Chemistry of Natural Compounds and Bioorganic Chemistry

Triterpenoids from *Abies* sp.

16.* Preparation and crystal structure of a new trinorlanostanoid

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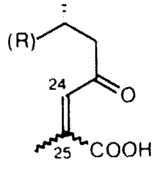
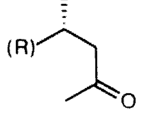
The structure of the molecule of a new trinorlanostane hydroxyketone obtained by alkali treatment of total triterpenoid acids from the ether extract of Siberian fir needles was established using NMR spectra and X-ray analysis.

Key words: triterpenoids; Siberian fir; retroaldol cleavage; two-dimensional NMR; X-ray analysis.

The known triterpenoids of woody plants of *Abies* Hill. genus (fir) make up a rather large group of neutral compounds (lactones) and carboxylic acids with lanostane, cycloarthane, and modified lanostane carbon skeletons.[2,3] The isolation of lactones is usually performed using the routine chromatographic procedures,[3,4] and the analysis of acidic triterpene metabolites, contained primarily in coniferous needles, is laborious due to the complexity of the mixture, similarity of their chromatographic properties, and the presence of interconverting pairs of (24*Z*)- and (24*E*)-isomers.[5,6] We found[7] the fragmentation reaction of the side chain of these acids, containing the common photolabile fragment of α-methyl-β-acylacrylic acid, which makes possible the selective transformation of monocarboxylic acids with fragment **A** to the neutral methyl ketones with fragment **B** (where R is the polycyclic part of the steroid molecule). Applying that procedure to a total acid mixture of Siberian fir (*Abies sibirica* Ledb.)[8], we obtained a mixture of neutral methyl ketones, in which we revealed, along with the major products formed from the known[2,6] triterpene acids with fragment **A**, a new methyl ketone arising from yet undescribed, biogenetically interesting native triterpene acids of this natural compound.



A



B

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* For Part 15 see Ref. 1.

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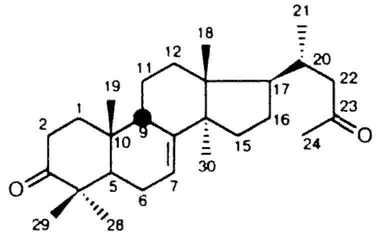
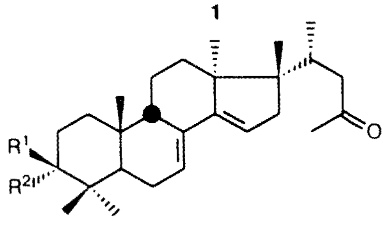
Since acids with (24*Z*)- as well as (24*E*)-configuration of the double bond in the side chain of their molecules undergo alkali-induced fragmentation,[7] the isolation of only "strong" acids[8] becomes unsuitable and we used the total amount of acids of the ethereal extract of needles. The reaction conditions were modified *vs.* those described in Ref. 7: a 5 % concentration of NaOH in EtOH was found to be quite sufficient, and the temperature was decreased to 60 °C. After 6 h, the total yield of methyl ketones was 60%. Chromatography of this mixture on SiO_2 gave a series of fractions, the major ones containing known compounds **1**—**3**.[7,9] In one of intermediate fractions, the easily crystallized new methyl ketone (**4**) was present; its content in the starting mixture was *ca.* 5% (GLC).

