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Photodegradation of chlorophyll phytyl chain in dead phytoplanktonic cells

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Abstract

The photodegradation rates of chlorophylls in their entirety and chlorophyll phytyl chains were compared in dead phytoplanktonic cells. The experimentally determined degradation rates were approximately five to eight times higher for the chlorophyll tetrapyrrolic structure than for the phytyl chain. Analysis of the photoproducts clearly established that the photodegradation of this isoprenoid chain involved mainly singlet oxygen. On the basis of the results obtained, it is predicted that a non-negligible part of the phytoplanktonic chlorophyll phytyl chain will be photodegraded during senescence in the upper portion of the euphotic zone of the oceans.

Keywords: Photodegradation; Chlorophyll phytyl chain; Phytoplanktonic cells

1. Introduction

Previous studies have shown that photo-oxidation processes strongly contribute to the disappearance of chlorophyll within the euphotic zone of the oceans [1,2]. In healthy cells, the chloroplast environment (carotenoids and other lipids) usually protects the chlorophyll from this fate [3,4]. This protection is lost in the early stages of senescence [5] or after grazing by zooplankton [1], and the chlorophyll molecule is quickly degraded by solar light [6] (Fig. 1). The mechanisms of chlorophyll breakdown are largely unknown [7], and only methyl ethyl maleimide [8,9] and some small hydrophilic photoproducts derived from the tetrapyrrolic structure [5,10] have been identified after chlorophyll *a* photodegradation.

The photo-oxidation of chlorophyll has been studied almost exclusively in terms of the porphyrin moiety of the molecule [11]. It is surprising that the phytol moiety has been neglected, because this unsaturated chain (considered to be the major source of acyclic isoprenoids with 20 or fewer carbon atoms in the biosphere) [12] is also susceptible to reaction with singlet oxygen or hydroxy and peroxy radicals, which are generated during chlorophyll photodegradation [3,13,14]. Indeed, it has been demonstrated recently that several free and esterified oxidized isoprenoid compounds are produced during the photodegradation of chlorophyll *a* in seawater (Fig. 2) [15–17]. On the basis of these results, we have determined the magnitude and rate of this phenomenon in dead phytoplanktonic cells.

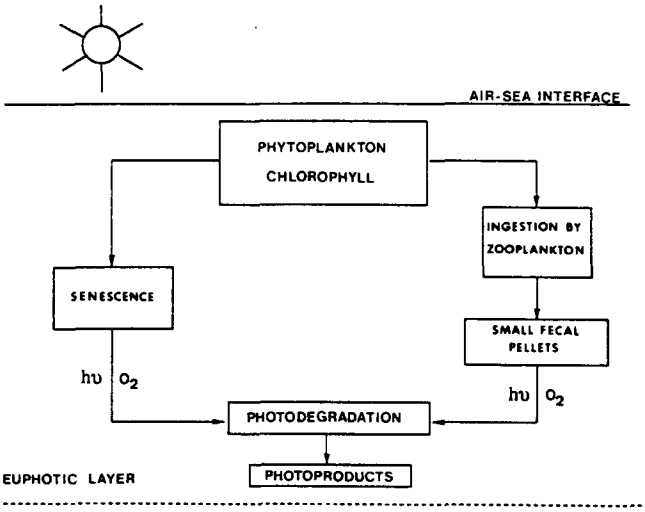

Fig. 1. Scheme for the photodegradation of chlorophyll in marine systems showing the contributions of grazing and senescence to this process.

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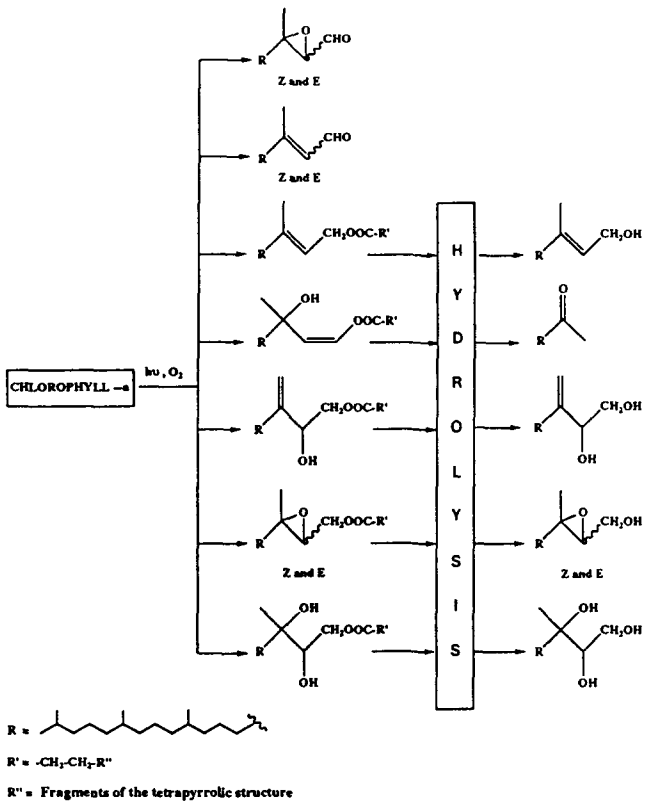


