PINUSOLIDE, A NEW DITERPENOID FROM Pinus sibirica AND P. korsensis

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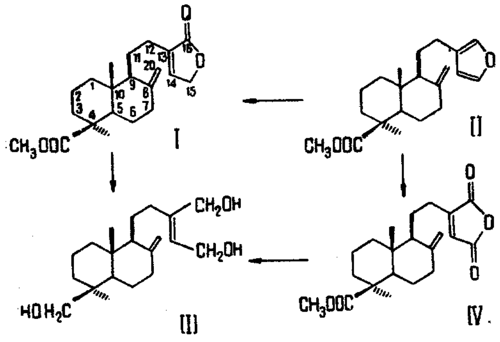
In a study of the diterpenoid components of the oleoresin of Pinus subg. Haploxylon (P. koraensis Sieb. et Zucc. and P. sibirica R. Mayr.), we isolated a diterpene lactone of the composition $C_{21}H_{30}O_4$,* which we have called "pinusolide." According to its IR spectrum, it contains an α-β-unsaturated γ-lactone ring[1], and methoxycarbonyl and exo-methylene groups. The NMR spectrum of the compound (Fig. 1) has signals at 4.52 ppm (1H), 4.82 ppm (1H), and 3.54 ppm (3H) due to the protons of the exo-methylene and the methoxycarbonyl groups. A broadened singlet at 7.02 ppm (1H) and a multiplet at 4.68 ppm (2H) are due, respectively, to a β-proton and to the protons of the methylene group of a $\Delta^{\alpha,\beta}$-butenolide ring [3, 4]. Two three-proton singlets (0.44 and 1.11 ppm) relate to two tertiary methyl groups. The unusually high shift of the signal of one of them (0.44 ppm) shows the 1,3-diaxial arrangement of the methyl and methoxycarbonyl groups [5]. The chemical shift of the signal of the second methyl group (1.11 ppm) shows the presence of a methoxycarbonyl group in the position which is geminal to it.

On the basis of its spectral characteristics and of biogenetic considerations, a labdane structure (I) may be proposed as the most probable for pinusolide. To prove this structure and establish its full configuration, we have made a correlation of the isolated lactone with methyl lambertianate (II).



When I was reduced with lithium aluminum hydride in diethyl ether, a triol (III) with mp 92-93°C was formed which, with respect to its IR, TLC, and optical rotatory dispersion properties, was identical with the triol obtained from methyl lambertianate (II) via the anhydride (IV).

We synthesized pinusolide using Takeda's method [6] by oxidizing methyl lambertianate (II) with perbenzoic acid and subsequent isomerization of the intermediate enol-lactone on alumina. This showed that pinusolide is methyl labd-8(20),13-dien-16,15-olid-19-oate.

Finding methyl lambertianate and pinusolide together in one source is of interest from the biogenetic point of view. The precursor of both diterpenoids is apparently methyl 16-hydroxy-15,16-epoxylabd-

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*We previously [2] reported the presence of a lactone in Pinus sibirica R. Mayr., but it was not an isolated substance.

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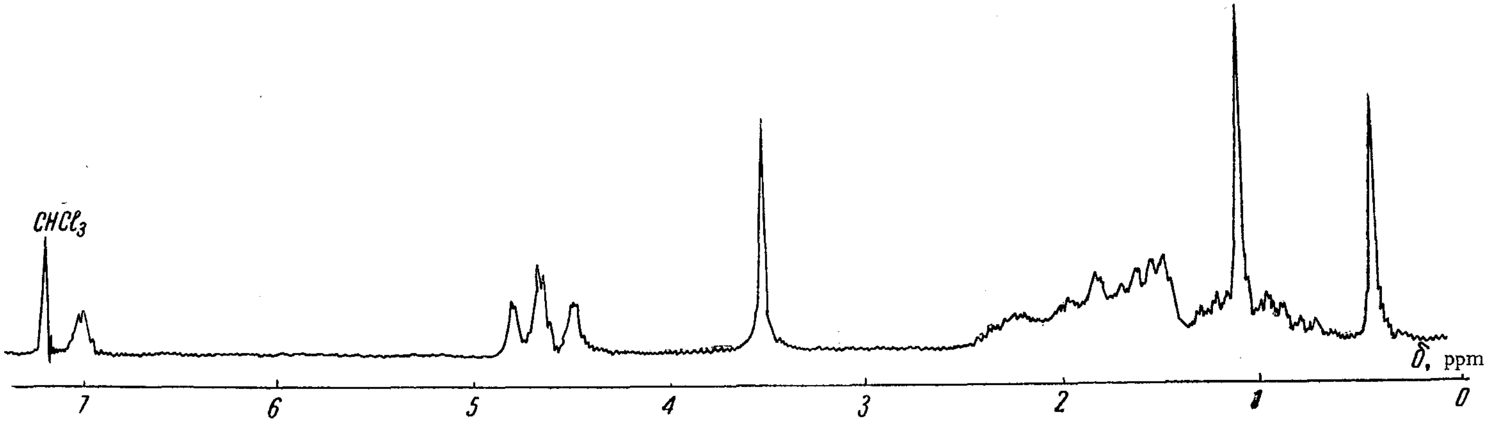
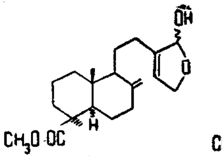
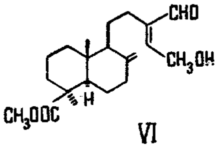


Fig. 1. NMR spectrum of pinusolide (I).

