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TWO ALKALOIDS FROM *NARCISSUS REQUIENII*

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Key Word Index—*Narcissus requienii*; Amaryllidaceae; pseudolycorine; 1-*O*-acetylpseudolycorine; 2-*O*-acetylpseudolycorine; 2D NMR spectra.

Abstract—The aerial part of *Narcissus requienii* (Amaryllidaceae) was found to contain pseudolycorine as the major alkaloid, and two new phenolic bases: 2-*O*-acetylpseudolycorine and 1-*O*-acetylpseudolycorine. The present communication reports the complete assignments of the NMR spectra of pseudolycorine, and the structure elucidation of the other compounds by means of 2D ^1H–^1H and ^1H–^{13}C NMR chemical shift correlation experiments.

INTRODUCTION

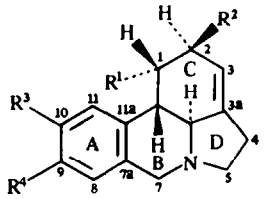
In our alkaloid screening program with about 600 plants [1, 2] we found that *Narcissus requienii* Roem. (Amaryllidaceae) yielded a strong positive result. This plant had not been essayed before (possibly due to its limited geographical distribution). Moreover, members of the Amaryllidaceae are known for their medicinal properties and several isolated alkaloids have shown a wide range of biological activities [3–5]. Thus, we considered a better chemical knowledge of the alkaloidal constituents of this plant to be of interest.

RESULTS AND DISCUSSION

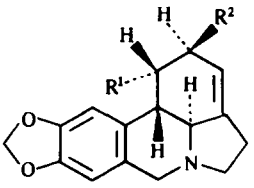
The plant material was collected in Montserrat (Barcelona, Spain) in April 1984 (flowering season) and treated according to the usual methods (see Experimental). Extract B contained the major alkaloid, pseudolycorine (**1**), and extract C had two new phenolic bases **2** and **3**. New spectroscopical studies on **1** and the structure elucidation of **2** and **3** are described below.

Compound **1**, $C_{16}H_{19}NO_4$, was identified as pseudolycorine on the basis of its physical and spectral properties, and by comparison of its chromatographic behaviour with that of a sample of authentic pseudolycorine [6]. Its ^1H NMR spectrum (Table 1) shows the characteristic singlets at δ6.89 and 6.71, corresponding to the aromatic protons, and a triplet at 5.60 assigned to the vinylic proton. These signals are in good agreement with the previously published results [6]. Further examination of the spectrum reveals: (i) two doublets at δ4.16 and 3.68 due to the β- and α-protons of the C-7 methylene between the amine group and the aromatic ring. The assignment of the β-proton at lower fields is in accordance to its *cis*-disposition with respect to axial lone pair of the nitrogen atom [7]; (ii) two broad doublets at δ2.74 and 3.02 assigned to the 11b and 11c protons, respectively. The magnitude of their coupling constant (J = 11.2 Hz) establishes a *trans*-union between rings B and C of the tetracyclic system.

The homonuclear correlated spectrum depicted in Fig. 1 in the form of a contour plot enabled us to perform the complete assignment of the ^1H NMR spectrum, which



1 $R^1 = R^2 = R^3 = OH$, $R^4 = OMe$
2 $R^1 = R^3 = OH$, $R^2 = OAc$, $R^4 = OMe$
3 $R^1 = OAc$, $R^2 = R^3 = OH$, $R^4 = OMe$
4 $R^1 = OAc$, $R^2 = R^4 = OH$, $R^3 = OMe$
5 $R^1 = R^2 = R^4 = OH$, $R^3 = OMe$



6 $R^1 = R^2 = OH$
7 $R^1 = OAc$, $R^2 = OH$
8 $R^1 = OH$, $R^2 = OAc$

Table 1. ^{1}H NMR data for pseudolycorine (**1**), 2-*O*-acetylpseudolycorine (**2**), and 1-*O*-acetylpseudolycorine (**3**) (200 MHz, $CDCl_3$–CD_3OD)

