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NARCISSUS ALKALOIDS, XIII.[1] COMPLETE ASSIGNMENT OF THE NMR SPECTRA OF PAPYRAMINE AND 6-*EPI*-PAPYRAMINE BY TWO-DIMENSIONAL NMR SPECTROSCOPY

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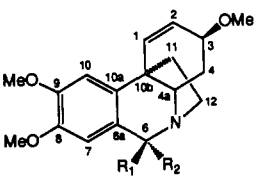
ABSTRACT.—Phytochemical studies on the aerial parts and bulbs of *Narcissus panizzianus* collected in Málaga, Spain, resulted in the isolation of five Amaryllidaceae alkaloids. Three of them were identified as homolycorine, pretazettine, and galanthine [**3**]. The fourth and fifth compounds were determined as papyramine [**1**] and 6-*epi*-papyramine [**2**] which have now been completely characterized by means of two-dimensional nmr experiments.

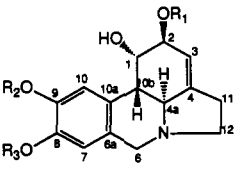
The genus *Narcissus* L. (Amaryllidaceae) is widely distributed in the Mediterranean area. During our study on the alkaloids of these plants, we have investigated *Narcissus panizzianus* Parl., for which no chemical work has been previously reported. The EtOH extract of the aerial parts and bulbs of *N. panizzianus* was found to contain the well known compounds homolycorine and pretazettine and the alkaloids papyramine [**1**] and 6-*epi*-papyramine [**2**], the major alkaloids of this species, and galanthine [**3**]. To our knowledge, this is the second reported isolation of papyramine epimers from nature. These alkaloids had been previously isolated only from *Narcissus papyraceus* Ker-Gawl. (2), but they are here completely characterized by 2D nmr techniques as two epimeric compounds. The structure of galanthine [**3**], another uncommon amaryllidaceous alkaloid, has been also completely assigned. The absolute stereochemical relationships of **1** and **2** are discussed below.

RESULTS AND DISCUSSION

The EtOH extract of the fresh aerial parts and bulbs of *N. panizzianus* was fractionated as described in the Experimental section. Each alkaloid-containing fraction was



1 R_1=H, R_2=OH
2 R_1=OH, R_2=H



3 R_1=R_2=R_3=Me
4 R_1=H, R_2=R_3=Me
5 R_1=Me, R_2+R_3=CH_2
6 R_1=H, R_2+R_3=CH_2

[1]For part XII in this series see Codina *et al.* (1).

separated by a combination of cc and preparative tlc, and five alkaloids were thus obtained. Extract A yielded papyramine [**1**], 6-*epi*-papyramine [**2**], and homolycorine, while extract C was found to contain additional papyramine epimers together with the alkaloids pretazettine and galanthine [**3**].

Compounds **1** and **2** crystallized together from EtOAc, and they were isolated as a mixture which was not easily separated by conventional chromatographic methods. Ms showed the molecular peak at *m/z* 317 consistent with a molecular formula $C_{18}H_{23}NO_4$. The base peak at *m/z* 262 $[M-C_3H_5N]^+$ is characteristic for the alkaloids of the haemanthamine-crinine series with a double bond between the C-1 and C-2 carbons and without hydroxy substituent at the C-11 position (3). The peaks at *m/z* 286 and 300 suggest the presence of an aliphatic OMe and a hydroxy group, respectively. The fragmentations of compounds **1** and **2** are both in agreement with those observed for papyramine (2) and 3-*epi*-papyramine, a very closely related compound, isolated from *Narcissus tazetta* L. var. *chinensis* Roem. (4).

The ^{1}H-nmr spectra of papyramine [**1**] and 6-*epi*-papyramine [**2**] recorded in $CDCl_3$ (Table 1) provided additional data. (a) Two singlets at δ 5.86 and 5.16 were found in the ratio 2:3, due to the benzylic H-6 protons for both epimers **1** and **2**, respectively. (b) One double doublet of an olefinic proton at δ 6.00 assignable to the H-2 protons of both epimers coupling with the H-1 doublets at 6.65 and 6.66 ppm was found. The value of the H-2/H-3 coupling constant ($J = 5.0$ Hz) was in agreement with a β position of the OMe group in C-3 and was identical to that of papyramine but not to that of 3-*epi*-papyramine ($J = 1.5$ Hz) (4). (c) Four singlet signals for aromatic protons were observed at δ 7.03, 6.89, 6.83, and 6.80. The assignment of these protons was made on the basis of the relative intensities of the signals of both epimers and the benzylic coupling between the H-6 (δ 5.86) and H-7 (δ 7.03) protons corresponding to the epimer **1**, observed by the 2D COSY experiment (Figure 1). (d) Three signals for the methoxy groups at δ 3.88, 3.37 and 3.31 were observed, the first of them integrating six protons while the sum of the other two only integrated three protons corresponding to the aliphatic methoxy group of both epimers. (e) A double doublet at δ 3.59 and a double doublet of doublets at δ 1.77 were assigned to the H-4a and H-4ax

