

Hydroxystilbenes in the roots of *Rheum rhaponticum*

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Abstract. Stilbene derivatives *trans*-3,4',5-trihydroxystilbene (resveratrol) and its 3-O- β -D-glucopyranoside (piceid), *trans*-3,5-dihydroxy-4'-methoxystilbene (deoxyrhapontigenin) and its 3-O- β -D-glucopyranoside (deoxyrhapontin), *trans*-3,3',5-trihydroxy-4'-methoxystilbene (rhapontigenin) and its 3-O- β -D-glucopyranoside (rhapontin) as well as two 3,3',4',5-tetrahydroxystilbene (piceatannol) glucosides were identified in the roots of common rhubarb *Rheum rhaponticum* cultivated in Estonia. The concentration of resveratrol in the roots of *R. rhaponticum* increased sharply in October, while the concentrations of other stilbenes maintained the values close to the concentrations at the beginning of the vegetation period in April–May. The roots of *R. rhaponticum* could be an effective raw material for producing natural resveratrol and other pharmacologically active stilbenoids.

Key words: resveratrol, piceatannol, hydroxystilbenes, *Rheum rhaponticum*, *Polygonum cuspidatum*.

INTRODUCTION

Several species of the genus *Rheum* (Polygonaceae) such as *Rheum palmatum*, *R. emodi*, and *R. officinale* are known for high contents of physiologically active anthraquinones (emodin, aloe-emodin, chrysophanol) in their roots [1–5]. In addition, a number of hydroxystilbenes including (OH)₃-stilbene resveratrol and (OH)₄-stilbene piceatannol have been identified in the rhizome and roots of Korean rhubarb *R. undulatum* (also *R. rhabarbarum*) [6–11]. In this respect,

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R. undulatum is similar to *Polygonum cuspidatum* (Japanese knotweed), a well-known Chinese medicinal plant used for the production of resveratrol [12]. The presence of stilbene derivatives has been reported also in the roots of *R. maximowiczii* [13, 14]. It is pertinent to note that in the literature there were no data available on bioactive components in the roots of *R. rhaponticum*, which is in many respects similar to *R. undulatum*.

In the present work we studied comparatively hydroxystilbene contents in the roots of *R. rhaponticum* and *P. cuspidatum* plants grown in Estonia.

MATERIALS AND METHODS

Extractions

To study the contents of stilbenoids in the roots of *Rheum rhaponticum* and *Polygonum cuspidatum*, root samples from plants grown at Harku, Estonia, were collected and dried at 45°C in a thermostat for 24 h. Then the samples were powdered in a coffee mill and macerated with 10-fold excess (by weight) of methanol for 72 h at room temperature in darkness. After filtration, the extracts were kept in a refrigerator at 4°C for use in analyses.

For the study of the dependence of the stilbenoid content in the roots of *R. rhaponticum* upon the plant growth stage, the root samples from plants cultivated in Narva-Jõesuu were collected monthly, from April to October 2002 (collection date within 6th to 10th day in every month), and dried at room temperature in darkness for two weeks. Then the air-dry sample was powdered in a mortar and macerated with 10-fold excess (by weight) of methanol for 72 h at room temperature. After centrifugation (4500 rpm, 10 min), the extracts were kept frozen at -20°C for use in analyses.

The plants used in the present study were botanically identified by comparison of their taxonomical features with authentic samples of *Rheum rhaponticum* L. and *Polygonum cuspidatum* Sieb. et Zucc. (also *Reynoutria japonica* Houtt.).

Chemicals

Deoxyrhapontin and *trans*-resveratrol, both 99% pure, were from Sigma. Rhapontin, 95% pure, was from Aldrich. β -Glucosidase was from Sigma. Acetonitrile, purity 99.9+, was from Rathburn Chemicals Ltd. Methanol was a standard preparation of 99.9% purity. Deionized water (Barnstead Easypure RF, 18.3 M Ω /cm) was used throughout the work.

Enzymatic hydrolysis of hydroxystilbene glucosides

In order to obtain aglucones for HPLC coelution experiments, authentic hydroxystilbene glucosides were hydrolysed by β -glucosidase (1 mg/mL) at pH 5 in the presence of not more than 10% (v/v) methanol. The incubation time at 30°C was 17 h. The details of the method were obtained from the literature [15].

Analytical procedures

For the identification of stilbene derivatives in the root methanolic extracts, an Agilent LC-MS 1100 Series HPLC system instrument consisting of an auto-sampler, a binary pump, a column thermostat, a diode array, and MS/MS detectors was used. The column temperature was 30°C. The sample volume injected was 5 μ L, and the flow rate was 0.5 mL/min. Elution was in linear gradient mode of two solvents: A – 0.1% formic acid in water and B – 0.1% formic acid in acetonitrile.

