caulerpenyne [4] (**1**) and 10,11-epoxycaulerpenyne [5] (**2**). In these tests, the stability and solubility of the very sensitive *C. taxifolia* terpenoids were carefully considered; their genuineness, both in EtOH solutions where they were stored and in EtOH/seawater solutions used for cytotoxicity assays, was checked by HPLC and NMR techniques before any set of experiments.

With the exception of two bacteria, either isolated from marine invertebrates like strain I9b or seaweeds like strain C21d which was resistant, **3a** had the highest antibacterial activity of all terpenes isolated from *C. taxifolia* ([5] and *Table 2*). Taking strain I6 (*Planococcus* sp.) as an example, the highest amounts of **1**, **2**, and **3a** not exerting any inhibitory effect on bacterial growth were 50, 30, and 5 μg/disk, respectively. This trend was observed also with the reference strain ATCC 25923 *Straphylococcus aureus*, the corresponding doses of **1**, **2**, and **3a** being 30, 20, and 10 μg/disk, respectively.

The susceptibility of the ciliates to the *C. taxifolia* terpenes shows an inter- and intra-specific variability ([5] and *Table 2*). This is not surprising considering the differences in geographical origin and phylogenetic relationships among the populations. What is worth noticing is the similarity in susceptibility between CM and other strains, even if the first ones were collected in an area colonized by *Caulerpa taxifolia*. Overall, like with bacteria, triacetate **3a** proved the most active of the terpenes of *C. taxifolia*, in some instances inducing toxic effects at a concentration as low as 0.5 μg/ml ([5] and *Table 2*). Thus, although sporadic resistance was observed with marine bacteria, the potential ecologic impact of triacetate **3a** on a microbial community appears ravaging, which may contribute to the success of this seaweed in invading the Mediterranean.

It is intriguing that, overall, the highest antibacterial and cytotoxic activities are displayed by epoxides of *C. taxifolia* terpenes, like **2**, or by allylic alcohols derived from them, like **3a**. Thus, one might be tempted to link the increase of cytotoxicity observed for

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[4]) The high reactivity of epoxides **6b** and **6a** must also be responsible for the incorporation of 3-$ClC_6H_4CO_3H$ during the $NaHCO_3$-buffered epoxidation of caulerpenyne, yielding hydroxy-esters **8a** and **8b** (*Scheme*). Their gross structures are fully supported by NMR and MS data (*Table 1* and *Exper. Part*). Acetylation to **9a** and **9b**, respectively, establishes that the free OH group in **8a** and **8b** is at C(6). However, the configuration at C(7) could not be assigned, and that at C(6) is only tentative. Cleavage of epoxides **6** to the 6-hydroxy 7-benzoates **8** is compatible with epoxide opening *via* the more stable carbonium ion at the tertiary C(7), in spite of the electron-withdrawing effect of the acetylenic group. Probably only partial carbonium-ion formation or a tight ion pair occurs to account for the generation of only two (or two major) diastereoisomers.

seawater/EtOH solutions of **2** on standing [5] to β-elimination leading to epoxide opening to form an allylic alcohol. This was not easy to assess for dilute solutions of terpenes in water where deprotection of enol acetates may be a competitive process.

We thank Mr. *S. Gadotti*, Mr. *A. Tomei*, and Mr. *A. Sterni* for excellent technical aid in synthesis/isolation, bioassays with ciliates, and mass spectra, respectively. We also thank the director and staff of *Consorzio Regionale di Idrobiologia e Pesca*, Livorno, for allowing *F. M.* and *F. P.* to take part in dredging campaigns to collect marine invertebrates, and MURST (Progetti 40%) and CNR, Roma, for financial support.

Experimental Part

1. *General.* See [4]. Moreover: For ^{1}H-NMR, the notation 'small' indicates $J < 0.5$ Hz.

2. *Collection and Isolation. C. taxifolia* was recollected on beginning August 1992 in the area of Cap Martin, Côte d'Azur. Following the procedure described previously [4], the FC fraction eluted with petroleum ether/AcOEt 5:1 was subjected to HPLC with hexane/AcOEt 49:1: *taxifolione* (**4**; t_R 13.5 min, 0.0027% rel. to freeze-dried seaweed). The FC fraction eluted with petroleum ether/AcOEt 1:4 was subjected to HPLC with hexane/AcOEt 3:1: *7,7-C-didehydro-6-hydroxy-6,7-dihydrocaulerpenyne* (**3a**; t_R 24.5 min, 0.0021% rel. to freeze-dried seaweed). These two terpenes, on examination of residual freeze-dried seaweed, were also present in the previous collection of *C. taxifolia* [4].

