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Photo- and Free Radical-Mediated Oxidation of Lipid Components During the Senescence of Phototrophic Organisms

Jean-François Rontani
Laboratory of Microbiology, Geochemistry and Marine Ecology (UMR 6117), Center of Oceanology of Marseille, Aix-Marseille University, Campus of Luminy, Marseille, France

1. Introduction

Recently, the role played by photochemical and free radical-mediated processes in the degradation of lipid components during the senescence of phototrophic organisms was investigated. The present paper reviews the results obtained in the course of these studies.

In a first part, visible and UV light-induced photooxidation of the main lipid cell components (chlorophylls, carotenoids, sterols, unsaturated fatty acids, highly branched isoprenoid and linear alkenes, alkenones, cuticular waxes …) in senescent phototrophic organisms (phytoplankton, cyanobacteria, higher plants, purple sulfur bacteria and aerobic anoxygenic phototrophic bacteria) is examined. Probably due to its long lifetime in hydrophobic micro-environments and thus in senescent cells, singlet oxygen plays a key role in the photodegradation of most of the lipid components.

The second part of this paper describes the free radical oxidation (autoxidation) of lipid components during the senescence of phototrophic organisms, which have been virtually ignored until now in the literature. In senescent phototrophic organisms, the mechanism of initiation of free-radical oxidation seems to be the homolytic cleavage (catalyzed by some metal ions) of photochemically produced hydroperoxides. It was also demonstrated recently that viral infection and autocatalytic programmed cell death could also lead to elevated production of reactive oxygen species (ROS) able to induce the degradation of cell components.

2. Photodegradation processes in phototrophic organisms

Several works suggested photo-oxidation as an important sink of organic matter in the photic layer of oceans (Zafiriou, 1977; Zafiriou et al., 1984). However, due to the lack of suitable markers this phenomenon has never been fully addressed. Owing to the problem of stratospheric ozone depletion, some studies have recently examined the degradative effects of enhanced UV-B doses on phytoplanktonic lipids (He and Häder, 2002). However, photochemical damages in phytoplanktonic cells are not a monopoly of UV radiation. In fact, due to the presence of chlorophylls (which are very efficient photosensitizers (Foote, 1976; Knox and Dodge, 1985)), numerous organic components of phytoplankton are susceptible to being photodegraded during senescence by photosynthetically active radiation (PAR).

2.1 Photodegradation of the main lipidic components of phytoplankton during senescence

When a chlorophyll molecule absorbs a quantum of light energy, an excited singlet state (^{1}Chl) is formed which, in healthy cells, leads predominantly to the characteristic fast reactions of photosynthesis (Foote, 1976). However, a small proportion (<0.1%) undergoes intersystem crossing (ISC) to form the longer lived triplet state (^{3}Chl; Knox and Dodge, 1985). ^{3}Chl is not only itself potentially damaging in type I reactions (hydrogen atom or electron abstraction) (Knox and Dodge, 1985), but can also generate highly reactive oxygen species (ROS) and, in particular, singlet oxygen ($^1O_2$), by reaction with ground state oxygen ($^3O_2$) via Type II processes. In order to avoid oxidative damage, there are many antioxidant protective mechanisms in chloroplasts. Carotenoids quench ^{3}Chl and $^1O_2$ by energy transfer mechanisms at very high rates (Foote, 1976) and tocopherols can remove $^1O_2$, $O_2^{\bullet-}$, $HOO^{\bullet}$ and $HO^{\bullet}$ by acting as sacrificial scavengers (Halliwell, 1987). Superoxide dismutase enzyme (SOD) and ascorbic acid may also scavenge $O_2^{\bullet-}$ (Halliwell, 1987), while catalase activity decreases H_2O_2 levels.

In senescent phototrophic organisms, the fast reactions of photosynthesis clearly do not operate, so an accelerated rate of formation of ^{3}Chl and $^1O_2$ would be expected (Nelson, 1993). The rate of formation of these potentially damaging species can then exceed the quenching capacity of the photoprotective system and photodegradation can occur (photodynamic effect; Merzlyak and Hendry, 1994). In phytodetritus, when the ordered structure of the thylakoid membranes has been disrupted, pigments tend to remain associated with other hydrophobic cellular components such as membrane lipids (Nelson, 1993). As a result, the photooxidative effect of chlorophyll sensitization might be strongly amplified within such a hydrophobic micro-environment. Moreover, the lifetime of $^1O_2$ produced from sensitizers in a lipid-rich hydrophobic environment could be longer, and its potential diffusive distance greater, than its behaviour in aqueous solution (Suwa et al., 1977). It is not surprising, therefore, that photodegradation processes act on the majority of unsaturated lipid components of senescent phytoplankton.

2.1.1 Chlorophylls

Irradiation of dead phytoplankton cells by PAR and UVR radiations results in rapid degradation of chlorophylls (Nelson, 1993; Rontani et al., 1995; Christodoulou et al., 2010). Photodegradation of chlorophyll-*a* and -*c* in killed cells of *E. huxleyi* appeared to be induced by both PAR and UVR (Christodoulou et al., 2010). The photochemical degradation of chlorophylls has so far been studied almost exclusively with respect to the macrocycle moiety of the molecule, which is the more reactive. Despite some progress regarding intermediary photoproducts (Engel et al., 1991; Iturraspe et al., 1994), no stable and specific markers for the chlorophyll macrocycle photodegradation have been characterised.

The isoprenoid phytyl side-chain of chlorophylls is also sensitive to photochemical processes. In fact, in phytodetritus, the photodegradation rates were only 3 to 5 times higher for the chlorophyll tetrapyrrolic structure than for the phytyl side-chain (Cuny et al., 1999; Christodoulou et al., 2010). Analysis of isoprenoid photoproducts of chlorophylls after irradiation of different dead phytoplanktonic cells by visible light clearly established that the photodegradation of the chlorophyll phytyl side-chain in phytodetritus involved mainly

$^1O_2$. The type II (i.e. involving $^1O_2$) photosensitized oxidation of the phytol moiety of chlorophylls leads to the production of photoproducts of structures **a** and **b** (Fig. 1), quantifiable after $NaBH_4$-reduction and alkaline hydrolysis respectively in the form of 6,10,14-trimethylpentadecan-2-one (**1**) (phytone) and 3-methylidene-7,11,15-trimethyl-hexadecan-1,2-diol (phytyldiol) (**2**) (Fig. 1) (Rontani et al., 1994).

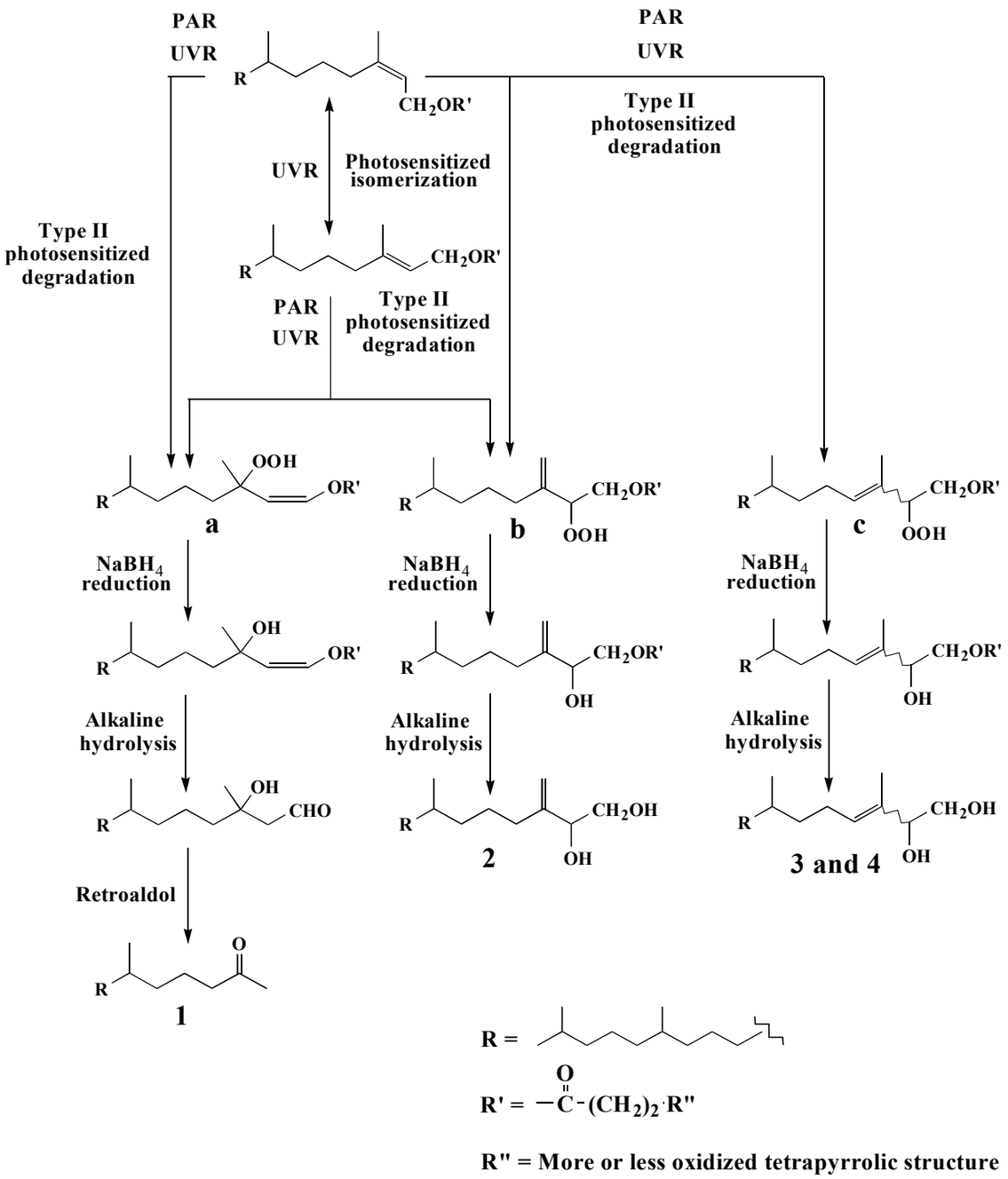


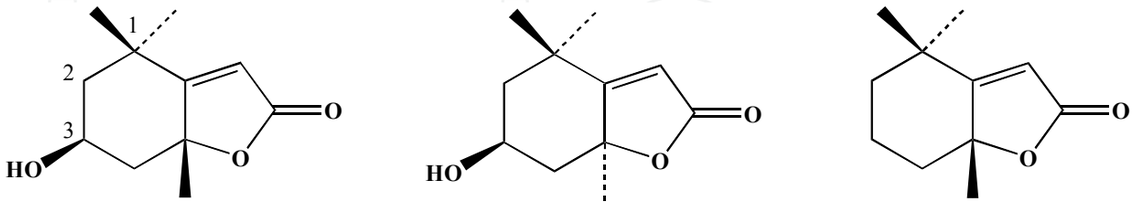
Fig. 1. Photooxidation of chlorophyll phytyl side-chain and reactions of oxidation products during alkaline hydrolysis.

