Chlorophyll-photosensitised Photodegradation of Caulerpenyne; a Potentially Harmful Sesquiterpenoid from Tropical Green Seaweeds in the Genus *Caulerpa*

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On exposure to daylight, caulerpenyne **1** in solution in the presence of oxygen and chlorophylls or pheophytins undergoes rapid, multifarious degradation involving a peculiar route through ynone **2**, thus providing a model for detoxification from these and similar food web and seagrass potentially harmful terpenoids of caulerpalean green seaweeds in Mediterranean areas invaded by the tropical *Caulerpa taxifolia*.

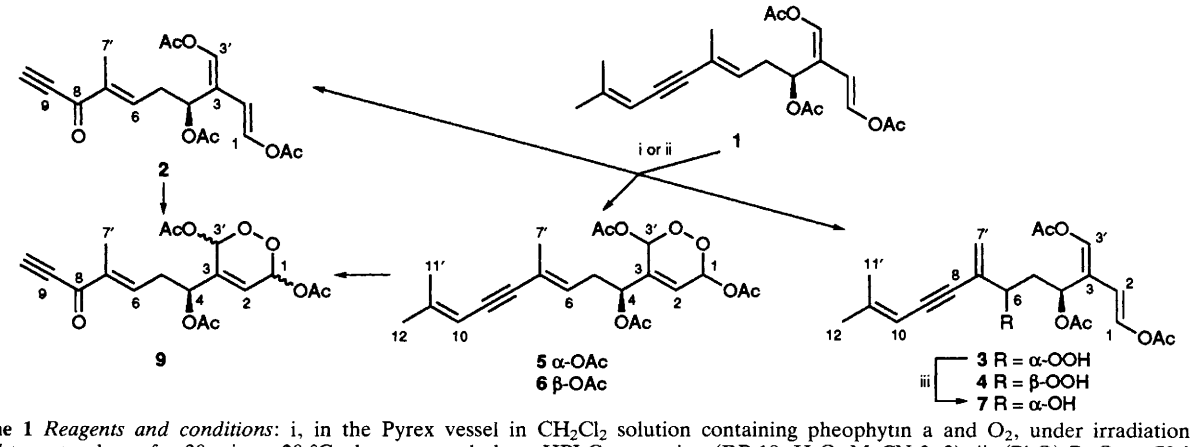
Tropical green seaweeds in the families Caulerpaceae and Udoteaceae produce strongly bioactive terpenoids as defensive agents against voracious grazers in the tropics.[1] Abnormally, however, as the result of continuing expansion of the recently imported tropical caulerpacean seaweed *Caulerpa taxifolia*, its main terpenoid, caulerpenyne **1**, occurs, as algal content in heavily colonized Mediterranean areas, at a dramatic 50–100 kg per hectare.[2a] Under such conditions, this and minor terpenoids of *C. taxifolia*[2a,b] may both adversely affect the marine food web[2b] and constitute a toxic hazard.[2c] It is thus urgent to evaluate which key organisms, besides marine bacteria and ciliates,[2b] may be in danger and how recycling of the algal toxins may occur.

We show here that these terpenoids are potentially harmful not only to the marine food web,[2b] but also to seagrass. However, they are photochemically rapidly degraded (involving a peculiar route) on daylight irradiation in the presence of oxygen and chlorophylls. Thus, irradiation at 20 °C for 30 min of a stirred solution of caulerpenyne (0.12 g) and *C. taxifolia* derived pheophytin a† (1.2 mg) in CH_2Cl_2 (40 cm^3) under O_2, with a 150 W tungsten lamp at 20 cm from the Pyrex reactor, followed by evaporation *in vacuo* and RP-18 HPLC with MeCN–H_2O (3:2, 5 cm^3 min^{-1}), gave products **2** (t_R 6.8 min, 0.0107 g),‡ **3** (t_R 9.3 min, 0.0069 g), **4** (t_R 8.3 min, 0.0064 g), **5** (t_R 16.3 min, 0.0044 g), **6** (t_R 17.1 min, 0.008 g) and **8**§ (t_R 5.8 min, 0.0015 g), besides unreacted **1** (t_R 20.3 min, 0.070 g) (Scheme 1). On prolonged irradiation, caulerpenyne disappeared completely while the above products were found in greatly diminished yield.

The same product distribution was observed both on irradiation of caulerpenyne in the presence of authentic chlorophyll a or b, albeit less efficiently,¶ and with replacement of the lamp with daylight irradiation, while without chlorophylls or pheophytins no reaction occurred.

That singlet-oxygen attack on the terpenoids must be involved was proven by obtaining the same product distribution on the treatment of caulerpenyne with thermally-generated[3] singlet oxygen (Scheme 1).

