Lipid Oxidation Products

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Useful Tools for Monitoring Photo-and Autoxidation in Phototrophs

By

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Cambridge Scholars Publishing

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PREFACE

The organic matter preserved in aquatic sediments provides an abundance of evidence for the environmental conditions prevailing at the time of deposition. Understanding the sources of organic matter and the processes that modify its composition is the foundation of the field of organic geochemistry. Many lipid biomarkers (compounds that can be related to specific organisms) have been identified that allow different sources (e.g., bacteria, archaea, vascular plants, microalgae, macroalgae, zooplankton, benthic animals etc) to be differentiated. However, only a small proportion of compounds biologically produced in the water column reach the sediment intact and many of the chemical structures are modified by chemical and biological processes in the water column and by processing through aquatic food webs.

Much attention has been paid to the microbial degradation of lipids, but abiotic processes can be particularly important in the surface layers of aquatic environments, where sunlight can penetrate, and also in oxic surface sediments. Through decades of research, Dr. J.-F. Rontani has shown that photooxidative degradation can destroy much of the unsaturated lipids in the water column and hence can strongly alter the lipid signature of organic matter reaching the seafloor. It is thus essential to take into account the potential effects of abiotic degradation when making palaeoenvironmental reconstructions from sedimentary organic matter. In this book, the main types of oxidation are examined in detail and specific degradation products have been identified as biomarkers for such reactions. These results are contrasted with the compounds produced by microbial degradation of lipids thus allowing a comprehensive understanding of the cycling of organic compounds in aquatic environments.

As an example of this approach, Dr. Rontani and his team incubated the geochemically important haptophyte *Emiliania huxleyi* and showed that free radical-mediated processes (autoxidation) were extensive and altered monounsaturated fatty acids, sterols and the chlorophyll phytyl side-chain giving rise to specific oxidation products. These included 11-hydroxyoctadec-*cis*-9-enoic and 8-hydroxyoctadec-*cis*-9-enoic acids, *Z*- and *E*-3,7,11,15-tetramethylhexadec-3-ene-1,2-diols and 3,7,11,15-tetramethylhexadec-2-ene-1,4-diols. Autoxidation also affects the composition of unusual very long-chain alkenones (unsaturated methyl and ethyl

ketones) produced by this species which can lead to small changes in the paleotemperature proxy values derived from alkenone abundances.

Photo- and autoxidation of lipids specific of terrestrial phototrophs are also covered in this book and several tracers for monitoring the abiotic degradation of these organisms on land and in the oceans are proposed.

Interactions between biotic and abiotic degradation processes are also discussed. Although complex, these interactions need to be taken into account during estimates of the balance between degradation and preservation of phototrophic organisms in the natural environment. Lipoxygenase (LOX)-induced autoxidative degradation of terrestrial particulate organic matter constitutes a nice example of such interactions. This process is shown to be widespread in estuaries and that this varies with latitude. At high latitudes, lower temperatures and irradiance favour photooxidative damage to higher plant debris and, consequently, hydroperoxide production. High hydroperoxide content strongly contributes to LOX activation in mixed waters. The high resulting LOX activity enhances alkoxyl radical production and thus autoxidation. At low latitudes, photooxidative effects are limited, but riverine autoxidation is enhanced by the high temperatures. This process affords high levels of hydroperoxides also inducing intensive LOX activity and autoxidation in estuaries.

In summary, this book provides, up-to-date and detailed information on oxidation of lipids and how carbon is cycled in aquatic environments which has until now been rather neglected in the literature. The analytical work is meticulous, and inferences are backed up by laboratory studies of geochemically relevant organisms. Multiple examples are provided drawn from scientific papers published over many decades spanning a variety of environments. The book should appeal to those new to the field and to experts alike.

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CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia

ACKNOWLEDGEMENTS

Ever since my childhood, it was my dream to do research in chemistry. If this dream has come true, it is thanks to the unwavering support of my parents Louis and Marie-Rose, who were never given the opportunity to get a real education but did everything they could to make it happen for me. The support of my wife Patricia and my two daughters Pauline and Mélanie has also been essential throughout my career.

Special thanks to my friend Claude Aubert, who allowed me to enjoy unfettered access to his facilities in my periods of scarcity and very limited equipment, and who enabled me to immerse myself in the fascinating study of the mechanisms of fragmentation of lipid compounds in mass spectrometry.

I would like to salute the memory of Professor François Blanc (ex director of the Marseilles Centre of Oceanology) who enabled me to join the CNRS and engage the world of research in organic geochemistry.

All these studies concerning lipid oxidation processes could not have been carried out without numerous fertile national and international collaborations. I especially want to thank Drs. John K. Volkman, Simon T. Belt, Thomas Brown, Lukas Smik, Stuart G. Wakeham, Frederick G. Prahl, Michal Koblizek, Ronald J. A. Wanders, Thomas Bianchi, Nicolas Ward, Patricia Medeiros, Patricia Bonin, Jean-Claude Marty, Juan-Carlos Miquel, Franck Pinot and Vincent Méjean (the list is not exhaustive)—it really was a joy working with you all.

The many PhD students that I have supervised during my career have also played a role in developing this sphere of research, and their contributions should not be overlooked.

I am also very grateful for all the financial support from the CNRS (EC2CO-microbien and LEFE-Cyber programs) and the University of Aix-Marseille throughout these years, and most recently the LabEx OT-Med (Objectif Terre: Bassin Méditerranéen) and the FEDER Oceanomed grant (No. 1166-39417), which allowed us to acquire PhD funding and invaluable GC-QTOF, LC-QTOF and GC-MS/MS systems, respectively.

Last but not least, the MALINA and GREENEDGE international projects (P.I. Marcel Babin) have enabled us to study our processes of interest in the fascinating but hard-to-reach areas of the Arctic.

