A NEW TRITERPENE FROM THE LEAVES OF *Betula mandschurica*

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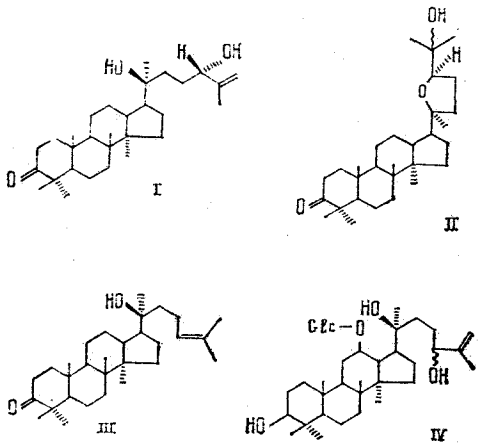
A new triterpene (I) has been isolated from the unsaponifiable fraction of an ethereal extract of the leaves of *Betula mandschurica* to which, on the basis of the results of a physicochemical investigation and a comparison of the ^{13}C spectra with the spectra of known triterpenes — ocotillone (II) and 20(S)-hydroxydammar-24-en-3-one (III) — the structure of 20(S),24(S)-dihydroxydammar-25-3n-3-one has been assigned. An approach to the determination of the configuration of the asymmetric center at C_{24} in 24-hydroxy derivatives of tetracyclic triterpenoids with an open side chain by the use of ^{13}C NMR spectroscopy is proposed.

Continuing investigations of the triterpenoids of Far Eastern species of birch [1-4], we have isolated a new triterpene $C_{30}H_{48}O_3$ (I) from the unsaponifiable fraction of an ethereal extract of the leaves of *Betula mandschurica*.

The IR spectrum of a solution of the triterpene (I) in $CHCl_3$ (c 2.5 mg/ml) shows the band of the stretching vibrations of the carbonyl group of a six-membered ring at 1693 cm^{-1}, three bands at 898, 1648, and 3076 cm^{-1} due to the vibrations of a $R_2C{=}CH_2$ group, and three bands at 3282, 3374, and 3601 cm^{-1} that are characteristic for the stretching vibrations of a hydroxy group. On five fold dilution of the solution, the band at 3282 cm^{-1} disappeared, while the bands at 3374 and 3601 cm^{-1} did not change their positions and the band at 3374 cm^{-1} did not change its intensity, either.

In the ^1H spectrum of the triterpene (I) the singlet signals of six tertiary methyl groups appear in the strong field (0.89, 0.94, 1.00, 1.04, 1.08, and 1.15 ppm) together with the singlet peak of a methyl group on a double bond (1.74 ppm), and in the weak field there are the signal of a carbonyl proton at 3.96 ppm (t, J = 5.2 Hz) and the singlet signals of two protons of a $R_2C{=}CH_2$ group at 4.84 and 4.96 ppm. The ^{13}C spectrum of the triterpene (I) (Table 1) contains the signals of a carbonyl C atom (218.6 ppm), of the two C atoms of an ethylene group [147.8 (s) and 110.9 (t) ppm], and of two carbonyl C atoms [76.4 (d) and 75.1 (s) ppm], indicating the presence of secondary and tertiary hydroxy groups in the molecule of the triterpene (I).



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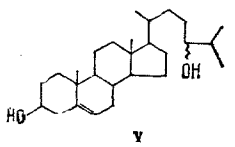

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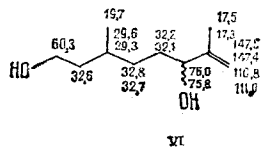


VI

The mass spectrum of the triterpene (I) contains a fragment with m/e 143 (12%) corresponding to the side chain ($C_8H_{15}O_2$) of the dammarane triterpenes [2-7]. Peaks with m/e 125 (100%) and 107 (19%) are obviously formed by the successive splitting out of two H_2O molecules from the fragment with m/e 143. The relatively low intensity of the peak with m/e 143 indicates that the side chain of the triterpene (I) is open [5-7]. This is also shown by the magnitude of the chemical shifts (CSs) of the carbonyl C atoms in the ^{13}C spectrum of the triterpene (I) [8, 9].

