refluxed for 3 min. On cooling, the benzaldehyde was extracted with ether (2×5 mL), and the aqueous layer was alkalized with a solution of NaOH to a strong alkaline reaction. The *N*-alkylaniline that formed was extracted with ether (3×5 mL), and the extract was analyzed by GLC with hexadecane as the internal standard. Calibration was performed with known mixtures for each monoalkylaniline obtained and the standard.

***N*-Butylaniline (6).** The reaction was carried out with the use of benzalaniline (**1a**) and *n*-butyl chloride (**2d**) according to the above procedure, except that the ethereal solution of *N*-butylaniline was dried with anhydrous $K_2CO_3$ and treated with dry HCl. The *N*-butylaniline hydrochloride that formed was recrystallized from ethyl acetate. Yield 76%, m.p. 114—115 °C (*cf.* Ref. 7: m.p. 115 °C).

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# Seasonal dynamics of distribution of isoprenologs of bound polyprenols and dolichols in leaves and branches of *Alnus glutinosa* (L.) Gartn.

***V. I. Roshchin,[a]* V. A. Raldugin,[b] O. Yu. Poverinova,[a] N. Yu. Nagibina,[a] L. I. Demenkova,[b] and T. P. Kukina[b]***

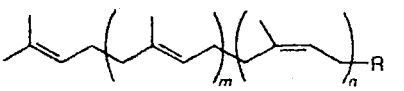
*[a]Saint Petersburg Academy of Forestry Engineering,*
*5 Institutsky per., 194018 Saint Petersburg, Russian Federation.*
*Fax: +7 (812) 550 0815*
*[b]Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences,*
*9 prosp. Akad. Lavrent'eva, 630090 Novosibirsk, Russian Federation.*
*Fax: +7 (383 2) 35 4752. E-mail: raldugin@nioch.nsc.ru*

Polyisoprenoid alcohols (polyprenols and dolichols) from leaves and branches of European alder (*Alnus glutinosa* (L.) Gartn.) were studied by $^1$H NMR spectroscopy and HPLC. Data on dynamics of relative monthly (May—August) content of each isoprenolog of polyprenols and dolichols were obtained.

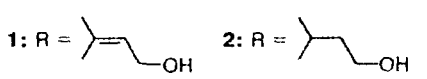
**Key words:** polyisoprenoids, polyprenols, dolichols, high performance liquid chromatography, $^1$H NMR spectra.

Acyclic polyisoprenoids, polyprenols, are usual constituents of a set of chemical compounds found in plant cells. Polyprenyl pyrophosphates play an important physiological function in plants, participating in biosynthesis of oligosaccharides and glycoproteins.[1] 2,3-Dihydroderivatives of polyprenols (dolichols),[2—4] which are present in animal but rarely in plant organisms, attract more and more attention of researchers due to the importance of these compounds for development of living organisms.[5] Polyprenols and their derivatives are characterized by a wide spectrum of biological activity, described in the review.[6]

The molecular structures of polyprenols and dolichols, which are usually found in nature as a mixture of isoprenologs, are represented by formulas **1** and **2** respectively, where *m* is usually 2 for polyprenols of conifers and dolichols and 3 for polyprenols of deciduous plants, and *n* varies within a rather wide range.[7] In plants polyprenols occur as free alcohols, acetates,[8] or esters of higher fatty acids,[3,9] and dolichols usually occur as esters.[2—4]




**1, 2**

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While doing phytochemical investigation of polyprenols and dolichols of ligneous plants[3,4] we studied novel polyisoprenoid compounds from leaves and defoliated branches of European alder (*Alnus glutinosa* (L.) Gartn.), widespread in Russia.[10] According to TLC data an ethanol extract from the above raw material contains polyisoprenoids as esters. To isolate a group of polyisoprenoid alcohols and to study the seasonal dynamics of distribution of isoprenologs of these alcohols in the mixture by HPLC we used a procedure described below. A weighed sample of ground freshly collected raw material was fully extracted with ethanol using Soxhlet apparatus, followed by isolation of the nonpolar part (soluble in petroleum ether) of the dried ethanol extract. The next steps were isolation of a fraction of polyisoprenoid esters from the petroleum ether extract, alkaline hydrolysis, and then chromatographic purification of the mixture of alcohols.

$^1$H NMR spectra of the isolated fractions were similar to those of polyprenols from leaves of sea buckthorn *Hippophae rhamnoides*[3] or needles of cedar.[4] The ratio of integral intensities of signals at δ 1.59, 1.67, and 1.72, which correspond to protons of Me groups at *trans*- and *cis*-double bonds and to protons of a Me group of the α-unit ($H_3C$—C(3)), was 3.2 : 7.7 : 1.0. Hence,[4,8] like betulaprenols,[11] polyprenols of the genus *Betula* (birch), botanically related to alder, molecules of the major polyprenols from leaves and branches of alder consist of 11—12 isoprene units and contain two *trans*-double bonds. The spectra under consideration also contain a signal of low intensity at δ 3.65, which indicates[12] the presence of dolichols in the isolated fractions.

The content of polyisoprenoid alcohols of alder is lower than that of conifers,[4,11] it varies from 0.001 to 0.009% in leaves and from 0.005 to 0.030% in branches, and from May to August it is gradually decreasing both in leaves and branches from the maximum value to the minimum one. Polyisoprenoid alcohols were not found in leaves collected in September. Fractions of polyisoprenoid alcohols isolated from leaves and branches at different collection times were analyzed by the generally accepted for these compounds HPLC method using a reversed phase column[8] (Table 1). Identification of components of the group of polyprenols was done by adding an authentic sample of pentadecaprenol ($C_{75}$); dolichols were identified by comparison of obtained chromatograms with those for leaves of sea buckthorn[3] and needles of cedar.[4]

As can be seen from the data in Table 1 the content of polyprenol mixture from branches insignificantly changes from May to August, and interesting age changes are observed in leaves: polyprenols with lower ($C_{55}$) and higher ($C_{70}$) molecular weights are disappearing. The relative content of the major polyprenol ($C_{65}$) of the fraction of polyisoprenoid alcohols is continuously increasing but it also disappears before leaf-fall (September). Although the total amount of polyprenols in branches is decreasing, their variety with respect to molecular weight remains practically unchanged within the observation period.

