Identification of di- and triterpenoid lipid tracers confirms the significant role of autoxidation in the degradation of terrestrial vascular plant material in the Canadian Arctic

Rontani, J-F

http://hdl.handle.net/10026.1/9234

10.1016/j.orggeochem.2017.03.011
Organic Geochemistry
Elsevier BV

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.
Identification of di- and triterpenoid lipid tracers confirms the significant role of autoxidation in the degradation of terrestrial vascular plant material in the Canadian Arctic

Jean-François Rontani*, Marie-Aimée Galeron, Rémi Amiraux, Lise Artigue, Simon T. Belt

*a Aix Marseille Université, Université de Toulon, CNRS/INSU/IRD, Mediterranean Institute of Oceanography (MIO) UM 110, 13288 Marseille, France

*b Laboratoire d'études en géophysique et océanographie spatiales (LEGOS), Université Paul Sabatier, 31400 Toulouse, France

*c Biogeochemistry Research Centre, School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, Devon PL4 8AA, UK

* Corresponding author. Tel.: +33-4-86-09-06-02; fax: +33-4-91-82-96-41.

E-mail address: jean-francois.rontani@mio.osupytheas.fr (J.-F. Rontani).
ABSTRACT

Autoxidation products of specific lipid components of angiosperms (betulin, α- and β-amyrians) and gymnosperms (dehydroabietic acid) and their parent compounds were quantified in surface sediments collected from different regions of the Canadian Arctic. The high autoxidation proportions observed in all the sediments investigated (mean values 60.1 ± 14.0%, 91.8 ± 1.1, 98.7 ± 2.2 and 96.6 ± 5.4% for dehydroabietic acid, betulin and α- and β-amyrians, respectively) confirms, unambiguously, the important role played by autoxidation in the degradation of vascular plant material in the region and its enhancement in seawater. The enhancement of these processes in Arctic waters could be the result of intense photooxidation during the senescence of the organisms within the terrestrial environment.

Key words. Canadian Arctic; Surface sediments; Autoxidation; Vascular plants; lipid tracers; Angiosperms; Gymnosperms.
1. Introduction

Understanding the alteration of organic matter (OM) discharged by rivers to the oceans has been an increasing area of research over the past few decades. Indeed, rivers play a major role in exporting terrestrial organic carbon (TerrOC) from the continents to the oceans (Burdige, 2005; Bianchi et al., 2009; Cui et al., 2016). TerrOC is a heterogeneous mixture of recent vascular plant detritus, associated soil OC, petrogenic OC and black carbon (Galy et al., 2007; Hedges, 1992). Vascular plant residues, characterized by a high content of recalcitrant biomacromolecules such as lignin, tanin, cutin and suberin, have been considered to be refractory with respect to further decomposition in the ocean (e.g. de Leeuw and Largeau, 1993; Wakeham and Canuel, 2006). However, it was estimated recently that the amount of C delivered to inland waters is about twice that delivered to the ocean (1.9 Pg C/yr vs. 0.9 Pg C/yr; Cole et al., 2007), suggesting that TerrOC in these systems is more labile than previously thought. Several recent studies have confirmed that, under some oceanographic conditions, particulate OM (POM) delivered by rivers may be sensitive to microbial remineralization in the Arctic shelf areas (van Dongen et al., 2008; Karlsson et al., 2010; Vonk et al., 2010). These observations are consistent with the role of estuaries as a source of CO₂ for the atmosphere (Raymond et al., 1997, 2000; Frankignoule et al., 1998; Cai et al., 2006, 2014). This unexpected microbial lability of terrestrial OM (TerrOM) may be attributed to: (i) the fact that bacterial assemblages in the marine environment can use specific parts of terrestrial POM more effectively than such assemblages in soils and rivers (Garneau et al., 2008), (ii) the involvement of a ‘priming effect’ (enhanced remineralization of terrestrial OM in the presence of fresh substrates from an algal source; Bianchi, 2011; Ward et al., 2016), or (iii) the formation of free radicals from extracellular non-enzymatic steps, including those generated during wood decomposition by certain Basidiomycotina fungi (i.e. brown-rot fungi; Goodell, 2003).
It is important to note, however, that the degradation of TerrOM is not restricted to biotic processes. Indeed, although often under-considered, abiotic processes such as photooxidation and autoxidation (spontaneous free radical reaction of organic compounds with $O_2$) can also play a role in the degradation of TerrOM. Due to the presence of chlorophyll, an efficient photosensitizer (Foote, 1976), visible light-induced photosensitized oxidation may be intense during the early senescence of vascular plants. Such photooxidation reactions involve mainly singlet oxygen ($^1O_2$) as the primary oxidant, which can act not only on the unsaturated lipid components of membranes (Rontani et al., 1996), but also on cutin (Rontani et al., 2005). In contrast, the mechanism by which autoxidation is initiated in senescent vascular plants seems to be via homolytic cleavage of photochemically produced hydroperoxides (Girotti, 1998; Rontani et al., 2003).

