Journal of Natural Products
Vol. 50, No. 2, pp. 199-202, Mar-Apr 1987

NARCISSUS ALKALOIDS, III. 9-*O*-DEMETHYLHOMOLYCORINE FROM *NARCISSUS CONFUSUS*[1]

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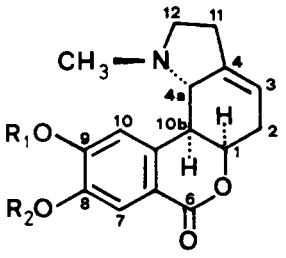
ABSTRACT.—From the bulbs of *Narcissus confusus* (Amaryllidaceae), homolycorine [**1**] and its new derivative, 9-*O*-demethylhomolycorine [**2**], were isolated and identified by means of 2D nOe.

As part of a continuing study of the flora of Catalonia (2,3) and especially on the alkaloidal constituents of the genus *Narcissus* L. (1,4), which elaborates some cytotoxic Amaryllidaceae alkaloids (5), we describe the isolation and characterization of homolycorine [**1**] and 9-*O*-demethylhomolycorine [**2**] from *Narcissus confusus* Pugsley.

Column chromatography of the alkaloid extract A (4) on neutral alumina afforded compound **1**, which was identified as homolycorine by comparison of its physical and spectroscopic data with those previously described (6,7). The most important signals of its ^{1}H-nmr spectrum are: (a) two singlets at δ 6.99 and 7.57 for the aromatic protons H-10 and H-7, respectively, according to the deshielding effect of the *peri*-carbonyl group on H-7 (7); (b) a broad signal at δ 5.50 due to the olefinic proton; (c) two doublets of doublets at δ 2.64 and 2.72 assigned to the methinic protons H-10b and H-4a, respectively; and (d) two signals at δ 2.24 (dd) and δ 3.14 (ddd) for the β and α protons of the C-12 positions, the latter being more deshielded due to its *cis*-relation with nitrogen lone pair (8). In **1** hydrochloride, the chemical shift of H-4a and Hα-12 appear at lower fields ($\sim$0.4 ppm) as a consequence of the protonation of the nitrogen atom (9). A large deshielding is observed on H-10 ($\Delta\delta$= 1.35), which is explained on the basis that compound **1** has the *N*-methyl group in its β-configuration [subject to diamagnetic shielding by the aromatic ring (6)], and, thus, the nitrogen lone pair (α-oriented) is directly affecting H-10.

Compound **2**, $C_{17}H_{19}NO_4$, shows in its mass spectrum a parent peak at *m/z* 301, and a characteristic fragment ion at *m/z* 109 originated from a retro-Diels Alder fragmentation, which constitutes a general process for these systems (10,11). The ir spec-



1 R_1=R_2=CH_3
2 R_1=H, R_2=CH_3
3 R_1=CH_3, R_2=H

[1]For paper II in this series, see Llabrés *et al.* (1).

trum is similar to that of compound **1** and 8-*O*-demethylhomolycorine [**3**] [named 9-*O*-demethylhomolycorine in (7), according to a different numbering system] and shows an absorption at 1710 cm^{-1} characteristic of the lactone carbonyl group.

The ^{1}H-nmr spectrum (Table 1) agrees with that of homolycorine but for the presence of a single methoxy group (δ 3.94) and a phenolic hydroxy group (δ 3.58). Moreover, the partially assigned spectrum of 8-*O*-demethylhomolycorine [**3**] (7) is similar to that of **2**, though H-4a is assigned at lower fields (δ 3.26).

TABLE 1. ^{1}H-nmr Chemical Shift Assignments in δ-Values (ppm) from TMS, for Homolycorine [**1**] ($CDCl_3$) and 9-*O*-Demethylhomolycorine [**2**] ($CDCl_3/CD_3OD$)

Proton	Compound	
	1	**2**
H-1	4.81 ddd (4.2, 1.8, 1.7)	4.80 ddd (4.2, 1.7, 1.6)
H-2	2.49 m	2.51 m
H-3	5.50 m (W½=7)	5.55 m (W½=7)
H-4a	2.72 dd (9.6, 2.0)	2.71 bd (10.0)
H-7	7.57 s	7.54 s
H-10	6.99 s	6.91 s
H-10b	2.64 dd (9.6, 1.8)	2.60 dd (10.0, 1.6)
H-11	2.61-2.67 m	2.49-2.64 m
Hα-12	3.14 ddd (9.2, 5.3, 4.1)	3.15 ddd (10.0, 7.0, 3.5)
Hβ-12	2.24 dd (18.0, 9.2)	2.30 dd (18.3, 9.2)
N-CH_3	2.00 s	2.01 s
O-CH_3	3.95 s 3.96 s	3.94 s

Both the confirmation of the position of the methoxy group in the aromatic ring and the assignment of the whole spectrum were possible by the application of the 2D nOe technique (Figure 1). Thus, while the proton at δ 7.54 shows nOe with the signal of the methoxy group, spatial proximity between the aromatic proton at C-10 (δ 6.91) with H-10b, and the *N*-methyl group is established, which, moreover, confirms the assignment of H-10b.