Irradiation with UVR resulted in the additional production of small amounts of *Z*-phytol and *Z* and *E*-3,7,11,15-tetramethylhexadec-3-en-1,2-diols (**3,4**) (Christodoulou et al., 2010). The detection of *Z*-phytol allowed to demonstrate the induction of *cis-trans* photosensitized isomerization by UVR. These reactions probably involve triplet states of ketones as sensitizers. Type II photosensitized oxidation of the *Z* configuration of phytol, which should lead to the production of photoproducts of structures **a**, **b** and **c** (Fig. 1) (Schulte-Elte et al., 1979), explains the detection of small amounts of *Z* and *E*-3,7,11,15-tetramethylhexadec-3-en-1,2-diols (**3,4**) after irradiation with UVR. Irradiation with UVR also resulted in a faster degradation of chlorophyll phytyl side-chain oxidation products (Christodoulou et al., 2010). This higher reactivity was attributed to UVR-induced homolysis of the peroxyl group of photoproducts of structures **a**, **b** and **c** (Fig. 1).

Phytyldiol (**2**) is ubiquitous in the marine environment and has been proposed as tracer for photodegradation of chlorophyll's phytyl side chain (Rontani et al. 1994; 1996a; Cuny and Rontani 1999). Further, the molar ratio phytyldiol:phytol (Chlorophyll Phytyl side-chain Photodegradation Index, CPPI) was employed to estimate the extent of chlorophyll photodegraded in natural marine samples by the empirical equation: chlorophyll photodegradation % = $(1-(CPPI + 1)^{-18.5}) \times 100$ (Cuny et al. 2002).

2.1.2 Carotenoids

In phytodetritus, chlorophylls and carotenoids remain in a close molecular-scale association at relatively high localized concentrations, even though the structure of the thylakoid membrane has been disrupted (Nelson, 1993). Thus, the sensitized photooxidation of carotenoids is enhanced. The photosensitized oxidation (involving $^1O_2$) of carotenoids in solvents has been studied (Iseo et al., 1972) and loliolide (**5**), *iso*-loliolide (**6**) and dihydroactinidiolide (**7**) (Fig. 2) were identified as major photoproducts, depending on the functionality of carotenoids at C-3. Loliolide (**5**) and *iso*-loliolide (**6**) have been detected in killed cells of *Dunaliella* sp. irradiated by visible light (Rontani et al., 1998). However, due to their apparent production by anaerobic bacteria (Repeta, 1989) and during dark incubations of killed phytoplanktonic cells (Rontani et al., 1998), these compounds cannot constitute unequivocal indicators of photooxidative processes.



5 **6** **7**

Fig. 2. Structure of the main carotenoid oxidation products.

2.1.3 Δ^5-sterols

As important unsaturated components of biological membranes, Δ^5-sterols are highly susceptible to photooxidative degradation during the senescence of phytoplankton. Irradiation by visible light of killed cells of *Skeletonema costatum*, *Dunaliella* sp.,

Phaeodactylum tricornutum and *Emiliania huxleyi* (Rontani et al., 1997a; 1997b; 1998) resulted in a quick photodegradation of the sterol components of these algae. The results obtained clearly established that the photooxidation of sterols in senescent cells of phytoplankton involves type II photoprocesses. These processes mainly produce Δ^6-5α-hydroperoxides (**8**) and to a lesser extent Δ^4-6α/6β-hydroperoxides (**9** and **10**) (Fig. 3) (Nickon and Bagli, 1961; Kulig and Smith, 1973). Δ^6-5α-hydroperoxysterols (**8**) are relatively unstable and may undergo allylic rearrangement to Δ^5-7α-hydroperoxysterols (**11**), which in turn epimerize to the corresponding 7β-hydroperoxides (**12**) (Fig. 3) (Smith, 1981). It was previously demonstrated that during singlet oxygen-mediated photooxidation of sterols in biological membranes (Korytowski *et al*., 1992) and senescent phytoplanktonic cells (Rontani *et al*., 1997a) the photogeneration of Δ^4-6α/6β-hydroperoxides (**9** and **10**) was more favourable than in homogeneous solution (ratio Δ^4-6α/6β-hydroperoxides/Δ^6-5α-hydroperoxysterols ranging from 0.30 to 0.35 instead of 0.1).

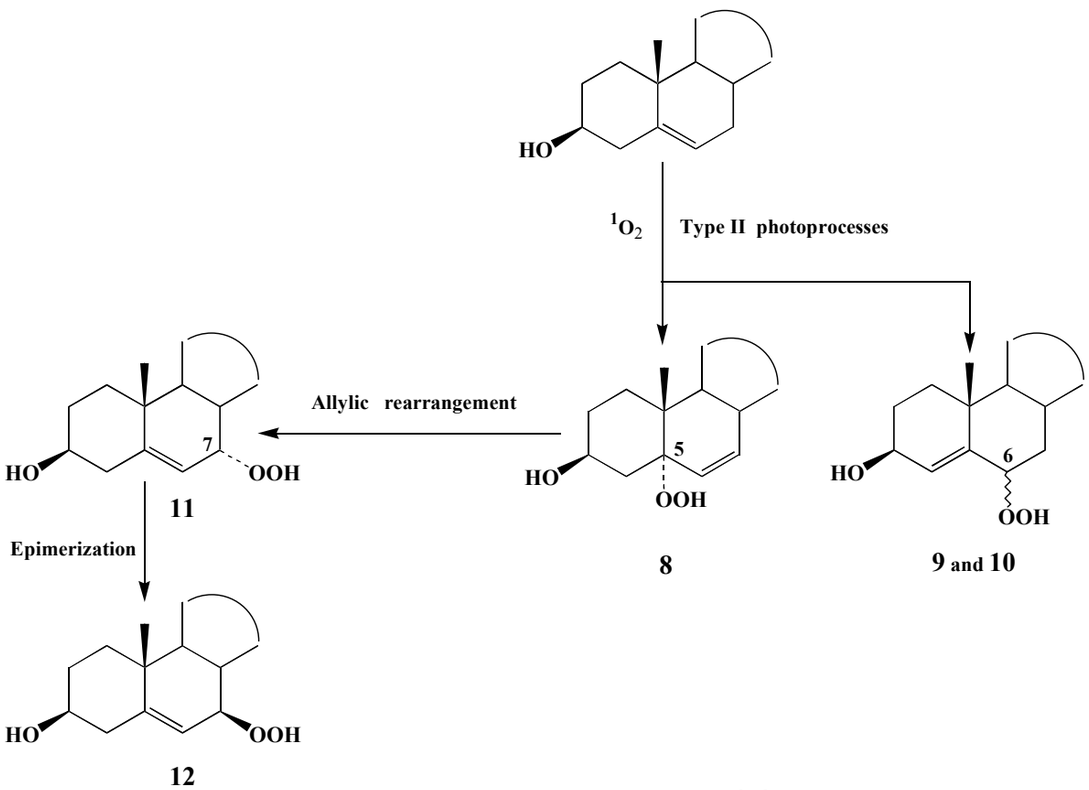


Fig. 3. Type II photosensitized oxidation of Δ^5 sterols.

Allylic rearrangement of Δ^6-5α-hydroperoxides (**8**) appeared to take place very weakly in senescent phytoplanktonic cells (Rontani et al., 1997a; 1997b; 1998). This surprising stability was attributed by Korytowski et al. (1992) either to hydrogen bonding between the unsaturated fatty acyl chain of phospholipids and Δ^6-5α-hydroperoxides (**8**) which could hinder the allylic rearrangement, or to differences of polarity in the carbon 7-10 zone of the fatty acyl chain (where sterols tend to localize in phospholipid/sterol bilayers (MacIntosch, 1978)). It is also interesting to note that the reduction of hydroperoxysterols to the corresponding diols weakly operates in killed phytoplanktonic cells (Rontani et al., 1997a).

Δ^6-5α-Hydroperoxysterols (**8**) are potential type II photodegradation markers, not only because they are the major products of singlet oxygen attack on the steroidal Δ^5-3β- system, but also because biological functionalization of steroids at C-5 is rare. Unfortunately, if these compounds are particularly stable in phytodetritus, they decay slowly in the sediment to their corresponding Δ^5-7α/β-derivatives (**11** and **12**) (Rontani and Marchand, 2000), which are not selective markers (see chapter 3.3). Moreover, according to the stability of the alkyl radicals formed during β-scission of the corresponding alkoxyl radicals, the following order of stability was proposed: Δ^4-6-hydroperoxysterols (**9** and **10**) > Δ^5-7-hydroperoxysterols (**11** and **12**) > Δ^6-5-hydroperoxy-sterols (**8**) (Christodoulou et al., 2009). Consequently, Δ^4-6α/β-hydroperoxysterols (**9** and **10**) (or their degradative products Δ^4-6α/β-hydroxysterols and Δ^4-6α/β-oxosterols) may be considered as more reliable *in situ* markers of type II photodegradation processes than Δ^6-5α-hydroperoxides (**8**).

