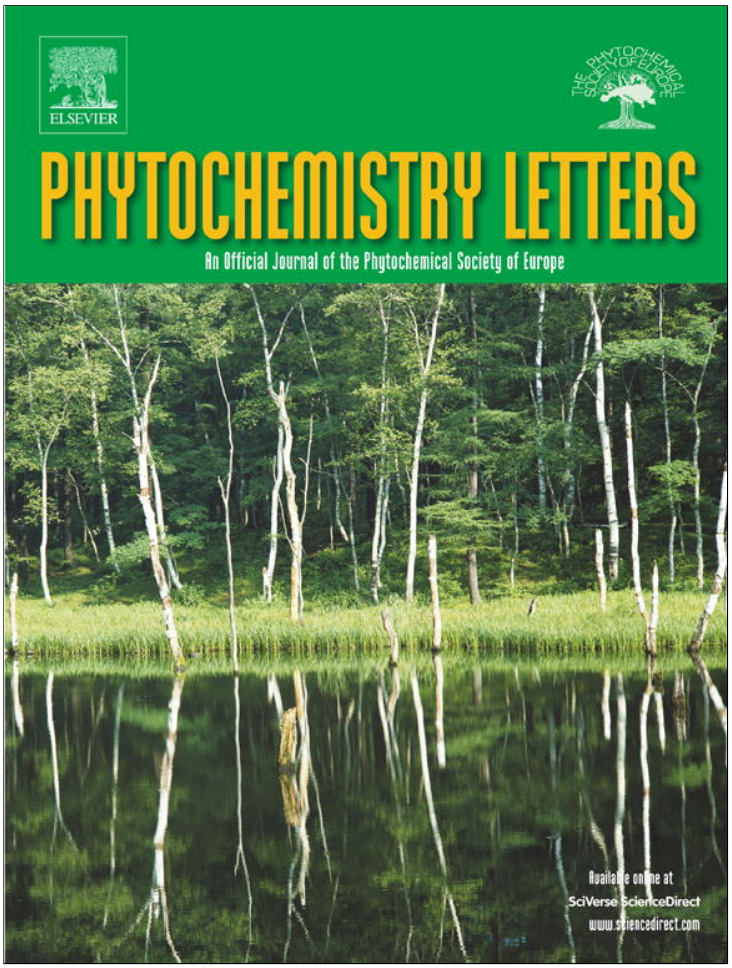

Phytochemistry Letters 8 (2014) 145–148

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Macamides from wild 'Maca', *Lepidium meyenii* Walpers (Brassicaceae)

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ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form 10 March 2014
Accepted 12 March 2014
Available online 1 April 2014

Keywords:
Lepidium
Meyenii
Brassicaceae
Maca
Macamides
NMR

ABSTRACT

The non-polar extract of the tubers of *Lepidium meyenii* Walpers yielded two benzylated alkamides (macamides), *N*-(3,4-dimethoxybenzyl)-hexadecanamide (**1**) and *N*-benzyltetracosanamide (**2**). The structure elucidation of the compounds was based on 1D and 2D NMR spectroscopic analyses, including ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC, ^{1}H–^{15}N HSQC and HMBC experiments.

1. Introduction

Lepidium meyenii Walpers (Brassicaceae), most commonly known as "maca", is an herbaceous plant that grows only in the Andean region of South-America, from Ecuador to northwestern Argentina. In South America, it is the only cruciferous crop that is cultivated for its starch content (Quirós and Aliaga Cardenas, 1997). Maca tubers were consumed for centuries by the indigenous population because of their nutritional and energizing values (Dini et al., 1994; Quirós and Aliaga Cardenas, 1997). Recently, maca root gained attention for its aphrodisiac properties as a sexual and fertility enhancer, giving maca an international notoriety that led this crop to be referred to as the "Ginseng of the Andes" in many western countries. Nowadays, maca can be found as part of dietary supplements or in combination with other crops. These products are marketed mainly in America, Europe and Japan and claim to possess invigorating and revitalizing effects (Hermann and Bernet, 2009). In addition to the supposed sexual enhancing properties, maca has been used for centuries in traditional medicine to treat or relieve menopause and rheumatism symptoms, to counter fertility problems associated with living at high altitudes in animals, to stimulate the metabolism and as a memory improver. Currently, none of these claims are supported by scientific data, as most of the research is focused on the sexual enhancing properties (Wang et al., 2007; Cicero et al., 2001; Gonzales et al., 2001; Zheng et al., 2000).