It is generally thought that the Arctic should provide the earliest and most dramatic manifestations of global change (Stroeve et al., 2007), with the destabilization of permafrost and its consequences for hydrology and plant cover expected to increase the input of terrigenous carbon to coastal seas (Benner et al., 2003; Schuur et al., 2009, 2015). However, before the influence of global change on the delivery and preservation of OC over the Arctic shelves can be predicted with greater confidence, a more complete understanding of the fundamental processes that control the degradation and preservation of terrigenous OM is required.

One approach to tacking this research theme is through the quantification of source specific lipids and their degradation products, especially if the latter are characteristic of unique transformation pathways. Recently, certain sterols and their biotic and abiotic degradation products in suspended particulate matter (SPM) from surface waters in the Mackenzie River mouth to the Beaufort Sea shelf (Canadian Arctic) were quantified (Rontani et al., 2014). Strong autoxidation of 24-ethylcholesterol (sitosterol) and 24-methylcholesterol
(campesterol) - components of vascular plants (Lütjohann, 2004) - was observed in some samples from the outer boundaries of the plume, suggesting that these radical processes play an important role in the degradation of vascular plant debris in near-coastal Arctic regions. However, since these sterols may be also produced by certain phytoplankton (Volkman, 1986; 2003), this conclusion remained equivocal. As such, in a subsequent study, we then identified (Rontani et al., 2015; Galeron et al., 2016a,b) autoxidation products of well-known di- and triterpenoid tracers of vascular plants (Fig. 1), i.e. dehydroabietic acid (8,11,13-abietatrien-18-oic acid; DHAA, 1), betulin (lup-20(29)-en-3β,28-diol, 2) and α- and β-amyrrins (urs-12-en-3β-ol and olean-12-en-3β-ol) (3 and 4).

Here, we aimed to quantify these same di- and triterpenoid lipids and their oxidation products in surface sediments from different regions of the Canadian Arctic, in order to confirm the key role played by autoxidation during the degradation of vascular plant material at high latitude settings. Indeed, often under-considered abiotic processes such as photooxidation and autoxidation may contribute to the degradation of the increasing amounts of organic carbon contained within permafrost released into Arctic waters.

2. Experimental

2.1. Sediment sampling

Sediment material was collected from 19 locations (Fig. 2) as part of the ArcticNet and IPY-CFL system studies on board the CCGS Amundsen in 2005 and 2008. In each case, surface samples (ca. 0–1 cm) were collected from box cores, freeze dried, and stored (< 4 °C) prior to analysis.
2.2. Sediment treatment

Sediments were placed in MeOH (15 ml) and hydroperoxides were reduced to the corresponding alcohols with excess NaBH$_4$ (70 mg, 30 min at 20°C). Due to their relatively high stability, hydroperoxides derived from autoxidation of amyrins and betulin (Fig. 1) were unaffected by this reduction step (Galeron et al., 2016a,b). Saponification was carried out on each reduced sample. After NaBH$_4$ reduction, water (15 ml) and KOH (1.7 g) were added and the mixture directly saponified by refluxing (2 h). After cooling, the contents of the flask were acidified (HCl, to pH 1) and extracted 3x with dichloromethane (DCM) (30 ml). The combined DCM extracts were dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to give the total lipid extract (TLE).

2.3. Derivatization

The TLE was derivatized by dissolving them in 300 µl pyridine/bis(trimethylsilyl)trifluoroacetamide (BSTFA; Supelco; 2:1, v/v) and silylated (50°C, 1 h). After evaporation to dryness under a stream of N$_2$, the derivatized residue was dissolved in hexane/BSTFA (to avoid desilylation) and analyzed using gas chromatography–electron ionization quadrupole time of flight mass spectrometry (GC–QTOF).