3. *Solubility and Stability of* C. taxifolia *Terpenes.* In abs. EtOH at –20°, both caulerpenyne (**1**) and 10,11-epoxycaulerpenyne (**2**; *ca.* 1 mg/ml) were stable for some months. Under the same conditions, the taxifolials [4] were less stable [5]. For all these terpenes, degradation, once started, ran as an autocatalytic reaction. In seawater/EtOH mixtures, the solubility decreased with the EtOH content, the limit being 20 or 5 μg/ml for **1** in seawater containing 2 or 0.5% EtOH, respectively, and 5 μg/ml for **2** in seawater containing 0.6% EtOH. Exceeding these limits led to the formation of oily droplets, which could be observed under the microscope. In seawater/EtOH solutions, these terpenes were far less stable than in 100% EtOH, *e.g.* **1** disappearing by 30, 45, and 55% in 1, 5, and 29 h, respectively, and **2** by 30% in 1 h and completely in less than 17 h. Triacetate **3a** is comprised within these limits.

4. *Biological Assays.* 4.1. *Antibacterial Assays.* Bacteria were selected from our collection as isolates of marine invertebrates, collected by dredging off the coast of Tuscany, and from *C. taxifolia* of Cap Martin. The antimicrobial activity was determined according to NCCLS's directions [10] for the agar-diffusion method, optimized as follows. Test bacteria were harvested from culture exponentially growing in *Difco Marine Broth 2216E*, washed twice, and diluted to 0.5×10^8 cells ml^{-1}, with sterile artificial seawater [11]. EtOH solns. of the test terpene (*ca.* 1 mg/ml) were absorbed on a standard paper disk (*BBL*; 7 mm diameter), which was dried so as to have 10–60 μg of terpene per disk, which were placed on a *Petri* agar plate (66.5 cm^2) containing the agarized *Marine Broth* (1.5% *Bacto Agar*, w/v), freshly seeded with the bacterial suspension (0.1 ml). The plate was incubated at 25° for 24–96 h, or longer for slow-growing marine bacteria. The test was run in triplicate for each terpene and bacterium, considering the bacterial growth inhibition zone diameter around the paper disk. Strains giving a diameter < 2.0 mm at 60 μg/disk of test terpene were considered to be resistant; for sensitive strains, the amount of test terpene resulting in a growth inhibition diameter of 2.0 mm was assumed as the highest dose of terpene not exerting any inhibitory effect on bacterial growth.

4.2. *Cytotoxicity Assays.* To minimize problems of degradation, *C. taxifolia* terpenes were used as freshly prepared solns. (from *ca.* 1 mg/ml stable solns. in abs. EtOH) in sterile, defined, artificial seawater [12], which thus had from 0.05 to 2% content of EtOH (ineffective *per se*, at these concentrations, on any of the ciliate stocks, as shown by control experiments). For each strain of ciliate and each terpene tested (*Table 2*), a series of consecutive steps in concentration was used to define LD_{100} and ED_{100} (see *Table 2* for definitions). Cytotoxic effects were assessed microscopically as complete loss of motility (LD_{100}) or as number of fission products that a single cell gave per time unit, from which the fission rate in fissions/day was calculated. Each strain was run 3 times in successive days. Cytotoxic effects were assessed in 6 single cells for each terpene concentration at each run for each strain. Effects were scored after 3 ± 1 and 16 ± 1 h, the latter being a longer generation time than any species analyzed. Controls were included for solvent-treated as well as untreated cells, and they were run simultaneously with terpene-treated cells.

