Polyphenolic components of knotwood extracts from *Abies sibirica*

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An extract from the knotwood of *Abies sibirica* (Siberian fir) was studied by HPLC, NMR spectroscopy, and mass spectrometry with the goal to identify new sources of phytoestrogens, which are promising medicinal agents, and to obtain new representatives of natural compounds of the indicated group. The following compounds were found in the extract and identified: 2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-4-[1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)methyl]tetrahydrofuran (**1**), 5′-(7-hydroxymatairesinyl)-5″-(7‴-hydroxymatairesinol) (**2**), 2,3-bis(4-hydroxy-3-methoxybenzyl)butane-1,4-diol (**3**, (−)-secoisolariciresinol), and 3-(4-hydroxy-3-methoxybenzyl)-5-(4-hydroxy-3-methoxyphenyl)-4-(hydroxymethyl)dihydrofuran-2(3*H*)-one (**4**). Compound **2** was previously unknown.

Key words: polyphenolic compounds, lignans, knotwood, *Abies sibirica*.

In recent years, it was shown that knotwood, which is derived from wood processing waste, contains various biologically active compounds,[1,2] in particular flavonoids,[3] lignans,[3,4] stilbenes,[5] and other classes of natural compounds that exhibit antioxidant[4,6—9] and bactericidal properties and act on the cells of hormonal (antiestrogens),[10] immune, and nervous systems.[11] These properties can be utilized to develop drugs and biologically active agents, which stimulates studying the biological activity profiles of these compounds.

The use of waste from wood processing plants as the raw material for preparing the above-indicated valuable phenolic derivatives would increase the degree of wood processing, decrease the environmental impact, and improve the economic and environmental performance of industrial processes. This accounts for the relevance of systematic studies of components of the extracts from knot areas of various types of wood. In this respect, it is reasonable to study the knotwood of *Abies sibirica* (Siberian fir), widely spread in the eastern and north eastern European area of Russia and in the central and south area of Siberia.[12] The wood of *Abies sibirica* serves as an important raw material for pulp and paper industry and for manufacture of merchantable and sound timber. Bark and needle extracts are used in medicine, optical industry, and microscopy.[13,14] The trunk of *Abies sibirica* can be up to 30 m long and has knots, which require development of efficient methods for obtaining deep processing products with high added value. This also refers to products that could be produced from the *Abies Sibirica* bark filled with transparent galipot (fir balsam) and needles of up to 3 cm length.[13]

It is noteworthy that the composition of the low-molecular-weight components of the extracts from *Abies Sibirica* needles, branches, and bark have been already analyzed in some laboratories.[5,15—17] High antioxidant,[15] antimicrobial,[15] antiestrogenic,[10] and antitumor activities[16] of both the extracts and isolated single components were demonstrated.

In order to study compounds present in the knotwood of *Abies sibirica* with natural moisture content, we obtained an extract by a previously described procedure.[5] Chromatogram of the extract is shown in Fig. 1.

Using preparative HPLC, six fractions characterized by high UV absorbance were isolated from the extract (Fig. 2). The fractions are designated according to the time intervals (min) of their elution from the column (see Fig. 2): 24—25, 29—31, 32—33, 34—35, 36—37, and 61—70.

The purity of the fractions obtained upon the preparative separation of the extract was evaluated using analytical HPLC under the conditions shown in Fig. 3.

According to analysis, fractions 24—25, 29—31, and 32—33 contained single compounds with 87—95% purity. Fractions 34—35 and 36—37, representing mixtures of several compounds with similar compositions (see Fig. 3), were combined and rechromatographed on a Supelcosil HS F5 column. This resulted in isolation of a ~90% pure component with the retention time of 13—14 min.

Fraction 61—70 with the longest retention time, eluted from the column only with 85% acetonitrile, and the precipitate that formed upon cooling a solution of the initial extract (see Experimental) were studied by GPC.

