TRITERPENOIDS FROM THE LEAVES OF *Betula lanata*

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From the unsaponifiable fraction of an ethereal extract of the leaves of *Betula lanata*, in addition to 3-epiocotillol (I) we have isolated a new triterpene, 20(S),24(R)-epoxydammarane-3α,11α,25-triol (V) and also derivatives of it — the monoacetates at C-3 (II) and C-11 (III), and the monoketone at C-3 (IV). The structures of compounds (I-V) have been established on the basis of the results of physicochemical investigations.

Continuing a study of the unsaponifiable fraction of ethereal extracts of the leaves of Far Eastern species of birch, from *Betula lanata* we have isolated five triterpenoids of the dammarane series (I-V in order of increasing polarity).

The IR spectrum of triterpene (I) shows two bands of the stretching vibrations of hydroxy groups at 3567 and 3621 cm^{-1}. Its PMR spectrum contains the signals of the protons of eight tertiary methyl groups in the 0.84-1.21 ppm region and, in the weak field, the signal of a carbinyl proton attached to C-3 at 3.32 ppm (1 H, triplet, $J < 4$ Hz) and a signal at 3.66 ppm (1 H, triplet) corresponding to a proton at C-24. The mass spectrum of compound (I) contains fragments with m/e 143 (100%), 125, and 50, which are characteristic for a side chain in the form of a substituted tetrahydrofuran ring [1].

In their physicochemical properties and spectral characteristics, triterpene (I) corresponds to the 3-epiocotillol isolated previously from the pollen of *Betula platyphylla* var. *japonica* [2].

The oxidation of compound (I) gave ocotillone (VII) the reduction and subsequent acetylation of which led to ocotillol (VIII) and 3-OAc-ocotillol (IX), respectively. The spectra characteristics and physicochemical properties of ocotillone and its derivatives (VII-IX) corresponded to those given in the literature [2, 3].

Triterpene (V) was found in the highest concentration (11%) in the unsaponifiable fraction of an ethereal extract of the leaves. The IR spectrum of (V) contained three bands of the stretching vibrations of hydroxy groups at 3450, 3570, and 3600 cm^{-1}.

The fragmentation in the mass spectrum of triterpene (V) was analogous to that in the mass spectrum of the triterpene (XII), 20(S),24(R)-epoxydammarane-3α,11α,25-triol, which we had isolated previously from the leaves of *Betula ermanii* [4, 5]. This permitted the assumption that triterpene (V) was an epimer of this compound (XII). The assumption was confirmed by the PMR spectrum of triterpene (V). In the 0.87-1.20 ppm region the signals of the protons of eight tertiary methyl groups were observed, and in the weak field the signals of a proton attached to C-24 at 3.73 ppm (1 H, triplet) at the signal of a proton attached to C-11 to 3.94 ppm (1 H, multiplet, $\Sigma J \approx 26$ Hz) which coincides with the corresponding signals in the PMR spectrum of triterpene (XII). Only the magnitudes of the chemical shift and of the spin–spin coupling constant of the C-3 proton — 3.36 ppm (1 H, triplet, $J = 3$ Hz) — indicate that the proton is equatorial and, consequently, that there is an axial α-OH group on this carbon atom, i.e., triterpene (V) is the epimer of triterpene (XII) at C-3.

When compound (V) was oxidized with chromium trioxide in pyridine, a diketone (X) was obtained which gave no depression of the melting point in admixture with the diketone obtained previously by the oxidation of triterpene (XII) [4]. On the basis of these facts, for triterpene (V) we suggest the structure of 20(S),24)R)-epoxydammarane-3α,11α,25-triol.

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TABLE 1. Chemical Shifts in the PMR Spectra of Triterpenes (II) and (III) (δ, ppm, relative to TMS)

Compound	Protons of tertiary Me groups	Protons of acetyl Me groups	C_3-H	C_{11}-H	C_{24}-H
Triterpene (II)	0,85 (3H, s), 0,95(6H, s) 0,97 (3H, s), 1,00 (3H. s) 1,12 (6H, s) 1,21 (3H, s)	1,96 (3H, s)	3,36 (1 H, triplet, J=3Hz)	5,12 (1 H, multiplet, $\Sigma J \cong 26$ Hz)	3,71 (1 H, triplet)
Triterpene (III)	0,85 (3H, s), 0,91 (3H, s) 0,96 (6H, s), 1,06 (3H, s), 1,13 (6H, s) 1,21 (3H, s)	2,08 (3H, s)	4,59 (1 H, triplet, J=3 Hz)	3,91 (1 H, multiplet, $\Sigma J \cong 22$ Hz)	3,74 (1 H, triplet)