INTRODUCTION

Lipids—from hydrocarbons, pigments and terpenoids to free fatty acids, acylglycerides, phospholipids, galactolipids, cutins, suberins and waxes; Harwood and Russell, 1984)—are important components of phototrophic organisms. To illustrate, lipids account for 16-26% of the organic content of phytoplankton (Jònasdòttir, 2019). Their relative stability (preservation in sediments for millions and even billions of years; Huang et al., 1995; Brocks et al., 1999) and specificity (restricted origins from individual or groups of organisms; Huang et al., 2004) means that lipids are often used as tracers of the origin of organic matter in terrestrial and marine environments (Volkman, 2006; Waterson and Canuel, 2008; Parrish, 2013; Nguyen Tu et al., 2017; Guo et al., 2020).

Compounds resulting from abiotic oxidation of unsaturated lipids can also prove very useful for discerning individual degradation processes such as photooxidation or autoxidation in specific phototrophic organisms. Unfortunately, most studies of the degradation of these organisms to date have focused on biotic degradation processes (Afi et al., 1996; Sun et al., 1999; Mäkinen et al., 2017), and investigations have only recently turned to the role played by photochemical and free radical-mediated processes in the degradation of lipid components during the senescence of phototrophic organisms (*e.g.* Walker et al., 2002; Ramel et al., 2012; Rontani et al., 2012a, 2014c, 2017; Amiraux et al., 2016). This book sets out to provide an instructive overview of: (i) the reactions involved during these abiotic degradation processes, (ii) the characterization and quantification of suitable lipid tracers of these processes, and (iii) the potential applications of such compounds.

Although complex lipids (such as acylglycerides, phospholipids, galactolipids and steryl esters) can be analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS; Roces et al., 2016; Pham et al., 2019) or by Iatroscan thin-layer chromatography-flame ionization detection (TLC-FID; Volkman et al., 1986; Parrish and Ackman, 1983), monitoring of lipids and their oxidation products is often carried out by gas chromatography-mass spectrometry (GC-MS) after $NaBH_4$ reduction and alkaline hydrolysis steps. Even though sample preparation for GC-MS analyses is relatively time-consuming, the technique is mostly used in electron ionization (EI) mode, which provides more structural information

than the soft ionization methods (such as ESI, APCI) utilized during HPLC-MS analyses (Xia and Budge, 2017). It is notably very useful to determine the position of functional groups of lipid oxidation products (Koek et al., 2011). The $NaBH_4$ reduction step serves to convert thermolabile hydroperoxides arising from lipid oxidation to the corresponding alcohols that are more amenable to analysis by GC-MS (Marchand and Rontani, 2001). The subsequent alkaline hydrolysis step then serves to (i) break complex lipids down into their constituent fatty acids, plus glycerol, phosphate, sterol or sugar groups, and (ii) separate fatty acids from 'neutral' lipid components such as hydrocarbons, sterols, alcohols and ketones (Volkman, 2006).

The first chapters of this book explain in detail the mechanisms (mainly involving singlet oxygen) and timing (during senescence or in response to a high stress) of type-II photosensitized oxidation processes in phototrophic organisms. We then go on to describe photooxidation of the main simple unsaturated lipid components of phototrophs (fatty acids, chlorophyll, carotenoids, sterols, triterpenoids, alkenones, *n*-alkenes, HBI alkenes, cuticular waxes, and more). In this part, we focus on the specificity of the photooxidation products formed and on their potential application as tracers of these processes.

The next chapters then discuss the transfer of photooxidative damage in non-phototrophic material (heterotrophic bacteria and zooplanktonic fecal pellets) and the effect of temperature and solar irradiance on the efficiency of type-II photooxidation processes.

We then explain the different steps (initiation, propagation and termination) of the free-radical oxidation (autoxidation) processes and look in detail at the autoxidation mechanisms of the main lipids of phototrophs, which can be affected by these processes, with a focus again on the selection of specific tracers of these processes.

The following chapters pay special attention to the characterization and quantification of the main photo- and autoxidation products of lipids selected as tracers. Mass spectrometry fragmentations of trimethylsilyl derivatives of these compounds are described, and selected fragment ions are proposed for monitoring abiotic degradation of specific phototrophic organisms in environmental samples. We anticipate that this part should be particularly useful for future users of lipid oxidation products. We also list published quantitative estimates of the degradation state of the main lipids of different phototrophic organisms (phytoplankton, phototrophic bacteria, terrestrial and aquatic higher plants) and in a variety of environmental samples (sinking and suspended particulate matter, sediments, sea ice and microbial mats).

We then describe some of the environmental hotspots of phototrophic organism photo- and autoxidation (e.g. polar and more particularly under-ice areas for type-II photosensitized oxidation and polar and equatorial estuaries for autoxidation).

The next chapter proposes several potential applications for lipid oxidation products (i.e. organism-specific indicators of stress, new proxies of paleoenvironmental changes, indicators of abiotic alterations of paleoproxies, ozone depletion and permafrost abiotic degradation, and use for determining the double bond position of monounsaturated fatty acids or *n*-alkenols).

The penultimate chapter uses several examples to discuss the very complex interactions between biotic and abiotic degradation processes that can substantially reshape the balance between degradation and preservation of phototrophic organisms in the natural environment.

The final chapter inventories and discusses the enzymatic oxidation processes liable to bias photo- and autoxidation estimates.

CHAPTER ONE

PHOTOOXIDATIVE REACTIONS

Direct photooxidative reactions

In the natural environment, direct photooxidation occurs when sunlight is absorbed by the chemical of interest to form excited or radical species, which then react with oxygen or water. These processes involve light-absorbing entities called chromophores (defined as a region in a molecule where the energy difference between two different molecular orbitals falls within the range of the solar spectrum; Shukla et al., 2016) that can undergo oxidative change as a direct consequence of absorbing photons (Zafiriou et al., 1984). In autotrophic organisms, only pigments (e.g. chlorophylls and carotenoids; Nelson, 1993), some polyunsaturated fatty acids (Collins et al., 2018), vitamins (e.g. thiamine and riboflavin; Ahmad et al., 2018, Golbach et al., 2014) and some amino acids (e.g. tryptophan, tyrosine, and histidine; Pattison et al., 2012) have absorption peaks in the UV and visible region of solar light (Zafiriou et al., 1984) and can thus be directly photooxidized under environmental conditions.