2.4. GC-QTOF

DHAA (1), betulin (2), α- and β-amyrins (3 and 4) and their oxidation products were identified and quantified using an Agilent 7890B/7200 GC-QTOF System (Agilent Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). A cross-linked 5%
phenyl-methylpolysiloxane (Macherey Nagel; Optima 5-MS Accent) column (30 m × 0.25 mm, 0.25 μm film thickness) was employed. Analysis was performed with an injector operating in pulsed splitless at 280 °C and the oven temperature programmed from 70 °C to 130 °C at 20 °C/min, then to 250 °C at 5 °C/min and then to 300 °C at 3 °C/min. The carrier gas (He) was maintained at 0.69 × 10^5 Pa until the end of the temperature program. Instrument temperatures were 300 °C for transfer line and 230 °C for the ion source. Accurate mass spectra were recorded across the range m/z 50-700 at 4 GHz. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12252 from m/z 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was utilized for daily MS calibration. Compounds were identified by comparison of their TOF mass spectra, accurate masses and retention times with those of standards, either purchased or synthesized in the laboratory (see following section). Quantification of each compound involved extraction of specific accurate fragment ions, peak integration and determination of individual response factors using external standards.

2.5. Standards

DHAA (1), betulin (2) and α- and β-amyrins (3 and 4) were obtained from Sigma-Aldrich. The synthesis of 7α/β-hydroxydehydroabietic acids (7 and 8), lupan-20-one-3β,28-diol (10), 3β,28-dihydroxy-lupan-29-oic acid (11), 3β-hydroxy-urs-12-en-11-one (13) and 3β-hydroxy-olean-12-en-11-one (15) (Fig. 1) was described previously (Galeron et al., 2016a, b; Rontani et al., 2015).

3. Results and discussion
3.1. Autoxidation of angiosperms

A variety of pentacyclic triterpenoids with structures based on the ursene (e.g. α-amyrin, 3), oleanene (e.g. β-amyrin,(4) or lupene (e.g. betulin, 2) skeletons are common in angiosperm material such as leaves, bark, roots and wood. Due to their ubiquity, such compounds are typically used as general tracers of vascular plant input (Pancost and Boot, 2004; Otto et al., 2005; Vàsquez et al., 2012). Diagenetic degradation processes of triterpenoids have been studied extensively in previous studies and involve initial loss of any oxygenated functionality at the C(3) position, which leads to the formation of the corresponding di-unsaturated counterparts (ten Haven et al., 1991), subsequent cleavage of ring A, and progressive aromatization of the skeleton (Diefendorf et al., 2015).

We demonstrated previously that autoxidation of betulin (2) affords two main products (Galeron et al., 2016a) (Fig. 1): 29-peroxy-20-hydroperoxy-lupan-3β,28-diol (9) and 3β,28-dihydroxy-lupan-29-oic acid (11). Compound 9 appeared to be unaffected by NaBH₄ reduction employed during the treatment of samples and was thermally cleaved to lupan-20-one-3β,28-diol (10) during GC injection. Compounds 10 and 11 could be detected in significant proportion in dry leaves of vascular plants and were thus selected as tracers of autoxidation of angiosperm material (Galeron et al., 2016a). Autoxidation of α- and β-amyrins (3 and 4) affords 11α-hydroperoxy-urs-12-en-3β-ol (12) and 11α-hydroperoxy-olean-12-en-3β-ol (14), respectively, which are also unaffected by NaBH₄ reduction and were cleaved to the corresponding ketones (13 and 15) during GC injection (Fig. 1; Galeron et al., 2016b). 3β-Hydroxy-urs-12-en-11-one (13) and 3β-hydroxy-olean-12-en-11-one (15) could be detected in dry leaves of vascular plants and in natural samples and thus were selected as tracers of autoxidation of angiosperm material (Galeron et al., 2016b).
GC-QTOF analysis allowed detection and quantification of these different tracers and their parent compounds in TLEs from all the surface sediment samples, despite the considerable spatial coverage (Figs. 3 and 4). The results, summarized in Tables 1 and 2, demonstrate, unambiguously, the extremely high efficiency of autoxidation towards lipid components of Arctic angiosperms. Thus, the mean autoxidation proportions for betulin (2) and α- and β-amyris (3 and 4) were 91.8 ± 1.1, 98.7 ± 2.2 and 96.6 ± 5.4%, respectively. Despite careful target analyses (± 10 ppm) of TOF chromatograms using accurate fragment ions of the main diagenetic degradation products of these triterpenoids (triterpenes, triterpadienes, triaromatic triterpenoids) (Diefendorf et al., 2014), we failed to detect significant amounts of such compounds in any of the TLEs.

