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Constituents of *Lepidium meyenii* 'maca'

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Abstract

The tubers of *Lepidium meyenii* contain the benzylated derivative of 1,2-dihydro-*N*-hydroxypyridine, named macaridine, together with the benzylated alkamides (macamides), *N*-benzyl-5-oxo-6*E*,8*E*-octadecadienamide and *N*-benzylhexadecanamide, as well as the acyclic keto acid, 5-oxo-6*E*,8*E*-octadecadienoic acid. The structure elucidation of the isolated compounds was based primarily on 1D and 2D NMR spectroscopic analyses, including ^{1}H–^{1}H COSY, ^{1}H–^{13}C HMQC, ^{1}H–^{13}C HMBC and ^{1}H–^{1}H NOESY experiments, as well as from ^{1}H–^{15}N NMR HMBC correlations for macaridine and *N*-benzylhexadecanamide. © 2002 Published by Elsevier Science Ltd.

Keywords: *Lepidium meyenii*; Brassicaceae; 1,2-Dihydro-*N*-hydroxypyridine; Macaridine; Macamide; *N*-Benzyl-5-oxo-6*E*,8*E*-octadecadienamide; *N*-Benzylhexadecanamide; 5-Oxo-6*E*,8*E*-octadecadienoic acid; 2D NMR; ^{1}H–^{15}N NMR

1. Introduction

Lepidium meyenii Walp. (Brassicaceae), commonly known as 'maca', is a nutritionally valuable native Peruvian plant that is used in the Andean diet (Leon, 1964). This plant was domesticated at least two centuries ago in the Andean mountains, where natives used its tubers as food and as a folk medicine. *L. meyenii* is known to contain valuable nutritional ingredients (Dini et al., 1994) and is used locally for the enhancement of fertility and sexual behavior in men and women, and as a traditional remedy of menopausal symptoms. The aphrodisiac activity of *L. meyenii* after oral administration in mice has recently been reported by Zheng et al. (2000). Earlier chemical work on the roots of this plant yielded mainly macaenes, macamides (alkamides), fatty acids, sterols and benzyl isothiocyanate (Zheng et al., 2000). However, other species of the genus *Lepidium* exhibit the presence of flavonoids, flavonoid glycosides (Fursa et al., 1970; KurKin et al., 1981) and alkaloids, including the *bis*-benzyl imidazole derivatives from *L. sativum* (Maier et al., 1998).

Several 'maca' dietary supplements[1] are currently available in the United States for the nutritional supplement of various sexual dysfunctions in men and women. As part of our continuing program to isolate marker compounds from traditional medicine and dietary supplements (Ganzera et al., 2001; Muhammad et al., 2001 a,b), the present study deals with the isolation and characterization of a 1,2-dihydro-*N*-hydroxypyridine derivative, macaridine (**1**), together with the hitherto unreported constituents *N*-benzyl-5-oxo-6*E*,8*E*-octadecadienamide (**2**), *N*-benzylhexadecanamide (**3**) and 5-oxo-6*E*,8*E*-octadecadienoic acid (**4**), from the tubers of *L. meyenii*.

2. Results and discussion

The petroleum ether extract of *L. meyenii* tubers was subjected to column chromatography (CC), followed by a short flash-CC and centrifugal preparative TLC (see

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[1] Maca Pure™ (Patent pending), Pure World Botanicals, Inc., 375 Huyler Street, South Hackensack, NJ 07606, USA, Web address <http://www.madis.com/news/macapure_spec.html>; Maca 750™, Medicine Plants, Web address: <http://www.maca750.com>.

