Polyphenolic components of knotwood extracts from *Populus tremula* (quaking aspen)*

D. E. Tsvetkov, A. S. Dmitrenok, Yu. E. Tsvetkov, A. O. Chizhov, and N. E. Nifantiev★

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (499) 135 6433. E-mail: nen@ioc.ac.ru

An extract from the knotwood of *Populus tremula* (quaking aspen) was studied by HPLC, NMR spectroscopy, and mass spectrometry in order to identify new sources of natural compounds as promising basis for the development of medicinal agents. The following compounds were detected and characterized in the extract: 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl *E*-3-(4-hydroxyphenyl)acrylate, 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl *Z*-3-(4-hydroxyphenyl)acrylate, *trans*-3-(4-hydroxyphenyl)acrylic acid, 7-*O*-(β-D-glucopyranosyl)naringenin, dihydrokaempferol, and naringenin.

Key words: polyphenolic compounds, flavanoids, knotwood, *Populus tremula*.

In recent years, it has been shown that knotwoods, which can be scooped from wood processing waste, contain a variety of biologically active compounds,[1,2] including those related to flavonoids,[3] lignans,[3,4] stilbenes,[5] and other classes of natural substances exhibiting antioxidant[4,6—9] and bactericidal properties, as well as acting on cells of the hormonal (antiestrogen),[10] immune, and nervous systems.[11] The presence of these properties opens up the possibility of developing appropriate drugs and biologically active additives, which stimulates research on the profile of biological activities.

The use of waste from wood processing enterprises as a raw material for obtaining the above valuable phenolic compounds will increase the degree of wood processing, which will lead to the development of wood biochemical production, reduce the burden on the environment, and improve the economic and environmental performance of technologies. The possibility of using waste makes it expedient to systematically study the components of extracts of different types of knotwood, including the knotwood components of *Populus tremula* (quaking aspen, a deciduous tree from the poplar genus, the willow family first described by Carl Linnaeus in the middle of the 18th century), one of the most widespread and fast-growing tree species of the European part of Russia, central and southern Siberia.[12,13] Aspen forests are characterized by fast postpyrogenic, postagrarian, and windfall successions; already after 50 years it is possible to obtain up to 400 m^3 of wood per hectare.[14] Aspen wood is an important raw material for pulp and paper industry and production of industrial lumber; bark and foliage extracts are used in medicine and veterinary medicine.[15,16]

Note that the composition of low molecular weight components of extracts of leaves, buds, bark, heartwood and sapwood has already been analyzed in a number of works.[17—26] The bark contains many biologically active compounds: aromatic and fatty acids, tannins, carbohydrates (raffinose, fructose), and phenolic glycosides. Aspen buds contain essential oils, resins, flavones, and mineral salts. Aspen leaves are rich in vitamin C, carotene, carotenoids, and various enzymes.[16] In addition, both extracts and isolated individual components have shown high antioxidant,[17] antimicrobial,[21] and anti-inflammatory[24] activities.

Results and Discussion

To study the compounds contained in the *Populus tremula* knotwood of natural moisture, we obtained an extract according to the previously described protocol.[5] Using preparative HPLC, the extract was separated into fractions (Fig. 1), which showed high UV absorption. The fractions were labeled according to the collection time intervals (min) during elution from the column: 10—11, 26—27, 28—30, 60—61, 62—63, 67—68, and 72—73.

* Dedicated to Academician of the Russian Academy of Sciences V. A. Tartakovsky on the occasion of his 90th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1777—1783, August, 2022.

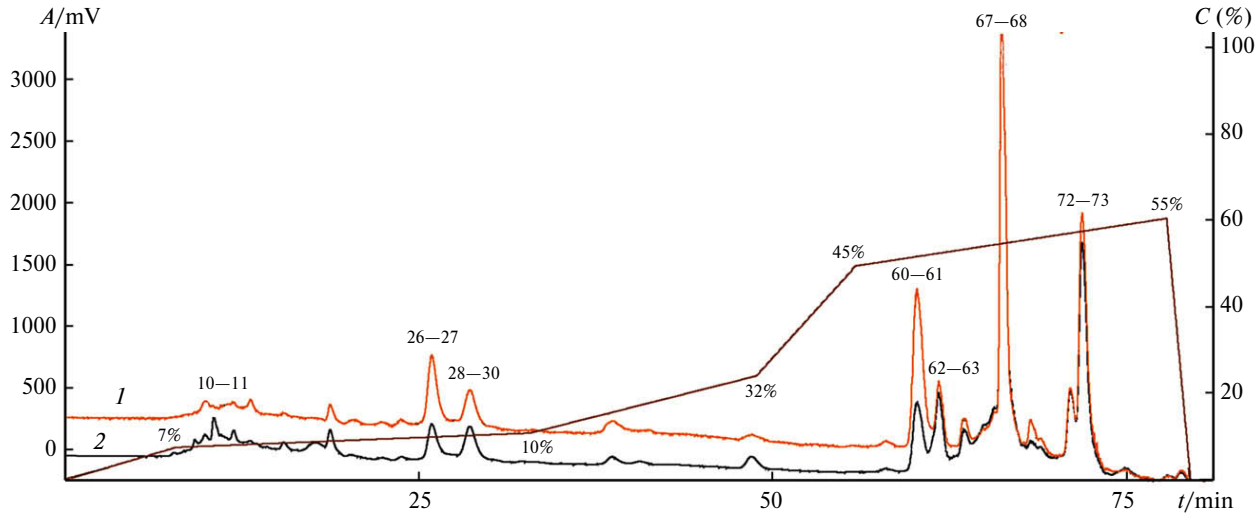


Fig. 1. Chromatogram of the separation of the crude extract on a preparative column filled with C-18 modified silica gel. Extract components were detected photometrically at 280 (*1*) and 225 nm (*2*); *A* is the photometer signal; the composition of the eluent is shown by a polygonal line indicating a linear change in the content of the organic modifier between the inflection points; the ranges above the peaks show the eluent collection intervals.

