# TRITERPENOIDS FROM THE LEAVES OF THE SIBERIAN SPECIES OF BIRCH Betula nana and B. exilis

N. D. Pokhilo, G. V. Malinovskaya,
V. V. Makhan'kov, V. A. Denisenko,
and N. I. Uvarova

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The compositions of the triterpene fractions of the unsaponifiable part of the ethereal extracts of the leaves of B. nana and B. exilis have been studied. The leaves of B. exilis have yielded a new triterpene for which the structure of 3α,20(S),25-trihyproxydammar-23-ene is proposed, and a four component mixture of epimers at C-3 and C-24 of the compound 3ξ,20(S),24ξ-trihyproxydammar-25-ene. The composition and ratio of the components of the mixture were determined on the basis of chemical and spectral characteristics.

Continuing a study of Siberian species of the genus Betula [1], we have investigated the triterpene fraction of the unsaponifiable part of ethereal extracts of the leaves of B. nana L. and B. exlis Sukacz., two close species of birch. The total amount of triterpenoids in the birches was the same, 0.3% (on the weight of the air-dry leaves), the main component being 3-epiocotillol (I) (0.15%), [2, 3]. Among the minor compounds in B. nana we detected ocotillol (II), [3, 4], betulafolienetriol oxide (III) [5], and the monoacetate at C-12 and the diacetate at C-3 and C-12 of betulafolienetriol oxide ((IV) [6] and (V) [2]). In the leaves of B. exilis, in addition to (I)-(III) we found betulafolienetriol (VI) [7], 3α,12β,-20(S),24-tetrahyproxydammar-25-ene (VII) [8], and a number of new compounds: the triterpene (VIII) and a complex mixture of triterpenes (IX) and (X).

The IR spectrum of the triterpene (VIII) showed the absorption band of a hydroxy group (3614 $cm^{-1}$). The mass spectrum of (VIII), showing peaks with m/z 424 ($M^+ - 2H_2O$), 361, 343, 317, 207, 189, 107, and 82, corresponded to the spectra of dammarane triterpenes with open side chains [2, 7-9].

In the PMR spectrum of (VIII) the signals of eight tertiary methyl groups appeared in the 0.84-1.33 ppm region, and the signal of a carbinyl proton in the weak field at 3.40 ppm (1 H, t, J = 3.0 Hz) which, for triterpenes, can unambiguously be assigned to $H_e^3$. Also in the weak field was a signal at 5.70 ppm (2 H, m), which is characteristic for olefinic protons. When the PMR spectrum of (VIII) was compared with the corresponding spectrum of 3β,20(S),25-trihyproxydammar-23-ene (isofouquierol) [9, 10], it was found that these compounds differed only by the orientation of the hydroxy group at C-3, i.e., the triterpene (VIII) was the 3α-epimer of isofouquierol. This was confirmed by the $^{13}C$ spectrum of (VIII) (Table 1). The spectrum contained the signals of three carbinyl C atoms at (ppm) 75.3 (C-3), 74.4 (C-20), and 69.8 (C-25) and of the two C atoms of an ethylene group at 123.2 ppm (C-23) and 142.6 ppm (C-24). The CSs of the C-20 and C-21 atoms showed the S configuration of the C-20 asymmetric center in triterpene (VIII) [11].

The $^1H$ and $^{13}C$ NMR spectra of (VIII) agreed completely with the structure of 3α,20(S),-25-trihyproxydammar-23-ene (3-epiisofouquierol).

The ethereal extract from the leaves of B. exilis also contained a complex mixture of triterpenes (IX) and (X) with close $R_f$ values. In spite of the fact that these substances could not be separated chromatographically, the composition of the mixture and the ratio of the components was established on the basis of an analysis of spectral characteristics and chemical transformations. The IR spectrum of the mixture contained characteristic absorption bands of a hydroxy group (3624 $cm^{-1}$) and of a $R_2C{=}CH_2$ group (1648 $cm^{-1}$). The presence in the mass spectrum of the mixture of (IX) and (X) of peaks with m/z 443, 361, 343, 317,

