NEW DITERPENOID COMPONENTS OF THE OLEORESIN OF *Pinus pumila*

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The Japanese stone pine [*Pinus pumila* (Pall.) Regel] differs sharply in respect of the diterpenoid components of its oleoresin from other *Pinus* species belonging to the Cembrae section [1]. We have made an investigation of the diterpenoids of Japanese stone pine oleoresin, for which purpose we investigated oleoresin collected in July, 1976, from trees growing in the north-eastern shore of Lake Baikal. Using separation schemes that have been described in the literature [1, 2], we isolated the same diterpenoids as were found previously, and also a number of new ones. In this oleoresin, as in that studied previously, the main component of the fraction of neutral oxygen-containing compounds was 13E-labda-8(20),13-dien-15-ol (~30%).

After the separation of aldehydes and other compounds of the fraction of carbonyl compounds [1], a mixture of esters was obtained. This mixture included the methyl esters of anticopalic [copaiferic, 13E-labda-8(20),13-dien-15-oic], dehydroabietic, abietic, isopimaric, and isopimara-8,15-dien-18-oic acids. They were identified from the PMR spectrum of the mixture of alcohols obtained by their reduction with lithium tetrahydroaluminate in diethyl ether, and also by comparison with authentic samples in GLC. In the sequence of esters given above, the R_f values in TLC on SiO_2 + 5% of $AgNO_3$ (eluent — petroleum ether containing 5-10% of diethyl ether) decreased from anticopalic to isopimara-8,15-dien-18-oic acid. This is the first time that methyl anticopalate has been detected in a natural source, although the acid itself has been found previously in two species of conifers [1, 3].

Isocembrol and its epimer, 4-epiisocembrol, the presence of which in the oleoresin of the Japanese stone pine has been recorded previously in the form of traces [1], was present in small amount (~10%) in a fraction the main component of which was the cis-abienol found previously [1]. As in other species of *Pinus*, in the mixture of the two epimers isocembrol predominates (70%), as was found by a comparison of the integral intensities of the H_3 signals of isocembrol and 4-epiisocembrol in the PMR spectrum of their natural mixture.

Abietinol, dehydroabietinol, isopimara-8,15-dien-18-ol, and also the components of the oleoresin that had been isolated previously — isopimarinol and 13E-labda-8(20),13-dien-15-ol [1] — were detected by means of NMR in the fractions of polar monohydric alcohols giving acetates on acetylation with acetic anhydride in pyridine.

The fraction described previously [1] as a mixture of two unknown compounds eluted from a column of SiO_2 before the cis-abienol was isolated again and was separated by chromatography into two components which, from their spectral and chemical characteristics, were identified as 13E-labda-8(20),13-dien-15-al and 13Z-labda-8(20),13-dien-15-al (I and II, respectively). The proof of their structures consisted in the formation of the known 13E-labda-8(20),13-dien-15-ol when the aldehyde (I) was reduced with lithium tetrahydroaluminate in diethyl ether, and in the production of a mixture of the aldehydes (I) and (II) (76:24, NMR spectrum) on the oxidation of 13E-labda-8(20),13-dien-15-ol with chromium trioxide in pyridine. This is the first time that the aldehydes (I) and (II) have been detected in conifers. We have found no literature information on their presence in other natural sources. Expoxy- and oxo-clerodane analogs of these compounds have been isolated by McCrindle [4] from *Solidago serotina* Ait.

The presence of the aldehyde (II) in the oleoresin studied is an interesting fact, since the corresponding alcohol and acid are not present in it in appreciable amounts. For the case of aliphatic terpene derivatives, it has been shown [5] that cis aldehydes of this type are formed by the enzymatic isomerization of the trans aldehydes. Thus, the cis aldehyde (II) is probably the product of the enzymatic isomerization of the trans aldehyde formed, in

