NEUTRAL COMPOUNDS OF THE EXTRACTIVE SUBSTANCES OF Larix gmelini

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The composition of the neutral fraction of the resinous substances of the wood of the Dahurian larch growing in the region of BAM [Baikal Amur Railway] has been investigated. About 40 terpenoids have been identified, and the compositions of the saturated hydrocarbons and aliphatic alcohols have been determined (GLC). The main components of the neutral substances are bicyclic compounds with the labdane structure: epimanool, and larixol and its monoacetate, making up about one-third of the neutral substances. In contrast to the species of larch studied previously, in the Dahurian larch we found squalene, cycloartenone, and pimosylvin dimethyl ether; in contrast to the Siberian larch, this species contained no epitorulosol and its derivatives.

The extractive substances of Larix gmelini (Rupr.) Rupr. (Dahurian larch) are attracting attention in connection with its use on an increasing scale for obtaining cellulose pulp by the sulfate method. It is known that the neutral components of the extractive substances of the wood may have an extremely powerful influence on the resin content of the pulp obtained from it and on the quality of the by-products of wood chemistry, which are becoming increasingly valuable and in short supply. In order to predict the nature of this influence, we must know the composition of the neutral substances, and this will also permit an estimation of the possibility of obtaining from them new products of wood chemistry, including biologically active substances: sterols, triterpene alcohols, etc.

At the present time, the oleoresins of various species of larch have been studied in detail [1-4]. There is information on the composition of the terpenoids of the extractive substances of the trunk part of the European larch [5] and the heart and sapwood [6] and the bark [7, 8] of the Dahurian larch.

TABLE 1. Fractional Composition of the Neutral Substances of the Wood of the Larch

Eluent*	Fraction No.	Amount in the extractive substance		Class of compounds
		g	%	
PE	1	0,1682	1,82	Hydrocarbons
PE—DE, 98:2	2	0,1303	1,41	The same
PE—DE, 96:4	3	2,6065	28,22	Esters, aldehydes
PE—DE, 90:10	4	0,4990	5,40	Alchohols
PE—DE, 85:15	5	2,6624	28,82	Alchohols and ether-alchohols
PE—DE, 75:20	6	0,6313	6,84	Alchohols
PE—DE, 65:35	7	0,4821	5,21	Sterols
PE—DE, 50:50	8	1,5618	16,91	Diols
DE	9	0,4967	5,37	Polyhydroxy compounds
Total		9,2383	100,00	
Weight of the initial substances		9,9722		

*PE — petroleum ether; DE — diethyl ether.

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TABLE 2. Component Composition of the Neutral Fraction of the Resinous Substances of the Larch Wood

Component	Amount, % by wt.
Hydrocarbons	4.36
Paraffins, including	1.69
n-Alkanes (C_{14}-C_{27})	1.48
Hexadecane	0.12
Heptadecane	0.21
Octadecane	0.25
Nonadecane	0.22
Eicosane	0.11
C_{14}-C_{15} and C_{21}-C_{27} (total)	0.57
Terpenes, including:	2.67
Monoterpenes	0.42
α-Pinene	0.22
β-Pinene	0.16
Δ^3-Carene	0.04
Sesquiterpenes	0.42
Longifolene	0.13
Bisabolene	0.03
δ-Cadinene	0.14
γ-Cadinene	0.04
Diterpenes	1.27
Abietadiene	0.55
Dehydroabietadiene	0.47
Cembrene	0.02
Triterpenes (squalene)	0.56
Aldehydes	3.40
Pimarinal	0.37
Δ^8-Isopimarinal	0.62
Levopimarinal (palustral)	1.22
Isopimarinal	0.02
Dehydroabietinal	0.05
Neoabietinal	0.68
Unidentified aldehydes	0.44
Ketones (cycloartenone)	1.45
Resin acid methyl esters, including:	0.25
Methyl dehydroabietate	0.21
Methyl isopimarate	traces
Oxides, including:	1.22
Epimanool oxide	0.69
Aromatic compounds, (pinosylvin dimethyl ether)	0.22
Products of the alkaline hydrolysis of the esters, including:	20.55
Acids	9.44
Alcohols, including:	11.11
Aliphatic saturated	0.59
Terpenic, including:	10.52
Cycloartenol	3.99
24-Methylenecycloartanol	0.22
Sterols, including:	4.40
β-Sitosterol	3.04
Campesterol	1.36
Unidentified alcohols	1.91
Larixyl acetate	20.08
Alcohols, including:	43.10
Saturated aliphatic (C_{13}-C_{28})	1.37
Behenyl (C_{22})	0.81
Lignoceryl (C_{24})	0.44