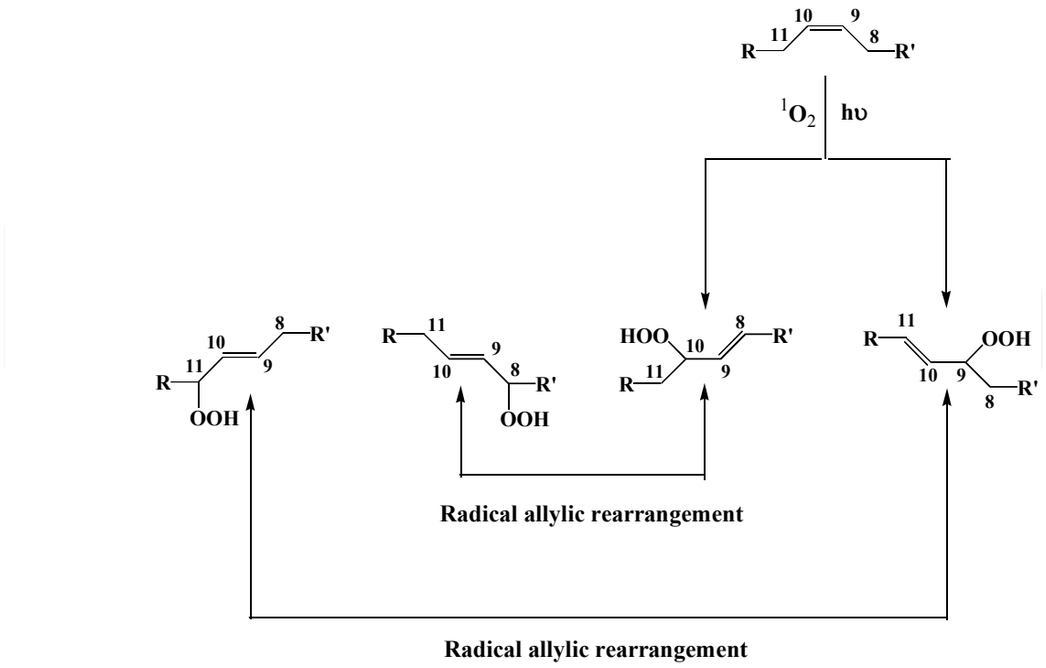
2.1.4 Unsaturated fatty acids

Chloroplast membrane components are particularly susceptible to type II photooxidation (Heath and Packer, 1968). This is the case notably for unsaturated fatty acids, which generally predominate in algal lipids, particularly in the photosynthetic membranes (Woods, 1974). In killed phytoplanktonic cells, the photodegradation rates of unsaturated fatty acids logically increase with their unsaturation degree (Rontani et al., 1998). Singlet oxygen-mediated photooxidation of monounsaturated fatty acids involves a direct reaction of $^1O_2$ with the carbon–carbon double bond by a concerted 'ene' addition (Frimer 1979) and leads to formation of hydroperoxides at each carbon of the original double bond. Thus, photooxidation of oleic acid produces a mixture of 9- and 10-hydroperoxides with an allylic *trans*-double bond (Frankel et al. 1979; Frankel, 1998), which can subsequently undergo stereoselective radical allylic rearrangement to 11-*trans* and 8-*trans* hydroperoxides, respectively (Porter et al. 1995) (Fig. 4).

The free radical nature of the allylic hydroperoxide rearrangement is supported by the observation that the rearrangement is catalysed by free radical initiators or light and inhibited by phenolic antioxidants (Porter et al., 1995). This allylic rearrangement weakly intervenes in most of the killed phytoplanktonic cells examined (Rontani et al., 1998). This was attributed to the relatively high localized fatty acid concentrations present in phytodetritus (Nelson, 1993), which favoured the dimerisation of hydroperoxides. Hydrogen atom abstraction to form allylperoxyl radicals does indeed occur readily from hydroperoxide monomers but not from hydroperoxide dimers (Porter et al., 1995).

During early diagenesis, isomeric hydroperoxyacids undergo heterolytic cleavage to aldehydes and ω-oxocarboxylic acids (Frimer, 1979) or homolytic cleavage and subsequent transformation to the corresponding alcohols or ketones (Fig. 5).

Taking into account the high amounts of photoproducts of mono-unsaturated fatty acids detected in the particulate matter samples (Marchand and Rontani, 2001; Christodoulou et al., 2009; Rontani et al., 2011a), and the well known increasing photooxidation rates of fatty acids with their degree of unsaturation (Frankel., 1998), it can be concluded that considerable amounts of poly-unsaturated fatty acids must be photooxidized during the senescence of phytoplankton in the marine environment. However, at this time photooxidation products of this kind of fatty acids could not be detected in natural samples.



R = -$(CH_2)_6$-CH_3

R' = -$(CH_2)_6$-COOH

Fig. 4. Type II photosensitized oxidation of oleic acid.

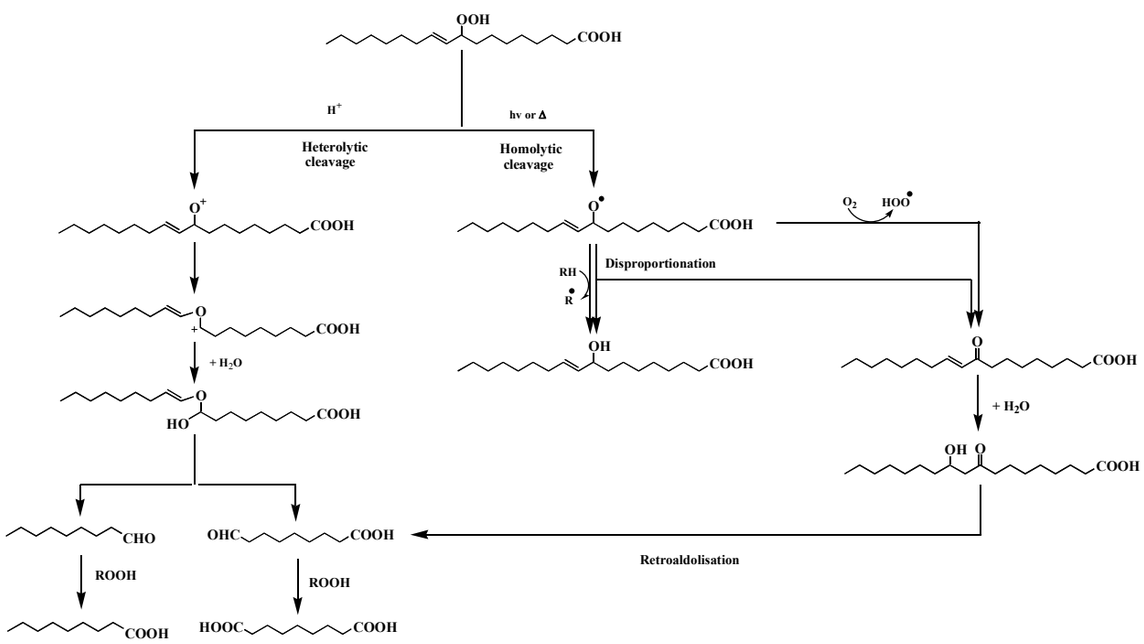


Fig. 5. Degradation of allylic hydroperoxides resulting from Type II photosensitized oxidation of monounsaturated fatty acids (the example given is this of 9-hydroperoxyoctadec-10-enoic acid) (RH = hydrogen donors, e.g. lipids or reduced sensitizers).

This is possibly due to: (i) the instability of the hydroperoxides formed, or (ii) the involvement of cross-linking reactions leading to the formation of macromolecular structures (Neff et al., 1988) non-amenable by gas chromatography.

2.1.5 Alkenones

Alkenones are a class of mono-, di-, tri-, tetra- and penta-unsaturated C_{35}-C_{40} methyl and ethyl ketones (Boon et al., 1978; Volkman et al., 1980; de Leeuw et al., 1980; Marlowe et al., 1984; Prahl et al., 2006; Jaraula et al., 2010), which are produced by certain marine haptophytes. *Emiliania huxleyi* and *Gephyrocapsa oceanica* are the major sources of alkenones in the open ocean (Volkman et al., 1980; 1995; Conte et al., 1994). The unsaturation ratio of C_{37} alkenones, defined as $U_{37}^{K'}$ = [$C_{37:2}$] / ([$C_{37:2}$] + [$C_{37:3}$]) where [$C_{37:2}$] and [$C_{37:3}$] are the concentrations of di- and tri-unsaturated C_{37} alkenones respectively, varies positively with the growth temperature of the alga (Prahl and Wakeham, 1987; Prahl et al., 1988). The $U_{37}^{K'}$ - growth temperature relationship in haptophyte algae and transferred to sinking marine particulate matter leads to a linear relationship between sedimentary C_{37} alkenone composition and mean annual SST records throughout the oceans (Rosell-Melé et al., 1995; Müller et al., 1998). The $U_{37}^{K'}$ index is now routinely used for paleotemperature reconstruction.

For alkenones to be useful as measures of sea surface temperature in the geological record, it is essential that any effects of degradation in the water column and in sediments either do not affect the temperature signal established during their initial biosynthesis by the alga (Harvey, 2000; Grimalt et al., 2000), or if there is a change its extent can be reasonably estimated.

Visible light-induced photodegradation of these compounds was thus previously investigated in order to determine if photochemical processes could appreciably modify $U_{37}^{K'}$ ratios during algal senescence (Rontani et al., 1997b; Mouzdahir et al., 2001; Christodoulou et al., 2010). Though potentially selective, photochemical degradation of alkenones is not fast enough in killed cells of *E. huxleyi* to induce strong modifications of the $U_{37}^{K'}$ ratio before the photodestruction of the photosensitizing substances (Rontani et al., 1997b; Mouzdahir et al., 2001). UVR also appeared to be inefficient to alter the $U_{37}^{K'}$ ratio (Christodoulou et al., 2010).

This stability was attributed to the *trans* configuration of alkenone double bonds (Rechka and Maxwell, 1988) that is 7 to 10 times less sensitive against singlet oxygen-mediated oxidation than the classical *cis* configuration of fatty acids (Hurst et al., 1985). This may explain the difference of photoreactivity observed between the alkenones and fatty acids with the same number of unsaturations. We also previously attributed the poor photoreactivity of alkenones to a localisation of these compounds elsewhere than in cell membranes (Rontani et al., 1997b; Mouzdahir et al., 2001), which could significantly decrease the likelihood of interaction between singlet oxygen and alkenones. Although this hypothesis is well supported by the recent results of Eltgroth et al. (2005), who demonstrated that alkenones are mainly localized into cytoplasmic vesicles, the migration of singlet oxygen from phytodetritus to attached heterotrophic bacteria previously observed (Rontani et al., 2003a; Christodoulou et al., 2010) strongly suggests a diffusion of this excited form of oxygen also in these cytoplasmic vesicles.

