Dissipation of pterosin B in acid soils - tracking the fate of the bracken fern carcinogen ptaquiloside

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Abstract Bracken ferns (Pteridium sp.) are well-known for their toxicity, especially the carcinogenic properties which are ascribed to their content of ptaquiloside and ptaquiloside-like substances. Ptaquiloside leach from the ferns and may cause contamination of drinking water. Pterosin B is formed by acid hydrolysis of ptaquiloside. Pterosin B is adsorbed more strongly and it is expected to have a slower turnover than ptaquiloside in soils. We thus hypothesized that pterosin B may serve as an indicator for any past presence of ptaquiloside. Pterosin B degradation was studied in acid forest soils from bracken-covered and bracken-free reference areas. Soil samples were incubated with pterosin B at 21°C at 3 and 8 μg g⁻¹ for 10 days, whereas sterile (autoclaved) samples were incubated for 23 days. Pterosin B showed unexpected fast degradation in soils with full degradation in topsoils of 2 to 5 days. Pterosin B dissipation followed the sum of two-first order reactions. The initial fast reaction with half-lives of 0.7 to 3.5 h contributed 11 to 59% of the total pterosin B degradation, while the slow reaction was 20 to 100 times slower than the fast reaction. Total dissipation half-lives were shorter for loamy sand (4 h) than for sandy loam soils (28 h). No degradation of pterosin B took place under sterile conditions assuming observed dissipation during the first 3 h could be attributed to irreversible sorption. Faster dissipation was observed in top soils compared to subsoils. Our results demonstrate that pterosin B is microbial degraded and that pterosin B is as unstable as ptaquiloside and hence cannot be used as an indicator for former presence of ptaquiloside in soil.

Keywords: Microbial degradation; Sorption; Soil; Degradation kinetics; Natural toxins
1. Introduction

Bracken ferns (Pteridium sp.) are found throughout the world inside forests, on moorlands and as a common weed on agricultural land. Bracken contains the carcinogenic compound ptaquiloside. Humans can be exposed to ptaquiloside via different routes such as milk, spores and drinking water (Alonso-Amelot et al., 1993; Rasmussen et al., 2003a; 2005; 2013; Clauson-Kaas et al., 2014) and a correlation has been indicated between human health and exposure to bracken (Alonso-Amelot and Avendaño, 2001; Recouso et al., 2003). Brackens are classified by WHO/IARC as ‘possibly carcinogenic to humans’ (WHO/IARC, 2014).

Ptaquiloside is a norsesquiterpene glucoside which is chemically unstable under acidic and alkaline conditions (Ojika et al., 1987, Ayala-Luis et al., 2006). Ptaquiloside can also be transformed by soil microorganisms (Engel et al., 2007). Ptaquiloside is readily hydrolysed to form the non-carcinogenic aromatic indanone pterosin B (Ojika et al., 1987; Nagao et al., 1989; Table 1). However, other reactions may occur depending on pH and the chemical composition of the matrix solution, e.g. leading to reactions with other nucleophiles such as alcohols, amines, thiols and sulphide groups or formation of chloro-pterosin in chloride rich environments (Ojika et al., 1987; Kushida et al., 1994; Cáceres-Peña et al., 2013;). Recently, Mohammad et al. (2016) identified a range of pterosins and pterosides in the bracken rhizomes indicating a more complex ptaquiloside-chemistry in the fern or the presence of a range of ptaquiloside-like substances like the well-known example of caudatoside. Pterosin A is known to be a radical scavenger suggesting such reactions as an alternative decomposition route for pterosins (Chen et al., 2015; Castillo et al., 1997). Pterosins are widely distributed among ferns, and today more than 30 pterosins have been identified.

