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(7*S*)-MACAUREAS A AND B, TWO UREA ANALOGUES FROM THE ROOTS OF *LEPIDIUM MEYENII*

Hui-Chun Geng,[1,2#] Shi-Yu Qiu,[1#] Shi-Wei Chen,[1] Song-Mei Lu,[1] Fu-Wen Yang,[1] Ji-Rui Wei,[1] Yong-Hui He,[1]* and Min Zhou[1]*

[1] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, Kunming 650031, P.R. China; [2] Institute of Quality Standard and Testing Technology,Yunnan Academy of Agricultural Science, Kunming 650223, Yunnan, P.R. China. E-mail: hee_csu@126.com; zhouminynun@163.com

Abstract – (7*S*)-Macaureas A and B (**1** and **2**), two urea derivatives were isolated from the roots of *Lepidium meyenii* (Maca) collected from Qujing, Yunnan Province of China. Their structures were established on the basis of extensive spectroscopic data, including 1D NMR, 2D NMR and HRESIMS techniques. The absolute configuration of (−)-**1** was corrected as 7*S* by a concise three-step synthesis from commercially available Boc-*L*-proline. Macaureas A and B were tested for their cytotoxicities against five human cancer cell lines.

Lepidium meyenii Walp. (commonly known as Maca), a plant indigenous to the Peruvian Andes, has been introduced as “Peruvian Ginseng” or “Plant Viagra” into many areas of China, such as Yunnan, Xinjiang, and Tibet provinces.[1] Maca has a long history of use in folk medicine to improve fertility in humans and cattle, enhance energy and vitality, treat menopausal syndrome, and cure other diseases such as respiratory disorders, malnutrition and anaemia.[1] Previously phytochemical and pharmacological studies on Maca found that the main bioactive components, macaenes and macamides, exhibited various biological functions, such as antioxidant, anti-fatigue, anti-osteoporosis, anti-cancer, and neuroprotective effects.[1] Interestingly, many benzyl isothiocyanate or benzyl cyanate derivatives with diverse structures and biological properties were recently discovered from Maca,[1] such as lepithiohydimerins A–D, four

[#] These authors contribute equally to this work.

neuroprotective thiohydantoin dimers bearing a disulfide bond,[2] meyeniins A–C, three rare hexahydroimidazo[1,5-*c*]thiazoles with cytotoxic activity,[3] macahydantoin A,[4] a novel thiohydantoin with a 1,3-diazabicyclo[3.3.1]nonane nucleus, and the revised macahydantoin B possessing an unusual 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole framework.[5] As a part of our continuing efforts on the bioactive principle components from Maca, two urea derivatives, (7*S*)-macaureas A and B (**1** and **2**), have been obtained. Their structures including absolute configurations were identified on the basis of extensive spectroscopic data and further confirmed by a concise three-step synthesis. Additionally, all of the compounds were screened for their cytotoxic activity against five human tumour cell lines. Herein, we mainly report the structural elucidation, the concise synthesis, and cytotoxic activity of the isolated compounds.

The methanol extract prepared from the roots of *L. meyenii* was purified repeatedly by silica gel column and RP-18 column, followed by preparative-HPLC and semi-preparative HPLC to yield two urea derivatives, (7*S*)-macaureas A and B (**1** and **2**) (Figure 1).

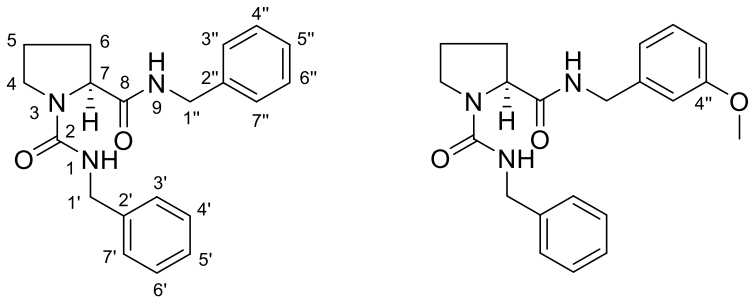

Figure 1. Chemical structures of compounds **1** and **2** from *Lepidium meyenii*

