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RESEARCH PAPERS

Morphological and Biochemical Characteristics of Genetically Transformed Roots of *Scutellaria andrachnoides*

I. N. Kuzovkina[a], M. Yu. Prokof'eva[a], A. R. Umralina[b], and T. P. Chernysheva[b]

[a] *Timiryazev Institute of Plant Physiology, Russian Academy of Sciences. Botanicheskaya ul. 35, Moscow, 127276 Russia; fax: 7 (499) 977-8018; e-mail: ikuz@mail.ru*

[b] *Institute of Biotechnology, National Academy of Sciences of Kyrgyz Republik, Bishkek, Kyrgyzstan*

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Abstract—Genetically transformed roots (hairy roots) and callus tissue of skullcap (*Scutellaria andrachnoides* Vved.) were for the first time introduced in the in vitro culture. *S. andrachnoides* is the endemic plant of the Kyrgyzstan. These cultures were characterized by active and stable growth in the hormone-free liquid Gamborg nutrient medium. The growth rate of undifferentiated callus tissue was higher than that of hairy roots, which were the source of this callus. The composition of secondary metabolites in hairy roots, callus tissue, and also the roots of seedlings and adult *S. andrachnoides* plants was analyzed. It was found that *S. andrachnoides* hairy roots and callus culture retained the ability for the synthesis of flavones typical for the roots of intact plants. Substantial quantitative differences in secondary metabolites were observed between the roots of juvenile and adult plants. In the seedling roots, which like hairy roots have no secondary thickening, wogonoside, a wogonin glucuronide, predominated among flavones. In the roots of adult plants growing due to the secondary thickening, balcalin, a baicalein glucuronide, was a dominating flavon. It is proposed to use the large-scale in vitro cultivation of roots and especially the rapidly growing callus tissue of *S. andrachnoides* with a profitable content of only one group of flavones for the development of the biotechnological method for producing wogonin and creating on its basis a new drug – a valuable anticancer agent of plant origin with selective cytotoxic activity.

Keywords: Scutellaria andrachnoides, hairy roots, flavones, baicalein, baicalin, wogonin, wogonoside, phenylethanoides, acteoside, secondary metabolism in vitro

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INTRODUCTION

Skullcap *Scutellaria andrachnoides* Vved. is a member of the genus *Scutellaria* (family Lamiaceae) comprising about 360 plant species. This skullcap species was identified by A.I. Vvedenskii, and in 1954 it was included in the catalog of plants of the USSR flora as a rare species growing in the Kyrgyzstan [1]. The distribution area of *S. andrachnoides* is very limited. The plant is met on the rocky slopes and rocks of Atoynok and Fergana ranges at an altitude of 900–1600 m above sea level and because of its small number is included not only in the international list of endangered species of medicinal plants [2] but also in the Red Book of endemic plants of the Kyrgyzstan [3]. There is no information about this plant cultivation.

S. andrachnoides is a perennial not tall plant with white with violet spots corollas of ornamental flowers with a structure typical for plants from the Labiaceae family. It flowers in May–June and bears fruits in July. The chemical composition of *S. andrachnoides* secondary metabolites is unknown because of its rare occurrence and growth in low accessible mountainous regions of Kyrgyzstan [3]. That is why this plant was not included in the list of other skullcap species, in which the composition of secondary compounds contained in the roots and aboveground parts was extensively studied [4]. Plant rare occurrence and complete absence of information about the chemical composition of secondary metabolites located in its organs and especially in the underground parts served as a prerequisite for the introduction into the in vitro culture of genetically transformed roots of *S. andrachnoides* and the study of their ability to synthesize flavonoids, flavones, and phenylethanoides, root-specific for all *Scutellaria* species and manifesting high physiological activity [5, 6].

The objective of this work was to study growth characteristics of *S. andrachnoides* roots cultivated in vitro and to compare qualitative and quantitative composition of secondary metabolites synthesized in genetically transformed roots, the roots of intact plants, not only adult but also juvenile ones.

Abbreviations: GI—growth index; IBA—indolylbutiric acid.

MATERIALS AND METHODS

Introduction of genetically transformed skullcap roots into the culture. To obtain sterile skullcap (*Scutellaria andrachnoides* Vved.) seedlings, the seeds were collected during the expedition in the summer of 2005 on the Mount Tahtalyk of the Fergana ridge. Stratified seeds were defatted with 96% ethanol, sterilized with diocide for 15 min, washed repeatedly with sterile water, and transferred to Petri dishes on modified nutrient Street medium [7]. After seed germination sterile seedlings were transferred into Petri dishes with fresh Street medium, which were placed in the chamber with illumination.