The purity of the fractions resulting from the preparative separation of the extract was evaluated using analytical HPLC (Fig. 2).

The results of the analyzes showed that the fractions 28—30, 60—61, 62—63, 67—68, and 72—73 contained the main components with 82—95% purity. The frac-

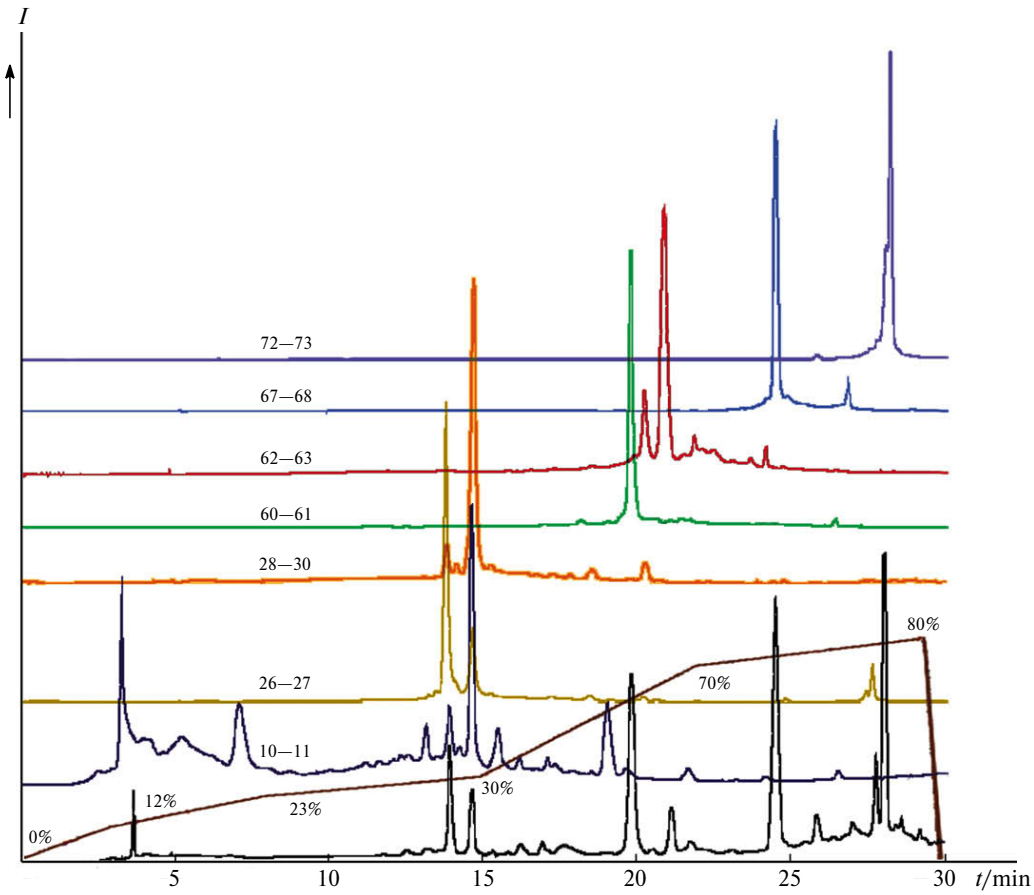


Fig. 2. Comparison of the chromatogram of the crude extract (*1*) with the chromatograms of the isolated fractions 10—11, 26—27, 28—30, 60—61, 62—63, 67—68, and 72—73 (HPLC on a Supelco HS F5 column; UV detection at 280 nm); the composition of the eluent is shown by a broken line indicating a linear change in the content of the organic modifier between the inflection points.

tions 10—11 and 26—27 consisting of a mixture of compounds were rechromatographed on a Supelcosil HS F5 column. As a result, the fraction 26—27 yielded 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl *E*-3-(4-hydroxyphenyl)acrylate (**1**, ~91% purity) with the retention time of 13—14 min (see Fig. 2), while the fraction 10—11 gave 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl *Z*-3-(4-hydroxyphenyl)acrylate (**2**, ~93% purity) with the retention time of 14—15 min, as well as a mixture of compounds with the retention time of 7—8 min. Based on the NMR data, it can be assumed that this mixture consists of oligosaccharides containing attached hydroxyphenyl (HOC_6H_4—) groups. This fraction was analyzed by GPC. It was shown (Fig. 3) that it contains compounds with a mass of 0.2—3.0 kDa with a predominance of compounds with a mass of about 1 kDa. The chromatogram of the crude extract shows a peak with a retention time of 23.5 min, which corresponds to compounds with a mass of about 0.5 kDa. For comparison, Fig. 3 shows the chromatogram of fraction 26—27, the main component of which is compound **1** with a mass of 472 Da. To determine the monosaccharide composition of fractions 10—11, we carried out exhaustive acid hydrolysis with subsequent reduction and acetylation of the hydrolysate;[27] the resulting mixture of polyol acetates was analyzed by GLC. A comparison with the authentic samples showed that fraction 10—11 contains rhamnose, xylose, and glucose in a ratio of 1 : 2 : 5. A significant extinction of the fraction components at 280 nm is explained by the presence of 4-hydroxyphenyl fragments, which is confirmed by NMR data. The ratio of rhamnose (Rha) and 4-HOC_6H_4 groups, which was determined from the integral intensities of the peaks at δ 7.26 and 6.85 related to the 4-HOC_6H_4 fragment and the peak at δ 1.19 corresponding to the Me group of the Rha residue, was found to be 1 : 2.