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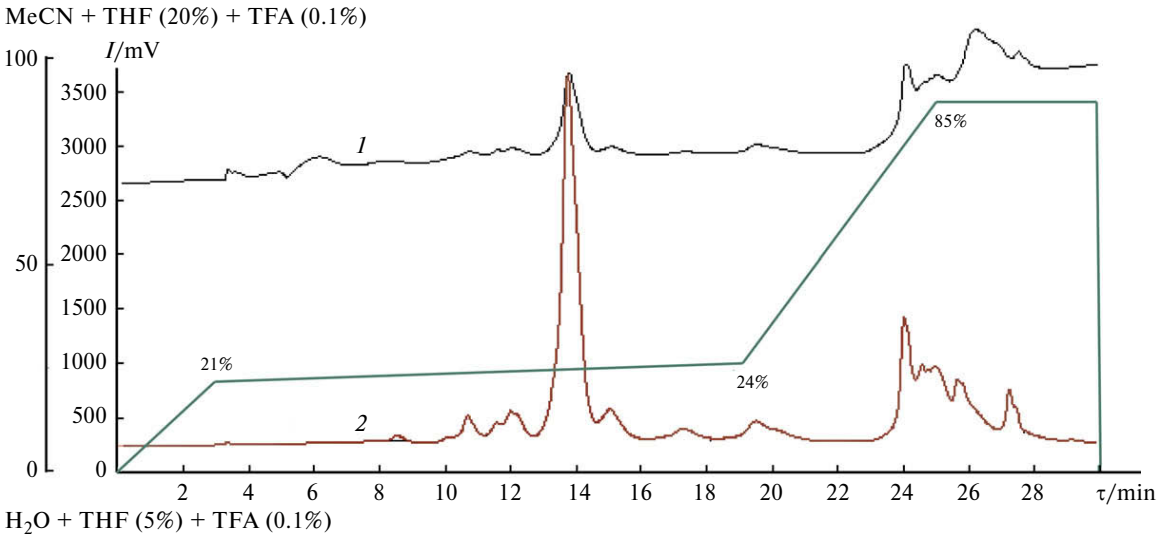


Fig. 1. Chromatogram of the extract from knotwood of *Abies sibirica* with a natural moisture content, obtained with gradient elution (here and in Figs 2 and 3 the eluent composition is indicated green color) on a column with modified C-18 silica gel. The components of the extract were detected by photometry at 215 (*1*) and 280 nm (*2*).

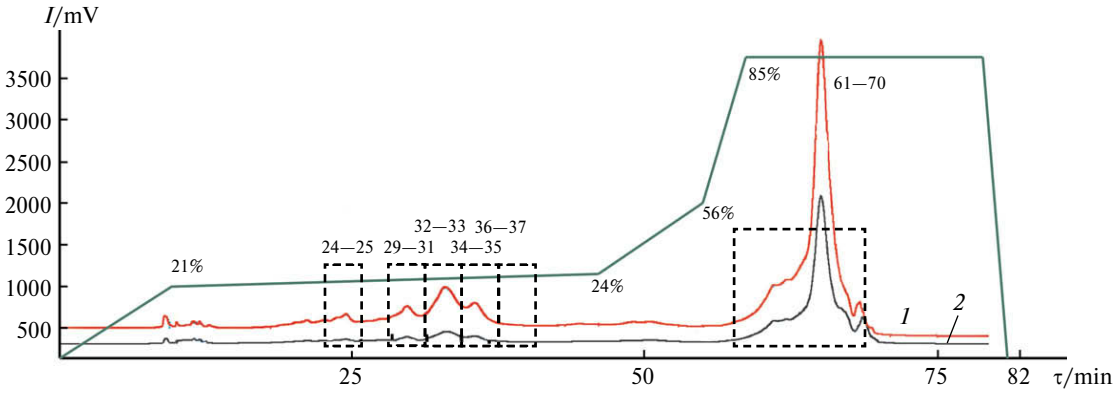


Fig. 2. Chromatogram for the separation of crude extract on a preparative column packed with modified C-18 silica gel. The components of the extract were detected by photometry at 215 (*1*) and 280 nm (*2*).

