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The occurrence of the stilbene piceatannol in grapes

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Summary

Piceatannol (*trans*-3,3',4,5'-tetrahydroxy-stilbene) is a natural stilbene occurring in a number of plant species, and it has been shown to have beneficial effects on human health. The compound can seldom be consumed by humans, because it occurs in non-food plants, or in non-edible organs. Here we show for the first time that grapes (*Vitis vinifera* L. cv. Cabernet Sauvignon) have significant amounts of piceatannol (0.052 μg g^{-1} fresh wt). The identity of piceatannol was confirmed by HPLC and LC-MS.

Key words: *Vitis vinifera*, grape berry, stilbene, piceatannol.

Introduction

Fruit (including grapes) intake in the diet is highly recommended because of beneficial effects on disease prevention due to the occurrence of health functional phytochemicals, such as phenolics (Kalt 2001). Phenolics are a large family of compounds showing mostly antioxidant activity, and some of those, like the grape stilbenes, are present in the fruit as phytoalexins, which are compounds produced by the plant in response to abiotic and biotic stress (Bavaresco and Fregoni 2001). Stilbenic phytoalexins have been identified in grapes, such as *trans*-resveratrol (*trans*-3,4',5-trihydroxy-stilbene) (Langcake and Pryce 1977), *trans*- and *cis*-piceid (*trans*- and *cis*-resveratrol-3-*O*-β-D-glucopyranoside) (Waterhouse and Lamuela Raventòs 1994; Mattivi *et al*. 1995; Romero-Pérez *et al*. 1999), ε-viniferin (trans-resveratrol dimer) (Bavaresco *et al.* 1997), pterostilbene (*trans*-3,5-dimethoxy-4'-hydroxy-stilbene) (Pezet and Pont 1988), but not piceatannol. Piceatannol (Fig. 1), or astringinin, has been detected in some non-food species like *Euphorbia lagascae* (Ferrigni *et al.* 1984), *Melaleuca leucadendron* (Tsuruga *et al.* 1991), *Picea abies* (Mannila and Talvitie 1992), *Picea sitchensis* (Aritomi and Donnelly 1976), *Cassia garrettiana* (Kimura *et al.* 2000) and in *Scirpus californicus* (Schmeda-Hirschmann *et al.* 1996), whose rhizomes are edible, *Rheum undulatum* (Ko and Ko 2000), whose rhizomes are used for traditional drugs, *Saccharum* sp. (Brinker and Seigler 1991) and in *Aiphanes aculeata* (Lee *et al.* 2001), while its glucoside (piceatannol 3-*O*-β-D-glucopyranoside) occurs in grape cell suspension cultures (Waffo-Teguo *et al.* 1996) and in wine (Carando *et al.* 1999; Landrault *et al*. 1999; Ribeiro de Lima *et al*. 1999). Nevertheless piceatannol has not been detected in grapes yet.

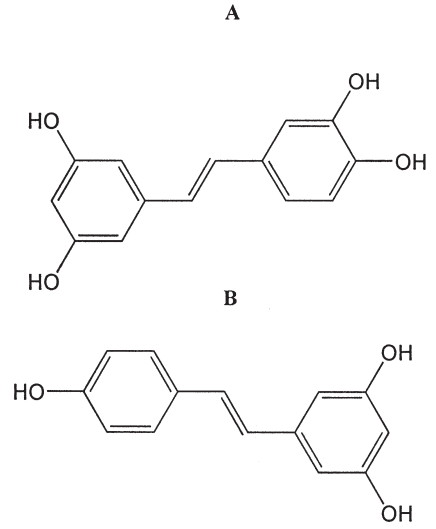

Fig. 1: Structure of piceatannol (**A**) and *trans*-resveratrol (**B**).

This compound is deserving interest because it is a known inhibitor of protein-tyrosine kinase (Geahlen and McLaughlin 1989; Oliver *et al.* 1994) and 5 alpha-reductase (Ko and Ko 2000), an antileukaemic agent (Ferrigni *et al.* 1984; Mannila *et al.* 1993), an antioxidant and a radical scavenger agent (Fauconneau *et al.* 1997). Recently it has been demonstrated that piceatannol prevents in B and T lymphocytes, in fibroblast and in HeLa cells interferon-α-induced Stat3 and Stat5 phosphorylation (Su and David 2000), as well as the progression of cell cycle in colerectal cancer cells (Wolter *et al.* 2002). According to Potter *et al.* (2002) resveratrol is converted to piceatannol by the cytochrome P450 enzyme CYP1B1 that is found in tumours, and piceatannol is claimed to be the active compound targeting and destroying cancer cells.

