Chemical Profiling and Standardization of *Lepidium meyenii* (Maca) by Reversed Phase High Performance Liquid Chromatography

Markus GANZERA,[a,1)] Jianping ZHAO,[a] Ilias MUHAMMAD,[a] and Ikhlas Ahmad KHAN*,[a,b]

[a] *National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi; University, MS 38677, U.S.A.: and* [b] *Department of Pharmacognosy, School of Pharmacy, The University of Mississippi; University, MS 38577, U.S.A.* Received February 12, 2002; accepted March 28, 2002

***Lepidium meyenii* (Maca) is one of the few plants that can be cultivated in the harsh climate of the Andes. Its nutritious hypocotyl is traditionally used as food and medicine, and Maca products are increasingly becoming popular in the western world as tonics. This paper describes the first analytical method allowing the determination of the main macamides and macaenes, the marker compounds of *L. meyenii*. A separation within 35 min was possible by using a C-12 stationary phase, an acidic mobile phase comprising of acetonitrile and water, and raising the column temperature to 40 °C. By monitoring the separation at 210 and 280 nm, the markers were detectable as low as 0.40 μg/ml. In order to validate the method, accuracy, precision, linearity, limit of detection and intra/inter day repeatability were determined. The analysis of several commercially available Maca products showed a similar qualitative pattern but significant differences in the quantitative composition. The percentage of total markers in the preparations varied from 0.15 to 0.84%, resulting in daily intakes for the consumer from 1.52 to 14.88 mg, respectively.**

Key words *Lepidium meyenii*; Cruciferae; Maca; HPLC; macamide; macaene

Maca, *Lepidium meyenii* WALP. (Cruciferae), a plant only found on the high plateaus (3800—4800 m) of the Puna region in Peru is one of the oldest crops of the Andeans.[2)] Evidences of an early cultivation of this plant have been found dating back as far as 1600 B.C., and today the pear-shaped tuberous hypocotyl of the plant ("root") is still cultivated because of its high nutritional value. Maca is the only known cruciferous crop of the Americas; it is rich in carbohydrates, lipids and proteins, and used in a similar fashion as potatoes.[3,4)] Comparative botanical studies suggest that today's cultivars may actually be a distinct species (*L. peruvianum*), but *L. meyenii* is still the commonly used name for Maca.[5,6)]

The native Indians used Maca to treat a number of conditions such as anemia, tuberculosis, sterility and fatigue.[7)] Currently, dietary supplements containing *L. meyenii* (often referred as "Ginseng of the Andes") are available throughout the United States and South America, with claims of anabolic effects as well as increased stamina and fertility; data from several pharmacological studies confirmed the latter indication.[7—9)] Earlier chemical investigations of Maca led to the isolation of characteristic macaenes and macamides (polyunsaturated fatty acids and their amides), along with other fatty acids (such as linolenic acid (**3**) and linoleic acid (**4**)), sterols and benzyl isothiocyanate.[9,10)] We recently reported the isolation of two new macamides (**2**, **5**) and a novel macaene (**1**), together with the *N*-hydroxypyridine derivative macaridine.[11)]

Even though the biologically active principles of Maca are not fully known, extracts rich in macamides and macaenes showed promising pharmacological activity.[9)] These compounds are therefore used as quality markers (some products are standardized for their content), but surprisingly no analytical method for their determination in Maca (plant material or extract) has been reported. This paper thus describes the first HPLC method suitable for the qualitative and quantitative determination of the main macamides and macaenes in *L. meyenii*, and combined with results of a market study, the status of Maca preparations in the U.S.A. is discussed.

Results

In the nonpolar region of *L. meyenii* methanolic extracts 5 dominating signals were observed (Fig. 2). Compounds **3**—**5** are detectable at 210 nm, whereas a wavelength of 280 nm is more suitable for a sensitive monitoring of **1** and **2**. Peaks **1**, **2** and **5** were isolated and identified as macamides and macaenes, compounds recently reported by our group.[11)] Peaks **3** and **4** were identified as linolenic acid and linoleic acid, respectively (for structures see Fig. 1).

