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ALKALOID *N*-OXIDES OF AMARYLLIDACEAE

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Key Word Index—*Pancratium maritimum*; *Lapiedra martinezii*; Amaryllidaceae; alkaloids; *N*-oxides.

Abstract—Ungiminorine *N*-oxide was isolated from *Pancratium maritimum*, and homolycorine *N*-oxide and *O*-methyl lycorenine *N*-oxide from *Lapiedra martinezii*. These compounds represent the first examples of naturally occurring *N*-oxides from the Amaryllidaceae.

INTRODUCTION

Over 100 alkaloids derived from phenylalanine and tyrosine have been isolated from members of the Amaryllidaceae [1, 2]. However, unlike other alkaloid-containing plants, *N*-oxides have never been isolated from this family [3], although lycorine *N*-oxide is easily prepared from its free base [4].

We now report on the isolation of ungiminorine *N*-oxide (**1**) from *Pancratium maritimum* L., a species from which several tertiary alkaloids have been isolated [5, 6], and of homolycorine *N*-oxide (**2**) and *O*-methyllycorenine *N*-oxide (**3**) from *Lapiedra martinezii* Lag., the only European species of the genus *Lapiedra*. This is the first time the alkaloids of this plant have been investigated.

RESULTS AND DISCUSSION

The IR spectrum of compound **1** exhibited an –OH band at 3350 cm^{-1} and a methylenedioxy absorption at 930 cm^{-1}. The mass spectrum (FAB) gave the formula $C_{17}H_{19}NO_6$. The ^1H NMR spectrum showed the presence of one olefinic proton, two *para*-oriented aromatic protons, a methylenedioxy group, an aliphatic *O*-methyl group and a doublet (J = 12.5 Hz) at δ 2.65 due to a benzyl proton, but no *N*-methyl signal. These data suggested that the compound had a lycorine-type skeleton. In addition, the ^1H NMR spectrum exhibited one signal for a low field proton (δ 3.68, *t*, J = 3.0 Hz) which was assigned to H-2 by 2D-COSY studies and comparison with a similar signal in the spectrum of ungiminorine (**4**), which was also isolated from *P. maritimum*. The coupling constants $J_{1,2}$, $J_{2,3}$ indicate that **1** has the configuration shown with the hydroxyl and *O*-methyl groups both in axial positions. The fact that the protons *alpha* to the nitrogen resonate at lower field than those of ungiminorine suggests the quaternary nature of the nitrogen atom, which is also supported by the deshielding of C-11c, C-5 and C-7 shown in the ^{13}C NMR spectrum (δ 84.0, 80.7 and 69.2, respectively). Together, these data indicate that **1** is the *N*-oxide of ungiminorine; and indeed treatment of **4** with H_2O_2 gave a single product which was shown to be identical to **1**.

The alpha configuration of the *N*-oxide group was deduced from the 2D-COSY results which reveal a long-range coupling (*W* rule) between H-11c (δ 4.23) and H-5 (δ 4.65), a phenomenon which is also observed in the case of ungiminorine.

Absolute stereospecificity in the quaternization of ungiminorine was also found when this compound was treated with methyl iodide, the single methiodide (**5**) being

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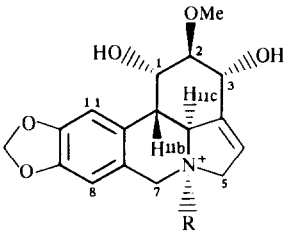
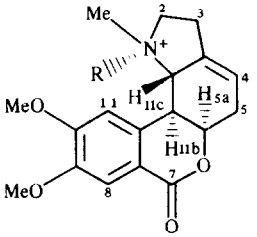
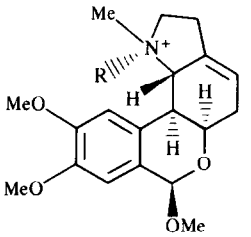


R	
1	—O⁻
4	free base
5	—MeI⁻



R	
2	—O⁻
6	free base



R	
3	—O⁻
7	free base

obtained. NOE experiments on **5** showed the *N*-methyl group to be on the same side as H-7α, H-5 and H-11c, thus proving it to be *alpha*-oriented.