Quantitative measurements at $\lambda = 306$ nm were performed on a Merck-Hitachi HPLC instrument, Model D-6000 (LH-4250 UV-VIS detector, L-6200 pump, L-5025 column thermostat) with the column Alltech GmbH, Platinum 100A EPS, 250 \times 4.6 mm, with particle size 5 μ M. The column temperature was 30°C. The sample volume injected through Rheodyne was 20 μ L. The flow rate was 1.0 mL/min in isocratic elution mode with either 25/75 or 40/60 v/v acetonitrile/water. In gradient mode, solvent A was methanol and B was 0.5% formic acid in water. The computer program Merck-Hitachi HPLC Manager, Version 2 was used.

Calibration curves for the quantitation of *trans*-resveratrol, rhapontin, and deoxyrhapontin in absorption units *versus* concentration were built up. The same curves were used for the quantitation of piceid (resveratrol glucoside), rhapontigenin (aglycon of rhapontin), and deoxyrhapontigenin (aglycon of deoxyrhapontin), respectively, taking into account the ratios of molecular weights and molar absorptivities.

In Fig. 1 a representative chromatogram of the HPLC analysis of methanolic extract of *R. rhaponticum* root obtained by diode array detection at 306 nm is

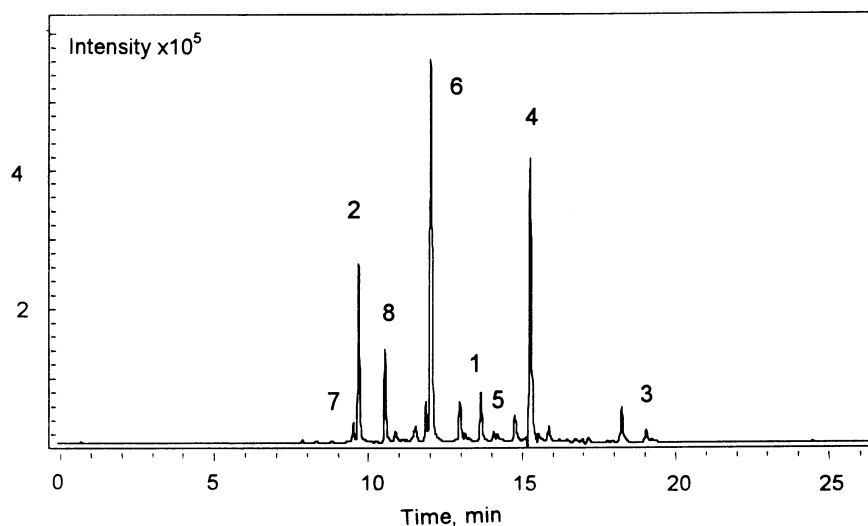


Fig. 1. UV chromatogram ($\lambda = 306.16$ nm) of methanolic extract of *Rheum rhaponticum* root. Numbers mark hydroxystilbenes as in Table 1.

shown. Figure 2 presents MS total ion chromatogram of the same sample together with the APCI negative-ion mass spectra of the individual compounds in Fig. 3. The hydroxystilbene derivatives identified are given in Table 1.

A chromatogram of the reversed-phase HPLC analysis of *R. rhaponticum* root methanolic extract in gradient mode chromatography for quantitative determination of major stilbenes in the roots is shown in Fig. 4. The same analysis was performed for a methanolic extract of *Polygonum cuspidatum* roots (chromatogram not shown). The roots of both *R. rhaponticum* and *P. cuspidatum* were collected at Harku in September 2002. The results of the analysis are shown in Table 2.

