DERIVATIVES OF ANTICOPALIC ACID AND OTHER NEW COMPOUNDS FROM THE OLEORESIN OF *Pinus pumila*

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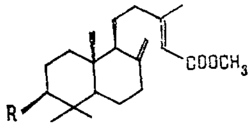
UDC: 547.595.9

From the oleoresin of the Japanese stone pine the previously known agatholic, agathalic, agathic, and 3β-hydroxyanticopalic acids, and also the new acids 19-methoxyanticopalic and 19-O-succinylagatholic acids have been isolated and identified in the form of their methyl esters. Two new esters of l-borneol have been isolated and characterized in the form of their acetates — the ferulate and the p-coumarate.

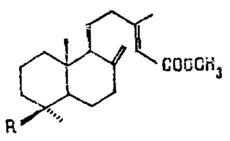
In preceding communications [1, 2] we have described a detailed investigation of the composition of the neutral part and of a mixture of the resin acids of the oleoresin of *Pinus pumila* (Pal.) Regel. (Japanese stone pine), which, as was shown, differs sharply from other *Pinus* species of the section Cembrae by the metabolism of the labdane diterpenoids and the very low content of cembrane compounds. In this communication we give information on the composition of the mixture of polar resin acids of the oleoresin under investigation, which contain, in addition to a carboxy group, other oxygen-containing functional groups. It has been established previously that the polar acids make up 7.5% of the total acids of the oleoresin and their bulk (about 80%) is represented by two acetoxy acids — O-acetylagatholic acid and an acetoxyanticopalic acid with an unknown position of the acetoxy group [1]. Both acids were isolated and characterized in the form of the methyl esters. After communication [1], a paper by Braun and Breitenbach [3] appeared in which the isolation of 3β-acetoxyanticopalic acid from the leaves of *Metasequoia glyptostroboides* Hu et Cheng was described. By saponifying a sample of our acetoxy esters with an ethanolic solution of caustic soda we obtained a crystalline hydroxy acid the constants of which were identical with those of the 3β-hydroxyanticopalic acid obtained by Braun and Breitenbach [3]. Consequently, the second acetoxy ester that we described in the previous paper is methyl 3β-acetoxyanticopalate (I).

By chromatography of the methylated mixture of polar acids we isolated another two esters, less polar than the acetoxy ester (I) — dimethyl agathate (II) and methyl agathalate (III) — and also two more polar esters — methyl agatholate and methyl 3β-hydroxyanticopalate ((IV) and (V), respectively). The esters (II), (III), and (V) were identified by comparing their constant and spectral characteristics with those given in the literature [4], and the ester (V) by comparison with an authentic sample obtained by methylating 3β-hydroxyanticopalic acid. The ester (V) was synthesized by Braun and Breitenbach [3] but we are the first to have detected 3β-hydroxyanticopalic acid itself in a natural source. The amounts of compounds (II)-(V) in the mixture of methyl esters of polar acids were approximately 3, 2, 8, and 2%, respectively. The acids corresponding to the methyl esters (II), (III), and (IV) are components of the resins of *Agathus* sp. [5] and of *Araucaria bidwilli* [4].



I. R = OCOCH$_3$

V. R = OH



II. R = COOCH$_3$

III. R = CHO

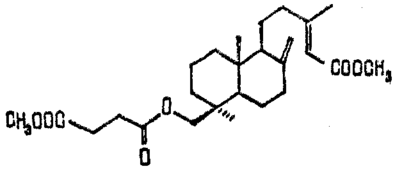
IV. R = CH$_2$OH

Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 158-163, March-April, 1983. Original article submitted April 8, 1982.

0009-3130/83/1902-0149$07.50

Since we studied the acidic fraction of the oleoresin after its methylation with diazomethane, the question of the presence of free agathic acid remained open, since its monomethyl ester is also found in nature [6]. In this connection, we performed the selective extraction of the strong acids, to which agathic acid may be assigned, with an aqueous solution of sodium bicarbonate from an ethereal solution of the acidic part of the oleoresin. After methylation of the acids that had passed into the aqueous bicarbonate solution and those remaining in the ether layer it was found by TLC that dimethyl agathate (II) was present only in the fraction obtained by methylation of the strong acids. The remaining acids passed into the aqueous bicarbonate solution to an insignificant extent. Consequently, the oleoresin contains free agathic acid.