Indirect photooxidative reactions

Indirect photooxidative reactions are common in the natural environment. They are crucial processes as they can alter molecules that resist photolysis, such as transparent species or chromophores whose reactive states are inefficiently populated by absorption (Zafiriou et al., 1984). The first step of these reactions involves the absorption of light by a substance called a 'photosensitizer', which is a molecule capable of producing a chemical change by transferring energy to an excited neighbouring molecule. These compounds have two systems of electronically excited states: singlet (^{1}S) and triplet (^{3}S) (Foote, 1976). The triplet state is usually longer-lived than the singlet, even though the singlet is the initial product of light absorption (Foote, 1976). Most of the photosensitized oxidation reactions in nature start from the photosensitizer in its triplet state (Gollnick, 1968). To be efficient under environmental conditions, sensitizers

need to: (i) absorb visible or near-UV light, (ii) afford a long-lived triplet state in high quantum yield, and (iii) be sufficiently stable. All photosensitizers contain chromophores, which are usually cyclic compounds with resonating conjugated double bond systems that enable them to absorb visible and UV-A light. However, some chromophores are bicyclic, some are tricyclic, and some, like tetrapyrroles (chlorophylls and hematoporphyrin), are polycyclic (Giese, 1980).

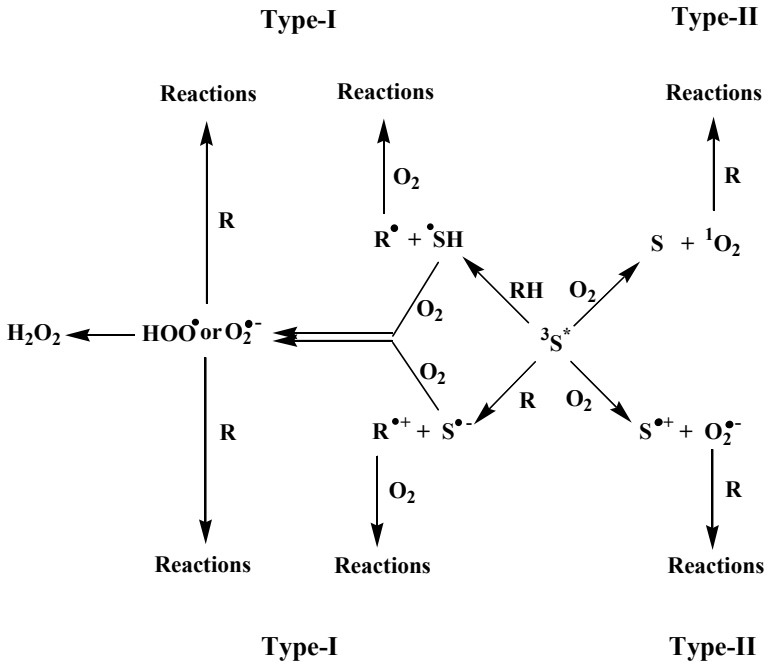


Figure 1. Reactions of a triplet sensitizer (Adapted from Rontani and Belt, 2020). (R or RH = reduced substrate, $^3S^*$ = triplet sensitizer, $^1O_2$ = singlet oxygen, $^3O_2$ = ground-state oxygen, $O_2^{\bullet -}$ = superoxide ion, HOO• = hydroperoxide radical, H_2O_2 = hydrogen peroxide)

In the presence of oxygen, triplet sensitizers can follow two main types of reactions (Fig. 1):

– In a type-I reaction, the excited triplet sensitizer reacts directly with a reducing substrate (RH or R) to afford free radicals (after hydrogen atom transfer) or radical ions (after electron transfer) (Schenck and Koch, 1960; Gollnick, 1968). These radicals can then: (i) abstract an electron or

a hydrogen atom to other molecules, (ii) react with oxygen to initiate free-radical chain autoxidation, or (iii) react with the reduced sensitizers (back-reactions). Reduced sensitizers (SH• and $S^{\bullet-}$) can also react with oxygen to afford superoxide ion ($O_2^{\bullet-}$) or hydroperoxide radical (HOO•), which can then disproportionate to H_2O_2 or react with the substrate (Fig. 1) (Foote, 1976).

– In a type-II reaction, the triplet sensitizer transfers its excitation energy to oxygen, forming an electronically excited singlet state of oxygen ($^1O_2$) (Fig. 1). Given the exceptionally high speed of this energy transfer, it is generally considered to account for most quenching of triplet sensitizers by oxygen (Foote, 1976). The $^1O_2$ thus formed is strongly electrophilic and can therefore only react with compounds that possess substituted double bonds or other electron-rich functionalities (Frimer, 1979). Less efficient electron transport from triplet sensitizer to oxygen can also occur, affording $O_2^{\bullet-}$ (Fig. 1) (Kasche and Lindqvist, 1964).

The mechanisms of (type-I or type-II) photosensitized oxidation depend on: (i) type of sensitizer and substrate, and (ii) concentrations of substrate and oxygen. Type-I photoprocesses are generally favoured in the case of readily-reduced sensitizers (e.g. quinones) and readily-oxidized substrates (e.g. phenols or amines; Saito et al., 1975), whereas less readily-reduced sensitizers (e.g. dyes or aromatics) and high oxygen concentrations will favour $^1O_2$ reactions (Nilson et al., 1972).

CHAPTER TWO

INDUCTION OF TYPE-II PHOTOSENSITIZED OXIDATION PROCESSES IN THE MEMBRANES OF PHOTOTROPHIC ORGANISMS

The photoprotective system of healthy cells

When a chlorophyll molecule absorbs a quantum of light energy, it forms an excited singlet state (^{1}Chl) which, in healthy cells, is mainly used in the characteristically fast photosynthesis reactions (Foote, 1976). The energy of this excited state gets transferred to the photosynthesis reaction centre where it drives photochemical reactions. However, a small proportion of ^{1}Chl (<0.1%) undergoes intersystem crossing (ISC) to form the longer-lived triplet state ^{3}Chl (Knox and Dodge, 1985; Fig. 2). ^{3}Chl is not only potentially damaging per se in type-I reactions (Knox and Dodge, 1985; Fig. 1), it can also generate damaging $^1O_2$ and $O_2^{\bullet-}$ by reaction with ground-state oxygen ($^3O_2$) via type-II photoprocesses (Fig. 1). $O_2^{\bullet-}$ may subsequently: (i) disproportionate to $^1O_2$ and H_2O_2 in the presence of a proton donor (Foote, 1976), and (ii) interact with H_2O_2 in the presence of non-heme Fe^{+3} to generate hydroxyl radicals (HO•) via the Haber–Weiss reaction (Leshem, 1988). Despite the production of these reactive oxygen species (ROS), the photoproduction of $^1O_2$ is mainly considered to be responsible for light-dependent reactions that damage plant cells (Triantaphylides et al., 2008).

