

TERPENOIDS OF THE OLEORESIN OF *Pinus pumila*

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For the investigation we took the oleoresin of *Pinus pumila* (Pall.) Regel. (Japanese stone pine) collected in August 1974 in the Poronai region of the Sakhalin oblast. It was separated into acid (80%) and neutral (20%) fractions by the usual method [1]. Chromatography of the neutral fraction on SiO_2 yielded a hydrocarbon fraction (50.5%) and a fraction of oxygen-containing compounds (49.5%).

The hydrocarbon fraction was investigated by the GLC method. Eleven monoterpenes were detected: α -pinene (71.1%), α -thujene (9.6%), camphene (2.1%), β -pinene (2.7%), Δ^3 -carene (1.8%), myrcene (~0.5%), limonene (2.7%), β -phellandrene (3.0%), γ -terpinene (~0.5%), terpinolene (6.0%), and p-cymene (traces). The sesquiterpenes were represented by 26 hydrocarbons of which we identified 17 compounds by the method of additives: cyclosativene, α -longipinene, longicyclene, longifolene, γ -elemene, sibirene, caryophyllene, α -humulene, β -ylangene, α -amorphene, α -, γ -, and ϵ -murolenes, α -, γ -, and δ -cadinenes, and α -calacorene. The main components of this mixture were longifolene, α -murolene, α -humulene, and caryophyllene. No diterpene hydrocarbons were detected.

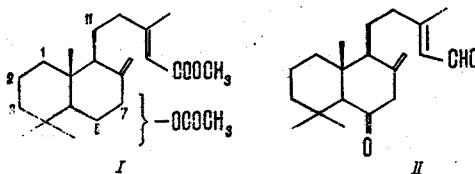
The acid fraction of the oleoresin was treated with diazomethane and the products were separated chromatographically into a mixture of methyl esters of resin acids (92.5%) and methyl esters of acetoxy-substituted and hydroxy-substituted resin acids (7.5%). As was found by GLC, the mixture of methyl esters of resin acids consisted of the esters of the following acids: (13E)-labd-8(20), 13-dien-15-oic (copaiferic) (49.3%), isopimara-8,15-dien-18-oic (17.1%), isopimaric (15.9%), abietinic (9.2%), neoabietinic (3.5%), levopimaric (2.8%), dehydroabietinic (1.2%), and sandaracopimaric (1.0%).

The composition found for the mixture of resin acids differs sharply from those of *Pinus sibirica* R. Mayer (Siberian pine) and *Pinus koraiensis* Sieb. et Zucc. (Korean pine), belonging to the same section (*Cembrae*) as the Japan stone pine. This difference is expressed in the absence of lambertianic acid from the oleoresin of the Japanese stone pine and also by the fact that in the latter the main components of the mixture of acids are copaiferic and isopimara-8,15-dien-18-oic acids, which are practically absent from the oleoresins of the above-mentioned species of *Pinus* L. Copaiferic acid was first found in conifers. We isolated its methyl ester by chromatographing the mixture of methyl esters of the resin acids on $\text{SiO}_2 + 5\%$ of AgNO_3 . Copaiferic acid was previously obtained by Oloff [2] from sclareole and it was then isolated by Bevan [3] and by Delle [4] from two plants of the family Leguminosae.

The fraction of the methyl esters of hydroxy-substituted and acetoxy-substituted resin acids formed a complex mixture, but the two least polar esters present in approximately equal amounts and making up about 80% of the fraction were isolated and purified by chromatography on SiO_2 . The first ester, having $[\alpha]_D^{22} + 39.2^\circ$ (c 1.03) and $n_D^{22} 1.5110$ was identical, according to its IR, UV, and NMR spectra with the methyl ester of acetyl-agatholic acid, previously synthesized by Carman [5] and then isolated by Caputo [6] from the oleoresin of *Copaifera multijuga* Hayne. The second ester, having the formula $\text{C}_{23}\text{H}_{36}\text{O}_4$ (high-resolution mass spectrometry) was an isomer of methyl acetyl-agatholate. Since the IR and UV spectra of the two esters were similar, it may be assumed that the second ester is an isomer of methyl acetyl-agatholate with respect to the acetoxy group. This is confirmed by the fact that the NMR spectrum of the second ester differs qualitatively from the NMR spectrum of methyl acetyl-agatholate by the presence of the signal of an additional tertiary methyl group, and also by the presence of a broad one-proton multiplet at 4.41 ppm in place of the AB system of protons that is present in the spectrum of methyl acetyl-agatholate. This multiplet can be assigned to a proton at a secondary group. The results obtained, and also biogenetic considerations, permit structure (I) to be proposed for the ester isolated.