From the fraction of methyl esters of strong acids, in which dimethyl agathate was the main component, we isolated another ester which proved to be a new compound, not previously described. Its PMR spectrum differed from that of methyl O-acetylagatholate [1] by the absence of the signals of the protons of an acetyl group and by the appearance in place of it of a singlet of another methoxycarbonyl group and of a four-proton singlet at 2.55 ppm. These facts, and also the formation of agathadiol on its reduction with lithium tetrahydroaluminate in diethyl ether permitted the structure of the dimethyl ester of O-succinylagatholic acid (VI) to be established for the compound isolated:

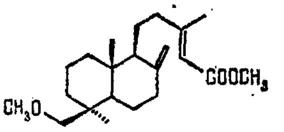


VI

The mass spectrum of compound (VI) showed the presence of an ion with m/z 316, which, by analogy with the mass spectra of other O-acyl derivatives [7], can be interpreted as the result of the splitting out of the $HOOCCH_2CH_2COOCH_3$ molecule from the molecular ion. This ion has the greatest intensity among ions with mass numbers greater than 190. The intensity of the molecular ion (M^+ 448) is only 13.3% of that of this ion (energy of the ionizing electrons 70 eV).

Compounds of this type have not been found previously in plants of the family Pinaceae. Compounds close in structure – 15-O-succinylcopalol and its 8,20-epoxide – have been isolated by Bohlmann and Jakupovic [8] from the plant *Relhania acerosa* (Compositae).

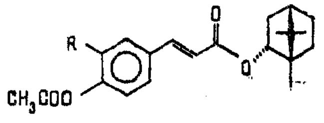
It was possible to isolate one more derivative of anticopalic acid, present in very small amounts (about 2% of the total polar acids) after the performance of chromatography on silica gel of the sum of all the acids of the oleoresin. Under these conditions, in the last fractions, which still contained a mixture of nonpolar resin acids, TLC showed the presence of an unknown component with a R_f value greater than those of the identified acetoxy acids but smaller than those of the nonpolar resin acids. Methylation of this fraction with diazomethane yielded a mixture of methyl esters the chromatography of which permitted the isolation of a new compound the spectral characteristics of which corresponded to those of methyl 19-methoxyanticopalate (VII). In order to confirm this structure we synthesized compound (VII) by the reaction of methyl agatholate (IV) with sodium hydride and methyl iodide in absolute tetrahydrofuran. The sample of (VII) obtained was identical in its constants and spectral characteristics to the specimen obtained from the oleoresin under investigation:



VII

When the chromatography of the mixture of acids was continued, after the fraction containing 19-methoxyanticopalic acid a fraction was eluted which contained two very similar compounds (yield, 0.3%). The IR spectrum of this fraction showed the bands of a phenolic hydroxyl (3560 cm^{-1}), of an aromatic ring (1520 cm^{-1}), of a trans-disubstituted double bond (990 cm^{-1}), and of an ester group (1720, 1270 cm^{-1}). Both its components were acetylated by acetic anhydride in pyridine, giving a mixture of acetates in the IR spectrum of which there were no longer any absorption bands in the 3100-3700 cm^{-1} region. On the basis of the results obtained, it may be assumed that these compounds were phenol esters. It was possible to separate them only in the form of their acetates, and these were characterized by their constants and spectral characteristics.

The IR, UV, and PMR spectra of the main component (50% of the mixture, mp 130-131°C, $[\alpha]_D^{17}$ − 21°) indicate that this compound was the acetate of l-bornyl ferulate (VIII).



VIII. R = OCH$_3$
IX. R = H

The correctness of the proposed structure was confirmed by the synthesis of compound [VIII] using Normant's general method [9] — by the reaction of ferulic acid with thionyl chloride and l-borneol in phosphoric hexamethyltriamide followed by acetylation of the product with acetic anhydride in pyridine.

The PMR spectrum of the second acetate, having mp 84-85°C, and $[\alpha]_D^{17}$ − 24.4°, differed from that of compound (VIII) only by the absence of a signal of the methoxy group and by the appearance, in place of the ABC system of protons of an aromatic ring, of an A$_2$B$_2$ system in the same region in the form of two doublets at 7.03 and 7.45 ppm, J_{AB} = 9 Hz. Such a system of protons is observed in the PMR spectra of alkyl p-coumarates [10]. In view of this, and also of the closeness of the optical properties of this compound and of the acetate (VIII), it may be concluded that the second component was the acetate of l-bornyl p-coumarate (IX).

Ferulic and p-coumaric acids and their esters are widely distributed in plants. Esters of ferulic and p-coumaric acids with fatty alcohols have been isolated from extracts of the bark of various species of confiers of the family Pinaceae [10, 11]. On the other hand, esters of borneol with benzoic acid derivatives are frequently found in plants of the family Umbelliferae [12]. d-Bornyl p-coumarate has been detected by Zinkel [13] in the needles of *Pinus bungeanna* Zucc.