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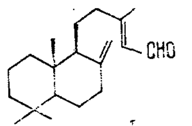
its turn, from 13E-labda-8(20),13-dien-15-ol, although isomerization without the participation of an enzyme, as has been observed for macrocyclic α-enones [6], is not excluded. The subsequent biological oxidation of these aldehydes takes place differently in the Japanese stone pine. This can be judged on the basis of the presence of more oxidized labdane diterpenoids in the oleoresin studied. While the trans aldehyde (I) is converted into the corresponding acid, which is the main component of the acid fraction of oleoresin [1], the cis aldehyde (II) may be converted, according to the scheme of the biosynthesis of the labdane derivatives pinusolide (III) and methyl lambertianate (IV, R = $COOCH_3$) which we have suggested previously [7], into the furan derivative (V). The first stage of such transformation must be the formation of the hydroxy aldehyde (VI) and its isomerization into a semiacetal, and the second stage will be the dehydration of the semiacetal to compound (V).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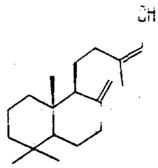
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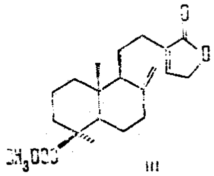
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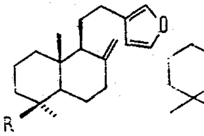
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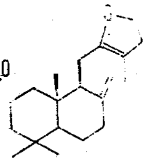
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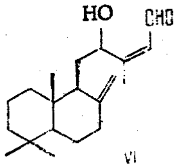
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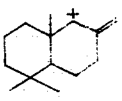
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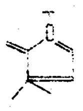
VIII

In actual fact, we have isolated a compound the spectral characteristics of which correspond completely to structure (V) from the hydrocarbon fraction of the oleoresin under investigation. The trivial name "pumiloxide" has been suggested for it. The compound forms a crystalline substance with mp 88-89°C (from pentane), the UV spectrum of which practically coincides with that of compound (IV, R = CH_3) (lambertiane), which we synthesized from lambertianol (IV, R = CH_2OH) via lambertianal (IV, R = CHO). The IR spectra of pumiloxide and lambertiane are very similar, and the NMR spectrum of pumiloxide differs qualitatively from that for lambertiane by the presence of the signal of a C_{13}-methyl group (δ 1.90 ppm) and of only two signals due to the protons of the furan ring (7.06 ppm, α-proton, and 6.01 ppm, β-proton). In the high-resolution mass spectrum, in addition to the peak of the molecular ions of pumiloxide corresponding to the formula $C_{20}H_{30}O$, two strong peaks of fragmentary ions are observed the elementary compositions of which correspond to formulas (VII) and (VIII). As is well known, cleavage at a C—C bond present in the β position to the furan ring is characteristic for alkylfuran derivatives [8], as is observed in this case. The absolute configuration shown in formula (V) is the most probable, since all labdane diterpenoids from plants of the family Pinaceae have the same configuration. The antipode of substance (V) (deoxyafricenol) was isolated by Halsall [9] from the wood of *Turraenthus africanus*, but the absence of experimental facts in his paper did not permit us to make a comparison of the spectral characteristics of these compounds. The identity of their structures is indicated by the closeness of the melting points of pumiloxide (88-89°C) and of deoxyafricenol (89-90.5°C).

It is interesting to note the absence of lambertianic acid (IV, R = COOH) and its methyl ester from Japanese stone pine oleoresin [1], while this compound is one of the main components of the oleoresins of other species of *Pinus* in the section Cembrae [2]. According to the scheme for the biosynthesis of methyl lambertianate [7, 10], from the most probable precursor — agathadiol — the oxidation of the methyl group at C_{13} in this precursor takes place with the retention of the hydroxy group at C_{15}. This explains the subsequent formation of the corresponding 15-hydroxylabdan-16-one derivative and its conversion into methyl lambertianate and pinusolide (III). However, the main feature of the biosynthesis of labdane diterpenoids in the Japanese stone pine is, as may be concluded from the structures of the compounds isolated, the predominant oxidation of the labdane precursor at C_{15}, which is an obstacle to the formation of pinusolide and of furan derivatives of type (IV) that are characteristic for other representatives of the section Cembrae.

