NEW DITERPENOID COMPONENTS OF THE OLEORESIN OF *Pinus koraiensis*

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UDC 547.595.9

In preceding papers we have described the isolation from the oleoresin of *Pinus koraiensis* Sieb et Zucc. (Korean pine) of 26 neutral diterpenoids belonging to the structural-stereochemical groups of cembrane [1-4], labdane [1, 5, 6], abietane [1, 6, 7], and isopimarane [7]. The diterpene acids from this oleoresin have been investigated by the GLC method [8]. To obtain a more complete pattern of the distribution of the diterpenoids over the groups mentioned, we have studied the neutral fraction of a fresh sample of the oleoresin collected in Khabarovsk territory. In this investigation it proved to be possible to confirm the native nature of a number of the compounds isolated previously.

The oleoresin was separated by the method described previously [9] into acidic and neutral fractions. Chromatography of the neutral fraction on alkaline alumina (activity grade II-III) gave a hydrocarbon fraction (57.5%) and a fraction of oxygen-containing compounds (42.5%). The latter were separated further by chromatography on air-dry SiO_2 into three fractions: nonpolar (9% — carbonyl compounds and oxides), a monohydric phenols fraction (28.5%), and a fraction of di- and polyfunctional compounds (5%).

Nonpolar Fraction. Its main components were diterpene aldehydes [7] and the methyl esters of resin acids. The ratio of these groups of compounds (5.6:1.0) was determined by comparing the integral intensities of the signals of the aldehydic protons and of the signals of methoxycarbonyl groups in the NMR spectra of the total nonpolar fraction.

To separate the aldehydes from the other substances, the nonpolar fraction was treated with $NaBH_4$ in aqueous methanol. After chromatography, an unchanged fraction (35%) and a mixture of primary alcohols — the products of the reduction of the aldehydes — were obtained. Analysis of the NMR spectra of this mixture of alcohols (both free and in the form of acetates) showed its composition: palustrol, 40%; dehydroabietinol, 20%; isopimarinol, 17%; isopamara-8,15-dien-18-ol, 15%; neoabietinol, about 2%; abietinol, 6%.

The unchanged part of the nonpolar fraction was then separated by chromatography on SiO_2 into three fractions: an oxide fraction, an ester fraction, and a fraction consisting almost completely of bornyl acetate. The main component of the oxide fraction, purified on SiO_2 + 5% $AgNO_3$, was identified as (+)-manoyl oxide. This fraction also yielded a very small amount of epimanoyl oxide, which was identified by comparison with an authentic sample.

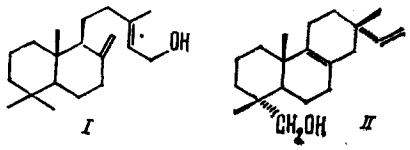
The ester fraction was a mixture of native methyl esters of resin acids, which we have isolated previously [1, 7]. Its main components were methyl isopimarate, methyl lambertianate, methyl dehydroabietate, and methyl abietate, present in a ratio of 6:5:5:4 (NMR spectrum).

Monohydric Alcohol Fraction. This fraction was separated into a mixture of acetylatable alcohols (acetic anhydride in pyridine, 20°C, 30 min) (13%) and a mixture of nonacetylatable tertiary alcohols (87%). By separating the tertiary alcohols on SiO_2 and then on SiO_2 + 5% $AgNO_3$, we obtained cis-abienol with mp 40-41°C and $[\alpha]_D$ +22°, isocembrol [1], and 4-epiisocembrol [4]. The isocembrol and its epimer amounted to 80% of the monohydric alcohol fraction and were the main diterpenoids of the neutral fraction of the oleoresin under investigation. When the oleoresin was stored, and also when the method of fractionating the neutral part of the oleoresin described previously [9] was used, the isocembrol and its epimer readily underwent dehydration to a mixture of cembrene and isocembrene.

Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 174-179, March-April, 1976. Original article submitted June 17, 1975.