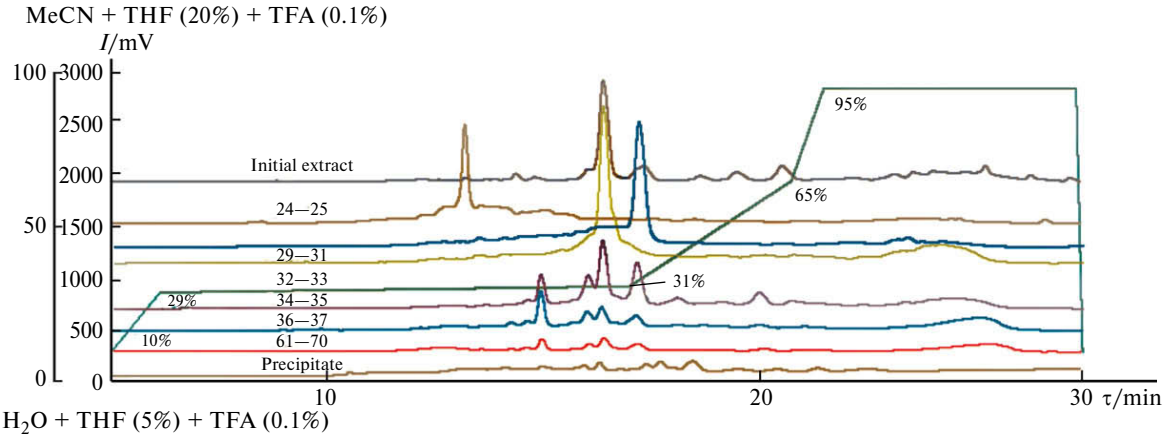


Fig. 3. Comparison of the chromatograms of the original extract with elution profiles of the isolated fractions (HPLC on a Supelco HS F5 column; UV detection at 280 nm).

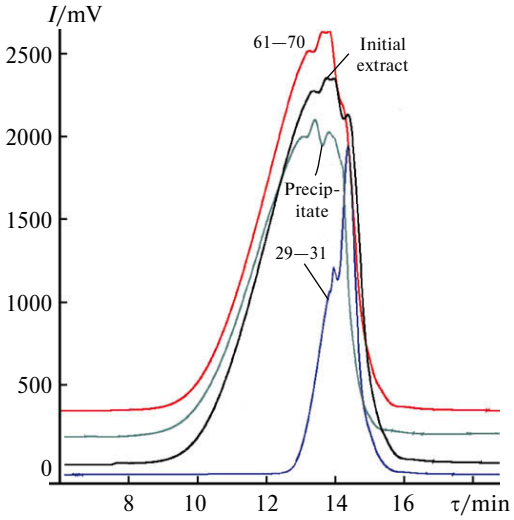
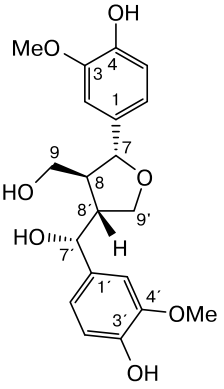


Fig. 4. Comparison of the chromatograms of the original extract from the knotwood of *Abies sibirica* with elution profiles of the isolated components (Tosoh Bioscience TSK 2000SW column; 0.7 mL min^{-1} flow rate; 0.03 *M* phosphate buffered saline (pH 7.2) with the addition of 35% MeCN; UV detection at 280 nm).

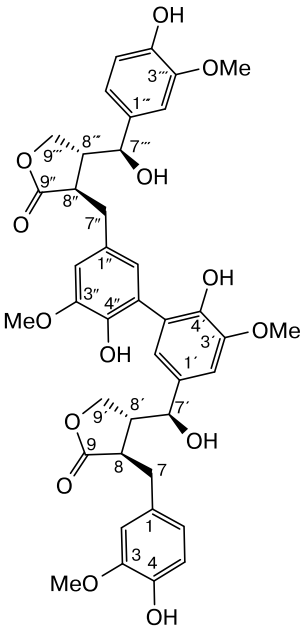
It was shown (Fig. 4) that the extract contained polymeric compounds with a molecular weight of up to 20 kDa. Comparison of chromatograms of the original extract, fraction 61—70, and the precipitate indicate that the molecular weight distributions almost coincided. The chromatogram of the original extract showed a peak with a retention time of 14.5 min, which apparently corresponded to lignan derivatives with a molecular weight of about 1 kDa. For comparison, the Figure shows the chromatogram of fraction 29—31, the major component of which was a compound with a molecular weight of 745 Da (see below); no such peak was present in the chromatograms of fraction 61—70 and the precipitate.

The structures of the isolated compounds were established by NMR spectroscopy and high-resolution mass spectrometry (ESI and MALDI-TOF). The NMR signals were assigned using homo- and heteronuclear 2D NMR procedures (HMBC, NOESY, COSY, and HSQC). As a result, it was shown that the major components of fractions 24—25, 29—31, and 32—33 and the sum of fractions 34—35 and 36—37 were 2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-4-[1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)methyl]tetrahydrofuran (**1**; (+/–)-tanegool), 5′-(7-hydroxymatairesinyl)-5″-(7‴-hydroxymatairesinol) (**2**), 2,3-bis(4-hydroxy-3-methoxybenzyl)butane-1,4-diol (**3**; (–)-secoisolariciresinol), and 3-(4-hydroxy-3-methoxybenzyl)-5-(4-hydroxy-3-methoxyphenyl)-4-(hydroxymethyl)dihydrofuran-2(3*H*)-one (**4**; (+)-isohydroxymatairesinol), respectively.

