TRITERPENOIDS FROM Abies SPECIES.

I. ABIESONIC ACID — A TRITERPENOID WITH A NEW CARBON SKELETON FROM THE OLEORESIN OF Abies sibirica

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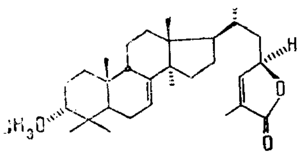
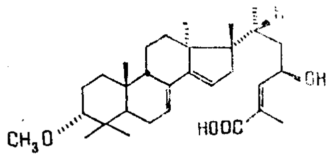
The structure and stereochemistry of abiesonic acid — a new triterpenoid with a modified 3,4-secolanostane carbon skeleton isolated in the form of the dimethyl ester from the oleoresin of the Siberian fir — has been established with the aid of x-ray structural analysis. On irradiation with UV light, this ester is converted reversibly into its 24Z- isomer.

A feature of the chemical composition of the needles and bark of some species of fir is the presence of highly polar neutral triterpenoids of the 9β-lanostane [1-4] or cycloartane [5, 6] series, the first of which to be described was abies lactone (I) [1, 2]. In the seeds of the fir Abies mariesii, Hasegawa has found a whole series of triterpene acids, for one of which (mariesiic A) he established structure II with a rearranged lanostane carbon skeleton.



I



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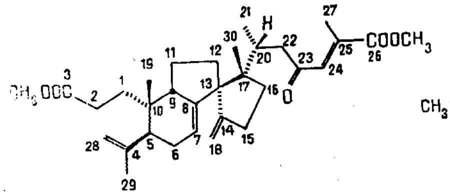
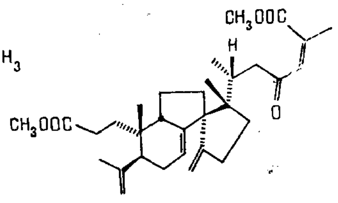


III



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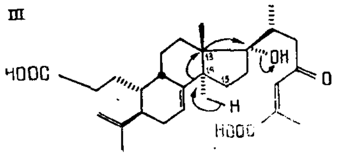

We have shown previously [8] that a substantial part of the acid fraction of the oleoresin of the Siberian fir is composed of triterpene acids, two of which were isolated in the form of methyl esters and were characterized by their constants and some spectral properties. In the present communication we give the results of the determination of the structure of one of them — abiesonic acid, obtained in the form of the dimethyl ester from a mixture of the dimethyl esters of the "strong acid" fraction [8] of the oleoresin by chromatography on silica gel.

Dimethyl abiesonate crystallizes well from acetonitrile or pentane. The structure and relative configuration of its molecule were established by x-ray structural analysis and are expressed by formula (3) and shown in Fig. 1. The six-membered ring has the usual half-

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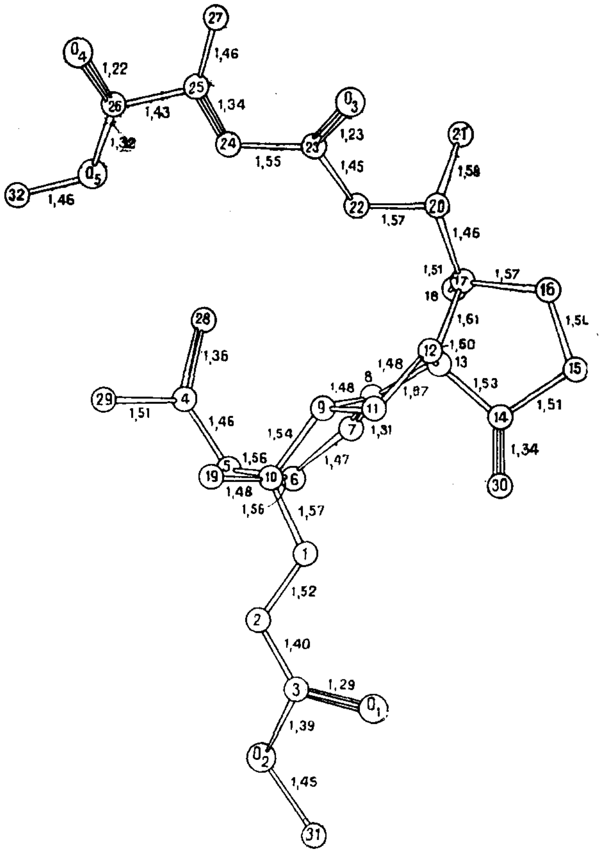

