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Article

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(+)-Meyeniins A–C, Novel Hexahydroimidazo[1,5-*c*]thiazole Derivatives from the Tubers of *Lepidium meyenii*: Complete Structural Elucidation by Biomimetic Synthesis and Racemic Crystallization

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ABSTRACT: (+)-Meyeniins A–C (**1**–**3**), a novel class of sulfur-containing hexahydroimidazo[1,5-*c*]thiazole derivatives, were isolated from the tubers of *Lepidium meyenii* cultivated in Lijiang, Yunnan Province, China. Guided by their biosynthetic hypothesis, a stereocontrolled biomimetic synthesis of meyeniins A–C and their individual enantiomers was efficiently accomplished by a combination of a condensation reaction and Edman degradation. The formation of high-quality crystals for X-ray crystallography occurred much more readily from a racemic mixture of (±)-meyeniin A than with the single enantiomer alone in this case. These extensive strategies, combined with circular dichroism (CD) spectra, allowed the complete structural assignments of (+)-meyeniins A–C. Among them, (+)-meyeniin A showed moderate selective cytotoxicities against the HL-60, A549 and MCF-7 human cell lines with IC_{50} values of 14.41, 32.22, and 33.14 μM, respectively. To some extent, these findings support traditional applications of maca as healthy nutritional supplements or functional foods for cancer prevention.

KEYWORDS: *(+)-meyeniins A–C, sulfur-containing hexahydroimidazo[1,5-c]thiazole, Lepidium meyenii, biomimetic synthesis, racemic crystallization, cytotoxicity*

INTRODUCTION

Lepidium meyenii Walp. (Brassicaceae), commonly known as 'maca', is a perennial herbaceous plant grown on Andean plateau in Peru under conditions of low temperature and high altitude (over 3500 m).[1] Maca tubers were consumed by the indigenous population as both a nutritional supplements and fertility medicine since pre-Columbian times.[1] Recently, pharmacological research on maca extracts provided evidence to support its diverse health-promoting properties, such as fertility-enhancing effect,[2] antioxidative activity,[3] immunomodulatory activity,[4] anticancer,[5,6] memory impairment,[7] regulation of hormonal secretion,[8] and effectiveness for decreasing serum lipid and blood sugar.[9] And, some of these activities have been closely linked to the lipidic constituents, especially the characteristic glucosinolates, macamides, and macaenes.[5,10-13] Due to these putative properties, maca often referred as "Peruvian ginseng" or "ginseng of the Andes", has gained worldwide attention as dietary supplements or functional foods with claims of anabolic effects since the 1990s.[1] In China, more than two hundred thousand tons of raw materials and a wide array of commercial products have been produced by the largest maca cultivation base located in northwest Yunnan province every year, which possesses a high diurnal temperature range and relatively low altitude (2800–3500 m) comparable to the Andean region.[14]

Considering that the bioactive principles of this important resource plant would plausibly be influenced by the ecological environment, we carefully examined the chemical components of the maca tubers collected from Lijiang, Yunnan. Interestingly, except for previously reported glucosinolates, macamides, and macaenes, a new class of

sulfur-containing hexahydroimidazo[1,5-*c*]thiazole derivatives, (+)-meyeniins A–C, **1**–**3** (Figure 1) were isolated as one of the major constituents from the lipidic fraction of the title plant. Their structures including absolute configurations were determined by extensive spectroscopic data, bioinspired synthesis combined with racemic crystallization and X-ray crystallographic analysis, as well as comparison of circular dichroism (CD) spectra. Herein, the complete structural assignments and biological properties of meyeniins A–C are described in detail.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter (Jasco, Tokyo, Japan). Circular dichroism spectra were measured on a Chirascan instrument (Applied Photophysics Limited, Leatherhead, England). Melting points were determined on a Yuhua melting point apparatus (Yuhua Instrument co., Gongyi, China). UV data were measured with a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). A BioRad FtS-135 spectrophotometer (Bio-Rad, Richmond, CA, USA) was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were realized on AV-400, DRX-500 NMR or Avance III 600 spectrometers (Bruker, Karlsruhe, Germany) with TMS as an internal standard. Mass spectra were recorded on a VG-Auto-Spec-3000 spectrometer (VG, Manchester, England). X-ray diffraction was realized on a Bruker SMART APEX II CCD crystallography system (Bruker, Karlsruhe, Germany). 5,5'-Dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent), *S*-butyrylthiocholine iodide, *S*-acetylthiocholine iodide, acetylcholinesterase derived from human erythrocytes, and

