



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



Marie-Aimée Galeron^{a,b}, John K. Volkman^c, Jean-François Rontani^{a,b,*}

^a Aix-Marseille University, Mediterranean Institute of Oceanography (MIO), 13288 Marseille, Cedex 9, France

^b Université du Sud Toulon-Var, CNRS-INSU/IRD UM 110, 83957, France

^c CSIRO Oceans and Atmosphere Flagship, GPO Box 1538, Hobart, Tasmania 7001, Australia

ARTICLE INFO

Article history:

Received 9 February 2015

Received in revised form 3 September 2015

Accepted 28 October 2015

Available online 6 November 2015

Keywords:

Betulin

Photo-oxidation

Autoxidation

Organic matter

Terrestrial higher plants

Tracers

ABSTRACT

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 trace biotic and abiotic degradation processes affecting terrestrial higher plant-derived OM in riverine environments. Samples of *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 β ,28,29-triol, lupan-20-one-3 β ,28-diol and the 20R and 20S epimers of 3 β ,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 β ,28,29-triol and lupan-20-one-3 β ,28-diol were also found in senescent leaves of *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.

© 2015 Elsevier Ltd. All rights reserved.

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 C₂₉ 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 $\delta^{13}\text{C}$ values can be used to distinguish between C₃ and C₄ 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 β -ol methyl ether) has been used as a marker for the cereal crop broomcorn millet (Jacob et al., 2008), methoxy serratenes (Le Miibeau 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

* Corresponding author at: Aix-Marseille University, Mediterranean Institute of Oceanography (MIO), 13288 Marseille, Cedex 9, France. Tel.: +33 4 86 09 06 02; fax: +33 4 86 09 06 41.

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

(Regnery et al., 2013) and betulin as a specific marker for birch-derived OM (Fine et al., 2001). This wide variety of structural types can be preserved in sediments ultimately to be found in crude oils as the corresponding alkanes (Rullkötter et al., 1994).

The dihydroxylated triterpenoid betulin (lup-20(29)-en-3 β ,28-diol; **1**) is an abundant constituent of birch wood (*Betula* spp.), where it occurs together with lesser amounts of lupeol, lupanone, betulinic acid and lupenone (Schnell et al., 2014 and refs. therein). It has the structural characteristics needed for a source-specific biomarker and was used as such by Fine et al. (2001) to trace particulate emissions from burning paper birch bark, since it dominated the emissions and was absent from the combustion products of other plants. Betulin has been found in a number of geological and archaeological settings, including alignite from the Czech Republic (Albrecht and Ourisson, 1971), 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 lupenone 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 hafting, 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 (3 β -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.

2. Material and methods

2.1. Standard compounds

Betulin (**1**) and betulinic acid (**2**) were obtained from Sigma-Aldrich and potential degradation products were synthesized.

20,29-Epoxy-lupan-3 β ,28-diol (**3**) was obtained after treatment of betulin (**1**) with 3-chloroperoxybenzoic acid in anhydrous dichloromethane (DCM) at room temperature for 1 h (yield 80%). Isomerization of epoxides to allylic alcohols in CHCl₃ has been observed (Belt et al., 2006), so **3** was shaken in CHCl₃ at room temperature for 10 days, yielding a rearranged product tentatively assigned as lup-19(20)-ene-3 β ,28,29-triol (**4**; yield 15%).

Lup-20(30)-ene-3 β ,28,29-triol (**5**) was produced in four steps after: (i) acetylation of betulin (**1**) in a mixture of pyridine/Ac₂O at room temperature overnight, (ii) epoxidation of the resulting 3,28-di-O-acetylbetulin as described above for the synthesis of **3**, (iii) treatment of the epoxide with lithium diethylamide in Et₂O (Rickborn and Thummel, 1969) and (iv) alkaline hydrolysis of the acetate groups (yield 20%).

OsO₄ oxidation of betulin (**1**) in anhydrous dioxane/pyridine (McCloskey and McClelland, 1965) afforded lupan-3 β ,20,28,29-tetraol (**6**; yield 85%). Treatment of the tetrol **6** with lead tetraacetate in toluene yielded lupan-20-one-3 β ,28-diol (**7**) (yield: 90%).

Oxidation of 3,28-di-O-acetylbetulin with CrO₃ AcOH at 70 °C (Vystrčil et al., 1973) and subsequent alkaline hydrolysis gave the 20R and 20S epimers of 3 β ,28-dihydroxy-lupan-29-oic acid (**8**; yield 15%).

The structures of **3**, **5**, **7** and **8** were confirmed by comparison of the mass spectra of their acetate derivatives with those described in the literature (Vystrčil et al., 1973; González et al., 1992; Huang et al., 1995).

2.2. Autoxidation and photooxidation experiments

Autoxidation experiments were performed under atmosphere of air in a 22 ml screw cap flask containing either betulin (**1**, 1.0 mg) or a mixture of betulin, methyl oleate, cholesteryl acetate, sitosterol and phytyl acetate (1.0 mg each), an internal standard (hexatriacontane, 1.0 mg), *tert*-butyl hydroperoxide (300 μ l of a 6.0 M solution in *n*-decane), di-*tert*-butyl nitroxide (2 mg) (Porter et al., 1995) and hexane (10 ml). After stirring, the flasks were incubated in the dark at 80 °C. Aliquots (300 μ l) were withdrawn after incubation for different times. Each sub-sample was evaporated to dryness and analyzed using gas chromatography-mass spectrometry with electron ionization (GC-EIMS) either directly (mainly for residual substrate quantification), or after NaBH₄ reduction and derivatization for identification of oxidation products.

Photooxidation of betulin (**1**) was carried out in the presence of hematoporphyrin (an artificial photosensitizer often employed to produce singlet oxygen). Solutions of **1** (1.0 mg) and hematoporphyrin (0.4 mg/0.1 mg of substrate) in pyridine were placed in 2 ml screw cap glass vials and irradiated at 17 °C with adjustable light cassettes of fluorescent lamps (Osram, Fluora, irradiance 36 W/m²) in a KBW 240 (E5.1) Binder plant growth chamber for one month. The lamps provided a spectral distribution of visible light suitable for photobiological processes involving chlorophyll-*a*. At the end of the irradiation, the samples were evaporated to dryness under N₂ and reduced with excess NaBH₄.

2.3. Reduction of betulin oxidation products

In order to identify the main degradation products formed during autoxidation and photooxidation, the samples were reduced with excess NaBH₄ in Et₂O:MeOH (5 ml; 4:1, v/v; 10 mg/mg extract) at room temperature (1 h). The operation was carried out in order to try to reduce labile hydroperoxides (resulting from photo- or autoxidation) to alcohols more amenable to analysis using GC-EIMS. After reduction, a saturated solution of NH₄Cl

Download English Version:

<https://daneshyari.com/en/article/5161432>

Download Persian Version:

<https://daneshyari.com/article/5161432>

[Daneshyari.com](https://daneshyari.com)