TABLE 1. ^{1}H-nmr Chemical Shift Values and Coupling Constants (in parentheses) for Compounds **1** and **2**.

Proton	Compound	
	1	**2**
H-1	6.65 d (10.0)	6.66 d (10.0)
H-2	6.00 dd (5.0, 10.0)	6.00 dd (5.0, 10.0)
H-3	3.88 m	3.85 m
H-4α	1.77 ddd (4.0, 13.4, 13.5)	1.60 ddd (4.0, 13.8, 13.9)
H-4β	2.21 brd (13.5)	2.09 m
H-4a	3.59 dd (4.0, 13.4)	3.90 m
H-6	5.86 s	5.16 s
H-7	7.03 s	6.89 s
H-10	6.80 s	6.83 s
H-11	2.00 m	2.00 m
H-12 endo	3.02 ddd (4.0, 9.5, 13.5)	2.84 ddd (6.5, 8.0, 14.0)
H-12 exo	3.73 ddd (4.5, 8.0, 13.5)	3.39 m
OMe	3.88 s	3.88 s
	3.88 s	3.88 s
	3.37 s	3.31 s

protons of **1**, respectively. These protons showed a large coupling ($J = 13.4$ Hz), due to their trans diaxial configuration, characteristic of the haemanthamine series (5). The ^{1}H-nmr data previously obtained for papyramine from *N. papyraceus* (2) are here completed by the 2D COSY experiment (Figure 1).

The ^{13}C-nmr spectra of papyramine [**1**] and 6-*epi*-papyramine [**2**], reported here for the first time (Table 2), confirms the proposed structures. Thus, the skeleton of compounds **1** and **2** contains 18 carbon atoms, eight of which show resonance in the shift range of $\delta > 90$ ppm. In the ^{13}C-nmr spectra of compounds **1** and **2**, most signals had the appearance of doublets, with one peak, associated with **2**, predominating. The low field signals are four singlets for the quaternary carbons C-6a, C-8, C-9, and C-10a and four doublets for the olefinic (C-1 and C-2) and the non-quaternary aromatic (C-7 and C-10) carbons. The aliphatic shift range is characterized by one singlet (C-10b), three doublets (C-3, C-4a, and C-6), and three triplets (C-4, C-11, and C-12). Three quartets for aliphatic and aromatic methoxy carbons were also found. The stereochemistry of the hydroxy group in the C-6 position is in agreement with the shieldings of C-4a and C-12 in **2** and **1**, respectively. All the signals have been unambiguously confirmed by means of the XCOR experiment.

