Analysis of content of (−)-secoisolariciresinol and related polyphenols in different morphological parts and anatomical structures of larch wood from Siberia*

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Productive efficiency of technology of polysaccharide and lignin manufacturing from wood raw materials can be significantly improved by integration of purification stages of low molecular weight wood components widely used in applied chemistry into the technological cycle. In this connection, phenols including lignans and flavonoids, which have a practical application potential, are of a special interest. In the present work the results of a study of the content of (−)-secoisolariciresinol, dihydroquercetin and related polyphenols in different morphological parts and anatomical structures of larch wood from the Siberia are analyzed. Analysis of the content of the listed products by reversed-phase HPLC provides the selection of optimal raw material for organizing the manufacture of the listed compounds with predictable efficiency. Increased content of (−)-secoisolariciresinol (up to 3—4%) observed in wood of the trunk knot areas of larch from Khakassia evidences the prospects of raw material processing in this region for production of (−)-secoisolariciresinol from the wastes of larch wood refinery.

Key words: (−)-secoisolariciresinol, dihydroquercetin, knot areas of the larch wood.

Wood of coniferous trees provides the significant quantity of a wide range of natural compounds with different biological activities.[1,2] The most abundant products are biopolymers, in particular polysaccharides and lignin. Among the most frequently used low molecular weight compounds from the wood of coniferous trees are polyphenols, primarily flavonoids, lignans, stilbenes and others. One of the most widely known compounds of this type is a flavonoid dihydroquercetin (**1**, DHQ), which is isolated from the butt area of Siberian larch (*L. sibirica*) and Dahurian larch (*L. gmelinii*) and is widely used as an efficient antioxidant in manufacture of over 200 products, which are mostly biological active additives, poultry fodder, cosmetics, food and other.[3] Recently we showed, that knots area of the wood of Siberian and Dahurian larches which can be extracted from the wastes of refinery of this high value wood contain, along with the indicated above flavonoid DHQ (**1**), considerable quantities of another useful polyphenol, namely, a lignan (−)-secoisolariciresinol (**2**) with a wider spectrum of biological activities as compared to DHQ. For example, it was found, that lignan **2** has, along with antioxidant activity, bactericidal, antiestrogen and immunomodulating properties,[4—6] and thus is regarded as a promising agent for prophylactics and cure of oncological diseases, inflammation, microbial infections, cardiovascular and other diseases, and also as a "green" natural bactericide for oilfield chemistry.[7]

* Dedicated to Yu. N. Bubnov, Academician of the Russian Academy of Sciences, on the occasion of his 80th birthday.

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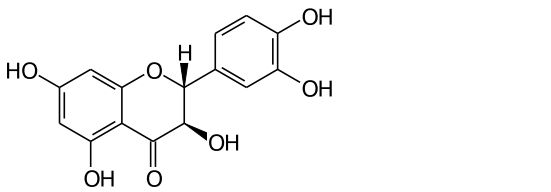

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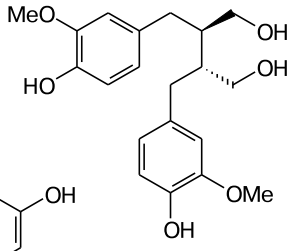

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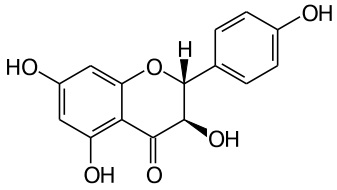

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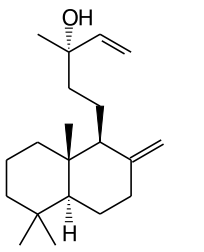
We developed an efficient technique[8] for isolation of lignan **2** from the wood extract, which provides good quality and preparative quantities sufficient for conduction of biological and clinical trails. To date, preclinical studies of a medicament on the basis of lignan **2** aimed at treatment of hormone dependent tumors and climacteric syndrome have been accomplished[9–11] during the fulfillment of a state contract funded by the federal targeted program "Farma 2020". Recently, rather promising results were obtained in the studies of the ability of polyphenols **1** and **2** to modulate the regeneration of neurons.[12]

