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BIOCHEMISTRY

The Phenolic Segment of the Metabolome of the Roots of *Scutellaria lateriflora*

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Abstract—The results of studying the metabolites of the roots and hairy roots of *S. lateriflora* using liquid chromatography–mass spectrometry are presented. It has been established that the main share of polyphenolic metabolites in the roots and hairy roots are phenylethanoids and flavonoids containing up to two and up to four methoxyl groups, respectively. Among flavonoids, wogonin, 6-OMe wogonin, and their glycosides are most abundant in the roots of the plant. Phenylethanoids are represented by a series of hydroxytyrosol caffeoyl rutinosides, with a content parity with flavonoids. In addition to polyphenols, a significant content of sucrose was found in the root system.

Keywords: *Scutellaria lateriflora*, roots, hairy roots, carbohydrates, flavonoids, methylation, glycosylation, LC-MS/MS

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INTRODUCTION

Plants of the genus *Scutellaria* are known for their flavonoids, which are used to treat various diseases. *S. lateriflora* is a perennial herb of this genus, which is widespread in wetlands of North America and Canada (Sherman and Nirmal, 2022). Genetic and phylogenetic analysis of 28 species of widely used medicinal plants confirmed the close relationship of *S. lateriflora* and *S. baicalensis* (Chen, 2019). The medicinal properties of the roots of *S. baicalensis*, which is included in many regional Red Books in Russia, are attributed to unique 4'-deoxy flavones, the molecules of which lack a hydroxyl group at the C4' atom of the B-ring: baicalein (3), wogonin (4), oroxylin A (5) (Zhao et al., 2016). These flavones have anti-inflammatory properties and also inhibit tumors in mice in vivo in various models.

The aerial part of *S. lateriflora,* which contains all three of the above-mentioned flavones (Islam et al., 2011; Li et al., 2012; Costine et al., 2022), was used as a sedative and anxiolytic agent by Native American Indians to treat various nervous disorders. Currently, the herb *S. lateriflora* is also in demand on the medicinal plant market of the North American continent. Among the nervous disorders identified in modern society, anxiety is the most common and potentially serious disorder, since it can lead to somatic and social dysfunction. For this reason, expanding the range of drugs used based on plant materials seems to be an urgent task, which in turn requires studying the phytochemical composition, patterns of synthesis, interconversion of substances, and the factors determining them (metabolomics).

The biosynthesis of flavones has specific pathways. The main step in biosynthesis starting from pinocembrin (0) is carried out by the enzyme FNSII with its conversion to chrysin (1) (5,7-di-OH-flavone) and subsequent oxidation of the A and B rings of the molecule by hydroxylases (Zhao et al., 2016, 2018; Cui et al., 2021). In the absence of the enzyme, pinocembrin (0) gives rise to the flavonone biosynthesis pathway (Pei et al., 2022). The biosynthesis of 4'-hydroxyflavones, with products of subsequent hydroxylation at the B ring such as scutellarein (5,6,7,4'-tetra-OH flavone) and apigenin (5,7,4'-tri-OH flavone), in the roots (Costine et al., 2022) and in the aerial parts of *S. lateriflora* (Zhang et al., 2009; Islam et al., 2011; Li et al., 2012) turned out to be less productive than the target flavones. Thus, the aerial part of *Scutellaria lateriflora* and the roots of *Scutellaria baicalensis* produce

Fig. 1. Roots of *S. lateriflora*. (1) White roots; (2) hairy roots; (3) dark roots. Photo by Yu.N. Yelkin.

the same 4'-deoxyflavones (baicalein and wogonin), which have pronounced biological activity and are widely used in medical practice. Meanwhile, preparations from the indicated parts of the plants have slightly different therapeutic effects, which indicates the presence of other metabolites or a higher content of known flavones.

It should be noted that the medicinal properties of the roots of *S. baicalensis* are largely associated with methylated 4'-deoxyflavones (Tsai et al., 2016; Zhao et al., 2016; Pei et al., 2022) synthesized by the hairy root culture (Elkin et al., 2018). Meanwhile, neither the above-ground part of *S. lateriflora* nor the its hairy root culture was found to contain significant amounts of methylated flavones, with the exception of wogonin (Wilczańska-Barska et al., 2012; Kim et al., 2014; Stepanova et al., 2021). The composition of metabolites of *S. baicalensis* has been studied in sufficient detail to date, while such information about *S. lateriflora* is very poorly covered. Thus, the purpose of the study is to determine the phenolic segment of the metabolome of *S. lateriflora* roots, the profile of which can largely be manifested in the metabolome of the above-ground part of the plant.

MATERIALS AND METHODS

S. lateriflora plants were obtained and successfully introduced from seeds (Prairie Moon, United States) in the conditions of southern Primorye, Tigrove tract, Nadezhdinskii district (43.390844, 132.006575). The plant vegetation took place under natural climatic conditions, without the introduction of additional nutrients. The roots were harvested at the end of October, cleaned of soil residue, and dried using the air-shadow method. White roots and hairy roots were selected for further extraction (Fig. 1). About 100 mg of the crushed material was extracted twice with 2 mL of 96% ethanol for 2 h at 50°C. The combined extract was centrifuged for 3 min at 15000 rpm, filtered through PTFE syringe filters (Phenomenex, pore size of 0.45 μm, diameter of 13 mm), and analyzed by LC-MS.

