Photoproducts of phytoplanktonic sterols: a potential source of hydroperoxides in marine sediments?

Jean-François Rontani *, Daphné Marchand

Laboratoire d’Océanographie et de Biogéochimie (UMR 6535), Centre d’Océanologie de Marseille (OSU), Campus de Luminy, case 901, 13288 Marseille, France

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Abstract

A detailed study of the lipid composition of Recent sediments of Carteau Bay (Gulf of Fos, Mediterranean Sea) has made possible the detection of significant amounts of Δ5-stenol photoproducts of phytoplanktonic origin. Photo-degradation of Δ5-stenols in senescent phytoplanktonic cells seems to play a role in the degradation of these compounds in the marine environment. These reactions lead to the production of Δ4-6α/β- and Δ6-5α-hydroperoxy-sterols mainly in esterified and bound forms, which appeared to be relatively well preserved in the sediments. This surprising stability could be attributed to: (i) the weak reducing properties of sulfides towards these hydroperoxides or (ii) the protection of these compounds in intact phytoplanktonic debris. Destruction of hydroperoxides and allylic rearrangement of Δ6-5α-hydroperoxy-sterols (to the corresponding Δ5-7α/β- derivatives) takes place at the bottom of the core analyzed. The detection of high amounts of 5,6-epoxy-24-ethylcholestan-3β-ol (52 ng/g dry sediment at 3.5 cm depth), (resulting probably from the oxidation of 24-ethylcholest-5-en-3β-ol by hydroperoxides in the absence of molecular oxygen) strongly suggests that hydroperoxy-sterols may play a role in the degradation of organic matter in anoxic sediments. Due to their greater stability in sediments, Δ4-6α/β-hydroperoxy-sterols will be more reliable in situ markers of type II photodegradation processes (i.e. those involving singlet oxygen) than Δ6-5α-hydroperoxy-sterols. Dehydration of the reduced sterol photoproducts described in the present work might constitute a potential source of steratrienes, which are often present in the sediments. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Type II photodegradation processes; Sterols; Senescent phytoplankton; Recent sediments; Tracers; Hydroperoxides

1. Introduction

Steroidal alcohols (sterols) are comparatively stable in sediments and hence they have a long geological record (Gagosian et al., 1982). Moreover, they possess structural features, such as positions of double bonds, nuclear methylation and patterns of side-chain alkylation, which can be restricted to a few groups of organisms (Volkman, 1986). Consequently, sterols in seawater act as good tracers of biogenic material and food chains (Steudler et al., 1977) and in Recent sediments they constitute excellent biomarkers for tracing diagenetic transformations (Mackenzie et al., 1982).

Though sterols are generally considered to be more stable than the majority of the organic compounds produced by phytoplankton (Gagosian et al., 1982), only a small part of the sterols produced in the euphotic zone (less than 1% in the equatorial Atlantic Ocean; Gagosian et al., 1982) reach the sediment. This disappearance is generally considered to be the result of biodegradation (Johannes and Satomi, 1966), bacterial degradation (Iturriaga, 1979) and coprophagy (Smayda, 1969).

We have recently demonstrated that Δ5-stenols (the predominant biogenic sterols in most environments) can...
also be quickly photodegraded in senescent cells of phytoplankton (Rontani et al., 1997, 1998). This photodegradation, which involves type II (i.e. involving singlet oxygen) photoprocesses (Rontani et al., 1997), results in the formation of 5α- and 6α/β-hydroperoxides. The surprising stability of these hydroperoxides observed in dead phytoplanktonic cells (no degradation products detected after 21 days of incubation at 20°C) strongly suggests that in the marine environment, measurable amounts of these photoproducts can survive the long transport times to the ocean floor.

In the present work, we provide evidence of the presence of such photoproducts in Recent sediments of the Gulf of Fos (Mediterranean Sea) in order to determine: (i) whether the photodegradation processes participate efficiently in the degradation of Δ5-stenols in the euphotic zone of the oceans, and (ii) whether these photoproducts are sufficiently stable to play the role of tracers of photodegradation processes.

