

TERPENOIDS OF THE OLEORESINS OF *Pinus pityusa*  
AND *P. eldarica*

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The chemical composition of the oleoresins of the pines *Pinus pityusa* and *P. eldarica* growing in the Caucasus has been studied. In relation to the amounts of the main components, the oleoresins of these species are similar. Characteristic for their resins is a high content of caryophyllene. Diterpenoids are represented mainly by tricyclic compounds, among which pimarinol and isopimarinol and methyl 15-hydroxydehydroabietate predominate.

Continuing a study of the resins of coniferous plants, we have investigated the chemical compositions of the oleoresins of the pines *Pinus pityusa* Stev. and *Pinus eldarica* Medw., of the subgenus *Diploxylon* growing in the Caucasus.

The question of the systematics of Crimean-Caucasian species of pines and, in particular *P. pityusa* and *P. eldarica* has been an object of discussion over a period of many years [1]. They have been separated into independent species, but the opinion exists that they are a single species — Calabrian pine — *Pinus brutia* Ten. (subgenus *Diploxylon*) with a fragmented area [1] the homeland of which is Asia Minor (Syria, Lebanon). An anatomical investigation of the needles of *P. pityusa*, *P. eldarica*, and *P. brutia* has shown that these species are close to one another [2].

It appeared of interest to make a comparative study of the chemical compositions of the oleoresins of the species mentioned.

There is information on the composition of the monoterpenes and resin acids of *P. pityusa* [3-6], and on the rosin of *P. eldarica* [5].

In the present paper we give the results of an investigation of the chemical compositions of the oleoresins of these species. The oleoresins were separated into neutral and acid fractions by treatment with a 5% solution of caustic soda at 45°C. The more severe saponification conditions in comparison with the procedure usually adopted [7] was due to the very small amount of neutral components in the oleoresins.

The neutral substances obtained in this way were separated by absorption chromatography into hydrocarbons and oxygen-containing compounds. Mono-, sesqui-, and diterpene fractions were obtained by the vacuum distillation of the hydrocarbons.

In the oleoresins of *P. pityusa* and *P. eldarica* the main components of the monoterpene hydrocarbons was  $\alpha$ -pinene (57 and 83%), while the oleoresins of *P. pityusa* also contained a considerable amount of  $\Delta^3$ -carene (34%).

The amounts of sesquiterpene hydrocarbons in the neutral fractions of the oleoresins proved to be very small (0.8 and 0.2% on the weight of the oleoresin). Among the sesquiterpenes of the oleoresin of *P. pityusa* caryophyllene (56%) and longifolene (23%) predominated. In the oleoresin of *P. eldarica* the amount of caryophyllene was greater, at 73.3%, and that of longifolene smaller, at 8.7%.

The oleoresins of both species were extremely poor in diterpene hydrocarbons and contained only two compounds — isopimaradiene and dehydroabietane.

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The neutral oxygen-containing compounds of the oleoresin consisted of mono- and diterpenoids. The compositions of the monoterpenoids of the oleoresins of *P. pityusa* and *P. eldarica* were completely identical: p-cymen-8-ol,  $\alpha$ -terpineol, terpineol-4, and sabinene hydrate.

The bulk of the oxygen-containing fractions of the oleoresins of both species consisted of diterpenoids, represented mainly by tricyclic esters, aldehydes, and alcohols, of the primarane and abietane types.

From the oleoresin of *P. pityusa* we isolated and identified isopimarinal, dehydroabietinal, methyl abietate, methyl dehydroabietate, methyl palustrate, pimarinal, isopimarinal, and 3,5-dimethoxystilbene, and also esters of hydroxy resin acids — methyl 15-hydroxyabietate and methyl 15-hydroxydehydroabietate.

We isolated two diterpene compounds of undetermined structure the spectra of which clearly showed the presence of an OH group and of a conjugated system of double bonds (in the UV spectrum,  $\lambda_{\max}$  295 and 241 nm). The identification of these substances is proceeding.

The compositions of the diterpene oxygen-containing compounds of the oleoresins of *P. pityusa* and *P. eldarica* were practically identical, only some quantitative differences being found; for example, the oleoresin of *P. eldarica* contained a larger amount of hydroxy esters.

Attention is attracted by the complexity of the separation of the mixture of diterpenoids in the oleoresins studied. This is probably due to the considerable amount of aldehydes which, as the result of autoxidation give a complex mixture of by-products. In addition, the diterpene primary alcohols present in the oleoresins have similar absorption properties, which makes it difficult to isolate individual components.

