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ALKALOIDS FROM *NARCISSUS TORTUOSUS**

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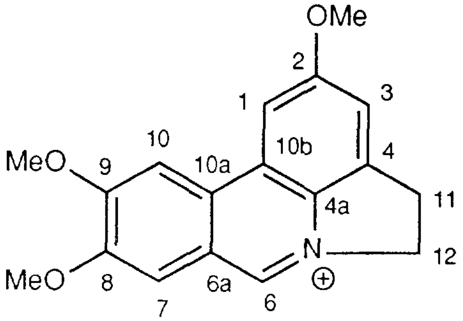
Key Word Index—*Narcissus tortuosus*; Amaryllidaceae; alkaloids; tortuosine; lycorine; 2D NMR spectroscopy.

Abstract—Whole plants of *Narcissus tortuosus* were found to contain tortuosine and lycorine. Tortuosine is reported for the first time and its structure was established by spectroscopic methods.

INTRODUCTION

Narcissus tortuosus, a species belonging to the *Pseudonarcissus* section, is notable for its pale and beautifully symmetrical flowers, regularly twisted. Morphologically, it is separable from *N. moschatus* by its stronger growth and broader foliage, and by its larger and less concolorous flowers [2]. Histological and caryological studies of *N. tortuosus* have been made recently [3, 4]. In the present paper, we describe the isolation and characterization of tortuosine (**1**) and lycorine (**2**) from whole plants of this species.



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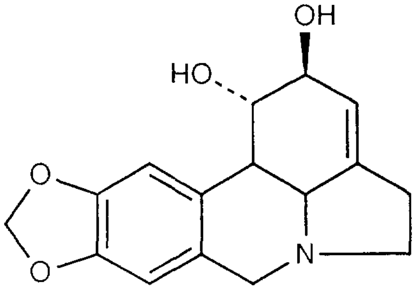

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RESULTS AND DISCUSSION

The alkaloids were extracted by routine acid-base work-up procedures and after CC (see Experimental) **1** was isolated as yellow crystals. Its HR mass spectrum ($[M]^+$ m/z 296.1292) indicated the molecular formula $C_{18}H_{18}NO_3$ which was consistent with a phenantridinium structure. Support for this assumption was obtained by comparison with spectroscopic data of the anhydrolycorinium ion, one of the biosynthetic products of *Amaryllis belladonna* most inhibitory to murine 3PS leukaemia [5], and vasconine, a quaternary alkaloid isolated from *N. vasconicus* [6].

The ^1H and ^{13}C NMR spectra were assigned taking into account the correlations observed in ^1H COSY, HMQC [7], HMBC [8] and ROESY [9] experiments. The most characteristic signals of the ^1H NMR spectrum (Table 1) were a one proton doublet at δ8.13 ($J = 1.4$ Hz) assigned to H-1 and one broad singlet at δ7.59 assigned to H-3, since they showed scalar coupling in the ^1H COSY spectrum. Also, the ROESY showed a spacial correlation between H-1 and the one-proton signal at δ8.34, and between H-1 and the methoxy group at δ4.20, allowing us to assign those signals to H-10 and H-2′, respectively. Moreover, in the ^1H COSY spectrum, the H-10 signal showed zig-zag coupling to H-6 at δ9.55 [10] and a NOE contour with the methoxy group at δ4.33 (H-9′) in the ROESY spectrum. After that, the signals at

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*Part 21 in the series '*Narcissus* alkaloids'. For Part 20 see ref. [1].

Table 1. ^{1}H–^{1}H scalar and spatial correlations of tortuosine

Proton	δ	COSY	ROESY
1	8.13 *d* (1.4)	H-3	H-10, H-2′
3	7.59 *br s*	H-11, H-1	—
6	9.55 *s*	H-12, H-10*	—
7	7.92 *s*	—	—
10	8.34 *s*	H-6*	H-1, H-9′
11	3.88 *t* (7.2)	H-12	—
12	5.35 *t* (7.2)	H-11	—
2′	4.20 *s*	—	H-1
8′	4.17 *s*	—	—
9′	4.33 *s*	—	H-10

Coupling constants in parentheses are in Hz.
*Zig-zag coupling.

