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NARCISSUS ALKALOIDS, XIV.[1] (+)-8-*O*-ACETYLHOMOLYCORINE AND VASCONINE, TWO NOVEL ALKALOIDS FROM *NARCISSUS VASCONICUS*

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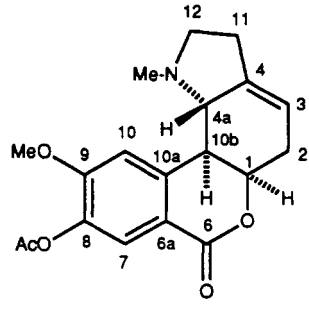
ABSTRACT.—From the MeOH extract of the whole plants of *Narcissus vasconicus*, two previously unreported alkaloids, (+)-8-*O*-acetylhomolycorine [**1**] and vasconine [**2**], have been isolated. The well-known alkaloids (−)-lycorine and (+)-homolycorine were also present.

During the course of our chemical investigations on *Narcissus* alkaloids, we have studied *Narcissus vasconicus* Fdez. Casas (Amaryllidaceae), a new recently described species growing in Spain (2). No work on the chemical constituents of this plant has been reported so far. The present investigation led to the isolation and structure elucidation of two novel alkaloids, (+)-8-*O*-acetylhomolycorine [**1**] and vasconine [**2**], along with the well-known alkaloids (−)-lycorine and (+)-homolycorine.

Separation and isolation of individual compounds from the MeOH extract of the fresh aerial parts and bulbs of *N. vasconicus* followed the steps described in the Experimental section. Each alkaloid-containing fraction was separated by a combination of cc and preparative tlc, and four alkaloids were thus obtained. Extract A yielded (+)-homolycorine, extract C was found to contain (−)-lycorine and (+)-8-*O*-acetylhomolycorine [**1**], and extract D afforded the alkaloid vasconine [**2**] as well as additional (−)-lycorine.

Compound **1** was obtained as a white crystalline solid, with hrms showing the molecular formula $C_{19}H_{21}NO_5$ and $[\alpha]^{20}D$ +70.4 (EtOH, $c = 0.54$). The eims showed a base peak at *m/z* 109, which is characteristic of the Amaryllidaceae alkaloids of the homolycorine series (3). The low abundance of the molecular ion at *m/z* 343 (<1) is charac-



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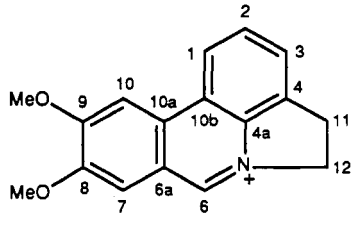

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[1]For part XIII in this series, see Bastida *et al.* (1).

teristic of all the homolycorine derivatives with a double bond between positions 3 and 4. The presence of a fragment at *m/z* 300 $[M-43]^{-1}$ could be due to the existence of an acetoxy group in this molecule. The ir spectrum showed an intense band at 1712 cm^{-1} for the carbonyl groups of aryl conjugated lactone and ester. The most important signals of the ^{1}H-nmr spectrum were: (a) two singlets at δ 7.47 and 7.13 for the aromatic protons H-7 and H-10, respectively [the shielding effect of the aromatic proton β to the acetyl group, in relation to the same proton of the 8-*O*-demethylhomolycorine (4), was similar to that observed between 9-*O*-acetyl-2α-hydroxyhomolycorine (5) and 9-*O*-demethyl-2α-hydroxyhomolycorine (6)]; (b) two broad doublets at δ 5.63 and 4.86 for the olefinic and H-1 protons; (c) a broad doublet and a doublet of doublets at δ 2.96 and 2.86 for the methine protons H-4a and H-10b, respectively, which were unambiguously assigned by means of the 2D COSY experiment; (d) two signals at δ 3.31 (ddd) and 2.45 (dd) for the H-12α and H-12β, the first of them more deshielded due to its cis relationship with the nitrogen lone pair (7); and (e) three singlets at δ 3.97, 2.16, and 2.00 for the *O*-Me, *N*-Me and acetoxy groups. The confirmation of the position of the acetoxy group in the aromatic ring was possible by application of the 2D nOe technique. Thus, the aromatic proton at C-10 (δ 7.13) showed nOe with the signals of the MeO group, H-10b, and *N*-Me group, while the proton at C-7 (δ 7.47) showed significant nOe only with the acetoxy group.