In the oleoresins of *P. pityusa* and *P. eldarica* resin acids predominate, their amount reaching 80-90%. The predominating components of the diterpene acids of the oleoresins of *P. pityusa* and *P. eldarica* are abietic acid (38.2 and 34%, respectively), and palustric acid (53 and 23.5%).

The investigation of the chemical compositions of the oleoresins of *P. pityusa* and *P. eldarica* has shown their closeness, and the quantitative differences in the individual components can be explained by different growth conditions. To confirm the identity of these pines with the Calabrian pine information is required on the chemical composition of the oleoresin of the latter.

#### EXPERIMENTAL

IR spectra were taken on UR-20 instrument in  $\text{CCl}_4$  solution and in KBr tablets, and PMR spectra on a Varian A-56/60A instrument using solutions of the substances in  $\text{CCl}_4$  with HMDS as internal standard, its chemical shift being taken as  $\delta$  0.05 ppm. Specific rotations were determined for solutions in chloroform.

The oleoresin of *P. pityusa* was collected in the region of the settlement of Gantiadi (Georgian SSR), and the oleoresin of *P. eldarica* on Mt. Ellyar-ougi, which is located on the boundary between the Georgian and Azerbaidzhan SSRs, in 1979 and 1980.

Treatment of the Oleoresins. Oleoresin of *P. pityusa*. The oleoresin (500 g) was treated with 5% caustic soda solution at 45-50°C. The neutral substances were extracted with diethyl ether. The liquid remaining after extraction with diethyl ether was acidified with 10% HCl, and the resin acids so formed were extracted with diethyl ether. The ethereal extracts, separately, were washed with water, dried with anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated. This gave 77 g of neutral substances and 386 g of resin acids. The neutral substances were chromatographed on alumina (activity grade III, 1:10). Petroleum ether eluted 69 g of hydrocarbons, and diethyl ether eluted 8.0 g of oxygen-containing compounds. The vacuum distillation of the hydrocarbons yielded 63 g of monoterpenes (bp 90-110°C/15 mm Hg), 4.2 g of sesquiterpenes (100-140°C/2 mm Hg), and 0.9 g of diterpenes (still residue). The oxygen-containing compounds (8.0 g) were separated by vacuum distillation into monoterpenoids (0.4 g) and diterpenoids (7.5 g).

Oleoresin of *P. eldarica*. The oleoresin (850 g) was treated as described above, this gave 655 g of resin acids and 130 g of neutral substances. After chromatography of the neutral substances on alumina, 112 g of hydrocarbons and 16.9 g of oxygen-containing compounds were obtained. The hydrocarbons were separated into 108 g of monoterpenes, 2.4 g of sesqui-

terpenes, and 1.02 g of diterpenes. From the oxygen-containing compounds, 0.6 g of monoterpenoids and 16.0 g of diterpenoids were isolated.

Monoterpene Hydrocarbons. The monoterpenes were analyzed qualitatively and quantitatively by GLC [8].

Oleoresin of *P. pityusa*. The monoterpene hydrocarbon fraction contained  $\alpha$ -pinene (57%),  $\beta$ -pinene (3.9%),  $\Delta^3$ -cadene (34%), and terpinolene (3.6%). Camphene, myrcene, limonene,  $\beta$ -phellandrene, and  $\gamma$ -terpinene were found in very small amounts.

Oleoresin of *P. eldarica*. In the monoterpene hydrocarbon fraction we found  $\alpha$ -pinene (83.5%),  $\beta$ -pinene (13%),  $\Delta^3$ -carene (3%), and in small amounts, camphene, myrcene, limonene,  $\beta$ -phellandrene, and  $\gamma$ -terpinene.

The sesquiterpene hydrocarbons were analyzed by the GLC method [8] in two capillary columns on a Chrom-4 instrument, the dimensions of the columns being 50 m  $\times$  0.2 mm with the stationary phases OV-17 and OV-225 at a rate of flow of the carrier gas, nitrogen, of 6 ml/min and with the column temperature programmed from 100 to 200°C at the rate of 20°C per minute.

The individual components isolated by adsorption chromatography were identified by IR and PMR spectroscopy.

Oleoresin of *P. pityusa*. Nine components were detected in the sesquiterpene hydrocarbon fraction. From their relative retention times and by the method of additives they were identified as caryophyllene (56%), longifolene (23%),  $\alpha$ -humulene (13.6%), longicyclene (3.5%), and  $\alpha$ -longipinene (2.2%).