The molecular formula of compound **1** was assigned as $C_{20}H_{23}N_3O_2$ by positive HRESIMS (360.1676 [M + Na]$^+$, calcd. for 360.1682), implying 11 degrees of unsaturation.The ^{1}H NMR spectrum (Table 1) showed obvious resonances for two symmetrical monosubstituted benzene moieties at δ_H 7.21–7.26 (10H, H-3'–H-7' and H-3"–H-7"), three heteroatom-bearing methylenes at δ_H 4.38 (H_2-1'), 4.35 (H_2-1") and 3.20–3.34 (2H, H_2-4) and one heteroatom-bearing methine at δ_H 4.45 (H-7). Its ^{13}C NMR and DEPT spectra (Table 1) revealed twenty carbon signals categorized into four quaternary carbons (including two olefinic carbons and two amide carbonyl), eleven methines (including one heteroatom-bearing and ten olefinic ones), and five methylenes (including three heteroatom-bearing ones). Except for the functional groups (two carbonyl carbons and two benzene rings) accounted for ten degrees of unsaturation, the presence of one ring was necessary to meet the degrees of unsaturation required. The aforementioned spectroscopic data coupled with literature reference[6] indicated compound **1** should be a urea derivative possessing a carbamothioylpyrrolidine-2-carboxamide nucleus and two *N*-benzyl substituents. The 2D

NMR spectra provided further evidence for this structural assignment (Figure 2). The carbamothioylpyrrolidine-2-carboxamide nucleus was established by ^{1}H-^{1}H COSY correlations of H_2-4/H_2-5/H_2-6/H-7 and HMBC correlations from H_2-4 (δ_H 3.34 and 3.20) to C-2 (δ_C 157.7) and C-7 (δ_C 60.3), from H-7 (δ_H 4.45) to C-2 (δ_C 157.7) and C-8 (δ_C 172.4), and from H_2-6 (δ_H 2.32 and 1.86) to C-4 (δ_C 46.5), C-7 (δ_C 60.3) and C-8 (δ_C 172.4) (Figure 2).

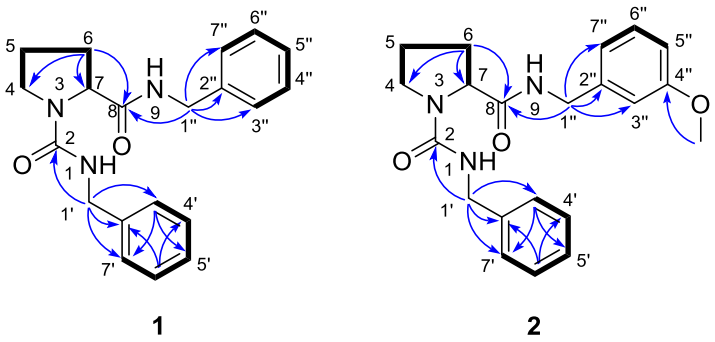

Figure 2. Selected HMBC and ^{1}H-^{1}H COSY NMR correlations of compounds **1** and **2**

Additionally, the location of the two *N*-benzyl groups was assigned at N-1 and N-9 based on HMBC correlations from H_2-1' (δ_H 4.38) to C-2 (δ_C 157.7), C-3' (δ_C 127.3) and C-7' (δ_C 127.3) and from H_2-1" (δ_H 4.35) to C-8 (δ_C 172.4), C-3" (δ_C 127.5) and C-7" (δ_C 127.5), respectively (Figure 2). Accordingly, the planar structure of compound **1** was determined as *N*-benzyl-1-(benzylcarbamothioyl)-pyrrolidine-2-carboxamide, which has the same planar structure with the reported macaurea A.[6] Previously, the absolute configuration of (−)-macaurea A was assigned to be 7*R* by comparison of their optical rotation with a synthetic analogue,