As chloroplasts are susceptible to oxidative damage, they possess an array of antioxidant-protective mechanisms. Carotenoids quench ^{3}Chl and $^1O_2$ by energy transfer mechanisms at very high rates (Fig. 2). These compounds have a dual role: preventing $^1O_2$ formation and helping to remove any $^1O_2$ that does manage to form (Foote, 1976). Tocopherols and ascorbic acid are also efficient quenchers of $^1O_2$ (Halliwell, 1987). Tocopherols can also remove $^1O_2$, $O_2^{\bullet-}$, HOO• and HO• by acting as sacrificial scavengers, i.e. in processes that induce irreversible oxidation of the tocopherol molecule (Halliwell, 1987). Superoxide dismutase (SOD) enzyme and ascorbic acid can scavenge $O_2^{\bullet-}$, while catalase

activity can decrease H_2O_2 levels, thus providing less substrate for HO• formation in the Haber–Weiss reaction (Leshem, 1988).

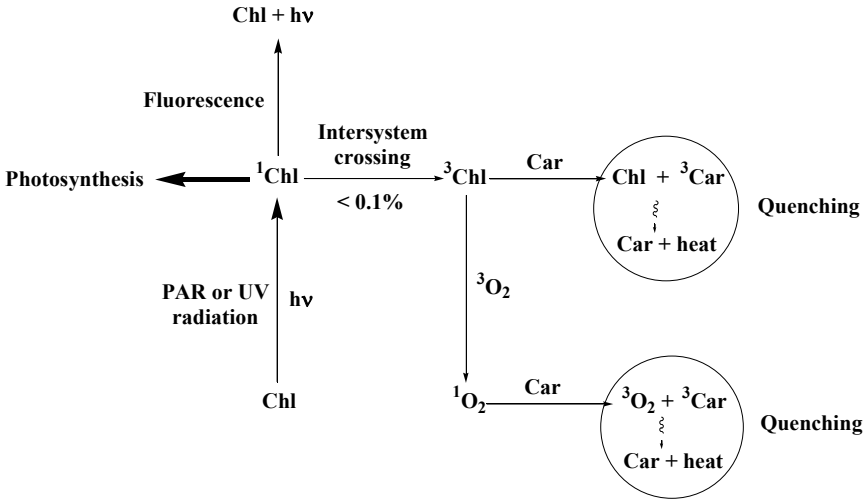


Figure 2. Potential pathways for chlorophyll excitation energy in healthy cells of autotrophic organisms (simplified scheme limited to the formation of $^1O_2$ and the photoprotective role of carotenoids).

The photodynamic effect in senescent cells

In senescent phototrophic organisms, the cessation of photosynthetic reactions results in an accelerated rate of formation of ^{3}Chl and ROS (mainly $^1O_2$) (Nelson, 1993). The rate of formation of these potentially damaging species can then exceed the quenching capacity of the photoprotective system, enabling the photodegradation of cell components to occur (photodynamic effect; Merzlyak and Hendry, 1994) (Fig. 3). Three sites in the photosynthetic apparatus are the major sources for generation of ROS: the photosystem II (PSII) reaction centre, the photosystem I (PSI), and the light-harvesting complex (LHC) of PSII (Pinnola and Bassi, 2018). The direct irreversible reaction of ^{3}Chl with ground-state triplet oxygen ($^3O_2$), i.e. direct photobleaching, gives photooxidation products (Harbour and Bolton, 1978) (Fig. 3). $^1O_2$ reacts very quickly with any nearby biomolecules at near-diffusion-controlled rates (Knox and Dodge, 1985; Cadenas, 1989). The very high reactivity of $^1O_2$ with numerous cell components such as unsaturated lipids, nucleic acids and some amino acids (Rontani, 2012; Devasagayam and Kamat,

2002) is a consequence of the loss of the spin restriction that normally prevents $^3O_2$ reaction with these biomolecules (Zolla and Rinalducci, 2002). $^1O_2$ also reacts with the sensitizer (chlorophyll), causing it to photobleach (Nelson, 1993; Rontani, 2012) (Fig. 3). Photobleaching of the sensitizer reduces $^1O_2$ production, and thus competes with the photodynamic effect.

