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REVIEW ARTICLE

Spiroflavonoid Compounds: Structure and Distribution in Nature Review

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This review summarizes the results of research aimed at isolation and structural characterization of flavonoid compounds belonging to a new class, namely, spirobiflavonoids, from different plant sources.

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INTRODUCTION

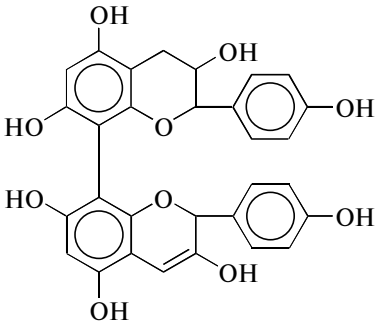
More than 9000 plant flavonoid compounds belonging to eight basic classes had been discovered and characterized by 2004 [1–9]. However, it is beyond doubt that even more flavonoids remain unknown and will be identified in the future [1] due to the improvement of the methods of isolation and analysis of natural compounds enabling the isolation of flavonoids belonging to new classes. Spirobiflavonoids, members of a relatively recently discovered class of flavonoids containing a spirane C-atom, serve as an example of this. Only 8 spirobiflavonoids from various organs of plants of four different families have been isolated.

Elucidation of the Structure of the Spirobiflavonoid Larixinol

L.T. Pashinina and colleagues isolated the bioflavonoid listvenol (**1**) from the bark of the Siberian larch *Larix sibirica* Ledeb. in 1973 [10]. The authors estimated the average content of listvenol in the bark as 0.1% and stated it to be the main bioflavonoid of Siberian larch bark. Using the results obtained by molecular spectroscopy methods available at that time (IR and ^{1}H NMR spectroscopy), the authors suggested a conjugated enol structure (1) for this compound. Pashinina et al. made the correct conclusion concerning the dimeric structure of this compound (judging by mass-spectrometry results) and the presence of (–)-epiafzelechin moiety and a second moiety containing a phloroglucin ring A and *para*-oxyphenyl ring B (judging by the results of alkaline cleavage). The presence of an unusual band at 1790 cm^{-1}, not characteristic of flavanes and absent from the spectrum of (–)-epiafzelechin in the IR spectrum of listvenol was attributed to the conjugated enol, and the presence of an additional one-proton singlet at δ 6.13 ppm in the ^{1}H NMR spectrum was attributed to the proton of the vinyl group.

Pashinina et al. are credited with isolating listvenol from the Siberian larch bark and determination of its content in the bark.

Z. Shen et al. succeeded in a more accurate determination of the listvenol structure in 1985, due to the use of more sophisticated analysis methods. They isolated it from the bark of Dahurian larch *Larix gmelinii* (Rupr.) Rupr. [11, 12] and named the bioflavonoid (**2**) larixinol, this being an exact translation of the name given by Pashinina et al.



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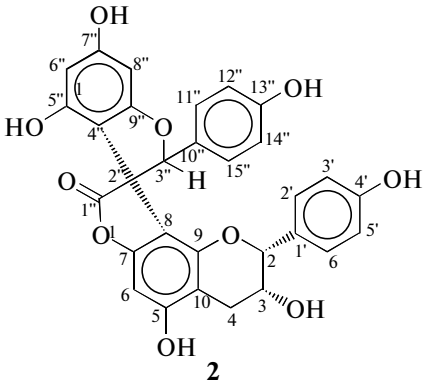