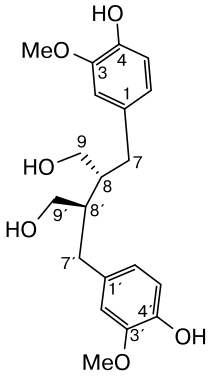
The ^{1}H and ^{13}C NMR chemical shifts and their assignments and specific optical rotations of compounds **1**, **3**, and **4** coincide with the data reported in the literature.



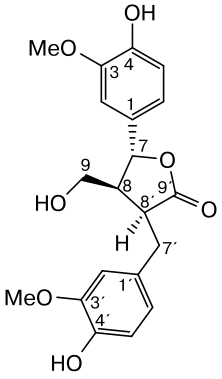
1
(24—25)



2
(29—31)



3
(32—33)



4
(34—35 and 36—37)

Indeed, the NMR chemical shifts of compound **1** agree with the values reported for (+/–)-tanegool,[18] those of compound **3** correspond to the values reported for (–)-secoisolariciresinol,[4,19] and those of compound **4** coincide with the spectral data for (+)-isohydroxymatairesinol.[20] Interpretation of the spectra and determination of the structure of the previously unknown compound **2** are given below.

The molecular formula $C_{40}H_{42}O_{14}$ of compound **2** (the major component of fraction 29—31) was established for the $[M - H]^-$ ion peak ($[C_{40}H_{41}O_{14}]^-$, *m/z* 745.2502; Δ 0.3 ppm), observed in the first order electrospray ionization negative ion mass spectra. The second order mass spectrum (collision induced dissociation (CID)) of this ion (Fig. 5) also exhibits the ions formed upon elimination of one (*m/z* 727.2270) and two (*m/z* 709.2192) water

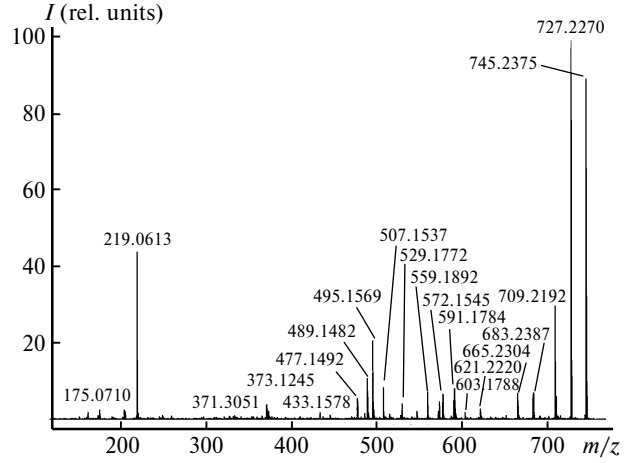


Fig. 5. Mass spectrum of compound **2** obtained upon collision induced dissociation (35 eV).

molecules from the analyte, which attests to the presence of hydroxy groups bound to the aliphatic moiety of the molecule. In addition, the spectrum shows the ions generated upon elimination of one and two carbon dioxide molecules (*m/z* 665.2304 and 621.2220, respectively). One more pair of ions observed in the CID mass spectrum presumably corresponds to elimination of methoxy groups as a formaldehyde molecule (*m/z* 591.1784).

The ^{1}H NMR spectrum of compound **2** in DMSO-d_6 (see Experimental) shows four broadened singlets with δ 8.90, 8.87, 8.81, and 8.77, which were assigned to the OH groups bound to the aromatic rings. In addition, the spectrum contains four doublets for aromatic protons at δ 6.80, 6.74, 6.59, and 6.56 with spin—spin coupling constants of 1.8—2 Hz; this is in line with the spectrum of a 1,3,4,5-tetrasubstituted aromatic ring. The multiplet at δ 6.73—6.59 correspond to four aromatic protons. Two doublets of doublets at δ 6.46 and 6.41 (J_1 = 8.1 Hz, J_2 = 2.0 Hz) belong to protons in position 2 of a 1,3,4-trisubstituted aromatic ring. The broadened doublets at δ 5.56 (J = 4.1 Hz) and 5.49 (J = 4.4 Hz) refer to the hydroxy groups bound to aliphatic carbon atoms. The multiplets at δ 4.57—4.50 and 4.29—4.24 correspond to hydroxylated CH groups. The multiplets at δ 4.16—4.09, 4.01—3.93, and 3.95—3.87 are due to the CH_2O moiety, while singlets at δ 3.72, 3.69, and 3.68 correspond to the protons of four MeO groups. The overlapping multiplets at δ 2.85—2.75 belong to one proton of the benzyl CH_2 group and two CH protons. The doublet of doublets at δ 2.65 (J_1 = 13.7 Hz, J_2 = 6.3 Hz) corresponds to the second proton of the benzyl CH_2 group. The multiplet at δ 2.59—2.52 belongs to one of the protons of one more benzyl CH_2 group, while the overlapping multiplets at δ 2.53—2.46, 2.50—2.42, and 2.46—2.40 refer to the second proton of the second benzyl CH_2 group and two CH protons.