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** (in $CDCl_3$ δ in ppm, 400 and 100 MHz)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1-NH	-	5.19 br s	-	5.02 t (5.6)
2	157.7 s	-	157.6 s	-
4	46.5 t	3.34 m, 3.20 m	46.5 t	3.37 m, 3.23 m
5	24.9 t	1.99 m, 1.92 m	25.0 t	2.01 m, 1.94 m
6	28.5 t	2.32 m, 1.86 m	28.4 t	2.35 m, 1.87 m
7	60.3 d	4.45 d (6.8)	60.3 d	4.48 d (8.0)
9-NH	-	7.63 br s	-	7.60 br s
8	172.4 s	-	172.3 s	-
1'	43.2 t	4.38 overlapped	43.2 t	4.38 overlapped
2'	138.5 s	-	139.2 s	-
3'	127.3 d	7.21 overlapped	127.6 d	7.26 overlapped
4'	128.6 d	7.24 overlapped	128.6 d	7.28 overlapped
5'	127.2 d	7.21 overlapped	127.4 d	7.26 overlapped
6'	128.6 d	7.24 overlapped	128.6 d	7.28 overlapped
7'	127.3 d	7.21 overlapped	127.6 d	7.26 overlapped
1"	44.6 t	4.35 overlapped	44.7 t	4.36 overlapped
2"	139.3 s	-	140.1 s	-
3"	127.5 d	7.26 overlapped	119.6 d	6.81 overlapped
4"	128.6 d	7.26 overlapped	159.8 s	-
5"	127.3 d	7.21 overlapped	112.8 d	6.78 overlapped
6"	128.6 d	7.26 overlapped	129.6 d	7.20 overlapped
7"	127.5 d	7.26 overlapped	112.8 d	6.78 overlapped
4"-OMe	-	-	55.2 q	3.76 s

N-Boc–aza-1,4-cyclohexadienylglycinyl-*L*-proline benzhydrylamide.[6] However, as compound **1** is likely to be biosynthesised from an *L*-proline and benzylamine, the proposed configuration is supported by the known configuration of its most likely biosynthetic precursor. In this study, the absolute configuration of **1** was further modified as 7*S* by a three-step synthesis from commercially available Boc-*L*-proline (Figure 3) and comparison of their optical rotation ($[\alpha]_D^{25}$ −41.0 for natural **1**, $[\alpha]_D^{25}$ −38.0 for synthetic **1**). Thus, the structure of **1** was established as shown in Figure 1 and named as (7*S*)-macaurea A.

The molecular formula of macaurea B (**2**) was determined to be $C_{21}H_{25}N_3O_3$ by positive HRESIMS at *m/z* 390.1780 $[M + Na]^+$ (calcd. for 390.1788). The 1D NMR data (Table 1) were quite similar to those of **1**, with the exception that one more methoxy group at C-4" position occurred in **2** instead of the hydrogen atom at the same position in **1**. This assignment was further supported by the key HMBC correlations of H-6" (δ_H 7.20) with C-2" (δ_C 140.1) and C-4" (δ_C 159.8), of H-3" (δ_H 6.81) with C-5" (δ_C 112.8) and C-7" (δ_C 112.8), and of the methoxy group (δ_H 3.76, s) with C-4" (δ_C 159.8). Accordingly, the planar structure of **2** was established. Similarly, the absolute configuration of C-7 in (−)-**2** was confirmed as *S* by comparison with the optical rotation of (7*S*)-macaurea A (**1**) ($[\alpha]_D^{25}$ −41.0 for **1**, $[\alpha]_D^{25}$ −53.2 for **2**). Accordingly, the structure of **2** was established as shown in Figure 1, and it was given a trivial name of (7*S*)-macaurea B.

