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Dolichols of the fern *Matteucia struthiopteris*✪

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Dolichols isolated from leaves of the fern *Matteucia struthiopteris* were present as a mixture of prenologues composed of 14 up to 20 isoprene units with Dol-16 dominating. They comprised approximately 0.004% of the fresh weight of fresh plant tissue and were accompanied by traces of polyprenols (Pren-14 up to Pren-17, Pren-16 dominating). Their structure was confirmed by electropray ionization mass spectrometry (ESI-MS). This is the first time that dolichols have been reported as dominating polyisoprenoid alcohols in plant photosynthetic tissue.

Matteucia struthiopteris, the Ostrich fern, is widely distributed within the northern hemisphere. It is registered as a strictly protected plant in Poland. On the other hand, in Japan the same plant known as kogomi is considered edible and its fiddleheads are grown and harvested commercially. In spite of it a use as a constituent of the human diet the chemical composition of *M. struthiopteris* has not been studied in detail. Seasonal changes of glyco-

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Abbreviations: Dol-n, dolichol composed of n-isoprene units; ESI-MS, electrospray ionization mass spectrometry; HPLC, high performance liquid chromatography; Pren, prenol; SIM, selected ion monitoring.

and phospholipid patterns have been reported (Rozentsvet *et al.*, 2002). Studies on the vicilin-like protein expressed during late stages of spore development considered as ancestors of seed storage globulins of gymnosperms and angiosperms have also been performed (Shutov *et al.*, 1998).

Studies on the occurrence of isoprenoids have revealed that these compounds are widespread among living organisms, both in eukaryotes and prokaryotes. These compounds are built of five-carbon isoprenoid units and they play important roles in cell metabolism. Dolichols (α-saturated isoprenoid alcohols) (Fig. 1) are found predominantly in animal

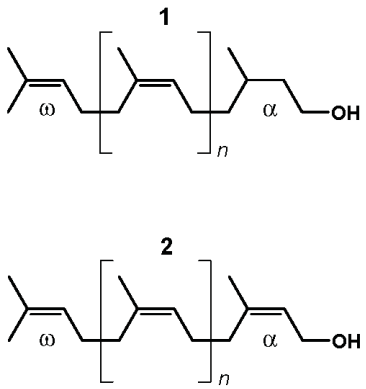

Figure 1. Structure of dolichol (1) and polyprenol (2)

n Indicates the number of internal isoprene residues, ω and α stand for the ω- and α-terminal isoprene residues, respectively.

cells, for example in rat and human organs, and also in yeast (Hemming, 1985). It has recently been shown that dolichols, and in lesser abundance, polyprenols are constituents of the shiitake mushroom *Lentinus edodes* (Wojtas *et al.*, 2004), the most popular edible mushroom used in Asian cuisine. Dolichols have also been identified as the predominant isoprenoid form in plant roots (Skorupińska-Tudek *et al.*, 2003). Polyprenols (α-unsaturated isoprenoid alcohols) (Fig. 1) have been found in bacteria and in plants where they occur mainly in green tissues but are also present in wood, seeds and flowers. Isoprenoid alcohols always occur as a mixture ("family") of compounds differing in the number of isoprene units per molecule and this pattern can be considered as a chemotaxonomic criterion (tissue specific variation being taken into consideration). Comparison of isoprenoid alcohol composition reveals that plant polyprenols exhibit great variability in chain length from 'narrow' mixtures consisting of five components (Pren-6 to Pren-9, with Pren-7 dominating) isolated from the wood of silver birch (*Betula verucosa*) up to extremely 'broad' two-family ones composed of more than 80 members (Pren-14 to Pren-100, Pren-17 and -25 dominating) isolated from leaves of *Lumnitzera racemosa*. The polyprenol spectrum is considered as a species-specific feature (Rezanka & Votruba, 2001). In contrast, dolichol families isolated from animal or yeast cells consist of seven or eight compounds irrespective of the organism. Dolichol-16, -18, and -19 are the predominating prenologues in yeast, rat and human, respectively (Krag, 1998).

In this study we describe the polyisoprenoid alcohols in the fern *Matteucia struthiopteris*. Electrospray ionization mass spectrometry (ESI-MS) was used to analyze the structure of these compounds.