For organizing of manufacture of lignan **2** systematic studies of its content in wood material are needed to establish its correlation with climatic, regional and other factors, as well as investigation of a variety of concurrent polyphenolic compounds and different impurities. The aim of the present work was the chromatography profile analysis of low molecular weight polyphenolic components of extracts of different morphological parts and anatomical structures of the larch wood, which is important for the selection of the type of the material and economic optimization of the manufacture using waste material of wood refinery instead of the line lumber.

Water-alcohol extract of the wood from the knot area of the larch is a complex mixture of polyphenolic compounds. Along with the target compounds DHQ (**1**) and secoisolariciresinol (**2**) together with a contaminant dihydrokaempferol (**3**), 13-epi-manool[13] (**4**), larixol acetate[13] (**5**), lariciresinol[14] (**6**), isolariciresinol[14] (**7**), quercetin[15] (**8**) and guaiacyl glycerol ether of secoisolariciresinol[16] (sesquimarocanol B) (**9**) were isolated by preparative chromatography on silica gel columns from the extracts of the wood of the knot area of the larch, and their structures were elucidated by NMR and mass spectroscopy, which were fully consistent with the published data.[13–16]

Characteristic chromatograms (methods A, B and C) of the samples of extracts are shown in Figs 1—3.

Chromatographic analysis of the wood samples (Tables 1—4) collected in Krasnoyarskij Krai conducted by methods A, B, and C showed that the content of lignan **2** can differ significantly in knot areas even in cases of



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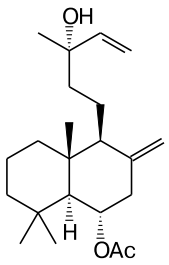

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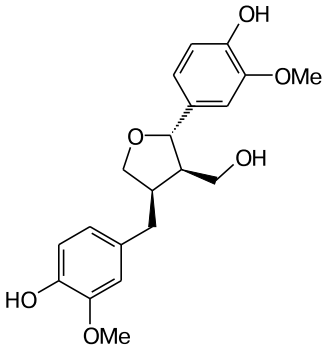

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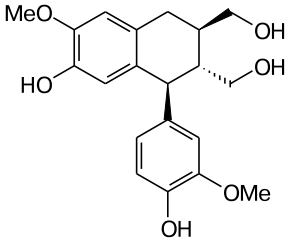

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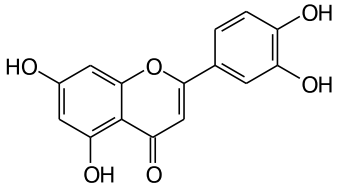

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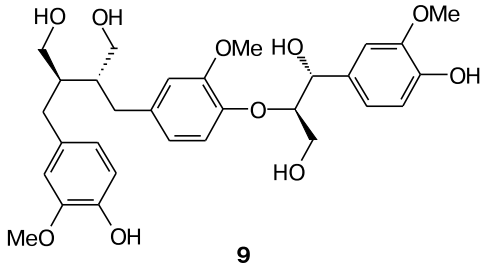