Maca roots contain several secondary metabolites of interest including glucosinolates, fatty acids esters, phytosterols, alkaloids and alkamides (macamides) (Wang et al., 2007; Piacente et al., 2002; Dini et al., 2002). This is the most relevant group because it is believed that the sexual enhancing activity observed in maca is closely related to the lipidic fraction, which is composed, in part, by macamides (Wang et al., 2007). These amides are also chemical markers as they are not found in any other *Lepidium* species (Hermann and Bernet, 2009; McCollom et al., 2005; Muhammad et al., 2002; Zhao et al., 2005). As a part of our ongoing research on plants used in Andean traditional medicine (González et al., 2012; Mercado et al., 2010; Coll Aráoz et al., 2010; Genta et al., 2010) we report the isolation and characterization of two novel macamides, i.e., *N*-(3,4-dimethoxybenzyl)-hexadecanamide (**1**) and *N*-benzil-tetracosanamide (**2**) from wild *Lepidium meyenii* Walpers collected in Tucumán, in northwestern Argentina.

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http://dx.doi.org/10.1016/j.phytol.2014.03.005
1874-3900/

2. Results and discussion

The hexane extract from roots of *L. meyenii* was saponified (Theodorou et al., 2007) and the residue was acetylated with acetic anhydride in dry pyridine. Column chromatography (CC) of the acetylated extract yielded a fraction containing a mixture of macamides. The LC–MS analysis of this fraction showed the presence of 12 amides (Table 1), two of which were not previously described in the literature reports concerning maca metabolites. The amide enriched fraction was subjected to a semi-preparative RP-HPLC in order to isolate the different amides. For the analysis, we obtained a good separation in 36 min using a C-18 stationary phase with a solvent system comprising water (+0.1% TFA) and acetonitrile at rt (see Section 3).

Compound **1** showed absorptions for a benzyl group at λ_{max} (hexane) 281 nm ($\log \varepsilon$ 3˙13), 231 nm ($\log \varepsilon$ 3˙58) and 208 nm ($\log \varepsilon$ 4˙06) in the UV spectrum, while the IR spectrum displayed bands at ν_{max} 3312 cm^{-1} (N–H) and 1635 cm^{-1} (C=O). The molecular formula $C_{25}H_{43}NO_3$ followed from HRMS. The 500 MHz ^{1}H NMR spectrum (Table 2) exhibited a primary methyl group at δ 0.89 (*t*, *J* = 6.9 Hz, H-16); a broadened resonance accounting for 24 protons at δ 1.27 corresponding to 12 methylene groups; a 2H multiplet at δ 1.66 (H-3), a 2H triplet at δ 2.22 (*J* = 7.8 Hz, H-2), and a 6H singlet at δ 3.88 corresponding to two methoxy groups. Two benzylic protons appeared as a doublet at δ 4.39 (2H, *J* = 5.6, H-1′), the N–H amide proton appeared as a broad singlet at δ 5.78 while the aromatic protons appeared as a 3H singlet at δ 6.83. The benzylic protons showed coupling to the amide proton in the ^{1}H–^{1}H COSY spectrum. The DEPT NMR spectrum showed an amide carbonyl (δ_C 172.9), two methoxy groups (δ_C 55.9), three aromatic C–H type carbons (δ_C 111.2, C-3′, δ_C 111.2, C-7′ and δ_C 119.9, C-6′) and three quaternary aromatic carbons (δ_C 149.2, C-4′, δ_C 148.5, C-5′ and δ_C 131.1, C-2′). The structure of **1** was rigorously established by 2D NMR spectroscopic studies including ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC experiments. HSQC experiment showed that the three aromatic protons (δ_H 6.83) were correlated to carbon signals at δ_C 111.2 (C-3′), δ_C 111.2 (C-7′) and δ_C 119.9 (C-6′). This, together with the data from the DEPT experiment, suggested the presence of a tri-substituted benzene ring. Two methoxy groups (δ_H 3.88, δ_C 55.9) showed a ^{1}H–^{13}C HMBC correlation to C-4′ (δ_C 149.2) and C-5′ (δ_C 148.5). In the same experiment, the benzylic protons showed three $^3J_{CH}$ correlations to C-3′ (δ_C 111.2), C-7′ (δ_C 111.2) and the carbonyl carbon (C-1, δ_C 172.9); also they showed a $^2J_{CH}$ correlation to C-2′ (131.1). All this data demonstrated that compound **1** features a *N*-(3,4-dimethoxybenzyl) fragment. The broad resonance that integrates for 24H in the ^{1}H spectrum was attributed to a saturated alkyl chain, and the ^{1}H–^{13}C HMBC correlations between C-1 (δ_C 172.9) to H-2 (δ_H 2.22) and H-3 (δ_H 1.66) showed that this was linked to the *N*-(3,4-dimethoxybenzyl) fragment. The presence of the secondary amide group in **1** was confirmed by ^{1}H–^{15}N HSQC and HMBC experiments that clearly showed the ^1J, ^2J and ^3J correlations between the nitrogen at δ_N 118.0 and the N–H (δ_H 5.78), H-1′ (δ_H 4.39) and H-2 (δ_H 2.22) protons, respectively. From the aforementioned spectroscopic data, compound **1** was unambiguously assigned as *N*-(3,4-dimethoxybenzyl)-hexadecanamide.