2.1.6 n-Alkenes

The visible light-induced degradation of *n*-alkenes was previously investigated in killed cells of the Prymnesiophycea *E. huxleyi* and the Eustigmatophycea *Nannochloropsis salina* (Mouzdahir et al., 2001).

In *E. huxleyi* killed cells, minor C_{31} and C_{33} *n*-alkenes were strongly photodegraded, while the major C_{37} and C_{38} *n*-alkenes appeared particularly recalcitrant towards photochemical processes. These strong differences of photoreactivity imply distinct biological syntheses and/or functions for these two groups of hydrocarbons in *E. huxleyi* cells. Interestingly, the stereochemistry of the internal double bonds in C_{31} and C_{33} n-alkenes has been established to be *cis*, while C_{37} and C_{38} alkenes internal double bonds exhibit a *trans* geometry (Rieley et al., 1998; Grossi et al., 2000). The photochemical recalcitrance of C_{37} and C_{38} n-alkenes could thus be partly attributed to the *trans* geometry of their internal double bonds.

Irradiation of dead cells of *N. salina* resulted in a strong modification of the hydrocarbon fraction. It did not provide evidence of a significant light-dependent degradation of monounsaturated hydrocarbons; this result was attributed to the terminal position of the double bond in these compounds (Gelin et al., 1997), which is poorly reactive towards singlet oxygen (Hurst et al., 1985). In contrast, di-, tri-, and tetraenes were strongly photodegraded during irradiation. The visible light-dependent degradation of phytoplanktonic *n*-alkenes showed apparent second-order kinetics with respect to light exposure and the half-life doses obtained logically decrease with increasing number of double bonds in these compounds (Mouzdahir et al., 2001).

2.1.7 Highly branched isoprenoid (HBI) alkenes

HBI alkenes are widely distributed in aquatic environments (Rowland and Robson, 1990; Sinninghe-Damsté et al., 2004), although they appear to originate from a relatively small number of diatomaceous algae including *Haslea* spp., *Rhizosolenia* spp., *Pleurosigma* spp. and *Navicula* spp. (Volkman et al., 1994; Sinninghe-Damsté et al., 2004; Belt et al., 2000, 2001; Allard et al., 2001; Grossi et al., 2004). Despite this, they have been commonly reported in marine sediments worldwide and provide some insight into the deposition of organic matter from the water column. One HBI alkene, a mono-unsaturated isomer termed IP_{25}, has been used as a proxy for the occurrence of spring sea ice in the Arctic (e.g. Belt et al., 2007, 2010; Massé et al., 2008).

Examination of the photoreactivity of several mono-, di-, tri- and tetra-unsaturated HBI alkenes in the presence of a photosensitizer solution and in dead cells of *H. ostrearia* allowed to show that HBI alkenes possessing at least one tri-substituted double bond may be photo-oxidized at similar or higher rates compared to other highly reactive lipids (e.g. PUFAs, vitamin E and chlorophyll *a*) during the senescence of diatom cells (Rontani et al., 2011b). As a consequence, it is proposed that HBI alkenes possessing trisubstituted double bonds are likely to be susceptible to photodegradation within the euphotic zone. In contrast, HBIs containing only mono- and di-substituted double bonds were found to be significantly less reactive towards $^1O_2$ and should, therefore, be relatively preserved during sedimentation through the water column (Rontani et al., 2011b). The kinetic experiments are supported by product analysis, which revealed that the main reaction with $^1O_2$ primarily occurs with the trisubstituted double bonds of HBI alkenes affording tertiary and secondary allylic hydroperoxides (Fig. 6). In contrast, the extremely low photoreactivity of the HBI monoene

IP_{25}, can be attributed to its containing only the least photochemically reactive double bond. This lack of reactivity supports (in part) the good preservation of IP_{25} generally observed in sediments (Belt et al., 2007, 2010; Massé et al., 2008).

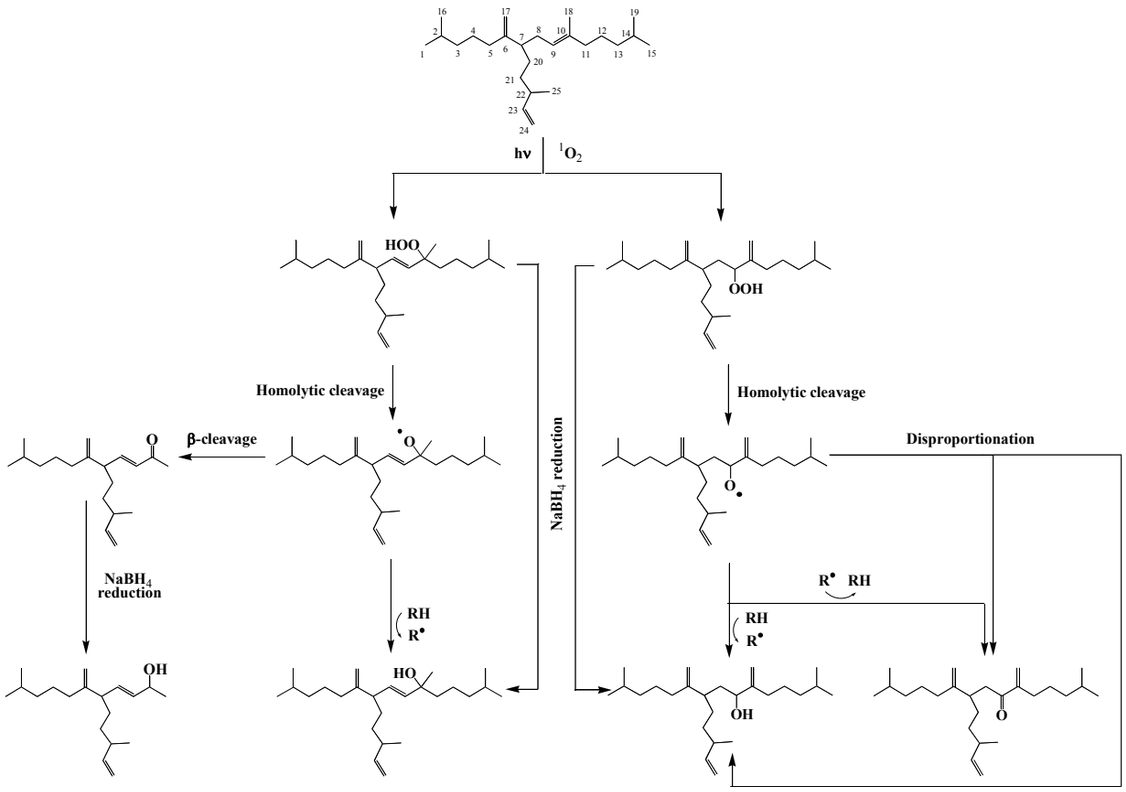


Fig. 6. Type II photosensitized oxidation of HBI alkenes (RH = hydrogen donors)

2.2 Photodegradation processes in other phototrophic organisms

Visible light-dependent degradation processes have been also studied in senescent cells of two purple sulfur bacteria (*Thiohalocapsa halophila* and *Halochromatium salexigens*) isolated from microbial mats from Camargue (France) (Marchand and Rontani, 2003). These reactions act intensively on the phytyl side chain of bacteriochlorophyll-*a* and lead to the production of phytone (**1**) and phytyldiol (**2**) as in the case of chlorophylls (Fig. 1). Palmitoleic and *cis*-vaccenic acids also undergo strong photodegradation, affording mainly isomeric allylic oxo-, hydroxy- and hydroperoxyacids.

These processes were also investigated in aerobic anoxygenic phototrophic bacteria (AAPs) (Rontani et al., 2003a). These organisms constitute a relatively recently discovered bacterial group (Yurkov and Beatty, 1998) and seem to be widespread in the open ocean (Kolber et al., 2000). They perform photoheterotrophic metabolism, requiring organic carbon for growth, but they are capable to use photosynthesis as an auxiliary source of energy (Kolber et al., 2001). Though sensitive to photochemical processes in senescent purple sulfur bacteria (Marchand and Rontani., 2003), the isoprenoid phytyl side-chain of bacteriochlorophyll -*a* is not significantly photodegraded in senescent cells of AAPs (Rontani et al., 2003a). In contrast, significant amounts of allylic hydroxyacids arising from the photo-oxidation of the

major unsaturated fatty acid of these organisms (*cis*-vaccenic acid) could be detected after irradiation (Rontani et al., 2003a).

As in the case of phytoplankton and cyanobacteria, visible light-dependent degradation processes act significantly on the chlorophyll phytyl side-chain (Rontani et al., 1996b), unsaturated fatty acids and sterols (Rontani, Unpublished results) during terrestrial higher plant senescence affording similar photoproducts. 9-Hydroperoxy-18-hydroxyoctadec-10(*trans*)-enoic (**13**) and 10-hydroperoxy-18-hydroxyoctadec-8(*trans*)-enoic (**14**) acids deriving from type II photooxidation of 18-hydroxyoleic acid (**15**) (Fig. 7) were detected after visible light-induced senescence experiments carried out with *Petroselinum sativum* and subsequent cutin depolymerisation (Rontani et al., 2005a).