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Experimental) to give compounds **1**–**4**. Compound **1**, analyzed for $C_{13}H_{13}NO_2$ by HRMS, gave a light pink color with aqueous $FeCl_3$, but was inactive with Dragendorff's reagent. The UV spectrum demonstrated α,β-unsaturated carbonyl and benzyl chromophores at λ_{max} 294 and 210 nm, respectively, and the IR spectrum showed hydroxyl, conjugated aldehyde and aromatic absorption bands [ν_{max} 3385 (*br.*), 1658, 781 and 725 cm^{-1}]. The ^1H NMR spectrum exhibited a deshielded proton at δ 9.52 (1H, *s*; $\delta_{C\text{-}8}$ 180.2, *d*) for an aldehyde group, two olefinic protons at δ 6.29 and 6.94 (each *d*, $J=4$ Hz; $\delta_{C\text{-}6}$ 111.2, $\delta_{C\text{-}5}$ 124.8) for a *cis*-disubstituted double bond and five aromatic protons (δ 6.98, 2H, *d*, $J=7.1$ Hz; 7.22-7.29, 3H, *m*; $\delta_{C\text{-}9}$ 138.2, $\delta_{C\text{-}10,14}$ 126.2, $\delta_{C\text{-}11,13}$ 129.1, $\delta_{C\text{-}12}$ 128.0) for a monosubstituted benzene ring. The ^{13}C NMR spectrum revealed two additional quaternary carbons at $\delta_{C\text{-}3}$ 142.5 and $\delta_{C\text{-}4}$ 133.2, accounting for a C-3(4)-tetrasubstituted double bond. In addition, the ^1H NMR spectrum demonstrated two 2H singlets at δ 4.54 ($\delta_{C\text{-}2}$ 57.0) and 5.73 ($\delta_{C\text{-}8}$ 48.9), indicating the presence of two sets of isolated methylene protons for –N–$\underline{CH_2}$–and C_6H_5–$\underline{CH_2}$– groups, respectively. The deshielding of the C-8–CH_2– protons was due to benzylic and allylic groups, as well as the anisotropic effect of C-7 carbonyl group. The ^{13}C NMR chemical shift value for the methylene carbon between nitrogen and oxygen atoms (–N–$\underline{CH_2}$–O–) was found to be highly deshielded (δ_C 80–83) (Linde et al., 1978; Hatfield and Maciel, 1987) compared to that observed for an *N*-methylene group ($\delta_{C\text{-}2}$ 57.0), which ruled out the possibility of an alternative dihydrooxazepine-type base structure. The ^1H–^{15}N NMR HMBC experiment established the presence of a single nitrogen atom at δ_N 159.7, suggesting the presence of an hydroxylamino group (–CH_2–$\underline{N(OH)}$–R), rather than an N–H group. (Hatfield and Maciel, 1987; Witanowski et al., 1993; Hadden et al., 1999) in a 1,2-dihydropyridine base structure. The presence of an hydroxylamino group was also supported by the HRMS using collision induced dissociation in the ESI source, which clearly demonstrated a strong fragment ion at *m*/*z* 198.0918 ($[C_{13}H_{12}NO]^+$, $[M-OH]^+$, calc. for 198.0913) due to the loss of OH^- ion, while no such fragment ion was observed under standard conditions. Furthermore, the lack of an NH proton and oxygenated carbon in the ^1H and ^{13}C NMR spectra, respectively, as well as the absence of a one bond correlation between the nitrogen atom and the NH proton in the ^1H–^{15}N NMR HMBC spectrum (with no low pass filter) could only suggest the presence of a hydroxyl group at the N-1 position. The above spectral data suggested the presence of benzyl and formyl groups in a 1,2-dihydro-*N*-hydroxypyridine nucleus and the placement of the substituents was established by gradient DQF-COSY, HMQC, gradient ^1H–^{13}C HMBC and ^1H–^{15}N HMBC NMR experiments.

The COSY and HMQC experiments established the systems –C–CH=CH–R– and C_6–H_5–, while the HMBC (Fig. 1) showed three-bond correlations between $\delta_{C\text{-}7}$ 180.2 and H-5, $\delta_{C\text{-}3}$ 142.5 and H-7, $\delta_{C\text{-}4}$ 133.2 and H_2-8, $\delta_{C\text{-}10,14}$ 126.5 and H_2-8, and $\delta_{C\text{-}2}$ 57.0 and H-6, confirming the relative placements of the *N*-methylene, benzyl, for-

Table 1
^1H NMR spectral data and coupling constants (in parentheses, in Hz) for compounds **2**–**4**[a]

