Oxidation products of betulin: New tracers of abiotic degradation of higher plant material in the environment

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\textbf{A B S T R A C T}

In order to fill the need for specific and stable tracers for monitoring the degradative state of particulate organic matter (OM), betulin and its degradation products were selected to track biotic and abiotic degradation processes affecting terrestrial higher plant-derived OM in riverine environments. Samples of \textit{Quercus ilex} leaves and suspended particulate matter were collected from the Marseille Luminy area and the Rhône River, respectively, and analyzed in order to validate the tracer potential of betulin oxidation products identified during in vitro simulations. Three degradation products were selected as tracers: lup-20(30)-ene-3,28,29-triol, lupan-20-one-3,28-diol and the 20R and 20S epimers of 3,28-dihydroxy-lupan-29-oic acid. They were deemed sufficiently stable for tracing the different degradative processes in aquatic systems affecting OM of terrestrial higher plant origin. All were found in riverine suspended particulate matter (SPM), evidencing the advanced degradation state of riverine particulate OM (POM), as well as the importance of autoxidation in the degradation. Lup-20(30)-ene-3,28,29-triol and lupan-20-one-3,28-diol were also found in senescent leaves of \textit{Q. ilex}, attesting to the involvement of photo- and autoxidation in the degradation of plant leaves. Alongside existing tracers, these compounds provide a better insight into the degradation state of riverine OM, as well as into the degradative processes at play, a knowledge that will be a necessary basis for further studies of the degradative state of particulate marine OM and sediments.

1. Introduction

Vascular plants can be significant contributors to the organic matter (OM) ultimately deposited in lacustrine and marine sediments, even in those deposited far from land (e.g. Volkman et al., 1987; ten Haven et al., 1992). Much of this OM is transported by rivers to estuaries and coastal areas. Because riverine particulate OM consists in part of already highly degraded residues from higher land plants (with a high content of lignin), it is generally considered to be refractory with respect to further decomposition in the ocean (e.g. de Leeuw and Largeau, 1993; Wakeham and Canuel, 2006). In order to check the validity of this paradigm, there is a need for tracers which are sufficiently stable and specific for monitoring the degradation of terrestrial higher plant material in lacustrine, riverine and marine environments. A number of biomarkers have been used successfully to recognize this material, including long chain n-alkanes with high odd carbon predominance, long chain n-alkanols and fatty acids with strong even carbon number predominance and C\textsubscript{29} sterols (e.g. Meyers and Ishiwatari, 1993; Diefendorf et al., 2011). The ratio of mid-chain alkanes to long chain alkanes has been used to differentiate between submerged and land plants (e.g. Ficken et al., 2000) and \textsuperscript{13}C values can be used to distinguish between C\textsubscript{3} and C\textsubscript{4} plants (e.g. Diefendorf et al., 2011), but in general these proxies do not provide information about the specific plants involved.

