# VOLATILE COMPONENTS OF THE OLEORESIN OF Abies alba

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The composition of the volatile components of the oleoresin of Abies alba Mill, has been studied. In this oleoresin 19 monoterpene compounds have been identified, the main ones of which are α- and β-pinenes and limonene. Among the 24 sesquitepene compounds identified in the oleoresin, 4 new terpenoids have been found: 10S,11S-himachala-2,3-diene, 10S,11S-himachala-3(12),4-diene, humula-4,9-dien-8-ol, and 4S,5S,10S-selin-6-en-4-ol. For the first time, α, β, and γ-himachalenes, 9-epicaryophyllene, intermedeol, and longiborneol have been found in the oleoresins of conifers of the genus Albies. A distinguishing feature of firs of the Albae series is the low content of oxygen-containing monoterpenoids and of sesquiterpenoids of the bisabolane series.

Continuing an investigation of the chemical composition of the oleoresins of conifers growing in the USSR, we have studied the mono- and sesquiterpene compounds of the oleoresin of the silver fir (Abies alba Mill.), which belongs to the species series Albae. It has been shown previously that in the composition of the mono- and sesquiterpenoids the oleoresins of the Far Eastern species of fir [1] belonging to the species series Veitchinae are close, while the species Abies sibiricae [2] and Abies semenovii [3], belonging to the same species series Sibiricae [4] differ considerably. It was therefore of interest to compare the compositions of the oleoresins of the silver fir and the Nordmann fir (Abies nordmaniana), also belonging to the Albae series and previously studied [5].

The monoterpene compounds of the oleoresin of Abies alba were represented mainly by hydrocarbons (22% on the oleoresin) among which α-pinene (58.8%), β-pinene (14.3) and limonene (23.5%) predominated. In addition to these, santene (traces) tricyclene (traces), camphene (0.7%), myrcene (0.6%), 3-carene (0.3%), α-fenchene (traces), β-phellandrene (2.9%), and p-cymene (traces) were identified by the GLC method. The main differences in the amounts of monoterpenes in the oleoresins of the silver fir and the Nordmann fir were connected with a considerable change in the β-phellandrene:linonene ratio. While in the oleoresin of the Nordmann fir, as in other USSR species of fir, a high amount of (21.4%) of β-phellandrene was found [5], among the monoterpenes of the silver fir this component was detected in very small amounts.

The amount of oxygen-containing monoterpenoids in the oleoresin investigated was small (∿0.2% on the oleoresin) and they were represented mainly by borneol, camphor, bornyl acetate, and α-terpineol. α-Terpenyl acetate, linalyl acetate, sabinene hydrate, and thymol methyl ether were detected in trace amounts. It must be mentioned that compounds of this group have not been detected at all in the oleoresin of the Nordmann fir. Apparently, the low amount of oxygen-containing monoterpenoids, especially bornyl acetate, in the oleoresins is a characteristic feature of firs of the Albae series.

The sesquiterpene compounds amounted to 3.6% of the oleoresin and were distributed uniformly between hydrocarbons (1.5%) and their oxygen-containing derivatives (2.1%). According to GLC the sesquiterpenes did not have a complex composition: in the hydrocarbon fraction 14-16 peaks were observed, and among them six compounds were represented in appreciable amounts – α-longipinene (9.2%), longifolene (24.7%), caryophyllene (11.7%), γ-humulene (8.0%), and two hydrocarbons not identified by GLC in amounts of 23% and 16%. We succeeded in isolating these components by adsorption chromatography, but their yields proved to be fairly low (∿12 and ∿7%, respectively), which indicated a labile nature of these sesquiterpenes. According to the results of PMR and UV spectroscopy, mass spectrometry, and isomerization into hydrocarbons of the longifolane series, the compounds isolated

