TERPENOIDS OF THE OLEORESINS OF *Picea orientalis* AND *Abies normanniana* GROWING IN THE CAUCASUS

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The terpenoid compositions of the oleoresins of the oriental spruce and of the Nordmann fir growing in the Caucasus have been studied. From the oleoresin of *Picea orientalis* (L.) Link, which belongs to the section *Omorica*, 32 mono-, sesqui-, and diterpene compounds have been identified, and from *Abies nordmanniana* Stev. 37 mono-, sesqui, and diterpene compounds. Together with other diterpenoids that are characteristic for the oleoresins of all species of fir, methyl dehydroabietate and methyl 15-hydroxydehydroabietate have been found in the oleoresin of the Nordmann fir. Three new sesquiterpene hydrocarbons have been isolated.

A considerable number of publications have been devoted to the study of the chemical compositions of the oleoresins of the spruces and firs growing in the territory of the Soviet Union. In these investigations, the main attention has been devoted to the species the area of which is located in Siberia, in the Far East, and in the European part of the USSR. The oleoresins of the conifers from the southern regions of the country have been studied inadequately.

It has been shown previously that the spruces belonging to two sections (*Morinda* and *Casicta*) differ in the chemical compositions of their resins [3, 6] and it was therefore of interest to determine whether this rule applied to representatives of the third section – *Omorica*.

We have investigated the chemical composition of the oleoresin of the oriental spruce (*Picea orientalis* (L.) Link) and the oleoresins of the Nordmann fir (*Abies nordmanniana* (Stev.) Spach.) collected in 1979 in the Borzhomi gorge in the R. Bordzhomula district and in the village of Akhaldaba, respectively.

The composition of the monoterpene hydrocarbons of the oleoresin of the oriental spruce was determined by the GLC method. The main components of this part of the resin were α- and β-pinenes and limonene, and in addition to these we found β-phellandrene, Δ^3-carene, myrcene, and camphene. On the whole, the composition of the monoterpene hydrocarbons was close to that for other species of spruce [8].

The sesquiterpene hydrocarbons were identified both by the GLC method through their relative retention times and by analysis of the PMR spectrum of the sesquiterpene fraction. The predominating components of this fraction were caryophyllene and α-copaene. We found smaller amounts of α-humulene, γ-murolene, α-murolene, sibirene, δ-cadinene, and longifolene. In its content of sesquiterpene hydrocarbons, the oriental spruce differed considerably from other species of spruce that have been studied. Thus, the oleoresins contained practically none of the products of I,II-cyclization of trans,cis-farnesyl pyrophosphate (longifolene, α-longipinene, longicyclene, which are the main sesquiterpenes of the Norway spruce [9], or of compounds of the cadalene series (cadinenes, and murolenes), which predominate in Far Eastern species of spruce [10]. The considerable amount of the products of the I,II-cyclization of trans,trans-farnesyl pyrophosphate, caryophyllene and α-humulene, is possibly a regional feature of the biogenesis of sesquiterpene compounds in the conifers of the Caucusus. The same compounds also predominate in the oleoresins of other species of this region – *Pinus pinea, P. eldarica,* and *P. pituysa*.

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The neutral diterpenoids of the oriental spruce are represented by bi- and tricyclic compounds.

Among the hydrocarbons we found pimaradiene, isopimaradiene, dehydroabietane, and an unidentified bicyclic hydrocarbon. The oxygen-containing diterpenoids were represented mainly by cis-abienol (∿50%). We also isolated epimannoyl oxide, isopimarinal, dehydroabietinal, methyl dehydroabietate, methyl isopimarate, an unidentified ketone, isopimarinol, and methyl 15-hydroxydehydroabietate. In contrast to the oleoresins of spruces of the section *Morinda*, in the resin of the oriental spruce we found no cembrene and compounds of the abietane type [3].

In the acidic fraction of the oleoresin of this species, as of other species of spruce, dehydroabietic acid predominated.

The chemical compositions of the oleoresins of the firs growing in the south of the country have hardly been investigated at all. In a study of the oleoresins of the Nordmann fir we have established that the monoterpene hydrocarbons include α-pinene, β-pinene, β-phellandrene, Δ^3-carene, limonene, camphene, and myrcene. The composition of the monoterpenes agrees with those of fir oleoresins studied previously [12].

