Phytochemistry, Vol. 24, No. 10, pp. 2423–2426, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

HOMOISOFLAVANONES FROM *MUSCARI COMOSUM* BULBS

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(*Received* 31 *January* 1985)

Key Word Index—*Muscari comosum*; Liliaceae; homoisoflavanones; 3-benzyl-4-chromanones; muscomosin; comosin; 8-*O*-demethyl-8-*O*-acetyl-7-*O*-methyl-3,9-dihydropunctatin; 3,9-dihydropunctatin.

Abstract—From the bulbs of *Muscari comosum* 3,9-dihydropunctatin and three novel homoisoflavanones were isolated.

INTRODUCTION

A number of phenolic compounds containing the basic 3-benzyl-4-chromanone skeleton have been isolated from bulbs of *Muscari comosum* and their structures have been elucidated [1, 2]. This further study has resulted in the isolation of three new compounds, muscomosin (**1**), comosin (**3a**) and 8-*O*-demethyl-8-*O*-acetyl-7-*O*-methyl-3,9-dihydropunctatin (**4**) along with known [3] 3,9-dihydropunctatin (**5**). The structures of **1**, **3a** and **4** are noteworthy since **1** is a homoisoflavanone of the rare scillascillin type [4], **3a** possesses a novel [5] homoisoflavanone skeleton since the 9-carbon is not benzylic, and **4** is the first acetyl 3-benzyl-4-chromanone derivative hitherto found in nature.

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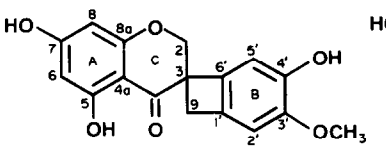
RESULTS AND DISCUSSION

The molecular formula $C_{17}H_{14}O_6$ was deduced for muscomosin (**1**) from the high resolution mass spectrum. The UV absorption at 291 nm was shifted upon both addition of sodium acetate (+35 nm) and of aluminium chloride (+22 nm), indicating the presence of hydroxyl groups at C-5 and C-7. Accordingly, the ^{1}H NMR spectrum (Table 1) exhibited the signals of the protons at C-6 and C-8 as an AB quartet with $J = 2.4$ Hz (*meta*-coupling) and those of the 5- and 7-hydroxyl groups at δ12.11 and δ8.5–9.3, respectively. The molecular formula implied the presence of a fourth ring in addition to rings A, B and C and to the unsaturated 4-carbonyl group [signal at δ196.2 in the ^{13}C NMR spectrum (Table 2)]. The proton signals of a third hydroxyl group and of methoxyl group were displayed at δ8.5–9.3 (overlapped with the 7-hydroxyl signal) and at δ3.79, respectively. Only two one-proton signals (δ6.90 and δ6.55) for the B-ring protons were



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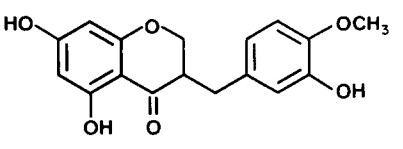

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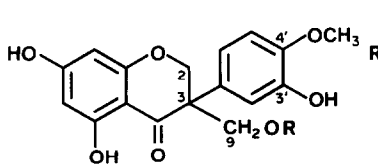
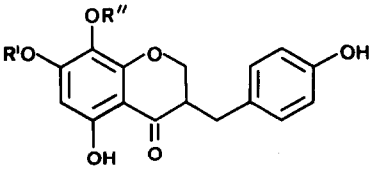


3a R = Ac
3b R = H



4 R′ = CH_3 R″ = Ac
5 R′ = H R″ = CH_3

Table 1. ^{1}H NMR (270 MHz) chemical shifts of homoisoflavanones from *Muscari* bulbs*

