Triterpenoids from *Abies* species

20.* Identification of new trinortriterpene methyl ketones in the products of alkaline degradation of total acids of *Abies sibirica* needles

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Four new compounds were identified in a mixture of tetracyclic methyl ketones obtained by alkaline cleavage of acids of Siberian fir needles. Two of them correspond to natural acids of the 17,13-fridolanostane series of yet unknown structure.

Key words: triterpenoids; Siberian fir; two-dimensional NMR.

Previously,[2] we described a new approach to the analysis of a complex natural mixture of triterpene acids from Siberian fir needles. This approach involves an alkaline treatment affording a simpler mixture of the corresponding neutral 25,26,27-trinorderivatives (methyl ketones), and each ketone corresponds to a pair of interconverting isomeric triterpene acids with the α-methyl-β-acylacrylic group in the molecule. The mixture of methyl ketones thus obtained contained at least 10 components (GLC), and four of them, *viz.*, compounds **1—4**, are known.[2] In a continuation of the study of the composition of this mixture, we isolated its new components, diketone **5** and hydroxy ketones **6** and **7** using adsorption chromatography on SiO_2 and SiO_2—5% $AgNO_3$. In addition, using GLC and the NMR spectrum, we identified diketone **8**, which is difficult to separate from the impurity of compound **4** described previously. This diketone is identical to the product of oxidation of hydroxy ketone **6** with pyridinium chlorochromate.

Hydroxy ketone **6** was identified by comparison of its ^{13}C (Table 1) and ^{1}H NMR spectra with the spectra of the known[3] ester **9**; the TLC, GLC, and ^{1}H NMR spectroscopic data for diketone **5** coincided with those for a sample of this compound prepared by alkaline cleavage of the known methyl ester **10** (see Ref. 4).

Triterpene acids, which correspond to isolated trinorderivatives **5** and **6**, are known as components of needles of *Abies sibirica*[4] and seeds of *A. firma*,[3] respectively. The natural precursor of diketone **8** studied has not yet been found among the triterpene acids of the *Abies* species.

The structure of hydroxy ketone **7** is proposed on the basis of spectral data. According to the high-resolution mass spectrum, it is the isomer of methyl ketone **6**. Its molecule contains two carbon—carbon double bonds (^{13}C NMR spectrum), which are not conjugated according to the UV spectrum. The ^{13}C and ^{1}H NMR spectral data (Table 2) are interpreted using $^{1}H—^{1}H$ (COSY), and $^{13}C—^{1}H$ (COSY, COLOC) NMR. Thus, in the two-dimensional $^{1}H—^{1}H$ (COSY) NMR spectrum, along with cross-peaks arising due to proton coupling through two and three σ-bonds, the following cross-peaks caused by long-range spin-coupling were observed: H(3)/H(1β), H(3)/C(28)H_3, H(1α)/C(19)H_3, C(7)H_2/H(11a), C(7)H_2/H(11b), C(30)H_3/H(15β), C(18)H_3/H(16α), and C(21)H_3/H(22a). In the COLOC spectrum, the following cross-peaks were observed: 3 H(18)/C(16), C(17), C(20); 3 H(19)/C(1), C(5), C(9), C(10), C(11); 3 H(28)/C(4), C(5), C(29); 3 H(29)/C(4), C(5), C(28); and 3 H(30)/C(8), C(13), C(14), C(15).

Treatment of hydroxy ketone **6** with HCl in MeOH—$CHCl_3$ results in gradual isomerization of **6** into **7** (^{1}H NMR).

Table 1. ^{13}C NMR spectra of compounds **6** and **9** (δ, *C* = 20 mg mL^{-1})

Atom	6	9
C(1)	29.22 t	29.4
C(2)	25.29 t	25.4
C(3)	76.42 d	76.5
C(4)	36.92 s	37.0
C(5)	37.98 d	38.0
C(6)	23.12 t	23.2
C(7)	122.13 d	122.5
C(8)	145.94 s	146.0
C(9)	50.93 d	51.0
C(10)	34.71 s	34.8
C(11)	28.05 t	28.1
C(12)	118.62 d	118.7
C(13)	156.02 s	156.0
C(14)	49.88 s	50.0
C(15)	36.68 t	36.8
C(16)	38.15 t	38.4
C(17)	46.27 s	46.4
C(18)	24.77 q	24.8
C(19)	22.06 q	22.2
C(20)	38.40 d	38.7
C(21)	15.60 q	15.8
C(22)	47.45 t	48.6
C(23)	209.20 s	202.4
C(24)	30.07 q	133.0
C(28)	28.01 q	28.1
C(29)	22.85 q	23.0
C(30)	25.83 q	25.8

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* For Part 19, see Ref. 1.