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). HL-60 (human promyelocytic leukemia cells), A549 (human lung adenocarcinoma epithelial cells), SMMC-7721 (human hepatocarcinoma cells), MCF7 (human breast adenocarcinoma cells), and SW480 (human colon cancer cells) tumor cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Column chromatography was performed with silica gel (100-200 mesh) (Qingdao Marine Chemical, Inc., Qingdao, China). Preparative HPLC was performed using a Shimadzu LC-8A preparative liquid chromatography (Shimadzu, Kyoto, Japan) with a Shimadzu PRC-ODS column. Semi-preparative HPLC was performed on an Agilent 1100 or 1200 semi-preparative liquid chromatography (Agilent Technologies, Foster City, CA, USA) with Zorbax SB-C18 (10 μm, 9.4 mm × 25 cm), Welch Ultimate XB-Phenyl (10 μm, 4.6 mm × 25 cm), or Welch Ultimate Cellu-D (5 μm, 4.6 mm × 25 cm) columns. Fractions were monitored by TLC (Qingdao Marine Chemical, Inc., Qingdao, China), and spots were detected by spraying with 8% H_2SO_4 in EtOH, followed by heating silica gel plates. All solvents were distilled prior to use.

Plant Materials. The tubers of *Lepidium meyenii* were collected from Lijiang, Yunnan Province, China, in September 2014, and identified by Prof. Ning Yuan. A voucher specimen (YNNI-14-09-25) has been deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission and Ministry of Education, Yunnan Minzu University.

Extraction and Isolation. The air-dried tubers of *L. meyenii* (25 kg) were powdered

and extracted with 80% aqueous acetone (3 × 60 L, 4 days each) at room temperature. After removal of the solvent by evaporation, the crude extract (5.8 kg) was suspended in H_2O, and then extracted successively with CH_2Cl_2 and EtOAc. The merged CH_2Cl_2 and EtOAc-soluble parts (280 g) were purified by CC (column chromatography on SiO_2 with CH_2Cl_2-Me_2CO gradient system 1:0, 9:1, 8:2, 7:3, 6:4 and 1:1) to yield six main fractions A-F. Fr. B (CH_2Cl_2/acetone 9:1, 35.0 g) eluted with PE/CH_2Cl_2 (10:1, 8:1 5:1, 2:1, and 0:1) and yielding subfractions B1-B5. Subfraction B4 (12.0 g, PE/ CH_2Cl_2 2:1) was fractionated on RP-18 with gradient elution with MeOH/H_2O (40:60 to 1:0) to yield fractions B41-B47, Subsequently fraction B45 (5.5 g) was further purified by preparative HPLC (20 mL/min, detector UV λ_{max} 202 and 254, MeOH/H_2O 75:15) and followed by semipreparative HPLC (3 mL/min, detector UV λ_{max} 202, 220, 254, and 280 nm, MeOH/H_2O 85:15) with an Ultimate XB-Phenyl (10 μm, 4.6 mm × 25 cm) column to yield **1** (155 mg, R_f = 12.5 min), **2** (189 mg, R_f = 17.2 min) and **3** (120 mg, R_f = 16.5 min), respectively.

(+)-Meyeniin A, **1**, $C_{14}H_{14}N_2O_3S_2$, obtained as colorless powder; mp 51 ^oC; $[\alpha]_D^{25.2}$ +235.8 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 276 (0.246), 241 (0.190), 203 (0.548) nm; CD (*c* 0.005, MeOH) $\Delta\varepsilon_{205}$ +13.0, $\Delta\varepsilon_{235}$ +11.8, $\Delta\varepsilon_{250}$ -4.81; IR (KBr) ν_{max} 3682, 3430, 3068, 2969, 2924, 2855, 2779, 2046, 1852, 1748, 1444, 1037 cm^{-1}; ^1H and ^{13}C NMR (400 and 100 MHz, in $CDCl_3$) (Table 1); ESIMS (positive ion mode) *m/z* 323 $[M + H]^+$; HRESIMS (positive ion mode) *m/z* 323.0520 $[M + H]^+$ (calcd 323.0519 for $C_{14}H_{15}N_2O_3S_2$).

(+)-Meyeniin B, **2**, $C_{13}H_{14}N_2OS_2$, obtained as colorless powder; mp 31 ^oC; $[\alpha]_D^{23.3}$

+258.8 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 274 (0.451), 246 (0.315), 204 (0.458) nm; CD (*c* 0.009, MeOH) $\Delta\varepsilon_{202}$ +19.8, $\Delta\varepsilon_{237}$ +21.4, $\Delta\varepsilon_{252}$ -10.1; IR (KBr) ν_{max} 3427, 3108, 3062, 2974, 2926, 2582, 1956, 1856, 1751, 1425, 938, 701 cm^{-1}; ^{1}H and ^{13}C NMR (400 and 100 MHz, in $CDCl_3$) (Table 2); ESIMS (positive ion mode) *m/z* 279 $[M + H]^+$; HRESIMS (positive ion mode) *m/z* 279.0627 $[M + H]^+$ (calcd 279.0620 for $C_{13}H_{14}N_2OS_2$).

