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 for 40 min at 50 °C and the sediment was washed twice with ethyl acetate. Supernatants were pooled, ethyl acetate was evaporated in a stream of nitrogen and then the samples were diluted in methanol. The subsamples from each sample used in the analysis were prepared in triplicate.

Table 1 Localities and data sampling.

	Locality	Data of collection	Leaf colour
A	Kutná Hora 49°56′54″N 15°16′6″E	20.9.2012	green; yellow-dark red
B	Hluboká nad Vltavou 49°3′8″N 14°26′3″E	9.10.2013	green; red; dark-red; yellow-green-dark-red; yellow-red
C	Lednice 48°47′60″N 16°48′12″E	10.10.2013 17.10.2014	green; green-dark red; green-yellow red
D	Starý Smolivec 49°31′39″N 13°45′7″E	18.8.2013 16.9.2013 5.10.2013	green; green-red border; green-dark red; red

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); that of 2,4-dime-

thoxy-6-hydroxyphenanthrene using a calibration curve for 9-phenanthrol (synonym for 9-hydroxyphenanthrene) using a fluorescence detector (Ex 315 nm, Em 395 nm). LOD and LOQ for *trans*-resveratrol were 0.055 µg/ml and 0.184µg/ml,respectively,for*trans*-pterostilbene,0.042µg/ml and 0.142 µg/ml, for 9-phenanthrol 0.042 µg/ml and 0.141 µg/ml. Each value was based on three measurements.

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 highest content of DMPH was recorded in the leaves from site A in 2012 and from site C in 2013. 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

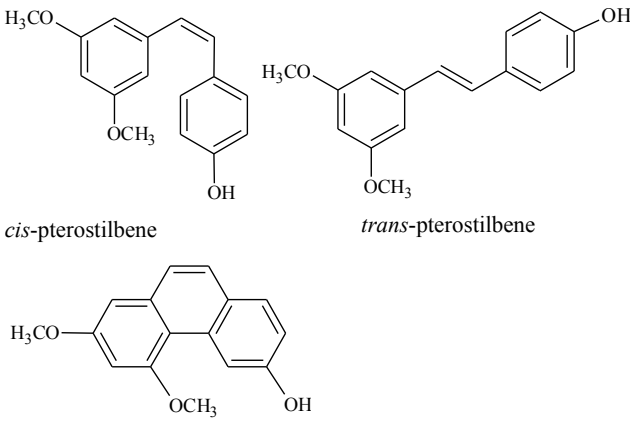

Fig. 1 The structure of analysed compounds.

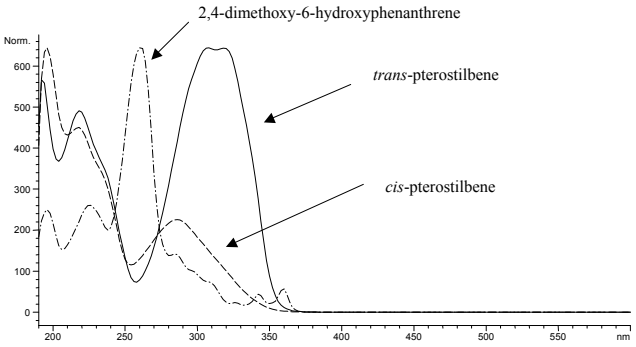

Fig. 2 DAD spectra of *trans*-pterostilbene and its derivatives.

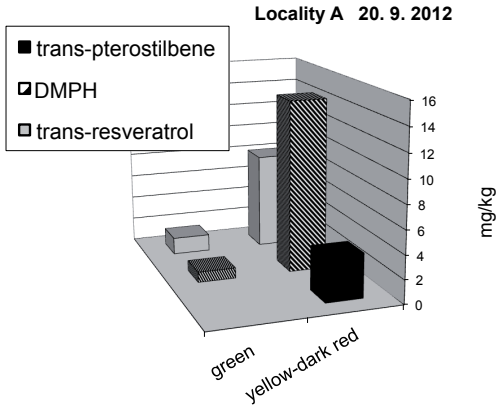

Fig. 3 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality A.