Pterosin B has been reported to be present in rather high concentrations in bracken fronds and rhizomes (>2,100 μg g⁻¹) (Alonso et al., 1996; Saito et al., 1989; Rasmussen, 2003; Mohammad et al., 2016). Quantification of pterosins in the living tissue is difficult, as e.g. pterosin B may form in substantial amounts during extraction and sample pre-treatment. In particular, drying temperature of the biomass has a profound effect on formation of pterosins, in particular pterosin B (Cáceres-Peña et al., 2013).

Ptaquiloside is released from bracken to soil either from dead bracken material or due to rainwater wash-off. Because of its high water solubility, it is readily leached to soil, surface water and ground water (Clauson-Kaas, 2016; Clauson-Kaas et al., 2014; O’Driscoll et al., 2016; Rasmussen et al., 2005). Ptaquiloside contamination of soils and surface waters have been found on a number of occasions in e.g. Denmark, Eire, Britain, New Zealand and Portugal, whereas similar studies in Italy resulted in negative findings (op.cit. and Zaccone et al., 2014). These different observations is a result of different analytical techniques but do also suggest marked variations in the fate of ptaquiloside in the environment, e.g. due to variations in soil composition and microbiological activity.
Ptaquiloside hydrolysis to pterosin B is strongly pH dependent (Ayala-Luis et al., 2006). This has been used by many researchers in developing analytical methods for ptaquiloside based on a 1:1 conversion into pterosin B, e.g. for analysis of ptaquiloside and ptaquiloside residues in plants, environmental samples, meat and milk (e.g. Zaccone et al., 2014 and Bonadies et al., 2011). As most soils are acid and as pterosin B is generally found to be stable under laboratory conditions, we hypothesize that the presence of pterosin B in soils may be used to track the previous presence of ptaquiloside (Hirono et al., 1987; Rasmussen et al. (2013); Clauson-Kaas et al., 2014).

If proved correct, this may prove useful in environmental monitoring of ptaquiloside. While ptaquiloside is highly water soluble and shows very low retention in soils and sediments pterosin B is considerably less polar (Table 1). Hence, pterosin B should be retained by sorption to soil organic matter and hence have low mobility in soil. If pterosin B is found in deeper soil layers and even in aquifer sediments it may be inferred that it has formed from the more mobile ptaquiloside in these compartments. However, the use of pterosin B as indicator of past ptaquiloside contamination is only possible if the compound does not degrade in the soil environment nor react with soil particles in a non-reversible manner through sorption or chemical bonding. Hence, in order to use pterosin B as an indicator of ptaquiloside contamination the main question is first of all: Is pterosin B stable in the soil environment?

To answer this question we investigated the abiotic and biotic degradation kinetics of pterosin B in top and sub-soils collected from a bracken infested area in Denmark (Pteridium aquilinum (L.) Kuhn). The soils investigated were acid forest soils with low microbial activity, as we expect pterosin B to be most stable in this type of natural environment. As part of the investigation we developed a new method for the formation, isolation and purification of pterosin B from aqueous bracken extracts.
Table 1: Physicochemical properties of pterosin B.

<table>
<thead>
<tr>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
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</table>

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>(±)-2,3-dihydro-6-(2-hydroxyethyl)-2,5,7-trimethyl-1H-inden-1-one</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS number</td>
<td>34175-96-7</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C_{14}H_{18}O_{2}</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>218.29 g mol^{-1}</td>
</tr>
<tr>
<td>Water solubility(^a)</td>
<td>162 mg L(^{-1})</td>
</tr>
<tr>
<td>LogK(_{oc})(^a)</td>
<td>2.6</td>
</tr>
<tr>
<td>LogK(_{ow})(^b)</td>
<td>3.3</td>
</tr>
</tbody>
</table>

\(^a\)Clauson-Kaas et al. (2014); \(^b\)Rasmussen et al. (2005).
2. Materials and Methods

2.1 Chemicals and reagents

Methanol (MeOH) and acetonitrile (AcN) were HPLC grade (Rathburn Chemicals Ltd). Analytical grade sodium hydroxide (J.T.Baker®) and trifluoroacetic acid (CF₃COOH; Sigma-Aldrich®) were used for conversion of ptaquiloside to pterosin B. Double deionized (DI) water with electrical conductivity (EC) < 0.1 μS cm⁻¹ was used throughout the experiments. Polyamide 6 resin was obtained from Fluka® (Sigma-Aldrich®).