2. Experimental

2.1. Sediment sampling

The top layer (20 cm) of the sediment was collected with a manual corer under 9 m of water. The sealed cores were maintained in isotherm bags (containing dry ice) during their transportation to Marseilles, where they were stored at −20°C until analysis. Station 35 (as numbered in the SEDIFOS program) in Carteau Bay (Gulf of Fos, Mediterranean Sea) was chosen as the site for this study. Its suitability for this work was based on: (i) minor variance in particle size distribution with depth (Bonin et al., 1999) suggesting minimal variation of sedimentary conditions over the profile studied, (ii) relatively high concentrations of chlorophyll phytyl sidechain photodegradation products (ratio photoproducts/unchanged chlorophyll phytyl chain = 0.3; Rontani et al., 1996) and (iii) very weak irradiance at the water-sediment interface owing to the resuspension of sediments (Barranguet, 1994). The oxic layer of the sediment was 4 mm deep (Bonin et al., 1999) and the sedimentation rate of this zone is approximately 0.5–1 cm year−1 (Grenz et al., 1990). The sediment contains a macrofaunal assemblage characteristic of muddy sand. Polychaetes dominated the benthic macrofauna (70%) and crustaceans were the second most dominant group (25%). More than 80% of the organisms were located in the upper 4 cm of sediment (Gilbert et al., 1998).

2.2. Treatment of sediments

All the manipulations were carried out with foil-covered vessels in order to exclude photochemical artifacts.

2.3. Extraction

The wet sediment slices (cut under dim light) were extracted ultrasonically with isopropanol:hexane (4:1, v/v) (Leeuw et al., 1977). Hexane extracts were combined and the isopropanol/water phase was filtered, concentrated under vacuum and then extracted three times with chloroform. The chloroform and hexane extracts were combined, dried over anhydrous Na2SO4, filtered and concentrated by rotary evaporation (at 40°C).

2.4. Reduction of hydroperoxides

Lipidic extracts of sediments were reduced in methanol (25 ml) by excess NaBH4 (10 mg/mg of extract) using magnetic stirring (15 min at 0°C) (Teng et al., 1973). During this treatment steroidal ketones are also reduced and the possibility of some ester cleavage cannot be excluded.

2.5. Alkaline hydrolysis

Saponification was carried out on both reduced and non-reduced samples (lipid extracts or sediments). After reduction, 25 ml of water and 2.8 g of potassium hydroxide were added and the mixture was directly saponified by refluxing for 2 h. In the case of non-reduced samples, an additional 25 ml of methanol was added before saponification. After cooling, the contents of the flask were extracted three times with hexane (sediments were filtered through Whatman qualitative filters before extraction). The combined hexane extracts were dried over anhydrous Na2SO4, filtered and concentrated.

2.6. Hydrogenation

Lipid extracts were hydrogenated overnight under magnetic stirring in methanol with Pd/CaCO3 (10–20 mg/mg of extract) (Aldrich) as a catalyst. After hydrogenation, the catalyst was removed by filtration and the filtrate was concentrated by rotary evaporation.

2.7. Derivatization

After evaporation of solvents, the residues were taken up in 400 μl of a mixture of pyridine and BSTFA (Supelco) (3:1, v/v) and silylated for 1 h at 50°C. After evaporation to dryness under nitrogen, the residues were taken up in ethyl acetate and analyzed by gas chromatography/electron impact mass spectrometry (GC/EIMS).

2.8. Identification and quantification of sterols and their oxidation products

These compounds were identified by comparison of retention times and mass spectra with those of standards
and quantified (calibration with external standards) by GC/EIMS. For low concentrations or in the case of coelutions, quantification was assessed by selected ion monitoring (SIM) with the diagnostic ions at $[M-90]^+$ for 7-hydroxysterols (Rontani et al., 1997), at $[M-143]^+$ for 6-hydroxysterols (Harvey and Vouros, 1979), at $[M-161]^+$ for 3,5,6-triols, at $[M-18]^+$ for 5-hydroxystanols, at $[M-15]^+$ for 7-hydroxystanols and at $M^+$ for the other compounds.

