TRITERPENES FROM BULBS OF *MUSCARI COMOSUM*, 1. ISOLATION OF NORTRITERPENES AND STRUCTURE OF (23S,24S)-17,23-EPOXY-24,31-DIHYDROXY-27-NOR-5α-LANOST-8-ENE-3,15-DIONE

M.ADINOLFI, G.BARONE, R. LANZETTA, G.LAONIGRO, L.MANGONI, and M.PARRILLI*

Istituto di Chimica Organica e Biologica della Universita' di Napoli
Via Mezzocannone 16, 80134 Napoli, Italy

ABSTRACT.—The occurrence of seven free nortriterpenes in the bulbs of *Muscari comosum* Mill. is reported. Of the three major compounds, two were known: eucosterol **1** and its 3-keto counterpart **2**, while the third (**5**) was a new compound, which is also characterized by a spirofused furanic ether linkage. The structure of the latter was assigned by spectral evidence and chemical correlation.

We recently reported (1-3) the isolation of a glycoside mixture from bulbs of *Muscari comosum* Mill. (Liliaceae) and the structural elucidation of the four nortriterpene aglycones (**1-4**) obtained by enzymatic hydrolysis of that mixture. The occurrence of eucosterol (**1**) as a free compound in several *Eucomis* species (4) prompted us to investigate the presence of a free nortriterpene fraction in the bulbs. This paper describes[1] the isolation (albeit in low amounts) of **1** and **2** and of five more nortriterpenes and the structural determination of one of the latter, **5**.

By an extractive and chromatographic procedure (see Experimental section), a nortriterpene fraction was isolated from the bulbs (yield: 40 mg/kg of fresh material). Repeated tlc separations and crystallizations of this fraction led to the isolation of seven pure compounds. The three major ones were identified as known **1** and **2** by comparison (mp, pmr) with authentic samples (1,2) and **5** by spectral and chemical evidence (see below). The structural determination of the four minor components, whose pmr spectra resembled those of **1-5**, will be reported in a forthcoming paper.

Compound **5** was crystallized from benzene as plates; mp, 194-5°, $[\alpha]^{rt}_{D}+67.2°$. The molecular formula, $C_{29}H_{44}O_5$, was deduced by hrms. The fragmentation pattern resembled that of **2**; the presence of the peak at *m/e* 341 of the ion **6** is particularly diagnostic (4) of a structure similar to that of **2**. The inspection of the ^{1}H-nmr spectrum of **5**, summarized in table 1, as well as that of **2**, reveals the structural relationship occurring between the two compounds. The relevant differences between the two spectra are well accounted for by the assumption that **5** is the 24-alcohol corresponding to **2**: the carbinolic proton resonates at δ 3.347 and is coupled with the protons 23-H and 25-H_2, as indicated by pertinent decoupling experiments. The signal of 25-H_2 in the spectrum of **5** appears at a higher field than in the spectrum of **2** because of the change in functionality at C-24. Accordingly, in the ^{13}C-nmr spectrum of **5**, the line of a hydroxylated methine carbon attributable to C-24 is displayed at δ 77.94, and the resonances of C-25 and C-26 are shifted[2] upfield and downfield, respectively, with respect to the spectrum of **2** (table 2).

Definitively convincing evidence for the structure **5** was readily obtained by the conversion of **2** into **5** through the selective reduction of the 24-ketonic function ($NaBH_4$-MeOH at 0°).

Finally, the glc modification of Horeau's method (6) applied to **5** allowed the determination of the chirality at C-24 as S.

[1]Preliminary communication: 13th International Symposium on the Chemistry of Natural Products, Pretoria, South Africa, 1982.

[2]See the resonances of C-3 and C-4 of *n*-butan-2-ol and butan-2-one (5).

TABLE 1. ^{1}H-nmr (270 MHz) chemical shifts (selected data) in $CDCl_3$[a]

	2	5
18-H_3	0.960 s	0.953 s
19-H_3	1.088 s	1.068 s
30-H_3	1,271 s	1,282 s
32-H_3	1.407 s	1.305 s
21-H_3	1.135 d	1.128 d
	$J_{20,21}$=6.62	$J_{20,21}$=6.80
31-H_2	3.469, 4.021. ABq, J_{AB}=11.40	3.462, 4.011, AB q, J_{AB}=11.21
20-H	2.35 m	2.20 m
22-H_2	1.949 dd[b]	1.51 m
23-H	4.698 t[c]	4.033 m[d]
24-H		3.347 m[e]
25-H_2	2.497 q	1.35 m
	$J_{25,26}$=6.99	
26-H_3	1.069 t	1.009 t
	$J_{25,26}$=6.99	$J_{25,26}$=7.35
16-H_2	2.227, 2.798, AB q, J_{AB}=19.12	2.358, 2.717, ABq, J_{AB}=19.49

[a]All chemical shifts values are given in δ (ppm) relative to TMS. Coupling constants are given in Hz and were inferred from pertinent decoupling experiments.