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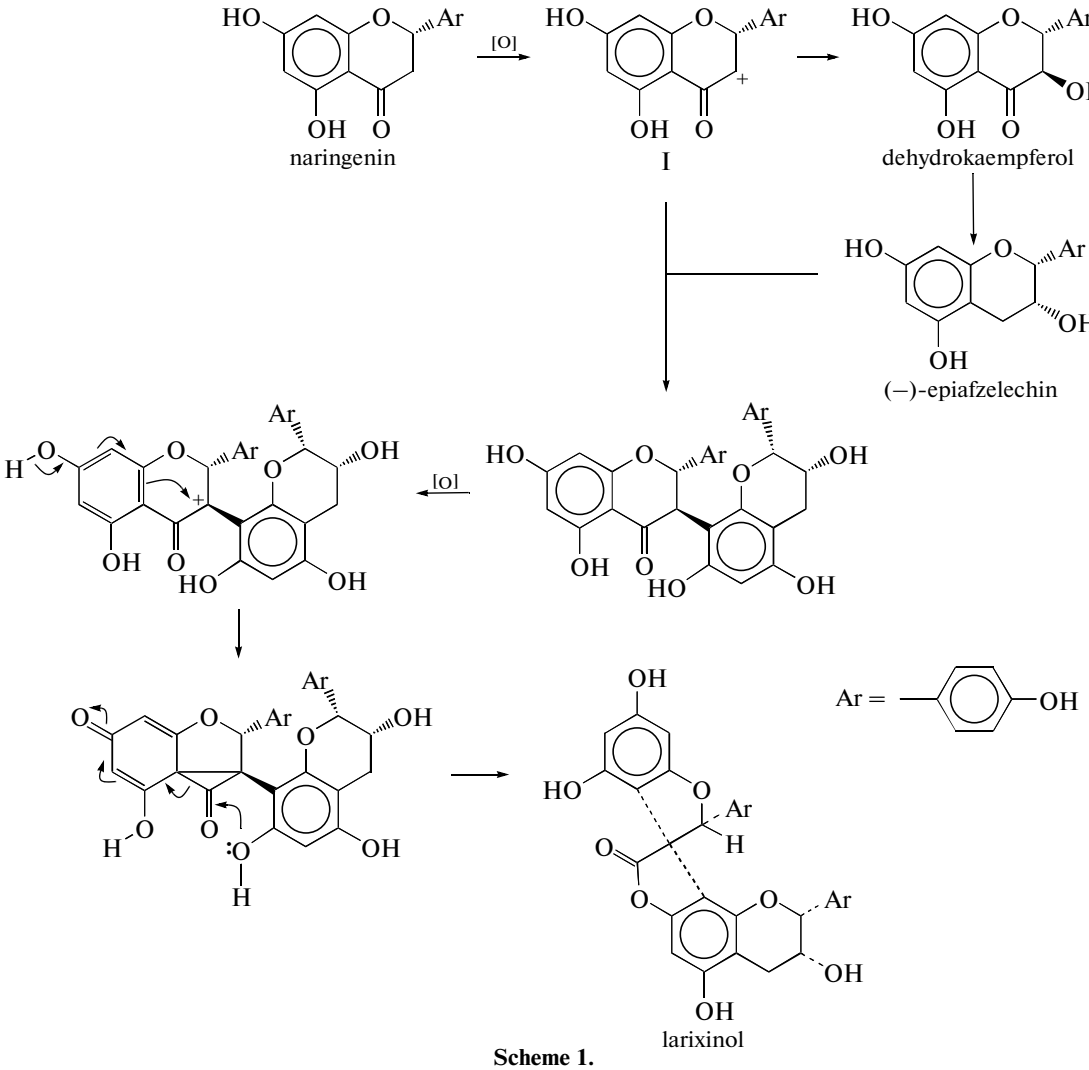
Shen et al. detected 30 signals of carbon atoms in the ^{13}C NMR spectrum of this compound and attributed 15 of these signals to the atoms of the (–)-epiafzelechin moiety of larixinol, while 6 of the 15 remaining signals were attributed to the monosubstituted phloroglucine ring and other 6 signals were attributed to the parahydroxysubstituted phenyl ring. An intense band at ~1810–1785 cm^{-1} in the IR spectrum of the spirobiflavonoid and its derivatives was attributed to the carbonyl group of γ-lactone cycle by the authors. Thus, the three remaining signals of carbon atoms were attributed to the carbon atom of the carbonyl group of the γ-lactone (C-1", signal at δ 179.1 ppm), to the methine carbon atom linked to the oxygen atom of the second heterocyclic moiety (C-3", signal at δ 91.0 ppm), and to the quaternary carbon atom of the spiro centre (C-2", signal at δ 61.1 ppm) [11, 12]. Consequently, the proton singlet at δ 6.13 ppm in the NMR spectrum of ^{1}H larixinol belongs to the proton bound to the methine carbon atom C-3", and not to the vinyl proton.

Thus, the structure of larixinol, the prototype spirobiflavonoid, was elucidated and the characteristic features of spectra of spirobiflavonoids containing a γ-lactone cycle were defined.