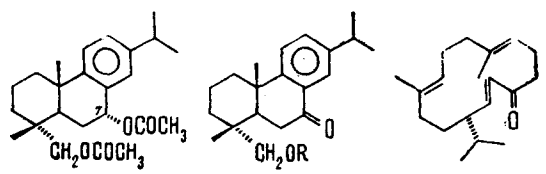
The isolation of cis-abienol from the oleoresin investigated confirms our hypothesis [6] according to which the alcohol isomeric with this, cis-neoabienol, isolated previously from the same source, is a product of the thermolysis of the cis-abienol taking place in the vacuum distillation of the neutral fraction of the oleoresin. A careful analysis (TLC, NMR) of the intermediate fractions from the isolation of cis-abienol and of isocembrol showed the absence of neoabienol from them. The other tertiary alcohols were mono- and sesquiterpenoid derivatives, as was shown by GLC. The norditerpene alcohols that we isolated previously (18-nordehydroabietan-4-ol and 18-norisopimary-7,15-dien-4-ol) [6, 10] were not found in the oleoresin investigated, which confirms the hypothesis that they were artefacts [10, 11]. The corresponding aldehydes, the precursors of these norditerpenoids, are present in large amounts in the nonpolar fraction.

The acetylatable alcohols were separated in the form of their acetates by chromatography on SiO_2 + 5% $AgNO_3$, and were then converted to the free alcohols by reduction with $LiAlH_4$. In this way we isolated the previously described isopimarinol and palustrol [7] and dehydroabietinol [1], and also, as new components, abietinol and labd-8(20),13-dien-15-ol (I). The main components of the fraction of acetylatable alcohols were isopimarinol and dehydroabietinol, present in approximately equal amounts and making up about 85% of this fraction. In an analysis of the minor components by GLC and NMR we established the presence among them of isopimara-8,15-dien-18-ol (II). This alcohol was first isolated by Zinkel [12] from the wood of *Pinus quadrifolia* Parl. In the oleoresin investigated, the alcohol (II) was accompanied by a large amount of isopimarinol, which is difficult to separate from it and which complicates the isolation of a pure sample of (II).



I II

Polar Fraction. The main components of the polar fraction were agathadiol [1] and isoagatholal [6], present in equal amounts and together making up about 60% of the fraction. A third compound, present in small amount, proved to be a new previously undescribed diol. It was isolated and characterized in the form of the diacetate with mp 105-106°C, $[\alpha]_D^{20}$ +32.2°, $C_{24}H_{34}O_4$ (high-resolution mass spectrometry). The IR and UV spectra of the diacetate obtained are very similar to those for the acetate of dehydroabietinol. The structural similarity of these compounds was confirmed by their NMR spectra. The most pronounced difference between the NMR spectrum of the diacetate and that of dehydroabietinol [1] is the presence in it of a narrow one-proton multiplet at 5.83 ppm, which can be assigned to a proton adjacent to a secondary acetoxy group, and the high value of its chemical shift shows that the corresponding proton is simultaneously a benzyl proton. The second acetoxy group forms part of an acetoxymethyl group (AB system with its center at 3.75 ppm, $J_{A,B}$ = 11 Hz). Thus, the structure of the diacetate of 7-hydroxydehydroabietinol (III) may be assumed for the diacetate isolated. To confirm the structure of (III), the diacetate was reduced with $LiAlH_4$ to the corresponding diol, which was immediately oxidized with active MnO_2 to the hydroxy ketone (IV). The acetate of the latter proved to be identical with the oxo acetate (V) obtained from the acetate of dehydroabietinol by Rowe's method [13]. The secondary acetoxy group is axial, since the signal of the $H_{(7)}$ proton in the NMR spectrum of (III) forms a narrow multiplet with $W_{1/2}$ = 5 Hz [14].



III IV: R = H V: R = COCH3 VI

Of the components of the polar fraction present in small amounts, we isolated the previously described pinusolide [5] and methyl 15-hydroxydehydroabietate [6].

From the diterpene fraction obtained by the vacuum distillation of the total neutral fraction of the oleoresin and stored at room temperature for a year we isolated a new norcembrane ketone (VI) with $[\alpha]_D^{21}$ +43° (c 1.28), $\lambda_{max}^{C_2H_5OH}$ 230 nm (log ε 3.85). The structure of this compound was shown by its formation from isocembrene on oxidation with $KMnO_4$ in aqueous pyridine. The ketone (IV) was absent from a fresh neutral fraction of the oleoresin and was formed in it on storage as a result of the autooxidation of isocembrene, as was confirmed in a model experiment.