The structures of the isolated compounds were established by NMR spectroscopy and high-resolution mass spectrometry. The signals in the NMR spectra were assigned using 2D homo- and heteronuclear resonance techniques (HMBC, NOESY, COSY, and HSQC). As a result, it was shown that the main components of fractions 10—11, 26—27, 28—30, 60—61, 62—63, 67—68, and 72—73 are esters **1** and **2**, *trans*-3-(4-hydroxyphenyl)acrylic acid (**3**), 7-*O*-(β-D-glucopyranosyl)naringenin (**4**), dihydrokaempferol (**5**), and naringenin (**6**), respectively.

The chemical shift values of the signals in the ^1H and ^{13}C NMR spectra, their assignments, and the specific optical rotation values of compounds **1**—**6** agree with the data described in the literature (references are given in the Experimental). Thus, the chemical shifts in the NMR spectra of compound **1** were assigned to 6-(α-L-rhamnosyl)-1-β-D-glucopyranosyl *E*-3-(4-hydroxyphenyl)acrylate and correspond to those described earlier[21] for the equilibrium mixture of *E*/*Z*- isomers. We isolated individual isomers and did not observe the formation of their equilibrium mixture.

A comparison of the content of phenolic compounds in the knotwood extracts of aspen wood grown in different regions is given in Table 1. It was found that the total content of phenolic compounds in aspen extracts from the Kostroma region is lower than in the case of aspen from Finland. In addition, the Finnish aspen extracts contained 7-*O*-β-glucoside of dihydrokaempferol (4.8% of the extract) and taxifolin (0.6%), which were absent in the samples studied by us. The content of compounds **1** and **2** in the extracts[21,22] was not determined.

Extensive chemical studies of tree knots have shown that the amount of polyphenols in knots of coniferous and hardwoods is usually increased in comparison with

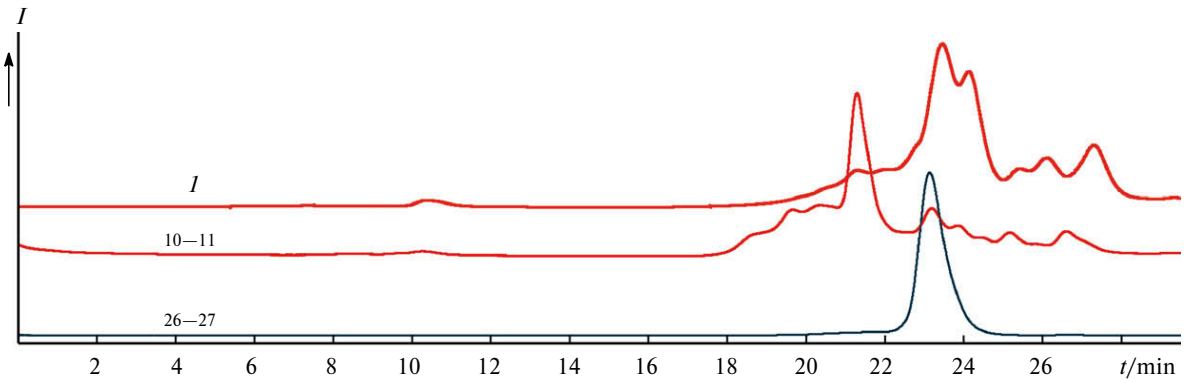
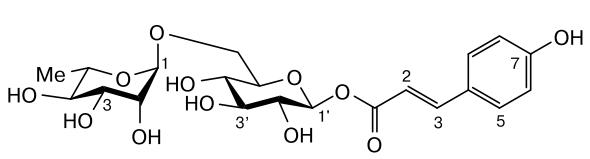
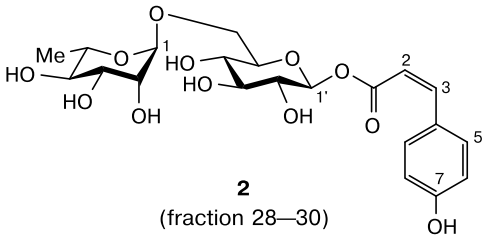


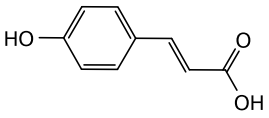
Fig. 3. Comparison of the chromatogram of the initial *Populus tremula* knotwood extract (*1*) with the chromatograms of the isolated fractions 10—11 and 26—27 (a TSK 2000SW column, the feed rate 0.5 mL min^{-1}, 0.03 *M* phosphate buffer (pH 7.2) with the addition of 15% of MeCN; UV detection at 280 nm).



