Imidazole Alkaloids from *Lepidium meyenii*

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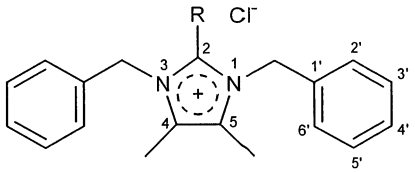
Two new imidazole alkaloids (lepidiline A and lepidiline B) have been isolated from a root extract of *Lepidium meyenii* with the common name Maca and identified as 1,3-dibenzyl-4,5-dimethylimidazolium chloride (**1**) and 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride (**2**), respectively. The structures of these two new compounds were determined by spectroscopic methods, as well as single-crystal X-ray diffraction performed on compound **1**.

Lepidium meyenii Walp (Solanaceae) is indigenous to the Andean Mountains in Peru and is found at an altitude of more than 10 000 ft. It was domesticated more than 2000 years ago and used by Andean Indians as food and folk medicine to enhance the fertility and sexual performance of men and women.[1] Our previous in vivo study on lipidic extracts showed the enhancement of sexual function of mice and rats, as evidenced by an increase in the number of complete intromissions and the number of sperm-positive females in normal mice, and a decrease in the latent period of erection in male rats with erectile dysfunction.[2] In a continuing search for biologically active components, a concentrated lipidic extract of the roots of *Lepidium meyenii* was investigated and found to be alkaloid-positive when tested with Dragendorff's reagent over silica gel TLC plates.

In 1981, Johns reported the presence of benzyl isothiocyanate and *p*-methoxybenzyl isothiocyanate in the roots.[3] Dini et al. also identified many fatty acids, amino acids, and sterols from the tubers in 1994.[4] Two classes of compounds, macaene and macamide, have been identified from the purified standardized products (MacaPure-01 and MacaPure-02), as well as other minor sterol and isothiocyanate constituents.[2,5] Recently, Muhammad reported the isolation of macaridine together with an acyclic keto acid and two macamides.[6] In this paper, we discuss the isolation and structural characterization of two new imidazole alkaloids, 1,3-dibenzyl-4,5-dimethylimidazolium chloride (**1**) and 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride (**2**), from the roots of *Lepidium meyenii*, as well as cytotoxic activity.



1 R=H
2 R=Me

Compound **1** was obtained as white needles, and its molecular formula of $C_{19}H_{19}N_2Cl$ was determined by HR-FABMS. The IR spectrum of **1** showed strong aromatic absorbances at 1621, 1605, and 1582 cm^{-1}. In the ^{13}C NMR spectrum of **1**, only eight carbon signals were observed at δ 8.1 (q), 49.6 (t), 127.2 (s), 127.8 (d), 128.6 (d), 129.1 (d), 134.3 (s), and 135.5 (d), suggesting that the molecule of **1** was symmetrical to match the mass spectral data. Analysis of the ^1H NMR signals at δ 5.41 (2H, s), 7.31 (4H, dd, J = 8.8, 2.2 Hz), and 7.37−7.46 (6H, m) and corresponding ^{13}C NMR signals (gHMQC) at δ 49.6 (t), 127.8 (d), 128.6 (d), 129.1 (d), and 134.3 (s) indicated the presence of an *N*-benzyl group.[7,8] This was supported by the COSY and gHMBC spectral data. The proton signal due to a methyl group appeared as a singlet at δ 2.18 (6H, s), but the correlative carbon was observed in unusually high field at δ 8.1 (q) in the gHMQC NMR experiment. This suggested the presence of a 4,5-dimethylimidazolium ring in **1** by comparing the proton and carbon chemical shifts with those of 1,3-di(4-fluorobenzyl)-4,5-dimethylimidazolium bromide, a synthesized product with application as a probe for intracellular pH determination.[8] In the gHMBC NMR experiment of **1**, a proton signal due to the methylene group in the benzyl unit at δ 5.41 (2H, s) exhibited other relevant cross-peaks at δ 127.2 (s) and 135.5 (d) attributable to C-2 and C-4 (5), respectively, apart from those of the benzyl moiety. On the other hand, H-2 showed two relevant cross-peaks at δ 49.6 (t) and 127.2 (s), assigned to the methylene group and C-4 (5), respectively.

The structure of compound **1** was confirmed by a single-crystal X-ray diffraction analysis.[9] In the crystal, **1** appears to be a quaternary ammonium chloride salt and the structure is symmetrical. A 2-fold symmetry axis passes through C-2, its hydrogen, and the chloride ion. The bond distances N1−C2 1.325 Å and N1−C5 1.393 Å and the carbon−carbon double bond 1.342 Å in the imidazole ring indicate that there is little delocalization in this ring (Tables 1−4, Supporting Information). A perspective drawing of the molecular structure of **1** is shown in the Figure 1.

Assignments of all protons and carbons of **1** were made by performing appropriate $^1H-^1H$ COSY, DEPT, gHMQC, and gHMBC NMR experiments (see Experimental Section). Thus, **1** was characterized as 1,3-dibenzyl-4,5-dimethylimidazolium chloride (lepidiline A).