In the IR spectra of triterpenes (II) and (III), in addition to the stretching vibrations of hydroxy groups at 3560 and 3618 cm^{-1} and at 3425, 3558, and 3591 cm^{-1}, there are the bands of stretching vibrations of ester carbonyls at 1715 and 1713 cm^{-} , respectively. When the triterpenes (II) and (III) were saponified with ethanolic caustic soda, in both cases the triterpene (V) giving no depression of the melting point in admixture with the (V) isolated directly from the leaves of *B. lanata* was obtained. A comparison of the PMR spectra of triterpenes (II) and (III) (Table 1) showed that compound (II) is the monoacetate of triterpene (V) at C-11 and (III) is the monoacetate at C-3.

Fischer and Seiler [6] have shown that in birch leaves the triterpene alcohols are present not in the free state but in the form of esters. To isolate the triperpenoids in the free state an ethereal extract of the leaves was saponified with alcoholic caustic soda. The appearance of monoacetates (triterpenes (II) and (III)) in the unsaponifiable part of the ethereal extract of the leaves of *B. lanata* is apparently due to the fact that the esters, namely, the acetates of triterpene (V), were not completely saponified under Fischer and Seiler's conditions. To confirm this hypothesis we obtained the diacetete (XI) which was then saponified under Fischer and Seiler's conditions. The reaction yielded a mixture of the monoacetates (II) and (III) (14%) and of the free alcohol (V) (86%). The quantitative ratio between the sum of the monoacetates (II) and (III) and the alcohols (V) obtained after saponification were the same as the ratio between these substances in the unsaponifiable part of the ethereal extract of the leaves. It is obvious that in spite of the fact that the acetate group at C-11 is equatorial but sterically hindered and the acetate group at C-3 is axial their rates of saponification are comparable.

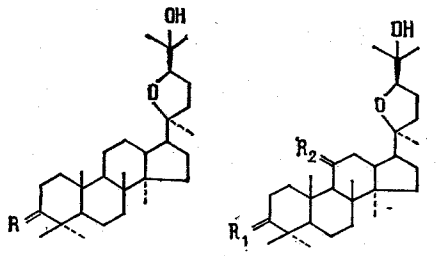
The IR spectrum of triterpene (IV) shows three bands of stretching vibrations of hydroxy groups at 3443, 3568, and 3599 cm^{-1}, and also the band of the stretching vibrations of a carbonyl group in a six-membered ring at 1698 cm^{-1}.

The presence of fragments with m/e 143 (100%), 125, and 59 in the mass spectrum of triterpene (IV) shows that its side chain is of the same type as in triterpenes (I) and (V).

The PMR spectrum of compound (IV) contains the signals of the protons of eighty tertiary methyl groups in the 0.92-1.21 ppm region, and in the weak field region the signal of a C-24 proton at 3.70 ppm (1 H, triplet) and the signal of a C-11 proton at 3.94 ppm (1 H, multiplet, $\Sigma J > 20$ Hz). The absence from the PMR spectrum of triterpene (IV) of a signal characteristic of a carbinyl proton at C-3 permits the assumption that the keto group is present at C-3, i.e., triterpene (IV) is the monoketone derivative of triterpene (V) at C-3. The oxidation of compound (IV) with chromium trioxide in pyridine gave the diketone (X) which was also obtained by the oxidation of triterpene (V) under the same conditions. Thus, for triperpene (IV) we propose the structure of 11α,25-dihydroxy-20(S),24(R)-epoxydammaran-3-one.

EXPERIMENTAL

IR spectra were recorded on a Specord 751 R spectrophotometer in $CHCl_3$ solution, and mass spectra on a LKB 9000 spectrometer at an ionizing voltage of 70 eV. PMR spectra were obtained on a Bruker HX-90 E instrument with TMS as internal standard. Chemical shifts are expressed on the δ scale.



