

Biochemistry

***Trans*-Piceid Stilbenoid in the Juice and Skin of the Red Grape (*Vitis vinifera* L.) Varieties Growing in Georgia**

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(Presented by Academy Member Shota Samsoniya)

ABSTRACT. *Trans*-resveratrol glucoside, *trans*-piceid (4',5-dihydroxylstilbene-3-O-β-D glucopyranoside) (polydatine), was identified and determined in the grape juice and skin of the red grape varieties growing in Georgia. These species are: Saperavi, Cabernet Sauvignon, Otskhanuri Sapere, Aleksandrouli, Mujuretuli, Shvkapito, Tavkveri, Aladasturi, Dzelshavi and Ojaleshi. Identification was done by means of acid hydrolysis, thin-layer and high-performance liquid chromatography (HPLC) and UV spectroscopy methods. Authentic *trans*-resveratrol was used for comparison purposes. The stilbenoids were extracted from the objects with ethyl acetate. A stilbenoid-containing fraction was isolated from the extracts obtained by treating on adsorbent (Sefadex G25). The study of stilbenoid dissolved in methanol in UV area is characterized by the absorption maximum at 308 nm- and 335 nm-long wave. Similar properties were identified for the study varieties: the concentration of *trans*-piceid in grape juice (2.20mg/l-12.41mg/l) much exceeds that in grape skin (0.12mg/kg-0.94mg/kg). In the varieties studied, the most amount of *trans*-piceid is found in the juice and skin of Saperavi variety (12.41 mg/l and 0.94 mg/kg, respectively). Identification of biologically active *trans*-piceid is an important basis to explain the curative and preventive properties of grape and wine. © 2013 Bull. Georg. Natl. Acad. Sci.

Key words: *trans*-piceid, *trans*-resveratrol, grape juice, grape skin.

The Georgian vine gene pool is made up of up to 525 white and red grape vine varieties. The vintage varieties are an important raw material for making different types of competitive high-quality wines having curative and preventive properties. Georgian vine varieties and wines made with them are characterized by a rich spectrum of phenol compounds represented by flavonoid (flavanols, flavonols,

proanthocyanidins, anthocyanins, etc.) and non-flavonoid (stilbenoids, phenol carbon acids, etc.) compounds. These phenol compounds by being localized in wine determine the wine quality and its functional designation in the curative and preventive respect[1,2]. Out of vine phenol compounds, the group of stilbenoids is important. Stilbenoids are biologically highly active substances and play the role of