HRMS analysis of compound **2** yielded $C_{31}H_{55}ON$ as molecular formula. In the UV spectrum it showed absorptions for a benzyl group at λ_{max} (hexane) 208 nm ($\log \varepsilon$ 3˙44). The ^{1}H NMR of **2** showed five aromatic protons between δ 7.19 and δ 7.27 (Table 2), a broad singlet attributed to the N–H proton at δ 5.66, a benzyl methylene doublet at δ 4.38 (H-1′, *d*, *J* = 5.6, 2H), a triplet at δ 2.14 (H-2, *t*, *J* = 7.9, 2H), a multiplet at δ 1.55 (H-3, 2H), a broad resonance that integrated for 40H at δ 1.18 (H-4–H-23, CH_2x20) and a primary methyl at δ 0.81 (H-24, *t*, *J* = 7.8). The DEPT experiment showed signals for 5 aromatic C–H type carbons at δ_C 127.9 (C-3′ and C7′, CHx2), 128.7 (C-4′ and C-6′, CHx2) and 127.5 (C-5′, CH), which were correlated to the aromatic proton signals in the ^{1}H–^{13}C HSQC spectrum; this clearly demonstrates the presence of a monosubstituted benzene ring. In addition, the ^{13}C spectrum showed a carbonyl carbon (δ_C 173.0, C-1) and an aromatic quaternary carbon at δ_C 138.5 (C-2′). The methylene proton signal (δ_H 4.38) showed a coupling to the N–H proton in the ^{1}H–^{1}H COSY spectrum; it also showed a $^3J_{CH}$ correlation to the carbonyl signal at δ_C 173.0 (C-1), to the aromatic carbons at δ_C 127.9 (C-3′ and C-7′) and a $^2J_{CH}$ correlation to the aromatic quaternary carbon at δ_C 138.5. All these data confirm the presence of a *N*-benzylamide fragment. As in **1**, the position of this secondary amide group was confirmed by ^{1}H–^{15}N HSQC and HMBC experiments, which showed ^1J, ^2J and ^3J correlations between the nitrogen at δ_N 118.0 and the N–H (δ 5.66), H-1′ (δ 4.38) and H-2 (δ 2.14) protons, respectively. The presence of a *N*-benzylamide fragment is also supported by the IR spectrum, which showed bands at ν_{max} 3303 (N–H) and ν_{max} 1638 (C=O). The remaining ^{1}H NMR signals arise from a saturated alkyl chain attached to the benzyl amide fragment via the carbonyl carbon as demonstrated from J_{CH} correlation between H-2 (δ_H 2.14) and C-1 (δ_C 173.0) in the ^{1}H–^{13}C HMBC. Thus, compound **2** could be unambiguously assigned as *N*-benzyltetracosanamide (Fig. 1).

These spectroscopic data are in close correlation with that reported in the literature about macamides (Wang et al., 2007; McCollom et al., 2005; Muhammad et al., 2002; Zhao et al., 2005) but to our knowledge this is the first report of compounds **1** and **2** from a natural source. It should be noted that **1** is the first macamide found in *Lepidium meyenii* Walp. with two methoxyl groups attached to the benzylamine ring.

Table 1
LC–MS analysis of the macamide fraction of wild *L. meyenii*.