Fig. 2. Main oxidized isoprenoid photoproducts produced during photodegradation of chlorophyll *a* in seawater. (In order to simplify the figure some minor photoproducts, such as 2-methylidene-6,10,14-trimethylpentadecanal, 4,8,12-trimethyltridecanol, 4,8,12-trimethyltridecanal and pristanal, were omitted.)

2. Experimental details

2.1. Biological material

The chlorophyte *Dunaliella tertiolecta* Butcher and the diatom *Phaeodactylum tricornutum* Bohlin [18] were grown under axenic conditions at constant illumination (150 μeinstein m^{-2} s^{-1}) in 500 ml f/2 medium [19]. The cultures were harvested by centrifugation (8000*g*) 10 days after subculturing. The concentrated cells were sonicated for 5 min at 0 °C (Sonifier 250, Bransonic) to provide some disruption of cellular structure.

2.2. Experiments

Dead phytoplanktonic cells were distributed in Pyrex flasks containing 100 ml of supernatant to which had been added 1 ml of a 0.1 M solution of mercuric chloride. The flask contents were irradiated for different times (with stirring) using a 30 W fluorescent lamp (Osram, daylight) at constant room temperature. Irradiance (as photosyntheticaly available radiations (PAR)) was measured from the flask centers using a LICOR LI 1000 data logger equipped with an LI 193SA spherical quantum sensor. Dark controls were carried out in parallel.

2.3. Treatment

After light or dark incubations, each sample was filtered on GF/F (Whatman) paper. Pigments were sonically extracted (15 min) with methanol [20] from a fraction of the sample (one-tenth) and the remainder was saponified (1 h) in 50% ethanolic potassium hydroxide (1 mol l^{-1}) in order to study the behavior of the phytyl chain. After saponification, the content of the flask was extracted three times with hexane and the combined hexane extracts were dried on Na_2SO_4, filtered and concentrated. These different manipulations were carried out in foil-covered vessels in order to exclude photochemical artifacts.

2.4. Pigment analysis

Chlorophylls were analyzed by high performance liquid chromatography (HPLC) using a binary gradient system (Gold Beckman) equipped with a UV–visible diode array detector. A C_{18} ultrasphere (Beckman) column was employed (5 μm, 250 mm×4.6 mm) and a gradient program was run between acetonitrile and isopropanol (90% to 50% acetonitrile during 15 min). Calibration factors for chlorophylls *a* and *b* were determined using pigment standards (Sigma).

2.5. Quantification of phytol and identification of its photoproducts

Gas chromatography (GC) analyses were performed on a Girdel series 30 chromatograph equipped with a Ross injector and a flame ionization detector (FID). The following operating conditions were employed: 25 m×0.3 mm (inside diameter) fused capillary column coated with SE 52; temperature programmed from 110 to 290 °C at 3 °C min^{-1}; carrier gas pressure (N_2), 0.9 bar; injector temperature, 290 °C; detector temperature, 280 °C.

Mass spectra were recorded on an HP 5987 mass spectrometer. The following operating conditions were used. Electron impact ionization: electron energy, 70 eV; source temperature, 200 °C. Positive chemical ionization: electron energy, 150 eV; source temperature, 150 °C; methane pressure, 1 Torr.

The degradation products were formally identified by comparison of their retention times and mass spectra with those of standards.