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TABLE 1. $^{13}C$ Chemical Shifts of Compounds (VIII), (X), (XV), and (XVII) (ppm, relative to TMS)*

| C atom | Compound | | | |
|---|---|---|---|---|
| | VIII | X | XV | XVII |
| 1 | 34,3 | 33,7 | 33,7 | 39,9 |
| 2 | 25,2 | 25,4 | 25,5 | 34,2 |
| 3 | 75,3 | 76,3 | 76,3 | 218,3 |
| 4 | 38,2 | 37,7 | 37,7 | 47,5 |
| 5 | 50,9 | 49 6 | 49.7 | 55,4 |
| 6 | 18 6 | 18,3 | 18 3 | 19.7 |
| 7 | 35,7 | 35 1 | 35.2 | 34,6 |
| 8 | 40 6 | 40,6 | 40,7 | 40.3 |
| 9 | 50,5 | 50,5 | 50 6 | 50,0 |
| 10 | 37,6 | 37,2 | 37,4 | 36,9 |
| 11 | 21,8 | 22,2 | 21.5 | 22.1 |
| 12 | 28,1 | 27,6 | 27,6 | 27,5 |
| 13 | 42,4 | 42,4 | 42,5 | 42,5 |
| 14 | 50 7 | 50,2 | 49 9 | 50,3 |
| 15 | 31,6 | 31,2 | 31,3 | 31,2 |
| 16 | 26,6 | 25,4 | 25,5 | 25,4 |
| 17 | 49,8 | 50,1(50 5)† | 49.7 | 50.2(50,0)† |
| 18 | 16,5 | 16,1 | 16 1 | 16,1 |
| 19 | 15,8 | 15.6 | 15,5 | 15 2 |
| 20 | 74,4 | 75,2 | 75,0 | 75,1(75,2) |
| 21 | 26,8 | 24 9 | 24,8 | 24,9 |
| 22 | 45,1 | 36 5(36.1) | 36,5 | 36,7(36,1) |
| 23 | 123,2 | 29,3(29.1) | 27,0 | 29,3(29,1) |
| 24 | 142,6 | 76 0(76,5) | 77.8 | 77,3(76,0) |
| 25 | 69,8 | 110,9(110.8) | 143,2 | 147,7 |
| 26 | 30,8 | 147,8 | 113.0(112,7)† | 111 0(110,9) |
| 27 | 30,8 | 17,8(18.1) | 18,3 | 17,7(18,1) |
| 28 | 29.4 | 28,3 | 28 3 | 26,7 |
| 29 | 22,5 | 21.5 | 22,2 | 21,1 |
| 30 | 16,7 | 16,6 | 16,7 | 16.4 |
| >C=O | | | 170,2 | |
| —OCOCH$_3$ | | | 21,1 | |

*The spectrum of (VIII) was taken in $C_5D_5N$, and those of (X), (XV), and (XVII) in $CDCl_3$.
†CSs for the 24(R)-epimers.

207, 189, 143 (32.4%), 125 (100%), 107, and 81 showed that they were dammarane triterpenes with open side chains [2, 7-9].

The assignment of the signals in the PMR spectrum of the mixture of (IX) and (X) was made by comparison with the corresponding spectra of fouquierol [9] and of 3α,12β,20(S),-24-tetrahyproxydammar-25-ene [8]. It must be mentioned that the authors concerned did not determine the configurations of the 3-, 20-, and 24-carbon atoms for fouquierol, which was first isolated from Fouquiera splendens Engelm. However, on the basis of the spectral information given in the paper it may be assumed that fouquierol is 3β,20ξ,24ξ-trihyproxydammar-25-ene. The singlet signals in the PMR spectrum of the mixture in the 0.78-1.75 ppm region belong to the protons of tertiary methyl groups, and signals in the weak field can be assigned in the following way: (ppm): 4.08 (m, $H^{24}$); 3.20 (dd, J = 5.5 and 10.5 Hz, $H_a^3$); 3.40 (t, J = 2.8 Hz, $H_e^3$); 4.85 (m, $H^{26}$ cis to 27-$CH_3$); 4.97 (m, $H^{26}$ trans to 27-$CH_3$).

On the basis of what has been said above, we assumed that we were dealing with a mixture of 3β,20ξ,24ξ-trihyproxydammar-25-ene (IX) (fouquierol) and its α-epimer at C-3 in a ratio of ~1:1. The results of the oxidation of the mixture of (IX) and (X) with chromium trioxide in pyridine confirmed this hypothesis and enabled the question of the configuration of the C-20 and C-24 asymmetric centers to te answered. On oxidation, the ketolactone (XVI), which was identical to its physicochemical properties and PMR spectrum with the ketolactone formed on the oxidation of fouquierol [9], and also 20(S),24ξ-dihyproxydammar-25-en-3-one (XVII) were obtained.