The compounds of interest show a wide polarity range, an unspecific UV spectrum (**3**—**5**) and usually tend to co-elute with compounds of similar polarity (especially **1**, **2**). Initial screening experiments showed that an acidic mobile phase enhances peak shape and separation, whereas raised tempera-

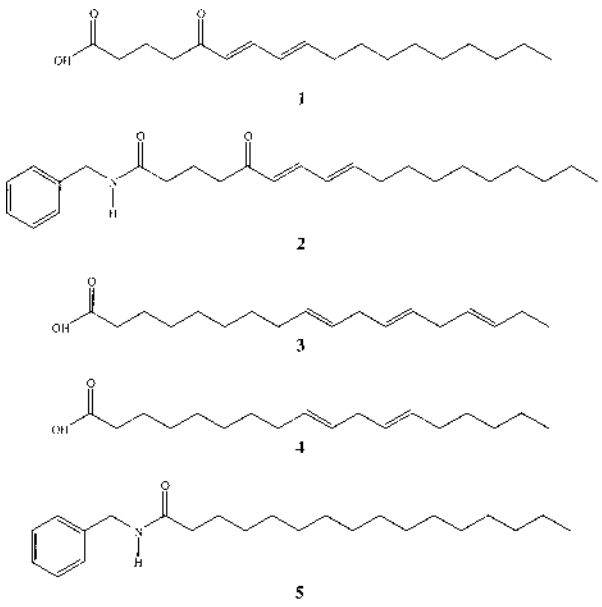

Fig. 1. Structures of Compounds **1**—**5**

* To whom correspondence should be addressed. e-mail: ikhan@olemiss.edu

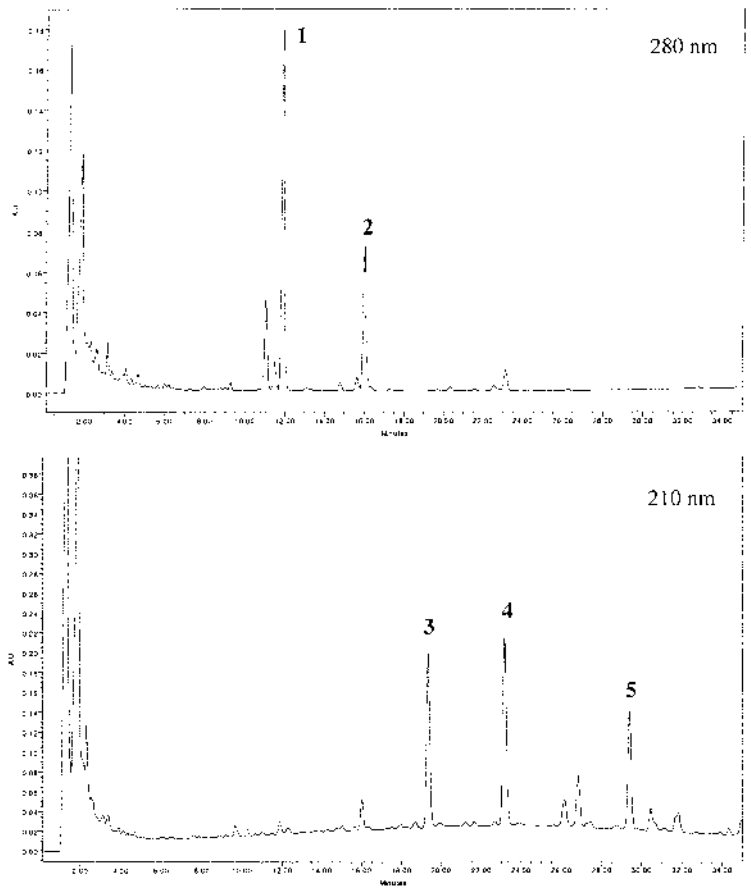

Fig. 2. Chromatogram of a Methanolic *L. meyenii* Extract (Sample NPC-LM-3) Separated under Optimized Conditions

Column: Synergi MAX-RP 80 Å, 4 μm particle size, 150×4.6 mm; mobile phase: water +0.025% TFA (A), acetonitrile +0.025% TFA (B), from 45A/55B to 5A/95B in 35 min; flow rate: 1.0 ml/min; detection: 210 and 280 nm; injected sample volume: 10 μl; temperature: 40 °C.