The sesquiterpene fraction (4 g) was chromatographed on silica gel (100 g). Petroleum ether eluted caryophyllene (1.45 g), longifolene (0.6 g), and  $\alpha$ -humulene (0.2 g).

Because of the small amount of longicyclene and longipinene, it was impossible to isolate them.

Oleoresin of *P. eldarica*. By the method given above, it was found that the mixture of sesquiterpene hydrocarbons contained caryophyllene (73.3%),  $\alpha$ -humulene (16.9%), longifolene (8.9%), and two components in very small amounts.

Diterpene Hydrocarbons. The Oleoresin of *P. pityusa*. The diterpene hydrocarbon fraction (0.9 g) was chromatographed on silica gel impregnated with 10% of  $\text{AgNO}_3$  (20 g). Petroleum ether with the addition of increasing amounts of diethyl ether eluted isopimaradiene (0.11 g) with  $n_D^{20}$  1.5185 and  $[\alpha]_D^{20}$   $-31.6^\circ$  (c 4.5) and dihydroabietane (0.06 g) with  $n_D^{20}$  1.5022 and  $[\alpha]_D^{20}$   $+10.3^\circ$  (c 3.6).

The Oleoresin of the *P. eldarica*. The total hydrocarbons were analyzed by the TLC method. The recording of the PMR spectrum of the total fraction and a direct comparison with the authentic samples showed the presence of dehydroabietane and isopimaradiene.

The oxygen-containing monoterpenoids were analyzed by the TLC method. On comparison with markers, p-cymen-8-ol, sabinene hydrate,  $\alpha$ -terpineol (the main component), and terpinol-4 were identified in the monoterpenoid fractions from the oleoresins of *P. pityusa* and *P. eldarica*. The PMR spectrum of the combined fraction showed the signal of the compounds mentioned.

Oxygen-Containing Diterpenoids. The Oleoresin of *P. pityusa*. The diterpenoids (5 g) were chromatographed on alumina (activity grade III, 1:10). By gradient elution in the petroleum ether-diethyl ether system, four fractions were obtained: A (1.5 g), B (1.4 g), C (0.9 g), and D (0.9 g).

GLC analysis [9] showed the presence in fraction A of the aldehydes dehydroabietinal and isopimarinal, and also of the methyl esters of abietic dehydroabietic, and palustric acids.

After rechromatography on silica gel (ratio 1:10), 3,5-dimethoxystilbene (0.8 g) and pimarinal (0.3 g) were isolated from fraction B.

Fraction C was rechromatographed on silica gel (ratio 1:30). Petroleum ether-dimethyl ether (75:25) isolated isopimarinal (0.2 g) and compound (I) (0.6 g), the latter having bands in its IR spectrum at 970, 1020, 1120, 1480, 1630, 3580, and 3620  $\text{cm}^{-1}$ . In the UV

spectrum,  $\lambda_{\max}$  295 nm ( $\epsilon$  350). PMR spectrum, ppm: 5.05; 3.7 (singlet 2 H); 1.56 (singlet, 6 H); 1.2 (singlet, 3 H). Methyl 15-hydroxydehydroabietate with mp 80–81°C was isolated from fraction D.

All the compounds isolated were identified by comparison with authentic samples in TLC and by spectral characteristics [10].

The Oleoresin of *P. eldarica*. The diterpenoids (10.4 g) were chromatographed on alumina (activity grade III, 1:15). Petroleum ether with the addition of from 10 to 100% of diethyl ether led to the isolation of three fractions: A (4 g), B (2.8 g), and C (1.9 g), while ethanol eluted fraction D (2.0 g).

The rechromatography of fraction A on silica gel (ratio 1:30) with petroleum ether–diethyl ether (90:10) led to the isolation of isopimarinal (0.1 g), dehydroabietinal (0.1 g), and 3,5-dimethoxystilbene (1.0 g). Petroleum ether–diethyl ether (80:20) eluted pimarinal (0.3 g) and a mixture of pimarinal and isopimarinal (0.59 g) from which by subsequent purification on silica gel pure isopimarinal was isolated. The substances were identified by means of their PMR spectra.

Fraction B (2.8 g) was chromatographed on silica gel (ratio 1:30). Gradient elution by petroleum ether with increasing amounts of diethyl ether led to the isolation of pimarinal (0.62 g) and a mixture of pimarinal and isopimarinal (0.8 g) from which by acetylation and purification isopimarinal acetate (0.25 g) was obtained. The most polar fraction (0.43 g) contained polyfunctional compounds.