The ^{13}C NMR spectrum of compound **2** shows signals for 38 carbon atoms, which are in line with the above-listed moieties. In particular, there are signals (δ 179.0 and 178.9) for carboxyl groups; three signals (δ 147.5, 147.4 (2 C), and 147.3) for the aromatic-ring quaternary carbon atoms bound to methoxy groups; four signals (δ 145.7, 145.6, 145.1, and 145.0) for the aromatic-ring quaternary carbon atoms bound to OH groups; two signals (δ 134.6 and 134.1) for the quaternary carbon atoms linking two aromatic moieties; and three signals (δ 128.8, 128.7, and 115.1) for the aromatic-ring quaternary carbon atoms bound to aliphatic groups. Five groups of signals (δ 121.8 and 121.7; 118.4 and 118.3; 115.3 and 115.2; 113.7 and 113.5; and 110.2 and 110.1) correspond to CH carbon atoms of the aromatic ring. In the region of aliphatic carbon atoms, the spectrum shows two signals (δ 72.4 and 72.3) for the CHOH groups; two signals (δ 68.3 and 67.7) for the CH_2O groups; two signals (δ 55.6 and 55.5) for the MeO groups; four signals (δ 45.9, 45.0, 42.9, and 42.5) for the CH groups bound to aliphatic substituents; and two signals (δ 34.3 and 33.8) corresponding to the benzyl carbon atoms of the CH_2Ar groups.

Thus, on the basis of spectral studies and the results obtained using HMBC, COSY, and HSQC procedures, compound **2** was found to contain four aromatic rings, two of them being 3-methoxy-4-hydroxyphenyl moieties, while other two being 3-methoxy-4-hydroxyphenyl moieties linked at position 5. In addition, two cyclic γ-butyrolactone residues were identified.

Since all signals in the ^{13}C NMR spectrum occur as pairs in which signals have very similar chemical shifts, it can be assumed that compound **2** is an unsymmetrical 7′-hydroxymatairesinol dimer. In the CID mass spectrum, this assumption is confirmed by low-intensity peaks for ions (*m/z* 373.1245) formed upon cleavage of the C(5′)—C(5″) bond between two aryl rings and an intense peak (*m/z* 495.1569) for the ion generated upon cleavage of the C(7″)—C(1″) bond.

Compound **2** was not described previously, but related matairesinol dimers from *Forsythia viridissima* were known.[21] Comparison with the published NMR data[21] for these compounds, considering the structures of compounds **1**, **3**, and **4**, which we studied here, suggest configuration of the asymmetric centers in compound **2** (see above).

Extensive chemical studies of knotwood demonstrated that the contents of polyphenols in the knots of both coniferous and deciduous trees are higher than in other parts of wood. In addition, similarity of the structures of components found in knots and in other parts was noted.[2,22] Compounds **1**—**4**, which we identified, either have been found[23] in other parts of *Abies sibirica* or have a "lignan" core, which confirms the assumption[2] of structural similarity between compounds found in knotwood extracts and in other parts of the trees. Due to

their structural similarity with endogenous estrogens, lignans can compete with enterolactone in physiological processes and inhibit the growth of breast cancer cells.[10] Epidemiological data also attest to a protective role of lignans.[24]

Thus, we demonstrated the possibility of using the wood of *Abies sibirica* as a raw material for the preparation of the above-indicated phenolic compounds exhibiting antioxidant,[15] antimicrobial,[15] antiestrogenic,[10] antitumor,[16] and anticoagulant[25] activities. This would increase the degree of processing of raw wood and reduce the environmental impact. It is also important that phenolic compounds from wood can also be used as the feedstock for chemical modification using simple reactions, for example sulfation (see, for example, Refs 26, 27), and for obtaining more active derivatives. This additionally substantiates the need for systematic analysis and study of the properties of the discussed types of natural compounds and for the search for raw materials for isolation of these compounds.