(+)-Meyeniin C, **3**, $C_{14}H_{16}N_2O_2S_2$, obtained as colorless powder; mp 33 °C; $[\alpha]_D^{25.1}$ +175.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 201 (0.319), 216 (0.186), 245 (0.128) nm; CD (*c* 0.004, MeOH) $\Delta\varepsilon_{204}$ +11.3, $\Delta\varepsilon_{237}$ +9.60, $\Delta\varepsilon_{253}$ -4.60; IR (KBr) ν_{max} 3679, 3427, 3054, 2925, 2854, 1749, 1604, 1424, 1341, 1267, 1158, 1042 cm^{-1}; ^{1}H and ^{13}C NMR (400 and 100 MHz, in $CDCl_3$) (Table 2); ESIMS (positive ion mode) *m/z* 309 $[M + H]^+$; HRESIMS (positive ion mode) *m/z* 309.0729 $[M + H]^+$ (calcd 309.0726 for $C_{14}H_{16}N_2O_2S_2$).

Synthesis Procedures.

Synthesis of (±)-Meyeniin A: a suspension of L-cysteine, **5** (1.21 g, 10.0 mmol) and KOAc (1.18 g, 12.0 mmol) was dissolved in aqueous methanol (1:1, 40 mL), and after 10 min, acetaldehyde (0.66 g, 15.0 mmol) was added. The reaction was performed by stirring the mixture under nitrogen at room temperature for 3h, and the solution then was reduced under a vacuum. The white precipitate was isolated by filtration, then, washed with methanol and dried under high vacuum overnight to yield **6** (1.35 g, 9.2 mmol, 92%) (Figure 2). The diastereomeric mixture of (2*R*,4*R*)-**6** and (2*S*,4*R*)-**6** (147 mg, 1.0 mmol) and 3,4-(methylenedioxy)benzyl isothiocyanate (232 mg, 1.2 mmol)

were further dissolved in dry pyridine (10 mL) under ambient conditions. The reaction mixture was stirred at room temperature for 12 h. The resulting solution was acidified with 1 M HCl, exhaustively extracted with EtOAc (3 × 20 mL), dried over sodium sulfate, and concentrated in vacuo. The resulting mixture was directly subjected to preparative HPLC to afford a white solid (264 mg, 0.82 mmol, 82%) (Figure 2). Analysis HPLC with an Ultimate Cellu-D chiral column combined with optical rotation measurements showed that the synthetic meyeniin A was a 1:1 enantiomers mixture.

Synthesis of (+)-Meyeniin A: the diastereomeric mixture of (2*R*,4*R*)-**6** and (2*S*,4*R*)-**6** (147 mg, 1.0 mmol) and 3,4-(methylenedioxy)benzyl isothiocyanate (232 mg, 1.2 mmol) were dissolved in dry EtOH (10 mL) and placed in a sealed tube under ambient conditions. The tube was placed into a 70 ^{o}C oil bath for 2 h. The resulting solution was exhaustively reduced under a vacuum. Concentration in vacuo gave meyeniin A (274 mg, 0.85 mmol, 78% over two steps) (Figure 3) as a white solid, which was further analysed by HPLC with chiral column. The result showed that the synthetic meyeniin A under this condition is an optically pure compound with positive absorption in optical rotations. The similar synthesis procedures led to the synthesis of (-)-meyeniin A (82%), (+)-meyeniin B (80%), (-)-meyeniin B (80%), (+)-meyeniin C (70%), and (-)-meyeniin C (74%), respectively.

X-ray Crystal Structure Analysis. The intensity data for a racemic mixture of synthetic or purified (+)-meyeniin A and synthetic (-)-meyeniin A was measured on a Bruker diffractometer equipped with an APEX II CCD, using graphite-monochromated Mo Kα radiation (Figure 4). The crystal structure was solved by direct methods using

SHELXS97, expanded by difference Fourier techniques, and refined by full-matrix least-squares calculations and the program NOMCSDP. The non-hydrogen atoms were refined using anisotropic displacement parameters, and hydrogen atoms were placed in calculated positions. The CIF files of crystallographic data for the structures of (±)-meyeniins A have been deposited in the Cambridge Crystallographic Data Centre database (deposition number CCDC 1518102). Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K. (fax + 44 1223 336033; e-mail: deposit@cced.cam.ac.uk).

