

MONO- AND SESQUITERPENOIDS OF THE OLEORESINS OF *Abies sachalinensis*, *A. mayriana*, and *A. gracilis*

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The compositions of the mono- and sesquiterpenoids of the oleoresins of the Sakhalin fir (*Abies sachalinensis* Fr. Schmidt), Mayr's fir (*Abies mayriana* Miyabe et Kudo), and the slender fir (*Abies gracilis* Kom.) have been studied. In the oleoresin of *A. gracilis* 37 compounds were identified, of which 11 were monoterpene hydrocarbons, seven were oxygen-containing monoterpenoids, 16 were sesquiterpene hydrocarbons, and three were sesquiterpene alcohols. In each of the oleoresins of *A. sachalinensis* and *A. mayriana* eight oxidized monoterpenoids, 18 sesquiterpene hydrocarbons, and a sesquiterpene alcohol - bisabolol - were identified.

The investigation of the chemical composition of the oleoresins of the Sakhalin fir (*Abies sachalinensis* Fr. Schmidt), Mayr's fir (*Abies mayriana* Miyabe et Kudo), and the slender fir (*Abies gracilis* Kom.) is part of a systematic study of the oleoresins of the coniferous trees of Siberia and the Far East.

The results of a study of the compositions of the monoterpene hydrocarbons and resin acids of the oleoresins of the Sakhalin fir and Mayr's fir have been published previously [1]. In the present paper we give the results of an investigation of the mono- and sesquiterpenoids of the oleoresin of the slender fir and the results of a further study of the oleoresins of the Sakhalin fir and Mayr's fir.

The composition of the monoterpene hydrocarbons of the fir was determined by gas-liquid chromatography (GLC) and proved to be similar to the compositions of the turpentines of the fir species studied previously. The main components are  $\beta$ -phellandrene (46.5%) and  $\alpha$ - and  $\beta$ -pinenes; they made up a total of  $\sim 50\%$  for the monoterpene fraction of the oleoresin studied. In addition to them, in the oleoresin of the fir we identified camphene, myrcene, limonene, fenchene,  $\gamma$ -terpinene, terpinolene, and p-cymene. The closeness of the compositions of the monoterpene hydrocarbons of the firs studied is evidence in favor of the hypothesis that the slender fir represents an isolated population of the Sakhalin fir [2], although the seasonal and geographical variations in the monoterpene hydrocarbons for firs may be considerable [3].

From the sesquiterpene hydrocarbon fractions we isolated a number of compounds by chromatography and identified them by their spectral characteristics. In addition, some components were identified by the GLC method from their relative retention times and by the use of additives.

We found the following sesquiterpene hydrocarbons in the oleoresin of the slender fir: longifolene, longicyclene,  $\alpha$ -longipinene,  $\beta$ -bisabolene,  $\alpha$ -ylangene,  $\alpha$ -copaene,  $\alpha$ - and  $\gamma$ -muurolene,  $\delta$ -cadinene, sibirene, caryophyllene,  $\alpha$ - and  $\beta$ -selinenes,  $\alpha$ -humulene,  $\gamma$ -elemene, and ar-curcumenene. All the above-listed components apart from  $\beta$ -selinene were found in the oleoresins of the Sakhalin fir and Mayr's fir, and also cyclosativene,  $\gamma$ -cadinene, and  $\epsilon$ -muurolene.

The main components of the sesquiterpene fractions of all three firs are longifoline and  $\beta$ -bisabolene. The amount of longifolene in the sesquiterpene hydrocarbon fractions of the oleoresin of the slender fir was 29%, and in the analogous fractions of the Sakhalin fir

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and Mayr's fir 20%. The amount of  $\beta$ -bisabolene in the oleoresins of the last two firs was the same, and in the oleoresin of the slender fir it was 18.7%. The compositions of the sesquiterpene hydrocarbons of the oleoresins of the Sakhalin fir and Mayr's fir were close to one another. The oleoresin of the slender fir differed from them by a higher combined content of  $\alpha$ -ylangene and  $\alpha$ -copaene (13%) and of  $\alpha$ - and  $\beta$ -selinenes (8%) and contained practically no  $\gamma$ -muurolene (of which there was 8% in the sesquiterpene fractions of the oleoresins of the Sakhalin fir and Mayr's fir, while  $\alpha$ -ylangene,  $\alpha$ -copaene, and  $\alpha$ -selinene were present in trace amounts).