5. *7,7-*C*-Didehydro-6-hydroxy-6,7-dihydrocaulerpenyne* (= *(4*S*,6*S*,1*E*)-3-[(*Z*)-Acetoxymethylidene]-6-hydroxy-11-methyl-7-methylidenedodeca-1,10-dien-8-yne-1,4-diyl Diacetate;* **3a**). $[\alpha]_D^{20} = -53.7$ ($c = 0.095$, EtOH). UV (EtOH): 242 (21500). ^{1}H-NMR (C_6D_6): 7.93 (*dd*, *J*(1,2) = 12.6, *J*(1, OCH=C(3)) = 0.6, H–C(1)); 1.554 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.77 (*dd*, *J*(2,1) = 12.6, *J*(2, OCH=C(3)) = 1.0, H–C(2)); 7.30 (br. *dd*, *J*(OCH=C(3),2) = 1.0, *J*(OCH=C(3),1) = 0.6, *J*(OCH=C(3),4) small, OC*H*=C(3)); 1.551 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 6.54 (br. *dd*, *J*(4,5β) = 10.5, *J*(4,5α) = 3.3, *J*(4, OCH=C(3)) small, H–C(4)); 1.69 (*s*, AcO–C(4)); 2.00 (*ddd*, J_{gem} = 14.7, *J*(5α,6) = 9.3, *J*(5α,4) = 3.3, H_α–C(5)); 2.68 (*ddd*, J_{gem} = 14.7, *J*(5β,4) = 10.5, *J*(5β,6) = 3.0, H_β–C(5)); 4.27 (*dddd*, *J*(6,5α) = 9.3, *J*(6,5β) = 3.0, *J*(6, CH_b–C(7)) = 1.5, *J*(6, CH_a=C(7)) = 1.2, H–C(6)); 5.45 (br. *s*, J_{gem} = 1.5, *J*(CH_a=C(7),6) = 1.2, CH_a=C(7)); 5.50 (*dd*, J_{gem} = *J*(CH_b=C(7),6) = 1.5, CH_b=C(7)); 5.38 (*qq*, *J*(10,12) = 1.4, *J*(10, Me–C(11)) = 1.2, H–C(10)); 1.84 (br. *s*, *J*(Me–C(11),10) = 1.2, *J*(Me–C(11),12) small, Me–C(11); 1.46 (br. *s*, *J*(12,10) = 1.4, *J*(12, Me–C(11)) small, Me(12)). MS: 348 (0.3, $[M - CH_2CO]^{+\cdot}$), 331 (0.2, $[M - AcO]^+$), 330 (0.4, $[M - AcOH]^{+\cdot}$), 288, (2.0, $[330 - CH_2CO]^+$), 271 (1.9, $[330 - AcO]^+$), 270 (2.2, $[330 - AcOH]^{+\cdot}$), 228 (8.2, $[270 - CH_2CO]^{+\cdot}$), 213 (6.0), 210 (3.1, $[270 - AcOH]^{+\cdot}$), 199 (7.1), 185 (5.6), 170 (6.5), 149 (3.4), 141 (7.6), 112 (13.2), 91 (12), 43 (100).

6. *Taxifolione* (= *6-Methylhept-5-en-3-yn-2-one;* **4**). UV (EtOH): 275 (12000). ^{1}H-NMR ($CDCl_3$): 2.37 (*s*, H–C(1)); 5.41 (*qq*, *J*(5,7) = *J*(5, Me–C(6)) = 1.2, H–C(5)); 1.90 (br. *s*, *J*(7,5) = 1.2, *J*(7, Me–C(6)) small, 3 H–C(7)); 1.99 (br. *s*, *J*(Me–C(6), 5) = 1.2, *J*(Me–C(6), 7) small, Me–C(6)). NOE: H–C(7)→13% on H–C(5). ^{1}H,^{13}C-COSY (^nJ): *H*–C(1)/C(2) and C(3); C*H*$_3$–C(6)/C(4), C(6), and C(7); *H*–C(7)/C(5) and C*H*$_3$–C(6).

7. *Treatment of* **3a** *with CF$_3$C(OMe)(Ph)COCl.* A soln. of (–)-(*R*)-CF_3C(OMe)(Ph)COCl [7] (8.0 mg) in anh. pyridine (0.1 ml) was added of a soln. of **3a** (2.05 mg) in CCl_4 (0.1 ml). The resulting mixture was stirred at r.t. for 14 h. After evaporation, the residue was dissolved in AcOEt and submitted to prep. TLC (petroleum ether/Et_2O 1:1; detection by UV): **5a** (1.8 mg, 60%; R_f 0.42). Under otherwise identical conditions, **5b** (57% yield; R_f 0.45) was obtained from **3a** and (+)-(*S*)-CF_3C(OMe)(Ph)COCl [7].

*(4*S*,6*S*,1*E*)-1,4-Diacetoxy-3-[(*Z*)-acetoxymethylidene]-11-methyl-7-methylidenedodeca-1,10-dien-8-yn-6-yl (2*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate* (**5b**): ^{1}H-NMR ($CDCl_3$): 7.62 (*dd*, *J* = 12.6, 0.6, H–C(1)); 2.05 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.81 (*dd*, *J* = 12.6, 0.9, H–C(2)); OCH=C(3) not det., probably under the solvent signal; 2.11 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 6.04 (*dd*, *J* = 11.0, 3.3, H–C(4)); 2.16 (*s*, AcO–C(4)); 2.418 (*ddd*, *J* = 14.8, 11.0, 3.0, H_α–C(5)); 2.175 (*ddd*, *J* = 14.8, 10.3, 3.3, H_β–C(5)); 5.54 (*dd*, *J* = 10.0, 3.0, H–C(6)); 5.51 (br. *s*, CH_2=C(7)); 5.32 (*qq*, *J*(10,12) = *J*(10, Me–C(11)) = 1.1, H–C(10)); 1.82 (br. *s*, Me–C(11)); 1.81 (br. *s*, Me(12)).