Fig. 1. Structure and relative configuration of the dimethyl abiesonate molecule (III). The probable errors in the bond lengths are 0.01-0.02 Å.

chair form and both five-membered rings have the envelope form, deviations of the C_{11} and C_{17} atoms from the planes formed by the other atoms of these rings being 0.62 and 0.64 Å, respectively. The carbon atoms and one oxygen atom of the $C_{32}{-}O{-}C_{26}({=}O){-}C_{25}({-}C_{27}){=}C_{24}$ fragment in one plane, while the carbonyl group at C_{23} departs from it. The $C_{25}{=}C_{24}{-}C_{23}{=}O$ torsional angle is 27°. The side chain at C_{10} is also practically planar.

Formula (III) also, apparently, expresses the absolute configuration of the dimethyl abiesonate molecule (Fig. 1 shows its enantiomeric structure), since the configuration at C_{10} and C_5 in it are the "natural" ones and correspond to the absolute configuration at these centers in the molecules of all known natural (not rearranged in the area of rings A and B) triterpenoids.

The UV spectrum of the compound under investigation has a maximum at 238 nm (log ε 2.54, in ethanol) corresponding to an electronic transition in an α-enone system. The corresponding Cotton effect on the circular dichroism curve has a negative sign.

In the PMR spectrum, the protons of the exomethylene groups give signals at 4.70 ppm (1 H, H'_{30}, 4.79 ppm (2 H, H''_{30} and H'_{28}), and 4.81 ppm (1 H, H''_{28}) in the form of narrow multiplets. Their assignment was confirmed by double resonance with the suppression of the signal of the C_4-methyl group appearing at 1.72 ppm. The J_{AB} value for the C_{28} protons was 2.5 Hz. The signal of the H_{24} proton is a quartet at 7.00 ppm (J = 1.2 Hz) and that of the protons of the C_{25}-methyl group protons a double at 2.17 ppm with the same spin-spin coupling constant (1.2 Hz). The signal of the protons of the C_{25}-methoxycarbonyl group is shifted downfield from its usual position for them because of the electron-accepting influence of the C_{23}-keto group and appears at 3.79 ppm (singlet). The signal of the protons of the second methoxycarbonyl group appears in the form of a similar singlet at 3.64 ppm. The H_7 proton gives a signal at 5.46 ppm in the form of a quartet (J ∿ 3 Hz) with poorly resolved lateral components. The angular methyl groups appear in the form of singlets at 0.87 and 0.89 ppm and the secondary methyl group (Me_{20}) in the form of a doublet (J = 6.0 Hz) at 0.81 ppm. The ^{13}C NMR spectrum (see the Experimental part) agrees with structure (III).

When a pentane solution of dimethyl abiesonate was irradiated with the light of a high-pressure mercury lamp, after 1.5 h a photoequilibrium mixture of (III) and its cis (24Z) isomer (IV) (3:7), readily separable by chromatography, had formed. The mass spectra of the two isomers practically coincided and the PMR spectra differed mainly only by the values of the chemical shifts for the signals of protons present in the side chains of their molecules. The H_{24} proton of the cis isomer (IV), in contrast to that of dimethyl abiesonate (III), was not under the descreening anisotropic influence of a C_{25}-methoxycarbonyl group, and its signal appeared in a stronger field (6.07 ppm). The same thing was observed for the C_{25}-methyl group, which in this case did not experience the descreening influence of a C_{23}-keto group and gave a signal at 2.00 ppm. This interpretation of the relative magnitudes of the chemical shifts is based on the assumption that the conformations of the α-enone systems in both the molecules under consideration was transoid. This assumption was confirmed by the IR spectra — in both compounds the band of the vibrations of the C_{24} double bond (1633 cm^{-1}) was considerably weaker than the band of the C=O vibrations of the conjugated carbonyl (1700 cm^{-1}) [9].

In spite of the unusual nature of the carbon skeleton, the biogenetic relationship between abiesonic acid and abies lactone (I) is quite obvious. Its direct biogenetic precursor in the plant is possibly a 3,4-secolanostane hydroxy keto acid of formula (V), which can give abiesonic acid on enzymatic dehydration. In this process the migration of the C_{13} methyl group, contraction of the ring with the formation of a spirane fragment of the molecule, and the ejection of a proton from the C_{14}-methyl group, shown by arrows in the formula, must take place successively. The fundamental difference of this route from that postulated for mariestic acid A (II) [7] consists in the occurrence of the contraction of ring C after the migration of the C_{13} methyl group. For the latter, in place of this stage, the migration of the C_{14} methyl group to the C_{13} atom with the subsequent loss of a proton from the C_{15} methyl group is suggested.