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By reducing the ester (I) with lithium tetrahydroaluminate, we obtained the corresponding diol with mp 160–163°C (from ethanol). The oxidation of this diol with chromium trioxide in pyridine gave an oxo aldehyde the UV spectrum of which was very similar to that of the (13E)-6-oxolabd-8(20),13-dien-15-al(II) which we synthesized from larixyl acetate [λ_{\max} 239 nm ($\log \epsilon$ 4.10) and λ_{\max} 230 nm ($\log \epsilon$ 4.17), respectively]. Consequently, the secondary acetoxy group in the ester (I) cannot be at C₇ or C₁₂. Since the oxo aldehyde from the ester (I) was not identical, according to TLC, with the oxo aldehyde (II), the position of the acetoxy group at C₆ is also excluded and one of four possible positions—C₁, C₂, C₃, or C₁₁—remains for it.

Monache [7] isolated from the oleoresin of *Copaifera multijuga* Haynem a hydroxy acid for which the most probable structure of 11-hydroxylabd-8(20),13-dien-15-oic acid was proposed. The NMR spectrum of the acetate of the methyl ester of this acid given in Monache's paper and the NMR spectrum of (I) differ by the magnitude of the chemical shift and the shape of the signal of the proton at the secondary acetoxy group, which shows the nonidentity of these two compounds. In view of the small amount of the oleoresin of the Japanese stone pine available to us, we were unable to obtain additional information on the position of the acetoxy group in the ester (I).

From the fraction of neutral oxygen-containing compounds of the oleoresin by chromatography we obtained the total carbonyl compounds of the fraction under investigation. These compounds consisted of diterpene aldehydes (95%) and methyl esters of resin acids (about 5%; determination from the integral intensities of the signals of the COOCH₃ and CHO groups in the NMR spectrum of the carbonyl fraction). The fraction of carbonyl compounds was treated with sodium tetrahydroborate in aqueous ethanol. This reduced the aldehydes to the corresponding alcohols while the methyl esters remained unchanged. The mixture of alcohols after the separation of the methyl esters on a column of SiO₂ was analyzed by NMR (100 MHz) both directly and in the form of the acetates. Isopimara-8,15-dien-18-ol, isopimarinal, levopimarinal, and neoabietinol were found in the same ratio as the corresponding acids in the acid fraction of the oleoresin, and also dehydroabietinol and palustrol. The relative amount of dehydroabietinol in this mixture was approximately twice that of the corresponding acid in the acid fraction of the oleoresin.

In addition to the diterpene aldehydes obtained in a mixture, from the fraction of the oxygen-containing compounds of the oleoresin investigated we isolated and identified manoyl oxide, cis-abienol, (13E)-labd-8(20),13-dien-15-ol, and isopimarinal, and also 13,14-bisnorlabd-8(20)-en-13-one, isolated previously by Cambie [8] from the wood of *Dacrydium kirkii* F. Muell. The main components of the fraction of oxygen-containing compounds were aldehydes (25%), cis-abienol (10%), and (13E)-labd-8(20),13-dien-15-ol (40%). Isocembrol and 4-epiisocembrol, the main neutral diterpenoids of the oleoresin of the Siberian pine and the Korean pine, were present in the oleoresin investigated only as traces. They were detected with the aid of TLC (SiO₂ + 5% of AgNO₃) in the crude cis-abienol fraction.

Thus, the composition of the mixture of neutral diterpenoids of the oleoresin of the Japanese stone pine also differs considerably from those of the Siberian pine and the Korean pine, which is expressed above all in the predominance of labdane derivatives and the very small amount of cembrane diterpenoids.

