TRANS-PTEROSTILBENE AND ITS DERIVATIVE 2,4-DIMETHOXY-6-HYDROXYPHENANTHRENE IN THE LEAVES OF PARTHENOCISSUS TRICUSPIDATA

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ABSTRACT

Trans-pterostilbene, cis-pterostilbene and 2,4-dimethoxy-6-hydroxyphenanthrene were detected in the leaves of *Parthenocissus tricuspidata* (Siebold et Zuccarini) Planchon. It was recorded for this plant for the first time that in autumn, when the leaves change in colour, there is an increase in content of 2,4-dimethoxy-6-hydroxyphenanthrene (DMPH), which is a derivative of trans-pterostilbene.

Keywords: *Parthenocissus tricuspidata*, trans-pterostilbene, 2,4-dimethoxy-6-hydroxyphenanthrene, trans-resveratrol

Introduction

*Parthenocissus tricuspidata* (Siebold et Zuccarini) Planchon, known as Japanese creeper, Boston ivy, Grape ivy or Japanese ivy, belongs to the family Vitaceae. It is native to eastern Asia.

The attention here is mainly on the detection and content of stilbenes, because they are known to be biologically active substances. In stem wood, in addition to trans-resveratrol and trans-piceid (Jeon et al. 2013), trans-ε-viniferin, pallidol, ampelopsin F, isoampelopsin F (Tanaka et al. 1998), parthenostilben A, parthenostilben B (Kim et al. 2005) and tricuspidatol A (Lins et al. 1991) are reported. In the leaves trans-piceid (Son et al. 2007; Park et al. 2008), longistylin A and longistylin B (Son et al. 2007) and trans-piceatannol (Kundaković et al. 2008) are reported.

The main goal of this study was to analyse the biologically active compounds present during the senescence of *Parthenocissus tricuspidata* leaves with the focus on trans-pterostilbene and its transformation products.

Materials and Methods

Plants material and preparation of extracts

The leaves of *Parthenocissus tricuspidata* (Siebold et Zuccarini) Planchon were collected at different locations in the Czech Republic in the years 2012–2014 (Table 1). The samples of leaves were frozen at −18 °C and then lyophilized. Finely ground samples were extracted with ethyl acetate and then the samples were diluted in methanol. The subsamples from each sample used in the analysis were prepared in triplicate.

Liquid chromatography

The extracts were analyzed using a HPLC (HP 1050 Ti-series, Hewlett Packard Palo Alto, CA, USA) and a Luna C18(2) column, 150 mm × 2 mm, 3 μm (Phenomenex, Torrance, CA, USA), G1315B diode array detector (DAD, Agilent) and G1321A fluorescence detector (FLD, Agilent). The compounds were identified by measurements made using a LC-MS (LCQ Accela Fleet (Thermo Fisher Scientific, San Jose, CA, USA)). Separations using HPLC and LC-MS (APCI) are described in detail in Tříska et al. (2012).

As standards trans-resveratrol and 9-phenanthrol from Sigma-Aldrich were used and trans-pterostilbene was kindly provided by prof. Jan Šmidrkal, University of Chemistry and Technology, Prague. Acetonitrile and methanol were from Merck, o-phosphoric acid and formic acid from Sigma-Aldrich.

Data analysis

Quantification of trans- and cis-pterostilbene using HPLC was done using a calibration curve for trans-pterostilbene (diode array detector, at 315 nm); quantification of trans-resveratrol using a calibration curve for trans-resveratrol (diode array detector, at 315 nm); of that 2,4-dime-
Trans-pterostilbene and 2,4-dimethoxy-6-hydroxyphenanthrene in the leaves of Parthenocissus tricuspidata

Results and Discussion

The samples were collected from four locations in autumn, when the leaves had begun to change in colour from green to yellow and red. The samples from one of these locations were collected on three dates in one year, the samples from the second location were collected in two consecutive years. The locations and dates of sampling are shown in Table 1.

In the samples trans-resveratrol, trans-pterostilbene and 2,4-dimethoxy-6-hydroxyphenanthrene (DMPH) were identified. It was possible to detect also cis-pterostilbene in some samples. The irradiated trans-pterostilbene standard served as a test substance to identify compounds present in the extracts.

By irradiating the methanol solution of trans-pterostilbene at 254 nm for 20 hours, a mixture of trans-pterostilbene, DMPH and cis-pterostilbene was obtained. The structures of these substances are shown in Fig. 1 and their DAD spectra in Fig. 2. The samples were also measured using LC-MS (APCI in positive mode): we recorded for 2,4-dimethoxy-6-hydroxyphenanthrene molecular ion at \( m/z \) 255 \([M+H]^+\) and for trans-pterostilbene and cis-pterostilbene molecular ion at \( m/z \) 257 \([M+H]^+\).

The content of DMPH and the stilbenes studied is very variable, but the DMPH content was always much greater in autumn “coloured leaves” compared to green leaves. The highest content of DMPH was in the samples of leaves that were either completely or partially dark red (Figs 3-6). The amount of trans-pterostilbene was only a few mg/kg; for many samples, the content of trans-pterostilbene was below the detection limit (e.g. in the green leaves from localities A, B and D). Only traces of cis-pterostilbene were detected in three samples: Locality A – yellow-dark red leaves, locality B – red leaves, locality C – green-dark red leaves 10.10.2013. In the green leaves from site C in 2014 the content of both stilbenes was below the detection limit.

Derivatives of phenanthrene are very common biologically active compounds in the plant kingdom (Kovács et al. 2008), but to our knowledge there is no information on the presence of DMPH in plants. Only the dihydro derivative of DMPH (double bond saturated in the positions 9, 10) is mentioned in the literature under the name orchinol (Kovács et al. 2008). DMPH was patented (Hashimoto et al. 1976) as a novel growth modifier useful for controlling growth, germination of seeds and regulating the dormant stages of seeds, bulbs and buds.

Formation of phenanthrene derivatives as final products in the UV photo isomerization of trans-resveratrol to cis-resveratrol and final cyclization to the derivative phenanthrene in the leaves of Vitis vinifera plants following attack by Plasmopara viticola is described in the literature (Tříska et al. 2012). Senescence of Parthenocissus tricuspidata leaves, visibly manifested by the colour change in autumn, may have a similar mechanism also...
Conclusions

During the senescence of the leaves of *Parthenocissus tricuspidata*, visibly manifested in autumn by the change in their colour, *trans*-pterostilbene, originally present in the leaves, is transformed into 2,4-dimethoxy-6-hydroxyphenanthrene, which is reported here for the first time in the leaves of *Parthenocissus tricuspidata*.

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REFERENCES