2.2 A novel method for purification of pterosin B

Conversion of ptaquiloside into pterosin B in an aqueous Bracken extract was found more time-efficient compared to conversion of pure ptaquiloside into pterosin B. Hence, ptaquiloside was extracted from milled bracken material. Sixty grams of dry bracken powder (Præstø, Denmark) were split in two, placed into shaking flasks, and extracted once with 300 mL of double deionized (DI) water each in the dark; shaking flasks were shaken at 14 strokes min⁻¹ for 1 h. Next, the extract was centrifuged for 20 min at 17,000 g and the supernatant filtered (Whatman® filter paper, ashless, 125 mm, Grade 42). The filtrate was passed through a polyamide dry packed column (50x5.0 cm I.D. glass Econo-Column) containing 2.2 g Polyamide 6S resin in order to remove fine particles and lipophilic substances. At this point, the bracken extract was divided into 100 mL flasks with a final extract volume in each flask of 71 mL. 1 M sodium hydroxide (5.32 mL) was added to the extract and incubated for 1 h at 40 °C. After cooling to room temperature, 5.32 mL 2M trifluoroacetic acid was added in order to complete the conversion of ptaquiloside to pterosin B (Agnew and Lauren, 1991). For isolation of pterosin B the extract was filtered using a regenerated cellulose (RC) membrane filter (25 mm diameter, 0.2 μm pore size, Mikrolab Aarhus A/S) in order to remove particulate impurities, before passing it through a wet packed polyamide column (50x5.0 cm I.D. glass Econo-Column) containing 3 g Polyamide 6S resin. Elution of pterosin B was performed with MeOH, by first washing the column with 10 mL which was discarded, then adding 4x5 mL which were collected independently. These four fractions containing pterosin B (determined using HPLC-UV) were bulked in a round-bottomed flask, evaporated at 45 °C until dryness at a rotary evaporator (approx. 30 min). The sample was then re-dissolved in 3.5 mL 40% (v/v) MeOH, before purification by preparative HPLC (40% (v/v) MeOH; 1 mL injection volume; flow rate 4 mL min⁻¹; 30°C (Merck T 6-300 Column thermostat); Phenomenex Luna C8 100 Å preparative column, 250×10 mm, 10 μm particle size; SemiPrep security guard cartridge, 10×10 mm). Pterosin B eluted at approx. 80 min (Shimadzu SPD-10AV UV-VIS detector; Shimadzu LC-10AT Pump; 220 nm). The pterosin B-containing fractions were collected and mixed in a round-bottomed flask. After evaporation at 45 °C for 15 min, a dry, semi-pure (50–70%) pterosin B material was obtained. The purity was determined against a pure pterosin B standard prepared from pure ptaquiloside (Agnew and Lauren, 1991; Aranha et al. 2014).
2.3 Quantification of pterosin B

Pterosin B was determined using an Agilent 1100 Series HPLC (Gemini C6-phenyl 50×2 mm column, 3 μm particle size; 35 °C; 214 nm; C6-phenyl guard column, 4×2 mm). Reverse phase separation was achieved using gradient elution, with mobile phase A as AcN/DI water (10:90 v/v) and pure AcN as mobile phase B. The chromatographic separation lasted 14 min (0–3 min: 92% A; 4–7 min: 70% A; 8–12 min: 20% A, hereafter returning to start conditions; flow rate 1 mL min⁻¹; injection volume 10 μL; retention time 4.9 min). The limit of detection (LOD) was 0.05 mg L⁻¹ (3 times the standard deviation of 7 replicates of the smallest pterosin B standard. The limit of quantification was 0.17 mg L⁻¹ (3.3 times LOD).

