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# PHENANTHRIDINE ALKALOIDS FROM *NARCISSUS ASSOANUS**

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**Key Word Index**—*Narcissus assoanus*; Amaryllidaceae; alkaloids; assoanine; oxoassoanine.

**Abstract**—The rare phenanthridine alkaloid assoanine and its new 7-oxo derivative, oxoassoanine, have been isolated from *Narcissus assoanus* collected at flowering. HPLC analysis of crude extracts of the plant has been carried out to ascertain that oxoassoanine is not an artefact.

## INTRODUCTION

Recently, we have described the isolation of the alkaloids pseudolycorine (**4**), 1-*O*-acetylpseudolycorine (**5**) and 2-*O*-acetylpseudolycorine (**6**) from extracts B and C of *Narcissus assoanus* [1]. Herein we report the isolation and characterization of two phenanthridine derivatives, assoanine (anhydromethylpseudolycorine) (**1**) and the new alkaloid oxoassoanine (7-oxoanhydromethylpseudolycorine) (**2**). Assoanine (**1**) has only been previously isolated from *Narcissus pseudonarcissus* bulbs [3]. Nevertheless, the $^1$H NMR and mass spectral data of **1** are provided in this work for the first time.

## RESULTS AND DISCUSSION

The mass spectrum of assoanine (**1**) suggested the molecular formula $C_{17}H_{17}NO_2$ ($[M]^+$ at *m/z* 267) and it gave a typical $^1$H NMR spectrum (Table 1) for this skeleton which showed the following features: (i) two singlets at $\delta$6.64 and 7.17 for the aromatic protons H-8 and H-11, respectively, according to earlier generalizations for this class of compounds [4]; (ii) an AMX system in the aromatic zone due to H-3, H-2 and H-1 established the aromatization of ring C (these assignments were carried out on the basis of the multiplicity and decoupling experiments); (iii) the singlets at $\delta$3.87, 3.93 and 4.09 assigned to the methoxyl groups and the benzylic protons of the 7-position, respectively; and (iv) two triplets at $\delta$3.00 and 3.32 for the methylene protons at the 4- and 5-positions, the latter being more deshielded due to its proximity to the nitrogen atom. The allylic coupling between the methylene group of the 4-position and H-3 was observed by decoupling experiments. Thus, irradiation of the H-5 signal ($\delta$3.32) simplified the triplet at $\delta$3.00 to a broad signal ($W_{\frac{1}{2}} = 1.2$ Hz). Irradiation at $\delta$3.00 not only simplified the H-5 triplet but also transformed the double triplet at $\delta$6.99 assigned to H-3 into a double doublet. These data agree with those reported for the structurally related alkaloid anhydrolycorine (**3**) [5]. The $^{13}$C NMR spectrum of **4** shows three characteristic methylenes at $\delta$29.16, 53.45 and 55.72 for C-4, C-5 and C-7, respectively, which agree with the proposed structure.

Spontaneous oxidation of assoanine into oxoassoanine (**2**) was observed when the alkaloid was allowed to stand at room temperature. Nevertheless, the latter was also isolated directly from the plant, and its presence in crude extracts, revealed by HPLC analysis, proved that it was not an artefact of the isolation procedure. Although this compound has already been obtained by several authors as a degradation product of other Amaryllidaceae alkaloids [4, 6, 7], its direct isolation from a natural source has not been reported before.

The UV and mass spectra of the compound isolated from *N. assoanus* agree with those reported for synthetic 7-oxoanhydromethylpseudolycorine. The IR spectrum of **2** shows an absorption at 1640 cm$^{-1}$ attributed to the conjugated carbonyl group. Moreover, its $^1$H NMR spectrum indicates that the signal at $\delta$4.09 corresponding to the C-7 protons of assoanine has vanished, and, in turn,

Table 1. $^1$H NMR spectral data of assoanine (**1**) and oxoassoanine (**2**) (200 MHz, $CDCl_3$)

| H | 1 | 2* |
|---|---|---|
| 1 | 7.31 *dd* (7.5, 1.2)† | 7.85 *br d* (7.5) |
| 2 | 6.75 *t* (7.5) | 7.27 *t* (7.5) |
| 3 | 6.99 *dt* (7.5, 1.2) | 7.33 *br d* (7.5) |
| 4 | 3.00 *br t* (8.0) | 3.45 *br t* (8.0) |
| 5 | 3.32 *t* (8.0) | 4.45 *br t* (8.0) |
| 7 | 4.09 *s* | — |
| 8 | 6.64 *s* | 7.57 *s* |
| 11 | 7.17 *s* | 7.81 *s* |
| MeO | 3.87 *s* | 4.03 *s* |
|  | 3.93 *s* | 4.09 *s* |

*With addition of $CD_3OD$.

†Values in parentheses are coupling constants in Hz.

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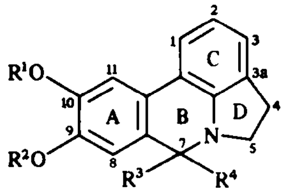
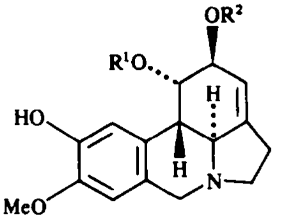
*Part 2 in the series "*Narcissus* Alkaloids". For Part 1 see ref. [1]. *N. requienii* Roem. and *N. juncifolius* Lag. are synonyms for *N. assoanus* Duf., although the latter has recently been proved to be a more accurate name [2].