TABLE 2 (continued)

Component	Amount, % by wt.
Monoterpenyl	0.38
Borneol	traces
α-Terpineol	0.38
Diterpenyl	8.13
Bicyclic (epimanool)	3.64
Tricyclic	3.96
Levopimarinol (palustrol)	1.19
Isopimarinol	2.24
Abietinol	0.11
Dehydroabietinol	0.42
Triterpenyl	1.23
Cycloartenol	0.98
24-Methylenecycloartanol	0.25
Sterols	2.36
β-Sitosterol	1.63
Campesterol	0.73
Unidentified tertiary alcohols	5.04
Diols	24.59
Larixol	4.00
Unidentified diols	20.59
Polyhydroxy compounds	5.37
Total	100.00

The present paper contains a more complete analysis of the chemical composition of the neutral fraction of the extractive substances of the Dahurian larch. Information has been published previously on the amount of extractive substances in the tree concerned, on the distribution of the extractive substances over the height of the trunk and over its cross section, and their group compositions [9, 10].

By column chromatography, the neutral substances were separated into nine fractions differing in the polarity of the compounds and consisting of hydrocarbons, aldehydes, esters, alcohols, and polyhdyroxy compounds (Table 1). By using the elements of a scheme for the group separation of conifer oleoresins [15], the fractions obtained were separated into groups of related compounds and individual components (Table 2).

Fraction 1 consisted of saturated hydrocarbons (93.1%) and terpene hydrocarbons. According to GLC, the saturated hydrocarbons formed a complex mixture containing more than 30 components. Alkanes with normal chains of carbon atoms amounted to 78.5%. The alkanes present in the greatest amount were the C_{17}, C_{18}, and C_{19} compounds (12.5, 14.5, and 12.7%, respectively). Fraction 2 contained terpene hydrocarbons, and these were separated by column chromatography into mono-, sesqui-, di-, and triterpenes. The last group was represented by a single component —squalene — which was isolated and identified by spectral methods. We have also detected squalene in the neutral substances of the black liquor from the boiling of larchwood [11]. Previously, so far as concerns coniferous species, squalene had been isolated only from the pine and the products of its processing [12, 13]. The main components of the monoterpene hydrocarbons were α-pinene, β-pinine, and Δ^3-carene. The sesquiterpenes consisted of six compounds, four of which were identified: longifolene, δ-cadinene, γ-cadinene, and bisabolene. The diterpenes comprised eight compounds, of which abietadiene and dehydroabietadiene made up more than 80%, the others, among which cembrene was identified, being present in very small amounts.

Fraction 3 had a complex composition. Its IR spectrum showed absorption bands characteristic for terpene aldehydes and esters. After its saponification with ethanolic alkali followed by chromatography on silica gel, three groups of substances were obtained: alcohols (41.0%) and acids (34.9%) — the products of the saponification of the esters — and unsapon-

ifiable substances (24.1%). The alcohols were separated by column chromatography on SiO_2/$AgNO_3$ into aliphatic and triterpene alcohols and sterols. According to GLC, the aliphatic alcohols consisted of compounds with odd and even numbers of carbon atoms in the chains of the molecules. The main components were alcohols with even numbers of carbon atoms – C_{16}, C_{18}, C_{22}, and C_{24}. From the triterpene alcohol fraction we isolated cycloartenol and 24-methylenecycloartanol. The sterols consisted of β-sitosterol and campesterol (69.01 and 30.99%, respectively).

The unsaponifiable part of fraction 3 was reduced with sodium tetrahydroborate, as a result of which the aldehydes were reduced to alcohols, and these were separated from the unreduced part by column chromatography. The main components of the aldehydes were levoprimarinal (palustral), Δ^8-isopimarinal, pimarinal, and neoabietinal (GLC). A considerable part of the reduction products consisted of a component the retention time of which was greater than that of the diterpene compounds. This compound was isolated by column chromatography and was identified as cycloartenol, which could have been obtained in the reduction of cycloartenone. There is information in the literature on the presence of a ketone of this structure – 24-methylenecycloartenone – in the extractive substances of the Siberian spruce [14].

Among the components of the unreduced part we identified epimanoyl oxide, methyl dehydroabietate, methyl isopimarate, and pimosylvin dimethyl ether.

