Phytochemistry Letters 25 (2018) 65–69

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Novel macamides from maca (*Lepidium meyenii* Walpers) root and their cytotoxicity

Chen Xia[a], Jian Chen[a,*], Jun-Lin Deng[a], Yong-Qing Zhu[a,*], Wan-Yi Li[b], Bu Jie[c], Tian-Yuan Chen[d]

[a] *Functional Food (Drug) Laboratories, Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu, 610066, Sichuan Province, China*

[b] *Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming, 650200, Yunnan Province, China*

[c] *Tibet Academy of Agricultural and Animal Husbandry Sciences, Lasa, 850002, Tibet Province, China*

[d] *Department of Molecular Cell Biology, University of California, Berkeley CA94720, USA*

ARTICLE INFO

Keywords:
Maca
Lepidium meyenii
Macamide
Isolation
Identification
Cytotoxicity

ABSTRACT

Maca, *Lepidium meyenii* Walpers (Brassicaceae), contains secondary metabolites, including macamides, which possess many bioactive properties. Two new macamides, namely, *N*-benzyl-9-oxo-10*E*,12*E*-octadecadienamide (**3**) and *N*-benzyl-9-oxo-10*E*,12*Z*-octadecadienamide (**4**), were isolated from maca root. In addition, two fatty acid derivatives, 9-oxo-10*E*,12*E*-octadecadienoic acid (**1**) and 9-oxo-10*E*,12*Z*-octadecadienoic acid (**2**), were found for the first time from maca. The structures of compounds (**3**) and (**4**) were elucidated by spectrometric and spectroscopic methods including UV, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and HR ESI–MS experiments. The novel macamides exhibit inhibitory activity against the proliferation of the HT-29 cancer cell line with IC_{50} values of 12.8 μmol/L (**3**) and 5.7 μmol/L (**4**). The two compounds were inactive (IC_{50} > 50 μmol/L) towards the proliferation of SGC7901, MCF7, NCI-H460 and HepG2 cancer cell lines.

1. Introduction

Maca, *Lepidium meyenii* Walpers (Brassicaceae), is a plant native to mountainous areas at 4000 m altitude in the Peruvian Central Andes. Maca root has been used as food and in traditional medicine for centuries (Wang et al., 2007; Gonzales et al., 2009; Gonzales et al., 2014). As a popular dietary supplement and functional food, it is grown and cultivated in many other countries. Laboratory experiments and/or clinical studies have shown that maca has many positive health and medical benefits, such as the improvement of sexual function (Zenico et al., 2009), spermatogenesis (Lee et al., 2016; Inoue et al., 2016; Melnikovova et al., 2015), benign prostatic hyperplasia reduction (Gonzales et al., 2012), anti-fatigue (Yang et al., 2016; Li et al., 2017; Choi et al., 2012), treatment of menopausal symptoms (Lee et al., 2011), anti-osteoporosis (Zhang et al., 2006), antidepressant and anxiety (Stojanovska et al., 2015; Ai et al., 2014), active against the human influenza virus (Del Valle Mendoza et al., 2014), and increases energy, quality of life and safe consumption (Gonzales-Arimborgo et al., 2016). These benefits must be a result of nutritional compounds and secondary metabolites of maca.

Macamides are a group of unique secondary metabolites and bioactive compounds that have been found only from maca (Zhao et al., 2005). These compounds can be used as markers for the authentication, standardization and quality control of maca (Zhao et al., 2005; Shu et al., 2015). Some studies have demonstrated that the macamides are fatty acid amide hydrolase (FAAH) inhibitors (Almukadi et al., 2013; Wu et al., 2013). They may act on the central nervous system by modulating the release of neurotransmitters to provide analgesic, anti-inflammatory, sexual performance or neuroprotective effects (Almukadi et al., 2013). They may also modulate energy metabolism and improve antioxidant status against exercise-induced fatigue (Yang et al., 2016). A further study has indicated that a macamide had 46 disease targets (Yi et al., 2016).