H	1	2	3
1	4.85 *br s*	4.43 *t* (1.6)*	5.58 (5.74)† *m*
2	4.50 *t* (1.6)	5.29 *dd* (1.6; 2.4)	4.15 (4.32) *dd* (1.4; 2.6)
3	5.60 *t* (2.4)	5.45 *t* (2.4)	5.69 (5.87) *dd* (1.6; 2.6)
4	2.6–2.7 *m*	2.63 *m*	2.66 (2.82) *t* (8.4)
5α	2.6–2.7 *m*	2.42 *dt* (9.6; 8)	2.59 (2.69) *br t* (8.4)
5β	3.37 *dd* (9.6; 2.4)	3.35 *dt* (9.6; 4.8)	3.33 (3.54) *m*
7α	3.68 *br d* (14.4)	3.54 *d* (14.4)	3.61 (3.75) *dd* (14.1; 1.4)
7β	4.16 *d* (14.4)	4.14 *d* (14.4)	4.17 (4.34) *d* (14.1)
8	6.71 *s*	6.61 *s*	6.70 (6.90) *s*
11	6.89 *s*	6.83 *s*	6.74 (6.92) *s*
11b	2.74 *br d* (11.2)	2.69 *d* (11.2)	2.90 (3.05) *br s*
11c	3.02 *br d* (11.2)	2.85 *d* (11.2)	2.90 (3.05) *br s*
OMe	3.84 *s*	3.82 *s*	3.84 (4.00) *s*
Ac	—	2.06 *s*	1.92 (2.08) *s*

*Figures in parentheses are coupling constants or line separations in Hz.
†Chemical shifts measured in CD_3OD.

agrees with the assignments for the structurally related compound lycorine (**6**) [8].

The ^{13}C NMR data reported in Table 2 are in agreement with the proposed structure. The heteronuclear chemical shift correlation experiment (XCOR) has been performed in order to: (i) assign all the signals of the ^{13}C NMR spectrum, and (ii) confirm the assignments made for the ^{1}H NMR spectrum. This can be achieved because the proton spectrum is separated through the large ^{13}C chemical shift dispersion. The XCOR spectrum of **1** is shown in Fig. 2. The signals at δ53.93 and 56.38 were assigned to C-5 and C-7, respectively, which are the opposite assignments from those previously described for lycorine (**6**) [8] and sternbergine (**4**) [9].

Table 2. ^{13}C NMR chemical shift assignments* for pseudolycorine (**1**) and 2-*O*-acetylpseudolycorine (**2**)

C	1	2
1	70.70	68.50
2	71.83	73.91
3	118.57	113.99
3a	141.71	144.62
4	28.34	28.77
5	53.93	53.95
7	56.38	56.74
7a	127.35	127.87
8	110.49	110.37
9	146.19	145.97†
10	145.11	145.69†
11	111.32	111.28
11a	126.52	127.20
11b	39.41	41.15
11c	61.41	60.88
OMe	56.11	56.02
Ac	—	21.28
Ac	—	170.82

*In ppm relative to TMS. Measured in $CDCl_3$–CD_3OD solution at 50.3 MHz. The assignments are in agreement with off-resonance spectra.
†The assignments may be interchanged.

Compound **2**, $C_{18}H_{21}NO_5$, was isolated from the $CHCl_3$–EtOH (9:1) eluates of a column chromatography on silica gel of extract C. Its mass spectrum showed the parent peak at *m/z* 331 and characteristic fragments at *m/z* 271, 270, 229 and 228, which point to the presence of an acetyl group attached either at position C-1 or C-2 of a pseudolycorine-type ring system. The loss of the C-1/C-2 moiety by a retro-Diels–Alder fragmentation is a general process for these systems [10].

The IR spectrum is similar to that of compound **1**, and shows an intense absorption at 1705 cm^{-1}, characteristic of the acetyl group. The ^{1}H NMR spectrum (Table 1) showed the characteristic signals of a pseudolycorine derivative and a singlet at δ2.06 corresponding to the methyl group of the acetate. The location of the acetoxy group on C-2 of the C ring, as well as the assignment of the whole spectrum, were made possible by the homonuclear correlated ^{1}H NMR spectrum (Fig. 3). Thus, the signal of the olefinic proton (δ5.45) is coupled to the signal at 5.29, corresponding to the proton of the C-2 position, which in turn, is coupled to the proton at the C-1 position (δ4.43). Further support for these assignments is obtained by the fact that the proton of the C-2 position of 2-*O*-acetyllycorine (**8**) presents the same chemical shift [8].

The NOE 2D spectrum (Fig. 4) allowed us to establish spatial proximity between the protons of the C-1 and C-11 positions, as well as between the methoxy group and the aromatic proton of the C-8 position, and between the latter and the C-7 methylene. These observations confirm that the methoxy group is attached to C-9, in the aromatic ring. In consequence, compound **2** was identified as 2-*O*-acetylpseudolycorine.