A comparison of the ^{13}C CCs of the triterpene (I), ocotillone (II) [9], and 20(S)-hydroxydammer-24-en-3-one (III) [8] permit one to speak of the identity of the spectra of the triterpenes (I-III), of the location of the carbonyl group in triterpene (I) at C_3, and, once again, of the presence of both hydroxy functions of the triterpene (I) in the side chain. The good agreement of the CSs of the C_{20} and C_{21} atoms in the ^{13}C spectra of the triterpenes (I) and (III) (Table 1) apparently shows the S-configuration of the C_{20} asymmetric center in the triterpene (I). This coincidence also indicates the location of the second hydroxy group of triterpene (I) at C_{24}, and not at C_{22}, as is confirmed by the considerable change in the CSs of the C atom of the ethylenic grouping in the ^{13}C spectrum of triterpene (I) as compared with known compounds [10].

Particular interest is presented by the elucidation of the configuration of the C_{24} asymmetric center in triterpene (I). In a number of 24-hydroxy derivatives of sterols, for example, compounds of the 24(R) series possess a considerably greater biological activity than compounds of the 24(S) series [11]. Previously, C. A. Meyer isolated from the flower buds of *Panax japonicus* a saponin (IV), the aglycone of which contained an oxygenated side chain similar to the side chain of the triterpene (I) [12]. It was shown by ^{13}C NMR spectroscopy that saponin (IV) was a mixture of epimers at C_{24} [13]. Since the ratio of the epimers in this mixture was different, itwas possible to isolate the signals of all the C atoms of the side chain for both the 24(S) and the 24(R) epimer (see Table 2) although, it is true, without their umambiguous assignment to one series or the other.

For triterpene (I) and equally for the saponin (IV) the most stable conformation of the side chain at C_{17} will be, theoretically, that in which the side chain has the form of a complex anti-zigzag. On the basis of modern ideas concerning the influence of electronic and steric factors on the CSs of the C atoms in the ^{13}C spectra of organic compounds [14-17], however, it is impossible for such a form (K1 and K2 conformations in Newman projections along the C_{22}-C_{23} bond) to explain the difference in the ^{13}C spectra of the epimers at C_{24} observed in the experimental ^{13}C spectrum of the saponin (IV). It probably appears when the side chain of the saponin (IV) passes into a different conformation. In actual fact, for compounds (I) and (IV) the side chain may exist in another favorable conformation (K3 or K4) in which eclipsings are also absent. In the K3 and K4 conformers there is a possibility for the formation of an intramolecular hydrogen bond between the hydroxy groups at C_{20} and C_{24}, which must stabilize these conformers in comparison with K1 and K2.

An investigation of the dependence of the positions and intensities of the absorption bands in the 3200-3600 cm^{-1} region of the IR spectrum of triterpene (I) on the concentration of the solution confirmed the presence of an intramolecular hydrogen bond. For a ring formed by such a bond, the most favorable conformation will be that resembling the "chair" conformation. In this case, the C_{22} and C_{24} resonances in the ^{13}C spectra of compounds of the 24(S) series should appear in a somewhat weaker field than the resonances of the same atoms for the compounds of the 24(R) series because of the, even if considerably weakened, syn-$\gamma H,H$ coupling [16] between H_{22} and H_{24}, which is absent in the K4 conformer. The K3 conformer (24(S) series) will also be somewhat more stable than the K4 conformer (24(R) series, because the isopropenyl radical at C_{24} occupies a position closer to the equatorial position in the K3 than in the K4 conformer.

It is interesting to note that a similar diagnostic characteristic relates to the C_{22} and C_{24} resonances in the ^{13}C spectra of the 24(S)- and 24(S)-hydroxycholesterols (V) [11]

TABLE 1. ^{13}C Chemical Shifts of the Compounds I-III (ppm relative to TMS)