LC-MS analyses of extracts of the root and hairy roots of *S. lateriflora* were conducted at the Instrumental Centre of Biotechnology and Gene Engineering of Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS. All components were identified using the Infinity 1260 analytical HPLC system (Agilent Technologies, Santa Clara, California, United States) equipped with a G1315D photodi-

ode array detector, a G1311C pump, a G1316A column thermostat, and a G1329B autosampler. The chromatographic system was coupled with an ion trap mass spectrometer (Bruker HCT ultra PTM Discovery System, Bruker Daltonik GmbH, Bremen, Germany) equipped with an electrospray ionization source (ESI). The MS analyses were carried out with negative ion detection. The following instrument parameters were used: the *m/z* detection range was 100–1200, the drying gas (N_2) flow rate was 10.0 L/min, the nebulizer gas (N_2) pressure was 241 kPa, the ion source potential was 4.0 kV, and the drying gas temperature was 365°C. Tandem mass spectra were acquired in the Auto–MS2 (intelligent fragmentation) mode using collision energy enhancement. The fragmentation amplitude was set to 1 V. Data were acquired with Bruker Daltonics Compass 1.3 esquire control software (version 6.2.581.3) and processed with Bruker Daltonics Compass 1.3 data analysis software (version 4.0.234.0).

An analytical column (Zorbax C18, 150 mm, i.d. 2.1 mm, 3.5 μm part size, Agilent Technologies, United States) was used for separation. Separation was performed under the following conditions: the column temperature was 40°C, the mobile phase consisted of 0.1% aqueous formic acid (A) and acetonitrile (B). The following elution gradient was used with a flow rate of 0.2 mL/min: 0 min 20% B; 3 min 20% B; 25 min 80% B, 30 min 100% B, and then eluent B for up to 40 min, with a wavelength of 275 nm. All solvents were of high-performance liquid chromatography (HPLC) grade.

RESULTS AND DISCUSSION

The composition of phenolic compounds in the root extract is roughly characterized by comparing the HPLC-UV profiles and the total ion current (Figs. 2a, 2b). The total ion current (TIC) profile demonstrates a more abundant composition of substances than the UV profile.

The results indicate an abundance of carbohydrates and a significant proportion of methylated flavone (MF) glycosides in the plant root extract. Indeed, in the roots of the related *S. baicalensis*, methylated flavones account for approximately half of the identified metabolites (Qiao et al., 2016). MS monitoring in the negative ion mode allowed us to record simultaneously several compounds that differ in polarity: carbohydrates, free and methylated flavones with their glycosides. The TIC profile is conventionally divided into four fractions, in accordance with the elution sequence reflecting the polarity of the substances. To determine their nature, selective ion monitoring (SIM) and collision-induced ion dissociation (MS2) were used. At each scanning cycle, the program selects the two most abundant ions to collect the MS2 spectrum (Qiao et al., 2016). This approach prompted us to present the measurement results in the form of a tree, where the MS2 spectra are like "leaves" on the "branch" fractions of the ion chromatograms (Supplements, Figs. S1–S4). Such visualization allows us to estimate the content of metabolites, since no relationship was found between the signal intensity of phenolate ions $[M-H]^-$ and the number of phenolic OH groups (Modelli and Pshenichnyuk, 2013; Xia and Attygalle, 2016). The detected metabolites are also presented in a more classical form in Table 1.

Flavone biosynthesis in the roots of *S. baicalensis*, a plant closely related to *S. lateriflora*, results in a significant accumulation of O-methylated derivatives and their diversity due to subsequent glycosylation (Qiao et al., 2016). Glycosylation of MFs modifies them, presumably for delivery and use in the aboveground organs during the growing season. Thus, methylation is the final result of the flavone biosynthesis pathway of *S. baicalensis* (Elkin et al., 2022). For this reason, the analysis of the metabolite composition of *S. lateriflora* roots was started with the nonpolar fraction F4. Based on the experience of studies of *S. baicalensis* roots, MFs (from mono- to penta-methylated) are mainly eluted in the range of 20–22 min (Elkin et al., 2018, 2022).

Methylated Flavones

LC-MS analysis of these compounds extracted from the roots of *S. lateriflora* revealed ten major MFs that give a characteristic fragmentation pattern with the cleavage of a methyl group and the formation of an odd electron ion. The ion chromatograms (ICs) of the MFs are displayed in the reverse order of increasing number of methyl groups on the carbon backbone of the flavone molecules and the *m/z* values of their anions $[M-H]^-$ (Fig. 3a). Among the ten MFs, five are predominant: wogonin (4, *m/z* 283 $[M-H]^-$), 6-OMe wogonin (10, *m/z* 313 $[M-H]^-$), presumably 6-OH-wogonin (6, *m/z* 299 $[M-H]^-$), and a precursor of flavone (10). The 1M peak of the phenolate ion $[M-H]^-$ *m/z* 267 at 16.9 min is due to 7-methoxy chrysin known as tectochrysin (Wang et al., 2018). The glycoside adduct with Na *m/z* 475 at 7.7 min also remains prominent in the TIC of flavone biosynthetic products (Supplements, Fig. S4). The ion (ss) *m/z* 301 could represent 5,7,4'-tri-hydroxy-6-methoxy flavonone (Takagi et al., 1980), but only signatures of flavonones were found in *S. lateriflora* roots. This pathway is implemented in plantation *S. baicalensis* roots and is manifested by seven ions of *m/z* 477, glucuronides of tri-OH-flavanone isomers (Wang et al., 2018). However, the retention time (18.3 min) for the compound