X-ray crystallographic analysis of (±)-meyeniins A: $C_{14}H_{14}N_2O_3S_2$, M = 322.39, a = 13.0549(13) Å, b = 6.8643(7) Å, c = 15.8088(15) Å, α = 90 °, β = 97.519(2) °, γ = 90 °, V = 1404.5(2) Å^3, T = 100(2) K, space group $P2/c$, Z = 4, μ(MoKα) = 0.390 mm^{-1}. The total number of reflections measured was 15102, of which 4170 were observed, R_1 = 0.0299 ($I > 2\sigma(I)$), $wR(F^2)$ = 0.0800 ($I > 2\sigma(I)$), R_1 = 0.0347 (all data), $wR(F^2)$ = 0.0835 (all data), F^2 = 1.085.

Acetylcholinesterase Inhibitory Activity. Acetylcholinesterase (AChE) inhibitory activity of the isolated (+)-meyeniins A–C was assayed by the spectrophotometric method in 96-well microplates developed by Ellman et al.[15] with slightly modification. Briefly, the reaction mixture (total 200 μL) contained test compound (50 μM), phosphate buffer (PB, pH 8.0), and acetyl cholinesterase solution (0.02 U/mL), was incubated at 37 ^{o}C for 20 min. Then, 40 μL of solution containing DTNB (0.625 mM) was added and the reaction was started by adding acetylthiocholine iodide (0.625 mM). The hydrolysis of acetylthiocholine was monitored by a 96-well plate reader at 405 nm

every 30 s for 1 h. Tacrine served as positive control (0.333 μM). All the reactions were measured three times. The percentage inhibition was calculated as follows: inhibition (%) = 1-S/E × 100 (S is the absorbance of the test compound and E is the absorbance of the blank).

Cytotoxicity Assay. The cytotoxicity of the isolated (+)-meyeniins A–C was tested using the MTT method, as reported previously.[16] All human tumor cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at 37 ^{o}C in 5% CO_2. Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Cells were seeded into 96-well plates 24 h before treatment and exposed to the test compound at different concentrations in triplicate for 72 h with DDP as positive control. The IC_{50} value of each compound was calculated by Reed and Muench's method.[17]

RESULTS AND DISCUSSION

Structure Elucidation. Compound **1** was obtained as colorless powder. Its molecular formula $C_{14}H_{14}N_2O_3S_2$ was established from HRESIMS and ^{13}C NMR spectra, possessing an index of hydrogen deficiency of nine. The IR spectrum showed absorption bands due to carbonyl group (1748 cm^{-1}). In the 1D NMR spectra (Table 1), a set of characteristic signals of a methylene group (δ_H 4.89 and 4.82, d, J = 14.4 Hz, H_2-7'; δ_C 45.0, t, C-7'), a methylenedioxy group (δ_H 5.92, s, H_2-1''; δ_C 101.1, t, C-1'') and a 1,3,4-trisubstituted benzene ring (δ_H 6.97, br s, H-2'; 6.73, d, J = 7.9 Hz, H-5'; 6.94, d, J = 7.9 Hz, H-6'; δ_C 129.2, s, C-1'; 109.5, d, C-2'; 147.4, s, C-3'; 147.7, s, C-4'; 108.2, d,

C-5'; 122.8, d, C-6') indicated the appearance of a 3,4-(methylenedioxy)benzyl substituent (ring C, partial molecular formula: $C_8H_7O_2$), which was further confirmed by the ^{1}H-^{1}H COSY cross peak of H-5'/H-6' (fragment **c**) and by the HMBC correlations observed between H_2-7' and C-1'/C-2'/C-6', between H-5' and C-1'/C-3'/C-4'/C-6', and between H_2-1" and C-3'/C-4' (Figure 5).

The remaining six carbon resonances belonged to the nucleus, comprising of one methyl at δ_C 23.8 (C-8), one methylene at δ_C 31.5 (C-1), two heteroatom-bearing methines at δ_C 60.4 and 65.7 (C-2 and C-7a), one amide carbonyl at δ_C 170.8 (C-7), and one amide thiocarbonyl at δ_C 183.7 (C-5), attributable to a multi-heteroatom-containing bicyclic ring system (rings A and B, partial molecular formula: $C_6H_7N_2OS_2$). Since nitrogen (3.04) is more electronegative than sulfur (2.58),[18] the two downfield methines at δ_C 60.4 and 65.7 might be connected by a nitrogen atom, respectively, while the upfield methylene at δ_C 31.5 should be linked with a sulfur atom. Analysis of the ^{1}H-^{1}H COSY spectrum belong to the nucleus of **1** exhibited two structural fragments [**a**: -CH_2(1)-CH(7a)-; **b**: -CH(3)-CH_3(8)] (Figure 5). Then, the HMBC spectrum was applied to assemble the two subunits with an amide carbonyl (C-7), an amide thiocarbonyl (C-5), two nitrogen atoms (N-4 and N-6), and a sulfur atom (S-2). Firstly, the HMBC correlations from protons of H_2-1 in fragment **a** to the methine at δ_C 60.4 (d, C-3) in fragment **b** allowed the connection between C-1 and C-3 by a sulfur atom (S-2). Meanwhile, the correlations from H-3 to the methine at δ_C 65.7 (d, C-7a) indicated that C-7a was linked with C-3 through a nitrogen atom (N-4). These data revealed that C-1, S-2, C-3, N-4, C-7a, and C-8 constructed a 2-methylthiazolidine moiety (ring A).