GC/EIMS analyses were carried out with a HP 5890 series II plus gas chromatograph connected to a HP 5972 mass spectrometer. The following operative conditions were employed: 30 m×0.25 mm (i.d.) capillary column coated with HPS (Hewlett Packard) (film thickness, 0.25 μm); oven temperature programmed from 60 to 130°C at 30°C min$^{-1}$ and then from 130 to 300°C at 4°C min$^{-1}$; carrier gas (He) pressure maintained at 1.04 bar until the end of the temperature program and then programmed from 1.04 to 1.5 bar at 0.04 bar min$^{-1}$; injector (on column with a retention gap) temperature, 50°C; electron energy, 70 eV; source temperature, 170°C; cycle time, 1.5 s.

2.9. Standard compounds

Cholest-5-en-3β-ol, 24-methylcholest-5-en-3β-ol, 24-ethylcholest-5-en-3β-ol and 24-ethylcholesta-5,24(28)E-dien-3β-ol were purchased from Aldrich and Sigma. 5α- and 6α/β-Hydroperoxides were obtained after photosensitized oxidation of the corresponding Δ5-stenols in pyridine in the presence of haematoporphyrin as sensitizer (Nickon and Bagli, 1961). Allylic rearrangement of 5α-hydroperoxides to 7α-hydroperoxides and epimerization of the latter to 7β-hydroperoxides was obtained at room temperature in chloroform (Teng, 1990). Subsequent reduction of these different hydroperoxides in methanol with excess NaBH₄ afforded the corresponding diols. Hydrogenation of these diols was carried out with Pd/CaCO₃ as catalyst. Treatment of Δ5-stenols with meta-chlororperoxy-benzoic acid in dry methylene chloride yielded a mixture of 5α,6α- and 5β,6β-epoxides. Heating of these epoxides in the presence of water afforded the corresponding 3β,5α,6β-triols (Holland and Diakow, 1979).

3. Results and discussion

Analyses of the top layer of the sediments revealed the presence of cholest-5-en-3β-ol (cholesterol), 24-methylcholesta-5,22E-dien-3β-ol and 24-ethylcholesta-5-en-3β-ol (sitosterol) as major Δ5-stenols. These compounds are present mainly in “bound” (i.e. non-solvent-extractable) form. Smaller amounts of 24-methylcholesta-5-en-3β-ol

![Fig. 1. SIM ion chromatograms (with the diagnostic ions at m/z 403, 431, 456, 458, 484 and 486) showing the sterol region of extracts: (A) E₃, (B) E₄ and (C) E₅ obtained after different treatments (as described in Fig. 3) of the slice (3–4 cm) of the sediment.](image-url)
(campesterol), 24-methylcholest-5,24(28)-dien-3β-ol and 24-ethylcholesta-5,24(28)-E-dien-3β-ol (fucosterol) were also detected. 24-Methylcholesta-5,22E-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol, not being commercially available, were identified by comparison of their electron impact mass spectra with mass spectral data described in the literature (Lee et al., 1979; Leeuw et al., 1983).

It was previously determined that the major part of the chlorophyll present in the sediments of station 35 in Carteau Bay originated from diatoms (Barranguet, 1994). The presence of 24-methylcholesta-5,22E-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol confirms that diatoms are important constituents of the phytoplankton in that environment (Volkman, 1986; Volkman et al., 1998). Sitosterol may also have a diatom origin (Volkman, 1986).

In the sediment analyzed, every sterol with a Δ5-double bond was accompanied by the equivalent Δ4,3-stenone, 5α(H)-3-stanone and 5α(H)-3β-stanol. The presence of these compounds can be attributed to the well known microbiological conversion of Δ5-stenols as demonstrated during several incubation experiments with labelled cholesterol in recent sedimentary environments (Gaskell and Eglinton, 1975; Mermoud et al., 1984).