Compound **2**, white plates, showed a molecular ion peak at *m/z* 291, 14 amu higher than that of **1**, in its low-resolution ESIMS. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, but with the absence of the H-2 signal. Analysis of the ^1H and ^{13}C NMR spectra of **2** indicated that there was a methyl group at the C-2 position of the imidazole ring from the observation of signals at δ 2.61 (3H, s) and 10.6 (q) in the ^1H and ^{13}C NMR, respectively. This inference was also supported by gHMQC and gHMBC NMR experiments. In a gHMBC NMR experiment performed on **2**, this proton signal showed one relevant cross-

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Table 1. Cytotoxic Activity of Compounds **1** and **2**[a]

	cell line[b]							
compd	A-549	UMUC3	HT-29	PC-3	PACA2	A498$_2$LM	MDA231	FDIGROV
1	>10	>10	>10	>10	>10	>10	>10	7.39
2	>10	6.47	>10	>10	1.38	>10	1.66	5.26

[a] Results are expressed as ED_{50} values (μg/mL). [b] Key: A495 = human lung carcinoma; UMUC3 = human bladder carcinoma; HT-29 = human colon adenocarcinoma; PC3 = human prostate adenocarcinoma; PACA2 = human pancreatic adenocarcinoma; A498$_2$LM = human kidney carcinoma; MDA231 = human breast carcinoma; FDIGROV = human ovarian carcinoma.

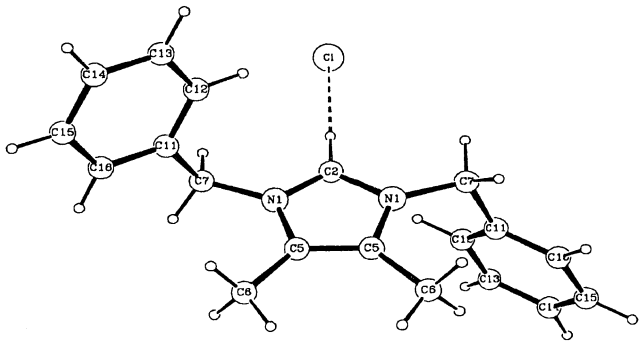

Figure 1. Perspective view of the molecular structure of **1** as determined by X-ray crystallography.

peak with the carbon signal at δ 145.1 (s), attributable to C-2. Thus, the structure of **2** was established as 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride (lepidiline B).

Compounds **1** and **2** were evaluated against a panel of human cancer cell lines as summarized in Table 1. Compound **1** was found to be weakly active only against the FDIGROV cell line (ED_{50} 7.39 μg/mL). Compound **2** showed cytotoxic activity against the UMUC3, PACA2, MDA231, and FDIGROV cell lines with ED_{50} values of 6.47, 1.38, 1.66, and 5.26 μg/mL, respectively. Both compounds were inactive against the A-549, HT-29, PC-3, and A4982LM cell lines.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA-400 instrument with tetramethylsilane (TMS) as internal standard. Low-resolution ESIMS spectra were measured with a Finnigan MAT-LCQ instrument. HRFABMS spectra were recorded on a VG Analytical ZAB 2SE high-field mass spectrometer using a PDP 11-250J data system. The X-ray data were measured on an Enraf-Nonius CAD4 diffractometer (graphite-monochromated Mo Kα radiation, $\omega-2\theta$ scans). Preparative HPLC was carried out using a normal-phase silica gel column (Dynamax-60 Å, 21 × 250 mm) on a Dynamax preparative HPLC system.

Plant Material. The roots of *Lepidium meyenii* (Solanaceae) were collected in the Andean Mountains of Peru in 1998. A voucher specimen representing this collection has been deposited at the Herbrio de Museo de Historia Natural "J. Prado" Un. H. S., Lima, Peru.

Extraction and Isolation. The air-dried roots (10 kg) of *L. meyenii* were washed with H_2O and then extracted with three changes of 100% EtOH. After removal of solvent, the resultant extract (2 kg) was dissolved in 10 L of MeOH, followed by adding 7 L of 1 N HCl solution slowly with stirring, and allowed to stand for 4 h. The acidic MeOH−H_2O solvent was separated from the precipitate, and the process was repeated twice. After removal of solvent, the combined aqueous phase was extracted with CH_2Cl_2 (3 × 4 L). The CH_2Cl_2 layers were combined and evaporated to dryness to afford extract AE (42 g). The remaining aqueous phase was adjusted to pH = 12 with 5 N NaOH solution and then extracted with CH_2Cl_2 (3 × 3 L) to afford extract BE (10 g). The partial extract AE (25 g) was absorbed and chromatographed on a Diaion HP-20MG column (i.d. 8 cm, 60 cm) and eluted with 10% MeOH in water, 40% MeOH in water plus 0.5% HOAc, MeOH, and acetone to yield fractions A1, A2, A3, and A4, respectively. After development using BuOH−AcOH−H_2O (5:1:4) as a solvent system, the TLC plate (silica gel) was dipped into Dragendorff's reagent solution. The alkaloid-positive spots were mostly found in fraction A2, which was subfractionationed over a normal-phase silica gel column using solvent systems CH_2Cl_2−MeOH−AcOH (20:1:0.1−9:1:0.1) to give seven fractions, B1−B7. Subfraction B5 was fractionationed over an Al_2O_3 (acid type) column eluted with CH_2Cl_2−MeOH−H_2O (20:1:0.1−9:1:0.1) mixtures to afford a crude alkaloid fraction, which was further purified by a combination of normal-phase column chromatography and preparative HPLC (silica gel-60 Å) to yield compounds **1** (13 mg) and **2** (10 mg). Compounds **1** and **2** were crystallized to afford white needles in acetone and white plates in 90% acetone−MeOH, respectively.