The Putative Pathway of Spirobiflavonoid Biosynthesis

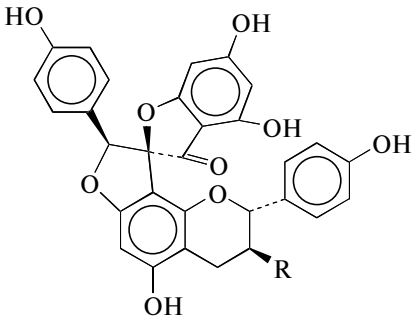
Shen and E. Haslam suggested a putative mechanism of larixinol formation from naringenin and (–)-epiafzelechin; this pathway is shown in Scheme 1 [12]. The authors showed that the spirobiflavonoid from larch bark belongs to the garcinium group of biflavonoids which contain a C-3–C-8' flavanon-flavanol bond. The biogenetic pathway suggested is based on the hypothesis stating that larixinol and biflavonoids of the garcinium type are formed due to an addition reaction involving the intermediate *I*; this reaction competes with the oxidation of the flavanone (naringenin in the case of larixinol) to yield a flavanonol (dihydrokaempferol). Subsequent recyclization of the biflavonoid containing the C-3–C-8' flavanon-flavanol bond yields biflavonoids of the spiro type (Scheme 1).



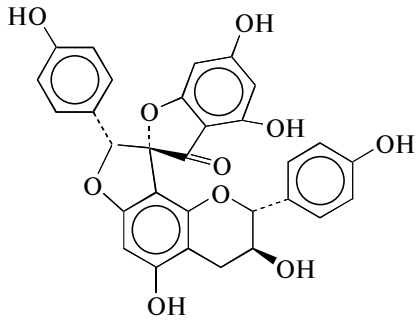
Scheme 1.

Spirobiflavonoid Distribution in Nature

By now, spirobiflavonoids have been detected in various organs of plants belonging to four families, namely, *Pinaceae*, *Thymelaeaceae*, *Vitaceae,* and *Agavaceae* (tribe *Yucceae*). The names of all spirobiflavonoids known by date were derived from the names of plants from which these compounds were isolated: daphnodorins C (**3**) and I (**4**) from the roots of *Daphne odora* Thunb. [13, 14], genquanol A (**5**) [13], vitisinol from the seeds of the grape *Vitis amurensis* Rupr. (**6**) [15], and yuccaone A (**7**) from the bark of *Yucca shidigera* [16].



R=H–**3**, R=OH–**4**



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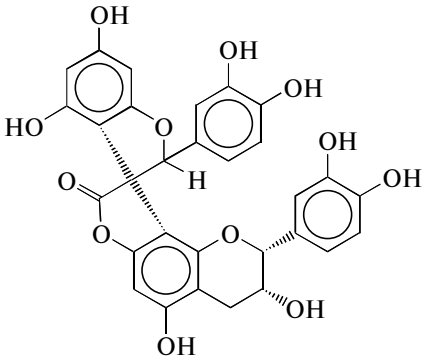

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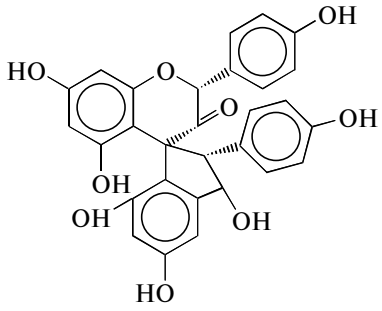

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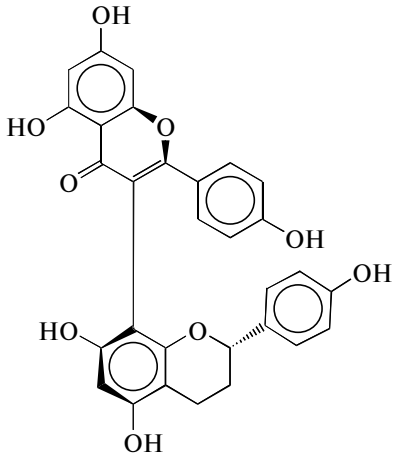
Beside spirobiflavonoids, atropoisomers daphnodorin D_1 (**8**) and daphnodorin D_2 (**9**), which may be the biogenetic precursors of daphnodorin C, were isolated from the roots of *Daphne odora* Thunb. Compounds **8** and **9** consist of apigenin and 5, 7, 4'-trihydroxyflavan connected by a C-3–C-8' flavone–flavane bond [14].



