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TRITERPENES FROM BULBS OF *MUSCARI COMOSUM*, 2. THE STRUCTURE OF TWO NOVEL NORTRITERPENES[1]

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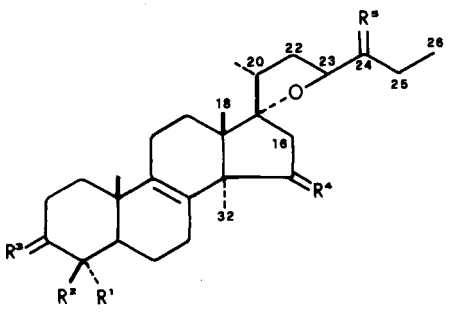
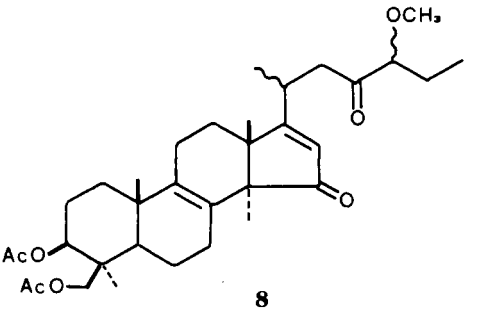
ABSTRACT.—The structure of two minor components of the free nortriterpene fraction isolated from bulbs of *Muscari comosum* has been established as (23*R*)-17,23-epoxy-3β,31-dihydroxy-27-nor-5α-lanost-8-ene-15,24-dione (**6**) and (23*R*)-17,23-epoxy-31-hydroxy-27-nor-5α-lanost-8-ene-3,15,24-trione (**7**).

The aglycone moieties obtained by the enzymatic hydrolysis of the glycoside mixture isolated from the bulbs of *Muscari comosum* Mill. have been assigned structures **1-4** (1-3), characterized by a spiro fused tetrahydrofuran ring in their norlanostane skeleton. From the bulbs, we have more recently isolated a free nortriterpene fraction (4), which consisted of **1, 2,** the new nortriterpene **5**, and four minor components. The structural elucidation of two of these latter components, **6** and **7**, is described in this paper.





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	R^1	R^2	R^3	R^4	R^5
1	23(S)CH_3	CH_2OH		O	O
2	23(S)CH_3	CH_2OH	O	O	O
3	23(S)CH_2OH	CH_2OH		H,H	O
4	23(S)CH_2OH	CH_2OH	O	H,H	O
5	23(S)CH_3	CH_2OH	O	O	
6	23(R)CH_3	CH_2OH		O	O
7	23(R)CH_3	CH_2OH	O	O	O
9	23(R)CH_3	CHO	O	O	O

Compound **6**, m.p. 196-8°, possesses the molecular formula $C_{29}H_{44}O_5$ (high resolution ms, *m/z* calcd 472.3189, found 472.3194). The fragmentation pattern was identical to that of **1**, except for minor differences in peak relative intensity. The ^{1}H-nmr signals (Table 1) also resembled those of **1**. Significant differences were observed only in the signals of 21-H_3, 20-H, 22-H_2, 23-H, and 25-H_2. In fact, the 21-H_3 doub-

[1]Part 1, see reference (4).

let and the 20-H multiplet were shifted upfield by about 0.2 ppm. The signal of the 23-H was shifted upfield by 0.4 ppm and appeared as a double doublet. The two protons at C-22 appear separately at δ 1.901 dd and δ 2.3 m. Furthermore, the signal of the 25-H_2 is shifted downfield by about 0.2 ppm and appears as the 16-line pattern of the AB part of a nearly first-order ABX_3 system (5). (In the spectrum of **1**, the corresponding signal appears as the A_2 q of an A_2X_3 system.) Correspondingly, in the ^{13}C-nmr spectrum of **6** (Table 2), the signals of 21-C, 32-C, and 24-C are shifted downfield by about 1 ppm and the signals of the 20- and 22-carbons, upfield by 2 and 0.7 ppm, respectively. These differences in ^{1}H- and ^{13}C-chemical shifts and ^{1}H-signal multiplicity of the nuclei of the D-ring, the tetrahydrofuran ring and the side chain (besides the similarity of the other spectral features, ms included, between **6** and **1**) suggested that the former differed from the latter in the configuration of C-23 and/or C-17. The fact that treatment with H^+/MeOH followed by acetylation converted both **6** and **1** into the same known acetate mixture of **8** (1) was in agreement with the above hypothesis.