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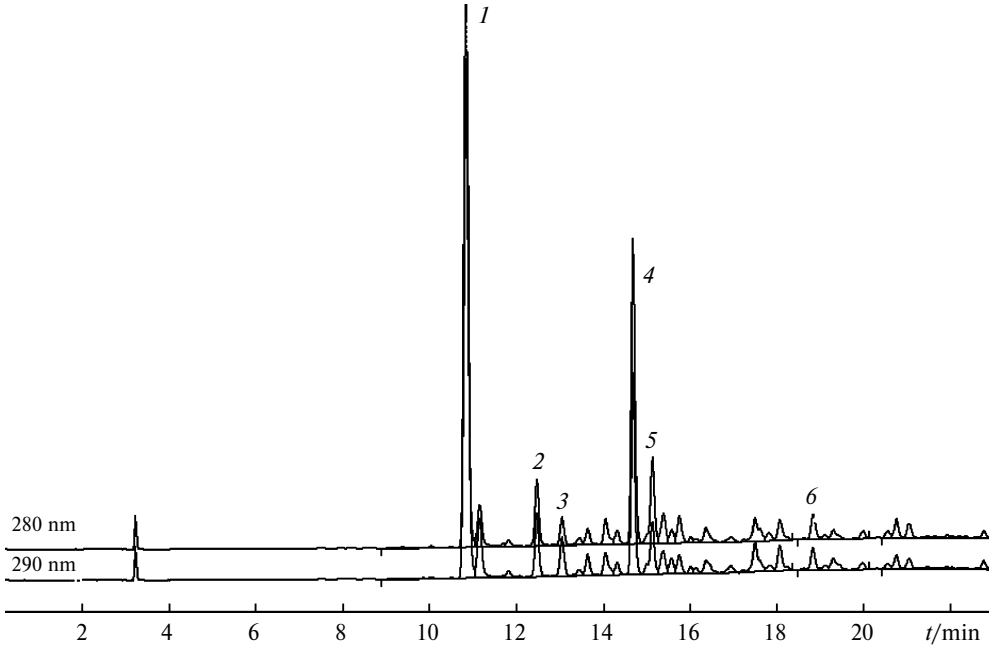

Fig. 1. HPLC analysis (method C) of the water-alcohol extract: peak *1*, DHQ (**1**); *2*, isolariciresinol (**7**); *3*, dihydrokaempferol (**3**); *4*, secoisolariciresinol (**2**); *5*, lariciresinol (**6**); *6*, guaiacyl glycerol ether of secoisolariciresinol (sesquimarocanol B) (**9**).

their close location on one sawn end. This difference was not observed for flavonoids **1** and **3** (see Table 1).

For the first time we analyzed the content of polyphenolic compounds **1**—**3** in larch wood which is the unique raw material for production of secoisolariciresinol **2**. The only representative of lignans, which content was analyzed in knot areas of coniferous trees, was 7-hydroxymateiresinol, which was isolated from some species of spruce. As reported earlier,[17] percentage of this compound is substantially decreased from the surface to the core of the trunk starting from the depth of 1.5—2 cm. For secoisolariciresinol (**2**) this type of dependence was not observed and its content can not be predicted based on the location of the wood sample. This is the reason why the wood from all knot areas of the larch (and not their separate parts) is advisable for industrial extraction.

Certain increase of the content of secoisolariciresinol (**2**) can be noted for the samples of knot areas from the upper parts of the trunk, thought not strictly (Table 2). Decreased content of secoisolariciresinol (**2**) was observed in tree butts (see Table 3) with concomitant increase of the content of DHQ (**1**). Notably, the content of secoisolariciresinol (**2**) in older and larger knots was substantially lower, than in younger ones (see Table 4). However, the knot areas of the youngest parts of the trunk (the treetops) are also characterized with low content of secoisolariciresinol (**2**). Thus, processing of the medium parts of larch trees (between 8 to 14 m) is advisable, where the average content of secoisolariciresinol (**2**) varies within the limits 1.5—2.1% (mass.) of the crushed knot areas.

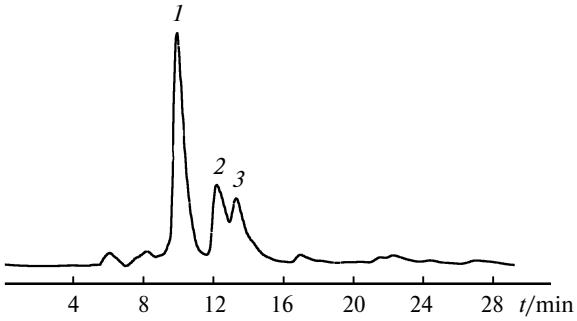

Fig. 2. HPLC analysis (method A) of the water-alcohol extract: peak *1*, DHQ (**1**); *2*, secoisolariciresinol (**2**); *3*, dihydrokaempferol (**3**).