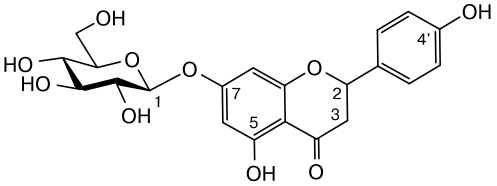
1
(fraction 26—27)



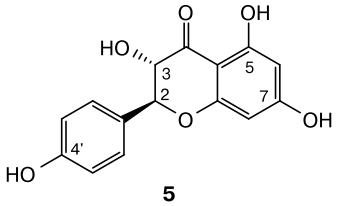
2
(fraction 28—30)



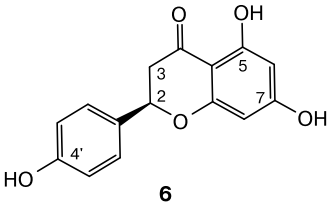
3
(fraction 60—61)



4
(fraction 62—63)



5
(fraction 67—68)



6
(fraction 72—73)

other tissues, in addition, the similarity of the structures of components found in knots and in other parts of the trees was noted.[2] Compounds **1**—**6** we identified were found[19—26] in other parts of *Populus tremula L.*, which confirms the assumption[2] about the structural similarity of compounds from knotwood extracts and compounds found in other parts of the tree. In a number of described cases,[2] only quantitative differences in the compositions of extracts were observed and in these cases, one can speak of general structural fragments.

In conclusion, this work shows the possibility of using *Populus tremula L.* wood as a raw material for obtaining the above phenolic compounds (specific yields are given in the Experimental) which exhibit antioxidant,[17,22] antimicrobial,[16] antiulcer,[16,24] and antitumor[18] activities. This allows one to increase the degree of processing of wood raw materials and reduce the burden on the environment. In addition, phenolic compounds from wood can be used for chemical modification through simple reactions, such as sulfation,[28,29] and for obtaining more active anticoagulants.[29] This additionally substantiates a necessity for a systematic analysis of the composition of wood extracts from different species, as well as the search for raw materials for their preparative isolation.

Table 1. Comparison of the content* of phenolic compounds in the knotwood extracts of aspen from the south of Finland and the Kostroma region

Compound	Content (%)	
	in the extract under study	in the extract from lit. data[22]
1	1.0	n.d.
2	1.1+0.9	n.d.
3	2.2	1.7
4	2.0	6.6
5	5.9	18.5
6	3.6	13.2

* n.d. stands for not determined.

Experimental

HPLC grade solvents (Sigma) were used for extraction and chromatographic separation. NMR spectra were recorded on Bruker AMX-III 400 (400 and 100 MHz) and Bruker Avance 600 (600 and 151 MHz) spectrometers using DMSO-d_6 as a solvent and tetramethylsilane (Me_4Si) as an internal standard. Signals were assigned using 2D $^1H—^1H$, NOESY, COSY and $^1H—^{13}C$ HSQC, HMBC NMR spectroscopy. Electrospray ionization (ESI) high-resolution mass spectra were recorded on a Bruker micrOTOF II instrument in positive (capillary voltage 4500 V) or negative ion mode (capillary voltage 3200 V). Analytical HPLC was carried out on Supelcosil HS F5 and Supelcosil LC-18 columns (4×250 mm,

5 μm, Supelco, USA), eluting with a gradient from THF (5%) in aqueous HCOOH (0.1%) to THF (20%) in acetonitrile, the eluent composition is shown in Figs 1 and 2, the flow rate was 1 mL min^{-1}. Preparative HPLC was carried out on Silasorb C-18 (22×250 mm, 5 μm, flow rate 9 mL min^{-1}) and Supelcosil HS F5 columns (10×250 mm, 5 μm, flow rate 4 mL min^{-1}). The eluate was monitored at two wavelengths (215 and 280 nm) using a 155 UV spectrophotometer (Gilson, France) as described earlier.[3—9,30,31] The purity was determined by chromatogram integration at 280 nm. Optical rotation was measured on a JASCO P-2000 digital polarimeter (Japan) at 24 °C. All values of specific rotation were measured for compounds with the specified purity, no correction was made.

Plant material, extraction. Wood samples collected at the industrial cutting area of the Dyukovsky forestry of the Kostroma region were obtained from live knots at a height of ~15 m of an aspen trunk about 70 years old with a diameter at the butt of 40 cm and a height of ~30 m. Knots with a diameter of 20—40 mm, in which heartwood is practically invisible, were drilled with a 20-mm drill to a depth of ~3 cm. The resulting chips were vacuum dried for 24 h (moisture content of the raw material 34%). A weighed portion (14.3 g) of dry chips was mixed with 70% aqueous propan-2-ol (200 mL) at ~25 °C and kept for ~14 h. The suspension was filtered, washed on the filter with 70% aqueous Pr^iOH (3×150 mL), the solvent was removed *in vacuo*, the residue was dried for 20 h (25 °C, 0.25 Torr) to obtain the extract (2.4 g, 17%).

Isolation of components. Optimized chromatography conditions were used for preparative separation on a Silasorb C-18 column (see Fig. 1 caption). The extract (0.74 g) was dissolved on heating in 65% aqueous MeCN (3 mL, 247 mg mL^{-1}) and cooled, the precipitate formed was filtered off (56 mg), the filtrate was applied in portions (1000 μL, ~250 mg) onto a Silasorb C18 column (20×250 mm) equilibrated by pumping (9 mL min^{-1}) with HCOOH(0.1%)—THF(5%), eluting with MeCN+20% THF gradient from 0 to 85% over 80 min at the eluent flow rate of 9 mL min^{-1}. The extract components were detected photometrically at 225 and 280 nm (see Fig. 1); the volume of collected fractions was 9 mL.