Macamides are generated in the post-harvest drying process of maca, through the synthesis of benzylamine and fatty acids with varying hydrocarbon chain lengths and degrees of unsaturation, and can sometimes contain a keto group (Esparza et al., 2015). There have been more than twenty macamide compounds isolated from maca (Zhao et al., 2005; Fernando et al., 2014). As part of our ongoing research on the quality, health and medical activities of maca, which is grown and processed in the high altitude areas of Western China, we report the isolation and identification of two new macamides from maca root, i.e., *N*-benzyl-9-oxo-10*E*,12*E*-octadecadienamide (**3**) and *N*-benzyl-9-oxo-10*E*,12*Z*-octadecadienamide (**4**). Additionally, two known fatty acid derivatives, 9-oxo-10*E*,12*E*-octadecadienoic acid (**1**) and 9-oxo-10*E*,12*Z*-octadecadienoic acid (**2**) (Dufour and Loonis,

* Corresponding authors.
E-mail address: jc_saas@yahoo.com (J. Chen).

https://doi.org/10.1016/j.phytol.2018.03.001
Received 1 December 2017; Received in revised form 9 February 2018; Accepted 1 March 2018

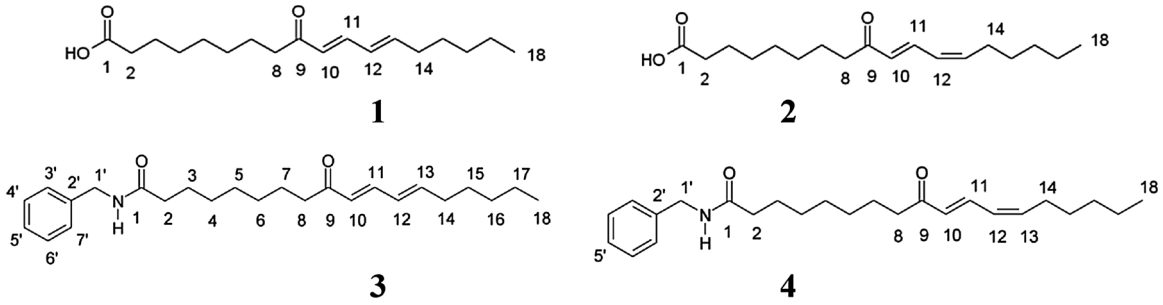

Fig. 1. The structures of compounds **1-4** isolated from maca root (*Lepidium meyenii* Walpers).

2005), were isolated for the first time from maca root (Fig. 1). Macamides **3** and **4** were also tested in terms of their inhibitory activities against the proliferation of five cancer cell lines, SGC7901 (human stomach cancer), HT-29 (human colon cancer), MCF7 (human breast cancer), NCI-H460 (human lung cancer) and HepG2 (human liver cancer).

2. Experimental

2.1. General experimental

NMR spectra were acquired on a Bruker Avance II-400 instrument (Bruker Corporation, USA) at 600 MHz (^{1}H). All spectra were recorded in $CDCl_3$, DMSO-d_6 or CD_3OD with TMS used as an internal standard. HRMS was obtained by direct injection using a Waters Q-TOF Premier (Waters Corporation, USA) and Waters Quattro Premier XE mass spectrometer (Waters Corporation, USA) equipped with an ESI source operating in positive mode. UV spectroscopy was recorded on a UV-6100 UV–vis spectrometer (Shanghai Metash Instrument Co. Ltd., China). IR spectroscopy was recorded on a Perkin Elmer FT-IR/NIR spectrometer (Perkin Elmer, Inc., USA). Silica gel 60 (0.070 ~ 0.200 mm, Qingdao Marine Chemical Inc., China) was used for column chromatography (60 cm × 5 cm, Jiangyin Jinque Chromatography Equipment Inc., China). Preparative RP-HPLC was performed on an axial compression column 100 chromatographic system (Beijing Hengke Innovation Technology Co. Ltd., China, APS-2004) with a Hengke Innovation horizontal pump (500 mL) and a UV detector (UV3000) using a XB-C18 column (50 cm × 8 cm, 10 μm particle size). The separation analysis was performed on an Agilent LC-1260 HPLC system equipped with a diode array detector (DAD) using a Zorbax XDB-C18 column (250 mm × 4.6 mm, 5 μm particle size). The mobile phase was comprised of water (A) containing 0.005% trifluoroacetic acid (TFA) and acetonitrile (B) containing 0.005% TFA. The separation was performed using linear gradient elution from 45:55 (A:B) to 10:90 over a period of 45 min at a flow rate of 1.0 mL/min and column temperature of 40 °C. The detection wavelength was 280 nm with a 10 μL sample injection volume. All chromatographic data were recorded and processed using ChemStation software from Agilent. TLC was conducted on silica gel GF_{254} plates (Qingdao Marine Chemical Inc., China). Reagents and solvents were of chemical, analytical or HPLC grade. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT, sigma, USA).