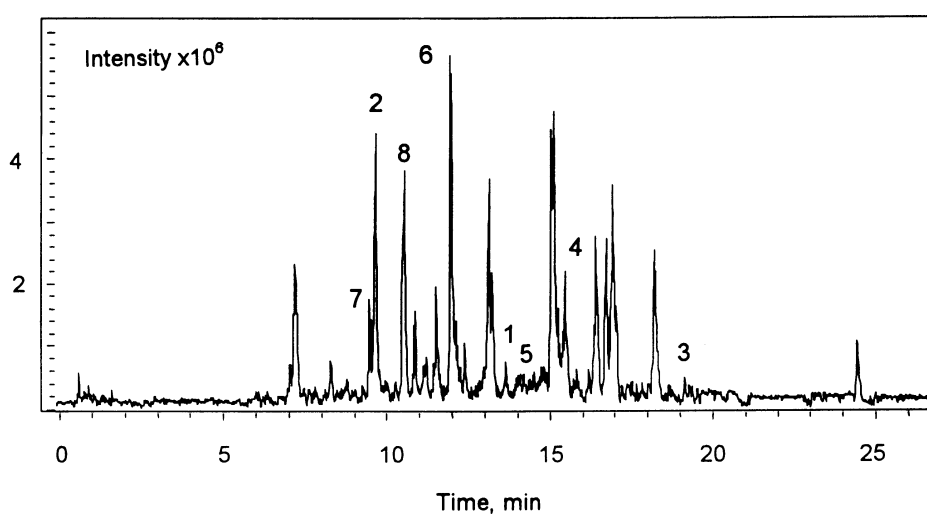
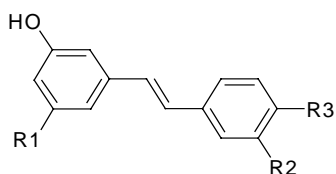


Fig. 2. Total ion chromatogram of the methanolic extract of *Rheum rhaponticum* root monitored by APCI LC-MS in negative ion mode. Numbers mark hydroxystilbenes as in Table 1.

Table 1. Stilbenoid constituents identified in the roots of *Rheum rhaponticum*



Compound	R1	R2	R3	$[M-H]^-$	R_f , min
Resveratrol (1)	OH	H	OH	227.2	13.6
Piceid (2)	OGlc	H	OH	389.2	9.7
Deoxyrhapontigenin (3)	OH	H	OCH ₃	241.1	19.0
Deoxyrhapontin (4)	OGlc	H	OCH ₃	403.1	15.2
Rhapontigenin (5)	OH	OH	OCH ₃	257.1	14.3
Rhapontin (6)	OGlc	OH	OCH ₃	419.2	12.0
Piceatannol-Glc (8)	OGlc	OH	OH	405.3	10.55
Piceatannol-Glc (7)	OH	OGlc	OH	405.3	9.5