In the composition of the sesquiterpenes, all the species of fir investigated were close to the balsam fir (*Abies balsamea* (L) Mill.) group [4], the biogenesis of the sesquiterpene compounds of which is characterized by a small contribution of the 1,10-cyclization of trans-cis-farnesyl pyrophosphate. The main directions of the hypothetical scheme for the biogenesis of sesquiterpenes in the firs studied are the 1,11-cyclization of trans-cis-farnesyl pyrophosphate (longifolene, longicyclene,  $\alpha$ -longipinene) and the 1,6-cyclization of trans-cis-farnesyl pyrophosphate ( $\beta$ -bisabolene, ar-curcumene). The oleoresin of the Siberian fir (*Abies sibirica* Ledeb.) studied previously [5] differs markedly from those of the species studied. In this, the products of the 1,11-cyclization of trans-trans-farnesyl pyrophosphate dominated —  $\alpha$ -humulene (41%) and caryophyllene (28%). Roughly the same total amount of these compounds (71%) was found by Smedman in the sesquiterpene hydrocarbons of *Abies bracteata* D. Don. [4].

From the fractions of mono- and sesquiterpene oxygen-containing compounds of the oleoresins of the species investigated we isolated and identified borneol,  $\alpha$ -terpineol, terpineol-4, bornyl acetate, and  $\alpha$ -terpenyl acetate. In addition to the compounds mentioned, in the oleoresin of slender fir we found nerolidol, geraniol,  $\beta$ -eudesmol, bisabolol, and sabinene hydrate, and in the oleoresins of the Sakhalin fir and Mayr's fir we also found thymol methyl ether, bisabolol, linalool, geraniol, and sabinene hydrate.

The great difference in the compositions of the sesquiterpene alcohols must be mentioned. While in the oleoresins of Sakhalin species bisabolol is the main component of the neutral oxygen-containing compounds, in the oleoresin of the slender fir nerolidol predominates and bisabolol is found only in trace amounts. It is possible that this is connected with the prolonged storage of the samples of oleoresin of the Sakhalin fir and Mayr's fir, as a result of which the labile sesquiterpene alcohols could undergo transformations.

#### EXPERIMENTAL

IR spectra were taken on a UR-20 instrument. PMR spectra were recorded in  $\text{CCl}_4$  on a Varian A-56/60 instrument using as internal standard HMDS, the chemical shift of which was taken as  $\delta$  0.05 ppm. Mass spectra were taken on a MS 902 instrument with a glass inlet system (120°C), 70 eV.

The analytical GLC of the sesquiterpene hydrocarbons was carried out on a Khrom-4 instrument with a 50 m  $\times$  0.2 mm column containing Tween-60 at a rate of flow of carrier gas, He, of 7 ml/min, with column temperatures of 120–180°C/2°C per minute, on a 50  $\times$  0.2 mm column containing Apiezon L at a rate of flow of carrier gas, He, of 5 ml/min and column temperatures of 110–190°C/2°C per minute, and on a Pye-105 instrument with a 5 m  $\times$  6 mm column with 10% of Tween-60 on Celite C-22 (45/60 mesh) as the stationary phase at column temperatures of 125–180°C/1°C per minute with a rate of flow of carrier gas of 150 ml/min.

The preparative GLC of the sesquiterpenes was carried out on the Pye-105 instrument, the conditions being somewhat varied according to the fraction being investigated. The oleoresin of the Sakhalin fir was collected in the Poronai region and that of Mayr's fir in the Korsakov region of the Sakhalin province in 1974, and the oleoresin of the slender fir in 1978 in the fir grove of the Kronotskii reserve.

Separation of the Resin Acids. The oleoresins of the Sakhalin fir and Mayr's fir (3 kg) were each dissolved in 6 liters of diethyl ether, the solution was treated with 30 liters of 1% NaOH, and the neutral fraction was extracted with 2 liters of diethyl ether. Polymeric products were precipitated by the addition of 2 liters of petroleum ether to each liter of ethereal extract. The precipitate was filtered off, the solvent was evaporated off from the filtrate, and the resulting mixture was dissolved in 6 liters of petroleum ether. The insoluble residue was filtered off and was added to the precipitate isolated previously, while the filtrate was evaporated in vacuum.