Table 1. Calibration Data for Compounds **1**—**5**, Including Regression Equation

Compound	Quantified at	Regression equation	R^2	LOD
1	280 nm	$y=3.62\times10^3X$	0.9996	0.28
2	280 nm	$y=1.23\times10^4X$	0.9994	0.15
3	210 nm	$y=8.55\times10^3X$	0.9994	1.13
4	210 nm	$y=4.07\times10^3X$	0.9996	2.02
5	210 nm	$y=3.94\times10^3X$	0.9997	0.40

y Reflects peak area, X the amount in μg/ml, correlation coefficient (R^2) and limit of detection (LOD) in μg/ml.

Table 2. Intra- and Inter-Day Precision of the Assay Determined by Analyzing Sample NPC-LM-8 on Three Consecutive Days

Compound	Intra-day ($n=5$)			Inter-day ($n=3$)
	Day 1	Day 2	Day 3	
1	2.03 (0.72)	2.10 (0.71)	2.07 (0.88)	2.07 (1.69)
2	0.27 (1.42)	0.27 (0.93)	0.27 (0.61)	0.27 (0.00)
3	1.17 (0.93)	1.18 (0.44)	1.19 (0.52)	1.18 (0.85)
4	2.50 (1.09)	2.52 (0.72)	2.57 (0.42)	2.53 (1.42)
5	2.31 (1.08)	2.33 (1.05)	2.35 (0.90)	2.33 (0.86)

Values in mg/g sample; relative standard deviations are given in parentheses.

ture facilitates separation in a reduced run time. The use of small particle size C-12 column material (Synergi MAX-RP) was advantageous over C-8 or C-18 materials (Luna C-8, Luna C-18, Hypersil C18, Discovery C18) in regard to resolution and peak symmetry. Acetonitrile was preferred over methanol as mobile phase as its use resulted in an improved separation as well as a significantly reduced column backpressure. The use of a buffer or the addition of modifiers like tetrahydrofuran (THF) or methyl *tert*-butyl ether (MBE) was not advantageous.

Several analytical and statistical parameters showed our systems compliance with the requirements for a validated method. The detector response was linear over the tested concentration range (2.1 to 500.0 μg/ml for **1**, **2** and **5**; 6.2 to 1500.0 μg/ml for **3** and **4**), with correlation coefficients higher than 0.999 for all quantified compounds (Table 1). The markers **1**, **2** and **5** had a detection limit of 0.40 μg/ml or lower, while the one of **3** and **4** was slightly higher with 1.13 and 2.02 μg/ml. Peak purity was confirmed by studying the photodiode array (PDA) data of all peaks of interest; no indications for impurities could be found. All samples were injected in triplicate, and the resulting standard deviations of max. 2.15% indicated the precision of the method. Intra- and inter-day variation of the assay was determined on 3 consecutive days with 5 repetitions each. All results were consistent with relative standard deviations lower than 1.7% (Table 2). Accuracy of the method was verified by spiking one sample (NPC-LM-8) with a known amount of standard compounds and calculating the recovery based on the theoretical amount.

Table 3. Quantitative Determination of Compounds **1**—**5** in Commercial Maca Products

Sample	**1**	**2**	**3**	**4**	**5**
NPC-LM-1	0.151 (0.72)	0.012 (0.51)	0.094 (0.61)	0.999 (0.65)	0.114 (0.72)
NPC-LM-2	0.143 (1.36)	0.019 (1.42)	0.039 (0.63)	0.123 (1.62)	0.047 (1.26)
NPC-LM-3	0.447 (0.63)	0.056 (0.62)	0.223 (0.76)	0.598 (0.46)	0.339 (0.70)
NPC-LM-4	0.143 (1.06)	0.026 (1.45)	0.159 (1.30)	0.343 (1.91)	0.457 (1.40)
NPC-LM-5	0.106 (1.27)	0.011 (1.46)	0.108 (0.78)	0.265 (0.19)	0.166 (1.42)
NPC-LM-6	0.093 (0.88)	0.005 (2.15)	0.055 (1.66)	0.119 (2.13)	0.049 (0.60)
NPC-LM-7	0.255 (0.57)	0.016 (2.19)	0.150 (0.75)	0.301 (0.53)	0.171 (0.53)
NPC-LM-8	0.217 (0.20)	0.027 (0.35)	0.119 (0.24)	0.251 (0.41)	0.233 (0.31)
NPC-LM-9	0.056 (0.43)	0.010 (0.36)	0.046 (0.32)	0.123 (1.10)	0.216 (0.59)