Experimental

Extraction and chromatographic separation were carried out using HPLC grade solvents (Sigma). NMR spectra were recorded on Bruker AMX-III 400 (400 and 100 MHz) and Bruker Avance 600 (600 and 126 MHz) spectrometers using DMSO-d_6 as the solvent and tetramethylsilane (Me_4Si) as an internal standard. The signals were assigned by $^1H—^1H$, NOESY, COSY, and $^1H—^{13}C$ HSQC, HMBC 2D NMR spectroscopy. High-resolution electrospray ionization (ESI) mass spectra were recorded on a Bruker micrOTOF II instrument in either positive ion (capillary voltage of 4500 V) or negative ion mode (capillary voltage of 3200 V). High-resolution mass spectra were obtained on a Bruker solariX XR instrument (ion cyclotron resonance mass analyzer, a superconducting magnet with 15 T field strength, Germany) equipped with a MALDI SmartBeam-II ionization source. The measurements were carried out in the negative ion mode using the *trans*-2-[3-(4-*tert*-butylphenyl)-2-(methyl-2-propenylidene]malononitrile matrix deposited on an MTP 384 Ground Steel target (Bruker Daltonics Inc., Germany) with the *m/z* scanning range of 50—1500. The data were treated using the Bruker Data Analysis 5.0 software package. Analytical HPLC was carried out on Supelcosil HS F5 and Supelcosil LC-18 columns (4×250 mm, 5 μm, Supelco, USA) at a flow rate of 1 mL min^{-1}. Preparative HPLC was performed using a Silasorb C-18 column (22×250 mm, 5 μm) at a flow rate of 10 mL min^{-1} and a Supelcosil HS F5 column (10×250 mm, 5 μm) at a flow rate of 4 mL min^{-1}. The eluate was monitored at two wavelengths (215 and 280 nm) on a 155 UV spectrophotometer (Gilson, France), as described previously.[3,4,6—9,28] Optical rotation power was measured on a JASCO P-2000 digital polarimeter (Japan) at 24 °C. All specific rotations were determined for compounds with the indicated purity and not corrected.

Plant material, extraction. Wood samples were obtained from living knots at a height of ~25 m of the trunk of a ~80 years old and ~35 m high Siberian fir with a diameter of 45 cm at the trunk bottom. The knots 30—40 mm in diameter in which the heartwood was almost unnoticeable were drilled out with a drill bit (30 mm) to a depth of 3—5 cm. The resulting chips were dried for 24 h *in vacuo* (the moisture content was 31%). A weighed portion (25.1 g) of dry chips was mixed with 200 mL of 70% aqueous Pr^iOH at ~25 °C and kept for ~14 h. The suspension was filtered and the precipitate was washed on the filter with 70% aqueous Pr^iOH (3×150 mL). The solvent was evaporated *in vacuo* and the residue was dried for 20 h (25 °C) at a pressure of 0.25 Torr to give 5.1 g (25%) of the extract. The extract composition is given in analytical chromatogram (see Fig. 1).

Isolation of components. For the preparative separation on the Silasorb C-18 column (see Fig. 2), the optimized conditions of chromatography presented in Fig. 1 were scaled up. The extract (1.0 g) was dissolved with heating in 3 mL of 65% aqueous MeCN (333 mg mL^{-1}), the solution was cooled down, the precipitate (94 mg) was collected on a filter, and the filtrate was applied on a column as 1000-μL portions (~300 mg) and eluted with a MeCN gradient from 0 to 85% within 80 min, with 9 mL min^{-1} flow rate of the eluent. The components of the extract were detected photometrically at 215 and 280 nm (see Fig. 2); the volume of the collected fractions was 9 mL.

After removal of the solvent *in vacuo* and freeze-drying of the residue, the following fractions were obtained: 24—25, 15.6 mg yield (1.6% of the taken extract; contains compound **1** with ~87% purity); 29—31, 19.1 mg yield (1.9%; contains compound **2** with ~92% purity); 32—33, 20.9 mg yield (2.1%; contains compound **3** with ~95% purity); 34—35, 23.9 mg yield (2.4%; contains a complex mixture); 36—37, 11.1 mg yield (1.5%; contains a complex mixture); 61—70, 351.0 mg yield (contains a complex mixture). The total yield was 53.8%. The fractions 34—35 and 36—37 were combined and chromatographed once again on a Supelcosil HS F5 column (10×250 mm, 5 μm) in the gradient presented in Fig. 3. A fraction with the retention time of 13—14 min was collected. After evaporation and freeze-drying, this gave 4.3 mg (0.4%) of compound **4** with a ~90% purity.

