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Original article

Codonopiloneolignanin A, a polycyclic neolignan with a new carbon skeleton from the roots of *Codonopsis pilosula*

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ABSTRACT

A neolignan codonopiloneolignanin A (**1**) with a novel 2,9:2′,9:7,7′-tricyclo-8,9′-neolignane skeleton was isolated from an aqueous extract of the *Codonopsis pilosula* roots. Its structure including the absolute configuration was elucidated by extensive spectroscopic analysis, including 2D NMR and electronic circular dichroism calculation. The proposed biosynthetic pathway of compound **1** is also discussed.

1. Introduction

The roots of *Codonopsis pilosula* (Franch.) Nannf. (Campanulaceae), named “dang shen” in Chinese, are among the most common drugs in Chinese traditional medicine, used for the treatment of body weakness, poor appetite, thirsty, indigestion, chronic diarrhea, archoptoma, chronic anemia, and leukemia, and usually used as a substitute of the Panax ginseng exhibiting similar therapeutic effects [1]. Pharmacological activities of the root extracts were reported in protecting against peptic ulceration and promoting its healing, enhancing immunity, and enhancing learning and memory behavior, as well as inhibiting inducible NO synthase and protein oxidation and attenuating the cardiac-impaired insulin-like growth factor II receptor pathway [2–6]. Meanwhile, different types of chemical principles were isolated from the extracts, including steroids, terpenoids, polyacetylenes, alkaloids, phenylpropanoids, lignanoids, and polysaccharides [7–12]. However, a literature survey shows that the previous investigations were mainly carried out on the ethanol or methanol extracts of the *C. pilosula* roots, which is inconsistent with a practical usage of decocting the drug and formulations. Therefore, an aqueous decoction of the *C. pilosula* roots was investigated, as part of a program to systematically study the chemical diversity of traditional Chinese medicines and their biological effects [13–23]. We previously described the characterization of eleven new C_{14}-polyacetylenes and a new unsaturated ω-hydroxy fatty acid from the decoction, as well as preliminary bioassays of those isolates [24,25]. A continuation of the investigation has resulted in the isolation and structural elucidation of a minor polycyclic neolignan with a novel carbon skeleton (**1**) (Fig. 1), and reported herein is the details.

2. Experimental

2.1. General experimental procedures

Optical rotation was measured on a P-2000 polarimeter. UV spectrum was recorded on a V-650 spectrometer. CD spectrum was measured on a JASCO J-815 CD spectrometer. IR spectrum was recorded on a Nicolet 5700 FT-IR Microscope spectrometer (FT-IR Microscope Transmission). 1D- and 2D-NMR spectra were obtained at 600 MHz for ^{1}H and 150 MHz for ^{13}C, respectively, on a Bruker 600 MHz spectrometer in acetone-d_6, with solvent peaks as references. ESIMS and HR-ESIMS data were obtained on an AccuToFCS JMS-T100CS spectrometer. Column chromatography (CC) was performed with HPD-110 (Cangzhou Bon Absorber Technology Co. Ltd, Cangzhou, China), MCI gel CHP 20P (Mitsubishi Chemical Inc., Tokyo, Japan), silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), or RP silica gel (Grace Davison Discovery Science, Deerfield, USA). HPLC separation was performed

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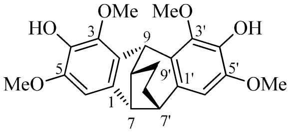

1

Fig. 1. Structure of compound **1**.

Table 1
NMR spectroscopic data for compound **1**[a].

Position	δ_H	δ_C	Position	δ_H	δ_C
1		132.6	1′		130.1
2		136.3	2′		126.2
3		141.7	3′		144.0
4		137.6	4′		137.1
5		146.8	5′		147.0
6	6.68 s	104.6	6′	6.27 s	107.2
7	3.32 dd (6.0, 4.2)	53.1	7′	2.93 dd (4.2, 3.6)	44.7
8	3.09 m	52.7	8′a	2.15 m	40.4
			8′b	1.81 m	
9	4.27 d (5.4)	43.4	9′a	1.78 m	22.3
			9′b	1.51 m	
O*Me*-3	3.84 s	59.5	O*Me*-3′	3.90 s	59.8
O*H*-4	6.91 s		O*H*-4′	7.02 s	
O*Me*-5	3.74 s	55.9	O*Me*-5′	3.70 s	55.5