According to IR spectroscopy, fraction 4 consisted of alcohols. After acetylation followed by chromatography, they were separated into acetylatable (primary and secondary) and unacetylatable (tertiary). The latter formed the bulk of the initial fraction and consisted mainly of two components, one of which was identified as epimanool. The acetylatable alcohols were analyzed together with the acetates of fraction 5.

Fraction 5 made up about one-third of the weight of the neutral substances. In its IR spectrum there were absorption bands at 1240, 1730, and 3620 cm^{-1}, which indicated the presence in it of alcoholic and ester groupings. In view of the chromatographic characteristics of the fraction, it was assumed that it could contain not only monohydric alcohols but also incomplete esters of polyols. The acetylation of the fraction gave two groups of substances: alcohol acetates (24.8%) and unacetylatable alcohols (75.2%). The former were separated into acetates of aliphatic, diterpenyl, and triterpenyl alcohols by column chromatography on SiO_2/$AgNO_3$. The aliphatic alcohols consisted of a complex mixture of compounds of normal structure with from 13 to 28 carbon atoms (GLC). The main components were behenyl (C_{22}) and lignoceryl (C_{24}) alcohols. Among the terpene alcohols levopimarinol (palustrol), isopimarinol, abietinol, dehydroabietinol, cycloartenol, and 24-methylenecycloartanol were identified (PMR and GLC). Larixol acetate (94%) and α-terpineol (identified by PMR) were isolated from the unacetylatable substances of the fraction.

After acetylation, fraction 6 was separated into three groups of substances: sterol acetates, diol acetates, and tertiary alcohols. The sterols consisted of β-sitosterol (68.9%) and campesterol (31.1%). The composition of the diols and tertiary alcohols was not established.

Fraction 7 consisted mainly of larixol (76.9%) which, after the acetylation of the fraction followed by chromatography on silica gel, was isolated in the form of its monoacetate. It was identified by spectral methods.

Fraction 8 consisted of a complex mixture of compounds which, on acetylation, gave diacetates, monoacetates, and unacetylatable tertiary diols. Their composition will be reported separately.

EXPERIMENTAL

Samples of larchwood were collected in the basin of the River Olekma, approximately 300 km northwest of the town of Tynda, on experimental plots the assessment characteristics of which corresponded to the average indices of the raw timber basis. From each of seven trunks we took samples consisting of transverse sections with a thickness of 20-30 mm, which were debarked and cut in a guillotine into chips with dimensions of 20 × 30 × 5 mm and were then comminuted in chopping machines to the state of sawdust. For the investigation we used the 0.5-1.0 mm fraction, making up 80% of the sawdust.

Isolation of the Extractive Substances. The amount of extractive substances was determined by extraction in Soxhlet apparatuses with a capacity of 40 ml for 8 h. The prepared

wood contained 1.5% of ether-soluble substances and 2.9% of substances extractable by ethanol–benzene (1:1). To study the component composition we used the ether-soluble fraction of the ethanol–benzene extract, which was isolated in the following way. Each portion of raw material (about 70 g) was extracted in Soxhlet apparatuses with a capacity of 500 ml for 48 h in ethanol–benzene (1:1). The solvent was distilled off from the extract obtained at 40°C under a residual pressure of 12-20 mm Hg until the volume of the residue in the flask was about 10 ml. To this was added 500 ml of diethyl ether and the resulting precipitate was filtered off, after which the filtrate was dried with anhydrous Na_2SO_4 and the solvent was distilled off at $t \leq 40°C$ under reduced pressure. The yield of the ether-soluble fraction of the ethanol–benzene extract amounted to 2.3% of the absolutely dry wood. To separate the free acids, the extract obtained was treated with a 5% solution of KOH. The amount of neutral substances was 28.3% of the ether-soluble fraction [9].

Column Chromatography. The neutral substances (9.97 g) were separated by column chromatography (glass column, 3 × 120 cm) on type L 40/100 silica gel (Czechoslovakia). Petroleum ether with increasing amounts (from 2 to 100%) of diethyl ether was used as the eluent. Samples of eluate (15 ml) were analyzed by TLC (Silufol plates with petroleum ether–diethyl ether as eluent) and were combined in accordance with the R_f values of the spots and their coloration on treatment with concentrated sulfuric acid (Table 1). According to IR spectroscopy, fractions 1 and 2 consisted of hydrocarbons (absence of absorption bands of oxygen-containing functional groups); fraction 3 had an absorption band at 1730 cm^{-1}, which is characteristic for esters and carbonyl compounds; fractions 4, 6, 7, and 8 consisted of alcohols (presence of absorption bands at 3610-3630 cm^{-1} corresponding to hydroxy groups); fraction 5 included ester-alcohols (absorption bands at 1730 and 3620 cm^{-1}). To separate the fractions obtained into narrow groups of substances and individual components, we used column chromatography on silica gel containing 5-10% of $AgNO_3$.