2-(4-Hydroxy-3-methoxyphenyl)-3-hydroxymethyl-4-[1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)methyl]tetrahydrofuran (1), $[\alpha]_D$ 0.5 (*c* 0.1, MeOH) (*cf.* Ref. 29: $[\alpha]_D$ 0.4). ^1H NMR (600 MHz), δ: 8.87, 8.85 (both s, 2 H, C(4)OH + C(4′)OH); 6.95—6.65 (m, 6 H, H(5) + H(5′) + H(2) + H(2′) + H(6) + + H(6′)); 5.48 (d, 1 H, C(7′)OH, J = 3.6 Hz); 5.01 (t, 1 H, C(9)OH, J = 5.2 Hz); 4.44 (d, 1 H, H(7), J = 8.2 Hz); 4.33 (dd, 1 H, H(7′), J_1 = 8.2 Hz, J_2 = 3.5 Hz); 3.76, 3.74 (both s, 6 H, C(3)OC$\underline{H}_3$ + C(3′)OC$\underline{H}_3$); 3.58—3.54, 3.47—3.43 (m, H(9)); 3.48—3.42 (m, H(9′)); 2.47—2.36 (m, H(8)); 2.13—2.05 (m, H(8′)). ^{13}C NMR (151 MHz), δ: 147.5, 147.4 (C(3) + C(3′)); 145.8, 145.7 (C(4) + C(4′)); 135.6 (C(1′)); 133.5 (C(1)); 119.3 (C(6′)); 119.0 (C(6)); 115.1 (C(5)); 115.0 (C(5′)); 110.8 (C(2)); 110.5 (C(2′)); 83.5 (C(7)); 75.1 (C(7′)); 69.5 (C(9′)); 61.7 (C(9)); 55.7 (C(3)O$\underline{C}H_3$ + C(3′)O$\underline{C}H_3$); 54.1 (C(8)); 50.8 (C(8′)). MS, found: *m/z* 377.1602 $[M + H]^+$; calcd. for $C_{20}H_{24}O_7$: 377.1595; found: *m/z* 399.1409 $[M + Na]^+$; calcd. for $C_{20}H_{24}O_7Na$: 399.1414; found: *m/z* 415.1159 $[M + K]^+$; calcd. for $C_{20}H_{24}O_7K$: 415.1154.

5′-(7-Hydroxymatairesinyl)-5″-(7‴-hydroxymatairesinol) (2), $[\alpha]_D$ −1.2 (*c* 0.23, MeOH). ^1H NMR (400 MHz), δ: 8.90, 8.87, 8.81, 8.77 (br.s, 4 H, C(4)OH + C(4′)OH + C(4″)OH + C(4‴)OH); 6.80 (d, 1 H, H(2′), J = 1.8 Hz); 6.74 (d, 1 H, H(2″), J = 1.8 Hz); 6.73—6.59 (m, 4 H, H(5) + H(5‴) + H(6′) + H(6″));

6.59 (d, 1 H, H(2) , J = 1.8 Hz); 6.56 (d, 1 H, H(2‴), J = 1.9 Hz); 6.46 (dd, 1 H, H(6) , J_1 = 8.0 Hz, J_2 = 1.9 Hz); 6.41 (dd, 1 H, H(6‴), J_1 = 8.1 Hz, J_2 = 2.0 Hz); 5.56 (d, 1 H, C(7′)OH, J = 4.1 Hz); 5.49 (d, 1 H, C(7‴)OH, J = 4.4 Hz); 4.57—4.50 (m, 1 H, H(7′)); 4.29—4.24 (m, 1 H, H(7‴)); 4.16—4.09, 4.01—3.93 (m, 2 H, H(9‴)); 3.95—3.87 (m, 2 H, H(9′)); 3.72 (s, 6 H, C(3)OCH_3 + C(3‴)OCH_3); 3.69, 3.68 (both s, 6 H, C(3′)OCH_3 + C(3″)OCH_3); 2.85—2.75 (m, 3 H, H(8) + H(8″) + + H_a(7)); 2.65 (dd, 1 H, H_b(7′), J_1 = 13.7 Hz, J_2 = 6.3 Hz); 2.59—2.52 (m, H_a(7″)); 2.53—2.46 (m, H(8′)); 2.50—2.42 (m, H_b(7″)); 2.46—2.40 (m, 1 H, H(8‴)). ^{13}C NMR (101 MHz), δ: 179.0, 178.9 (C(9″) + C(9)); 147.5, 147.4, 147.4, 147.3 (C(3) + + C(3′) + C(3″) + C(3‴)); 145.7, 145.6 (C(4) + C(4‴)); 145.1, 145.0 (C(4′) + C(4″)); 134.6 (C(5′)); 134.1 (C(5″)); 128.8, 128.7 (C(1) + C(1″)); 121.8, 121.7 (C(6) + C(6‴)); 118.4, 118.3 (C(5) + + C(5‴)); 115.3, 115.2 (C(6′) + C(6″)); 115.1 (C(1′) + C(1‴)); 113.7, 113.5 (C(2) + C(2‴)); 110.2, 110.1 (C(2′) + C(2″)); 72.4, 72.3 (C(7′) + C(7‴)); 68.3, 67.7 (C(9′) + C(9‴)); 55.6, 55.5 (C(3)O$\underline{C}H_3$ + C(3′)O$\underline{C}H_3$ + C(3″)O$\underline{C}H_3$ + C(3‴)O$\underline{C}H_3$); 45.9 (C(8′)); 45.0 (C(8‴)); 42.9 (C(8)); 42.5 (C(8″)); 34.34, 33.84 (C(7) + C(7″)). MS, found: m/z 745.2502 $[M - H]^-$; calcd. for $C_{40}H_{41}O_{14}$: 745.2504.

