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ALKALOIDS FROM *NARCISSUS CANTABRICUS**

JAUME BASTIDA,† JOSÉ LUIS CONTRERAS, CARLES CODINA, COLIN W. WRIGHT‡ and J. DAVID PHILLIPSON‡

Departament de Productes Naturals, Facultat de Farmàcia, Universitat de Barcelona, 08028 Barcelona, Spain; ‡Department of Pharmacognosy, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, U.K.

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Key Word Index—*Narcissus cantabricus*; Amaryllidaceae; alkaloids; cantabricine; structural determination.

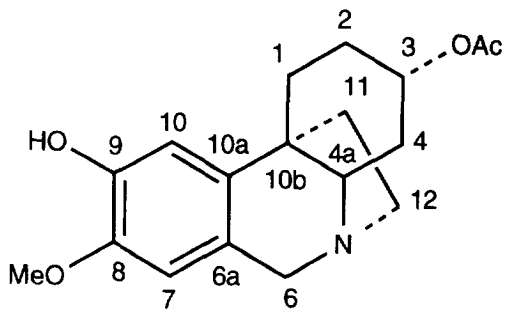
Abstract—Phytochemical studies on *Narcissus cantabricus* resulted in the isolation of six Amaryllidaceae alkaloids. Five of them, vittatine, crinamine, 6α-hydroxybuphanisine, 6β-hydroxybuphanisine and tazettine, are well known in this plant family, whereas cantabricine is reported for the first time.

INTRODUCTION

As a part of a continuing study on the alkaloid constituents of the genus *Narcissus*, we describe the chemical examination of *N. cantabricus*, belonging to the *Bulbocodii* section. This species may be associated with the Cantabric mountains, north of Spain, but, actually, it is widely distributed in the south of the Iberian Peninsula, Balearic Islands and Morocco, found in concentrated groups, which is indicative of its active vegetative propagation [2]. Histological and anatomical studies of this species have recently been carried out [3].

RESULTS AND DISCUSSION

The methanol extract of *N. cantabricus* was fractionated as described in the Experimental section. Each alkaloid-containing fraction was separated by conventional chromatographic methods. Compound 1 ($C_{18}H_{23}NO_4$) showed in its mass spectrum a $[M]^+$ at *m/z* 317 and characteristic fragments at *m/z* 258, 228, 204, and 187, following the pattern of the crinane compounds with no bridge substituent and a saturated C-ring [4]. The IR spectrum exhibited a hydroxyl band at 3450 cm^{-1}, as well as an intense absorption at 1734 cm^{-1} characteristic of an ester carbonyl group. The absolute configuration of the alkaloid, with an α-ethano bridge, was determined from the CD spectrum, which was close in shape and sign to that of the known (−)-epimaritinamine [5] and included a minimum at 233 nm. In the ^1H NMR spectrum (Table 1), four singlets at δ6.74, 6.48, 3.82 and 2.02 were assigned to the two aromatic protons, the aromatic methoxy group and the acetoxy group, respectively, and two doublets at δ4.50 and 3.91 were attributed to the AB-system of the benzylic position. The disposition of the MeO group in the aromatic ring was clarified by a ROESY experiment. Thus, H-7 (δ6.48) showed spatial correlation with the methoxyl group and both H-6 protons, whereas H-10 (δ6.74) showed a NOE contour with both H-1 protons. The aliphatic zone was completely assigned by means of a 2D COSY experiment; the plot showed correlation between H-1 equatorial and a *ddd* at δ1.77 assigned to H-1 axial, and also with a multiplet at δ2.06 (H-2 equatorial), as well as with a *dddd* (δ1.61) assigned to H-2 axial. The large coupling constant between both axial protons of the C-1 and C-2 positions (J = 13.5 Hz) give support for this assumption. The H-12 protons were observed as two multiplets at δ3.60 and 2.94. The low-field signal was assigned to H-12 *exo* because of its coplanarity with the N lone pair electrons and the NOE contour correlation with H-4 axial (δ1.44, *ddd*). The large vicinal coupling



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*Part 22 in the series '*Narcissus* alkaloids'. For part 21 see Ref. [1].

†Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) spectral data of cantabricine*

Proton	δ(p.p.m.)	
H-1ax	1.77	*ddd* (13.5, 13.5, 4.5)
H-1eq	2.39	*dt* (13.5, 3.5)
H-2ax	1.61	*dddd* (13.5, 13.5, 11.5, 3.5)
H-2eq	2.06	*m*
H-3ax	4.67	*tt* (11.5, 4.5)
H-4ax	1.44	*ddd* (12.5, 12, 11.5)
H-4eq	2.23	*m*
H-4a	3.18	*dd* (12, 5)
H-6α	3.91	*d* (16.5)
H-6β	4.50	*d* (16.5)
H-7	6.48	*s*
H-10	6.74	*s*
H-11exo	2.30	*ddd* (12, 11.5, 7)
H-11endo	1.83	*ddd* (12, 9, 5)
H-12exo	3.60	*m*
H-12endo	2.94	*m*
OMe	3.82	*s*
COMe	2.02	*s*

*Coupling constants in parentheses are in Hz.