2.2. Plant material

Fresh tubers of maca (*Lepidium meyenii* Walpers, Brassicaceae) (maca roots) were grown at the Lasa farm (3700 m altitude) of Tibet Province located in Northwest China and harvested in November 2015. The fresh tubers were regularly processed and dried under natural sunlight by the workers of the Tibet Academy of Agricultural and Animal Husbandry Sciences. The sample has been deposited in the herbarium of Institute of Agro-product Processing Science and Technology, Sichuan Academy of Agricultural Sciences.

2.3. Extraction and isolation

Dry maca roots were cut to small pieces and milled to 40–60 mesh powder. The maca powder (3.2 kg) was extracted using aqueous 95% EtOH at 60 °C for 2 h (9.6 L × 4). The combined extracts were concentrated at 50 °C under reduced pressure to yield 480 g of the crude extract. The crude extract was partitioned between EtOAc (1.0 L) and H_2O (1.0 L) two times. The EtOAc solution was evaporated at 50 °C under reduced pressure to obtain 288 g of residue. The residue was first subjected to separation analysis using HPLC and then fractionated using column chromatography over silica gel using petroleum ether (A, b.p. 60–90 °C) and EtOAc (B) as the eluent with an elution gradient from 5:1 (A:B) to 1:1 to obtain 5 fractions. Fractions 2–4 were subjected to preparative RP-HPLC using water and acetonitrile (25:75) to elute at a detection wavelength of 280 nm. The collected solutions of the same absorbance were combined and concentrated to about 50 mL volume under reduced pressure at 45 °C. The concentrated solutions were then allowed to stand for 24 h at room temperature to precipitate any solids. The solids were collected by filtration and dried under vacuum to obtain two macamides, i.e., compound **3** (300 mg) and **4** (60 mg). The two compounds were identified by spectrometric and spectroscopic methods including UV, IR, HR ESI–MS, ^{1}H NMR, ^{13}C NMR and 2D NMR experiments. Two known compounds, **1** (173 mg) and **2** (36 mg), were also isolated and identified from the maca root. Their details are deposited in the Supporting information.

N-Benzyl-9-oxo-10*E*,12*E*-octadecadienamide (**3**): White powder; UV (MeOH) λ_{max} (logε) = 207 (3.91), 276 (4.12) nm; IR (powder) ν_{max} = 3285 (N−H), 2926, 2850, 1681 (C=O), 1630 (C=O), 1593, 1551, 1235, 994, 723, 693 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), see Table 1; HR ESI–MS, *m/z* found 384.2900 ($[M + H]^+$) (calcd. for $C_{25}H_{38}NO_2$ $[M + H]^+$, 384.2895), 260.1651 $[M–CH_3(CH_2)_4CH=CHCH=CH + H]^+$.

N-Benzyl-9-oxo-10*E*,12*Z*-octadecadienamide (**4**): White powder; UV (MeOH) λ_{max} (logε) = 207 (3.86), 279 (4.06) nm; IR (powder) ν_{max} = 3293 (N−H), 2927, 2851, 1681 (C=O), 1630 (C=O), 1549, 1236, 724, 694 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), see Table 1; HR ESI–MS, *m/z* found 384.2898 ($[M + H]^+$) (calcd. for $C_{25}H_{38}NO_2$ $[M + H]^+$, 384.2898), 260.1648 $[M–CH_3(CH_2)_4CH=CHCH=CH + H]^+$, 125.0964 [M- $PhCH_2NHCO(CH_2)_7CO + 1]^+$.

2.4. Cell lines and cell cultures

SGC7901 cells (CL-0206 human stomach cancer), HT-29 cells (CL-0118 human colon cancer), MCF7 cells (CL-0149 human breast cancer), NCI-H460 cells (CL-0299 human lung cancer) and HepG2 cells (CL-0103 human liver cancer) were purchased from Wuhan Procell Life Science & Technology, China, and were cultured in RPMI-1640 medium