Table 2. ^{13}C NMR, HMQC and HMBC data of tortuosine

Proton	Correlated carbon	
	HMQC	HMBC
1	102.2 *d*	C-2, C-3, C.4a, C-10, C-10a
	164.5 *s* (C-2)	
3	118.0 *d*	C-1, C-4a, C-11
	139.7 *s* (C-4)	
	133.0 *s* (C-4a)	
6	142.6 *d*	
	122.9 *s* (C-6a)	
7	111.0 *d*	C-6, C-9, C-10a
	153.1 *s* (C-8)	
	159.1 *s* (C-9)	
10	104.6 *d*	C-6a, C-8, C-9, C-10b
	131.4 *s* (C-10a)	
	126.1 *s* (C-10b)	
11	27.8 *t*	C-4
12	57.0 *t*	
2′	57.4 *q*	C-2
8′	57.0 *q*	C-8
9′	57.8 *q*	C-9

Carbon multiplicities were established by DEPT pulse sequence.

δ3.88 (2H, *t*, $J = 7.2$ Hz), 5.35 (2H, *t*, $J = 7.2$ Hz) and 4.17 (3H, *s*) were easily assigned to H-11, H-12 and H-8′, respectively. The ^{13}C NMR signals of **1** were assigned considering the connectivities from HMQC and HMBC spectra (Table 2). The carbon singlets at δ164.5, 159.1 and 153.1 were assigned to C-2, C-9 and C-8, respectively, because of their three-bond correlation with their corresponding methoxy protons in the HMBC spectrum. The carbon singlet at δ133.0 was ascribed to C-4a due to its three-bond connectivities to H-1 and H-3. H-1 and H-7 also gave a three-bond correlation with the carbon singlet at δ131.4, allowing us to assign this signal to C-10a. The carbon singlets at δ126.1 and 122.9 were tentatively assigned to C-10b and C-6a, according to their three-bond correlation with H-10 shown in the HMBC experiment. Finally, the two-proton triplet at δ3.88 (H-11) gave a two-bond connectivity with the carbon singlet at δ139.7 for the C-4 carbon resonance.

EXPERIMENTAL

General. Mps uncorr. IR were recorded in KBr discs. ^{1}H, ^{13}C NMR, DEPT and ^{1}H COSY spectra were recorded in a Varian VXR 500 and the HMQC, HMBC ($J = 7$ Hz) and ROESY (spin lock 1000 msec) spectra in a Bruker AMX 400 using CD_3OD as solvent with TMS as int. standard. Chemical shifts are reported in δ units (ppm). 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. Silica gel 60 F_{254} (Merck) was used for analytical (0.25 mm) and prep. (1 mm) TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

Plant material. Narcissus tortuosus Haworth was collected in May 1989 in Peña Mayor, Asturias, Spain. Samples were authenticated by Dr Herminio Nava, Facultad de Biologia, Universidad de Oviedo, and a voucher specimen (No. 890183) is deposited at the Jardí Botànic de Barcelona.

Extraction and isolation of alkaloids. F. whole plants (aerial parts and bulbs 1.2 kg) were extracted with EtOH in a Soxhlet apparatus for 10 hr. The EtOH soln was evapd under red. pres. and the residue acidified with 2% HCl. The filtered acidic soln was extracted with Et_2O to remove neutral materials and was made basic (pH 8–9) with Na_2CO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 soln was concd *in vacuo* and taken to dryness to yield extract C (1.68 g). The former aq. phase was then extracted with CH_2Cl_2–EtOH (3:2) and evapd to provide extract D (0.79 g). The aq. phase was found to be free of alkaloids. Extract C was first chromatographed by flash CC eluting with CH_2Cl_2–MeOH (19:1), increasing the gradient for the last steps until (4:1) and 2 frs were obtained. Fr. 1 yielded **2** by direct recrystallization (327 mg). Fr. 2 was chromatographed by CC on silica gel, eluting with CH_2Cl_2–MeOH (19:1), giving **2** (121 mg) and **1** (95 mg), after purifying the last one by prep. TLC eluting twice with CH_2Cl_2–MeOH (9:1). Additional **2** (59 mg) was obtained from extract D.

Tortuosine (**1**). HRMS *m/z* 296.1292 (calcd 296.1287 for $C_{18}H_{18}NO_3$). Mp 242–243°. IR ν_{max} cm^{-1}: 3450, 2923, 1630, 1515, 1266. EIMS 70 eV *m/z* (rel. int.): 296 $[M]^+$ (4), 294 (8), 280 (35), 267 (18), 239 (12), 180 (22), 167 (23), 58 (21), 44 (34), 43 (100). ^{1}H and ^{13}C NMR: Tables 1 and 2, respectively.

(−)-*Lycorine* (**2**). This alkaloid was identified by direct comparison of its chromatographic and spectroscopic data with those of an authentic sample [11].

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