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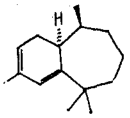
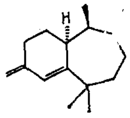
were conjugated dienes of the himachalane series (I) and (II). The determination of the structures of these dienes and also of two other new sesquiterpenoids from the oleoresin of the silver fir will be discussed in one of our following papers. In addition to the main components mentioned, α, β, and γ-himachalenes, longicyclene, α- and γ-humulenes, epicaryophyllene, sibirene, β-bisabolene and β-farnesene were isolated from this fraction and were identified from their PMR spectra. The results obtained show that the biosynthesis of sesquiterpenes in the oleoresin of the silver fir takes place with a predominance of the I,II-cyclization of farnesyl pyrophosphate: the proportion of compounds of the himachalane-longifolane series amounts to ~75% and that of sesquiterpenes of the humulane-caryophyllane series to ~20%. A comparison of the chromatograms of the sesquiterpene fractions of the oleoresins of the silver fir and of the Nordmann fir indicated a closeness of the species. In these fractions, longifolene predominated while α-longipinene, caryophyllane, and α-humulene were present in appreciable amounts. The hydrocarbons A, B, and C, previously unidentified in the Nordmann fir [5] are himachala-2,4-diene (I), γ-himachalene and γ-humulene, respectively. A comparative analysis of the composition of the sequiterpenes of the silver fir and of the Nordmann fir indicates that the sesquiterpenes of the oleoresins are a fairly reliable chemotaxonomic characteristic of conifers of the genus Abies.



I



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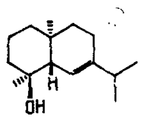

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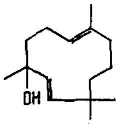

IV

According to GLC results, the fraction of oxygen-containing mono- and sesquiterpenoids contained an appreciable amount of six sesquiterpene compounds. Adsorption chromatography on silica gel and on silica gel impregnated with silver nitrate led to the isolation and identification from their spectral characteristics of cubebol and epicubebol (1:1, PMR 30%), longiborneaol (12%), himachalol (6%), two new sesquiterpenoids – selin-6-en-4β-ol (III) (~9%) and humula-4,9-dien-8-ol (IV) (4%) epibubenol (2%), caryophyllene oxide (2%), and in-termedeol (2%). In spite of the practical absence of compounds of the cadalene and selinane series in the hydrocarbon fraction of the oleoresin, representatives of these biogenetic groups made up a considerable part of the oxygen-containing sesquiterpenoids. Apparently, the biosynthesis of the sesquiterpene compounds among the hydrocarbon and the oxygen-containing derivatives in the silver fir takes place by different routes. The absence of α-bisabolol – the sesquiterpenoid that is most characteristic for the fir species studied [1-3] - must be mentioned. The practical absence of compounds of the bisabolene series, together with the low amount of bornyl acetate, is the main distinguishing characteristic of fir of the Albae series.

## EXPERIMENTAL

IR-spectra were recorded on a UR-20 instrument using solutions in $CCl_4$ and UV-spectra on a Specord UV-VIS instrument using solutions in ethanol. Mass spectra were taken on a MS-902 spectrometer at a temperature for direct introduction of 120°C at an ionizing energy of 70 eV.

PMR spectra were obtained on Varian A 56/60 A and Bruker WP 200 SY instruments using solutions in $CCl_4$ and $CDCl_3$ respectively. $^{13}C$ NMR spectra were recorded on a Bruker WP 200 SY instrument in $CDCl_3$. Angles of optical rotation were measured on a Zeiss polarimeter (GLR) for solutions in chloroform.

Analytical GLC was performed on a Chrom-41 chromatograph (Czechoslovakia) with glass capillary columns containing PMS (31 m) and XE-60 (50 m) at a rate of flow of the carrier gas, nitrogen, of 2 ml/min. The temperature for the analysis of the monoterpenes was 40-80°C/2°C per minute, that for sesquiterpenes 80-140°C/2°C per minute, and that for the oxygen-containing mono- and sesquiterpenoids 80-160°C/2°C per minute, the components being identified from their relative retention times with the aid of additives, and quantitative analysis being performed by the method of simple normalization.

Preparative GLC was performed on a Pye-105 instrument with a 6 mm × 4 m column, containing 5% of SE-30, on Chromaton N, 0.250-0.315 mm, at a thermostat temperature of 120°C with a rate of flow of the carrier gas, nitrogen, of 60 ml/min.

Air-dry silica gel 0.040-0.140 mm and silica impregnated with 20% of silver nitrate ($SiO_2$ + $AgNO_3$) were used for adsorption chromatography. The eluent was petroleum ether (bp 40-60°C) with the gradient addition of 0-50% of diethyl ether.

The silver fir oleoresin was collected in the Ust'-Chornya forest area of the Transcarpathian province in August, 1985.

