Phytochemistry, Vol 27, No 11, pp 3657–3660, 1988
Printed in Great Britain

0031-9422/88 $3.00+0.00
Pergamon Press plc

DUBIUSINE FROM *NARCISSUS DUBIUS**

JAUME BASTIDA, JOSE M. LLABRES, FRANCESC VILADOMAT, CARLES CODINA, MARIO RUBIRALTA† and MIGUEL FELIZ‡

Laboratori de Fisiologia Vegetal, Facultat de Farmàcia, †Laboratori de Química Orgànica, Facultat de Farmàcia, ‡Departament de Química Orgànica, Facultat de Química, Universitat de Barcelona, 08028-Barcelona, Catalonia, Spain

(*Received* 5 *January* 1988)

Key Word Index—*Narcissus dubius*; Amaryllidaceae, alkaloid, dubiusine; homolycorine series; 3-hydroxybutyryl radical, 2D NOE NMR

Abstract—A single alkaloid, dubiusine, has been isolated from *Narcissus dubius* growing in Candasnos (Spain). The structure 2α-acetoxy-9-(3-hydroxybutyryl)homolycorine is proposed for the compound from spectroscopic analysis and chemical correlation.

INTRODUCTION

As part of our continued interest in the alkaloids of plants belonging to the genus *Narcissus* we undertook the chemical examination of *N. dubius*, a quite rare species growing in La Serreta Negra de Fraga (Spain), a unique floristic area which acts as a link between Catalonia and Aragon floras [2]. The present paper reports on the alkaloid composition of the aerial parts of this plant for which no chemical studies have been reported so far. Surprisingly, only one alkaloid was present at the time of collection, namely dubiusine (**1**). The structure of this new alkaloid shows a 3-hydroxybutyryl radical which esterifies the 9-hydroxy group Only three other Amaryllidaceae alkaloids, clivatine, leucotamine and *O*-methylleucotamine have previously been reported to bear this biogenetically interesting radical [3, 4]. It has been pointed out the possible role of *O*-methylleucotamine as a galanthamine precursor due to its ready conversion and the isolation of free galanthamine from the same plant. However, the ester bond between the alkaloid moiety and the radical in dubiusine should present a different stability, for no free alkaloid has been found in the plant.

RESULTS AND DISCUSSION

Compound **1**, $C_{23}H_{27}NO_8$, showed in its mass spectrum a parent peak at *m/z* 445 and a base peak at *m/z* 125, which together with the fragment at *m/z* 96 is characteristic of the Amaryllidaceae alkaloids of the homolycorine series with an hydroxy group in position 2 [5]. Moreover, fragmentations at *m/z* 402 and 316, indicate the existence of acetoxy and 3-hydroxybutyryl groups. The IR spectrum of **1** shows a band at 3200–3600 cm^{-1} for the hydroxy group and two bands at 1710 and 1730 cm^{-1} for the carbonyl groups of an aryl conjugated lactone and an ester, respectively. The ^1H NMR spectrum (Table 1) is analogous to that of 2α-hydroxyhomolycorine [6], but showing a single methoxy group (δ3.94), a singlet at δ2.0 for the acetoxy group, and additional signals at δ1.29, 2.5–2.6 and 5.24, corresponding to the methyl, methylene and methyne protons of an additional substituent.

The substituent was identified as a 3-hydroxybutyryl radical on the basis of the comparison of the chemical shifts in the ^1H NMR spectrum with those of other Amaryllidaceae alkaloids having the same group. On the other hand, the mass spectrum exhibits a $[M]^+$ at *m/z* 445 and the fragments at 402 and 316 confirm the presence of the acetoxy and 3-hydroxybutyryl groups. However, the acetoxy group must be located at position 2 due to the presence of a peak at *m/z* 167 which, by loss of 43 mass units must be the origin of the base peak at *m/z* 125, characteristic of the pyrrolidine fragment [6].

Similar to the effect observed in 9-*O*-demethylhomolycorine [7], the confirmation of the position of the methoxy group in the aromatic ring was possible by application of the 2D NOE technique (Fig 1). Thus, the aromatic proton at C-7 (δ7.52) shows NOE with the signal of the methoxy group, and the proton at C-10 (δ6.97) shows NOE with H-10b and the *N*-methyl group. From these observations it can be inferred that the position of the acetoxy and 3-hydroxybutyryl groups are C-2 and C-9, respectively.

