2. A change in the absolute configuration at C_{11} in saturated C_6-trans lactones — balchanolide and mucronin — affects only the intensity of the Cotton effect at 220 nm without causing an inversion of its sign.

3. Geissman's rule is not observed in C_8-trans germacranolides with 6α-hydroxy groups.

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TRITERPENOIDS OF THE LEAVES OF Betula pendula FROM DIFFERENT GROWTH SITES

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A comparative study has been made of the triterpene fraction of the unsaponifiable part of ethereal extracts of five samples of the leaves of Betula pendula collected in various regions of the Soviet Union. Together with known sterols and triterpenoids, two new compounds have been isolated — 12β,20(S),25-trihydroxydammar-23-en-3-one and dammar-25-ene-3α,12β,17α,20(S),24ξ-pentaol, the structures of which have been determined on the basis of spectral characteristics and chemical transformations.

The leaves of Betula pendula Roth. were first studied for their triterpenoid content by the German chemists Fischer and Seiler. They detected two previously unknown triterpene alcohols of the dammarane series — betulafolienetriol (dammar-24-ene-3α,12β,20(S)-triol) (I) and betulafolienetetraol (dammar-24-ene-3α,12β,17α,20(S)-tetraol) (II), the total amount of which was 0.8% of the weight of the crude birch leaves. The main component was the betulafolienetriol, the amount of which was seven times that of the betulafolienetetraol [1]. Triterpene alcohols of this type are particularly interesting because of their structural similarity to the aglycons of the panaxosides — the glycosides of ginseng. It must be mentioned that betulafolienetriol differs from the protopanaxadiol [2] only by the orientation of the hydroxy group at C-3. From these two alcohols (I) and (II) synthetic analogs of the pamaxosides have been obtained which have exhibited physiological activity equal to the activity of the native panaxosides [3].

B. pendula grows over the major part of the territory of the USSR. However, the leaves of B. pendula collected in the boundary area of this birch, in Eastern Siberia that we inves-

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TABLE 1. ^{13}C Chemical Shifts of Compounds (XIII) and (XIV) (ppm relative to TMA, $CDCl_3$)

C atom	Compound		C atom	Compound	
	XIII	XIV		XIII	XIV
1	39,8	33,8	18	15,9	16,1
2	34,1	25,3	19	15,5	15.9
3	217,4	76,2	20	73.8	77,0
4	47,4	37,2	21	27,3	20,7
5	55,4	49,5	22	38,0	32,6 (31,5)*
6	19,6	18,3	23	122,3	30,0 (29,0)
7	34,1	34,2	24	142,1	76,8 (75,3)
8	39,8	40.6	25	70,5	147,8 (147.5)
9	49.5	50.0	26	30,0	110,7
10	36,8	37,3	27	29,0	17,9 (18,3)
11	31,5	32,0	28	26,6	28.3
12	70.8	67,1	29	21,1	22,1
13	48,4	50,3	30	16,8	17.4
14	51,6	51,1			
15	30,9	32,0			
16	26,3	36,9			
17	52,7	85,7			

*CS for the 24(R)-epimers.

tigated contained no triterpenoids (I) and (II). Three triterpene alcohols were detected in the sample mentioned (1): dammar-24-ene-3α,17α,20(S)-triol (III), 20(S),24(R)-epoxy-dammarane-3α,17α,25-triol (IV), and 20(S),24(S)-dihydroxydammar-25-en-3-one (V) [4]. It is obvious that, within a large area having different climatic conditions, B. pendula does not remain homogeneous in chemical composition.

In a search for the richest source of betulafolienetriol and betulafolienetetraol, we have made a comparative study of the triterpene fraction of the unsaponifiable part of the ethereal extracts of another five samples (2-6) of B. pendula leaves collected in different regions of the Soviet Union (Western Siberia, Kazakhstan, and the Sverdlovsk and Leningrad provinces). In so doing, we isolated and identified two sterols – β-sitosterol (VI) and citrostadienol (VII) – and 11 triterpenoids of the dammarane series – (I), (II), (III), (IV), betulafolienetriol oxide (VIII) [5], 12β,20(S)-dihydroxy-dammar-24-en-3-one (IX) [5], dammar-25-ene-3α,12β,20(S)-tetraol (XII) and the new triterpenoids 24(R)-tetraol (XI) [7], and dammar-23-ene-3α,12β(S),25-tetraol (XII) and the new triterpenoids (XIII) and (XIV).

The citrostadienol, found for the first time in birch leaves, was identified by comparing its physicochemical properties and spectral characteristics with those given in the literature [8].