Material and Methods

Chemicals: Acetonitrile, methanol, and acetic acid were HPLC grade and purchased from Carlo Erba (Milan, Italy), ethyl acetate from BDH (Poole, Dorset, England), phosphoric acid from Merck (Darmstadt, Germany). The *trans*-resveratrol (*trans*-3,4',5-trihydroxy-stilbene) and piceatannol (*trans*-3,3',4,5'-tetrahydroxy-stilbene) standards were purchased from Sigma (Milan, Italy), *cis*-resveratrol was prepared from the standard of *trans*-resveratrol by photoisomerization, *trans*-piceid (*trans*-resveratrol-3-*O*-β-D-

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glucopyranoside) was isolated from the roots of *Polygonum cuspidatum*. The purity of each stilbene was controlled by HPLC and the identity was confirmed according to MATTIVI *et al*. (1995).

Sample preparation: Berries from clusters of 5 potted grapevines (*V. vinifera* L., cv. Cabernet Sauvignon, clone R5) were sampled at maturity (average sugar concentration 18.3 ° Brix). About 20 g of fresh berries (without seeds) from each grapevine were ground in a mortar with 30 ml methanol 95 %, and vigorously shaken for 20 min, at room temperature, according to BAVARESCO *et al*. (1997). A filtration by GF/A Whatman filters followed, the liquid was evaporated *in vacuo* at 40 °C and the water fraction was extracted twice with 5 ml ethylacetate and 5 ml $NaHCO_3$ (5 %), by phase partitioning. The organic phase was evaporated to dryness and stilbene compounds were recovered by 2 ml plus 1 ml methanol 100 % and stored in adiactinic vials at -18 °C. An aliquot of 600 µl of sample extract was evaporated to dryness under a gentle flow of nitrogen. The residue was immediately redissolved in 100 µl methanol and 200 µl 1 % acetic acid in H_2O. The sample was filtered through a 0.22 µm PVDF filter (Millipore, Bedford, MA) into an HPLC vial, and then analyzed by HPLC.

HPLC-DAD conditions: An HP 1090 series HPLC (Agilent, Palo Alto, CA) with a gradient pump and diode array detector was used for this analysis. RP-HPLC analyses were performed using ODS Hypersil 200 x 2.1 (5 µm) with guard ODS Hypersil 20 x 2.1 mm (5 µm) (Agilent, Palo Alto, CA). The mobile phases consisted of 0.001 M phosphoric acid (A) and acetonitrile (B). Separation was carried out at 40 °C under the following conditions: linear gradients from 0 to 50 % B in 25 min, to 100 % B in 1 min. The column was equilibrated with 100 % A for 5 min prior to each analysis. The flow rate was set to 0.6 ml min^{-1} and the injection volume to 6 µl. The UV spectra were recorded from 200 to 400 nm. Detection was made at 310 nm for *trans*-resveratrol, piceatannol and *trans*-piceid, and at 282 nm for *cis*-resveratol and *cis*-piceid. All compounds were identified on the base of their UV spectra and retention time, compared to authentic standards. Samples were quantified by the external standard method. The *trans*-piceid was expressed as *trans*-resveratrol equivalents, µg g^{-1} .

HPLC-MS conditions: Mass spectrometric analysis were carried out on Micromass ZQ LC-MS system (Micromass, Manchester, UK), equipped with a Waters 2690 HPLC system and a Waters 996 DAD detector (Waters Corp., Miliford, MA) and MassLynx Software version 3.5 (Micromass, Manchester, UK). The conditions and gradient of separation were the same as for HPLC-DAD, with the only exception of solvent A, where phosphoric acid was replaced by 1 % acetic acid in H_2O due to its higher volatility. Capillary voltage was 3000 V, cone voltage 25 V, extractor voltage 5 V, source temperature 105 °C, desolvation temperature 200 °C, cone gas flow (N_2) 30 l h^{-1} , desolvation gas flow (N_2) at 450 l h^{-1} . The outlet of the HPLC system was split (9:1) to the ESI interface of the mass analyzer. Electrospray mass spectra ranging from *m/z* 100 to 500 were taken in positive mode with a dwell time of 0.1. Piceatannol was quantified in the SIR mode, *m/z* 245.3.