8(20),13-dien-19-oate (V) which is the hemiacetal form of 16-formyl-15-hydroxylabd-8(20)-1,3-dien-19-oate (VI). The dehydration of V in vivo can lead to the formation of methyl lambertianate, whose oxidation can lead to pinusolide. The conversion of the methyl ester (VI) into methyl lambertianate has been effected by Miyasaka [5] by heating it in acetic acid.


V


VI

Pinusolide is the first diterpenoid $\Delta^{\alpha,\beta}$-butenolide found in the family Pinaceae; two similar lactones isolated from plants of other families have been described in the literature [4, 7].

EXPERIMENTAL

The melting points were determined on a Kofler block. The UV spectra were taken in ethanol on a Unicam sp. 700C instrument, the IR spectra on a UR-20 instrument, the mass spectra on an MKh-1303 instrument (with an energy of the ionizing electrons of 70 eV), the NMR spectra on Varian A-60 and Varian HA-100 instruments (with hexamethyldisiloxane as internal standard, δ scale), and the ORD curves and angles of rotation were obtained for solutions in methanol on a Spectropol I spectropolarimeter.

Pinusolide (I). The neutral oxygen-containing diterpene fraction of the oleoresin was chromatographed on alkaline Al_2O_3 (activity grade II) using petroleum ether with increasing concentrations of diethyl ether as the eluents. The pinusolide fraction was eluted with diethyl ether; on standing it crystallized. After three recrystallizations from petroleum ether, pinusolide was obtained in the form of fine needles with mp 83-84°C, $[\alpha]_D^{23}$ + 24°, $[\alpha]_{460}^{23}$ + 48°, $[\alpha]_{250}^{23}$ + 384°, and $[\alpha]_{230}^{23}$ + 600° (c 0.05), UV spectrum: λ_{max} 205 nm (log ε 4.22); IR spectrum (in KBr, cm^{-1}): 900, 1660, and 3090 ($>C=CH_2$), 1160 and 1730 ($COOCH_3$), and 1410 and 1754 ($\Delta^{\alpha,\beta}$-butenolide). Found %: C 72.43, 72.61; H 8.64, 8.62. Mol. wt. 346 (mass spectrometry). $C_{21}H_{30}O_4$. Calculated %: C 72.8; H 8.73.

Reduction of Pinusolide with Lithium Aluminum Hydride. Excess of a suspension of $LiAlH_4$ in absolute ether was added to a solution of 90 mg of pinusolide in the same solvent, and the mixture was heated under reflux for 2 h. After the usual working up, 50 mg of the triol (III) was obtained with mp 92-93°C (recrystallized twice from diethyl ether), $[\alpha]_D^{23}$ + 25.4° (c 0.118); IR spectrum (in KBr, cm^{-1}): 890, 1650, and 3080 ($>C=CH_2$), 1010 and 1030 (C–O of primary OH groups), and 850 ($>C=CH-$).

Preparation of Labd-8(20)-1,3-dien-15,16,19-triol (III). Methyl lambertianate (900 mg) was converted into the anhydride (IV) by a published method [8]. Yield 70%; IR spectrum (film): 1787 and 1855 cm^{-1}. Without additional purification IV was reduced with lithium aluminum hydride in absolute ether. After recrystallization of the product from diethyl ether, 190 mg of the triol (III) was obtained with mp 92-93°C, $[\alpha]_D^{23}$ + 25° (c 0.03), giving no depression of the melting point with the III obtained from I.

Triacetate of Labd-8(20)-1,3-dien-15,16,19-triol (III). Acetylation of the triol (III) with acetic anhydride in pyridine give a liquid triacetate with n_D^{24} 1.4960; IR spectrum (film, cm^{-1}): 900, 1655, and 3084 ($>C=CH_2$), 855 ($>C=CH-$), 1040, 1250, and 1750 (CH_2OOCCH_3); NMR spectrum (in CCl_4): 0.66 and 0.90 ppm (3H each), methyl groups at C_{10} and C_4, respectively, 4.77 and 4.55 ppm, protons of an exo-methylene group, 1.93 ppm (3H) and 1.96 ppm (6H), the methyls of the three acetyl groups, and 5.41 ppm, triplet (J = 7 Hz), a proton at C_{14}. Found %: C 70.24; H 9.22. $C_{26}H_{40}O_6$. Calculated %: C 69.61; H 8.99.

Synthesis of Pinusolide. A solution of 600 mg of II in chloroform cooled to 0°C was treated dropwise with 7 ml of chloroform solution of perbenzoic acid (content of perbenzoic acid in the working solution 58 mg/ml), and the mixture was left for 2 h with the temperature being allowed to rise gradually to +15°C. After being washed with aqueous sodium carbonate solution and with water, the solution was evaporated, and the residue was dissolved in petroleum ether, transferred to a column containing 200 g of Al_2O_3 (activity grade II), and left for 12 h. A mixture of petroleum ether and diethyl ether (1 : 1) then eluted two unidentified substances (220 mg), and diethyl ether eluted pinusolide (150 mg) with mp 83-84°C giving no depression of the melting point with an authentic sample and having IR, NMR, and mass spectra completely identical with the corresponding spectra for I.

CONCLUSIONS

From the neutral fraction of the oleoresin of *Pinus sibirica* R. Mayr and *P. koraensis* Sieb. et Zucc. a new diterpenoid has been isolated which we have called "pinusolide," whose structure has been shown to be that of methyl labd-8(20)-1,3-dien-16,15-olid-19-oate.

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