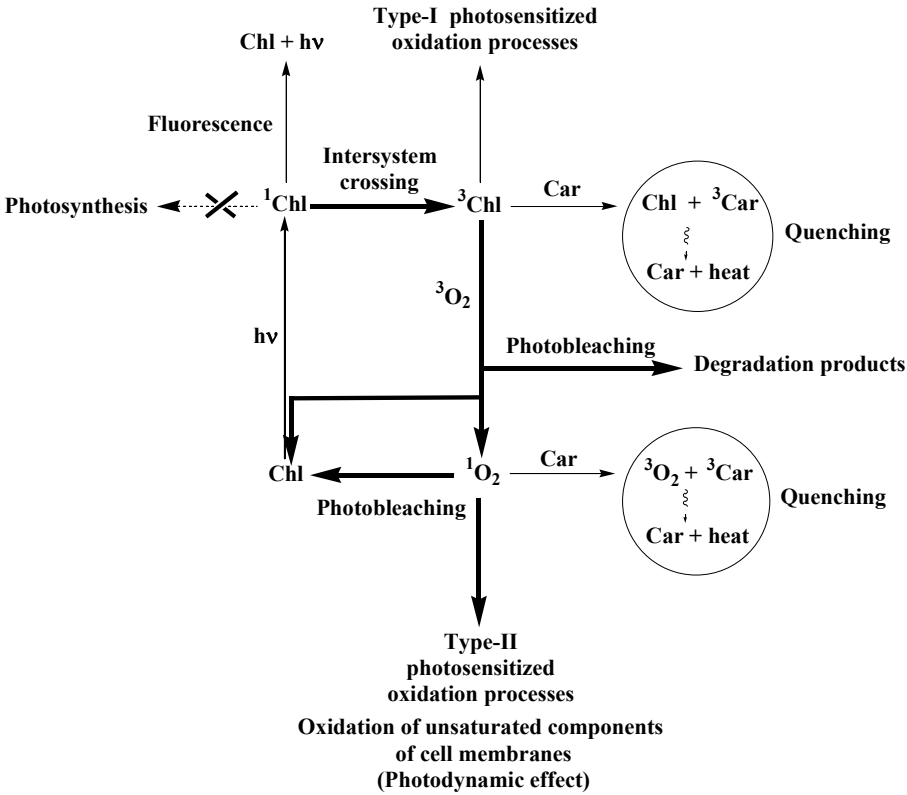


Figure 3. Potential pathways for chlorophyll excitation energy in senescent cells of autotrophic organisms (simplified scheme limited to the formation of $^1O_2$ and the photoprotective role of carotenoids). Adapted from Rontani et al. (2021b).

Due to its high reactivity and short lifetime, it is generally thought that $^1O_2$ can mostly interact with molecules in its closest environment (Krasnovsky, 1998). However, $^1O_2$ produced from sensitizers in a lipid-rich hydrophobic environment could have a longer lifetime and greater

potential diffusive distance than its behaviour in aqueous solution (Suwa et al., 1977). In biological membranes, the lifetime of $^1O_2$ ranges between 13 and 35 μs (Ehrenberg et al., 1998; Sokolov and Pohl, 2009), which equates to a calculated diffusion length of about 400 nm (Baier et al., 2005). It has been observed in the photosynthetic apparatus of *Chlamydomonas reinhardtii* that $^1O_2$ produced in thylakoid membranes under high light conditions is able to reach the cytoplasm or even the nucleus (Fisher et al., 2007). It is not surprising, therefore, that type-II photosensitized oxidation of the majority of unsaturated lipid components has been observed in numerous senescent autotrophic organisms ranging from phytoplankton, cyanobacteria and purple sulphur bacteria to terrestrial and aquatic higher plants (Marchand and Rontani, 2003; Rontani et al., 1996a; 2005a; Rontani, 2012; 2019).

Note that in autotrophic organisms, the physiological state of the cells plays a key role in the induction of type-II photosensitized oxidation processes. Indeed, $^1O_2$ production can only exceed the quenching capacities of the photoprotective system (and thus induce cell damage) when the photosynthetic pathways are inoperative, as is the case in senescent or highly-stressed cells (Nelson, 1993).

The problem of stratospheric ozone depletion has prompted numerous studies to examine the degradative effects of enhanced UV-B doses on lipids in autotrophic organisms (e.g. He and Häder, 2002; Nawkar et al., 2013). However, UV radiation does not hold a monopoly on photochemical damage in autotrophs. In fact, the presence of chlorophylls, which are very efficient photosensitizers (Foote, 1976; Knox and Dodge, 1985), means that numerous organic components of senescing autotrophs are susceptible to photodegradation by visible photosynthetically active radiation (PAR).

CHAPTER THREE

REACTION OF SINGLET OXYGEN WITH OLEFINS

Different types of reactions

Due to its strong electrophilic character and the lack of spin restriction (Dmitrieva et al., 2020), $^1O_2$ readily reacts with molecules containing double bonds. The reactions of $^1O_2$ with olefins can be collapsed into three classes, which are outlined in Fig. 4. The first class involves a [2 + 2] cycloaddition to the double bond affording a dioxetane. This reaction mainly takes place in the case of electron-rich or sterically-hindered double bonds (Frimer, 1979). The dioxetane thus formed, which is not very stable, is generally cleaved into two carbonyl fragments under the action of temperature or light. In the presence of allylic hydrogen atoms, a direct reaction of $^1O_2$ with the carbon–carbon double bond by a concerted "ene", also named Schenk-ene reaction addition, results in the formation of allylic hydroperoxides at each end of the original double bond while shifting it to the adjacent position (Frimer, 1979) (Fig. 4). In the case of conjugated dienes, the addition of $^1O_2$ produces cyclic peroxides (endoperoxides) (Clennan, 1991).

Numerous theoretical and experimental studies have investigated the mechanism involved in $^1O_2$-mediated allylic oxidation (e.g. Alberti and Orfanopoulos, 2008; Sheppard and Acevedo, 2009). It now seems likely that a perepoxide is a viable intermediate in the $^1O_2$ addition to simple alkenes (Alberti and Orfanopoulos, 2010). Note, however, that this is not a free radical process.

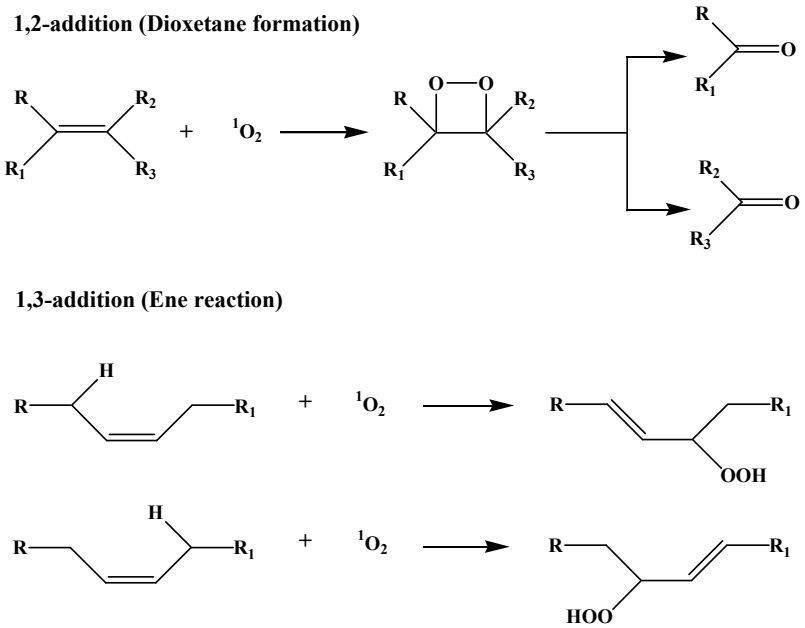
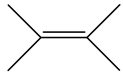
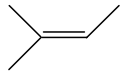
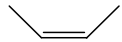
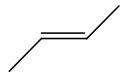


Figure 4. Main classes of $^1O_2$ reactions with olefins. Adapted from Frimer (1979).