Protons	**2**	**3**	**4**
2	2.17 *m*	2.11 *t* (9.3)	2.29 *m*
3	1.61 *br m*	1.55 *m*	1.57 *br m*
4	2.50 *br t* (7.3)	–	2.49 *br t* (7.0)
6	6.04 *d* (15.4)	–	6.03 *d* (15.5)
7	7.09 *dd* (3.0, 9.7, 15.4)	–	7.07 *dd* (2.8, 8.8, 15.5)
8	6.14 *m*	–	6.14 *m*
9	6.12 *m*	–	6.12 *m*
10	2.13 *m*	–	2.13 *m*
15	–	1.17 *m*[b]	–
16	–	0.81 *t* (6.9)	–
17	1.24 *m*[b]	–	1.26 *m*[b]
18	0.87 *t* (7.0)	–	0.85 *t* (7.0)
1′	4.41 *d* (5.6)	4.31 *d* (7.0)	–
3′	7.24 *d* (8.4)	7.17 *d* (8.0)	–
4′	7.30 *m*	7.22 *m*	–
5′	7.30 *m*	7.22 *m*	–
6′	7.30 *m*	7.22 *m*	–
7′	7.24 *d* (8.4)	7.17 *d* (8.0)	–
Other protons	1.29–1.43 *m*	1.18 *br s*, 1.94 *m*	1.21–1.58 *m*
	12H (H-11–H-16)	22H (H-4–H-14)	12H (H-11–H-16)
N—H	5.76 *br s*	6.03 *br s*	–

[a] Spectra for **2**–**4** were recorded at 500 MHz in $CDCl_3$.
[b] Superimposed with other CH_2 protons.

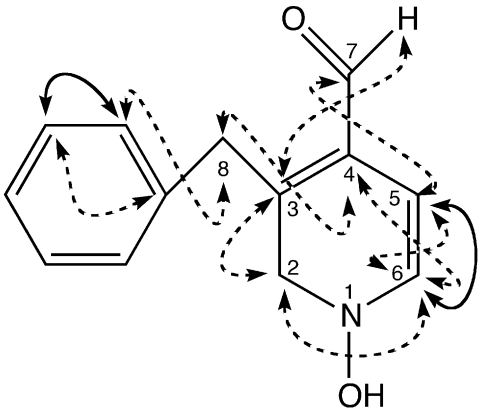
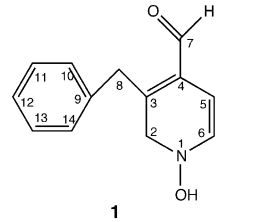
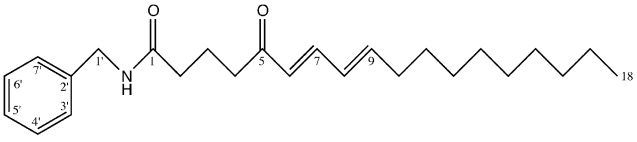


Fig. 1. 2D NMR ^{1}H–^{13}C HMBC (broken lines) and COSY (solid lines) correlations for compound **1**.

myl and olefinic substituents at C-2–C-4 and C-5(6) positions, respectively. The HMBC also showed correlations between $\delta_{C\text{-}3}$ 142.5 and $\delta_{H\text{-}2}$ 4.54, as well as correlations between $\delta_{C\text{-}4}$ 133.2 and $\delta_{H\text{-}6}$ 6.29. This establishes the position of $\delta_{C\text{-}2}$ 57.0 between the nitrogen atom and C-3. The assignment of the nitrogen atom at the N-1 position was confirmed using an ^{1}H–^{15}N HMBC experiment which showed ^{2}J correlations between the signals at $\delta_{N\text{-}1}$ 159.7, $\delta_{H\text{-}2}$ 4.54 and $\delta_{H\text{-}6}$ 6.29, ^{3}J correlations between $\delta_{N\text{-}1}$ 159.7 and $\delta_{H\text{-}5}$ 6.94, ^{4}J correlations between $\delta_{N\text{-}1}$ 159.7 and $\delta_{H\text{-}8}$ 5.73; as well as ^{5}J correlations through double bonds with $\delta_{H\text{-}7}$ 9.52. From the foregoing data the structure of **1**, named macaridine, was assigned as shown.



1



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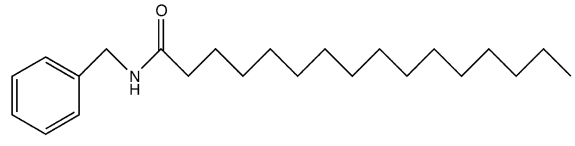

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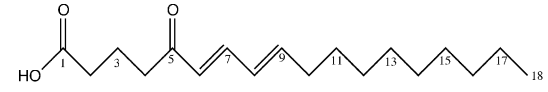

