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SCHEME FOR THE GROUP SEPARATION OF THE COMPONENTS OF CONIFER OLEORESINS. TERPENOIDS OF THE OLEORESIN OF Pinus cembra

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The oleoresins of conifers consist of very complex mixtures of terpenoids sometimes also containing paraffinic hydrocarbons [1]. Their analysis usually includes preliminary separation into acidic and neutral fractions. The acidic fraction is methylated with diazomethane and investigated by GLC, and the neutral fraction is separated by chromatography into a hydrocarbon fraction and a fraction of oxygen-containing compounds or is subjected directly to vacuum distillation. In both cases, the neutral substances are then subjected to repeated chromatographic separation in order to isolate individual components. The closeness of their adsorption properties and the lability of the terpene compounds considerably complicate the process of chromatographic separation and make it difficult to evaluate quantitatively the amounts of the characteristic types of terpenoids and of individual compounds. The latter aspect of the problem is particularly pronounced with the use of high-temperature vacuum distillation for the preliminary separation of the neutral fraction.

The presence in all conifer oleoresins of certain classes of neutral and acidic terpene components (hydrocarbons, aldehydes, resin acids, etc.) makes it possible to separate the oleoresin into groups of compounds by using the differences in the adsorption and chemical properties of these groups. Such a separation can be performed by a scheme which we have tried out on the oleoresins of the Korean pine [2] and the Japanese atone pine [3]. It will probably be suitable for the separation of the oleoresins of other types of conifers, as well.

A reduction in the probability of the formation of by-products from the native compounds is achieved by using air-dry SiO_2 for chromatography at all stages of this scheme. To separate the hydrocarbons from the fraction of neutral oxygen-containing compounds it is possible to use Al_2O_3 (activity grade III). In all cases, the eluent is petroleum ether (bp 40-70°C) with increasing concentrations (from 0 to 100%) of diethyl ether. Analytical TLC on SiO_2, which is used to check the separation of the fractions of neutral oxygen-containing compounds and of the methylated acid fraction of the oleoresin, is performed with the same solvents. This separation is performed with the aid of marker substances. All the substances having R_f values greater than the marker substance isocembrol (or manool, epimanool, or cis-abienol, which have the same R_f value on SiO_2) pass into the fraction of oxides, esters, and carbonyl compounds. All substances having the same R_f as isocembrol pass into the second fraction (nonpolar monohydric alcohols. The fraction of polar monohydric alcohols includes substances having R_f values lower than that of isocembrol and greater than that of the second marker substance, pinusolide. The separation of the monohydric alcohols into two fractions is due to the fact that the less polar tertiary diterpene alcohols are frequently comparatively well separated from the more polar primary diterpene alcohols. These two groups of alcohols are common components of conifer oleoresins and their separation is therefore justified. All substances having R_f values equal to or smaller than that of pinusolide pass into the fraction of di- and polyfunctional compounds. (See scheme on following page).

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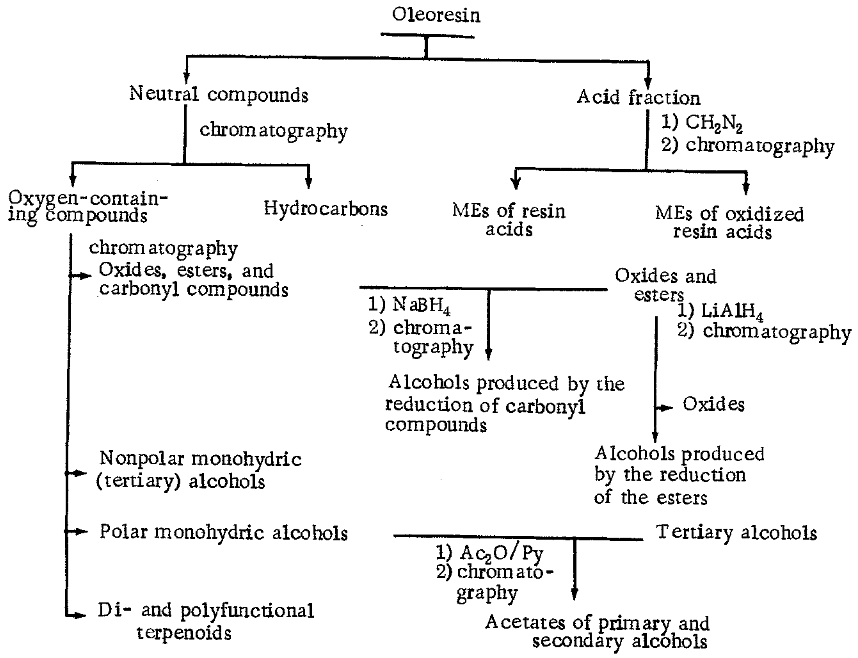