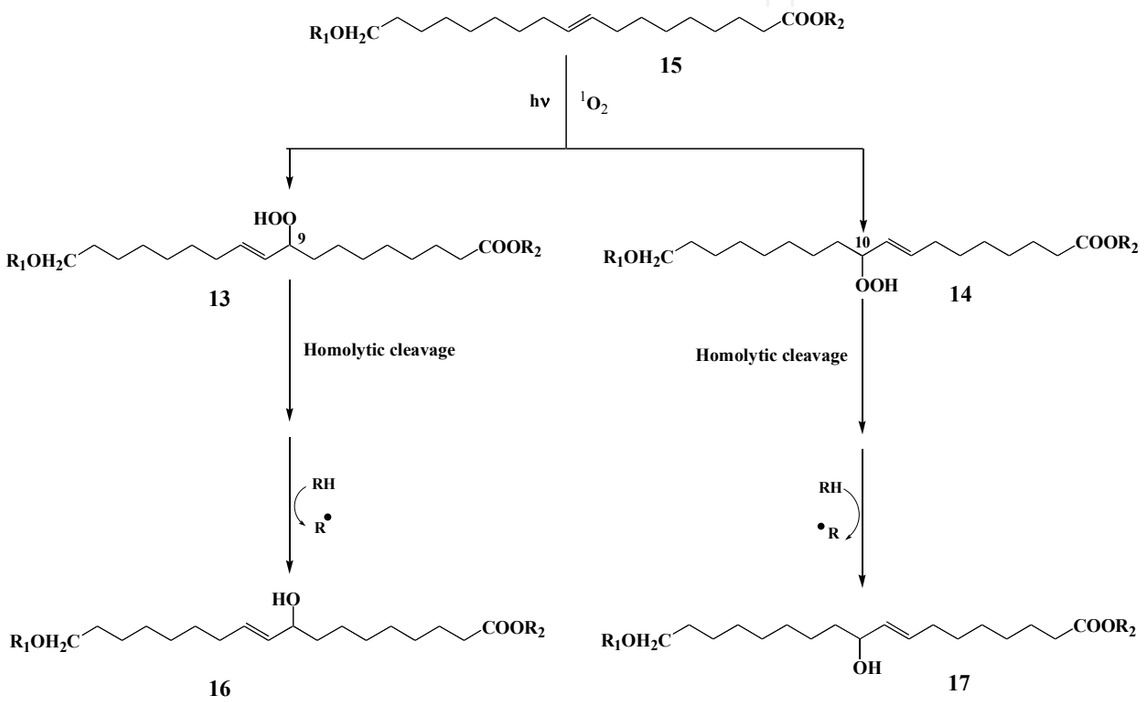


Fig. 7. Type II photosensitized oxidation of 18-hydroxyoleic acid in cutin polymers.

These results showed that in senescent plants, where the $^1O_2$ formation rate exceeds the quenching capacity of the photoprotective system, $^1O_2$ can migrate outside the chloroplasts and affect the unsaturated components of cutins. Significant amounts of 9,18-dihydroxyoctadec-10(*trans*)-enoic (**16**) and 10,18-dihydroxyoctadec-8(*trans*)-enoic (**17**) acids resulting from the reduction of these photoproducts of 18-hydroxyoleic acid were also detected in different natural samples (Rontani et al., 2005a). These results well support the significance of the photooxidation of the unsaturated components of higher plant cutins in the natural environment.

3. Free radical degradation (autoxidation) processes in phototrophic organisms

Autoxidation is the direct reaction of molecular oxygen with organic compounds under mild conditions. The autoxidation of organic compounds (in particular, lipids) involves free

radical reaction chains and thus includes an initiation, a propagation and a termination phase. Mechanisms of initiation for the free radical processes have been the subject of many studies. In senescent phytoplanktonic cells, initiation seems to result from the decomposition of hydroperoxides produced during photodegradation of cellular organic matter (Rontani et al., 2003b). Until now, autoxidative degradation in the marine environment has been largely ignored. Specific markers of these reactions have been highlighted by *in vitro* studies (Frankel, 1998; Rontani et al., 2003b; Rontani and Aubert, 2005). Using these markers, it was demonstrated *in situ* that autoxidation plays a very significant role in the degradation of particulate organic matter (Marchand et al., 2005; Rontani et al., 2006; Christodoulou et al., 2009; Rontani et al., 2011a).

Although the occurrence of autoxidation processes was clearly demonstrated *in situ*, it is not easy to induce these processes in laboratory cultures. Indeed, the mechanism of initiation of lipid radical oxidation, which has been debated for many years, seems to be the homolytic cleavage of photochemically produced hydroperoxides in phytodetritus (Rontani et al., 2003b). Redox-active metal ions are generally considered as the initiators of perhaps greatest importance for lipid oxidation in biological systems (Pokorny, 1987; Schaich, 1992). They may direct the cleavage of hydroperoxides either through alkoxyl or peroxyl radicals. In classical culture media (such as f/2) the metal chelator EDTA, which is present in high amounts, tightly binds free catalytic metal ions and thus renders them unavailable. EDTA thus acts in the culture media as an antioxidant and strongly limits radical oxidation processes.

Recently, autoxidative damages in cells of *E. huxleyi* strain CS-57 could be induced after incubation of this strain under an atmosphere of air + 0.5% CO_2 (Rontani et al., 2007a). The presence of additional CO_2 allowed: (i) to induce a stress that favoured oxidative damage and (ii) to decrease the pH of the culture medium releasing metal ions from EDTA complexes, which can act as catalysts of hydroperoxide homolysis.

It was also demonstrated recently that viral infection (Evans et al., 2006) and autocatalytic programmed cell death (Bidle and Falkowski, 2004) of phytoplanktonic cells could also lead to elevated production of reactive oxygen species (ROS) able to induce the degradation of cell components.

3.1 Chlorophyll phytyl side-chain

Autoxidation of the esterified chlorophyll phytyl chain involves either addition of peroxyl radicals to the double bond or hydrogen abstraction at the allylic carbon 4 (Rontani and Aubert, 1994; Rontani and Aubert, 2005). Classical addition of peroxyl radical to the double bond gives a tertiary radical (Fig. 8). This radical can then: (i) lead to *Z* and *E* epoxides (**18** and **19**) by fast intramolecular homolytic substitution (Fossey et al., 1995), or (ii) react with molecular oxygen affording (after hydrogen abstraction on another molecule of substrate) a diperoxide (**20**) (Fig. 8). Subsequent $NaBH_4$-reduction and alkaline hydrolysis of these compounds gives 3,7,11,15-tetramethylhexadecan-1,2,3-triol (**21**) (Fig. 8). In contrast, abstraction (by photochemically-produced peroxyl radicals) of a hydrogen atom at the allylic carbon 4 of the phytyl chain and subsequent oxidation of the allylic radicals thus formed affords (after $NaBH_4$-reduction and alkaline hydrolysis) *Z* and *E* 3,7,11,15-tetramethylhexadec-3-en-1,2-diols (**3** and **4**) and *Z* and *E* 3,7,11,15-tetramethyl-hexadec-2-en-1,4-diols (**22** and **23**) (Fig. 8). Compounds **22 and 23** (which are well specific markers of free radical oxidation) could

be detected in particulate matter samples (Marchand et al., 2005) and *E. huxleyi* cells (Rontani et al., 2007a) attesting to the involvement of such processes in senescent phytoplanktonic cells.

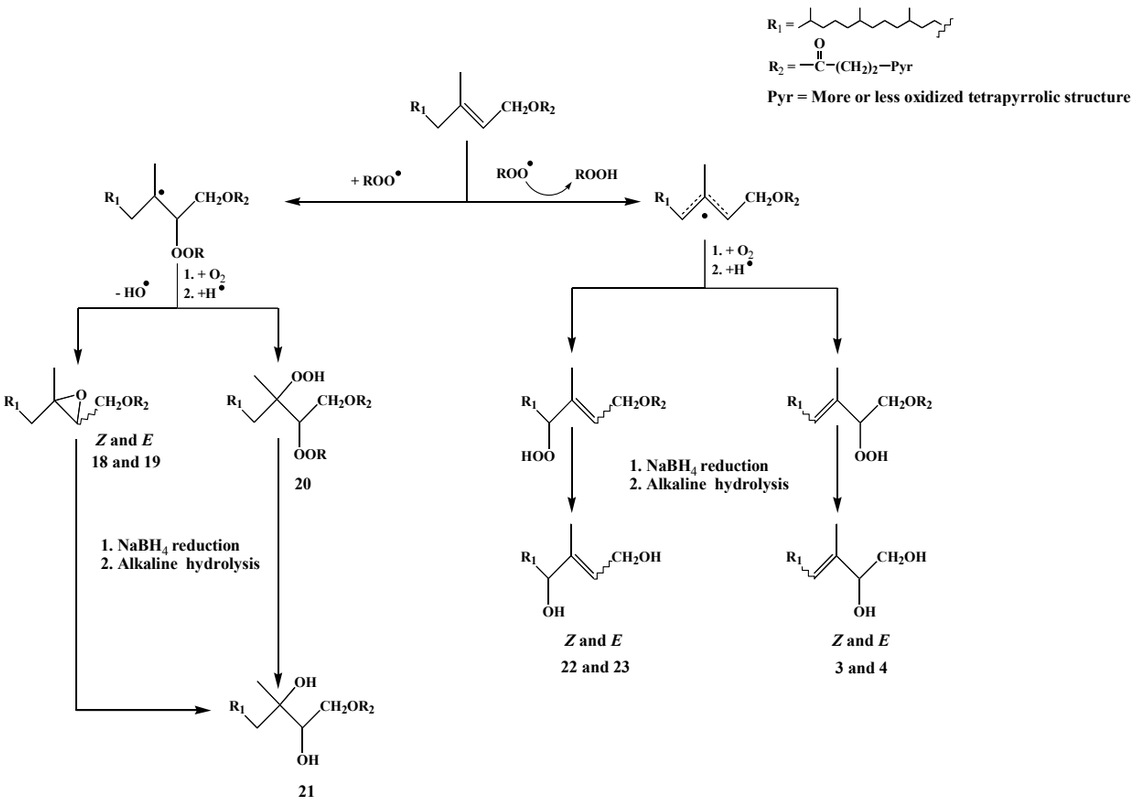
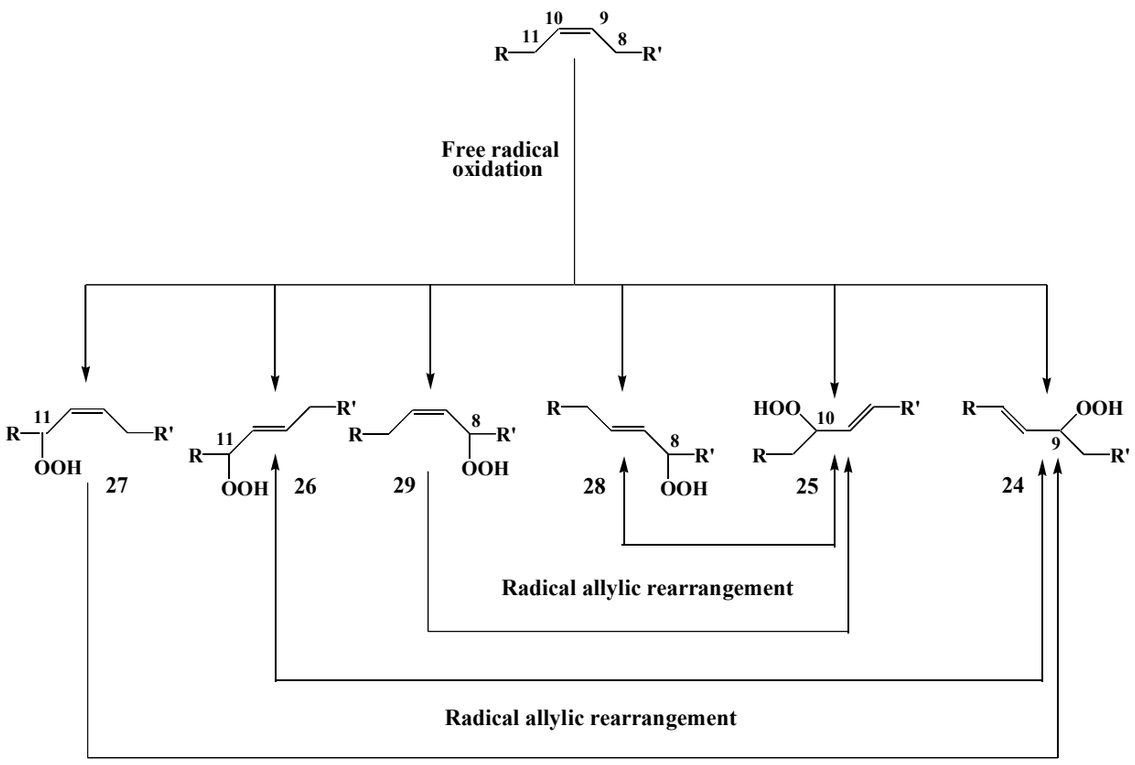