Hydrocarbon Fraction. After the distillation of the monoterpenes and the bulk of the sesquiterpenes from the hydrocarbon fraction, a mixture of compounds was obtained which gave by separation on SiO_2 + 5% $AgNO_3$ only two diterpenes out of those found previously — isopimara-7,15-diene and neocembrene. Cembrene and isocembrene [1] were not detected in appreciable amounts in this product. However, these hydrocarbons did appear in the hydrocarbon fraction if Al_2O_3 of increased activity (activity grade I-II according to Brockmann) was used for the chromatography of the neutral fraction of the oleoresin. Under these conditions, intensive dehydration of the isocembrol and 4-epiisocembrol takes place.

The pinacenes [3] are also secondary products formed from the epimeric isocembrols in the vacuum distillation of the neutral fraction. The latter contains traces of diterpene acids which, at an elevated temperature, may cause the dehydration of the isocembrols to cembrene, and then the isomerization of the cembrene to the stereoisomeric pinacenes. In actual fact, when the neutral fraction of the oleoresin investigated was subjected to vacuum distillation as described previously [9], after chromatography of the mixture of hydrocarbons, in which cembrene predominated, we obtained isocembrene and the pinacenes. Under these conditions, the amount of isocembrol and 4-epiisocembrol in the fraction of oxygen-containing compounds decreased sharply.

EXPERIMENTAL METHOD

The melting points were determined on a Kofler block, the IR spectra were recorded on a UR-20 instrument (in CCl_4), the UV spectra on a "Specord UV-VIS" instrument, and the NMR spectra on Varian A-56/60A and Varian HA-100 instruments (in CCl_4, internal standard HMDS, the signal of which was taken as 0.05 ppm, δ scale). The optical rotations were determined for solutions in $CHCl_3$ on a Zeiss polarimeter. The elementary analyses of the previously undescribed compounds corresponded to the calculated figures. The isolation of the initial fractions from the oleoresin has been described in the discussion part of the paper.

Separation of the Nonpolar Fraction. The nonpolar fraction (3.7 g) was dissolved in 30 ml of a mixture (5:1) of methanol and water, and 3 g of dry $NaBH_4$ was added. After being stirred at 20°C for 30 min, the reaction mixture was diluted with 100 ml of hot water and was extracted with a mixture of petroleum ether and diethyl ether (1:1; 3 × 100 ml). After drying over Na_2SO_4, the extract was evaporated and the product was chromatographed on 40 g of SiO_2, giving 1.3 g of unchanged fraction (checked by TLC), and 2.35 g of a mixture of alcohols.

The chromatography of the unchanged fraction on 20 g of SiO_2 yielded 0.4 g of an oxide fraction, 0.5 g of an ester fraction, and 0.35 g of bornyl acetate.

(+)-Manoyl Oxide and Epimanoyl Oxide. The oxide fraction (0.4 g) was chromatographed on 20 g of SiO_2 + 5% $AgNO_3$. Petroleum ether—dimethyl ether (99:1 and 98:2) eluted successively 0.005 g of epimanoyl oxide, identical according to TLC and NMR and IR spectroscopy with an authentic sample, 0.1 g of a mixture of three unknown oxides, and 0.23 g of (+)-manoyl oxide with mp 24.5-26°C, $[\alpha]_D^{20}$ +25° (c 1, 40). Literature data [15]: mp 25.5-26°C, $[\alpha]_D^{20}$ +30°.

Separation of the Fraction of Acetylatable Alcohols. The mixture of the acetates of the acetylatable alcohols (4 g) was chromatographed on 80 g of SiO_2 + 5% $AgNO_3$. Petroleum ether with increasing concentrations (from 15 to 25%) of diethyl ether eluted successively the acetates of borneol (0.06 g), of dehydroabietinol (1.3 g), of labd-8(20),13-dien-15-ol (0.5 g), of palustrol (0.2 g), of abietinol (0.05 g, identified by TLC, GLC, and NMR and UV spectroscopy), and of isopimarinol (1.3 g), and a mixture of the acetates of isopimarinol and isopimara-8,15-dien-18-ol (0.5 g), present in a ratio of 8:1 (NMR spectrum).