Isolation of the Mono- and Sesquiterpenoids. A solution of 100 g of the oleoresin in 200 ml of diethyl ether was treated twice with 500-ml portions of a 1% solution of NaOH. The ethereal layer was separated off and the aqueous layer was twice extracted with 100 ml of diethyl ether. The ethereal extracts were combined and the solvent was distilled off. The neutral fraction so obtained (57 g) was chromatographed on silica gel (600 g), whereupon petroleum ether eluted hydrocarbons (24 g), and diethyl ether eluted the oxygen-containing compounds (33 g). Fractional distillation of the hydrocarbons (24 g) gave 22 g of monoterpenes (60-110°C/10 mm Hg), 1.5 g of sesquiterpenes (120-150°C/3 mm Hg), and 0.5 g of diterpenes (still residue). Vacuum distillation of the oxygen-containing compounds yielded 2.3 g of mono- and sesquiterpenoids (80-170°C/3 mm Hg).

Isolation of the Individual Sesquiterpenes. The sesquiterpene fraction (1.05 g) was separated by chromatography on $SiO_2$ + $AgNO_3$ (30 g) into fractions I-X.

Longicyclene. According to GLC, fraction I (0.02 g) was an individual substance and its PMR spectra coincided with that of an authentic sample of longicyclene.

10S,11S-Himachala-2,4-diene (I). According to spectral characteristics, fraction II (0.12 g) was the individual compound (I), $[\alpha]_D^{20}$ +42.5° (c 3.0). UV spectrum: 256 nm ($\varepsilon$ 4800).

IR spectrum ($cm^{-1}$): 1660, 1600, 1465, 1385, 1370, 1202, 1110.

Mass spectrum: 204 ($C_{15}H_{24}$, $M^+$, 68%), 189(23), 161(48), 133(100), 119(87); 105(78).

PMR spectrum (ppm): 0.83 (3 H, doublet, J = 6.5 Hz); 1.03 (3H, singlet); 1.09 (3H, singlet); 1.72 (3H, br. singlet); 5.23 (1H, br. $\omega$ = 11 Hz); 5.54 (1H, br. singlet, $\omega$ = 3 Hz).

$^{13}$C NMR spectrum (ppm): quartets – 21.1, 22.30, 26.60, 32.55; triplets – 23.04, 27.99, 39.76, 40.91; doublets – 34.21, 37.57, 115.96, 119.69; singlets – 38.47, 132.59, 153.45.

Longifolene and α-Longipinene. The hydrocarbons isolated by preparative GLC from fraction III (0.3 g) had $[\alpha]_D^{20}$ +20° (c 14.0) and +22° (c 3.0) and, according to their spectral and chromatographic characteristics were identical with authentic samples of longifolene and α-longipinene.

Siberene and α-Copaene were identified from the PMR spectra of the components isolated from fraction IV (0.25 g) by preparative GLC; sibirene; $[\alpha]_D^{20}$ +110° (c 2.0).

α- and β-Himachalenes. The hydrocarbons isolated from fraction IV (0.25 g) by preparative GLC had $[\alpha]_D^{20}$ −137° (c 1.4) and +132° (c 6.0) and were identified by a comparison of their IR and PMR spectra with those given in the literature [6] as α- and β-himachalenes, respectively.

10S,11S-Himachala-3(12),4-diene. According to the results of PMR, UV spectroscopy, and mass spectrometry, fraction V (0.07 g) corresponded to himachala-3(12),4-diene (II), $[\alpha]_D^{20}$ +2.7° (c 22.0).

UV spectrum: 244 nm ($\varepsilon$ 6200).

IR spectrum ($cm^{-1}$): 3080, 1632, 1601, 1460, 1385, 1370, 1110, 895, 874.

Mass spectrum: 203 ($C_{15}H_{24}$, $M^+$, 98%), 189 (78), 161(100), 147(45), 133(64), 119(40), 105(63), 91(60).

PMR spectrum (ppm): 0.94 (3H, doublet, J = 6.5 Hz); 0.96 (3H, singlet); 1.13 (3H, singlet); 4.65 (1H, multiplet, ω = 4 Hz); 4.70 (1H, multiplet, ω = 4 Hz); 5.94 (1H, singlet).

γ-Himachalene and γ-Humulene. The spectral characteristics (IR, PMR) of fractions VI (0.03 g) and VII (0.08 g) coincided with those given in the literature for γ-himachalene [7] and γ-humulene [8].

β-Bisabolene and β-Farnesene. Components isolated by preparative GLC from fraction VIII (0.05 g) proved by their PMR spectra to be β-bisabolene, $[\alpha]_D^{20}$ +9.9° (c 2.7), and β-farnesene.