GC/EIMS analyses allowed detection of significant amounts of Δ4,3β,6- and Δ4,3β,7-epimeric unsaturated diols probably arising from cholesterol and sitosterol in the sediments (Fig. 1A). Mass spectra of (disilylated) Δ5,3β,7-diols exhibit strong [M – HOSiMe3]+ peaks (Fig. 2A), whereas those of (disilylated) Δ4,3β,6-diols show characteristic [M – 143]+ ions associated with A-ring loss (Fig. 2B). Photooxidation of Δ5-stenols in senescence phytoplanktonic cells involves type II (i.e. involving singlet oxygen) photoprocesses and gives rise to Δ6-5α- and Δ6-5β-allylic hydroperoxides (Rontani et al., 1997). Δ6-5α-Hydroperoxides are relatively unstable and may undergo allylic rearrangement to Δ5-7α-hydroperoxides, which in turn epimerize to Δ5-7β-hydroperoxides (Smith, 1981). Such conversions can occur during the photoreaction itself or during product isolation and analysis (Korytowski et al., 1992). We have previously demonstrated that allylic rearrangement does not occur significantly in dead cells of phytoplankton nor during the extraction, reduction and saponification processes employed (Rontani et al., 1997). It occurs quantitatively in the chromatograph during the GC analysis of (disilylated) Δ6-3β,5α-diols when an on column injector is used, whereas splitless injection results in a complete desilylation (Rontani et al., 1997). To avoid this inconvenient gas chromatographic allylic rearrangement, sterol photoproducts must be hydrogenated before GC/EIMS analyses (Rontani et al., 1998).

Different treatments (Fig. 3) were applied to a slice of superficial sediments (3–4 cm) in order to determine: (i) whether Δ5-3β,7-diols arise from allylic rearrangement of the corresponding Δ6-3β,5α-diols during GC analyses or are actually present in the sediment, and (ii) whether the sediments contain hydroperoxides or alcohols. Comparison of the results obtained in extracts E1, E2 and E3 (Table 1) clearly show that most of the Δ5-3β,6- and Δ5-3β,7-diols are in esterified and bound (i.e. non-extractable) forms. After hydrogenation of double bonds (extracts E4), we detected mainly saturated 3β,5α-diols (Fig. 1B) with lesser amounts of saturated 3β,6αβ-diols (Table 1). Saturated 3β,5α-diols are silylated only at position 3 by the mixture BSTFA/pyridine and easily lose a water molecule upon electron impact. Consequently, their EI mass spectra (Fig. 2C) closely resemble those of Δ4-stenols and comparison of retention times is needed to identify them unambiguously. Saturated 3β,7αβ-diols are present in extracts E4 at trace level (Table 1) and represent only 2% of the Δ5-3β,7-diols content of the corresponding extract E3. It appears that the most of the Δ5-3β,7-diols detected in non-hydrogenated extracts is formed during GC injection and that the allylic

![Fig. 2. Electron impact mass spectra of (A) cholest-5-en-3β,7α-diol (disilylated); (B) cholest-4-en-3β,6αβ-diol (disilylated) and (C) cholesta-3β,5α-diol (silylated at position 3 only).](Image)
rearrangement of 5α-hydroperoxy- and/or 5α-hydroxy-sterols is not significant in the first 3 cm of the sediment.

The quantities of unsaturated 3β,6- and 3β,7-diols decrease considerably if reduction with NaBH₄ is omitted before the alkaline hydrolysis (comparison between extracts E₃ and E₅) (Fig. 1A and C, Table 1). This decrease is accompanied by increased amounts of the corresponding Δ⁵-3β-ol-7-ones and Δ³,5-7-ones (Fig. 4, Table 1) in extracts E₅. The formation of these ketones, which are well known products of biradical oxygen autoxidation of sterols (Smith, 1981), can be attributed to: (i) the involvement of autooxidative processes in the water column or in the aerobic zone of the sediments affording Δⁿ-7α/β-hydroperoxides, (ii) the oxidation of the corresponding sterols during the saponification procedure employed, or (iii) the degradation of Δ⁶-5α-hydroperoxysterols during alkaline hydrolysis (Balci, 1981) (Fig. 5).

