

BIOTRANSFORMATION OF HYDROQUINONE AND 4-HYDROXYBENZOIC ACID IN *Schisandra chinensis* (CHINESE MAGNOLIA VINE) *in vitro* CULTURES

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ABSTRACT

Optimization of the process of biotransformation of hydroquinone into its β -D-glucoside – arbutin, was performed in agitated shoot cultures of *Schisandra chinensis*. The optimisation involved testing various concentrations of the precursor and different ways of administering it. Arbutin was accumulated mainly in the *in vitro* cultured biomass (85.2–98.6%). By optimizing the process, a 2.26-fold increase in the overall product content was obtained. The highest amount (17.8 mg·g⁻¹ DW) was found after administering 384 mg·l⁻¹ hydroquinone in a dose divided into two portions. An experiment with the biotransformation of 4-hydroxybenzoic acid did not produce arbutin but a mixture of two products of glucosylation of the precursor – hydroxybenzoic acid 4-O- β -glucopyranoside and 4-hydroxybenzoic acid β -glucopyranosyl ester. The identity of all biotransformation products was confirmed by ¹H-NMR analysis. The results for the production of arbutin by the biotransformation of hydroquinone are of potential practical importance. On the other hand, the fact of confirming the presence of two glucosylation products has a great cognitive value.

Key words: schizandra, β -glucosylation, arbutin production, agitated shoot cultures, HPLC analysis

INTRODUCTION

Arbutin (β -D-glucoside of hydroquinone) is a known plant metabolite important in pharmacy and cosmetology. Disinfecting properties of this compound are used in phytotherapy, especially in the treatment of urinary tract inflammation [Stammwitz 1998, WHO... 2002, Quintus et al. 2005, Wichtl 2007]. The cosmetics industry, on the other hand, makes use of the skin-lightening effect of arbutin because arbutin inhibits melanin formation due to its tyrosinase inhibitory activity [Akiu et al. 1988]. The new directions of the biological activity of arbutin proven in recent times, its antioxidant, anti-

inflammatory, antifungal and antitumour properties, greatly extend the range of possible applications of this compound [Azadbakht et al. 2004]. Recently, Migas and Krauze-Baranowska [2015] summarized the latest findings and significance of arbutin and its derivatives in therapy and cosmetics.

Arbutin is present in selected plant taxa, mainly in the species of the families Ericaceae, Saxifragaceae, and Rosaceae [WHO... 2002, Wichtl 2007]. The classic pharmacopoeial material, authorized for use for medicinal purposes in the European Union countries, are the leaves of the bearberry plant (*Uvae ursi*

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folium) [European Pharmacopoeia 2016b]. In Asia, a rich source of this compound are species of the genera *Bergenia* and *Pyrus* [Ekiert et al. 2012]. Acquisition of the European material mentioned above is difficult due to, among other things, the protection of this species in Central European countries and special habitat requirements of this dwarf forest shrub (adequate soil acidity and the presence of mycorrhizal fungi) [Pękoś-Mirkowa et al. 2003, Recasens et al. 2008]. Some solution has been provided by the successful attempts at cultivation in Europe of the arbutin-rich Asian species *Bergenia cordifolia*. Another possibility is chemical synthesis of arbutin. However, it is a complex, three-step process. That is why other alternative sources of arbutin, including biotechnological solutions, are still being awaited [Ekiert et al. 2012]. Taking advantage of the enzymatic potential of microorganisms, it is possible to obtain α -arbutin, an isomer of the plant metabolite β -arbutin [Kurosu et al. 2002]. More relevant work in this field of plant biotechnology is thus being undertaken. The most promising solution is the possibility of obtaining β -arbutin by biotransformation of simple phenolic compounds in plant cultures *in vitro* (fig. 1) [Ekiert et al. 2012]. In these processes, advantage is taken of the enzymatic potential of plant cells.

Hydroquinone – the precursor of arbutin is formed as a result of oxidative decarboxylation of 4-hydroxybenzoic acid. In plants, this acid can be synthesized from both phenylalanine and tyrosine in the presence of phenylalanine ammonia lyase (PAL) or tyrosine ammonia lyase (TAL) [Mandal et al. 2010]. The best-known biosynthetic pathway leads *via* cinnamic acid and *p*-coumaric acid, and its active form *p*-coumaroyl-CoA (fig. 1).