2.6. Standard compounds

E phytol was purchased from Riedel de Haën. 6,10,14-Trimethylpentadecan-2-one was synthesized by oxida-

tion of phytol with $KMnO_4$ in acetone [21]. 3-Methylidene-7,11,15-trimethylhexadecan-1,2-diol was produced in two steps from E phytol according to a previously described procedure [16]. Epoxidation of the two isomers of phytol (Fluka) with *meta*-chloroperoxybenzoic acid in dry methylene chloride yields *Z* and *E* 3,7,11,15-tetramethyl-2,3-epoxyhexadecanols [22].

2.7. Pigment dispersion experiment

Chlorophyll *b* (0.1 mg) was dispersed (with 1 ml of acetone) in 50 ml of synthetic seawater [23] and irradiated with magnetic stirring.

3. Results and discussion

In accord with previous investigations [6,20], HPLC analyses of pigments revealed the presence of chlorophyll *a* in *Phaeodactylum tricornutum* (Fig. 3) and chlorophylls *a* and *b* in *Dunaliella tertiolecta* (Fig. 4). We determined whether or not the photochemical degradation of the phytyl chain differs between chlorophylls *a* and *b*. After irradiation of an aqueous suspension of chlorophyll *b* and subsequent saponification, we identified the same main isoprenoid photoproducts as in the case of chlorophyll *a* [17], i.e. 6,10,14-trimethylpentadecan-2-one (**1**), 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol (**2**), *Z* and *E* 3,7,11,15-tetramethyl-2,3-epoxyhexadecanols (**3** and **4**) and 3,7,11,15-tetramethylhexadecan-1,2,3-triol (**5**) (Fig. 5). The major part of the isoprenoid ketone **1** originates from the attack of singlet oxygen on the olefinic C-3 atom of the phytyl chain [15]; a small quantity of this ketone is probably produced by hydrolysis of free isoprenoid photoproducts such as phytenals, which do not survive the saponification [16]. The diol **2** arises from a similar photo-oxygenation of the olefinic C-2 atom of the phytyl chain [16], and the triol **5** and the isomeric epoxyalcohols **3** and **4** result from the addition of hydroxyl and peroxy radicals respectively to the phytol double bond [17].

First-order kinetics adequately describe the photooxidation of pigments in marine particles [1]. Therefore we calculated the first-order rate constants (k_1) for the photodegradation of chlorophylls in their entirety and the chlorophyll phytyl chain in dead phytoplanktonic cells. The rate constants are represented by the slopes of the regression lines determined as $\ln(C/C_0) = -k_1 D$ where C is the concentration at the time of sampling, C_0 is the initial concentration and D is the light dose [6].

The results obtained for chlorophylls show a good fit to first-order kinetics (Table 1) and clearly establish that they are photodegraded more slowly in chlorophyte