Macamides	t_R [min]	MW
N-(3-methoxybenzyl)-(9Z,12Z,15Z)-octadecatrienamide	4.273	397
N-benzyl-(9Z,12Z,15Z)-octadecatrienamide	4.405	367
N-(3-methoxybenzyl)-(9Z,12Z)-octadecadienamide	5.145	399
N-benzyl-(9Z,12Z)-octadecadienamide	5.457	369
N-(3,4-dimethoxybenzyl)-hexadecanamide (**1**)	5.785	405
N-(3-methoxybenzyl)-hexadecanamide	5.901	375
N-benzylhexadecanamide	6.068	345
N-benzyl-9Z-octadecenamide	6.247	371
N-benzylheptadecanamide	6.729	359
N-benzyloctadecanamide	7.359	373
N-benzyl-15Z-tetracosenamide	9.038	455
N-benzyltetracosanamide (**2**)	9.162	457

3. Experimental

3.1. General experimental procedures

NMR spectra were acquired on a Bruker Avance III instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C). All spectra were recorded in $CDCl_3$ with the solvent used as an internal reference (δ_H 7.26; δ_C 77.3, 77.0, 76.7). Multiplicity determinations (DEPT 135) and 2D NMR spectra (^{1}H–^{1}H gCOSY, ^{1}H–^{13}C gHSQC, ^{1}H–^{13}C gHMBC) were acquired using standard Bruker programs. ^{1}H–^{15}N HSQC and HMBC NMR spectra were recorded at 50.7 MHz. HRMS mass spectra were obtained by direct injection using a Bruker microTOF-Q II mass spectrometer, equipped with an ESI source operating in positive mode. LC–MS analysis was performed in an Agilent 1100 series HPLC, with quaternary pump, DAD and single quadrupole MS detector type VL with an API-ES source operating in positive mode, using a Phenomenex Kinetex C18 column (100 mm × 2.1 mm, 2.6 μm particle size). RP-HPLC system consisted of an Agilent ProStar binary pump, UV–Vis detector (DAD)

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **1** and **2**[a] (δ ppm, in $CDCl_3$).

Compound **1**				Compound **2**		
Position	δ_c	Type	δ_H (*J* in Hz)	δ_c	Type	δ_H (*J* in Hz)
1	172.9	C	–	173.0	C	–
2	36.9	CH_2	2.22 *t* (7.8)	36.9	CH_2	2.14 *t* (7.9)
3	25.8	CH_2	1.66 *m*	25.8	CH_2	1.55 *m*
14	31.9	CH_2	–	–	–	–
15	22.7	CH_2	–	–	–	–
16	14.1	CH_3	0.89 *t* (6.9)	–	–	–
22	–	–	–	31.9	CH_2	–
23	–	–	–	22.7	CH_2	–
24	–	–	–	14.1	CH_3	0.81 *t* (7.8)
1′	43.4	CH_2	4.39 *d* (5.6)	43.6	CH_2	4.38 *d* (5.6)
2′	131.1	C	–	138.5	C	–
3′	111.2	CH	6.83 *s*	127.9	CH	7.19 *d* (7.57)
4′	149.2	C	–	128.7	CH	7.23 *m*
5′	148.5	C	–	127.5	CH	7.23 *m*
6′	119.9	CH	6.83 *s*	128.7	CH	7.23 *m*
7′	111.2	CH	6.83 *s*	127.9	CH	7.19 *d* (7.57)
OMe x 2	55.9	CH_3	3.88 *s* 6H	–	–	–
Other carbons	29.1–29.7 (12xCH_2)	12 CH_2	1.27 *m* 24H (H-4–H-15)	29.4–29.7 (20xCH_2)	20 CH_2	1.18 *br s* 40H (H-4–H-23)
N–H	–	–	5.78 *br s*	–	–	5.66 *br s*

[a] Multiplicities were determined by DEPT, COSY and m.e.gHSQC experiments.

with automatic sample injector. For the separation a Phenomenex Luna C-18(2) column (250 mm × 10 mm, 5 μ particle size) was used. The mobile phase consisted of water (A) containing 0.1% TFA and acetonitrile (B). Separation was performed by linear gradient elution from 20A:80B to 0:100B over a period of 24 min, after which the column was washed with 100% B for 6 min. The flow rate was adjusted to 4.5 ml/min, with detections wavelengths of 210 nm and 214 nm. The column operated at rt and 25 μl of sample was injected. All chromatographic data was recorded and processed by ChemStation software from Agilent. IR spectra were recorded on a Bruker IFS 66/S spectrometer. TLC: silica gel F_{254} plates, solvent: Hexane:EtOAc (5:1); CC: Silica gel Merck 230–400 mesh. For compound detection the plates were sprayed with p-anisaldehyde.