It was previously observed that the rate of reaction of $^1O_2$ with olefins is controlled by entropy (ΔS) and thus by the degree of substitution and the configuration (*cis*- or *trans*-) of the double bond (Table 1) (Hurst et al., 1985). This means that terminal and *trans* olefins are weakly reactive to $^1O_2$.

Substrate	ΔS (e.u.)*	k (mol^{-1} s^{-1})	References
	-23	2.2×10^7	Hurst et al. (1985)
	-30	7.2×10^5	Hurst et al. (1985)
	-32	4.8×10^4	Hurst et al. (1985)
	-42	7.2×10^3	Hurst et al. (1985)
	-43	4.0×10^3	Hurst et al. (1985)
		2.3×10^2	Kopecky and Reich, 1965

* Entropy units

Table 1. Relative rate constants for the reaction of $^1O_2$ with isolated acyclic double bonds in solvents.

Features of the "ene" reaction

As seen in Chapter 5, the "ene" reaction plays a key role in the photosensitized oxidation of natural unsaturated lipids bearing allylic hydrogen atoms. A special feature of this reaction is its remarkable side specificity (named *cis* effect), where the more substituted side of trisubstituted double bonds is also the most highly reactive (Stratakis and Orfanopoulos, 2000; Griesbeck et al., 2003) (Fig. 5). Houk et al. (1981) attributed this specificity to lower rotational barriers in the more highly congested environment.

The "ene" reaction appears to be particularly sensitive to electronic effects and to a lesser extent to the steric hindrance when bulky substituents are present (Morales et al., 2012). Indeed, in some cases, the presence of a

bulky substituent may diminish reactivity by partially blocking the singlet oxygen attack on the double bond. This is notably the case in some polycyclic structures such as Δ^5-sterols and pentacyclic triterpenes (Beutner et al., 2000; Galeron et al., 2016a; 2016b).

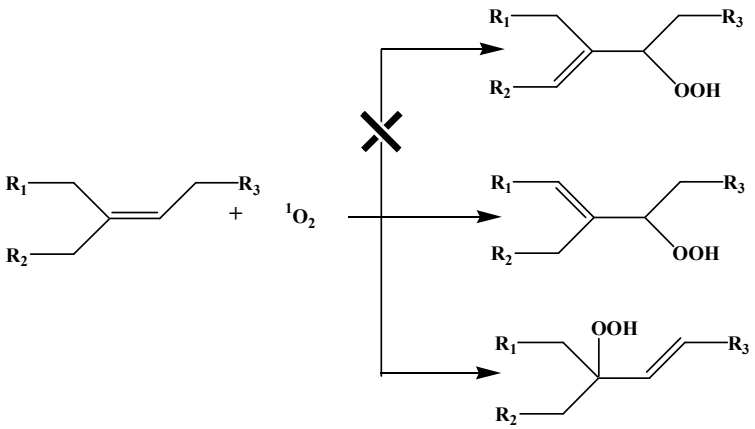


Figure 5. *Cis* effect in the reaction of $^1O_2$ with trisubstituted alkenes.

CHAPTER FOUR

DEGRADATION AND REARRANGEMENT OF ALLYLIC HYDROPEROXIDES UNDER ENVIRONMENTAL CONDITIONS

Allylic rearrangement

The rearrangement of allylic hydroperoxides (Fig. 6), which produces exclusively *trans* allyl products, has been extensively studied over the years (for reviews, see Porter et al., 1995; 2013). It is known to act on allylperoxyl radicals (Frimer, 1979), but the mechanism involved is still open to debate. The latest studies now seem to indicate that it proceeds through an oxygen-allyl radical complex (Porter, 2013). The intensity of this rearrangement is very sensitive to the hydrogen donor properties of the surrounding molecules (Porter et al., 1995). The rearrangement should be slow in biological membranes that are rich in polyunsaturated fatty acids (PUFAs), which are good hydrogen donors, but fast in membranes that are rich in monounsaturated fatty acids (MUFAs) or saturated fatty acids, which are poor hydrogen donors (Rontani et al., 2021a). The extent of the allylic rearrangement of the hydroperoxides present in environmental samples could therefore reflect the composition and ageing of the organisms present (Rontani et al., 2021a).

Heterolytic cleavage

Heterolysis of the hydroperoxide O–O bond leads to the formation of two carbonyl fragments (Hock cleavage). This proton-catalyzed cleavage is initiated by the migration of groups to positive oxygen, which then induces a series of skeletal changes (Fig. 6; Frimer, 1979). The migratory aptitude follows the order: cyclobutyl > aryl > vinyl > hydrogen > cyclopentyl or cyclohexyl >> alkyl (Frimer, 1979). In the particular case of allylic hydroperoxides, the migrating group will be the vinyl group and the resulting fragments will be two aldehydes (Fig. 6). These cleavages are

generally acid-catalyzed, but several of them have been reported to occur in the absence of any added acid (Turner and Herz, 1977; Frimer, 1979), and notably in senescent phytoplanktonic cells (Rontani, 1998) and seawater (Rontani et al., 2007c).