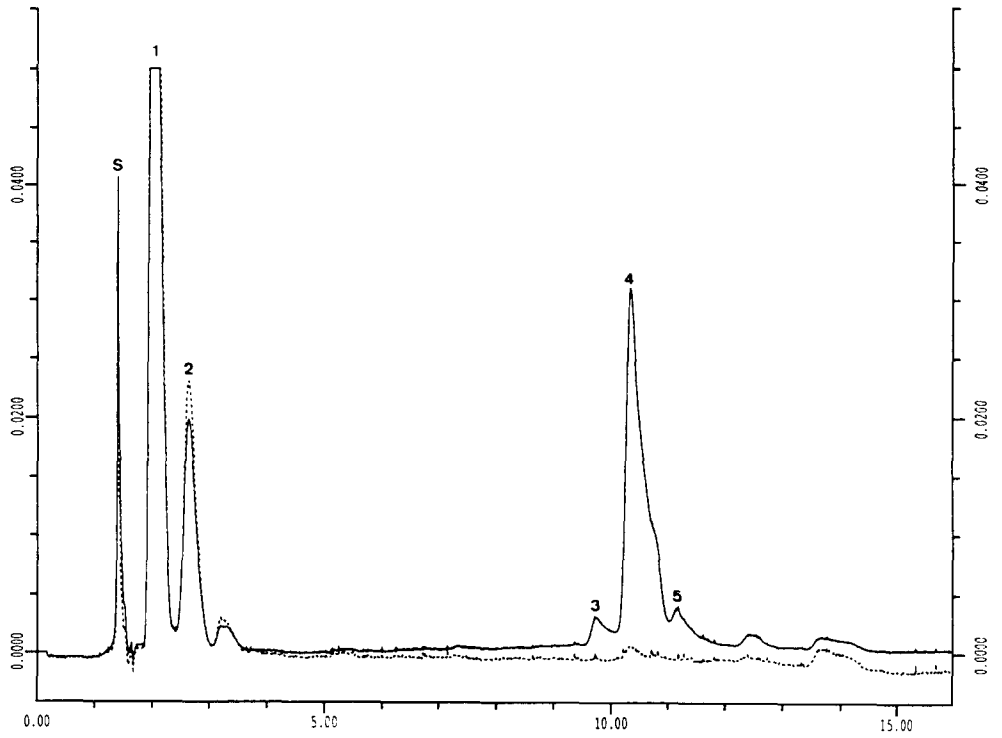


Fig. 3. High performance liquid chromatogram for pigments of *Phaeodactylum tricornutum* scanned at 457 nm (broken line) and 428 nm (full line). Peak identifications: (S) solvent peak from injection; (1) fucoxanthin; (2) diadinoxanthin; (3) chlorophyll *a* allomer; (4) chlorophyll *a*; (5) chlorophyll *a* epimer.

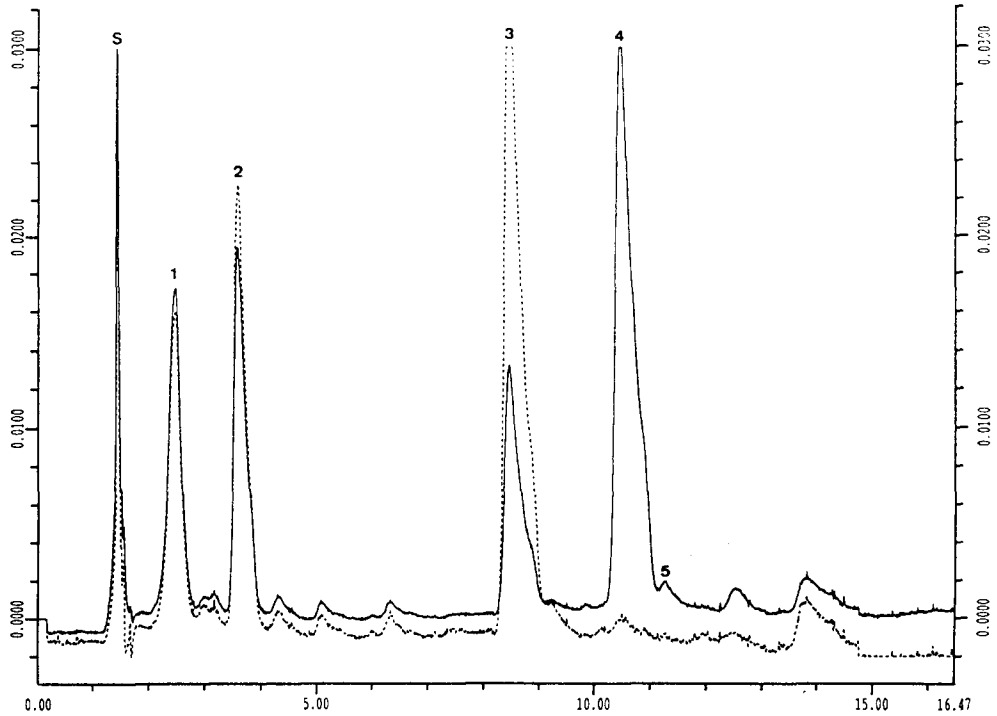


Fig. 4. High performance liquid chromatogram for pigments of *Dunaliella tertiolecta* scanned at 457 nm (broken line) and 428 nm (full line). Peak identifications: (S) solvent peak from injection; (1) neoxanthin; (2) lutein; (3) chlorophyll *b*; (4) chlorophyll *a*; (5) chlorophyll *a* epimer.