It is important to note, however, that diagenetic remineralization processes could also act to varying extent on biological triterpenoids and their autoxidation products, thus influencing our autoxidation estimates. For example, it was shown previously that the simultaneous presence of an oxygenated functionality and a Δ12 double bond in the ring C of triterpenoids (as is the case for compounds 13 and 15) can facilitate their aromatization (Poinsot et al., 1995). The diagenetic remineralization of autoxidation products of α- and β-amyris (3 and 4) should thus be enhanced relative to their parent compounds, with consequential underestimation of autoxidation extent. The same influence is also likely for the autoxidation products of betulin (2), due to the presence of oxygenated functionalities (ketone or acid) on the isopropyl group.

3.2. Autoxidation of gymnosperms

Although DHAA (1) is only a minor component of fresh resin conifers, its abundance increases with age, at the expense of the corresponding abietadienic acids. DHAA in
sediments is thus often used as a biomarker of gymnosperms (Brassell et al., 1983; Otto et al., 2005), although its selectivity in paleobotanical and geochemical studies was challenged recently by Costa et al. (2015), following its detection in several cyanobacteria. However, in the environment, the amount of DHAA (1) of plant origin is likely to be much higher than that of cyanobacterial origin, and the validity of most of the studies using it as tracer of gymnosperms is unlikely to be called into question by these recent findings. In oxidizing environments, diterpenoids are decarboxylated and/or dehydrated and then progressively aromatized (Diefendorf et al., 2014). Thus, the degradation of DHAA (1) leads to the formation of abietatetraenoic acids, norabietatrienes and norabietatetraenes (Otto and Simoneit, 2001).

Autoxidation of DHAA (1) mainly involves the formation of hydroperoxide groups at the thermodynamically favored allylic (C-7) position (Fig. 1; Rontani et al., 2015). 7α/β-Hydroperoxydehydroabietic acids (5 and 6) could therefore act as potential tracers of the autoxidation of OM from gymnosperms in the environment. However, due to their thermal instability, it is necessary to first reduce these primary oxidation products to the corresponding 7α/β-hydroxydehydroabietic acids (7 and 8) (Fig. 1) in order to quantify them in natural samples using GC-MS. It may be noted that some bacteria are also able to oxidize DHAA (1) to 7-α/β-hydroxydehydroabietic acids (7 and 8; Doménech-Carbó et al., 2006). However, they generally do not accumulate these metabolites and so should not significantly bias the use of 7 and 8 as tracers of autoxidation of gymnosperm material.

Target analyses (± 10 ppm) of TOF chromatograms using accurate fragment ions of the main diagenetic degradation products of DHAA (1) (abietatetraenoic acids, norabietatrienes and norabietatetraenes) (Otto and Simoneit, 2001), clearly showed that these processes did not act significantly on DHAA (1) in the surface sediment samples investigated.
In contrast, and consistent with our lipid autoxidation data for angiosperms, 7-α/β-hydroxydehydroabietic acids (7 and 8) could also be detected in significant amounts in the TLEs of all the Arctic surface sediment samples (Table 3, Fig. 5), although the extent of autoxidation for gymnosperms (mean 60.1 ± 14.0%), was somewhat lower than that for angiosperms (see above). This difference of autooxidative degradation state could potentially be attributed to: (i) the trapping of DHAA (1) and other diterpenoids within the resinous tissue of the conifers (Otto and Simoneit, 2001), which could limit exposure to O₂, or (ii) the expected greater aqueous solubility of products 7 and 8 relative to their parent compounds, which would result in an underestimation of autoxidation extent.