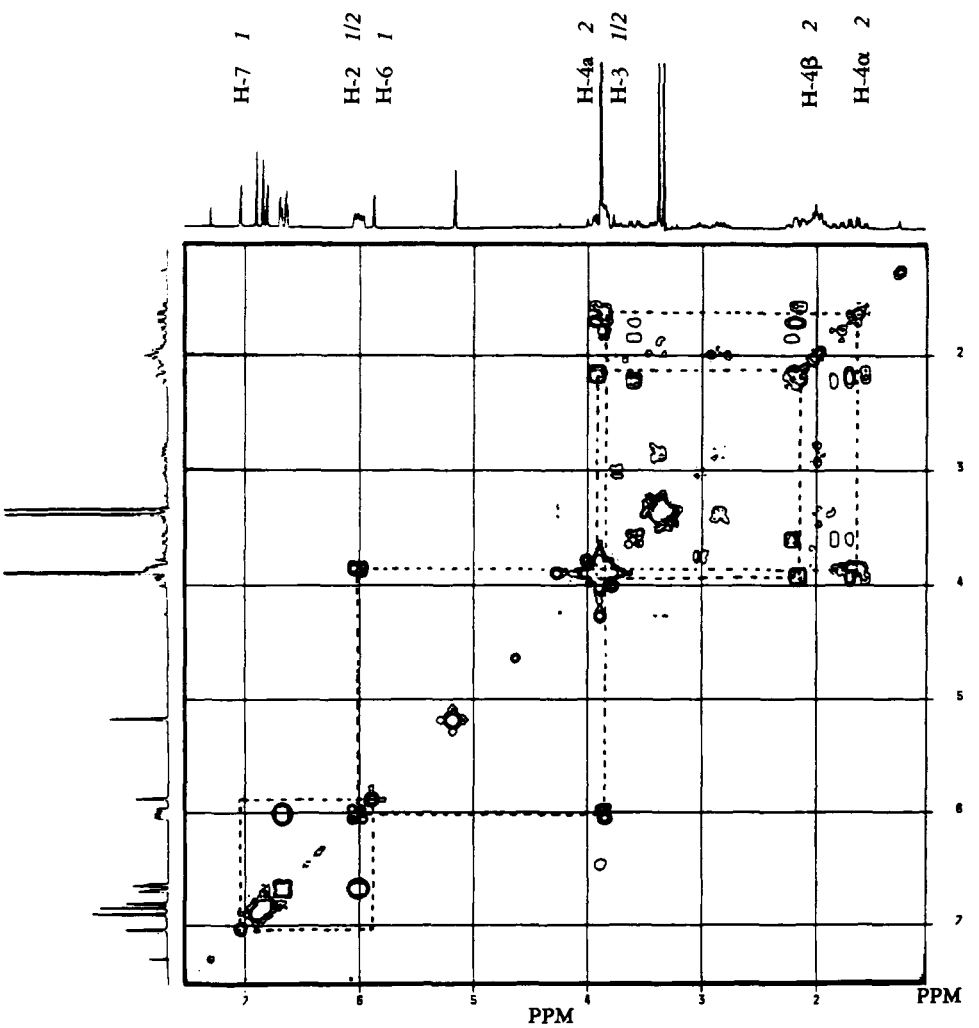


FIGURE 1. 2D COSY of papyramine epimers **1** and **2**.

TABLE 2. ^{13}C-nmr Chemical Shifts for Compounds **1** and **2**.

Carbon	Compound	
	1	**2**
C-1	132.4 d	132.0 d
C-2	125.6 d	125.9 d
C-3	72.4 d	72.4 d
C-4	28.7 t	28.1 t
C-4a	62.2 d	56.3 d
C-6	87.0 t	88.9 t
C-6a	127.4 s	126.5 s
C-7	111.0 d	112.5 d
C-8	147.8 s	147.8 s
C-9	148.5 s	148.7 s
C-10	105.4 d	105.4 d
C-10a	136.4 s	137.4 s
C-10b	44.7 s	44.1 s
C-11	42.3 t	40.9 t
C-12	42.0 t	47.9 t
OMe	56.6 q	56.6 q
	56.6 q	56.6 q
	56.1 q	56.0 q

The third compound isolated, $C_{18}H_{23}NO_4$, was identified from spectral evidence as galanthine [**3**]. Its ^{1}H-nmr spectrum showed the characteristic singlets at δ 6.78 and 6.52 corresponding to the aromatic protons, two doublets at δ 4.05 and 3.40 for the AB system corresponding to the C-6 position, three singlets at δ 3.78, 3.74, and 3.40 for the two aromatic methoxy groups and the aliphatic one, respectively, and a broad singlet at δ 5.55 assigned to the vinylic proton. These signals are in good agreement with previously published data (6). Further examination of the spectrum revealed: (a) a broad signal at δ 4.55 for the H-1, and two multiplets at δ 3.72 and the interval of 2.45–2.60, assigned to the H-2 and H-11 protons, respectively; (b) a double doublet and a double doublet of doublets for the α and β protons of the C-12 position, respectively; and (c) an apparent singlet at δ 2.65 integrated by two protons, assignable to the 4a and 10b positions. The convergence of these two protons with respect to 9-*O*-methylpseudolycorine (7) [**4**] is in agreement with the shielding effect of the C-2 methoxy group on the H-4a proton, which is also observed in other structurally similar compounds such as hippamine (8) [**5**] and lycorine (9) [**6**]. Correlation of this signal (δ 2.65) with H-3 (by allylic coupling with H-4a), H-1, and H-6α (by benzylic coupling with H-10b) (Figure 2) enabled us to make the assignment. Confirmation was secured by the XCOR experiment, in which correlation between H-4a/H-10b (δ 2.65) and both C-4a (δ 60.9) and C-10b (δ 41.5) was observed. The aliphatic methoxy group in the C ring was assigned to the C-2 position by means of the nOe experiment (Figure 3). Likewise, this technique allowed the unambiguous positional assignment of the aromatic protons and the two aromatic methoxy groups. Furthermore, the ir, ms, and ^{13}C-nmr spectral data of **3** were fully in agreement with those reported in the literature (6,10).