<table>
<thead>
<tr>
<th>Compound</th>
<th>E₁</th>
<th>E₂</th>
<th>E₃</th>
<th>E₄</th>
<th>E₅</th>
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<tr>
<td>24-Ethylcholest-5-en-3β,7α/β-diols</td>
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<td>20</td>
<td>31</td>
<td>tr</td>
<td>7</td>
</tr>
<tr>
<td>24-Ethylcholest-4-en-3β,6α/β-diols</td>
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<td>12</td>
<td>18</td>
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<tr>
<td>24-Ethylcholesta-3β,7α/β-diols</td>
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<td>24-Ethylcholesta-3β,5α-diols</td>
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<td>7</td>
<td>35</td>
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<tr>
<td>24-Ethylcholesta-3β,6α/β-diols</td>
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<td>15</td>
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<tr>
<td>24-Ethyl-5,6-epoxycholestan-3β-ols (β + α)</td>
<td>tr</td>
<td>52</td>
<td>tr</td>
<td>tr</td>
<td>21</td>
</tr>
<tr>
<td>24-Ethylcholesta-3β,5α,6β-triol</td>
<td>tr</td>
<td>7</td>
<td>tr</td>
<td>tr</td>
<td>7</td>
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<tr>
<td>24-Ethyl-3β-hydroxycholestan-5-en-7-one</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>9 (nd&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
<tr>
<td>24-Ethylcholesta-3,5-dien-7-one</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>17 (25&lt;sup&gt;d&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Accuracy estimated to be ± 5 ng/g dry sediment.

<sup>b</sup> tr = traces (amounts < 5 ng/g dry sediment).

<sup>c</sup> nd = not detected.

<sup>d</sup> Cold saponification overnight under nitrogen.
The first hypothesis can be discarded. In fact, if \( \Delta^5-3\beta\text{-ol-7-ones} \) and \( \Delta^{5,7}\text{-ones} \) or their parent \( \Delta^5-7\alpha/\beta\)-hydroperoxides were initially present in the sediment analyzed, we would recover relatively large amounts of saturated \( 3\beta,7\)-diols and \( 7\)-stanols after reduction and hydrogenation. This is not the case, since the quantities of saturated \( 3\beta,7\)-diols detected in extracts \( E_4 \) represent only a small proportion (4%) of the amounts of \( \Delta^5-3\beta\text{-ol-7-ones} \) present in extracts \( E_5 \) and we failed to detect \( 7\)-stanols. Metal ions present in the sediments can promote autooxidation of sterols during the hot saponification procedure employed. This hypothesis can be

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**Fig. 4.** Electron impact mass spectra of (A) \( 3\beta\)-hydroxy-24-ethylcholest-5-en-7-one (silylated) and (B) 24-ethylcholesta-3,5-dien-7-one.

**Fig. 6.** Electron impact mass spectra of (A) 5,6-epoxy-24-ethylcholestan-3\( \beta\)-ol (silylated) and (B) 24-ethylcholesta-3\( \beta,5\alpha,6\beta\)-triol (disilylated at positions 3 and 6).

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**Fig. 5.** Proposed degradation pathways of \( 5\alpha\)-hydroperoxysterols during alkaline hydrolysis.
discarded since the production of $\Delta^5$-3$\beta$-ol-7-ones was also observed after an attempt of cold saponification (overnight) carried out under nitrogen (Table 1). The lack of $\Delta^{3,5}$-7-ones observed after this treatment demonstrates that the dehydration of $\Delta^3$-3$\beta$-ol-7-ones during saponification requires heating. The greater part of $\Delta^3$-3$\beta$-ol-7-ones and $\Delta^{3,5}$-7-ones results probably from the degradation of the corresponding $\Delta^6$-5$\alpha$-hydroperoxides during the hot saponification treatment (Fig. 5). These different results seem to indicate that the degradation of sterol hydroperoxides operates slowly in the sediment. Though sulfides produced by sulfate reducing bacteria in the anoxic zone of the sediments are known to easily reduce hydroperoxides (Mihara and Tateba, 1986), we observed that these species are not very efficient reducers of 5$\alpha$-hydroperoxysterols (Rontani 1999, (unpubl. data). This result could explain the surprising preservation of these hydroperoxides observed in the core analyzed.