EXPERIMENTAL

The IR spectra were recorded for solutions in CCl₄ on a UR-20 instrument, the UV spectra for solutions in ethanol on a Specord UV-VIS instrument, and the NMR spectra were obtained on Varian HA-100 and Varian A-56/60A instruments using solutions in CCl₄ with TMS as internal standard, δ scale. The optical rotations were measured on a Zeiss polarimeter using solutions in chloroform.

Gas-liquid chromatography was performed on a Khrom 4 instrument with a flame-ionization detector. Conditions for the analysis of the monoterpenes: column 3.7 m \times 3 mm; 10% of TCEP on Chromosorb P; t° = 74°C; carrier gas nitrogen, 24 ml/min. Conditions for the analysis of the sesquiterpenes: a) column 50 m \times 0.2 mm; Tween-60; t° = 100–190°C at a rate of programming of 2 deg/min; carrier gas nitrogen, 5 ml/min; b) column 50 m \times 0.2 mm; Apiezon L; t° = 130–200°C at a rate of programming of 2 deg/min; carrier gas

nitrogen, 7 ml/min. Conditions for the analysis of the methyl esters of the resin acids: column 2.5 m × 3 mm; 5% of XE = 60 on Chromaton with a grain diameter of 0.200-0.250 mm; $t^\circ = 214^\circ\text{C}$; carrier gas nitrogen, $P_{\text{exc}} = 0.46$ atm.

For adsorption chromatography, unless specially mentioned, we used air-dry silica gel and as eluent in all cases petroleum ether with increasing concentrations of diethyl ether.

Separation of the Neutral Fraction of the Oleoresin. The neutral fraction of the oleoresin (2.45 g) was chromatographed on 40 g of SiO_2 . Petroleum ether eluted 1.24 g (50.5%) of hydrocarbons, and diethyl ester eluted 1.21 g (49.5%) of a fraction of the oxygen-containing compounds of the oleoresin. The hydrocarbon fraction was analyzed directly by GLC.

The fraction of oxygen-containing compounds (1.21 g) was chromatographed on 30 g of SiO_2 . The following compounds were eluted successively: 15 mg of manoyl oxide (identified by its NMR and IR spectra); 300 mg of a carbonyl fraction; 20 mg of 14,15-bisnorlabd-8(20)-en-13-one (identical according to NMR and IR spectra, and also TLC, with an authentic sample synthesized from epimanol by Shenk's method [9]; 60 mg of a mixture of two unknown compounds free from hydroxy groups according to their IR spectrum; 120 mg of a fraction the main component of which was cis-abienol, which was then isolated by chromatography on $\text{SiO}_2 + 5\%$ of AgNO_3 ; 330 mg of a fraction the rechromatography of which on $\text{SiO}_2 + 51$ of AgNO_3 yielded 60 mg of isopimarinal, identified by comparison with an authentic sample; and 230 mg of (13E)-labd-8(20),13-dien-15-ol, $n_D^{22} 1.5210$, $[\alpha]_D^{20} + 27.2^\circ$ (c 4.4), p-nitrobenzoate with mp $112-113^\circ\text{C}$ (from CCl_4 -ethanol); literature data [2]: p-nitrobenzoate—mp $107-108.5^\circ\text{C}$. Then another 230 mg of (13E)-labd-8(20),13-dien-15-ol was eluted (total yield of the latter, 40%).

Methyl Ester of (13E)-Labd-8(20),13-dien-15-oic (Copaiferic) Acid. When the mixture of methyl esters of the resin acids was chromatographed on $\text{SiO}_2 + 5\%$ AgNO_3 , methyl copaiferate was isolated first. It had $[\alpha]_D^{24} + 44.5^\circ$ (c 2.02), $n_D^{23} 1.5150$; literature data [4]: $[\alpha]_D^{26} + 46.5^\circ$ (c 1.8). The NMR, IR, and UV spectra of methyl copaiferate corresponded to those given in the literature [4].

