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Review

Phytol degradation products as biogeochemical tracers in aquatic environments

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Abstract

Phytol (the ester-linked side-chain of chlorophyll-*a*) is perhaps the most studied biomarker of those found in modern aquatic environments. This paper reviews recent studies of phytol degradation and provides an update on more classical studies. After a discussion of the biosynthesis and isotopic fractionation of phytol, we examine the different biotic and abiotic processes which may play a significant role during the diagenesis of this widely distributed isoprenoid alcohol: (i) photodegradation of the chlorophyll phytyl chain in senescent phytoplanktonic cells, (ii) hydroperoxide-induced oxidation of the chlorophyll phytyl chain in senescent phytoplanktonic cells, (iii) degradation of the chlorophyll phytyl chain during marine invertebrate feeding, (iv) aerobic and anaerobic biodegradation of phytol, (v) clay-catalysed degradative processes, and (vi) sulfur incorporation. Emphasis is given to the mechanisms of these processes and to the structures of the products formed. We conclude with some remarks on the potential and constraints of the main phytol degradation products as biogeochemical tracers and highlight some areas requiring further work.

1. Introduction

Acyclic isoprenoids are compounds formed by condensation of isoprene skeletal units. An isoprene unit is a 5-carbon unit (2-methylbutyl), derived from isopentenyl diphosphate (IDP or IPP in the older literature), which can be formed via two alternative pathways, the mevalonate (MVA) and the deoxyxylulose/methylerythritol (MEP) pathway (Flesch and Rohmer, 1988; Lichtenthaler et al., 1997); (see Section 3). Most naturally occurring acyclic isoprenoids have chain-lengths of C_{10}, C_{15}, C_{20}, C_{30} and C_{40} (Volkman and Maxwell, 1986), although polyisoprenoids with longer chain-lengths are known. Some compounds diagenetically related to acyclic isoprenoid precursors [e.g. 2,6,10,14-tetramethylpentadecane (pristane) and 6,10,14-trimethylpentadecan-2-one], which do not contain multiples of 5 carbon atoms and hence do not fit the strict definition of an isoprenoid structure, are generally considered as isoprenoids by organic geochemists.

Naturally occurring acyclic isoprenoids exhibit many functionalities and degrees of unsaturation. This gives rise to a range of compound classes including alcohols, ketones, carboxylic acids, hydrocarbons, ethers, esters and other derivatives (Volkman and Maxwell, 1986). There are also variations in the way isoprene skeletal units are linked together, which may be "head to head", "head to tail" or "tail to tail", with more than one mode occurring in some compounds. Isoprenoid compounds are well suited as biological markers since they are often abundant and widely distributed. Moreover, the relatively stable isoprane skeletal unit is readily identified and permits these compounds to be used as tracers over long periods of geological time (Volkman and Maxwell, 1986).

Phytol (3,7,11,15-tetramethylhexadec-2*E*-enol) (the ester-linked side-chain of chlorophyll-*a*), which is

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probably the most abundant acyclic isoprenoid compound in the biosphere, is generally considered to be the major source of isoprenoids with 20 or fewer carbon atoms in geological samples. However, other sources have been proposed. For example, Azevedo et al. (2001) attributed C_{14} and C_{19} isoprenoid methyl ketones in the tasmanite oil shale to oxidation of double bonds in the side chain of a tricyclooctaprenol or the corresponding unsaturated hydrocarbon, tricyclooctaprenene. Carotenoid pigments (Volkman and Maxwell, 1986), tocopherols (Goossens et al., 1984) and methyltrimethyltridecylchromans (MTTCs; Li et al., 1995) are other potential sources of isoprenoids in sediments. An unusual source of C_{20} isoprenoids is suggested by the report by Kobayashi et al. (2000) that the primary electron acceptor of green sulfur bacteria, bacteriochlorophyll (BChl) 663 has the chlorophyll-*a* macrocycle esterified to 2,6-phytadienol instead of phytol. These bacteria are found at the interface between oxic and anoxic waters in environments where the anoxic waters are within the photic zone.

The possibility that bacteria can be sources of many acyclic isoprenoids was raised by the isolation of pristane, 2,6,10,14-tetramethylhexadecane (phytane) and complex lipids containing the dihydrophytyl (phytanyl) moiety from halophilic bacteria (Kates et al., 1966; Han and Calvin, 1969). Diphytanylglycerol diether (archaeol) is the dominant membrane lipid in most methanogens and all extreme halophiles (Kates, 1993; Koga et al., 1993). In contrast, the cell membranes of hyperthermophilic archaea and a few methanogens contain caldarchaeol, a dibiphytanyldiglycerol tetraether (Tornabene and Langworthy, 1978; Sprott, 1992) which occurs as part of a variety of polar lipids (e.g. Sprott et al., 1997). Considering the relative stability of such ether lipids (after loss of the polar head groups) and the ubiquity and abundance of archaea in marine habitats, these organisms must be considered as significant sources of acyclic isoprenoids in marine sediments (DeLong et al., 1998).

Phytol and its degradation products have been frequently used as biomarkers of chemical and biological processes (Volkman and Maxwell, 1986). However, some recent studies of phytol degradation processes plus the recognition that many acyclic isoprenoids in sediments are derived from sources other than phytol, suggest a lack of specificity for many common biomarkers. The present paper reviews recent studies of the biotic and abiotic degradation of phytol. In the light of these results, the potential and constraints of the main phytol degradation products as biomarkers are reconsidered.

2. Sources of phytol

Phytol, as an integral part of chlorophyll-*a*, is ubiquitous in the marine environment (e.g. Boon et al., 1975; Johns et al., 1980; Sun et al., 1998). Chlorophyll-*b*, chlorophyll-*d* and bacteriochlorophyll-*a* constitute other minor contributors of phytol (Gillan and Johns, 1980). It also occurs in the chlorophyll-*c* of some of the haptophyte microalgae that are abundant in some marine environments (e.g. Zapata and Garrido, 1997). There are reports of phytol esterified to fatty acids in leaves (Csupor, 1971; Suga and Aoki, 1974) and mosses (Gellerman et al., 1975). Isophytol has been reported as a minor constituent of seagrass *Zostera marina* (Kawasaki et al., 1998) and of some plants (Munoz et al., 1998).

Some marine dinoflagellates contain phytol esterified to saturated and unsaturated fatty acids (Withers and Nevenzel, 1977). Since dinoflagellates can be major sources of lipids in sediments (e.g. de Leeuw et al., 1983), these phytyl esters could be an additional source of phytol in some environments (Volkman and Maxwell, 1986). Phytyl esters have been also detected in krill (Sargent and Falk-Petersen, 1981). Other possible sources of phytol in the environment include bacterial phospholipids and glycerol derivatives (Kates et al., 1965; Nissenbaum et al., 1972), esters present in mammals (Su and Schmidt, 1975) and vitamin K (Hansen, 1980).

The hydrolysis of chlorophyll-*a* to free phytol and chlorophyllide-*a* or pheophorbide-*a* in marine and lacustrine waters is thought to be mainly associated with herbivore grazing (Daley, 1973; Shuman and Lorenzen, 1975; Ma and Dolphin, 1999), or phytoplanktonic (notably diatom) senescence (Jeffrey and Hallegraeff, 1987). In sediments, the early-stage degradation of chlorophyll-*a* is generally assumed to involve dechelation and hydrolysis to yield respectively pheophytin-*a* and pheophorbide-*a* plus free phytol (Didyk et al., 1978). However, Johns et al. (1980) showed that degradation of chlorophyll-*a* with depth in a contemporary temperate intertidal sediment involved conversion to non-chlorin colourless phytyl esters prior to incorporation of the phytol into a bound fraction; at depth this bound phytol was hydrolysed to free phytol. In sediments, hydrolysis of the phytyl chain in chlorophyll can result from macrofaunal digestion (Hawkins et al., 1986; Abele-Oeschger and Theede, 1991) and clay-catalysed reactions (Rontani and Grossi, 1995).

3. Biosynthesis of phytol and isotopic fractionation

Acetogenic lipids are formed from acetyl-CoA, where the C_2 unit is produced by oxidative decarboxylation of pyruvate. In contrast, the biosynthesis of the universal C_5 building block of isoprenoids, isopentenyl diphosphate (IDP), proceeds via two distinct pathways. In the classical Bloch-Lynen pathway, IDP is formed from three molecules of acetyl-CoA via mevalonate (the MVA pathway). This mechanism was thought to be universal, but research by the groups of Arigoni and

Rohmer identified a different pathway (now called the DOXP/MEP or simply the MEP pathway) in which IDP is formed from pyruvate and glyceraldehyde (Rohmer et al., 1993; 1996; Rohdich et al., 2001). In this pathway, the C_5 product is formed by reaction of thiamine-activated acetaldehyde (from pyruvate oxidation) which attacks the carbonyl carbon of glyceraldehyde-3-phosphate. The product is the linear molecule 1-deoxyxylulose 5-phosphate (DOXP) which internally rearranges to give 2-C-methylerythritol 4-phosphate (MEP) and then ultimately IDP. The biosynthesis of plastidic isoprenoids by this MEP pathway appears to be widely distributed in photosynthetic organisms (Bach, 1995; Lichtenthaler et al., 1997; Schwender et al., 1997; reviewed by Rohmer, 1999). It is also widespread in bacteria, but not present in fungi, animals or man (Rosa Putra et al., 1998). No evidence has been found for the simultaneous occurrence of both MVA and MEP pathways in eubacteria (Rosa Putra et al., 1998). Note that leucine may also be incorporated intact into sterols without first being broken down to acetyl-CoA through the intermediary formation of HMG-CoA and conversion to MVA (see Ginger et al., 2001 for leading references). In plants and animals this occurs in the mitochondria.

In higher plants, sterols in the cytosol are formed via acetate and mevalonate (i.e. the MVA pathway), whereas in the plastids the isoprenoids are formed by the MEP pathway (Disch et al., 1998; Schwender et al., 2001). The pathways which operate in algae are only now becoming clearer, with a proliferation of papers in the late 1990s (Table 1). Macroalgae from the Charophyceae, being close relatives of land plants, use the same IDP biosynthetic pathway as higher plants (i.e. sterols are formed via MVA and the phytol-moiety of chlorophylls via the MEP pathway), but microalgae from the Chlorophyceae exclusively use the MEP pathway (Schwender et al., 2001). In the red alga *Cyanidium caldarium* and in the chrysophyte *Ochromonas danica* sterols are formed via the MVA route, whereas chloroplast isoprenoids such as phytol are synthesized via the MEP route. In contrast, the euglenophyte *Euglena gracilis* synthesizes phytol via the MVA route. In the cyanobacterium *Synechocystis* PCC 6714, phytol and β-carotene were shown to have the typical labelling pattern derived from the MEP route (Disch et al., 1998). Cvejic and Rohmer (2000) showed that CO_2 was the main source for phytol biosynthesis in the chloroplasts via the MEP pathway in the two diatoms *Phaeodactylum tricornutum* and *Nitzschia ovalis* grown in mixotrophic conditions under low light. This compartmentalisation of isoprenoid biosynthesis is the same as in higher plants, the red alga *Porphyridium cruentum* and the chrysophyte *O. danica* (Table 1).

Table 1
Biosynthetic pathways of sterols and phytol in different photosynthetic organisms

Organisms	Sterols	Phytol
Higher plants	MVA[a]	DOXP/MEP[b]
Charophyceae	MVA	DOXP/MEP
Chlorophyceae	DOXP/MEP	DOXP/MEP
Cyanidium caldarium	MVA	DOXP/MEP
Ochromonas danica	MVA	DOXP/MEP
Euglena gracilis		MVA
Synechocystis PCC 6714		DOXP/MEP
Phaeodactylum tricornutum		DOXP/MEP
Nitzschia ovalis		DOXP/MEP
Porphyridium cruentum		DOXP/MEP

[a] Mevalonate pathway.
[b] 1-Deoxyxylulose 5-phosphate/2-C-methylerythritol 4-phosphate pathway.

The occurrence of distinct pathways for isoprenoid biosynthesis may give rise to different carbon, hydrogen and oxygen isotope patterns in the products. Additional effects on isotope fractionation can be due to: (i) synthesis of different lipids in different compartments in the cell; (ii) the presence of active carbon uptake in some species; (iii) multiple uses for some of the intermediate compounds and (iv) translocation of the products. Sakata et al. (1997) noted that lipid biomarkers derived from eukaryotes can be depleted relative to dissolved CO_2 by as little as 8‰ or by as much as 32‰ and perhaps greater. While isotope analyses may have potential for discriminating different sources of lipids, the interpretation of such data is not necessarily straightforward. As an example, Sakata et al. (1997) observed that isoprenoid compounds in a cyanobacterium produced by the MEP pathway had, by a combination of effects, similar isotope values to those of compounds expected from the MVA pathway.

A clear picture of isotope differences between phytol and acetogenic and other isoprenoid lipids in different organisms is yet to emerge, but useful sets of data are now available. For example, Collister et al. (1994) showed that phytol isolated from C_3 plants was, on average, 1.5‰ enriched in ^{13}C (range 0.9–2.0‰) relative to the average $\delta^{13}C$ values for long-chain wax *n*-alkanes. In the cyanobacterium *Synechocystis* UTEX 2470, Sakata et al. (1997) found that polyisoprenoid lipids fell into two isotopic groups, with phytol, diplopterol, and diploptene depleted by 6.4–6.9‰ and bishomohopanol (produced from bacteriohopanepolyol) depleted by 8.4‰ relative to average biomass. Schouten et al. (1998) observed that phytol was consistently enriched in ^{13}C by 2–5‰ compared with the C_{16} fatty acid (an acetogenic lipid) in a range of microalgae. The sterols were enriched in ^{13}C by 0—8‰ compared to the C_{16} fatty acid, indicating a diversity of isotope fractionation within the isoprenoid lipids. A detailed study of isotope fractionation in the haptophyte *Emiliania huxleyi* by Riebesell et al. (2000) showed that, relative to biomass, individual fatty acids were depleted in

^{13}C by 2.3–4.1‰, phytol was depleted by 1.9‰, and the major sterol 24-methylcholesta-5,22*E*-dien-3β-ol was depleted by 8.5‰.

The ability to measure the isotope compositions of the wide range of compounds found in Recent sediments using isotope-ratio capillary gas chromatography–mass spectrometry (irm–GC–MS), has considerably expanded our abilities to assign the origins of compounds in sediments. A recent example is the work by Schouten et al. (2001) who examined a range of acetogenic and isoprenoid lipids in sediments from Ace Lake, Antarctica. Saturated and unsaturated 2,6,10,15,19-pentamethylicosane, archaeol ($\delta^{13}C$ of −17.1‰) and sn2-hydroxyarchaeol were attributed to methanogenic archaea. Chlorobactene and isorenieratene derived from the green- and brown-coloured strains of the green sulfur bacteria (Chlorobiaceae) were ^{13}C-enriched ($\delta^{13}C$ of −18.1 to −19.7‰). Phytenes ($\delta^{13}C$ of −29.5 to −32.1‰) were derived from algal and cyanobacterial chlorophylls, whereas phytane ($\delta^{13}C$ of −19.6 to –24.0‰) was likely derived from species within the archaea. The most ^{13}C-depleted compounds (ca.−55‰) were the cyclic polyisoprenoids 4-methyl-5α-cholest-8(14)-en-3β-ol and co-occurring 4-methylsteradienes derived from methanotrophic bacteria. Lipids of photoautotrophic origin were relatively depleted (ca.−28 to −36‰), whilst archaeal biomarkers were relatively enriched in ^{13}C (ca. −17 to −25‰).

4. Diagenesis of phytol in the marine environment

4.1. Photodegradation of the chlorophyll phytyl chain in senescent phytoplanktonic cells

To date, due to the lack of adequate tracers, the role played by photochemical processes during the degradation of phytoplankton in the euphotic layer of the oceans has been almost completely ignored. To address possible effects due to stratospheric ozone depletion, some studies have recently examined the degradative effects of enhanced UV-B doses on phytoplanktonic lipids (e.g. Skerratt et al., 1998). However, photochemical damage in phytoplanktonic cells is not restricted to UV radiation (Nelson, 1993). In fact, due to the presence of chlorophylls (which are efficient photosensitizers: Foote, 1976), many of the organic components of phytoplankton are susceptible to being photodegraded during senescence by photosynthetically active radiation (PAR; Rontani et al., 1998; Rontani, 2001).