After removal of the solvent *in vacuo* and lyophilization of the residue, the following fractions were obtained: 10—11, the yield was 16.6 mg (2.2% of the applied extract; contains a mixture of compounds); 26—27, the yield was 12.2 mg (1.7% of the applied extract; contains a mixture of compounds with a predominance of compound **1** (67%); 28—30, the yield was 8.4 mg (1.1% of the applied extract; contains compound **2**, ~82% purity); 60—61, the yield was 16.5 mg (2.2% of the applied extract; contains compound **3**, ~92% purity); 62—63, the yield was 14.6 mg (2.0% of the applied extract; contains compound **4**, ~92% purity); 67—68, the yield was 43.6 mg (5.9% of the applied extract; contains compound **5**, ~96% purity); 72—73, the yield was 26.3 mg (3.6% of the applied extract; contains compound **6**, ~98% purity). The total yield was 36.7%.

The fraction 10—11 (16.6 mg) was dissolved in water and rechromatographed on a Supelcosil HS F5 column in the gradient presented in the caption to Fig. 3. The eluate was collected with a retention time of 7—8 min (see Fig. 2), concentration and lyophilization of which gave a mixture of carbohydrates Rha—Xyl—Glc—4-HOC_6H_4 (1 : 2 : 5 : 2, 6.1 mg, 0.9% of the original extract; Xyl is xylose, Glc is glucose). We also collected an eluate with the retention time of 14—15 min, concentration and lyophilization of which gave compound **2** with ~93% purity (6.5 mg, 0.9% of the crude extract).

The fraction 26—27 (12.2 mg) was dissolved in water and rechromatographed on a Supelcosil HS F5 column to collect an eluate with the retention time of 13—14 min, concentration and lyophilization of which gave compound **1** with ~91% purity (7.4 mg, 1.0% of the crude extract).

6-*O*-(α-L-Rhamnopyranosyl)-β-D-glucopyranosyl *E*-3-(4-hydroxyphenyl)acrylate (1); $[\alpha]_D$ 83 (*c* 0.01, EtOH) (*cf.* Ref. 21: $[\alpha]_D$ 76.9). ^{1}H NMR, δ: 7.64 (d, 1 H, H(3), $J_{2,trans\text{-}3}$ = 15.9 Hz); 7.57 (d, 2 H, H(5) + H(9), *J* = 8.8 Hz); 6.81 (d, 2 H, H(6) + H(8), *J* = 8.6 Hz); 6.39 (d, 1 H, H(2), $J_{3,trans\text{-}2}$ = 15.9 Hz); 5.45 (d, 1 H, H(1′), *J* = 8.1 Hz); 4.65 (d, 1 H, H(1″), *J* = 11.5 Hz); 3.80 (dd, 1 H, H(6′), J_1 = 11.3 Hz, J_2 = 1.4 Hz); 3.59 (dd, 1 H, H(2″), J_1 = 3.5 Hz, J_2 = 1.7 Hz); 3.47—3.37 (m, H(6′) + H(3″) + H(5″)); 3.30—3.24 (m, H(3′)); 3.22—3.12 (m, 2 H, H(2′) + H(4″)); 3.10 (m, 1 H, H(5′)); 1.11 (d, 3 H, H(6″), $J_{6'',5''}$ = 6.2 Hz). ^{13}C NMR (151 MHz), δ: 165.3 (C(1)), 160.1 (C(7)), 146.0 (C(3)), 130.5 (C(5) + C(9)), 125.0 (C(4)), 115.9 (C(6) + (C(8)), 113.6 (C(2)), 100.7 (C(1″)), 94.2 (C(1′)), 76.4 (C(5′) + C(3′)), 72.5 (C(4″)), 72.0 (C(2′)), 70.7 (C(3″)), 70.4 (C(2″)), 69.7 (C(4′)), 68.4 (C(5″)), 66.5 (C(6′)), 17.9 (C(6″)). MS, found: *m/z* 495.1448 $[M + Na]^+$; calculated for $C_{21}H_{28}O_{12}$ 495.1473; found: *m/z* 511.1166 $[M + K]^+$; calculated for $C_{21}H_{28}O_{12}$ 511.1212.

6-*O*-(α-L-Rhamnopyranosyl)-β-D-glucopyranosyl *Z*-3-(4-hydroxyphenyl)acrylate (2); $[\alpha]_D$ 83 (*c* 0.01, EtOH). ^{1}H NMR, δ: 7.32 (d, 2 H, H(5) + H(9), *J* = 8.0 Hz); 6.93 (d, 1 H, H(3), $J_{2,trans\text{-}3}$ = 12.2 Hz); 6.79 (d, 2 H, H(6) + H(8), *J* = 8.0 Hz); 6.14 (d, 1 H, H(2), $J_{3,cis\text{-}2}$ = 12.2 Hz); 5.11 (d, 1 H, H(1′), *J* = 11.6 Hz); 5.03 (d, 1 H, H(1″), *J* = 3.5 Hz); 3.70—3.57 (m, H(6′)); 3.50—3.32 (m, H(6′)); 3.47—3.34 (m, H(6′) + + H(3″) + H(5″)); 3.31—3.19 (m, H(3′) + H(2″)); 3.28—3.16 (m, 2 H, H(2′) + H(4″)); 3.20—3.10 (m, 1 H, H(5′)); 1.10 (d, 3 H, H(6″), $J_{6'',5''}$ = 6.3 Hz). ^{13}C NMR (151 MHz), δ: 165.1 (C(1)), 162.5 (C(7)), 146.1 (C(3)), 129.5 (C(5) + C(9)), 127.3 (C(4)), 114.9 (C(6) + C(8)), 114.7 (C(2)), 99.7 (C(1″)), 94.3 (C(1′)), 76.5 + 76.4 (C(5′) + C(3′)), 73.0 (C(4″)), 71.7 (C(2′)), 69.6 (C(3″)), 69.5 (C(2″) + C(4′)), 68.3 (C(5″)), 66.1 (C(6′)), 17.9 (C(6″)). MS, found: *m/z* 495.1475 $[M + Na]^+$; calculated for $C_{21}H_{28}O_{12}$ 495.1473.