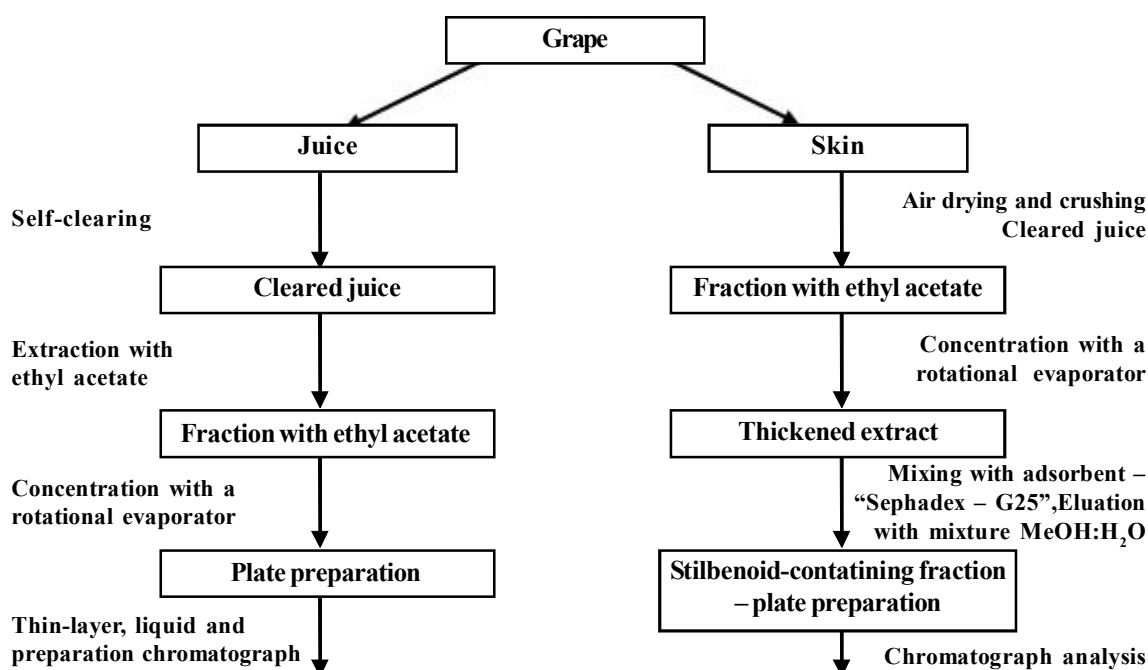


Fig. 1. Diagram of isolating stilbene-containing fractions from grape juice and skin

phytoalexin in the plant. Such stilbenoids are: resveratrol, ϵ -viniferin, etc. [3, 4]. Studying stilbenoids, owing to the biological activity of these substances, and first of all, in red wines to explore their functional designation is a topical issue at present. Such stilbenes as *trans*-resveratrol and its dimer: ϵ -viniferin and two tetrameric stilbenes were isolated and identified from vine in Georgia [5]. Studies certify that the red grape varieties growing in Georgia, such as Saperavi, Saperavi Budeshurisebri, Cabernet Sauvignon, Otskhanuri Sapere, Aladasturi, Asuretuli Shavi, Chkhaveri, Aleksandrouli, Mujuretuli, Ojaleshi and their wines contain *trans*-resveratrol and its derivatives: dimeric ϵ -viniferin and tetrameric stilbene, with *trans*-resveratrol being ranked first in terms of its proportion [6].

Out of stilbenoids in the red grape varieties and wines of a number of countries, *trans*- and *cis*-isomers of resveratrol, ϵ -, δ -, α -viniferins, palidol, piceatanole, stilbenoid glucosides: *trans-cis*-piceids, atringin, etc. are obtained [7,8]. Following the studies of red Georgian wines and grape, while continuing the study of stilbenoids, we aimed at studying stilbenoid glucosides in the juice and skin of red-grape varieties.

Materials and methods

Sound grape juice and skin of red-grape varieties growing in Georgia were obtained in the technical ripeness period. Such varieties are: Saperavi, Cabernet Sauvignon, Otskhanuri Sapere, Aleksandrouli, Mujuretuli, Shavkapito, Tavkveri, Aladasturi, Dzelshavi and Ojaleshi. We prepared the objects of study with grape juice and skin according to plan and used it for analysis (Fig. 1); We used grape juice of Saperavi to obtain acid hydrolysate, added concentrated HCL to it to reach 10% acid concentration in the reaction area and accomplished hydrolysis at the temperature of 80°C for 3 hours. Under the given conditions, we carried out acid hydrolysis of the individual study substances isolated by means of preparations. The hydrolysates were extracted with ethyl acetate, concentrated with rotational over-flow and analyzed.

UV spectrum of the compound under study was shot with spectrometer "Specord". We used paper chromatography for qualitative analysis of sugars in acid hydrolysates. We used a mixture of solvents: N-butanol-ethyl acetate-propanol-acetic acid-water (35:100:62:35:30) as a system. We developed a chro-

matogram with the mixture of water solutions of ammonium molybdate and ammonium chloride in acid area.

Qualitative analysis was accomplished with thin-layer chromatography (Sorbfil, ПТСХ- П-А 10x20; system – chloroform:methanol, 80:20). The chromatograms were developed with diazotized sulfanilic acid.

HPLC analysis: chromatograph “Varian”, column Supelcosil™ LC18, 250X4.6 (mm); solvent A, 0.025%, trifluoroacetic acid; solvent B, acetonitrile (ACN)/A, 80/20, gradient regime 0-35 min. 20-50% B; 35-40 min. 50-100% B; 41-46 min. 100% B; 46-48 min. 100-20% B; 48-53 min. 20% B. Eluent transfer velocity 1 ml/min, wavelength 306nm [9]. The samples for chromatography were filtered with a membrane filter (0.45µm) in advance.

