**Organic Chemistry** 

## Content of the Biologically Active Trans-Resveratrol and ε-Viniferin in Color Vine Varieties Growing in Georgia

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ABSTRACT. Stilbene-containing plate preparations have been extracted from the skin of industrial color-grape vine (*Vitis vinifera* L.) growing in Georgia. These varieties are as follows: Saperavi, Saperavi Budeshurisebri, Cabernet Sauvignon, Otskhanuri Sapere, Ojaleshi, Aladasturi, Chkhaveri, Aleksandrouli, Mujuretuli, Asuretuli Shavi. With the use of the high-performance liquid chromatograph (HPLC), their diversified stilbene spectra have been identified, with the trans-resveratrol and its dimer  $\varepsilon$ -viniferin was identified in them. There is a common regularity identified in the skin of all grape varieties, in particular, the concentration of trans-resveratrol much exceeds that of its dimer  $\varepsilon$ -viniferin. The quantities of identified stilbenes vary depending on the varieties and habitats of the vine. Studying the stilbene spectrum is the theoretical basis to explain the functional designation of grape and wines in respect of their curative and nutritional value. © 2011 Bull. Georg. Natl. Acad. Sci.

Key words: trans-resveratrol,  $\varepsilon$ -viniferin, skin of red grape.

The gene pool of the Georgian vine contains up to 525 varieties of white- and color-grape vine, technical and table varieties. As for the wine quality, it depends on the generic features of the grape and technological treatment of wine. Red wine deserves particular attention due to its organoleptic characteristics, curative and nutritional value and is the subject of intense consideration. These properties of red wine are mainly the result of the phenol compounds of a wide spectrum with antioxidant activity and with stilbenes being the most significant. We started to study stilbenes in 1991, by extracting trans-resveratrol, its dimer  $\epsilon$ -viniferin and two tetramer stilbenes from the annual shoots of Rkatsiteli vine varieties. We identificated and established their biological activity [1]. The  $\varepsilon$ -viniferin, like trans-resveratrol, is a biologically active natural substance and plays a particular role in the vine and wine. It is a phytoalexin and its effect against chlorosis and mildew is proved [2, 3]. The viniferin and resveratrol extracted from the vine pruning are characterized by oxidation and apoptotic effects against chronic leukaemia lymphocytes [4]. The viniferin extracted from red wine showed much stronger inhibiting effect against human liver microsomes than resveratrol [5]. Natural viniferin is characterized by anti-inflammatory property – by PDE4 inhibiting [6]. It is also an inhibitor during the oxidation of lipids with superoxide anion [7].

By continuing the study of stilbenes, we determined the quantity of  $\varepsilon$ -viniferin of trans-resveratrol in the colorgrape vine varieties growing in West and East Georgia.

Materials and methods. The objects of the study were the fractions extracted with ethyl acetate from the skin of 2009 harvest of color-grape vine varieties (*Vitis vinifera* L.) growing in Georgia. These varieties include Saperavi, Saperavi Budeshurisebri, Cabernet Sauvignon, Otskhanuri Sapere, Ojaleshi, Aladasturi, Chkhaveri, Aleksandrouli, Mujuretuli, Asuretuli Shavi. The Saperavi grape was harvested in certain districts of Kakheti (East Georgia), in its habitat – Akhasheni, Kardenakhi, Kindzmarauli and Tsinandali.

**Obtaining stilbene plate preparations and qualitative analysis.** We extracted fractions with ethyl acetate from air-dried grape skin by using step extraction in hot. The duration of each step was 30 minutes. We grouped and concentrated the obtained fractions. In order to divide prepared plate preparations, we placed them on a column, which was filled with adsorbent Sephadex G25. We conducted elution with the mixture methanol: water [8], and we obtained the stilbene plate preparations as a result. In the purified plate preparations, trans-resveratrol and  $\varepsilon$ -viniferin were automatically fixed with thin-layer chromatography conducted with Siluphol plates (20x20 cm). We used the system chloroform: methanol (80:20) as a solvent. We developed the chromatograms with diazotized sulfanilic acid.

**HPLC analysis.** Quantitative determination of åviniferin and trans-resveratrol was conducted at the central laboratory of our institute by the HPLC method [9] (high-performance liquid chromatograph produced by Varian, pillar module - Prostar 500; transfer module of solvents - Prostar 210, detector – ultra-violet/visible light spectrum - Uv/Vis spectrometer Prostar 325, pillar -Microsorb 100 C 18; 250x4,6 LxId (mm); 5µm – particle size; terms of the gradient regime: solvent A, 0.025% water solution TFA (trifluoroacetic acid); solvent - ACN/A, 80/20 (v/v));

0-35 min 20-50% B;



I. trans-resveratrol C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>



II. ε-viniferin C28H22O6

35-40 min 50-100% B; 41-46 min 100% B; 46-48 min 100-20% B; 48-53 min 20% B.

The speed of eluent supply was 1 ml/min; injecting amounted to 20  $\mu$ l at room temperature at wave length of 306 nm.

**Results and discussion.** Thin-layer and liquid chromatograms of stilbene-containing plate preparations are evidence of the variety of the stilbene spectrum in the experimental vine varieties. They include a monomer representative of stilbenes trans-resveratrol(I) and  $\varepsilon$ viniferin(II), which we aimed to identify.

