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HOMOISOFLAVANONES FROM *MUSCARI NEGLECTUM*

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Key Word Index—*Muscari neglectum*; Liliaceae; homoisoflavanones.

Abstract—From the bulbs of *Muscari neglectum* a novel scillascillinoid homoisoflavanone was isolated. The quite unprecedented oxygenation pattern of its ring B was elucidated mainly by long-range 2D carbon 13-proton and proton–proton shift correlation experiments. Also isolated were scillascillin and four known 3-benzyl-4-chromanones.

INTRODUCTION

In connection with our study on the homoisoflavanone content of *Muscari* species [1–4], we now report the results of an investigation on *Muscari neglectum* Guss bulbs. From this source we have isolated, besides known compounds **1–8** [1–5], compound **9**, that exhibits a novel substitution pattern in the B-ring [cf. 6].

RESULTS

The known compounds **1–7** were identified by comparison of their physical properties with those of authentic samples [1–4]. The structure of **8** was derived from comparison with spectroscopic and physical data reported in the literature [5]. For this latter we report here the ^{13}C NMR chemical shifts (Table 1), not yet described.

The elucidation of structure **9** was achieved by spectral analysis (^{1}H, ^{13}C NMR, MS) of the native product and of its permethylated derivative **10**. A molecular formula $C_{18}H_{16}O_6$ was deduced for compound **9** from the high resolution mass spectrum. The two ABq's in the ^{1}H NMR spectrum (Table 2) for the methylene protons at C-2 and C-9 as well as the quaternary-carbon signal in the ^{13}C spectrum at δ54.3, attributable to the C-3 spirocarbon, indicate the absence of a proton at C-3 and suggest [4] a scillascillin skeleton for **9**.

Mass spectral fragments at *m/z* 167 and at *m/z* 162 indicate [2, 4] the presence of one methoxyl and one hydroxyl function both on the A- and B-ring. The hydroxy-proton NMR signal at δ12.10 ($CDCl_3$, Table 2) indicates an OH chelated at 5-C, while a 7-OMe substitution is deduced from the NOE enhancements measured for the signals of both the 6- and the 8-proton at δ6.06 and δ6.19 ($CDCl_3$, Table 2), respectively, upon irradiation of the methoxyl protons at δ3.84. This substitution pattern is in agreement with the chemical shifts of the A-ring proton and carbon atoms [7].

As for the B-ring substitution pattern, an *ortho* relationship between the protons whose signals appear at δ6.62 and δ6.88 (CD_3OD, Table 2) was easily deduced from the $J^3_{H,H}$ value (7.9 Hz). The same relationship was found to occur between the proton at δ6.88 and the methoxyl group responsible for the signal at δ3.80, on the

Table 1. Carbon shifts of **8–10** in the ^{13}C NMR spectra measured in CD_3OD*

Carbon	8†	9	10	Carbon	8†	9	10
2	75.7	74.2	75.0	1′	136.2	135.8	136.5
3	56.6	54.3	56.8	2′	106.5	115.8	118.9
4	197.7	198.1	192.3	3′	149.6	114.8	116.1
4a	102.5	103.2	105.9	4′	148.9	148.6	151.5
5	165.9	165.5	164.3	5′	104.7	142.7	146.0
6	97.4	95.7	94.0	6′	137.4	127.2	130.3
7	168.7	169.5	168.3	7′	101.7	—	—
8	96.1	94.7	94.8	5-OMe	—	—	56.3
8a	165.0	164.9	166.9	7-OMe	—	56.9	56.3
9	35.7	38.0	37.8	4′-OMe	—	56.2	57.2
				5′-OMe	—	—	59.2

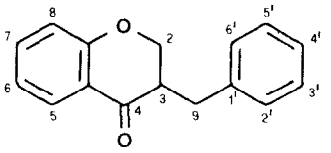
*Chemical shifts are given in δ (ppm) relative to TMS.

†Assignments are based on comparison with the spectra of **9**, **10** and other scillascillinoid homoisoflavanones [7].