In the IR spectrum of the triterpene (XIII), $C_{30}H_{50}O_4$, a band of the stretching vibrations of a carbonyl group in a six-membered ring at 1698 cm^{-1} and bands of the stretching vibrations of a hydroxy group at 3611 and 3361 cm^{-1} were observed.

The mass spectrum of the triterpene (XIII), which had the characteristic peaks of ions with m/z 456 ($M^+ - H_2O$), 438, 420, 375, 357, 339, 313, 143, 125, and 107, corresponded to the spectra of dammarane triterpenes with an open side chain [7, 9].

In the PMR spectrum of (XIII) in the 0.902-1.323 ppm region appeared the signals of the protons of eight tertiary methyl groups and, in the weak field, the signal of a carbinyl proton at 3.64 ppm (d-t, J = 4.5, 9 Hz, H_a^{12}) and signals characteristic for olefinic protons at 5.68 ppm (m, H^{24}) and 5.71 ppm (m, H^{23}). The absence from the PMR spectrum of (XIII) of a signal that could be assigned to a proton at C-3 permitted the assumption that (XIII) was a 3-keto derivative of a triterpene alcohol. This was confirmed by the ^{13}C NMR spectrum of (XIII) (Table 1) in which the signals were observed of three carbinyl C atoms at (ppm) 70.8 (C-12), 73.8 (C-20), and 70.5 (C-25), the signal of a carbonyl C atom at 217.4 ppm, unambiguously assigned to C-3 [10], and of the two C atoms of an ethylene group at 122.3 ppm (C-23) and 142.1 ppm (C-24). A fragment with m/z 205 (d^+) in the mass spectrum of (XIII) also showed the presence of a 3-keto group.

When (XIII) was acetylated, a monoacetate (XV) was obtained the IR spectrum of which contained the absorption bands of a hydroxy group at 1598 and 3522 cm^{-1}. The oxidation of (XIII) with chromium trioxide in pyridine gave a diketone (XVI) identical with the diketone obtained by oxidizing the triterpene alcohol (XII) under the same conditions.

According to these results, the triterpene (XIII) has the structure of 12β,20(S),25-trihydroxydammar-23-en-one.

Triterpene (XIV), $C_{30}H_{52}O_5$, the most polar of the compounds isolated, was detected in only one sample of B. pendula leaves (2). The IR spectra of (XIV) showed a narrow absorption

TABLE 2. Amounts of Triterpenoids in the Leaves of B. pendula Roth. (% of crystalline substance on the weight of the air-dry leaves)

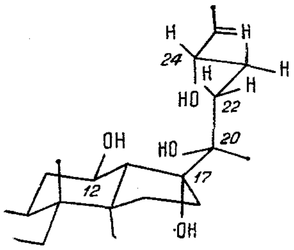
Sample No.	Site and date of collecting the leaves	Compound												Sum of the triterpenoids
		I	II	III	IV	V	VIII	IX	X	XI	XII	XIII	XIV	
1	BurASSR, Selenga Region July 2, 1981	—	—	0,009	0,005	0,007	—	—	—	—	—	—	—	0,021
2	KazSSR. environs of Semipalatinsk, July 15, 1982	0,02	0,09	—	0.02	—	0,05	—	—	—	0,02	—	0,02	0,22
3	Novosibirsk privince, environs of Novosibirsk, June 25, 1983	0,02	0,09	0.002	—	—								0,112
4	Sverdlovsk province, village of Verkhoturka, June 28, 1984	0,19	0,01	—	—	—	0,007	0,05	0,01	0,01	0,05	0,02	—	0,337
5	Leningrad province, Kavgolovskie heights, June 24, 1983	0,15	0,01	—	—	—	0,008	0,01	0,005	0,01	0,04	0.005	—	0,238
6	Leningrad province, village of Lembolovo, July 6, 1983	0.11	0,01	—	—	—	0,002	0.01	0,005	0,01	0,04	0,005	—	0,192

band at 3617 cm^{-1}, characteristic of the stretching vibrations of a hydroxy group, and a broad band at 3339 cm^{-1} which was assigned to the stretching vibrations of hydroxy groups bound by an intramolecular hydrogen bond (intraHB), since on 12-fold dilution it remained practically unchanged.