Measuring a series of nuclear Overhauser effects (Table 3) allowed us to establish spatial proximity among some relevant protons in compound **6** and led us to the conclusion that this compound is actually the 23-epimer of **1**. In fact, results *a* and *c* (Table 3; see also perspective formulas in Figure 1) identified the signals of the 16-H_α (δ 2.658) and of the 16-H_β (δ 2.244). Thus, results *e* and *b* ruled out the possibility that, in **6**, the configurations both at C-17 and C-23 were inverted with respect to **1** (Fig. 1c), and results *f* and *d* led us to reject the hypothesis that **6** was the 17-epimer of **1** (Fig. 1b). On the contrary, every observed nOe accorded with the structure depicted in Figure 1a.

The occurrence of the same 23-epimeric relationship between **7** and **2** was easily shown. Compound **7**, m.p. 184-6°, possesses the molecular formula $C_{29}H_{42}O_5$ (high resolution ms, *m/z* calcd 470.3032, found 470.3026). The fragmentation pattern strictly resembled that of **2**. As shown by the data displayed in Table 1, the ^{1}H signals of **7** appear analogous to those of **2**, except for the 21-H_3, 20-H, 22-H_2, 23-H, and 25-H_2 signals. These are instead similar to those of **6**. Furthermore, the signals of the 19-H_3, 31-H_2, and 30-H_3 differ from those of **6** in the same way as the corresponding signals of **2** differ from those of **1**. Analogous observations may be made about the ^{13}C signals (Table 2) of **7** as compared with those of **2**, **6**, and **1**. This evidence strongly suggested that **7** was the 3-keto counterpart of **6**, *i.e.*, the 23-epimer of **2**. In fact, direct chemical correlation of **7** and **6** was readily achieved by oxidation of both compounds separately with pyridinium chlorochromate to the same aldehyde (**9**) (identical ^{1}H spectrum (Table 1) and tlc behavior).[2]

The fact that the isolated nortriterpenes are epimers at a center adjacent to a carbonyl group can lead one to suppose that **6** and **7** originate from the major components **1** and **2** by epimerization during the isolation process. However, both **1** and its 23-epimer, **6**, were recovered unaltered, both after they were left for 1 month on a silica gel column and after treatment in acid conditions (H_2SO_4/MeOH, pH 5) at room temperature for 1 month. On the other hand, occurrence of basic conditions during isolation is hardly assumable and, in any case, it is known that bases instead cause easy opening of the tetrahydrofuran ring (6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler block and are uncorrected. Mass spectra were measured on an AEI 902 instrument. ^{1}H- and ^{13}C-nmr spectra were recorded on a Fourier transform Brüker WH 270 spectrometer with Aspect 2000 computer with 48 K memory (32 K data).

[2]The ^{1}H-spectrum of **9** differs from that of its known 23-epimer (**6**) in the same way the spectra of **6** and **7** differ from those of **1** and **2**, respectively.

TABLE 1. ^{1}H-nmr (270 MHz) Chemical Shifts (Selected Data) in $CDCl_3$.[a]