*(4*S*,6*S*,1*E*)-1,4-Diacetoxy-3-[(*Z*)-acetoxymethylidene]-11-methyl-7-methylidenedodeca-1,10-dien-8-yne-6-yl (2*S*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate* (**5a**): ^{1}H-NMR ($CDCl_3$): 7.57 (*dd*, *J* = 12.9, 0.9, H–C(1)); 2.05 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.78 (*dd*, *J* = 12.9, 1.1, H–C(2)); 7.20 (br. *s*, OCH=C(3)); 2.09 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 5.86 (*dd*, *J* = 11.0, 3.6, H–C(4)); 2.15 (*s*, AcO–C(4)); 2.345 (*ddd*, *J* = 14.9, 11.0, 3.5, H_α–C(5)); 2.192 (*ddd*, *J* = 14.9, 10.2, 3.6, H_β–C(5)); 5.64 (*dd*, *J* = 10.2, 3.5, H–C(6)); 5.62 (br. *s*, CH_a=C(7)); 5.56 (br. *s*, CH_b=C(7)); 5.39 (*qq*, *J*(10,12) = *J*(10, Me–C(11)) = 1.2, H–C(10)); 1.84 (br. *s*, Me–C(11), Me(12)).

8. *Epoxydation of Caulerpenyne* [4] (**1**) *with 3-Chloroperoxybenzoic Acid.* 8.1. *Buffered with NaHCO$_3$.* To a soln. of **1** (52 mg, 0.139 mmol) in CH_2Cl_2 (5 ml) were added 80% 3-$ClC_6H_4CO_3H$ (35.9 mg) and $NaHCO_3$ (17.5 mg, 0.21 mmol). On stirring at 0° for 1 h, all **1** disappeared. The mixture was filtered and evaporated. Et_2O was added, the org. phase washed with $NaHSO_3$ and then $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated and the residue (62 mg) subjected to HPLC (silica gel, gradient hexane/AcOEt 85:15→7:3) to give, in order of increasing polarity, fractions *a–g*. *Fr. a*, **6a** (2.2 mg, 4%); *Fr. b*, **6b** (3.0 mg, 5%); *Fr. c*, 1:1 diastereoisomer mixture of 10,11-epoxycaulerpenyne (**2**; 10.3 mg, 19%); *Fr. d*, diastereoisomer mixture **7** (7.8 mg, 13%) which was not separated; *Fr. e*, 2:5 mixture **8b**/**8a** (14.6 mg); *Fr. f*, *ca.* 1:1 mixture **8b**/**3b** (6.0 mg) which was separated by HPLC (hexane/AcOEt 7:3); *Fr. g*, **3a** (3.8 mg, 7%). Yields: **8a** 11%, **8b** 9%, and **3b** 5%.

Both **8a** and **8b** were acetylated with excess Ac_2O in pyridine (overnight at r.t. to give **9a** and **9b**, respectively quant.).

*(4*S*,6*R*,1*E*)-3-[(*Z*)-Acetoxymethylidene]-6-hydroxy-11-methyl-7-methylidenedodeca-1,10-dien-8-yne-1,4-diyl Diacetate* (**3b**): ^{1}H-NMR (C_6D_6): 7.94 (*dd*, *J*(1,2) = 12.6, *J*(1, OCH=C(3)) = 0.6, H–C(1)); 1.56 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.78 (br. *dd*, *J*(2,1) = 12.6, *J*(2, OCH=C(3)) = 1.4, *J*(2,4) small, H–C(2)); 7.33 (*dd*, *J*(OCH=C(3), 2) = 1.4, *J*(OCH=C(3), 1) = 0.6, OCH=C(3)); 1.62 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 6.44 (br. *dd*, *J*(4,5β) = *J*(4,5α) = 7.5, *J*(4,2) small, H–C(4)); 1.68 (*s*, AcO–C(4)); 2.369, 2.372 (2 *ddd*, *J* = 15.0, 7.5, 7.5 and *J* = 15.0, 7.5, 6.0, resp., CH_2(5)); 3.5 (br. *s*, OH), 4.14 (br. *dd*, *J* = 7.5, 6.0, H–C(6)); 5.41 (br. *s*, J_{gem} = 1.5, *J*(CH_a–C(7), 6) small, CH_a=C(7)); 5.38 (*dd*, J_{gem} = *J*(CH_b=C(7), 6) = 1.5, CH_b=C(7)); 5.37 (*qq*, *J*(10,12) = 1.5, *J*(10, Me–C(11)) = 1.2, H–C(10)); 1.79 (br. *s*, *J*(Me–C(11), 10) = 1.2, *J*(Me–C(11), 12) small, Me–C(11)); 1.45

(br. *s*, $J(12,10) = 1.5$, $J(12$, Me–C(11)) small, Me(12)). NOE: H–C(6)→6% on CH_a=C(7). MS: 331 (0.2, $[M - AcO]^+$), 330 (0.4, $[M - AcOH]^{+\cdot}$), 288 (2.2), 271 (4.8), 270 (4.2), 255 (4.2), 246 (3.0), 229 (9.1), 228 (13.8), 213 (10.5), 199 (12.6), 43 (100).