3.3. Biogeochemical implications of the results

Autoxidation, largely ignored until recently for the marine realm, proceeds by a radical chain reaction and acts mainly on organic compounds possessing C=C or C-H bonds whose bond energies are relatively low (e.g. allylic, tertiary, α to oxygen etc.; Fossey et al., 1995). It can act not only on unsaturated lipids (e.g. sterols, unsaturated fatty acids, chlorophyll phytlyl side chain, alkenes, tocopherols and alkenones; Rontani, 2012), but also (and often at a similar or higher rate; Davis, 2005) on amino acids (Seko et al., 2010), nucleic acids (Pryor, 1982) and carbohydrates (Lawrence et al., 2008). Consequently, the strong autoxidation state of di- and triterpenoid components of vascular plants in Arctic surface sediments described here, suggests strongly that numerous other organic components of these organisms should also be strongly affected. Moreover, it is interesting to note that autoxidation can also affect biopolymers (Schmid et al., 2007), lignin (Palmer et al., 1987; Waggoner et al., 2015) and kerogen (Fookes and Walters, 1990), inducing ring opening and chain cleavage, which may then enhance bacterial degradation of these (generally considered) recalcitrant substrates.
(Bianchi, 2011; Bianchi and Bauer, 2011). Such interactions could play a role in the loss of lignin often observed during export of terrestrial OM from points of deposition in soil to DOM in natural waters (Opsahl and Benner, 1997).

The high efficiency of autoxidation in vascular plant debris from the Canadian Arctic might be attributed to enhanced photooxidation of senescent vascular plants in the region. Indeed, homolytic cleavage of photochemically-produced hydroperoxides (relatively stabilized at low temperature) can initiate free radical oxidation chains (Girotti, 1998; Rontani et al., 2003). Moreover, photooxidation processes can degrade phenols (Opsahl and Benner, 1993), which are present in significant concentrations in higher plants (Zapata and McMillan, 1979), and can inhibit autoxidation processes due to their strong antioxidant properties. This is supported by the enhancement of Type II (i.e. involving $^{1}\text{O}_2$) photosensitized oxidation of phytoplankton lipids observed in the Arctic (Rontani et al., 2012). This apparent paradox (i.e. increased photooxidation despite relatively low temperature and solar irradiance) has been attributed recently by Amiraux et al. (2016) to: (i) the relative preservation of the sensitizer (chlorophyll) at low irradiance, which permits a longer production time for $^{1}\text{O}_2$, and (ii) the slower diffusion rate of $^{1}\text{O}_2$ through the cell membranes at low temperature (Ehrenberg et al., 1998), thereby favoring the intracellular involvement of type II photosensitized reactions.

Autoxidation of vascular plant debris can be initiated within their native terrestrial setting or during their riverine or atmospheric transport towards the marine environment as demonstrated by the recent detection of significant proportions of autoxidation products of α- and β-amyrins (3 and 4) in particles collected in the Mackenzie River (41.5 ± 17.7% and 20.1 ± 6.4% for α- and β-amyrins, respectively) (Galeron, 2016). Interestingly, these proportions were considerably lower than those observed at the stations close to the mouth of the Mackenzie River (i.e. stations 434 and 428) (Table 2). The use of specific lipid tracers therefore shows that autooxidative degradation processes are strongly enhanced in vascular
plant debris following their discharge from Arctic rivers into the adjacent seas, thus
confirming our previous conclusions based on the use of sterol tracers (Rontani et al., 2014).

In summary, it is becoming increasingly clear that biodegradative, autoxidative and
photooxidative degradation processes within such systems are inextricably linked, and that an
understanding of their interactions, although complex, is fundamental to the precise
identification of the balance between degradation and preservation of vascular plant material
during sedimentation.

4. Conclusion

Quantification of specific di- and triterpenoid lipid tracers (betulin, α- and β-amyrin
and DHAA) and of their autoxidation products in a suite of surface sediment samples from
across the Canadian Arctic allowed us to confirm preliminary results obtained from the
Beaufort Sea (Rontani et al., 2014) and to demonstrate, unambiguously, the strong tendency
for vascular plant debris to undergo autoxidation in the region. This strong autoxidation has
the potential to increase the bioavailability of the detrital fragments of higher plants. It is
proposed that this enhancement of autoxidation results from a very intense photooxidation of
senescent vascular plants on land and in Arctic rivers. These processes may be especially
significant for such regions in the future, since climatically induced destabilization of
permafrost is expected to increase the input of terrigenous carbon to coastal seas.