4

Alkamides **2** and **3** analyzed for the molecular formulas $C_{25}H_{37}NO_2$ and $C_{23}H_{39}NO$, respectively, by HRMS. The alkamides were homogenous on TLC, but were inactive with Dragendorff's reagent. The UV spectrum of **2** showed chromophores for a benzyl group and an α,β-unsaturated ketone (λ_{max} 210, 274 nm), and the IR spectrum exhibited absorption bands at ν_{max} 3311 and 1638 cm^{-1}, for N–H and carbonyl group(s), respectively. The NMR spectra revealed a carbonyl (δ_C 201.4), an amide carbonyl (δ_C 173.3) and a monosubstituted benzene ring (Tables 1 and 2), as well as two disubstituted double bonds (δ_C 127.9, 143.4, 131.7, 146.1; each *d*; C-6–C-9, respectively), indicating that compound **2** is a benzylamide of an oxo-octadecadienoic acid. The ^{1}H NMR spectrum exhibited two *trans*-coupled olefinic protons at δ 7.09 (*ddd*, $J = 3.0$, 9.7, 15.4 Hz, H-7) and 6.04 (*d*, $J = 15.4$ Hz, H-6), two other olefinic protons between δ 6.14 and 6.12 (*m*, H-8 and H-9), a primary methyl group at δ 0.87 (*t*, $J = 7.0$, H-18), as well as 22 protons between δ 1.29–2.43, attributed to eleven methylene groups. Due to the complexity of the H-8 and H-9 signals, the coupling constants could not be established. A 2D NMR ^{1}H–^{1}H COSY experiment of **2** established the diene system –CH=CH–CH=CH–CH_2–, which was further substantiated by a series of double resonance experiments. Thus, irradiation of the protons at δ 6.12 and 6.14 (H-8 and H-9) resulted in a doublet at δ 7.09 ($J = 13$ Hz, H-7), confirming the presence of a *trans*-olefin at the C-6(7) position. The geometry of

Table 2
^{13}C NMR spectral data for compounds **2**–**4**[a]

Carbon	**2**	**3**	**4**
1	173.3 *s*[b]	173.3 *s*	179.5 *s*
2	37.1 *t*	36.9 *t*	34.3 *t*
3	24.7 *t*	–	24.7 *t*
4	40.8 *t*	–	40.8 *t*
5	201.4 *s*	–	201.5 *s*
6	127.9 *d*	–	128.2 *d*
7	143.4 *d*	–	143.4 *d*
8	131.7 *d*	–	130.8 *d*
9	146.1 *d*	–	146.0 *d*
10	33.5 *t*	–	33.4 *t*
15	–	22.8 *t*	–
16	–	14.3 *q*	–
17	22.8 *t*	–	22.7 *t*
18	14.4 *q*	–	14.3 *q*
1′	44.0 *t*	43.7 *t*	–
2′	138.8 *s*	138.6 *s*	–
3′	128.2 *d*	127.9 *d*	–
4′	129.2 *d*	128.8 *d*	–
5′	129.1 *d*	127.5 *d*	–
6′	129.2 *d*	128.8 *d*	–
7′	128.2 *d*	127.9 *d*	–
Other carbon	25.0–31.7 (6 *t*) 6×CH_2	22.8–36.9 (11 *t*) 11×CH_2	25.0–31.7 (6 *t*) 6×CH_2

[a] Spectra for **2**–**4** were recorded at 125 MHz in $CDCl_3$.
[b] Multiplicities were determined by DEPT 135°, also aided by 2D NMR COSY and HMQC experiments.

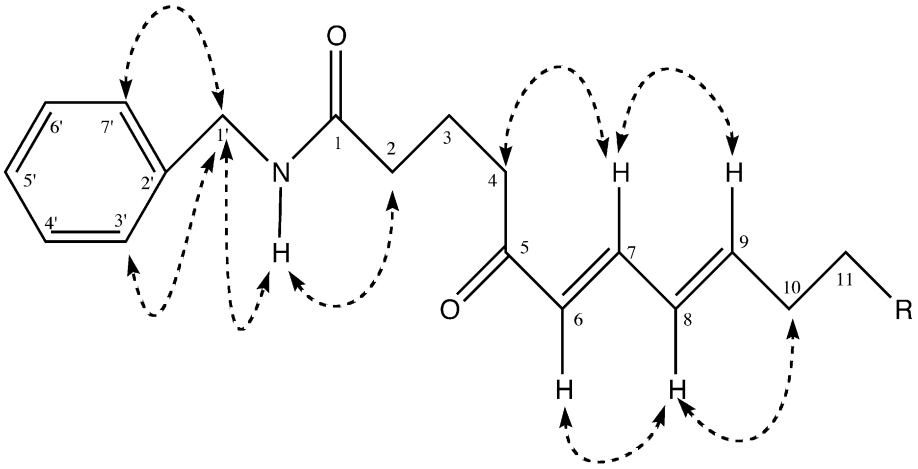

Fig. 2. 2D NMR ^{1}H–^{1}H NOESY correlations for compound **2**.

