TRITERPENOIDS FROM Abies SPECIES

X. TWO NEW 3,4-SECOTRITERPENE ACIDS FROM THE OLEORESIN OF THE SILVER FIR

T. V. Leibyuk, É. N. Shmidt,
and V. A. Raldugin

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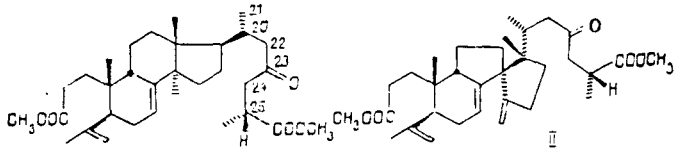
Two new 3,4-secotriterpene acids have been isolated in the form of dimethyl esters from the acid fraction of the oleoresin of the silver fir Abies alba Mill. On the basis of spectral characteristics and the results of a chemical correlation with the known triterpenoids methyl abiesolidate, dimethyl abiesonate and methyl firmanoate it has been established that they are (25R)-3,4-seco-9β-lanosta-4(28),7-dien-23-one-3,26-dioic acid and (25R)-3,4-seco-abiesa-4(28),7,14(30)-trien-23-one-3,26-dioic acid.

The silver fir Abies alba Mill., having a small area in Transcarpathia in the USSR [1], has already been the object of chemical study [2-4]. Continuing an investigation of the triterpenoids from fir species [5], we have found that in the oleoresin of the silver fir the amount of "strong" [6] acids is low (3.7%), and their composition differs appreciably from that for the oleoresin of the Siberian fir. When the total methyl wsters of these acids were chromatographed on silica gel, we succeeded in detecting only a small amount of the known dimethyl abiesonate [7] (yield 3.7% on the total material analyzed), and two of the main components proved to be the methyl esters of two new acids having the structures expressed by formulas (I) and (II). They were obtained with yields of 24.8 and 14.5%, respectively, on the sum of the methyl esters of the strong acids.



I II

The crystalline ester (I) had the empirical formula $C_{32}H_{50}O_5$ (high-resolution mass spectrometry). Its PMR spectrum (Table 1 and the Experimental part) proved to be close to the PMR spectrum of the known triterpenoid methyl abiesolidate (III) [8] but differed from the latter by the presence of the signal of the proton of a second methoxycarbonyl group, the absence of the signal of a lactone proton (H-23) and a different form of the signal for H-25. In the light of the empirical formula it was possible to suggest that the molecule of the compound under investigation differed from that of methyl abiesolidate by the presence of a carbonyl group at C-23 and a methoxycarbonyl group at C-25 in place of the lactone ring. The correctness of this hypothesis was shown by the synthesis of the ester (I) from methyl abiesolidate (III) by the successive performance of the following chemical transformations: alkaline hydrolysis, isolation and methylation of the free hydroxycarboxylic acid (IV), and oxidation of the dimethyl ester of the acid (IV) with pyridinium chlorochromate.

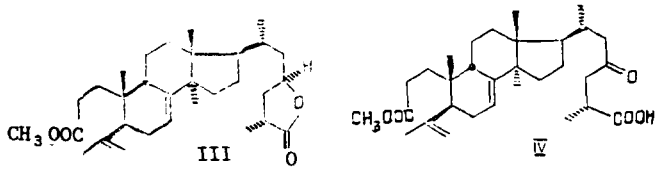


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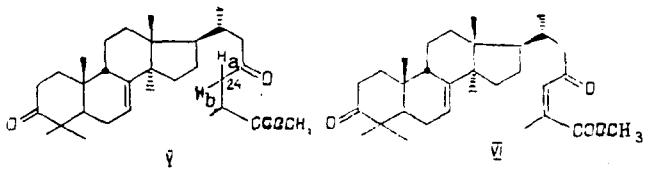
TABLE 1. Chemical Shifts (ppm) and Spin-Spin Coupling Constants (Hz, shown in parentheses) of Some Signals in the PMR Spectrum of Compounds (I) and (V)[a] (400 MHz)