C atom	Compound		
	I	II	III
1	40,2; 40,0*	39,9; 39 9	39,8; 39,8
2	34,3; 34,2	34,2; 34,2	34,0; 34,2
3	218,6; 214,6	216,2; 216,2	217,6; 216,2
4	47,6; 47,4	47,4; 47,4	47,3; 47,3
5	55,7; 55,3	55,5; 55,3	55,3; 55 3
6	19,9; 19,9	19,8; 19,8	19,6; 19,8
7	34,8; 34,9	34,7; 34,8	34,5; 34,8
8	40,6; 40,6	40,4; 40,4	40,2; 40,5
9	50,5; 50,3	50,3; 50,0	49,9; 50,2
10	37,1; 36,9	36,9; 36,9	36,7; 36,8
11	22,3; 22,4	22,2; 22,2	22,0; 22,2
12	27,7; 28,1	27,6; 27,4	27,5; 28,0
13	42,8; 42,7	43,3; 43,3	42,3; 42,6
14	50,4; 50,7	50,0; 50,0	50,2; 50,5
15	31,4; 31,6	31,6; 31,7	31,1; 31,5
16	25,0; 25,4	25,7; 26,0	25,4; 25,8
17	50,5; 50,3	49,7; 50,0	49,7; 50,2
18	16,1; 16,1	16,1; 16,0	15,9; 16,0
19	15,3; 15,4	15,2; 15,1	15,2; 15,2
20	75,1; 74,0	86,1; 86,2	75,1; 73,9
21	25,0; 26,4	23,7; 23,3	24,7; 25,2
22	37,0; 38,3	35,9; 36,2	40,5; 41,7
23	29,4; 30,6	26,9; 26,8	22,5; 23,2
24	76,4; 76,3	83,4; 84,1	124,7; 126,0
25	147,8; 150,3	71,5; 71,1	131,3; 130,5
26	110,9; 110,2	27,6; 26,0	25,7; 26,1
27	17,6; 18,1	24,4; 26,8	17,6; 17,7
28	26,9; 26,8	26,3; 26,8	26,6; 26,7
29	21,1; 21,2	21,1; 21,1	21,0; 21,0
30	16,5; 16,7	16,4; 16,4	16,2; 16,6

*First value, in $CDCl_3$; second, in C_5D_5N.

TABLE 2. ^{13}C Chemical Shifts of the C Atoms of the Side Chain of the Saponin (IV) and of 24-Hydroxycholesterol (V) (ppm relative to TMS)

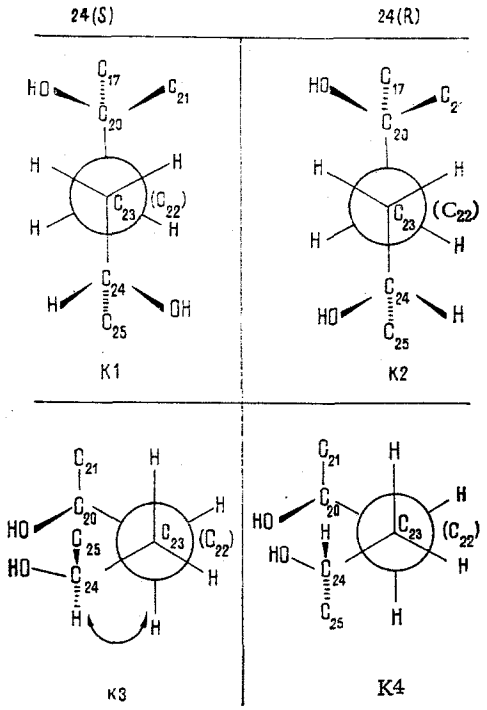
Series	C atom							
	20	21	22	23	24	25	26	27
	Compound (IV)							
24(S)	72,9	27,0	32,5	30,4	76,2	150,1	110,0	18,2
24(R)	73,2	27,0	32,2	30,0	75,9	150,1	109,7	18,6
	Compound (V)							
24(S)	35,8	19,0	32,1	30,6	77,0	33,0	16,7	18,8
24(R)	35,6	18,8	31,9	30,4	76,6	33,4	17,2	18,6

Note. The spectrum of (IV) was taken in C_5D_5N and that of (V) in $CDCl_3$.

(Table 2), but the authors concerned incorrectly explained this by $\delta_{H,H}$ coupling [18] between H_{20} and H_{24}, which was basically rejected in the paper that they cited.

On the basis of what has been said, the ^{13}C CSs of the C atoms of the side chain of saponin (IV) in the upper part of Table 2 can be assigned to the 24(S) series and those in the lower part to the 24(R) series. By then comparing the CSs of C_{24} in the ^{13}C spectra of the saponin (IV) and the triterpene (I) it is possible to deduce the S-configuration of the C_{24} asymmetric center in triterpene (I). Unfortunately, it is impossible to carry out a similar comparison of the C_{22} CSs, since the C_{22} resonance in the ^{13}C spectrum of the saponin (IV) appears in a considerably weaker field than for the triterpene (I), because of the formation in the saponin (IV) of a strong intramolecular hydrogen bond between the C_{20}-OH and the C_{12}-β-OGlc, which, as shown previously [8], has a particular effect on the C_{22} resonance.