The fractions of hydrocarbons and methyl esters of resin acids were analyzed by GLC. All the other groups of compounds obtained by the treatment of the oleoresin by the Scheme given were subsequently analyzed by GLC, NMR, and adsorption column chromatography on SiO_2 and SiO_2 + 5% $AgSO_3$.

We have used the above-described scheme of group separation to investigate the terpenoids of the oleoresin of the Swiss stone pine (Pinus cembra L.). This oleoresin was collected in Transcarpathia in August, 1970, from trees growing at a height of 1340 m above sea level. The yield of the acid fraction of the oleoresin was 67.8% and of the neutral fraction 31.4%.

The total hydrocarbons (56.8% of the neutral fraction) were analyzed directly by GLC. They contained monoterpene (78%), sesquiterpene (20.5%), and diterpene (1.5%) hydrocarbons: α-pinene, fenchene, camphene, β-pinene, Δ^3-carene, limonene, terpinolene, myrcene, β-phellandrene, γ-terpinene, p-cymene, α-longipinene, cyclosativene, β-elemene, sibirene, longicyclene, longifolene, caryophylene, ε-murolene, α-humulene, γ-murolene, α-amorphene, α-murolene, δ-cadinene, γ-cadinene, α-cadinene, cembrene, isopimara-7,15-diene, and abietadiene. The methyl ether of thymol was detected in the same fraction. Among the monoterpenes the predominating compounds were α-pinene (47.1%) and Δ^3-carene (23.8%). In the sesquiterpene group the main compounds were sibirene, caryophyllene, and α-murolene, and the main components among the diterpenes were cembrene, isopimara-7,15-diene, and an unidentified hydrocarbon. Haagen-Smith [4], who investigated the monoterpenes of the oleoresin of the European stone pine, identified only α- and β-pinenes.

The fraction of oxides, esters, and carbonyl compounds (yield 20%; here and below the yields are given in relation to the total amount of neutral oxygen-containing compounds) consisted, according to the NMR spectrum, mainly of diterpene aldehydes and methyl esters, which were present in a ratio of 20 : 21 (determined from the integral intensities of the signals of the aldehyde and methoxycarbonyl groups). Reduction of this fraction with sodium tetrahydroborate in ethanol gave a mixture of alcohols produced by the reduction of the carbonyl compounds (aldehydes) (yield 9.5%) and a fraction of oxides and esters (yield 9.5%). According to the PMR spectrum, the mixture of alcohols consisted of dehydroabietinol, isopimara-7,15-dien-18-ol, abietinol, palustrol (main components), and isopimara-8,15-dien-18-ol.

In the oxide and ester fractions, the oxides were present in very small amount, and therefore this fraction was analyzed directly by GLC. The following native esters of resin acids were found: methyl isopimarate (1.6%), methyl dehydroabietate (3.3%), methyl lambertianate (83.6%), and an unidentified ester (retention time relative to methyl isopimarate 0.70) (11.5%). The presence of these esters was confirmed by the NMR spectrum. The oxide fraction (yield 0.5%) obtained after the elimination of the methyl esters by reduction with lithium tetrahydroaluminate and chromatography contained nine compounds, according to GLC. Its main component – manoyl oxide – was identified by the method of additives, and also by TLC.