Diterpenoid and triterpenoid alcohols, ketones and hydrocarbons have been shown to be useful and specific markers for OM from vascular plants in air, water, soil and sediments (e.g. Rowland and Maxwell, 1984; Volkman et al., 1987; Rieley et al., 1991). For example, miliacin (olean-18-en-3\textsuperscript{β}-ol methyl ether) has been used as a marker for the cereal crop broomcorn millet (Jacob et al., 2008), methoxy serratene (Le Milbeau et al., 2013) and abietic acid derivatives for conifers (e.g. Sanchez-Garcia et al., 2008), pentacyclic methyl ethers as indicators of the Gramineae (Jacob et al., 2005) and des-A derivatives of lupanes...
having two OH groups. For example, Koch et al. (2005) showed that 2-hydroxy-lup-20(29)-en-28-oic acid; 2013) and results mainly in the production of betulinic acid (3 has been studied (Chen et al., 2009; Liu et al., 2011; Feng et al., Schnell et al., 2014). The degradation of betulin by several fungi and aromatic hydrocarbons (e.g. ten Haven and Rullkötter, 1988; 2003, 2005). where mangroves are the dominant vegetation (Koch et al., 2013) and subsequent alkaline hydrolysis gave the mass spectra of their acetate derivatives with those described (Vystrčil et al., 1973) and in north German peats (Köller, 1998). It has also been used as a marker for OM derived from basal woodland peat in coastal sediments of the Wadden Sea (Volkman et al., 2000). Zocatelli et al. (2014) found that it and its derivatives could be used as specific markers for Betula pendula in soil underlying woody vegetation in France. Birch bark tar found in archaeological samples contains a high abundance of betulin, with lupene and lupeol as minor constituents (Dudd and Evershed, 1999). Such tar (or pitch) appears to have been used for a wide range of purposes, including haunting, waterproofing and repairing. Betulin also occurs in other plants, such as some mangroves species, such as Avicennia germinans (Koch et al., 2003, 2005) and is a useful marker of OM derived from specific mangroves in sediments where mangroves are the dominant vegetation (Koch et al., 2003, 2005). The diagenetic fate of a biomarker must be assessed before it can be used as a quantitative marker for a particular source (Simoneit et al., 2009). Triterpenoid alcohols undergo a variety of diagenetic reactions in sediments, leading to ketones, alkenes and aromatic hydrocarbons (e.g. ten Haven and Rullkötter, 1988; Killops and Frewin, 1994; Rullkötter et al., 1994; Tay et al., 2013; Schnell et al., 2014). The degradation of betulin by several fungi has been studied (Chen et al., 2009; Liu et al., 2011; Feng et al., 2013) and results mainly in the production of betulinic acid (3β-hydroxy-lup-20(29)-en-28-oic acid; 2). Betulin in particular has been shown to be quite labile, perhaps because it is more polar, having two OH groups. For example, Koch et al. (2005) showed that it was degraded completely after 40 days when leaves of the mangrove A. germinans were incubated with surface sediment. The fact that it can be observed in sediments (Volkman et al., 2000; Koch et al., 2003; Silva and Madureira, 2012) suggests that bioprotection by association with macromolecular plant matter (e.g. peat) or adsorption to clay (Volkman et al., 2000) may be important. In contrast, the abiotic degradation of betulin has not been reported. In the present work, we investigated the photooxidation and autoxidation of this compound to assess its robustness as a conservative tracer of input from terrestrial plants such as birch trees and as a tool for studying the processes by which triterpenoids are degraded in the environment. In particular, we hoped to identify specific oxidation products that might be used as tracers for degradation of OM from vascular, non-coniferous plants in air, water and sediments.
(10 ml) was added cautiously to remove unreacted NaBH₄; the pH was adjusted to 1 with dilute HCl (2 N) and the mixture shaken and extracted with hexane:CHCl₃ (5 ml, 4:1, v/v; × 3). The combined extracts were dried over anhydrous Na₂SO₄, filtered and evaporated to dryness under a stream of N₂.

2.4. Sampling

Suspended particulate samples (21) were collected between May 2012 and May 2013 at the Rhône River reference station of Arles, 40 km upstream from the river mouth. Suspended particulate material (SPM) was collected using a high speed centrifuge (CEPA Z61) coated with Teflon to avoid contamination. Samples were immediately frozen at −20 °C until analysis.

Fresh and senescent leaves of Quercus ilex were collected in a forest near the Lumiñy campus. This species was selected on the basis of the presence of significant amounts of betulin in its leaves, where photosensitized oxidation induced by chlorophyll should be intensive during senescence (Rontani et al., 1996). The samples were freeze-dried, placed in a mortar and ground to a fine powder.