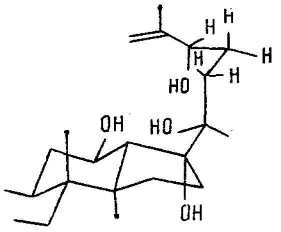
The mass spectrum of (XIV) contained the characteristic peaks of ions with m/z 456, 438, 348, 207, 143, 125, and 107. This gave grounds for assuming that the compound (XIV) was a tetracyclic triterpenoid of the dammarane series with an open side chain [7, 9]. The assignment of the signals in the PMR and ^{13}C NMR spectra of (XIV) was made in comparison with the spectra of compounds (II), (III), (IX), (X), and (XI). A comparative analysis of the spectra showed that the triterpene (XIV) consisted of a mixture of two epimeric pentaols in approximately equal amounts. In the strong-field region of the PMR spectrum of (XIV) at 0.845-1.233 ppm appeared the signals of the protons of six tertiary methyl groups and two singlet peaks of the protons of a methyl group on a double bond, one of which at 1.747 ppm was assigned to the 24(S) epimer and the other, at 1.729 ppm, to the 24(R) epimer. In the weak field there were the signals of carbinyl protons at (ppm) 3.41 (t, J = 2.7 Hz, H_a^3); 3.84 (t, J ~ 11 Hz, H_a^{12}); 4.128 (d-d, J = 8.2 and 2.8 Hz, H^{24} of the (R) epimer); and 4.037 ppm (d-d, J = 5.0 and 6.0 Hz, H^{24} of the (S) epimer), and the signals of the protons of a $R_2C{=}CH_2$ group at (ppm) 4.866 (t, J = 1.4 Hz, H^{26} cis to 27-CH_3, (R) epimer); 4.817 (t, J = 1.7 Hz, H^{26} cis to 27-CH_3 (S) epimer); and 4.966 ppm (br.s, H^{26} trans to 27-CH_3).

The ^{13}C NMR spectrum of (XIV) (Table 1) contained the signals of the two C atoms of an ethylene group (ppm) 110.7 (C-26) and 147.8, 147.5 (C-25) and of carbonyl C atoms at (ppm) 76.2 (C-3), 67.1 (C-12), 85.7 (C-17), 77.0 (C-20), and 76.8, 75.3 (C-24), indicating the presence of five hydroxy groups in the molecule of the triterpene (XIV). The magnitudes of the C-20 and C-21 chemical shifts showed the S configuration of the C-20 asymmetric center in the triterpene (XIV) [10].

An interHB between the OH groups at C-12, C-20, and C-24 is the cause of the predominance of conformations A and B of the side chain over its other possible conformations in the 24(S) and 24(R) epimers (XIV). By making use of empirical rules for the calculation of the magnitudes of ^{13}C CSs [11] it is possible to assume that these CSs will be greater for the C-22 and C-24 atoms in the case of the 24(S)-epimer than in the case of the 24(R)-epimer.



A

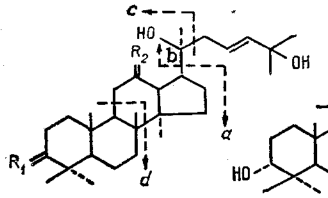


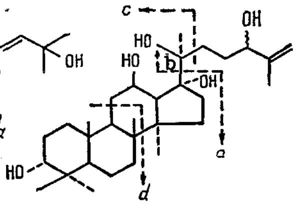
B

We used these characteristics in an assignment of the signals of the carbon atoms of the side chain in the ^{13}C spectrum of the epimeric mixture (XIV).

On the basis of what has been said, it may be concluded that triterpene (XIV) is a mixture of epimers at C-24 of dammar-25-ene-3α,12β,17α,20(S),24ξ-pentaol.

Triterpene alcohols of the dammarane series having five hydroxy groups are found extremely rarely in birch leaves for which the presence of triols and tetraols and, more rarely, of diols is more characteristic. Only one pentaol has been isolated previously, and this differed from (XIV) in the structure of the side chain — dammar-23-ene-3α,12β,17,20,25-pentaol (betulafolienepentaol) [9].





XIV

XII. $R_1 = <^{OH}_{H}$, $R_2 = <^{OH}_{H}$

XIII. $R_1 = O$, $R_2 = <^{OH}_{H}$

XV. $R_1 = O$, $R_2 = <^{OAc}_{H}$

XVI. $R_1 = O$, $R_2 = O$

Sterols (VI) and (VII) were present in all the leaf samples studied (the total amount of (VI) and (VII) was approximately 0.04% of crystalline substances on the weight of the air-dry leaves). Information on the amounts of the individual triterpenoids in the leaf samples is given in Table 2). For comparison, it also includes information on the composition of the triterpene fraction of the leaves of B. pendula gathered in Easter Siberia (sample 1) [4]. As can be seen from Table 2, the qualitative and quantitative compositions of the triterpenoids depended substantially on the side at which the leaves were collected. Samples 2 and 3 contained betulafolienetetraol (II) as the main component, thereby differing from specimens growing in the Ukraine and in the European part of the USSR, where the predominating substance was betulafolienetriol (I), and the tetraols were represented by several compounds – (II) and (X-XIII). The quantitative compositions of samples 4-6 were identical and some difference was observed only in the quantitative amounts of the individual components.