The fraction of nonpolar monohydric alcohols (yield 55.8%) proved to be a mixture of two compounds – isocembrol and 4-epiisocembrol [5] present in a ratio of 95 : 5 (determined from the integral intensities of the

H_3 signals in the NMR spectrum of this mixture). In this mixture we detected no cis-abienol or bisabolol, which are present in the analogous fraction of the oleoresin of the Korean pine [2].

The yield of the polar monohydric alcohol fraction was 14.7% Its unacetylatable part (yield 7.9%; tertiary alcohols) formed a crystalline mass consisting mainly of the δ-cadinol. The latter was isolated from this fraction by preparative TLC. The acetylatable part of the monohydric alcohol fraction (yield 6.3%) consisted, according to the NMR spectrum of their combined acetates, of (13E)-labd-8(17),13-dien-15-ol, isopimara-7,15-dien-18-ol (main components), dehydroabietinol, abietinol, isopimara-8,15-dien-18-ol, and a small amount of unidentified components.

Rechromatography of the fraction of di- and polyfunctional terpenoids on SiO_2 gave pinusolide (yield2%), an unresolvable mixture of isoagatholal and methyl isocupressate (1%) in a ratio of 4:1 (NMR spectrum), and agathadiol (1%). When the mixture of isoagatholal and methyl isocupressate was treated with sodium tetrahydroborate in ethanol, a mixture of agathdiol and unchanged methyl isocupressate was obtained, each of these components being identified by TLC in comparison with authentic samples.

The methylated acidic fraction of the oleoresin was separated by chromatography into a mixture of esters of resin acids (3% of diethyl ether in the eluent, yield 95%) and a mixture of esters of oxidized resin acids (100% of diethyl ether in the eluent, yield 5%). Analysis of the mixture of methyl esters of resin acids by GLC showed the presence of esters of the following acids: Δ^8-isopimaric (1.9%), sandaracopimaric (0.5%), levopimaric + palustric (?) (3.5%), isopimaric (21.4%), dehydroabietic (4.6%), abietic (30.3%), lambertianic (35.6%), and neoabietic (2.2%). No methyl pimarate was found in the mixture.

The oxidized resin acids were obtained in the form of a very complex mixture of methyl esters, the main component of which was isolated by chromatography on SiO_2 + 5% $MgNO_3$ and was identified by NMR and IR spectroscopy as methyl isocupressate [6, 7].

Thus, the composition that has been found for the oleoresin of the European stone pine is very close to that of the Korean pine [2] and the Siberian pine [8, 9]. The botanical closeness of these species is reflected in the closeness of the chemical composition of their oleoresins.

EXPERIMENTAL

The instruments and methods have been described previously [3]. The analysis of the diterpenoid hydrocarbons by GLC was performed under the same conditions as for the sesquiterpene hydrocarbons [3]. The separation of the oleoresin into acidic and neutral fractions was performed as described elsewhere [10]. All the chromatographic separations were performed at a ratio of substance and adsorbent of 1:20-30.

Separation of the Fractions of Neutral Compounds. The neutral compounds (4.48 g) obtained from 14 g of oleoresin were chromatographed on 90 g of alkaline alumina (activity grade III). Petroleum ether eluted 2.52 g (56.2%) of hydrocarbons, and ethanol eluted 1.9 g (42.4%) of a fraction of neutral oxygen-containing compounds. Chromatography of the latter gave 0.28 g of a fraction containing oxides, esters, and carbonyl compounds, 1.06 g of a nonpolar monohydric alcohol fraction, 0.28 g of a polar monohydric alcohol fraction and 0.14 g of a fraction of di- and polyfunctional compounds. The concentrations of diethyl ether in the eluents for these fractions were 4, 15, 20, and 100%, respectively.