Table 2. ^{13}C NMR (50 MHz, $CDCl_3$) spectral data of cantabricine

Carbon	δ(p.p.m.)	
C-1	25.4	*t*
C-2	26.4	*t*
C-3	69.9	*d*
C-4	30.3	*t*
C-4a	66.0	*d*
C-6	59.1	*t*
C-6a	118.4	*s*
C-7	109.0	*d*
C-8	146.3	*s*
C-9	145.4	*s*
C-10	109.6	*d*
C-10a	137.1	*s*
C-10b	42.4	*s*
C-11	35.5	*t*
C-12	50.8	*t*
OMe	55.8	*q*
COMe	170.5	*s*
COMe	21.0	*q*

constants between H-4 axial and H-4a (J = 12 Hz) and between H-4 axial and H-3 (J = 11.5 Hz) denoted their *trans*-diaxial relationship. Consequently, the acetoxy substituent on C-3 should be assigned the equatorial disposition.

The ^{13}C NMR spectrum was consistent with a structure of the crinane series with an acetoxy substituent (Table 2). The skeleton contains 18 carbon atoms, seven of which showed resonance in the shift range of δ >90 ppm. The low-field signals were five singlets for the acetoxy carbonyl group and the quaternary carbons of ring A, and two doublets for the non-quaternary aromatic (C-7 and C-10) carbons. The aliphatic shift range is characterized by one singlet (C-10b), two methine carbons (C-3 and C-4a), six triplets (C-1, C-2, C-4, C-6, C-11 and C-12) and two quartets for the methoxyl and acetoxy groups. The assignments of the carbon signals were confirmed by a heteronuclear (XCOR) shift-correlation spectrum.

EXPERIMENTAL

General. Mp uncorr. IR spectra were recorded in KBr discs or $CHCl_3$. EIMS at 70 eV. NMR spectra were recorded in the solvent specified and with TMS as int. standard; chemical shifts are reported in δunits (ppm). Silica gel SDS Chromagel 60 A CC (230–400 mesh and 70–230 mesh) were used for flash CC and VLC, Sephadex LH-20 (Pharmacia) for gel filtration. Silica gel 60 F_{254} (Merck) was used for analytical and prep. (1 mm) TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

Plant material. Whole plants of *N. cantabricus* DC were collected in March 1992, during the flowering period, near the Puerto de la Virgen, Almeria, Spain. Samples were authenticated by Dr Alfonso Susanna and a voucher specimen (no 34612) has been deposited at the Herbarium of the Faculty of Pharmacy, University of Barcelona, Spain.

Extraction and isolation of alkaloids. Freshly collected aerial parts and bulbs (5 kg) were crushed and extracted with MeOH in a Soxhlet apparatus for 10 hr. The extract was evapd under red. pres. and acidified to pH 4. After removing neutral material with Et_2O, the acidic soln was extracted with $CHCl_3$ to give extract A (0.92 g). After basifying to pH 8–9, the soln was extracted with $CHCl_3$, and later with $CHCl_3$–MeOH (3:2), affording 1.9 g of a gummy extract C. Extract A was chromatographed by VLC; three frs were obtained. Fr. I was purified by prep. TLC using EtOAc–MeOH (9:1) and a non-separable mixt. of two compounds was isolated, the epimers 6α/6β-hydroxybuphanisine (4 mg). Fr. II was chromatographed by prep. TLC eluting twice with MeOH–EtOAc (7/3) yielding **1** (37 mg) and fr. III gave crinamine (58 mg). Extract C was chromatographed by flash CC and elution was made with $CHCl_3$ gradually enriched with MeOH up to 20%. After combination of similar frs, final purification was by Sephadex LH-20, giving vittatine (13 mg), tazettine (56 mg) and more crinamine (28 mg).

Cantabricine (**1**). HRMS *m/z* 317.1635, $C_{18}H_{23}NO_4$ requires 317.1627. Mp: 75–76°. $[\alpha]_D^{20}$ − 7.14 (MeOH; *c* 0.52). IR ν_{max} cm^{-1}: 3450, 2924, 1734, 1576, 1406, 1243. EIMS 70 eV, *m/z* (rel. int.): 317 $[M]^+$ (89), 258 (100), 229 (52), 228 (63), 204 (82), 203 (37), 202 (26), 187 (50). ^{1}H and ^{13}C NMR data in Tables 1 and 2.

(+)-*Vittatine.* Mp 206–208°. $[\alpha]_D$ + 26 (MeOH; *c* 0.25). ^{1}H NMR and ^{13}C NMR spectra in agreement with the lit. [5, 6].

(+)-6α/6β-*Hydroxybuphanisine.* Mp 128–130°. $[\alpha]_D$ + 37 (MeOH; *c* 0.25). ^{1}H NMR in agreement with the lit. [7].

(+)-*Crinamine and* (+)-*tazettine*. Identified by direct comparison of spectral data [8, 9].

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