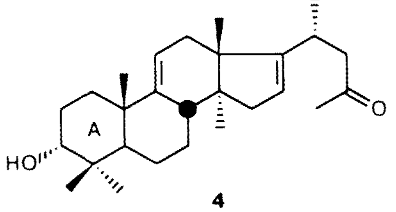


1



2: $R^1 + R^2 = O$

3: $R^1 = H$; $R^2 = OH$



4

The structure of the new methyl ketone **4** was established using ^1H and ^{13}C NMR spectral data (Table 1), two-dimensional spectra $^1H—^1H$ and $^{13}C—^1H$ NMR (COSY), and $^{13}C—^1H$ (COLOC) NMR. The following cross-peaks in the $^1H—^1H$ COSY are important for interpretation of one-dimensional NMR spectra: $CH_3(19)/H(1\alpha)$, $CH_3(18)/H(12\alpha)$, $CH_3(28)/H(3)$, $CH_3(30)/H(15\beta)$, $H(15\beta)/H(20)$. The ^{13}C NMR signal at 50.88 ppm was assigned to the C(13) atom on the basis of the presence of cross-peaks C(13)/H(11) and C(13)/H(20) in the COLOC spectrum. The NMR spectra were recorded for the solution in a mixture of C_6D_6 and $CDCl_3$ (1:1 vol.), because in the $CDCl_3$ solution the H(20) and H(22a) signals in the ^1H NMR spectrum coincide. It should be noted that the H(8) and H(12α) signals overlap for $CDCl_3$ as well as for a mixture of $CDCl_3$ and C_6D_6.

Table 1. ^{13}C and ^1H NMR spectral data on compound **4** ($CDCl_3$ + C_6D_6 (1:1), tetramethylsilane, δ, *c* = 40 mg/mL)

Atomic number	δ_C	δ_H
1	30.37 t	1.38; 1.80
2	25.61 t	1.52; 1.84
3	75.66 d	3.22 t
4	37.63 s	—
5	46.43 d	1.33
6	20.98 t	1.38; 1.50
7	27.86 t	1.37; 1.59
8	39.92 d	2.31
9	149.45 s	—
10	39.46 s	—
11	114.02 d	5.27 br.d
12	31.06 t	1.68 (H(12β)); 2.32 (H(12α))
13	50.79 s	—
14	46.62 s	—
15	40.66 t	1.79 dd (H(15α)); 2.03 br.d (H(15β))
16	120.15 d	5.12 ddd
17	155.65 s	—
18	19.29 q	0.77 s
19	21.75 q	0.99 s
20	27.84 d	2.61 br.sext.
21	20.87 q	0.96 br.d
22	49.98 t	2.21 dd (H(22b)); 2.44 dd (H(22a))
23	206.50 s	—
24	29.84 q	1.81 s
28	28.13 q	0.85 s
29	22.31 q	0.76 s
30	19.83 q	0.82 s

Note. J (Hz) in the ^1H NMR spectrum are as follows: $^3J_{3,2a} = {}^3J_{3,2b} = 2$; $^3J_{11,12\beta} = 5.8$; $^2J_{15\alpha,15\beta} = 15.0$; $^3J_{16,15\alpha} = 3.2$; $^3J_{16,15\beta} = {}^3J_{15\beta,20} = 1.5$; $^3J_{20,21} = 7.0$; $^2J_{22a,22b} = 16.5$; $^3J_{22a,20} = 6.5$; $^3J_{22b,20} = 8.0$.

Confirmation of structure **4** and the assignment of the relative configuration of its chiral centers at C(8) and C(20) was performed by X-ray analysis (Fig. 1). Rings A and B have chair conformations, and ring C has the half-chair conformation (the deviations of the C(13) and C(14) atoms from the plane of the double bond are 0.493(9) and — 0.367(9) Å, respectively). Ring D has the envelope conformation with the deviation of the C(14) atom from the plane of the double bond equal to 0.590(8) Å.

We cannot find structures with the same backbone in the Cambridge Crystallographic databank (version of 1991). However, three compounds without a double bond in the five-membered cycle in the polycyclic skeleton were found. The interatomic distances in molecule **4** are in good agreement with the corresponding bond lengths in these compounds.[10—12] The $C_{sp^3}—C_{sp^3}$ bond lengths in the polycyclic part of the molecule are within the 1.511 to 1.561 Å interval characteristic of strained