2.5. Treatment of SPM and leaf samples

Wet samples of SPM (100 mg) and ground Q. ilex leaves (120 mg) were treated with excess NaBH₄ in MeOH (25 ml; 30 min) to reduce labile hydroperoxides (resulting from photosensitization to alcohols more amenable to analysis using GC–EIMS (Marchand and Rontani, 2001). After NaBH₄ reduction, water (25 ml) and KOH (2.8 g) were added and the resulting mixtures saponified under reflux (2 h). After cooling, the resulting solutions were acidified (HCl, 2 N) to pH 1 and extracted with DCM (3 × 20 ml). The combined DCM extracts were dried over anhydrous Na₂SO₄, filtered and concentrated using rotary evaporation (40 °C).

An experiment was carried out on SPM with deuterons instead of protons in order to determine the source of compound 7 during the treatment. Reduction was carried out with NaBH₄/CD₃OD, alkaline hydrolysis with NaOD (produced from Na and D₂O) and acidic hydrolysis with 2 N DCl in D₂O.

2.6. GC–EIMS

Before GC–EIMS, the samples were dissolved in 300 μl of a mixture of pyridine and bis (trimethylsilyl) trifluoroacetamide (BSTFA; Supelco; 2:1, v/v) and silylated (1 h) at 50 °C. After evaporation to dryness under a stream of N₂, the derivatized residue was dissolved in a mixture of EtOAc and BSTFA (to avoid desilylation of some easily silylated compounds). GC–EIMS was carried out with an Agilent 6890 gas chromatograph coupled to an Agilent 5973 Inert mass spectrometer. The following conditions were employed: 30 m × 0.25 mm (i.d.) fused silica column coated with SOLGEL-1 (SGE; 0.25 μm film thickness); 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; carrier gas (He) maintained at 0.69 × 10⁻⁶ Pa until the end of the temperature program and then programmed from 0.69 × 10⁻⁶ Pa to 1.49 × 10⁻⁶ Pa at 0.04 × 10⁵ Pa min⁻¹; injector (on column) temperature 50 °C; electron energy 70 eV; source temperature 170 °C; cycle time 1.5 s. Betulin degradation products were formally identified by comparison of their retention times and EI mass spectra with those of synthesized compounds.

3. Results

3.1. Photooxidation of betulin

Due to the presence of chlorophyll, which is a very efficient photosensitizer (Foote, 1976; Knox and Dodge, 1985), Type II photosensitized processes (i.e. involving the formation of singlet oxygen) act intensively during the senescence of leaves of terrestrial higher plants (Rontani et al., 1996). Since betulin (1) is present in the leaves of white birch (Yin et al., 2013) and other higher plants (Hayek et al., 1989), we thus irradiated a pyridine solution of this compound in the presence of hematoporphyrin (an artificial photosensitizer often employed to produce singlet oxygen). The pseudo-first order rate constant (k) for betulin photodegradation was obtained from the gradient of a regression line determined according to the relationship ln(C/C₀) = −kt, where C is the concentration of betulin at the time of sampling, C₀ its initial concentration and D the light dose. The degradation constant obtained (k = 4.3 × 10⁻⁶ m²/s; r² = 0.93, n = 4) is of the same magnitude as that for 2,6,10,14-tetramethyl-7-(3-methylpent-4-enyl)pentadecane (IP25; k = 1.7 × 10⁻⁵ m²/s; Rontani et al., 2011), also possessing a dissubstituted methylene group, but almost two orders of magnitude lower than that of cholesteryl acetate (k = 3.0 × 10⁻⁴ m²/s; Rontani et al., 2011) which possesses a trisubstituted double bond.

After NaBH₄ reduction and silylation, only one photoprod of 1 could be detected. On the basis of a comparison of its retention time and EI spectrum (Fig. 1A) with those of a synthesized standard, this compound was unambiguously assigned as lup-20(30),28,29-triol (5).