We also isolated from *N. panizzianus* the very common amaryllidaceous alkaloids homolycorine and pretazettine, which were identified by their chromatographic and spectroscopic properties.

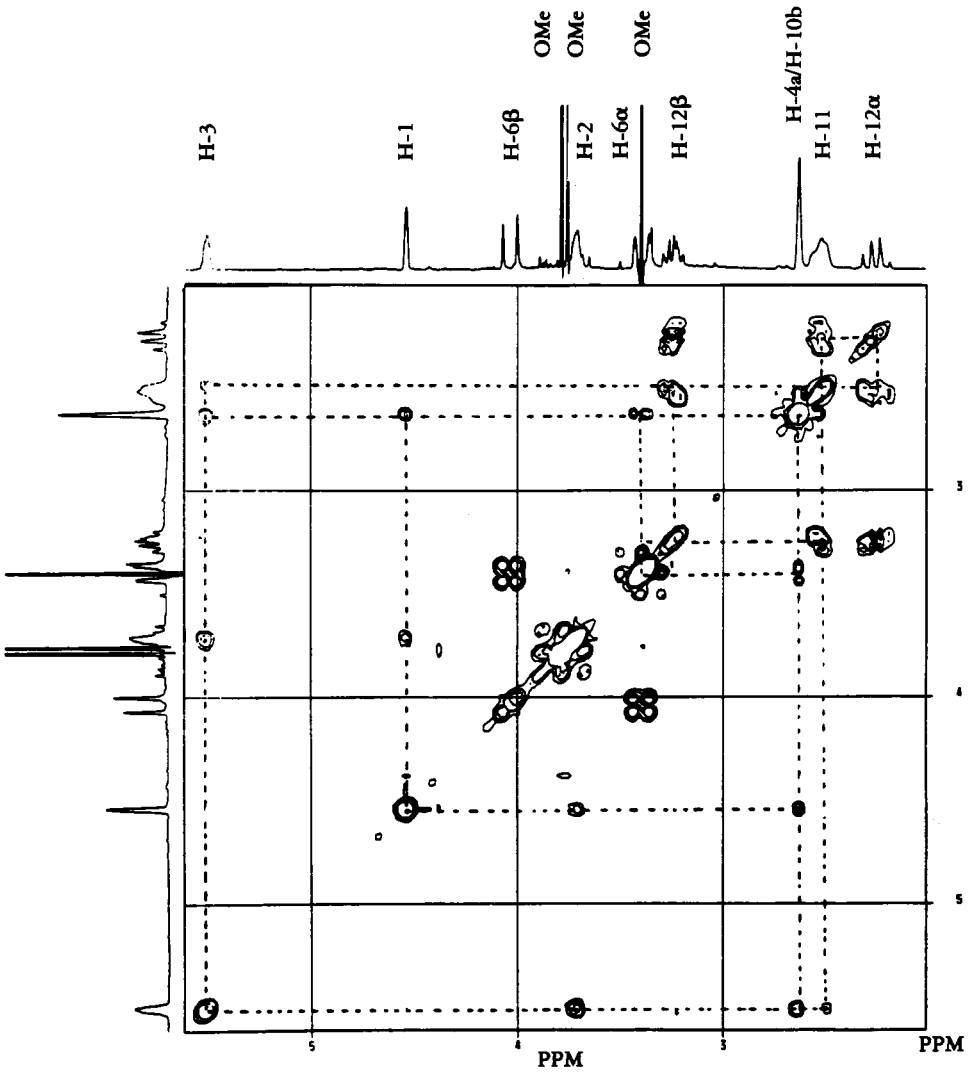


FIGURE 2. 2D COSY of galanthine [3].