From the sesquiterpene compounds we isolated longifolene, caryophyllene, longipinene, α-humulene, γ-murolene, γ-cadinene, sibirene, β-bisabolene, a mixture of δ-cadinene and α-murolene, and three unidentified hydrocarbons, A, B, and C. In the structure of the bicyclic hydrocarbon A (mol. wt. 204) according to PMR spectroscopy, there are two trisubstituted bonds (5.20 ppm, 1 H; 5.55 ppm, 1 H), a methyl group at a double bond (1.70 ppm, 3 H), two tertiary methyl groups (1.1 and 1.05 ppm, 3 H each), and a secondary methyl group (0.86 ppm, doublet, $J = 6$ Hz, 3 H). The presence of two double bonds was also confirmed by the ^{13}C NMR spectrum — singlets at 152.7 and 132.0 ppm, and doublets at 119.2 and 115.5 ppm.

The PMR spectrum of the bicyclic hydrocarbon B (mol. wt. 204) showed the signals of the protons of two trisubstituted double bonds (5.45 and 5.52 ppm, 1 H each), of two methyl groups on double bonds (1.67 ppm, 6 H), and of two other methyl groups (0.95 and 1.0 ppm, 3 H each).

The IR and UV spectra of hydrocarbon B show the existence of conjugation of the double bonds (1610 and 1562 cm^{-1}, λ_{max} 220 nm (log ε 4.12)). According to the PMR spectrum, the hydrocarbon has a trans-disubstituted double bond (AB system: 5.53 ppm, doublet, $J = 16$ Hz, 1 H; 5.82 ppm, doublet, $J = 16$ Hz, 1 H), a trisubstituted double bond (5.23 ppm, broadened triplet, $J = 7$ Hz, 1 H), an exomethylene double bond (4.80 ppm, 2 H), two methyl groups at a double bond (1.42 ppm, 6 H), and two tertiary methyl groups (0.95 ppm, 6 H). The presence of trans-disubstituted and exomethylene double bonds was confirmed by the IR spectrum (976 and 888 cm^{-1}). Consequently, hydrocarbon B is a monocyclic sesquiterpene (mol. wt. 204) with three double bonds.

In addition to the sesquiterpene hydrocarbons mentioned, α-ylangene, α-copaene, longicyclene, β-farnesene, and γ-elemene were identified by GLC from their relative retention times.

The composition of the sesquiterpene hydrocarbons of *Abies nordmanniana* is intermediate between the sesquiterpene hydrocarbon of *Abies sibirica* [9] (the main components of which are caryophyllene and α-humulene) and the Far Eastern species *Abies sachalinensis* and *Abies gracilis* [12] (the main component of which is longifolene). On the whole, the biogenesis of the sesquiterpenes of *Abies nordmanniana* is characterized by the predominance of the products of the I,II-cyclization of farnesyl pyrophosphate.

With respect to the composition of the diterpenoids of the oleoresin, the Nordmann fir differs sharply from the species of fir growing in Siberia and in the Far East studied previously [9, 13], primarily by its low content of cis-abienol (≈ 4%) and the presence of tricyclic compounds.

The diterpene hydrocarbons of the oleoresin were represented by dehydroabietane and abietadiene. In the neutral oxygen-containing fraction of the oleoresin we found the methyl abietate, methyl dehydroabietate, abietinal, and dehydroabietinal). From the same fraction we isolated abietinol, which is characteristic for fir oleoresins [13]. We have found methyl 15-hydroxydehydroabietate in the oleoresins of the genus *Abies* for the first time.

The acid fraction of the oleoresin of the Nordmann fir is close in its quantitative composition to those of the oleoresins of other species of fir [4] and contains mainly abietic acid, but differs by the presence of hydroxy acids.

With respect to the composition of the diterpenoids, the Nordmann fir is close to *Abies alba* [14, 15], from the oleoresin of which, likewise, dehydroabietane derivatives have been isolated. It must be mentioned that these two species belong to the same section of *Abies* [16].

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument, PMR spectra in CCl_4 on a Varian A-56/60A instrument, ^{13}C NMR spectra (22.63 MHz) on a Bruker Physik AG HX-90 instrument in the pulsed regime with Fourier transformation (with HMDS and CCl_4 as internal standards), and mass spectra on MS-902 instrument.