For genetic transformation of *S. andrachnoides* seedlings, cotyledon and hypocotyl fragments were used; they were detached from sterile juvenile three-week-old plants. Wild stain 15834 of the soil bacterium *Agrobacterium rhizogenes* was used for transformation. Transformation was performed as described in the earlier published paper [8]. Some part of obtained and typical in their morphology genetically transformed *S. andrachnoides* roots (so called hairy roots) were transferred after the second subculturing into liquid nutrient B5 medium [9], whereas other roots remained to be cultivated under conditions of surface culture on the agar-solidified B5 medium. By the end of the third passage, the signs of rhizogenesis started to appear: small calli arose and then fused into rather large undifferentiated growing structures. An attempt to stabilize normal root growth via the addition of IBA (0.5 mL/L) was inefficient, and callusogenesis in the obtained culture continued. When the callus produced (about 2 g fr wt) was transferred to 100 mL of liquid B5 medium not containing growth substances, the suspension-callus *S. andrachnoides* culture of root origin was obtained, which grows stably in vitro already during seven years.

During two-year-long cultivation of another part of transformed roots growing under conditions of continuous surface culture, no signs of undifferentiated growth were noted and explants retained morphology of isolated root culture. The transfer of such roots into liquid B5 nutrient medium after their two-year-long growth on agar-solidified nutrient B5 medium did not change the type of root culture growth, and it retained morphological characteristics typical for hairy roots (rapid growth, intense branching, and plagiotropic growth of the root ends).

Thus, as a result of genetic transformation of sterile *S. andrachnoides* seedlings, two lines of the root culture were produced: one retaining morphological characteristics typical for hairy root culture and another with the root transformation into the rapidly growing suspension-callus culture. Both cultures were used in experiments in this study. At the analysis of the secondary compound composition in obtained callus and root cultures, the roots of juvenile *S. andrachnoides* in vitro grown plants and the roots of adult plants collected during the expedition were used as controls.

Conditions of culturing and determination of growth parameters. Similar conditions were used for the cultivation of two obtained cultures, calli and roots: cultivation in liquid hormone-free B5 medium (with 2% sucrose), on the shaker (90 rpm), in darkness, at 25–26°C. In both cases, subculturing was performed every four weeks. Inoculates for callus culture subculturing were about 2 g fr wt per 100 mL of nutrient medium, and those for root culture were about 1 g fr wt per 40 mL of medium. To produce a greater mass of skullcap root culture, root prolonged growing was applied: after three weeks of root growth in 100-mL flasks with 40 mL of nutrient medium, root explants were transferred into 300-mL flasks with 100 mL of nutrient medium and cultivation was prolonged for further four weeks. By the end of the third week of root growth in flasks of increased volume, 30–50 mL of fresh nutrient medium was added and this increased the duration of hairy root growth to seven weeks and provided for the production of a greater root mass.

In the end of subculturing rounds of root and callus cultures, fresh weights of final explants were determined. To this end, these explants were placed on filter paper in the Büchner funnel and the nutrient medium excess was removed at small vacuum in the Bunzen flask. Thereafter the cultures were frozen at –20°C, lyophilized, and their dry weights were determined. The growth index (GI) and the content of dry matter in the plant mass (%) were calculated. Along with growth parameters of roots and calli, the state of nutrient medium, where cultures were growing, was assessed: pH and electric conductivity were determined by the end of subculturing, which served as an indicator of mineral medium component uptake by the roots.

For the chemical analysis of secondary metabolites in roots and calli cultivated in vitro, lyophilized and ground with a mortar and pestle plant material was used. The roots of seedlings and intact adult plants served as control in the chemical analysis. To obtain the mass of juvenile plant roots sufficient for the chemical analysis, 3–4-cm stem segments of sterile 6–8-week-old skullcap seedlings were rooted and grown for three months under illumination on agar-solidified B5 nutrient medium containing 0.25 mg/L IBA for rhizogenesis activation. The roots detached from the rooted cuttings and the roots of plants growing under natural conditions were dried at 60°C for 48 h, ground in the mortar, and used for chemical analysis.

Chemical analysis of plant materials. To prove the efficiency of genetic transformation of obtained plant materials, along with the observation of typical hairy root morphological features, opine presence was determined, which was performed by the method of Petit et al. [10]. Opines were separated electrophoretically after their extraction from fresh plant material

with 10 volumes of water-ethanol (50%) at heating at 80°C for 15 min.

Secondary compounds were extracted with methanol from powdered dried plant samples. Extraction was performed in DURAN 10-mL tubes with tight screw caps on a magnetic stirrer for 16 h at room temperature. Samples (25–50 mg) of plant material were weighed to the nearest four decimal and extracted with 100 volumes of methanol. After extraction of secondary plant metabolites, aliquots of the extracts (2.0 mL) were transferred into Eppendorf tubes and after centrifugation the supernatant was used for the chemical analysis by analytical HPLC.

In the case of preparative isolation of one of flavones, wogonoside, secondary compounds were extracted from *S. andrachnoides* callus also with methanol but in DURAN 250-mL flasks on a magnetic stirring for 18 h. The ratio of methanol to sample volume was 50 : 1. Some portion of the extract (about 0.5 of volume) filtered through a paper filter was evaporated in the rotor evaporator at 40°C to 25–30 mL. Such evaporation resulted in the appearance of yellow-white pellet, which was separated by centrifugation, and the supernatant was used for preparative chromatographic separation for isolation and successive identification of the flavon wogonoside. The pellet obtained after centrifugation was washed with methanol, and the isolated compound was identified by HPLC (the retention time, UV spectrum of the absorption peak, and co-chromatography with the standard supposed compound, acteoside).