The most characteristic signals of the ^{13}C-nmr spectrum of **1** were: (a) two signals at δ 166.3 and 174.9 for the C-6 and acetoxy carbonyl groups; (b) two peaks at δ 138.7 and 116.5, corresponding to the olefinic carbons; (c) two signals at δ 115.9 and 110.9, assignable to the C-7 and C-10 carbons of the aromatic ring, respectively; and (d) three quadruplets at δ 56.0, 42.9, and 21.0, corresponding to the *O*-Me, *N*-Me, and acetoxy groups. A signal at δ 42.2 was clearly identified as the C-10b, which could be considered as a key carbon to elucidate the presence or not of an hydroxyl group at the C-2 position in the alkaloids of the homolycorine series. This carbon appeared about 4.5 ppm deshielded in the dehydroxylated compounds in comparison with the hydroxylated compounds, since a "γ-gauche" effect does not exist between C-2 and C-10b. The ^{13}C-nmr spectrum supported the proposed structure. The assignment of certain signals, however, was hindered by their overlapping; at δ 56.0 are found the signals corresponding to both the C-12 and OMe groups. The reversed-phase heteronuclear ^{1}H-^{13}C correlated spectrum of **1** was also obtained, thus allowing the unequivocal assignment of the C-3, C-7, and C-10 signals, as well as the full assignment of the rest of carbons of the molecule.

Compound **2**, $C_{17}H_{16}NO_2$ from hrms, was isolated from extract D and was similar to the anhydrolycorinium ion, one of the biosynthetic products of *Amaryllis belladonna* most inhibitory to murine 3 PS leukemia (8). Its ms showed a parent base peak at *m/z* 266. The ^{1}H nmr of **2**, recorded in $CDCl_3$/MeOH-d_4, exhibited: (a) a singlet at δ 10.17 belonging to the proton of the imminium salt; (b) two singlets at δ 4.16 and 4.05 assignable to the MeO groups; (c) two singlets at δ 8.00 and 7.89 for the aromatic protons of ring A; (d) two pseudo triplets at δ 5.36 and 3.75 for the protons at the positions 12 and 11, respectively; and (e) two doublets at δ 8.35 and 7.70 and one triplet at δ 7.85, belonging to a 1,2,3-three-substituted aromatic ring.

The 2D nOe experiment allowed the unambiguous assignment of the aromatic protons of ring A on the basis of the spatial proximity between the protons of the C-10 and C-1 positions. Thus, the singlet at δ 7.89 showed an nOe with the doublet at δ 8.35 and with the

singlet at δ 4.16, providing evidence that this MeO group is located at the C-9 position. The nOe between the H-7 and one of the MeO groups (δ 4.05) clearly determined the A ring substitution.

The ^{13}C-nmr spectrum of **2** exhibited 17 carbon atoms. The DEPT spectra revealed the presence of seven quaternary carbons, six methine carbons, two methylene carbons, and two methyl carbons. The deshielding of the methine carbon C-6 of **2** in relation to assoanine (9) was similar to that observed for the same carbon in bicolorine and 5,6-dihydrobicolorine (10), as a consequence of an imminium salt effect. Likewise, a deshielding of the C-12 in α position with respect to the quaternary nitrogen was also observed.

We also isolated from *N. vasconicus* the very common amaryllidaceous alkaloids lycorine and homolycorine, which were identified by direct comparison with authentic samples.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—[α]D was recorded in a Perkin-Elmer 241 polarimeter. Ir spectra were measured on a Perkin-Elmer 1430 spectrophotometer. ^{1}H- (250 MHz) and ^{13}C- (62.5 MHz) nmr spectra were recorded on a Brüker AC250 spectrometer using TMS as internal standard. Chemical shifts are reported in δ (ppm) values and coupling constants (*J*) in Hz. Eims was obtained with a Hewlett-Packard 59865 A instrument operating at 70 eV. Analytical and preparative tlc was carried out on Si gel 60 F_{254} plates (Merck). 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. Spots on chromatograms were detected by Dragendorff's reagent and under uv light (254 nm).

PLANT MATERIALS.—The whole plants of *N. vasconicus* (*Narcissus jacetanus* Fdez. Casas subsp. *vasconicus* Fdez. Casas = *Narcissus asturiensis* var. *brevicoronatus* Pugsley) were collected in April 1987 during the flowering period, from Alava province, Spain. The plant identification was authenticated by Prof. Javier Fernández Casas, Real Jardín Botánico de Madrid, and a voucher specimen (No. 33619) has been deposited at the Herbarium of the Faculty of Pharmacy, University of Barcelona, Spain.

EXTRACTION AND ISOLATION.—Fresh aerial parts and bulbs (6 kg) were crushed and extracted with MeOH in a Soxhlet apparatus for 10 h. The extract obtained by evaporation of the MeOH was dissolved in 2% HCl, and the mixture was filtered. After removal of the neutral material with Et_2O, the acidic solution was extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated under reduced pressure and then washed with Na_2CO_3, dried with anhydrous Na_2SO_4, and filtered. After evaporation, 2.68 g of extract A was obtained. The former aqueous solution was made basic with Na_2CO_3 and extracted with $CHCl_3$. The organic phase was treated as for extract A, affording 1.99 g of extract C. The aqueous alkaline solution was extracted with $CHCl_3$-EtOH (3:2), yielding 2.98 g of extract D.