Acknowledgements

This work is a contribution to the Labex OT-Med (n° ANR-11-LABX-0061) funded
by the French Government «Investissements d’Avenir» program of the French National
Research Agency (ANR) through the A*MIDEX project (n° ANR-11-IDEX-0001-02). It was supported by the LEFE-CYBER (Les Enveloppes Fluides et l'Environnement) national program, as part of the MORTIMER (Matière ORganique Terrestre rejetée par les fleuves et les rivieres en MER) research program. Thanks are due to the FEDER OCEANOMED for the funding of the apparatus employed during this work. We are also grateful to G. Massé, A. Rochon and the officers and crew of the CCGS Amundsen for help with obtaining box core sediment material. Finally, we thank Dr. T.S. Bianchi and an anonymous reviewer for their useful and constructive comments.

References


dehydroabietic and abietic acids are widespread in cyanobacteria. Nature Scientific Reports, doi: 10.1038/srep23436.


Lawrence, G.D., Mavi, A., Meral, K., 2008. Promotion by phosphate of Fe(III) and Cu(II)-catalyzed autoxidation of fructose. Carbohydrate Research 343, 626-635.


Figure captions

Fig. 1. Summary of formation of lipid tracers of autoxidation employed in the present work.

Fig. 2. Summary map showing sampling locations.

Fig. 3. Partial ion chromatograms (m/z 365.3208, 395.3310, 455.3709, 496.4149 and 498.3915) showing the presence of betulin (2) and its degradation products (lupan-20-one-3β,28-diol (10) and 3β,28-dihydroxy-lupan-29-oic acid (11)) in surface sediment from sample location NOW.

Fig. 4. Partial ion chromatograms (m/z 218.2035, 232.1839, 273.2228, 383.3329 and 512.4063) showing the presence of α- and β-amyrins (3 and 4) and their degradation products (3β-hydroxy-urs-12-en-11-one, 13 and 3β-hydroxy-olean-12-en-11-one, 15) in surface sediment from sample location NOW.

Fig. 5. Partial ion chromatograms (m/z 191.0886, 234.0886, 237.1637 and 239.1794) showing the presence of DHAA (1) and its degradation products (7α/β-hydroxydehydroabietic acids, 7 and 8) in surface sediment from sample location NOW.
*Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.*
Dehydroabietic acid

Retention time (min)

- m/z 239.1794
- m/z 191.0886
- m/z 234.1433
- m/z 237.1637
### Table 1
Autoxidation of betulin (2) in the surface sediments investigated

<table>
<thead>
<tr>
<th>Sampling station</th>
<th>Betulin (2)</th>
<th>Lupan-20-one-3β,28-diol 10 ng/g dw</th>
<th>3β,28-Dihydroxy-lupan-29-oic acid 11 ng/g dw</th>
<th>Betulin auto (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.2</td>
<td>272.7</td>
<td>109.1</td>
<td>90.3</td>
</tr>
<tr>
<td>0</td>
<td>24.2</td>
<td>179.0</td>
<td>96.8</td>
<td>91.1</td>
</tr>
<tr>
<td>0</td>
<td>20.0</td>
<td>140.0</td>
<td>70.0</td>
<td>91.3</td>
</tr>
<tr>
<td>0</td>
<td>24.6</td>
<td>177.1</td>
<td>63.9</td>
<td>90.0</td>
</tr>
<tr>
<td>0</td>
<td>26.3</td>
<td>236.8</td>
<td>105.3</td>
<td>92.1</td>
</tr>
<tr>
<td>b</td>
<td>20.5</td>
<td>148.1</td>
<td>75.9</td>
<td>91.9</td>
</tr>
<tr>
<td>W</td>
<td>36.6</td>
<td>329.3</td>
<td>197.6</td>
<td>93.3</td>
</tr>
<tr>
<td>2</td>
<td>22.7</td>
<td>190.9</td>
<td>63.6</td>
<td>91.5</td>
</tr>
<tr>
<td>2</td>
<td>17.8</td>
<td>182.8</td>
<td>42.2</td>
<td>92.2</td>
</tr>
<tr>
<td>4</td>
<td>20.7</td>
<td>185.2</td>
<td>107.4</td>
<td>93.6</td>
</tr>
<tr>
<td>2</td>
<td>32.0</td>
<td>238.4</td>
<td>127.9</td>
<td>92.8</td>
</tr>
<tr>
<td>4</td>
<td>26.9</td>
<td>182.1</td>
<td>41.4</td>
<td>89.3</td>
</tr>
<tr>
<td>4</td>
<td>25.9</td>
<td>197.8</td>
<td>65.9</td>
<td>91.3</td>
</tr>
<tr>
<td>3</td>
<td>15.6</td>
<td>161.3</td>
<td>60.5</td>
<td>93.2</td>
</tr>
<tr>
<td>6</td>
<td>28.0</td>
<td>189.1</td>
<td>84.8</td>
<td>90.1</td>
</tr>
<tr>
<td>4</td>
<td>32.4</td>
<td>270.0</td>
<td>126.0</td>
<td>92.0</td>
</tr>
<tr>
<td>6</td>
<td>31.8</td>
<td>252.9</td>
<td>135.3</td>
<td>92.4</td>
</tr>
<tr>
<td>4</td>
<td>32.9</td>
<td>214.3</td>
<td>109.5</td>
<td>90.1</td>
</tr>
</tbody>
</table>