3.2. Autoxidation of betulin

In order to compare the rate of autoxidation of betulin with those of other well known lipid components of vascular plants, a mixture of betulin, methyl oleate (as a model for glycerides), phytol acetate (as a model of the chlorophyll phytol side chain), cholesteryl acetate (as a model for esterified sterols), sitosterol and hexatriacontane (internal standard) in hexane was incubated in the presence of a radical enhancer (tert-butyldihydroperoxide) and a radical initiator (di-tert-butylnitroxide) (Porter et al., 1995) at 80 °C in the dark. The pseudo-first order rate constant (k) for the autoxidation of each lipid was obtained from the gradient of the regression lines determined according to the relationship ln(C/C₀) = −kt, where C is the concentration of the analyte at the time of sampling, C₀ its initial concentration and t the duration of the incubation. Degradation rates of all the lipids incubated showed a good fit with pseudo-first order kinetics (Table 1). Betulin reacted at a similar or higher rate than monounsaturated fatty esters and chlorophyll phytol side chain, respectively, but slower than free and esterified sterols (Table 1).

Betulin was oxidized under similar conditions for 4 days. After subsequent NaBH₄ reduction and silylation, 20,29-epoxy-lupan-3β,28-diol (3), lup-19(20)-ene-3β,28,29-triol (4), 3β,28-dihydroxy-lupan-29-0ic acid (8) and lupan-20-one-3β,28-diol (7) were identified from GC–EIMS (Table 2). The assignments were based on comparison of the EI spectra (Figs. 1 and 5) and retention times with those of synthesized standards.

3.3. Degradation of betulin during the senescence of Q. ilex leaves

The lipid content of fresh and senescent leaves of Q. ilex (containing a significant amount of betulin) was examined after NaBH₄ reduction and alkaline hydrolysis. Lup-20(30)-ene-3β,28,29-triol (5) and lupan-20-one-3β,28-diol (7) could be formally identified in senescent leaves by comparison of retention times and mass
3.4. Degradation of betulin in SPM from the Rhône River

Betulin (1), lup-20(30)-ene-3β,28,29-triol (5), lupan-20-one-3β,28-diol (7) and the 20R and 20S epimers of 3β,28-dihydroxy-lupan-29-oic acid (8) could be detected in most of the samples (Table 3, Fig. 2 Supplementary Section).

In order to determine if the ketodiol 7 was also produced in significant proportion from heterolytic cleavage of 29-hydroperoxy-lup-20(30)-ene-3β,28-diol (14), resulting from photooxidation of betulin (Fig. 2), a sample of SPM from the Rhône River was hydrolyzed with NaOD in D₂O/CD₃OD and with DCl in D₂O) respectively. Under such conditions heterolytic cleavage of 14 should afford monodeuteriated 7, while homolytic and heterolytic cleavage of 11 should give an unlabelled product (Fig. 3 Supplementary Section).

spectra with those of standards (Fig. 1, Supplementary Section), while we failed to detect these compounds in fresh leaves of Q. ilex.

Table 1
First order rate constants for betulin and model lipids incubated in hexane in the presence of tert-butyl hydroperoxide and di-tert-butyl nitroxide at 80 °C in the dark.

<table>
<thead>
<tr>
<th>Compound</th>
<th>k [h⁻¹]</th>
<th>i²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl oleate</td>
<td>4.7 × 10⁻³</td>
<td>0.97</td>
<td>5</td>
</tr>
<tr>
<td>Phytol acetate</td>
<td>2.5 × 10⁻³</td>
<td>0.99</td>
<td>4</td>
</tr>
<tr>
<td>Cholesterol acetate</td>
<td>9.5 × 10⁻³</td>
<td>0.97</td>
<td>5</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>9.2 × 10⁻³</td>
<td>0.96</td>
<td>5</td>
</tr>
<tr>
<td>Betulin</td>
<td>3.7 × 10⁻³</td>
<td>0.91</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2
Main autoxidation products of betulin (1) after incubation in hexane in the presence of peroxides at 65 °C for 4 days.