***trans*-3-(4-Hydroxyphenyl)acrylic acid (3).** ^{1}H NMR (400 MHz), δ: 7.54—7.44 (m, 3 H); 6.80 (d, 2 H, *J* = 7.8 Hz); 6.28 (dd, 1 H, J_1 = 16.1 Hz, J_2 = 1.1 Hz). ^{13}C NMR (151 MHz), δ: 168.0 (C(1)), 159.7 (C(4′)), 144.3 (C(3)), 130.2 (C(2′) + C(6′)), 125.36 (C(1′)), 115.9 (C(3′) + C(5′)), 115.41 (C(2)). MS, found: *m/z* 163.0861 $[M - H]^+$; calculated for $C_9H_7O_3$ 163.0395; found: *m/z* $[M + Na]^+$ 187.0363; calculated for $C_9H_8O_3$ 187.0371; found: *m/z* 203.0014 $[M + K]^+$; calculated for $C_9H_8O_3$ 203.0111.

7-*O*-(β-D-Glucopyranosyl)naringenin (4); $[\alpha]_D$ −28 (*c* 0.1, MeOH) (*cf.* Ref. 22: $[\alpha]_D$ −31). ^{1}H NMR (400 MHz), δ: 12.04 (br.s, 1 H, C(5)OH); 9.63 (br.s, 1 H, C(4′)OH); 7.32 (d, 2 H, H(2′) + H(6′), $J_{2',3'}$ = 8.3 Hz); 6.80 (d, 2 H, H(3′) + + H(5′), $J_{3',2'}$ = 8.3 Hz); 6.15 (d, 1 H, H(6), $J_{6,8}$ = 1.8 Hz); 6.13 (d, 1 H, H(8), $J_{8,6}$ = 1.9 Hz); 5.50 (dd, 1 H, H(2), $J_{2,3}$ = 12.7 Hz, $J_{2,1}$ = 3.1 Hz); 5.34 (br.s, 1 H, C(2″)OH); 5.10—5.01 (m, C(6″)OH); 4.95 (d, 1 H, C(1″)H, $J_{1'',2''}$ = 7.3 Hz); 3.67 + 3.43 (m, C(6″)H_2); 3.37 + 3.26 + 3.14 (C(5″)H, C(4″)H, C(3″)H); 3.34 (C(3)H_2); 3.26 (d, 1 H, C(2″)H, *J* = 9.1 Hz); 2.74 (dd, 1 H, C(3)H_2, $J_{3,3}$ = 17.2 Hz, $J_{3,2}$ = 3.1 Hz). ^{13}C NMR (151 MHz), δ: 197.3 (C(4)), 165.4 (C(7)), 163.0 (C(5)), 162.8 (C(9)), 157.9 (C(4′)), 128.8 (C(1′)), 128.5 (C(6′) + C(2′)), 115.3 (C(5′) + C(3′)), 103.5 (C(10)), 99.7 (C(1″)), 96.6 (C(6)), 95.5 (C(8)), 78.7 (C(2)), 77.13 + 76.35 + 69.6 (C(5″), C(4″), C(3″)), 73.1 (C(2″)), 60.6 (C(6″)), 42.1 (C(3)). MS, found: *m/z* 435.1276 $[M + H]^+$; calculated for $C_{21}H_{22}O_{10}$ 435.1286; found: *m/z* 457.1099 $[M + Na]^+$; calculated for $C_{21}H_{22}O_{10}$ 457.1105.

Dihydrokaempferol (5); $[\alpha]_D$ 31 (*c* 0.01, MeOH) (*cf.* Ref. 22: $[\alpha]_D$ 28.4). ^{1}H NMR (400 MHz), δ: 11.88 (br.s, 1 H, C(5)OH); 9.66 (br.s, 1 H, C(4′)OH); 7.31 (d, 2 H, H(6′) + + H(2′), $J_{2',3'}$ = 8.4 Hz); 6.79 (d, 2 H, H(3′) + H(5′), $J_{5',6'}$ = 8.4 Hz); 5.93 (d, 1 H, H(6), $J_{6,8}$ = 2.3 Hz); 5.87 (d, 1 H, H(8), $J_{8,6}$ = 2.3 Hz); 5.04 (d, 1 H, H(2), $J_{2,3}$ = 11.2 Hz); 4.57 (d, 1 H, H(3), $J_{3,2}$ = 11.2 Hz). ^{13}C NMR (151 MHz), δ: 198.0 (C(4)), 166.8 (C(9)), 163.2 (C(5)), 162.8 (C(7)), 157.8 (C(4′)), 129.7 (C(6′) + C(2′)), 127.8 (C(1′)), 115.1 (C(5′) + C(3′)), 100.7 (C(10)), 96.1 (C(6)), 95.2 (C(8)), 83.1 (C(2)), 71.6 (C(3)). MS, found: *m/z* 289.0713 $[M + H]^+$; calculated for $C_{15}H_{12}O_6$ 289.0713; found: *m/z* 311.0526 $[M + Na]^+$; calculated for $C_{15}H_{12}O_6$ 311.0526.