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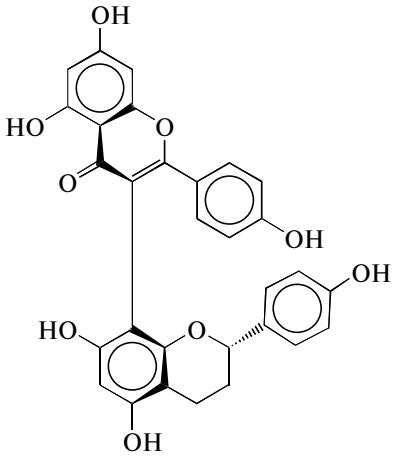

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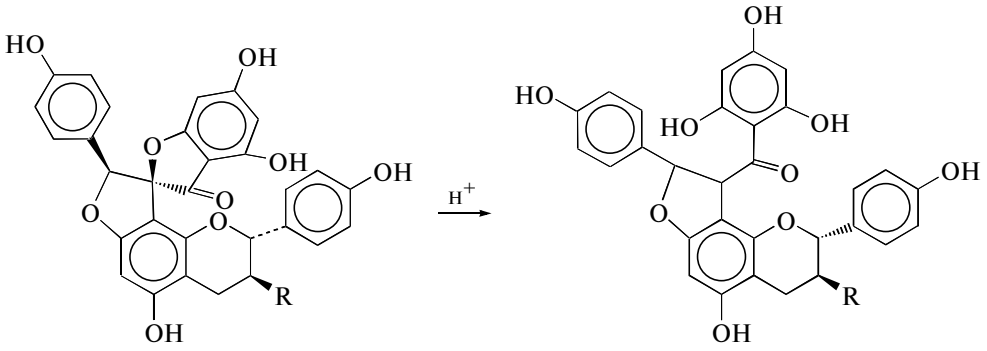
In addition, the authors showed that the spirobiflavonoids (**3**) and (**4**) could be transformed into biflavonoids—daphnodorins A (**10**) and B (**11**), respectively—upon heating in methanol acidified by small amounts of HCl; the abovementioned daphnodorins were also detected in *Daphne odora* Thunb. [13].



3–R=H,
4–R=OH

10–R=H
11–R=OH

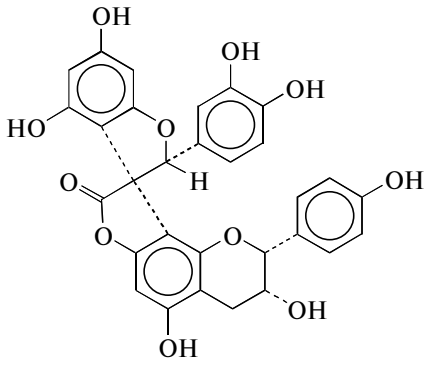
Thus, the authors succeeded in isolating the bioflavonoid compounds which probably are biogenetically related to spirobiflavonoids (daphnodorins), while only monomeric flavonoid precursors of spiro compounds were isolated from other plants. For example, larixinol precursors naringenin and (–)-epiafzelechin were detected in the bark of Siberian larch [17, 18].

Yuccaone A, as noted in [16], is a unique example of phenolic spiro derivatives composed of C_{15} and C_{14} structural subunits.

Among the above-listed spirobiflavonoids, only vitisinol is a structural analog of larixinol. The difference between these two compounds is in the type of substituent in the B ring: in larixinol this is *p*-hydroxyphenyl moiety, and in vitisinol, a pyrocatechine moiety; i.e., larixinol contains naringenin as the "upper" module and (–)-epiafzelechin as the "lower" module, while vitisinol contains eriodictyol and (–)-epicatechin, respectively.

Spiroflavonoid Compounds From the Bark of Siberian Larch and Dahurian Larch

The bark of Siberian larch (*Larix sibirica* Ledeb.), as well as the bark of Dahurian larch (*Larix gmelinii* (Rupr.) Rupr.), is a rich source of spirobiflavonoids. In addition to larixinol (**2**) [19], we have isolated three spirobiflavonoid compounds which are much less abundant in the bark than (**2**), namely, spirobiflavonoids larixidinol (**12**) [20] and larisinol (**13**) [21] and the trimer triflarixinol (**14**) [22], from the bark of these larch species.