The oleoresin of the Sakhalin pine yielded 750 g (25%) of polymeric products and 558 g (18.6%) of neutral compounds soluble in petroleum ether; the oleoresin of Mayr's fir yielded 750 g (25%) and 582 g (19.4%), respectively. The oleoresin of the slender fir (2.17 kg) was treated with 20 liters of 1% NaOH with vigorous stirring and the mixture was left until the separation of the layers was complete. The upper layer, consisting of neutral substances, was partially crystallized. The crystals (200 g) — cis-abienol (IR, PMR) — were separated off, pressed out on the filter, and recrystallized from petroleum ether, mp 41–42°C. The solvent was distilled off from the filtrate, giving 594 g (27.4%) of neutral compounds (the percentage is given without taking the cis-abienol isolated into account).

Isolation of the Hydrocarbons. The fraction of neutral compounds was chromatographed on  $\text{Al}_2\text{O}_3$  (neutral, activity grade I–II) at a ratio of substance to sorbent of 1:10. Petroleum ether eluted hydrocarbons, and ethanol eluted oxygen-containing compounds. From the oleoresin of the Sakhalin fir we isolated 232 g (7.7%) of hydrocarbons and 244 g (8.1%) of oxygen-containing compounds, from the oleoresin of the Mayr fir 243 g (8.1%) and 216 g (7.2%), and from the oleoresin of the slender fir 284 g (13%) and 224 g (10.3%), respectively.

Fractionation of the Hydrocarbons. The hydrocarbon fractions were subjected to vacuum distillation to give monoterpenes (bp 70–110°C/20 mm Hg), sesquiterpenes (bp 80–140°C/2 mm Hg), and diterpene compounds (still residue). From the hydrocarbon fraction of the oleoresin of the Sakhalin fir we obtained 174 g (5.8%) of monoterpenes and 14.8 g (0.5%) of sesquiterpene hydrocarbons, from Mayr's fir 172 g (5.7%) and 13 g (0.4%), and from the oleoresin of the slender fir 229 g (10.5%) and 17.5 g (0.9%), respectively.\*

Analysis of the Monoterpenes. The GLC of the monoterpenes was carried out under conditions given previously [1]. From their relative retention times we identified in the monoterpene fraction of the oleoresin of the slender fir  $\alpha$ -pinene ( $28.0 \pm 0.9\%$ ), camphene ( $0.3 \pm 0.1\%$ ),  $\beta$ -pinene ( $22.2 \pm 1.6\%$ ), myrcene ( $0.8 \pm 0.1\%$ ), limonene ( $1.8 \pm 0.2\%$ ),  $\beta$ -phellandrene ( $46.5 \pm 1.6\%$ ), and traces of fenchene,  $\gamma$ -terpinene, terpinolene, and p-cymene.

Identification of the Sesquiterpene Hydrocarbons. The sesquiterpene hydrocarbon fraction of the oleoresin of the slender fir (15 g) was chromatographed on  $\text{SiO}_2$  impregnated with 20%  $\text{AgNO}_3$  (100 g). Gradient elution (petroleum ether–diethyl ether) yielded seven fractions: A (4.2 g), B (2.1 g), C (2.19 g), D (0.41 g), E (0.6 g), F (0.41 g), and G (0.6 g). By the preparative GLC of fraction A we obtained a mixture of  $\alpha$ -ylangene and  $\alpha$ -copaene (IR and PMR),  $\alpha$ -muurolene (PMR), and longifolene (PMR). The column chromatography of fraction B (1.5 g) on  $\text{SiO}_2$  (50 g) with elution by petroleum ether led to a mixture of  $\alpha$ -longipinene and longifolene (0.04 g, PMR), a mixture of longifolene, sibirene, and  $\alpha$ -ylangene (0.03 g, PMR),  $\alpha$ -selinene (0.15 g, IR, PMR), ar-curcumene (0.5 g, IR, PMR), and an unidentified compound (0.12 g). IR spectrum,  $\text{cm}^{-1}$ : 893, 1375, 1450, 1645, 3085, mass spectrum: 204 ( $\text{M}^+$ ), 161, 147, 134, 133, 121, 120, 119 (100%); PMR spectrum, ppm: 0.86 (doublet,  $J = 7$  Hz, 3 H), 1.64 (broadened, 3H), 1.7 (broadened, 3H); 4.56 (1 H), 4.75 (1 H), and 5.27 (broadened, 1H).