The cleavage of ring A in the biosynthesis of triterpenoids is fairly common [10], although 3,4-secotriterpenoids have not previously been found in conifers. The investigation of the triperpenoids of the Siberian fir is continuing.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Bruker WP-200 SY instrument (200.13 MHz for ^1H and 50.327 MHz for ^{13}C) using solutions in $CDCl_3$ (δ scale; internal standard chloroform, the signal of which was taken as 7.24 ppm). High-resolution mass spectra were obtained on a MS-902 instrument (70 eV, direct introduction), UV spectra on a Specord UV-VIS instrument using solutions in ethanol, and IR spectra on a UR-20 spectrophotometer using solutions in carbon tetrachloride. For chromatography, type L silica gel (Czechoslovakia) with a grain size of 0.100-0.160 mm was used in a ratio of substance to sorbent of ∿1:20 with, as eluent, petroleum ether containing increasing (from 0 to 40%) amounts of diethyl ether.

The oleoresin of the Siberian fir was collected in the Maslyanino region of Novosibirsk province.

Dimethyl Abiesonate (III). A solution of 87.2 g of the oleoresin in 2 liters of diethyl ether was shaken vigorously with 1 liter of saturated aqueous sodium bicarbonate solution. The aqueous layer was taken off and it was washed with 0.2 liter of diethyl ether, and was acidified to pH 2 with hydrochloric acid and reextracted with diethyl ether (1 liter). The ethereal extract was washed with saturated aqueous sodium chloride solution (3 × 200 ml), dried with sodium sulfate, and evaporated to dryness. This gave 9.6 g (11% on the oleoresin) of the combined "strong" acids in the form of a pale yellow amorphous powder, to an ethereal solution of which an excess of an ethereal solution of diazomethane was added (until the appearance of a permanent yellow coloration), after which the mixture was kept for 10 min. Then the ether was driven off and the mixture of methyl esters obtained was chromatographed. Petroleum ether containing 27% of diethyl ether eluted a fraction (3 g) the crystallization of which from pentane gave the ester (III) (0.77 g) with mp 93-94°C, $C_{32}H_{46}O_5$ (M^+ 510.3328; calculated, 510.3345), $[\alpha]_D^{20}$ −37.3° (c 4.84; chloroform).

^{13}C NMR spectrum (ppm): singlets at 201.00 (C_{23}), 174.37, 167.81, 160.89, 148.81, 143.23, 139.11, 63.32, 50.05, 36.66; doublets at 133.24, 122.05, 49.28, 43.90, 33.67; triplets at 111.74, 106.58, 48.05, 35.85, 31.03, 30.85, 30.30, 28.96, 27.56, 22.32; and quartets at 52.20, 51.28, 25.77, 24.42, 17.45, 16.17, and 13.96.

TABLE 1. Coordinates of the Nonhydrogen Atoms of the Dimethyl Abesionate Molecule (III) in Fractions of the Elementary Cells

