BICYCLIC DITERPENE ACIDS FROM THE OLEORESIN

OF Pinus sibirica

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In the oleoresins of conifers, in addition to neutral substances and resin acids there is a considerable amount of diterpene hydroxy acids. An investigation of the latter is of interest both from the biogenetic and from the chemotaxonomic points of view.

In the present paper we give the results of a study of the diterpene hydroxy acids from the oleoresin of <u>Pinus sibirica</u> R. Mayr. To separate them from the neutral substances, we converted the acids into their sodium salts. After regeneration from the salts, the hydroxy acids were separated from the resin acids in the form of a precipitate insoluble in petroleum ether. The precipitate was treated with diazomethane to convert the hydroxy acids into the methyl esters. From the complex mixtures of esters we isolated by chromatography on alumina and identified methyl labda-8(20), 13-dien-16,15-olid-19-oate (Ia) – which we have found previously [1] in the neutral fraction of the oleoresin and have called pinusolide – and methyl isocupressate (IIa) [2].

A third compound consisted of a liquid methyl ester of a dihydroxy resin acid with the empirical formula $C_{21}H_{34}O_4$, $[\alpha]_D^{23} + 36.0^\circ$. The NMR spectrum of this ester was similar to that of methyl sciadopate (IIIa), isolated from Sciadopitys verticillata Sieb. et Zucc. [3-6], but it differed from the latter by its constants. Literature data: mp 108.5°C, $[\alpha]_D^{-0.7^\circ}$ [4] and +0.36 [6]. The reduction of this methyl ester gave the same triol (IV) as that formed from pinusolide (Ia) and methyl lambertianate (Va) [1].



The triol exists in two crystalline forms with different melting points (92-93°C and 150.5-151°C). A similar phenomenon has been described for other diterpenoids of complex structure [7, 8].

The NMR spectrum of the diacetate of the ester of the dihydroxy acid differed from that of the diacetate of methyl sciadopate considered by Japanese workers: the signal of two acetate methyls forms a sin-

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• 1974 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$15.00. glet (2.03 ppm, 6H), while in the literature [4] the presence of two singlets (2.06 and 2.08 ppm) is reported and a difference was observed in the nature of the signals in the olefinic region.*

The IR spectrum of the methyl ester of the dihydroxy acid shows an intramolecular hydrogen bond (3540 cm⁻¹, 0.01% solution in CCl₄). The above facts permitted us to conclude that the compound isolated is the methyl ester of trans-sciadopic acid (IIIa) with the cis position of the C_{15} and C_{16} substituents.

It is known [9] that in bicyclic diterpenoids with the trans configuration of the double bond in the side chain the value of the specific rotation is greater than that of the compound with a cis double bond. The compound described by the Japanese workers probably has the cis configuration of the double bond with the trans orientation of the carbinol groups. The same suggestion has been put forward by other authors [7, 10].

The presence in the Siberian pine oleoresin of agathadiol (IIc) [11], pinusolide (Ia) [1], and lambertianic acid (Vb) [12] has been reported previously. The presence in the same raw material of isocupressic (IIb), trans-sciadopic (IIIb), and pinusolic† (Ib) acids shows the undoubted biogenetic connection of the compounds isolated; agathadiol (IIc) is apparently a precursor of the hydroxy acids; oxidation may take place in accordance with the following scheme:

> agathadiol → isocupressic acid trans-sciadopic acid → pinusolic acid tambertianic acid

EXPERIMENTAL

The IR spectra were recorded on a UR-20 instrument (CCl₄) and the NMR spectra on an A56-60A instrument (standard TMS). The molecular weights of the compounds were determined mass-spectrometrically on MKh-1303 and MI-1305 instruments. The melting points were measured on a Kofler block. Neutral alumina (activity grade III-IV) and silica gel (KSK, 140 μ , air-dry) were used for chromatography. The oleoresin of the pine (6.5 kg) was treated with a 2% aqueous solution of caustic soda. The aqueous solution of the sodium salts of the acids (after the separation of the neutral substances) was acidified with 10% HCl, and the hydroxy acids were extracted with diethyl ether. The yield of acids was 3.9 kg. The acids were dissolved in a large excess of petroleum ether (10 liters per kg of acids). The precipitate that deposited (oxidized products) amounted to 1% of the initial mixture of acids.

The oxidized products were methylated with diazomethane in diethyl ether-methanol (9:1) solution. The completeness of methylation was checked by thin-layer chromatography. Column chromatography of the methyl esters of the hydroxy acids was performed on alumina (1:50). The eluents were petroleum ether containing increasing amounts of ethyl acetate, and mixtures of ethyl acetate and ethanol. Petroleum ether with 10% of ethyl acetate eluted pinusolide and then methyl isocupressate; a mixture of ethyl acetate and 10% of ethanol eluted methyl sciadopate.

<u>Pinusolide (Ia)</u>. The recrystallization of the crude pinusolide fractions from petroleum ether gave a crystalline substance $C_{21}H_{30}O_4$, mp 82-83°C, $[\alpha]_D^{22} + 54.4^\circ$ (c 1.56; ethanol), \ddagger mol. wt. 346. There was no depression of the melting point (mp 82-83°C) of a mixture with an authentic sample of pinusolide isolated from the neutral fraction of the oleoresin of the Siberian pine. The IR and NMR spectra of the two samples were identical.