I. R = OH, H

II. R_1 = OH, H ; R_2 = OAc, H

VI. R = OAc, H

III. R_1 = OAc, H ; R_2 = OH, H

VII. R = O

IV. R_1 = O ; R_2 = OH, H

VIII. R = OH, H

V. R_1 = OH, H ; R_2 = OH, H

IX. R = OAc, H

X. R_1 = O ; R_2 = 0

XI. R_1 = OAc, H ; R_2 = OAc, H

XII. R_1 = OH, H ; R_2 = OH, H

For chromatography we used type KSK silica gel. The individuality of the substances was checked by thin-layer chromatography on silica gel in the following systems: 1) benzene–ethanol (10:1); 2) hexane–acetone (2:1); and 3) chloroform–ethanol (10:1). To detect the triterpene on the chromatograms we used a 10% solution of H_2SO_4 in methanol.

The elementary analyses of all the compounds corresponded to the calculated figures.

Isolation of the Triterpenes (I-V). The air-dry leaves of *B. lanata* collected on July 6, 1978 in the environs of Vladivostok (10 kg) were treated by Fischer and Seiler's method [6]. This gave 70 g of unsaponifiable fraction of the ethereal extract, which was chromatographed on silica gel with elution by hexane–acetone solvent systems. The hexane–acetone (45:1) system yielded triperpene (I), (35:1) gave (II), (30:1) gave (III), (16:1) gave (IV), and (8:1) gave (V).

Triterpene (I), 3.6 g, $C_{30}H_{52}O_3$, mp 160-162°C (acetone), $[\alpha]_D^{20}$ + 18.5° (c 1.0; chloroform). Mass spectrum, m/e: 445 (M^+-CH_3), 401 ($M^+-C_3H_7O$), 383 ($M^+-C_3H_7O-H_2O$), 143 (100%), 125, 59 (C_3H_7O).

Triterpene (II), 1.2 g, $C_{32}H_{24}O_5$, mp 176-178°C (hexane–acetone); $[\alpha]_D^{20}$ –5.3° (c 1.0 chloroform). Mass spectrum, m/e: 503 (M^+-CH_3), 500 (M^+-H_2O), 485 ($M^+-CH_3-H_2O$), 459 ($M^+-CH_3H_6O$), 143 (100%), 125, 59 (C_3H_7O).

Triterpene (III), 0.1 g, $C_{32}H_{54}O_5$, mp 198-200°C (hexane–acetone), $[\alpha]_D^{20}$ –6.2°, (c 0.5; chloroform). Mass spectrum, m/e: M^+ 518, 503 (M^+-CH_3), 500 (M^+-H_2O) 485 ($M^+-CH_3-H_2O$), 482 (M^+-2H_2O), 143 (100%), 125, 59 (C_3H_7O).

Triterpene (IV), 1.3 g, $C_{30}H_{40}O_4$, mp 90-97°C (acetone), $[\alpha]_D^{20}$ +75.4° (c 1.0; chloroform). Mass spectrum, m/e: M^+ 474, 459 (M^+-CH_3), 456 (M^+-H_2O), 441 ($M^+-CH_3-H_2O$), 438 (M^+-2H_2O), 143 (100%), 125, 59 (C_3H_7O).

Triterpene (V), 8 g, $C_{30}H_{52}O_4$, mp 159-160°C (hexane), $[\alpha]_D^{20}$ +15.6° (c 1.0; chloroform). Mass spectrum, m/e: 461 ($M^+ - CH_3$), 443 ($M^+ - CH_3 - H_3O$), 440 ($M^+ - 2H_2O$), 143 (100%), 125, 59 (C_3H_7O).

Acetylation of (I). A solution of 418 mg of (I) in 6 ml of pyridine was treated with 3 ml of acetic anhydride. The mixture was left overnight at 20°C. After the usual working up, 380 mg of compound (VI) was obtained with mp 136-137°C (hexane), $[\alpha]_D^{20}$ −1.5° (c 1.0; chloroform). IR psectrum, cm^{-1}: 3564, 3305 (O–H), 1711 (–$OCOCH_3$). PMR spectrum, ppm: 0.83 (3 H, s) 0.86 (3 H, s), 0.88 (3 H, s), 0.91 (3 H, s), 0.96 (e H, s), 1.12 (3 H, s), 1.13 (3 H, s), 1.20 (3 H, s) – the protons of tert-Me groups, 2.08 (3 H, s) – the protons of an acetate Me group, 4.62 ppm (1 H, triplet, J = 3 Hz, C -H), 3.72 (1 H, triplet, C_{24}-H). Mass spectrum, m/e: M^+ 502, 487 ($M^+ - CH_3$), 442 (M^+ –AcOH), 143 (100%), 125, 59.