Compound	Solvent	C-2	C-3	C-5	C-6, C-8	C-7	C-9	C-2′, C-6′	C-3′, C-5′	C-4′
1	$(CD_3)_2SO$	4.58 4.63 ABq J = 11		12.11†s	5.96 5.98 ABq J = 2.4	8.5–9.3†	3.05 3.45 ABq J = 13.4	6.90 s (C-2′)	6.55 s (C-5′) 3.79 s (C-3′)	8.5–9.3†
3a‡	$CDCl_3$	4.61 4.89 ABq J = 12.1		12.03†s	5.85 5.95 ABq J = 2.2	5.63†s	4.26 4.70 ABq J = 11.5	7.07 d (C-2′) J = 2.7 6.95 dd (C-6′) J = 2.7 J = 8.8	6.81 d (C-5′) J = 8.8 6.34†br (C-3′)	3.86 s
3b	$(CD_3)_2CO$	4.71 4.92 ABq J = 12.1		12.05†s	5.76 5.82 ABq J = 2.3		3.66 4.22 ABq J = 11.4	7.01 d (C-2′) J = 2.4 6.92 dd (C-6′) J = 2.4 J = 8.4	6.87 d (C-5′) J = 8.4	3.79 s
4§	$(CD_3)_2SO$	4.19 m 4.37 m AB of ABX	3.10 m	12.14†s	6.36 s (C-6)	3.88 s	2.66 m 3.0 m	7.06 d J = 8.5 AA′BB′	6.74 d	9.28†s
5	$(CD_3)_2CO$	4.30 m 4.40 m AB of ABX	2.94 m	12.01†s	5.97 s (C-6) 3.72 s (C-8)		2.70 m 3.14 m	7.12 d J = 8.5 AA′BB′	6.80 d	

*All chemical shifts are given in δ (ppm) relative to TMS. Coupling constants are given in Hz.
†Protons exchangeable with D_2O.
‡(9)–OAc signal at δ2.04.
§(8)–OAc at δ2.28.

Table 2. ^{13}C NMR (67.88 MHz) chemical shifts of homoisoflavanones from *Muscari* bulbs*

Carbon	1†	3a‡	4†	5‡
2	72.9	71.8	69.5	70.5
3	53.7	52.7	45.7	48.0
4	196.2	197.7	198.3	199.3
4a	100.8	103.0	101.6	102.8
5	163.9§	166.0	159.4§	161.0§
6	96.0	97.2	92.9	97.2
7	167.9§	168.5	160.9§	161.6§
8	94.9	95.8	119.4	130.1‖
8a	163.1§	164.2	152.0§	157.1§
9	35.0	66.5	30.8	33.1
1′	131.8	128.8	127.8	129.8‖
2′	108.8‖	115.3§	129.9	131.1
3′	149.5¶	149.2‖	115.3	116.4
4′	147.0¶	147.9‖	155.9	155.6
5′	109.5‖	113.0§	115.3	116.4
6′	135.4	119.6§	129.9	131.1
OMe	56.0	56.5	56.5	61.5
OCOMe		172.3	168.2	
OCOMe		20.5	19.9	

*Chemical shifts are given in δ (ppm) relative to TMS. The assignments are based on on- and off-resonance spectra and on comparison to data from refs [1] and [2].

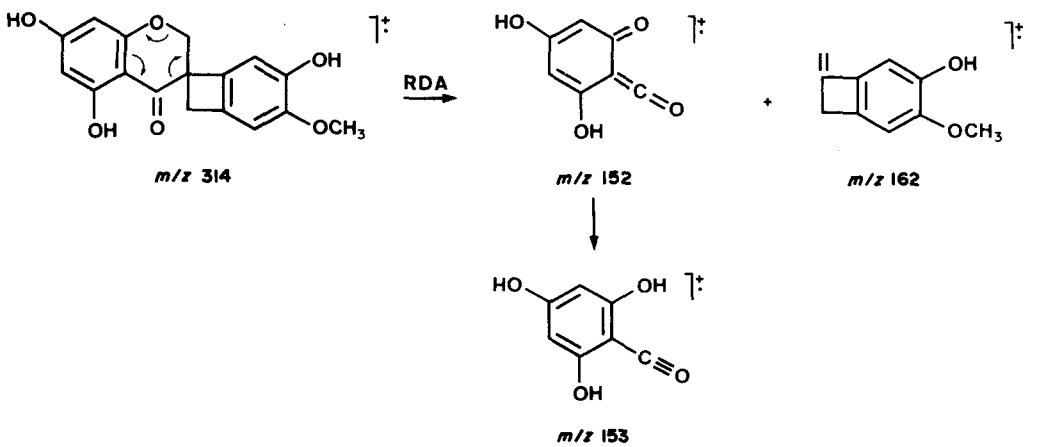
†In $(CD_3)_2SO$.

‡In CD_3OD.