	18-H_3	19-H_3	30-H_3	32-H_3	21-H_3	31-H_2	3-H	20-H	22-H_2	23-H	25-h_2	26-H_3	16-H_2
1[b]	0.931s	0.951s	1.267s	1.384s	1.125d $J_{20,21}$=6.62	3.375 4.242 ABq J_{AB}=11.27	3.468dd[c]	2.35m	1.950dd[d]	4.674t[e]	2.495q $J_{25,26}$=7.35	1.076t	2.207 2.774 ABq J_{AB}=19.42
2[f]	0.960s	1.088s	1.271s	1.407s	1.135d $J_{20,21}$=6.62	3.469 4.021 ABq J_{AB}=11.40		2.35m	1.949dd[g]	4.698t[h]	2.497q $J_{25,26}$=6.99	1.069t	2.227 2.798 ABq J_{AB}=19.12
6	0.938s	0.958s	1.260s	1.343s	0.903d $J_{20,21}$=6.99	3.353 4.221 ABq J_{AB}=11.40	3.448dd[i]	2.10m	22-H_S[m]:1.901dd $J_{22S,23}$=2.94 $J_{22S,22R}$=13.24 22-H_R[m]:2.3[j]	4.276dd[k] $J_{22S,23}$=2.94 $J_{22R,23}$=10.66	ν_A=2.667 ν_B=2.754 J_{AB}=18.02 J_{AX}=7.35 J_{BX}=7.35 [l]	1.087t	2.244(16-H_β)[m] 2.658(16-H_α)[m] J_{AB}=19.12
7	0.968s	1.081s	1.298s	1.369s	0.912d $J_{20,21}$=6.99	3.470 4.006 ABq J_{AB}=11.40		2.12m	22-H_S[n]:1.902dd $J_{22S,23}$=2.57 $J_{22S,22R}$=13.24 22-H_R[n]:2.3[j]	4.292dd[k] $J_{22S,23}$=2.57 $J_{22R,23}$=10.66	ν_A=2.677 ν_B=2.751 J_{AB}=18.36 J_{AX}=7.35 J_{BX}=7.35 [l]	1.091t	2.263(16-H_β)[n] 2.680(16-H_α)[n] ABq J_{AB}=19.12
9	0.983s	1.162s	1.307s	1.358s	0.915d $J_{20,21}$=6.99	−CHO: 9.721s		2.12m	22-H_S[n]:1.902dd $J_{22S,23}$=2.57 $J_{22S,22R}$=13.24 22-H_R[n]:2.3[j]	4.291dd[k] $J_{22S,23}$=2.57 $J_{22R,23}$=10.66	ν_A=2.669 ν_B=2.752 J_{AB}=18.02 J_{AX}=7.35 J_{BX}=7.35 [l]	1.089t	2.263(16-H_β)[n] 2.680(16-H_α)[n] ABq J_{AB}=19.12

[a]All chemical shift values are given in δ (ppm) relative to TMS. Coupling constants are given in Hz and were inferred from pertinent decoupling experiments.
[b]The spectrum of **1** (3,6) has been remeasured at 270 MHz in this occasion.
[c]X part of an ABX system ($J_{AX}+J_{BX}$=15.20).
[d]AA′ part of an AA′X system, further split (J=3.10) by coupling with 20-H.
[e]X part of an AA′X system ($J_{AX}+J_{A'X}$=18.92).
[f]The spectrum of **2** is taken from reference (4).
[g]AA′ part of an AA′X system, further split (J=3.31) by coupling with 20-H.
[h]X part of an AA′X system ($J_{AX}+J_{A'X}$=19.86).
[i]X part of an ABX system ($J_{AX}+J_{BX}$=15.81).
[j]Buried with the 16-H_β signal.
[k]X part of an ABX system (A=22-H_S, B=22-H_R).
[l]Sixteen lines of the AB part of a nearly first-order ABX_3 system (X_3=26-H_3) (5).
[m]Assignments by nOe experiments (Table 3).
[n]Assignments by analogy to **6**.

TABLE 2. ^{13}C-nmr (67.88 MHz) Chemical Shifts of Compounds **1**,**2**,**6**, and **7** in $CDCl_3$.[a]

Carbon	**1**	**2**	**6**	**7**
1	35.39	35.43	35.59	35.46
2	28.28	34.47	28.51	35.05
3	80.73	219.93	80.75	220.01
4	42.86	51.85	43.13	50.37
5	50.78	51.62	49.54	49.50
6	18.26	18.92	18.42	18.48
7	26.68	26.24	27.04	26.68
8	133.19	134.14	133.39	134.42
9	135.99	134.48	135.89	134.56
10	37.30	37.17	37.47	38.81
11	20.52	20.59	20.68	20.87
12	22.97	23.01	23.35	23.39
13	47.57	47.56	47.46	47.22
14	57.84	57.88	57.96	58.18
15	215.08	215.13	215.54	215.47
16	51.85	51.75	51.08	51.82
17	91.19	91.12	90.84	89.31
18	20.43	20.59	20.51	20.51
19	19.80	19.45	19.96	19.10
20	43.46	43.49	41.27	41.30
21	17.17	17.18	18.42	18.48
22	36.78	36.77	36.06	35.86
23	81.71	81.72	81.04	81.77
24	212.03	211.92	213.05	212.80
25	32.40	32.44	32.11	32.18
26	7.38	7.28	7.69	7.66
30	22.28	21.88	22.45	22.04
31	64.41	65.81	64.60	65.89
32	23.78	23.78	24.68	24.67

[a]Chemical shift values are given in δ (ppm) relative to TMS. The assignment of the signals of **6** and **7** are based on the comparison with the spectra (3) of **1** and **2**.