The ^{13}C NMR spectum of **1** is consistent with a structure of the homolycorine series with an acetoxy substituent. Moreover, the peaks at δ19.9 (*q*), 40.8 (*t*), 66.7 (*d*), and 170.8 (*s*) confirm the presence of the 3-hydroxybutyryl group (Table 2).

Treatment of compound **1** with hydrochloric acid–methanol (see Experimental) yielded compound **2**, which did not react with ferric chloride. The ^1H NMR spectrum indicated the absence of the 3-hydroxybutyryl group, but the acetoxy substituent seemed to be still present. This was confirmed by a singlet at δ1.94. Furthermore, whilst most peaks remained almost unchanged with respect to **1**, H-2 shifted to higher fields ($\Delta\delta$ = 1.26 ppm). This indicated the presence of a free hydroxy group at C-2, instead of an ester function. The ^{13}C NMR spectrum of **2** brought further support for this hypothesis. The absence of the acetoxy radical on the 2α-hydroxy

*Part 7 in the series '*Narcissus* alkaloids'. For part 6 see ref [1]

Table 1 ^{1}H NMR data of dubiusine (1) and compounds 2 and 3 (200 MHz, $CDCl_3$ + CD_3OD, TMS int standard)

H	1	2	3*
1	4 63 *dd* (3 3, 1 6)†	4 57 *dd* (3 3, 1 6)	4 63 *dd* (3 6, 1 6)
2	5.46 *dd* (3.3, 1 6)	4 20	4 32 *m*
3	5 62 *m* ($W_{1/2}$ = 7 Hz)	5 67 *m* ($W_{1/2}$ = 7 Hz)	5 74 *m* ($W_{1/2}$ = 8 Hz)
4a	2 74 *br d* (9 6)	2 65 *br d* (9 4)	2 5–2 7 *m*
7	7 52 *s*	7 38 *s*	7 56 *s*
10	6 97 *s*	6 70 *s*	7 00 *s*
10b	2 79 *br d* (9 6)	3 28 *br d* (9 4)	3 32 *br d* (9 5)
11	2 5–2 7 *m*	2 4–2 6 *m*	2 5–2 7 *m*
12α	3 19 *ddd* (9 6, 6.4, 3 1)	3 14 *m*	3 23 *ddd* (9 6, 6 4, 3 1)
12β	2 37 *dd* (9 6, 18 8)	2 25 *dd* (9 4, 18 8)	2 36 *dd* (9 6, 18 8)
NMe	2 06 *s*	2 13 *s*	2 14 *s*
OMe	3 94 *s*	3 87 *s*	3 98 *s*
COMe	2 00 *s*	1 94 *s*	—
CHMe	1 29 *d* (6.3)	—	—
$COCH_2$	2 53 *dd* (16, 5 5) 2 65 *dd* (16, 7 5)	—	—
CHOH	5 24 *m*	—	