4.1.1. Induction of photodegradative processes in phototrophic organisms

In healthy phytoplankton cells the primary route for energy conversion from the excited chlorophyll singlet state (^1Chl) is via the fast photochemical reactions of photosynthesis (Foote et al., 1970). In dead phytoplanktonic cells this pathway is obviously not functional; thus accelerated production of the longer living triplet state (^3Chl; by intersystem crossing) and of toxic oxygen species (singlet oxygen, hydrogen peroxide, superoxide ion and hydroxyl radical) by reaction of ^3Chl with ground state oxygen (Rontani, 1999a) might be expected. The rate of formation of these potentially damaging chemicals can then exceed the quenching capacity of the photoprotective system of the cells and photodegradation can occur (Merzlyak and Hendry, 1994). Chlorophylls would tend to remain associated with other hydrophobic cellular compounds, such as membrane lipids, in phytodetritus (Nelson, 1993). The photooxidative effect of chlorophyll sensitization can be strongly amplified within such a micro-environment. Moreover, the lifetime of singlet oxygen ($^1O_2$) produced from sensitizers in a lipid-rich hydrophobic microenvironment could be longer and its potential diffusive distance greater, than if produced by sensitizers in an aqueous solution (Suwa et al., 1977).

4.1.2. Visible light-dependent degradation of chlorophylls in senescent phototrophic organisms

Irradiation of dead phytoplanktonic cells by the light normally used for growth results in rapid degradation of chlorophylls (Nelson, 1993; Rontani et al., 1995). 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 recent progress regarding intermediary photoproducts, no stable and specific markers for the chlorophyll macrocycle photodegradation have been characterised.

The isoprenoid phytyl side-chain of chlorophyll is also sensitive to photochemical processes. In fact, in phytodetritus the visible light-dependent degradation rates were only 3–4 times higher for the chlorophyll tetrapyrrolic structure than for the phytyl side-chain (Cuny et al., 1999). It was previously demonstrated that the type II (i.e. involving $^1O_2$) photosensitized oxidation of the phytol moiety of chlorophyll-*a* or-*b* leads to the production of photoproducts of structures **a** and **b** (Fig. 1), quantifiable after alkaline hydrolysis respectively in the form of 6,10,14-trimethylpentadecan-2-one (phytone) and 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol (phytyldiol; Fig. 1; Rontani et al., 1994). Small amounts of 4,8,12-trimethyltridecan-1-ol can also be formed by heterolytic cleavage (Frimer, 1979) of photoproducts of structure **b**. In contrast, type I (i.e. involving oxy free radicals) photooxidation of the phytyl chain affords mainly *Z* and *E* 3,7,11,15-tetramethyl-2,3-epoxyhexadecan-1-ols, 3,7,11,15-tetramethylhexadecan-1,2,3-triol and phytone after subsequent alkaline hydrolysis (Fig. 1; Rontani and Aubert, 1994). Analysis of isoprenoid photoproducts of chlorophyll after irradiation

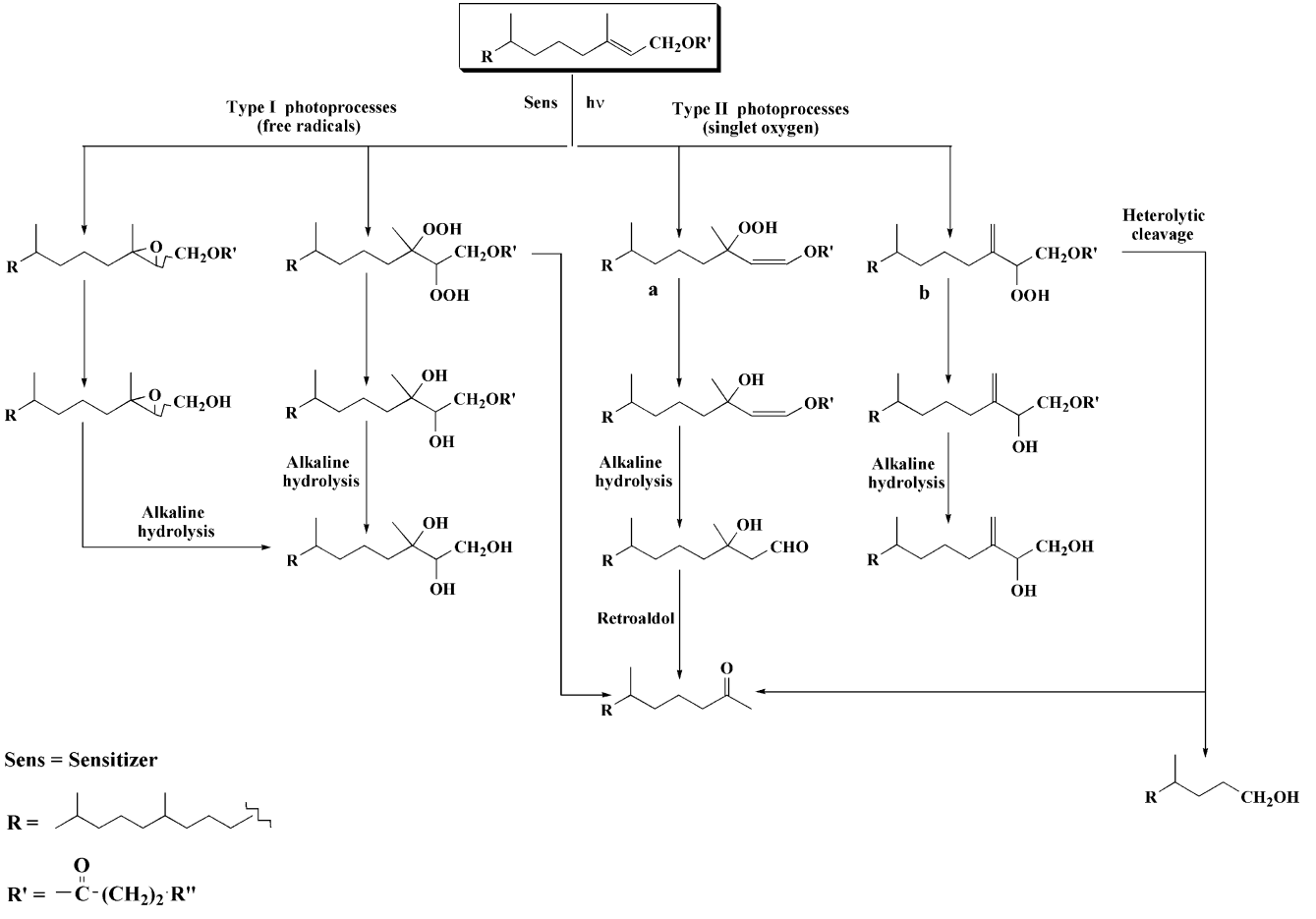


Fig. 1. Photooxidation of chlorophyll phytyl chain and reactions of photoproducts during alkaline hydrolysis (adapted from Rontani, 2001).

of different dead phytoplanktonic cells by visible light clearly established that the photodegradation of the chlorophyll phytyl side-chain in phytodetritus involved mainly $^1O_2$ and to a small degree free radicals (Rontani et al., 1995; 1998).

Using several phytoplanktonic strains, Cuny et al. (1999) showed that the major part of the photooxidized phytyl chain was recovered in the form of phytone and phytyldiol. However, this was not the case after irradiation of broken cells of the diatom *Phaeodactylum tricornutum* in which, phytone and phytyldiol represented only 58% of the amount of phytol photodegraded (Rontani et al., 2000). The other 42% loss of phytyl chain was attributed to the involvement of photooxidative cross-linking of the intact and/or photooxidized phytyl chain with itself and/or with other phytoplankton lipids. The hydrophobic microenvironment of phytodetritus should provide high, but localised, concentrations of unsaturated lipids and visible light-absorbing photosensitizers which would favour such processes (Nelson, 1993). It was suggested that photochemical condensations must be favoured by the presence of a relatively high proportion of free phytol in killed cells of *P. tricornutum* (Rontani et al., 1998).

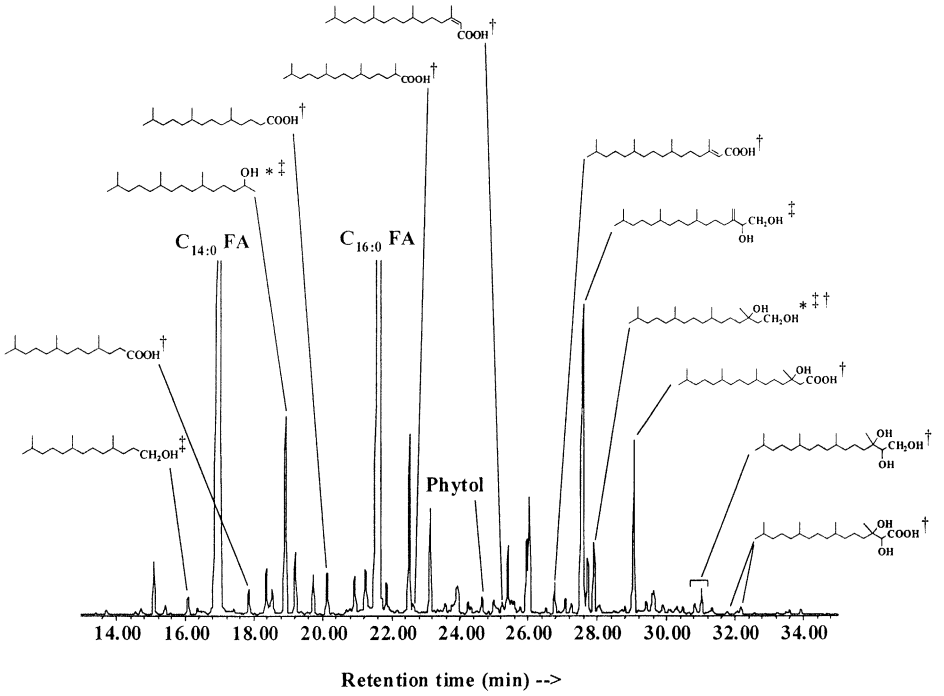
It was previously demonstrated that the pathways of photodegradation of the phytyl chain do not differ significantly among algae (Rontani, 2001) and higher plants (Rontani et al., 1996a). In the marine medium, it is generally considered that approximately 95% of the total particulate organic matter consists of particles <20 μm possessing settling velocities less than 1 m day^{-1} (McCave, 1975). Consequently, most of the organic particulate matter has a considerably long residence time within the euphotic zone. Assuming a surface irradiance of 60 mol of photons m^{-2} day^{-1} (PAR; a value representative of mid-latitude waters under a clear summer sky; Nelson, 1993), it is apparent that the phytoplanktonic chlorophyll phytyl chain (half-life doses ranging from 4 to 24 mol of photons m^{-2}; Rontani, 2001) could be significantly photodegraded in the upper portion of the euphotic zone during senescence. A non-negligible proportion of phytol could be photodegraded even near the lower limit of the euphotic zone (at a daily irradiance of 0.6 mol of photons m^{-2}). However, under such low light conditions, particle sinking or biologically mediated degradation may have a greater impact on lipid concentration than photooxidation processes (Vernet and Mitchell, 1990; Nelson, 1993).

4.2. Hydroperoxide-induced aerobic and anaerobic oxidation of the chlorophyll phytyl chain in senescent phytoplanktonic cells

In phytodetritus, the photodegradation of the phytyl chain stops when all the photosensitizers (i.e. the tetrapyrrole moiety of chlorophylls or phaeopigments) have been destroyed or after the cells settle out of the euphotic zone. At this time, significant amounts of hydroperoxides could be produced by photochemical reactions. Though these compounds are generally considered to be labile, it was recently demonstrated that significant amounts of hydroperoxides deriving from phytoplanktonic sterols (Rontani and Marchand, 2000) and monounsaturated fatty acids (Marchand and Rontani, 2001) were present in particulate matter and Recent sediment samples.

Under mild conditions very many organic compounds react with molecular oxygen to give different oxygen-containing species such as peroxides, hydroperoxides, alcohols, ketones, aldehydes and acids (Fossey et al., 1995). These processes, which are called autoxidation, proceed by a radical chain mechanism and act mainly on compounds possessing hydrogens whose bond energies are relatively low (e.g. allylic, tertiary or α to oxygen). It is well known that autoxidation is enhanced by the presence of a peroxide or hydroperoxide initiator (Fossey et al., 1995). In senescent phytoplanktonic cells, photochemically-produced hydroperoxides could thus induce autoxidation of unsaturated lipid components such as phytol. This assertion is supported by the detection of several isoprenoid compounds deriving from the autoxidation of phytol and of its photoproducts after dark incubation of prior-irradiated senescent cells of the diatom *Skeletonema costatum* (Fig. 2). It was previously demonstrated that autoxidation of the chlorophyll phytyl chain involves mainly the addition of hydroxyl or peroxyl radicals to the double bond and to a lesser extent abstraction of allylic hydrogen atoms (Rontani and Aubert, 1994), in a similar fashion to type I photoprocesses (Fig. 1). This is in good agreement with the results of Huyser and Johnson (1968), who demonstrated that esterification of allylic alcohols strongly favours addition processes rather than abstraction. The presence of significant amounts of phytol oxidation products deriving from abstraction processes (e.g. phytenic and 4,8,12-trimethyltridecanoic acids) in senescent cells of *S. costatum* can be attributed to the well documented high chlorophyllase activity of senescent diatoms (Jeffrey and Hallegraeff, 1987) catalysing the hydrolysis of chlorophyll to free phytol and chlorophyllide. The main oxy-free radical reactions deriving from allylic hydrogen abstraction on free phytol are shown in Fig. 3. Autooxidative processes appear to be a source of numerous acyclic isoprenoids in the marine environment, but more work is required to elucidate the mechanisms of these complex processes.

The effects of hydroperoxides need not be limited to aerobic marine zones. Indeed, the formation of isomeric 5,6-epoxy-24-ethylcholestan-3β-ols in anoxic sediments was recently attributed to the oxidation of 24-ethylcholest-5-en-3β-ol by hydroperoxides in the



† Oxy-free radical oxidation products of chlorophyll phytyl chain

‡ Type II photoproducts of chlorophyll phytyl chain (see Fig. 1)

* Compounds formed by reduction of the corresponding carbonyl compounds during $NaBH_4$ treatment

Fig. 2. Total ion chromatogram of $NaBH_4$-reduced total lipid extract showing the production of several isoprenoid oxidation products after dark incubation of first irradiated killed cells of *Skeletonema costatum*.

absence of molecular oxygen (Rontani and Marchand, 2000). These results suggest that some hydroperoxides can be sufficiently stable in sediments to play a role in the degradation of unsaturated organic matter under anoxic conditions (Mouzdahir et al., 2001). The actual extent of this phenomenon on the chlorophyll phytyl chain remains to be determined.

4.3. Degradation of the chlorophyll phytyl chain during marine invertebrate feeding

It is well recognised that many different marine invertebrates contribute to the flux of lipids through the water columns of the oceans (Bradshaw and Eglinton, 1993). To provide detailed information concerning invertebrate processing of dietary lipids, a limited number of laboratory experiments have been carried out (e.g. Avigan and Blumer, 1968; Prahl et al., 1984a,b; Harvey et al., 1987; Bradshaw et al., 1990a). Most of these experiments have concentrated on pelagic crustaceans (cited as important mediators in the flux of organic carbon; Corner et al., 1986 and references therein), particularly the copepod *Calanus helgolandicus* (Prahl et al., 1984a,b; Harvey et al., 1987) which is important in North Sea waters. Some experiments have used benthic animals, extending the investigation to groups such as molluscs (Bradshaw et al., 1991) and annelids (Bradshaw et al., 1990b). However, the range of predator and prey species studied is still quite limited.