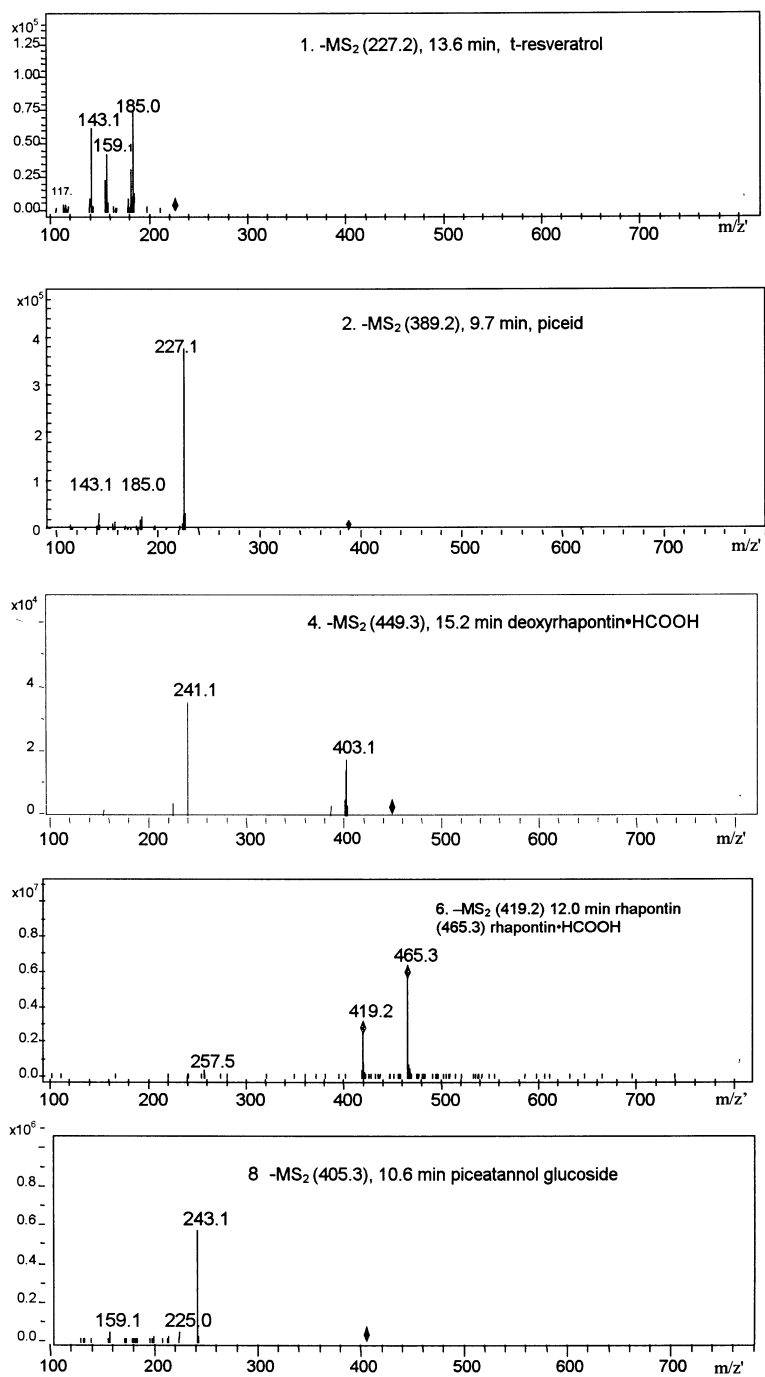


Fig. 3. APCI negative-ion mass spectra of hydroxystilbenes from Fig. 2. Compound ·HCOOH indicates formic acid adduct of the compound from mobile phase. ♦ mark the parent ion(s) [M-H]⁻.

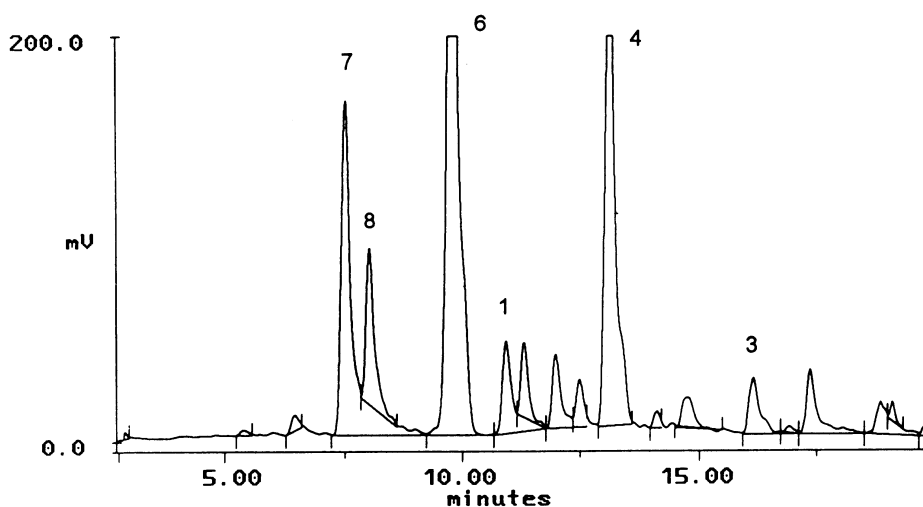


Fig. 4. Reversed-phase HPLC profile of *Rheum rhaponticum* root methanolic extract at 306 nm. Numbers mark hydroxystilbenes as in Table 1.

RESULTS AND DISCUSSION

Comparison of the data in Table 1 for *R. rhaponticum* with data in the literature [6, 10] on the stilbenoid contents in *R. undulatum* shows that these two species of rhubarb are indeed similar in this respect. However, the Japanese authors [6, 10] did not find piceid in *R. undulatum* while it was definitely present in *R. rhaponticum* (see also Table 3). On the other hand, we identified in the roots of *R. rhaponticum* only glucosides of piceatannol while in *R. undulatum* aglucon piceatannol was also found [6, 10].

The contents of the major hydroxystilbenes, comparatively, in the roots of *R. rhaponticum* and *P. cuspidatum* are presented in Table 2. It can be seen that the content of resveratrol in the roots of *R. rhaponticum* is about 8 times and the total content of hydroxystilbenes about 40 times higher than in the roots of *P. cuspidatum*.

Table 2. Major hydroxystilbenes in the roots of *Rheum rhaponticum* and *Polygonum cuspidatum*

Stilbene	Concentration, mg/g dried root		Ratio R/P
	<i>R. rhaponticum</i>	<i>P. cuspidatum</i>	
(OH) ₃ -stilbenes			
Resveratrol	3.9	0.5	7.8
Deoxyrhapontin	7.44	0.5	14
Deoxyrhapontigenin	1.11	0	
(OH) ₄ -stilbenes			
Rhapontin	14.38	0	
Piceatannol-Glc	11.7	0	

In the roots of *R. rhaponticum*, derivatives of piceatannol deserve special attention, as their content in *P. cuspidatum* was practically nil. Piceatannol is known as an antileukaemic agent [16], it has been shown to reduce the proliferation rate of a human colon carcinoma cell culture [17], and it can be used for suppressing rejection responses of organ transplant recipients [18].