The ^{13}C NMR spectrum of compound **2** (Table 2) supports the proposed structure. The acetyl group at C-2

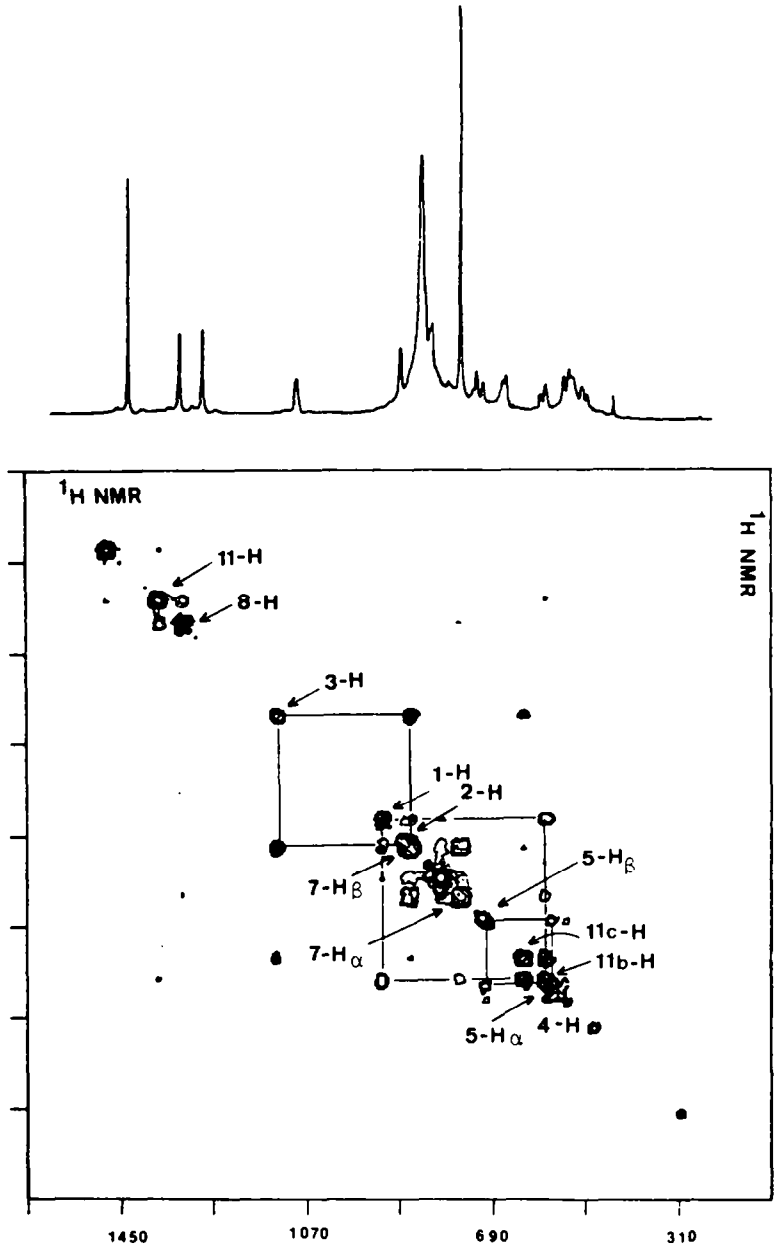


Fig. 1. Homonuclear correlated ^{1}H NMR spectrum (200 MHz) of pseudolycorine (1) in $CDCl_3$.

deshields this carbon (δ 73.91, $\Delta\delta$ = 2 ppm with respect to 1), shields the β-carbons (C-1 and C-3), and deshields the γ-carbons (C-11b and C-3a). These shifts are in agreement with the observations reported for the acetylation of an allylic hydroxy group in diterpene derivatives [11].