Hydrocarbons. Fractions 1 and 2 and also the hydrocarbons from fraction 3 (1.13% of the neutral substances) were separated by column chromatography on $SiO_2/AgNO_3$ into mono-, sesqui-, di-, and triterpenes, and their compositions were determined by the GLC method.

Saponification of the Esters. The saponification of fraction 3 was carried out with 0.5 N ethanolic alkali at 80°C for 3.5 h. The ethanolic solution after saponification was diluted with a tenfold amount of water and was acidified with 12% H_2SO_4 to pH 1, and the saponification products and unsaponifiable substances were extracted with diethyl ether. To separate the acids, the ethereal solution was washed with 5% KOH solution and was dried and evaporated. The extract obtained was chromatographed on a column of silica gel to separate the unsaponifiable substances from the alcohols produced by the saponification of the esters. The composition of the alcohols was determined by GLC.

Reduction of the Aldehydes. The part of fraction 3 that was not saponified by the 0.5 N ethanolic alkali was dissolved in 25 ml of ethanol, and 800 mg of $NaBH_4$ was added; the mixture was stirred at room temperature for 15 min and was then transferred to a separatory funnel containing 150 ml of petroleum ether. Extraction was performed with petroleum ether. The ethereal extracts were dried with anhydrous Na_2SO_4 and the solvent was distilled off. The extract obtained was chromatographed on silica gel with the separation of the alcohols produced by the reduction of the aldehydes from the unreduced fraction. The alcohols were analyzed gy GLC. The main component of the unreduced part was epimanoyl oxide (GLC).

Acetylation of the Alcohol Fractions. The alcohol fractions were acetylated with acetic anhydride in pyridine at room temperature. The acetates of the primary and secondary alcohols obtained were separated from the tertiary alcohols by column chromatography. Both groups of substances were chromatographed on silica gel containing 10% of $AgNO_3$.

Analytical gas-liquid chromatography was performed on Tsvet-65, Chrom-4, and LKhM 8 MD chromatographs under the following conditions.

Paraffins: Chrom-4 chromatograph, 3 mm × 2.5 m glass column, pressure 0.4 atm, liquid phase Lukopren, programming of the temperature from 160 to 220°C and isothermal regime at 270°C. The components were identified by the method of markers, using the C_{16} and C_{20} normal alkanes as standards. Making use of the linear relationship between the logarithm of the relative retention time and the number of carbon atoms, components of normal structure with from 14 to 27 carbon atoms were identified.

Terpenes. Chrom-4 chromatograph, steel capillary column 0.25 cm × 45 m; stationary phase OV-17; carrier gas nitrogen; pressure at the inlet 3.0 atm; programming of the temperature

from 130 to 220°C at the rate of 2°C/min. The components of the mixture were identified from their relative retention times.

Acetates of Aliphatic Alcohols. Tsvet-65 chromatograph; steel column 3 mm × 2 m; stationary phase 5% of SE-30 on Chromaton N-AW; flame-ionization detector; carrier gas nitrogen at the rate of 30 ml/min; programming of the column temperature from 100 to 300°C at the rate of 10°C/min; identification by the marker method of acetates of aliphatic alcohols with 20-26 carbon atoms.

Acetates of Diterpene Alcohols. Chrom-4 chromatograph, 3 mm × 25 m glass column; liquid phase OV-17; programming of the temperature from 155 to 215°C. Acetates of known diterpene alcohols were identified by the additive method.

Acetates of Triterpene Alcohols and of Sterols. LKhM 8 MD chromatograph; 3 mm × 3 m steel column; carrier gas nitrogen (30 ml/min); liquid phase 5% of SE-30 on Inerton AW-DMSS; programming of the temperature from 170 to 250°C at the rate of 6°C/min.

IR spectra were taken on a UR-20 instrument; PMR spectra on a Varian A-55/60 A instrument (internal standard – hexamethyldisiloxane; δ-scale; solvent CCl_4); and UV spectra on a Specord spectrophotometer in ethanol.