Compound	Me-20	Me-25	H-24	H-24	H-25
I[b]	0,92 d (6)	1,04 d (7)	2,03 dd (17,5; 5)	2,67dd (17,5; 8,5)	3,00 ddq (8,5; 5; 7)
(25R)-V[b]	0,96 d (6)	1,050 d (7)	2,03 dd (17,5; 5)	2,67dd (17,5; 8,5)	3,30 m
(25S)-V[b]	0,96 d (6)	1,045 d (7)	1,86 dd (17,5; 5)	2,74 dd (17,5; 8,5)	3,30 m
I[c]	0,84 d (6)	1,15 d (7)	2,45 dd (17,5; 5,5)	2,81 dd (17,5; 8)	2,92 ddq (8; 5,5; 7)
(25R)-V[c]	0,847 d (6)	1,16 d (7)	— d	2,81 dd (17,5; 8)	2,92 m
(25S)-V[c]	0,843 d (6)	1,16 d (7)	— d	2,87 dd (17,5; 8)	2,92 m

[a]Mixture of the 25R- and 25S- epimers present in a ratio of 2.4:1.
[b]Solution in C_6D_6.
[c]Solutionin $CDCl_3$; [d]signal masked by other multiplets.

The PMR spctrum of the ester (I) that had been synthesized coincided with that for the natural compound (the spectra were recorded for solutions in deuterochloroform at 200 MHz). Here, however, the question arose as to whether the natural acid was an individual compound or a quasi-racemic mixture of epimers at C-25, since the hypothesis was quite probable that the epimers of the ester (I) at C-25 had identical PMR spectra. To answer this question, we used a model compound – the crystalline dihydroester (V) obtained previously by the catalytic hydrogenation of the ester (VI) [9]. It proved to be a mixture of epimers at C-25, as was established by PMR spectroscopy recorded for a solution in C_6D_6 (200 or 400 MHz). In this spectrum, the signals of the protons at C-24 were observed for each epimer separately (Table 1), and the ratio of the epimers was 2.4:1 (determined from the relative integral intensities of the signals for H-24a). The assignment of the H-25, H-24a and H-24b signals was made on the basis of double-resonance experiments (on the suppression of the H-25 signal, the Me-25 doublet was converted into a singlet, and the H-24a and H-24b signals into doublets with the geminal constant). The fact that the signals observed were due just to epimers at C-25 of the general formula (V) was confirmed by the formation of the same components, but in a ratio of 1:2, on the catalytic hydrogenation of the 24E- isomer of the ester (VI) (methyl firmanoate [10, 11]).



V VI

The PMR spectrum of the methyl ester (I) obtained from the oleoresin under investigation unambiguously showed that the compound concerned was individual, since this spectrum revealed only one signal for H-24a, coinciding in form and position with that for the main component of the mixture of epimers of the general formula (V) obtained by the hydrogenation of the ester (VI). In the light of the results of a chemical correlation with the lactone (III) it was possible to conclude that the methyl ester (I) was diastereomerically pure and had the R-configuration of the asymmetric center at C-25. When the PMR spectrum of a solution of the ester (I) in C_6D_6 was recorded, its 25S-epimer was detected as an impurity (10%) (two signals were observed for H-24 coinciding in form and position with that for the ester (V)). This impurity through partial epimerization at C-25 taking place on the alkaline hydrolysis of methyl abiesolidate [8].

Thus, the PMR spectra for solutions in C_6D_6 (200 MHz and more) permit the reliable determination of the diastereoisomeric purity of compounds (I) and (V) and, as may be assumed, of other compounds with the same side chain. The spectra for solutions in $CDCl_3$ are sufficiently informative if they are recorded in an instrument with a working frequency of the order of 400 MHz. Then, for the epimer with the 25R-configuration the signal due to the H-24 proton is likewise present in a higher field (by 0.07 ppm) than in the spectrum of the 25S- epimer (see Table 1).

The methyl ester (II) of the second new acid isolated from the oleoresin under investigation had the empirical formula $C_{32}H_{48}O_5$ (high-resolution mass spectrometry). A comparison of its PMR spectrum (see the Experimental part) with the spectra of dimethyl abiesonate (VII) [7] and the dihydro ester (I) permitted the assumption that it was the 24,25-dihydro derivative of dimethyl abiesonate. In actual fact, the catalytic hydrogenation of compound (VII) and its 24-cis-isomer (VIII) [7] over palladium in ethyl acetate yielded specimens of 24,25-dihydro derivatives coinciding on TLC with one another and with the methyl ester of the natural acid. However, the PMR spectrum (400 MHz, $CDCl_3$) showed that the synthetic dihydro derivatives were mixtures of epimers at C-25, their ratio for (VII) being 3:1 and for (VIII) 1:2. They were distinguished by the chemical shifts of the Me-17, Me-25, and H-24a signals, the difference being most considerable for H-24a (0.07 ppm).