Fig. 8. Free radical-mediated oxidation of chlorophyll phytyl side-chain.

Free radical oxidation of chlorophyll phytyl chain appeared to be different in senescent cells of *S. costatum* (Rontani et al., 2003b). The differences observed were attributed to the well documented high chlorophyllase activity of this strain (Jeffrey and Hallegraeff, 1987) catalysing the hydrolysis of chlorophyll to free phytol and chlorophyllide. Indeed, in the case of free allylic alcohols hydrogen abstraction at carbon 1 is strongly favoured to the detriment of addition reactions (Huyser and Johnson, 1968).

3.2 Unsaturated fatty acids

Free radical oxidation of isolated classical 1,2-disubstituted double bonds generally involved mainly allylic hydrogen abstraction. Addition of peroxyl or alkoxyl radicals to the double bond becomes competitive only in the case of conjugated, terminal, or trisubstituted double bonds (Schaich, 2005). Effectively, autoxidation of mono-unsaturated fatty acids appears to mainly involve allylic hydrogen abstraction and subsequent oxidation of the allylic radical thus formed. For example, autoxidation of oleic acid mainly results in the formation of 9-hydroperoxyoctadec-*trans*-10-enoic (**24**), 10-hydroperoxyoctadec-*trans*-8-enoic (**25**), 11-hydroperoxyoctadec-*trans*-9-enoic (**26**), 11-hydroperoxyoctadec-*cis*-9-enoic (**27**), 8-hydroperoxyoctadec-*trans*-9-enoic (**28**) and 8-hydroperoxyoctadec-*cis*-9-enoic (**29**) acids (Fig. 9) (Frankel, 1998).



R = -$(CH_2)_6$-CH_3

R' = -$(CH_2)_6$-COOH

Fig. 9. Free radical-mediated oxidation of oleic acid.

Free radical oxidative processes can be easily characterised based on the presence of *cis* allylic hydroperoxyacids, which cannot be produced photochemically (see Fig. 4) and are specific products of these degradation processes (Porter et al., 1995; Frankel, 1998).

Large amounts of oxidation products of oleic acid could be detected in cells of *E. huxleyi* grown under an atmosphere of air + 0.5% CO_2 for 10 days (Rontani et al., 2007a). The presence (after $NaBH_4$-reduction) of a high proportion of 11-hydroxyoctadec-*cis*-9-enoic (**27**) and 8-hydroxyoctadec-*cis*-9-enoic (**29**) acids (Fig. 10) showed that under these conditions the degradation of oleic acid mainly involved free radical oxidation processes.

3.3 Δ^5-sterols

Free radical autoxidation of Δ^5-stenols yields mainly 7*a*- and 7*β*-hydroperoxides and, to a lesser extent, 5*a/β*,6*a/β*-epoxysterols and 3*β*,5*a*,6*β*-trihydroxysterols (Smith, 1981; Morrissey and Kiely, 2006) (Fig. 11).

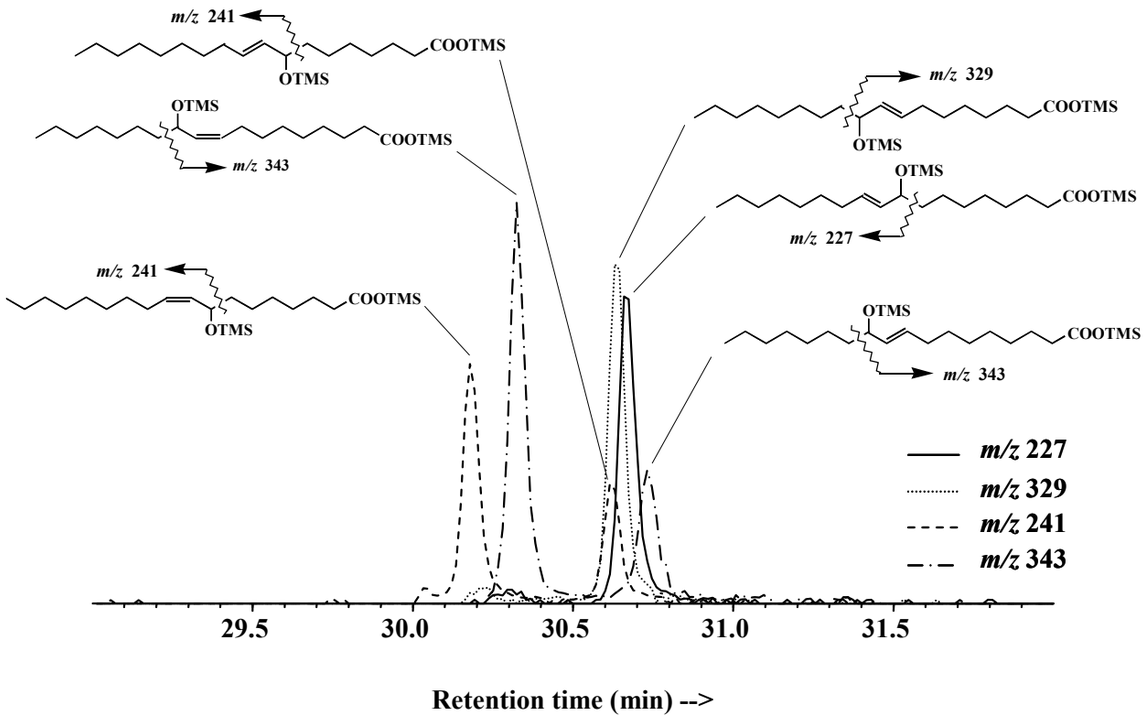


Fig. 10. Partial mass chromatogram of *m/z* 227, 329, 241 and 343 revealing the presence of oxidation products of oleic acid in the saponified fraction of *E. huxleyi* strain CS-57 grown under an atmosphere of air + 0.5% CO_2.

Owing to: their lack of specificity (possible formation by allylic rearrangement of photochemically-produced 5-hydroperoxides (see chapter 2.1.3), 7-hydroperoxides cannot be employed as tracers of autoxidation processes in phytodetritus. In contrast, it is generally considered that 5*α/β*,6*α/β*-epoxysterols arise mainly from peroxidation processes (Breuer and Björkhem, 1995; Giuffrida et al., 2004). Unfortunately, these compounds are not very stable and may be easily hydrolysed to the corresponding triol in seawater and during the treatment of the samples. 5*α/β*,6*α/β*-Epoxysterols and the corresponding 3*β*,5*α*,6*β*-trihydroxysterols were thus finally selected as tracers of sterol autoxidation..

5*α/β*,6*α/β*-Epoxysterols and 3*β*,5*α*,6*β*-trihydroxysterols corresponding to sitosterol, stigmasterol and campesterol were previously detected in young and old cell cultures of *Chenopodium rubrum* (Meyer and Spiteller, 1997). The results showed that the increase of these oxidation products well correlated with the age of the culture.