We have previously isolated 20(S),24(S)-dihydroxydammar-25-en-3-one (XVIII) from the leaves of B. mandschurica Nakai [12]. The good agreement of the CS values of the C-20 and C-21 atoms in the $^{13}C$ spectra of the triterpenes (XVII) (Table 1) and (XVIII) [12] showed the S configuration of the C-20 asymmetric center in the triterpene (XVII). In addition, a study of the $^1H$ and $^{13}C$ NMR spectra of compound (XVII) showed that it was a mixture of epimers at C-24 in a ratio of ~1:1. A comparison of the CSs in the spectra of the triterpenes

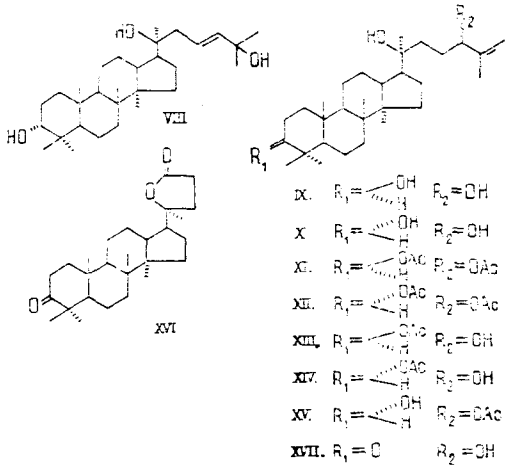
(XVII) and (XVIII) permitted the observation in the spectra of (XVII) of the signals for both the 24(S) and the 24(R) epimers. In the PMR spectrum of (XVII), together with singlet signals in the 0.89-1.74 region characteristic for the protons of tertiary methyl groups and a multiplet at 2.3-2.60 ppm assigned to the proton at C-2, the following signals were observed for the 24(S) epimer (ppm) 4.047 (t, J = 6.2 Hz, $H^{24}$); 4.960 (d-q, J = 1.0 and 2,0 Hz, $H^{26}$ trans to 27-$CH_3$); and 4.842 (d-q, J = 2.0 and 2.0 Hz, $H^{26}$ cis to 27-$CH_3$); and for the 24(R)-epimer (ppm): 4.079 (d-d, J = 6.4 and 5.4 Hz, $H^{24}$); 4.969 (d-q, J = 1.0 and 2.0 Hz, $H^{26}$ trans to 27-$CH_3$); and 4.854 ppm (d-q),J = 2.0 and 2.0 Hz, $H^{26}$ cis to 27-$CH_3$).

By using differences in the rates of acetylation of the hydroxy groups of the epimers (IX) and (X) we succeeded in isolating compound (X). When the mixture of (IX) and (X) was treated with acetic anhydride in pyridine for 3 h, a mixture of diacetates and monoacetates at C-24 was obtained. The chromatographic separation of the acetates on silica gel led to the isolation of a mixture of monoacetates in which the monoacetate (XV) largely predominated, which enabled us to obtain it by crystallization from acetone.

The IR spectrum of (XV) showed the absorption bands of an ester group (1730 $cm^{-1}$) and of a hydroxy group (3620 $cm^{-1}$). Its PMR spectrum, unlike the spectrum of the mixture of (IX) and (X) described above, revealed the signal of the protons of an acetyl group (2.09 ppm, s), the signal at 3.20 ppm characteristic for $H_a^3$ was absent, and the $H^{24}$ signal had undergone a downfield shift (5.17 ppm, t, J = 6.5 Hz). This indicated that (XV) was the 24-acetyl derivative of the 3α-epimer.

The alkaline hydrolysis of (XV) gave the free alcohol (X) the PMR spectrum of which likewise lacked the $H_a^3$ signal. Analysis of the $^{13}C$ spectra of the triterpene (X) and its monoacetate (XV) confirmed that they were mixture of epimers at C-24, and that the C-20 atom had the S configuration (Table 1). On the basis of these facts, the structure of 3α,20(S),24ξ-trihyproxydammar-25-ene is proposed for (X).

Thus, the mixture contained four components in approximately equal proportions – 3α,20-(S),24(S)-trihyproxydammar-25-ene, 3β,20(S),24(R)-trihyproxydammar-25-ene, 3α,20(S),24(S)-trihyproxydammar-25-ene, and 3α,20(S),24(R)-trihyproxydammar-25-ene.




## EXPERIMENTAL

IR spectra were recorded on a Specord 75 IR spectrophotometer in $CHCl_3$ solution, mass spectra on an LKB 9000 spectrometer at an ionizing energy of 70 eV, and $^1H$ and $^{13}C$ NMR spectra on a Bruker WM 250 instrument. Chemical shifts are expressed in 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 Boëtius stage.