*(4*S,*6*S,*7*S,*1*E*)-3-[(*Z*)-Acetoxymethylidene]-6,7-epoxy-7,11-dimethyldodeca-1,10-dien-8-yne-1,4-diyl Diacetate* (**6a**): ^{1}H-NMR (C_6D_6): 7.91 (br. *d*, $J(1,2) = 12.6$, $J(1$, OCH=C(3)) small, H–C(1)); 1.56 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.71 (br. *d*, $J(2,1) = 12.6$, $J(2$ OCH=C(3)) small, H–C(2)); 7.34 (br. *s*, J(OCH=C(3), 2) and J(OCH=C((3), 1) small, OCH=C(3)); 1.57 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 6.34 (br. *dd*, $J(4,5\beta) = 6.6$, $J(4,5\alpha) = 8.4$, H–C(4)); 1.68 (*s*, AcO–C(4)); 2.00 (*ddd*, $J_{gem} = 14.1$, $J(5\alpha,4) = 8.4$, $J(5\alpha,6) = 6.6$, H_α–C(5)); 1.92 (*ddd*, $J_{gem} = 14.1$, $J(5\beta,4) = 6.6$, $J(5\beta,6) = 6.6$, H_β–C(5)); 3.34 (*dd*, $J(6,5\alpha) = J(6,5\beta) = 6.6$, H–C(6)); 1.52 (*s*, Me–C(7)); 5.22 (*qq*, $J(10,12) = J(10$, Me–C(11)) = 1.1, H–C(10)); 1.73 (br. *s*, J(Me–C(11), 10) = 1.1, J(Me–C(11), 12) small, Me–C(11)); 1.40 (br. *s*, $J(12,10) = 1.1$, $J(12$, Me–C(11) small, Me(12)).

*(4*S,*6*R,*7*R,*1*E*)-3-[(*Z*)-Acetoxymethylidene]-6,7-epoxy-7,11-dimethyldodeca-1,10-dien-8-yne-1,4-diyl Diacetate* (**6b**): ^{1}H-NMR (C_6D_6): 7.91 (br. *d*, $J(1,2) = 12.8$, $J(1$, OCH=C(3)) small, H–C(1)); 1.576 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.69 (br. *d*, $J(2,1) = 12.8$, $J(2$, OCH=C(3)) small, H–C(2)); 7.36 (br. *s*, J(OCH=C(3), 2), J(OCH=C(3), 1), and J(OCH=C(3), 4) small, OCH=C(3)); 1.583 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 6.36 (br. *dd*, $J(4,5\beta) = 6.6$, $J(4,5\alpha) = 8.7$, $J(4$, OCH=C(3)) small, H–C(4)); 1.70 (*s*, AcO–C(4)); 1.89 (*ddd*, $J_{gem} = 14.1$, $J(5\alpha,4) = 8.7$, $J(5\alpha,6) = 6.6$, H_α–C(5)); 2.00 (*ddd*, $J_{gem} = 14.1$, $J(5\beta,4) = J(5\beta,6) = 6.6$, H_β–C(5)); 3.29 (*t*, $J(6,5\alpha) = J(6,5\beta) = 6.6$, H–C(6)); 1.43 (*s*, Me–C(7)); 5.21 (*qq*, $J(10,12) = J(10$, Me–C(11)) = 1.2, H–C(10)); 1.73 (br. *s*, J(Me–C(11), 10) = 1.2, J(Me–C(11), 12) small, Me–C(11)); 1.38 (br. *s*, $J(12,10) = 1.2$, $J(12$, Me–C(11)) small, Me(12)).