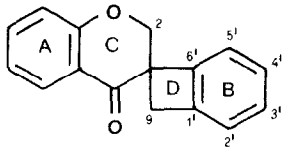
Table 2. Proton shifts in the ^{1}H NMR spectra of **8**–**10***

Compound	Solvent	2-H_2	5-H/H_3	7-H_3	9-H_2	6-H	8-H	2′-H	3′-H	4′-H_3	5′-H/H_3	7′-H_2
8	CD_3OD	4.50, 4.53 ABq (11.8)	—	—	2.97, 3.45 ABq (13.7)	5.90, 5.92 ABq (2.1)		6.56 s‡	—	—	6.73 s‡	5.87 s
8	$(CD_3)_2SO$	4.56, 4.57 ABq (11.6)	12.10 s	—	3.06, 3.35 ABq (13.4)	5.94, 5.98 ABq (2.3)		6.75 s	—	—	6.92 s	5.98 s
9	CD_3OD	4.46, 4.76 ABq (11.6)	—	3.81 s	3.16, 3.30 ABq (13.4)	6.04 s		6.62 6.88 ABq (7.9)		3.80 s	—	—
9	$CDCl_3$	4.52, 4.77 ABq (12.6)	12.10 s	3.84 s	3.16, 3.51 ABq (13.7)	6.06, 6.19 ABq (2.8)		6.70 6.84 ABq (7.7)		3.85 s	5.60 s	—
10	CD_3OD	4.50, 4.77 ABq (11.5)	3.82 s	3.85 s	3.20†, 3.22 ABq (13.4)	6.21, 6.19 ABq (2.2)		6.76‡ 6.92 ABq(1.0) (8.1)		3.78 s	3.71 s	—

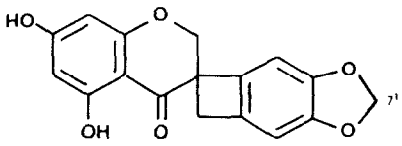
*Chemical shifts are given in δ (ppm) relative to TMS. Coupling constants (in parentheses) are given in Hz.
†Further split by coupling with the 2′-proton ($J = 1.0$ Hz).
‡Further split by coupling with one 9-proton ($J = 1.0$ Hz).



	5	6	7	8	3′	4′
1	OH	OMe	OH			OH
2	OH	OMe	OMe	OH		OH
3	OH		OH		OH	OMe
4	OH		OH			OH



	5	7	3′	4′	5′
5	OH	OH	OMe	OH	
6	OH	OMe	OH	OMe	
7	OH	OMe	OH	OH	
9	OH	OMe		OMe	OH
10	OMe	OMe		OMe	OMe



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basis of the NOE enhancement measured for the former upon irradiation of the latter. These spatial relationships accord with four possible B-ring substitution patterns. That one depicted in structure **9** was supported by the results of a long-range 2D proton–proton shift correlation experiment that showed a link between the methylene protons at C-9 and the proton at δ6.62, thus assigned at the 2′-C position.

Conclusive evidence for structure **9** was obtained from the NMR data of the permethylated derivative **10**. The ^{1}H NMR in CD_3OD spectrum (Table 2) of this compound displays four methoxyl group signals, three of which (δ3.85, 3.82 and 3.78) were assigned to the methoxyl groups at C-5, C-7 and at the B-ring, respectively, on the basis of their correlation with *ortho* protons, measured by long-range 2D proton-proton shift correlation experiment. The fourth methoxyl group, that exhibited no correlation with an *ortho* proton, was found to be in close proximity with one of the two 2-methylene protons as a NOE enhancement of the signal of this latter (δ4.77) was observed upon irradiation at δ3.71. This indicates its location at the 5′-position and therefore the presence of an hydroxyl group at that position in compound **9**. The ^{13}C chemical shift signals of **9** and **10** (Table 1), assigned by both one-bond and long-range carbon-proton shift correlation experiments, are in agreement with the pro-

posed structures. Particularly, the low field (δ 59.2) chemical shift value of the 5'-methoxyl of **10** accords with its crowded location [8].