Turning now to homolycorine *N*-oxide (**2**), the IR spectrum of this compound exhibited a C=O band at 1720 cm^{-1} due to a lactone group. The high resolution mass spectrum indicated the formula $C_{17}H_{21}NO_5$.

The ^1H NMR spectrum showed two *para*-oriented aromatic protons, two vicinal aromatic methoxyl groups, one olefinic proton and one *N*-methyl signal. These data were consistent with the skeleton being of the lycorenine type. The strong coupling ($J = 10$ Hz) of H-11b and H-11c constitutes strong evidence for their *trans*-diaxial relationship. The assignment of the hydrogens on C-2, C-3 and C-5 was made by means of a 2D-COSY experiment, in which H-2, H-3 and H-4, H-5 correlations were observed. However, the low field shift of the *N*-methyl signal (δ 2.95) suggested quaternization of the nitrogen atom. The chemical shifts for C-2, C-3 and N-Me were also paramagnetically deshielded with respect to the values observed in the ^{13}C NMR spectrum of the free base, homolycorine (**6**), which was also isolated from *L. martinezii* [7].Treatment of homolycorine with hydrogen peroxide gave **2** in quantitative yield, confirming compound **2** as homolycorine *N*-oxide.

The stereochemistry of the nitrogen atom was deduced from a 2D-NOESY experiment. The N-Me, C-10 O-Me and H-11 correlation can be explained by assuming a *beta* configuration for the *N*-methyl group.

Compound **3** showed no carbonyl absorption band in its IR spectrum. High resolution mass spectrometry indicated the formula $C_{19}H_{25}NO_5$. The ^1H NMR spectrum of **3** was very similar to those recorded for **2**. The main spectral difference between the two was apparent mainly in the ^{13}C NMR spectrum were the presence of a quaternary carbon at very low field (δ 99) and an aliphatic methoxyl group (δ 55) were indicative of a ketal functionality in **3**. Furthermore, since the general features of the ^1H NMR spectrum were similar to those of the spectrum of **2**, compound **3** must also have a lycorenine-type skeleton and must be an *N*-oxide.

Compound **3** was prepared by treatment of *O*-methyllycorenine (**7**) (also present in *L. martinezii*) with MCPBA. Attempts to prepare the *N*-oxide with hydrogen peroxide gave **2** as the sole product.

Once again a 2D-NOESY experiment was used to establish the stereochemistry of the nitrogen. Correlation between the *N*-methyl and the *O*-methyl protons was observed, confirming the *beta*-orientation of the *N*-methyl group.

Confirmation that **1**–**3** were genuine natural products and not artifacts, was provided by the demonstration that the free bases **4**–**7** were not converted into N-oxides when subjected to the same extraction procedure. Furthermore, freshly prepared crude extracts showed by TLC the presence of **1**–**3**.

The results obtained may have chemotaxonomic implications since the new classification of the Mediterranean Amaryllidaceae, which is based on morphology and floral characteristics, includes *Pancratium* and *Lapiedra* within the tribe Pancratiae [8].

EXPERIMENTAL

General. Mps: uncorr.; NMR: 250 or 200.13 MHz for ^1H and 50.32 or 62.85 MHz for ^{13}C in the solvent specified. CC: neutral alumina (Merck 70–230 mesh ASTM; 0.063–0.200 mm) and silica gel (Merck 70–230 mesh ASTM).

Plant collection. Lapiedra martinezii Lag. was collected near 'Pinares de San Antón' (Málaga), in December 1984. *Pancratium maritimum* L. was collected in the northwest of Spain (Barrañan and Corrubedo beaches, La Coruña), in July, 1984. Voucher specimens have been deposited at the Department of Botany, University of Málaga, with registry numbers MGC-14123 and MGC-18232, respectively.

Extraction and isolation. (a) *L. martinezii.* The powdered dried plant (3.75 kg, aerial parts) was extracted in a Soxhlet apparatus with MeOH. The solvent was concd to *ca* 2 l and 2 l of 5% HCl added. After filtration, the acid soln was shaken with Et_2O. The aq. layer was brought to pH 8 with NH_4OH and extracted with CH_2Cl_2 and EtOAc. Removal of the organic solvent gave 200 g of a syrup which by titration against 0.0092 N *p*-toluene-sulphonic acid was shown to have an alkaloid content of 57g.

