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ALKALOIDS FROM *NARCISSUS* CV. SALOME*

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Key Word Index—*Narcissus* cv. Salome; Amaryllidaceae; bulbs; alkaloids; pseudolycorine; crinamine; hippeastrine; norgalanthamine; vasconine; tortuosine; 2α-hydroxy-6-*O*-methyloduline.

Abstract—Seven alkaloids have been isolated from bulbs of *Narcissus* cv. Salome. 2α-Hydroxy-6-*O*-methyloduline is reported here for the first time; its structure and stereochemistry were established by physical and spectroscopic methods. ^{1}H and ^{13}C NMR spectra of vasconine and hippeastrine were completely assigned by means of two-dimensional NMR techniques. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In 1958, Lionel Richardson bred *Narcissus* cv. Salome from *Narcissus* varieties Salmon Trout × Rose Caprice [2] and, at present, it is largely grown in Britain and Holland. The present paper deals with the isolation and structural elucidation of seven alkaloids from the bulbs of this unhitherto studied plant. The principal constituents, norgalanthamine, pseudolycorine and crinamine, arise from the three main biosynthetic pathways [3]. The new alkaloid 2α-hydroxy-6-*O*-methyloduline (**1**), just as hippeastrine (**2**), belongs to the homolycorine series, whereas the presence of the quaternary phenantridinium alkaloids (tortuosine and vasconine (**3**)) is noteworthy because of their structural similarity to the pharmacologically interesting compound, anhydrolycorinium chloride [4].

RESULTS AND DISCUSSION

Fractionation of the crude alkaloid extract of *Narcissus* cv. Salome (see Experimental) yielded seven alkaloids. Compound **1** ($C_{18}H_{21}NO_5$) shows in its mass spectrum a parent peak at *m/z* 331 (2%) and two fragment ions at *m/z* 125 and 96 originating from a retro-Diels–Alder cleavage of ring C, characteristic of the Amaryllidaceae alkaloids of the homolycorine series, with an hydroxyl group in position 2 [5, 6]. A less intense peak at *m/z* 206 indicates the presence of one methoxyl group in the hemiacetalic moiety. The characteristic signals in the ^{1}H NMR spectrum (500 MHz, $CDCl_3$) (Table 1) were: (1) singlets at δ 6.94, 6.73 and 5.43 for the two aromatic protons H-10 and H-7, and for the methine proton of the carbinol moiety, respectively. The assignment of the two signals belonging to the aromatic ring was carried out by 2D NOE experiments. This technique also allows the confirmation of the stereochemistry of the methoxyl group at C-6. Thus, the spatial proximity between H-1 (in α-configuration) and the methoxyl group at C-6 was observed, allowing the assignment of the proposed structure, which has the same stereochemistry as the related alkaloid, lycorenine [7]; (2) three intense signals at δ 5.91, 3.51 and 2.24 corresponding to the methylenedioxy, methoxyl and *N*-methyl groups, respectively; (3) three broad signals at δ 5.69, 4.21 and 4.15 for the olefinic protons H-3, H-2 and H-1, respectively. The small magnitude of the coupling constant between H-1 and H-10b clearly indicates the *cis*-junction of the B and C rings. A coupling constant of 12.0 Hz was reported for the alkaloid clivimine [8], an analogous compound with a *trans* B–C ring configuration: (4) two doublets at δ 2.87 and 2.85 assigned to the methinic protons H-4a and H-10b, respectively; their coupling constant ($J_{4a/10b}$ = 10.0 Hz) constitutes strong evidence for a *trans*-diaxial relationship; (5) two signals at δ 3.33 (*m*) and 2.38 (*dt*) for the α- and β-protons of the C-12 position, the first being more deshielded due to its *cis*-relation with the nitrogen lone pair [9], and (6) one multiplet centred at δ 2.54, assignable to the protons of the C-11 position.

The ^{13}C NMR spectrum of compound **1**, using $CDCl_3$ as a solvent, is consistent with a structure of the homolycorine series without the carbonyl group. The most characteristic signals were: (1) one methylenedioxy group at δ 101.1 and two methyl groups at δ 55.5 and 43.8 for the methoxyl group and *N*-methyl group, respectively; (2) three methine carbons at δ 119.4, 109.8 and 107.6 assignable to the

*Part 23 in the series '*Narcissus* alkaloids'. For part 22 see ref. [1].

