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Survey of volatile oxylipins and their biosynthetic precursors in bryophytes

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ABSTRACT

Oxylipins are metabolites which are derived from the oxidative fragmentation of polyunsaturated fatty acids. These metabolites play central roles in plant hormonal regulation and defense. Here we survey the production of volatile oxylipins in bryophytes and report the production of a high structural variety of C5, C6, C8 and C9 volatiles of mosses. In liverworts and hornworts oxylipin production was not as pronounced as in the 23 screened mosses. A biosynthetic investigation revealed that both, C18 and C20 fatty acids serve as precursors for the volatile oxylipins that are mainly produced after mechanical wounding of the green tissue of mosses.

1. Introduction

The addition of molecular oxygen to polyunsaturated fatty acids and the further transformations of the resulting hydroperoxides lead to the production of a large family of oxygenated products, collectively known as oxylipins (Feussner and Wasternack, 2002). Oxylipins are found in most living beings and the diversity of oxylipin-producing enzymes is high (Andreou et al., 2009). This compound class comprises oxidized long chain fatty acids, cyclic structures, such as jasmonates or prostaglandins, volatile oxygenated short chain hydrocarbons and short chain ω-oxo-acids. Oxylipins are an important class of signaling molecules in plants, especially related to plant stress responses and innate immunity (Wasternack, 2007). In addition these metabolites can also be directly involved in the chemical defense (Matsui, 2006). Due to their central role in plant defense and as hormones, the biosynthesis of oxylipins has attracted much attention (Delker et al., 2006; Matsui, 2006). In higher plants oxylipins are mainly formed from the unsaturated C18 fatty acids linolenic and linoleic acid with multiple pathways that are activated or induced during stress or mechanical disruption of the tissue (Feussner and Wasternack, 2002; Noordermeer et al., 2001). Oxylipins in algae play similar roles in the chemical defense, but these organisms rely predominantly on metabolites derived from polyunsaturated C20 fatty acids (Gerwick, 1994; Pohnert and Boland, 2002).

In contrast to higher plants and algae, the role and biosynthesis of oxylipins in bryophytes is rather poorly understood (Dembitsky, 1993; Toyota et al., 1997). The most recent comprehensive analysis of bryophyte relationships resolves hornworts as most likely sister group to the vascular plants and liverworts as the sister group to all other land plants (Qiu et al., 2006). Thus an insight into oxylipin formation gives information on the overall distribution and biosynthesis of these central metabolites in plants.

First studies have been undertaken with *Physcomitrella patens*, a moss that serves as a model species due to the availability of its genome (Quatrano et al., 2007; Rensing et al., 2008). In *P. patens* both C18 and C20 fatty acids are substrates for lipoxygenases and a structurally diverse group of oxylipins is formed (Anterola et al., 2009; Wichard et al., 2005). *P. patens* lipoxygenases and hydroperoxide lyases were cloned and studied in further detail revealing unusual mechanisms of fatty acid transformation (Senger et al., 2005; Stumpe et al., 2006). Interestingly, *P. patens* uses motives from both, algae and higher plants for the biosynthesis of its oxylipins. Despite this progress on the model species, little is known about the nature and distribution of oxylipins in bryophytes in general. Notable exceptions are acetylenic oxylipins from

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the moss *Dicranum scoparium* and volatile C5 and C10 oxylipins from the liverwort *Chiloscyphus pallidus* (Ichikawa et al., 1983; Toyota and Akakawa, 1994). We show here that mosses are a generally rich source of volatile oxylipins and that oxylipin formation depends generally on both, C18 and C20 fatty acids as biosynthetic precursors.

2. Results and discussion

For this survey mosses, liverworts and hornworts have been collected in the area of Lac Léman in Switzerland and in the Thuringian Forest in Germany. Extraction of volatile oxylipins could be easily carried out with only 1–6 mg of biomass using solid phase microextraction (SPME). For this purpose, moss samples were transferred into 1.5 ml glass vials, which were sealed with a Teflon septum. Extraction was carried out with a polydimethylsiloxane fiber (Maier et al., 1996). Mosses were collected as entire plantlets and transported to the lab with the substratum. In order to remove substratum that can act as a source for volatiles, mosses were washed immediately before the start of the experiment. Independent experiments confirmed that washing and cultivation in the lab did not significantly affect oxylipin formation. GC–MS analysis revealed that mosses that were transferred directly into the vials did not release any detectable amounts of oxylipins. If entire plantlets were transferred into the sample vials and volatiles were enriched over 20 h no oxylipins were detected as well. It is only after mechanical damage of the green tissue that a rapid onset of the production of volatiles was observed. Tissue disruption can be obtained by clipping of the leave like structures with scissors or by mechanical disruption of shock frozen samples in a mortar. The latter protocol was used to quantify and compare results from the different mosses. A wound activated release of volatile oxylipins is commonly found in higher plants and algae as well (Matsui, 2006; Pohnert, 2000).