The syrup was chromatographed on a neutral alumina column using a CH_2Cl_2–EtOAc step gradient (0–100% EtOAc) and EtOAc–EtOH (0–100% EtOH). 12 fractions were collected. Fractions 3–6 were rechromatographed in the same conditions as above to give homolycorine *N*-oxide (**2**) (150 mg), *O*-methyllycorenine *N*-oxide (**3**) (90 mg) and the known Amaryllidaceae alkaloids tazettine (20 mg), ismine (15 mg) and licorenine (650 mg).

(b) *P. maritimum.* The powdered dried bulbs of the plant (5.3 kg) were extracted in a Soxhlet apparatus with MeOH. The solvent was concd, and the syrup dissolved in 5% HCl and extracted with hexane. The aq. layer was brought to pH 9 with Na_2CO_3 and extracted with CH_2Cl_2–$CHCl_3$. Removal of the organic solvent gave 9 g of a syrup, which was chromatographed on a silica gel column using a CH_2Cl_2–MeOH step gradient (0–100% MeOH). Further purification of the fractions obtained by prep. TLC (silica gel, CH_2Cl_2–MeOH, 17:3) afforded ungiminorine *N*-oxide (**1**).

Ungiminorine N-*oxide* (**1**). This material was purified by recrystallization from MeOH, mp 182–184°; UV λ_{max}^{MeOH} nm: 214, 240, and 290; IR ν_{max}^{KBr} cm^{-1}: 3300, 2900, 1480, 1360, 1100, 1040, 980, 930; MS (FAB) *m/z* (rel. int.): 667 $[2M+1]^+$, (45), 334 $[M+1]^+$, (80), 195 (85), 155 (45) and 119 (100); ^1H NMR (250 MHz, $CDCl_3$): δ 6.97 (1H, *s*, H-11), 6.84 (1H, *s*, H-8), 5.97 and 5.95 (2 × 1H, 2*d*, $J = 1.6$ Hz, $-OCH_2O-$), 5.63 (1H, *br d*, $J = 1.5$ Hz, H-4), 4.65 (4H, *m*, H-1, H-3, H-5), 4.49 [1H, *d*, J (*gem*) = 12.6 Hz, H-7], 4.23 (1H, *br d*, $J_{11b,11c} = 12.6$ Hz, H-11c), 4.15 [1H, *d*, J (*gem*) = 12.5 Hz, H-7'], 3.68 (1H, *t*, $J_{1,2} = J_{2,3} = 2.9$ Hz, H-2), 3.34 (3H, *s*, $-OMe$), and 2.65 (1H, *br d*, $J_{11b,11c} = 12.5$ Hz, H-11b); ^{13}C NMR (62.85 MHz, $CDCl_3$): δ 148.5, 146.4, 138.3, 130.0, 124.0, 116.2, 109.5, 106.0 (arom. and C=C), 101.3 ($-OCH_2O-$), 84.0 (C-11c), 80.7 (C-5), 80.0 (C-2), 69.2 (C-7), 68.2 (C-3), 68.0 (C-1), 58.0 ($-OMe$), 42.7 (C-11b).

Synthesis of **1**. To a soln of ungiminorine (**4**) (10 mg) in EtOH, were added 0.7 ml 36% H_2O_2 and 4 drops aq. Na_2CO_3 satd. After 2 days, the reaction mixture was worked-up by adding Pd-C. After filtration and removal of the solvent only one product was obtained whose spectral data and R_f were identical to that of **1**.