(−)-2,3-Bis(4-hydroxy-3-methoxybenzyl)butane-1,4-diol (3; (−)-secoisolariciresinol), $[\alpha]_D$ −30 (*c* 0.15, MeOH) (*cf.* Ref. 19: $[\alpha]_D$ −32). ^{1}H NMR (600 MHz), δ: 8.63 (br.s, 2 H, C(4)OH + + C(4′)OH); 6.68—6.61 (m, 4 H, H(5) + H(5′) + H(2) + H(2′)); 6.50 (d, 2 H, H(6) + H(6′), J = 8 Hz); 4.55 (br.s, 2 H, C(9)OH + + C(9′)OH); 3.61 (s, 6 H, C(3)OCH_3 + C(3′)OCH_3); 3.38 (H(9) + + H(9′)); 2.49 (H(7) + H(7′)); 1.86—1.77 (m, 2 H, H(8) + + H(8′)). ^{13}C NMR (151 MHz), δ: 147.3 (C(3) + C(3′)); 144.3 (C(4) + C(4′)); 132.3 (C(1) + C(1′)); 121.2 (C(6) + C(6′)); 115.1 (C(5) + C(5′)); 113.0 (C(2) + C(2′)); 60.3 (C(9) + C(9′)); 55.5 (C(3)O$\underline{C}H_3$ + C(3′)O$\underline{C}H_3$); 42.5 (C(8) + C(8′)); 34.0 (C(7) + + C(7′)). MS, found: m/z 363.1803 $[M + H]^+$; calcd. for $C_{20}H_{26}O_6$: 363.1802; found: m/z 385.1630 $[M + Na]^+$; calcd. for $C_{20}H_{26}O_6Na$: 385.1622; found: m/z 401.1296 $[M + K]^+$; calcd. for $C_{20}H_{26}O_6K$: 401.1361.

3-(4-Hydroxy-3-methoxybenzyl)-5-(4-hydroxy-3-methoxyphenyl)-4-(hydroxymethyl)dihydrofuran-2(3*H*)-one (4), $[\alpha]_D$ 50 (*c* 0.2, MeOH) (*cf.* Ref. 20: $[\alpha]_D$ +51). ^{1}H NMR (400 MHz), δ: 8.35 (br.s, 2 H, C(4)OH + C(4′)OH); 6.79—6.56 (m, 4 H, H(5) + + H(5′) + H(2) + H(2′)); 6.47 (d, 2 H, H(6) + H(6′), J = 8.1 Hz); 6.07 (br.s, 1 H, H(7)); 3.70, 3.69 (both s, 6 H, C(3)OCH_3 + + C(3′)OCH_3); 3.46—3.38 (m, H(9)); 3.26—3.15, 2.70—2.62 (m, H(7′)); 1.85—1.76 (m, 1 H, H(8′)); 1.65—1.57 (m, 1 H, H(8)). ^{13}C NMR (151 MHz), δ: 165.9 (C(9′)); 147.7 (C(3′)); 147.6 (C(3)); 144.20 (C(4) + C(4′)); 127.4 (C(1) + C(1′)); 121.6 (C(6) + C(6′)); 116.4 (C(7)); 115.5 (C(5) + C(5′)); 113.1 (C(2) + + C(2′)); 63.8 (C(9)); 55.7 (C(3)O$\underline{C}H_3$ + C(3′)O$\underline{C}H_3$); 46.0 (C(8)); 38.2 (C(8′)); 32.4 (C(7′)). MS, found: m/z 375.1381 $[M + H]^+$; calcd. for $C_{20}H_{22}O_7$: 375.1438; found: m/z 397.1258 $[M + Na]^+$; calcd. for $C_{20}H_{26}O_6Na$: 397.1257.

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