Formation of the allylic hydroperoxides **3** and **4** at the central double bond of caulerpenyne conforms to the typical behaviour of allylic systems towards singlet oxygen. Peroxidation at 1,3′ of the previously unexplored 1,4-diacetoxybutadiene system, which is typical of these seaweeds, is also in line with the reactivity of conjugated dienes towards singlet oxygen. But reactivity at the enyne system is most unusual. We can envisage a reaction pathway going through a strained six-membered peroxy allene intermediate of attack by singlet oxygen at C-8/C-11 of the enyne system. Such an intermediate finds analogy in six-membered 1,2-cyclohexadienes.[4] Alternatively, singlet oxygen attack at C-8 in the enyne system may give a diradical allene intermediate that adds oxygen at C-11 while breaking down to **2** and acetone. In any case, **8** can arise from either singlet-oxygen peroxidation of **2** or enyne attack on **5**/**6**, or both.‖ While the peroxy and hydroperoxy products of Scheme 1 proved ineffective, intermediate **2**, like 10,11-epoxycaulerpenyne,[2b] **2**, at doses as low as 1 μg cm^{-3}, proved to depress markedly the rate of division of the marine ciliated protists *Euplotes vannus* and *Euplotes crassus*,†† and to cause their death at higher doses (5 μg cm^{-3}). Moreover, on *Nicotiana glauca, in vitro* callus cultures,[5] **2** at 1 μg cm^{-3} induced marked necrotization,‡‡ while 10,11-epoxycaulerpenyne,[2a] at similar concentrations, caused a dramatic dedifferentiated cell proliferation, which was determined[5] as a model for flower plants, to depend on satellite DNA amplification. Whether this reflects a role of 10,11-epoxycaulerpenyne as plant growth promoter which might explain the enhanced vigorousness of *C. taxifolia* in the Mediterranean areas,§§ or whether it indicates the presence of tumorous cells, is under study. In either case, these terpenoids, when present at the abnormally high concentrations of the Mediterranean areas invaded by *C. taxifolia*, are potentially harmful to both the marine food web and seagrass.



Scheme 1 *Reagents and conditions*: i, in the Pyrex vessel in CH_2Cl_2 solution containing pheophytin a and O_2, under irradiation with a 150 W tungsten lamp for 30 min at 20 °C, then reversed-phase HPLC separation (RP-18, H_2O:MeCN 2:3); ii, $(PhO)_3P$, O_3, −70 °C, then warm from −70 °C to room temp; iii, PPh_3

However, if decompartmentalization of chlorophylls and terpenoids occurs at some stage of development of *C. taxifolia*, or the pheophytins become involved, even after senescence of the alga, reactions of the type in Scheme 1 may provide a mechanism for detoxification. These multiple reaction modes may provide inactivation of most types of bioactive terpenoids of these algae, where the 1,4-diacetoxybutadiene and olefinic functionalities commonly occur.

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Footnotes

† Two pigments, 'aged chlorophyll a' and 'aged chlorophyll b', were isolated in April 1994 from the 1991 *C. taxifolia* ethanolic extracts[2a] that had been stored at −20 °C throughout. They gave NMR spectra quite similar to authentic chlorophyll a or b, respectively, but proved to be less polar (RP-18 HPLC with EtOH 5 ml min^{-1}, t_R 7.0 and 6.3 min; silica gel TLC with hexane–AcOEt 3 : 1, R_F 0.5 and 0.3; authentic chlorophyll a and b (Aldrich) had TLC R_F 0.2 and 0.1, respectively, under the same conditions). These data, and the absence of detectable magnesium in flame emission spectroscopy, agree for pheophytin a and b (H. Scheer, *Chlorophylls*, CRC Press, Boca Raton, Florida, 1991) respectively.

‡ *Selected data* for **2**: $[\alpha]_D^{20}$ −41.0 (*c* 0.89, EtOH); UV (EtOH) λ_{max}/nm 250 (26000); ^{13}C NMR (75.43 MHz, C_6D_6) δ_c (rel. to $SiMe_4$) 137.51 (d, C–1), 167.24 (s) and 19.87 (q, AcO–1), 109.35 (d, C–2), 118.49 (s, C–3), 135.08 (d, C–3′), 166.26 (s) and 19.74 (q, AcO–3′), 68.11 (d, C–4), 169.04 (s) and 20.28 (q, AcO–4), 32.89 (t, C–5), 143.37 (d, C–6), 140.73 (s, C–7), 10.57 (q, C–7′), 178.48 (s, C–8), 79.87 (s, C–9), 78.93 (d, C–10); ^1H NMR (299.94 MHz, C_6D_6) δ_H (rel. to $SiMe_4$) 7.89 (br. d, $J_{1,2}$ 12.7 Hz, 1–H), 1.58 (s, 1–AcO), 5.70 (dd, $J_{2,1}$ 12.7, $J_{2,3'}$ 0.9 Hz, 2–H), 7.31 (br. s, 3′–H), 1.52 (s, 3′–AcO), 6.19 (br. t, $J_{4,5}$ 7.4 Hz, 4–H), 1.68 (s, 4–AcO), 2.56 and 2.47 (ABXYM$_3$, J_{AB} 15.0 Hz, 5–H_2), 7.02 (tq, $J_{6,5}$ 7.3, $J_{6,7'}$ 1.5 Hz, 6–H), 1.69 (ddd, $J_{7',6}$ = $J_{7',5\alpha}$ = $J_{7',5\beta}$ = 1.5 Hz, 7′–H_3), 2.36 (s, 10–H). Assignments were mainly from one-bond and long-range ^1H–^{13}C correlations, in particular revealing the position of the carbonyl group at C–8 by heterocorrelation with 7′–H_3 and 6–H. EI–MS *m/z* 348 ($M^{+\cdot}$, 0.2%), 288 ([M − AcOH]$^{+\cdot}$, 0.6), 246 (288 − CH_2CO, 5.3), 228 ([M − 2 AcOH]$^{+\cdot}$, 2.1), 186 (24), 43 (100).