2.4 Stock solution and calibration standards for analytical HPLC

Stock solutions of pterosin B (15-36 mg L⁻¹) was prepared by dissolution of purified pterosin B in MeOH. A stock solution of 92.6 mg L⁻¹ pterosin B for use in calibration standards was made by hydrolysis of ptaquiloside isolated according to Aranha et al. (2014). Calibration standards (0.55-5.50 mg L⁻¹) were prepared by dilution with DI water. Standards were kept at -18 °C in the dark when not used.

2.5 Soil sampling and geochemical characterization of soils

Mineral soil materials were collected in October 2014 at two different forested sites in Grib Skov (Zealand, Denmark; Table 2). The forest is a mixture of deciduous and coniferous tree species. Bracken (Pteridium aquilinum (L.) Kuhn) occurs as dense stands in larger parts of the forest. At each site, soils were collected from two neighbouring areas at the same soil type after removal of the O-horizons: One covered by bracken, and another not covered by bracken situated 15 m and 100 m apart (site A and B respectively). The depth of the topsoil (A horizon) was up to 20 cm while the subsoil (B horizon) had a thickness of 20 to 40 cm. Site A was situated in a glade surrounded by spruce (Picea abies (L.) H. Karst.) and larch (Larix kaempferi (Lamb.) Carrière). Site B was located on a large north-facing slope on open land with a dense bracken population (no significant surface flow). Both sites were well-drained acid old forest soils with low microbial activity developed on morainic sandy material. Bulk soil samples of the central parts of the A- and B-horizons were collected from shallow soil pits into polyethylene bags. Field moist soil samples were sieved (2 mm mesh) and stored in polyethylene bags at 5°C for one mth prior to use. Samples to be used for geochemical characterization of the soils were dried at room temperature for 1 mth before sieving (2 mm MESH). Apart from texture and C/N ratio, the soils showed no major differences and the bracken-covered and reference soils were highly similar (Table 2).
<table>
<thead>
<tr>
<th>Area</th>
<th>Site</th>
<th>UTM Coordinates</th>
<th>Soil type</th>
<th>Horizon</th>
<th>Texture (%)</th>
<th>Water content (%)</th>
<th>pH</th>
<th>Organic C (%)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bracken-covered area</td>
<td>A</td>
<td>55.977694N</td>
<td>Loamy sand</td>
<td>Top</td>
<td>86</td>
<td>6</td>
<td>8</td>
<td>22.0</td>
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<tr>
<td></td>
<td></td>
<td>12.325833E</td>
<td></td>
<td>Sub</td>
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<td>6</td>
<td>8</td>
<td>14.5</td>
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</tr>
<tr>
<td></td>
<td>B</td>
<td>56.004825N</td>
<td>Sandy loam</td>
<td>Top</td>
<td>74</td>
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<td>14</td>
<td>11.1</td>
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<td></td>
<td>Sub</td>
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<td>6</td>
<td>14</td>
<td>3.7</td>
<td>3.7</td>
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<tr>
<td>Bracken-free reference area</td>
<td>A</td>
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<td>Loamy sand</td>
<td>Top</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>19.5</td>
<td>3.4</td>
</tr>
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<td></td>
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<td>Sandy loam</td>
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<td>12</td>
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<td>Sub</td>
<td>84</td>
<td>6</td>
<td>10</td>
<td>5.9</td>
<td>3.5</td>
</tr>
</tbody>
</table>

1Universal Transverse Mercator; 2Soil texture was determined according to Bouyoucos (1926; single determination) and Soil Survey Staff (2003); 3Water content determined after drying of soils at 110 °C for 24 h (w/w), single analysis; 41:2.5 (soil:0.01 M CaCl₂; duplicate analysis; STDEV≤0.02 pH units); 5Carbon and nitrogen were measured by Dumas combustion at an Vario Macro Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany; single analysis; CV(reference compound sulfanilamide) ≤ 0.7 % (w/w)).
2.6 Recovery of pterosin B from spiked soil samples