Grimalt et al. (1991) previously detected different cholesta-3,5-dien-7-ones in sediments of the Santa Olalla Lagoon. These authors considered that the formation of these compounds is mediated via hydroperoxides and attributed their production to water column oxidation processes. Our results support these conclusions and suggest that the dienones detected in the surface sediments of this area probably resulted from the degradation during the treatment (Soxhlet extraction + alkaline hydrolysis) of the corresponding $\Delta^6$-5$\alpha$-hydroperoxysterols produced in the euphotic zone of the water column.

The presence of hydroperoxides in the sediment is supported by the detection of relatively high amounts of isomeric 5,6-epoxy-24-ethylcholestan-3$\beta$-ols [with a large proportion of the $\beta$ isomer (ratio $\beta/\alpha = 8$)] in extracts E2 (Table 1, Fig. 6A). It is well known that attack of sterols by the first-formed sterol hydroperoxides yields both isomeric 5,6-epoxides (5$\alpha$,6$\alpha$-epoxides and 5$\beta$,6$\beta$-epoxides in the ratio 1:8 to 1:11; Smith and Kulig, 1975). The formation of these epoxides was previously observed after heating of aqueous dispersions of cholesterol under aerobic conditions (Chicoye et al., 1968; Smith and Kulig, 1975) or under nitrogen in the presence of 5$\alpha$- and 7$\alpha$/7$\beta$-hydroperoxides (Smith and Kulig, 1975).

It is important to note that under aerobic conditions 7-hydroperoxides constitute major sterol degradation products (ratio 7-hydroperoxides/5,6-epoxides = 18) (Smith

Fig. 7. Depth profiles of cholesterol and its photoproducts (quantified in extracts E3) in sediment core sections of Carteau Bay. (* The proportions of $\Delta^5$-3$\beta$,7$\alpha$/7$\beta$-diols and $\Delta^6$-3$\beta$,5$\alpha$-diols were determined after hydrogenation).
and Kulig, 1975). As was demonstrated above after hydrogenation, 7-derivatives are present in very low amounts in the slice of sediment analyzed (ratio saturated 3β,7-diols/5,6-epoxides = 0.02). Consequently, we attributed the production of these epoxides to oxidation of Δ5-stenols by their own 5- and 6-hydroperoxides under anaerobic conditions. It is generally considered that in sediments, spontaneous oxidation of Fe(II) and other metal ions at the surface of oxide minerals generates a superoxide radical, which decomposes to H₂O₂ (Sawyer, 1991). Although attack of H₂O₂ on Δ5-stenols can constitute an additional source of 5,6-epoxides (Smith et al., 1978), the absence of significant amounts of 7-hydroperoxides does not support the involvement of such a pathway. We also detected 24-ethylcholesta-3β,5α,6β-triol (Table 1; Fig. 6B), arising probably from hydration of the corresponding 5,6-epoxides (Smith, 1981). We failed to detect significant amounts of 5,6-epoxycholestan-3β-ol and cholesta-3β,5α,6β-triol in the different extracts analyzed.

Significant amounts of cholesta-3β,5α-diols and traces of cholesta-3β,6αβ-diols were also detected in extracts E₃ (Table 1). We attributed the production of these compounds to an anaerobic microbial hydrogenation of the corresponding 5- and 6-hydroxysterols similar to that responsible for the conversion of Δ5-stenols to stanol (Gagosian et al., 1980; Leeuw and Baas, 1986). It can be noted that the quantities of cholesta-3β,5α-diols decreases if the reduction with NaBH₄ is omitted before alkaline hydrolysis (comparison between extracts E₃ and E₄).
E5; Table 1). Some hydrogenation seems to take place before reduction of the 5-hydroperoxy group afforded cholestan-3β,5α,6β-hydroperoxides. Alkaline hydrolysis of such compounds must result in the formation of tertiary alkoxyl radicals, which can either abstract a hydrogen atom (from other molecules) to give the corresponding diols or undergo β-fragmentation processes leading to A- or B- ring cleavage.