Pelagic crustaceans assimilate the chlorophyll phytyl chain when feeding herbivorously (Prahl et al., 1984a;b; Harvey et al., 1987; Bradshaw et al, 1990a). Benthic invertebrates such as molluscs and annelids are also capable of removing this compound from food while feeding both herbivorously and coprophagously (Bradshaw et al., 1990b, 1991). Any phytol remaining in the faeces of pelagic zooplankton or benthic invertebrates will be quickly removed on subsequent reprocessing of the faecal material (Bradshaw and Eglinton, 1993).

Several phytol degradation products have been identified during these experiments, including pristane, isomeric pristenes, isomeric phytadienes, dihydrophytol and phytanic, pristanic, 4,8,12-trimethyltridecanoic and isomeric phytenic acids.

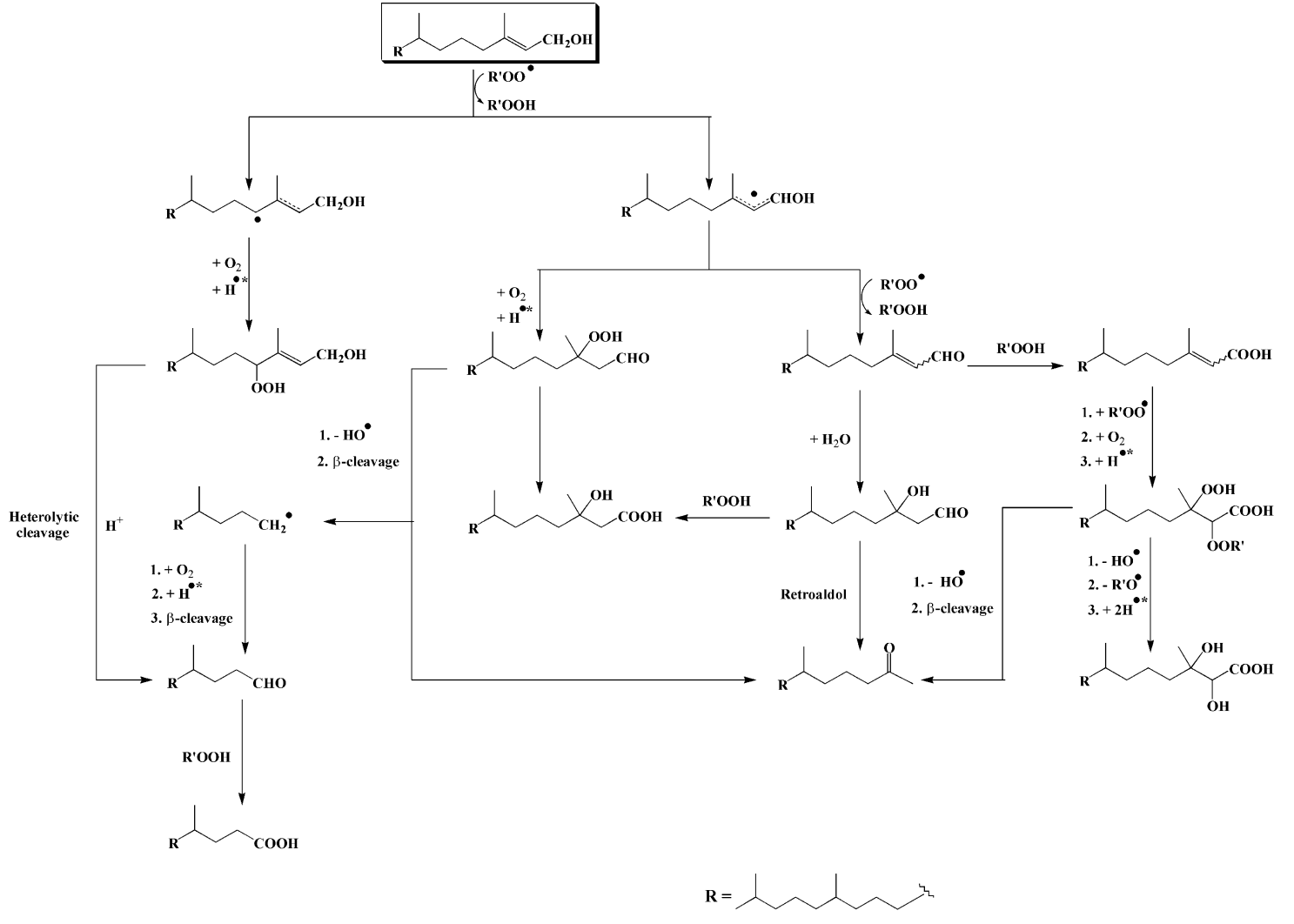


Fig. 3. Autoxidation of free phytol induced by hydrogen abstraction.

2,6,10,14-Tetramethylpentadecane (pristane) is found in many marine organisms and it is highly enriched in calanoid copepods (Blumer et al., 1963; 1964). In addition, 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) is also an important lipid in species of *Calanus* (Blumer and Cooper, 1967; Avigan and Blumer, 1968; Prahl et al., 1984b). Avigan and Blumer (1968) investigated the product–precursor relation between pristane and phytanic acid and dietary phytol in two *Calanus* species using uniformly labelled ^{14}C phytol as a tracer. These authors proposed a pathway involving decarboxylation of phytanic acid for the conversion of phytol to pristane in these organisms (Fig. 4). The detection of significant amounts of 3,7,11,15-tetramethylhexadecanol (dihydrophytol) in faecal pellets of *C. helgolandicus* (Prahl et al., 1984a) provided further support for this metabolic route. However, is biological reduction of phytol to dihydrophytol mediated microbially by gut flora or enzymatically by the animal? Evidence that the animal is responsible was obtained from experiments with *C. helgolandicus* treated with antibiotics in which dihydrophytol was still detected in the faecal pellets (Prahl et al., 1984a). These results are in good agreement with the lack of dihydrophytol observed after anaerobic biodegradation of phytol by sulfate-reducing (Grossi et al., 1998) and denitrifying bacteria (Rontani et al., 1999b; see Section 4.4), but not with the biological formation of dihydrophytol recently demonstrated by Schulze et al. (2001) in a slurry of lacustrine anoxic sediments. The ability of zooplankton to produce metabolites like pristane, dihydrophytol and phytanic acid seems to be species-dependent (Prahl et al., 1984a).

Classical oxidative metabolism of phytanic acid (Mize et al., 1969) may afford 2,6,10,14-tetramethylpentadecanoic (pristanic) and 4,8,12-trimethyltridecanoic acids (Fig. 4; see also Section 4.4), which have been detected in different species of *Calanus* (Avigan and Blumer, 1968; Prahl et al., 1984b). The occurrence of such metabolism is in good agreement with the detection by Avigan and Blumer (1968) of radioactive linear fatty acids resulting from a de novo synthesis from labelled 2- and 3-carbon fragments produced by degradation (β-oxidation) of the isoprenoid structure.

Isomeric pristenes have been isolated from mixed zooplankton of the Gulf of Maine (Blumer and Thomas, 1965). The formation of such compounds could be attributed to the decarboxylation of isomeric phytenic acids, which were detected in small amounts during the experiments of Avigan and Blumer (1968).

It has been taken for granted that isomeric phytadienes are biological alteration products of phytol occurring as a consequence of grazing of phytoplankton by herbivorous organisms (Blumer, 1965; Blumer et al., 1969). However, phytadienes may be generated artificially during some laboratory procedures used for lipid analysis (de Leeuw et al., 1977a; van de Meent et al., 1977). In a recent reinvestigation of the possible formation of phytadienes in the marine food web (using procedures avoiding dehydration reactions), Grossi et al. (1996) were unable to detect these compounds in copepods (*Temora longicornis*) feeding on different species of algae (*Rhodomonas* sp. and *Thalassiosira weissflogii*) or in a heterotrophic flagellate (*Oxyrrhis marina*), or in their faecal pellets. Although the presence of phytadienes in marine pelagic organisms cannot be totally ruled out, these observations suggest that many reports on the occurrence of phytadienes in organisms living in the water column may refer to artifacts.

4.4. Aerobic and anaerobic biodegradation of phytol

Microbial degradation of phytol is often considered to be an important source of acyclic isoprenoid compounds in the marine environment (Volkman and Maxwell, 1986). These processes seem to act mainly on free phytol (Brooks and Maxwell, 1974; Brooks et al., 1978; Gillan et al., 1983). Indeed, during non-sterile incubation of senescent cells of diatoms possessing a strong chlorophyllase activity, no significant biodegradation of esterified phytol could be observed even though more than 90% of free phytol was biodegraded (Rontani et al., 2000). We have summarised here recent work on the biodegradation of phytol under both aerobic (Rontani and Acquaviva, 1993; Rontani et al., 1999a) and anaerobic (Grossi et al., 1998; Rontani et al., 1999b; Schulze et al., 2001) conditions.

4.4.1. Aerobic biodegradation

Pure strains (Rontani and Acquaviva, 1993; Rontani et al., 1999a) and communities (Rontani et al., 1999b) of aerobic marine bacteria degrade free phytol efficiently. Aerobic bacterial degradation involves the transient production of the corresponding aldehyde (*E*)-3,7,11,15-tetramethylhexadec-2-enal ((*E*)-phytenal; Gillan et al., 1983), which in turn can be converted by biotic and abiotic processes in seawater to (*E*)-3,7,11,15-tetramethylhexadec-2-enoic acid [(*E*)-phytenic acid)] and abiotically to 6,10,14-trimethylpentadecan-2-one (Rontani and Acquaviva, 1993). The production of this C_{18} ketone involves addition of water to the activated double bond of phytenal and a subsequent retro-aldol reaction.

6,10,14-Trimethylpentadecan-2-one can be aerobically metabolised by two different pathways (pathways II and III in Fig. 5). Pathway II involves oxidation to 4,8,12-trimethyltridecanol acetate (Fig. 5) and subsequent hydrolysis of this ester to 4,8,12-trimethyltridecanol, which can be metabolised (after oxidation to the corresponding acid) via classical β-oxidation sequences. In contrast, pathway III involves oxidation of the keto-terminal methyl group of the ketone and subsequent decarboxylation and oxidation of the resulting C_{18} α-keto acid to the

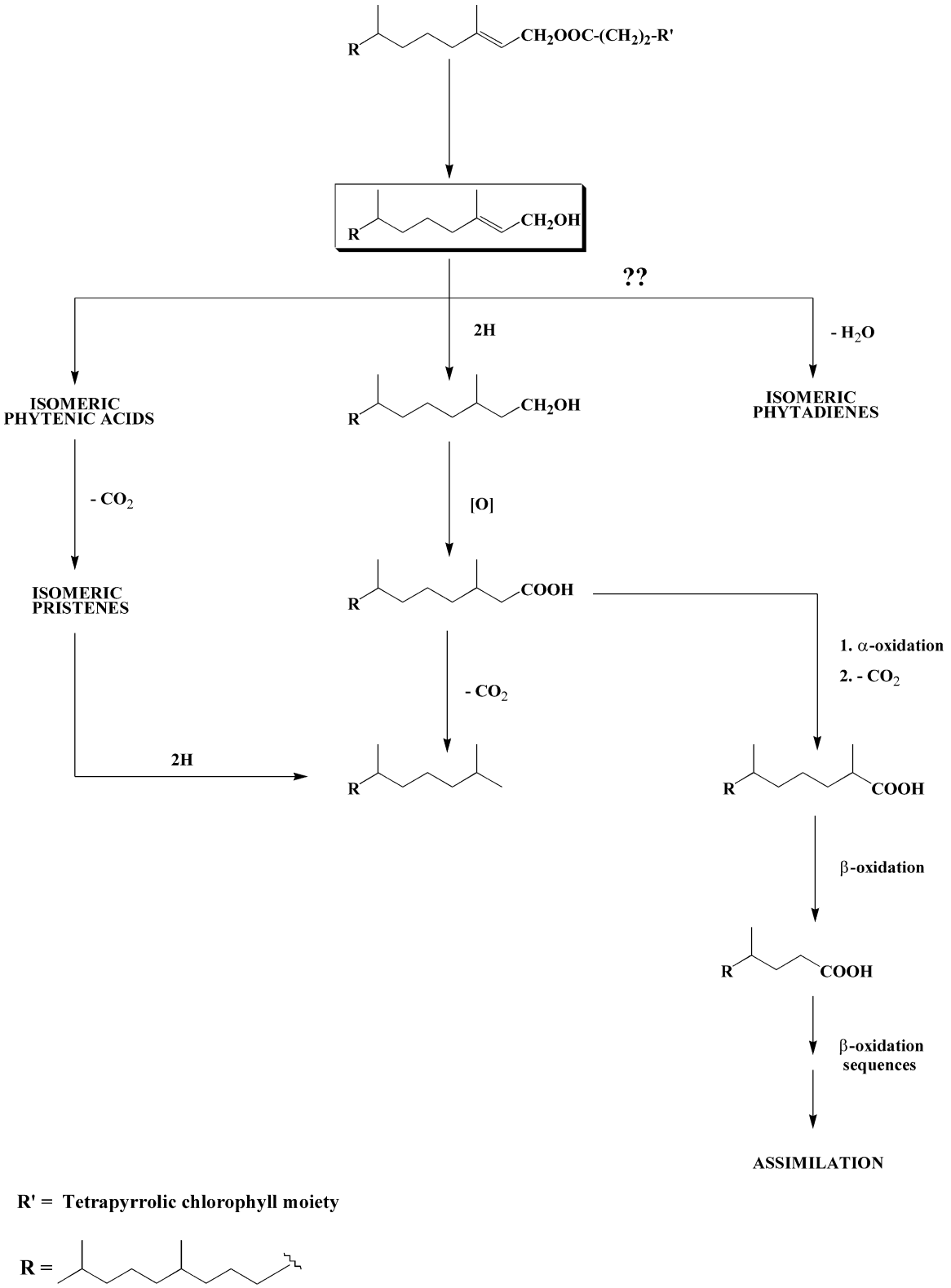


Fig. 4. Proposed pathways for the degradation of the chlorophyll phytyl chain during invertebrate feeding (adapted from Avigan and Blumer, 1968; Prahl et al., 1984a,b).