Conditions of chromatography. Analytical HPLC was performed using an Agilent 1100 chromatograph (Germany) with the Multohigh 100 RP-18 column (250 × 4.6 mm, 5 μm). Solvents: A—twice deionized H_2O with 0.1% trifluoroacetic acid; B—methanol (MeOH), also with added 0.1% trifluoroacetic acid. Linear solvent gradient: 0–1 min 10% B, 1–25 min 100% B, 25–30 min 10% B. Loaded extract volume was 10 μL; the rate of solvent flow – 1 mL/min; compounds were detected at 276 nm.

Chromatographic separation of extracts for the identification of wogonoside was performed by preparative HPLC in combination with subsequent mass spectrometry (MS) and NMR spectroscopy. Conditions of HPLC performance: chromatograph – Shimadzu LC-20A Prominence (Japan) with autosampler and a FRC-10A automatic fraction collector, and also with LC controller SP 1. Conditions of separation: column – Purospher STAR RP-18e (Merck, Germany) (250 × 10 mm, 5 μm); the rate of separation – 3.5 mL/mimn; solvents: A – H_2O with 0.1% trifluoroacetic acid, B – MeOH with added 0.1% trifluoroacetic acid. Linear solvent gradient: 0–1 min 10% B, 1–38 min 100% B, 38–45 min 10% B. Compounds were detected at 254 and 280 nm. Wogonoside fraction was collected in the interval between 28 and 29 min (the area of the main peak – 34.9% at 254 nm).

Conditions of mass spectrometry: mass spectrometer – Bruker Esquire 3000 (Germany) with Esquire controller; injection of the methanol extract of isolated compounds – in the regime of negative ionization; capillary voltage – 4000 V, nebulizer – 30 psi, the rate of drying gas-reagent – 12 L/min, temperature of drying gas – 330°C.

Conditions of NMR spectroscopy: instrument – Bruker Avance 2400 MHz (Germany); controller – TopSpin, v. 2.1, with the standard program Bruker; ^1H – at the frequency of 400.13 MHz; ^{13}C – at the frequency of 100.62 MHz; 5 mm PABBI 1H-BB Z-GRD; sample – 10 mg dissolved in DMSO-d6.

Quantification of flavonoids in plant samples was performed using calibration curves built for each flavon (baicalin, wogonoside, baicalein, and wogonin) and also phenylethanoid (acteoside). For calibration curve building, HPLC standard compounds (AppliChem, Germany) were used. Determination of secondary metabolite contents was performed in four replications. Calibration curves for flavones and acteoside were constructed and the calculations of the phenolic compound content were performed on the basis of peak areas on chromatograms using Origin 7.0 and Microsoft Office Excel 2010 softwares.

The content of secondary compounds in plant samples was expressed in mg/g dry wt. The mean values and their standard errors are presented.

RESULTS

To date the introduction to the culture of genetically transformed *S. andrachnoides* roots and callus tissue of root origin represent two steadily growing plant materials under the same conditions (Figs. 1, 2). Root culture was characterized by root plagiotropic growth, intense branching, and not very well expressed apical growth of root ends, and this led to a very dense interweaving of formed lateral roots. As a result, by the end of the seventh week, the root explants represented a mass of firmly entangled roots, which could be difficult to extract from the culture flasks with medium.

The *S. andrachnoides* root culture grew slower than that of another skullcap species *S. baicalensis.* One more difference was that, during prolonged cultivation, *S. andrachnoides* root mass separated clearly into the two parts: submerged in nutrient medium and positioned in the center of the flask in air above the medium surface (Fig. 1). On the ends of explant roots in the air phase, drops of root exudates were usually seen. Complete root submersion in the liquid nutrient medium resulted in the reduced contact between apical root ends and the air, and this led to the appearance of yellowish coloration of the medium because of excretion of secondary metabolites from the roots; this accelerated culture perishing because of root intoxication by the excretion products.

Growth index (GI) of *S. andrachnoides* root culture was about 25 by the end of the 7th week of pro-

Fig. 1. Appearance of 7-week-old genetically transformed roots of skullcap (*S. andrachnoides*) at long-term cultivation.

longed cultivation; the content of dry matter in the tightly packed explants attained 8.5%, which was much higher than the corresponding characteristic of *S. baicalensis* hairy roots (about 6%) and the root cultures of other plants (5–7%). Nutrient medium acidity was about 7.0 by the end of *S. andrachnoides* subculturing (at the initial value of 5.7); electrical conductivity by the end of subculturing reduced from 3.5 to 2.0 mS/cm at the single addition of a small portion of fresh medium.