Compound 3, $C_{18}H_{21}NO_5$, an isomer of 2, was isolated also from extract C. Its mass spectrum showed the same parent peak (m/z 331) and main fragmentation peaks as compound 2, thus indicating that they are positional isomers at C-1 and C-2. Concordantly, its IR spectrum showed a strong absorption at 1720 cm^{-1} most likely due to an acetyl group, together with other pseudolycorine-type absorptions. The data of the ^{1}H NMR spectrum are reported in Table 1. The attachment of the acetate at the C-1 position of the pseudolycorine ring system produces a downfield shift of H-1 (δ 5.58) with respect to compound 1 ($\Delta\delta$ = 0.73 ppm), and an upfield shift of H-11 (δ 6.74), as was previously reported for 1-*O*-acetyllycorine (7) [8] and sternbergine (4) [9], when compared with their respective deacetyl derivatives. In the case of 1-*O*-acetylisopseudolycorine (4), convergence of the signals of H-11b and H-11c, with respect to isopseudolycorine (5), was also noticed, due to the opposite effect of the acetate on each proton [9]. This

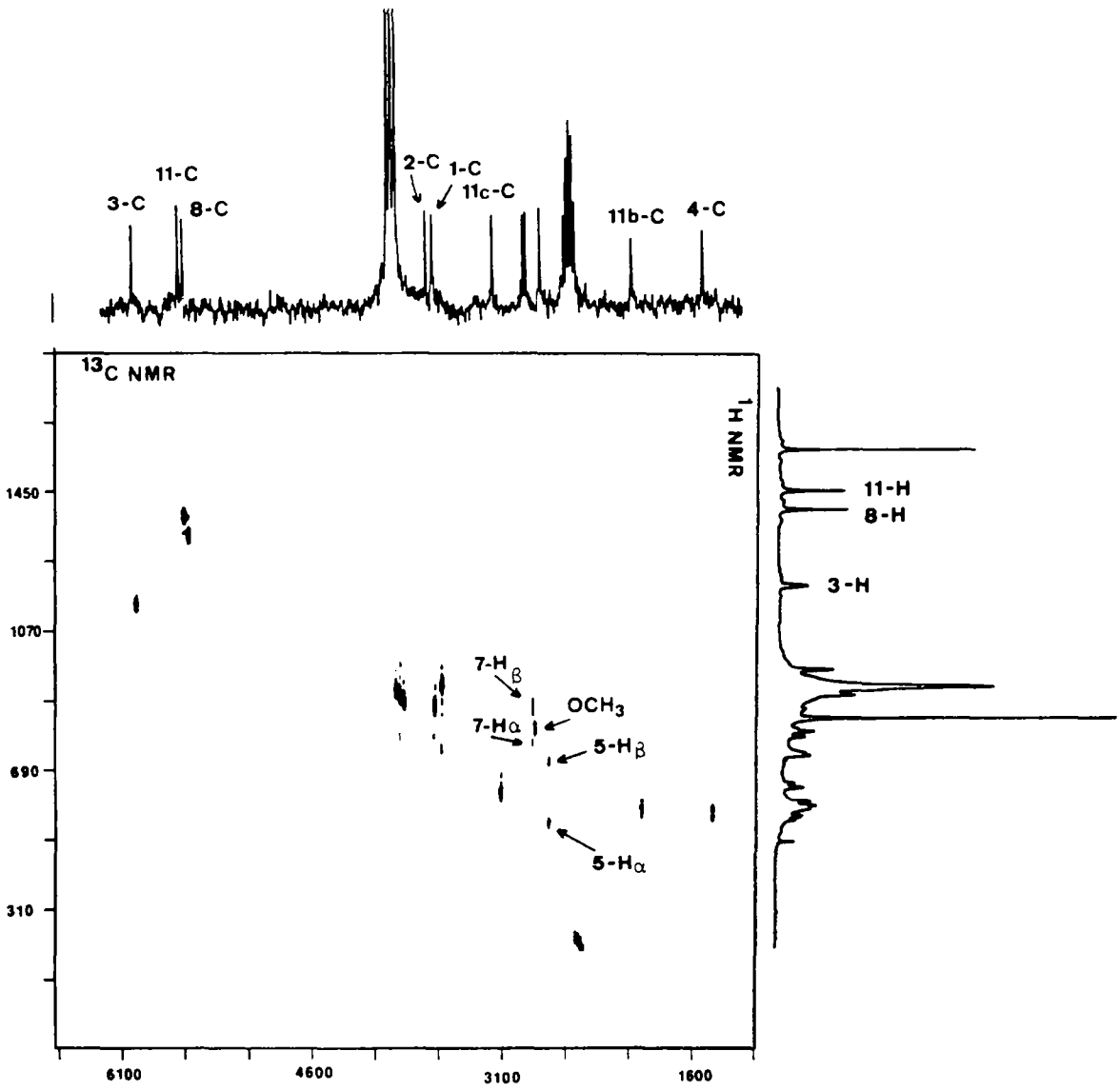


Fig. 2. Heteronuclear correlated spectrum of pseudolycorine 1 in $CDCl_3$–CD_3OD.

effect is also apparent in compound 3 where both signals have coalesced to yield a single signal at δ2.90. In view of these results, compound 3 was identified as 1-*O*-acetylpseudolycorine.