Without taking the two bornyl esters into account, all the polar acids of the oleoresin under investigation are derivatives of anticopalic acid. Of hydroxy acids of other structural types it was possible to detect and identify (in the form of methyl esters) by comparison with authentic samples through PMR spectra and TLC only the difficulty separable 15-hydroxyabietic and 15-hydroxydehydroabietic acids, which have been isolated previously by Carman [5] from the resins of *Agathus* sp. They made up about 1% of the total polar acids of the oleoresin.

EXPERIMENTAL

The instruments and methods have been described in communication [1].

For chromatography we used air-dry KSK silica gel having partical dimensions of 0.140-0.315 mm with a ratio of substance and sorbent in all cases of ∿1:20, the eluent being petroleum ether with increasing (from 0 to 100%) amounts of diethyl ether.

The PMR spectra of compounds (I-VII) (solvent carbon tetrachloride, internal standard hexamethyldisiloxane, δ scale) contained the same signals — a doublet (J = 1 Hz) of the methyl group at C$_{13}$ (2.05 ppm), a singlet (3.55 ppm) of the protons of a methoxycarbonyl groups, and three multiplets identical in form (width at half-height 4 Hz each) due to the protons of an exomethylene group and H$_{14}$ (4.4, 4.7, and 5.5 ppm, respectively). Their IR spectra also contained identical bands at 3090, 1660, and 900 cm^{-1} (exomethylene group), and 1730, 1250, 1150 cm^{-1} (COOCH$_3$).

The Methyl Esters (II), (III), (IV), and (V). The chromatography of 40.0 g of the mixture of methyl esters of the acids of the oleoresin of the Japanese stone pine [1] yielded 36.5 g of a mixture of methyl esters of resin acids and 3.00 g of a mixture of methyl esters of polar acids, the rechromatography of which gave successively 3.00 g of a mixture of methyl esters containing substance (VII), 0.09 g of dimethyl agathate (II) (oil) with n_D^{20} 1.5150 and $[\alpha]_D^{20} + 65.5°$ (c 3.02) (according to the literature [4]: $[\alpha]_D + 52°$; [14]: $[\alpha]_D + 63°$, n_D^{25} 1.5139), 0.06 g of methyl agathalate (III) (oil) with $[\alpha]_D^{18} + 34.3°$ (c 4.18) (according to the literature [4]: $[\alpha]_D + 33°$), 2.28 g of a mixture of the ester (I) and of methyl O-acetylagatholate, 0.15 g of a mixture of substances, 0.24 g of methyl agatholate (IV) with mp 78-79°C (from a mixture of pentane and diethyl ether), $[\alpha]_D^{20} + 42.5°$ (c 2.38) (according to the literature [4]: mp 74-76°C, $[\alpha]_D + 45°$; [14]: mp 77-79°, $[\alpha]_D + 44°$), and 0.09 g of a mixture of the ester (V), methyl 15-hydrodehydroabietate, and methyl 15-hydroxyabeitate. Rechromatography of the last fraction yielded 0.06 g of the ester (V) with the composition $C_{21}H_{34}O_3$, mp 39-40°C (from petroleum ether with diethyl ether) (according to the literature [3]: mp 39-41°C). PMR spectrum, ppm: 0.63, 0.70, 0.91 (3 H each, singlets, tertiary methyl groups at C_4 and C_{10}), 3.08 (1 H, doublet of doublets, J = 5.5 and 9.5 Hz, H_3). IR spectrum: 3620 cm^{-1} (OH).

Dimethyl Ester of 19-O-Succinylagatholic Acid (VI). A solution of 60 g of the acidic part of the oleoresin of the Japanese stone pine in 500 ml of diethyl ether was treated in a separatory funnel with 100 ml of saturated aqueous sodium bicarbonate solution. The aqueous bicarbonate solution was separated off, washed with diethyl ether, acidified with hydrochloric acid to pH 2, and extracted with diethyl ether. The ethereal solution was washed with saturated aqueous sodium chloride solution, and an ethereal solution of diazomethane was added to it until a permanent yellow coloration (showing an excess of diazomethane) appeared. After the solution had been dried with sodium sulfate, it was evaporated to dryness, and the product (0.22 g) was chromatographed on silica gel. This gave 0.16 g of dimethyl agathate (II) and 0.04 g of the ester (VI) in the form of an oil with n_D^{21} 1.5145 and $[\alpha]_D^{21} + 27°$ (c 3.4). PMR spectrum, ppm: 0.62, 0.87 (3 H each, singlets, tertiary methyl groups); 2.45 (4 H, singlet, $OOC{-}\underline{CH_2}{-}\underline{CH_2}{-}COOCH_3$); 3.55, 3.57 (3 H each, singlets, 2 $COOCH_3$). The protons at C_{19} form a AB system with J_{AB} = 11 Hz and the components at 3.55, 3.79, 4.01, and 4.25 ppm.