As distinct from the roots, *S. andrachnoides* callus tissue of root origin cultivated in liquid nutrient medium not containing growth substances grew much faster, and its GI was about 40 by the end of the 4th week (Fig. 2). Undifferentiated growing callus tissue of *S. andrachnoides* grew well at complete submersion into nutrient medium and contained more water than the root culture. The content of dry matter in callus was much lower than in the *S. andrachnoides* root culture (about 2.0–2.5%). Electrical conductivity of the nutrient medium for callus cultivation reduced by the end of the 4th week from 3.5 to 0.5 mS/cm; this indicates the almost complete exhaustion of mineral components in medium and a necessity of tissue subculturing. A specific feature of skullcap callus tissue is its capability of greening during cultivation on agar-solidified nutrient medium in the light.

During the first two years of cultivation of *S. andrachnoides* surface callus culture, mighty shoot organogenesis was observed, which ceased completely after its three-year-long growing as a submerged culture in darkness. Conversion of genetically transformed skullcap roots into callus culture may mean that we deal with untransformed cells, and this occurs sometimes during the initial stages of rhizogenesis at genetic transformation. However, in this case callusogenesis occurred on typical genetically transformed roots of the third subculturing, and the primary site of its manifestations was the region of lateral root formation. This excluded the possibility that obtained callus

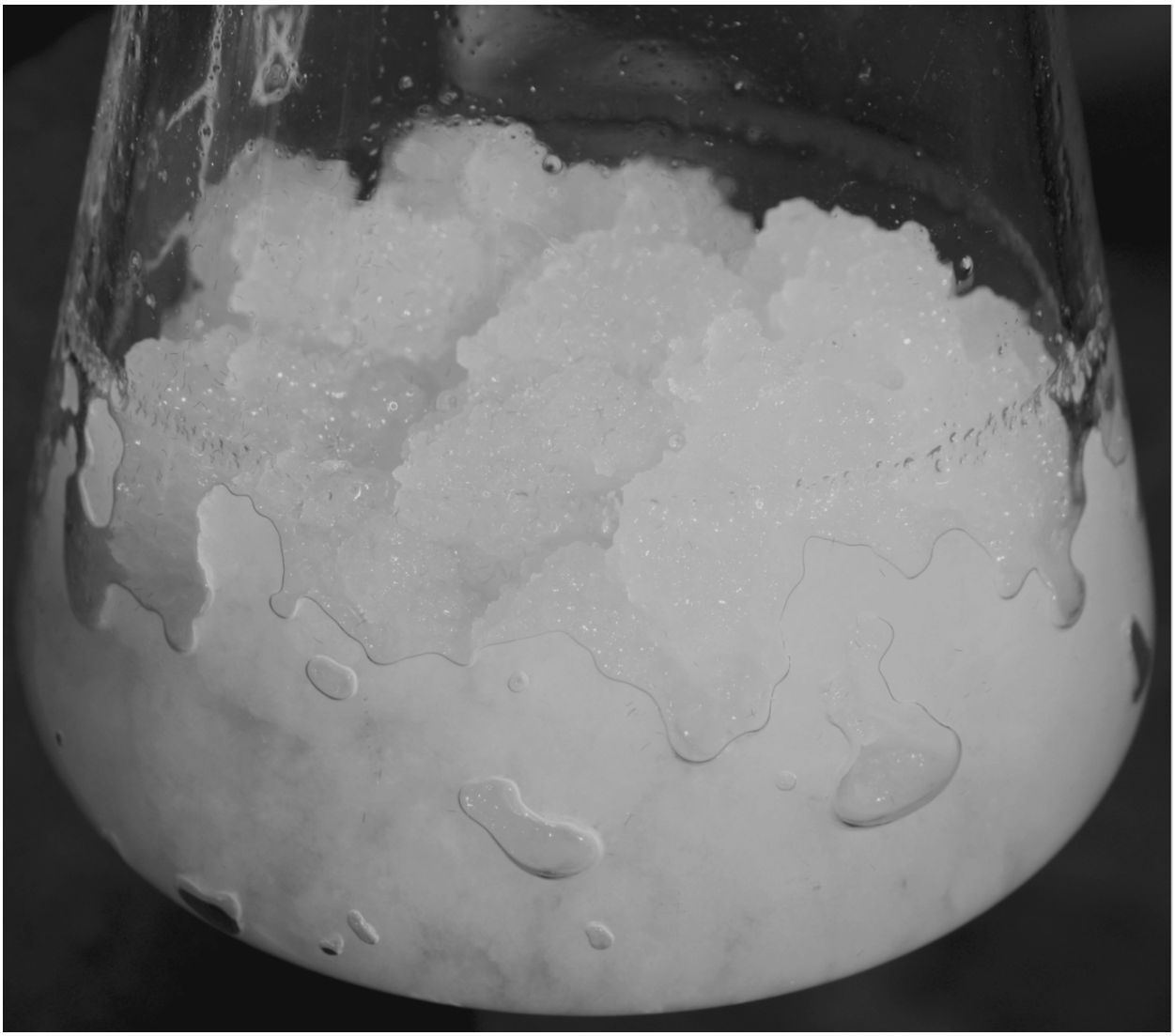

Fig. 2. Appearance of skullcap (*S. andrachnoides*) callus tissue by the end of the 4th week of growth.

culture was genetically untransformed. This conclusion is also confirmed by intense callus growth in nutrient medium devoid of growth substances.