EXPERIMENTAL

All mps are uncorr. EIMS were recorded at 70 eV. NMR spectra were recorded on a Varian XL-200 spectrophotometer working at 200 MHz and 50.3 MHz, for proton and carbon, respectively. Chemical shifts are in ppm. The ^1H–^1H homonuclear correlation experiment (HOMCOR) was performed using the standard sequence [12], and 32 transients were accumulated for 256 values of evolution period, with a spectral width of 1600 Hz in both dimensions. The time between transients was 2 sec, and the acquisition time was of 0.160 sec A 512 × 512 points data matrix was measured with pseudoecho [13] data for improved peak definition and triangular folding for improved sensitivity. In the NOE 2D experiment, the sample was degassed by a nitrogen stream, and was performed using the standard sequence [14]. The mixing time was 0.150 msec, and 64 transients were accumulated for 256 values of evolution period with a spectral width of 1300 Hz in both dimensions, and a delay of 2 sec was employed. A 512 × 512 data matrix was used with pseudoecho and triangular folding. The ^{13}C NMR and ^1H–^{13}C heteronuclear shift correlation experiment (the XCOR sequence written by D. L. Foxal, Varian, Palo Alto, was used) were recorded using a microcell (0.3 ml) and a high sensitivity ZENS probe. For the XCOR experiment 1760 transients were used for each one of the 64 values of the evolution period. The spectral width of ^{13}C NMR was 5500 Hz with an acquisition time of 0.185 and a delay of 0.900 sec. The spectral width for ^1H NMR was 2000 Hz, 2048 × 512 data points were used.

Plant material. The plant material was identified by Professor Oriol de Bolòs i Capdevila from the Botanic Institute of Barcelona. This plant might also be referred to as *N. juncifolius* Lag. or *N. assoanus* Dufour. There is a voucher specimen