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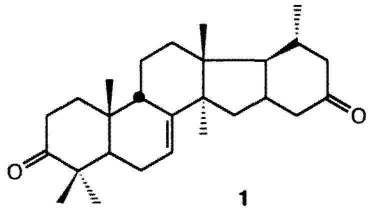

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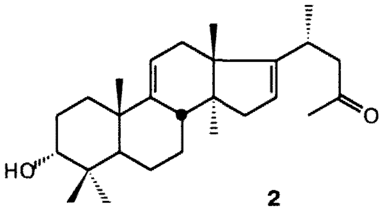

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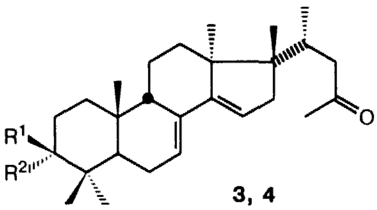
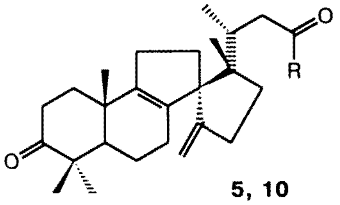


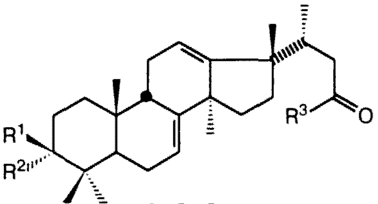
3, 4

3: R^1 = H; R^2 = OH
4: R^1 + R^2 = O



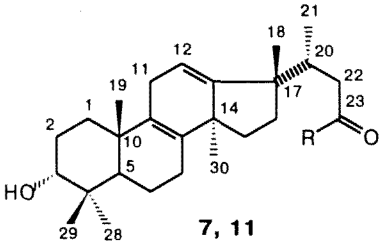
5, 10

5: R = Me
10: R = CH=C(Me)COOMe-(*Z*)



6, 8, 9

6: R^1 = H; R^2 = OH; R^3 = Me
8: R^1 + R^2 = O; R^3 = Me
9: R^1 = H; R^2 = OH; R^3 = CH=C(Me)COOMe-(*E*)



7, 11

7: R = Me
11: R = CH=C(Me)COOH

Table 2. ^{13}C and ^{1}H NMR spectra of compound **7** (δ, *J*/Hz, *C* = 54 mg mL^{-1})

Atom number	δ_C	δ_H
1	29.46 t	1.49* (H(1β)); 1.53* (H(1α))
2	25.64 t	1.60*; 1.94 m
3	75.81 d	3.416 (t, ^3J = 2.5)
4	37.43 s	—
5	44.65 d	1.51*
6	18.22 t	1.45*; 1.61*
7	25.49 t	2.06*; 2.09*
8	134.58 s	—
9	136.33 s	—
10	36.88 s	—
11	26.18 t	2.49 (ddt, H(11b)); 2.54 (ddt, H(11a), ^2J = 20.0, $^3J_{11a,12}$ = 6.0; $^5J_{11a,7a}$ = $^5J_{11a,7b}$ =1.6, $^5J_{11b,7a}$ = $^5J_{11b,7b}$ = 3)
12	116.30 d	5.366 (dd, $^3J_{12,11a}$ = 6.0, $^3J_{12,11b}$ = 2.2)
13	155.29 s	—
14	47.59 s	—
15	34.77 t	1.50* (H(15α)); 1.52* (H(15β))
16	33.90 t	1.26 (ddd, H(16β), ^2J = 12.0, ^3J = 6.0, ^3J = 1.6); 1.74 (dt, H(16α), ^2J = 12.0, ^3J = 7.5)
17	46.79 s	—
18	26.60 q	0.911 (d, $^5J_{18,16\alpha}$ = 0.7)
19	18.95 q	0.915 (d, $^5J_{19,1\alpha}$ = 0.5)
20	38.01 d	2.08*
21	15.92 q	0.84 (dd, ^3J = 6.5, $^4J_{21,22a}$ = 1.2)
22	47.19 t	2.14* (H(22b)); 2.70 (m, H(22a))
23	209.63 s	—
24	30.23 q	2.10 s
28	27.89 q	0.96 s
29	22.05 q	0.85 s
30	23.46 q	0.89 (d, $^5J_{30,15\beta}$ = 0.4)

* These values were obtained from the two-dimensional ^{13}C—^{1}H COSY NMR spectrum.

Natural acid **11**, the hypothetical precursor of methyl ketone **7**, has not yet been found among the acids from *Abies sibirica* needles. The percentages of methyl ketones **1—8** in the initial mixture are 17, 5, 32, 7, 4, 16, 2, and 4%, respectively. Other components of the mixture have not been isolated yet.

Experimental

The melting points were measured with a Kofler block. The IR spectra were recorded with a UR-20 spectrometer, and the UV spectrum of compound **7** was recorded with a Specord UV-VIS spectrophotometer. The NMR spectra were recorded with a Bruker AM-400 instrument in $CDCl_3$ (tetramethylsilane was used as the internal standard) using standard Bruker programs for recording two-dimensional COSY and COLOC spectra. The EI mass spectra (70 eV) were obtained with a Finnigan MAT 8200 instrument. Optical rotations were measured with a Polamat A polarimeter at 580 nm in $CHCl_3$.