We were previously unable to detect diterpene hydrocarbons in Japanese stone pine oleoresin because of the absence of a sufficient amount of the oleoresin [1]. In the performance of the present investigation, by distilling off the mono- and sesquiterpenes in vacuum we succeeded in concentrating the high-boiling residue of the hydrocarbon fraction, with a yield of ~0.6% (0.83 g) of the total hydrocarbon fraction of the oleoresin. When this residue was chromatographed on SiO_2 + 5% of $AgNO_3$, the successive elution was observed of 0.2 g of a mixture of unidentified hydrocarbons, 0.1 g of (+)-dehydroabietane, 0.1 g of (−)-abietadiene, 0.2 g of a complex mixture of hydrocarbons containing, according to TLC, isopimaradiene and cembrene, 0.08 g of the furan compound (V) (pumiloxide), and 0.05 g of a mixture of unidentified hydrocarbons. After additional chromatographic purification on SiO_2, the dehydroabietane and abietadiene gave optical rotatory dispersion curves identical with those for authentic samples of (+)-dehydroabietane and (−)-abietadiene. They were identified from their IR and NMR spectra. The presence of cembrene and of isopimaradiene in the initial mixture of hydrocarbons was confirmed by GLC.

EXPERIMENTAL

The apparatus, the separation of the oleoresin, and the conditions of chromatography have been described in our previous paper [1]. The angles of optical rotation were measured for solutions in chloroform. The PMR spectra (δ scale) were obtained for solutions in carbon tetrachloride. The optical rotary dispersions of abietadiene and dehydroabietane were recorded on a Spectropol I instrument for solutions in chloroform. The molecular formulas of compounds (I), (II), and (V) were determined by the use of high-resolution mass spectrometry. The analyses of lambertianal and lambertiane corresponded to the calculated figures.

Isolation of the Mixture of Aldehydes (I) and (II). The chromatography of 4.2 g of the total neutral oxygen-containing compounds of the oleoresin on 80 g of SiO_2 yielded 0.22 g of a mixture of the aldehydes (I) and (II), the rechromatography of which on 20 g of SiO_2 gave 0.08 g of the aldehyde (II) and 0.11 g of the aldehyde (I).

13Z-Labda-8(20),13-diene-15-al (II). Colorless oil with n_D^{20} 1.5280 $[\alpha]_D^{24}$ −8.5° (c 4.22). UV spectrum: λ_{max} (in ethanol): 240 nm (log ε 4.13). IR spectrum (in CCl_4), cm^{-1}: 1620, 1680 (C=C–C=O), 890, 1640, and 3080 (>$C=CH_2$). NMR spectrum, ppm: 0.65, 0.76, 0.82 (singlets, 3 H each, tertiary methyl groups at C_4 and C_{10}), 1.90 (doublet, 3 H, J = 1.3 Hz, methyl group at C_{13}, present in the trans position to an aldehyde group [4]), 4.51 and 4.85 (narrow multiplets, 1 H each, >$C=CH_2$), 5.75 (1 H, doublet, J = 8 Hz, H_{14}), 9.55 (1 H, doublet, J = 8 Hz, CHO).

13E-Labda-8(20),13-dien-15-al (I). Colorless oil with n_D^{16} 1.5305, $[\alpha]_D^{16}$ +40.5° (c 6.42). UV spectrum: λ_{max} (in ethanol) 241 nm (log ε 4.20). The main bands in the IR spectrum were the same as in the IR spectrum of aldehyde (II). NMR spectrum, ppm: 0.65, 0.76, 0.82 (singlets, 3 H each, tertiary methyl groups at C_4 and C_{10}), 2.10 (singlet, 3 H, methyl group at C_{13} present in the cis position to the aldehyde group [4]), 4.48 and 4.85 (narrow multiplets, 1 H each, >$C=CH_2$), 5.75 (doublet, 1 H, J = 7.5 Hz, CHO).