To confirm the efficiency of genetic transformation of both in vitro cultures, the content of opines in them was determined. The electrophoregram demonstrated clearly the presence of mannopin in both *S. andrachnoides* root and callus cultures (Fig. 3).

Chromatographic analysis of methanol extracts from the roots of adult plants and seedlings of *S. andrachnoides* revealed the presence in them of secondary metabolites, which, as judged from their characteristics (retention time and UV-spectra of absorption peaks), belong to the group of flavones typical for the roots of all skullcap species. In both the roots of adult and juvenile *S. andrachnoides* plants, this group was represented by two pair of related flavones: lipophilic aglicones, baicalein and wogonin, and glucuronides, baicalin and wogonoside, respectively, which were usually present together in the skullcap roots (Fig. 4).

These flavones differ from each other by the presence of methoxy group in the position 8 in wogonin and the additional hydroxyl group in the position 6 in baicalein. A comparison of the qualitative composition of secondary metabolites revealed by HPLC in the extracts from the roots of adult and juvenile plants (Figs. 5a, 5b) and those in the in vitro cultivated roots (Fig. 5c) demonstrated the presence in hairy roots of all four main flavones typical for the roots of intact *S. andrachnoides* plants. However, in undifferentiated *S. andrachnoides* callus tissue only two flavones were found, supposedly wogonoside and its aglicon wogonin (Fig. 5d). Just this unusual flavon composition of skullcap callus tissue motivated us to perform preparative isolation of wogonoside from the methanol

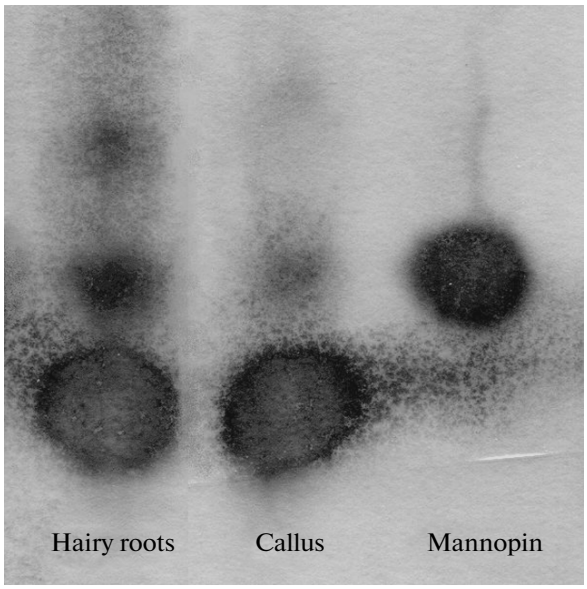

Fig. 3. Electrophoregram of water-ethanol extracts from skullcap (*S. andrachnoides*) hairy roots and callus tissue. Analysis of opine content.

extract of undifferentiated growing callus tissue for its complex identification. The results of mass-spectrometry and also ^{1}H and ^{13}C NMR-spectroscopy confirmed that the main flavon of skullcap callus tissue was wogonoside (Figs. 6, 7). Thus, the presence of its aglycon, wogonin, in the extract from the callus tissue identified by the retention time and UV-spectrum of absorption peaks of compound detected by HPLC seems quite logical.

It should be noted that, in the extracts of all four plant samples (roots of adult plants and seedlings, hairy roots, and callus tissue), the secondary metabolite with more hydrophilic properties than flavones was found; its peak on chromatograms is designated by the figure 1 (Fig. 5). It was noted above (see Materials and Methods section) that, during the preparation of skullcap methanolic extract from callus tissue for the preparative isolation of wogonoside, after its evaporation in vacuum the pellet was precipitated. After its separation and purification, a compound was isolated, which, according to the results of chromatographic analysis, was identical to acteoside (=verbascoside). This fact was confirmed by co-chromatography of methanolic extracts of callus tissue, hairy root, seedling roots, and the roots of adult plants with the standard acteoside.

Acteoside belongs to the group of phenylethanoides; it is a phenylpropanoid comprising two molecules of coffeic acid linked by ester bonds with ramnose. It was detected in the roots of intact *S. baicalensis* roots as a minor secondary metabolite [11] and also in genetically transformed roots of this skullcap species [12]. According to literature data, phenylethanoides belong to secondary metabolites, which are met most frequently and abundantly in plants from families Loganiaceae, Plantaginaceae, Scrophulariaceae, and Verbenaceae [13]. It is also established that phenylethanoides, acteoside in particular, have a rather wide range of physiological activities, including cytotoxic one, in connection with which they represent some practical interest for their possible use in medicine [14].