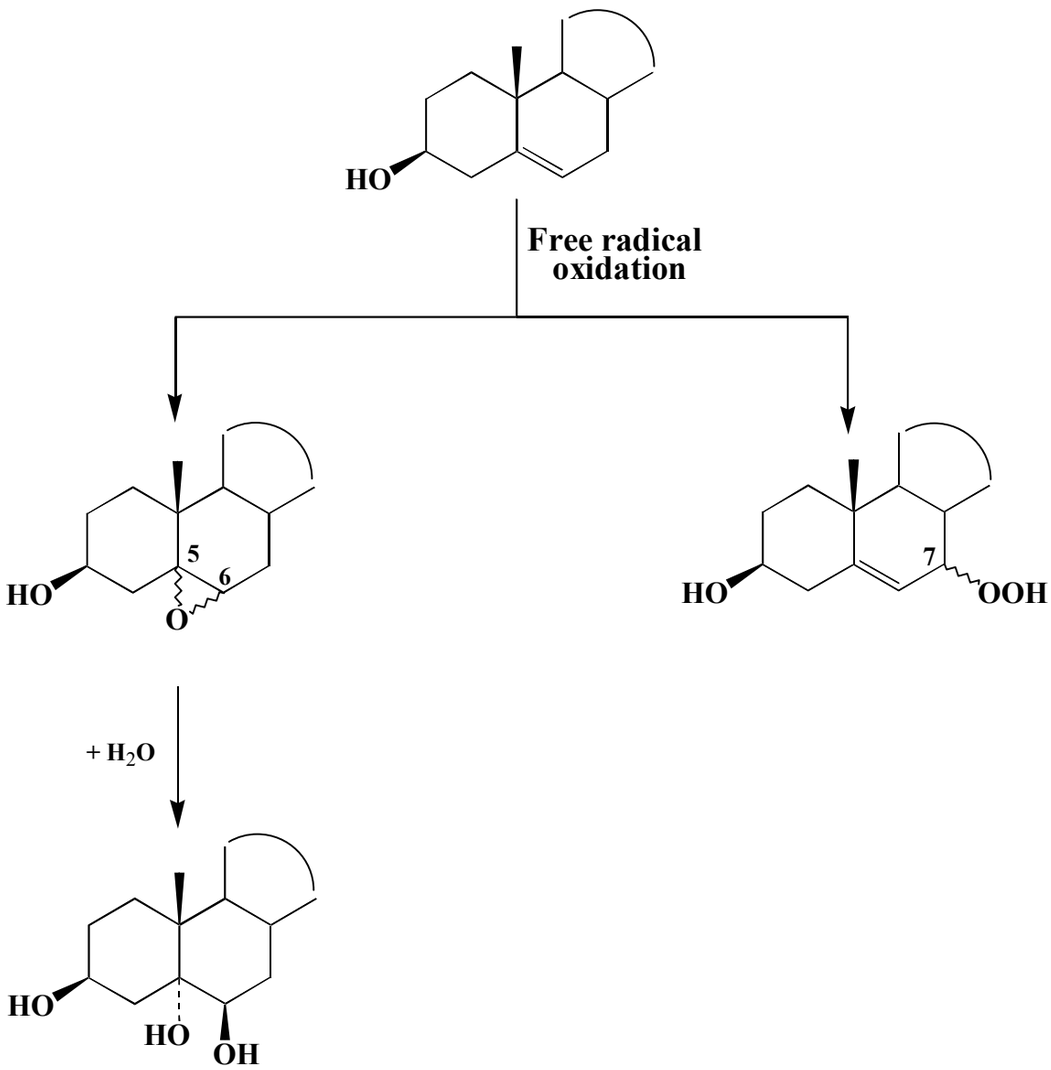


Fig. 11. Free radical-mediated oxidation of Δ^5 sterols.

3.4 Vitamin E

Vitamin E is relatively abundant in most photosynthetic organisms, such as higher plants (Rise et al., 1988; Schultz, 1990), cyanobacteria (Dasilva and Jensen, 1971), microalgae (Brown et al., 1999) and macroalgae (Sanchez-Machado et al., 2002), where it plays an essential role in the removal of toxic forms of oxygen (singlet oxygen, superoxide anion, hydroxyl and peroxyl radicals), by acting as sacrificial chemical scavenger (Halliwell, 1987); the process results in the irreversible oxidation of the tocopherol molecule. Vitamin E reacts rapidly with peroxyl radicals, affording small amounts of phytone (**1**), 4,8,12,16-tetramethylheptadecan-4-olide, α-tocopherylquinone and epoxy-α-tocopherylquinones, and dimers and trimers as major oxidation products (Liebler, 1994; Frankel, 1998; Rontani et al., 2007b) (Fig. 12).

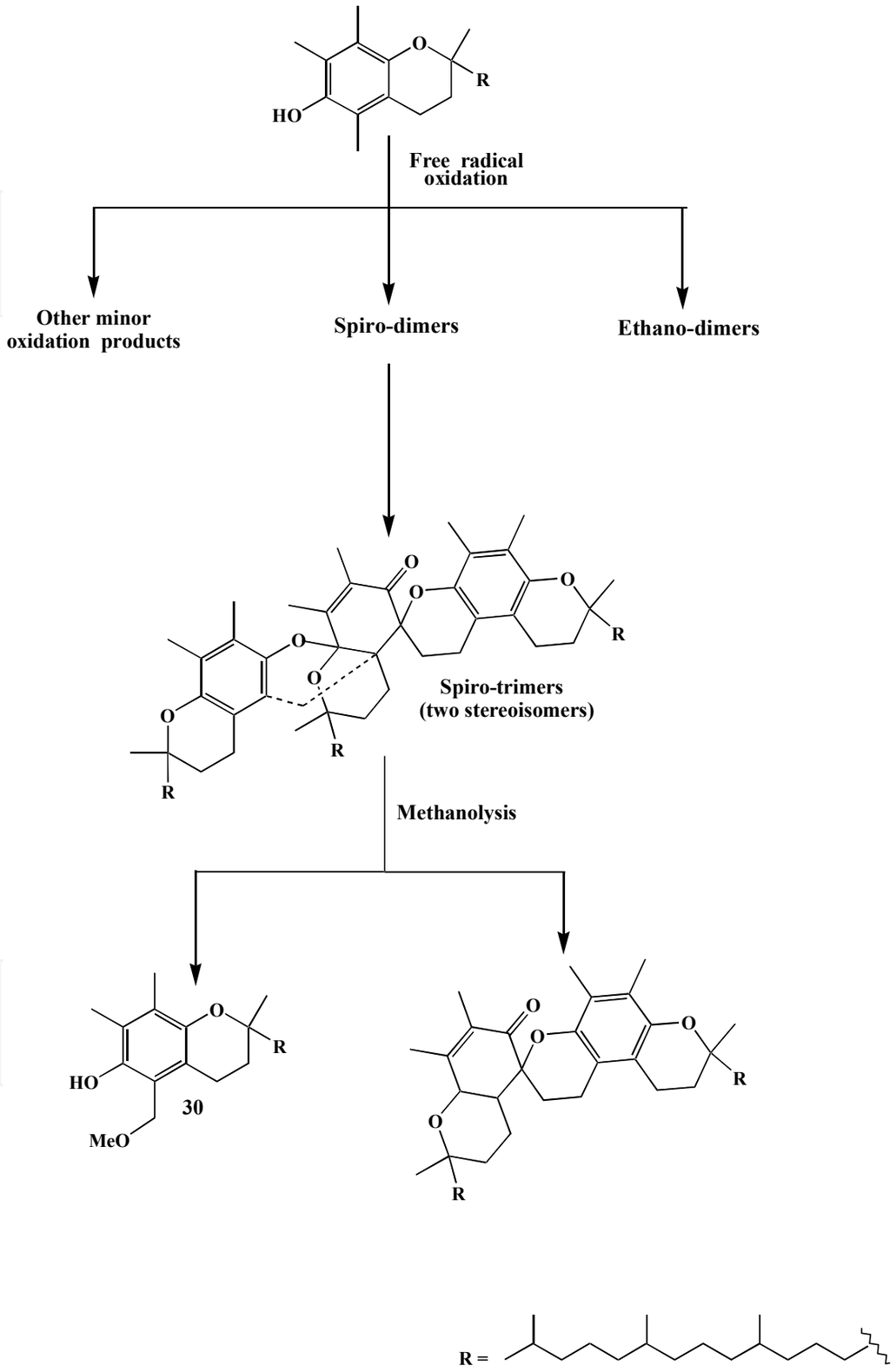


Fig. 12. Autoxidation of vitamin E and methanolysis of the foregoing trimers.

Isomeric trimers have been previously observed as products in numerous oxidations of vitamin E (e.g. Suarna et al., 1988; Krol et al., 2001). Such compounds cannot be easily detected since they are too heavy to be amenable by gas chromatography. However, methanolysis of the residues obtained resulted to the formation of high amounts of 5a-methoxytocopherol (**30**) arising from the methanolysis of the ketal group of trimers (Fig. 12) (Yamauchi et al., 1988). ESI-TOF MS analyses of oxidation products were also carried out in order to confirm the presence of high proportions of trimers (Nassiry et al., 2009).

Despite the intensive study of vitamin E oxidation since several decades, trimeric oxidation products could be detected in plants only very recently by Row et al. (2007). These authors detected these trimers in seeds of *Euryale ferox* containing extraordinarily high content of tocopherols. It is interesting to note that trimers were previously obtained as the major reaction products of vitamin E autoxidized under mild conditions in solution (1%) in methyl linoleate (Yamauchi et al., 1988). In plastoglobules, which are lipid monolayer subcompartments of the thylakoid membranes of chloroplasts (Maeda and Dellapenna, 2007), the concentration of tocopherols can reach 10% of the total fatty acids (Vidi et al., 2006). At such a concentration, the formation of a high proportion of trimers during photodynamic damages is thus very likely. In order to check this hypothesis, we searched for the presence of 5a-methoxytocopherol (**30**) after methanolysis of $NaBH_4$-reduced and non-reduced lipid extracts obtained from cells of *Emiliania huxleyi* strain TWP1 and *Chrysotila lamellosa* strain HAP17. The detection of significant amounts of this methanolysis product of trimers (Yamauchi et al., 1988) in these extracts (Nassiry et al., 2009) well supported the presence of such trimeric oxidation products of vitamin E in these algae.

3.5 Alkenones

The autoxidative reactivity of alkenones was studied in the laboratory in the presence of a radical initiator (di-*tert*-butyl nitroxide) and a radical enhancer (*tert*-butyl hydroperoxide) (Rontani et al., 2006). Alkenones appeared to be more sensitive towards oxidative free radical processes than analogues of other common marine lipids such as phytyl acetate, methyl oleate and cholesteryl acetate, and their oxidation rates increase in proportion with their number of double bonds. As the result of this increasing reactivity with degree of unsaturation, the $U_{37}^{K'}$ ratio increased significantly (up to 0.20) during the incubation.

Autoxidation of alkenones appears to mainly involve allylic hydrogen abstraction and subsequent oxidation of the allylic radical thus formed (Fig. 13). According to these processes, oxidation of each double bond of alkenones and subsequent $NaBH_4$ reduction affords four positional isomeric alkenediols. These compounds could be very useful indicators of autoxidation of alkenones but, unfortunately, they did not accumulate during the incubation. Indeed, due to the presence of additional reactive double bonds, hydroperoxyalkenones may undergo subsequent oxidation reactions affording, di-, tri- and tetrahydroperoxyalkenones according to the degree of unsaturation of the starting alkenone. In seawater, these different hydroperoxides may undergo two main degradative processes: (i) homolysis of the O-O bond leading to carbonyl (dehydration), alcoholic (reduction) and fragmentation (β-scission) products (Rontani et al., 2007c) and (ii) heterolysis of the O-O bond leading to the formation of two carbonyl fragments (Hock cleavage), this proton-catalysed cleavage being initiated by migration of groups to positive oxygen (Frimer, 1979). Dimeric and oligomeric compounds cross-linked through either peroxide or ether linkages (Frankel, 1998) may also be formed during autoxidation of alkenones.