Caryophyllene. According to PMR and GLC results, fraction IX (0.11 g) proved to be caryophyllene, $[\alpha]_D^{20}$ −6.5° (c 1.7).

Epicaryophyllene and α-Humulene. These were isolated by preparative GLC from fraction X (0.05 g), obtained by extracting the aqueous ammoniacal eluate with diethyl ether. The PMR spectra of the compounds coincided with those for epicaryophyllene [9] and α-humulene.

Oxygen-containing Mono- and Sesquiterpenoids. The fraction of oxygen-containing mono- and sesquiterpenoids (1.5 g) was separated by chromatography on $SiO_2$ + $AgNO_3$ (50 g) into fractions I-V.

The rechromatography of fraction I (0.4 g) on silica gel (20 g) yielded 0.1 g of hydrocarbons, 0.1 g of a mixture on bornyl acetate and α-terpinyl acetate (3:1, PMR), 0.03 g of caryophyllene oxide, and 0.1 g of longiborneol, mp 110-112°C, $[\alpha]_D^{20}$ +6.3° (c 4.8).

By chromatography on silica gel (20 g), fraction II (0.4 g) yielded 0.05 g of longiborneol, 0.03 g of epicubenol, mp 57-59°C, 0.09 g of himachalol, mp 64-66°C, $[\alpha]_D^{20}$ +28° (c 5.8) (according to the literature, mp 67-68°C [10]), and 0.19 g of a mixture of cubebol and epicubebol (1:1, PMR).

The chromatography of fraction III (0.2g) on silica gel (10 g) gave 0.1 g of a mixture of cubebol and epicubenol, and 0.03 g of intermedeol with mp 45-47°C, $[\alpha]_D^{20}$ +21° (c 3.4) (according to the literature, mp 47-48°C [11]).

By rechromatography on silica gel (10 g) fraction IV (0.15 g) yielded 0.03 g of a mixture of borneol and α-terpineol (3:1, PMR) and 0.09 g of 4S,5S,10S-selin-6-en-4-ol (III).

4S,5S,10S-Selin-6-en-4-ol. mp 89-90°C (acetonitrile) $[\alpha]_D^{20}$ +15.5° (c 4.3). IR spectrum ($cm^{-1}$): 3600, 1460, 1382, 1110, 1095, 920. Mass spectrum: 222 ($C_{15}H_{26}O$, $M^+$, 17%), 204(92), 189(43), 161(87), 137(40), 135(42), 121(48), 105(52), 81(100).

PMR spectrum: 0.79 (3H, singlet); 0.99 (6H, doublet, J = 7 Hz); 1.09 (3H, singlet); 2.19 (1H, septet, J = 7 Hz); 5.52 (1H, br. ω = 5 Hz). $^{13}C$ NMR spectrum (ppm); quartets – 17.26, 21.29, 21.48, 21.94; triplets – 20.12, 23.08, 39.08, 40.33, 42.84; doublets – 34.64, 54.04, 116.80; singlets – 33.74, 76.08, 143.46.

The chromatography of fraction V (0.15 g) on silica gel (10 g) gave 0.04 g of silin-6-en-ol (III), 0.02 g of α-terpineol, and 0.06 g of humula-4,9-dien-8-ol (IV).

Humula-4,9-dien-8-ol (IV). mp 67-68°C (acetonitrile). Mass spectrum; 222 ($C_{15}H_{26}O$, $M^+$, 2%), 204(47), 189 (22), 161(28), 121(23), 119(22), 109(100).

PMR spectrum (ppm); 0.93, 0.94, 1.25, 1.57 (singlets, 3H); 4.94 (1H, triplet, J = 8 Hz); 5.22 and 5.32 (doublets, J = 16 Hz, 1 H each).

$^{13}C$ NMR spectrum (ppm); quartets – 18.48, 27.99, 27.99, 28.99; triplets – 22.28, 23.78, 39.46, 40.54, 44.27; doublets – 126.58, 134.18, 136.26; singlets – 34.97, 72.77, 132.92.

## SUMMARY

1. The composition of the volatile components of the oleoresin of the Abies alba Mill has been studied. In it 19 monoterpene and 24 sesquiterpene compounds have been identified.

2. Among the sesquiterpenoids of the oleoresin of the silver fir, 4 new compounds have been found – 10S,11S-himachala-2,4-diene, 10S,11S-himachala-3(12),4-diene, 4S,5S,10S-selin-6-en-4-ol, and humula-4,9-dien-8-ol, and the amounts and structures of these terpenoids have been determined.