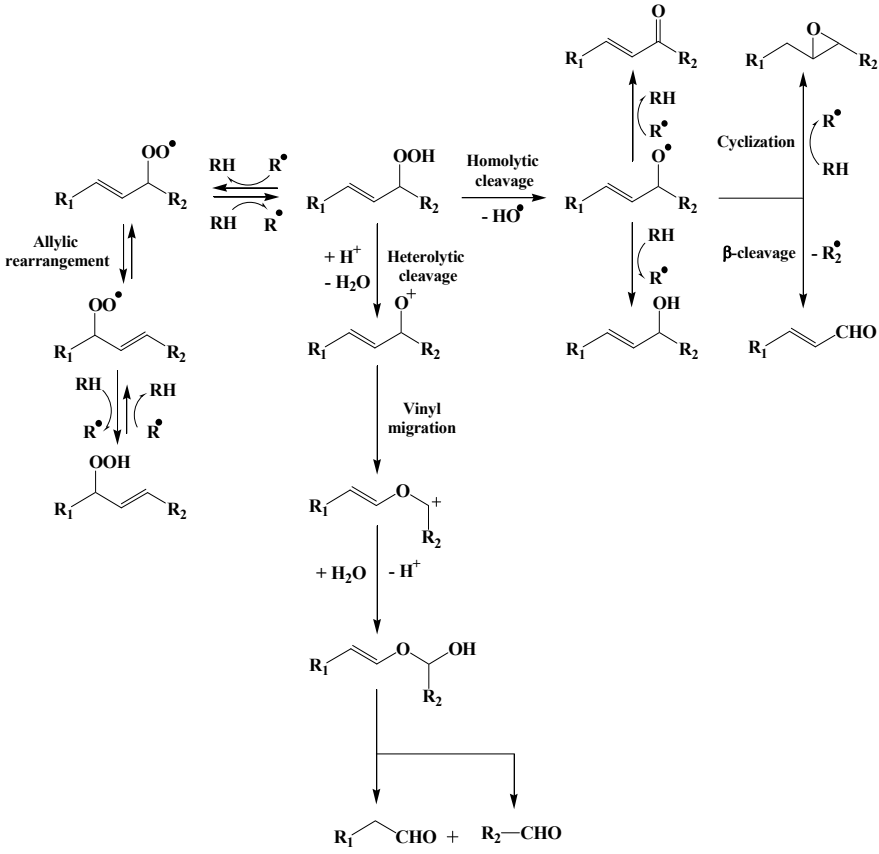


Figure 6. Rearrangement process and main degradation pathways of allylic hydroperoxides.

Homolytic cleavage

Homolysis of the O–O bond of hydroperoxides is induced by enzymes (e.g. lipoxygenases), redox-active metal ions undergoing one-electron

transfer, heat, or light (Schaich, 2005) (see Chapter 8 for further details). It leads to carbonyl (dehydration), alcohol (reduction), fragmentation (β-scission) or oxirane (radical cyclization) products (Fig. 6; Frimer, 1979). Note that β-scission acts mainly on the side of the hydroperoxy group affording an alkyl radical, since cleavage on the other side leads to the formation of an unstable and unlikely vinyl radical. Homolytic cleavage also produces several radicals, and notably the very reactive hydroxyl radical ($HO^{\bullet}$), which may be at the origin of the initiation of free-radical-mediated oxidation chain-reactions (See Chapter 8).

Further oxidation or condensation

Lipid hydroperoxides can also be oxidized to epoxyhydroperoxides, oxohydroperoxides, bihydroperoxides, cyclic peroxides, and bicyclic endoperoxides (Frankel, 1984), or else undergo condensation reactions forming dimers and polymers cross-linked through either peroxide or ether linkages and containing hydroperoxy, oxo- or hydroxy groups (Neff et al., 1988; Frankel, 1998; Pignitter and Somoza, 2012). These condensation reactions mainly act in the case of polyunsaturated substrates (Frankel, 1998).

Occurrence under natural environmental conditions

Despite these various degradation processes, literature nevertheless features several reports of intact allylic hydroperoxides in environmental samples or organisms, including phytoplankton (Orefice et al., 2015), bacteria (Petit et al., 2013), marine and terrestrial angiosperms (Rontani et al., 2014a; Rontani, 2019), particulate matter (Rontani et al., 2012; Galeron et al., 2015) and marine sediments (Rontani and Marchand, 2000). The unexpected stability of allylic hydroperoxides in sediments (where the residence time of organic matter may be relatively long) could result from protection of these compounds in well-silicified diatoms or higher plant debris (Rontani and Marchand, 2000).

CHAPTER FIVE

TYPE-II PHOTOSENSITIZED OXIDATION OF THE MAIN UNSATURATED LIPIDS OF AUTOTROPHIC ORGANISMS: SELECTION OF PROCESS SPECIFIC TRACERS

Chlorophylls

$^1O_2$ produced by chlorophyll photosensitization may act directly on the sensitizer, inducing chlorophyll degradation (photobleaching) (Fig. 3). In the literature, the photodegradation of chlorophylls has mainly been studied with respect to the tetrapyrrolic moiety of the molecule (Fig. 7), which is the more reactive. Although promising intermediate photoproducts were identified (Engel et al., 1991; Iturraspe et al., 1994), they are not sufficiently stable and specific to serve as specific tracers for the chlorophyll macrocycle photodegradation in the natural environment.

The trisubstituted double bond of the phytyl side-chain of chlorophyll-a or -b (Fig. 7) can also react with $^1O_2$. The rate of this reaction is 3–5 times slower than that of the tetrapyrrolic structure (Cuny et al., 1999; Christodoulou et al., 2010). Due to the well-known *Syn* selectivity of the "ene" reaction (*cis* effect) (Alberti and Orfanopoulos, 2006), this reaction affords photoproducts of structures **a** and **b** (Fig. 8), which are quantifiable after reduction and alkaline hydrolysis, respectively, in the form of 6,10,14-trimethylpentadecan-2-ol and 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol (more concisely named 'phytyldiol'; Fig. 8; Rontani et al., 1994).

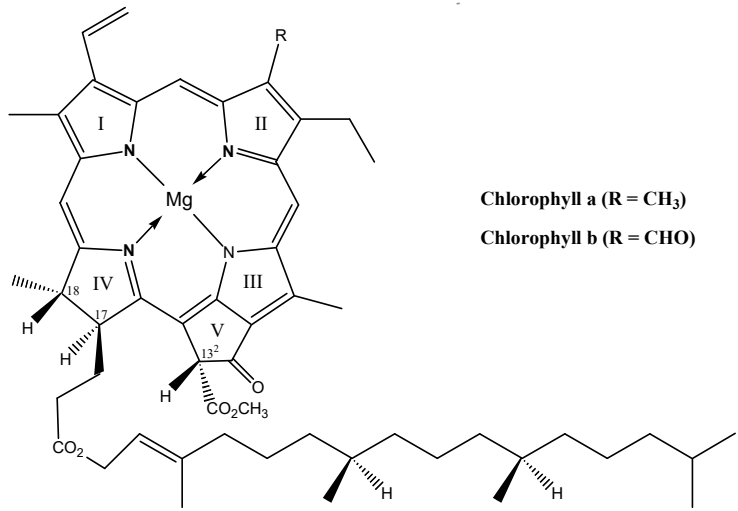


Figure 7. Structures of chlorophyll-a and chlorophyll-b.