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20,29-Epoxy-lupan-3β,28-diol</td>
<td>25.4</td>
</tr>
<tr>
<td>Lup-19(20)-ene-3β,28,29-triol</td>
<td>31.5</td>
</tr>
<tr>
<td>3β,28-Dihydroxy-lupan-29-oic acid⁴</td>
<td>27.0</td>
</tr>
<tr>
<td>Lupan-3β,20,28,29-tetraol</td>
<td>-</td>
</tr>
<tr>
<td>Lupan-20-one-3β,28-diol</td>
<td>16.1</td>
</tr>
<tr>
<td>Lup-20(29)-ene-3β,28,19-triol</td>
<td>-</td>
</tr>
</tbody>
</table>

⁴ Mixture of 20R and 20S epimers.
The lack of labelling of 7 after this test allowed us to exclude significant production of this ketodiol from photoproduct 14.

4. Discussion

4.1. Photooxidation of betulin

The rate constant for reaction of a monoolefin with singlet oxygen is sensitive to the ionization potential of the substrate (Monroe, 1981). In general, alkyl substitution of double bonds decreases their ionization potential and increases their reactivity towards singlet oxygen (Monroe, 1981). The lower degradation rate of 1 relative to cholesteryl acetate may be thus attributed to the nature of their double bonds (disubstituted in the case of betulin and trisubstituted in the case of Δ5-sterols).

The very high regio selectivity of the attack by singlet oxygen, as shown by the lack of lup-19(20)-ene-3β,28,29-triol (4; Fig. 2), is in agreement with previous results obtained in the case of gem-disubstituted alkenes possessing a methyl and a bulky substituent. Indeed, in this case photooxygenation shows a strong preference for hydrogen abstraction from the methyl group that is geminal to the larger substituent of the alkene (Alberti and Orfanopoulos, 2006).

4.2. Autoxidation of betulin

On the basis of: (i) the similar or faster antioxidative degradation rate of betulin relative to methyl oleate and phytyl acetate (Table 1) and (ii) the previous detection of significant proportions of autoxidation products of monounsaturated fatty acids and chlorophyll...
phytyl side chain in suspended particles (Christodoulou et al., 2009; Rontani et al., 2011, 2012a) and sediment samples (Rontani et al., 2012b), intense autoxidation of betulin in the marine environment would be expected.

Epoxidation of olefins under autoxidation conditions is well known (Fossey et al., 1995) and arises from the addition of peroxyl radicals (ROO•) to the C=C bond, followed by ring closure and elimination of an alkoxyl radical (RO•). During autoxidation of olefins, ROO• addition to the C=C bond competes with allylic hydrogen abstraction only when there is a double bond that is 1,1-disubstituted (Schaich, 2005) as is the case for betulin. The lack of lup-20(29)-ene-3β,28,19-triol (9) among the betulin oxidation products (Table 2) confirms that allylic hydrogen abstraction at C-19 is not significant. In contrast, ROO• addition takes place at C-29 and results in formation of the more stable tertiary radical (Fig. 3). This radical can then: (i) lead to epoxide 3 by fast intramolecular homolytic substitution, (ii) lose a hydrogen atom, affording peroxide 10 or (iii) react with O2 to form a peroxyl radical that can then abstract a hydrogen atom from another molecule, leading to the formation of 11 (Fig. 3).