Results and discussion

Unknown substances were found in Saperavi grape juice, which were not in evidence after the acid hydrolysis of the grape juice. Their chromatographic properties are shown in Fig.2 and Table 1.

One of the individual substances originated as a result of the acid hydrolysis of Saperavi grape juice, with its Rf (0.68), brownish-orange coloration of lacquer, bluish-violet illumination in the UV area and absorption maximum in the UV spectrum (300nm), is identified as *trans*-resveratrol. Substance -IV, through acid hydrolysis, forms *trans*-resveratrol, and glucose is found in hydrolysate. This evidences the presence of *trans*-resveratrol glucoside. Substance -IV yields an absorption maximum of 300-350 nm in the UV area typical of stilbenoids of *trans*-form. In particular,

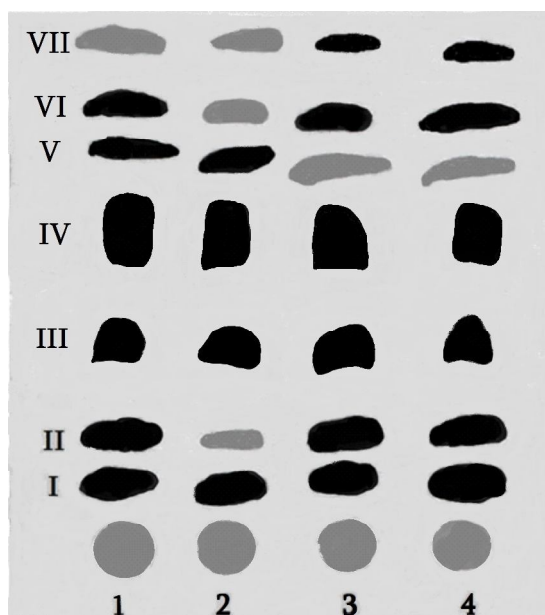


Fig. 2. Thin-layer chromatogram of fractions with grape juice ethyl acetate (system: Chloroform: methanol (80:20); 1. Aleksandrouli, 2. Aladasturi, 3. Mujuretuli, 4. Dzelshavi; IV-Required substance

λ_{\max} (MeOH) – 308 nm; 335 nm. The study substance, after comparing the above-mentioned properties with the present ones was identified as *trans*-piceid (4'-5-dihydroxystilbene-3-O- β -D glucopyranoside) (polydatine).

It is interesting that intense coloration is evidenced in the area of UV rays on a thin-layer chromatogram of the fraction with ethyl acetate under strict conditionals of acid hydrolysis of substance – IV (by boiling) in the relevant state of *trans*-resveratrol, but a stain is not developed with diazotized sulfanilic acid. This evidences the absence of phenol hydroxyl in the given substance. The given compound, isolated individually by means of the preparation in UV spec-

Table 1. Chromatographic properties of the study substance

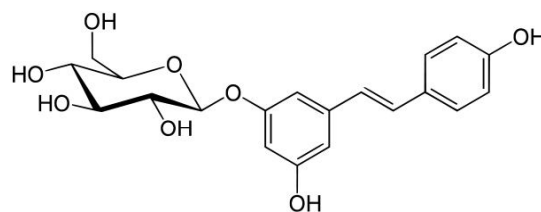
Substance, N	Rf	Lacquer coloration	Developer	System
I	0.093	Brownish-orange	Diazotized sulfanilic acid	Chloroform : methanol (80:20)
II	0.187	Brownish-orange	“-----“	“-----“
III	0.26	Yellow	“-----“	“-----“
IV	0.39	Brownish-orange	“-----“	“-----“
V	0.49	Wine-colored	“-----“	“-----“

Table 2. Content of *trans*-piceid in grape juice and skin

No	Variety	Juice,mg/l	Skin,mg/kg
1	Saperavi	12.40	0.94
2	Cabernet Sauvignon	6.96	0.26
3	Otskhanuri Sapere	5.11	0.22
4	Aleksandrouli	5.75	0.28
5	Mujuretuli	9.70	0.30
6	Shavkapito	4.35	0.17
7	Tavkveri	3.82	0.12
8	Aladasturi	5.25	0.46
9	Dzelshavi	2.20	0.18
10	Ojaleshi	2.50	0.30

trum yields an absorption maximum at 278 nm wavelength corresponding to the structure of a stilbene.