Fraction C (1.9 g) was rechromatographed on silica gel (ratio 1:30). Petroleum ether–diethyl ether eluted from the column methyl 15-hydroxydehydroabietate (0.14 g) and two unidentified compounds – (I) (0.18 g) and (II) (0.2 g).

In its spectral characteristics, compound (I) was similar to the compound (I) isolated from the oleoresin of *P. pityusa*.

Compound (II), with mp 159–162°C gave a UV spectrum with absorption bands at 1055, 1150, 1395, and 1650  $\text{cm}^{-1}$ . In the UV spectrum,  $\lambda_{\max}$  238 (shoulder) and 241 nm. PMR spectrum, ppm: 6.0 (singlet, 2 H); 1.25 (singlet, 6 H); 0.87 (singlet, 3 H).

Fraction D contained a complex mixture of polyfunctional compounds, and these are the subject of further investigations.

Resin Acids. The compositions of the resin acids in the form of their methyl esters were studied by the GLC method [9].

Oleoresin of *P. pityusa*. In the mixture of resin acids we identified abietic (38.2%), neoabietic (9.5%), palustric or levopimaric (33.3%), isopimaric (15.1%), dehydroabietic (3.7%), and sandaracopimaric (traces).

Oleoresin of *P. eldarica*. In this oleoresin we found the acids abietic (34.5%), neoabietic (15.0%), isopimaric (17.9%), palustric or levopimaric (23.5%), dehydroabietic (7.0%), and sandaracopimaric (2.0%).

#### SUMMARY

1. The chemical compositions of the oleoresins of *P. pityusa* and *P. eldarica* have been studied. It has been shown that the oleoresins of these species are similar, differing only by their contents of individual components, which may be due to different ecogeographical conditions.

2. Among the monoterpenes,  $\alpha$ -pinene predominates. The sesquiterpene hydrocarbons contain mainly caryophyllene.

3. The bulk of the neutral oxygen-containing compounds consists of diterpenoids, which are represented by pimarinal and isopimarinal and the corresponding aldehydes, and also methyl 15-hydroxydehydroabietate.

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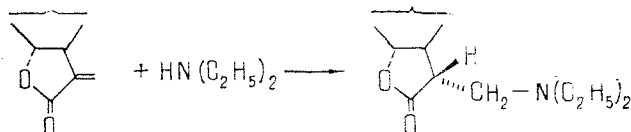
# CIRCULAR DICHROISM OF THE PRODUCTS OF THE AMINATION OF LACTONES

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The circular dichroism method has been used to study the products of the amination of sesquiterpene lactones containing  $\alpha$ -methylene- $\gamma$ -lactone groupings. Analysis of the results obtained has shown that in all the lactones considered the amination reaction takes place with the predominant formation of one of the possible stereochemical forms of the product of the addition of the amine to the exomethylene group of the lactone ring, namely, that having the 11S configuration.

Sesquiterpene lactones with a  $\gamma$ -lactone ring conjugated with an exomethylene group readily add secondary amines by the Michael reaction [1]:



This reaction takes place with the hydrogen atom adding to the more substituted carbon atom and the N-ethyl residue to the less substituted one, forming a new asymmetric center at C<sub>11</sub>, the configuration of which is unknown. This reaction is frequently used to obtain soluble forms of this group of lactones and in the separation of mixtures of lactones.

In stereochemical investigations the stereospecific reactions of reduction by sodium tetrahydroborate and diazomethane of an exomethylene bond conjugated with a  $\gamma$ -lactone grouping are used with success [2, 3]. It appeared of interest to establish whether the amination reaction is stereospecific and to determine the configuration of the newly formed asymmetric center at C<sub>11</sub>. With this aim, we have considered the circular dichroism (CD) spectra of guaianolides containing the  $\alpha$ -methylene- $\gamma$ -lactone grouping and the products of their amination at room temperature. The CD spectra of the following guaianolides were recorded: rupicolin A (1), rupicolin B (2), ajanin (3), ajadin (4), arteglaasin (5), cumambrin (6), chrysartemin (7), the eudesmanolide artecalin (8), tetrahydrorupicolin B (9), dihydroajanin (10), and dihydrochrysartemin (11). In the spectra of these lactones in the 250-270 nm region a Cotton effect (CE) is observed which is due to an  $n \rightarrow \pi^*$  transition in the  $\alpha$ -methylene- $\gamma$ -lactone chromophore the sign of which depends on the nature of the linkage of rings B/C [4]. All the compounds studied had the trans linkage of the lactone rings closed at C<sub>6</sub>. This

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