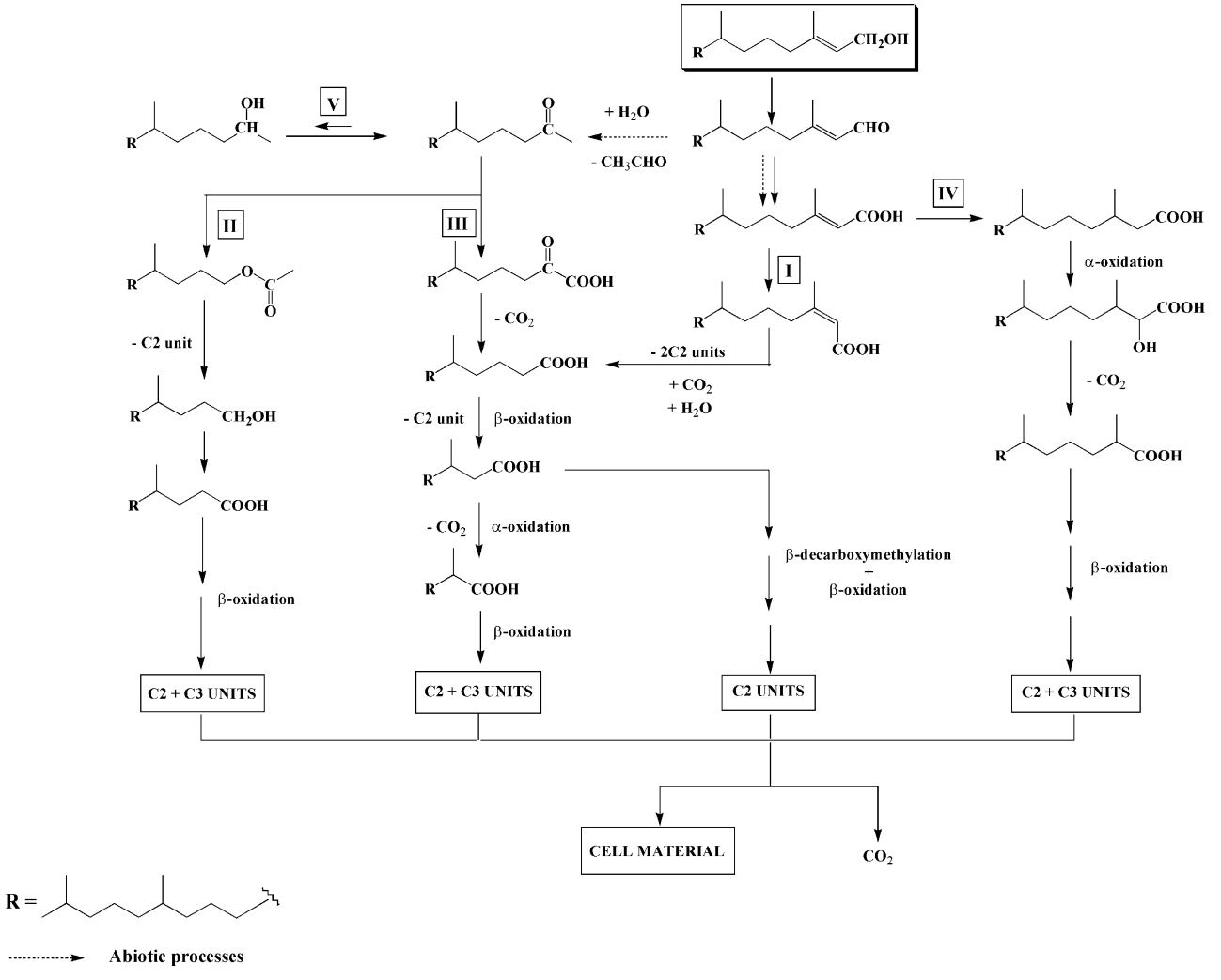


Fig. 5. Proposed pathways for the metabolism of phytol by a marine aerobic bacterial community (the broken arrows indicate abiotic processes). (Reprinted from Rontani et al., 1999b: Biodegradation of free phytol by bacterial communities isolated from marine sediments under aerobic and denitrifying conditions, Applied Environmental Microbiology, 65, 5484–5492, with permission from The American Society for Microbiology.)

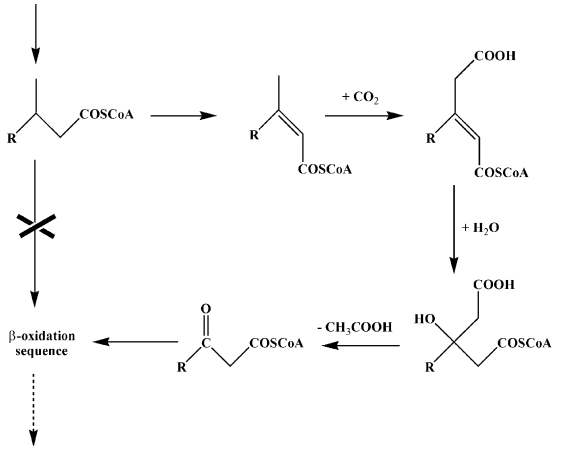


Fig. 6. β-Decarboxymethylation reaction sequence (COSCoA, acyl CoA thioester). (Reprinted from Rontani et al., 1999b: Biodegradation of free phytol by bacterial communities isolated from marine sediments under aerobic and denitrifying conditions, Applied Environmental Microbiology, 65, 5484–5492, with permission from The American Society for Microbiology.)

5,9,13-trimethyltetradecanoic acid (Gillan et al., 1983). Subsequently, the β-oxidation cycle can proceed only for one complete sequence before a metabolic blockage occurs (β-methyl branch). The assimilation of the resulting 3,7,11-trimethyldodecanoic acid requires the involvement of an additional strategy such as α-oxidation (Mize et al., 1969) or β-decarboxymethylation (Cantwell et al., 1978; Fig. 6). Traces of 6,10,14-trimethylpentadecan-2-ol were also formed during the aerobic incubation of phytol (pathway V in Fig. 5), probably by a dehydrogenase (Platen and Schink, 1989). The involvement of this ''blind alley'' pathway suggests that this process results from non-specific enzyme activity that is not related specifically to phytol degradation.

Concurrently, phytol is metabolised via (*E*)-phytenic acid by two different pathways (pathways I and IV in Fig. 5). Pathway I involves isomerization to (*Z*)-phytenic acid and subsequent β-decarboxymethylation (Fig. 6) and β-oxidation sequences. The 5,9,13-trimethyltetradecanoic acid thus formed is then metabolised according to the mechanisms described above. Pathway IV, which was previously proposed by Gillan et al. (1983), consists of a reduction to phytanic acid, followed by α-oxidation to 2-hydroxy-3,7,11,15-tetramethylhexadecanoic acid, which is then converted to pristanic acid by decarboxylation (Mize et al., 1969). The pristanic acid thus formed can be subsequently metabolised via classical β-oxidation reactions (McKenna and Kallio, 1971).

4.4.2. Anaerobic biodegradation

Owing to the abundance of sulfate in anoxic marine sediments, bacterial sulfate-reduction is generally considered to be essential for organic matter mineralisation. The degradation of phytol has thus been studied in slurries of anoxic sediments under sulfate-reducing conditions (Grossi et al., 1998). Under these conditions phytol was rapidly biodegraded (80–95% degradation after 3 months of incubation at 30 °C) to *Z* and *E* isomers of 3,7,11,15-tetramethylhexadeca-1,3-diene (phyta-1,3-dienes), 3,7,11,15-tetramethylhexadeca-1,3(17)-diene (neophytadiene) and isomeric phytenes (3,7,11,15-tetramethylhexadecenes). Although numerous studies have already considered phytadienes as compounds of biogeochemical interest (e.g. Kohnen et al., 1991b; Spooner et al., 1994), they are also easily generated during laboratory procedures associated with lipid analysis (de Leeuw et al., 1977a; Grossi et al., 1996) unless appropriate care is taken (Grossi et al., 1996; Schulze et al., 2001) as noted in Section 4.3. Available data suggest that phytol is mainly mineralised by sulfate-reducing bacteria via phytadienes and phytenes (Fig. 7), although alternative pathways cannot be excluded. The assimilation of phytenes seems to involve hydration of double bonds and subsequent oxidation of the alcohols thus formed to ketones, which are then mineralised via unknown pathways. Neither phytane, nor compounds usually considered as anoxic biotransformation products of phytol such as dihydrophytol, were detected by Grossi et al. (1998). In contrast, Schulze et al. (2001) unambiguously demonstrated the biological reduction of phytol to dihydrophytol in anaerobic lacustrine sediments. It would be interesting to determine the type of organism responsible for this transformation.

The anaerobic degradation of phytol by a denitrifying bacterial community isolated from Recent marine sediments was studied by Rontani et al. (1999b). Denitrifiers involved in the mineralisation of organic matter are generally aerobic species that can use nitrate as an alternative electron acceptor to oxygen when the latter is depleted. Despite the weak nitrate concentration of marine sediments, the role played by denitrifying bacteria in the mineralisation of acyclic isoprenoids cannot be neglected (Rontani, 1999b), since these aerobic facultative microorganisms possess higher metabolic and adaptive capacities than sulfate-reducing bacteria. This denitrifying bacterial community efficiently degrades phytol under anaerobic conditions (83% degradation after 30 days of incubation at 20 °C). Grossi et al. (1998) detected relatively high quantities of phytadienes and phytenes (41–74% of the amount of phytol that had disappeared), after 3 months of incubation of phytol under sulfate-reduction conditions, whereas the different metabolites identified at the end of the experiment with the denitrifying community represented only 2% of the degraded substrate. As aerobic

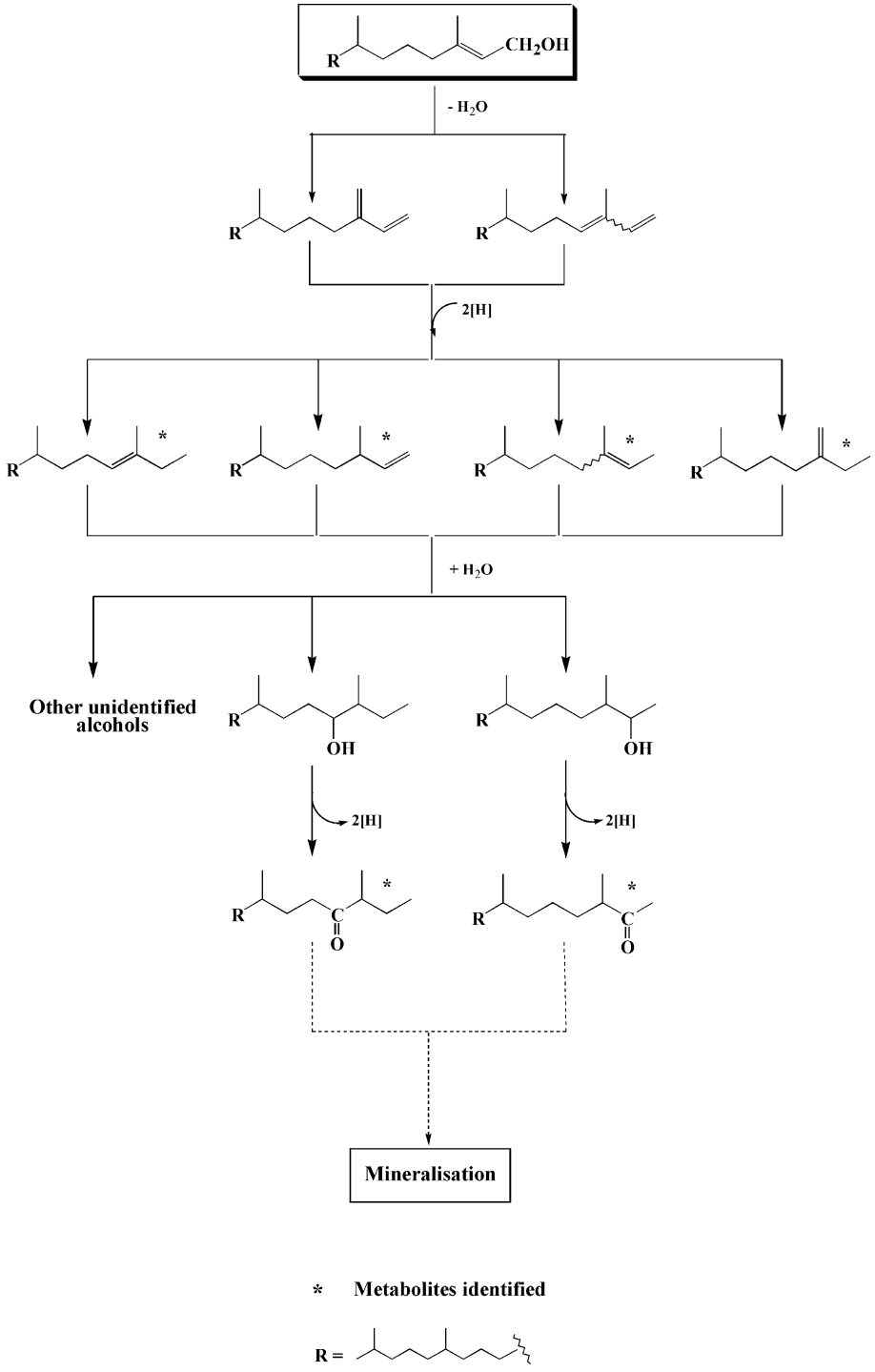


Fig. 7. Proposed pathways for the biodegradation of phytol under sulfate-reducing conditions (adapted from Grossi et al., 1998).

facultative bacteria, denitrifiers possess the powerful enzymatic suite of aerobic bacteria (Blackburn, 1986) and can use some of these enzymes (which are independent of molecular oxygen) under anaerobic conditions.

As under aerobic conditions, the first step of the anaerobic degradation of phytol by denitrifying bacteria involves the production of (*E*)-phytenal, which is then partially converted abiotically to 6,10,14-tri-

methylpentadecan-2-one. Anaerobic biodegradation of this C_{18} ketone may involve either a carboxylation reaction (pathway II in Fig. 8), or hydration of the enol forms of this ketone (pathways III and IV in Fig. 8). Anaerobic degradation of acetone and higher ketones by different denitrifying strains of the genus *Pseudomonas* involves an initial carboxylation reaction (Platen and Schink, 1989). In the case of 6,10,14-trimethylpentadecan-2-one, such a pathway affords 5,9,13-trimethyltetradecanoic acid, which may be subsequently metabolised via alternating β-decarboxymethylation and β-oxidation sequences as described above. A mechanism involving hydration of the enol form under kinetic control was previously proposed for the metabolism of 6,10,14-trimethylpentadecan-2-one by the denitrifying strain *Marinobacter* sp CAB (Rontani et al., 1997). This pathway produces 6,10,14-trimethylpentadecan-1,2-diol, which is then metabolised to 5,9,13-trimethyltetradecanoic acid (pathway III in Fig. 8). The hydration can also take place on the enol form of 6,10,14-trimethylpentadecan-2-one under thermodynamic control (pathway IV in Fig. 8), which leads to the production of 4,8,12-trimethyltridecanoic acid that is easily assimilable via a classical β-oxidation sequence. Reduction of 6,10,14-trimethylpentadecan-2-one to the corresponding alcohol (pathway VIII in Fig. 8) appears to be more intense under anaerobic conditions.

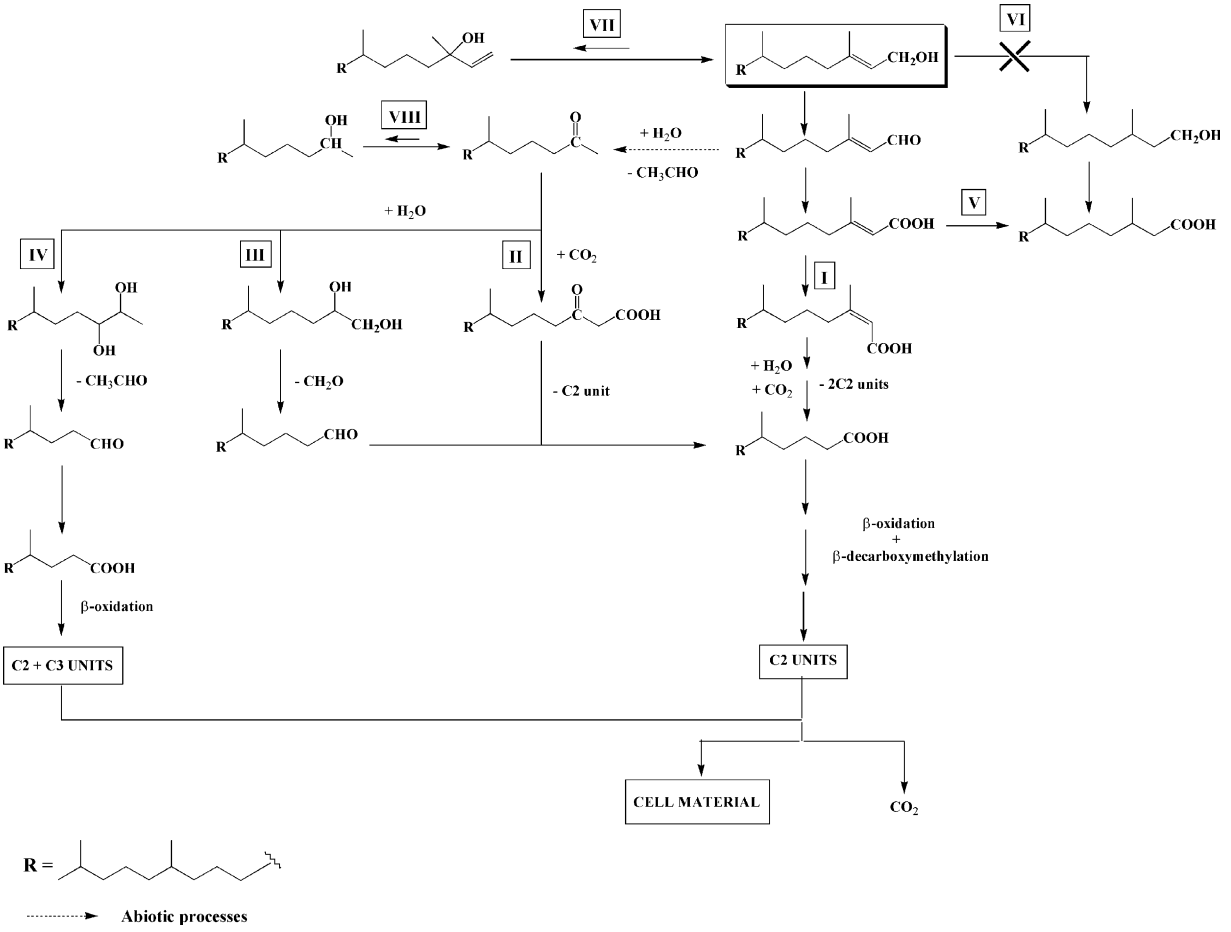


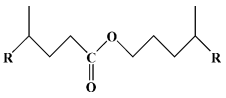
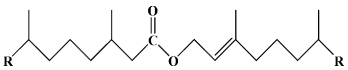
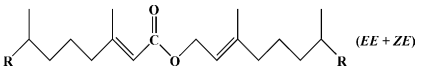
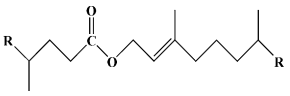
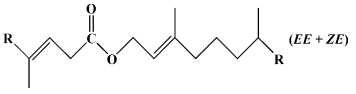
Fig. 8. Proposed pathways for the anaerobic metabolism of phytol by a marine denitrifying bacterial community (the broken arrows indicate abiotic processes). (Reprinted from Rontani et al., 1999: Biodegradation of free phytol by bacterial communities isolated from marine sediments under aerobic and denitrifying conditions, Applied Environmental Microbiology, 65, 5484–5492, with permission from The American Society for Microbiology.)