Fraction C consisted mainly of  $\beta$ -bisabolene, which was freed from impurities by preparative GLC. In fraction D we found  $\beta$ -selinene and  $\beta$ -bisabolene (IR, PMR). According to IR and PMR spectroscopy, fraction E consisted of a mixture of caryophyllene,  $\alpha$ -humulene, and  $\beta$ -selinene. Fractions F and G consisted of oxidized compounds of low polarity (IR, GLC) and were not investigated further.

By analytical GLC of the sesquiterpene fraction of the oleoresin of the slender fir, using relative retention times, we identified  $\gamma$ -elemene,  $\delta$ -cadinene, and  $\gamma$ -muurolene.

From the sesquiterpene hydrocarbon fraction of the oleoresin of the Sakhalin fir (8 g) by chromatography under similar conditions we isolated and identified from their IR and PMR spectra longifolene (0.78 g),  $\beta$ -bisabolene (0.71 g),  $\alpha$ -humulene (0.11 g),  $\alpha$ -muurolene (0.14 g),  $\gamma$ -muurolene (0.07 g), caryophyllene (0.04 g), longicyclene (0.03 g),  $\alpha$ -longipinene (0.04 g), and ar-curcumene (0.06 g).  $\alpha$ -Selinene,  $\gamma$ -elemene,  $\delta$ - and  $\gamma$ -cadinenes, sibirene,  $\epsilon$ -muurolene,  $\alpha$ -ylangene, and  $\alpha$ -copaene were identified in this fraction by their relative retention times (GLC).

From the sesquiterpene hydrocarbon fraction of the oleoresin of Mayr's fir we isolated longifolene (IR, PMR),  $\alpha$ -muurolene (PMR),  $\beta$ -bisabolene (IR, PMR), and  $\alpha$ -longipinene (IR) by preparative GLC; the other components were identified by their relative retention times (GLC).

\*All the percentage contents are given on the weight of the initial oleoresin. Then the percentage contents in the fractions are given.

Identification of the Oxygen-containing Mono- and Sesquiterpenoids. The fraction of oxidized mono- and sesquiterpenoids from the oleoresin of the slender fir (6.5 g) was chromatographed on a SiO<sub>2</sub> (140 g), and gradient elution yielded fractions I-VII. Fraction I (0.25 g) consisted of a mixture of bornyl acetate and  $\alpha$ -terpenyl acetate (PMR), while fraction II (0.13 g) was a mixture of bornyl acetate,  $\alpha$ -terpenyl acetate, and bisabolol (PMR, TLC).

Fraction III (1.71 g) was separated by column chromatography on SiO<sub>2</sub> (50 g) impregnated with 10% AgNO<sub>3</sub>. Petroleum ether with the addition of 20% of diethyl ether eluted terpineol-4 (0.04 g, PMR), petroleum ether-diethyl ether (1:1) eluted cis-abienol (0.15 g, PMR), nerolidol (0.46 g, IR, PMR,  $[\alpha]_D^{20} +5.2^\circ$  (in the pure form)).

From fraction IV (0.15 g) by chromatography on SiO<sub>2</sub> (7.2 g) impregnated with 10% AgNO<sub>3</sub>, we isolated  $\beta$ -eudesmol (0.02 g, IR). Fraction V was borneol (0.1 g), mp 110-111°C,  $[\alpha]_D^{20} -38.3^\circ$  (c 3.91, CHCl<sub>3</sub>), fraction VI (0.25 g) was  $\alpha$ -terpineol (TLC, PMR), and fraction VII (0.18 g) was sabinene hydrate (IR, PMR).

The fractions of oxygen-containing mono- and sesquiterpenoids of the oleoresins of the Sakhalin fir and Mayr's fir were treated similarly. From them we isolated and identified by IR and PMR spectroscopy bisabolol, thymol methyl ether, bornyl acetate,  $\alpha$ -terpenyl acetate, terpineol-4,  $\alpha$ -terpineol, and borneol. Geraniol, linalool, and sabinene hydrate were identified in these fractions by analytical GLC using the method of additives.

#### SUMMARY

1. The compositions of the mono- and sesquiterpenoids of the oleoresins of *Abies sachalinensis* Fr. Schmidt, *A. mayriana* Miyabe et Kudo, and *A. gracilis* Kom. have been studied.
2. From the compositions of the sesquiterpene hydrocarbons all three species can be assigned to the balsam fir group which is characterized by a low amount of compounds of the cadalene series.

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