Compounds 3, 10 and 11 are affected by a number of degradative processes during incubation, treatment (NaBH4 reduction and acidic hydrolysis) and GC injection (thermal cleavage); they are summarized in Fig. 4. Incubation of epoxide 3 in CHCl3 at room temperature for several days allowed us to demonstrate that the lup-19(20)-ene-3β,28,29-triol (4) detected after betulin
autoxidation (Fig. 1C; Table 2) resulted from the well known rearrangement of epoxides in chlorinated solvents (Belt et al., 2006). Epoxide 3 can also easily rearrange to the aldehyde 12 (Tolstikov et al., 2005; Fig. 4), which may be quickly oxidized to the corresponding acid 8 in the presence of O$_2$ (McNesby and Heller, 1954; Hansen, 1977; Fig. 5A, Table 2). It may be noted that the aldehyde 12 may also be formed after homolytic cleavage of the peroxide 10 and subsequent β-cleavage of the foregoing alkoxy radical (Fig. 4). Surprisingly, among the betulin oxidation products (Table 2) we failed to detect lupan-3,20,28,29-tetraol (6), which should result from NaBH$_4$ reduction of 11. The unexpected presence of lupan-20-one-3,28-diol (7) after NaBH$_4$ reduction (Fig. 5C; Table 2) and the total lack of the corresponding lupan-3β,20,28-triol (13) allowed us to attribute the formation of this ketodiol to homolytic (during GC injection) or heterolytic (during acidic hydrolysis) cleavage of the C-20 tertiary hydroperoxy group of 11 (Fig. 4). It may be noted that tetrat 6 could be detected in significant proportion after reduction of autoxidized betulin with a stronger reductant (LiAlH$_4$). This observation allowed us to confirm the presence of unreduced 11 after NaBH$_4$ reduction. The unexpected stability of 11 is attributed to the involvement of intramolecular six membered hydrogen bonding between the hydrogen atom of the hydroperoxy group and the first oxygen atom of the peroxy group (Aoki and Seebach, 2001; Fig. 4).

4.3. Selection of degradation tracers

As described in Section 3.1, Type II photosensitized oxidation of betulin selectively produces (after NaBH$_4$ reduction) lupan-20β(30)-ene-3β,28,29-triol (5). To our knowledge, the presence of this compound in plants has only been reported by González et al. (1992) in Maytenus canariensis. Due to the very restricted distribution of this species (endemic to the Canary Archipelago), 5 may be proposed as a specific tracer of photooxidation of vascular plant material in the environment (Fig. 6).

Among the degradation products of betulin described in Section 3.2, we selected 3β,28-dihydroxy-lupan-29-oic acid (8) and lupan-20-one-3β,28-diol (7) as specific tracers of autodative degradation of OM from vascular plants (Fig. 6). Although produced in significant proportions (Table 2), epoxide 3 was discarded as a proxy due to its high relative lability. Indeed, epoxides are generally considered to be readily degraded in the environment (Stephanou and Stratigakis, 1993). Moreover, they may be easily converted to the corresponding diols, methoxyhydrins and chloro-hydrins during alkaline hydrolysis and subsequent acidification steps employed during sample treatment (Marchand and Rontani, 2001) and rearrange easily to allylic alcohols in chlorinated solvents (Belt et al., 2006). It may be noted that lupan-20-one-3β,28-diol (7), named messagerin, was detected in Melilotus messanensis (Macías et al., 1994). This ‘biosynthetic’ compound should be converted to the corresponding alcohol 13 during NaBH$_4$ reduction employed during the treatment of environmental samples and should thus not hinder the use of ‘degradative’ (i.e. resulting from cleavage of 11 during the treatment and GC injection) compound 7 as a tracer of betulin autoxidation.

Since betulonic acid (3β-hydroxy-lup-20(29)-en-28-oic acid; 2) is known to result from the biotransformation of betulin by several fungi (Chen et al., 2009; Liu et al., 2011; Feng et al., 2013), it could
constitute a potential tracer for biotic degradation of vascular plants in the environment. Unfortunately, it is also naturally present in low proportion in the plant kingdom (Jäger et al., 2009), so was discarded as an unambiguous tracer for biodegradative processes affecting betulin (1).

4.4. Degradation of betulin during senescence of Q. ilex leaves

The presence of triol 5 (6% of the residual betulin) in senescent leaves of Q. ilex attests to the involvement of Type II photosensitized oxidation of betulin. Despite its relative weak reactivity towards singlet oxygen, betulin is thus significantly photodegraded during the senescence of terrestrial higher plants. The ketodiol 7 (4% of residual betulin) likely results from the cleavage of 11 unaffected by NaBH₄ reduction (Fig. 4). This shows that autoxidation also intervenes during the senescence of terrestrial higher plants. The process is probably induced by homolytic cleavage of photochemically produced hydroperoxides, which should be strongly favoured by the strong UV irradiance and the relatively high temperature in Mediterranean zones.