Phytyldiol appeared to be relatively stable under environmental conditions (Rontani et al., 1996b) and yet highly specific. This specificity results from the strong preference for *Syn* ene addition of $^1O_2$ at the disubstituted side of the double bond (*cis* effect; Alberti and Orfanopoulos, 2006; Fig. 8). Phytyldiol compound was thus proposed as biogeochemical marker of chlorophyll photodegradation in the natural environment (Cuny and Rontani, 1999).

The molar ratio phytyldiol:phytol (defined as chlorophyll phytyl side-chain photodegradation index, or 'CPPI') was proposed as an estimator of the extent of photooxidation of chlorophylls possessing a phytyl side-chain in natural samples, using the empirical Eq. 1 (Cuny et al., 1999).

$$(\text{chlorophyll photooxidation } \% = (1 - [\text{CPPI}+1]^{-18.5}) \times 100 \qquad (1)$$

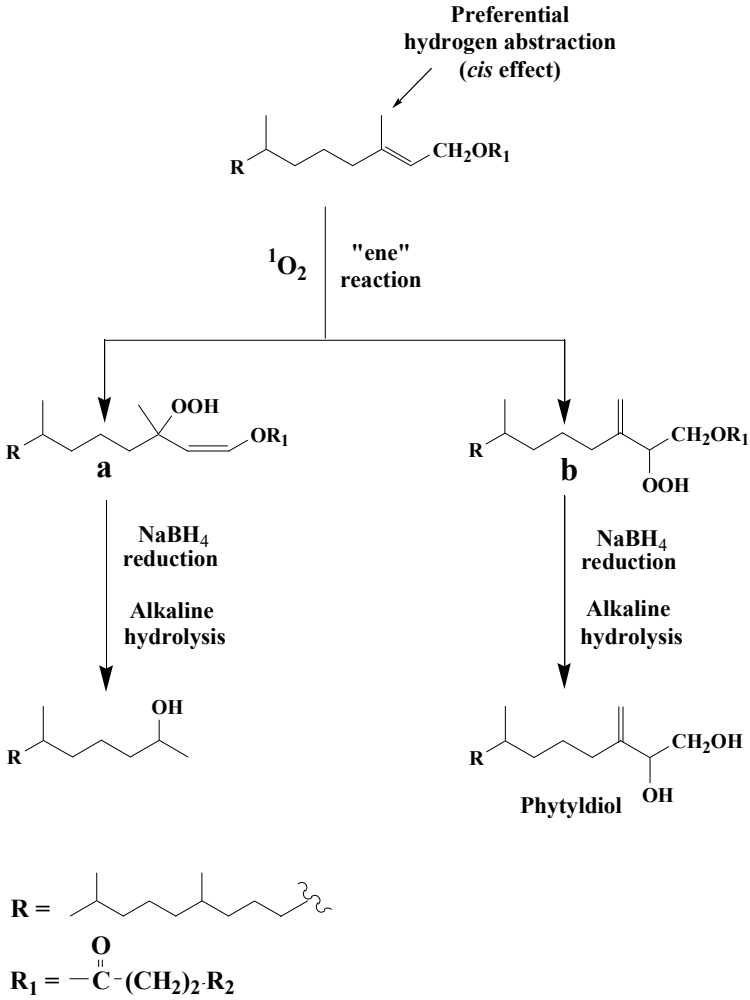


Figure 8. Type-II photosensitized oxidation of the chlorophyll phytyl side-chain and subsequent reduction and alkaline hydrolysis of the resulting hydroperoxides. Adapted from Rontani and Belt (2020).

Carotenoids

Carotenoids are tetraterpenes containing a conjugated system of double bonds with delocalized π-electrons (Fig. 9). As seen in Chapter 2, these constituents of thylakoid membranes play special roles in the protection of tissues against damage caused by light and oxygen (Britton, 1995). The physical quenching pathway described in Eqs. 2 and 3 is thought to be the most favoured mechanism for carotenoid and $^1O_2$ interactions (Boon et al, 2010), but carotenoids can also quench $^1O_2$ by chemical reaction (scavenging) (Eq. 4).

$$Car + {}^1O_2 \rightarrow {}^3Car^* + {}^3O_2 \quad (2)$$

$${}^3Car^* \rightarrow Car + Heat \quad (3)$$

$$Car + {}^1O_2 \rightarrow \text{Carotenoid oxidation products} \quad (4)$$

The reaction of $^1O_2$ with carotenoids in biological membranes is not well understood (Boon et al., 2010). In the case of β-carotene, which appeared to be a preferred *in vivo* target of $^1O_2$ compared to xanthophylls (Ramel et al., 2012), it seems that β-carotene-5,8-endoperoxide (Fig. 9) was the primary oxidation product formed (Fiedor et al., 2005). Further degradation of this compound affords several aldehydes and ketones (β-ionone, β-apo-14′-carotenal, β-apo-10′-carotenal, β-apo-8′-carotenal, and more; Stratton et al., 1993; Yamauchi et al., 1998; Ramel et al., 2012). While β-carotene-5,8-endoperoxide makes a useful early signal of $^1O_2$ production in plant leaves (Ramel et al., 2012), it is clearly not stable enough to serve as a viable environmental tracer. Unfortunately, most of the shorter oxidation products resulting from the degradation of this endoperoxide can be also produced by enzymatic and autoxidative degradation of β-carotene (Boon et al., 2010) and thus do not make unequivocal indicators of type-II photosensitized oxidation of carotenoids in phototrophic organisms.