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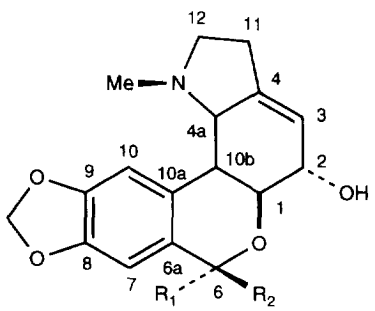
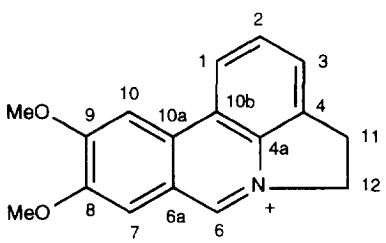
Table 1. ^{1}H NMR, HMQC and HMBC data for compound **1** (*J* given in Hz in parentheses)

H	δ	HMQC	HMBC
1α	4.15 *br s*	72.8 *d*	C-3, C-4a, C-6, C-10a
2β	4.21 *br s*	68.3 *d*	
3	5.69 *br s*	119.4 *d*	
		144.0 *s* (C-4)	
4a	2.87 *d* (10.0)	68.3 *d*	
6	5.43 *s*	98.5 *d*	C-1, C-6a, C-7, C-10a, OMe
		126.9 *s* (C-6a)	
7	6.73 *s*	107.6 *d*	C-6, C-8, C-9, C-10a
		147.1 *s* (C-8)	
		147.1 *s* (C-9)	
10	6.94 *s*	109.8 *d*	C-6a, C-8, C-9, C-10b
		131.2 *s* (C-10a)	
10b	2.85 *d* (10.0)	39.2 *d*	C-2
11	2.54 *m*	27.7 *t*	C-4a
12α	3.33 *m*	56.3 *t*	
12β	2.38 *dt* (9.5, 9.5)	56.3 *t*	C-4a
-OCH$_2$O-	5.91 2*d* (2)	101.1 *t*	C-8, C-9
NMe	2.22 *s*	43.8 *q*	C-4a, C-12
OMe	3.51 *s*	55.5 *q*	C-6

olefinic and the aromatic carbons C-10 and C-7, assignments which were confirmed on the basis of HMQC information; (3) one methine carbon at δ 98.5 assignable to C-6; this carbon responds to the change from hydroxylation (oduline) to methoxylation (**1**) by a low-field shift of *ca* 8 ppm [10]; (4) four methine carbons at δ 72.8, 68.3 and 39.2 for the C-1, C-2, C-4a and C-10b, δ 68.3 being the assignment of C-2 and C-4a, which was confirmed by correlation with the signals of the corresponding protons (HMQC); (5) two methylene carbons at δ 56.3 and δ 27.7 for C-12 and C-11, respectively, and (6) four singlets at lower field for the quaternary carbons C-8, C-9, C-4, C-10a and C-6a, which were assigned taking into account HMBC correlations (Table 1). The signal at δ 147.1 was ascribed to both carbons C-8 and C-9, due to their three-bond correlations with H-10 and H-7, respectively, as well as with the methylenedioxy protons.



1: R_1=OMe, R_2=H
2: R_1+R_2=O



3

Compound **2**, ($C_{17}H_{17}NO_5$), shows, in its mass spectrum, a very weak $[M]^+$ at *m/z* 315 (<1%) and two fragment ions at *m/z* 125 and 96, together with a less intense fragment from the other part of the molecule which encompasses the aromatic lactone moiety (*m/z* 191). The ^{1}H NMR and ^{13}C NMR spectra are in agreement with the structure of hippeastrine [11] and only minor assignment changes were made at C-4 and C-10a, applying the HMBC technique (Table 2).

Compound **3**, ($C_{17}H_{16}NO_2$) is chromatographically and spectrally identical to vasconine, previously isolated from *N. vasconicus* [12]. However, several ^{13}C NMR spectrum assignments corresponding to the pair C-2/C-3, and also the quaternary carbons, have been changed in accordance with HMQC and HMBC data (Table 3).

EXPERIMENTAL

General. Mps uncorr. IR spectra were measured in KBr discs or as dry films. EI-MS at 70 eV. ^{1}H, ^{13}C NMR, DEPT, 2D COSY, 2D NOESY, HMQC and HMBC spectra were recorded in a Varian VXR 500, using the solvent specified and TMS as int. standard. Chemical shifts are reported in δ units (ppm) and coupling constants (*J*) in Hz. Silica gel Merck (70–230 mesh) and silica gel SDS chromagel 60 A CC (230–400 mesh) were used for CC and flash CC, respectively.

Table 2. ^{1}H NMR, HMQC and HMBC data for compound **2** (*J* given in Hz in parentheses)

H	δ	HMQC	HMBC
1α	4.58 *br s*	82.2 *d*	C-3, C-4a, C-10a
2β	4.38 *br s*	66.2 *d*	
3	5.63 *br s*	119.4 *d*	
		142.4 *s* (C-4)	
4a	2.62 *d* (9.5)	67.1 *d*	C-3, C-4, NMe
		165.0 *s* (C-6)	
		118.0 *s* (C-6a)	
7	7.45 *s*	109.8 *d*	C-6, C-8, C-9, C-10a
		148.0 *s* (C-8)	
		151.9 *s* (C-9)	
10	6.92 *s*	108.5 *d*	C-6a, C-8, C-9, C-10b
		138.8 *s* (C-10a)	
10b	2.85 *dd* (9.5, 2.5)	38.4 *d*	C-2, C-6a, C-10, C-10a
11	2.48 *m*	27.3 *t*	C-3, C-4
12α	3.13 *ddd* (10, 8, 3)	55.9 *t*	C-4, C-4a
12β	2.23 *ddd* (10, 9, 9)	55.9 *t*	C-11, NMe
-OCH_2O-	6.04 2*d* (1.5)	102.1 *t*	C-8, C-9
NMe	2.03 *s*	42.9 *q*	C-4a, C-12

Silica gel 60 G Merck (mean particle size 15 μm) was used for VLC. Sephadex LH-20 was used for gel filtration and silica gel 60 F_{254} Merck (0.25) for TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

Plant material. Bulbs of *Narcissus* cv. Salome were supplied by the Servei de Parcs i Jardins, Ajuntament de Barcelona, in April 1994.