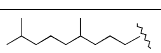
The detection of significant amounts of (*Z*)-phytenic acid in these experiments showed that, like aerobic bacteria, the denitrifying bacterial community metabolises phytol via alternating β-decarboxymethylation and β-oxidation sequences (pathway I in Fig. 8). This pathway (which is independent of molecular oxygen; Hylemon and Harder, 1999) is used by denitrifying bacteria under anaerobic conditions. The growth of *Pseudomonas citronellolis* on 3,7-dimethyloctan-1-ol or citronellol under anaerobic conditions in the presence of nitrate as electron acceptor (Harder and Probian, 1995) is consistent with this view. Substantial amounts of phytanic acid were also detected after the growth of the denitrifying community on phytol. This acid can be anaerobically produced from phytol by two pathways: one by way of dihydrophytol (pathway VI in Fig. 8) and another by (*E*)-phytenal and (*E*)-phytenic acid (pathway V in Fig. 8). The lack of dihydrophytol among the metabolites isolated indicates that the first mechanism is not involved. 3-Hydroxy-3,7,11,15-tetramethylhexadecene (isophytol) was also detected at the end of the experiment. The production of this compound was attributed to the involvement of a reversible enzyme-catalysed allylic rearrangement of phytol (pathway VII in Fig. 8) analogous to that proposed by Foss and Harder (1997) for the reverse transformation of linalool to geraniol by the denitrifying strain *Thauera linaloolentis*.

4.4.3. Bacterial production of isoprenoid wax esters

During incubation of uniformly labelled ^{14}C phytol in sediments, Brooks and Maxwell (1974) observed the formation of phytyl esters and suggested that esterification of the introduced phytol is brought about by enzymatic processes, possibly microbial. Volkman and Maxwell (1986) also suggested that esterification may be a significant process in microbially active sediments. Recently, the production of isoprenoid wax esters was unambiguously demonstrated during growth of aerobic and denitrifying marine bacteria on phytol (Table 2) (Rontani et al., 1999a,b) and in slurries of anaerobic lacustrine sediments (Schulze et al., 2001). These compounds are formed by condensation of phytol metabolites with themselves or with phytol (Rontani et al., 1999a) and constitute energy storage components of bacteria (Fixter et al., 1986; Alvarez et al., 1997). The amount of the esters formed increased considerably in N-limited cultures, where the ammonium concentration corresponds to conditions often found in the marine sediments. This suggests that the bacterial formation of isoprenoid wax esters might be favoured in such environments.

Table 2
Isoprenoid wax esters produced during bacterial growth on phytol

Metabolites (%)[a]	*Pseudomonas nautica*[b]	*Marinobacter Hydrocarbonoclasticus*[b]	*Marinobacter* sp. strain CAB[b]	Aerobic bacterial community[c]	Denitrifying bacterial community[c]
	0.43	0.02	0.04	–	–
	3.71	0.39	0.25	0.66	1.78
 (*EE* + *ZE*)	–tr	0.11	0.15	0.25	0.60
	–	0.41	0.43	1.66	–
 (*EE* + *ZE*)	–	0.05	0.12	–	-

R = 
tr = traces.

[a] Relative percentages were based on the amount of degraded substrate.
[b] Rontani et al., 1999a.
[c] Rontani et al., 1999b.

4.4.4. Effect of temperature and particle association on the bacterial metabolism of phytol

As shown above, the production of 6,10,14-trimethylpentadecan-2-one during the biodegradation of phytol under aerobic or denitrifying conditions involves addition of water to the activated double bond of (*E*)-phytenal and a subsequent retro-aldol reaction. At relatively high temperatures (e.g. 30 °C) this abiotic process is quite rapid, and consequently much of the phytol is metabolised through this C_{18} ketone. On the other hand, at low temperatures (e.g. 15 °C), (*E*)-phytenal is more stable and phytol is biodegraded mainly through (*E*)-phytenic acid (Rontani and Acquaviva, 1993). Recently, it was also demonstrated that sorption to mineral particles hinders the addition of water to the activated double bond of (*E*)-phytenal and consequently strongly favours the degradation of phytol through (*E*)-phytenic acid (Rontani and Bonin, 2000). Consequently, it was concluded that in the aerobic and the denitrifying zones of Recent temperate marine sediments phytol is probably metabolised mainly via (*E*)-phytenic acid. This agrees with the previous detection of high amounts of (*Z* and *E*)-phytenic acids in the top layer of Recent sediments of Carteau Bay (Rontani et al., 1999b). The labile intermediate (*E*)-phytenal appears to play a key role during the bacterial metabolism of phytol in seawater. Depending upon temperature and sorption to mineral particles, different routes can be selected in the bacterial metabolism of this isoprenoid alcohol. Consequently, results obtained in vitro at relatively high temperatures and with free cell cultures may not be comparable to the marine environment.

4.5. Clay-catalysed degradative processes

In order to simulate the catalytic breakdown of the chlorophyll phytyl chain during early diagenesis, a series of experiments under simulated geological conditions was carried out with free phytol (de Leeuw et al., 1974, 1977a) and phytylpropionate (a model for the chlorophyll phytyl chain; Rontani and Grossi, 1995). A simulation experiment is a compromise between two paradoxical objectives: on the one hand the reaction conditions have to be as natural as possible, but on the other the reaction products must be produced in a reasonably short period of time (de Leeuw et al., 1974). Consequently, most of the simulation experiments have involved incubation of free phytol or phytylpropionate at relatively low temperature (60 °C or less) in the presence of montmorillonite (as a representative clay) and water under oxic or anoxic conditions. Under oxic conditions, autooxidative processes act in parallel to clay-catalysed reactions. In this section we focus on clay-catalysed reactions since autoxidation of the chlorophyll phytyl chain has already been described (see Section 4.2).

During the incubation of phytylpropionate with montmorillonite, acid-catalysed hydrolysis rapidly releases free phytol (Rontani and Grossi, 1995). Consequently, the degradation products detected are very similar to these previously identified during thermo-catalytic simulations with free phytol (de Leeuw et al., 1974, 1977a). Clay-catalysed degradation of phytol affords mainly isophytol, isomeric phytadienes, isomeric phytenes, pristenes, phytanal, phytenal, phytone, diphytylether and several dimeric and trimeric compounds. Different pathways have been proposed in order to explain the formation of these compounds (Fig. 9).

Proton-catalysed dehydration of phytol results in the formation of isomeric phytadienes (pathway I in Fig. 9). This reversible reaction, which involves intermediate allylic-stabilised carbenium ions, may result in the isomerisation of phytol to isophytol. Phytadienes constitute short-lived geochemical intermediates which react further either with other reactive organic molecules or by incorporation into a high molecular weight network. Reaction of phytadienes with each other or reaction of phytadiene molecules with phytol molecules (probably via Diels–Alder reactions) result in the formation of dimeric and probably higher molecular weight compounds (de Leeuw et al., 1977a). The dimeric and trimeric compounds present in these simulation experiments consist of a variety of isomers with elemental composition $C_{40}H_{76}$, $C_{40}H_{78}O$, $C_{60}H_{114}$ and $C_{60}H_{116}O$ (de Leeuw et al., 1977a). The relatively large amounts of dimeric products in the simulation experiments is of course a consequence of the relatively high concentration of monomers. Therefore, the phytadienes and the dimeric products can only be expected to be present in sediments in minor amounts (de Leeuw et al., 1977a).

A simulation carried out in the presence of methanol (instead of water) in order to trap unstable intermediates allowed significant amounts of 1,3-dimethoxy-3,7,11,15-tetramethylhexadecane to be detected (Rontani and Grossi, 1995). This result suggested the formation of 3,7,11,15-tetramethylhexadecan-1,3-diol in the presence of water by a process involved protonation of the olefinic carbon-2 of phytol and subsequent addition of water to the tertiary carbocation thus formed. The lack of this compound in simulation experiments with water can be explained by its instability in acidic media. Indeed, in addition to classical dehydration reactions, 1,3-diols (where one OH group is tertiary) undergo a particular cleavage under acidic conditions (Zimmerman and English, 1954). In the present case, this cleavage leads to the production of 2,6,10,14-tetramethylpentadecene (prist-1-ene) after elimination of water and formadehyde (pathway II in Fig. 9; Rontani and Grossi, 1995). The formation of this alkene during thermal simulations on phytol was previously attributed either to a decarboxylation of phytenic acid

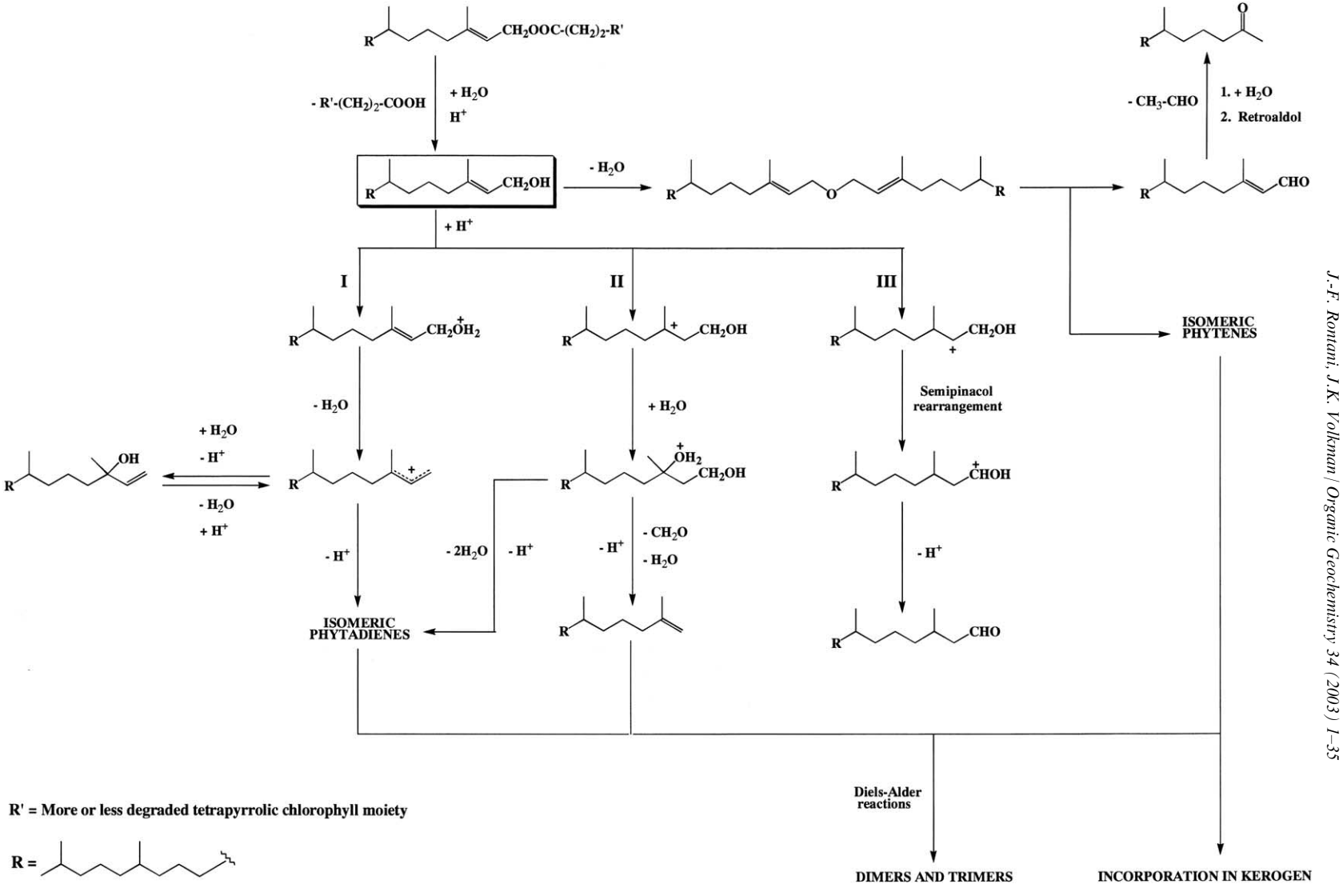


Fig. 9. Proposed pathways for the clay-catalysed degradation of chlorophyll phytyl chain (adapted from de Leeuw et al., 1974, 1977a; Rontani and Grossi, 1995).

(Ikan et al., 1975), or to an intramolecular rearrangement of isomerised phytol (de Leeuw et al., 1974).

It is interesting to note that if protonation occurs on olefinic carbon-3, the carbocation thus formed can undergo a semipinacol rearrangement (March, 1985) leading to the formation of 3,7,11,15-tetramethylhexadecanal (phytanal; pathway III in Fig. 9). This aldehyde may represent a potential intermediate in the transformation of phytol to phytanic acid in sediments, but this is still uncertain.

The formation of diphytyl ether involves the clay-catalysed loss of water from two molecules of phytol (de Leeuw et al., 1974). This ether can account for the occurrence of both 3,7,11,15-tetramethylhexadec-2-enal (phytenal) and 3,7,11,15-tetramethylhexadecene (phyt-1-ene) via an intramolecular rearrangement (de Leeuw et al., 1974). The presence of phytene isomers was attributed to the involvement of simple double bond isomerisation processes. Phytenal can be then easily attacked by water in a Michael addition, affording an unstable β-aldol, which in turn can undergo a retroaldol reaction producing 6,10,14-trimethylpentadecan-2-one (phytone; Fig. 9).

One potential difficulty in relating the results of these clay-catalysed simulation experiments to the natural environment is the use of free phytol based on the assumption that chlorophyll hydrolysis proceeds rapidly in the water column or sediments. Though biologically mediated hydrolysis of chlorophyll has been demonstrated in senescent diatoms (Cuny et al., 1999; Rontani et al., 2000) and in copepods (Shuman and Lorenzen, 1975), in sediments the ester bond between phytol and the tetrapyrrolic macrocycle can resist hydrolysis as shown by the isolation of intact phytyl esters from sediments several million years old (Baker and Smith, 1974).