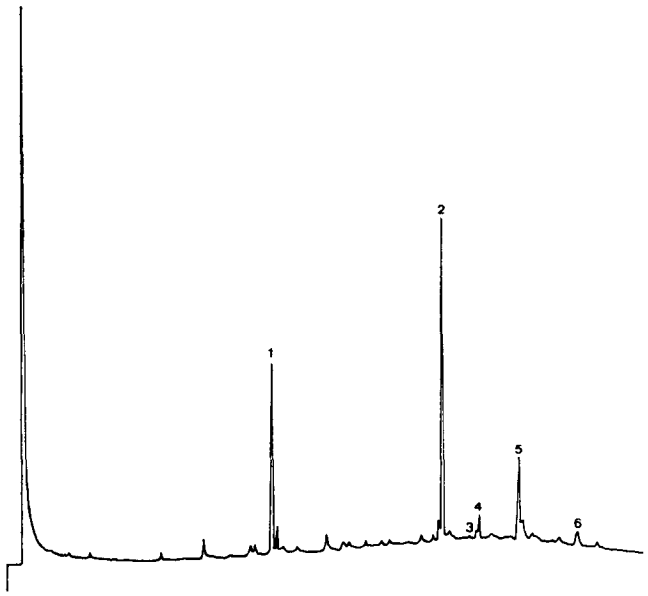


Fig. 5. Gas chromatogram obtained after saponification and acetylation of chlorophyll *b* photodegraded in seawater. Peak identifications: (1) 6,10,14-trimethylpentadecan-2-one; (2) phytol; (3) *Z* 3,7,11,15-tetramethyl-2,3-epoxyhexadecanol; (4) *E* 3,7,11,15-tetramethyl-2,3-epoxyhexadecanol; (5) 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol; (6) 3,7,11,15-tetramethylhexadecan-1,2,3-triol.

cells than in diatom cells; this is in good agreement with the observations of Nelson [6].

For the chlorophyll phytyl chain, the experimental points deviate significantly from a linear dependence (especially in the case of *Phaeodactylum tricornutum*). In contrast it can be seen in Figs. 6 and 7 that plots of 1/[phytol] against light exposure present a good linear fit ($r^2 = 0.997$ for the two algae). These results suggest that the photodegradation of the chlorophyll phytyl chain is a second-order process, which fits the equation $-\mathrm{d[phytol]}/\mathrm{d}D = k_2[\mathrm{phytol}]^2$.

If we consider $D_{1/2}$ (light exposure under which the compounds are reduced to one-half of their initial concentrations) (Table 1), it appears that the rates of photodegradation are approximately five to eight times higher for the chlorophyll tetrapyrrolic structure than for the phytyl chain. These differences in reactivity are not sufficient to justify the lack of investigations of the photochemical degradation of the chlorophyll phytyl chain in the literature.

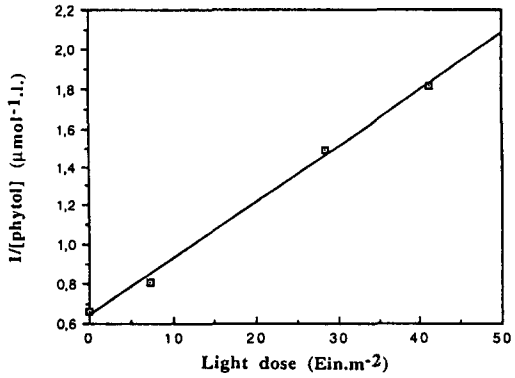


Fig. 6. Fit to apparent second-order kinetics for the photodegradation of *Dunaliella tertiolecta* chlorophyll phytyl chain.

Table 1
Calculated rate constants and $D_{1/2}$

Sample	k_1 (einstein^{-1} m^2)	r^2	k_2 (μmol^{-1} einstein^{-1} m^2)	r^2	$D_{1/2}$ (einstein m^{-2})
Dunaliella tertiolecta					
Chlorophylls ($a+b$)	0.23	0.99			3.0
Phytol			0.029	0.997	23.0
Phaeodactylum tricornutum					
Chlorophyll *a*	0.56	[a]			1.2
Phytol			0.161	0.997	5.6

[a]Two points only.

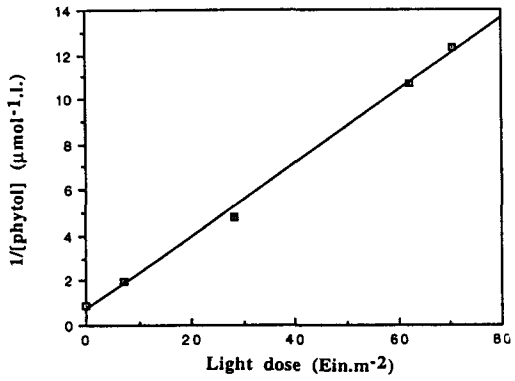
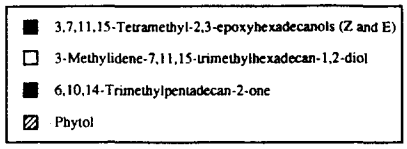


Fig. 7. Fit to apparent second-order kinetics for the photodegradation of *Phaeodactylum tricornutum* chlorophyll phytyl chain.