MeOH-water solutions were used to extract pterosin B from the soils in the degradation experiments (modified from Jensen et al., 2008). The recovery of the method was tested at two levels: 15/300 μL 10 mg L⁻¹ pterosin B solution were added to the test soil (1 g topsoil, site A; 50 mL centrifuge tubes (Duran®-glass; lid with rubber/PTFE); triplicate analysis). MeOH were evaporated after spiking with a gentle air flow for 3hrs. Each recovery test was compared to a no-soil control spiked with the same amount of pterosin B. Extraction was performed using 2 mL of 80 % (v/v) MeOH (shaking for 1 h; shaking table, 14 rpm). Subsequently, the soil-MeOH samples were centrifuged for 10 min at 1500 g. The supernatants were filtered using regenerated cellulose membrane filters (25mm Ø; 0.2 μm; Mikrolab Aarhus A/S) and were transferred to amber LC vials for subsequent determination of pterosin B. The recovery of pterosin B was 100±3% (ratio between samples and controls).

2.7 Degradation of pterosin B

The experiment was set-up according to the principles in OECD Test No. 301: Ready Biodegradability (OECD, 1992):

Degradation in non-sterile soils. Pterosin B degradation was investigated for soils from bracken-covered areas and non-bracken reference areas at 21 ºC (Sanyo Versatile Environmental Test chamber MLR-351; incubation in 100 mL un-capped glass flasks wrapped in aluminium foil; aerobic conditions). Twenty grams of soil kept at 75 % of the water holding capacity (adjusted with DI water), was amended in each flask with 125μL 15 mg L⁻¹ pterosin B stock solution (to ensure optimal mixing of pterosin B into the soil, the pterosin B was initially mixed with 2 g soil and 500 μL DI water for 30 seconds, before being mixed with the remaining soil sample for 3min using a micro spatula of stainless steel. Pterosin B was extracted with 80% MeOH at time zero, 2hrs, 5hrs and 1, 2, 3, 5, 7 and 10 days after pterosin B addition (new flasks at each time point). In addition, an experiment with higher temporal resolution was performed in the soils from bracken covered areas (every hour during the first 10hrs).

Degradation in sterile soils. A degradation experiment using sterile samples was applied to the soils from the bracken-covered areas. This experiment followed the method described above, except that moist soils were initially sterilized by autoclaving for at least 1 h at 121 ºC repeated on two or more consecutive days (Berns et al., 2008). The number of colony forming units (CFU) in the incubated soils was determined on the first day of the experiment and at day 3, 10, 15 and 22 to test if soils remained sterile. In brief, 0.5 g soil was added 4.5 mL sterile MilliQ-water, vortexed for 2 min and left to settle for 20 min. Appropriate dilutions of supernatant (100μL) were plated on Luria-Bertani agar plates, (Eisenstadt et al., 1994), incubation at 21 ºC for 2 days before finally counting visible colonies.

2.8 Data handling
Degradation kinetics was described as a sum of two first-order reactions in non-sterile soils:

\[
\%PTB = a \times \exp(-k_{fast}t) + (100 - a) \times \exp(-k_{slow}t) \tag{1}
\]

where \%PTB is the percentage of pterosin B remaining in soil at time t (h), a is the percentage of pterosin B degraded in the initial fast reaction, and \(k_{fast}\) and \(k_{slow}\) are the rate constants for the first and second first-order reactions, respectively.

Curves were fitted using all data points (SigmaPlot, version 13.0; Systat Software Inc., San Jose, California). All data were used for modelling in the situations where both long- and short-term experiments had been carried out. The kinetic parameters and their uncertainties were estimated by the fitting programme. Data for sterile soils were not fitted as no degradation reaction took place.