Liverworts and hornworts contained only minor amounts of oxylipins and thus they were not further included in the survey (data not shown). It is interesting to note that these sister groups of land and vascular plants apparently do not rely on an explicit release of volatile oxylipins after wounding. In contrast, all investigated mosses produce volatile oxylipins after tissue disruption, and a total of 17 fatty acid derived metabolites were identified (Fig. 1, Table 1). All structures were verified by comparison of mass spectra and retention times with synthetic or commercially available standards. Among the products were the octenols **11** and **12** as well as the octadienol **13** that are associated with the typical smell of mushrooms. In mushrooms octenols are produced via oxygenase mediated oxidation of linoleic acid and subsequent cleavage of an intermediate 10-hydroperoxy linoleic acid while the moss *P. patens* produces these metabolites using a bi-functional lipoxygenase (Brodhun et al., 2009; Wichard et al., 2005) and the octatriene **9** that is found in the volatile blend released by brown algae and diatoms (Pohnert, 2000). The C6 metabolites **5**–**8**, which are known as green leave volatiles from higher plants (Matsui, 2006) as well as other metabolites (e.g. **1**, **2**, **14**–**17**) that are commonly released after mechanical wounding of plants were also detected (Noordermeer et al., 2001). Some of these metabolites (**11**, **12**, **15**, **16**) have also been found in the volatile blend of *P. patens* and as products from a cloned *P. patens* lipoxygenase and a hydroperoxide lyase (Senger et al., 2005; Stumpe et al., 2006; Wichard et al., 2005). Table 1 summarizes the distribution of volatile oxylipins in bryophytes. It is evident that with few exceptions C8 oxylipins are the dominant volatiles from the mosses. With one exception, oct-1-en-3-ol (**12**) is found in all investigated mosses and represents often the major metabolite. *Neckera crispa* and *Leucodon sciuroides* release predominantly (*Z*)-non-2-enal (**14**). *Brachythecium rutabulum* and *Hylocomium splendens* produce 1*E*,3*E*,5*Z*-octatriene (**9**) as dominant metabolite. Only *D. scoparium*, which is the moss releasing the highest structural diversity of oxylipins, produces more C5

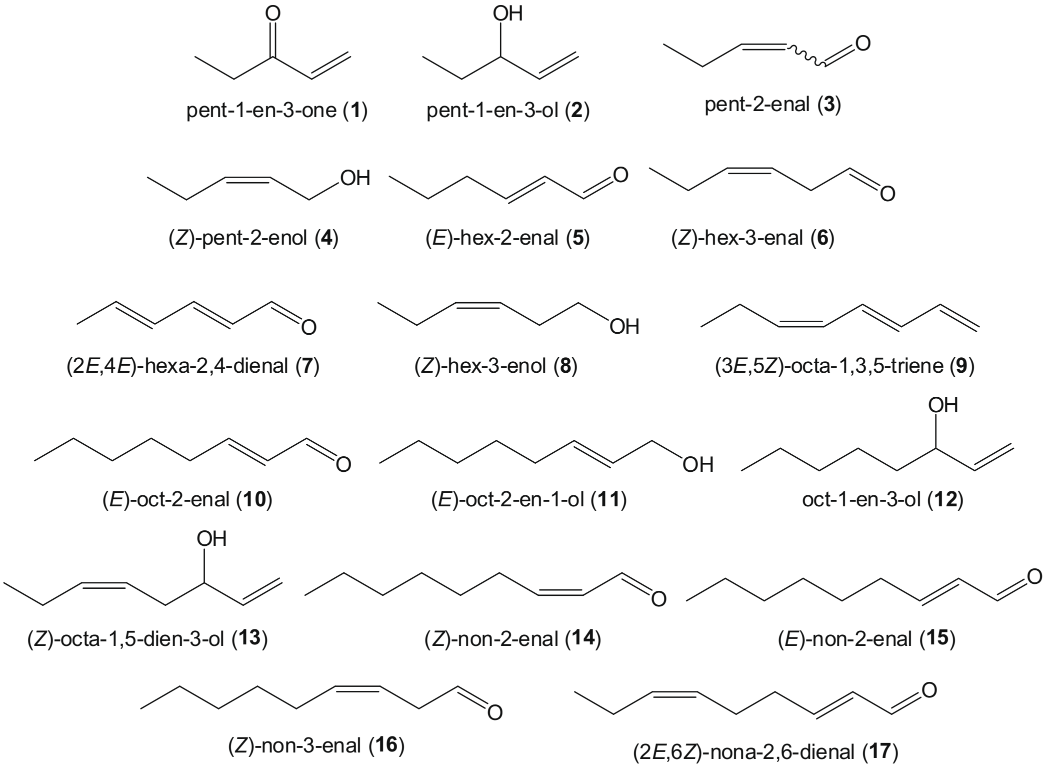

Fig. 1. Structures of oxylipins from the investigated bryophytes.