Results and Discussion

The average concentrations of the known stilbenes (on the basis of berry fresh weight) were as follows: *trans*-resveratrol 0.297 µg g^{-1} and *trans*-piceid 0.097 µg g^{-1} , while

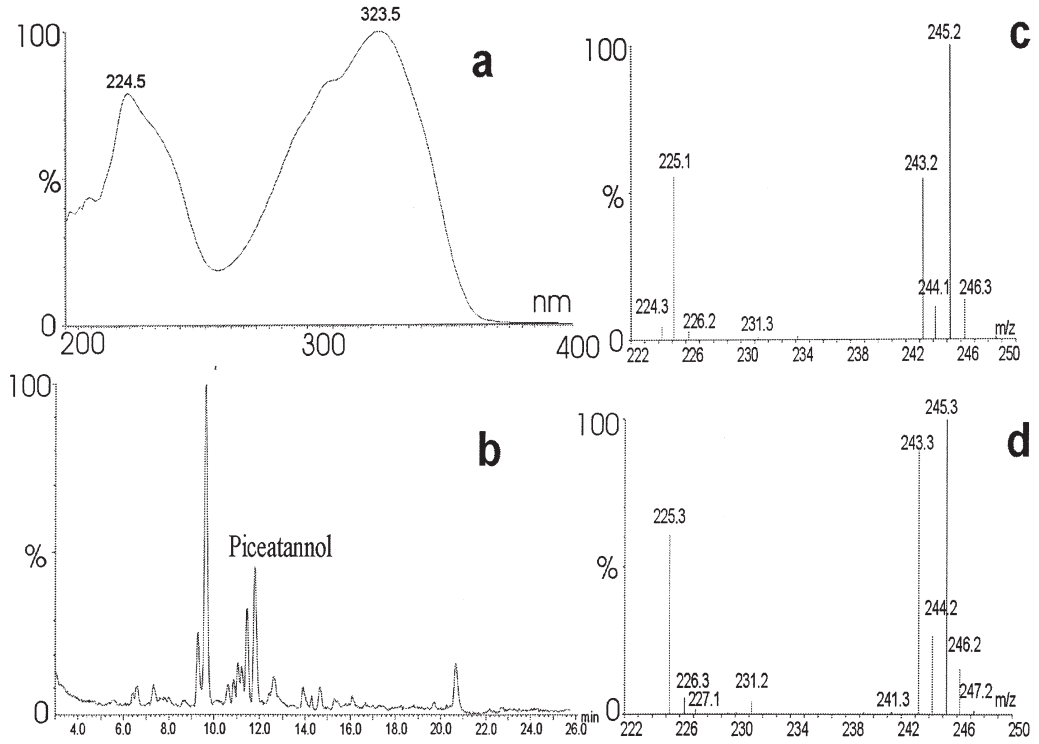

Fig. 2: **a**) UV spectrum of piceatannol; **b**) LC-ESI-MS chromatogram of grape extract, in positive mode, at *m/z* = 245.3; **c**) MS spectrum of piceatannol standard; **d**) MS spectrum of piceatannol in the grape extract.

the *cis*-resveratrol and *cis*-piceid were below the limit of detection in all the samples considered. The HPLC-DAD analysis showed the possible presence of a stilbene derivative which was found to coelute with an authentic sample of piceatannol, and which had an UV spectrum with maxima at 323.5 nm (Fig. 2 a) and profile matching exactly that of piceatannol. It was impossible to obtain an accurate quantification of this compound from the UV trace since this peak overlapped partially with some unidentified phenolics, with the spectra of flavonols glycosides and interferring with the quantification. The presence of such compounds could be the reason that piceatannol was not found in previous studies on grape stilbenes. Each grape extract, and finally a bulk sample obtained by pooling all the samples together, were analysed also by LC-MS in order to confirm the tentative identification of piceatannol and to obtain more accurate quantitative data (Fig. 2 b). The MS spectra allowed us to confirm the identity of peaks at 12.1 min to be piceatannol. The MS spectra of this peak (Fig. 2 c, d) presented the molecular ions and related fragments typical for piceatannol, that is *m/z* 245.2 (M+1), 243.2 (M-1), 225.2, 215.2 and 197.2. The partially overlapping peak in the chromatogram was found to be a quercetin-3-glycoside, the sugar being a hexose. Quantitative analysis by LC-ESI-MS allowed us to estimate an average concentration of piceatannol in grape of 0.052 μg g^{-1} fresh wt. In light of previous literature data (Carando *et al*. 1999; Landrault *et al*. 1999; Ribeiro da Lima *et al*. 1999) we would expect the possible presence of *trans*-astringin (a 3-glucoside of *trans*-piceatannol) in the extracts. Its presence would be evidenced in the chromatograms by a peak having a retention time shorter than that of piceatannol, appearing both in the UV trace at 310 nm and in the SIR trace at *m/z* = 245.2 due to the base peak originated by the loss of the sugar, possibly with the presence in the total ion chromatogram of the molecular ion at *m/z* = 407. In our samples, no peak with such features was observed. Moreover, it is unlikely that the piceatannol in the extract would have resulted from hydrolysis of *trans*-astringin during the sample processing, because the conditions of extraction, analysis and storage of stilbenes from grape were not hydrolytic, being routinely used for the quantification of the glucosides of *trans*-resveratrol. We can therefore suggest piceatannol as a new stilbene in grape.

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