Ungiminorine (**4**). Mp 204–206°, lit. [9] 206–208°; EIMS *m/z* (rel. int.): 317 $[M]^+$ (14), 316 (24), 299 (61), 268 (100), 250 (29), 242 (51), 214 (72), and 212 (67); ^1H NMR (250 MHz, $CDCl_3$): δ 6.87 (1H, *s*, H-11), 6.65 (1H, *s*, H-8), 5.90 and 5.87 (2 × 1H, 2*d*, $J = 1.5$ Hz, $-OCH_2O-$), 5.57 (1H, *d*, $J = 1.4$, H-4), 4.80 (2H, *br s*, OH), 4.64 (1H, *br d*, $J_{3,2} = 2$ Hz, H-3), 4.60 (1H, *br t*, H-1), 4.09 (1H, *d*, J (*gem*) = 14 Hz, H-5), 4.04 [1H, *d*, J (*gem*) = 13 Hz, H-7], 3.83 (1H, *m*, H-11c), 3.76 (1H, *t*, $J_{2,3} = J_{2,1} = 2.9$ Hz, H-2), 3.5 [2H, *br dd*, J (*gem*) = 13 Hz, H-5 and H-7], 3.42 (3H, *s*, OMe), and 2.68 (1H, *dd*, $J_{11b,1} = 1.6$ Hz, $J_{11b,11c} = 11.2$ Hz, H-11b); ^{13}C NMR

(62.85 MHz, $CDCl_3$): δ 147.5, 145.9, 140.2, 131.1, 127.2, 120.6, 107.8, 105.6 (arom. and C=C), 101.0 (–OCH_2O–), 80.3 (C-2), 68.4 (C-3), 68.1 (C-1), 63.4 (C-11c), 62.3 (C-5), 58.1 (OMe), 54.6 (C-7), and 41.5 (C-11b).

Synthesis of 5. Ungiminorine (10 mg) was dissolved in Me_2CO, MeI added and the mixture left overnight. The solvent was removed under red. pres. to give a yellow solid; mp 207–212° (decomp., Me_2CO), lit. [8] 246–248°; ^{1}H NMR [250 MHz, $(CD_3)_2CO$]: δ 7.22 (1H, *s*, H-11), 7.15 (1H, *s*, H-8), 6.07 and 6.04 (2 × 1H, 2*d*, *J* (*gem*) = 1 Hz, –OCH_2O–), 5.96 (1H, *br s*, H-4), 5.10 [1H, *d*, *J* (*gem*) = 13.6 Hz, H-7α], 4.95 (1H, *br s*, H-3), 4.87 (2H, *m*, H-5), 4.76 (1H, *d*, $J_{1,2}$ = 2.5 Hz, H-1), 4.67 [1H, *d*, *J* (*gem*) = 13.6 Hz, H-7β], 4.29 (1H, *ddd*, $J_{11b,11c}$ = 12 Hz, $J_{11c,4}$ = 4 Hz, $J_{11c,5}$ = 2 Hz, H-11c), 3.82 (1H, *t*, $J_{2,1} = J_{2,3}$ = 2.5 Hz, H-2), 3.45 (3H, *s*, –OMe), 3.35 (3H, *s*, N-Me), and 3.11 (1H, *d*, $J_{11b,11c}$) = 12 Hz, H-11b).

Homolycorine N-*oxide* (**2**). This alkaloid was purified by recrystallization from MeOH–H_2O; mp 134–136°. $[\alpha]_D^{25}$ +27.4 (MeOH, *c* 0.1); found: M_r 331.1419; $C_{18}H_{21}NO_5$ requires 331.1419; UV λ_{max}^{MeOH} (ε) nm: 210 (12700), 226 (6500), 270 (1940), and 302 (4200); IR ν_{max}^{KBr} cm^{-1}: 3300 (due to H_2O of crystallization), 2800, 1725, 1612, 1475, 1450, 1300, 1250, 1150, and 1050; MS *m/z* (rel. int.): 331 (1.2), 313 (14.0), 256 (12.4), and 109 (100); ^{1}H NMR (200.13 MHz, $CDCl_3$, int. ref. $CDCl_3$, 7.24) δ 7.52 (1H, *s*, H-8), 7.43 (1H, *s*, H-11), 5.76 (1H, *br d*, *J* = 1.9 Hz, H-4), 4.85 (1H, *m*, H-5a), 4.01 (3H, *s*, –OMe), 3.91 (3H, *s*, –OMe), 3.81 (1H, *br d*, *J* = 9.8 Hz, H-11c), 3.75 (1H, *m*, *J* = 10.7 Hz, H-2), 3.72 (1H, *dd*, *J* = 9.8, 1.9 Hz, H-11b), 3.43 (1H, *ddd*, *J* = 10.7, 10.7, 8.6 Hz, H-2'), 3.10 (1H, *m*, H-3), 2.99 (3H, *s*, N-Me), and 2.65 (3H, *m*, H-3, 2H-5); ^{13}C NMR (50.32 MHz, CD_3OD–$CDCl_3$): δ 165.5 (*s*, C-7), 153.6 (*s*, C-9), 149.4 (*s*, C-10), 136.0 (*s*, C-3a), 133.6 (*s*, C-7a), 120.4 (*d*, C-4), 116.4 (*s*, C-11a), 112.2 (*d*, C-11), 111.8 (*d*, C-8), 77.6 (*d*, C-11c), 77.6 (*d*, C-5a), 69.8 (*t*, C-2), 56.5 (*q*, –OMe), 56.0 (*q*, –OMe), 55.5 (*q*, –NMe), 37.0 (*d*, C-11b), 30.6 (*t*, C-5), and 25.4 (*t*, C-3).