More detail analysis of the HPLC results for plant sample extracts and especially of the quantitative analysis of individual flavones and acteoside revealed substantial differences in the content of secondary metabolites in the roots of adult and juvenile plants and in the in vitro cultivated genetically transformed roots and callus skullcap tissue (table). Firstly, differences

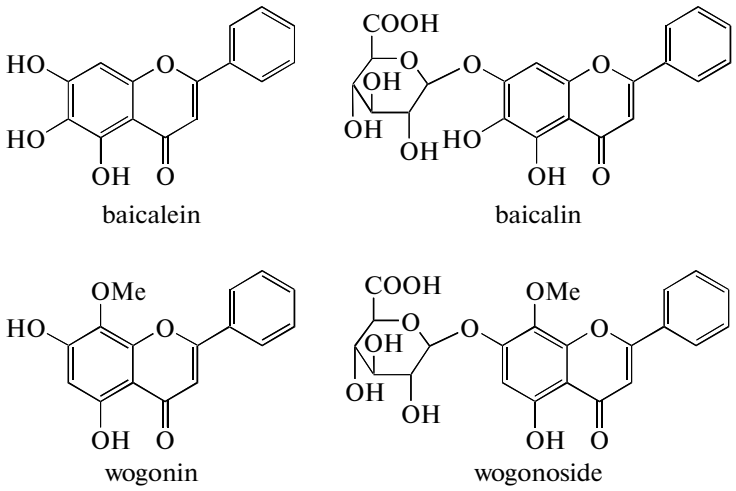

Fig. 4. Structural formulas of main flavones of skullcap (*S. andrachnoides*).
OMe—metoxy group.

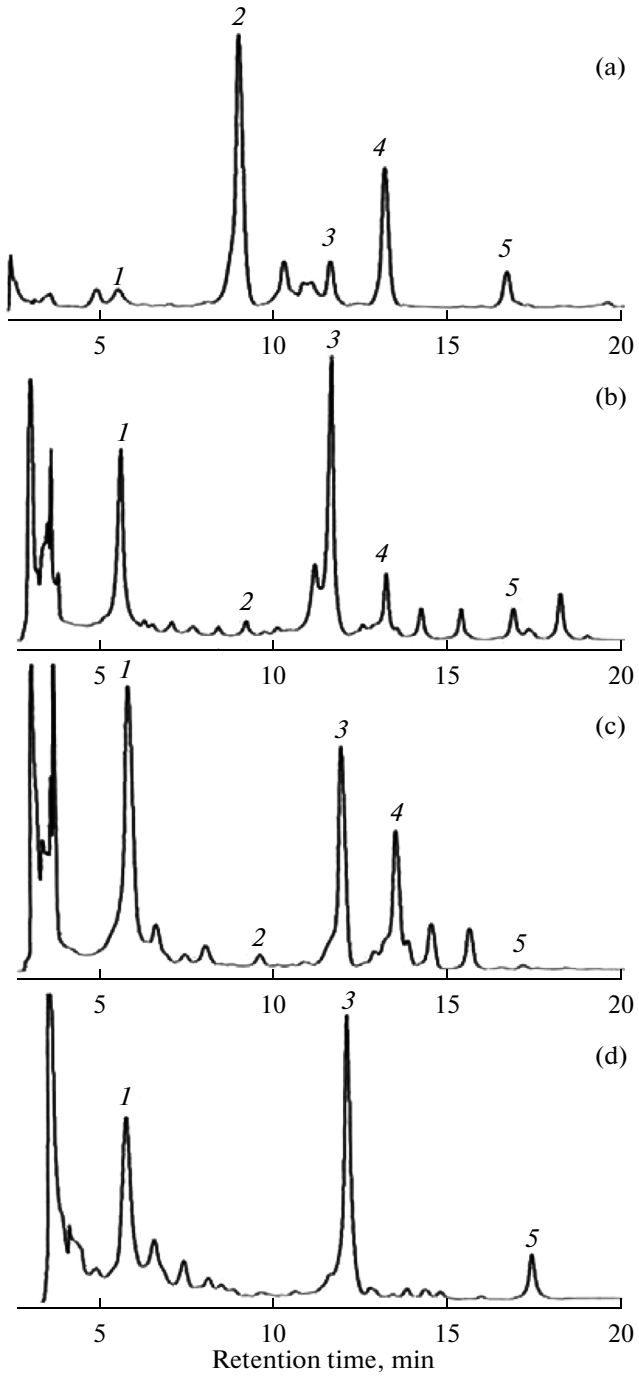

Fig. 5. HPLC profile of methanol extracts from the roots of adult plants (a), seedling roots (b), hairy roots (c), and callus tissue (d) of *S. andrachnoides*.