The labd-8(20),13-dien-18-ol, obtained by the reduction of its acetate with $LiAlH_4$ in diethyl ether, had n_D^{20} 1.5215, $[\alpha]_D^{20}$ +32° (c 2, 5); p-nitrobenzoate, mp 108.5-109.5°C (from ethanol—CCl_4). Literature data [16]: mp 107-108.5°C; the NMR spectrum corresponded to that given in the literature [17].

Diacetate of 7α-Hydroxydehydroabietinol (III). The polar fraction (5 g) was chromatographed on 70 g of SiO_2. Petroleum ether containing increasing amounts (from 20 to 75%) of diethyl ether eluted successively 0.1 g of pinusolide, 0.5 g of methyl 15-hydroxydehydroabietate, 1.5 g of isogatholal, and 1.6 g of agathadiol. Then 0.6 g of a fraction consisting mainly of a single substance was eluted. The IR spectrum of this fraction lacked the absorption bands of carbonyl groups. Its acetylation with acetic anhydride in pyridine followed by purification in SiO_2 gave the diacetate (III) (0.4 g) with mp 105-106°C (from ethanol), $[\alpha]_D^{20}$ +32.2° (c 0.55). IR spectrum (in KBr), cm^{-1}: 840, 860, 1510 (1,2,4-trisubstituted benzene ring), 1035, 1255, 1730 ($OCOCH_3$); NMR spectrum, ppm: 0.90 and 1.06 (3 H each, singlets, $C_{(4)}$—CH_3 and $C_{(10)}$—CH_3), 1.14 [6 H, doublet, J = 6.8 Hz, —$CH(CH_3)_2$], 1.94 (singlet, 6 H, 2 $OCOCH_3$), 2.83 (1 H, sextet, J = 6.8 Hz, $H_{(15)}$), 3.72 (2 H, AB system with J_{AB} = 11 Hz, —CH_2OCOCH_3), 5.83 (1 H, $H_{(7)}$), and 6.92-7.19 ppm (3 H, multiplet, protons of a benzene ring).

Preparation of the Oxo Acetate (V) from the Diacetate (III). The crude product from the reduction of 0.1 g of the diacetate (III) with $LiAlH_4$ was dissolved in 10 ml of absolute diethyl ether, 0.1 g of active MnO_2 was added, and the mixture was left at 22°C for 1 h. The residue after filtration and the evaporation of the solution was acetylated with acetic anhydride in pyridine. The resulting oxo acetate (V) (0.04 g), after purification on SiO_2, had mp 61-62°C (from ethanol), $[\alpha]_D^{20}$ +22° (c 0.112). A mixture with the oxo acetate (V) obtained by Rowe's method [13] melted at 61-62°C.

18-Norcembra-2,7,11-trien-4-one (VI). From an SiO_2 column, the ketone (VI) was eluted immediately after the nonpolar fraction (yield 0.01% of the diterpene fraction obtained by the method described previously [9]). After additional purification on SiO_2 + 5% $AgNO_3$ the ketone (VI) had n_D^{23} 1.5060, $[\alpha]_D^{21}$ +43° (c 1, 28); IR spectrum, cm^{-1}: 993 (trans-disubstituted double bond), 1624, 1690 (α-enone); UV spectrum (in ethanol): λ_{max} 230 nm (log ε 3.85); NMR spectrum, ppm: 0.83 and 0.87 (3 H each, doublets, J = 6.5 and 6.5 Hz, methyls of an isopropyl group), 1.50 and 1.56 (3 H each, methyl groups on nonconjugated double bonds), 4.65-5.22 ppm (2 H, multiplet, $H_{(7)}$ and $H_{(11)}$ protons); the protons of the trans-disubstituted double bond form an AB system with J_{AB} = 15.5 Hz with the centers of the A and B parts at 5.92 ($H_{(3)}$) and 6.41 ($H_{(2)}$), respectively. The B part was split ($J_{1,2}$ = 8.0 Hz) because of $H_{(2)}$ and $H_{(1)}$ coupling.