Values in g/100g; relative standard deviations are given in parentheses ($n=3$).

The resulting recovery rates were between 97.11 (**2**) and 102.56% (**1**); these data also confirms the integrity of the extraction procedure, *e.g.* the sample filtration.

The efficiency of our extraction procedure with methanol was studied in a separate experiment. One gram of sample NPC-LM-8 was extracted five times with 3 ml of methanol, and each of the extracts was analyzed separately. Compounds **1**—**5** were detectable up to the fourth extraction. A minimum of 96.3% (**1**) of each standard compound was already in solution after a 3-fold repetition, thus the extraction procedure was considered to be exhaustive.

Nine commercial Maca preparations were analyzed, and compounds **1**—**5** were consistently assignable (Table 3). Compounds **3** and **4** are not typical markers for this species, but being main compounds and indicators for the nutritional value of the material, were quantified as well. The dominating macamides and macaenes were either **1** (0.09 to 0.45%) or **5** (0.05 to 0.46%); macamide **2** was only present in minor amounts (0.01 to 0.06%). The total of the three markers in the products varied from 0.15% in sample NPC-LM-6 to 0.84% in NPC-LM-3. The ratio of linolenic acid to linoleic acid showed variations from 1 : 2.0 (NPC-LM-7) to 1 : 10.6 (NPC-LM-1), the highest content of linoleic acid was found in sample NPC-LM-1 (0.99%).

Discussion

Since *L. meyenii* is increasingly becoming popular as a dietary supplement in the western world, methods for the standardization of this plant are in demand. These products have no regulatory standard in the U.S.A., thus their analysis is essential to ensure safety and efficacy for the consumer. Furthermore, analytical methods like these might also serve agricultural purposes in order to produce higher yielding crops. The method presented in this paper allows the first accurate determination of the main quality markers in Maca. By optimizing extraction and separation conditions, a reliable, reproducible and accurate methodology was obtained, which fulfills the requirements of a validated method. A study of commercial Maca products revealed remarkable quantitative variations, resulting in nearly 10 fold differences in the daily uptake of the total marker compounds. One product (NPC-LM-3) claimed the use of a standardized plant extract (0.6% macamides and macaenes). Our results confirmed this statement, as 0.84% of the marker compounds was found.

Experimental

Materials Authenticated plant material (*L. meyenii*, dried hypocotyl) for the isolation of the standard compounds was purchased in February 1999 in Lima/Peru, and supplied by Chromadex (Irvine, CA, U.S.A.).[11] The dietary supplements analyzed in this study (NPC-LM-1 to NPC-LM-9) were purchased from Mississippi and California Supermarkets in 2001. Voucher specimens of all samples are deposited at the NCNPR.

Standard compounds **1**, **2** and **5** were isolated in our laboratories by column chromatography and HPLC; identity and purity of each compound were confirmed by chromatographic (TLC, HPLC) and spectroscopic methods (IR, 1D- and 2D-NMR, high resolution electrospray ionization mass spectroscopy (HR-ESI-MS)). Reference compounds **3** (linolenic acid) and **4** (linoleic acid) were purchased from Aldrich (Milwaukee, WI, U.S.A.).

Solvents (water, acetonitrile and methanol) were of HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.); trifluoroacetic acid was bought from Sigma (St. Louis, MO, U.S.A.).