*a Oxidation product / (parent compound + oxidation product) * 100
### Table 2

Autoxidation of α- and β-amyrins (3 and 4) in the surface sediments investigated

<table>
<thead>
<tr>
<th>Sampling station</th>
<th>β-Amyrin 4 (ng/g dw)</th>
<th>3β-hydroxy-olean-12-en-11-one 15 (ng/g dw)</th>
<th>β-Amyrin autoxidation (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>α-Amyrin 3 (ng/g dw)</th>
<th>3β-hydroxy-urs-12-en-11-one 13 (ng/g dw)</th>
<th>α-Amyrin autoxidation (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.49</td>
<td>8.07</td>
<td>84.4</td>
<td>3.90</td>
<td>36.92</td>
<td>90.5</td>
</tr>
<tr>
<td>10</td>
<td>0.48</td>
<td>6.70</td>
<td>93.3</td>
<td>0.80</td>
<td>25.10</td>
<td>96.9</td>
</tr>
<tr>
<td>8</td>
<td>0.13</td>
<td>6.34</td>
<td>98.0</td>
<td>0.19</td>
<td>29.07</td>
<td>99.3</td>
</tr>
<tr>
<td>1</td>
<td>0.09</td>
<td>4.89</td>
<td>98.3</td>
<td>0.11</td>
<td>29.63</td>
<td>99.6</td>
</tr>
<tr>
<td>0</td>
<td>0.03</td>
<td>4.67</td>
<td>99.3</td>
<td>0.11</td>
<td>20.16</td>
<td>99.5</td>
</tr>
<tr>
<td>0.05</td>
<td>0.02</td>
<td>6.95</td>
<td>99.3</td>
<td>0.10</td>
<td>32.71</td>
<td>99.7</td>
</tr>
<tr>
<td>0.02</td>
<td>3.77</td>
<td>99.4</td>
<td>0.04</td>
<td>20.18</td>
<td>99.8</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>5.25</td>
<td>99.5</td>
<td>0.13</td>
<td>22.78</td>
<td>99.4</td>
<td></td>
</tr>
<tr>
<td>3W</td>
<td>0.01</td>
<td>4.48</td>
<td>99.7</td>
<td>0.14</td>
<td>19.37</td>
<td>99.3</td>
</tr>
<tr>
<td>0.05</td>
<td>0.05</td>
<td>4.73</td>
<td>98.9</td>
<td>0.17</td>
<td>23.78</td>
<td>99.3</td>
</tr>
<tr>
<td>5</td>
<td>0.12</td>
<td>6.86</td>
<td>98.3</td>
<td>0.16</td>
<td>31.86</td>
<td>99.5</td>
</tr>
<tr>
<td>4</td>
<td>0.12</td>
<td>5.80</td>
<td>98.0</td>
<td>0.52</td>
<td>25.57</td>
<td>98.0</td>
</tr>
<tr>
<td>0.10</td>
<td>0.10</td>
<td>5.75</td>
<td>98.4</td>
<td>0.19</td>
<td>27.83</td>
<td>99.3</td>
</tr>
<tr>
<td>14</td>
<td>0.13</td>
<td>3.98</td>
<td>96.9</td>
<td>0.24</td>
<td>26.92</td>
<td>99.1</td>
</tr>
<tr>
<td>8</td>
<td>0.06</td>
<td>6.39</td>
<td>99.0</td>
<td>0.17</td>
<td>25.89</td>
<td>99.3</td>
</tr>
<tr>
<td>0.02</td>
<td>6.36</td>
<td>99.6</td>
<td>0.06</td>
<td>32.40</td>
<td>99.8</td>
<td></td>
</tr>
<tr>
<td>0.23</td>
<td>0.23</td>
<td>7.29</td>
<td>96.9</td>
<td>0.34</td>
<td>37.12</td>
<td>99.1</td>
</tr>
<tr>
<td>16</td>
<td>0.08</td>
<td>6.90</td>
<td>98.9</td>
<td>0.27</td>
<td>36.19</td>
<td>99.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Oxidation product / (parent compound + oxidation product) * 100
Table 3