A good exogenous substrate most often used for the reaction that leads to the formation of arbutin is hydroquinone. This is because of the prevalence in plants of enzymes from the group of β -glucosyl transferases and the lack of substrate specificity shown by them. The β -D-glucosylation reaction can therefore occur in *in vitro* cultures of many plant species that under natural conditions do not produce arbutin [Skrzypczak-Pietraszek et al. 2005, Piekoszewska et al. 2010].

Schisandra chinensis (Turczaninow) Baillon (*Schizandra chinensis*, Chinese magnolia vine), Schisandraceae, is a Far-Eastern species native to China, Korea, Japan and Russia, with medicinal, cosmetic, and culinary qualities [Hancke et al. 1999, Panossian and Wikman 2008, Szopa and Ekiert 2014, Szopa et al. 2017a]. *Schisandrae chinensis fructus* has been listed in European pharmacopoeial documents since 2008 as an important material that exhibits hepatoprotective, antioxidant, anticancer, and adaptogenic activities, among others [European Pharmacopoeia 2008, 2016a]. The fruit is also recognized by the official medicine in Japan, Korea, China and Russia [Szopa and Ekiert 2014, Szopa et al. 2016a], as well as in the USA [United States Pharmacopoeia 1999]. Since 2007, it has been listed in Pharmacopoeia Internationalis published by WHO... [2007]. The main group of compounds characteristic of this plant species, especially in the fruits, are dibenzocyclooctadiene lignans. The material also contains terpene compounds and anthocyanin glycosides. Additionally, the presence of various glycosidic conjugates, including flavonoids, has been confirmed in the leaves [Szopa et al. 2017a]. Recently, the value of *S. chinensis* has also been recognized in cosmetology [Szopa et al. 2016b].

The *in vitro* cultures of *S. chinensis* established in our laboratory, with different degrees of differentiation, maintained as both solid and agitated cultures, had shown the capacity for the production of lignans and phenolic acids [Szopa and Ekiert 2014, Szopa et al. 2016a, 2017a, 2017b]. Those results and the confirmed presence of glycoside conjugates in the parent plant had encouraged us to investigate the efficiency of β -D-glucosylation of hydroquinone to arbutin.

Our preliminary studies had shown that the cells in the agitated shoot cultures did convert exogenous hydroquinone into arbutin and that a good production medium was the Murashige and Skoog (MS) [1962] medium with 3 mg·l⁻¹ BA and 1 mg·l⁻¹ NAA. This medium was selected for further studies.

The aim of the current work was optimization of the conversion process, which consisted in testing different doses of the precursor (96–384 mg·l⁻¹) and different methods of administering it (a single dose, or divided into 2 or 3 portions).