3. Distinguishing features of the amounts of lower terpenoids in the oleoresins of firs of Albae section has been ascertained. It has been shown that the composition of the sesquiterpenoids can be used as a chemotaxonomic characteristic of the firs.

## LITERATURE CITED

1. T. F. Titova, V. A. Khan, V. I. Bol'shakova, L. I. Demenkova, Zh. V. Dubovenko, and V. A. Pentegova, Khim. Prir. Soedin., 195 (1980).
2. V. A. Pentegova, Zh. V. Dubovenko, L. M. Vol'skii, S. M. Vasilyuk, M. A. Chirkova, and É. N. Shmidt, Izv. Sibirskogo Otd. Akad. Nauk SSSR, Ser. Khim. Nauk., Issue No. 4, Series No. 2, 114 (1968).
3. V. A. Khan, N. A. Pankrushina, É. N. Shmidt, Zh. V. Dubovenko, and V. A. Pentegova, Khim. Prir. Soedin., 115 (1984).
4. E. G. Bobrov, Forest-Forming Conifers of the USSR [in Russian], Nauka, Leningrad (1978).
5. É. N. Shmidt, V. A. Khan, Z. A. Isaeva, T. D. Drebushchak, Zh. V. Dubovenko, É. P. Kemertelidze, and V. A. Pentegova, Khim. Prir. Soedin., 189 (1982).
6. T. C. Joseph and Sukh Dev., Tetrahedron, 24, 3808 (1968).
7. R. Tabacchi, J. Garnero, and P. Buil, Helv. Chim. Acta, 57, 849 (1974).
8. Yano Katzuini and Nishima Tamiio, Phytochemistry, 13, 1207 (1974).
9. F. Bohlmann and C. Zdero, Phytochemistry, 17, 1135 (1978).
10. S. C. Bisarja and Dev. Sukh, Tetrahedron, 24, 3861 (1968).
11. G. L. Chetty, V. B. Zalkow, L. H. Zalkow, Tetrahedron Lett., 3223 (1968).

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# CYCLIZATION OF SOME LABDANE ALCOHOLS WITH A HYDROXY GROUP AT C-13 BY A SUPERACID

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On the superacid cyclization of the labdane alcohols manool, isomanool, and sclareol the tetracyclic hydrocarbon (1S,2R,11S,12R,15R)-2,7,7,11,15-pentamethyltetracyclo[10.2.1.$0^{2,11}$.$0^{3,8}$]pentadec-3(8)-ene with a new carbon skeleton is formed.

It was established previously [1] that on the superacid cyclization of a number of labdane alcohols with a tertiary hydroxy group at C-13 [manool (I), isomanool (II), and sclareol (III)] a mixture of two new tetracyclic hydrocarbons was formed. The structure of one of them – (1R,2S,7S,11S,12R,13R)-2,6,6,11,13-pentamethyltetracyclo[10.2.1.$0^{1,10}$.$0^{2,7}$]pentadec-9-ene (IV) – was shown by x-ray structural analysis. The structure of the second, liquid, hydrocarbon remained unelucidated.

In the present communication we give the results of an investigation of this new compound, which is (1S,2R,11S,12R,15R)-2,7,7,11,15-pentamethyltetracyclo[10.2.1.$0^{2,11}$.$0^{3,8}$]pentadec-3(8)-ene (V).

When hydrocarbon (V) was ozonized it gave a mixture of a crystalline diketone and a liquid epoxide. The structure of the diketone was determined by x-ray structural analysis. It proved to be (1S,2R,11S,12R,15R)-2,7,7,11,15-pentamethyltricyclo[10.2.1.$0^{2,11}$]pentadecane-3,8-dione (VI). Consequently, the initial hydrocarbon possessed the structure shown in formula (V). The absolute configuration of the latter followed from the fact that on the migration of a methyl group from the C-10 to the C-9 position of the labdane skeleton it remains on the β-side of the molecule. The structure of hydrocarbon (V) was also confirmed by $^{13}$C NMR results (see [1]).

The second product of the oxidation of hydrocarbon (V) by ozone was (1S,2R,11S,12R,15R)-2,7,7,11,15-pentamethyl-3α,8α-epoxytetracyclo[10.2.1.$0^{2,11}$.$0^{3,8}$]pentadecane (VII). Its

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