Naringenin (6); $[\alpha]_D$ 12 (*c* 0.01, MeOH) (*cf.* Ref. 22: $[\alpha]_D$ 16.6). ^{1}H NMR (400 MHz), δ: 12.13 (br.s, 1 H, C(5)OH); 10.83 (br.s, 1 H, C(7)OH); 9.62 (br.s, 1 H, C(4′)OH); 7.32 (d, 2 H, H(6′) + H(2′), $J_{2',3'}$ = 8.6 Hz); 6.79 (d, 2 H, H(3′) + H(5′), $J_{5',6'}$ = 8.6 Hz); 5.89 (s, 2 H, H(8) + H(6)); 5.43 (dd, 1 H, H(2), $J_{2,trans\text{-}3}$ = 12.8 Hz, $J_{2,cis\text{-}3}$ = 3.0 Hz); 3.26 (dd, 1 H, H(3), $J_{trans\text{-}3,cis\text{-}3}$ = 17.1 Hz, $J_{trans\text{-}3,2}$ = 12.8 Hz), 2.68 (dd, 1 H, H(3) , $J_{trans\text{-}3,cis\text{-}3}$ = 17.2 Hz, $J_{2,cis\text{-}3}$ = 3.1 Hz). ^{13}C NMR (151 MHz), δ: 196.39 (C(4)), 166.7 (C(9)), 163.5 (C(5)), 163.0 (C(8)), 157.8 (C(4′)), 128.8 (C(1′)), 128.3 (C(6′) + C(2′)), 115.2 (C(5′) + C(3′)), 115.0 (C(1′)), 101.8 (C(10)), 95.8 (C(6)), 95.0 (C(8)), 78.46 (C(2)), 42.01(C(3)). MS, found: *m/z* 273.0757 $[M + H]^+$; calculated for $C_{15}H_{12}O_5$ 273.0763.

No human or animal subjects were used in this research.

The authors declare no competing interests.

References

1. J. D. Ford, L. B. Davin, N. G. Lewis, in *Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology*, Eds G. G. Gross, R. W. Hemingway, T. Yoshida, Kluwer Academic/ Plenum Publishers, New York, 1999, 675 pp.
2. R. Kumar, D. E. Tsvetkov, V. Varshney, N. E. Nifantiev, *Ind. Crops Prod.*, 2020, **145**, 112077; DOI: 10.1016/ j.indcrop.2019.112077.
3. D. V. Yashunsky, V. M. Menshov, D. E. Tsvetkov, Yu. E. Tsvetkov, A. A. Belko, G. G. Vasiyarov, E. V. Titova, A. V. Pimenov, A. A. Onuchin, V. A. Dokichev, Yu. V. Tomilov, N. E. Nifantiev, *Russ. Chem. Bull.*, 2014, **63**, 2571; DOI: 10.1007/s11172-014-0780-7.
4. D. E. Tsvetkov, R. Kumar, R. Devrani, A. S. Dmitrenok, Yu. E. Tsvetkov, A. O. Chizhov, A. Ya. Yashin, Ya. I. Yashin, V. K. Varshney, N. E. Nifantiev, *Russ. Chem. Bull.*, 2019, **68**, 2298; DOI: 10.1007/s11172-019-2703-0.
5. N. E. Nifantiev, D. V. Yashunsky, V. M. Menshov, Yu. E. Tsvetkov, D. E. Tsvetkov, *Sposob vydeleniya sekoizolaritsirezinola i digidrokvertsetina iz drevesiny* [*Method of Isolation of Secoisolariciresinol and Dihydroquercetin from Wood*], Patent RF 2 359 666, published: 27.06.2009, *Byul. Izobret.* [*Invention Bull.*], 2009, 18 (in Russian).
6. D. E. Tsvetkov, A. S. Dmitrenok, Yu. E. Tsvetkov, V. M. Menshov, D. V. Yashunsky, A. Ya. Yashin, Ya. I. Yashin, R. Kumar, V. K. Varshney, N. E. Nifantiev, *J. Biol. Act. Prod. Nat.*, 2016, **6**, 272; DOI: 10.1080/22311866.2016. 1253502.
7. D. E. Tsvetkov, R. Kumar, A. S. Dmitrenok, Yu. E. Tsvetkov, A. O. Chizhov, A. Ya. Yashin, Ya. I. Yashin, V. K. Varshney, N. E. Nifantiev, *Russ. Chem. Bull.*, 2019, **68**, 1756; DOI: 10.1007/s11172-019-2621-1.
8. D. E. Tsvetkov, A. S. Dmitrenok, Yu. E. Tsvetkov, R. Kumar, V. K. Varshney, N. E. Nifantiev, *Russ. J. Bioorg. Chem.*, 2021, **47**, 103. DOI: 10.1134/S1068162021010271.
9. D. E. Tsvetkov, A. S. Dmitrenok, Yu. E. Tsvetkov, A. O. Chizhov, N. E. Nifantiev, *Russ. Chem. Bull.*, 2019, **69**, 2390; DOI: 10.1007/s11172-020-3050-x.
10. O. S. Zhukova, L. V. Fetisova, A. V. Trishin, N. Yu. Anisimova, A. E. Shcherbakov, D. V. Yashunskii, D. E. Tsvetkov, V. M. Men′shov, M. V. Kiselevskii, N. E. Nifantiev, *Bull. Exp. Biol. Med.*, 2010, **149**, 511; DOI: 10.1007/s10517-010-0981-5.
11. G. Loers, D. V. Yashunsky, N. E. Nifantiev, M. Schachner, *J. Nat. Prod.*, 2014, **77**, 1554.
12. S. Ya. Sokolov, O. A. Svyazeva, V. A. Kubli, *Arealy derev′ev i kustarnikov SSSR* [*Areas of Trees and Shrubs of the USSR*], Nauka, Leningrad, 1977, V. **1**, p. 84 (in Russian).
13. I. A. Gubanov, K. V. Kiseleva, V. S. Novikov, V. N. Tikhomirov, *Illyustrirovannyi opredelitel′ rasteniy sredney Rossii* [*Illustrated Guide to Plants of Central Russia*], T-vo nauchnykh izdaniy, Moscow, 2003, V. **2**, p. 11 (in Russian).
14. M. A. Makarova, *Botanicheskii Zh.* [*Botanical J.*], 2020, **105**, 957; DOI: 10.31857/S0006813620100063 (in Russian).
15. Ya. Ya. Smilga, *Osina* [*Aspen*], Nauch.-proizv. ob-nie "Silava", Latv. Research Institute of Forestry Probl., Zinatne, Riga, 1986 (in Russian).
16. V. F. Turetskova, I. Yu. Lobanova, S. S. Rassypnova, N. M. Talykova, *Byul. Sibir. Med.* [*Bull. Siber. Med.*], 2011, **10**, No. 5, 106 (in Russian).
17. P. N. Diouf, T. Stevanovic, *Wood Sci. Technol.*, 2009, **43**, 457; DOI:10.1007/s00226-009-0240-y.