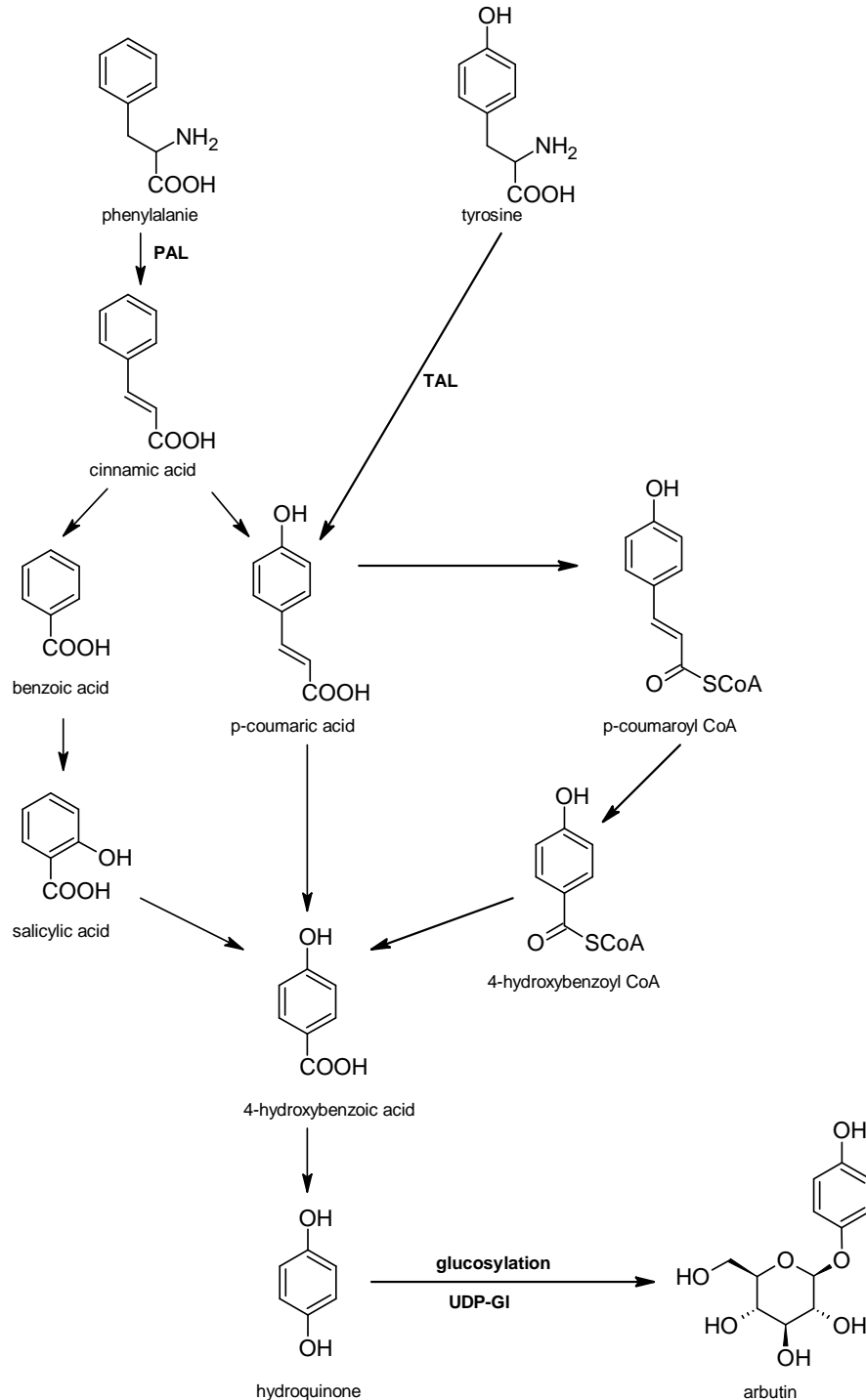


Fig. 1. Possible ways of arbutin biogenesis

Source: acc. Ekiert et al. [2012]

In *in vitro* cultures of selected plant species, a good arbutin substrate, often better than hydroquinone, is 4-hydroxybenzoic acid (fig. 1) [Dušková et al. 1999]. An additional objective of our study was therefore to assess the possibility of converting 4-hydroxybenzoic acid into arbutin by *S. chinensis* cells cultured *in vitro*. The products of this biotransformation could be of interest from a cognitive and practical point of view.

MATERIALS AND METHODS

Initial *in vitro* cultures

Cultures of *Schisandra chinensis* were initiated from leaf buds of the plants growing in Rogów Arboretum, Warsaw University of Life Sciences, Forest Experimental Station in Rogów (Poland) [Szopa et al. 2016a]. The resulting shoot cultures were grown on the MS medium (1962) supplemented with cytokinin – 3 mg·l⁻¹ BA (6-benzylaminopurine), and auxin – 1 mg·l⁻¹ NAA (α -naphthaleneacetic acid) (pH 5.7). The cultures were maintained under constant artificial light conditions (16 μ mol·m⁻²·s⁻¹, LF-40 W lamp, daylight, Piła), at 25 \pm 2°C.

Experimental agitated shoot cultures

For agitated shoot cultures, 500 ml Erlenmeyer flasks were filled with 100 ml of the MS medium [1962] supplemented with BA (3 mg·l⁻¹) and NAA (1 mg·l⁻¹) (pH 5.7). The inoculum for the cultures was 4 g of fresh biomass per flask. The cultures were maintained under the same light and temperature conditions as the initial cultures. The flasks were mounted on an Altel rotary shaker operating at 140 rpm with a vibration amplitude of 35 mm.