Sample Preparation A 1.00 g portion of the plant material or commercial product (capsules and tablets containing root powder or extract) was extracted three times with 3 ml of methanol by sonication for 10 min. After centrifugation at 3000 rpm for 10 min, the supernatants were combined in a 10 ml volumetric flask and adjusted to the final volume with methanol. Prior to use, all samples were filtered through a 0.45 μm Acrodisc syringe filter from Gelman (Ann Arbor, MI, U.S.A.). Every sample solution was injected in triplicate; relative standard deviations were below 2.2% for all experiments.

Calibration 5.0 mg of compound **1**, **2** and **5**, and 15.0 mg of **3** and **4** were dissolved in 10.0 ml methanol (stock standard solution); further calibration levels were prepared by serial dilution with methanol. The range of concentrations injected varied from to 2.1 to 500.0 μg/ml (**1**, **2** and **5**) and 6.2 to 1500.0 μg/ml (**3**, **4**), respectively. All six calibration levels were injected in triplicate. The calibration data (Table 1) indicates linearity of the detector response in the range mentioned above. Standard solutions were stored in darkness at 4 °C and remained stable for at least 2 months (verified by re-assaying the standard solutions).

Accuracy For recovery experiments, 1.00 g of sample NPC-LM-8 was spiked with 100.0 μl of the stock standard solution. The sample was extracted by the above procedure, and the obtained recovery rates were 102.56% (**1**), 97.11% (**2**), 100.02% (**3**), 100.37% (**4**) and 99.28% (**5**).

Repeatability Intra- and inter-day assay precision was determined by analyzing 5 individual samples of one specimen (sample NPC-LM-8) on 3 consecutive days. The samples were extracted and assayed under optimized conditions; for results see Table 2.

Analytical Method HPLC experiments were performed on a Waters Alliance 2690 HPLC system, equipped with a 996 photodiode array detector (Waters, Milford, MA, U.S.A.). For all separations a Synergi MAX-RP 80 Å column (150×4.6 mm, 4 μm particle size) from Phenomenex (Torrance, CA, U.S.A.) was used. The mobile phase consisted of water (A) and acetonitrile (B), both containing 0.025% trifluoroacetic acid (TFA). Separations were performed by linear gradient elution from 45A/55B to 5A/95B over a period of 35 min. The flow rate was adjusted to 1.0 ml/min, with detection wavelengths of 210 nm and 280 nm, respectively. The column oven was set to 40 °C and 10 μl of sample was injected. Peaks were assigned by spiking the samples with standard compounds, and comparison of the UV-spectra and retention times. All data was recorded and processed by Millennium 32 software from Waters.

Acknowledgements This work was funded in part by the United States Department of Agriculture, ARS Specific Cooperative Agreement No. 58-6408-7-012. The authors would like to thank Chromadex for partial support of this project.

References and Notes

1) Present address: *Institut für Pharmazie, Abteilung Pharmakognosie, Universität Innsbruck, 6020 Innsbruck, Austria*.
2) Leon T., *Econ. Bot.*, **18**, 122—127 (1964).
3) Dini A., Migliuolo G., Rastrelli L., Saturino P., Schettino O., *Food Chemistry*, **49**, 347—349 (1994).
4) Johns T., *J. Ethnobiol.*, **1**, 208—212 (1981).
5) Chacon R. C., *Rev. Peruana Biol.*, **3**, 171—172 (1990).
6) Toledo J., Dehal P., Jarrin F., Hu J., Hermann A., Al-Shebaz I., Quiros C. F., *Ann. Bot.*, **82**, 523—539 (1998).
7) Cicero A. F., Bandieri E., Arletti R., *J. Ethnopharmacol.*, **75**, 225—229 (2001).
8) Gonzalez G. F., Cordova A., Gonzales C., Chung A., Vega K., Villena A., *Asian J. Andrology*, **3**, 301—303 (2001).
9) Zheng B. L., He K., Khim C. H., Rogers L., Shao Y., Huang Z. Y., Lu Y., Yan S. J., Qien L. C., Zheng Q. Y., *Urology*, **55**, 598—602 (2000).
10) Comas M., Miquel X., Arias G., de la Torre M. C., *Alimentaria*, **286**, 85—90 (1997).
11) Muhammad I., Zhao J., Dunbar D. C., Khan I. A., *Phytochemistry*, **59**, 105—110 (2002).