18. J.-J. Lu, J.-L. Bao, X.-P. Chen, M. Huang, Y.-T. Wang, *Evid.-Based Complement. Altern. Med.*, 2012, **2012**, 12; DOI: 10.1155/2012/485042.
19. I. N. Abreu, M. Ahnlund, T. Moritz, B. R. Albrectsen, *J. Chem. Ecol.*, 2011, **37**, 857; DOI: 10.1007/s10886-011-9991-7.
20. S. Pietarinen, J. Hemming, S. Willför, F. Vikström, B. Holmbom, *J. Wood Chem. Technol.*, 2005, **25**, 27; DOI: 10.1081/WCT-200058235.
21. M. Neacsu, V. Micol, L. Pérez-Fons, S. Willför, B. Holmbom, R. Mallavia, *Molecules*, 2007, **12**, 205; DOI: 10.3390/12020205.
22. M. Neacsu, P. C. Eklund, R. E. Sjöholm, S. P. Pietarinen, M. O. Ahotupa, B. R. Holmbom, S. M. Willför, *Holz Roh Werkst*, 2007, **65**, 1; DOI: 10.1007/s00107-006-0121-0.
23. A. St-Pierre, D. Blondeau, A. Lajeunesse, J. Bley, N. Bourdeau, I. Desgagné-Penix, *Molecules*, 2018, **23**, 1739; DOI: 10.3390/molecules23071739.
24. S. G. Krylova, V. F. Turetskova, O. G. Makarova, L. A. Efimova, E. P. Zueva, O. Yu. Rybalkina, *Tikhookean. Med. Zh.* [*Pacific Med. J.*], 2015, No. 2, 18 (in Russian).
25. A. M. Martynov, M. N. Tokareva, E. V. Chuparina, *Sibir. Med. Zh.* [*Siber. Med. J.*], 2011, No. 6, 235 (in Russian).
26. O. V. Kotsupii, Yu. V. Zagurskaya, V. I. Ufimtsev, *Izv. Vuzov. Prikladnaya Khimiya i Biotekhnologiya* [*Univ. Bull. Applied Chemistry and Biotechnology*], 2020, **10**, 470; DOI: 10.21285/2227-2925-2020-10-3-470-478 (in Russian).
27. M. I. Bilan, A. A. Grachev, N. E. Ustuzhanina, A. S. Shashkov, N. E. Nifantiev, A. I. Usov, *Carbohydr. Res.*, 2002, **337**, 719; DOI: 10.1016/S0008-6215(02)00053-8.
28. V. B. Krylov, N. A. Ushakova, N. E. Ustyuzhanina, M. E. Preobrazhenskaya, D. V. Yashunsky, V. M. Menshov, D. E. Tsvetkov, G. V. M. Sharma, P. Radha Krishna, N. E. Nifantiev, *Russ. Chem. Bull.,* 2010, **59**, 232—235; DOI: 10.1007/s11172-010-0067-6.
29. V. B. Krylov, N. E. Ustyuzhanina, A. A. Grachev, N. E. Nifantiev, *Tetrahedron Lett.*, 2008, **49**, 5877—5879; DOI: 10.1016/j.tetlet.2008.07.135.
30. D. E. Tsvetkov, A. S. Dmitrenok, Yu. E. Tsvetkov, Yu. V. Tomilov, V. A. Dokichev, N. E. Nifantiev, *Russ. Chem. Bull.*, 2015, **64**, 1413—1418; DOI: 10.1007/s11172-015-1025-0.
31. D. E. Tsvetkov, A. S. Dmitrenok, Yu. E. Tsvetkov, V. M. Men´shov, N. E. Nifantiev, *Russ. Chem. Bull.*, 2021, **70**, 1356—1362; DOI: 10.1007/s11172-021-3223-2.

Received April 22, 2022;
in revised form May 25, 2022;
accepted June 7, 2022