4.6. Sulfur incorporation

The occurrence of numerous organic sulfur compounds (OSC) in crude oils and ancient sediments was well established by some of the earliest studies in petroleum geochemistry. However, the mechanisms of their formation were largely unknown and it was assumed that high temperature reactions must be involved. Important advances were made in the 1980s with the identification of a novel sulfur-containing C_{35} hopanoid thiophene (Valisolalao et al., 1984), isoprenoid thiophenes (Brassell et al., 1986) and highly branched isoprenoid thiophenes (Sinninghe Damsté et al., 1989a) in immature sediments. By 1990, over 1500 OSCs had been identified (Sinninghe Damsté and de Leeuw 1990). The results of these studies implied that sulfur could be incorporated into labile lipids during the early stages of diagenesis, which has since been confirmed by many simulation studies (e.g. de Graaf et al., 1992; Rowland et al., 1993; Krein and Aizenshtat, 1994, Adam et al., 1998), and by analyses of modern sediments deposited under anoxic bottom waters (e.g. Robertson et al., 1995; Wakeham et al., 1995; Grossi et al., 1998; Adam et al., 2000; Kok et al., 2000; Werne et al., 2000; Schouten et al., 2001).

This incorporation of reduced inorganic sulfur into organic matter is an important mechanism for the preservation of functionalized organic compounds in the sedimentary environment. For OSC to form there must be an abundant supply of labile organic matter, plus an available supply of inorganic sulfides, which implies anoxic conditions and bacterially-mediated sulfate reduction together with low amounts of reactive iron. It was generally thought that reactive iron minerals can outcompete organic compounds for sulfur incorporation but clearly this need not be the case (e.g. Hartgers et al., 1997).

Only alkenes, ketones and aldehydes are known to react to form organic sulfur compounds (Schouten et al., 1994). Compounds possessing carbon-carbon double bonds generally undergo an addition reaction following the Markovnikov rule. The oxo-group of ketones and aldehydes is substituted by sulfur such that polysulfide-linked dimers are formed. Polymers or cyclic sulfides are derived from substrates with more than one functionality depending on the spacing between functional groups (Fig. 10). Given the breadth of research in this area and diversity of compounds studied, the following discussion is mainly restricted to sulfurization of phytol and its derivatives in modern sediments.

The full identification of two C_{20} isoprenoid thiophenes in a range of relatively young sediments by Brassell et al. (1986) provided clear evidence that sulfur is readily incorporated into isoprenoid lipids. One compound was identified as 3-methyl-2-(3,7,11-trimethyldodecyl)-thiophene which could be derived from sulfur incorporation into phytol or phyta-1,3-diene. The second was 3-(4,8,12-trimethyltridecyl)-thiophene which could be derived from phytol or phyta-1,3(17)-diene. The form of sulfur was not known, but was postulated to be either H_2S or polysulfides. Note that these compounds involve an intramolecular S bond, but these authors also recognised the likely importance of intermolecular incorporation giving rise to sulfur-linked polymeric material.

This work was followed up by Fukushima et al. (1992). These authors identified three C_{20} isoprenoid thiophene isomers in diverse modern sediments including brackish water lake and inland bay sediments. The likely formation reaction was postulated to be from chlorophyll-derived phytol and hydrogen sulfide (H_2S) via phytadiene intermediates formed by phytol dehydration (Fig. 10). Indeed, two of the isomers could be produced by reacting phytol in sealed tubes with H_2S-saturated seawater. Acid dehydration of phytol in aqueous media yielded four phytadiene isomers and subsequent reaction of the phytadiene mixtures in the H_2S-saturated seawater gave the same two thiophene iso-

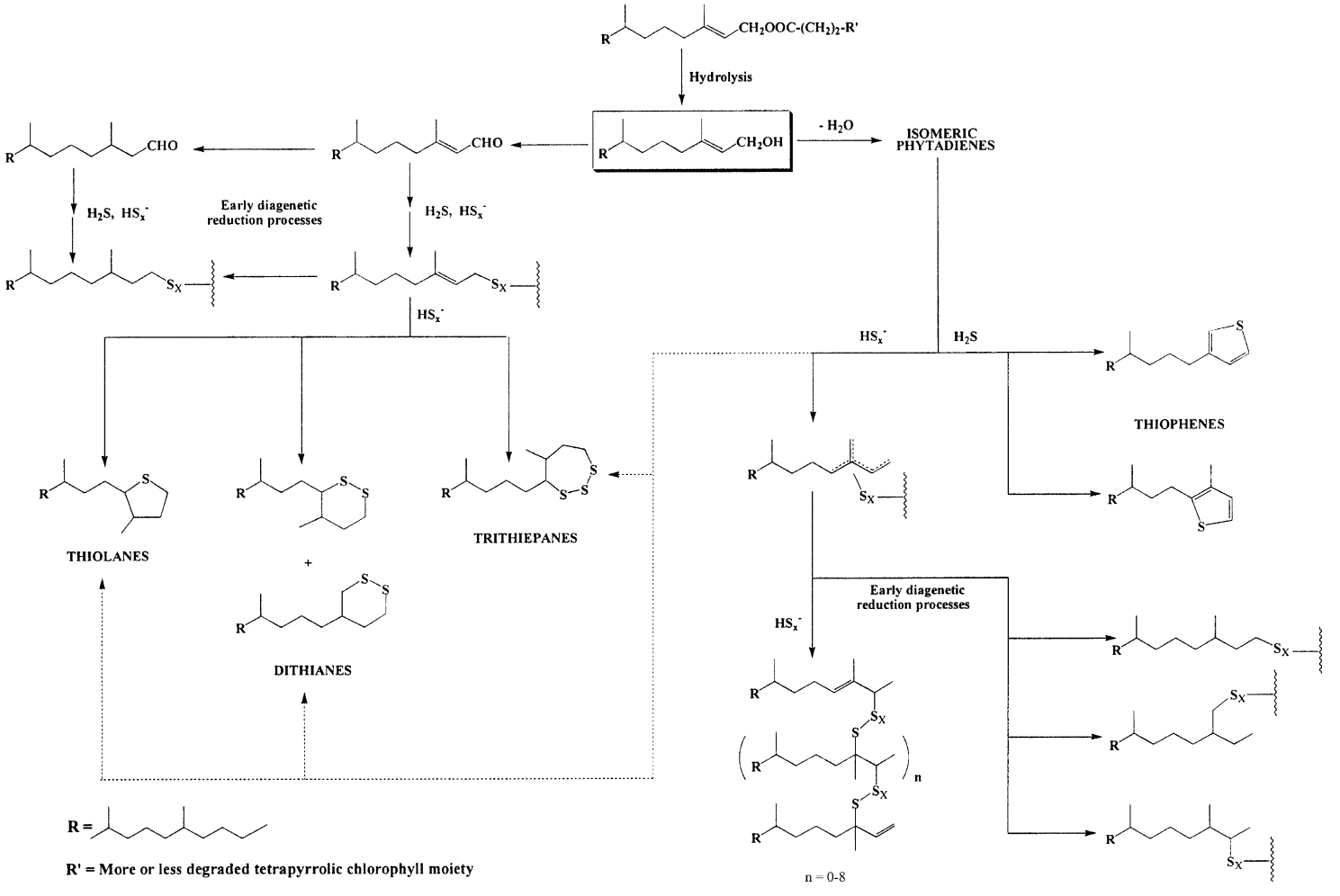


Fig. 10. Proposed pathways for the sulfurization of the chlorophyll phytyl chain (broken arrows indicate minor processes). (Adapted from Sinninghe-Damsté and de Leeuw, 1990; Kohnen et al., 1991a,b,c; de Graaf et al., 1992; Fukushima et al., 1992, Adam et al., 2000).

mers. Isoprenoid thiophenes with 20 carbon atoms are probably the most abundant extractable OSC in sediments with 7 isomers now known (Sinninghe Damsté and de Leeuw, 1990).

Other types of organic sulfur compound include the saturated dithianes, trithiepanes and thiolanes (Fig. 10). 1,2-Dithianes are compounds with a 6 membered ring containing two adjacent S atoms, while 1,2,3-trithiepanes are compounds with a 7 membered ring containing three adjacent S atoms (Sinninghe Damsté and de Leeuw, 1990). Kohnen et al. (1989) reported cyclic di- and tri-sulfides with a C_{20} isoprenoid skeleton in sediments from Quaternary to Pliocene in age. Phytadienes derived from phytol were suggested as possible precursors. The disulfide could be formed by H_2S reaction via intermediate thiols and subsequent oxidation, but the presence of the cyclic trisulfide provided the first clear evidence for the involvement of polysulfides perhaps including HS_4^-, HS_5^-, S_4^{2-} and S_5^{2-}. Much less is known about isoprenoid thiolanes which typically occur in complex mixtures. A number of isomers have been tentatively identified with one confirmed by synthesis (Sinninghe Damsté et al., 1987).

Sulfur is also incorporated into a variety of macromolecular substances through intermolecular reactions (Fig. 10; Sinninghe Damsté and de Leeuw 1990; Kohnen et al., 1993; Adam et al., 2000). A number of C_{20} isoprenoid thiophenes were described in pyrolysates of protokerogens by Kenig and Huc (1990) and in resin fractions from Rozel Point crude oil by Sinninghe Damsté et al. (1990). Raney-Ni has been used to cleave C–S bonds selectively to release molecules attached to the macromolecular matrix. The common occurrence of phytane in the reaction products attests to the ready incorporation of phytol-derived compounds into this fraction (e.g. Sinninghe Damsté et al., 1988). Adam et al. (2000) used a mild cleavage reagent which produces mainly methylthioethers to demonstrate the ready incorporation of phytyl and steroid skeletons through polysulphide bonds into the macromolecular fraction of Recent sediments. The main products included *cis* and *trans* 1-methylthiophyt-2-enes, which is consistent with the view that the first sulfurization steps produce polysulphide-bound unsaturated thiols, which are subsequently altered to yield their saturated counterparts, and that sulfurization occurs mainly through the carbonyl group of aldehydes and ketones (Fig. 10).

Numerous laboratory simulations have been conducted to assess the impact of natural sulfurization on phytol and its derivatives (de Graaf et al., 1992; Fukushima et al., 1992; Rowland et al., 1993; Schouten et al., 1994). The large set of experimental conditions used by these authors showed that the sulfurization of C_{20} isoprenoids can occur under relatively mild conditions. However, these experimental conditions prevented any bacterial process occurring. The very low amounts of phytol or phytadienes preserved as OSC in slurry experiments conducted under sulfate-reducing conditions relatively close to those of natural systems (Grossi et al., 1998) indicates that anaerobic biodegradation and sulfurization should be regarded as two competing diagenetic processes, particularly in Recent anoxic sediments. Evidence for this comes from the identification of a wide variety of reduced and/or sulfurized diagenetic products in modern sediments deposited under anoxic conditions in Ace Lake, Antarctica (Robertson et al., 1995; Kok et al., 2000; Schouten et al., 2001).

Work by Adam et al. (1998) provided a new perspective on sulfurization reactions by demonstrating through simulation experiments that organic matter is efficiently sulfurized under natural sunlight leading to thiophenes and sulfur cross-linked macromolecules similar to those occurring in sediments deposited in anoxic environments. Their results suggest that photochemically induced sulfurization of organic matter in the water column might be important in environments with photic zone anoxia.

5. The potential of phytol degradation products as biogeochemical tracers

The known formation processes of the different phytol degradation products are summarised in Table 3. From these relationships, we discuss the potential and constraints of various phytol-related compounds as biogeochemical markers. Unfortunately, as our understanding has developed about the many pathways by which some of these compounds can be formed, the specificity of many of these markers and ratios of markers is now called into doubt.

5.1. Isoprenoid ketones

There are several reports of free 6,10,14-trimethylpentadecan-2-one (phytone) in sediments (Simoneit, 1973; Ikan et al., 1973; de Leeuw et al., 1974, 1977a; Rontani et al., 1999b) and water column particulate matter (Volkman et al., 1983). It appears that this isoprenoid ketone can be produced in several ways: (i) from free phytol, by aerobic (Brooks and Maxwell, 1974; Brooks et al., 1978, Gillan et al., 1983; Rontani and Acquaviva, 1993) and anaerobic (Rontani et al., 1999b) bacterial degradation (Figs. 5 and 8 in Section 4.4) and autoxidation (Fig. 3 in Section 4.2), (ii) by photosensitised oxidation of some isoprenoid hydrocarbons such as pristane (Rontani and Giusti, 1987) and phytane (Rontani and Giral, 1990), (iii) by hydrolysis of chlorophyll-*a* photoproducts (Rontani et al., 1991) (Fig. 1 in Section 4.1) and by alkaline hydrolysis of tocopherols (Rontani et al., 1996b). In view of the numerous possible precursors and

Table 3
Known formation processes of phytol deriving isoprenoid compounds

Compound	Aerobic biodegradation of phytol	Anaerobic biodegradation of phytol	Autoxidation of phytol[a]	Photooxidation of chlorophylls	Clay-catalysed degradation of phytol[a]	Degradation of phytol during grazing
6,10,14-Trimethylpentadecan-2-one (free)	+	+	+	+	+	
6,10,14-Trimethylpentadecan-2-one (bound)				+		
3-Methylidene-7,11,15-trimethylhexadecan-1,2-diol				+		
3,7,11,15-Tetramethylhexadecan-1-ol						+
3,7,11,15-Tetramethylhexadec-1-en-3-ol		+			+	
6,10,14-Trimethylpentadecan-2-ol	+	+				
(*Z* and *E*) 3,7,11,15-Tetramethylhexadec-2-enal	+	+	+	+	+	
(*Z* and *E*) 3,7,11,15-Tetramethylhexadec-2-enoic acids	+	+	+	+		+
3,7,11,15-Tetramethylhexadecanal					+	
3,7,11,15-Tetramethylhexadecanoic acid	+	+				+
2,6,10,14-Tetramethylpentadecanoic acid	+		+			+
5,9,13-Trimethyltetradecanoic acid	+	+	+			
4,8,12-Trimethyltridecanoic acid	+	+	+			+
Isomeric phytadienes		+			+	?
Isomeric phytenes		+			+	
Isomeric pristenes					+	+
2,6,10,14-Tetramethylhexadecane						
2,6,10,14-Tetramethylpentadecane						+
Isoprenoid wax esters	+	+				
(Z) 3,7,11-Trimethyldodec-2-enoic acid	+	+				
3,7,11,15-Tetramethylhexadeca-1,2,3-triol[b]			+			
3,7,11,15-Tetramethyl-2,3-hydroxyhexadecanoic acid[b]			+			
(*Z* and *E*) 3,7,11,15-Tetramethyl-2,3-epoxyhexadecan-1-ol			+			

[a] In this table "pure clay-catalysed degradation processes" are separated from autoxidative ones.
[b] Mixtures of diastereoisomers.

formation processes for phytone (Table 3), it is evident that this compound in its free form is not a specific biogeochemical marker.

In 1987 ten Haven et al. detected unexpectedly large quantities of this isoprenoid ketone in extracts obtained after alkaline and acidic hydrolysis of a Soxhlet-extracted sapropel residue. At the time, the production of this "bound" ketone was attributed to the hydrolysis of chlorophyll phytyl chain photoproducts of type **a** (Fig. 1 in Section 4.1), which could not be extracted from sediments during Soxhlet extraction owing to their high polarity (Rontani et al., 1992). "Bound" phytone was thus proposed as a marker for the photodegradation of chlorophylls having a phytol ester group in the marine environment (Rontani et al., 1992). Unfortunately, the rapid hydrolysis of chlorophyll phytyl chain photoproducts of type **a** in the presence of clays (Rontani and Grossi, 1995), and the production of "bound" phytone by alkaline hydrolysis of tocopherols (Rontani et al., 1996b) considerably hinder such a use.