The methyl ester of the natural acid was an individual compound and the signals in its PMR spectrum coincided with those for the main epimer formed on the hydrogenation of dimethyl abiesonate (VII) having an upfield shift of the signal for the H-24a atom (2.75 ppm, while for the minor epimer it was 2.82 ppm). In this respect, the situation was completely analogous to that observed for the ester (I) and the epimers of the general formula (V), and in view of the similar structures and stereochemistries at C-20 of the side chains of the molecules under consideration it was possible to conclude that the methyl ester of the natural acid from the oleoresin under investigation was dimethyl 24,25-dihydroabiesonate with the 25R-configuration of the epimeric center at C-25 (formula (II)) while its epimer had the structure (IX).

The epimers of compounds (I) and (II) at C-25 were absent from the mixture of methyl esters of the acids from the oleoresin under investigation. It may therefore be assumed that the stage of hydrogenation in the process of biosynthesis of the corresponding acids is completely stereoselective. On the other hand, the possibility of the existence of the free hydroxy acid (IV) permitted it to be considered as a general biogenetic precursor of abiesolidic acid [8] and of the dicarboxylic keto acid corresponding to the dimethyl ester (I).

EXPERIMENTAL

The PMR spectra were recorded on Bruker AC-200 (200 MHz) and Bruker AM-400 (400 MHz) instruments for solutions in deuterochloroform and hexadeuterobenzene, the signals of which were taken as 7.240 and 7.150 ppm, δ-scale. High-resolution mass spectra were obtained on a Finnigan MAT 8200 instrument, and IR spectra (for solutions in CCl_4) on a UR-20 spectrometer. Angles of optical rotation were measured for solutions in chloroform on a Polamat A polarimeter. Melting points were determined on a Kofler stage.

For chromatography we used air-dry silica gel of type KSK with grain sizes of 0.05-0.14 mm, the eluent being mixtures of petroleum ether and diethyl ether containing increasing concentrations, from 10-30%, of the latter. DE is an arbitrary abbreviation for diethyl ether. The oleoresin of the silver fir was gathered in August, 1985, in the Ust'-Chorna Reserve, Transcarpathia province.

Isolation of the Individual Compounds. A solution of 10.62 g of the oleoresin in 200 ml of DE was shaken with 100 ml of a saturated aqueous solution of sodium bicarbonate. The aqueous layer was separated off, washed with DE, acidified with hydrochloric acid to pH 2, and extracted with DE. The ethereal extract was washed with aqueous sodium chloride and was dried with sodium sulfate. After the elimination of the DE, 0.39 g (3.7%) of fatty acids was obtained which wre methylated by the addition of an excess of an ethereal solution of diazomethane followed by the slow removal of the excess of diazomethane under vacuum. A portion of the resulting mixture of methyl esters (0.242 g) was chromatographed. This gave successively 0.009 g of methyl abiesonate (VII) (identified from its PMR spectrum and by TLC), 0.004 g of an unidentified methyl ester, 0.035 g of the ester (II), 0.060 g of the ester (I), and 0.13 g of a mixture of unidentified compounds.

Dimethyl Ester of (25R)-3,4-seco-9β-lanosta-4(28),7-dien-23-one-3,26-dioic acid (I). Crystals with mp 73-76°C (from pentane), $[\alpha]_{5\,94}^{22}$ −27° (c 0.15). IR spectrum, cm^{-1}: 915, 1640, 3080 (C=CH_2); 1250, 1740 ($COOCH_3$); 1730 cm^{-1} (C=O). Mass spectrum (m/z): 514.3613 (100%; calculated from $C_{32}H_{50}O_5$ − 514.3658), 427 (30%, $(M - CH_2CH_2COOCH_3)^+$). In the PMR spectrum (for a solution in $CDCl_3$), in addition to the signals shown in Table 1, singlets were observed at (ppm) 0.75, 0.81, and 1.00 (3 H, angular methyl groups), and signals at 1.77 (3 H, broadened singlet, Me-4); 3.64 and 3.65 (3 H each, s, s 2 COOCH.); 4.79 and 4.85 (1 H each, narrow multiplet, C=C$\underline{H}_2$); and 5.27 (1 H, m, H-7).

For a solution in C_6D_6, in addition to signals shown in Table 1, the PMR spectrum contained signals at (ppm) 0.74, 0.75, and 1.01 (3 H each, singlets, protons of angular Me groups); 1.74 (3 H, broadened singlet, Me-4); 2.55 (1 H, m, unidentified proton); 3.36 and 3.38 (3 H, each, s, s, $2COOCH_3$); 4.97 (2 H, narrow m, C=C$\underline{H}_2$); and 5.27 (1 H, m, H-7).