*Recorded in CD_3OD

†Values in parentheses are coupling constants in Hz

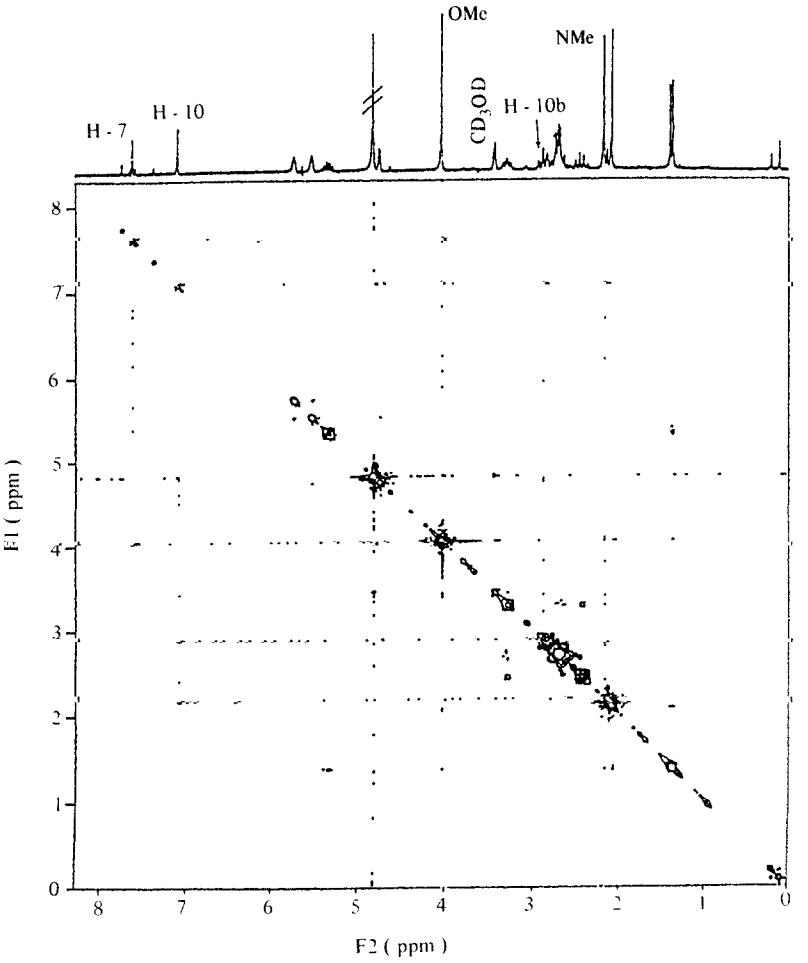
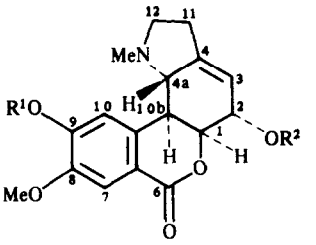


Fig 1 NOE 2D spectrum of dubiusine (1).

Table 2. ^{13}C NMR chemical shift assignments for dubiusine (**1**) and compounds **2** and **3**

C	1	2	3
1	79 7	82 8	83 6
2	69 2	66.9	67.8
3	115.1	118 9	118.4
4	147 4	144 2	145 2
4a	67.5	66 9	67.6
6a	115 4	117 7	119 4
7	115.0	117 7	119.4
8	148 2	144 3	145 2
9	152 0	151.0	151.6
10	112.7	110 9	111.9
10a	136 7	139 6	140.4
10b	39 9	39 0	40.0
11	28 0	27.6	28 3
12	56 3	56 3	56 9
NMe	43 3	42.9	43.4
OMe	56.2	55 2	55 8
COMe	21.1	23 8	—
CHMe	19 9	—	—
$COCH_2$	40.8	—	—
CHOH	66 7	—	—
C=O	165 4	165 0	165.1
	169.4	168.1	—
	170.8	—	—



1 R^1 = ——$COCH_2CHOHMe$, R^2 = Ac
2 R^1 = Ac, R^2 = H
3 R^1 = H, R^2 = H

group of **2** is confirmed by the shifts of the α, β, and γ carbons, as compared to those of **1**, which show similar effects as the ones observed in the ^{13}C NMR spectra of pseudolycorine and 2-*O*-acetylpseudolycorine [8]. Then, it must be assumed that a transacetylation reaction has taken place, and the acetoxy group is now located in the aromatic ring. As a matter of fact, the aromatic protons have experienced some slight changes in their shifts (Table 2).

Treatment of **1** with potassium hydroxide–ethanol (see Experimental) afforded a strongly polar compound **3**, which gave a positive reaction with ferric chloride. The ^1H NMR spectrum of **3** is quite similar to that of **2**, except for the absence of a singlet assignable to an acetoxy group and minor changes in the aromatic region. The shifts of the aromatic protons H-7 and H-10 coincide with those of 9-*O*-demethylhomolycorine [7].

Thus, the ^{13}C NMR spectrum of **3** (Table 2) bears a strong resemblance to those of 9-*O*-demethylhomolycorine, and 2α-hydroxyhomolycorine [6]. The structure of 2α-hydroxy-9-*O*-demethylhomolycorine is proposed for compound **3**, and compound **1**, dubiusine, is identified as 2α-acetoxy-9-(3-hydroxybutyryl)homolycorine.

EXPERIMENTAL

General Mps uncorr EIMS were recorded at 70 eV NMR spectra were recorded at 200 MHz and 50.3 MHz, for proton and carbon, respectively Chemical shifts are reported in ppm downfield (δ) from TMS. In the 2D NOE expt, the sample was degassed with N_2 and was performed using the standard sequence [8] The mixing time was 0 150 msec, 8 repetitions were accumulated for 256 values of evolution period with a spectral width of 1657 Hz in both dimensions, a delay of 3 sec was employed. A 1K × 1K data matrix was used with pseudo-echo shafcd.