MATERIALS AND METHODS

Extraction and isolation. Leaves of the kogomi fern *M. struthiopteris* (510 g) were obtained fresh from the local market (Sendai, Japan). Lipids were extracted with chloroform/methanol 1:1 (v/v) and after alkaline hydrolysis isoprenoid alcohols were extracted with hexane. For analytical purposes a small aliquot of this extract was purified on an RP-18 Sep-Pak cartridge (Waters). The polyisoprenoid alcohol content and pattern in the mixture was analyzed by two-plate thin-layer chromatography (Sagami *et al.*, 1992). For preparative purposes, the polyprenol fraction after hydrolysis was isolated by flush chromatography on a silica gel col-

umn with a stepwise gradient of diethyl ether in hexane from 0 to 25%. Fractions containing detectable amounts of polyprenols (according to thin-layer chromatography, TLC) were pooled and analyzed by HPLC using a Waters dual pump (Waters 510) apparatus and a Hypersil ODS column with the solvent system: A – methanol/water 9:1 (v/v); B – methanol/propan-2-ol/hexane 2:1:1 (by vol.) and a flow rate of 1.5 ml/min controlled by a Waters gradient programmer. The UV detector (Waters 484) was set at 210 nm. Dol-23 (Collection of Polyprenols, Intitute Biochemistry and Biophysics, Poland, PAS, Warsaw) was applied as an external quantitative standard.

HPLC/ESI-MS measurements. HPLC/ESI-MS experiments were carried out as described previously (Skorupinska-Tudek *et al.,* 2003) using an HP 1100 series HPLC system (Agilent Technologies) equipped with a 2 × 200 mm HPLC column filled with Nucleosil 100-C18 (5 μm) coupled to an API 365 triple quadrupole mass spectrometer (Applied Biosystems). Gradient elution was employed. Solvent A was a methanol/propan-2-ol/water mixture 12:8:1 (by vol.) and solvent B was hexane/propan-2-ol 7:3 (v/v). The linear gradient started from 100% of solvent A and changed to 70% of solvent B in 40 min. The flow rate was 0.2 ml/min. The UV detector was set at 210 nm.

Sodium acetate dissolved in solvent B (400 μg/ml) was introduced post-column by a syringe pump (flow rate 5 μl/min) through a T union into the LC flow before entering the mass spectrometer. The final concentration of sodium acetate in the effluent was 10 μg/ml.

Mass spectra were acquired using a standard TurboIon Spray ion source. Nitrogen was used as the nebulizing and curtain gas. Electrospray ionization was carried out in a positive ion mode. The spray tip voltage was kept at 4500 V, the declustering potential was set to 85 V and the focusing potential to 380 V. These parameters were optimized for the highest intensity of the peak for the $[M+Na]^+$ ion of dolichol-16. Data acquisition was performed in the selected ion monitoring (SIM) mode for the $[M+Na]^+$ ions of dolichols and prenols from 12 to 24 isoprene units.

RESULTS AND DISCUSSION

A chloroform/methanol extract of the leaves of *M. struthiopteris* was hydrolyzed and the nonsaponifiable lipid fraction was extracted with hexane and analyzed by two-plate thin-layer chromatography (not shown) (Sagami *et al.*, 1992). Initial identification of a family of dolichols within the range of Dol-15 to Dol-18 was confirmed by HPLC/UV analysis indicating the domination of Dol-16 (Fig. 2a). The approximate dolichol content was found to be 0.004% of the fresh weight. In order to identify the structure of the putative dolichols, HPLC/ESI-MS was used. A recently established method (Skorupińska-Tudek *et al.*, 2003) permitted the observation of $[M+Na]^+$ peaks of polyprenols and dolichols. To enhance the intensity of these peaks, sodium acetate solution was added through a T union to the LC effluent before it entered the ion source of the mass spectrometer. The results are presented in Fig. 2. The UV trace (Fig. 2a) allowed peaks in the range from approx. 22 to 30 min to be assigned as potential members of the polyprenol and dolichol series. The full scan technique did not, however, permit the identification of any polyprenol alcohol due to the complexity of the mixture, so the selected ion monitoring (SIM) technique was used. Combined SIM traces for the $[M+Na]^+$ ions of dolichols and prenols, covering the retention time range given above, are shown in Figs. 2b and 2c, respectively. A series of peaks corresponding to the $[M+Na]^+$ ions of five dolichols from Dol-14 to Dol-18 with Dol-16 predominating, are clearly visible. Careful inspection of individual SIM traces indicates the presence of Dol-13 and Dol-19 as well. Combined SIM

traces for the $[M+Na]^+$ ions of polyprenols (Fig. 2c) show the presence of the Pren-14 to Pren-16 alcohols with Pren-15 dominating, however, their concentration is very low which results in a rather poor signal-to-noise ratio. The amount of polyprenols appears to be less than 5% of that of the dolichols.