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

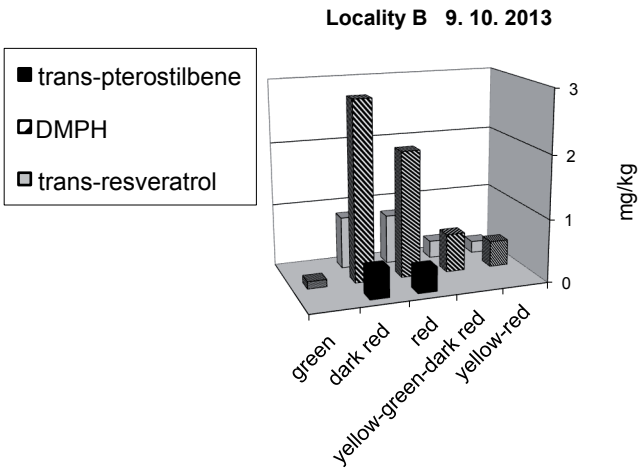

Fig. 4 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality B.

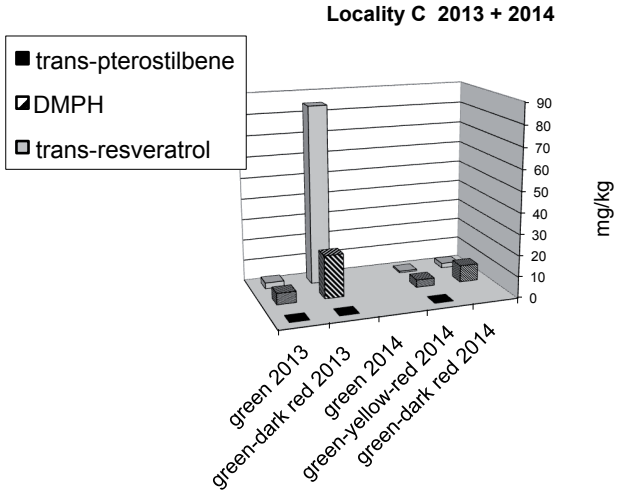

Fig. 5 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality C.

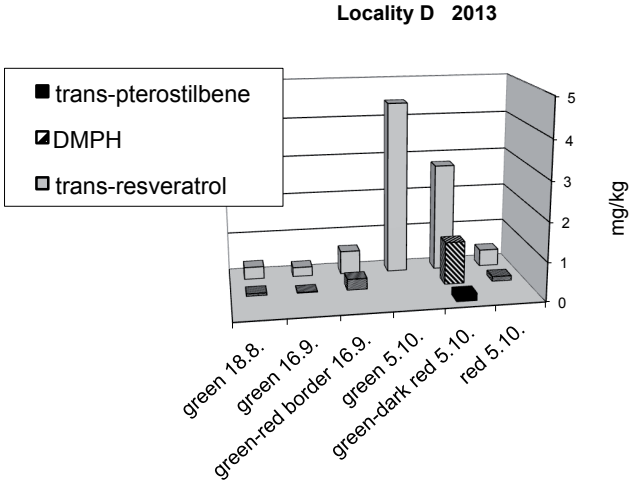

Fig. 6 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality D.

ending with phenanthrene derivative, in this case mainly with DMPH.

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 tranformed into 2,4-dimethoxy-6-hydroxyphenanthrene, which is reported here for the first time in the leaves of *Parthenocissus tricuspidata*.

Acknowledgements

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic within the National Sustainability Program I (NPU I), grant no. LO1415. We also wish to thank I. Soural and K. Matějková for assistance in collecting plant material and thank J. Šmidrkal for providing *trans*-pterostilbene.

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