3. Results and discussion

Pterosin B degraded rapidly at 21 °C in all non-sterile soil samples from bracken-covered areas (Figs 1C, D). In the loamy sand soil (Site A), pterosin B was fully degraded in the topsoil after 27 h and the rate of the fast reaction was four times faster in the topsoil as compared to the subsoil (half-lives of 4 and 17 h, respectively). For the sandy loam soil (site B), the degradation for the top soil was seven times slower than for the loamy sand top soil (site A) (Figs 1C, D). Subsoil degradation followed a similar pattern. A higher clay content of the sandy loam may cause sorption of pterosin B hence retarding biodegradation. Results indicate higher clay content. However, the difference in the clay content is not significant and does not warrant final conclusions in this respect. In addition, the sandy loam had a slightly higher C/N ratio and it was slightly more acidic which could also explain why degradation was slower in this soil. In general, soil organic matter biodegradation is limited by N availability in temperate forest soils. But again, differences are minute and more variation in the geochemical composition of the examined soils is needed for more general conclusions.

The incubations in sterilised soil resulted in a fast initial dissipation of pterosin B during the first 3 h after which very little dissipation took place (Figs 1A, B). Monitoring of cultivatable bacteria in the sterilised samples showed that they were consistently below the detection limit of the cultivation assay (~100 CFU g\(^{-1}\)) except for one of the triplicates in loamy sand subsoils at day 10, and the same soil sample day 15. Hence, the repeatedly autoclaved soils were properly sterilized. The fact that no dissipation of pterosin B took place after 3 h strongly indicates that soil microbial activity was responsible for the dissipation observed in the natural non-sterilized soils. The fast initial drop in pterosin B level in all the sterilized soils is most likely due to irreversible sorption of pterosin B to reactive organic matter formed during autoclaving as no such fast dissipation was seen for the non-sterilized soils. Sterilization of soils by autoclaving is well known to cause breakdown of organic
matter and formation of new structural moieties (e.g. Berns et al., 2008; Slizovskiy et al., 2010; Mahmood et al., 2014).

The degradation study did also include soils from non-bracken covered reference areas in order to test if bracken coverage was required to select for a microbial community using pterosin B as a substrate. In the reference soils, half of pterosin B degraded after 16 h in the topsoil and after 46 h in the subsoil for the loamy sand (Site A), while for the sandy loam (Site B) half-lives of 28 and 69 h were obtained in the top- and subsoils, respectively (Figs 1E/F). Hence, pterosin B degradation was 2.7-4.0 times faster in the presence than in the absence of bracken cover in the loamy sand, while negligible effects of bracken cover on pterosin B degradation was observed for the sandy loam. Nevertheless, pterosin B degraded within days in both bracken-covered and reference areas. Hence, there was no clear trend to indicate that bracken cover in general selects for pterosin B-degrading microorganisms although it may have happened at one of the sites.

Pterosin B degradation in the non-sterilised soils was fitted as a sum of two first-order reactions; for most of the degradation experiments with sandy loam soils a better fit was obtained using a simple first-order reaction only, and as the rate constants were low they are referred to the slow reaction. The rate constants were estimated based on fitting all data from both short and long term experiments (Figs 1C, D; inserts); rate constants are listed in Table 3. The fast reaction displayed rate constants between 0.3 and 1 h⁻¹ and contributed 11–59% of the total pterosin B degradation. The contribution of the initial fast reaction to total degradation tended to be higher in top soils than in subsoils probably due to higher microbial activity in the top soils. There was no apparent lag phase suggesting that microbial degrader organisms were initially present. The slow reaction was 6 to 90 times slower than the fast reaction and displayed rate constants in the range 0.01-0.05 h⁻¹. We tentatively attribute the fast reaction to degradation of pterosin B pools that are readily bioavailable, while the slow reaction may be limited by desorption of bioaccessible pterosin B pools slowly becoming bioavailable for microbial uptake over time (Semple et al., 2004).