Treatment of the Oleoresins. The Oleoresin of the Oriental Spruce. The resin (42.2 g) was dissolved in 1 liter of 1% aqueous caustic soda solution. After the resin had dissolved completely, apart from the neutral components (15 g), the latter were removed and the solution was extracted with diethyl ether. The sodium salts were converted by acidification with 10% hydrochloric acid into the free acids (24.6 g). The neutral substances were chromatographed on alumina (activity grade II, 1:10). Petroleum ether eluted 7 g of hydrocarbons, and ethanol eluted 4.5 g of oxygen-containing compounds. The hydrocarbons were separated by vacuum distillation into monoterpenes (3.8 g; mp 60-100°C/10 mm Hg), sesquiterpenes (0.53 g, bp 70-100°C/$4 \cdot 10^{-2}$ mm Hg), and a still residue consisting of diterpenes (2.6 g).

Oleoresin of the Nordmann Fir. The fir oleoresin (375 g) was treated as described above. The neutral substances (153 g) were chromatographed on alumina. This gave 60 g of hydrocarbons and 46 g of oxygen-containing compounds. The hydrocarbons were separated by vacuum distillation into monoterpenes (45 g), sesquiterpenes (9.5 g), and diterpenes (2.5 g).

The Monoterpene Hydrocarbons. The qualitative and quantitative analysis of the monoterpenes was carried out by GLC [8].

Oriental Spruce. The monoterpene hydrocarbon fraction contained (%): α-pinene, 19.3; β-pinene, 38.7; limonene, 15.9; β-phellandrene, 14.5; Δ^3-carene, 6.8; myrcene, 2.5; camphene, 2.2.

Nordmann Fir. The following were found in the monoterpene hydrocarbon fraction (%): α-pinene, 46.1; β-pinene, 14.9; β-phellandrene, 21.4; Δ^3-carene, 10.4; limonene, 3.8; camphene, 1.7; myrcene, 1.5.

Sesquiterpene Hydrocarbons. The sesquiterpene hydrocarbons were analyzed by GLC in a Khrom-4 instrument with two capillary columns, 50 × 0.2 mm containing the stationary phases OV-17 and OV-225 at a rate of flow of carrier gas, nitrogen, of 6 ml/min and with the temperature of the column programmed from 100 to 200°C at the rate of 2°C per minute.

Oriental Spruce. The sesquiterpene hydrocarbon fraction was found on the basis of relative retention times and by the method of additives to contain (%): caryophyllene, 52.0, α-copaene, 21.0; α-humulene, 10; α-murolene, 7.5; γ-murolene, 1.6; sibirene 2.2; δ-cadinene, 2.4; longifolene, 0.9.

Nordmann Fir. It was established similarly that the sesquiterpene hydrocarbon fraction of the oleoresin contained (%): longifolene, 28.4; caryophyllene, 18.1; α-humulene, 12.6; longipinene, 9.3; γ-cadinene, 4.0; sibirene, 3.1; δ-cadinene, 4.5; γ-murolene, 2.0; α-murolene, 1.1; β-bisabolene, 0.5; longicyclene, 1.7; γ-elemene, 1.3; α-ylangene, 1.0; α-copaene, 0.9; β-farnesene, 0.9; hydrocarbon A, 4.0; hydrocarbon B, 2.1; and hydrocarbon C, 1.2.

The sesquiterpene fraction (6 g) was chromatographed on silica gel (150 g). Elution with petroleum ether gave fractions 1 (2.4 g), 2 (1.2 g), 3 (1.2 g), and 4 (0.4 g). According to their PMR spectra, fractions 2 and 4 consisted of caryophyllene and α-humulene, respectively.

The rechromatography of fraction 1 on SiO_2 + 20% $AgNO_3$ (60 g) led to the isolation of α-longipinene (0.12 g), longifolene (1.15 g), sibirene (0.06 g), hydrocarbon A (0.13 g), a mixture of δ-cadinene and γ-murolene (0.18 g), γ-murolene (0.04 g), and γ-cadinene (0.05 g).

By chromatography on SiO_2 + 20% $AgNO_3$ (25 g), fraction 3 yielded β-bisabolene (0.04 g), hydrocarbon B (0.04 g), hydrocarbon C (0.07 g), caryophyllene (0.03 g), and α-humulene (0.21 g).

The hydrocarbons were identified by their IR and PMR spectra.

Hydrocarbon A $[\alpha]_D^{20}$ −136° (in the pure form). IR spectrum, cm^{-1}: 880, 1207, 1372, 1380, 1385, 1445, 1466, 1602, 1667. ^{13}C NMR spectrum (ppm): 21.5, 22.7, 23.5, 27.0, 28.3, 33.0, 34.5, 37.9, 38.8, 40.1, 41.4, 115.5, 119.2, 132.0, 152.7. Mass spectrum: 204 M^+ (38%), 189 (17%), 161 (30%), 133 (100%), 119 (76%), 105 (71%).