Silica gel L 100/160 μm was used for chromatography. The individuality of the substances was monitored by thin-layer chromatography in systems: 1) benzene–ethanol (10:1), and 2) benzene–ethyl acetate–ethanol (10:10:1). The triterpenes were detected on the chromatograms with a 10% solution of $H_2SO_4$ in ethanol.

Isolation of Triterpenes from the Leaves of B. nana. The air-dry leaves gathered at the end of June, 1983, in the environs of the village of Kruglikovo, Novosibirsk province, (5.5 kg) were treated by the method of Fischer and Seiler [7]. This gave 100 g of unsapon-

ifiable fraction fo the ethereal extract. The individual substances (I-V) were isolated by repeated chromatography on silica gel with elution by the following solvent systems: 1) hexane–acetone; 2) chloroform–ethanol; and 3) benzene–ethyl acetate. All the triterpenoids isolated were identified by comparison with authentic samples in terms of melting points and IR spectra.

Isolation of Triterpene from the Leaves of B. exilis. The air-dry leaves, collected in July, 1982, in the environs of the settlement of Nizhneangarska, Buryat ASSR, (4 kg) were treated by the Fischer–Seiler method [7]. This gave 52 g of unsaponifiable fraction of the ethereal extract. Triterpenoids (I-III, VI-X) were isolated as above. Compounds (I-III, VI, and VII) were identified by direct comparison with authentic samples.

Triterpene (VIII) (3-epiisofouquierol), $C_{30}H_{52}O_3$, mp 162-165°C (acetone), $[\alpha]_D^{14}$ +11.6° (c 0.5; chloroform). $\nu_{max}^{CHCl_3}$ 3614 $cm^{-1}$. PMR spectrum ($CDCl_3$, ppm): 0.84, 0.86, 0.89, 0.94, 0.96, 1.13 (3 H, s), 1.33 (6 H, s) (the protons of tertiary Me groups); 3.40 (1 H, s, J = 30 Hz, $H_e^3$); 5.70 (2 H, m, $H^{23}$ and $H^{24}$); 2.20 (2 H, m, 2 $H^{22}$). Mass spectrum, m/z (%): 424 ($M^+ - 2H_2O$), 361 (1.5), 343 (3.2), 207 (6.1), 189 (13.6), 150 (10.4), 107 (43.6), 82 (59.5).

Oxidation of (IX) and (X). A solution of 140 mg of the mixture in 2 ml of pyridine was added dropwise to a solution of 200 mg of $CrO_3$ in 2 ml of pyridine. Oxidation was carried out at room temperature for 2 days. After the usual working up, the mixture of products obtained was chromatographed on silica gel with elution by petroleum ether–acetone. This gave 11 mg of (XVII) and 20 mg of the crystalline ketolactone (XVI) with mp 175-176°C (methanol). PMR spectrum ($CDCl_3$, ppm): 0.90, 0.94, 1.00, 1.04, 1.08, 1.38 (each 3 H, s); 2.35-2.80 (4 H, m, 2 $H^2$ and 2 $H^{23}$).

Acetylation of (IX) and (X). 1. This was performed by keeping 96 mg of the mixture of (IX) and (X) in 2 ml of pyridine and 1 ml of acetic anhydride at room temperature overnight. After the usual working up, 103 mg of a mixture of the diacetates (XI) and (XII) was obtained which it was impossible to separate by chromatographic methods. $\nu_{max}^{CHCl_3}$ ($cm^{-1}$); 3611, 1721, 1651. PMR spectrum ($CDCl_3$, ppm): 0.85, 0.87, 0.89, 0.90, 0.93, 0.98, 1.15, 1.73 (the protons of tertiary Me groups); 2.05, 2.07, 2.10 (the protons of acetyl groups); 4.49 (dd, J = 5.5 and 10.5 Hz, $H_a^3$); 4.63 (t, J = 2.8 Hz, $H_e^3$); 4.90 (m, $H^{26}$ cis to 27-$CH_3$); 4.97 (m, $H^{26}$ trans to 27-$CH_3$); 5.16 (t, J = 6.5 Hz, $H^{24}$).