Separation of the Fraction of Oxides, Esters, and Carbonyl Compounds. This fraction (0.38 g) was dissolved in 10 ml of ethanol, and 0.5 g of dry sodium tetrahydroborate was added to the resulting solution, with sitrring. After continued stirring at room temperature for 20 min, the mixture was poured into 100 ml of water and extracted twice with petroleum ether. The usual working up followed by chromatography gave 0.18 g of a mixture of alcohols produced by the reduction of the aldehydes and 0.18 g of an unchanged fraction consisting of a mixture of esters and oxides. The reduction of the latter with lithium tetrahydroaluminate in diethyl ether (15 min at room temperature) followed by chromatography gave 0.17 g of a mixture of alcohols produced by the reduction of the methyl esters and 0.009 g of an oxide fraction.

Isocembrol and 4-Epiisocembrol. The chromatography of 1.06 g of the nonpolar monohydric alcohol fraction on 30 g of SiO_2 + 5% of $AgNO_3$ gave 0.9 g of isocembrol with n_D^{20} 1.5020, $[\alpha]_D^{20}$ + 75.5° (c 2.5; chloroform); literature data [11]: $[\alpha]_D^{20}$ + 80.1° (chloroform) and 0.05 g of 4-epiisocembrol, identified from its IR spectrum and by TLC with an authentic sample.

Separation of the Polar Monohydric Alcohol Fraction. This fraction (0.28 g) was dissolved in 5 ml of pyridine, and 2 ml of acetic anhydride was added to the resulting solution. After being kept for 3 h, the usual working up and chromatography yielded 0.12 g of a mixture of acetates and 0.15 g of a mixture of unacetylatable alcohols. Crystallization of the latter from diethyl ether gave 0.1 g of δ-cadinol with $[\alpha]_D^{20}$ + 110° (c 0.5; chloroform); literature data [12]: $[\alpha]_D^{20}$ + 118.4° (ethanol). A mixture with an authentic sample of δ-cadinol melted without depression.

Separation of the Fraction of Di- and Polyfunctional Compounds. The chromatography of 0.14 g of this fraction on 30 g of SiO_2 yielded: 0.04 g of pinusolide with mp 82-83°C (from diethyl ether), $[\alpha]_D^{20}$ + 55° (c 1.5; chloroform) giving no depression of the melting point with an authentic sample; 0.02 g of a mixture of methyl isocupressate and isoagathol (25% of diethyl ether in the eluent); and 0.02 g of agathadiol (50% of diethyl ether in the eluent) with mp 108-109°C (from ethanol), giving no depression of the melting point with an authentic sample.

SUMMARY

A scheme has been proposed for the group separation of the components of conifer oleoresins, and the mono-, sesqui- and diterpenes of the oleoresins of the European stone pine have been investigated by means of this scheme.

2. The composition of the oleoresin of the European stone pine is very similar to those of the Siberian pine and of the Korean pine, but differs from the latter by the absence of cis-abienol and bisabolol.

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A CHROMATOGRAPHIC INVESTIGATION OF THE n-HEPTANE IN THE ESSENTIAL OIL OF REPRESENTATIVES OF THE FAMILY PINACEAE

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There is no information in the literature on the presence of n-heptane, C_7H_{16} in the essential oils of the family Pinaceae. Heptane has been found previously in an investigation of the turpentine [1] in nine species of pine.

We have analyzed the oil of the needles and 1-yr shoots of the following larches: European larch (Larix decidua Mill.), Sukaczev's larch (L. sukaczewii Djil.), Siberian larch (L. sibirica Ledeb.), Dahurian larch (L. dahurica, Turcz.), Japanese larch (L. leptolepis Gord.), eastern larch (L. americana Michx. [L. laricina]), western larch (L. occidentalis Nutt.), and the Altai variety of the Siberian larch (L. sibirica var. altaica).

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