§‖¶Interchangeable values.

observed, indicating a tetrasubstitution pattern for the ring B. All these data strongly suggested the presence of a 3-spirocyclobutane ring as in scillascillin [4]. Additional evidence was obtained from ^{1}H and ^{13}C NMR spectra. The methylene protons at C-2 and at C-9 appeared as two AB quartets, due to the absence of a proton at C-3. The quaternary-carbon resonance at δ53.7 could be assigned to the 3-carbon and the signals of the C-2 and C-9 methylene carbons exhibited low-field shifts compared to the corresponding carbons of 3′-hydroxy-3,9-dihydroeucomin 2 [2]. The position of the hydroxyl and of the methoxyl group in ring B remained to be established. The singlet appearance of the signals at δ6.90 and δ6.55 indicated the *para* location (C-2′ and C-5′) of the B-ring protons. An NOE enhancement was measured for the δ6.90 singlet upon irradiation at δ3.79, thus indicating that the methoxyl group was *ortho* to the ring-B proton responsible for the δ6.90 signal (and consequently that the hydroxyl group was *ortho* to the B-ring proton responsible for the δ6.55 signal). Since in two long range selective proton decoupling (LSPD) [6] experiments weak-power irradiation at δ6.55 and at δ6.90 eliminated the fine splitting of the 3-carbon (δ53.7) and of the 9-carbon (δ35.0) signals, respectively, in consequence of the disappearance of the $J^3_{C,H}$ coupling, the hydroxyl group should be at C-4′ and the methoxyl group at C-3′. These LSPD experiments allowed us also to assign the ^{13}C-line at δ131.8 to the 1′-carbon and the ^{13}C-line at δ135.4 to the 6′-carbon owing to the disappearance of the *meta*-couplings with the protons at δ6.55 (H-5′) and at δ6.90 (H-2′), respectively. Therefore, a heteronuclear ^{13}C–{^{1}H} NOE [7] enhancement measured for the carbon signal at δ131.8 upon irradiation at the 9-proton frequencies yielded further support to the location of the ring-B hydroxyl and methoxyl groups deduced as above. Based on these results, the structure of muscomosin is **1**, which also accords with the mass spectrum (Scheme 1) [5].

Comosin (**3a**) possesses the molecular formula $C_{19}H_{18}O_8$ (high resolution mass spectrum). In the ^{1}H NMR spectrum the signals of three hydroxyl, one methoxyl and one acetoxyl group were displayed. A mass peak at *m/z* 153, the NMR signal of the *meta*-coupled C-6 and C-8 protons (δ5.85 and δ5.95, AB*q*, J = 2.2 Hz) and of the C-5 hydroxyl proton (δ12.03 *s*), and the bathochromic shifts of the UV absorption maximum at 292 nm induced both by $AlCl_3$ (+24 nm, presence of a 5-hydroxyl group) and NaOAc (+35 nm, presence of a 7-hydroxyl group) indicated the A-ring substitution pattern. In the ^{1}H NMR spectrum the signals of the protons at C-2′ (δ7.07 *d*, J_{meta} = 2.7 Hz), C-5′ (δ6.81 *d*, J_{ortho} = 8.8 Hz) and C-6′ (δ6.95 *dd*, J_{ortho} = 8.8 Hz, J_{meta} = 2.7 Hz) were clearly discerned, indicating substitution at the 3′ and 4′ positions. The assignment of the third hydroxyl group at the 3′-position and of the methoxyl group at the 4′-position was based on the NOE enhancement observed



Scheme 1. Mass spectrum of muscomosin (**1**).

for the signal at δ6.81 (C-5′ proton) upon irradiation of the methoxyl protons at δ3.86. In the mass spectrum the base peak was not the B-ring tropylium fragment as is usual with 3-benzyl-4-chromanones [5], but a peak at *m/z* 222. This result and the appearance of the C-2 and the C-9 protons as AB quartets in the ^{1}H NMR spectrum indicated the non-benzylic nature of the C-9 methylene group and the absence of a proton at C-3, and suggested structure **3a** for comosin. The *m/z* 222 fragment could thus be assumed to originate from the retro-Diels–Alder cleavage of ring C which is characteristic of isoflavanones [8]. Structure **3a** was also supported by the presence in the mass spectrum of the *m/z* 301 peak of fragment **7** originating from β-cleavage of the acetylated primary alcoholic function at C-9. The absence in the ^{13}C NMR spectrum of the benzylic carbon resonance in the range δ31–34 [1, 2] and the appearance of a signal at δ66.5, consistent with the presence of a methylene carbon carrying an acetoxy group, also accorded fully with structure **3a**. Confirmatory evidence was obtained upon deacetylation of comosin to the primary alcohol **3b**, whose ^{1}H NMR spectrum exhibited the expected $-C\underline{H}_2OH$ signal at δ3.66, 4.22, AB*q*, J = 11.4 Hz. Therefore, comosin must be 5,7-dihydroxy-3-acetoxymethyl-3-(3′-hydroxy-4′-methoxyphenyl)-4-chromanone (**3a**).