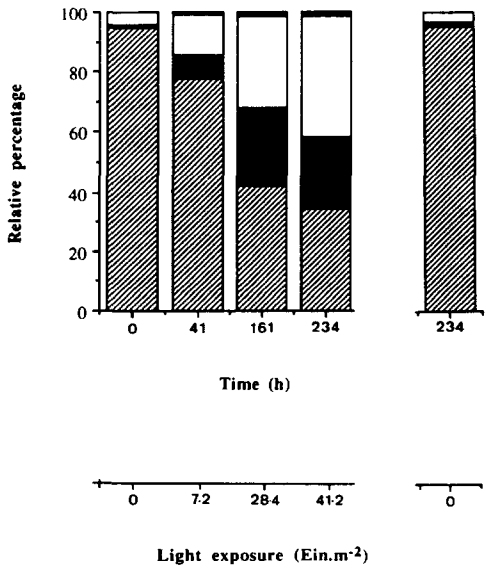
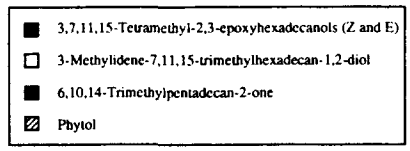


Fig. 8. Changes in chlorophyll phytyl chain during *Dunaliella tertiolecta* incubations.



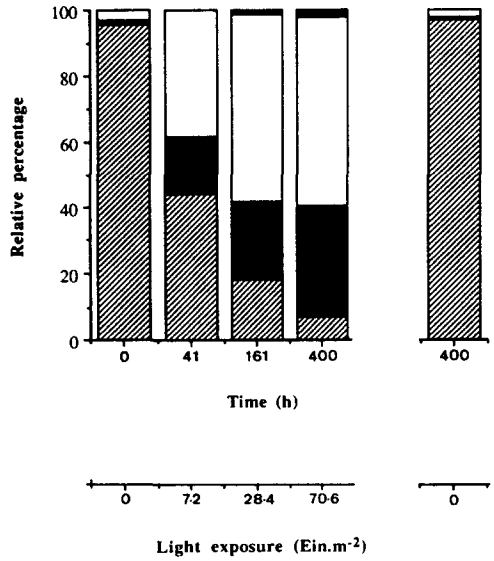


Fig. 9. Changes in chlorophyll phytyl chain during *Phaeodactylum tricornutum* incubations.

As in the case of chlorophyll aqueous dispersions [15] (Fig. 5), the chlorophyll phytyl chain is strongly degraded in dead cells after visible light exposure. Dark controls under a similar mixing regime show only negligible changes in phytol concentration. These processes result in the formation of 6,10,14-trimethylpentadecan-2-one (**1**) and 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol (**2**) as major products, with small amounts of *Z* and *E* 3,7,11,15-tetramethyl-2,3-epoxyhexadecanols (**3** and **4**) (Figs. 8 and 9). The lack of 3,7,11,15-tetramethylhexadecan-1,2,3-triol (**5**) [17] shows that in dead cells HO$^{\cdot}$ radicals do not react significantly on

the chlorophyll phytyl chain. During algal senescence, the photochemical degradation of this isoprenoid chain involves mainly singlet oxygen (production of compounds **1** and **2**) [16]. Peroxy radicals participate slightly in these processes (production of compounds **3** and **4**) [17].

On the basis of the results of this study, we can predict that, in dead cells, the chlorophyll phytyl chain will be reduced to one-half of its initial concentration after exposure to 23.0 einstein m^{-2} (for *Dunaliella tertiolecta*) and 5.6 einstein m^{-2} (for *Phaeodactylum tricornutum*).

Considering a settling velocity of 0.24 m day^{-1} (for particular organic carbon (POC) in the size range 1–10 μm) [24] and a surface irradiance (representative of mid-latitude waters) of 60 einstein m^{-2} day^{-1} [6], we conclude that a non-negligible part of the phytoplanktonic chlorophyll phytyl chain must be photodegraded during senescence in the upper portion of the euphotic zone of the oceans. This assumption is confirmed by the recent detection of high quantities of 6,10,14-trimethylpentadecan-2-one (**1**) [25,26] and 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol (**2**) [16] after alkaline hydrolysis of marine sediments.

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