GLC was 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^{-1}).

Chromatography was carried out on KSK silica gel, the substance : sorbent ratio was *ca.* 1:20, and a hexane—ether mixture was used as the eluent (from 10 to 40% of Et_2O). Silufol plates were used for TLC, and the compounds were detected by spraying with concentrated H_2SO_4. The initial total mixture of methyl ketones was prepared from the acidic fraction of the extract of Siberian fir needles using the procedure described previously.[2]

Separation of methyl ketones. The mixture of methyl ketones (4.83 g) was chromatographed on SiO_2 affording fractions I—V (0.81, 0.75, 0.43, 2.38, and 0.39 g, respectively). Fraction I is pure methyl ketone **1**. Compound **5** (0.19 g) and a mixture of components **4** and **8** (0.51 g, 2:1, according to ^1H NMR) were isolated by repeated chromatography of fraction II. Crystallization of fraction III from hexane gave compound **2** (0.24 g), and chromatography of the mother liquor yielded methyl ketone **7** (0.10 g). Chromatography of fraction IV on SiO_2 impregnated with $AgNO_3$ (5%) gave successively compound **3** (1.56 g) and compound **6** (0.78 g) (a 9:1 chloroform—hexane mixture was used as the eluent). Fraction V contained unidentified components.

Diketone 5. Crystals, m.p. 117.5—118 °C (hexane), $[\alpha]^{20}_{580}$ +137.3° (*c* 0.67). Mass spectrum, *m/z* (I_{rel} (%)): 396.30336 $[M]^+$ (66). Calculated for $C_{27}H_{40}O_2$: 396.31846. ^1H NMR, δ: 0.83 (d, 3 H, $C(21)H_3$, $J_{20,21}$ = 6.0 Hz); 0.86, 1.03, 1.06, and 1.10 (all s, 4 Me); 2.08 (s, 3 H, $C(24)H_3$); 4.48 and 4.73 (both narrow m, 1 H, $H_2C{=}C$).

Diketone **5** was prepared from ester **10** according to the published procedure[4] in a 90% yield.

Hydroxy ketone 6. Oil, $[\alpha]^{15}_{580}$ −130.7° (*c* 2.74). IR (CCl_4), ν/cm^{-1}: 1720 (C=O); 3620 (OH). Mass spectrum, *m/z* (I_{rel} (%)): 398.31736 $[M]^+$ (63). Calculated for $C_{27}H_{42}O_2$: 398.31846. ^1H NMR, δ: 0.85 (d, 3 H, $C(21)H_3$, $J_{20,21}$ = 6.0); 0.897, 0.904, 0.907, 0.95, and 1.15 (all s, 5 Me); 2.08 (s, 3 H, $C(24)H_3$); 2.75 (br.d, 1 H, H(22a), $J_{22a,22b}$ = 12); 3.49 (narrow m, 1 H, H(3)); 5.44 (dd, 1 H, H(12), *J* = 8.4 and 2.4 Hz); 5.61 (br.d, 1 H, H(7), *J* = 5.5 Hz). The ^{13}C NMR spectral data are presented in Table 1.

Hydroxy ketone 7. Oil, $[\alpha]^{15}_{580}$ +36° (*c* 0.5). IR (CCl_4), ν/cm^{-1}: 1710 (C=O); 1620, 3030 (C=C); 3630 (OH). UV (EtOH): no significant absorption in the region from 210 to 400 nm. Mass spectrum, *m/z* (I_{rel} (%)): 398.31657 $[M]^+$ (28). Calculated for $C_{27}H_{42}O_2$: 398.31846. The ^{13}C and ^1H NMR spectral data are presented in Table 2.

Synthesis of diketone 8. Pyridinium chlorochromate (0.20 g) was added to a solution of hydroxy ketone **6** (0.04 g) in CH_2Cl_2 (2 mL) at ambient temperature and the mixture was stirred at 20 °C for 24 h. After the usual workup, compound **8** (0.03 g) was isolated as an oil. ^1H NMR, δ: 0.86 (d, 3 H, $C(21)H_3$, $J_{20,21}$ = 6.5 Hz); 0.90, 1.06, 1.07, 1.08, and 1.16 (all s, 5 Me); 2.08 (s, 3 H, $C(24)H_3$); 2.67—2.76 (m, 2 H, $C(2)H_2$); 5.44 (dd, 1 H, H(12), *J* = 8.5 and 3.5 Hz); 5.65 (m, 1 H, H(7)).

Isomerization of compound 6. Three drops of a saturated solution of HCl in MeOH were added to a solution of hydroxy ketone **6** (0.13 g) in $CHCl_3$ (5 mL), and the mixture was allowed to stay at *ca.* 20 °C in the dark for 4 h. After the usual workup and chromatography on SiO_2, a mixture of methyl ketones **6** and **7** (0.06 g) in the ratio of 2:1 (^1H NMR) and a mixture of two less polar products (0.05 g), whose IR spectrum have no bands of carbonyl groups and which are presumably ketals of compounds **6** and **7**, were obtained.

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