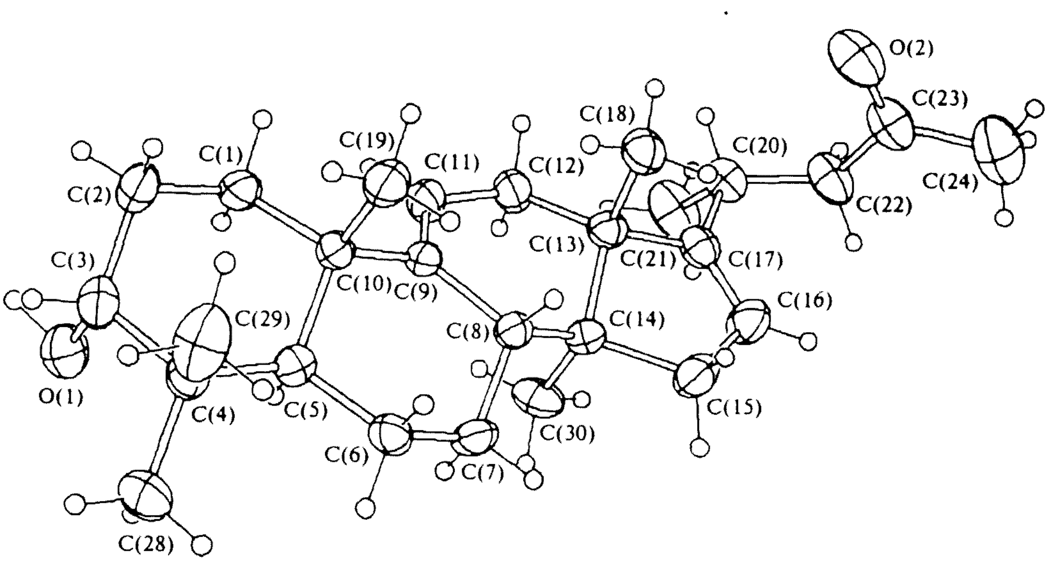


Fig. 1. Molecular structure of **4**.

steroid systems.[13] The other bond lengths were also close to the expected values.[14] The hydroxyl group in the crystal participates in the intermolecular hydrogen bond having the following parameters: O(1)...O(2) 2.857(6) Å, O(1)—H 0.98(8) Å, H...O(2) 1.88(8) Å, thus forming chains along the *b* axis.

At first glance, the chair-like conformation of ring A of the molecule of ketone **4** is in contradiction with the weak cross-peak of $CH_3(28)/H(3)$ observed in the two-dimensional $^1H-^1H$ NMR spectrum (COSY). We assigned the appearance of this cross-peak to the aforementioned interaction assuming that ring A of the ketone molecule **4** can accept another conformation ("twist") in solution, in which the H(3)—C(3) and C(4)—C(28) bonds are antiparallel, thus creating conditions for the appearance of spin coupling through four σ-bonds. The molecular mechanics calculations by the MMX program for the model compound (the substituent at C(17) in the molecule of ketone **4** is replaced by the isopropyl group) show that this conformation virtually exists; it is 2.5 kcal/mol less stable than the conformation with the chair-like ring A, and its percentage is *ca.* 1.5%. The calculated $\Delta H^{\neq}$ value for the conformational transition "chair" → "twist" is 9.2 kcal/mol.

The distinguishing feature of the structure of methyl ketone **4** is the presence of the double bonds at positions 9(11) and 16(17) of the steroid system. Whereas the first bond is present in the molecule of the lanostane hydroxylactone found in needles of *Abies alba* Mill.,[15] the second was found for the first time in the molecule of lanostane derivative from *Abies* species. As supposed, the biosynthesis of triterpenoids with a modified lanostane carbon skeleton includes the intermediate hypothetical carbocation bearing a positive charge at the C(17) atom.[16] The existence of methyl ketone **4** with the double bond at C(16)—C(17) in its molecule can be considered as an indirect confirmation of this hypothesis.

Experimental

Melting points were determined with a Kofler block. The IR spectrum was recorded with a UR-20 instrument. The NMR spectra were recorded with a Bruker AM-400 using standard Bruker programs (for recording two-dimensional COSY and COLOC spectra). MS (EI, 70 eV) was obtained with a Finnigan MAT 8200 instrument. Optical rotations were measured with a Polamat A polarimeter (at 580 nm) in $CHCl_3$. GLC analyses were carried out with a Chrom-5 chromatograph (carrier gas N_2, 5% SE-30 on Chromaton N, 2500×3 mm column, linear temperature program from 210 to 265 °C (2 deg/min)). Chromatography was carried out on KSK silica gel, the substance:sorbent ratio was 1:20, and hexane—ether (9:1 → 3:2) gradient elution was used.