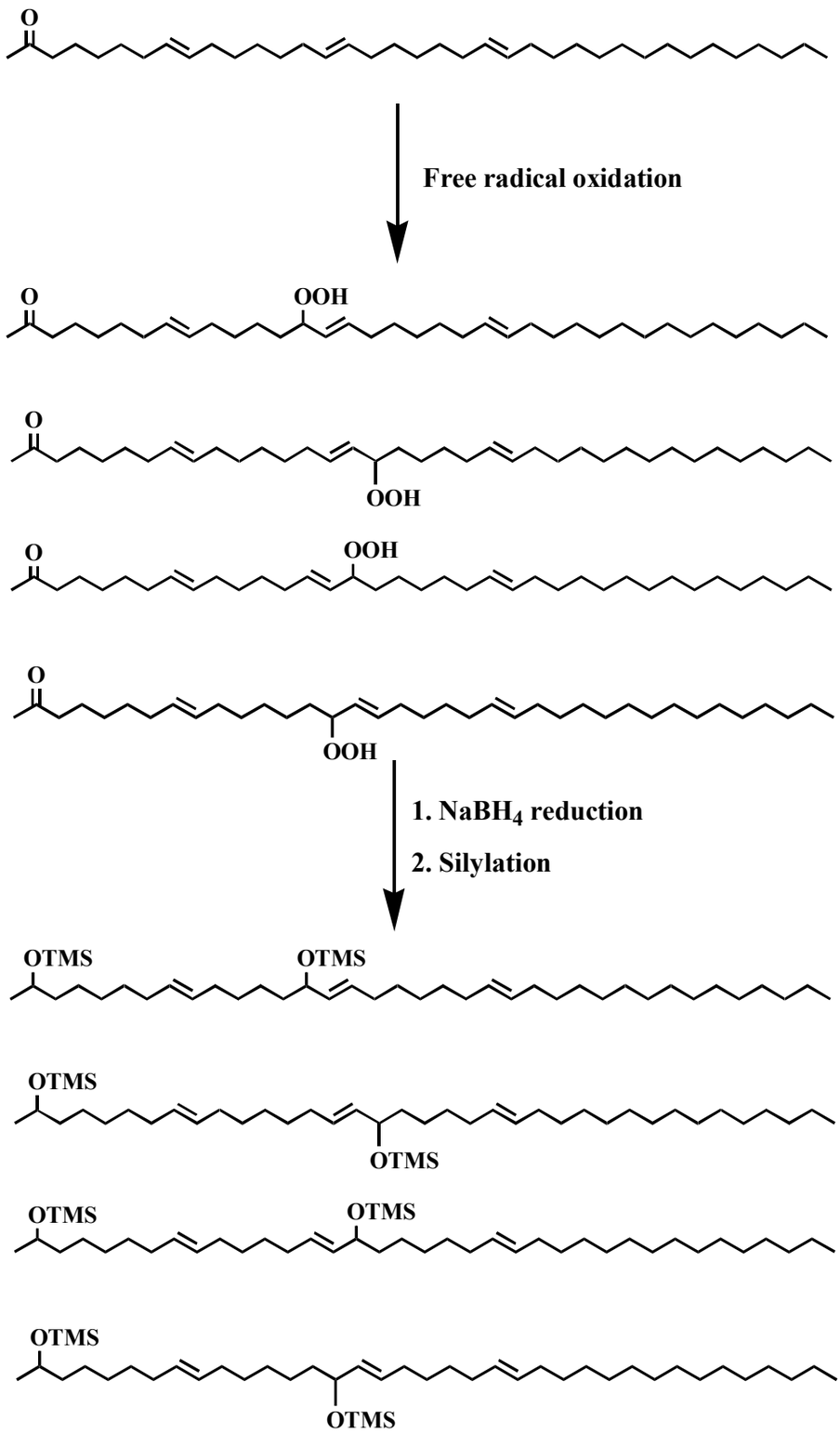


Fig. 13. Characterization of oxidation products derived from the autoxidation of the ω22 double bond of the $C_{37:3}$ alkenone (TMS = trimethylsilyl).

These results were corroborated by the further finding of significant amounts of alkenediols arising from $NaBH_4$-reduction of the corresponding hydroperoxyalkenones in cultures of *E. huxleyi* strain CS-57 grown under an atmosphere of air + 0.5% CO_2 (Rontani et al., 2007a) and more recently after incubation of a culture of the strain *E. huxleyi* TWP1 under darkness (Rontani, Unpublished results) (Fig. 14) both exhibiting an anomalously high unsaturation ratio It seems thus that autoxidation processes have the potential to affect alkenone distributions leading to a warm bias in estimates of palaeotemperatures derived from alkenone ratios in sediments.

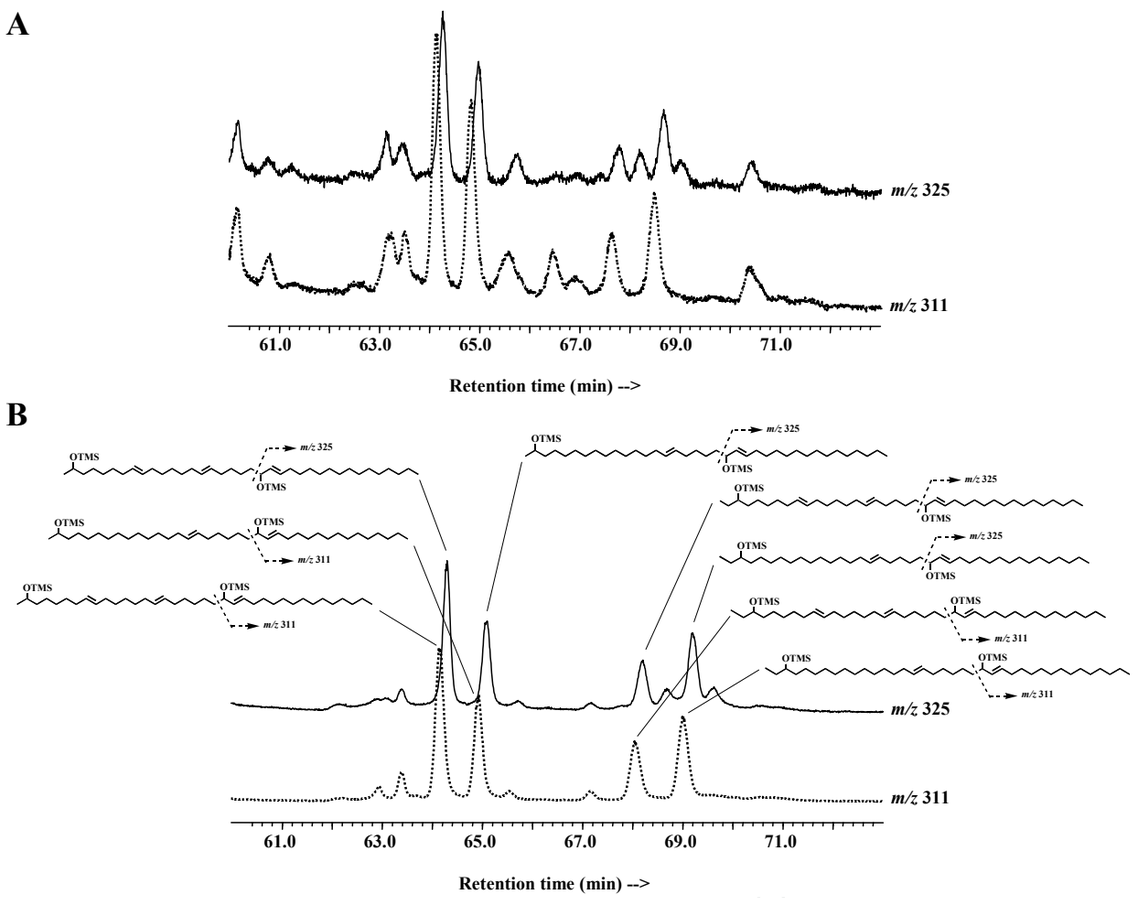


Fig. 14. Partial mass fragmentograms of *m/z* 311 and 325 revealing the presence of silylated C_{37} and C_{38} alkenediols after $NaBH_4$-reduction and silylation of the total lipid extract of *E. huxleyi* cells incubated under darkness (A) and standard autoxidation products of alkenones (B).

4. Conclusions

Due to the lack of adequate tracers, the role played by light-induced photochemical and free radical-mediated (autoxidative) processes during the degradation of lipid components of phototrophic organisms has been virtually ignored until now.

It was recently demonstrated that most of the unsaturated lipid components of these organisms (chlorophylls, carotenoids, unsaturated fatty acids, sterols, *n*-alkenes and HBI alkenes) could be photodegraded by visible and UV radiations during the senescence. This degradation mainly involves type II (i.e. involving $^1O_2$) photoprocesses. Singlet oxygen appeared to be sufficiently stable in this hydrophobic micro-environment to migrate outside the chloroplasts and affect the unsaturated components of cutins of higher plants.

Free radical-mediated oxidation (autoxidation) processes also intervene intensively during the senescence of phototrophic organisms. Induction of these processes seems to mainly result from the homolytic cleavage (catalyzed by some metal ions) of photochemically produced hydroperoxides. Unsaturated fatty acids, chlorophyll phytyl side-chain, vitamin E, sterols and alkenones appeared to be strongly affected by these degradative processes. In the case of alkenones, it is very important to note that autoxidative degradation processes may alter significantly their unsaturation ratio and thus constitute a potential source of biases during paleotemperature reconstruction.

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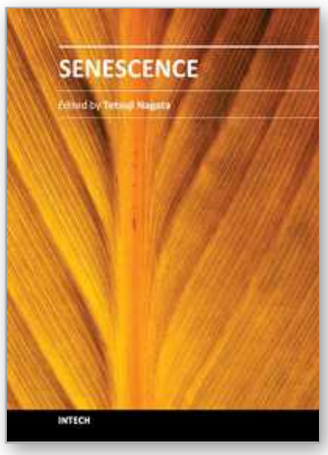

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The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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