Synthesis of 18-Norcembra-2,7,11-trien-4-one from Isocembrene. A solution of 0.3 g of $KMnO_4$ in 20 ml of a mixture of pyridine and water (1:1) was added to a solution of 0.3 g of isocembrene in 20 ml of pyridine that had been cooled to 0°C and stirred. The stirred mixture was left at 0°C for 3 h, after which a 5% aqueous solution of Na_2SO_3 was added to it and it was extracted with petroleum ether (2 × 100 ml). Chromatography of the product on 15 g of SiO_2 yielded 0.04 g of the ketone (VI) (yield 13%), $[\alpha]_D^{20}$ +40° (c 1,5); n_D^{20} 1.5070.

Isolation of the Ketone (VI) from the Mixture of the Products of the Autooxidation of Isocembrene. Isocembrene (2 g) was left at room temperature for three days in the form of a film on the walls of a flask. The product was dissolved in petroleum ether and chromatographed on 20 g of SiO_2. Petroleum ether with 4% of diethyl ether eluted 0.03 g of the crude ketone (VI) which, after purification on SiO_2 + 5% $AgNO_3$, had $[\alpha]_D^{21}$ +41° (c 2, 0) and was identical according to its IR and NMR spectra with the sample obtained from the oleoresin.

SUMMARY

1. The following compounds have been isolated from the oleoresin of *Pinus koraiensis* Sieb. et Zucc., in addition to those described previously: cis-abienol, labd-8(20),13-dien-15-ol, (+)-manoyl oxide, epimanoyl oxide, and 7α-hydroxydehydroabietinol (in the form of the diacetate). The structure of the last-mentioned, previously unknown, diterpenoid has been shown by chemical transformations and by spectroscopy.

2. It has been established that the cembrene, isocembrene, pinacenes, neoabienol isolated previously and the 18-norcembra-2,7,11-trien-4-one now isolated for the first time are secondary products formed in the storage and treatment of the oleoresin.

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REDUCTION OF 17β-ACETOXY-17α-ETHYNYL-3-METHOXYESTRA-1,3,5(10)-TRIENE WITH METALS IN LIQUID AMMONIA

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UDC 615.357.631.012.1

One of the methods for obtaining 17-alkyl- or 17-alkenyl-substituted estranes, which are used as active hormonal compounds or intermediates in their synthesis, is the reduction of 17-ethynylestradiol and its derivatives. Particular interest is presented by the reduction of the latter by metals in liquid ammonia (the Birch reaction [1]), since in this process not only the ethynyl group but also ring A of the steroid is reduced [2-5].

We have reduced the 3-methyl ether of ethynylestradiol with sodium in liquid ammonia [6] and have found that in the case of the 17-acetate (Ib) the reaction takes place differently from the reduction of the 17-alcohol (Ia) [2, 3]. The main product of the reduction of the acetate (Ib) was 3-methoxy-19-norpregna-2,5(10),20-triene (III), isolated with a yield of 65% when tetrahydrofuran was used as the solvent for the steroid. When diethyl ether was used, a mixture of the triene (III) and the hydroxy vinyl compound (II) was obtained.

The IR spectrum of the enol ether (III) has the absorption bands of the C_{21}-methylene group at 3080 and 905 cm^{-1} and of double bonds at 1700 and 1670 cm^{-1}. The NMR spectrum of this compound has the signals of four ethylene protons: a weakly resolved, broadened, signal at 4.57 ppm (1 H at C_2), a group of signals in the 4.81-4.98-ppm region (2 H at C_{21}), and a multiplet with its center at 5.74 ppm (1 H at C_{20}), and also the isolated signals of the C_{19} methyl group at 0.54 ppm and of the methoxy group at 3.47 ppm, and a group of signals of the C_1 and C_4 methylene protons with an intensity of four proton units in the 2.5-2.64-ppm region.

The hydrolysis of the enol ether (III) in the presence of hydrochloric acid gave the α,β-unsaturated ketone (IV). The NMR spectrum of this compound did not show the signals of methoxy and methylene allyl protons of ring A, but it had a signal at 5.63 ppm (1 H at C_4) and the signals of the C_{17}-vinyl group remained unchanged. In the mass spectrum of the

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 180-184, March-April, 1976. Original article submitted July 30, 1975.