*(4*S,*6*R,*1*E*)-1,4-Diacetoxy-3-[(*Z*)-acetoxymethylidene]-6-hydroxy-7,11-dimethyldodeca-1,10-dien-8-yn-7-yl 3-Chlorobenzoate* (**8a**; configuration at C(6) tentative): ^{1}H-NMR (C_6D_6): 7.99 (br. *d*, $J(1,2) = 12.6$, $J(1$, OCH=C(3)) small, H–C(1)); 1.57 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.81 (*dd*, $J(2,1) = 12.6$, $J(2$, OCH=C(3)) = 0.9, H–C(2)); 7.35 (br. *s*, J(OCH=C(3), 2) = 0.9, J(OCH=C(3), 1) and J(OCH=C(3), 4) small, OCH=C(3)); 1.61 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 6.64 (br. *dd*, $J(4,5\alpha) = 2.9$, $J(4,5\beta) = 11.1$, $J(4$, OCH=C(3)) small, H–C(4)); 1.77 (*s*, AcO–C(4)); 1.98 (*ddd*, $J_{gem} = 14.4$, $J(5\alpha,4) = 2.9$, $J(5\alpha,6) = 10.4$, H_α–C(5)); 2.84 (*ddd*, $J_{gem} = 14.4$, $J(5\beta,4) = 11.1$, $J(5\beta,6) = 1.5$, H_β–C(5)); 4.39 (*dd*, $J(6,5\alpha) = 10.4$, $J(6,5\beta) = 1.5$, H–C(6)); 1.89 (*s*, Me–C(7)); 5.26 (*qq*, $J(10,12) = J(10$, Me–C(11)) = 1.2, H–C(10)); 1.86 (br. *s*, J(Me–C(11), 10) = 1.2, J(Me–C(11), 12) small, Me–C(11)); 1.41 (br. *s*, $J(12,10) = 1.2$, $J(12$, Me–C(11) small, Me(12)); arom. H: 8.10 (br. *dd*, $J(2,4) = J(2,6) = 1.8$, $J(2,5)$ small, H–C(2)); 7.05 (*ddd*, $J(4,5) = 8.0$, $J(4,2) = 1.8$, $J(4,6) = 1.1$, H–C(4)); 6.76 (*dd*, $J(5,4) = 8.0$, $J(5,2) = 7.8$, $J(5,2)$ small, H–C(5)); 7.80 (*ddd*, $J(6,5) = 8.0$, $J(6,2) = 1.8$, $J(6,4) = 1.1$, H–C(6)). MS: 446 (0.5), 444 (1.1), 288 (5.6), 271 (16.2), 246 (8.7), 245 (9.3), 228 (19.5), 158 (9.1), 156 (22.4), 141 (38.3), 139 (79.8), 123 (48.6), 43 (100).

*(4*S,*6*S,*1*E*)-1,4-Diacetoxy-3-[(*Z*)-acetoxymethylidene]-6-hydroxy-7,11-dimethyldodeca-1,10-dien-8-yn-7-yl 3-Chlorobenzoate* (**8b**; configuration at C(6) tentative): ^{1}H-NMR (C_6D_6): 7.97 (br. *d*, $J(1,2) = 12.4$, $J(1$, OCH=C(3)) small, H–C(1)); 1.58 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.85 (br. *d*, $J(2,1) = 12.4$, $J(2$, OCH=C(3)) small, H–C(2)); 7.39 (br. *s*, J(OCH=C(3), 2) and J(OCH=C(3), 1) small, OCH=C(3)); 1.65 (*s*, AcOOCH=C(3) or AcO–C(1)); 6.62 (*dd*, $J = 10.0$, 5.1, H–C(4)); 1.68 (*s*, AcO–C(4)); 2.67, 2.28 (2 *ddd*, $J = 13.9$, 10.0, 1.7 and $J = 13.9$, 10.7, 5.1, resp., CH_2(5)); 4.10 br. *ddd*, $J = 10.7$, 1.7, $J(6$, OH) = 6.6, H–C(6)); 2.48 (br. *d*, J(OH, 6) = 6.6, OH), 1.88 (*s*, Me–C(7)); 5.22 (*qq*, $J(10,12) = J(10$, Me–C(11)) = 1.2, H–C(10)); 1.82 (br. *s*, J(Me–C(11), 10) = 1.2, J(Me–C(11), 12) small, Me–C(11)); 1.39 (br. *s*, $J(12,10) = 1.2$, $J(12$, Me–C(11)) small, Me(12)); arom. H: 8.06 (br. *dd*, $J(2,4) = J(2,6) = 2.0$, $J(2,5)$ small, H–C(2)); 7.02 (*ddd*, $J(4,5) = 7.9$, $J(4,2) = 2.0$, $J(4,6) = 1.2$, H–C(4)); 6.71 (br. *dd*, $J(5,4) = J(5,2) = 7.9$, $J(5,2)$ small, H–C(5)); 7.77 (*ddd*, $J(6,5) = 7.9$, $J(6,2) = 2.0$, $J(6,4) = 1.2$, H–C(6)). MS: 330 (0.6), 305 (0.4), 288 (3.0), 287 (2.2), 271 (5.2), 270 (3.7), 246 (6.5), 231 (6.9), 229 (6.2), 158 (8.5), 156 (24.2), 141 (19.5), 139 (60.5), 43 (100).

8.2. *Buffered with Na_2HPO_4.* Under otherwise identical conditions to those in *Exper. 8.1*, **2**, **6a**, and **6b** were obtained in 25, 20, and 20% yield, respectively.

9. *Acetylation of* **8a** *and* **8b** with Ac_2O in pyridine at r.t. overnight gave **9a** and **9b**, respectively (quant.).