EXPERIMENTAL

PLANT MATERIAL.—The whole plants of *N. panizzianus* were collected by us in February 1989, during the flowering period, from the Málaga province, Spain. The plants were identified by Dr. Alfonso Susanna, Institut Botànic de Barcelona, and a voucher specimen (no. 35195) has been deposited at the Herbarium of the Faculty of Pharmacy, University of Barcelona, Spain.

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded in KBr on a Perkin-Elmer 1430 spectrophotometer. Mass spectra were obtained with a Hewlett-Packard 59865 A spectrometer operating at 70 eV. Nmr spectra were recorded in $CDCl_3$ at 200 MHz (^{1}H nmr) and 50.3 MHz (^{13}C nmr) on a Brüker AC200 instrument using TMS as internal reference. Chemical shifts are reported in δ (ppm) values and coupling constants (*J*) in Hz. Si gel 60 Merck (70–230 mesh) and Si gel SDS Chromagel 60 A CC (230–400 mesh) were used for cc and flash cc, respectively, and pre-coated Si gel plates (Kieselgel 60 F_{254}, 70–230 mesh, Merck) were used for analytical and preparative tlc. The chromatograms were examined under uv light (254 nm), and the developed plates were visualized by spraying with Dragendorff's reagent.

EXTRACTION AND ISOLATION OF ALKALOIDS.—The freshly collected aerial parts and bulbs of *N. panizzianus* (10 kg) were crushed and extracted with EtOH in a Soxhlet apparatus for 10 h. The extract ob-

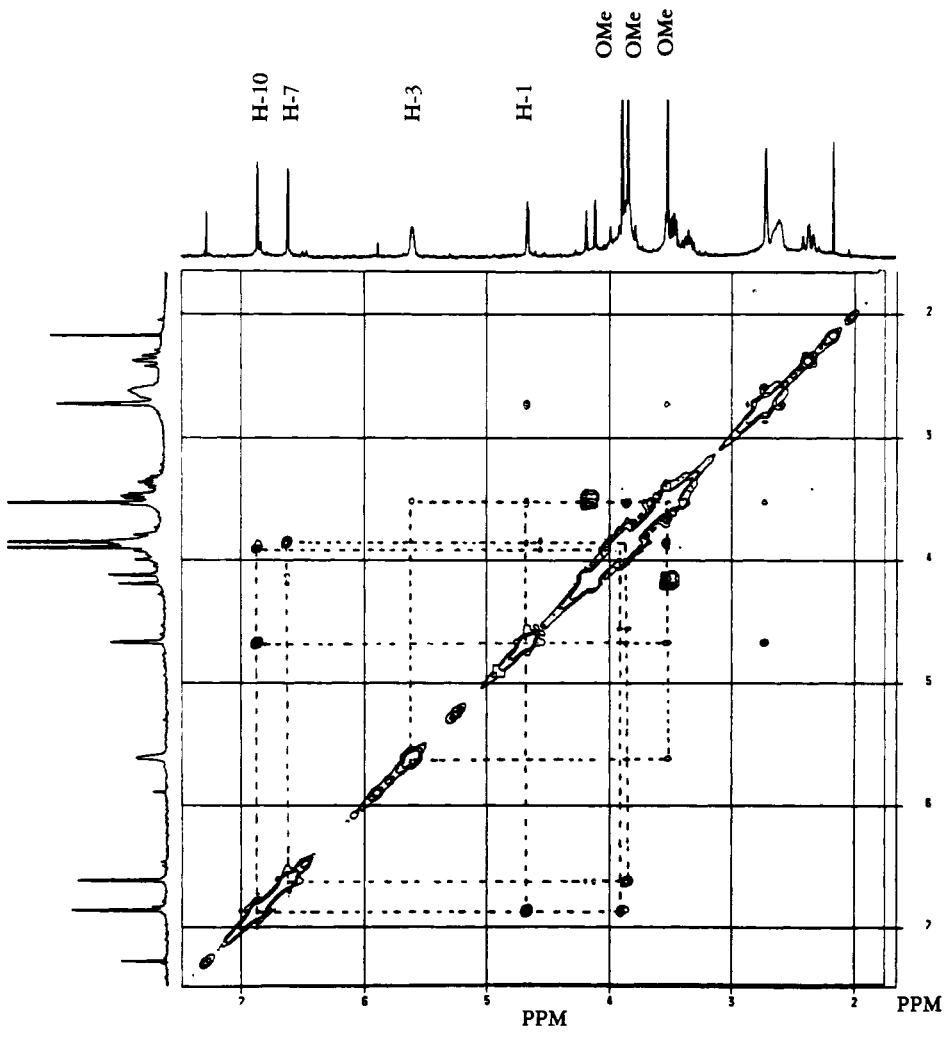


FIGURE 3. 2D nOe spectrum of galanthine [**3**].