Oxidation of (I). A solution of 739 mg of (I) on 10 ml of pyridine was added to 1.31 g of CrO_3 in 15 ml of pyridine. Oxidation was carried out at 20°C for one day. After the usual working up, 650 mg of ocotillone (VII) was obtained with mp 160-163°C (methanol), $[\alpha]_D^{20}$ +62.2° (c 1.0; chloroform). IR spectrum, cm^{-1}: 3570 (O–H), 1697 (C=O). PMR spectrum, ppm: 0.88 (3 H, s), 0.94 (3 H, s), 0.99 (3 H, s), 1.04 (3 H, s), 1.08 (3 H, s), 1.13 (3 H, s), 1.21 (3 H, s), 1.44 (3 H, s), the protons of tert-Me groups, 3.74 (1 H, triplet, C_{24}-H). Mass spectrum, m/e: 445 ($M^+ - CH_3$), 440 ($M^+ - H_2O$), 425 ($M^+ - H_2O - CH_3$), 399 ($M^+ - C_3H_7O$), 143 (100%), 125, 59 (C_3H_7O).

Reduction of (VII). A solution 183 mg of (VII) in 8 ml of isopropanol was added to 350 mg of $NaBH_4$ in 8 ml of isopropanol. The mixture was kept overnight at 20°C. The reaction yielded 170 mg of ocotillol (VIII) with mp 196-198°C (acetone), $[\alpha]_D^{20}$ +35.2° (c 0.5; chloroform). IR spectrum, cm^{-1}: 3556, 3605 (O–H). PMR spectrum, ppm: 0.77 (3 H, s), 0.83 (3 H, s), 0.87 (3 H, s), 0.95 (3 H, s), 0.97 (3 H, s), 1.12 (6 H, s), 1.21 (3 H, s), the protons of tert-Me groups, 3.73 (1 H, triplet, C_{24}-H), 3.13 (1 H, quartet, $J_{a,a}$ = 10 Hz, $J_{a,e}$ = 6 Hz, C_3-H). Mass spectrum, m/e: 445 ($M^+ - CH_3$), 401 ($M^+ - C_3H_7O$), 383 ($M^+ - C_3H_7O - H_2O$), 143 (100%), 125, 59 (C_3H_7O).

Acetylation of (VIII). A solution of 112 mg of (VIII) in 1.5 ml of pyridine was treated with 0.8 ml of acetic anhydride. The mixture was left overnight at 20°C. This gave 98 mg of (IX) with mp 256-258°C (hexane–acetone), $[\alpha]_D^{14}$ +43.6° (c 0.5; chloroform). IR spectrum, cm^{-1}: 3556 (O–H), 1718 (–$OCOCH_3$). PMR spectrum, ppm: 0.87 (9 H, s), 0.95 (3 H, s), 1.13 (3 H, s), 1.21 (3 H, s), 1.26 (6H, s), the protons of tert-Me groups, 2.04 ppm (3 H, s), the protons of an acetate Me group, 3.73 (1 H, triplet, C_{24}-H), 4.51 (1 H, quartet, $J_{a,a}$ = 5.8 Hz, C_3-H). Mass spectrum, m/e: 487 ($M^+ - CH_3$), 484 ($M^+ - H_2O$), 443 (M^+ –AcOH), 427, 383 (M^+ –AcOH–H_2O), 143 (100%), 125, 59 (C_3H_7O).

Saponification of (II). A mixture of 100 mg of (II) and 14 ml of 1 N ethanolic KOH solution was heated under reflux for 2 h. After the usual working up, 93 mg of (V) was obtained with mp 158-160°C (hexane), which gave no depression of the melting point in a mixture with the (V) obtained directly from the leaves of *B. lanata*.

Saponification of (III). A mixture of 40 mg of (III) and 6 ml of 1 N ethanolic KOH solution was heated under reflux for 2 h. This gave 36 mg of (V) with mp 158-160°C (hexane) showing no depression of the melting point in admixture with the (V) isolated from the leaves of *B. lanata*.