Secondly, the HMBC correlations from H-3 and H-7a to the amide thiocarbonyl C-5, and from H_2-1' and H-7a to the amide carbonyl C-7, led to the N-4 and C-5 linkage and the C-7 and C-7a linkage, respectively. This structural assignment also revealed that N-4, C-5, C-7, C-7a, together with the remaining nitrogen atom (N-6) formed a 2-thioxoimidazolidin-4-one moiety (ring B), which is coupled to 2-methylthiazolidine moiety (ring A) at N-4 and C-7a. Finally, the direct linkage between the hexahydroimidazo[1,5-*c*]thiazole nucleus (rings A and B) and 3,4-methylenedioxy-benzyl substituent through the nitrogen atom N-6 could be readily established by the key HMBC correlations observed between H_2-7' and C-5/C-7 (Figure 5). The above evidence led to the planar structural assignment of **1** as depicted in Figure 1. Unfortunately, no reliable NOESY correlation could be observed to determine the relative or absolute stereochemistry at C-3 and C-7a.

The ^{1}H and ^{13}C NMR data of **2** (Table 2) were similar to those of **1**, with the exception that the 3,4-(methylenedioxy)benzyl substituent in **1** was replaced by a benzyl group in **2**. This inference was further proved by the HMBC correlations observed between H_2-7' and C-1'/C-2'/C-6', between H-2' or H-6' and C-1'/C-3'/C-4'/C-5', and between H-3' or H-5' and C-1'/C-2'/C-4'/C-6' in **2**. Similarly, a direct comparison of the NMR data of **1** and **3** (Tables 1 and 2) suggested that 3,4-(methylenedioxy)benzyl moiety in **1** were replaced by 3-methoxybenzyl moiety in **4**. Such assignment was further supported by the HMBC correlations observed from H_2-7' to C-1', C-2', and C-6', and from H-2' to C-1', C-3', C-4', C-6' and C-7', and from H_3-1" to C-3'.

The relative configuration at C-3 and C-7a in **2** and **3** was determined to be identical to that of **1** based on the same ^{1}H and ^{13}C chemical shifts of the hexahydroimidazo[1,5-*c*]thiazole nucleus. However, their relative stereochemistry could not be assigned by spectroscopic data alone, and all the efforts to obtain single crystals for X-ray crystallography analysis also failed. Finally, the absolute configuration of natural compounds **1**−**3** was determined as (3*S*,7a*R*) by biosynthetic prediction, biomimetic synthesis combined with racemic crystallization and X-ray cry-stallographic analysis (Figure 4), as well as comparison of circular dichroism (CD) spectra. Thus, compounds **1**−**3** were elucidated as shown (Figure 1) and they were given trivial names of (+)-meyeniins A−C, respectively.

Hypothetical Biogenetic Pathway of (+)-meyeniins A−C. Though several synthetic counterparts have been reported previously,[19 and 20] meyeniins A−C represent the first examples of naturally occurring hexahydroimidazo[1,5-*c*]thiazole derivatives. This is an interesting case that the basic skeleton of the synthetic analogues were discovered from natural origins. Our postulated biosynthetic pathway of (+)-meyeniins A−C involves the construction of the 2-methylthiazolidine ring by condensation reaction between acetaldehyde, **4**, and L-cysteine, **5**, followed by the formation of the 2-thioxoimidazolidin-4-one moiety *via* the Edman degradation reaction between a diastereomeric mixture of 2-methylthiazolidine-4-carboxylic acid, **6**, and related isothiocyanate, **7** (Figure 6).[19 and 20] Especially, in the second key step, the intermediate **E** must undergo a ring opening and subsequent epimerization at C-2, followed by ring re-closure. This process may lead exclusively to the formation of (+)-meyeniins A−C

possessing the less strained ring system with *trans*-configuration between C-3 and C-7a.[21]