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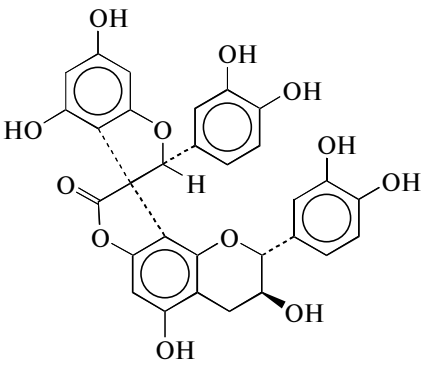

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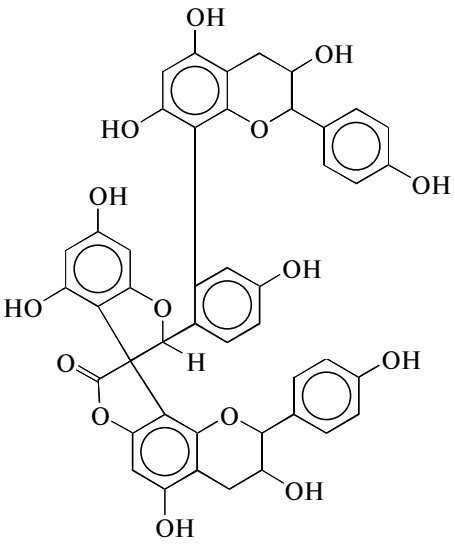

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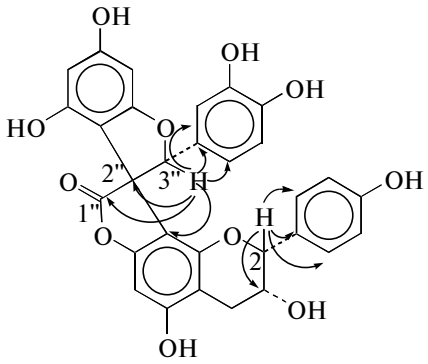
Chemical shifts of signals in the ^{13}C NMR spectra of the compounds 2, 6* and the acetate derivatives of larch bark spirobiflavonoids

Carbon atom	δ ^{13}C, ppm				
	2	6*	Ac 2	Ac 12	Ac 13
2	78.1	78.6	76.9	76.4	77.8
3	65.9	66.4	65.2	66.3	68.4
4	28.6	28.6	26.3	25.6	29.7
5	154.2	157.4	152.5	150.9	150.5
6	93.2	90.9	99.0	98.2	98.5
7	151.6	152.1	150.5	150.7	150.3
8	104.9	105.9	109.8	108.9	109.3
9	151.9	152.8	151.2	151.1	150.8
10	103.7	104.1	109.2	108.7	110.2
1'	129.7	131.2	133.6	134.1	135.0
2'	128.0	114.2	126.9	127.1	121.9
3'	114.4	145.3	121.8	121.0	141.4
4'	156.8	145.1	151.2	150.4	141.9
5'	114.4	115.6	121.8	121.0	122.9
6'	128.0	119.4	126.9	127.1	124.1
1"	179.9	179.1	176.8	176.0	175.8
2"	60.4	61.2	59.6	59.8	59.6
3"	90.4	94.2	89.3	90.8	90.6
4"	105.5	106.5	115.5	114.7	115.0
5"	156.9	154.8	146.8	146.8	146.9
6"	95.7	96.8	109.8	109.5	109.8
7"	160.2	160.9	152.9	152.8	152.9
8"	90.1	91.2	102.9	102.2	102.3
9"	163.9	163.5	162.0	161.3	161.2
10"	127.4	128.8	131.5	134.7	134.2
11"	126.6	113.9	126.9	120.6	120.6
12"	114.8	145.5	121.7	141.6	141.6
13"	156.8	145.2	151.2	141.8	141.8
14"	114.8	115.3	121.7	122.7	123.6
15"	126.6	118.1	126.9	123.1	123.1
*CH_3CO			19.4–21.1 (6 sign.)	19.7–21.2 (7 sign.)	19.9–21.1
CH_3*CO			168.2–171.3 (6 sign.)	166.6–170.4 (7 sign.)	165.9–169.6 (8 sign.)

* Published data [15].



Scheme 2.

The spirobiflavonoid larixidinol has a different type of side ring substitution and represents a derivative of eriodictyol and (–)-epiafzelechin. Larisinol is a stereoisomer of the spirobiflavonoid vitisinol, namely, a derivative of eriodictyol and (+)-catechin. The trimer triflarixinol is a product of condensation of the spirobiflavonoid larixinol and (–)-epiafzelechin. It is worth noting that all the sprirobiflavonoids detected fit the scheme of biogenetic relations of the flavonoids of larch biomass, since all their precursors were found in larch bark and wood [18, 19].

The spectral characteristics of the novel spirobiflavonoids of larch bark are similar to those of larixinol and vitisinol (see table) [15, 19–21]. An intense characteristic band of the carbonyl group of the γ-lactone cycle is present in the IR spectra of the acetates of compounds **12** and **13** in the 1785–1810 cm^{-1} region.