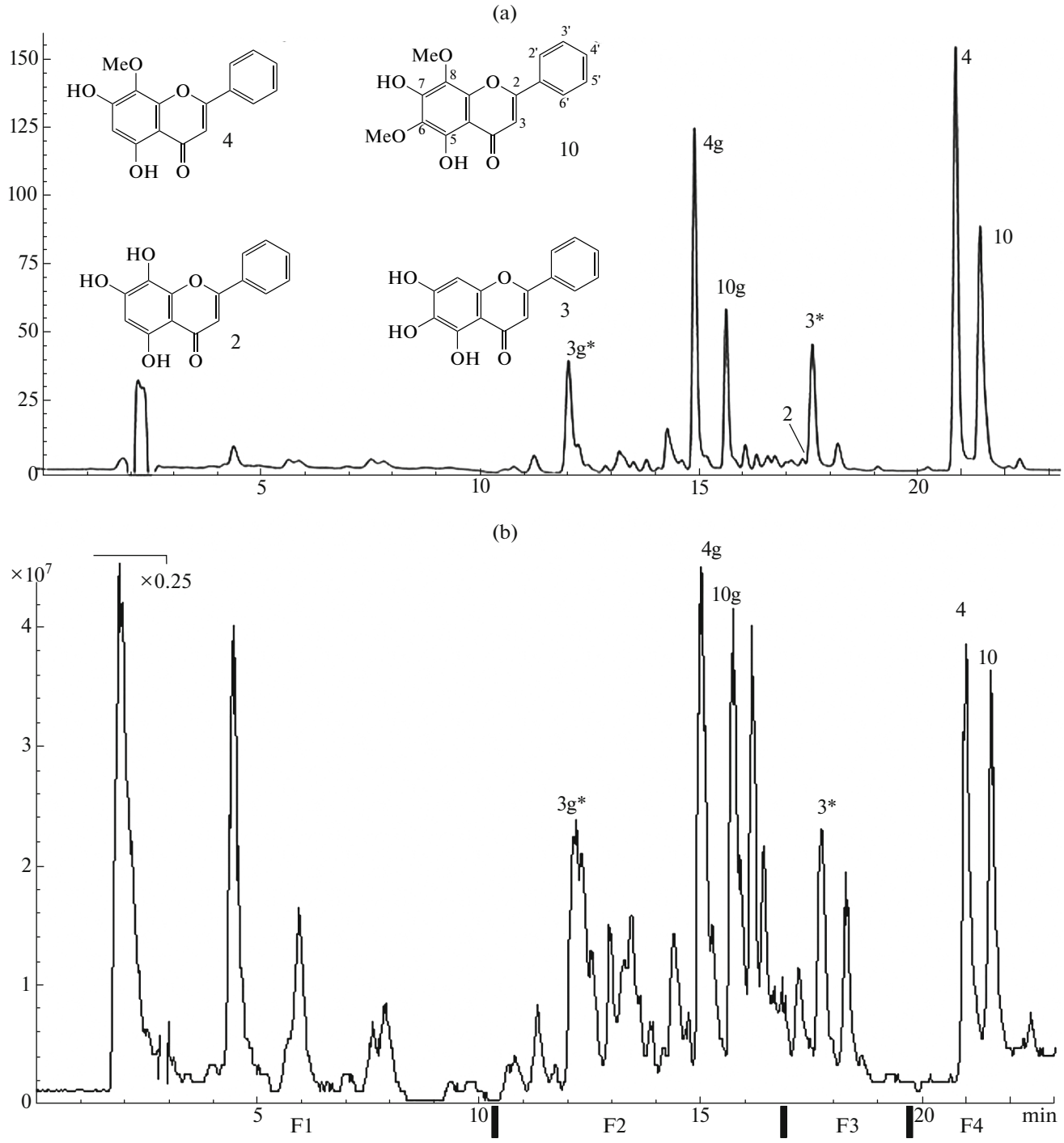

Fig. 2. (a) UV chromatogram and (b) total ion current of the extract of *S. lateriflora* roots. Fraction annotation: F1 carbohydrates and phenylethanoloids; F2 glycosides of flavones; F3 flavones; F4 methylated flavones. 3*, baicalein; 4, wogonin; 10, 6-OMe wogonin and their glucuronides 3g*, 4g, 10g, respectively.