[b]AA′ part of an AA′X system, further split (J=3.31) by coupling with 20-H.

[c]X part of an AA′X system ($J_{AX}+J_{A'X}$=19.86).

[d]Buried with a 31-H_2 signal. When shifted by Eu(dpm)$_3$ it appears as a six-line pattern: X quartet ($J_{AX}+J_{BX}$=15.5) of an ABX system, further split (J=7.92) by coupling with 24-H.

[e]Six-line pattern: X quartet ($J_{AX}+J_{BX}$=12.19) of an ABX system, further split (J=7.92) by coupling with 23-H.

TABLE 2. ^{13}C-nmr (67.88 MHz) chemical shifts in $CDCL_3$[a]

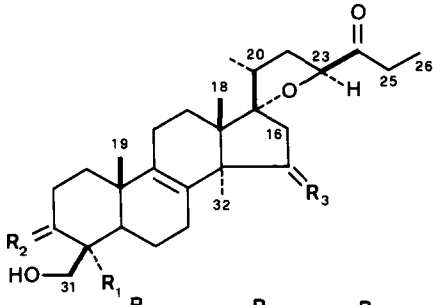
	2	5		2	5
C-1	35.43	35.67	C-16	51.75	53.62
C-2	34.47	35.03	C-17	91.12	90.04
C-3	219.93	220.20	C-18	20.59	20.91
C-4	51.35	51.61	C-19	19.45	19.65
C-5	51.62	51.95	C-20	43.49	44.36
C-6	19.82	19.18	C-21	17.18	17.72
C-7	26.24	26.57	C-22	36.77	36.37
C-8	134.14	134.28	C-23	81.72	82.02
C-9	134.48	134.94	C-24	211.92	77.94
C-10	37.17	37.50	C-25	32.44	30.01
C-11	20.59	20.91	C-26	7.28	10.16
C-12	23.01	23.24	C-30	21.88	22.21
C-13	47.56	48.09	C-31	65.81	66.14
C-14	57.88	58.31	C-32	23.78	24.27
C-15	215.13	215.97			

[a]Chemical shifts are given in δ (ppm) relative to TMS. The assignments of the signals of **5** are based on the comparison with the spectrum of **2**, discussed in reference (2).

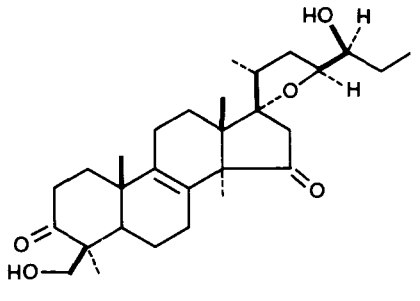
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—The equipment used in this experiment consisted of: m. ps, Kofler block; rotations, Perkin-Elmer model 141; nmr, Brüker model WH270; glc, Carlo Erba model Fractovap 4160; silica gel plates (thickness 0.25 mm) and silica gel for cc, from Merck; ms, AEI model 902.

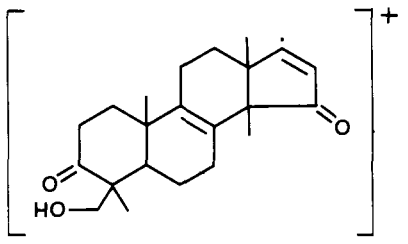
ISOLATION PROCEDURE.—Sliced, fresh bulbs (1 kg) of *Muscari comosum* Mill. (Liliaceae) (collected in



	R_1	R_2	R_3
1	CH_3	β-OH, α-H	O
2	CH_3	O	O
3	CH_2OH	β-OH, α-H	H, H
4	CH_2OH	O	H, H



5



6

autumn in Puglia, Italy, and authenticated by the Botanical Garden of the University of Naples) were boiled in acetone (4 liters) for 8 h. Acetone was removed from the extract by evaporation at reduced pressure. The resulting aqueous slurry was centrifuged. The solid was dried under reduced pressure and then extracted in a Soxhlet apparatus with hexane (1 liter, 8 h) and then with ether (1 liter, 24 h). The ether extract was evaporated to dryness, and the residue (3 g) was chromatographed on a silica gel (100 g) column with hexane containing increasing percentages of ether. The fraction (1.8 g) eluted with ether alone was chromatographed on a silica gel (60 g) column with hexane containing increasing percentages of acetone. Tlc (silica gel, ethyl acetate-chloroform, 85:15, two runs) of the fraction (40 mg) eluted with hexane-acetone, 70:30, afforded four fractions; (*a*) 12 mg; (*b*) 8 mg; (*c*) 14 mg; (*d*) 6 mg (decreasing Rf order).