Autoxidation of DHAA in the surface sediments investigated

<table>
<thead>
<tr>
<th>Sampling station</th>
<th>DHAA 1 (ng/g dw)</th>
<th>7β-hydroxydehydroabietic acid 8 (ng/g dw)</th>
<th>7α-hydroxydehydroabietic acid 7 (ng/g dw)</th>
<th>DHAA autoxidation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>71.5</td>
<td>124.7</td>
<td>57.4</td>
<td>71.</td>
</tr>
<tr>
<td>11</td>
<td>53.7</td>
<td>94.4</td>
<td>30.8</td>
<td>70.</td>
</tr>
<tr>
<td>300</td>
<td>36.0</td>
<td>34.9</td>
<td>7.9</td>
<td>54.</td>
</tr>
<tr>
<td>28</td>
<td>25.1</td>
<td>15.3</td>
<td>7.4</td>
<td>47.</td>
</tr>
<tr>
<td>31</td>
<td>34.3</td>
<td>42.4</td>
<td>26.0</td>
<td>66.</td>
</tr>
<tr>
<td>300</td>
<td>25.5</td>
<td>28.9</td>
<td>17.2</td>
<td>64.</td>
</tr>
<tr>
<td>420.4</td>
<td>330.1</td>
<td>97.5</td>
<td></td>
<td>50.</td>
</tr>
<tr>
<td>35.5</td>
<td>73.1</td>
<td>13.7</td>
<td></td>
<td>71.</td>
</tr>
<tr>
<td>35b</td>
<td>31.7</td>
<td>36.2</td>
<td>16.6</td>
<td>62.</td>
</tr>
<tr>
<td>OW</td>
<td>123.6</td>
<td>221.9</td>
<td>69.7</td>
<td>70.</td>
</tr>
<tr>
<td>122</td>
<td>23.7</td>
<td>16.7</td>
<td>10.8</td>
<td>53.</td>
</tr>
<tr>
<td>15</td>
<td>68.6</td>
<td>145.9</td>
<td>47.6</td>
<td>73.</td>
</tr>
<tr>
<td>14</td>
<td>20.5</td>
<td>14.5</td>
<td>6.3</td>
<td>50.</td>
</tr>
<tr>
<td>611.8</td>
<td>559.5</td>
<td>134.9</td>
<td></td>
<td>53.</td>
</tr>
<tr>
<td>214</td>
<td>36.1</td>
<td>10.1</td>
<td>6.1</td>
<td>30.</td>
</tr>
<tr>
<td>38</td>
<td>29.5</td>
<td>94.4</td>
<td>31.7</td>
<td>81.</td>
</tr>
<tr>
<td>1432.8</td>
<td>458.6</td>
<td>184.6</td>
<td></td>
<td>31.</td>
</tr>
<tr>
<td>2</td>
<td>200.1</td>
<td>386.4</td>
<td>140.6</td>
<td>72.</td>
</tr>
<tr>
<td>116</td>
<td>42.3</td>
<td>64.4</td>
<td>17.4</td>
<td>65.</td>
</tr>
</tbody>
</table>

*Oxidation product / (parent compound + oxidation product) * 100