The Acetoxy Ester (I). The ester (I) was eluted from an SiO_2 column immediately after methyl acetyl-agatholate. It had $[\alpha]_D^{20} + 40^\circ$ (c 3.65), $n_D^{22} 1.5080$; UV spectrum: $\epsilon_{\text{max}} 222$ nm ($\log \lambda 4.08$); IR spectrum, cm^{-1} :

899, 3080 (>C=CH_2), 1030, 1155, 1250, and 1730 (COOCH_3 and OCOCH_3); NMR spectrum, ppm: 0.70, 0.81, and 0.85 (3 H each, methyl groups at C_4 and C_{10}); 1.95 (3 H, singlet, OCOCH_3); 2.12 (3 H, singlet, $\text{C}_{13}-\text{CH}_3$); 3.60 (3 H, singlet, COOCH_3); 4.41 (1 H, multiplet, $W_{1/2} = 14$ Hz, proton in the geminal position to a secondary acetoxy group); 4.54 and 4.85 (1 H each, narrow multiplets, >C=CH_2); and 5.57 (1 H, narrow multiplet, H_{14}).

$M^+ 376.2612$; calculated for $\text{C}_{23}\text{H}_{36}\text{O}_4$, 376.2613.

SUMMARY

1. The composition of the acid fraction of the oleoresin of *Pinus pumila* (Pall.) Regel. has been studied, and from it has been isolated, in addition to known compounds, the methyl ester of a new labdane acetoxy acid.
2. In the hydrocarbon fraction of the oleoresin 11 monoterpenes and 26 sesquiterpenes have been found (and 17 of the latter have been identified).
3. The composition of the fraction of neutral oxygen-containing diterpenoids from *P. pumila* differs considerably in its diterpenoid components from the corresponding fractions of the oleoresins of botanically related species—*P. sibirica* and *P. koraiensis*.

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DEVELOPMENT OF A METHOD FOR DETERMINING BORNYL ACETATE IN THE PRODUCTS OF THE PROCESSING OF *Abies sibirica*

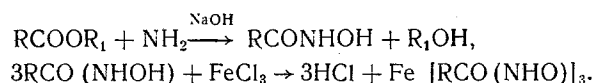
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A number of investigations has been published recently on the development of a technology for the production of extracts from coniferous raw material [1]. The main component of the essential oil of *Abies sibirica* (Siberian fir), responsible for the smell of the oil, is bornyl acetate [2].

Several methods of determining the amount of esters are used: saponification [3], gas chromatography [2], and crystallization out from the essential oil (for bornyl acetate) [2]. The standard method of saponification based on the titration of the acids, is inapplicable to a CO₂ extract of the fir, since the latter has a pronounced color and titration of the acids does not show a distinct change in the color of the indicator. The gas chromatographic method is unsuitable for working with small amounts of CO₂ extracts, since these extracts contain a large amount of nonvolatile substances, and working with small samples does not enable the amount of nonvolatile fraction to be determined. Crystallization out from the essential oil cannot be used in the case of CO₂ extracts because the presence of waxes, sterols, and resin acids in the CO₂ extracts imparts a viscous consistency to it which interferes with the formation of crystals and is associated with large losses.

We have used the reaction of esters with an alkaline solution of hydroxylamine [4]. This reaction forms hydroxamic acids. The product of the reaction of these acids with salts of trivalent iron gives the solution a pink-yellow color (pH 2.0 ± 0.2). The reaction takes place in accordance with the equations



EXPERIMENTAL

By using a scheme for the planning of experiments by the method of steepest ascent, we found the optimum conditions for performing the reaction [5]. As the optimization parameter we used the optical density of the colored product Y formed. As the independent variables we chose the following:

- X₁—the amount of hydroxylamine hydrochloride, ml;
- X₂—the amount of NaOH, ml;
- X₃—the time for the first stage of the process, min;
- X₄—the amount of HCl, ml;
- X₅—the amount of FeCl₃, ml;
- X₆—the time for the second stage of the process, min.

We used a 20% solution of hydroxylamine hydrochloride, a 0.5 N solution of NaOH, a 5 N solution of HCl, and a 6% solution of FeCl₃ prepared in 0.1 N hydrochloric acid. All the solutions were used in the freshly prepared state. The planning matrix for the experiment and the results are shown in Table 1.

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