Compound **4** possesses the molecular formula $C_{19}H_{18}O_7$ (high resolution mass spectrum). The ^{1}H NMR spectrum displayed the signals of the $-(2)CH_2-(3)CH-(9)CH_2-$ grouping and of the AA′BB′ system of the B-ring protons. The presence of the hydroxyl group at C-4′ was indicated by the appearance of the hydroxytropylium peak (*m/z* 107) in the mass spectrum. The main feature of both mass and ^{1}H NMR spectra was relative to the presence of an acetoxy group in the structure of **4** (mass peak at *m/z* 316, $[M - CH_2{=}C{=}O]^+$, and proton signal at δ2.28, 3H, Ac, respectively). Based on the following data, this group could be located at C-8. The δ12.14 singlet in the ^{1}H NMR spectrum should be due to the 5-OH proton, involved in a strong hydrogen bond. Accordingly, the UV absorption (285 nm) underwent a bathochromic shift (30 nm) upon addition of aluminium chloride. Significant NOE enhancements were measured between the signal at δ12.14 and the signal at δ6.36 (A-ring aromatic proton) and between this latter and the methoxyl signal at δ3.88, indicating the complete substitution pattern of the A ring as depicted in structure **4**, 8-*O*-demethyl-8-*O*-acetyl-7-*O*-methyl-3,9-dihydropunctatin. This was supported by the ^{13}C NMR spectrum.

Compound **5**, $C_{17}H_{16}O_6$ (high resolution mass spectrum), was found to be identical to known 3,9-dihydropunctatin [3] from its physical properties (mp, rotation, UV, MS, ^{1}H NMR). The ^{1}H NMR spectrum in Me_2CO-d_6 is reported in Table 1. The as yet unreported ^{13}C NMR spectrum is summarized in Table 2.

EXPERIMENTAL

Isolation of homoisoflavanones. Compounds **1**, **3a** and **5** and compound **4** were obtained from fractions *d* and *a*, respectively, isolated from the bulbs of *M. comosum* Mill. by the procedure described previously [1]. Prep. TLC (silica gel, hexane–Et_2O–dioxane, 5:3:2, 3 runs) of fraction *d* (85 mg) yielded pure compounds **1** (42 mg), **5** (16 mg) and **3a** (4 mg) (increasing polarity order). Compound **1** had mp 196–198° (from $CHCl_3$–hexane), $[\alpha]_D$ +68° (MeOH, *c* 0.2). UV λ_{max}^{MeOH} nm (log ε): 230 (4.27), 291 (4.38), 336 (3.70). EIMS, 70 eV, *m/z* (rel. int.): 314.0796 ($[M]^+$; calc. for $C_{17}H_{14}O_6$: 314.0790) (100), 162 (42), 153 (82). Compound **3a** was a vitreous solid. UV λ_{max}^{MeOH} nm (log ε): 227 (4.39), 292 (4.37), 327 (4.01). EIMS, 70 eV, *m/z* (rel. int.): 374.1012 ($[M]^+$; calc. for $C_{19}H_{18}O_8$: 374.1001) (45), 301 (12), 222 (100), 180 (28), 162 (12), 153 (25), 152 (8). Compound **5** had mp 205–206° (from $CHCl_3$–hexane), $[\alpha]_D$ −37° (MeOH, *c* 0.3). UV λ_{max}^{MeOH} nm (log ε): 291 (4.42), shifted to 330 and 313 upon addition of NaOAc and $AlCl_3$, respectively. EIMS, 70 eV, *m/z* (rel. int.): 316.0955 ($[M]^+$; calc. for $C_{17}H_{16}O_6$: 316.0947) (49), 210 (44), 195 (22), 107 (100). Compound **4** was a minor component (6 mg) of fraction *a* (50 mg) and was isolated from this latter by prep. TLC (silica gel, C_6H_6–Et_2O, 7:3, 3 runs). It had mp 155–156° (from $CHCl_3$–hexane), $[\alpha]_D$ −18° (MeOH, *c* 0.3), UV λ_{max}^{MeOH} nm (log ε): 228 (4.38), 286 (4.35), 337 (3.60). EIMS, 70 eV, *m/z* (rel. int.): 358.1042 ($[M]^+$; calc. for $C_{19}H_{18}O_7$: 358.1052) (6), 316 (58), 210 (46), 182 (8), 107 (100).

Deacetylation of compound **3a**. A sample of **3a** was treated with 5% KOH–MeOH (room temp., 2 hr). Usual work-up gave compound **3b** (1 mg). ^{1}H NMR: Table 1.

Acknowledgements—This work was supported by Ministero della Pubblica Istruzione. NMR spectra were performed at the Centro di Metodologie Chimico-Fisiche della Università di Napoli.

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