Atom	$x\cdot10^3$	$y\cdot10^3$	$z\cdot10^4$	Atom	$x\cdot10^3$	$y\cdot10^3$	$z\cdot10^4$
C_1	264 (2)	137 (1)	8363 (3)	C_2	228 (2)	280 (1)	8330 (3)
C_3	134 (2)	309 (1)	9021 (4)	C_4	622 (2)	142 (1)	9101 (3)
C_5	534 (1)	162 (1)	8762 (2)	C_6	654 (2)	131 (1)	8440 (3)
C_7	646 (2)	−006 (1)	8342 (3)	C_8	535 (1)	−092 (1)	8462 (2)
C_9	390 (1)	−052 (1)	8700 (2)	C_{10}	352 (1)	093 (1)	9719 (3)
C_{11}	239 (1)	−147 (1)	8593 (2)	C_{12}	332 (1)	−270 (1)	8532 (2)
C_{13}	507 (1)	−232 (1)	8321 (3)	C_{14}	473 (1)	−242 (2)	7925 (2)
C_{15}	534 (2)	−371 (1)	7788 (3)	C_{16}	601 (2)	439 (1)	8110 (3)
C_{17}	662 (1)	−335 (1)	8385 (3)	C_{18}	835 (1)	−277 (1)	8264 (3)
C_{19}	226 (1)	124 (1)	9004 (3)	C_{20}	677 (1)	−396 (1)	8730 (2)
C_{21}	826 (2)	−503 (1)	8740 (3)	C_{22}	708 (2)	−298 (1)	9038 (2)
C_{23}	651 (2)	−343 (1)	9380 (1)	C_{24}	653 (2)	−243 (1)	9682 (3)
C_{25}	657 (2)	−274 (2)	10023 (4)	C_{26}	662 (2)	−165 (2)	10252 (3)
C_{27}	663 (3)	−404 (2)	10169 (4)	C_{28}	745 (2)	051 (1)	9172 (4)
C_{29}	580 (2)	225 (1)	9417 (3)	C_{30}	400 (2)	−151 (2)	7722 (4)
C_{31}	055 (2)	477 (1)	7595 (3)	C_{32}	651 (3)	065 (2)	10356 (4)
O_1	083 (3)	242 (1)	7752 (4)	O_2	139 (1)	438 (1)	7922 (2)
O_3	588 (2)	−451 (1)	9433 (2)	O_4	618 (2)	−180 (1)	10569 (3)
O_5	653 (2)	−047 (1)	10123 (3)				

The x-ray structural experiment. (λ Mo K_α, ω-scanning, $2\theta < 45°$) was performed on a Syntex $P2_1$, diffractometer. The crystallographic parameters for the ester (III) were: a = 7.562(2), b = 10.376(3), c = 38.004(13) Å; space group $P2_12_12_1$, z = 4, d_{calc} = 1.14 g/cm^3. The intensities of the reflections from the two independent regions were averaged, and 2254 reflections were obtained of which 1599 with $I > 3\sigma$ were used in the calculations. The structure was interpreted by the RANTAN procedure [11] and was refined in the usual way to R = 0.093. The coordinates of the nonhydrogen atoms so obtained are given in Table 1.

It must be mentioned that the atoms of the side chains had high amplitudes of the thermal vibrations and it is apparently for this reason that the values of some bond lengths are low.

Dimethyl 24-cis-Abiesonate (IV). A solution of 0.070 g of the ester (III) in 20 ml of methane was irradiated for 1.5 h with the light of a DRSh-1000 high-pressure mercury lamp. Chromatography of the product yielded 0.015 g of the initial ester (III) and 0.052 g of the ester (IV) in the form of an oil with $[\alpha]_D^{20}$ −38° (c 1.05; chloroform). M^+ 510. IR spectrum (cm^{-1}): 900, 880, 1645, and 3080 ($C{=}CH_2$); 1633, 1700 ($C{=}C{-}C{=}O$), and 1740 (broad, C=O of methoxycarbonyl group). PMR spectrum: 0.83 (3 H, doublet, J = 6.5 Hz, Me_{20}); 0.86 and 0.89 (3 H, singlets, angular methyl groups); 1.75 (3 H, slightly broadened singlet, Me_4); 2.00 (3 H, doublet, J = 1.2 Hz, Me_{25}); 2.64 and 2.76 (3 H each, singlets, 2 $COOCH_3$), 3.64 and 3.76 (1 H each, narrow multiplets, $C(_{14}){=}CH_2$); 4.76 and 4.79 (1 H each, narrow multiplets $C(_4){=}CH_2$; on double resonance with the suppression of the signals of the C_4-methyl group they were converted into two doublets forming a strongly coupled AB system with J_{AB} = 2.8 Hz); 5.44 (1 H, quartet with poorly resolved lateral components; J ∿ 3 Hz); 6.07 (1 H, quartet, J = 1.2 Hz, H_{24}).

Photoisomerization of the Ester (IV). A solution of 0.070 g of the ester (IV) in 25 ml of pentane was irradiated with the light of a DRSh-1000 mercury lamp for 1.5 h. Chromatography of the product yielded 0.048 g of the initial ester (IV) and 0.018 g of the ester (III) with mp 93-94°C (from acetonitrile).

SUMMARY

1. The oleoresin of the Siberian fir has yielded, in the form of its dimethyl ester, a new triterpenoid – abiesonic acid – the structure of which has been established with the use of x-ray structural analysis.

2. Under the action of ultraviolet radiation, dimethyl abiesonate is reversibly isomerized to dimethyl 24-cis-abiesonate.

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