The reduction of the ester (VI) with lithium tetrahydroaluminate in diethyl ether (room temperature, 1 h) yielded agathadiol with mp 108-109°C (from diethyl ether), $[\alpha]_D^{20} + 30°$ (c 3.5) (according to the literature [14]: mp 107-108°C, $[\alpha]_D + 31°$).

Methyl 19-Methoxyanticopalate (VII). When 40.0 g of the combined acids of the oleoresin was chromatographed, petroleum ether containing 15-20% of diethyl ether eluted the bulk of the resin acids, and petroleum ether containing 25% of diethyl ether eluted a fraction containing resin acids and 19-methoxyanticopalic acid (monitored by TLC). Methylation of the latter with diazomethane and chromatography yielded 0.06 g of the ester (VII) in the form of an oil with n_D^{19} 1.5070, $[\alpha]_D^{19} + 40°$ (c 2.0). IR spectrum, cm^{-1}: 1120 (C—O). PMR spectrum, ppm: 0.62, 0.88 (3 H each, singlets, methyl groups at C_{10} and C_4, respectively); 3.16 (3 H, singlet, OCH_3). The methylene protons at C_{19} formed an AB system with J_{AB} = 9 Hz and components at 2.90, 3.05, 3.20, and 3.35 ppm.

Petroleum ether containing 30% of diethyl ether eluted a mixture (0.12 g) of 1-bornyl p-coumarate and 1-bornyl ferulate.

Synthesis of the Ester (VII). Sodium hydride (0.1 g) was added to a stirred solution of 0.3 g of methyl agatholate (IV) in 20 ml of absolute tetrahydrofuran. After 20 min, 0.5 ml of methyl iodide was added to the resulting mixture and it was stirred at room temperature for 2 days. After the usual working up and chromatography of the product, 0.1 g of the ester (VII) was obtained with $[\alpha]_D^{25} + 36.2°$ (c 3.37), its IR and PMR spectra coinciding with those of the specimen obtained from the oleoresin.

The Acetate (IX). The mixture of 1-bornyl p-coumarate and 1-bornyl ferulate (0.12 g) was acetylated with an excess of acetic anhydride in pyridine (20°C, 12 h). After the usual working up and chromatography, 0.05 g of the acetate (IX) and 0.05 g of the acetate (VIII) were obtained.

The acetate (IX): colorless needles from petroleum ether, mp 84-85°C, $[\alpha]_D^{17}$ − 24.4° (c 4.93). UV spectrum, λ_{max} 283 nm (log ε 4.39). IR spectrum, cm^{-1}: 1780, 1210 (aryl acetate group); 1730 (COOC); 1640, 1620, 1510, 990, 920 (aromatic ring); 1460, 1430, 1380, 1320, 1280, 1170, 1120, 1030, 950. PMR spectrum, ppm: 0.80, 0.82, 0.88 (3 H each, singlets, tertiary methyl group of the bornyl moiety); 2.13 (3 H, singlet, $OOCCH_3$); 4.90 (1 H, doublet of multiplets. J ∿ 10 Hz, CHOOC). The protons of a trans-disubstituted double bond and of a benzene ring appeared in the form of two AB systems at 6.31 and 7.48 ppm (1 H each, J_{AB} = 16 Hz) and 7.03 and 7.45 ppm (1 H each, J_{AB} = 9 Hz), respectively.

The Acetate (VIII). Colorless needles from a mixture of petroleum ether and diethyl ether (2:1) with mp 130-131°C, $[\alpha]_D^{17}$ − 21° (c 2.89). UV spectrum, λ_{max}: 282 (log ε 4.35), 313 (shoulder) (log ε 4.08). The IR spectrum contained, in addition to the bands present in the spectrum of the acetate (IX), bands at 1470, 1270, 1220, 1045 cm^{-1}. PMR spectrum, ppm: 0.80, 0.82, 0.88 (3 H each, singlets, tertiary methyl groups of the bornyl moiety); 2.13 (3 H, singlet, $OOCCH_3$); 3.75 (3 H, singlet, methoxy group); 6.31, 7.48 (1 H each, doublets, forming an AB system with J_{AB} = 16 Hz); 6.74-7.18 (3 H, multiplet with two main signals forming an unsymmetrical doublet at 6.29 ppm [11], protons of an aromatic ring).