(*1*) Acteoside; (*2*) baicalin; (*3*) wogonoside; (*4*) baicalein; (*5*) wogonin.

between the roots of adult and juvenile *S. andrachnoides* plants in the contents of secondary metabolites should be noted (Figs. 5a, 5b). As evident from HPLC analysis, in the roots of adult skullcap plants (Fig. 5a) predominated flavones were baicalin and its aglycon baicalein, whereas in the roots of juvenile plants (Fig. 5b)

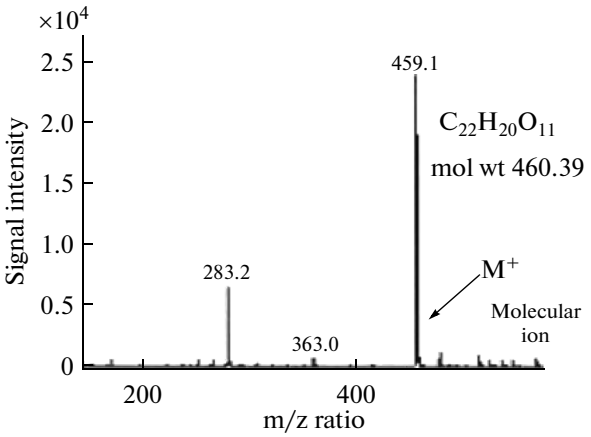

Fig. 6. Mass spectrum of wogonoside isolated from the callus tissue of *S. andrachnoides*.

On the ordinate axis is the ratio of the molecular ion peak value to the maximal peak of the fragment obtained during ionization of indentifying compound. Multiplier $\times 10^4$ characterizes an increase in the instrument sensitivity. Figures above peaks correspond to m/z values.

predominated flavon was wogonoside, a glucuronide of wogonin, which was represented on the chromatogram of seedling root extracts by a small peak. The total content of flavones in the seedling roots was only 1.4-fold lower than the flavon content in the roots of adult plants, on the background of the fourfold higher content of wogonoside.

The second difference between the roots of *S. andrachnoides* seedlings and adult plants was the presence in the seedling roots a substantial amount of acteoside, which was in the roots of adult plants only in minor quantities. As evident from the table and HPLC profiles of extracts, the contents of secondary metabolites in *S. andrachnoides* hairy roots were close to those in the seedling roots (Figs. 5b, 5c). They differed only slightly in the total content of flavones (table), and wogonoside was the main component in both cases, whereas a typical dominant component in the roots of adult plants was baicalin. The latter is represented on chromatograms by a little noticeable peak, as distinct from clearly expressed peak of its aglycon baicalein, the amount of which in hairy roots was comparable with the content of this aglycon in the roots of adult plants. In addition, a great amount of acteoside was detected in hairy roots; its content exceeded that in the seedling roots.

Most unexpected was the detection in the *S. andrachnoides* callus tissue of only a single group of flavones, e.g., wogonoside and small amount of its aglycon wogonin; their total content was essentially comparable with the content of these flavones in hairy roots (5.2 and 6.5 mg/g, respectively). However, the content of acteoside in callus tissue was almost three times lower than in hairy roots. It was established that, during callus tissue subculturing, the content of

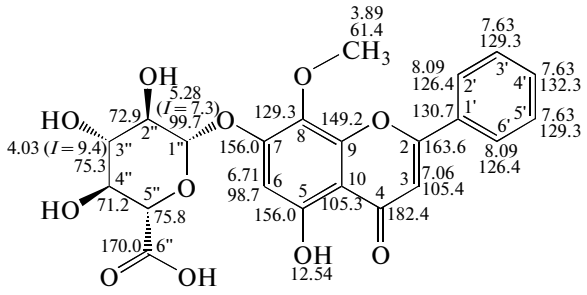

Fig. 7. Structural formula of wogonoside as the result of data analysis obtained by ^{1}H and ^{13}C NMR spectroscopy of flavon isolated from *S. andrachnoides* callus tissue.

wogonoside in it decreased gradually. However, taking into account the fact that *S. andrachnoides* callus tissue had the higher GI and the cycle of its cultivation was twice shorter than the cycle of hairy root cultivation, the total callus wogonoside production was essentially similar to that of cultivated roots. In addition, an obvious advantage of callus tissue is the presence in it of only a single flavon, wogonin.

Acteoside quantification in the in vitro cultivated roots and callus tissue showed that the highest concentration of this phenylethanoid was in the in vitro cultivated *S. andrachnoides* roots followed by seedling roots and callus tissue. The acteoside concentration in the roots of intact adult *S. andrachnoides* plants was almost 50-fold lower than its averaged concentration in materials grown in vitro.

DISCUSSION

Since the literature data on the chemical composition of secondary metabolites of *S. andrachnoides* roots are absent, the present work can be considered as the first publication on this subject. It is useful to compare the obtained results with those obtained in experiments with the in vitro cultured roots of another, the most common and therefore well-studied skullcap species, *S. baicalensis* [15]. An undoubted fact is that the total content of flavonoids in the roots of adult *S. andrachnoides* plants was by an order of magnitude lower than in the roots of *S. baicalensis* [16], which may be explained by unfavorable growth conditions of *S. andrachnoides* plants. A common phenomenon for the roots of both *Scutellaria* species is the dominant content in them of baicalin but not wogonoside. The ratio of baicalin to wogonoside content in *S. andrachnoides* roots was 4 : 1.