tained by evaporation of the EtOH was dissolved in 2% HCl, and the mixture was filtered. After removal of the neutral material with Et_2O, the acidic solution was basified with NH_4OH and evaporated under reduced pressure. The crude alkaloidal residue (224 g) was acidified with 2% HCl and extracted with $CHCl_3$. Two solutions were thus obtained. The $CHCl_3$ solution was washed with Na_2CO_3, dried with anhydrous Na_2SO_4, and filtered. After evaporation, 2.53 g of extract A was obtained. The former aqueous solution was made basic with Na_2CO_3 and extracted with CH_2Cl_2. The aqueous phase was found to be free of alkaloids, and the organic phase was treated as for extract A affording 3.34 g of extract C.

TREATMENT OF EXTRACT A.—The brown gum was chromatographed by flash cc and eluted with a $CHCl_3$/MeOH mixture, gradually increasing the MeOH concentration up to 10%. Fractions (100 ml) were combined on the basis of their tlc patterns. Further purification through preparative tlc afforded papyramine epimers **1** and **2** (324 mg) and homolycorine (74 mg), which crystallized from EtOAc and Me_2CO, respectively.

TREATMENT OF EXTRACT C.—Extract C was subjected to flash cc, eluting with a CH_2Cl_2/MeOH mixture. The MeOH concentration was gradually increased up to 20%. Further purification by preparative tlc was applied to the eluate and yielded pretazettine (754 mg), galanthine [**3**] (34 mg), and additional papyramine epimers **1** and **2** (486 mg).

Papyramine epimers **1** *and* **2**.—$C_{18}H_{23}NO_4$ (found C 67.53, H 7.18, N 4.37; requires C 68.12, H 7.30, N 4.41); mp 110–112°; ir ν max cm^{-1} 3420, 2935, 1610, 1512, 1464, 1403, 1268, 1223, 1142,

1116; ms *m/z* (rel. int.) $[M]^+$ 317 (62), 300 (9), 286 (19), 268 (11), 262 (100), 247 (53), 246 (17), 233 (26), 229 (21), 203 (42), 128 (26), 115 (37) [lit. (2)]; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Galanthine [3].—$C_{18}H_{23}NO_4$ (found C 67.94, H 7.29, N 4.38; requires C 68.12, H 7.30, N 4.41); mp 160–162°; ^{1}H nmr ($CDCl_3$) δ 6.78 (1H, s, H-10), 6.52 (1H, s, H-7), 5.55 (1H, brs, H-3), 4.55 (1H, s, H-1), 4.05 (1H, d, 14.0, H-6β), 3.78 (3H, s, OMe), 3.74 (3H, s, OMe), 3.72 (1H, m, H-2), 3.40 (3H, s, OMe), 3.40 (1H, brd, 14.0, H-6α), 3.25 (1H, ddd, 6.0, 10.0, 16.4, H-12β), 2.65 (2H, s, H-4a and H-10b), 2.45–2.60 (2H, m, H-11), 2.25 (1H, dd, 8.2, 16.4, H-12α); ^{13}C nmr ($CDCl_3$) δ 147.8 (s, C-8), 147.6 (s, C-9), 143.9 (s, C-4), 129.3 (s, C-6a), 126.6 (s, C-10a), 115.1 (d, C-3), 110.8 (d, C-7), 108.0 (d, C-10), 81.0 (d, C-2), 68.3 (d, C-1), 60.9 (d, C-4a), 57.3 (q, OMe), 56.6 (t, C-6), 56.0 (q, OMe), 55.9 (q, OMe), 53.8 (t, C-12), 41.5 (d, C-10b), 28.5 (t, C-11) [lit. (10)] ms *m/z* (rel. int.) $[M]^+$ 317 (8), 257 (18), 243 (43), 242 (45), 149 (11), 137 (12), 129 (11), 125 (29), 124 (13), 123 (16), 111 (41), 43 (100) [lit. (6)].

Homolycorine and pretazettine.—These alkaloids were chromatographically (tlc) and spectrally (ir, ms, ^{1}H nmr) identical with authentic compounds (5,11).

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