*(4*S,*6*R,*1*E*)-1,4,6-Triacetoxy-3-[(*Z*)-acetoxymethylidene]-7,11-dimethyldodeca-1,10-dien-8-yn-7-yl 3-Chlorobenzoate* (**9a**; configuration at C(6) tentative): ^{1}H-NMR (C_6D_6): 7.98 (*dd*, $J(1,2) = 12.9$, $J(1$, OCH=C(3)) = 0.6, H–C(1)); 1.55 (*s*, AcO–C(1) or *Ac*OCH=C(3)); 5.79 (*dd*, $J(2,1) = 12.9$, $J(2$, OCH=C(3)) = 0.9, H–C(2)); 7.36 (br. *s*, J(OCH=C(3), 2) = 0.9, J(OCH=C(3), 1) = 0.6, OCH=C(3)); 1.62 (*s*, *Ac*OCH=C(3) or AcO–C(1)); 6.35 (*dd*, $J(4,5\alpha) = 4.2$, $J(4,5\beta) = 10.5$, H–C(4)); 1.81 (*s*, AcO–C(4) or AcO–C(6)); 2.18 (*ddd*, $J_{gem} = 14.7$, $J(5\alpha,4) = 4.2$, $J(5\alpha,6) = 10.8$, H_α–C(5)); 2.96 (*ddd*, $J_{gem} = 14.7$, $J(5\beta,4) = 10.5$, $J(5\beta,6) = 2.1$, H_β–C(5)); 6.09 (*dd*, $J(6,5\alpha) = 10.8$, $J(6,5\beta) = 2.1$, H–C(6)); 1.84 (*s*, AcO–C(6) or AcO–C(4)); 1.87

(*s*, Me–C(7)); 5.28 (*qq*, $J(10,12) = J(10,$ Me–C(11)$) = 1.2$, H–C(10)); 1.87 (br. *s*, J(Me–C(11), 10) = 1.2, J(Me–C(11), 12) small, Me–C(11)); 1.40 (br. *s*, $J(12,10) = 1.2$, $J(12,$ Me–C(11) small, Me(12)); arom. H: 8.19 (br. *dd*, $J(2,4) = J(2,6) = 1.8$, $J(2,5)$ small, H–C(2)); 7.03 (*ddd*, $J(4,5) = 7.9$, $J(4,2) = 1.8$, $J(4,6) = 1.2$, H–C(4)); 6.77 (*dd*, $J(5,4) = 7.9$, $J(5,6) = 7.8$, $J(5,2)$ small, H–C(5)); 7.93 (*ddd*, $J(6,5) = 7.8$, $J(6,2) = 1.8$, $J(6,4) = 1.2$, H–C(6)). ^{13}C-NMR (C_6D_6): 137.32 (*d*, C(1)); 19.85 (*q*, $MeCO_2C(1)$ or $MeCO_2CH{=}C(3)$); 167.12 (*s*, $MeCO_2C(1)$ or $MeCO_2CH{=}C(3)$); 109.72 (*d*, C(2)); 119.45 (*s*, C(3)); 134.74 (*d*, $OCH{=}C(3)$); 19.91 (*q*, $MeCO_2CH{=}C(3)$ or $MeCO_2C(1)$); 166.43 (*s*, $MeCO_2CH{=}C(3)$ or $MeCO_2CH{=}C(1)$); 66.05 (*d*, C(4)); 20.41 (*q*, $MeCO_2C(4)$ or $MeCO_2C(6)$); 169.77 (*s*, $MeCO_2C(4)$ or $MeCO_2C(6)$); 34.15 (*t*, C(5)); 72.98 (*d*, C(6)); 20.47 (*q*, $MeCO_2C(6)$ or $MeCO_2C(4)$); 169.49 (*s*, $MeCO_2C(6)$ or $MeCO_2C(4)$); 78.11 (*s*, C(7)); 21.69 (*q*, Me–C(7)); 88.43 (*s*, C(8)); 86.93 (*s*, C(9)); 104.95 (*d*, C(10)); 150.87 (*s*, C(11)); 21.12 (*q*, Me–C(11)); 24.48 (*q*, C(12)); arom. C: 163.02 (*s*, CO), 133.14 (*s*, C(1)); 130.09 (*d*, C(2)); 134.78 (*s*, C(3)); 132.89 (*d*, C(4)); 129.95 (*d*, C(5)); C(6) submerged by the solvent signals. MS: 428 (0.1), 426 (0.3), 390 (1.4), 330 (3.8), 288 (11.2), 271 (10.8), 270 (8.4), 229 (9.4), 228 (26.9), 213 (5.2), 199 (7.0), 158 (2.1), 156 (7.0), 141 (16.5), 139 (47.8), 43 (100).