From these observations can be inferred: (a) that the methoxy group is attached at C-8 in the aromatic ring, and in consequence compound **2** is identified as 9-*O*-demethylhomolycorine; and (b) H-10b and H-4a are unambiguously assigned, which permits their reassignment in homolycorine (6) and 8-*O*-demethylhomolycorine, (7).

The ^{1}H-nmr spectrum of **2** hydrochloride shows deshieldings of the aromatic proton at C-10 (δ 7.53), as well as of the *N*-methyl group (δ 2.57), which agree with the above mentioned observations for homolycorine.

The ^{13}C-nmr spectrum of compound **2** supports the proposed structure and agrees with the literature for homolycorine and 8-*O*-demethylhomolycorine (7). Nonetheless, the mp 270-272° found for **2** is in contrast with that previously described for a demethylhomolycorine (mp 213-214°) (12), which also differs from that of 8-*O*-demethylhomolycorine (mp 138-140°) (7).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—All mps are uncorrected. Eims were recorded at 70 eV. Nmr spectra were recorded on a Varian XL-200 spectrometer working at 200 MHz and 50.3 MHz for proton and carbon, respectively. Chemical shifts are in ppm. In the 2D nOe experiment, the sample was degassed by a N_2 stream and was performed using the standard sequence (13). The mixing time was 0.150 msec, and 32 transients were accumulated for 256 values of evolution period with a spectral width of 1315 Hz in both dimensions, and a delay of 2 sec was employed. A 512×512 data matrix was used with pseudoecho and triangular folding (14).