Biotransformation of hydroquinone

Hydroquinone (Merck, Darmstadt, Germany) was introduced as an aqueous solution (1 or 2 mg·ml⁻¹) 14 days after inoculation. It was administered into the culture flasks aseptically through a 0.22 μ m membrane filter (Merck-Millipore, Darmstadt, Germany). Fresh medium (100 ml) of was also added to each flask. The final concentrations of hydroquinone were: 96, 144, 192, 288, and

384 mg·l⁻¹ of medium. Hydroquinone was administered in a single dose or in split doses as two or three portions added at 24 h intervals. Harvesting of the biomass and culture media was carried out separately 24 h after the last dose of the precursor. The biomass was dried and the media lyophilized. The experimental combinations were run independently in three replications.

Biotransformation of 4-hydroxybenzoic acid

The reaction substrate, 4-hydroxybenzoic acid (Sigma-Aldrich, St. Louis, USA), was introduced as an aqueous solution (1 mg·ml⁻¹) 14 days after inoculation. It was administered into the culture flasks aseptically through a 0.22 μ m membrane filter (Merck-Millipore, Darmstadt, Germany). Fresh medium (100 ml) was also added to each flask. The final concentration of 4-hydroxybenzoic acid was 200 mg·l⁻¹ of medium. The acid was administered in a single dose. Harvesting of the biomass and culture media was carried out separately 3, 6, 9, 24, 72, 96 and 120 h after the addition of the precursor. The biomass was dried and the media lyophilized.

Extraction and chromatographic analysis

Dried biomass (1 g) was ground and subjected to extraction twice with boiling methanol (2 \times 50 ml) for 4 h (2 \times 2 h). The methanol was evaporated and the residue was dissolved in HPLC grade methanol. The lyophilized media were also dissolved in methanol.

The methanolic extracts were analyzed by HPLC [Štambergová et al. 1985]. An HPLC-system (Hitachi, Tokyo, Japan) and Purospher RP-18e analytical column (4 \times 250 mm, 5 μ m) (Merck, Darmstadt, Germany) with the mobile phase consisting of methanol/water (1 : 9, v/v) were used for separation at a flow rate of 1 ml·min⁻¹. Arbutin and hydroquinone were detected at 285 nm using a diode array detector (retention times: 3.7 and 5.5 min, respectively). Qualification and quantification were based on comparisons with reference standards. The amounts of the compounds were calculated from calibration curves by comparing the mean peak areas with those for standard concentrations.

Biotransformation efficiency

The following formula was used to calculate the efficiency of the biotransformation of hydroquinone into arbutin: $\text{efficiency} = a/b \times 100\%$ (a – overall amount of arbutin produced per Erlenmeyer flask (in the biomass and in 250 ml of medium); b – maximum amount of arbutin that can theoretically be obtained from a given amount of hydroquinone (calculated from the molar masses of the compounds)).

Isolation and identification of arbutin

Methanolic extract ($384 \text{ mg}\cdot\text{l}^{-1}$, 2 portions of precursor) was chromatographed on TLC plates (Merck, Darmstadt, Germany, No. 1.05553.0001) with ethyl acetate : formic acid : water (88 : 6 : 6, v/v/v) solvent system. Fractions containing arbutin were eluted with methanol and analyzed by TLC with n-hexane : ethyl acetate : methanol (2 : 1 : 2, v/v/v) as eluent. $^1\text{H-NMR}$ spectrum was prepared (AVANCE III Bruker, Ettlingen, Germany, 500 MHz, CD_3OD).

Isolation and identification of 4-hydroxybenzoic acid biotransformation products

Methanolic extract (72 h after addition of the precursor at $200 \text{ mg}\cdot\text{l}^{-1}$) was chromatographed on TLC plates (Merck, Darmstadt, Germany, No. 1.05553.0001) with ethyl acetate : methanol : water (100 : 17 : 13, v/v/v) solvent system. Fractions containing the biotransformation product were eluted with methanol and analyzed by TLC with n-hexane : ethyl acetate : methanol (2 : 1 : 2, v/v/v) as eluent. $^1\text{H-NMR}$ spectrum was prepared (AVANCE III Bruker, Ettlingen, Germany, 500 MHz, CD_3OD).

Statistical analysis

Results are presented as the mean \pm SD of three independent determinations. Statistical significance of the differences was assessed using a two-way ANOVA followed by the Least Significant Difference Test for post-hoc comparisons. The differ-

ences were statistically significant when $p < 0.05$. The analyses were performed using Statistica ver. 10.