The red grape vine varieties were found to be different depending on the content of *trans*-piceid. In addition, they were found to have a common feature - the concentration of *trans*-piceid in grape juice of each vine variety much exceeds that in the skin of varieties. Large amount of *trans*-piceid is found in Saperavi grape juice (12.40 mg/l), while Saperavi grape skin contains an amount of *trans*-

**Scheme.** *Trans*-piceid C₂₀H₂₂O₈; M_r-390

piceid of all vine species (0.94 mg/kg) (Table 2).

The studies have proved the biological activity of *trans*-piceid: it inhibits thrombocyte aggregation, reduces oxidation of low density lipoproteins in the human body, reduces the content of lipids and is an inhibitor of eicosanoids synthesis[10].

Conclusion

Thus, as a result of the accomplished experiment, *trans*-piceid, *trans*-resveratrol glucoside (4',5'-dihydroxystilbene-3-O-β-D glucopyranoside) (polydatine) was identified and determined in the grape juice and skin of red grape vine varieties (*Vitis vinifera* L.) growing in Georgia. The presence of biologically active stilbenoid glucoside is an important condition for forming curative and preventive properties in grape and wine.

ბიოქიმია

სტილბენოიდი ტრანს-პიცეიდი საქართველოში გავრცელებული წითელყურძნიანი ვაზის (*Vitis vinifera* L.) ჯიშების ყურძნის წვენსა და კანში

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(წარმოდგენილია აკადემიკოს შ. სამსონიას მიერ)

საქართველოში გავრცელებული ვაზის წითელყურძნიანი ჯიშების ყურძნის წვენსა და კანში პირველად იდენტიფიცირდა და განისაზღვრა ტრანს-რეზვერატროლის გლუკოზიდი ტრანს-პიცეიდი (4',5-დიჰიდროქსისტილბენ-3-O-β-D- გლუკოპირანოზიდი) (პოლიდატინი). ეს ჯიშებია: საფერავი, კაბერნე სოვინიონი, ოცხანური საფერე, ალექსანდროული, მუჯურეთული, შაკაპიტო, თავკვერი, ალადასტური, ძელშავი და ოჯალეში. იდენტიფიკაცია ჩატარებულია მჟავური ჰიდროლიზის, მაღალეფექტური სითხური და თხელფენოვანი ქრომატოგრაფიის, ულტრაიისფერი სპექტროსკოპიის მეთოდების საფუძველზე. აგლიკონთან შესადარებლად გამოყენებულია ავთენტიკური ტრანს-რეზვერატროლი. სტილბენოიდები ობიექტებიდან გამოწვლილული იქნა ეთილაცეტატით. მიღებული ექსტრაქტიდან სტილბენოიდებშემცველი ფრაქცია გამოიყო აღსორბენტზე (Sephadex G25) დამუშავებით. მეთანოლში გახსნილი საკვლევი სტილბენოიდი ულტრაიისფერ უბანში ხასიათდება შთანთქმის მაქსიმუმით 308ნმ და 335ნმ ტალღის სიგრძეზე. საკვლევი ჯიშებისთვის გამოვლინდა ერთნაირი მახასიათებელი: ტრანს-პიცეიდის კონცენტრაცია ყურძნის წვენში (2.20 მგ/ლ-12.41 მგ/ლ) მნიშვნელოვნად აღემატება მის რაოდენობას ყურძნის კანში (0.12მგ/კგ-0.94მგ/კგ). საკვლევი ჯიშებს შორის ტრანს-პიცეიდი ყველაზე მეტი რაოდენობით აღმოჩნდა საფერავის წვენსა (12.41 მგ/ლ) და კანში (0.94 მგ/კგ). ბიოლოგიურად აქტიური ტრანს-პიცეიდის იდენტიფიკაცია მნიშვნელოვანი საფუძველია ყურძნის და ღვინის სამკურნალო-პროფილაქტიკური თვისებების ასახსნელად.

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