Biomimetic Synthesis and Racemic Crystallization for Complete Structural Elucidation of (+)-meyeniins A−C. Guided by this biosynthetic consideration, a two-step stereocontrolled synthesis of (+)-meyeniins A–C and their individual enantiomers was efficiently accomplished. Briefly, treatment of L-cysteine (**5**) and acetaldehyde (**4**) with KOAc in carboxylic acid, **6**, as a diastereomeric mixture (Figure 2).[19-21] Surprisingly, the diastereoisomers further condensed with 3,4-(methylenedioxy)benzyl isothiocyanate in pyridine to form a racemic mixture of (±)-meyeniin A in 75% overall yield,[21] which was determined by HPLC analysis with a chiral column and optical rotation measurement (Figure 3). We propose a mechanism involving a racemization at C-4 catalyzed by pyridine and an epimerization at C-2 by Edman degradation reaction as stated previously (Figure 6).

With this in mind, the same reaction materials were dissolved in anhydrous ethanol and placed in a sealed tube under ambient conditions, which led to optically pure (+)-meyeniin A with a large positive optical rotation. Similarly, (+)-meyeniin B, (+)-meyeniin C and (-)-meyeniins A−C were easily prepared under the same reaction conditions (Figure 6). The NMR, optical rotation, and circular dichroism data of the synthetic (+)-meyeniins A−C were identical in all respects to those of the natural counterparts, indicating that the cysteine residues in the isolated structures are the L-(+)-form. Although the synthetic meyeniins A−C were readily available, we still failed to obtain their single crystals for confirming their absolute configuration at C-3

even after extensive trials.

Research has suggested that the racemic crystallization of a 1:1 enantiomeric mixture from solution occurred much more readily than the formation of homochiral crystals from a solution of the single enantiomer in some cases (an approximate 9:1 ratio).[22 and 23] This propensity might be attributed to the new possibilities for favorable packing arrangements available in racemic space groups.[21] Accordingly, diffraction quality crystals appeared rapidly from a solution of acetone/methanol (1:4) containing a racemic mixture of synthetic or purified (+)-**1** and synthetic (-)-**1** in only ten minutes. X-ray crystallographic analysis was realized, which clarified not only the planar structure but also the absolute configuration to be 3*S*,7a*R* (Figure 7). In the CD spectra of compounds **1**−**3**, the Cotton effects were all negative at 205 and 235 nm and positive at 250 nm, suggesting the same absolute configuration for these three compounds.

Acetylcholinesterase Inhibitory Activity and Cytotoxicity Assay. Considering the medicinal applications of the plant species,[5-7] compounds **1**–**3** were tested for acetylcholine esterase inhibitory activity using the Ellman method.[15] No significant activity of these compounds was detected at concentrations up to 50 μM. Compounds **1**–**3** were also evaluated for cytotoxic activity against a panel of human cancer cell lines using the MTT method with DDP as positive control.[16] (+)-Meyeniin A showed selective cytotoxicities against the HL-60, A549 and MCF-7 human cell lines with IC_{50} values of 14.41, 32.22, and 33.14 μM, respectively. To some extent, these findings support maca's traditional applications as healthy nutritional supplements or functional foods for cancer prevention.

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SUPPORTING INFORMATION

1D and 2D NMR, and CD data of natural (+)-meyeniins A–C, 1D NMR and CD data of synthetic (±)-meyeniins A–C, and X-ray structure of synthetic racemic meyeniin A are supplied as supporting information, which is available free of charge via the Internet at http: //pubs.acs.org.

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FIGURE CAPTIONS

Figure 1. The structures of isolated (+)-meyeniins A–C (**1**–**3**).

Figure 2. Synthesis of a racemic mixture of meyeniin A in pyridine.

Figure 3. Synthesis of (+)/(-)-meyeniins A−C in ethanol.

Figure 4. Key ^{1}H-^{1}H COSY and selected HMBC correlations of compound **1**.

Figure 5. ORTEP diagram of compound **1**.

Figure 6. Hypothetical biogenetic pathway of (+)-**1**–**3**.