5.2. Phytyldiol (3-methylidene-7,11,15-trimethylhexadecan-1,2-diol)

The detection of significant amounts of phytyldiol in sediments and particulate matter samples of various origins (e.g. Mediterranean Sea, North Sea, Antarctica and Atlantic Ocean; Rontani et al., 1994; Cuny and Rontani, 1999) attests to the widespread occurrence of this compound in the marine environment. Phytyldiol results from hydrolysis of chlorophyll phytyl chain photoproducts of type **b** (Fig. 1 in Section 4.1, Table 3). Photo-oxygenation of the olefinic C-2 of the chlorophyll phytyl chain in dead phytoplanktonic cells involves the selective hydrogen abstraction by singlet oxygen on the allylic methyl group *Z*-orientated to the carbinol group (Rontani et al., 1994). This strong regioselectivity, which is in accord with the results obtained by Schulte-Elte et al. (1979) in the case of 3-methyl-3-alkyl-substituted allylic alcohols, suggests that phytyldiol can be used as a specific tracer of chlorophyll photodegradation processes (Rontani et al., 1994; Cuny and Rontani, 1999).

A theoretical equation of chlorophyll photodegradation has been developed based on light-dependent degradation rates of intact chlorophylls and of chlorophyll phytyl chain determined during incubation experiments with different senescent phytoplanktonic strains (Cuny et al., 1999). This equation, which links the Chlorophyll Phytyl side-chain Photodegradation Index (CPPI; molar ratio phytyldiol/phytol) to the amounts of chlorophyll photodegraded, was applied for the first time to a seasonal survey of chlorophyll photodegradation in a marine environment (Cuny et al., 2002). Although free phytol would be more reactive toward singlet oxygen than the esterified phytyl chain, it could be demonstrated that the CPPI calculated with total phytyldiol and phytol concentrations allowed a good estimation of the chlorophyll photodegradation state in senescent cells of the diatom *Phaeodactylum tricornutum* (Rontani et al., 2000), which has a strong chlorophyllase activity (Jeffrey and Hallegraeff, 1987). This work also demonstrated that aerobic bacterial degradation processes do not significantly alter the CPPI. The next step to validate CPPI as a tool for the monitoring of chlorophyll photodegradation in the marine environment must be to study the relative fate of phytol and phytyldiol during the grazing of phytoplankton by zooplankton.

Chlorophyll phytyl chain photoproducts of type **b** (Fig. 1 in Section 4.1) are degraded in Recent sediments showing high bacterial activity at a similar rate to an unchanged chlorophyll phytyl chain (Rontani et al., 1996b). This non-selective degradation permits CPPI to be used to monitor the past photodegradation of chlorophylls with a phytyl ester group in sediments. The temporal and spatial limits of these tracers remain to be determined, but their use is encouraging given the well known preservation of tetrapyrrole pigment ester linkages in sediments several million years old (Baker and Smith, 1974; Brassell et al., 1983) and the detection of "esterified + bound" phytyldiol in sediments of different origins ranging from 5000 to 25,000 years (Cuny and Rontani, 1999). Since photooxidative degradation of lipid components of phytoplankton intervenes mainly in senescent cells, CPPI could be also indicative of the physiological state of phytoplanktonic communities (Cuny et al., 2002).

5.3. Dihydrophytol (3,7,11,15-tetramethylhexadecan-1-ol)

Dihydrophytol has been reported as a minor constituent of the lipids of a few Recent sediments (Sever and Parker, 1969; Brooks and Maxwell, 1974; Van Vleet and Quinn, 1979; Rontani et al., 1999b). This compound has often been considered as a reduction product of free phytol (Brooks et al., 1978; Van Vleet and Quinn, 1979) and has been proposed as a marker for reducing conditions during early diagenesis (de Leeuw et al., 1977a). The absence of dihydrophytol formation during bacterial incubations of (*E*)-phytol under sulfate-reducing (Grossi et al., 1998) and denitrifying conditions (Rontani et al., 1999b) strongly suggests that the presence of this compound in the sediments is more probably due to a direct input of lipids from Archaebacteria (Volkman and Maxwell, 1986; Franzmann et al., 1988), or to its production from the chlorophyll phytyl side-chain during macrofaunal (Sun et al., 1998) or copepod (Prahl et al., 1984a,b) digestion. However, the recent demonstration of a biological formation of

dihydrophytol from phytol in a slurry of anoxic lacustrine sediments (Schulze et al., 2001) opens up the debate once again.

5.4. 6,10,14-Trimethylpentadecan-2-ol

Small amounts of 6,10,14-trimethylpentadecan-2-ol have been detected in sediments, both ancient (Cox et al., 1972) and Recent (Grimalt et al. 1991; Rontani et al., 1999b). Brooks et al. (1978) previously showed that a microbial population enriched from a lake sediment and grown on phytol under anaerobic conditions produced this C_{18} alcohol, probably by reduction of the corresponding ketone produced during phytol biodegradation. The production of significant amounts of 6,10,14-trimethylpentadecan-2-ol observed during the biodegradation of phytol by bacterial communities isolated from marine sediments (Rontani et al., 1999b) supports this hypothesis. This compound could be used as a tracer of bacterial activity in sediments (Table 3), but its low abundance considerably limits its use.

5.5. Isophytol (3,7,11,15-tetramethylhexadec-1-en-3-ol)

To our knowledge there is no report in the literature of the presence of isophytol in sediments. However, Brooks and Maxwell (1974) observed the formation of this isoprenoid alcohol during incubation of uniformly labelled ^{14}C-phytol with sediment from Esthwaite Water and attributed its formation to a rearrangement of phytol by an unknown mechanism. The enzyme-catalysed allylic rearrangement observed during biodegradation of phytol under denitrifying conditions (Rontani et al., 1999b) (Fig. 8 in Section 4.4), or the involvement of carbenium ions during clay-catalysed dehydration of phytol (de Leeuw et al., 1974; Fig. 9 in Section 4.5) could account for this isomerisation.

5.6. Phytenals ((Z and E)-3,7,11,15-tetramethylhexadec-2-enals)

There are few reported occurrences of phytenals in sediments (Rowland, 1982; Rowland and Maxwell, 1990; Grimalt et al., 1991) and particulate matter (Rontani et al., 1990). Great care must be taken in the identification of these compounds due to their possible formation from phytol (Rowland, 1982) and easy degradation during some isolation procedures (Rontani et al., 1990). In the marine environment, these compounds may be produced from phytol by aerobic (Rontani and Acquaviva, 1993; Rontani et al., 1999b) and anaerobic (Rontani et al., 1999b) bacterial degradation (Figs. 5 and 8 in Section 4.4), clay-catalysed degradation (de Leeuw et al., 1974; Fig. 9 in Section 4.5) and free radical oxidation (Rontani and Aubert, 1994; Fig. 3 in Section 3.2). They also constitute minor photoproducts of chlorophyll-*a* photodegradation (Rontani et al., 1991). The high lability of these aldehydes and numerous potential formation processes (Table 2) considerably limits their potential as tracers.

5.7. Phytenic acids ((Z and E)- 3,7,11,15-tetramethylhexadec-2-enoic acids)

There are few reports of (*E*)-phytenic acid in sediments (Boon et al., 1975; Grimalt et al., 1991; Rowland and Maxwell, 1990), although it may not have been recognised in many earlier studies (Volkman and Maxwell, 1986). The presence of (*Z*)-phytenic acid in Recent sediments has only been demonstrated recently (Rontani et al., 1999b). It is generally considered that phytenic acids do not accumulate in sediments because they are readily degraded or converted to phytanic acid (Volkman and Maxwell, 1986). During a quantification of the abundances of isoprenoid compounds in Recent sediments of the Carteau Bay (Golf of Fos, Mediterranean Sea; Rontani et al., 1999b), it was observed that the phytenic acid content declined rapidly with depth in the core analysed, but this degradation took place without any significant production of phytanic acid. The amount of phytanic acid produced corresponded to only 2% of the amount of phytenic acids that had disappeared. Due to the presence of a high proportion of the *Z* isomer, the rapid degradation of phytenic acids was attributed to the involvement of alternating β-decarboxymethylation and β-oxidation sequences induced by denitrifiers (Fig. 6 in Section 4.4).

Phytenic acids have been proposed as indicators of oxic conditions during sedimentation (Boon et al., 1975; de Leeuw et al., 1977a). However, in some lake sediments a direct input of phytenic acid in the form of phytylphytenate from mosses is possible (de Leeuw et al., 1977b), although never proven. A difficulty with the use of these compounds as markers of oxic conditions is the possibility that they may be formed via microbially mediated reactions which may not reflect the redox conditions of the sediment as a whole (Volkman and Maxwell, 1986). Indeed, during aerobic microbial degradation of organic matter, oxygen serves two different functions: that of a terminal acceptor of electrons which are released during oxidation of organic carbon and that of a reactant in a primary attack on the substrate molecules themselves (Schink, 1988). The first function may be transferred in the absence of oxygen to other oxidized compounds, such as nitrate, metal ions, carbon dioxide, or sulfate (although with smaller energy gains), while water and carbon dioxide may fulfil its functions as a reactant in the primary transformation of some substrates, leading to the incorporation of oxygen atoms in the substrate molecules under anoxic conditions. This is the case for the production of phytenic acids

previously observed during the biodegradation of phytol under denitrifying conditions (Rontani et al., 1999b; see Section 4.4), which involves incorporation of the oxygen atom of a water molecule (Schink, 1988). Owing to the involvement of such processes, which seem to play a key role in the degradation of phytol in marine sediments (Rontani et al., 1999b), the use of phytenic acids as tracers of oxic conditions of sedimentation is not recommended.

5.8. Phytanal (3,7,11,15-tetramethylhexadecanal)

This aldehyde was initially observed by de Leeuw et al. (1977a) during thermo-catalysed experiments on phytol and incubation of uniformly labelled ^{14}C-phytol in a sediment core. Its formation during thermo-catalysed simulations was then attributed by Rontani and Grossi (1995) to a pinacolic rearrangement of phytol after protonation of its olefinic carbon-3 (Fig. 9 in Section 4.5). Phytanal might constitute a useful tracer of clay-catalysed processes in sediments but, to our knowledge, there is only one report of phytanal in sediments (Rowland and Maxwell, 1990).

5.9. Phytanic (3,7,11,15-tetramethylhexadecanoic), pristanic (2,6,10,14-tetramethylpentadecanoic), 5,9,13-trimethyltetradecanoic and 4,8,12-trimethyltridecanoic acids

Phytanic, pristanic and 4,8,12-trimethyltridecanoic acids are common in both Recent and ancient sediments (e.g. Blumer and Cooper, 1967; Brooks et al., 1977; Grimalt et al., 1991; Sun et al., 1998; Rontani et al., 1999b). Owing to its numerous formation processes (Table 3), 4,8,12-trimethyltridecanoic acid presents limited potential as a tracer. Simoneit (1977, 1978) noted that in a Cretaceous black shale and in an Aptian black sapropelic mudstone the pristanic acid/phytanic acid ratio was less than 1 which he attributed to an anoxic depositional environment. This hypothesis was supported by the values of the pristane/phytane ratio (see Section 5.13) and by the high concentration of porphyrins found. All the known formation processes of pristanic acid involve aerobic reactions (Table 3), while phytanic acid may be formed during anaerobic biodegradation of phytol (Rontani et al., 1999b). Consequently, unless the anaerobic bacterial production of pristanic acid (notably from pristenes or pristane) can be demonstrated, the use of this ratio as tracer of oxic conditions of sedimentation seems reasonable.

Grimalt et al. (1991) detected relatively high amounts of 5,9,13-trimethyltetradecanoic acid in Santa Olalla sediments and water column particulate matter and considered that the presence of this compound was indicative of oxic decomposition processes. The production of this acid during the anaerobic biodegradation of phytol (Rontani et al., 1999b; Fig. 8 in Section 4.4) does not support this hypothesis.

5.10. Isomeric phytadienes

The production of these dienes has been demonstrated unambiguously only during clay-catalysed degradation (de Leeuw et al., 1977a) and anaerobic biodegradation (Grossi et al., 1998; Schulze et al., 2001) of phytol. The regioselective bacterial dehydration of phytol under sulfate-reducing conditions results in the predominant formation of (*Z* and *E*)-phyta-1,3-dienes (Grossi et al., 1998), while thermal and acid-catalysed dehydration processes produce mixtures of phytadiene isomers containing significant amounts of neophytadiene. Analysis of phytadienes in recently deposited sediments collected at the same site as the sediments used for the in vitro bacterial incubation on phytol (Grossi et al., 1998) showed a strong similarity of the distribution of phytadiene isomers in the incubated slurries and in the fresh sediment core. Consequently, the predominance of (*Z* and *E*)-phyta-1,3-dienes has been proposed as a marker for the anaerobic biodegradation of phytol in sediments provided that the sediments are analysed using an analytical procedure that avoids phytol dehydration (Grossi et al., 1996).

5.11. Isomeric phytenes

Isomeric phytenes are often detected in marine sediments (e.g. Volkman and Maxwell, 1986; Grimalt et al., 1991) and are usually considered as degradation products of phytol (Ikan et al., 1975; de Leeuw et al., 1977a; Grossi et al., 1998) or as lipids of methanogenic bacteria (Risatti et al., 1984). These compounds may be produced from phytol by clay-catalysed degradation (de Leeuw et al., 1977a; Fig. 9 in Section 4.5) or by biodegradation under sulfate-reducing conditions (Grossi et al., 1998; Schulze et al., 2001; Fig. 7 in Section 4.4). Recently, it was also demonstrated that the reduction of phytenethiols by H_2S under mild conditions led to the formation of *Z* and *E* phyt-2-enes as well as isomeric phyt-3-enes (Hebting et al., 2001). The distributions of phytene isomers in recently deposited sediments showed a strong similarity to those collected at the same site used for the in vitro bacterial incubations of phytol (Grossi et al., 1998), particularly the predominance of phyt-2(*E*)-ene. Consequently, such a distribution of phytenes might constitute a potential marker for the anaerobic biodegradation of phytol in sediments.

5.12. Isomeric pristenes

Isomeric pristenes have been isolated from mixed zooplankton and from the liver oils of various marine fishes and mammals (Blumer and Thomas, 1965). The

formation of these compounds was attributed to the degradation of chlorophyll phytyl chain in the diet of the zooplankton (Blumer et al., 1969). Because of their stability in the digestive tract of carnivorous copepods and in marine fishes which feed on zooplankton, these isoprenoid hydrocarbons have been proposed as tracers of food-web processes (Blumer et al., 1969). Moreover, since they are not present in ancient sediments or in petroleum, it was proposed that they could be used to distinguish between hydrocarbons derived from organisms and those from oil pollution (Blumer et al., 1969). However, there are two major problems in the use of these compounds in this way: (i) isomeric pristenes may be formed during clay-catalysed degradation of phytol in sediments (de Leeuw et al., 1977a; Rontani and Grossi, 1995) and (ii) the mechanism proposed for the formation of prist-1-ene during clay-catalysed degradation of phytol (Rontani and Grossi, 1995; Fig. 9 in Section 4.5) could also intervene during chromatographic separations of lipidic extracts on water-deactivated silica gel, affording artificial isomeric pristenes (after isomerisation).

5.13. Pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethyl-hexadecane)

Pristane is present throughout the biosphere where it is either directly introduced during oil spills, or produced during diagenesis from different precursors such as: chlorophyll phytyl chain, bound tocopherols (Goossens et al., 1984), ether lipids of archaea (Rowland, 1990) and bound methylated 2-methyl-2-(5,8,12-trimethyltridecyl)chromans (Li et al., 1995). The formation of pristane from the chlorophyll phytyl chain has been demonstrated during invertebrate feeding (Avigan and Blumer, 1968; Fig. 4 in Section 4.3). Clay-catalysed degradation of phytol produces significant amounts of pristene isomers (de Leeuw et al., 1977a; Fig. 9 in Section 4.5), which may be subsequently reduced pristane in reducing environments.