RESULTS

Biotransformation of hydroquinone

Identification of the product – arbutin. Biotransformation of exogenous hydroquinone in *Schisandra chinensis* cultures *in vitro* resulted in the formation of arbutin. The identity of the compound, which was isolated from methanolic extracts from the biomass from *in vitro* cultures, was confirmed by comparing the chromatographic parameters (Rf-TLC, Rt-HPLC), and UV-Vis and $^1\text{H-NMR}$ spectra with those of the standard substance – arbutin (Sigma-Aldrich, St. Louis, USA). The same resonance signals were present in the $^1\text{H-NMR}$ spectrum of the test sample and that of the arbutin standard [Kwieceń et al. 2013, Chen et al. 2014].

Optimization of the biotransformation of hydroquinone

In order to optimize the process of biotransformation of hydroquinone, the precursor was administered in different doses, in the range $96\text{--}384 \text{ mg}\cdot\text{l}^{-1}$. According to the literature data and results of our own studies, higher doses of hydroquinone (above $100 \text{ mg}\cdot\text{l}^{-1}$) have an adverse effect on both the morphological appearance of the *in vitro* cultured biomass and its growth and biosynthetic capacity [Yokoyama and Inomata 1998]. In the present experiment, there were no adverse effects of single high doses of hydroquinone ($192\text{--}384 \text{ mg}\cdot\text{l}^{-1}$) on biomass growth ($0.5\text{--}0.52 \text{ g DW}$). Only in the case of the highest dose of the precursor administered in portions did we observe slight inhibition in the growth of biomass (0.43 g DW). The increase in biomass in agitated cultures of *S. chinensis* resulting from addition of precursors has been included in the graph in Figure 2.

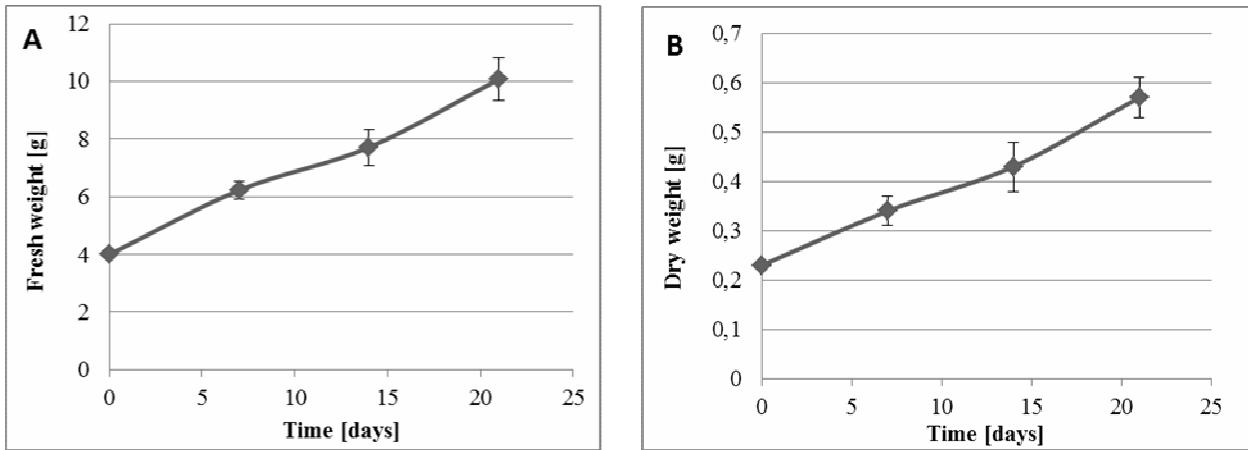


Fig. 2. The growth curve of fresh (A) and dry biomass (B) in agitated cultures of *S. chinensis*