Cholesterol and sitosterol photoproducts were quantified in extracts E3 of different core sections covering 18 cm of sediment. Figs. 7 and 8 depict the concentration profiles with depth of sterols and their photoproducts in the core sections investigated. Concentrations of Δ⁴,6αβ-hydroperoxides and Δ⁴,6αβ₃-3β,5α,6β₂-diol (calculated for the first 10 cm of sediment) are relatively close to that of their parent sterol (Table 2). Consequently, information relative to the photo-degradation state of phytoplankton carried by the ratio 5- or 6-hydroxysterols/intact sterols must not be strongly altered during early diagenesis. However, hydrogenation experiments showed that at the bottom of the core Δ⁴,6αβ₃-3β,5α,6β₂-diol and Δ⁴,6αβ₃-3β,5α,6β₂-diols decay via allylic rearrangement to their Δ⁵,6β₃,7αβ-derivatives (Figs. 7 and 8). This can be attributed to: (i) a progressive destruction of the structural integrity of cell membranes with increasing core depth or (ii) the well known efficient microbial degradation of unsaturated fatty acids in sediments (Cranwell, 1976). The absence of allylic rearrangement of Δ⁴,5α-hydroperoxyxysterols in phytodetritus was in fact previously attributed to the high Δ⁹ unsaturated fatty acid content of phytoplanktonic cell membranes (Rontani et al., 1997). Korytowski et al. (1992) have previously observed that 5α-hydroperoxides are more stable in membranes containing unsaturated phospholipids than in those containing saturated phospholipids. This stability was attributed either to hydrogen bonding between the unsaturated fatty acyl chain of phospholipids and 5α-hydroperoxides which could hinder the allylic rearrangement (Nakano et al., 1980), or to differences of polarity in the carbon 7-10 zone of the fatty acyl chain (where sterols tend to localise in phospholipid/sterol bilayers; MacIntosh, 1978).

7-Hydroxyxysterols cannot constitute specific tracers of photooxidative processes, since they may also be formed by autoxidation (Van Lier and Smith, 1970; Smith, 1981) and bacterial degradation (Krischenoski and Kieslich, 1993; Mahato and Garai, 1997) of Δ⁵-stenols. Consequently, due to their relative stability in sediments, Δ⁴,6αβ₃-3β,5α,6β₂-hydroperoxy- and Δ⁴,6αβ₃-3β,5α,6β₂-hydroxysterols [which are produced in higher proportion in cell membranes than in homogeneous solutions (Korytowski et al., 1992)] may be considered to be better tracers of photooxidative processes than Δ⁴,5α-hydroperoxy- and Δ⁴,5α-hydroxysterols.

The destruction of the integrity of cell membranes must also favour hydroperoxide degradation. The decrease of 5,6-epoxides and 3β,5α,6β-triol concentrations observed at the bottom of the core (Fig. 9) supports this hypothesis; however, this decrease can also be explained by a simple reduction of interactions between Δ⁵-stenols and their corresponding hydroperoxides after destruction of cell membranes.

Photoproducts of cholesterol are present throughout the core in lower proportion than those of sitosterol (Figs. 7 and 8). This can be explained by the fact that (i) zooplankton faecal pellets are considered to be important sources of cholesterol in marine sediments (Volkman, 1986) and (ii) photodegradation rates are slower in copepod faecal pellets than in senescent phytoplanktonic cells (Nelson, 1993). Such an origin of cholesterol and its photoproducts could also explain the lack of 5,6-epoxycholestan-3β-ol in the sediments. Indeed, the modification of the structural integrity of phytoplanktonic cells during the feeding of zooplankton might result in a decrease in the proximity of hydroperoxides to their parent sterol. The very high concentrations of cholesterol observed in the sublayer (0-2 cm) (Fig. 7) may also be attributed to the macrofauna (Parrish et al.,...
1996; Sun et al., 1998), which is mainly located in the upper 4 cm of sediment (Gilbert et al., 1998).