Oxidation of 13E-Labda-8(20),13-dien-15-ol. With stirring, an oxidizing mixture obtained by adding 0.5 g of chromium trioxide to 20 ml of pyridine at 0°C was run into a solution of 0.5 g of 13E-labda-8(20),13-dien-15-ol in 20 ml of pyridine. After the reaction mixture had been allowed to stand for a day at 0°C, it was diluted with a tenfold volume of water and was extracted with petroleum ether. According to TLC, the mixture of reaction products consisted of the aldehydes (I) and (II) and a small amount of the initial compound. The ratio of the aldehydes (I) and (II) in it (76:24) was determined from the integral intensities of the signals due to the protons of the aldehyde groups. Chromatography of this mixture on 20 g of SiO_2 yielded 0.1 g of the aldehyde (II) and 0.3 g of the aldehyde (I), identical with the samples of these compounds from the Japanese stone pine oleoresin.

Pumiloxide (V). Colorless needles from pentane, mp 88-89°C $[\alpha]_D^{20}$ −20° (c 2.20). UV spectrum: λ_{max} (in ethanol) 219 nm (shoulder) (log ε 3.92). IR spectrum (in CCl_4), cm^{-1}: 890, 1650, 3090 (>$C=CH_2$), 1070, 1090, 1155, 1510 (furan ring). NMR spectrum, ppm: 0.72, 0.80, 0.85 (singlets, 3 H each, tertiary methyl groups present at C_4 and C_{10}), 1.90 (singlet, 3 H, methyl group at C_{13}), 4.50 and 4.66 (narrow multiplets, 1 H each, $>C=CH_2$), 6.01 and 7.06 (1 H each, narrow multiplets, β and α protons of a furan ring).

Lambertianal (IV, R = CHO). Lambertianol (0.5 g) was oxidized with chromium trioxide in pyridine by a method similar to that described for 13E-labda-8(20),13-dien-15-ol. The yield of lambertianal was 0.4 g, mp 84-85°C (from ethanol), $[\alpha]_D^{23}$ +28.3° (c 3.81). IR spectrum (in CCl_4), cm^{-1}: 1726, 2700 (CHO). NMR spectrum: 9.68 ppm (1 H, singlet, CHO).

Lambertiane (IV, R = CH_3). The reduction of 0.2 g of lambertianal by a procedure similar to that described by Cambie et al. [11] gave 0.1 g (52% of theoretical) of lambertiane in the form of a colorless oil with n_D^{20} 1.5125, $[\alpha]_D^{20}$ +33° (c 1.65). IR spectrum (in CCl_4), cm^{-1}: 890, 1650, 3090 ($>C=CH_2$), 880, 1034, 1070, 1170, 1510 (furan ring). UV spectrum (in ethanol): λ_{max} 219 nm (shoulder) (log ε 3.4). PMR spectrum, ppm: 0.65, 0.77, 0.83 (singlets, 3 H each, tertiary methyl groups at C_4 and C_{10}), 4.52 and 4.80 (narrow multiplets, 1 H each, $>C=CH_2$), 6.11 (narrow multiplet, 1 H, α proton of a furan ring), 7.08 and 7.21 (narrow multiplets, 1 H each, β protons of a furan ring).

SUMMARY

1. Five new diterpenoid components have been isolated from the oleoresin of the Japanese stone pine — (+)-dehydroabietane, (—)-abietadiene, 13E-labda-8(20),13-dien-15-al, 13Z-labda-8(20),13-dien-15-al, and pumiloxide, which is probably an enantiomer of deoxyafricenol. The presence of methyl anticopalate (copaiferate) and of the methyl esters of abietic, dehydroabietic, isopimaric, and isopimara-8,15-dien-18-oic acids has been established in this resin.

2. From the components of its oleoresin, the Japanese stone pine differs from other species of *Pinus* present in the section Cembrae by its low content of cembrane diterpenoids, the absence of lambertianic acid and its methyl ester, and the specific biosynthesis of labdane diterpenoids consisting in the accumulation in the oleoresin of 13E-labda-8(20),13-dien-15-ol and its subsequent oxidation predominantly at C_{15}.

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