Methyl Isocupressate (IIa). The rechromatography on silica gel of the fractions containing methyl isocupressate (0.7 g) gave 0.6 g of a colorless oily substance, $C_{21}H_{34}O_3$, $[\alpha]_D^{22} + 48.5^\circ$ (c 2.27; ethanol), n_D^{22} 1.5000, mol. wt. 334. IR spectrum, cm⁻¹: 890, 1650, 3090 (exomethylene double bond), 1160, 1240, 1735 (ester group), and 1000 and 3630 cm⁻¹ (hydroxyl). NMR spectrum (CCl₄), ppm: 0.48, 1.16, and 1.64 (3H each) – methyl groups at C_{10} , C_4 , and C_{13} , respectively; 3.58 (3H) protons of a methoxycarbonyl group; 4.52 and 4.82 – exomethylene protons; 4.01 (doublet, J = 7 Hz, 2H) – C_{15} methylene protons; 5.31 ppm (triplet, J = 7 Hz, 1H) – proton at C_{14} .

* The sample of methyl sciadopate was kindly given to us by Dr. M. Sumimoto.

[‡]The value of $[\alpha]$ given previously [1] was low because of the low concentration of the solution. A value of $[\alpha]_D^{24}$ +55° is given in a later paper [13].

[†]Name derived from pinusolide.

Methyl Isocupressate p-Nitrobenzoate. When 200 mg of methyl isocupressate and 200 mg of p-nitrobenzoyl chloride were boiled in pyridine for 2 h, the p-nitrobenzoate (170 mg) was obtained, with mp 99-101°C (from petroleum ether). Literature data: mp 102-103°C [2].

Reduction of Methyl Isocupressate. An excess of LiAlH_4 in absolute diethyl ether was added to 200 mg of methyl isocupressate in 2 ml of the same solvent, and the mixture was boiled under reflux for 2 h. After the usual treatment, the reaction mixture yielded 180 mg of agathadiol, $C_{20}H_{34}O_2$, mp 108-109°C, mol. wt. 306, giving no depression of the melting point in admixture with an authentic sample. The IR and NMR spectra of the agathadiol obtained and of an authentic sample were identical.

<u>Methyl trans-Sciadopate (IIIa)</u>. The crude methyl sciadopate fraction (2.1 g) was chromatographed on silica gel (150 g) with elution by a mixture of petroleum ether and ethyl acetate (1:1). A colorless oil was obtained (0.9 g), $C_{21}H_{34}O_4$, $[\alpha]_D^{23}+36.0^\circ$ (c 2.5; ethanol), mol. wt. 350 (m/e 332, M-18).

IR spectrum, cm⁻¹: 890, 1650, 3090 (exomethylene double bond), 1160, 1240, 1735 (ester group), 3630, 1000 (hydroxyl). NMR spectrum (CCl₄), ppm: 0.48 and 1.17 (3H each) – methyl groups at C_{10} and C_4 ; 3.59 (3H) protons of a methoxycarbonyl group; 4.53 and 4.58 – protons of an exomethylene group; 4.04 (3H) and 4.12 (1H) protons of the C_{15} and C_{16} methylene groups; 5.48 (triplet, 1H) – proton at C_{14} .

The liquid methyl sciadopate diacetate was obtained as described by Sumimoto et al. [5] with $[\alpha]_D^{20}$ + 33.2° (c 1.22; ethanol). NMR spectrum (CHCl₃), ppm: 0.50 and 1.17 (3H each) – methyls at C₁₀ and C₄; 2.03 (6H) methyls of two acetate groups; 3.60 (3H) – protons of a methoxycarbonyl group; 4.49 and 4.85 – exomethylene protons; 4.61 (3H) and 4.69 (1H) – C₁₅ and C₁₆ methylene protons; 5.45 (triplet, 1H, J = 7 Hz) – proton at C₁₄.

Literature data: NMR spectrum (CDCl₃), ppm: 0.50, 1.17; 2.06; 2.08; 3.56; 4.46; 4.51; 4.82 and 5.54 [5].

<u>Reduction of Methyl Sciadopate</u>. The reduction of 300 mg of methyl sciadopate by a suspension of lithium tetrahydroaluminate in diethyl ether gave 270 mg of the triol (IV). Recrystallization from diethyl ether formed plates with mp 92-93°C, $([\alpha]_D^{22} + 24.2^\circ)$, which on further recrystallization from the same solvent were converted into needles with mp 150.5-151°C, $[\alpha]_D^{22} + 25.0^\circ$ (c 1.99; ethanol); when the crystals with mp 92-93°C were mixed with a sample of triol that we had obtained from pinusolide, there was no depression of the melting point (92-93°C).

Found: %: C 74.47; H 10.63. Mol. wt 322 (m/e 304, M - 18). C₂₀H₃₄O₃. Calculated: %: C 74.49; H 10.63. Mol. wt. 322.

NMR spectrum (CD₃OD), ppm: 0.60 and 0.87 (3H each) – methyls at C_{10} and C_4 ; 3.45 (AB system, J = 11 Hz, 2H) – protons of the carbinol group at C_4 ; 4.01 (2H) and 4.09 (doublet, J = 7 Hz, 2H) – protons of the C_{16} and C_{15} carbinol groups; 4.50 and 4.74 – exomethylene protons; 5.40 ppm (triplet, J = 7 Hz, 1H) – proton at C_{14} .

SUMMARY

The acid fraction of the oleoresin of <u>Pinus sibirica</u> R. Mayr. contains pinusolic acid (labda-8(20),13dien-16,15-olid-19-oic acid), and isocupressic and trans-sciadopic acids. The hydroxy acids were isolated and identified in the form of the methyl esters.

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