Oxidation of (IV). A solution of 70 mg of (IV) in 1 ml of pyridine was added to 125 mg of CrO_3 in 1.5 ml of pyridine. Oxidation was carried out at 20°C for one day. The reaction yielded 60 mg of (X) with mp 173-175°C (hexane) which gave no depression of the melting point in admixture with an authentic sample of (X).

Oxidation of (V). A solution of 70 mg of (V) in 1 ml of pyridine was added to 125 mg of CrO_3 in 1.5 ml of pyridine. Oxidation was carried out at 20°C for 1 day. This gave 65 mg of (X) which showed no depression of the melting point in admixture with an authentic sample of (X).

Acetylation of (V). A solution of 250 mg of (V) in 4 ml of pyridine was treated with 2 ml of acetic anhydride. The mixture was left overnight at 20°C. After the usual working up, the residue was chromatographed on silica gel in the hexane–acetone (40:1) system. This gave 29 mg of (II) with mp 175-177°C (hexane–acetone), and 200 mg of a homogeneous noncrystalline substance (IX) with $[\alpha]_D^{20}$ −6.6°C (c 0.5; chloroform). IR spectrum, cm^{-1}: 3548 (O–H), 1714 (–$OCOCH_3$). PMR spectrum, ppm: 0.86 (3 H, s), 0.90 (3 H, s), 0.99 (6H, s), 1.02 (3 H, s),

1.12 (6 H, s), 1.21 (3 H, s), the protons of tert-Me groups, 2.09 (3 H, s), 1.96 (3 H, s), the protons of acetate Me groups, 3.72 (1 H, triplet, C_{24}-H), 4.59 (1 H, triplet, J < 4 Hz, C_3-H), 5.16 (1 H, multiplet, $\Sigma J \sim 20$ Hz, C_{11}-H). Mass spectrum, m/e: 542 ($M^+ - H_2O$), 545 ($M^+ - CH_3$), 501 ($M^+ - 59$), 500 ($M^+ - AcOH$), 143 (100%), 125, 59.

Saponification of the Diacetate (XI). A mixture of 150 mg of (XI) and 5 ml of 0.9 N methanolic KOH was heated under reflux for 2 h. After the usual working up, the residue was chromatographed on silica gel in the hexane–acetone (10:1) system. The reaction yielded 15 mg of a mixture of (II) and (III), and also 95 mg of the triterpene (V).

SUMMARY

1. From the unsaponifiable fraction of an ethereal extract of the leaves of *Betula lanata*, in addition to 3-epiocotillol (I) we have isolated a new triterpene of the dammarane series 29(S),24(R)-epoxydammarane-3α,11α,25-triol (V), and also derivatives of it: 11α-acetoxy-20(S),24(R)-epoxydammarane-3α,25-diol (II), 2α-acetoxy-20(S),24(R)-epoxydammarane-11α, 25-diol (III), and 11α,25-dihydroxy-20(S),24(R)-epoxydammaran-3-one (IV).

2. It has been established that the triterpenoids are present in the leaves of *B. lanata* in the form of acetates.

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CHARACTERISTICS OF THE MASS SPECTRA OF ECDYSTEROIDS WITH DIFFERENT NUMBERS OF –OR GROUPS

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The mass spectra of 15 phytoecdysteroids and acetyl derivatives have been compared. With a decrease in the number of –OR groups, the contribution of cleavages of the bonds of the steroid skeleton increases. 20,22-Diols are characterized by the greatest significance of fragmentation at the C-20–C-22 bond. In all the spectra, clear indications of fragmentation of the side chain at the C-22–C-23, C-23–C-24, and C-24–C-25 bonds are observed.

The detection in the plant *Silene praemixta* M. Pop. of several phytoecdysones belonging to the subgroup of 2-deoxy-α-ecdysone [1, 2] has substantially supplemented the general pattern of distribution of compounds of this type in the plants of Central Asia. To ecdysteroids found previously the molecules of which contain seven or eight hydroxy groups (integristerones A and B [3, 4]) compounds with three or four hydroxy functions have been added. The necessity for an all-sided study of the ecdysteroids of the latter group follows, for example, from the work E. Ohnishi et al. [5], who have shown that 2-deoxy-α-ecdysone is not only one of the main metabolites of α-ecdysone but also plays an independent role in the metamorphosis of insects.

The analysis of mass-spectrometric characteristics is important for establishing the structures of the ecdysones [6]. The basic laws of fragmentation of these compounds have

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