Squalene. PMR spectrum: 1.58 ppm (18 H, singlet, 6-trans-CH_3); 1.66 ppm (6 H, singlet, 2 ring cis-CH_3); 5.13 ppm (6 H, broadened singlet, C=CH–); 2.03-2.11 ppm (20 H, singlets, $-CH_2-CH_2-$).

Epimanool. PMR spectrum: 0.62, 0.75, 0.80, 1.15 ppm (3 H each, singlets, methyl groups); 4.50 and 4.57 ppm (1 H each, narrow multiplets, $C=CH_2$); 4.93 (1 H, doublet of doublets J = 2 and 10 Hz); 5.09 ppm (1 H, doublet of doublets, J = 2 and 17 Hz); 5.86 ppm (1 H, doublet of doublets, J = 10 and 17 Hz) – the signals of a $-CH=CH_2$ fragment.

Cycloartenol Acetate. PMR spectrum: 0.28 and 0.55 ppm (1 H each, doublets J = 3.5 Hz, cyclopropane ring); 0.8-0.9 ppm (15 H, singlets, methyl groups); 1.55 and 1.60 ppm [6 H, doublet, J = 15 Hz, $C=C(CH_3)_2$]; 4.50 ppm (1 H, multiplet, $-\underline{CH}-OH$); 5.03 ppm [1 H, multiplet, $\underline{CH}=(CH_3)_2$].

Larixol Acetate. PMR spectrum: 0.7, 0.83, 0.95, 1.17 ppm (3 H each, singlets, methyl groups); 1.92 ppm (3 H, singlet, $-O-CO-CH_3$); 2.58 ppm (1 H, multiplet of signals with its center at 2.58 ppm; signals of the proton at C_6); 4.58-6.07 ppm (5 H, signals of the protons of double bonds).

SUMMARY

1. The component composition of the neutral part of the resinous substances of the wood of the Dahurian larch growing in the region of BAM [Baikal-Amur Railway] in the basin of the River Olekma has been studied.

2. About 40 terpenoids have been identified, and the compositions of saturated hydrocarbons and aliphatic alcohols have been determined. The main components of the neutral substances are compounds of the labdane type of structure –epimanool and larixol and its monoacetate – making up about one-third of the neutral substances.

3. In contrast to species of larch investigated previously, in the Dahurian larch we have detected squalene, cycloartenol, and pinosylvin dimethyl ether. In contrast to the Siberian larch, this species contains no epitorulosol and its derivatives.

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TRITERPENE GLYCOSIDES OF Silphium perfoliatum.

V. THE STRUCTURE OF SILPHIOSIDE A

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From the epigeal part of Silphium perfoliatum L. we have isolated glycoside F, identified as oleanolic acid 3-O-β-D-glucopyranosiduronic acid and a new triterpene glycoside – silphioside A – for which the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-(methyl β-D-glucopyranosiduronate) has been established.

Continuing a study of the triterpene glycosides of Silphium perfoliatum L. [1, 2], we have isolated compounds A and F from the epigeal part of this plant. This is the first time that glycoside A has been described, and we have called it silphioside A (I).

The acid hydrolysis of glycoside (I) gave a genin – oleanolic acid (II) – and in the carbohydrate fraction D-glucose and D-glucuronic acid were identified.

In the PMR spectrum of silphioside A the signals of two anomeric protons appeared in the form of doublets at 4.92 and 6.23 ppm, which showed the presence of two sugar residues in the compound under investigation. The downfield shift of one of the signals (6.23 ppm) showed the addition of one of the monoses to the carboxy group of the aglycone, and the spin-spin coupling constants for both signals of 7.5 Hz showed the β configuration of the glycosidic bonds.

The results obtained showed that the qualitative and quantitative compositions of the sugars of glycoside (I) and of glycoside G (III) isolated previously from the same plant were identical (see following page).

A comparison of the PMR spectra of silphiopsides A (I) and G (III) likewise showed a similarity of their main spectral characteristics (Table 1). However, in the PMR spectrum of (I), unlike that of compound (III), there was a three-proton singlet at 3.72 ppm showing the presence of a methoxy group in the molecule of glycoside A.

To determine the site of attachment of the methoxy group, we used the method of selective homonuclear H_i–{H_j} double resonance in the usual and extended variants. An assignment of the signals of the carbohydrate protons of glycosides (I) and (III) was made with its aid.

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