2. In another experiment, 400 mg of the mixture of (IX) and (X) in 2 ml of pyridine and 1 ml of acetic anhydride was left at room temperature for 3 h. After the usual working up, the reaction mixture was chromatographed on silica gel with elution by the benzene–ethanol system. This gave 170 mg of the diacetates (XI) and (XII) and 130 mg of monoacetates, from which, after to recrystallizations from acetone, 50 mg of (XV) was isolated with mp 169-172°C (acetone), $[\alpha]_D^{21}$ + 16.0° (c 0.5; chloroform). $\nu_{max}^{CHCl_3}$ ($cm^{-1}$): 3620, 1730. PMR spectrum ($CDCl_3$, ppm): 0.86, 0.88, 0.91, 0.96, 0.98, 1.15, 1,78 (protons of tertiary Me groups); 2.09 (protons of an acetyl group); 3.43 (t, J = 3.0 Hz, $H_e^3$); 5.17 (t, J = 6.5 Hz, $H^{24}$); 4.91 (m, $H^{26}$ cis to 27-$CH_3$); 4.97 (m, $H^{26}$ trans to 27-$CH_3$).

3. Similarly, 474 mg of the mixture of (IX) and (X), 3 ml of pyridine, and 1.5 ml of acetic anhydride was left at room temperature for 1 h. After the usual working up, the reaction mixture was chromatographed on silica gel with the elution by the benzene–ethanol system. This gave 100 mg of the initial mixture, 93 mg of a mixture of (XI) and (XII), 220 mg of (XV), and 42 mg of a mixture of the monoacetates at C-3 (XIII) and (XIV). PMR spectrum $CDCl_3$, ppm); 0.86, 0.87, 0.89, 0.90, 0.93, 0.97, 0.98, 1.15, 1.75 (the protons of tertiary Me groups); 2.05, 2.10 (the protons of acetyl groups); 4.50 (dd, J = 5.5 and 10.5 Hz, $H_a^3$); 4.64 t, J = 2.8 Hz, $H_e^3$); 4.07 (m, $H^{24}$); 4.88 (m, $H^{26}$ cis to 27-$CH_3$); 4.98 (m, $H^{26}$ trans to 27-$CH_3$).

Saponification of (XV). A mixture of 45 mg of (XV) in 5 ml of methanol and 0.5 g of KOH was heated for 2 h. After the usual working up, 40 mg of the free alcohol (X) was obtained with mp 152-155°C (acetone), $[\alpha]_D^{28}$ +22.2° (c 0.5; chloroform). $\nu_{max}^{CHCl_3}$ ($cm^{-1}$): 3624, 1648. PMR spectrum ($CDCl_3$, ppm), 0.85, 0.88, 0.91, 0.95, 0.97, 1.15, 1.75 (the protons of tertiary Me groups); 3.41 (t, J = 3.0 Hz, $H_e^3$); 4.08 (m, $H^{24}$); 4.87 (m, $H^{26}$ cis to 27-$CH_3$); 4.97 (m, $H^{26}$ trans to 27-$CH_3$).

## SUMMARY

1. The composition of the triterpene fractions of the unsaponifiable parts of ethereal extracts of the leaves of the birches B. nana and B. exilis have been studied. It has been shown that they can serve as a source of 3-epicotillol.

2. The leaves of B. exilis have yielded a new triterpene, for which the structure of 3α,20(S),25-trihydroxydammar-23-ene is proposed, and a four-component mixture of epimers at C-3 and C-24 of the compound 3ξ,20(S),24ξ-trihydroxydammar-25-ene. The composition of the mixture and the ratio of the components were determiend n the basis of chemical and spectral characteristics.

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# A NEW STEROID GLYCOSIDE FROM THE STARFISH Patiria pectinifera


A. A. Kicha, A. I. Kalinovskii,
É. V. Levina, Ya. B. Rashkes,
V. A. Stonik, and G. B. Elyakov



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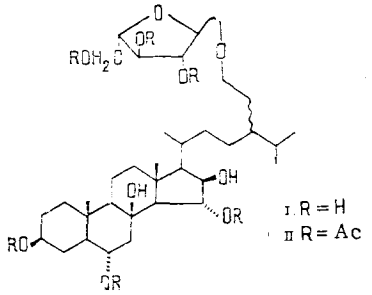
From the glycoside fraction of the starfish Patiria pectinifera, after its desulfation, an artefactural glycoside has been obtained: 29(α-L-arabinofuranosyloxy)-5α-stigmastane-3β,6α,8,15α,16β-pentaol (I). The structure of (I) was shown by $^{1}H$ and $^{13}C$ NMR spectra and mass spectra and by acetylation and high-temperature hydrogenation.


Continuing an investigation of the steroid glycosides of the Far Eastern starfish Patiria pectinifera, among the products of mild desulfation of these compounds we have detected and isolated a new steroid derivative: 29-(α-L-arabinofuranosyloxy)-5α-stigmastane-3β,6α,8,15α,16β-pentaol (I).





Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Institute of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.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