404 Table 1. NMR Spectroscopic Data of Natural and Synthetic (+)-**1** in $CDCl_3$ (δ ppm)

no.	**1** (natural)			**1** (synthetic)	
	δ_C	δ_H	HMBC	δ_C	δ_H
1β	31.5 t	2.91, t (11.0)	3, 7a, 7	31.6 t	2.91, t (11.0)
1α		3.27, dd (11.0, 6.8)			3.27, dd (11.0, 6.8)
3α	60.4 d	5.63, q (6.3)	1, 7a, 5	60.5 d	5.63, q (6.3)
5	183.7 s	-	-	183.8 s	-
7	170.8 s	-	-	170.9 s	-
7aβ	65.7 d	4.56, dd (11.0, 6.8)	1, 5, 7	65.8 d	4.56, dd (11.0, 6.8)
8β	23.8 q	1.63, d (6.3)	3	23.9 q	1.63, d (6.3)
1'	129.2 s	-	-	129.2 s	-
2'	109.5 d	6.97, br s	1', 3', 4', 6', 7'	109.6 d	6.97, br s
3'	147.4 s	-	-	147.5 s	-
4'	147.7 s	-	-	147.8 s	-
5'	108.2 d	6.73, d (7.9)	1', 3', 4', 6'	108.4 d	6.73, d (7.9)
6'	122.8 d	6.94, d (7.9)	1', 2', 4', 5', 6', 7'	122.9 d	6.94, d (7.9)
7'	45.0 t	4.89, d (14.4)	5, 7, 1', 2', 6'	45.1 t	4.89, d (14.4)
		4.82, d (14.4)			4.82, d (14.4)
1"	101.1 t	5.92, s	3', 4'	101.2 t	5.92, s

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406 Table 2. NMR Spectroscopic Data of Compounds **2** and **3** in $CDCl_3$ (δ ppm)

no.	**2** (natural)			**3** (natural)		
	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
1β	31.7 t	2.92, dd (11.0, 9.9)	3, 7a, 7	31.7 t	2.93, dd (11.0, 9.9)	3, 7a, 7
1α		3.28, dd (11.0, 6.8)			3.28, dd (11.0, 6.8)	
3α	60.5 d	5.65, q (6.3)	1, 7a, 5	60.5 d	5.65, q (6.3)	1, 7a, 5
5	183.9 s	-	-	183.9 s	-	-
7	170.9 s	-	-	170.9 s	-	-
7aβ	65.8 d	4.58, dd (9.9, 6.8)	1, 5, 7	65.7 d	4.58, dd (9.9, 6.8)	1, 5, 7
8β	23.9 q	1.64, d (6.3)	3	23.9 q	1.64, d (6.3)	3
1'	135.5 s	-	-	136.9 s	-	-
2'	128.9 d	7.24, m	1', 3', 4', 7'	114.5 d	7.00, d (1.2)	1', 3', 4', 7'
3'	128.7 d	7.11, m	1', 2', 4', 6'	159.7 s	-	-
4'	128.2 d	7.11, m	2', 3', 5', 6'	113.6 d	6.82, dd (8.0, 1.2)	2', 3', 5', 6'
5'	128.7 d	7.11, m	1', 2', 4', 6'	129.7 d	7.23, t (8.0)	1', 2', 4', 6'
6'	128.9 d	7.24, m	1', 3', 4', 7'	121.1 d	7.01, d (8.0)	1', 3', 4', 7'
7'	45.3 t	5.01, d (14.5)	5, 7, 1', 2', 6'	45.2 t	4.97, d (14.5)	5, 7, 1', 2', 6'
		4.91, d (14.5)			4.88, d (14.5)	
1"	-	-	-	55.3 q	3.79, s	3'

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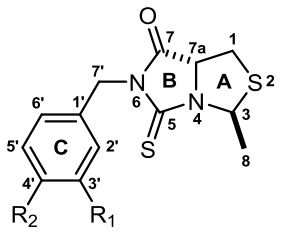


(+)-meyeniin A (**1**) $R_1 + R_2 = OCH_2O$
(+)-meyeniin B (**2**) $R_1 = H$ $R_2 = H$
(+)-meyeniin C (**3**) $R_1 = OMe$ $R_2 = H$

Figure 1. The structures of isolated (+)-meyeniins A–C (**1**–**3**).

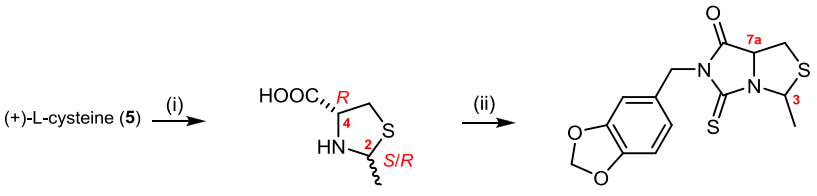


Figure 2. Synthesis of a racemic mixture of meyeniin A in pyridine[a].

[a]Reagents and conditions: (i) L-cysteine (**5**) (1 equiv), acetaldehyde (**4**) (1.5 equiv), KOAc (1.2 equiv), rt, H_2O:MeOH = 1:1, 3 h; (ii) 2-methyl-thiazolidine-4-carboxylic acid (**6**) (1 equiv), 3,4-(methylenedioxy)benzyl isothiocyanate (1.2 equiv), rt, pyridine, 12 h, 75% for (+)-**1**:(-)-**1** = 1:1 over two steps.