§ *Selected data* (NMR: C_6D_6, limited to differences with respect to caulerpenyne). For **3**: δ_H 4.69 (brdd, *J* 9.0, 4.2 Hz, 6–H), 8.10 (br s, 6–OOH), 5.51 and 5.44 (br AB, J_{AB} 1.4 Hz, 7′–H_2); δ_c 84.65 (d, C–6), 131.69 (s, C–7), 122.25 (t, C–7′). For **4**. δ_H 4.55 (brdd, *J* 8.4, 5.7 Hz, 6–H), 8.12 (br s, 6–OOH), 5.51 and 5.42 (br AB, J_{AB} 1.5 Hz, 7′–H_2); δ_c 85.09 (d, C–6), 131.53 (s, C–7), 122.88 (t, C–7′). For **5** (or **6**): δ_H 6.67 (ddd, *J* 2.1, 1.2, 0.9 Hz, 1–H), 5.58 (ddd, *J* 2.1, 1.2, 1.1 Hz, 2–H),6.82 (ddd, *J* 1.2, 1.2, 0.6 Hz, 3′–H); δ_c 90.67 (d, C–1), 124.17 (d, C–2), 137.78 (s, C–3), 89.76 (d, C–3′). For **6** (or **5**): δ_H 6.61 (ddd, *J* 2.1, 1.2, 1.2 Hz, 1–H), 5.57 (ddd, *J* 2.1, 1.2, 1.1 Hz, 2–H), 6.94 (ddd, *J* (0.6, 0.6, 0.6 Hz, 3′–H); δ_c 90.67 (d, C–1), 124.82 (d, C–2), 138.62 (s, C–3), 89.89 (d, C–3′). For **8**: (5 : 3 diastereoisomeric mixture); major diastereoisomer: δ_H 6.65 (ddd, *J* 2.1, 1.2, 1.2 Hz, 1–H), 5.53 (ddd, *J* 2.1, 1.2, 1.2 Hz, 2–H), 6.90 (ddd, *J* 1.2, 1.2, 0.6 Hz, 3′–H), 2.40 (s, 10–H); δ_c 90.61 (d, C–1), 124.96 (d, C–2), 138.37 (s, C–3), 89.77 (d, C–3′), 178.44 (s, C–8), 92.32 (s, C–9), 79.44 (d, C–10); minor diastereoisomer: δ_H 6.71 (ddd, *J* 1.9, 1.2, 0.9 Hz, 1–H), 5.67 (ddd, *J* 1.9, 1.2, 1.2 Hz, 2–H), 6.78 (ddd, *J* 1.2, 1.2, 0.5 Hz, 3′–H), 2.41 (s, 10–H); δ_c 90.55 (d, C–1), 124.86 (d, C–2), 137.26 (s, C–3), 89.59 (d, C–3′), 178.48 (s, C–8), 92.35 (s, C–9), 79.72 (d, C–10).

¶ The relative efficiency of these sensitizers in terms of produced **2** at the expenses of **1**, observed after 0.5 h of irradiation, was: pheophytin a : chlorophyll b : chlorophyll a, 24 : 11 : 4. Under these irradiation conditions the green colour due to chlorophyll b turned rapidly to pale rose, while the green colour of chlorophyll a and the green–yellow colour of pheophytin a shaded, albeit less quickly, to pale yellow.

‖ Easy deoxygenation of hydroperoxide **3** (Scheme 1) affords an alternative to the epoxide route[2b] to caulerpenynol **7**, a minor, strongly bioactive product of *C. taxifolia*, scarcely available from nature.[2b]

†† Which are otherwise by no means fragile unicellular organisms, having proven to be insensitive to such powerfully cytotoxic agents as sphinxolide[6] or colchicine[7] at high doses.

‡‡ This recalls necrotization observed in field in the region of Monaco-Cap Martin in Cote d'Azur of the seagrass *Posidonia oceanica* in contact with *C. taxifolia*.[8]

§§ So far, 10,11-epoxycaulerpenyne has only been described from the Mediterranean *C. taxifolia*. We are currently studying whether this reflects genetic modifications undergone by the Mediterranean *C. taxifolia*.

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