Considering the higher concentrations of stilbenoids in the roots of *R. rhaponticum* as well as the fact that the roots of rhubarb are easier to process, it can be concluded that, as a raw material for hydroxystilbene production, the roots of *R. rhaponticum* are much better than the roots of *P. cuspidatum*, relevant at least to the growing conditions for these plants in Estonia.

In order to find out the optimum harvesting time of *R. rhaponticum* roots, we monitored the concentrations of the studied stilbene derivatives in the roots during the whole vegetation period from April to October. The results of the measurements by reversed-phase HPLC with isocratic elution and coelution with authentic standard compounds *trans*-resveratrol as well as deoxyrhapontin and rhapontin (and their enzymatic hydrolysis products) at UV detection at $\lambda = 306$ nm are presented in Table 3.

It can be seen from Table 3 that the concentration of resveratrol in the roots of *R. rhaponticum* increased sharply in October. At the same time, concentrations of rhapontigenin and deoxyrhapontigenin were rather stable, with their glucosides having the same trend, with an exception of an about two-fold increase of the concentration of deoxyrhapontin in August. Although the total amount of stilbene derivatives in August and in October was about the same, considering the highest concentration of resveratrol, the optimum harvesting time of the roots should be October.

Table 3. Content of hydroxystilbenes in the roots of *Rheum rhaponticum* depending on the plant growth stage

Month*	Concentration, mg/g of air-dry root					
	Piceid	Resveratrol	Rhapontin	Rhapontigenin	Deoxyrhapontin	Deoxy-rhapontigenin
April	15.85	0.15	38.07	1.40	24.81	1.76
May	17.75	0.34	41.78	1.29	22.68	1.82
June	19.06	0.45	39.77	1.21	25.03	1.99
July	17.79	0.21	36.48	1.24	25.20	1.95
August	28.42	0.32	43.20	1.34	42.56	3.80
September	15.26	1.12	30.93	1.17	16.65	1.65
October	36.66	4.99	40.83	1.62	23.47	2.53

* The samples were collected from the 6th to the 10th day of each month.

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Hüdroksüstilbeenid rabarberi *Rheum rhaponticum* juurtes

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Tatraliste (*Polygonaceae*) sugukonna rabarberi (*Rheum*) perekonna mitmete liikide (*Rheum palmatum*, *R. emodi*, *R. officinale*) juured paistavad silma farmakoloogiliselt aktiivsete antrakinoonide (emodiin, aaloe-emodiin, krüsofanool) sisalduse poolest. Sealjuures on kurdlehise rabarberi (*R. undulatum*) juurikates ja juurtes identifitseeritud ka hüdroksüstilbeene, nagu (OH)₃-stilbeen resveratrool ja (OH)₄-stilbeen pitseatannool. Selles mõttes on kurdlehine rabarber sarnane hiina rahvameditsiinis tuntud taimega vooljas pargitatar (*Polygonum cuspidatum*), mille juured on Hiinas kasutusel resveratrooli tootmises. Stilbeeniderivaate on leitud ka *R. maximowiczii* juurtest. Kurdlehise rabarberiga mitmes mõttes sarnase hariliku rabarberi (*R. rhaponticum*) juurtes olevate bioaktiivsete komponentide kohta kirjanduses andmed puuduvad.

Eelöeldust lähtudes määrati võrdlevalt hüdroksüstilbeenide sisaldus Eestis kasvanud hariliku rabarberi ja voolja pargitatra juurte metanooliekstraktides, kasutades kõrgefektiivset vedelikkromatograafiat pöördfaas (C₁₈)-kolonnis UV ja MS/MS detekteerimisega. Selgus, et resveratrooli sisaldus oli hariliku rabarberi juurtes kuni 8 korda ja summaarne hüdroksüstilbeenide hulk kuni 40 korda suurem kui voolja pargitatra juurtes. Resveratrooli kõrval pakuvad erilist huvi hariliku rabarberi maa-alustes osades leiduvad pitseatannooli derivaadid, mida vooljas pargitatras peaaegu ei ole. Pitseatannoolil on leukeemiavastane toime ja see aine on kasutatav immunodepressandina organite siirdamisel. Arvestades ka rabarberi juurematerjali paremat töödeldavust võrreldes pargitatraga, võib öelda, et harilik rabarber on hüdroksüstilbeenide saamiseks vähemalt Eesti kasvutingimustes parem kultuur kui vooljas pargitatar. Rabarberi maa-aluste osade parim kogumisaeg on käesoleva töö andmetel oktoober.