Source: own research

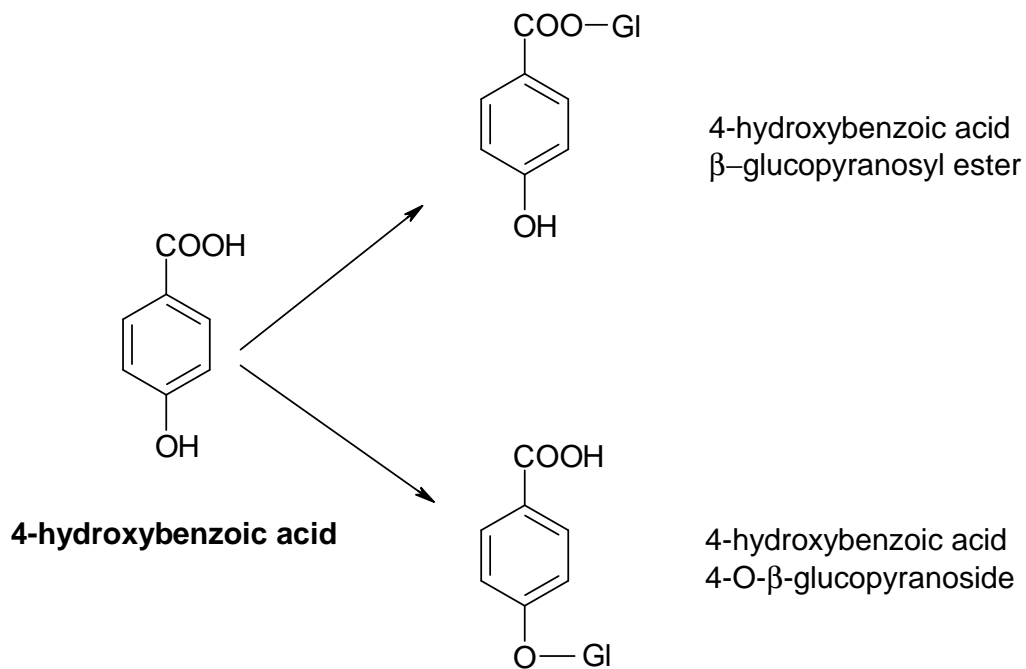


Fig. 3. 4-Hydroxybenzoic acid biotransformation products

Source: based on own research

Table 1. Amounts of arbutin and the efficiency of the biotransformation process in *in vitro* shoot cultures of *Schisandra chinensis* grown on MS medium (BA – 3 mg·l⁻¹ and NAA – 1 mg·l⁻¹). Statistically significant differences p < 0.05 (n = 3)

Concentration of precursor (mg·l ⁻¹)	Method of precursor supply	Arbutin in biomass (mg·flask ⁻¹)	Arbutin in medium (mg·flask ⁻¹)	Total arbutin content (mg·flask ⁻¹)	Biotransformation efficiency (%) ± SD
96	a) single dose	8.6	0.12	8.7 ± 2.15 ^{efhklmno}	14.7 ± 3.62 ^{cdghijklmno}
	b) 2 portions	9.6	0.74	10.3 ± 1.04 ^{klno}	17.4 ± 1.75 ^{dghijklmno}
	c) 3 portions	11.5	0.37	11.9 ± 1.80 ^{kn}	20.0 ± 3.04 ^{adefghijklmno}
144	d) single dose	9.0	0.29	9.3 ± 1.76 ^{efhklmno}	10.4 ± 1.97 ^{abcefjmo}
	e) 2 portions	13.1	0.27	13.3 ± 1.87 ^{adn}	15.0 ± 2.10 ^{cdghijklmno}
	f) 3 portions	12.4	0.87	13.2 ± 1.58 ^{adkn}	14.9 ± 1.77 ^{cdghijklmno}
192	g) single dose	11.5	0.14	11.7 ± 0.43 ^{kno}	9.8 ± 0.36 ^{abcefjmo}
	h) 2 portions	11.0	0.30	11.3 ± 0.34 ^{adkn}	9.5 ± 0.29 ^{abcefmo}
	i) 3 portions	10.0	0.58	10.6 ± 1.54 ^{kno}	8.9 ± 1.29 ^{abcef}
288	j) single dose	10.6	0.65	11.2 ± 1.04 ^{kno}	6.3 ± 0.58 ^{abcddefg}
	k) 2 portions	16.4	0.30	16.7 ± 4.92 ^{abcdefghij}	9.4 ± 2.76 ^{abcef}
	l) 3 portions	12.9	0.99	13.9 ± 4.94 ^{abdn}	7.8 ± 2.78 ^{abcef}
384	m) single dose	13.4	0.70	14.1 ± 2.24 ^{adn}	6.0 ± 0.94 ^{abcddefgh}
	n) 2 portions	16.3	1.59	17.8 ± 5.91 ^{abcddefghijlm}	7.5 ± 2.49 ^{abcef}
	o) 3 portions	12.0	2.09	14.1 ± 5.65 ^{abdgiij}	5.9 ± 2.38 ^{abcddefgh}