Crystallization of fraction *a* from ethanol gave **2** (8 mg), identical (mp, rotation, pmr) to an authentic sample (2). The mother liquors were evaporated. Tlc (silica gel, benzene-ether, 35:65, five runs) of the residue gave two pure compounds (3 and 1 mg, respectively) whose pmr resembled those of **1-5**.

Tlc (silica gel, benzene-ether, 35:65, five runs) of fraction *b* gave two other pure compounds (1 and 3 mg, respectively) whose spectra resembled those of **1-5**.

Crystallization of fraction *c* from ethanol gave **1** (10 mg), identical (mp, rotation, pmr) to an authentic sample (1).

Crystallization of fraction *d* from benzene yielded **5** (5 mg), mp, 194-5°, $[\alpha]^{rt}D$ +67.2° (chloroform, c=0.2); ms, *m/e* 472.3177 (M^+, calcd. for $C_{29}H_{44}O_5$ 472.3189), 454 (M^+-18, H_2O), 414 (M^+-58, CH_3CH_2CHO), 384, 341, 301, 288; ^{1}H-nmr: table 1; ^{13}C-nmr, table 2.

REDUCTION OF **2**.—A sample of **2** (3 mg) was dissolved in methanol (0.5 ml) and treated with $NaBH_4$ (traces) at 0° for 30 min. Usual work-up gave a solid (3 mg); tlc (silica gel, benzene-ether, 35:65, six runs) gave **5** (1 mg), identical (mp, pmr) to the sample isolated from the bulbs, as above.

DETERMINATION OF THE ABSOLUTE CONFIGURATION OF C-24 IN **5**.—24-Alcohol **5** (0.5 mg) was treated with (±)α-phenylbutyric anhydride, and the excess of anhydride was analyzed by glc at 200° on a 25-m glass capillary coated with OV-101 as (+)-R-α-phenylethylamides of (−)R- and (+)S-phenylbutyric acid, following the procedure described in (6). A peak increment of +6 for R-acid was calculated, indicating S configuration of C-24 in **5**.

ACKNOWLEDGMENTS

This work was supported by Consiglio Nazionale delle Ricerche, Rome. Nmr spectra were performed at the Centro di Metodologie Chimico-Fisiche della Universita' di Napoli.

LITERATURE CITED

1. M. Parrilli, R. Lanzetta, V. Dovinola, M. Adinolfi, and L. Mangoni, *Can. J. Chem.*, **59**, 2261 (1981).
2. M. Parrilli, M. Adinolfi, and L. Mangoni, *Gazz. Chim. It.*, **109**, 611 (1979).
3. M. Parrilli, R. Lanzetta, M. Adinolfi, and L. Mangoni, *Tetrahedron*, **36**, 3591 (1980).
4. R. Ziegler and C. Tamm, *Helv. Chim. Acta*, **59**, 1997 (1976).
5. J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, NY, 1972, pp 140, 146.
6. C.J. Brooks and J.D. Gilbert, *J. Chem. Comm.*, 194 (1973).

Received 15 October 1982

ERRATUM

R.J. Hamilton has requested the following erratum:

Dr. E.A. Bernays should be added as a co-author to both the following papers:

The Effects of Plant Waxes on Insects [*J. Nat. Prod.*, **45**, 694 (1982)] and The Changes with Age in the Epicuticular Wax of Sorghum Bicolor [*J. Nat. Prod.*, **45**, 697 (1982)].

The authors for each paper should be: D.S.J. Atkin and R.J. Hamilton, Chemistry and Biochemistry Department, Liverpool Polytechnic, and E.A. Bernays, Centre for Overseas Pest Research, Wright's Lane, London.

The following acknowledgment should be added to each of the above papers:

One of us, D.S.J. Atkin, is grateful to Science and Engineering Research Council for a CASE studentship and to the Centre for Overseas Pest Research, Wright's Lane, London, for their support and for the facilities to perform the bioassays. We would like to thank Dr. Sue Woodhead and Dr. Reg Chapman for helpful discussions.