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Dissipation of pterosin B in acid soils – Tracking the fate of the bracken fern carcinogen ptaquiloside

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HIGHLIGHTS

- Pterosin B degraded within days in both loamy sand and sandy loam soils.
- Microbial activity appears to be the main cause of degradation.
- Indications of adapted soil microbiota under bracken.
- Soil degradation of pterosin B as fast as parent compound ptaquiloside.

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ABSTRACT

Bracken ferns (*Pteridium* spp.) are well-known for their carcinogenic properties, which are ascribed to the content of ptaquiloside and ptaquiloside-like substances. Ptaquiloside leach from the ferns and may cause contamination of drinking water. Pterosin B is formed by hydrolysis of ptaquiloside. In soil, Pterosin B is adsorbed more strongly and it is expected to have a slower turnover than ptaquiloside. 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 areas. Soil samples were incubated with pterosin B 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 in 2–5 days. Pterosin B dissipation followed the sum of two-first order reactions. The initial fast reaction with half-lives of 0.7–3.5 h contributed 11–59% of the total pterosin B degradation, while the slow reaction was 20–100 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. Our results demonstrate that pterosin B is microbially 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.

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., 2003; 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).

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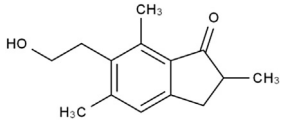
Table 1
Physicochemical properties of pterosin B.

Chemical structure	
Chemical name	(±)-2,3-dihydro-6-(2-hydroxyethyl)-2,5,7-trimethyl-1H-inden-1-one
CAS number	34175-96-7
Chemical formula	$C_{14}H_{18}O_2$
Molecular weight	218.29 g mol^{-1}
Water solubility[a]	162 mg L^{-1}
$LogK_{oc}$[a]	2.6
$LogK_{ow}$[b]	3.3

[a] Clauson-Kaas et al. (2014).
[b] Rasmussen et al. (2005).

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 (>2100 μg g^{-1}) (Alonso-Amelot et al., 1995; 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 (Clausson-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; 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 and Yamada, 1987; Rasmussen et al., 2013; Clauson-Kaas et al., 2014).

If shown to be 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.

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 (CF_3COOH; 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 (50 × 5.0 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