Several authors have mentioned the presence of steratrienes with the three double bonds in the AB-ring system in sediments (Gagosian and Farrington, 1978; Wardroper, 1979; Grimalt et al., 1991). The formation of these compounds has been attributed either to a dehydrogenation of the 3,5-steradienes (Leeuw and Baas, 1986), or to a microbial hydroxylation of 5-ste-nols (Holland and Diakow, 1979) followed by dehydration (Gagosian et al., 1980). Dehydration of the reduced sterol photoproducts described in the present work constitutes another potential source of these trienes (Fig 10).

4. Conclusions

Significant amounts of photoproducts resulting from the addition of $^1$O$_2$ (Frimer, 1979) to the $^5$-double bond of cholesterol and sitosterol have been detected in the sediment core analyzed. These results show that photodegradation of $^5$-stenols in senescent or dead phytoplanktonic cells plays a role in the degradation of these compounds in the marine environment. Though 24-methylcholesta-5,22$E$-dien-3$\beta$-ol, 24-methylcholesta-5,24(28)-dien-3$\beta$-ol and 24-ethylcholesta-5,24(28)$E$-dien-3$\beta$-ol are also present in this sediment, we failed to detect significant amounts of their photoproducts. This can be attributed to the addition of $^1$O$_2$ to the double bond localised on the side-chain of these sterols, which likely leads to the production of unidentified photoproducts.

The surprising preservation of hydroperoxysterols observed throughout the core could be attributed to the weak reducing properties of sulfides towards these compounds. These photoproducts, which are present mainly in esterified and bound forms, could also be protected in intact phytoplanktonic membranes of well-silicified diatoms. Indeed, it is generally considered that most of the bound materials observed in sediments represent the contents of intact biological debris (Cranwell, 1978; Sun et al., 1993).

$\Delta^5$-$\Delta$Hydroperoxysterols (or the corresponding $\Delta^6$-$\Delta$-hydroxyesters) are potential type II photodegradation markers, not only because they are the major products of singlet oxygen attack on the steroidal $\Delta^5$-$\Delta$-system, but also because biological functionalization of steroids at C-5 is rare. If these compounds are particularly stable in phytodetritus (Rontani et al., 1997, 1998), they decay slowly in the sediment to their corresponding $\Delta^5$-$\Delta$- derivatives, which are not selective markers. Microbial C-6 hydroxylation of some $\Delta^4$-steroidal compounds is known (for reviews see Mahato and Majumdar, 1993; Mahato and Garai, 1997) but results only to the formation of $\Delta^4$-epimers. Consequently, $\Delta^4$-$\Delta$-hydroperoxysterols (or
the corresponding $\Delta^4$-6$\alpha$/6$\beta$-hydroxysterols) may be considered as more reliable in situ markers of type II photodegradation processes than $\Delta^6$-5$\alpha$-hydroperoxides (Korytowski et al., 1992).

High amounts of isomeric 5,6-epoxy-24-ethylcholestan-3$\beta$-ols have been detected in the sediments analyzed. The formation of these compounds was attributed to the oxidation of 24-ethylcholest-5-en-3$\beta$-ol by hydroperoxides in the absence of molecular oxygen. These results suggest that hydroperoxyesters can be sufficiently stable in sediments to play a role in the degradation of organic matter under anoxic conditions. In future studies, we intend to use HPLC/MS–MS analyses in order to confirm these results and to directly prove the existence of sterol hydroperoxides in the sedimentary material.

Steroidal $\Delta^4$-3$\beta$,6$\alpha$/6$\beta$-diols could be associated with some photoproducts of the chlorophyll phytyl chain (Cuny and Rontani, 1999) and monounsaturated fatty acids (Rontani, 1998) to constitute a “pool” of useful indicators of photooxidative alterations of phytoplankton. This “pool” could be used to validate photoinduced oxidative processes in Recent sediments, providing new ways to gain information on current environmental problems related to ozone depletion.

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