(ss) shows that this substance, with a molecular weight of 302, is closer to the nonpolar MFs 4 and 10. Its LC-UV peak is several times smaller than the neighboring peak of baicalein (3), while they are comparable in the TIC profile (Fig. 2), indicating its non-aromatic nature. The fragmentation result of the ion m/z 301 also indicates a compound of a different nature, low intensity of the demethylated fragment ion, and high values of m/z 154 (Supplements, Fig. S3). It can be assumed that this is C15 sesquiterpene with OMe and

Table 1. LC-MS characteristics of detected metabolites of *Scutellaria lateriflora* roots

Metabolite	Molecular weight, g/mol	Retention time, min	$[M-H]^-$	(–) ESI-MS/MS, *m/z*
Methylated flavones				
7-Methoxy chrysin (tectochrysin) (1M)	268	16.9	267	252 224 168
Wogonin (4)	284	21	283	268 239 138
Oroxylin A (5)	284	21.5	283	268 239 151 137
6-OH-wogonin (6)*	300	17.8	299	284 240 165 137
C15 sesquiterpene with OMe (ss)*	302	18.3	301	286 155 140
6-OMe wogonin (10)	314	21.6	313	298 281 240 138
Precursors of flavones 4 and 10	316	22.5	315	300 272 241 179
5.7.2'(6')-Tri-ON-6.8.6'(2')-tri-OMe flavone*	360	17.3	359	344 329 267 183
5-ON-6.7.8.6'-tetra-OMe (8)	374	21.5	373	358 343 314 267 195
Methylated flavone glycosides				
Wogonin glycoside (4g)	460	14.5	459	283 268 175
Oroxylin A glycoside (5g)	460	15.1	459	283 268 175
Sesquiterpene glucuronide (ssg)	478	12.4	477	367 331 301 286 175 155 140
Wogonin 6-OMe glycoside (10g)	490	15.8	489	313 298 175
Tri-OH-mono-OMe flavone glycoside (9g)	492	16.5	491	315 300 175
Dimethyl flavone glycoside	506	13.7	505	329 314 175
		14.8	505	329 314 290 175
Tri-OH-tri-OMe flavone glucuronide (7g)	536	16.3	535	359 344 329 175
Free flavones and their glycosides				
Chrysin (1)	254	21.2	253	225 210 194 183 165
Baicalein (3)	270	17.8	269	251 241 223 195 169
Nor-wogonin (2)	270	17.4	269	241 223
Baicalin (3g)	446	12.2	445	269 175
Glycosides of tri-OH flavones (3a, 3b, 3c)	446	10.8	445	399 285 269 175
		14.2	445	283 269
		14.8	445	269 175
C-glucuronide of wogonin (4c)	446	13.5	445	430 326 283 268
Glucuronide of nor-wogonin (2g)	446	13.7	445	430 379 283 269
Carbohydrates and phenylethanoids				
Sucrose	342	1.9	341	179
Parent phenylethanoids	624	4.5	623	461 315
		6.1	623	461 415 315
Monomethylated phenylethanoids	638	8.0	637	593 491 461
		9.9	637	622 491 461 315
Dimethylated phenylethanoids	652	13.0	651	608 505 475 458 329 284 265 193
		13.5	651	529 505 475 457 443 421 193
Glycosylated phenylethanoids	784	12.3	783	651 607 505 475
		12.7	783	651 607 589 505 475

* The substance was identified presumptively. Substances were identified in accordance with the work of Qiao et al., 2016.