Plant material N dubius Gouan was collected in Candasnos (Huesca, Spain) in April 1985, when flowers were already fading. The species was authenticated by Dr Julià Molero [Professor of Botany at the Faculty of Pharmacy, Barcelona University in which herbarium a voucher specimen (No 32928) is deposited].

Alkaloid isolation. Fr. plant material was processed in the usual manner [9] separating aerial parts (4 kg) from bulbs (3 2 kg). Bulbs were devoid of alkaloids, only extract A (850 mg) of the aerial parts gave a positive reaction with Dragendorff's reagent It was then subjected to CC on 100 g of silica gel and eluted with $CHCl_3$–EtOH (9:1). On crystallization from $CHCl_3$, 256 mg of pure compound **1** were obtained.

Dubiusine **1**. $C_{23}H_{27}NO_8$ (Found C, 61 09, H, 6.30, N, 3.11. $C_{23}H_{27}NO_8$ 1/2 H_2O requires: C, 60.79; H, 6.17; N, 3.08); mp 226–228°, IR ν_{max}^{KBr} cm^{-1}: 3600–3200, 1730, 1710, 1595, 1450; UV λ_{max}^{EtOH} nm (log ε) 210 (4 15), 232 (4.24), 276 (3.80), 308 (3.66), MS *m/z* (rel. int.) 445 $[M]^+$ (< 1), 254 (6), 253 (38), 167 (< 1), 126 (10), 125 (100), 124 (21), 96 (7), 43 (9), 42 (1).

Compound **2**. A soln of **1** (60 mg, 0.13 mmol) in MeOH–HCl (4 ml) was refluxed under N_2 with stirring for 2 hr The resulting mixt was evapd and resuspended in 5% Na_2CO_3 and $CHCl_3$ The organic phase was sepd and the aq layer extd with $CHCl_3$–EtOH (4.1) The combined organic solvent exts were dried and evapd to give **2** (45 mg, 94%): $C_{19}H_{21}NO_6$, mp 192–195°; IR ν_{max}^{KBr} cm^{-1} 3600–3100, 1705, 1580, 1420

Compound **3**. A soln of **1** (60 mg, 0.13 mmol) in EtOH–5% KOH (5 ml) was refluxed with stirring for 30 min The cooled soln was adjusted to pH 3 with 1 M HCl and then made alkaline with Na_2CO_3, followed by extn with $CHCl_3$–EtOH (4:1). The combined organic solvent exts were dried and evapd to give **3** (37 mg, 86%) $C_{17}H_{19}NO_5$, mp 272–275°, IR ν_{max}^{KBr} cm^{-1}. 3600–3100, 1700, 1585, 1505, 1465

Acknowledgments—This work was financially supported by a grant from the University of Barcelona (Ajut a la Investigació, 1984). We gratefully acknowledge Dr. Julià Molero, Faculty of Pharmacy, Barcelona University, for his assistance with the collection of plant material.

REFERENCES

1 Bastida, J., Llabrés, J M., Viladomat, F., Codina, C. and Rubiralta, M (1988) *Planta Med.* (in press)
2. Bolòs, O. (1973) *Mem Real Acad Ci. Artes (Barcelona), Ser.* 3, **42**, 289.
3 Döpke, W. (1977) *Heterocycles* **6**, 551.
4 Kobayashi, S, Yuasa, K, Sato, K., Imakura, Y. and Shingu, T. (1982) *Heterocycles* **19**, 1219

5. Schnoes, H R , Smith, D H , Burlingane, A L , Jeffs, P W and Dopke, W (1968) *Tetrahedron* **24**, 2825
6 Jeffs, P W , Abou-Donia, A , Campau, D and Saiger, D (1985) *J Org Chem* **50**, 1732
7 Bastida, J , Llabrés, J M , Viladomat, F , Codina, C Rubiralta, M and Feliz, M (1987) *J Nat Prod* **50**, 199
8 Macura, S , Wutrich, K and Ernst, R R (1982) *J Magn Reson* **46**, 269.
9 Llabrés, J M , Viladomat, F , Bastida, J , Codina, C , Serrano M , Rubiralta, M and Feliz, M (1986) *Phytochemistry* **25**, 1453