The ubiquity of phytane in the marine environment, and its structural similarity with phytol, led to the suggestion that phytol is the major precursor of phytane (Didyk et al., 1978; Volkman and Maxwell, 1986), although alternative precursors such as archaebacterial ether lipids (Volkman and Maxwell, 1986; Rowland, 1990) or sulfur-bound phytane (Koopmans et al., 1999) have also been proposed. However, the formation of phytane from phytol in modern sediments has never been demonstrated. Didyk et al. (1978) associated the transformation of phytol to phytane with anoxic sedimentary environments, assuming that phytol would be dehydrated to isomeric phytadienes and then reduced to phytane via phytene isomers. Although the production of phytadienes and phytenes during the biodegradation of phytol by sulfate-reducing bacteria has recently been demonstrated (Grossi et al., 1998; Schulze et al., 2001), the lack of phytane among the degradation products of phytol does not support this hypothesis.

Based on the assumption that pristane is formed from phytol by an oxidative pathway, while phytane is generated through various reductive pathways, the ratio of pristane to phytane has been used as an indicator of the oxicity of the depositional environment (Brooks et al., 1969; Didyk et al., 1978). However, there are several problems with the use of this ratio: (i) the chlorophyll phytyl chain is not the only source of these compounds (see above), (ii) thermal maturation of immature sedimentary rocks containing S-bound phytane may result to the formation of free phytane (Krein and Aizenshtat, 1994; Schouten et al., 1994),) (iii) this ratio has been demonstrated to change significantly with increasing thermal stress (e.g. Tissot et al., 1971; Radke et al., 1980) and (iv) moreover the production of pristene isomers during clay-catalysed degradation of phytol described in the Fig. 9 can proceed in the absence of oxygen. For these different reasons the ratio pristane to phytane must be used very cautiously (and in conjunction with data for other biological marker compounds) as an indicator of oxic or/ anoxic stages of diagenesis.

5.14. Wax esters

Phytyl esters have been detected in a few Recent sediments (Boon and de Leeuw, 1979; Cranwell, 1986). This can be attributed to rapid hydrolysis of these compounds in the sediments (de Leeuw et al., 1977b), or perhaps to the difficulty of detecting these high-boiling compounds using common chromatographic procedures. Proposed sources have included mosses (de Leeuw et al., 1977b), bryophytes (Gellerman et al., 1975; Buchanan et al., 1996), dinoflagellates (Withers and Nevenzel, 1977) or zooplankton (Sargent and Falk-Petersen, 1981). Phytyl esters have been also reported in cultures of a few marine microalgae (Withers and Nevenzel, 1977; Findlay and Patil, 1984), and small amounts are also present in senescent cultures of the green microalga *Tetraselmis chui* (J.K. Volkman, unpublished results). These limited examples suggest that microalgae are not likely to be major sources of phytyl esters in sediments. Recently, it was demonstrated that bacterial degradation of phytol under aerobic (Rontani et al., 1999a,b) and denitrifying conditions (Rontani et al., 1999b) might constitute another potential source of these compounds in sediments (Table 2) and that this process might be enhanced under low ammonium concentrations. During incubation of uniformly labelled ^{14}C phytol in sediments, Brooks and Maxwell (1974) observed the formation of phytyl esters and suggested that esterification of the introduced phytol is brought about by enzymatic processes, possibly microbial. These recent results strongly support this

hypothesis and are also in good agreement with Volkman and Maxwell (1986), who suggested that esterification may be a significant process in microbially active sediments.

Despite these observations, isoprenoid wax esters are rarely abundant in sediments, probably due to their rapid hydrolysis during early diagenesis. Indeed, de Leeuw et al. (1977b) analysed a lake sediment which received inputs of the moss *Fontinalis antipyretica* (in which phytylphytenate is a major wax ester) and failed to detect this ester. They concluded that a rapid hydrolysis of the ester occurred in the detritus, resulting in its absence in the underlying sediment. However, it is interesting to note that despite the occurrence of these hydrolytic processes, Cranwell (1986) detected phytylphytenate in lacustrine sediments up to 40,000 years old. Similarly, esters of sterols are thought to be more stable in sediments than the corresponding free compounds (de Leeuw et al., 1983), suggesting that, under some conditions, esterification can enhance the preservation potential of labile compounds such as phytol.

There is a demonstrable need to identify bacterial metabolites that have sufficient structural specificity to act as biological markers for microbial degradation in the aquatic environment. Some of the isoprenoid wax esters produced during the bacterial metabolism of phytol (Rontani et al., 1999a,b; Schulze et al., 2001; Table 2), which result from the condensation of phytol metabolites between themselves or with phytol could play this role. Consequently, it would be useful to search for these compounds in marine sediments and particulate matter samples.

5.15. *Thiophenes and other sulfurized derivatives of phytol*

The presence of abundant organic sulfur compounds (OSC) in sediments implies that the depositional environment was anoxic and that conditions favoured the sequestration of sulfur by functionalised lipids rather than by detrital iron. Although OSC are found in many environments their formation tends to be favoured in non-clastic environments such as carbonates, siliceous oozes and evaporites (Sinninghe Damsté and de Leeuw, 1990). OSC generally retain the carbon skeletons of their lipid precursors and thus have an excellent potential as paleoenvironmental markers. Moreover, the positions of the S atoms provide a strong indication of the positions of functionalities in the precursor lipids (or in their degradation products as in the case of phytadienes derived from phytol). Although bound lipids can be more abundant than free lipids (e.g. Kohnen et al., 1991c; Putschew et al., 1996) in sediments, the extent of sulfurization depends on the presence of appropriate functional groups. Thus, this selective preservation can lead to a bias in the types of compounds preserved and hence the distributions of OSC may not be fully representative of the original suite of deposited biomarkers (e.g. Kohnen et al., 1991c).

Isoprenoid thiophenes, especially those containing 20 carbon atoms, are probably the most ubiquitous OSC (for a review see Sinninghe Damsté and de Leeuw, 1990) in geological samples. They occur in Recent sediments (e.g. Fukushima et al., 1992; Adam et al., 2000 and refs therein), deep-sea sediments (e.g. Rullkötter et al., 1988; ten Haven et al., 1990), in Recent lacustrine sediments (e.g. Cranwell, 1988; Putschew et al., 1996), in Recent microbial mats (e.g. Kenig and Huc, 1990), in bitumens (e.g. Rullkötter et al., 1990), in crude oils (e.g. Sinninghe Damsté et al., 1987) and in pyrolysates of (proto)kerogens (Kenig and Huc, 1990) and of polar fractions (Sinninghe Damsté et al., 1990). The presence of two structural isomers of isoprenoid 1,2-dithianes and one isoprenoid 1,2,3-trithiepane (Fig. 10 in Section 4.6) in immature sediments from the Peruvian upwelling region has been reported by Kohnen et al. (1989, 1991a). Although a variety of chemical degradation methods (e.g. Raney Ni, Li-EtNH_2, nickel boride, methyl iodide) have now been employed to release these S-bound lipids (see Adam et al., 2000 for leading references), it is a common observation that phytane predominates in the hydrocarbons released by desulfurization of polar fractions (e.g. Putschew et al., 1996).

The sulfurized products derived from phytol are quite different from those derived from phytadienes (Fig. 10; de Graaf et al., 1992). Phytol gives rise to phytenethiols which on further reaction produces various monomeric polysulphides containing 2 and 3 S atoms in a ring system (dithianes, trithiepanes). Phytadienes produce different thiol isomers which can react together to form intermolecular S-linked high molecular weight compounds. The relative proportions of these various products have the potential to provide information about the initial degradation pathways occurring in a particular depositional environment.

C_{20} isoprenoid distributions have been proposed as an indicator of paleosalinity (de Leeuw and Sinninghe Damsté, 1990). Sulfur incorporation into chlorophyll-derived phytol (from algae or vascular plants) yields 3-methyl-2-(3,7,11-trimethyltridecyl)-thiophene and 3-(4,8,12-trimethyltridecyl)-thiophene (Fig. 10 in Section 4.6), while reaction of sulfur with $\Delta^{2,6}$-phytadienol and geranylgeraniol (from some photosynthetic sulfur bacteria and archaebacteria) yields 5 other isomeric thiophenes with an internal thiophenic group. The observed differences in distribution patterns of the C_{20} isoprenoid thiophenes (expressed as an "isoprenoid thiophene ratio" ITR) may thus be explained by the abundance of different species in the depositional environment as a response to paleosalinity, with values of less than 0.5 for ITR indicative of hypersaline paleoenvironments (Sinninghe Damsté et al. 1989b; de Leeuw and Sinninghe Damsté, 1990). A possible complication in this inter-

pretation is that many halophilic purple sulfur bacteria contain bacteriochlorophyll-*a* with a phytyl ester group, so that some hypersaline environments may contain more thiophene isomers of the phytol-type.

Barakat and Rullkötter (1997) tested this index in Miocene lake sediments and proposed a new index (ITTR) that included the thiolane abundances. In most cases, the two indices provided the same ranking of paleosalinity, but in one case the ITTR suggested a higher salinity in agreement with inferences from the chroman distributions. Interestingly, although all samples from highly saline environments showed characteristic low pristane/phytane ratios (ten Haven et al., 1988), none showed an even-over-odd *n*-alkane distribution and only two showed a dominance of C_{35} hopanes. Schreiber et al. (2001) have also noted that supposed indicators of paleosalinity such as gammacerane, 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane, C_{20} isoprenoid thiophenes or chromans are not always present in sediments from hypersaline environments and that their distributions can be affected by both maturation and the type of mineral matrix.

OSC distributions can also provide information about thermal maturity since there is a general trend from thiolanes/thianes to thiophenes to benzo[b]thiophenes to dibenzothiophenes with increasing thermal maturity (Sinninghe Damsté and de Leeuw, 1990 and refs therein). However, exceptions are known and the precise mechanisms and timing of these reactions remain elusive. For example, ten Haven et al. (1990) reported C_{20} isoprenoid thiolanes in sediments from the Falkland Plateau and yet less mature samples from elsewhere in the Atlantic Ocean contained isoprenoid thiophenes and negligible amounts of thiolanes. Rather than thiolanes and thiophenes being chemically interconverted during diagenesis it is possible that they simply derive from the same functionalised precursor (Barakat and Rullkötter, 1997), for which there is evidence from isotope data (Kohnen et al., 1992).

5.16. Other phytol degradation products with potential as tracers

Some of the autoxidation products of phytol (e.g. (*erythro* and *threo*)-3,7,11,15-tetramethylhexadecan-1,2,3-triol, (*erythro* and *threo*)-3,7,11,15-tetramethyl-2,3-hydroxyhexadecanoic acid and (*Z* and *E*)-2,3-epoxy-3,7,11,15-tetramethylhexadecan-1-ol) have been recently detected in Recent sediments (Rontani, unpublished results). The presence of pairs of diastereoisomers and of *Z* and *E* configurations, which is the result of non-stereospecific oxy-free radical processes (Rontani and Aubert, 1994; Fig. 1 in Section 4.1), argues against enzymatic formation since enzymatic processes are generally highly stereospecific. There is a need to search for these compounds in sediments from contrasting sedimentation conditions in order to validate their role as tracers of oxic deposition conditions.

It is noteworthy that small amounts of (*Z*)-3,7,11-trimethyldodecen-2-enoic acid have been also detected in Recent sediments of the Carteau Bay (Gulf of Fos, Mediterranean Sea) (Rontani, unpublished results). This compound, which is produced during the bacterial metabolism of 5,9,13-trimethyltetradecanoic acid (an intermediate in the biodegradation of phytol; Rontani et al., 1999b; Figs. 5 and 8 in Section 4.4) by β-decarboxymethylation processes (Fig. 6 in Section 4.4; Rontani et al., 1997), could turn out to be a highly specific tracer of microbial activity in sediments.

6. Conclusions

The chlorophyll phytyl chain is generally considered to be the main source of isoprenoids with 20 or fewer carbon atoms in geological samples (Volkman and Maxwell, 1986). The present review has shown the great diversity of compounds that may result from the diagenesis of phytol. The relatively great abundance of chlorophylls in photosynthetic organisms does not imply however that other compounds, such as tocopherols or archaebacterial ether lipids, can be ruled out as significant precursors of isoprenoids in the geosphere. The potential survival of a compound during early diagenesis may be also important. Indeed, a high percentage of the phytol biosynthesised by primary producers is probably degraded within the food web and in Recent sediments by the different processes reviewed in the present paper. In tocopherols, for example, the isoprenoid moiety is bonded via a carbon-carbon bond to an aromatic nucleus, favouring survival during early diagenesis. Moreover, these compounds may escape biodegradation once incorporated into macromolecular structures (Goossens et al., 1984). Acyclic archaebacterial ether lipids, which have been detected in a wide variety of marine sediment samples (e.g. Michaelis and Albrecht, 1979; Chappe et al., 1982; Pauly and Van Vleet., 1986), have also been reported to be more resistant to degradation and turnover than are the ester-linked lipids of bacteria and eukaryotes (Harvey et al., 1986). Consequently, planktonic archaea may well contribute to carbon deposition in the sea in part through production, sedimentation and accumulation of their biphytanyl tetraether lipids in marine sediments (DeLong et al., 1998).

Numerous investigations have recently been carried out on the degradation of phytol in sediments. These include aerobic (Rontani et al., 1999a) and anaerobic (Grossi et al., 1998; Rontani et al., 1999b) biodegradation, photodegradation (for a review see Rontani, 2001) and sulfurization (Fukushima et al., 1992; de Graaf et al., 1992; Kok et al., 2000; Adam et al., 2000; Schouten

et al., 2001). Although the results obtained in the course of these studies have significantly increased our understanding of the diagenesis of this widespread isoprenoid compound, there are still many gaps in our knowledge.

Further studies are required to define the role played by hydroperoxide-induced autoxidative processes in the degradation of phytol in senescent phytoplanktonic cells and to elucidate the mechanisms of these complex processes. Some of the autoxidation products formed could turn out to be useful tracers of oxic conditions of sedimentation. These compounds could also give useful indications about the physiological state of phytoplanktonic communities and the residence time of organic matter in the water column.

Interactions and competition between biotic and abiotic degradation processes have also to be better understood. This is particularly the case for autoxidative and bacterial processes, which may be intimately linked (Mouzdahir, 2001) and for sulfurization and biodegradation under sulfate-reducing conditions, which can be considered as two competitive processes (Grossi et al., 1998).

The mechanism of the loss of the phytyl chain observed after visible light irradiation of senescent cells of *Phaeodactylum tricornutum*, which has been attributed to the involvement of photooxidative cross-linking of the intact and/or photooxidized free phytol with itself and/or with other lipids of phytoplankton (Rontani et al., 1998), also needs further investigation. Indeed, it was previously proposed that the photooxidative cross-linking of unsaturated lipids could play a role in the formation of humic acids (Harvey et al., 1983). The hydrophobic microenvironment of phytodetritus, which provides high localised concentrations of unsaturated lipids and visible-light-absorbing photosensitizers (Nelson, 1993), could constitute an ideal site for such reactions.

It was recently demonstrated that several isoprenoid wax esters may be formed during aerobic and anaerobic biodegradation of phytol (Rontani et al., 1999a,b), this process being enhanced at the low ammonium concentrations found in marine sediments. It would be useful to search for these compounds in a range of sedimentary environments in order to determine if bacterial esterification can really enhance the preservation potential of labile compounds such as phytol.

We hope that this review has provided a useful perspective on the strengths and weaknesses of specific biomarkers as indicators of oxic or anoxic conditions of deposition. The situation is undoubtedly complicated by the multiplicity of isoprenoid sources and the potential of anaerobic microorganisms to incorporate oxygen atoms from water or carbon dioxide molecules (Schink, 1988). Although a good understanding of all the main diagenetic pathways involved in organic matter degradation is emerging, many details are still elusive. Isoprenoids were amongst the first compounds proposed as biomarkers for paleoenvironmental purposes (the pristane/phytane ratio being a classic example), but they have also been the most controversial as new studies reveal apparent exceptions to the rules previously proposed. This should not be seen as particularly negative, but rather demonstrates the need to continually refine these proxies and combine information from a variety of sources (biomarkers, sedimentology, palaeontology etc) to provide a consistent picture of the organic matter sources and pathways of degradation in sediments.

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