SUMMARY

The protolytic properties of flavonoids isolated from pea chloroplasts have been studied. It has been shown that flavonol triglucosides have two to four dissociation constants, which permits them to exhibit protonophoric properties over a wide pH range.

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TERPENOIDS OF THE OLEORESIN OF THE LARCH GROWING IN KAMCHATKA

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UDC 547.595.9.913.2.596/599

The complete chemical composition of the oleoresin of the larch *Larix cajanderi* M. growing in Kamchatka has been studied. Fifty-four terpene compounds have been isolated and identified. It has been shown that among the monoterpenes the main constituents are α- and β-pinenes, their amounts being 66.3% and 18.3%, respectively. Among the sesquiterpene hydrocarbons the main components are δ-cadiene and γ-elemene (15 and 17%). Diterpenoids are represented mainly by bicyclic labdane compounds — epimanool (∼15%), larixylacetate (∼28%), and larixol (∼40%). In the acidic fraction of the oleoresin, isopimaric acid (40%) predominates.

Continuing a systematic investigation of the oleoresins of conifers, we have studied the terpenoids of the larch growing in Kamchatka. In the opinion of investigators, the central Kamchatkan populations of the larch belong to the species *Larix cajanderi* M. (Cajander's larch) and are considered as relict populations [1].

We have studied the complete chemical composition of the oleoresin of this species of larch. The oleoresin was treated by a method described previously [2] for separating the acidic components.

The neutral substances (50% of the weight of the oleoresin) were separated by adsorption chromatography into hydrocarbons and oxygen-containing compounds (16 and 34%, respectively). The hydrocarbons consisting of a mixture of monoterpenes, sesquiterpenes, and diterpenes, were subjected to fractionation, which yielded 65% of monoterpenes, 5% of sesquiterpenes, and 25% of diterpenoids.

The monoterpene hydrocarbons were analyzed by gas—liquid chromatography (GLC). The main components were α-piene (66.3%) and β-piene (18.3%).

In the sesquiterpene fraction, 13 hydrocarbons were found by adsorption chromatography and gas—liquid chromatography, the main ones being δ-cadiene (15%) and γ-elemene (12%). The individual sesquiterpenes were identified by their IR and PMR spectra (see the experimental part).

In the diterpenoid fraction, hydrocarbons and aldehydes were detected. Of the hydrocarbons, isopimaradiene, abietadiene, and dehydroabietadiene were identified, their amounts being very small.

The neutral oxygen-containing compounds consisted of mono-, sesqui-, and diterpenoids. Among the monoterpenoids we found bornyl acetate, α-terpinyl acetate, thymol methyl ether, α-terpineol, terpineol-4, borneol, linalool, geraniol, sabinene hydrate and p-cymene-8-ol.

Sesquiterpenoids were present in the oleoresin in trace amounts. We succeeded in isolating only one alcohol, of unknown structure, the study of which is continuing.

More than 80% of the diterpenoids, which made up the bulk of the neutral oxygen-containing fraction of the resin, consisted of bicyclic compounds of the labdane type: epimanool, ∼15%; larixylacetate, ∼28%; and larixol, ∼40%. We also found the tricyclic alcohols abietinol and dehydroabietinol, and the aldehydes corresponding to them. No methyl esters of resin acids were found in the neutral fraction of the oleoresin.

The analysis of the acidic fraction of the oleoresin was performed by the GLC method. It was established that the composition of the acids of the oleoresin of Cajander's larch was the usual one for conifer resins [3] the main acids present being isopimaric (45%) and abietinic (∼28%).

The results of the investigation have shown that the chemical composition of the oleoresin of the larch growing in Kamchatka is very close to the composition of the oleoresin of the Dahurian larch, an exception being formed by the sesquiterpene hydrocarbons [4, 5]. The acid fraction of this oleoresin lacks the communic acid found previously in the oleoresins of the Siberian and Dahurian larches (*Larix sibirica* L. and *L. dahurica* T.) [6].

EXPERIMENTAL

The IR spectra of the compounds were recorded on a UR=20 instrument in carbon tetrachloride, and the PMR spectra on a Varian A 56/60 A instrument with HMDS as internal standard. The specific rotations of the substances were determined in chloroform, the concentrations being shown in the text. The larch oleoresin was collected in the region of the settlement of Kozyrevsk (Kamchatka province) in August, 1978, the age of the tapped trees being 80–100 years.

Treatment of the Oleoresin. The oleoresin (700 g) was treated with 1% aqueous caustic soda (7000 ml). The neutral compounds were obtained by extraction with diethyl ether (350 g). The sodium salts of the acids were treated with 5% hydrochloric acid to pH 2 and the free acids were then extracted with diethyl ether. After elimination of the solvent, a mixture of resin acids was obtained (300 g).