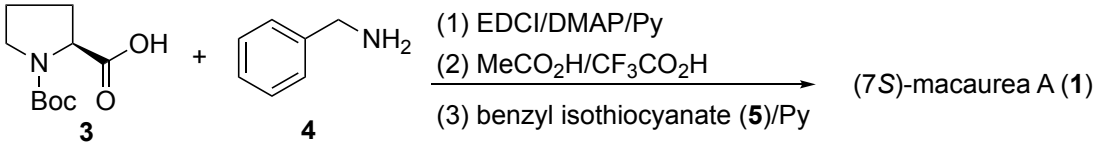

Figure 3. Concise synthesis of (7*S*)-macaurea A (**1**) from Boc-*L*-proline

The isolated urea derivatives (**1** and **2**) were tested for their cytotoxicities on five human cancer cell lines, including human leukaemia (NB4), human neuroblastoma (SHSY5Y), carcinomic human alveolar basal epithelial (A549), human prostate cancer (PC3), and human breast adenocarcinoma (MCF7) tumor cells, as reported previously.[7] However, no significant activities were detected at concentrations up to 40 μM.

EXPERIMENTAL

General. Column chromatography was performed with silica gel (80-100, 100-200, and 200-300 meshes) (Qingdao Marine Chemical, Inc., Qingdao, China). Preparative HPLC was performed on a Agilent 1260 HPLC (Agilent Technologies, Wilmington, DE, USA) with a Welch Ultimate XB-C18 (10 μm, 4.6 × 25 cm, Welch Technologies, Shanghai, China). Semi-preparative HPLC was performed on an Agilent 1200 HPLC (Agilent Technologies, Wilmington, DE, USA) with a Zorbax SB-C18 column (10 μm, 2.12 × 25 cm, Agilent Technologies, Wilmington, DE, USA). Fractions were monitored by thin-layer

chromatography (TLC) (Qingdao Marine Chemical, Inc., Qingdao, China), and spots were visualized by heating SiO_2 plates sprayed with 10% H_2SO_4 in EtOH and heating. Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter (Jasco International Co., Ltd., Tokyo, Japan). Ultraviolet (UV) spectra were collected on a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). Infra-red (IR) spectra were carried out on a Tenor 27 spectrophotometer (Bruker, Karlsruhe, Germany) with KBr pellets. Nuclear magnetic resonance (NMR) spectra were performed on a Bruker AV-400 NMR spectrometer (Bruker, Karlsruhe, Germany) with the deuterated solvent as the internal standard. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded on a Thermo UltiMate 3000-Q Exactive spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

Plant material. The roots of *Lepidium meyenii* were collected from Qujing, Yunnan Province of China in September 2021. The samples were identified by Prof. Qing-Song Yang of Yunnan Minzu University. The voucher specimen (No. 20210912) has been deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources of Yunnan Minzu University.

Extraction and Isolation. The dried and powdered roots of *Lepidium meyenii* (12 kg) were extracted three times with MeOH (3 d, each 30 L) at room temperature and filtered. The extract was evaporated to get a resulting residue (2.8 kg), which was suspended in water (10 L) and extracted with CH_2Cl_2 (3 times, each 10 L). The CH_2Cl_2 extract (approximately 200 g) was applied to silica gel column chromatography with gradient petroleum/EtOAc from 1:0 to 1:1 to yield five fractions A-E. The fraction C (petroleum/EtOAc 8:2, 28.0 g) was fractionated on SiO_2 eluting with petroleum/CH_2Cl_2 from 10:1 to 0:1 to give five subfractions C1-C5. The subfraction C4 (petroleum/CH_2Cl_2 5:1, 5.5 g) was separated into six main fractions C31-C36 by an RP-18 column with gradient elution with MeOH/water (1:1 to 1:0). Subsequently, fraction C33 (1.8 g) was further performed on a preparative HPLC (20 mL/min, MeOH /water 75:25) and followed by a semi-preparative HPLC (3 mL/min, MeCN/water 65:45) to afford **1** (75 mg). Similarly, fraction C32 (0.6 g) was further purified by a preparative HPLC (20 mL/min, MeOH /water 75:25) and followed by a semi-preparative HPLC (3 mL/min, MeCN/water 60:40) to afford **2** (12 mg).