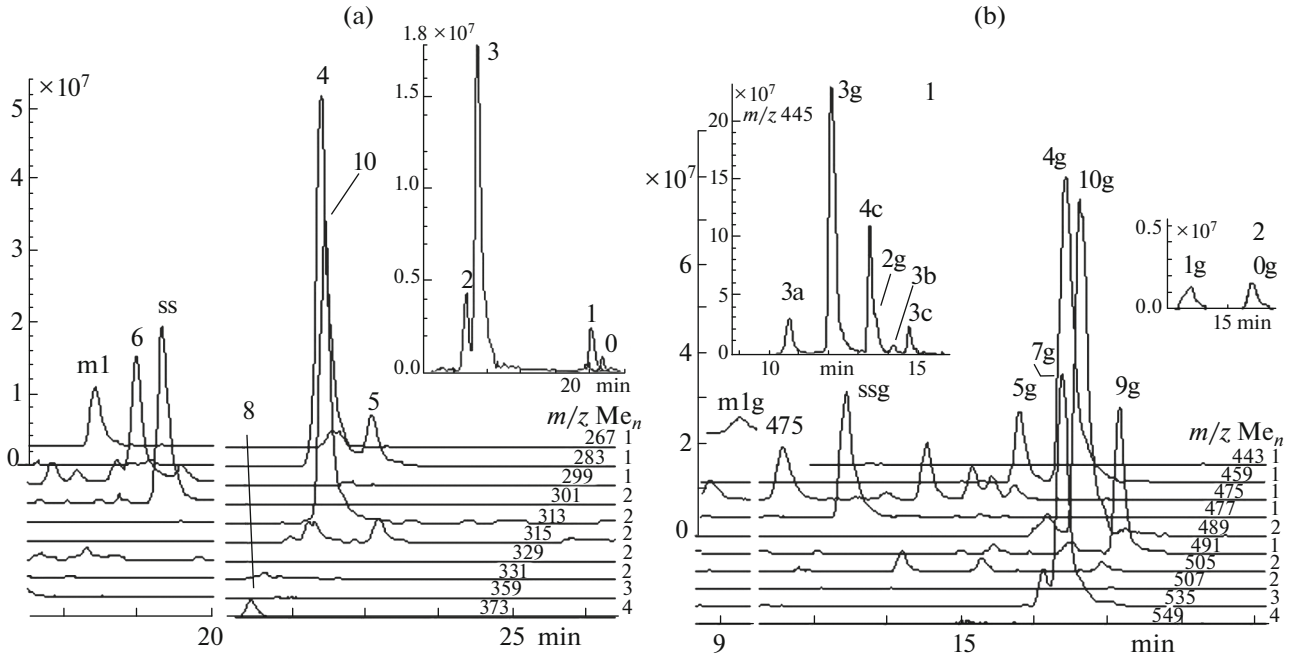

Fig. 3. Ion chromatograms of (a) ten major methylated flavones and (b) their glucuronides in the roots of *S. lateriflora*. (a) 1M, 7-OMe chrysin (tectochrysin); 4, wogonin; 5, oroxylin A; 6, 6-OH wogonin; ss, terpene; 10, 6-OMe wogonin; 8, 5-OH-6,7,8,6'-tetra-OMe flavone. Free flavones: 0, pinocembrin; 1, chrysin; 2, nor-wogonin; 3, baicalein. (b) 7g, tri-OH-tri-OMe flavone; 9g, tri-OH-mono-OMe flavone. Insert 1: glucuronides of free flavones: 2g, nor-wogonin; 3g, baicalein; 3a, 3b, 3c, tri-OH flavones; 4c, C-glucuronide of wogonin. Insert 2: glucuronides: 0g, pinocembrin; 1g, chrysin.

OH groups, since the root contains its glucuronide *m/z* 477 at 12.4 min, and C15 terpenes were also found in the roots of *S. baicalensis* (Wang et al., 2018).

The predominance of 6-OMe wogonin (10) among MFs in the roots of *S. lateriflora* can be considered a species-specific feature. This flavone is also present in the roots of *S. baicalensis*, with a parity content with the 7-OMe isomer of oroxylin A (5), but with a significantly lower content of both compared to wogonin (4) (Qiao et al., 2016; Elkin et al., 2018). An interesting fact is the noticeable proportion of tetra-OMe flavone (8), which emphasizes the need of the plant to synthesize a hydrophobic flavone. For example, in the root bark of *S. baicalensis*, flavone 8 is very abundant and comparable in this to wogonin (4) (Elkin et al., 2022). MFs 4 and 10 essentially determine the ion current of fraction 4, which also contains chrysin (1) and pinocembrin (0), which initiate the flavone biosynthesis pathway (Supplements, Fig. S1). Along with them, the fraction contains trace amounts of compounds the ions of which (*m/z* 315) can be attributed to flavonones, precursors of flavones 4 and 10, allowing for the advanced activity of O-methyltransferases (OMTs). The rate of dehydrogenation by the FNSII enzyme is apparently inferior to that of 6- and 8-OMTs. Tetra-OH-di-OMe flavonone (*m/z* 331) with an additional OH group on ring B is even less abundant. The phenomenon of the need of the plant for 6-OMe wogonin (10) apparently has deep roots in the evolution of plant biochemistry. Flavone 6 (*m/z* 299) is among the major MFs and belongs to the group of second-line hydroxylation products of flavonoxidases, for which there is a choice of four C-atoms of ring B, excluding C-4'. Taking into account the high intensity of the 6-OMe wogonin signal (10), peak 6 of the selective ion chromatogram of the *m/z* 299 ion may be its precursor, 5,6,7-tri-OH-8-OMe flavone. In addition, 6-OMe chrysin known as oroxylin A (5) is also among the most major MFs of the roots of the studied plant, carriers of the 6-OMe group.

Glycosides of Methylated Flavones

Flavone biosynthesis products from chrysin to final methyl esters of flavones are glycosylated (Zhao et al., 2016) and, acquiring hydrophilicity, can then enter the above-ground organs of the plant. Biosynthesis of MF glycosides in roots demonstrates an interesting pattern. Wogonin (4g) and 6-OMe wogonin (10g) glycosides also remain the most abundant, as are the original MFs, ions *m/z* 459 and *m/z* 489 (Fig. 3b). It is noteworthy that glycoside 10g was slightly more abundant than glycoside 4g, while the ratio of the original aglycones 4 and 10 was the opposite. The ratio of the proportions of the glycosides of oroxylin A (5g) and wogonin (4g) increased when compared with the ratio