Isolation of the Hydrocarbons. The neutral compounds (350 g) were chromatographed on alumina (activity grade I-II, at a ratio of 1:10). Petroleum ether eluted hydrocarbons (117 g) and ethanol eluted oxygen-containing compounds (180 g). After vacuum distillation of the petroleum ether fraction, 76 g of mo-oterpenes (bp 80–120, 12 mm), 6 g of sesquiterpenes (bp 110–145, 2 mm) and 30 g of still residue (diterpenoids) were obtained. Part of the ethanol fraction (45 g) was subjected to distillation, giving 1.8 g of a mixture of mono- and sesquiterpenoids and 42 g of diterpenoids.

Monoterpene Hydrocarbons. The qualitative and quantitative analysis of the monoterpenes was performed by GLC using the procedure described previously [7]. The following were identified from their relative retention times: α-piene (66.3%), β-piene (18.3%), camphene (1%), Δ^3-carene (6.2%), myrcene (0.6%), limonene (1.7%), β-phellandrene (2.2%), γ-terpiene (2.5%), terpinolene (0.5%), and traces of p-cymene.

Sesquiterpene Hydrocarbons. The sesquiterpene hydrocarbon fraction (3 g) was chromatographed on silica gel (60 g) impregnated with silver nitrate (10%). Gradient elution (petrolium ether—diethyl ether) yielded logifolene [0.074 g, $[\alpha]_D^{20}$ +41.3° (c 2.7)], δ-cadinene + thymol methyl ether (0.18 g), bisabolene (0.11 g), β-farnesene (0.08 g), γ-elemene (0.46 g) and a fraction of oxidized monoterpene compounds (a mixture of bornyl acetate and α-terpinyl acetate, 2:1, 0.32 g).

Preparative GLC on a Pye–105 instrument (column 5 m × 6 mm, stationary phase 10% of Tween-60 on Celite C-22 (45/60 mesh), column temperature 125–180°C/1°C per minute, rate of flow of carrier gas 150 ml/min) yielded the following compounds, which were identified by their IR and PMR spectra: cyclosativene, logicyclene, β-cadiene [$[\alpha]_D^{20}$ −31.2° (c 5.4)], γ-cadiene, γ-muurolene, γ-amorphene, sibirene, α-muurolene, calamenene, ar-curcumene, and α-selinene.

Identification of the Oxygen-Containing Mono- and Sesquiterpenoids. The fraction of oxygen-containing mono- and sesquiterpenoids (1.8 g) was chromatographed on silica gel (45 g). Elution with petroleum ether containing increasing amounts of diethyl ether yielded a mixture of bornyl acetate and α-terpenyl acetate (0.08 g, PMR), fraction 2 (0.26 g), fraction 3 (0.12 g), fraction 4 (0.42 g), and sabinene hydrate (0.11 g). The chromatography of fraction 2 (0.26 g) on silica gel containing silver nitrate (10%, 15 g) with petroleum ether containing increasing amounts of diethyl ether yielded terpineol-4 (0.07 g), borneol (0.04 g), linalool (0.06 g), and an unidentified sesquiterpene alcohol (0.08 g) with mp 149-150°C.

Mass spectrum of the alcohol: 222 (M^+, 61.8%), 219 (25.9%), 204 (95.8%), 189 (100%), 161 (80.7%). IR spectrum, cm^{-1}: 915, 1110, 1380, 1455, 3640.

By chromatography on silica gel impregnated with silver nitrate (8 g), fraction 3 (0.12 g) yielded borneol (0.04 g), p-cymen-8-ol (0.05 g), and α-terpineol (0.03 g). Fraction 4 similarly yielded α-terpineol (0.09 g) and geraniol (0.29 g).

All the compounds isolated were identical with authentic samples in relation to spectral characteristics.

Isolation of Diterpene Aldehydes. The diterpenoids (30 g) were chromatographed on alumina (activity grade I-II, 300 g); petroleum ether eluted 20 g of a fraction consisting of a viscous colorless oil, and diethyl ether isolated 9 g of oxygen-containing compounds. The petroleum ether fraction (1 g) was chromatographed on silica gel impregnated with silver nitrate at a ratio of 1:40. Petroleum ether yielded 0.1 g of a substance in the form of a mobile oil with n_D^{20} 1.5028, and IR and PMR spectra of which were completely identical with those of isopimaradiene. Petroleum ether—diethyl ether (99:1) eluted 0.32 g of dehydroabietinal with mp 78-80°C, $[\alpha]_D^{20}$ +69°; according to the literature [8]: mp 86-87°, $[\alpha]_D^{20}$ +66.5°.