the C-8(9) double bond was established as *trans* by using 2D NMR ^{1}H–^{1}H NOESY experiment (vide infra; Fig. 2). Furthermore, the ^{1}H NMR spectrum revealed five aromatic protons (δ 7.24, 2H, *d*, $J=8.4$ Hz, H-3′,7′; 7.30, 3H, *m*, H-4′-6′), *N*-benzyl methylene protons at δ 4.41 (2H, *d*, $J=5.6$ Hz, H-1′) and a broad one proton singlet at δ 5.76, attributable to an N–H group. These spectral data are in close agreement with those observed for *N*-benzylhexadecanamide (**3**) (Tables 1 and 2). Thus, a close comparison of the ^{1}H and ^{13}C NMR spectral data of **2** with those of **3** led to the conclusion that indeed compound **2** was a benzylated alkamide of oxo-octadecadienoic acid.

The geometry of the double bonds at C-6(7) and C-8(9) was inferred from 2D NMR ^{1}H–^{1}H NOESY experiments, which showed cross peaks between H-4 (δ 2.50) and H-7 (δ 7.09). On the other hand, no NOESY correlation was observed between H-7 and H-10 (δ 2.13), while H-7 was correlated with H-9, suggesting *E* configurations for both the olefins [at C-6(7) and C-8(9)]. Furthermore, the NOESY spectrum showed cross peaks between H-6 (δ 6.04) and H-8 (centered at δ 6.14), the latter proton being correlated with H-10 (δ 2.13), thereby confirming the assignment of a *trans*-C-8(9) olefin. Other key NOESY correlations are depicted in Fig. 2. Molecular modeling[2] indicated that the NOESY correlation between H-7 and H-9 (2.4 Å), and H-8 and H-10 (2.3 Å) are consistent with a *trans* C-8(9) double bond. From the foregoing data alkamide **2** was assigned as *N*-benzyl-5-oxo-6*E*,8*E*-octadecadienamide.

The structure of alkamide **3** was unambiguously established by rigorous 2D NMR COSY, HMQC, and HMBC experiments. In addition, the placement of the secondary amide group system (–CH_2–NH–CO–CH_2–) in **3** was confirmed by a ^{1}H–^{15}N NMR HMBC experiment, which showed the ^1J, ^2J and ^3J correlations between the signals at δ_N 118.6 (–NH–CO–) and $\delta_{N\text{-}H}$ 6.03, $\delta_{H\text{-}1'}$ 4.31, $\delta_{H\text{-}2}$ 2.11, respectively.

Compound (**4**) displayed the molecular formula $C_{18}H_{30}O_3$ from its HRMS, indicating four degrees of unsaturation. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) of **4** were found to be generally similar to those observed for **2**, except for the differences associated with the absence of an *N*-benzyl group at C-1. Its UV spectrum had a chromophore expected for an α, β-unsaturated ketone (λ_{max} 274 nm), and the IR spectrum showed a strong and broad carbonyl absorption band (ν_{max} 1708 cm^{-1}). The^{13}C NMR spectrum revealed carbonyl (δ_C 201.5) and carboxylic acid (δ_C179.5) groups, as well as two disubstituted double bonds (δ_C 128.2, 143.4 and δ_C 130.8, 146.0; each *d*), indicating that compound **4** is an oxo-octadecadienoic acid. The pattern of ^{1}H NMR chemical shift values of the four olefinic protons [δ 7.07 (*ddd*, $J=2.8$, 8.8, 15.5 Hz, H-7), 6.03 (*d*, $J=15.5$ Hz, H-6), 6.14 and 6.12 (each 1H, *m*; H-8 and H-9)] were in clear agreement with those reported for a 6*E*,8*E*- diene system of **2**, as well as the analogous *E*,*E*-diene system of alkamides, isolated from *Echinacae spp*. (Bauer et al., 1988). Structure **4** was unambiguously established by detailed 2D NMR spectroscopic studies, including the application of COSY, HMQC and HMBC experiments. The HMBC experiment showed three-bond correlations between $\delta_{C\text{-}5}$ 201.5 and H-7, $\delta_{C\text{-}9}$ 146.0 and H-7, $\delta_{C\text{-}8}$ 130.8 and H-6, and $\delta_{C\text{-}1}$ 179.5 and H-3 (δ 1.57), confirming the placement of carboxylic acid, carbonyl and the two olefinic groups at the C-1, C-5, C-6(7) and C-8(9) positions, respectively. In addition, the HMBC revealed two-bond correlations between $\delta_{C\text{-}5}$ 201.5 and H-4 (δ 2.49), $\delta_{C\text{-}9}$ 146.0 and H-10 (δ 2.13), and $\delta_{C\text{-}1}$ 179.5 and H-2 (δ 2.29), which served to establish structure **4** as 5-oxo-6*E*,8*E*-octadecadienoic acid.