EXPERIMENTAL

IR spectra were recorded on a Specord 75 IR spectrophotometer in $CHCl_3$ solution. The mass spectra of the triterpenes (XIII) and (XV) were obtained on a LKB 9000 spectrometer and those of the triterpenes (XIV) and (XV) on a MKh-1320 at an ionizing energy of 70 eV. PMR spectra were obtained on a Bruker WM-250 instrument and ^{13}C NMR spectra on a Bruker HX-90E instrument. Chemical shifts are expressed on the δ scale relative to TMS. Optical rotations were determined on a Perkin-Elmer instrument in a cell 10 cm long, and melting points on a Boetius stage.

For chromatography we used silica gel L 80/160 μm. The individuality of the substances was checked by thin-layer chromatography in the benzene–ethanol (10:1) system. A 10% solution of H_2SO_4 in ethanol was used to reveal the triterpenes on the chromatograms.

Isolation of the Triterpenes from the B. pendula Leaves. Samples of air-dry leaves (2-6) collected in various regions (Table 2) were treated in accordance with [1]. The individual substances (I-XIV) were isolated from the unsaponifiable part of ethereal extracts of the leaves by repeated chromatography on silica gel with elution by the following solvent systems: 1) hexane–acetone; 2) chloroform–methanol; 3) benzene–ethyl acetate. Compounds (I-IV), (VI), and (VIII-XII) were identified by direct comparison with authentic samples.

Citrostadienol (V). mp 165-168°C, (methanol), $[\alpha]_D^{20}$ + 10° (c 0.5; chloroform). According to the literature [8]: mp 166-167°C; 165°C, 162-164°C (depending on the source of isolation); $[\alpha]_D$ +24°.

Triterpene (XIII). $C_{30}H_{50}H_4$, noncrystalline, $[\alpha]_D^{20}$ +32.2° (c 0.5; chloroform). $\lambda_{max}^{CHCl_3}$ (cm^{-1}): 3611, 3363, 1698. Mass spectrum, m/z: 456 (M^+-H_2O), 438 (M^+-2H_3O), 420 (M^+-3H_2O), 375 (c^+), 357 (c^+-H_2O), 339 (c^+-2H_2O), 331 (a^+), 313 (a^+-H_2O), 205 (d^+), 143 (b^+), 125 (b^+-H_2O), 107 (b^+-2H_2O). PMR spectrum ($CDCl_3$, ppm): 0.902, 0.989, 1.024, 1.045, 1.085, 1.167, 1.299, and 1.323 (3 H, s), the protons of tert-Me groups; 3.64 (1 H, d-t, J = 4.5 and 9.0 Hz, H_a^{12}); 5.68 (1 H, m, H^{24}); 5.71 (1 H, m, H^{23}; and 4.80 (1 H, s, OH).

Acetylation of (XIII). A mixture of 63 mg of (XIII), 1 ml of pyridine, and 0.5 ml of acetic anhydride was left overnight at room temperature. After the usual working up and chromatography of the reaction mixture on silica gel in the chloroform–ethanol (100:1) system, 40 mg of the noncrystalline monoacetate (XV) was obtained; $C_{32}H_{52}O_5$, $[\alpha]_D^{20}$ + 12° (c 0.5; chloroform). $\lambda_{max}^{CHCl_3}$ (cm^{-1}: 3598, 3522, 1744, 1702. Mass spectrum, m/z: 456(M^+ – OAc), 438 (M^+-H_2O-OAc), 417 (c^+), 373 (a^+), 357 (c^+ – OAc), 339 ($c^+-OAc-H_2O$), 313 (a^+ – OAc), 205 (d^+), 125 (b^+-H_2O). PMR spectrum ($CDCl_3$, ppm): 0.942, 0.967, 1.043, 1.049, 1.091, 1.113, 1.335, 1.342 (3 H, s) – the protons of tert-Me groups; 2.08 (3 H, s), the protons of an acetate group: 4.75 (1 H, d-t, J = 4.9; 10.7 Hz, H_a^{12}); 5.69 (1 H, d, J = 15.7 Hz, H^{24}); 5.80 (1 H, d-d-d, J = 5.5, 7.9, and 15.7 Hz, H^{23}); 3.18 (1 H, s, OH).