Source: own research

Arbutin was accumulated mainly in the *in vitro* grown biomass. At hydroquinone doses in the range 96–288 mg·l⁻¹, the amount of the product in the biomass was from 8.6 to 16.4 mg per flask⁻¹, and in the media it ranged from 0.12 to 0.87 mg·flask⁻¹ (tab. 1). At the highest hydroquinone dose tested (384 mg·l⁻¹), the concentration of arbutin was higher and varied between 12.0 and 16.3 mg·flask⁻¹, depending on the method of administering the precursor. The overall arbutin content (in the biomass and culture medium) varied over a wide range, depending on the concentration of the precursor, from 8.7 to 17.8 mg·flask⁻¹ (tab. 1).

The arbutin content ranged from 8.6 to 16.4 mg·flask⁻¹ in the biomass, and from 0.12 to 2.09 mg·flask⁻¹ in the culture media (tab. 1).

When the concentration of exogenous hydroquinone administered in single, undivided doses-in-

creased from 96 to 384 mg·l⁻¹, the arbutin content in the biomass increased from 8.6 to 13.4 mg·flask⁻¹ in the biomass, and from 0.12 to 0.70 mg·flask⁻¹ in the media. When hydroquinone was administered in split doses, the overall arbutin content (in the biomass and medium) increased by as much as 7.5 mg (from 10.3 to 17.8 mg·flask⁻¹, 2 portions), and by 3.5 mg (from 10.6 to 14.1 mg·flask⁻¹, 3 portions) (tab. 1). Administering hydroquinone in doses divided into two portions caused the amount of the product to increase from 9.6 to 16.4 mg·flask⁻¹ in the biomass, and from 0.27 to 1.59 mg·flask⁻¹ in the media. When hydroquinone was administered in 3 portions, the arbutin content in the biomass increased to a lesser extent, from 10.0 to 12.9 mg·flask⁻¹, but the amount of the product in the media increased markedly, from 0.37 to 2.09 mg·flask⁻¹ (tab. 1). Statistically significant differences (p < 0.05) in arbutin content were found

between hydroquinone concentration and hydroquinone supply.

The efficiency of the process of biotransformation of hydroquinone into arbutin was low, ranging from 5.9 to 20.0%, depending on the concentration of the precursor and the method of administering it (tab. 1). The highest efficiencies of 17.4% and 20.0% were obtained after applying the lowest dose of hydroquinone ($96 \text{ mg}\cdot\text{l}^{-1}$) in 2 and 3 portions, respectively (tab. 1). With the increase in the concentration of a single-hydroquinone dose (from 96 to $384 \text{ mg}\cdot\text{l}^{-1}$), the efficiency of the process decreased markedly from 14.7 to 6.0%. When increasingly higher doses of the precursor were administered in 2 or 3 portions, there was also a marked decrease in the efficiency of the process, from 17.4 to 7.5% (2 portions) and from 20.0 to 5.9% (3 portions) (tab. 1). In terms of reaction efficiency, statistically significant differences ($p < 0.05$) between hydroquinone concentration and hydroquinone supply were found.

Biotransformation of 4-hydroxybenzoic acid

Identification of the products. Following the biotransformation of exogenous 4-hydroxybenzoic acid in *Schisandra chinensis* cultures *in vitro*, the presence of arbutin was not confirmed. The cells did convert the supplied substrate into one main product, but with chromatographic parameters different from those of arbutin ($R_f = 0.73$, $R_t = 11.05 \text{ min}$). That product was isolated and purified. Then its identity was determined by $^1\text{H-NMR}$ spectral analysis. The product was found to be a mixture of two compounds, products of glucosylation of 4-hydroxybenzoic acid: 4-hydroxybenzoic acid 4-O- β -glucopyranoside and 4-hydroxybenzoic acid β -glucopyranosyl ester (1 : 0.56) (fig. 3) [Klick and Herrmann 1988, Keller et al. 1996].