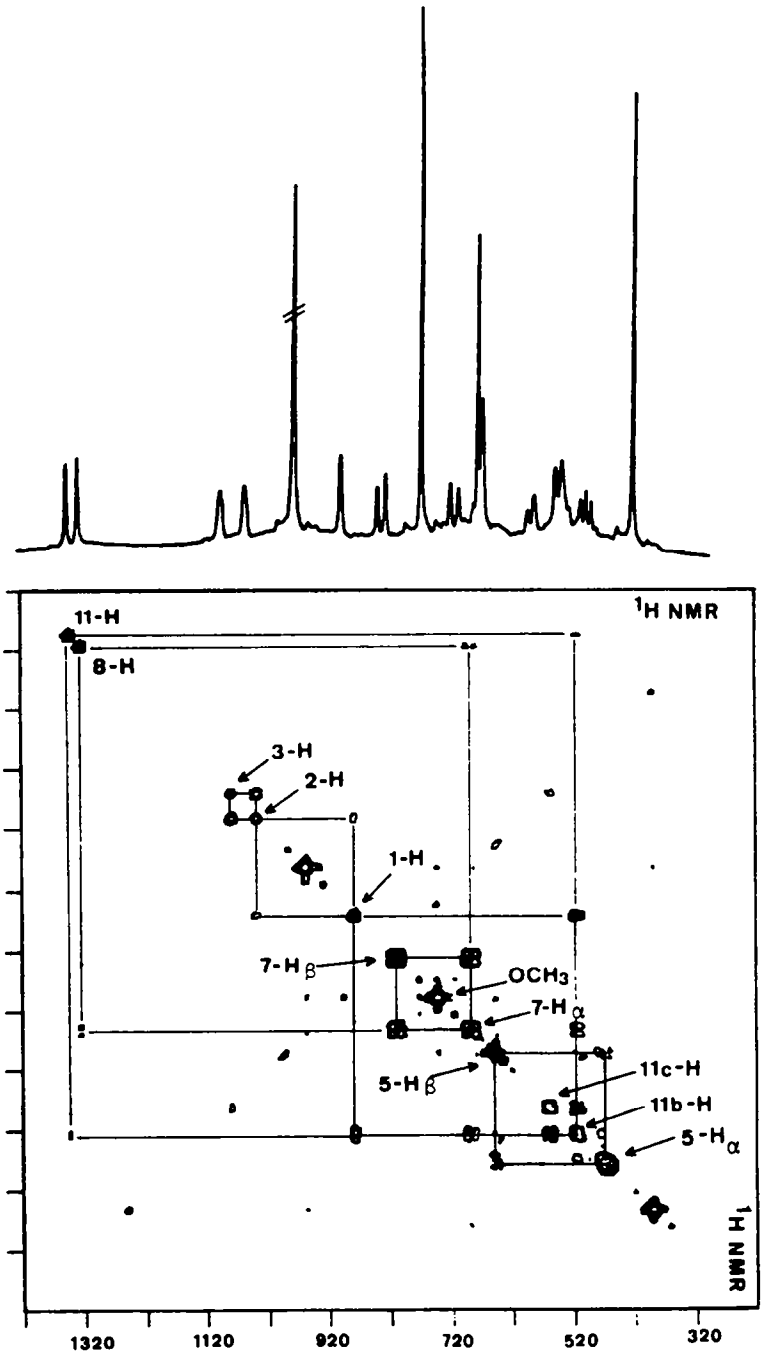


Fig. 3. Homonuclear correlated ^{1}H NMR spectrum (200 MHz) of 2-*O*-acetylpseudolycorine (2) in $CDCl_3$.

deposited in the herbarium of the Department of Botany, Faculty of Pharmacy, University of Barcelona.

Alkaloid isolation. Fresh aerial parts (8.1 kg) of *Narcissus requienii* were ground in 96% EtOH and macerated for 24 hr. The ethanolic soln was filtered, concd *in vacuo* and then made acid (pH 4) with glacial HOAc. The filtered acidic soln was extracted ×4 with Et_2O to remove neutral material, and extracted ×4 $CHCl_3$ to provide 1.80 g of $CHCl_3$-soluble alkaloid acetates (extract A). The aq. acidic soln was made basic (pH 8–9) with Na_2CO_3 and extracted ×4 with $CHCl_3$. This extract was washed with a Na_2CO_3 soln, then dried and concd *in vacuo* to a vol. of 200 ml and left overnight at 4°. The floating yellow ppt formed was removed by filtration and dried (extract B, 1.75 g). The filtrate was dried and taken to dryness to yield a brown gum (extract C, 1.44 g).

Extract A contained many impurities and some minor alkaloids, which have not been identified hitherto, due to insufficient material.

Treatment of extract B. The yellow residue was dissolved in 5% NaOH, filtered, and the soln satd with solid NH_4Cl. A pale yellow