It should be noted that the biogenetic pathway [9] for 3-*spiro*-cyclobutene homoisoflavanones must account also for the occurrence of the novel oxygenation pattern of **9**.

EXPERIMENTAL

^{13}C and ^{1}H NMR spectra were recorded in CD_3OD (when not otherwise specified) solutions at 30° and 75.47/300.14 MHz ($^{13}C/^{1}H$) with an AM-300 FT NMR spectrometer (Brucker) equipped with a dual-probe. One-dimensional spectra were typically obtained with 3000 Hz (^{1}H) and 13000 Hz (^{13}C) spectral widths. Nuclear Overhauser enhancements were obtained in the difference mode as reported [in ref-10]. Long-range 2D carbon-proton shift correlation [11] experiments were performed with the Brucker XHCORR microprogram using delay $D_3 = 71.4$ msec, corresponding to $J_{C,H} = 7$ Hz. Long-range 2D proton–proton shift correlation [12] experiments were performed with the Brucker COSYLR microprogram using delay $D_2 = 80$ msec, corresponding to $J_{H,H} = 3$ Hz.

Isolation of homoisoflavanones. Fresh bulbs (600 g) of *Muscari neglectum* Guss (collected in March 1986 near Lecce, Italy, and authenticated by the staff of the Botanical Garden of the University of Naples) were homogenized in a macerator, freeze-dried and extracted in a Soxhlet with light petrol (12 hr), with Et_2O (12 hr) and then with MeOH (12 hr). The Et_2O extract was evaporated (1 g) and chromatographed on a silica gel (50 g) column to yield fractions a (9:1 $CHCl_3$–AcOEt; 200 mg), b (7:3; 110 mg), c (1:1; 207 mg) and d (1:4; 73 mg).

Fraction a yielded by chromatography on PLC (silica gel, 49:1 C_6H_6–EtOAc, three runs) three further fractions. The two less polar fractions gave compounds **6** (8 mg) and **9** (22 mg) by crystallization from MeOH. The third one gave **8** (3 mg) by TLC (silica gel, 49:1 C_6H_6–EtOAc, three runs).

Fraction b was further purified by PLC (silica gel, 95:5 C_6H_6–EtOAc, three runs). Compound **3** (20 mg) and a fraction that, upon further PLC (silica gel, 19:1 $CHCl_3$–Me_2CO, four runs) gave **5** (15 mg) and **7** (11 mg), were obtained.

Fraction c gave **2** (90 mg) by crystallization from $CHCl_3$. PLC (silica gel, 85:15 C_6H_6–EtOAc, three runs) of the mother liquor gave **1** (14 mg) and **4** (5 mg). Finally, fraction d again gave **2** (10 mg) and **1** (3 mg) by PLC (silica gel, 9:1 $CHCl_3$–MeOH, two runs).

Compound **8** had mp 209–211° (from MeOH) (lit. (5): 210–211°). EIMS, 70 eV, *m/z* (rel. int.): 312.0649 (M^+; calc. for $C_{17}H_{12}O_6$ 312.0634) (30), 149 (100). ^{1}H and ^{13}C NMR: see Table 2 and Table 1, respectively.

Compound **9** had mp 155–156° (from MeOH), $[\alpha]_D$ −16° (MeOH; *c* 0.6). EIMS, 70 eV, *m/z* (rel. int.): 328.0959 (M^+; calc. for $C_{18}H_{16}O_6$ 328.0947) (50), 167 (100), 162 (20). ^{1}H and ^{13}C NMR: see Table 2 and Table 1, respectively.

Methyl derivative **10**. Compound **9** (6 mg) was treated with CH_2N_2–MeOH (room temp., overnight) to give methyl derivative **10** (6 mg). ^{1}H and ^{13}C NMR: see Table 2 and Table 1, respectively.

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