Petroleum ether—diethyl ether (97:3) gave palustral (0.05 g) with mp 51-53°C, $[\alpha]_D^{20}$ +59°. Trace amounts of abietinal were found among the aldehydes. All the aldehydes were identified by direct comparison with authentic samples by means of TLC and IR, UV, and PMR spectroscopy.

Oxygen-Containing Diterpenoids. The ethanol fraction (see above) (3 g) was chromatographed on silica gel (60 g). Petroleum ether—diethyl ether (95:5) eluted 0.44 g of epimanool with n_D^{20} 1.5180, $[\alpha]_D^{20}$ +55° (c 2.0), the IR and PMR spectra of which were identical with those of an authentic sample.

Petroleum ether—diethyl ether (90:10) eluted 0.5 g of crude larixylacetate and after its recrystallization from petroleum ether a product was obtained with mp 78-80°C, $[\alpha]_D^{20}$ +60° (c 3.1); according to the literature [10]: mp 82°C, $[\alpha]_D^{20}$ +67°.

The main component (1.34 g) was isolated with petroleum ether—diethyl ether (75:25). From its spectral characteristics and physical constants, the compound was identified as larixol, mp 99-100°C, $[\alpha]_D^{20}$ +55° (c 2.0); according to the literature [10]: mp 101°C, $[\alpha]_D^{20}$ +57°.

Resin Acids of the Larch Oleoresin. The acids (6 g) were dissolved in diethyl ether and treated with an ethereal solution of diazomethane. After the elimination of the solvent, the methyl esters obtained were analyzed by GLC using the procedure described previously [11]. The following composition of the resin acids was found (%): sandaracopimaric, 1.2; palustric, 9.8; isopimaric, 44.7; dehydroabietinic, 4.4; abietinic, 27.5; neoabietinic, 12.2.

SUMMARY

1. The complete chemical composition of the oleoresin of the larch *Larix cajanderi* M. growing in Kamchatka has been studied.

2. It has been established that the compositions of the monoterpenes and resin acids are close to those from the oleoresin of the Dahurian larch.

3. The oxygen-containing diterpenoids of the oleoresin are represented by the bicyclic compounds epimanool, lamixyl acetate, and larixol.

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DETERMINATION OF THE ABSOLUTE CONFIGURATION OF THE ASYMMETRIC CENTER AT C_{11} IN THE EUDESMANOLIDES

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UDC 547.314+582.998

It has been shown that it is possible to use the method of circular dichroism to determine the absolute configuration of the asymmetric center at C_{11} in the eudesmanolides. The orientations of the hydroxy group at C_1 and of the methyl group at C_4 in arabsin have been established.

In demonstrating the spatial structures of eudesmanolides, the determination of the absolute configuration of the asymmetric center at C_{11} is a fairly complex problem. The existing chemical method of determination, which is based on the treatment of the substance with phenyl selenide and sodium tetrahydroborate is laborious and requires the use of a large amount of material. It is also difficult to assign the signals of the proton at C_{11} from the NMR spectra.

Analysis of the results of circular dichroism (CD) measurements have shown that the sign of the Cotton effect (CE) in the 230–210 nm region, which is due to a $n \to \pi^*$ transition in the lactone chromophore, is determined by two factors: the nature of the linkage of the lactone ring with ring B and the configuration of the asymmetric center at C_{11} in the lactone ring [1, 2]. Since natural sesquiterpene lactones of the eusdesmanolide type with the trans-linkage of rings A/B were considered, the influence of this factor on the parameters of the lactone CE was not taken into account.

Below we give the results of measurements of the circular dichroism of eudesmanolide:

Compound		Configuration	λ, nm	$\Delta\varepsilon$	Compound		Configuration	λ, nm	$\Delta\varepsilon$
Arabsin	(I)	11R	283	+0.66	Tetrahydro-α-santonin	(III)	11S	285	+0.83
			218	−0.59				215	−1.03
Arabsin	(Ia)*		340	−0.26	Tetrahydro-β-santonin	(IV)	11R	285	+0.91
			283	+0.54				215	−0.72
			238	+1.16	(Arsubin)	(V)	11R	214	−0.41
			207	−2.17	(Artemin)	(VI)	11S	218	+1.84
Arsanin	(II)	11S	282	+0.64	(Artesin)	(VII)	11S	218	+2.10
			215	+1.33	(Taurin)	(VIII)	11S	294	−1.70
Arsanin	(IIa)*		340	−0.28				223	+1.13
			283	+0.54				202	−5.70
			226	+2.10	(Ashurbin)	(IX)	11S	223	+0.19
			207	−0.82	(Tetrahydroalantolactone	(X)	11S	233	−0.10

*The CD measurements were made an hour after acidification.