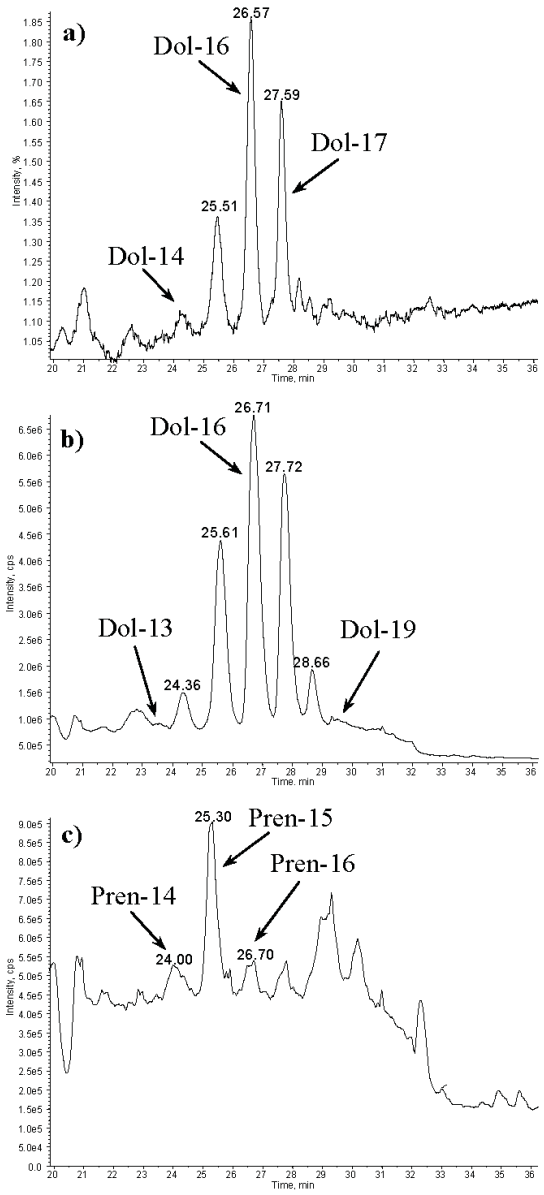

Figure 2. HPLC records of the unsaponifiable lipid fraction from *M. struthiopteris*.

a) HPLC/UV (210 nm) chromatogram. Two lower panels present overlayed selected ion monitoring (SIM) chromatograms for the masses of $[M+Na]^+$ ions of dolichols (b) and prenols (c) containing 13 to 19 isoprene units. In all cases only a selected part of the chromatogram, corresponding to the retention times (indicated) of the expected polyisoprenoid alcohols, is presented.

To confirm whether other ferns contain dolichols and/or polyprenols two species, warabi (bracken, *Pteridium aquilinum*) and zenmai (osumund, royal fern, flowering fern, *Osumunda japonica*) that are popular edible ferns in Japan, were analyzed by the 2D-TLC method. Both contained a family of dolichols within the range from Dol-15 to Dol-19, with Dol-17 dominating. No detectable amount of polyprenols was observed on the TLC chromatogram, unlike in the case for *M. struthiopteris,* although the presence of traces of these alcohols should not be excluded.

To our knowledge this is the first report describing the occurrence of polyisoprenoid alcohols in ferns. It is worth underlining that ferns accumulate dolichols (α-saturated alcohols) in their photosynthetic tissues, which is in contrast to other plants studied so far, where accumulation of mainly polyprenols was observed in leaves and needles. In a number of green plant green tissues only trace amounts of dolichols have been detected, not exceeding 1–2% of the total amount of polyisoprenoid alcohols. One exception is the case of *Capparis coriacea* from which a mixture containing equal amounts of dolichols and polyprenols was extracted (Jankowski *et al.*, 1994). A similar phenomenon was recently observed in leaves of wheat (J. Mikoszewski, personal communication).

The dolichol spectrum found in the three fern samples studied agrees very well with that found upon normal physiological conditions in the yeast *Saccharomyces cerevisiae* (dolichols 14–18, Dol-16 dominating) (Szkopinska *et al.*, 2002) and *Schizosaccharomyces pombe* (dolichols 14-18, Dol-16 and -17 dominating) (Quellhorst *et al.*, 1998). Similar dolichol spectrum was also described for the shiitake mushroom *Lentinus edodes* (Wojtas *et al.*, 2004). The meaning of these observations requires further studies, however, the occurrence of dolichols in Sporophyta might indicate their significant biological role.

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