**1** $R^1 = R^2 = Me$; $R^3 = R^4 = H$ Assoanine
**2** $R^1 = R^2 = Me$; $R^3 + R^4 = O$ Oxoassoanine
**3** $R^1 + R^2 = CH_2$; $R^3 = R^4 = H$ Anhydrolycorine



**4** $R^1 = R^2 = H$ Pseudolycorine
**5** $R^1 = Ac$; $R^2 = H$ 1-*O*-Acetylpseudolycorine
**6** $R^1 = H$; $R^2 = Ac$ 2-*O*-Acetylpseudolycorine

the H-4 and H-5 protons have been strongly shifted downfield, as well as the aromatic protons (Table 1). The $^{13}C$ NMR spectrum of oxoassoanine confirms the proposed structure. The signal at $\delta 157.51$ has been assigned to the C-7 carbonyl group, and the signal at $\delta 153.33$, characteristic of an aromatic carbon attached to a methoxyl group, *ortho* to another methoxyl and *para* with respect to the lactame function, corresponds to C-10 [4, 8].

## EXPERIMENTAL

*General.* Mps are uncorr. IR spectra were recorded in $CHCl_3$. UV spectra were recorded in EtOH, and $^1H$ NMR and $^{13}C$ NMR using TMS as internal standard. EIMS were recorded at 70 eV. Analytical HPLC: Novapack $C_{18}$ column (15 × 0.39 cm), UV detection 280 nm; flow: 1 ml/min. Elution was begun with 90% of soln A (10% of MeCN, 90% of 1% $NH_4OAc$ soln, pH 5.8), and 10% of soln B (80% of MeCN, 20% of the same $NH_4OAc$ soln) and finished with 20% of soln A and 80% of soln B, in 20 min.

*Plant material.* Aerial parts of *N. assoanus* Duf. were collected at flowering in Montserrat (Barcelona, Spain) and identified by Prof. Oriol de Bolòs of the Institut Botànic de Barcelona. A voucher specimen has been deposited at the herbarium of the Departament de Botànica, Facultat de Farmàcia, Universitat de Barcelona.

*Alkaloid extraction.* Fresh plant material (8.1 kg) was treated as described previously [1]. The 1.8 g residue constituting the $CHCl_3$-soluble alkaloid acetates (extract A) was redissolved in $CHCl_3$ and shaken with a 5% $NH_4OH$ soln and dried to yield a brown gum, which was resuspended in 2 N HCl, filtered and extracted with $CHCl_3$. The $CHCl_3$ extract was shaken with a 5% $NH_4OH$ soln and dried, affording 1.32 g of residue, which was chromatographed by CC on 100 g of silica gel. Elution with $CHCl_3$–EtOH (19:1) yielded oxoassoanine (17 mg, crystallized from EtOH), and $CHCl_3$–EtOH (9:1) yielded assoanine (25 mg, crystallized from EtOH).

*Sample preparation.* Alkaloid samples for HPLC analysis were obtained according to the alkaloid isolation method, working at 4°. The corresponding extract A was repurified by solvent changes as described above, and the residue redissolved in MeOH. Samples were kept in the freezer until HPLC analysis to avoid oxidation of assoanine.

*Assoanine* (**1**). IR $\nu_{max}$ cm$^{-1}$: 1630, 1595, 1505; UV $\lambda_{max}$ nm (log $\varepsilon$): 251 (4.10), 254 (3.96), 325 (3.70), 347 (3.68); MS *m/z* (rel. int.): 267 $[M]^+$ (51), 226 (100), 265 (10), 264 (10), 252 (5), 251 (10), 250 (29), 222 (16), 193 (12), 180 (13); $^{13}C$ NMR ($CDCl_3$–$CD_3OD$): $\delta 139.02$ (C-11c), 136.39 (C-7a), 120.23 (C-1), 119.56 (C-2), 111.23 (C-11a), 110.74 (C-8), 57.09, 56.81 (MeO), 55.72 (C-7), 53.45 (C-5), 29.16 (C-4).

*Oxoassoanine* (**2**). $C_{17}H_{15}NO_3$ (Found: C, 73.94; H, 5.42; N, 4.90. Requires: C, 72.60; H, 5.34; N, 4.98%). Mp 247–250° (lit. 260–270° [3], 229–232° [5], 232–234° [7]); IR $\nu_{max}$ cm$^{-1}$: 1640, 1605, 1515; UV $\lambda_{max}$ nm (log $\varepsilon$): 252 (4.36), 272 (4.27), 326 (3.65), 342 (3.67); MS *m/z* (rel. int.): 281 $[M]^+$ (100), 279 (18), 266 (18), 239 (10), 236 (10), 140 (8); $^{13}C$ NMR ($CDCl_3$–$CD_3OD$): $\delta 157.51$ (C-7), 153.33 (C-10), 149.85 (C-9), 131.29 (C-11c), 129.00 (C-7a), 124.31 (C-3a), 123.90 (C-2 and C-3), 120.32 (C-11a), 119.45 (C-1), 117.01 (C-11b), 108.64 (C-8), 103.32 (C-11), 56.29, 56.22 (MeO), 46.78 (C-5), 27.50 (C-4).

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