Oxidation of Triterpene (XII). A mixture of 140 mg of (XII), 220 mg of CrO_3, and 3.5 ml of pyridine was left overnight at room temperature. After the usual working up, the mixture of products obtained was chromatographed on silica gel with elution by the chroloform–ethanol (100:1) system. This gave 63 mg of the diketone (XVI), $C_{30}H_{48}O_4$, mp 165-172°C (acetone). $[\alpha]_D^{20}$ + 58° (c 0.5; chloroform). $\lambda_{max}^{CHCl_3}$ (cm^{-1}; 3605, 3400, 1700. Mass spectrum, m/z: 454 (M^+-H_2O), 436 (M^+-2H_2O), 373 (c), 329 (a^+), 205 (d^+), 143 (b^+), 125 (b^+-H_2O), 107 (b^+-2H_2O). PMR spectrum ($CDCl_3$, ppm): 0.804, 1.035, 1.069, 1.094, 1.104, 1.222 (3 H, s), and 1.318 (6 H, s), the protons of tert-Me groups; 5.66 (1 H, d, J = 15.8 Hz, H^{24}); 5.74 (1 H, d-t, 2 J = 2×7.0, J = 15.8 Hz, H^{23}); 3.27 (1 H, br. s, OH).

Oxidation of Triterpene (XIII). A mixture of 117 mg of (XIII), 190 mg of CRO_3, and 3.5 ml of pyridine was left overnight at room temperature. The fraction mixture was worked up as described above, giving 43 mg of a substance with mp 165-172°C (acetone) showing no depression of the melting point in admixture with a sample of (XVI).

Triterpene (XIV). $C_{30}H_{52}O_5$, noncrystalline, $[\alpha]_D^{20}$ − 3° (c 0.5; chloroform), $\lambda_{max}^{CHCL_3}$ (cm^{-1}: 3615, 3300. Mass spectrum, m/z: 456 ($M^+ - 2H_2O$), 438 ($M^+ - 3H_2O$), 420 ($M^+ - 5H_2O$), 402 ($M^+ - 5H_2O$), 375 ($c^+ - H_2O$), 357 ($c^+ - 2H_2O$), 349 (a^+), 331 ($a^+ - H_2O$), 313 ($a^+ - H_2O$), 313 ($a^+ - 2H^2$), 295 ($a^+ - 3H_2O$), 207 (d^+), 189 ($d^+ - H_2O$), 125 ($b^+ - A_2O$), 107 ($b^+ - 2H_2O$). PMR spectrum ($CDCl_3$, ppm): the 24(S)-epimer: 0.845, 0.890, 0.941, 0.960, 1.165, 1.233, and 1.747 (s), the protons of tert-Me groups; 3.41 (t, J = 2.7 Hz, $H_a{}^3$); 3.84 (t, J ~ 11.0 Hz, $H_a{}^{12}$); 4.037 (d-d, J = 5.0 and 6.0 Hz, H^{24}), 4.817 (t, J = 1.7 Hz, H^{26} cis to 27-CH_3); 4.966 (br.s, H^{26} trans to 27-CH_3; the 24(R)-epimer: 0.845, 0.890, 0.941, 0.970, 1.165, 1.201, and 1.729 (s) − the protons of tert-Me groups; 3.41 (t, J = 2.7 Hz, $H_e{}^3$); 3.84 (t, J ~ 11.0 Hz, $H_a{}^{12}$); 4.128 (d-d, J = 8.2 Hz, H^{24}); 4.866 (t, J = 1.4 Hz, H^{26} cis to 27-CH_3); 4.966 (br.s, H^{26} trans to 27-CH_3).

SUMMARY

1. A comparative study has been made of the triterpene fraction of the unsaponifiable part of ethereal extracts of five samples of *B. pendula* leaves collected in various regions of the Soviet Union. It has been shown that the yields and the numbers of components of the mixtures of triterpenoids in the leaves rise in the series of samples studied in the following sequence: Eastern Siberia, Western Siberia, the Urals.

2. The most promising source of betulafolienetriol is formed by the leaves of birch trees growing in the Sverdlovsk and Leningrad provinces, and that of betulafolienetetraol those growing in the regions of Western Siberia and Kazakhstan.

3. The structures of new triterpenoids of the dammarane series have been established —dammar-23-en-12β,20(S),25-trihydroxydammar-25-en-3-one, and the 14(S) and 24(R) epimers of dammar-25-en-3α,12β,17α,20(S),24ξ-pentaol.

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