3.2. Plant material

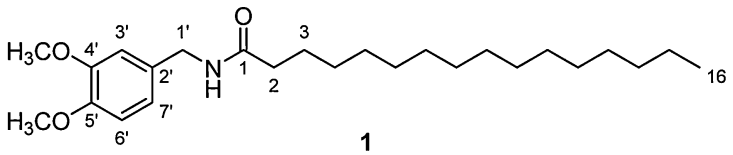
Tubers from *Lepidium meyenii* were collected in March 2009 from Chalcaqui Valleys, Tucuman, Argentina (Lat. S 26° 39,612′; Long. W 65° 44,639′, 4339 meters above sea level). A voucher specimen was deposited in the Herbarium of the Miguel Lillo Foundation (LIL. 1604). The material collected was positively identified as *Lepidium meyenii* Walpers.

3.3. Extraction and isolation of compounds

180 g of dried ground tubers of wild *L. meyenii* were extracted at room temperature by Soxhlet with *n*-hexane and the solvent was evaporated under reduced pressure to yield 2.0685 g of crude extract. The extract was subjected to a selective alkaline hydrolysis using NaOH 1N in MeOH, to remove fatty acid esters (Theodorou et al., 2007) The residue fraction (329 mg), composed mostly by fatty acid amides (macamides) and phytosterols, was acetylated with acetic anhydride in dry pyridine for 24 h at rt. The acetylated extract (310 mg) was subjected to CC over Silica gel (230–400 mesh, 9 g), using *n*-hexane followed by increasing concentrations of EtOAc (0.5–3%) in *n*-hexane as eluent, to afford 30 fractions which were pooled based on TLC characteristics. Fractions 1–9 (150 mg, R_f 0.82) contained mostly waxes and sterols acetates, while fractions 16–30 (60 mg, R_f 0.43) contained almost pure fatty acid amides when analyzed by LC–MS. Fraction 16–30 was subjected to semi-preparative RP-HPLC using water (A) with 0.1% TFA and acetonitrile (B) using a linear gradient of 20:80 (A:B) to 0:100. After evaporation of the solvent under reduced pressure, the procedure afforded 12 different macamides (see Table 1), including *N*-(3,4-dimethoxybenzyl)-hexadecanamide (**1**) (6 mg) and *N*-benzyltetracosanamide (**2**) (3 mg).

3.4. N-(3,4-dimethoxybenzyl)-hexadecanamide (1)

Solid (white powder); UV (Hexane) λ_{max} (log ε) 208 (3.48), 281 (2.59), 231 (3.05) nm; IR ν 3312 (N–H) 2920, 2850, 1635, 1521, 1469, 1420, 1266, 1240, 1156, 1139, 1027, 804, 719, 639 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectrum: Table 2; ESI-HRMS *m/z* 406.3305 ($[M+H]^+$); (calc. for $[C_{25}H_{43}NO_3 + H]^+$ 406.3310).



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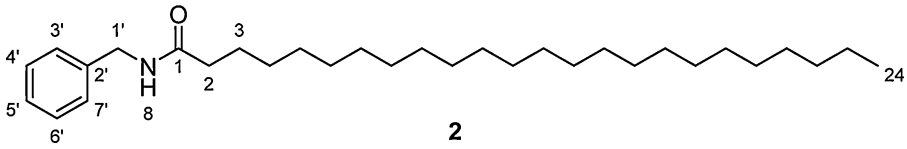

2

Fig. 1. Chemical structures of compounds **1** and **2**.

3.5. N-*benzyltetracosanamide* (**2**)

Solid (white powder); UV (Hexane) λ_{max} (log ε) 208 (3.44) nm; IR ν 3306 (N–H) 2918, 2849, 1639, 1551, 1455, 730, 698 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectrum: Table 2; ESI-HRMS *m*/*z* 458.4348 ($[M+H]^+$); (calc. for $[C_{31}H_{55}NO + H]^+$ 458.4350).

Acknowledgments

We thank Lic. M. Arias for the FT-IR spectra recordings and Dr. J. Van den Begin for the RP-HPLC assistance. The NMR equipment used was funded by a Hercules II grant attributed to Dr. J.C. Martins (AUGE/09/006). This work was supported with grants from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) PIP 0225 and Erasmus Mundus Action 2 Strand 1 Lot 16.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2014.03.005.

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