The dolichol of animal tissue contains from 16 to 21 isoprene units (it is a mixture of isoprenologs with a number of C atoms in a molecule from 80 to 105).[13] It is interesting to note, that the dolichol of alder is less similar to the dolichol of animal tissue with respect to distribution of isoprenologs than the polyisoprenoids of this type of conifers.[3,4]

**Table 1.** Polyprenol and dolichol content (%) of fractions of polyisoprenoid alcohols from leaves and branches of *Alnus glutinosa* (L.) Gartn. during a vegetation period from May (V) to August (VIII)

| Isoprenolog* | Leaves | | | | Branches | | | |
|---|---|---|---|---|---|---|---|---|
| | V | VI | VII | VIII | V | VI | VII | VIII |
| $C_{25}$ | 0.7 | 4.0 | — | — | 0.2 | 0.6 | 2.3 | — |
| $C_{30}$ | 2.6 | 0.4 | — | — | 0.6 | 1.2 | 2.5 | — |
| $C_{35}$ | 4.6 | 0.3 | — | — | 2.3 | 2.0 | 3.9 | Traces |
| $C_{40}$ | 8.7 | 1.6 | — | — | 4.7 | 6.2 | 8.2 | Traces |
| $C_{45}$ | 7.5 | 0.5 | Traces | — | 10.7 | 7.0 | 5.1 | 1.9 |
| $C_{50}$ | 15.6 | 10.3 | Traces | — | 8.5 | 10.1 | 12.7 | 5.9 |
| $C_{55}$ | 12.1 | 2.9 | 4.5 | 6.0 | 15.1 | 14.0 | 12.0 | 11.8 |
| $C_{55}$(D) | 0.5 | Traces | — | — | 0.4 | 0.5 | 0.7 | Traces |
| $C_{60}$ | 8.7 | 13.5 | 14.4 | 9.2 | 18.0 | 17.8 | 15.3 | 14.7 |
| $C_{60}$(D) | 0.5 | Traces | — | — | 0.8 | 1.0 | 1.4 | Traces |
| $C_{65}$ | 21.1 | 54.3 | 74.6 | 79.6 | 25.2 | 24.9 | 18.8 | 30.3 |
| $C_{65}$(D) | 0.4 | Traces | — | — | 2.3 | 3.0 | 3.7 | Traces |
| $C_{70}$ | 5.6 | 10.9 | 6.5 | 5.2 | 8.5 | 8.5 | 8.2 | 29.5 |
| $C_{70}$(D) | 2.3 | Traces | — | — | 1.3 | 2.0 | 3.7 | Traces |
| $C_{75}$ | 2.5 | 1.2 | Traces | — | 1.3 | 1.2 | 1.6 | 5.9 |
| $C_{75}$(D) | 1.4 | Traces | — | — | — | — | — | — |

* D — dolichol.

## Experimental

Adsorption chromatography was performed on air-dried silica gel L (Czech Republic, 100—160 μm, $SiO_2$), eluent — petroleum ether (b.p. 40—60 °C) with the addition of $Et_2O$ (from 0 to 10%).

$^1H$ NMR spectra were recorded for solutions in $CDCl_3$ with a Bruker WP-200 spectrometer (δ $CHCl_3$ 7.24 relative to $Me_4Si$). HPLC was performed with a Milichrom microcolumn liquid chromatograph using a column with a reversed phase of Lichrosorb RP-18 (5 μm, Merck), eluent — a mixture of $Me_2CO$—MeOH (60 : 40), UV detection at λ = 210 nm. TLC was performed on Silufol plates; spots were visualized with conc. $H_2SO_4$.

**Sample selection and preparation of extracts.** Samples of leaves and branches of European alder were collected in Leningrad Region from May to August 1988 (on the 25—28 of each month) from the same 20 model trees. Branches were defoliated manually; leaves and branches were separately ground with a laboratory grinder. Moisture content of the ground samples was determined by drying them in an oven at 105—110 °C to a constant weight.

**Isolation of a mixture of polyisoprenoid alcohols (general procedure).** Extraction of a weighed portion of crude ground leaf (branch) material (500—700 (±0.5) g) with 96% ethanol was carried out in Soxhlet apparatuses for 10 h. Ethanol extract was filtered, concentrated to ~0.1 L, and treated with petroleum ether (3×0.1 L). The extracts were combined and concentrated to dryness. The residue was subjected to chromatography on 30 g of $SiO_2$ to give a mixture of nonpolar components of the extract (eluent — petroleum ether + 4% $Et_2O$); the latter was stirred under reflux with 10 mL of 3% KOH in EtOH for 1 h. The hydrolyzate was cooled to ~20 °C, diluted with water (40 mL), and extracted with $Et_2O$ (3×50 mL). The combined extract was concentrated to dryness and the residue was subjected to chromatography on $SiO_2$; an authentic mixture of polyprenols and dolichols from leaves of sea buckthorn[3] was used as the TLC reference. Eluate containing polyisoprenoid alcohols (petroleum ether + 10% $Et_2O$) was concentrated to dryness; the residue was weighed and used for HPLC. The results are given in Table 1.

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