Saponification of the acetate (VIII) with a 10% ethanolic solution of sodium hydroxide (1 h at 100°C) yielded n-borneol with $[\alpha]_D^{18}$ − 31.2° (c 2.24), the PMR spectrum of which coincided with that of an authentic sample.

Synthesis of the Acetate (VIII). At 0°C, 0.7 ml of thionyl chloride was added to a solution of 1.18 g of ferulic acid in 40 ml of phosphoric hexamethyltriamide, and the mixture was left at the same temperature for 12 h, after which 0.77 g of *l*-borneol was added to it and the new mixture was kept for another 12 h at the same temperature. After the usual working up, the product was dissolved in 20 ml of pyridine and 5 ml of acetic anhydride was added to the solution. The reaction mixture was kept at 20°C for 6 h and after the usual working up the products were separated by chromatography. This gave 0.50 g of bornyl acetate and 0.20 g of the acetate (VIII) with mp 131-132°C, the IR, UV, and PMR spectra of which coincided with those for the specimen obtained from the oleoresin.

3β-Hydroxyanticopalic Acid. A solution of 0.20 g of the ester (I) [1] in 5 ml of ethanol was treated with 10 ml of a 10% ethanolic solution of sodium hydroxide, and the mixture was heated at 80°C for 30 min. After the usual working up, 0.14 g of 3β-hydroxyanticopalic acid was obtained with mp 156-158°C (from diethyl ether), $[\alpha]_D^{18}$ + 39.2° (c 2.56); according to the literature [3]: mp 157.5-158.5°C, $[\alpha]_D$ + 40.7°.

SUMMARY

1. From the acidic fraction of the oleoresin of the Japanese stone pine agatholic acid, its 19-O-methyl and 19-O-succinyl derivatives, agathalic acid, agathic acid, 3β-hydroxyanticopalic acid, and 3β-acetoxyanticopalic acid have been isolated in the form of their methyl esters. New esters of borneol — *l*-bornyl ferulate and *l*-bornyl p-coumarate — have been isolated in the form of their acetates.

2. The metabolism of anticopalic acid in the Japanese stone pine is connected with its biological oxidation at carbon atoms 8 and 19.

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THE OXYMERCURATION—DEMERCURATION OF CARYOPHYLLENES

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UDC: 547.912.5:539.26:546.492

The oxymercuration—demercuration of caryophyllene (I) at a molar ratio of olefin to mercury salt of 1:2 has been performed. Using various chromatographic methods, the following substances have been isolated from the crude product: clovene (II), 1%; caryophyllane 4,8-α-oxide (III), 52%; 9-epicaryophyllane 4,8-β-oxide (IV), 11%; caryolan-1-ol (V), 5.3%; dihydrocaryophyllen-4α-ol (VI), 11%; caryophyllan-4-ol 5,8-oxide (VII), 6.5%; the symmetrical mercury derivative of the 9-epi-4,8-β-oxide (VIII), 2.1%; and an unknown hydrocarbon, A, 1%. The structure of the oxide (IV) has been established by x-ray structural analysis.

Oxymercuration followed reductive demercuration is a simple method for hydrating olefins [1]. Under the action of mercury(II) salts, as a rule, di- and triolefins cyclize [2]. Cases are known of cyclization with the participation of the oxygen atom and the formation of intracyclic ethers as, for example, in the case of α-humulene [3] and of Δ^3-carene [4].

According to literature, the oxymercuration—demercuration of caryophyllene (I) at a molar ratio of olefin to mercury salt of 1:1 leads to caryolan-1-ol (V) with a yield of 61% [5], while the same reaction at a ratio of 1:2 gives caryophyllane 4,8-α-oxide (III) with a yield of 83% [6]. It appears unlikely that the ratio of the reactants would affect the direction of cyclization in this way. In the present paper we give the results of a more detailed study of the reaction of caryophyllene with mercury(II) acetate.

The reaction was carried out at room temperature by a standard procedure [1], and the mixture of products was separated into hydrocarbon, ether, and alcohol fractions (Table 1).

Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR. Lenin Komsomol Novosibirsk State University. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 164-172, March-April, 1983. Original article submitted April 8, 1982.

0009-3130/83/1902-0154$07.50