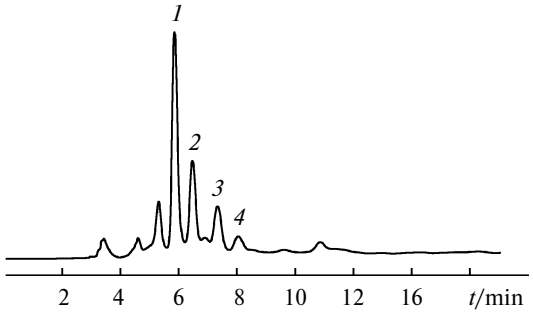

Fig. 3. HPLC analysis (method B) of the water-alcohol extract: peak *1*, DHQ (**1**); *2*; secoisolariciresinol (**2**); *3*, dihydrokaempferol (**3**); *4* , quercetin (**8**).

Table 1. Content (*C*) of DHQ (**1**), secoisolariciresinol (**2**) and dihydrokaempferol (**3**) in samples of knot areas of larch collected in Krasnoyarskij Krai (batch No.1)

h/m	*N*	*n*	*C* (%)		
			1	**2**	**3**
8.4—10.4	1	1	0.71	2.37	0.26
		2	0.83	0.61	0.18
		3	1.05	2.51	0.27
		4	0.89	1.56	0.19
	2	1	0.73	0.99	0.14
		2	0.71	1.64	0.17
		3	1.47	2.60	0.20
		4	0.62	2.30	0.23
	3	1	1.25	2.31	0.19
		2	0.37	2.33	0.22
		3	0.34	1.54	0.25
		4	0.70	2.62	0.35
10.4—12.4	4	1	0.51	1.19	0.10
		2	0.49	0.77	0.08
		3	0.47	0.28	0.04
		4	0.51	2.24	0.26
		5	0.61	3.13	0.40
		6	0.43	1.75	0.21
		7	0.44	1.33	0.09
		8	0.44	1.86	0.20
10.4—12.4	5	1	0.54	1.79	0.20
		2	0.47	1.78	0.18
	6	1	0.55	2.32	0.25
		2	0.59	2.73	0.32
		3	0.47	0.77	0.06
		4	0.54	1.66	0.15
	7	1	0.49	0.40	0.05
		2	0.49	0.94	0.095
12.4—14.4	8	1	0.31	2.23	0.25
		2	0.50	1.16	0.14
		3	0.19	2.02	0.17
		4	0.47	1.89	0.21
	9	1	0.52	2.85	0.30
		2	0.68	3.19	0.39
		3	0.38	1.90	0.16
		4	0.43	1.83	0.18
	10	1	0.70	2.96	0.33
		2	0.48	2.47	0.26
		3	0.47	2.68	0.28
		4	0.32	2.39	0.25

Note: *h* is height of the trunk, *N* is number of the plate, *n* is number of the knot, *C* (% of crushed wood mass).

Table 2. Content (*C*) of DHQ (**1**), secoisolariciresinol (**2**) and dihydrokaempferol (**3**) in samples of knot areas of larch collected in Pogorelskii stationary of Krasnoyarskij Krai (batch No.2)

h/m	*N*	*n*	*C* (%)		
			1	**2**	**3**
8.1—10.2	1	1	1.04	1.38	0.260
		2	1.61	0.41	0.180
	2	1	1.28	1.11	0.150
		2	3.14	0.90	0.290
		3	2.47	0.27	0.210
	3	1	1.07	0.63	0.130
		2	1.28	1.11	0.150
10.2—13.0	4	1	0.22	0.24	0.015
		2	0.93	0.26	0.075
		3	0.56	0.44	0.030
	5	1	0.65	0.25	0.060
		2	0.52	0.35	0.100
		3	1.00	1.32	0.190
	6	1	1.46	0.39	0.110
		2	0.77	0.48	0.090
13.0—15.2	7	1	0.35	0.15	0.035
		2	0.26	0.20	0.030
		3	0.22	0.20	0.035
	8	1	0.33	0.20	0.060
		2	0.46	0.55	0.060
		3	0.37	0.57	0.070

Note: *h* is height of the trunk, *N* is number of the plate, *n* is number of the knot, *C* (% of crushed wood mass).