4.5. Degradation of betulin in SPM from the Rhône River

As mentioned in Section 4.2, we expect betulin to be intensely autooxidized in the marine environment, but it appears that autoxidation is already a major degradative process in riverine SPM. In all our samples, 7 was present in large quantity, greater even than that of betulin itself (samples 25, 27, 28; Table 3). When both compounds selected as autoxidation tracers are added (7 and 8), it becomes clear that autoxidation is intense in riverine SPM of terrestrial higher plant origin, and is the major degradative process year round. Photooxidation was also apparent in our samples, although the process seems to be much less important. There is little variation in the amounts of degradation products quantified, but their presence is in line with the amounts of photo- and autooxidation products of sitosterol previously found in SPM from the Rhône (Galeron et al., 2015). It appears that these compounds

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*Fig. 5. EI spectra of: (a) 3β,28-dihydroxy-lupan-29-oic acid (8), (b) lupan-3β,20,28,29-tetraol (6) and (c) lupan-20-one-3β,28-diol (7) trimethylsilyl derivatives.*
can be successfully used to trace the degradation state of SPM derived from terrestrial higher plants.

Ketodiol 7 was detected in all the samples (Table 3). The lack of lupan-3β,20,28-triol (13), which should be produced from NaBH₄ reduction of ketodiol 7 during sample treatment, was attributed to both a lack of ketodiol 7 in the particulate matter and post-NaBH₄ reduction production of this compound during alkaline/acidic hydrolysis or even during GC analysis. As suggested in Section 4.2., this production likely results from heterolytic or homolytic cleavage of 11 (strongly stabilized by hydrogen bonding; Fig. 4).

While a high proportion of acid 8 (relative to ketodiol 7) was obtained after autoxidation of betulin in n-hexane (Table 2) in the particulate matter samples, 7 was strongly dominant (Fig. 2 Supplementary material). We attribute the differences to the nature of the solvent. Indeed, if the good hydrogen donor properties of n-hexane should favor the formation of hydroperoxide 10 and thus of acid 8 from radical a (Fig. 3), in water these processes should be strongly disfavoured and radical a mainly reacts with oxygen, affording 11 and then ketodiol 7.

5. Conclusions

In light of the need for specific tracers to monitor the degradative state of terrestrial higher plant-derived OM in aquatic environments, we propose the use of betulin and its degradation products alongside used tracers such as sterols. Lup-20(30)-ene-3β,28,29-triol could be used as a specific tracer of photooxidation of vascular plant-derived OM, while we propose the use of 3β,28-dihydroxy-lupan-29-oic acid and lupan-20-one-3β,28-diol to trace autoxidation of this material. These compounds allowed us to demonstrate the high autoxidation state of terrestrial higher plant residues carried by the Rhône River – particulate OM that will ultimately form a component of marine sediments deposited in coastal areas. Based on the recent observations carried out on the Mackenzie Shelf (Rontani et al., 2014), further studies of the degradative state of terrestrial POM of higher plant origin upon its arrival at sea are needed in order to better quantify coastal carbon fluxes.

Acknowledgements

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Appendix A.

Betulin (1)  
Betulonic acid (2)  
20,29-Epoxy-lupan-3β,28-diol (3)

Lup-19(20)-ene-3β,28,29-triol (4)  
Lup-20(30)-ene-3β,28,29-triol (5)  
Lupan-3β,20,28,29-tetraol (6)

Lupan-20-one-3β,28-diol (7)  
3β,28-Dihydroxy-lupan-3-oic acid (8)  
Lup-20(29)-ene-3β,28,19-triol (9)

29-Peroxy-lupan-3β,28-diol (10)  
29-Peroxy-20-hydroperoxy-lupan-3β,28-diol (11)  
3β,28-Dihydroxy-lupan-29-al (12)

Lupan-3β,20,28-triol (13)  
29-Hydroperoxy-lup-20(30)-ene-3β,28-diol (14)
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orgeochem.2015.10.010.

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References