The total acidic ethereal extract of needles of Siberian fir collected near Novosibirsk city in December, 1994 was prepared as described in Ref. 8, mixing together "weak" and "strong" acids.

Preparation and separation of a mixture of methyl ketones. The total acidic ethereal extract of needles (6.92 g) was dissolved in 5 % ethanolic NaOH (70 mL), and the solution was heated at 60 °C for 6 h. The solution was cooled to 20 °C, diluted with water (400 mL), and the product was extracted with ether (2×200 mL). The extract was washed with water and dried over Na_2SO_4. After removal of the solvent, a mixture of neutral compounds (4.12 g) was obtained; it was separated by chromatography into four fractions (yields 0.41, 0.42, 0.48, and 2.50 g, respectively). The first and last fractions contained compounds **1** and **3** as the major components, respectively, and the second and third contained compounds **2** and **4** and other methyl ketones. Crystallization of the second fraction from hexane afforded compound **4** (0.08 g).

The relative GLC retention times of methyl ketones **1**, **2**, **3**, and **4** are 1.00, 0.95, 0.96, and 0.93, respectively.

3α-Hydroxy-(20*R*)-25,26,27-trinor-8β-lanosta-9(11),16-dien-24-one (4). Crystals, m.p. 181—182 °C, $[\alpha]^{20}_{580}$ +33.7° (*c* 0.95). IR ($CHCl_3$), ν/cm^{-1}: 1710 (C=O), 1620, 3030 (C=C), and 3624 (OH). The ^{13}C and ^1H NMR spectra are presented in Table. 1. MS (*m/z*): 398.31814 $[M]^+$. Calculated for $C_{27}H_{42}O_2$: 398.31846.

Table 2. Coordinates (× 10^4) and equivalent thermal factors (× 10^3/Å) of nonhydrogen atoms of compound **4**

Atom	x	y	z	U_{eq}
C(1)	117(7)	5873(4)	3412(2)	58(2)
C(2)	−492(8)	5994(5)	3925(2)	66(2)
C(3)	1165(9)	6009(5)	4255(2)	66(2)
C(4)	2584(8)	6931(5)	4130(2)	62(2)
C(5)	3100(7)	6850(4)	3602(2)	47(1)
C(6)	4535(7)	7737(4)	3443(2)	52(1)
C(7)	5261(7)	7463(5)	2957(2)	58(2)
C(8)	3739(7)	7386(4)	2584(2)	43(1)
C(9)	2184(6)	6592(4)	2752(2)	42(1)
C(10)	1443(7)	6817(4)	3249(2)	45(1)
C(11)	1405(8)	5830(4)	2453(2)	59(2)
C(12)	1790(9)	5739(4)	1932(2)	61(2)
C(13)	2780(7)	6828(4)	1768(2)	47(1)
C(14)	4458(6)	7039(4)	2098(2)	47(1)
C(15)	5554(8)	7933(5)	1813(2)	67(2)
C(16)	5140(8)	7572(5)	1313(2)	68(2)
C(17)	3683(8)	6893(4)	1281(2)	54(1)
C(18)	1342(8)	7811(4)	1771(2)	63(2)
C(19)	348(8)	7967(4)	3207(2)	64(2)
C(20)	2811(9)	6353(4)	851(2)	61(2)
C(21)	3419(13)	5096(5)	796(2)	98(2)
C(22)	3309(11)	7002(5)	404(2)	77(2)
C(23)	2338(11)	8143(5)	363(2)	72(2)
C(24)	3244(15)	9028(6)	66(3)	120(3)
C(28)	4348(10)	6715(8)	4431(2)	89(2)
C(29)	1816(13)	8102(5)	4287(2)	92(2)
C(30)	5654(8)	5946(5)	2134(2)	69(2)
O(1)	2063(6)	4897(4)	4245(1)	80(1)
O(2)	869(7)	8325(4)	556(1)	90(2)