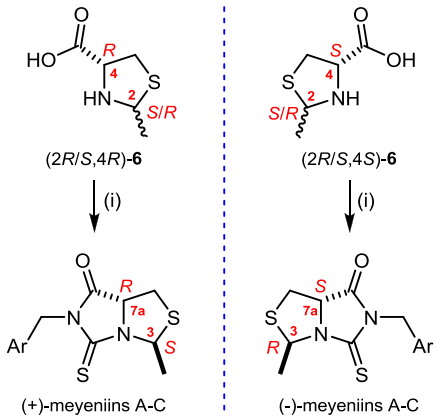
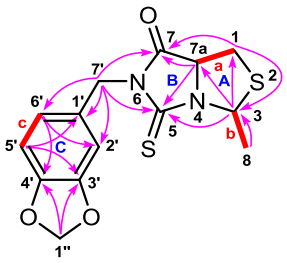


Figure 3. Synthesis of (+)/(-)-meyeniins A−C in ethanol[a].

[a]Reagents and conditions: (i) 2-methylthiazolidine-4-carboxylic acid (**6**) **(**1 equiv**)**, related isothiocyanates **(**1.2 equiv**)**, rt, ethanol, 2 h, 78% for (+)-**1**, 82% for (-)-**1**, 80% for (+)-**2**, 80% for (-)-**2**, 70% for (+)-**3**, and 74% for (-)-**3** over two steps.



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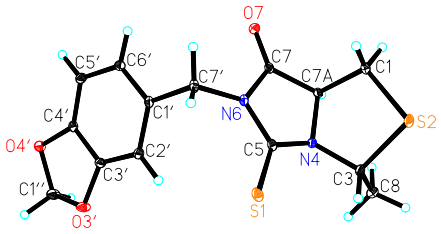
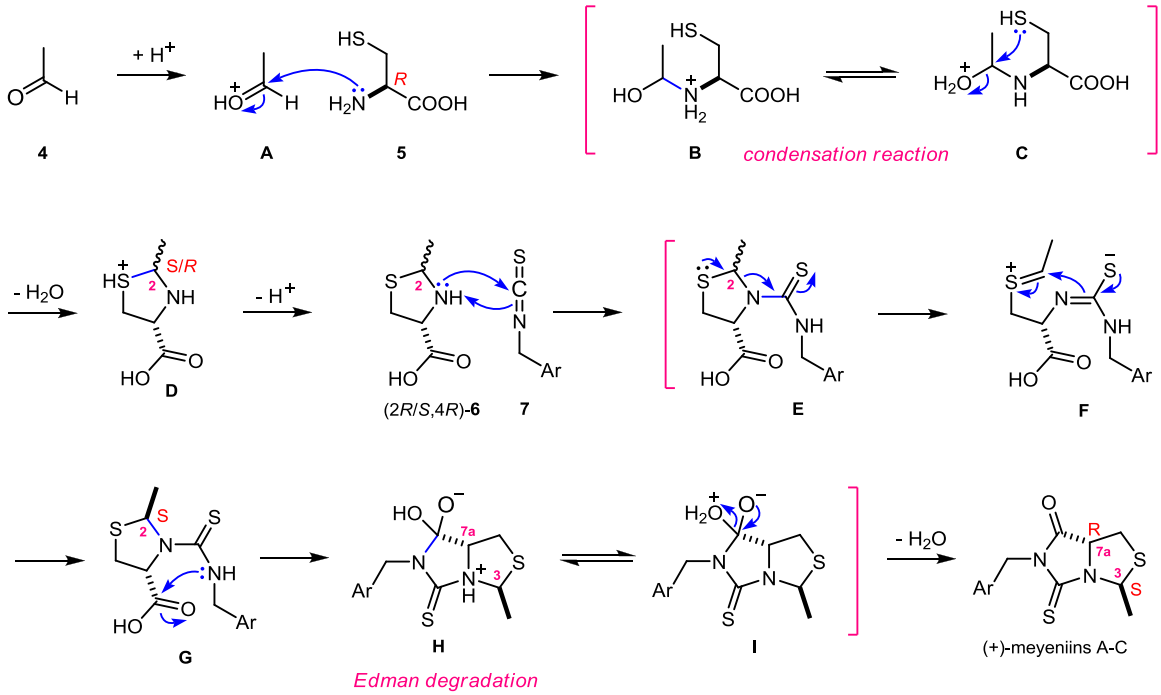
424 Figure 4. Key ^{1}H-^{1}H COSY and selected HMBC correlations of compound **1**.

425 

426 Figure 5. ORTEP diagram of compound **1**.



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428 Figure 6. Hypothetical biogenetic pathway of (+)-**1**–**3**.

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