Hydrocarbon B. IR spectrum (cm^{-1}): 1150, 1370, 1382, 1391, 1456. Mass spectrum: 204 (M^+, 64%), 189 (26%), 161 (43%), 134 (35%), 133 (76%), T 121 (46%), 119 (74%), 105 (82%), 93 (100%).

Hydrocarbon C. IR spectrum (cm^{-1}): 875, 888, 976, 1370, 1388, 1455, 1610, 1652, 3030, 3080. Mass spectrum: 204 (M^+, 57%), 189 (26%), 161 (60%), 148 (25%), 147 (28%), 134 (71%), 133 (71%), 119 (73%), 105 (76%), 93 (100%).

Diterpene Hydrocarbons. Oriental Spruce. The diterpenes (0.71 g) were chromatographed on SiO_2 + 5% $AgNO_3$ (30 g). Elution was carried out with petroleum ether containing increasing concentrations of diethyl ether. Pimaradiene (0.1 g), isopimaradiene (0.16 g), and dehydroabietadiene (0.1 g) were isolated and were identified from their spectral characteristics. The same fraction yielded an unidentified hydrocarbon (0.08 g) and epimannoyl oxide (0.25 g) with mp 98-99°C, which gave no depression of the melting point with an authetic sample.

Nordmann Fir. The diterpene hydrocarbons (1 g) were chromatographed on SiO_2 + 5% $AgNO_3$ (40 g), and a mixture of petroleum ether and diethyl ether eluted dehydroabietane (0.2 g) and abietadiene (0.4 g), which were identified by TLC and by their PMR, IR, and UV spectra.

Oxygen-Containing Diterpenoids. Oriental Spruce. The oxygen-containing diterpenoids (2.5 g) were chromatographed on silica gel (60 g) with elution by petroleum ether containing from 0 to 25% of diethyl ether. The following were isolated successively isopimarinal (0.1 g), pimarinal (0.08 g), dehydroabietinal (0.2 g), cis-abietinol with mp 40-41°C (1.3 g), isopimarinol (0.23 g), an unidentified ketone (0.1 g) (1690 cm^{-1}), and methyl 15-hydrodehydroabietate (0.15 g) with mp 81-83°C. The compounds were identified by their spectral characteristics in comparison with authentic samples.

Nordmann Fir. The oxidized compounds (7 g) were chromatographed on silica gel (140 g). A mixture of petroleum ether and 1% diethyl ether eluted abietinal (0.1 g), dehydroabietinal (0.13 g), methyl abietate (0.23 g), and methyl dehydroabietate (0.23 g) (a ratio of aldehydes and methyl esters of 2:3 was established by the PMR method). When the polarity of the solvent was increased (10% of diethyl ether), cis-abienol (1.6 g) abietinol (2.5 g) and methyl 15-hydroxydehydroabietate (1.0 g) were isolated.

The resin acids of the spruce and fir oleoresins were analyzed by the GLC method in the form of their methyl esters [11].

In the oleoresin of the oriental spruce we found the acids dehydroabietic (43.2%) isopimaric (19.0%), palustric and/or levopimaric (18.0%), abietic (5.2%), neoabietic (8.3%), sandarocopimaric (4.2%), and pimaric (0.5%).

In the oleoresin of the Nordmann fir we identified the acids abietic (41.0%), palustric and/or levopimaric (26.5%), dehydroabietic (17.1%), neoabietic (8.0%), sandacopimaric (1.2%), and trace amounts of isopimaric acid, and also the hydroxy acids 15-hydroxydehydroabietic (3.0%), and 15-hydroxyabietic (1.9%).

CONCLUSION

1. The terpenoid composition of the oleoresin of *Picea orientalis*, belonging to the section *Omorica* has been studied and 32 mono-, sesqui-, and diterpene compounds have been identified.

2. The chemical composition of the oleoresin of *Abies nordmanniana* has been studied and 37 mono-, sesqui-, and diterpene compounds have been identified. Three new susquiterpene hydrocarbons have been isolated. In addition to the diterpenoids characteristic for the oleoresins of all species of fir, methyl dehydroabietate and methyl 15-hydroxydehydroabietate have been found in the oleoresin of the Nordmann fir.

LITERATURE CITED

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