Table 3. Content (*C*) of DHQ (**1**), secoisolariciresinol (**2**) and dihydrokaempferol (**3**) in samples of tree butts of larch collected in Krasnoyarskij Krai (batch No.1)

N′	*C* (%)		
	1	**2**	**3**
1	1.70	0.78	0.02
2	2.07	0.69	0.10
3	4.47	0.56	n.d.
4	3.23	0.84	0.03
5	4.01	0.69	n.d.
6	1.70	5.48	1.00
7	4.93	1.06	0.07

Note: *N*′ is number of the butt, *C* (% of crushed wood mass).

For more representative evaluation of the content of secoisolariciresinol (**2**) in different larch samples it is reasonable to select the wider parts of sawn ends in the form of crushed chips.

Similar analysis of the content of the basic phenolic components in wood samples collected in Khakassia showed (Tables 5 and 6) that the content of secoisolariciresinol (**2**) in these samples was somewhat higher, than in samples from Krasnoyarskij Krai. As in the previous study, characteristic increase of content of secoisolariciresinol (**2**) in the upper parts of the trunk for these sam-

Table 4. Content (*C*) of DHQ (**1**), secoisolariciresinol (**2**) and dihydrokaempferol (**3**) in samples of large old knot areas of larch collected in Krasnoyarskij Krai (batch No.1)

n	*C* (%)			*n*	*C* (%)		
	1	**2**	**3**		**1**	**2**	**3**
1	0.17	0.33	0.045	10	0.21	0.26	0.045
2	0.08	0.13	0.020	11	0.28	1.55	0.110
3	0.11	0.18	0.035	12	0.12	0.29	0.040
4	0.09	0.23	0.030	13	0.11	0.20	0.030
5	0.99	0.76	0.190	14	0.07	0.17	0.020
6	0.21	0.29	0.050	15	0.18	0.67	0.050
7	0.60	0.12	0.060	16	0.52	0.21	0.095
8	0.13	0.12	0.030	17	0.30	0.09	0.070
9	0.06	0.20	0.020	18	0.42	0.11	0.080

Note: *N´* is number of the knot, *C* (% of crushed wood mass).

ples was observed (compare data in Tables 5 and 6 to data in Tables 1 and 2).

Hence, elevated content of secoisolariciresinol (**2**) (an average of about 3—4%) in the wood of knot areas of larch trunks from Khakassia evidences the potential of processing of wood refinery wastes of larch on wood processing plants of Khakassia for production of secoisolariciresinol.

Table 5. Content (*C*) of DHQ (**1**), secoisolariciresinol (**2**) and dihydrokaempferol (**3**) in samples of knot areas of larch collected in Khakassia (batch No. 3)

h/m	*N*	*n*	*C* (%)		
			1	**2**	**3**
8.0—10.3	1	1	2.62	3.01	0.820
		2	1.72	1.89	0.580
		3	1.47	1.81	0.570
		4	1.97	1.63	0.630
	2	1	1.40	1.59	0.540
		2	2.71	5.86	1.580
		3	0.83	2.69	0.840
10.3—13.6	3	1	0.76	2.26	0.050
		2	0.73	0.85	0.160
		3	0.64	1.88	0.320
	4	1	0.62	2.46	0.320
		2	0.79	2.36	0.320
		3	0.92	2.98	0.480
13.6—15.2	5	1	1.58	4.68	0.860
		2	1.23	4.75	0.650
	6	1	1.24	4.35	0.690
		2	0.66	2.77	0.470
		3	1.34	4.10	0.740
Tree-top	7	1	0.02	0.03	0.005
		2	0.02	0.060	0.005

Note: *h* is height of the trunk, *N* is number of the plate, *n* is number of the knot, *C* (% of crushed wood mass).