^{13}C NMR spectra of acetate derivatives of spirobiflavonoids **12** and **13** (see table) exhibit signals of carbon atoms of the lower flavane moiety: (–) epiafzelechin in the case of larixidinol and (+)-catechin in the case of larisinol, as well as the signals of the upper recyclized flavanone moiety, namely, signals of the phloroglucine ring (A-ring), 3',4'–dihydroxyphenyl B-ring, and the signals of three carbon atoms of the γ-lactone cycle [20, 21].

Complete attribution of signals in ^{1}H and ^{13}C NMR spectra was performed using two-dimensional spectroscopy, namely, the methods of HMQC, HMBC, COSY, and NOESY.

The key element of the HMBC spectrum is the correlation between the signal of the methine hydrogen atom H-3'' of the recyclized moiety and the H-2 proton of the flavane module (Scheme 2). Scheme 2 shows the key HMBC correlations of the signals of protons H-3'' and H-2 in larixidinol acetate. The H-3'' proton exhibits cross-peaks with the signals of quaternary carbon atoms of the carbonyl group of γ-lactone (C-1'') and the spirocenter (C-2''). In addition, cross peaks of this proton signal and the signals of carbon atoms, i.e., C-8 of the lower flavane-3-ol moiety and atoms of the B-ring of the upper recyclized module, are observed. The H-2 hydrogen atom gives cross-peaks with the carbon atoms C-1', C-2', and C-6' of the B-ring, and the C-3 carbon atom of the hetero ring of the lower moiety.

Upon the elucidation of vitisinol structure J.-N. Wang and coauthors [15] noted that the angular structure of spirobiflavonoids **12** and **13** is proven by the presence of a cross-peak between the H-2 and H-15'' protons in the NOESY spectra (δ 4.55 and 6.73 ppm, respectively, in the case of larixidinol [20]).

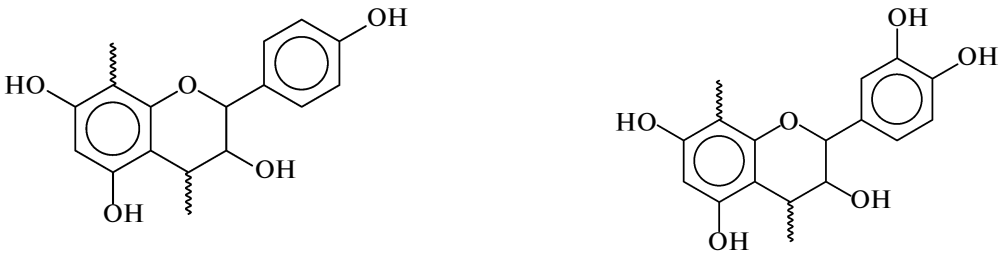
The IR spectrum of triflarixinol acetate also exhibits an intense characteristic band of the carbonyl group of the γ-lactone cycle ν(C=O) in the 1785–1810 cm^{-1} range.

^{1}H and ^{13}C NMR spectra of the acetate of the trimer **14** contain all signals characteristic of larixinol acetate, as well as some additional signals indicative of the presence of a flavan-3-ol moiety—(–)-epiafzelechin—in the molecule **14** also known to contain a larixinol moiety [22]. The interflavane bond in triflarixinol involves the C-8 carbon atom of the *A*-ring of the (–)-epiafzelechin module and the C-11'' (or C-15'') carbon atom of the B-ring of larixinol upper module.

Structural Peculiarities of Oligomeric and Polymeric Flavonoid Compounds from the Bark of Siberian Larch and Dahurian Larch