Of the objects under study, Saperavi and Aleksandrouli grape skin is distinguished for a higher concentration of  $\varepsilon$ -viniferin (0.67mg/100g and 0.57mg/ 100g). Monomer trans-resveratrol and its dimer a-viniferin in the skin of all varieties under study are localized by the same regularity: the concentration of trans-resveratrol much exceeds that of  $\varepsilon$ -viniferin (Table). Saperavi, Otskhanuri Sapere and Aladasturi are distinguished for higher content of trans-resveratrol.

It should be noted that the experimental varieties grow in different vine-making regions of Georgia with different agro-climatic conditions. Under the influence of this factor, it is known that stilbenes are accumulated through peculiar biosynthesis and the result is seen in the difference in concentration. According to the literature data [10], the accumulation of stilbenes is affected by several factors, which may be divided into two groups -(1) those before harvesting and (2) those after harvesting. The first group includes the vine generic genetics, methods of vine

#### Table

Content of trans-resveratrol and  $\epsilon$ -viniferin in grape skin (mg/100g)

Varieties	ε-viniferin	<b>Trans-resveratrol</b>
Saperavi	0.67	6.67
Saperavi Budeshurisebri	0.38	1.86
Cabernet Sauvignon	0.40	2.96
Otskhanuri Sapere	0.34	6.07
Chkhaveri	0.26	1.73
Ojaleshi	0.43	2.92
Aladasturi	0.30	3.96
Aleksandrouli	0.57	3.21
Mujuretuli	0.26	2.26
Asuretuli Shavi	0.45	2.06
Regions of habitats of Saperavi in Kakheti		
Akhasheni	0.67	6.67
Kardenakhi	0.69	4.46
Kindzmarauli	0.48	3.52
Tsinandali	0.98	4.63

treatment, weather - climatic conditions, light effects, vine pests and pests control, soil, time of harvesting. The principal factors in group (2) are the technological methods of storing and processing the grape. As shown by the results of the experiments, the varieties growing in Western Georgia, in terms of the content of  $\varepsilon$ -viniferin in them, take the following order: Aleksandrouli > Ojaleshi > Otskhanuri Sapere > Aladasturi > Chkhaveri > Mujuretuli. As for the content of trans-resveratrol, the same varieties were ordered as follows: Otskhanuri Sapere > Aladasturi > Aleksandrouli > Ojaleshi > Mujuretuli. As to the concentration of  $\varepsilon$ -viniferin in the skin of Saperavi grape, the following order was established by considering the habitat of the varieties: Tsinandali > Kardenakhi > Akhasheni > Kindzmarauli. As to the content of trans-resveratrol, the order is as follows: Akhasheni > Tsinandali > Kardenakhi > Kindzmarauli.

**Conclusions.** Thus, the experiments conducted have helped to identify the versatile stilbene spectrum in the skin of color-grape industrial vine varieties (*Vitis vinifera* L.) growing in Georgia. It has been established that the quantitative content of biologically active monomer transresveratrol and its dimer  $\varepsilon$ -viniferin varies depending on the varieties of the vine and its habitat. In addition, there is a common regularity observed – the concentration of trans-resveratrol much exceeds that of  $\varepsilon$ -viniferin. The presence of certain biologically active stilbenes serves as evidence of the functional designation of the experimental vine varieties and the wines made of them.

#### ორგანული ქიმია

## ბიოლოგიურად აქტიური ტრანს-რეზვერატროლის და ε-ვინიფერინის შემცველობა საქართველოში გავრცელებულ ფერადყურძნიან ვაზის ჯიშებში

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საქართველოს სახელმწიფო აგრარული უნივერსიტეტი მევენახეობის, მეღვინეობისა და მებაღეობის ინსტიტუტი, თბილისი

(წარმოღგენილია აკაღემიის წევრის შ.სამსონიას მიერ)

საქართველოში გავრცელებული ვაზის საწარმოო ფერადყურძნიანი ჯიშების ყურძნის კანიღან გამოყოფილია სტილბენეშემცველი ჯამური პრეპარატები. ეს ჯიშები შემდეგია: საფერავი, საფერავი ბუდეშურისებრი, კაბერნე სოვინიონი, ოცხანური საფერე, ჩხავერი, ოჯალეში, ალაღასტური, ალექსანდროული, მუჯურეთული, ასურეთული შავი. მაღალეფექტური სითხური ქრომატოგრაფიის საფუძველზე გამოვლენილია მათი მრავალფეროვანი სტილბენეური სპექტრები, რომელთა შორის განსაზღვრულია ტრანს-რეზვერატროლი ღა ε-ვინიფერინი. თითოეულ მათგანში შეინიშნება ერთი კანონზომიერება – მონომერული სტილბენეს ტრანსრეზვერატროლის კონცენტრაცია მნიშვნელოვნად აღემატება ღიმერული ε-ვინიფერინის კონცენტრაციას. მათი რაოდენობანი ცვალებადობს ჯიშის გავრცელების ადგილის მიზედვით. სტილბენეური სპექტრის შესწავლა თეორიული საფუძველია სამკურნალო-კვებითი ღირებულების თვალსაზრისით როგორც ყურძნის, ასევე მათგან დამზადებული ღვინოების ფუნქციური ღანიშნულების ასახსნელად.

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