Lepidiline A (1): white needles (acetone); mp 231−3 °C; UV (MeOH) λ_{max} (log ϵ) 200 (3.33), 258 (2.29), 278 (2.12) nm; IR (film) ν_{max} 3400, 1648, 1525, 1454, 1358, 742, 712 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.04 (1H, s, H-2), 7.37−7.46 (6H, m, H-3′, H-4′, H-5′), 7.31 (4H, dd, J = 8.8, 2.2 Hz, H-2′, H-6′), 5.41 (4H, s, PhCH_2N); 2.18 (6H, s, CCH_3-4/5); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 135.5 (CH, C-2), 134.3 (C, C-1′), 129.1 (CH, C-3′, C-5′), 128.6 (CH, C-4′), 127.8 (CH, C-2′, C-6′), 127.2 (C, C-4, C-5), 49.6 (CH2, PhCH_2N), 8.1 (CH_3, C-6, C-7); gHMBC (DMSO-d_6, 400 MHz) H-2→C−PhCH_2N, 4/5; CCH_3-4/5→C-4/5; H-2′/6′→C-2′/6′, 4′, PhCH_2N; PhCH_2N→C-2, 4/5, 2′/6′; EIMS m/z 277 [M − Cl]$^+$ (100), 186 [M − Cl-benzyl]$^+$ (16), 91 [benzyl]$^+$ (25); HRFABMS m/z 277.1717 (calcd for $C_{19}H_{21}N_2$, 277.1704).

Lepidiline B(2): white plates (90% acetone/MeOH); mp 220−2 °C; UV (MeOH) λ_{max} (log ϵ) 201 (3.32), 258 (2.29), 278 (2.10) nm; IR (film) ν_{max} 3401, 2360, 1648, 1525, 1454, 742, 712 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.36−7.44 (6H, m, H-3′, H-4′, H-5′), 7.14 (4H, dd, J = 8.8, 2.2 Hz, H-2′, H-6′), 5.46 (4H, s, PhCH_2N); 2.61 (3H, s, CH_3-2), 2.23 (6H, s, CCH_3-4/5); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 145.1 (C, C-2), 135.3 (C, C-1′), 130.5 (CH, C-3′,C-5′), 129.6 (CH, C-4′), 128.0 (C, C-4, C-5), 127.4 (CH, C-2′, C-6′), 49.5 (CH2, PhCH_2N), 10.6 (CH_3, CCH_3), 8.1 (CH_3, CCH_3); gHMBC (DMSO-d_6, 400 MHz) CCH_3-4/5→C-4/5; H-2′/6′→2′/6′, C4′, PhCH_2N; PhCH_2N→C-2, 4/5, 2′/6′; ESIMS m/z 291 [M − Cl]$^+$ (100), 200 [M − Cl − benzyl]$^+$ (12), 91 [benzyl]$^+$ (34); HRFABMS m/z 291.1851 (calcd for $C_{20}H_{23}N_2$ 291.1861).

X-ray Structure Analysis of 1: *Crystal data.* $C_{19}H_{21}N_2Cl$, M_r = 312.846, monoclinic, *C2/c*, a = 18.799(4) Å, b = 10.101(2) Å, c = 9.612(2) Å, β = 111.11(2)°, V = 1702.7(6) Å^3, Z = 4, D_c = 1.22 g cm^{-3}, μ(Mo Kα) = 2.203 cm^{-1}. *Data Collection and Processing.* The size of the crystal used for data collection was approximately 0.24 × 0.36 × 0.88 mm. The structure was solved by a multiple-solution procedure and was refined by full-matrix least squares.[10] In the final refinement, the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included in the structure-factor calculations, but their parameters were not refined. The final discrepancy indices are R = 0.049, R_w = 0.049 for the 850 observed reflections. The final difference map has no peaks greater than ±0.21 e Å^{-3}. Of the 1498 reflections for θ < 25°, 850 were considered observed [$I > 3.0\sigma(I)$]. See Tables 1−4, Supporting Information.

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Supporting Information Available: X-ray crystallographic data of compound **1**. This material is available free of charge via the Internet at http//pubs.acs.org.

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(9) Crystallographic data for compound **1** reported in this paper have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 212005. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Rd., Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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