DISCUSSION

In our study on the optimization of the bioconversion of hydroquinone into arbutin we did not observe what is often described in the literature as a very unfavourable, damaging effect of higher doses of that precursor (above $100 \text{ mg}\cdot\text{l}^{-1}$) on the cells cultured *in*

vitro [Yokoyama and Inomata 1998]. Only at the highest dose of the precursor ($384 \text{ mg}\cdot\text{l}^{-1}$) did we observe inhibition in the growth of the biomass of *Schisandra chinensis* shoots. The high degree of organogenesis of the *in vitro* grown biomass used in the experiment (shoot cultures) guaranteed that it was less sensitive to the damaging effect of hydroquinone. Likewise, in *in vitro* cultures of other plant species with a high degree of differentiation, carried out in our laboratory, such as shoot cultures of *Ruta graveolens*, *Hypericum perforatum* and *Aronia melanocarpa*, and shoot-differentiating callus cultures of *Ruta graveolens* ssp. *divaricata*, there had been no evidence of the damaging effect of hydroquinone on the biomass [Dušková et al. 1999, Zubek et al. 2009, Piekoszewska et al. 2010, Kwiecień et al. 2013].

As a result of the optimization of the biotransformation process, we obtained a 2.26-fold increase in the arbutin content in the cultured biomass. Similar increases in the arbutin content in the biomass had also been obtained by us in the studies with *in vitro* cultures of *Ruta graveolens* ssp. *divaricata* and *Aronia melanocarpa* (2.6- and 2.4-fold, respectively) [Zubek et al. 2009, Kwiecień et al. 2013].

The highest overall arbutin content (total content in the biomass and medium) obtained by us was $3.90 \text{ g}\cdot 100 \text{ g}^{-1}\text{DW}$. In a study conducted by Dušková et al. [1999], the arbutin content was higher – $5.01 \text{ g}\cdot 100 \text{ g}^{-1}\text{DW}$. Our determinations of arbutin content were made in samples collected 24 hours after the last dose of the precursor. The results obtained by Dušková et al. [1994] were measurements after 7 days (168 hours). It can thus be assumed that by continuing the optimization of the process we could have obtained even larger amounts of the product. Other studies on the biotransformation of hydroquinone into arbutin had indicated that the dynamics of β -D-glucosylation in *in vitro* cultures of different plant species were different. The maximum amounts of the product had been obtained by us at different times after administering the precursor: 18 h (*Ruta graveolens*, *Melittis melissophyllum*), 24 h (*Echinacea purpurea*), 48 h (*Exacum affine*), and 72 h (*Ruta graveolens* ssp. *divaricata*) [Skrzypczak-Pietraszek et al. 2005].

The maximum efficiency of the process of biotransformation of hydroquinone into arbutin obtained by us in the present study (approx. 20%) was low, lower than what we had obtained with *in vitro* cultures of other plant species – *Ruta graveolens*, *Hypericum perforatum* and *Aronia melanocarpa* [Dušková et al. 1999, Piekoszewska et al. 2010].

In *in vitro* cultures of some plant species, another arbutin precursor that is as good as, or better than, hydroquinone is 4-hydroxybenzoic acid. This has been demonstrated for cultures of *Bellis perennis*, *Bergenia crassifolia*, and *Leuzea carthamoides*, among others [Štambergová et al. 1985].

In our experiment with 4-hydroxybenzoic acid, we found that in *S. chinensis in vitro* cultures this compound was not converted into arbutin. Two compounds were formed as biotransformation products – hydroxybenzoic acid 4-O- β -glucopyranoside and 4-hydroxybenzoic acid β -glucopyranosyl ester in a ratio of 1 : 0.56 [Klick and Herrmann 1988, Keller et al. 1996]. The fact of confirming the presence of hydroxybenzoic acid 4-O- β -glucopyranoside and 4-hydroxybenzoic acid β -glucopyranosyl ester in *S. chinensis* cultures is extremely important from a cognitive point of view. It is an indication of a considerable enzymatic potential of those cultures. At the same time, it is yet another proof of how easily the β -glucosylation reaction proceeds in plant cultures *in vitro*.

CONCLUSIONS

Although the metabolism of the cells from our *in vitro* cultures of *Schisandra chinensis* with a high degree of organogenesis is mainly directed towards the production of lignans, as evidenced by the results of our research [Szopa and Ekiert 2014, Szopa et al. 2016a, 2017b, 2017c], the current study has demonstrated other biosynthetic capabilities of cells from these cultures, leading to the formation of arbutin – an important product in phytotherapy and cosmetology. Moreover, the fact of confirming the presence of two glucosylation products as the result of bioconversion of 4-hydroxybenzoic acid is of significant cognitive importance.

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