This study clearly indicates that pterosin B will not accumulate in acid forest soils sustaining active microbial communities. Pterosin B will degrade as soon as it has been formed with degradation rates similar to ptaquiloside (Rasmussen et al., 2005; Engel et al., 2007; Ovesen et al., 2008, Zaccone et al., 2014). Given the higher chemical stability of pterosin B as compared to ptaquiloside under laboratory conditions, it is however still possible that pterosin B may be used as an indicator for past presence of ptaquiloside in environments with very low microbial activities such as ground waters.

This is the first study to demonstrate that pterosin B is degraded rapidly in the soil environment. Hence, the hypothesis that pterosin B may be used to track the prior presence of ptaquiloside and used as an indicator of ptaquiloside contamination of soils can thus clearly be negated.
4. Conclusion

Pterosin B degraded within days in loamy sand and sandy loam forest soils. In most cases pterosin B degradation could be fitted as sum of two first-order reactions; degradation half-lives were in general 2 to 4 times higher for top than subsoils. Degradation was limited in the sterile soils with the majority of dissipation occurring within the first 3 h which is ascribed to fast irreversible sorption to reactive organic material. Though the experiments were carried out using soils of acidic and nutrient-depleted conditions, degradation of pterosin B appeared to be entirely due to microbial activity. In conclusion, soil degradation of pterosin B is as fast as degradation of ptaquiloside as documented in studies from Denmark, Italy and New Zealand. Hence, pterosin B formed from ptaquiloside does not accumulate in the soil environment and therefore, pterosin B cannot be used as a measure to track the prior presence of ptaquiloside in natural soil. This is the first study of pterosin degradation in the soil environment.
Table 3: Kinetic parameters for pterosin B degradation at 21°C described as the sum of two first-order reactions (Eq. 1) in bracken-covered areas and reference areas of both sites and horizons.

<table>
<thead>
<tr>
<th>Area</th>
<th>Soil Type</th>
<th>Horizon</th>
<th>A</th>
<th>k_{fast} (h^{-1})</th>
<th>k_{slow} (h^{-1}) \times 10^{-2}</th>
<th>R^2</th>
<th>t_{1/2} (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bracken-covered area</td>
<td>Loamy</td>
<td>Top</td>
<td>59±21</td>
<td>0.3±0.1</td>
<td>5.0±3.0</td>
<td>0.937</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>Sub</td>
<td>16±3</td>
<td>1.0±0.6</td>
<td>4.0±0.4</td>
<td>0.969</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Sandy</td>
<td>Top¹</td>
<td></td>
<td></td>
<td>2.5±0.2</td>
<td>0.985</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>loam</td>
<td>Sub¹</td>
<td></td>
<td></td>
<td>1.2±0.1</td>
<td>0.962</td>
<td>58</td>
</tr>
<tr>
<td>Bracken-free reference area</td>
<td>Loamy</td>
<td>Top</td>
<td>23±9</td>
<td>0.8±1.0</td>
<td>3.0±0.5</td>
<td>0.959</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>Sub</td>
<td>11±5</td>
<td>1.0±2.0</td>
<td>1.0±0.2</td>
<td>0.930</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Sandy</td>
<td>Top</td>
<td>23±11</td>
<td>0.2±0.1</td>
<td>2.0±0.3</td>
<td>0.973</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>loam</td>
<td>Sub¹</td>
<td></td>
<td></td>
<td>1.0±0.1</td>
<td>0.933</td>
<td>69</td>
</tr>
</tbody>
</table>

¹Best fit obtained by simple first-order reaction. ²Half-lives were calculated using the methodology in FOCUS Degradation Kinetics Report (2011).
Figure 1: Dissipation of pterosin B in acid forest soils. A: Sterile loamy sand (site A); B: Sterile sandy loam (site B); C and D: Natural soils from bracken-covered areas (non-sterile); E and F: Natural soils from bracken-free reference areas. All studies kept at 21°C. Insets show the data for the first 20hrs. Error bars represent the standard error of the mean (n = 3).
References


sesquiterpene glucosides from *Pteridium aquilinum* var. *caudatum*. Phytochemistry 44:5:901-906.