[2] Molecular modeling was done using CS Chem3D Pro Version 5.0 MM2 molecular dynamics minimization followed by MM2 steric minimization. The software was obtained from CambridgeSoft Corporation, 100 Cambridge Park Drive, Cambridge, MA 02140-2312, USA.

This appears to be the first report of compounds **1**–**4** from a natural source. Various hydroxamic acid derivatives were previously isolated, including benzoxazinoids-cyclic hydroxamic acids from the genus *Aphelandra* (Baumeler et al., 2000) and fusarinines A and B from *Fusarium roseum* (Sayer and Emery, 1968), but to our knowledge there is no report regarding hydroxylamino-type derivative, such as 1,2-dihydro-*N*-hydroxypyridines, as natural products. Furthermore, it is intriguing to note that the benzylated derivatives of imidazole alkaloids (including lepidine and lepidine B), analogous to benzylated derivative 1,2-dihydro-*N*-hydroxypyridine (**1**), were previously isolated from *L. sativum* (Bahroun and Damak, 1985; Maier et al., 1998). Finally, several benzylated alkamides (macamides) are currently regarded as chemical markers for 'maca' dietary supplements (Zheng et al., 2000), but to our knowledge macamides **2** and **3** appear to be new markers for *L. meyenii* (maca).

3. Experimental

3.1. General

NMR spectra were acquired on a Bruker Avance DRX-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C), in $CDCl_3$, using the residual solvent signal as int. standard; Multiplicity determinations (DEPT 135°) and 2D NMR spectra (gradient DQF-COSY, HMQC, gradient HMBC and NOESY) were acquired using standard Bruker pulse programs; ^{15}N NMR spectra were recorded at 50.7 MHz using the HMBC pulse program with no low pass filter; chemical shift values are reported relative to liquid NH_3 by calibrating nitromethane to 380.2 ppm; HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with an Analytica Electro-Spray Ionization (ESI) source and the capillary voltage was increased from 80 to 130 to generate collision induced dissociation; TLC: silica gel GF_{254} plates, solvent: CH_2Cl_2:EtOAc (8:2); CC: flash-silica gel G (J.T. Baker, 40 μM Flash); Centrifugal preparative TLC (CPTLC, using Chromatotron®, Harrison Research Inc. Model 8924): 1, 2 or 4 mm Si gel GF Chromatotron™ rotors, (Analtech, Inc.) using a N_2 flow rate of 4 ml min^{-1}. The isolated compounds were visualized by observing under UV-254 nm, followed by spraying with anisaldehyde–H_2SO_4/neutral and acidic aqueous $FeCl_3$/ and Dragendorff's spray reagents.

3.2. Plant material

The tubers of *L. meyenii* were collected in February 1999 from Lima, Peru. A voucher specimen has been deposited at the Herbarium of the University of Mississippi. The material was collected, identified and provided by Frank L. Jaksch Jr.