Extraction and isolation of alkaloids. Bulbs (5.6 kg) were crushed and macerated with EtOH for 48 hr. The extract was evapd under red. pres. and the residue acidified to pH 4. After removing neutral material with Et_2O, the acidic soln was extracted with $CHCl_3$ to provide extract A. Basifying this soln to pH 8–9 and extracting it with $CHCl_3$ gave extract C. Finally, $CHCl_3$–MeOH (3:2) extraction of the basic soln gave extract D. VLC from extract A on silica gel eluting with CH_2Cl_2–MeOH (10:0), increasing the gradient for the last steps to (4:1), led to the isolation of crinamine (214 mg), which was purified by further recrystallization from MeOH. Extract C was chromatographed by flash CC using CH_2Cl_2–MeOH (9:1), followed by further CC eluting with CH_2Cl_2 and a CH_2Cl_2–MeOH step gradient; after purification on Sephadex LH-20, hippeastrine (21 mg), 2α-hydroxy-6-*O*-methyloduline (38 mg), tortuosine (18 mg), norgalanthamine (569 mg), vasconine (110 mg) and additional crinamine (26 mg) were isolated. Extract D was purified in a similar manner to that described for extract A and pseudolycorine (417 mg) was isolated.

2α-Hydroxy-6-O-methyloduline (**1**). Found: C, 65.08; H, 6.11; N, 4.31. Calc. for $C_{18}H_{21}NO_5$: C, 65.23; H, 6.39; N, 4.23%. Mp 245–248. $[\alpha]_D^{22}$ +91.1° ($CHCl_3$; *c* 0.34). IR ν_{max} cm^{-1}: 3500–3100 (-OH), 2919, 1650–1620, 1484, 1237, 1038, 932 (-OCH_2O-). EI-MS 70 eV, *m/z* (rel. int.): 331 $[M]^+$ (2), 300 (2), 206

Table 3. ^{1}H NMR, HMQC and HMBC data for compound **3** (*J* given in Hz in parentheses)

H	δ	HMQC	HMBC
1	8.32 *d* (7)	120.0 *d*	C-3, C-4a, C-10a
2	7.84 *t* (7)	131.2 *d*	C-4, C-10b
3	7.70 *d* (7)	125.6 *d*	C-1, C-4a
		123.2 *s* (C-4)	
		136.0 *s* (C-4a)	
6	10.42 *s*	144.7 *d*	C-4a
		121.3 *s* (C-6a)	
7	8.09 *s*	110.5 *d*	C-6, C-8, C-9, C-10a
		151.7 *s* (C-8)	
		157.9 s (C-9)	
10	7.86 *s*	102.4 *d*	C-6a, C-8, C-9, C-10b
		130.6 *s* (C-10a)	
		136.0 *s* (C-10b)	
11	3.76 *t* (7)	27.4 *t*	C-4a
12	5.42 *t* (7)	55.4 *t*	
8-OMe	4.08 *s*	56.4 *q*	C-8
9-OMe	4.19 *s*	56.7 *q*	C-9

(<1), 175 (31), 125 (100), 134 (3), 96 (43). ^{1}H and ^{13}C NMR: Table 1.

Hippeastrine (**2**). Found: C, 64.82; H, 5.29; N, 4.52. Calc. for $C_{17}H_{17}NO_5$: C, 64.74; H, 5.43; N, 4.44%. Mp 214–216°.$[\alpha]_D^{22}$ +142° (EtOH; *c* 0.5). IR ν_{max} cm^{-1}: 3500–3100 (-OH), 1713, 1614, 1479, 1250, 1120, 1038, 938 (-OCH_2O-). EIMS 70 eV, *m/z* (rel. int.): 315 $[M]^+$ (<1), 190 (1), 162 (2), 134 (1), 125 (100), 96 (35). ^{1}H and ^{13}C NMR: Table 2.

Vasconine (**3**). Mp 232–234°. ^{1}H and ^{13}C NMR spectra: Table 3.

(−)*Pseudolycorine* [13], (+)*crinamine* [14], (−)*norgalanthamine*[15], *and tortuosine* [16]. These compounds were identified by comparison of their chromatographic and spectroscopic properties (TLC, IR, CD, MS, ^{1}H and ^{13}C NMR) with those of authentic samples.

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