Concise synthesis of (7*S*)-macaurea A (1). Boc-*L*-proline (**3**, 1 equiv, 108 mg, 0.5 mmol) and benzylamine (**4**, 1 equiv, 54 mg, 0.5 mmol) were dissolved in anhydrous pyridine (5 mL), and after 10 min, EDCI (1.5 equiv, 144 mg, 0.75 mmol), and DMAP (1.5 equiv, 92 mg, 0.75 mmol) were added, and the reaction was performed by stirring the mixture under nitrogen at room temperature for 12 h. Then, the solution was reduced under a vacuum. Subsequently, removal of Boc group in acetic acid and trifluoroacetic acid (TFA) (1:1, 5 mL) at room temperature for 10 h yielded (*S*)-*N*-benzylpyrrolidine-2-carboxamide TFA salt. Finally, the unpurified TFA salt and benzyl isothiocyanate (**5**, 1.0 equiv, 75 mg, 0.5 mmol) were further dissolved in anhydrous pyridine (5 mL), then the reaction

was performed by stirring the mixture under nitrogen at room temperature for 2 h. The solution was reduced under a vacuum and the resulting mixture was directly subjected to silica gel column eluted with petroleum/CH_2Cl_2 from 5:1 to 0:1 to afford synthetic (7*S*)-macaurea A (**1**) as a colourless powder (85 mg, 0.25 mmol, 50%).

Cytotoxicity Assays. The cytotoxicity of the isolates was investigated using a previously reported procedure.[7] The five human cancer cell lines, including human acute promyelocytic leukemia (NB4), human lung adenocarcinoma epithelial (A549), human neuroblastoma (SHSY5Y), human prostate cancer (PC3), and human breast adenocarcinoma (MCF7) tumour cells, were all obtained from the American Type Culture Collection (ATCC). All cells were cultured in RMPI-1640 (Hyclone, Logan, UT) or DMEM medium (Biological Industries, Kibbutz Beit-Haemek, Israel) supplemented with 10% fetal bovine serum (Biological Industries, Kibbutz Beit-Haemek, Israel) in 5% CO_2 at 37 ℃. The assay was performed according to 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS) (Promega, Madison, WI, USA) method. Briefly, cells were seeded into each well of a 96-well cell culture plate. After 12 h of incubation at 37 ℃, the test compound (40 μM) was added. Then, incubated for 48 h, cells were subjected to the MTS assay. Compounds with a growth inhibition rate of 50% were further evaluated under the concentrations of 40, 8, 1.6, 0.32, and 0.064 μM in triplicate, with paclitaxel as positive control (Sigma, St. Louis, MO, USA). The IC_{50} value of each compound was calculated by Liang's method.[8]

(7*S*)-Macaurea A (1): $C_{20}H_{23}N_3O_2$, obtained as a colourless powder; $[\alpha]_D^{25}$ −41.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.32), 256 (2.62) nm; IR (KBr) ν_{max} 3412, 1733, 1624, 1436 and 960 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 400 and 100 MHz), see Table 1. Positive ESIMS *m/z* 360 $[M + Na]^+$; Positive HRESIMS *m/z* 360.1676 $[M + Na]^+$ (calcd. for $C_{20}H_{23}NaN_3O_2$, 360.1682).

(7*S*)-Macaurea B (2): $C_{21}H_{25}N_3O_3$, obtained as a colourless gum; $[\alpha]_D^{25}$ −53.2 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.32), 258 (2.45) nm; IR (KBr) ν_{max} 3442, 1735, 1623, and 1439 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 400 and 100 MHz), see Table 1. Positive ESIMS *m/z* 390 $[M + Na]^+$; Positive HRESIMS *m/z* 390.1780 $[M + Na]^+$ (calcd. for $C_{21}H_{25}N_3NaO_3$, 390.1788).

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