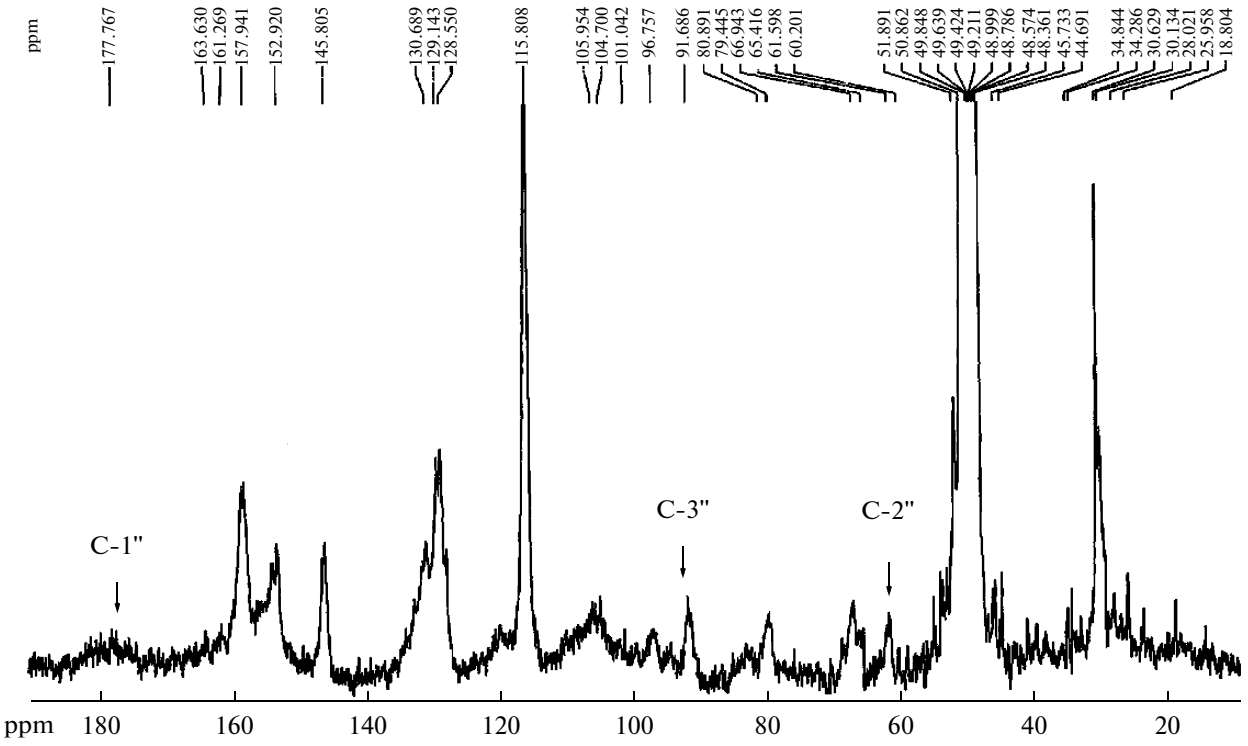
Analysis of the fractions of oligomeric and polymeric flavonoid compounds constituting 40–60 mass % of the extractive compounds of larch bark shows that the oligomeric fraction consists of propelargonidine (*PP*) modules with a *p*-hydroxyphenyl type of B ring substitution and procyanidine (*PC*) modules with a pyrocatechin type of B ring substitution [19]. This is proven by the presence of signals at 115.8, 128.6, and 157.8 ppm of the B-rings of *p*-hydroxyphenyl type and signals at 115.8, 145.8, and 119.6 ppm of the B-rings of the pyrocatechin type of the flavonoid modules in the ^{13}C NMR spectra of the oligomeric and polymeric fractions (see figure).

A study of the oligomeric and polymeric fractions of the polyphenol complex of larch bark by IR spectroscopy and ^{13}C NMR spectroscopy revealed the presence of spirobiflavonoid diagnostic signals (ν(C=O) in the 1785–1810 cm^{-1} range and signals with chemical shifts (CS) of 61.9, 91.6, and 177.8 ppmin the ^{13}C NMR spectra), this being indicative of the presence of spirobiflavonoid moieties in these compounds (see figure).



PP

PC



^{13}C NMR spectrum of the oligomeric flavonoid compounds from Siberian larch bark.

The discovery of triflarixinol (*14*) and elucidation of its structure are, consequently, important steps in structural research on those biologically active natural polymers, because they demonstrate one of the ways of spirobiflavonoid module incorporation into polymers.

Therefore, the presence of spirobiflavonoid modules (beside the monomeric flavan-3-ols) in the oligomeric and the polymeric flavonoid compounds (condensed tannins) of Dahurian larch and Siberian larch bark are a distinctive feature of this tree species demonstrated by us for the first time [19].

Methods of Spirobiflavonoid Isolation

Spirobiflavonoids are usually extracted by polar solvents from plant raw material, such as bark, seeds, and roots, together with other phenolic compounds. Various chromatographic methods are used for the isolation of spirobiflavonoids from total phenol extracts.