of their aglycones 5 and 4. Whether the specificity constant of glucuronosyltransferase is higher for highly methylated flavones remains to be determined, but a preference for 6-OMe flavones can be noted. The tendency for glycosylation of more lipophilic MFs resulted in an abundance of the glucuronide of tri-OH-tri-OMe flavone (7g) *m/z* 535 (Fig. 3b), whereas the proportions of either of its two precursors *m/z* 359 are negligible (Fig. 3a). Presumably, this is one of two possible 5,7,2'(6')-tri-OH-6,8,6'(2')-tri-OMe flavones with a free C7 OH group between the two OMe groups for glycosylation. This phenomenon also concerns the methylated flavonone *m/z* 315, the glycoside *m/z* 491 of which was among the five most abundant. Glycoside 8g *m/z* 549 was found in minor amounts compared to aglycone 8 *m/z* 373, in which the C7-OH group is methylated (Fig. 3a). Sesquiterpene glucuronide (ssg) ion *m/z* 477 also remains among the abundant glycosides. Among the glycosides of the second-line hydroxylation MFs, ions *m/z* 475, there were only three with a free C7 OH group (Supplements, Fig. S5). The first two of them are the most abundant and probably originate from 6-OMe flavones with additional OH groups in the B ring at different carbon atoms. The peak of the third, in the mixture with the C-glycoside of di-OH-di-OMe flavonone, appears in a ratio of 4 : 1. Another noticeable ion is the ion with *m/z* 475 (m1g), the glycoside of methylated chrysin, as a cluster with Na at 7.7 min also remains noticeable in the TIC of the flavone biosynthesis products (Supplements, Fig. S5). Evaluation of the total ion current of the fractions by the method of selective ion monitoring and the MS2 fragments revealed numerous MFs and their glycosides with low signal intensity: clusters of Na and formate, penta-, and hexosides, acetyl hexosides, sulfates, and C-glycosides (Supplements, Fig. S1–S4). All together, these fractions are almost represented by methylated flavones.

Free Flavones and Their Glycosides

The content of nor-wogonin (2) (5,7,8-tri-OH-flavone) and baicalein (3) in the roots of *S. lateriflora* is lower than that of their methylated derivatives, and the precursors pinocembrin (0) and chrysin (1) are present only in trace amounts (insert in Fig. 3a). The ratio of flavones 2 and 3, the first-line chrysin hydroxylation products, in the roots of *S. lateriflora* is the same as in the roots of *S. baicalensis* (Elkin et al., 2018), indicating a common origin of the flavonoid biosynthesis pathway in both plant species. Glycosylation of free flavones 2 and 3 is also preferential, like their methylation; however, it occurs less selectively, as evidenced by the presence of five peaks in the ion profile of *m/z* 445 (insert 1 in Fig. 3b). In addition to baicalein and nor-wogonin, random products of phenoloxidases, tri-OH flavones 3a, 3b, 3c, are apparently immediately glycosylated and then transported to the above-ground plant organs. The most abundant of them, ion 3a, may be the glucuronide of 2'-hydroxychrysin found in the roots of *S. baicalensis* (Wang et al., 2018), since the OH groups in ring B increase the polarity of flavones, causing earlier elution than glucuronides 2g and 3g. The product of 4g is represented by the $[M-CH_4]^-$ ion of the C-glycoside of wogonin, as follows from its MS2 profile (Supplements, Fig. S6).

Carbohydrates and Phenylethanoids

The carbohydrate fraction F1 eluted with aqueous acetonitrile consists of sucrose and derivatives of phenylethanoide (PE), hydroxytyrosol caffeoyl rutinoside (Supplements, Fig. S4). Among them, there are methylated ones and those additionally glycosylated by pentose (Supplements, Fig. S7). Each of the nine PEs has two catechol motifs in the structure of the molecule, which provide equal ionization efficiency with the flavonoids of fractions 2–4. Thus, their proportions in the extracts can be compared by the phenolate anion current. The abundance of PEs, which is comparable to that of flavonoids, is apparently associated with their protective function as catechol carriers (Pshenichnyuk et al., 2015; Elkin et al., 2023). The peak of the alcoholate anion $[M-H]^-$ *m/z* 341 of sucrose has a lower ionization efficiency, but at the same time demonstrates the largest area of the ion current, which indicates its higher content than is reflected in the TIC profile, which indicates that the plant has accumulating a huge reserve of sucrose in the roots.

Hairy Roots

Soil as one of the plant habitats determines its morphology and physiology, including through the development of hairy roots. The latter, being an endosymbiotic consortium of root cells and the soil microbiome, require carbohydrate nutrition. Indeed, the TIC of the hairy roots extract is due almost entirely to the sucrose ion *m/z* 341 (Fig. 4). The IC profile of PE derivatives (F1) differs significantly from the profile of the roots (Supplements, Fig. S7), in which the abundance of ions *m/z* 623, 637, 783, and 651 is comparable with the glycosides of MFs. However, methylated derivatives dominate among the PEs. The production of methylated (F4) and free flavones (F3) by hairy roots is three orders of magnitude less, with the exception of baicalein (3), and the relative amount of flavone glycosides is an order of magnitude higher. It is noteworthy that baicalin (3g) is among the major glycosides of MFs. In contrast to the roots, the glycosides