The nuclear Overhauser effect difference FIDs were obtained by gated decoupling (decoupler on for 10s before each scan) with a microprogram virtually identical with the one described in the Brüker Aspect 2000 NMR Softaware Manual 1 (4). For each measurement, 200 scans with irradiation off resonance were substracted from those with irradiation on resonance. The sample concentration was 2 mg in 0.5 ml $CDCl_3$ with TMS as internal standard.

TABLE 3. Nuclear Overhauser Effects Measured on **6** ($CDCl_3$).

Irradiate	Observe		
1.343 (32-H_3)	*a*	2.658	16-H_α
	b	4.273	23-H
0.938 (18-H_3)	*c*	2.244	16-H_β
	d	2.10	20-H
4.276 (23-H)	*e*	2.658	16-H_α
0.903 (21-H_3)	*f*	2.267	25-H_2
		2.754	
	g	1.901	22-H_S

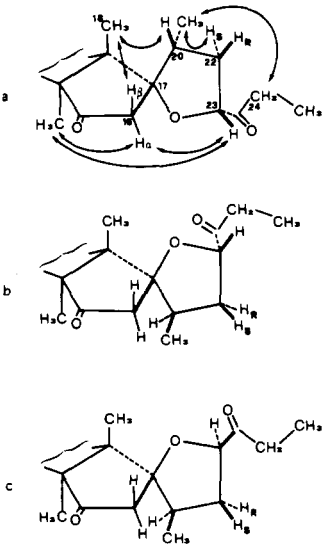

FIGURE 1. Perspective view of the D- and tetrahydrofuran-rings of compound **6**, with the configuration inverted at (a) C-23, (b) C-17, and (c) C-23 and C-17, with respect to **1**; nOe were measured (Table 3) between the protons indicated by the arrows.

ISOLATION.—Nortriterpenes **6** and **7** were obtained from fractions *b* and *a*, respectively, isolated from the bulbs of *M. comosum* by the procedure described previously (4).

Compound **6** was the major component isolated by tlc from fraction *b* (4). It had m.p. 196-8° (from Me_2CO); ms *m/z* 472.3194 (M^+, calcd for $C_{29}H_{44}O_5$ 472.3189), 454 (M^+-18, H_2O), 439 (M^+-33, H_2O+CH_3), 415 (M^+-57, CH_3CH_2CO), 414 (M^+-58, CH_3CH_2CHO), 371, 343, 303, 290, 181, 155; ^{1}H-nmr, Table 1; ^{13}C- nmr, Table 2.

Compound **7** was the major component isolated by tlc from the mother liquors of the crystallization of fraction *a* (4). It had m.p. 184-6° (from EtOH); ms *m/z* 470.3026 (M^+, calcd for $C_{29}H_{42}O_5$ 470.3032), 440 (M^+-30, CH_2O), 413 (M^+-57, CH_3CH_2CO), 412 (M^+-58, CH_3CH_2CHO), 341, 271, 155; ^{1}H-nmr, Table 1; ^{13}C-nmr, Table 2.

ACETATES (**8**).—Samples of **1** and **6** were subjected separately to methanolysis (2.5% HCl/MeOH, 4 h, reflux) and subsequently to acetylation (Ac_2O/Pyridine, 24 h, room temperature). Usual work-up gave, in both cases, the same known acetate mixture **8** (6).

ALDEHYDE (**9**).—Compound **6** (0.5 mg) was treated in dry CH_2Cl_2 (0.5 ml) with pyridinium chlorochromate (2 mg) for 6 h at room temperature. Filtration and evaporation afforded the aldehyde (**9**) (^{1}H-nmr, see Table 1). By the same treatment, compound **7** (0.5 mg) yielded the aldehyde (**9**), identical (tlc, ^{1}H-nmr) to the sample obtained above.

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