For example, acetone extraction of *Larix sibirica* bark deresinated with benzene was used as the first step of listvenol isolation [10]. The residue from acetone extract evaporation was dissolved in an aqueous solution of sodium bicarbonate, and the flavonoids were extracted by ether. The ether extract was chromatographed on cellulose columns, and a fraction containing listvenol together with small quantities of catechins was obtained. Listvenol purification was achieved by rechromatographing this fraction several times under the same conditions. The average listvenol content in the bark equaled 0.1%.

The procedure for larixinol (2) extraction from the bark of *Larix gmelinii* differed from the one described above. The authors of [11, 12] obtained the acetone

extract of larch bark in a similar way, but then they dissolved it in water and heated it in order to evaporate acetone. The aqueous solution was extracted by chloroform and then by ethyl acetate. Further fractionation of the ethyl acetate extract by column chromatography on Sephadex LH-20 and rechromatography on silicagel yielded larixinol. The yield of **2** equaled 0.14% of the bark mass.

Daphnodorins C (**3**) and I (**4**) were isolated from the ethyl acetate extract of the roots of *Daphne odora* Thunb. [13, 14] by column chromatography on silicagel with hexane–ethyl acetate as the eluent and sequential rechromatography of the fractions on silicagel with chloroform–methanol as the eluent and then on Sephadex LH-20 with methanol as the eluent. The daphnodorin (**4**) yield from 4.5 kg of *Daphne odora* roots equaled 0.3 g [13].

The preparation of vitisinol (**6**) [15] involved fractionation of the ethanol extract of the seeds of grapes *Vitis amurensis* Rupr. in water–chloroform and then in water–ethyl acetate systems. Sequential fractionation on Diaion HP-20 and gel filtration of the ethyl acetate-soluble part of the extract with subsequent HPLC purification enabled the authors to isolate several compounds including vitisinol. The yield of the spirobiflavonoid *6* equaled 0.01 g per 10 kg of the raw material.

The scheme of yuccaon A (**7**) isolation from the bark of *Yucca shidigera* [16] involved the extraction of the plant raw material by methanol and subsequent fractionation of the concentrated methanol extract on a column with Sephadex LH-20 with methanol as the eluent. Yuccaon A was obtained from these fractions by chromatography on a reverse-phase (C_{18}) column. Acetonitrile solution (22% in H_3PO_4) was used as the eluent. The yield of compound **7** equaled 0.012 g from 0.1 kg of bark.

Spirobiflavonoids larixinol (**2**), larixidinol (**12**), and larisinol (**13**) and the trimer triflarixinol (**14**) were isolated from the ethyl acetate extracts of the bark of Siberian larch and Dahurian larch [19–22]. For this, the ethyl acetate extract was fractionated on a silica gel column with gradient elution by methanol (1–100%) in chloroform. One of the fractions obtained contained spirobiflavonoids. Rechromatography of this fraction was used to isolate substances enriched by various components. The substances were acetylated and separated by column chromatography on silica gel in the benzene–aceton system. All spirobiflavonoids, excluding larixinol, were isolated and identified as completely acetylated derivatives (~15–20 mg) [20–22].

The Physiological Activity of Spirobiflavonoids

Information on the biological activity of individual spirobiflavonoid compounds is still missing, probably due to the low content of these compounds in plants [13–15].

Detailed studies of the chemical composition of the polyphenol complex (PFC) extracted by ethyl acetate from larch bark showed that it is a complex mixture of phenolic compounds [19]. The PFC can be chromatographically fractionated into the following fractions: I, phenolic acids and their esters; II, monomeric flavonoids; III, spiroflavonoids; and IV, oligomeric and polymeric flavonoid compounds. The content of these fractions in PFC from larch bark equaled 7–10% for fraction I, 12–15% for fraction II, 35–40% for fraction III, and 40–45% for fraction IV [24].

Toxicological and pharmacological studies performed in the laboratory of pharmacology of NIOCh, Siberian Branch, Russian Academy of Sciences (Novosibirsk) revealed a strong strengthening action of PFC on capillaries; this action was 1.2–1.4 times stronger than that of dihydroquercetin (DQ), which is well known as a powerful antioxidant and capillary protector [25, 26]. The antioxidant effect of PFC was slightly smaller than or equivalent to that of DQ, and the hepatoprotective effect was much higher, with the anticholestase properties being on average two times higher than those of DQ.

The PFC of larch bark was also shown to have gastroprotective properties. A biologically active food additive, Piknolar, based on this complex was patented [25].

The high spirobiflavonoid content in larch bark will allow for the isolation of these compounds in sufficient quantities and research on the biological activity of this unique class of flavonoid compounds in the future.

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