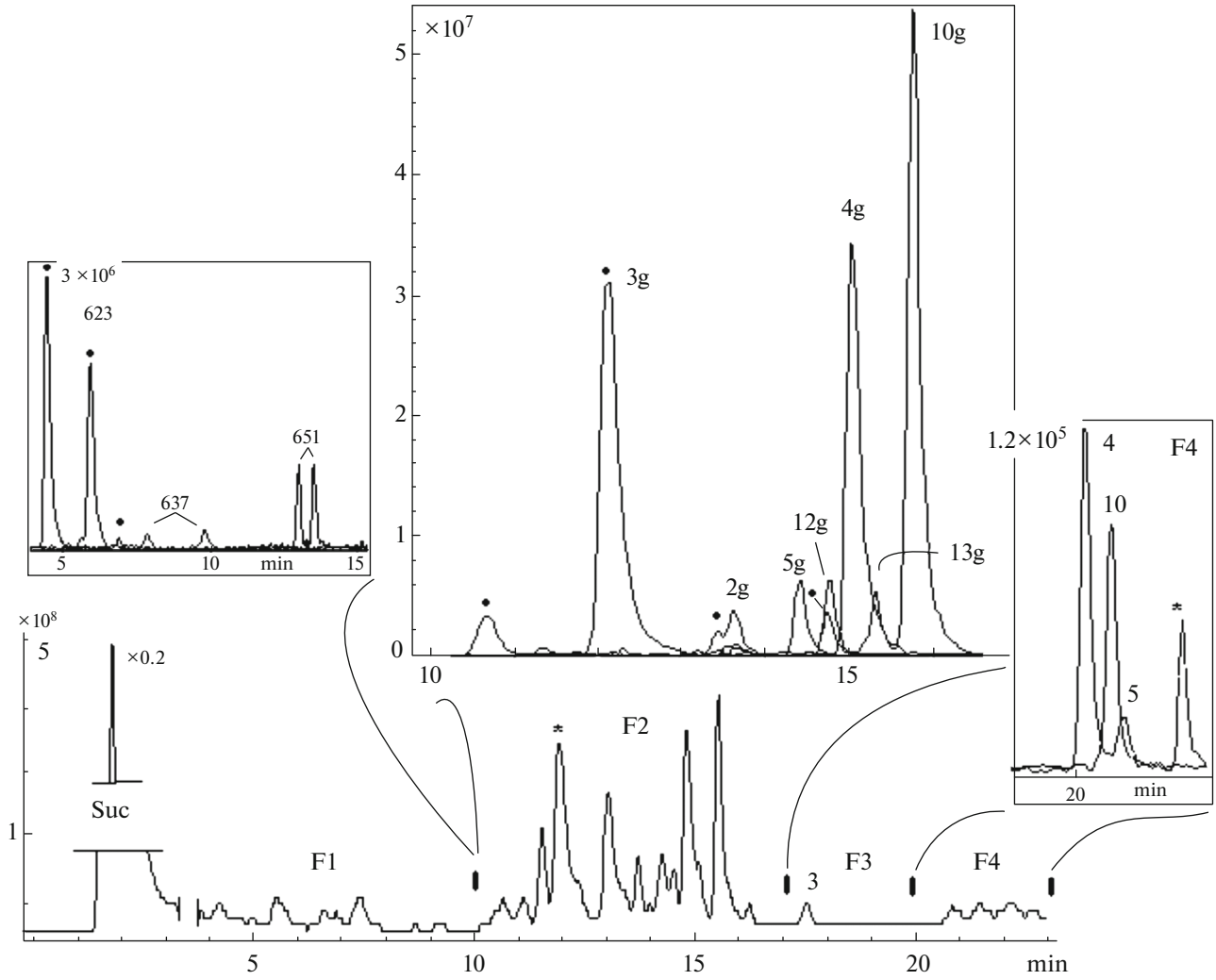

Fig. 4. Total ion current profile of *S. lateriflora* hairy roots extract and ion chromatograms by fractions; Suc, sucrose. Insert F1: •, *m/z* 623 derivatives of rutinose, mono-, and di-methylated *m/z* 637 and *m/z* 651, respectively. Insert F2: glycosides (g) of flavones and methylated flavones. •, ions *m/z* 445. 3g, baicalein; 2g, nor-wogonin; 5g, oroxylin A; 12g, 3OH-2OMe flavone; 4g, wogonin; 13g, 5,8-di-OH-6,7-di-OMe flavone; 10g, 6-OMe wogonin. F3: 3, baicalein. Insert F4: 4, wogonin; 10, 6-OMe wogonin; 5, oroxylin A. * Compounds are not identified.

of hairy roots do not contain glycosides of dimethylflavonone *m/z* 491 and trimethyl flavone *m/z* 535 (Fig. 3b). However, the hairy roots produce a noticeable yield of dimethyl flavone glycoside *m/z* 505. In general, hairy roots also produce hydrophobic flavones and ensure their mobility by glycosylation.

Oxidation of flavonoids in plants is mainly catalyzed by polyphenol oxidases and peroxidases, which occurs during plant development as well as due to biotic and abiotic stress. The detrimental effects of stress on plants are manifested as the action of "leakage electrons" (Li et al., 2021), reactive oxygen species (ROSs), primarily the superoxide anion (O_2^-). MFs with catechol and pyrogallol motifs under the protection of methyl groups interact with O_2^- by the *o*-quinone anion (reductant) with the cleavage of a CH_4 molecule (Pshenichnyuk et al., 2015; Elkin et al., 2023). This process clearly demonstrates the stability of the corresponding reductants R_4^- and R_{10}^- upon activation of flavones 4 and 10 in the ion source (Fig. 5). The described anion radicals suppress ROSs in plant cells, which is what explains the significant content of MFs in the roots of *S. lateriflora*. It is noteworthy that the abundance of MF glycosides in the above-ground organs of the plant has been previously described (Li et al., 2012). Thus, it can be assumed that the root system of *S. lateriflora* forms a spectrum of MFs that provides protection for the entire plant during the growing season.