3.3. Extraction and isolation of compounds

Dried ground tubers of *L. meyenii* (1 kg) were percolated successively at room temperature with petroleum ether (60–80°), $CHCl_3$ and EtOH to yield 138, 21 and 25 g of crude extract, respectively. The dried petroleum ether extract was re-extracted by percolation with $CHCl_3$ (250 ml ×3) that afforded a 16 g $CHCl_3$ soluble fraction. A portion of the $CHCl_3$ fraction (4 g) was subjected to flash-chromatography over Si gel (40 μM, 120 g), using *n*-hexane followed by increasing concentrations of EtOAc (30–70%) in *n*-hexane as eluent, to give four fractions (A–D) after pooling by TLC analysis. Fraction A (110 mg) was subjected to short-flash CC, using 2% EtOAc in CH_2Cl_2 to give **4** (38 mg, R_f 0.68, silica gel, solvent: CH_2Cl_2:EtOAc, 8:2), while fraction B (600 mg) was purified by repeated CPTLC (2mm and 1 mm Si-gel GF disc), using 2% MeOH in CH_2Cl_2 to afford **2** (40 mg, R_f 0.36,), followed by palmitic acid (250 mg) and β-sitosterol (50 mg). Finally, mixture C (80 mg) and D (800 mg) were separately subjected to CPTLC (1 and 4 mm Si-gel GF disc), using 1% MeOH in CH_2Cl_2 and 10% EtOAc in CH_2Cl_2, respectively, to yield **1** (15 mg, R_f 0.56,) and **3** (10 mg, R_f 0.49), respectively.

3.4. Macaridine (3-benzyl-1,2-dihydro-N-hydroxypyridine-4-carbaldehyde) (*1*)

Solid; UV λ_{Max}^{MeOH} (log ε) 208 (4.07), 255 sh (3.76), 294 (4.14) nm; IR ν_{Max}^{film} 3385, *br* (OH), 1658 (CHO), 1494, 1453, 1402, 1372, 1179, 1035, 725, 781 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.52 (1H, *s*, H-7), 7.26 (2H, *m*, H-11,13), 7.22 (1H, *m*, H-12), 6.98 (2H, *d*, *J* = 7.1 Hz, H-10,14), 6.94 (1H, *d*, *J* = 4.0 Hz, H-5), 6.29 (1H, *d*, *J* = 4.0 Hz, H-6), 5.73 (2H, *s*, H-8), 4.54 (2H, *s*, H-2); ^{13}C NMR ($CDCl_3$) δ_C 180.2 (*d*, C-7), 142.5 (*s*, C-3), 138.2 (*s*, C-9), 133.2 (*s*, C-4), 129.1 (*d*, C-11,13), 128.0 (*d*, C-12), 126.5 (*d*, C-10,14), 124.8 (*d*, C-5), 111.2 (*d*, C-6), 57.0 (*t*, C-2), 48.9 (*t*, C-8); ESI–HRMS *m*/*z* 216.1021 ($[M+H]^+$); (calc. for $[C_{13}H_{13}NO_2+H]^+$, 216.10188).

3.5. N-Benzyl-5-oxo-6E,8E-octadecadienamide (*2*)

Gum, UV λ_{Max}^{MeOH} (log ε) 210 (4.08), 276 (3.99) nm; IR ν_{Max}^{film} 3311 (N-H), 2928, 2845, 1638, 1545, 1239, 1000, 731, 697 cm^{-1}; for ^{1}H NMR spectrum: Table 1; for ^{13}C NMR spectrum: Table 2; ESI–HRMS *m*/*z* 384.3034 ($[M+H]^+$); (calc. for $[C_{25}H_{37}NO_2+H]^+$, 384.2903).

3.6. N-Benzylhexadecanamide (*3*)

Solid; UV λ_{Max}^{MeOH} (log ε) 208 (4.03) nm; IR ν_{Max}^{film} 3303 (N-H), 2917, 2849, 1639, 1549, 1454, 730, 696 cm^{-1}; for ^{1}H NMR spectrum: Table 1; for ^{13}C NMR spectrum:

Table 2; ESI–HRMS m/z 346.3142 ($[M+H]^+$); (calc for $[C_{23}H_{39}NO+H]^+$, 346.3104).

3.7. 5-Oxo-6E,8E-octadecadienoic acid (*4*)

Gum; UV λ_{Max}^{MeOH} (log ε) 224 (3.70), 274 (3.49) nm; IR ν_{Max}^{film} 3300–2800 (*br*), 2930, 2857, 1708, 1461, 1410 cm^{-1}; for ^1H NMR spectrum: Table 1; for ^{13}C NMR spectrum: Table 2; ESI–HRMS m/z 295.2319 ($[M+H]^+$); (calc. for $[C_{18}H_{30}O_3+H]^+$, 295.2273).

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