Synthesis of the Ester (I) from Methyl Abiesolidate. A solution of 0.24 g of compound (III) in 50 ml of ethanol was treated with 100 ml of a 5% ethanolic solution of KOH and the mixture was heated at 60-70°C for 2 h and was then evaporated under vacuum to a volume of 50 ml. This reaction mixture was cooled to room temperature, diluted with water (50 ml), acidified with hydrochloric acid to pH 4, and extracted with DE (3 × 30 ml). The extract was

washed with water to neutrality, and an ethereal solution of diazomethane was added to it until the mixture had acquired a permanent yellow coloration, and then the ether and the excess of diazomethane were driven off under vacuum. This gave 0.15 g of the dimethyl ester of the acid (IV) with mp 63-70°C. IR spectrum: 3620 cm^{-1} (OH). Mass spectrum (m/z): 516.3793 (M^+, 10%, calculated for $C_{32}H_{52}O_5$: 516.3815), 484 (100%), 397 (50%).

A solution in 10 ml of CH_2Cl_2 of 0.15 g of the compound obtained was added to a stirred suspension of 0.1 g of pyridinium chlorochromate and 0.1 g of sodium acetate in 10 ml of CH_2Cl_2, and stirring was continued at room temperature for 2.5 h. After the usual working up, chromatography, and crystallization from a mixture of DE and petroleum ether, 0.01 g of the ester (I) was obtained with mp 73-75°C, its IR and PMR ($CDCl_3$, 200 MHz) spectra coinciding with those for the ester (I) obtained from the oleoresin. The PMR spectrum recorded for a solution in C_6D_6 showed at 2.74 ppm the signal of the 25S-epimer of (I) (H-24a). The ratio of the integral intensities of the signals at 2.74 and 2.67 ppm was 1:9.

Catalytic Hydrogenation of Methyl Firmanoate. The hydrogenation of methyl firmanoate and the isolation of the product were carried out by the procedure described in [9] for the ester (VI). The product obtained was identical with the ester (V) according to TLC, while its PMR spectrum (solution in C_6D_6) showed that it was a mixture of the epimers 25S-(V) and 25R-(V) present in a ratio of 2:1.

Dimethyl Ester of (25R)-3,4-secoabiesa-4(28),7,14(30)-trien-23-one-3,26-dioic acid (II). Viscous oil with $[\alpha]_{594}^{20}$ −5.6° (c 3.4). IR spectrum (cm^{-1}): 890, 910, 1650, 3090 (C=CH_2); 1720 (shoulder, C=O); 1270, 1740 ($COOCH_3$). Mass spectrum (m/z): 512.3499 (M^+ 100%; calculated for $C_{32}H_{48}O_5$: 512.3502); 425 (40%). PMR spectrum ($CDCl_3$, 400 MHz), ppm: 0.78 (3H, d, J = 6 Hz Me-20), 0.86 (3H, s, Me-10), 0.89 (3H, s, Me-17), 1.138 (3H, d, J = 7 Hz, Me-25), 1.75 (3H, br.s, Me-4), 2.30 (1H, dd, J = 17.5 and 5.5 Hz, H-24b), 2.75 (1H, dd, J = 17.5 and 8.0 Hz H-24a), 2.92 (1H, m, H-25), 3.64 and 3.65 (3 H each, s, s, $2COOCH_3$), 4.69, 4.72, 4.75, and 4.80 (1H each, narrow multiplets, $2C{=}C\underline{H}_2$); 5.43 (1H, m, H-7).

Catalytic Hydrogenation of the Methyl Esters (VII) and (VIII). The hydrogenation of these esters and the isolation of their 24,25-dihydro derivatives (mixtures of epimers at C-25) were carried out as described in [9] for the ester (VI). The signals in the PMR spectra ($CDCl_3$, 400 MHz) of the products that were assigned to the 25S-epimer (IX) were: 0.92 (3 H, s, Me-17); 1.146 (3 H, d, J = 7 Hz, Me-20); 2.82 (1 H, dd, J = 18.0 and 7.5 Hz, H-24a); 4.82 (1 H, narrow m, proton of one of the C=CH_2 groups). The signal of a H-24b proton was not recorded, while the other signals coincided with those for the ester (II), including signals at 4.69, 4.72, and 4.75 ppm.

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