Thus, the synthesis of polyhydroxy flavonoids by epidermal cells of the cambium of plant roots is carried

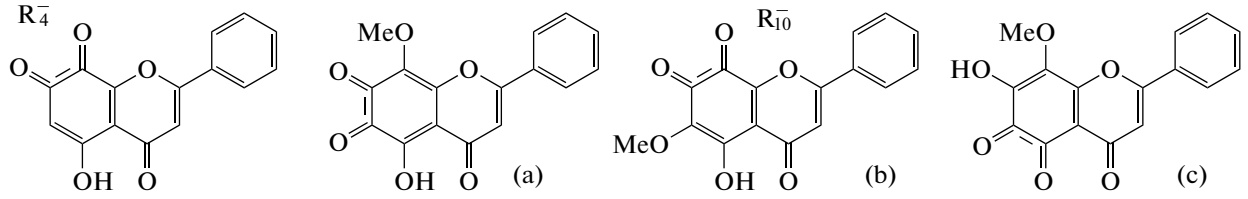

Fig. 5. Schemes of reductants R_4^- *m/z* 268 and R_{10}^- *m/z* 298 upon activation by collision of ions $[M-H]^-$ of flavones 4 and 10 in the ion source. (a, b, c) Putative structures of 6-OMe wogonin.

out for the purpose of protection from ultraviolet radiation, herbivores, and bioinvasions, representing an evolutionarily emerged biochemical mechanism of adaptation. It is the root bark of *S. baicalensis* that was found to contain flavones carriers of the catechol (wogonin) and pyrogallol (oroxylin A) motif (Elkin et al., 2022), which are a means of chemical protection (Xia et al., 2016; Elkin et al., 2023). However, it is largely they in methylated form that determine the therapeutic effect of plants, like spice flavones (curcumin, girgyrin, capsin) (Bolton et al., 2018).

CONCLUSIONS

Detection of the dominant synthesis of methylated flavones and the high content of sucrose and glycosides of hydroxytyrosol in the roots of *S. lateriflora* has a physiological basis for the viability of the plant. Being located on the A ring after glycosylation, methoxyl groups of the flavone molecules of the plant roots impart amphiphilicity to them, providing hydrophobic protection of the root and the possibility of delivery to the above-ground organs of the plant. The need for sucrose accumulation by the root system is obvious, while the role of modified phenylethanolides remains to be clarified. Presumably, two catechol sites of the molecule serve as specific protection against bioinvasion. The increased content of MFs and their glycosides in relation to free flavones (2) and (3) is due to the danger to the plant of catechol and pyrogallol sites in their molecules, respectively, which is due to their high chemical activity. For this reason, flavones of the plant root system accumulate in a protected methylated form. This view of methylation is also confirmed in the case of hairy roots, in the PEs of which more than half of the catechol groups are methylated. Using this approach, the plant protects its own cells from the threat of autophagy caused by di- and tri-OH groups of free flavones, especially those of nor-wogonin.

SUPPLEMENTARY INFORMATION

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Fig. S1. Section of fraction 4 of the total ion current profile with MS2 spectra of $[M-H]^-$ ions detected in the profile.

Fig. S2. Section of fraction 3 of the total ion current profile with MS2 spectra of $[M-H]^-$ ions detected in the profile.

Fig. S3. Section of fraction 2 of the total ion current profile with MS2 spectra of $[M-H]^-$ ions detected in the profile.

Fig. S4. Section of fraction 1 of the total ion current profile with MS2 spectra of $[M-H]^-$ ions detected in the profile with superposition of the ion chromatogram of this phenylethanoid ion.

Fig. S5. Selective ion chromatograms of eight $[M-H]^-$ *m/z* 475, the MS2 spectra of which demonstrate multiple ion overlaps and ambiguity of nominal ion masses. Among the eight $[M-H]^-$ *m/z* 475 ions, only three are derivatives of second-line glycosylation flavones (*m/z* 299).

Fig. S6. Superimposed selective ion chromatograms of $[M-H]^-$: (a) glycosides of flavones and glycosides of dimethylated phenylethanoids *m/z* 783, (b) flavones and monomethylated flavones of the second-line hydroxylation *m/z* 299.

Fig. S7. Selective ion chromatograms $[M-H]^-$ of three types of phenylethanoids (a) roots, (b) hairy roots: original (*m/z* 623), monomethylated (*m/z* 637), dimethylated (*m/z* 651), and with subsequent glycosylation (*m/z* 783). Abstract: flavones. 2, oroxylin A; 3, baicalein. 2g and 3g, their glucuronides; 4c, C-glycoside of wogonin; 6, 6-OH wogonin. 3a, 3b, 3c, tri-OH-flavones.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human or animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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