Table 6. Content (*C*) of DHQ (**1**), secoisolariciresinol (**2**) and dihydrokaempferol (**3**) in samples of knot areas of larch collected in Khakassia (batch No. 4)

h/m	*N*	*n*	*C* (%)		
			1	**2**	**3**
8.2—10.3	1	1	0.44	3.89	0.42
		2	0.47	2.88	0.25
		3	0.37	2.87	0.26
	2	1	1.07	3.99	0.54
		2	1.48	5.02	0.93
		3	0.98	4.02	0.64
		4	0.95	4.29	0.94
10.3—13.2	3	1	0.64	3.88	0.50
		2	0.46	3.96	0.50
		3	0.40	2.68	0.22
		4	0.52	4.02	0.62
		5	0.94	4.40	0.60
	4	1	0.94	3.86	0.51
		2	0.93	3.35	0.39
		3	1.24	4.10	0.63
13.2—15.4	5	1	0.75	4.25	0.67
		2	0.66	3.06	0.40
	6	1	0.41	3.87	0.66
		2	0.33	2.55	0.28
		3	0.93	3.53	0.44
Tree-top	7	1	0.15	1.36	0.08
		2	0.10	0.60	0.05

Note: *h* is height of the trunk, *N* is number of the plate, *n* is number of the knot, *C* (% of crushed wood mass).

The study revealed regularities of the content of secoisolariciresinol (**2**) in wood material which provide the production of this compound with guaranteed predictable yield. At the same time, the obtained data evidences the demand for the continuation of systematic studies of wood materials collected in different regions and in different seasons in order to optimize the selection of the material and propose suitable recommendations on the material selection with the further reduction of production cost price of secoisolariciresinol (**2**).

Experimental

Samples of the wood of Siberian larch used as raw material were collected in Krasnoyarskij Krai (batches 1 and 2) and Khakassia (batches 3 and 4). Cross section knot areas were collected on different levels of the trunks and sawn lengthwise at a distance of 2.5 cm. The resulting sawn ends of the specified wood piece were marked depending on the level of sampling of the cross section sawn end and the number of the lengthwise sawn piece. Using the electric drill with a joiner´s gimlet the knot areas were drilled out to produce 1—3 mm chips, which were extracted in the following conditions: to a specimen of crushed wood an aliquot of 70% aqueous propan-2-ol (optimal proportion of extragent components was found earlier[8]) and the mix-

ture was kept at room temperature for c 2—3 h with occasional stirring; the extract was filtered off and analyzed by reversed phase HPLC[18] using the column ODSRegis "Regis Technologies Inc." (5 μm, 0.45×25 cm) (method A) or using a column ECC8 Analyt IBM (5 μm, 0.45×25 cm) (method B) in acetonitrile — water (1 : 4) which contained 1 ml L^{-1} trifluoroacetic acid at rate of 0.8 mL min^{-1} using UV-detector (280 nm), loading 0.01 mL onto a column. Qualititative characterization of the content of known components in the extract, which are DHQ (**1**), lignan **2** and flavonoid dihydrokaempferol (**3**) was based on the areas of corresponding peaks with the use of calibration curves plotted for analytically pure samples of these components. Using conversion equivalents which characterize the intensity of the absorption of each analyzed component the percentage of these compounds was calculated for extracts and starting wood samples.

For more detailed elucidation of the composition of the extractive mixture the reversed-phase HPLC was used (method C): column Diasfer-160-C18 (5 μm, 25×0.46 cm, BioKhimMak), gradient of organic solvent from 10 to 60% for 20 min (2 : 1 mixture of acetonitrile and methanol was used as organic phase and 0.05% aqueous trifluoroacetic acid as the aqueous phase), flow rate 0.8 mL min^{-1}, UV-detection (280/290 nm), temperature of the column 60 °C, volume of the sample 20 μL.

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