It is not excluded that the approach described above to determining the configuration of the C_{24} asymmetric center in an open side chain of 24-hydroxy triterpene derivatives from their ^{13}C spectra can be used not only for 24-hydroxy sterols [11], but also for aliphatic diastereomeric alcohols of the type of (VI) [19], in which, even when there is no intramolecu-



lar hydrogen bond, conformations of types K3 and K4 can be realized with a fairly high relative population.

Thus, the triterpene (I) apparently has the structure of 20(S),24(S)-dihydroxygammer-25-en-3-one.

EXPERIMENTAL

The IR spectra were recorded on a Specord 751R spectrophotometer in $CHCl_3$ solution. The mass spectra were obtained on a LKB 9000 spectrometer at an ionizing voltage of 70 V. The ^1H and ^{13}C NMR spectra were measured on a Bruker HX-90E instrument using 0.5% solutions of the triterpene (I) in $CDCl_3$ and 5% solutions in C_5D_5N, with TMS as internal standard. The chemical shifts (CSs) are expressed on the δ scale. The accuracy of measurement was ±0.15 Hz for ^1H and ±1.5 Hz for ^{13}C. The assignment of the signals in the ^{13}C spectra of triterpene (I) was carried out by the method of off-resonance spin decoupling and by the method of selective double heteronuclear resonance on the basis of the PMR results. Since C_5D_5N exerts a considerable influence on the CSs of the C atoms of the side chain of triterpene (I), for the purposes of better comparison the ^{13}C CSs of triterpenes I-III are given for both solvents (Table 1).

The individuality of the substances was checked by TLC on silica gel (KSK) in the chloroform–ethanol (10:1) and petroleum ether–acetone (2:1) systems. To reveal the triterpenes on the chromatograms we used a 10% solution of H_2SO_4 in methanol.

Isolation of the Triterpene (I). At room temperature, 17.6 kg of the air-dry leaves of *Betula mandschurica* (collected in June, 1978, in the neighborhood of the R. Narva, Khasan region, Maritime Territory) was exhaustively extracted with diethyl ether. The extract was evaporated to dryness, and the subsequent treatment of the residue was carried out by the method for Fischer and Seiler [20]. The unsaponifiable fraction of the ethereal extract (186 g) was chromatographed on a column of SiO_2 with elution by benzene–chloroform systems. In addition to known triterpenes — betulafolienetriol [20], betulafolienetriol oxide [7], and 12β,20(S)-dihydroxydammer-24-en-3-one [7] — 162 mg of the triterpene (I) was eluted by the benzene–chloroform (5:1) system. After crystallization from petroleum ether–acetone (1:1), 116 mg was obtained of the chromatographically homogeneous triterpene (I) having the composition $C_{30}H_{48}O_3$, mp 216-218°C, $[\alpha]_D^{20}$ +44° (c 0.5 $CHCl_3$).

^1H spectrum ($CDCl_3$, ppm): 0.89 (s, 3 H), 0.94 (s, 3 H), 1.00 (s, 3 H), 1.04 (s, 3 H), 1.08 (s, 3 H), 1.15 (s, 3 H, C_{20}-CH_3), 1.74 (s, 3 H, C_{25}-CH_3), 3.96 (t, 1 H, J = 5.2 Hz, H_{24}), 4.84 (broadened singlet, 1 H, H_{26}), and 4.96 (s, 1H, H_{26}).

Mass spectrum, m/e (%): 441 (1.3), 440 (2.3), 438 (0.6), 359 (1.9), 316 (2.5), 315 (2.3), 313 (2.4), 245 (3.2), 206 (3.2), 205 (7.7), 175 (2.5), 163 (4.2), 161 (4.2), 149 (6.5), 143 (12.3), 125 (100.0), 107 (18.8), 95 (19.4), 81 (20.3), 43 (41.2), 41 (14.2).

SUMMARY

1. A new triterpene of the dammarane series has been isolated from the unsaponifiable fraction of an ethereal extract of the leaves of *Betula mandschurica* which apparently has the structure of 20(S),24(S)-dihydroxydammar-25-en-3-one (I).

2. The approach to the determination of the configuration of the C_{24} asymmetric center in 24-hydroxy derivatives of tetracyclic triterpenes with an open side chain is proposed on the basis of the ^{13}C NMR spectra.

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