TREATMENT OF EXTRACT A.—Extract A was subjected to flash cc, eluting with a CH_2Cl_2/MeOH mixture. The MeOH concentration was gradually increased up to 20%. The eluate was further purified by preparative tlc and yielded (+)-homolycorine (664 mg).

TREATMENT OF EXTRACT C.—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. Through subsequent purification of the crude fraction on precoated plates, pure (−)-lycorine (35 mg) and (+)-8-*O*-acetylhomolycorine [**1**] (15 mg) were obtained.

TREATMENT OF EXTRACT D.—Extract D was also chromatographed by flash cc, employing gradient elution with $CHCl_3$/MeOH, gradually increasing the MeOH proportion up to 10%. Further purification was by tlc, and afforded vasconine [**2**] (13 mg) as well as additional (−)-lycorine (69 mg).

(+)-8-O-Acetylhomolycorine [**1**].—Hreims *m/z* 343.1423 (calcd for $C_{19}H_{21}NO_5$ *m/z* 343.1419); mp 186–188°; ir ν max ($CHCl_3$) cm^{-1} 1712, 1600, 1510, 1452, 1348, 1309, 1265, 1218, 1159, 1076, 1056, 752; eims *m/z* (rel. int.) $[M]^+$ 343 (<1), 302 (8), 300 (6), 164 (18), 136 (14), 135 (8), 121 (8), 115 (9), 110 (76), 109 (100), 108 (78), 107 (16), 94 (17), 93 (10), 82 (15), 81 (8); ^{1}H nmr ($CDCl_3$/CD_3OD) δ 7.47 (1H, s, H-7), 7.13 (1H, s, H-10), 5.63 (1H, br d, 2.0, H-3), 4.86 (1H, br d, 4.7, H-1), 3.97 (3H, s, OMe), 3.31 (1H, ddd, 9.3, 6.0, 4.2, H-12α), 2.96 (1H, br d, 10.0, H-4a), 2.86 (1H, dd, 10.0, 2.0, H-10b), 2.5–2.7 (4H, m, H-11, H-2), 2.45 (1H, dd, 18.0, 9.3, H-12β), 2.16 (3H, s, NMe), 2.00 (3H, s, *Me*CO); ^{13}C nmr ($CDCl_3$/CD_3OD) δ 174.9 (s, Me*C*O), 166.3 (s, C-6), 152.2 (s, C-9), 146.3 (s, C-8), 138.7 (s, C-4), 135.7 (s, C-10a), 116.5 (s, C-6a), 116.5 (d, C-3), 115.9 (d, C-7), 110.9 (d, C-10), 77.5 (d, C-1), 66.8 (d, C-4a), 56.0 (t, C-12), 56.0 (q,

OMe), 42.9 (q, NMe), 42.2 (d, C-10b), 30.9 (t, C-2), 27.4 (t, C-11), 21.0 (q, *Me*CO).

Vasconine [**2**].—Hreims *m/z* 266.1183 (calcd for $C_{17}H_{16}NO_2$ *m/z* 266.1181); mp 233–235°; ir ν max (KBr) cm^{-1} 3421, 1606, 1517, 1486, 1390, 1276, 1240, 1209, 1166, 1027, 989; eims *m/z* (rel. int.) $[M]^+$ 266 (100), 265 (13), 264 (4), 252 (8), 251 (43), 250 (58), 222 (14), 221 (10), 220 (7), 205 (6), 194 (16), 193 (8), 192 (9), 180 (11), 178 (5), 154 (5), 152 (5), 50 (11); ^1H nmr ($CDCl_3/CD_3OD$) δ 10.17 (1H, s, H-6), 8.35 (1H, d, 7.5, H-1), 8.00 (1H, s, H-7), 7.89 (1H, s, H-10), 7.85 (1H, t, 7.5, H-2), 7.70 (1H, d, 7.5, H-3), 5.36 (2H, t, 7.0, H-12), 4.16 (3H, s, OMe), 4.05 (3H, s, OMe), 3.75 (2H, t, 7.0, H-11); ^{13}C nmr ($CDCl_3/CD_3OD$) δ 159.7 (s, C-8), 153.4 (s, C-9), 146.0 (d, C-6), 137.8 (s, C-4a and C-10b), 132.5 (d, C-3), 132.2 (s, C-4), 126.9 (d, C-2), 124.8 (s, C-6a), 122.6 (s, C-10a), 121.6 (d, C-1), 111.4 (d, C-7), 104.4 (d, C-10), 57.7 (q, OMe), 57.0 (t, C-12), 56.9 (q, OMe), 28.6 (t, C-11).

(−)-Lycorine and (+)-homolycorine.—These alkaloids were chromatographically (tlc) and spectrally (ir, ms, ^1H nmr) identical with authentic compounds (11,12).

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