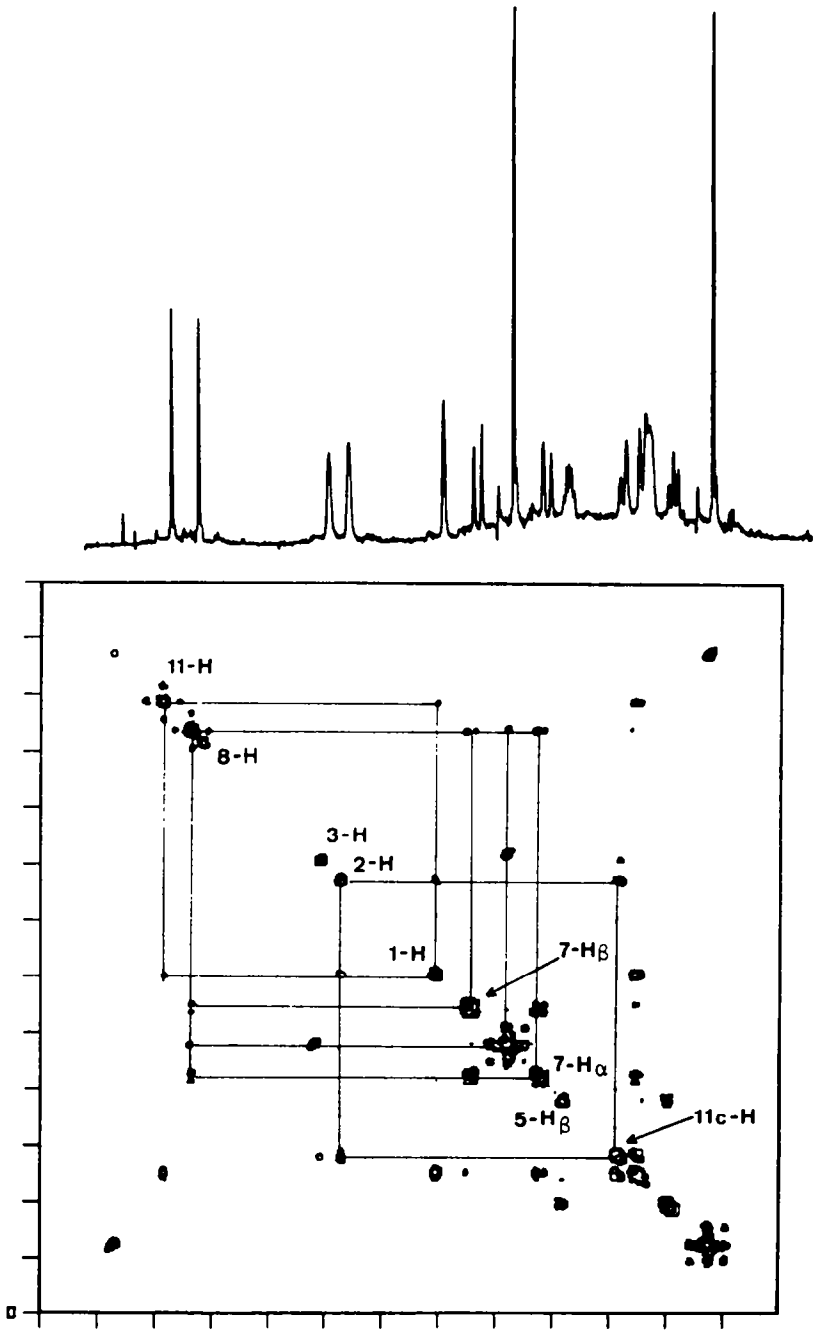


Fig. 4. NOE 2D spectrum of 2-*O*-acetylpseudolycorine (2).

residue was separated by filtration (1.39 g) containing alkaloid 1, which crystallized from MeOH (0.86 g).

Pseudolycorine (1). Mp 237–240° (lit. 247–249° [6]); IR ν_{max}^{KBr} cm^{-1}: 3400, 1580, 1510, 1460; UV λ_{max}^{EtOH} nm (log ε): 212 (4.22), 288 (3.61); adding one drop of a 0.1 N NaOH soln: 252 (3.85), 302 (3.73); MS *m/z* (rel. int.): 289 $[M]^+$ (39), 288 (16), 270 (24), 252 (13), 230 (15), 229 (82), 228 (100).

Treatment of extract C. The brown gum was chromatographed by CC on 100 g of silica gel. On elution with $CHCl_3$–EtOH (9:1) alkaloids 2 and 3 were obtained. Compound 2 crystallized from MeOH (160 mg of yellow needles) and compound 3 crystallized from $CHCl_3$–EtOH (20 mg of yellow needles).

2-*O*-*Acetylpseudolycorine* (2). $C_{18}H_{21}NO_5$; (Found: C, 63.51; H, 6.36; N, 4.03. $C_{18}H_{21}NO_5 \cdot 1/2H_2O$ requires: C, 63.50; H, 6.47; N, 4.12%); mp 168–170°; IR ν_{max}^{KBr} cm^{-1}: 3540, 3320, 1705, 1585, 1510; UV λ_{max}^{EtOH} nm (log ε): 210 (4.26), 286 (3.62), adding NaOH soln: 254 (3.84), 298 (3.73); MS *m/z* (rel. int.): 331 $[M]^+$ (23), 272 (33), 271 (56), 270 (56), 254 (45), 253 (50), 252 (100), 229 (21), 228 (38).

1-*O*-*Acetylpseudolycorine* (3). (Exact mass: *m/z* 331.1432, calc.

for $C_{18}H_{21}NO_5$, *m/z* 331.1414), mp 248–250°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3515, 1720; UV λ_{max}^{EtOH} nm (log ε): 212 (4.26), 286 (3.64), adding NaOH soln: 254 (3.84), 304 (3.74); MS *m/z* (rel. int.): 331 $[M]^+$ (48), 271 (15), 270 (25), 252 (15), 230 (28), 229 (100), 228 (97), 43 (28).

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