(4S,6S,1E)-1,4,6-Triacetoxy-3-[(Z)-acetoxymethylidene]-7,11-dimethyldodeca-1,10-dien-8-yn-7-yl 3-Chlorobenzoate (**9b**; configuration at C(6) tentative): ^{1}H-NMR (C_6D_6): 7.97 (*dd*, $J(1,2) = 12.9$, $J(1,$ OCH=C(3)$) = 0.6$, H–C(1)); 1.57 (*s*, AcO–C(1) or $AcOCH{=}C(3)$); 5.81 (*dd*, $J(2,1) = 12.9$, $J(2,$ OCH=C(3)$) = 0.6$, H–C(2)); 7.50 (*dd*, $J($OCH=C(3), 2$) = 0.9$, $J($OCH=C(3), 1$) = 0.6$, OCH=C(3)); 1.70 (*s*, $AcOCH{=}C(3)$ or AcO–C(1)); 6.39 (br. *dd*, $J(4,5\alpha) = 9.6$, $J(4,5\beta) = 5.1$, $J(4,2)$ small, H–C(4)); 1.75 (*s*, AcO–C(4) or AcO–C(6)); 2.83 (*ddd*, $J_{gem} = 14.1$, $J(5\alpha,4) = 9.6$, $J(5\alpha,6) = 1.5$, H_α–C(5)); 2.39 (*ddd*, $J_{gem} = 14.1$, $J(5\beta,4) = 5.1$, $J(5\beta,6) = 10.2$, H_β–C(5)); 5.75 (*dd*, $J(6,5\alpha) = 1.5$, $J(6,5\beta) = 10.2$, H–C(6)); 1.76 (*s*, AcO–C(6) or AcO–C(4)); 1.86 (*s*, Me–C(7)); 5.25 (*qq*, $J(10,12) = 1.5$, $J(10,$ Me–C(11)$) = 1.2$, H–C(10)); 1.84 (*dq*, $J($Me–C(11), 10$) = 1.2$, $J($Me–C(11), 12$) = 0.6$, Me–C(11)); 1.40 (*dq*, $J(12,10) = 1.5$, $J(12,$ Me–C(11)$) = 0.6$, Me(12)); arom. H: 8.14 (*ddd*, $J(2,4) = J(2,6) = 1.8$, $J(2,5) = 0.6$, H–C(2)); 7.00 (*ddd*, $J(4,5) = 7.8$, $J(4,2) = 1.8$, $J(4,6) = 1.2$, H–C(4)); 6.71 (*ddd*, $J(5,4) = J(5,2) = 7.8$, $J(5,2) = 0.6$, H–C(5)); 7.87 (*ddd*, $J(6,5) = 7.8$, $J(6,2) = 1.8$, $J(6,4) = 1.2$, H–C(6)). ^{13}C-NMR (C_6D_6): 137.57 (*d*, C(1)); 19.94 (*q*, $MeCO_2C(1)$ or $MeCO_2CH{=}C(3)$); 167.08 (*s*, $MeCO_2C(1)$ or $MeCO_2C(3)$); 109.48 (*d*, C(2)); 117.50 (*s*, C(3)); 136.56 (*d*, $OCH{=}C(3)$); 19.98 (*q*, $MeCO_2CH{=}C(3)$ or $MeCO_2C(1)$); 166.50 (*s*, $MeCO_2CH{=}C(3)$ or $MeCO_2C(1)$); 67.17 (*d*, C(4)); 20.47 (*q*, $MeCO_2C(4)$ or $MeCO_2C(6)$); 169.53 (*s*, $MeCO_2C(4)$ or $MeCO_2C(6)$); 34.20 (*t*, C(5)); 73.37 (*d*, C(6)); 20.66 (*q*, $MeCO_2C(6)$ or $MeCO_2C(4)$); 169.04 (*s*, $MeCO_2C(6)$ or $MeCO_2C(4)$); 78.29 (*s*, C(7)); 21.53 (*q*, Me–C(7)); 88.98 (*s*, C(8)); 86.75 (*s*, C(9)); 104.88 (*d*, C(10)); 150.71 (*s*, C(11)); 21.17 (*q*, Me–C(11)); 24.51 (*q*, C(12)); arom. C: 163.02 (*s*, CO), 133.10 (*s*, C(1)); 130.07 (*d*, C(2)); 134.74 (*s*, C(3)); 132.84 (*d*, C(4)); 129.84 (*d*, C(5)); C(6) submerged by solvent signals. MS: 449 (0.3, $[M - ClC_6H_4CO]^+$), 428 (0.3), 427 (0.4), 426 (0.4), 330 (5.3), 288 (16.2), 271 (17.3), 270 (15.3), 229 (14.5), 228 (25.7), 213 (8.5), 139 (11.4), 158 (4.8), 156 (11.3), 141 (21.0), 139 (60.2), 43 (100).

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