Synthesis of compound **2**. A soln of **6** (100 mg) in MeOH (2 ml) was mixed with 33% H_2O_2. White crystals (92 mg) were deposited when the soln was allowed to stand overnight at room temp. Recrystallization from MeOH–H_2O furnished white crystals which were shown to be identical with compound **2** by TLC and spectral comparison.

O-*Methyllycorenine* N-*oxide* (**3**). This oxide was purified by recrystallization from Me_2CO; mp 136°; $[\alpha]_D^{25}$ +86.8 (MeOH; *c* 0.1); found: M_r 347.1735; $C_{19}H_{25}NO_5$ requires 347.1732; UV λ_{max}^{MeOH} (ε) nm: 210 (5100), 234 (2100), and 282 (900); IR ν_{max}^{KBr} cm^{-1}: 3300 (due to H_2O crystallization), 1510, 1475, 1450, 1250, and 1050; MS *m/z* (rel. int.): 347 (15.3), 330 (13.8), 288 (45.6), 256 (62.5), and 109 (100); ^{1}H NMR (200.13 MHz, $CDCl_3$, int. ref. $CDCl_3$, 7.24): δ 7.35 (1H, *s*, H-11), 6.80 (1H, *s*, H-8), 5.72 [1H, *d* (*br*), *J* = 2 Hz, H-4], 5.50 (1H, *s*, H-7), 4.33 (1H, *d*, *J* = 5.9 Hz, H-5a), 3.91 (3H, *s*, ArOMe), 3.87 (3H, *s*, ArOMe), 3.80 (1H, *d*, *J* = 9.5 Hz, H-11c), 3.61 (1H, *ddd*, *J* = 10.8, 8.9, 1.7 Hz, H-2), 3.51 (3H, *s*, –OMe), 3.40 (1H, *ddd*, *J* = 10.8, 10.8, 8.5 Hz, H-2'), 3.40 (1H, *dd*, *J* = 9.5, 1.8 Hz, H-11b), 3.10 (1H, *m*, H-3), 3.05 (3H, *s*, –NMe), 2.60 (2H, *m*, H-5, H-3'), and 2.35 (1H, *m*, H-5'); ^{13}C NMR (50.32 MHz, CD_3OD–$CDCl_3$): δ 149.8 (*s*, C-10, C-9), 134.2 (*s*, C-3a), 129.8 (*s*, C-7a), 126.8 (*s*, C-11a), 121.4 (*d*, C-4), 114.1 (*d*, C-8), 111.7 (*d*, C-11), 99.1 (*d*, C-7), 79.5 (*d*, C-11c), 71.1 (*d*, C-2), 67.8 (*d*, C-5a), 56.5 (*m*, 3 –OMe), 55.8 (*q*, –NMe), 37.9 (*d*, C-11b), 31.7 (*t*, C-5), and 26.1 (*t*, C-3).

Synthesis of compound **3**. MCPBA (100 mg) was added to a soln of **7** (155 mg) in $CHCl_3$ (3 ml) and the mixture kept for 48 hr at room temp. After shaking with a soln of 10% Na_2CO_3 in H_2O, the organic layer was separated, dried with Na_2SO_4 and concd *in vacuo*. Purification of the residue by prep. TLC on silica gel ($CHCl_3$–EtOAc–MeOH, 2:2:1) afforded 50 mg *O*-methyllycorenine *N*-oxide (**3**) (TLC and spectral data).

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