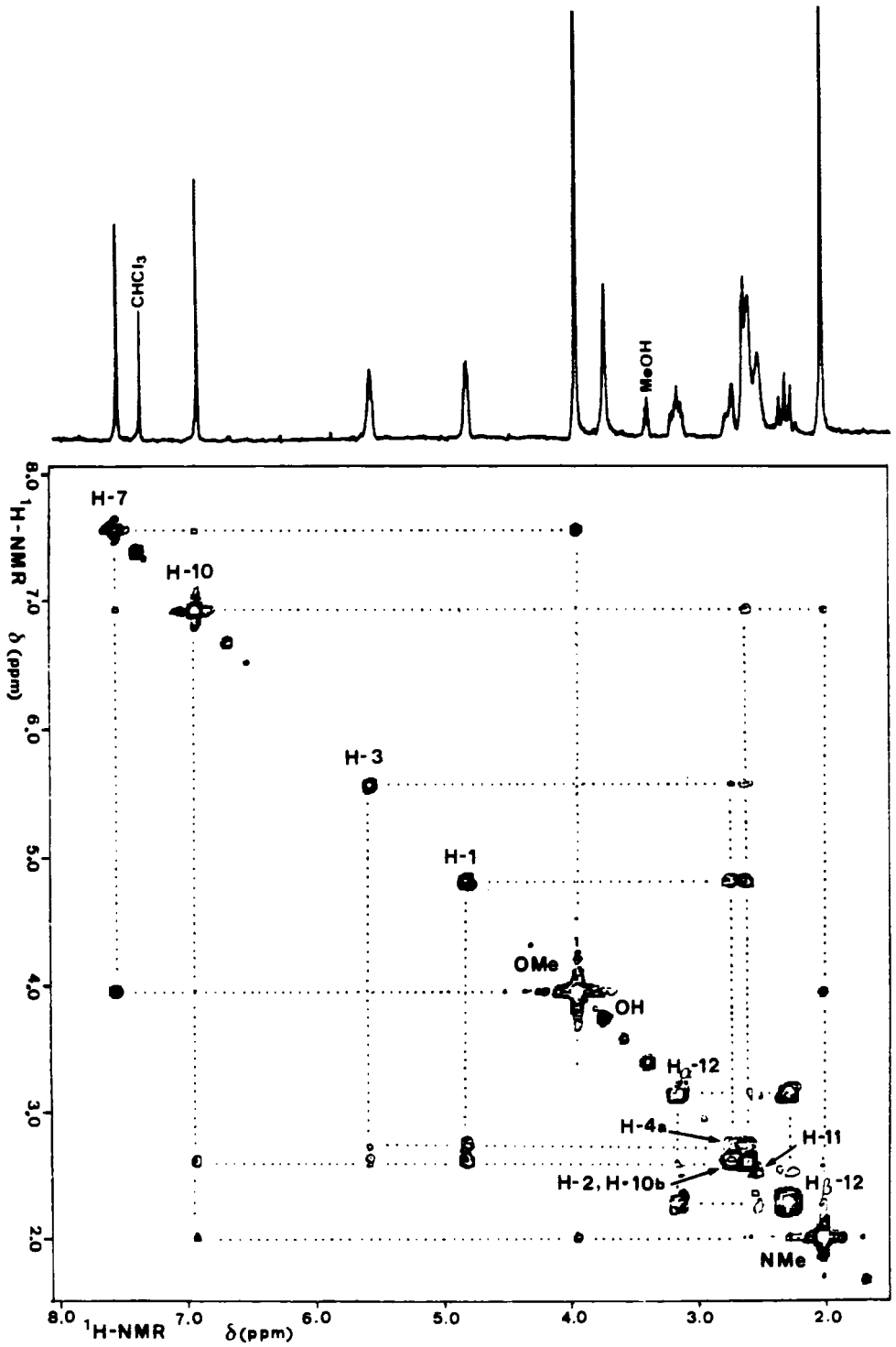


FIGURE 1. 2D nOe spectrum of 9-*O*-demethylhomolycorine [**2**]

PLANT MATERIAL.—*N. confusus* was collected in Béjar, Salamanca, in 1985. The species was authenticated by Dr. Javier Fernández Casas from the Botanic Institute of Madrid, and a voucher specimen is deposited in the herbarium of the Department of Botany, Faculty of Pharmacy, University of Barcelona.

PLANT EXTRACTION.—In a typical experiment (4) extracts A (2.70 g) and C (22.42 g) were obtained from 5550 g of fresh bulbs of *N. confusus* (no extract B was obtained). Further extraction of the aqueous alkaline solution with $CHCl_3$-EtOH (3:2) afforded extract D (0.31 g).

Fresh aerial parts (5150 g) were treated likewise, extracts A, C, and D having the same main alkaloidal composition as in the bulbs, as shown by co-tlc analysis in different solvents. Extract C is still under investigation.

TREATMENT OF EXTRACT A.—The brown gum was chromatographed by cc on 60 g of neutral alumina and eluted with $CHCl_3$, affording compound **1** which was crystallized from hexane/$CHCl_3$ and recrystallized several times with Me_2CO (392 mg).

Homolycorine [**1**].—Mp 169-171° [lit. 173-175° (7)]; ir ν max (KBr) cm^{-1} 1710, 1600, 1510, 1460, 1080; uv λ max (EtOH) nm (log ϵ) 226 (4.22), 230 (4.22), 270 (3.92), 306 (3.70); ms *m/z* (rel. int.) 206 (1), 178 (3), 109 (100), 108 (29), 94 (6), 82 (8), 42 (13); ^{13}C nmr ($CDCl_3$) in agreement with lit. (7).

1 *Hydrochloride.*—Mp 284-287° [lit. 285° (15)]; ^1H nmr $CDCl_3/CD_3OD$) δ 2.59 (3H, s, NMe), 3.13 (1H, m, H-4a), 3.56 (1H, m, Hα-12), 3.94 (3H, s, OMe), 4.01 (3H, s, OMe), 5.03 (1H, m, H-1), 5.88 (1H, m, H-2), 7.52 (1H, s, H-7), 8.34 (1H, s, H-10).

TREATMENT OF EXTRACT D.—Evaporation of the solvent yielded white crystals of compound **2**, which was recrystallized from EtOH/$CHCl_3$ (103 mg).

9-O-Demethylhomolycorine [**2**].—$C_{17}H_{19}NO_4$ (Found: C, 67.53; H, 6.31; N, 4.59; $C_{17}H_{19}NO_4$ requires: C, 67.77; H, 6.31; N, 4.65); mp 270-272°; ir ν max (KBr) cm^{-1} 3390, 1710, 1590, 1460, 1290, 1050; uv λ max (EtOH) nm (log ϵ) 230 (4.25), 274 (3.84), 306 (3.65), adding one drop of NaOH 0.1N soln: 216 (4.30), 250 (4.13), 334 (4.24); ms *m/z* (rel. int.) 301 $(M)^+$ (2), 227 (5), 225 (5), 181 (5), 109 (100), 108 (37), 69 (7), 55 (8), 44 (20); ^{13}C nmr ($CDCl_3/CD_3OD$) δ 27.9 (t, C-11), 31.3 (t, C-2), 43.0 (d, C-10b), 43.3 (q, NMe), 56.2 (q, OMe), 56.5 (t, C-12), 66.9 (d, C-4a), 77.9 (d, C-1), 112.7 (d, C-7), 115.0 (d, C-10), 115.6 (s, C-6a), 116.1 (d, C-3), 138.0 (s, C-10a), 140.0 (s, C-4), 148.1 (s, C-8), 152.0 (s, C-9), 167.0 (s, C-6).

2 *Hydrochloride.*—Mp 220-222°; ^1H nmr ($CDCl_3/CD_3OD$) δ 2.57 (3H, s, NMe), 3.58 (1H, m, Hα-12), 3.94 (3H, s, OMe), 4.89 (1H, m, H-1), 5.83 (1H, m, H-3), 7.53 (1H, s, H-10), 7.56 (1H, s, H-7).

ACKNOWLEDGMENTS

This work was financially supported by a grant of the University of Barcelona.

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Received 3 July 1986