Although the total content of flavones in *S. andrachnoides* seedling roots and in the roots of adult plants differed insignificantly, the ratio between the basic flavones was different in these roots. In the roots of adult plants, baicalin but not wogonoside dominated, and the baicalin/wogonoside ratio was sharply changed. As evident from the table, the total content of major flavones in the in vitro cultivated *S. andrachnoides* roots differed little from the content of flavones in seedling roots. Like in the roots of juvenile plants, the dominating flavon in them was not baicalin but wogonoside, and the ratio between them was 1 : 60. Taking into account that cultivated *S. andrachnoides* roots and seedling roots had similar primary growth type, wogonoside may be considered as juvenile secondary metabolite, predominant occurrence of which in the skullcap hairy roots can be determined by such root incapability to grow due to the secondary thickening characteristic of the roots of adult plants.

Similar differences in the manifestation of secondary metabolism between seedlings, undifferentiated growing callus tissues, plant cells, and intact plants were observed for some other secondary plant compounds, for example, for benzophenanthridine alkaloid sanguinarine and morphine-like alkaloid thebaine [17]. It should be noted that a common feature of all tested plant materials (roots of adult plants and seedlings, hairy roots, and callus tissue) is the predomination in the living undamaged plant tissues and cells of flavones as their glucuronides (baicalin and wogonoside) but not their lipophilic aglycons (baicalein and wogonon). According to literature data, the anomalous accumulation of flavon aglycones was observed only under stress influence on skullcap tissues, as it was in the case of mechanical root damage of intact *S. baicalensis* plants or at elicitation of cultivated cells of this skullcap species [18] and also hairy roots of *S. lateriflora* plants [19]. Unexpected picture found when analyzing extracts of undifferentiated growing skullcap callus tissue, where only one group of root-specific flavones, namely wogonoside and wogonin, confirms the assumption about the juvenile nature

Content of phenolic compounds (acteoside and flavones) in the roots of adult plants, seedlings, and also hairy roots and callus culture of *S. andrachnoides*, mg/g dry wt

Plant material	Acteoside	Baicalin	Wogonoside	Baicalein	Wogonin	Sum of flavones
Roots of adult plant	0.26 ± 0.02	8.82 ± 0.27	2.09 ± 0.01	3.55 ± 0.04	2.04 ± 0.03	16.50 ± 0.09
Roots of seedlings	7.48 ± 0.92	0.16 ± 0.01	8.65 ± 0.05	1.89 ± 0.02	1.37 ± 0.03	12.07 ± 0.03
Genetically transformed roots (hairy roots)	10.36 ± 1.57	0.09 ± 0.04	6.49 ± 0.42	4.32 ± 0.22	0.01 ± 0.05	10.91 ± 0.18
Callus tissue	2.86 ± 1.04	–	3.74 ± 0.11	0.02 ± 0	1.40 ± 0.02	5.16 ± 0.07

of the glucuronide wogonoside. It should be noted that wogonoside and wogonin always were a kind of "adjutants" of baicalin and baicalein, and the data about their autonomous occurrence as secondary metabolites in plant roots are absent.

Despite the fact that the content of flavones in callus tissue was twice lower than in hairy roots, the preservation by the callus tissue a capability to synthesize root-specific metabolites is of great interest. Monocomponent flavon composition in callus tissue growing in hormone-free nutrient medium is its extreme useful feature because rapidly growing *S. andrachnoides* callus culture may be considered as a potential producer of flavones from the group of wogonin.

At present, a great attention of leading biochemist and pharmacologs is attracted by the wogonin property to selectively induce apoptosis of only oncogenic cells, which was discovered in 2007 [20]. The selective wogonin action on the growth of only malignant tumor cells is now confirmed by numerous experiments performed under in vitro and in vivo conditions by the researchers in several laboratories in Germany [21], Japan [22], China [23], South Korea [24], and USA [25]. This established fact indicates a necessity to study the mechanism of wogonin selective action and its undoubtedly perspective using as a basis for the creation of a new cytotoxic drug that does not cause myelosuppression during chemotherapy of patients with malignant deceases. The investigation of the mechanism of wogonin selective cytotoxic action, but the realization of its application as a new anti-tumor drug is limited by its low concentration in the roots of perennial skullcap plants of various species, which is not exceed 1% of root dry weight. In addition, an important role is played by a sharp decline in the natural population of all skullcap species and difficulties of their introduction.

The chemical synthesis of wogonin was so far unsuccessful. Some attempts were undertaken to use wogonoside hydrolysis, which concentration in the roots of intact skullcap plants is much higher than wogonin concentration. To this end, a complex method of enzymatic wogonoside hydrolysis by β-glucuronidase, constitutively present in skullcap tissues, was developed with the application of cavitation during flavon extraction from plant material [26]. In any case, the applicability of this method is limited by the low content of plant row material, i.e., naturally growing skullcap plants, most of which are endangered species. Therefore, the large-scale in vitro cultivation of *S. andrachnoides* roots and especially rapidly growing callus tissue with the advantageous content of only one flavonoid group can be used for the development of a biotechnology of wogonin production and creation on its basis of a new drug of plant origin manifesting unique selective cytotoxic activity.

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