X-ray analysis of compound **4** was carried out with a Syntex P2_1 diffractometer (Cu-Kα radiation, graphite monochromator). The crystals were of rhombic syngony: a = 7.220(1), b = 11.657(2), c = 28.403(6) Å, V = 2390.5(7) Å^3, space group $P\,2_12_12_1$, Z = 4, $C_{27}H_{42}O_2$, μ = 0.512 mm^{-1}, d_{calc} = 1.108 g/cm^3. The intensities of 1903 independent reflections with $2\theta < 115°$ were measured by $\theta/2\theta$-scanning of the crystal (0.06×0.35×0.56 mm^3). After correction on the absorption and the real form of the crystal (transmission 0.83—0.97) using the DIFABS program (absorption 0.75—1.19), the structure was solved using the SHELX-86 program by the direct method, and refinement was carried out by the least-square method in full-matrix anisotropic-isotropic (for hydrogen atoms) approximation using the SHELXL-93 program to wR_2 = 0.1339, S = 1.0 for all reflections (R = 0.0497 for 1248 reflections with $I > 2\sigma$). The positions of hydrogen atoms were calculated geometrically. The coordinates of nonhydrogen atoms are presented in Table. 2.

References

1. V. A. Raldugin, M. M. Shakirov, and S. A. Shevtsov, *Sib. Khim. Zhurn.* (*Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk*), 1992, No. 3, 65 (in Russian).
2. V. A. Raldugin and S. A. Shevtsov, *Khim. Prir. Soedin.*, 1990, 443 [*Chem. Nat. Comp.*, 1990, 373 (Engl. Transl.)].
3. R. Tanaka and S. Matsunaga, *J. Nat. Prod.*, 1991, **54**, 1337.
4. A. F. Barrero, J. F. Sanchez, E. J. Alvarez-Manzaneda, M. Munoz, and A. Haidour, *Phytochemistry*, 1992, **31**, 615.
5. S. A. Shevtsov and V. A. Raldugin, *Khim. Prir. Soedin.*, 1988, 364 [*Chem. Nat. Comp.*, 1988, 426 (Engl. Transl.)].
6. V. A. Raldugin, M. M. Shakirov, T. V. Leibyuk, and S. A. Shevtsov, *Khim. Prir. Soedin.*, 1991, 511 [*Chem. Nat. Comp.*, 1991, 444 (Engl. Transl.)].
7. V. A. Raldugin, S. A. Shevtsov, and T. V. Leibyuk, *Khim. Prir. Soedin.*, 1991, 206 [*Chem. Nat. Comp.*, 1991, 176 (Engl. Transl.)].
8. V. A. Raldugin, S. A. Shevtsov, N. I. Yaroshenko, Yu. V. Gatilov, I. Yu. Bagryanskaya, L. I. Demenkova, and V. A. Pentegova, *Khim. Prir. Soedin.*, 1987, 824 [*Chem. Nat. Comp.*, 1987, 684 (Engl. Transl.)].
9. V. A. Raldugin, M. M. Shakirov, Yu. V. Gatilov, and S. A. Shevtsov, *Sib. Khim. Zh. (Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk)*, 1992, No. 2, 101 (in Russian).
10. L. G. Vorontsova, *Zh. Strukt. Khim.*, 1979, **20**, 882 [*J. Struct. Chem. USSR.*, 1979, **20** (Engl. Transl.)].
11. V. M. Tseikinskii, V. B. Rybakov, V I. Simonov, A. V. Kamernitskii, V. N. Ignatov, and I. S. Levina, *Bioorgan. Khim.*, 1980, **6**, 752 [*Sov. J. Bioorg. Chem.*, 1980, **6** (Engl. Transl.)].
12. Yu. V. Gatilov, I. Yu. Bagryanskaya, V. I. Lutskii, A. S. Gromova, and A. A. Semenov, *Khim. Prir. Soedin.*, 1987, 533 [*Chem. Nat. Comp.*, 1987 (Engl. Transl.)].
13. J. E. Berg, B. Karlson, A.-M. Pilotti, and A.-C. Soderholm, *Acta Crystallogr.*, 1977, **B33**, 2927.
14. F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen, and R. Taylor, *J. Chem. Soc. Perkin Trans. 2*, 1987, S1.
15. J.-C. Muller and G. Ourisson, *Phytochemistry*, 1974, **13**, 1615.
16. S. Hasegawa, T. Miura, N. Kaneko, Y. Hirose, and Y. Iitaka, *Tetrahedron*, 1987, **43**, 1775.

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