[a] NMR data (δ) were measured in acetone-d_6 at 600 MHz for ^1H and at 150 MHz for ^{13}C, respectively. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on DEPT, ^1H–^1H COSY, HSQC, and HMBC experiments.

on an instrument consisting of an Agilent ChemStation for LC system, an Agilent1200 pump, and an Agilent 1100 single-wavelength absorbance detector with a Welch ultimate XB-Phenyl (250 × 10 mm i.d.) semi-preparative column packed with phenyl-silica gels (5 μm) (Welch Materials inc. Shanghai, China) and a Chiralpak AD-H column (250 × 10 mm i.d.) packed with amylose tris(3,5-dimethylphenylphenylcarbamate) coated on silica gel (5 μm) (Daicel Corporation, Osaka, Japan). TLC was conducted on precoated silica gel GF_{254} plates. Spots were visualized under UV light (254 or 356 nm) or by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

2.2. Plant material

The roots of *C. pilosula* (Franch.) Nannf. were collected in October 2012 from the culture field in Weiyuan, Gansu Province, China. Plant identity was verified by Mr. Lin Ma (Institute of Materia Medica, Beijing 100050, China). A voucher specimen (No. ID-S-2503) was deposited at the herbarium of the Department of Natural Medicinal Chemistry, Institute of Materia Medica, Beijing 100050, China.

2.3. Extraction and isolation

The dried and powered roots of *C. pilosula* (50 kg) were decocted with H_2O (150 L, 3 × 0.5 h). The decoction was evaporated under reduced pressure to yield a brown residue (26 kg). The residue was dissolved in H_2O (100 L), loaded on a macroporous adsorbent resin (HPD-110, 20 L) column (20 × 200 cm), and eluted successively with H_2O (100 L), 50% EtOH (120 L), and 95% EtOH (80 L) to yield three corresponding fractions A–C. Fraction B (270 g) was chromatographed over MCI gel CHP 20P (5.5 L), successively eluting with H_2O (20 L), 30% EtOH (30 L), 50% EtOH (30 L), and 95% EtOH (8 L), to give B1–B4. Fraction B3 (22 g) was subjected to flash chromatography over RP silica gel, eluting with a gradient of increasing MeOH (40%–100%) in H_2O, to yield subfractions B3-1–B3-5. Fraction B3-4 (2.5 g) was separated by CC over silica gel, eluting with a gradient of increasing MeOH concentration (0–100%) in CH_3Cl, to give subfractions B3-4-1–B3-4-10. Fraction B3-4-1 (100 mg), was purified by RP HPLC (XB-Phenyl column, 31% MeOH in H_2O, flow rate 1.5 mL/min) to yield **1** (0.8 mg, 0.0000016%, t_R = 47 min).

Codonopiloneolignanin A (**1**): Yellowish amorphous powder; $[\alpha]^{20}_D + 19.3$ (*c* 0.06, MeOH); UV(MeOH) λ_{max} (log ε) 203 (4.72), 288 (3.57) nm; CD (MeOH) 211 ($\Delta\varepsilon$ + 7.07), 239 ($\Delta\varepsilon$ − 0.70) nm; IR ν_{max} 3444, 2926, 2854, 1677, 1612, 1491, 1466, 1431, 1343, 1300, 1219, 1188, 1110, 1011, 908, 843, 799, 721, 697 cm^{-1}; ^1H NMR (acetone-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 1; (+)-ESIMS *m/z* 385 $[M + H]^+$, 407 $[M + Na]^+$; (+)HR-ESIMS *m/z* 385.1647 $[M + H]^+$ (calcd. for $C_{22}H_{25}O_6$, 385.1646), 407.1470 $[M + Na]^+$ (calcd. for $C_{22}H_{24}O_6Na$, 407.1465).

2.4. ECD Calculation of 1

Conformational analysis of **1** was performed by using the MMFF94 molecular mechanics force field *via* the MOE software package [26]. Only one lowest energy conformer of **1** with relative energy within 2 kcal/mol was obtained (Fig. S1 in Supporting information), which was further optimized at the B3LYP/6-31 g (d, p) level in the gas phase. The energies, oscillator strengths, and rotational strengths of the first 60 electronic excitations were calculated using the TDDFT methodology at the B3LYP/6-311G (2d, 2p) level for ECD and at the B3LYP/6-311G/aug-*cc*-pVDZ level for optical rotation calculation. ECD spectrum of the conformer was simulated using a Gaussian function with a half-bandwidth of 0.33 eV. The corresponding theoretical ECD spectrum of the enantiomeric **1** was depicted by inverting that of **1** (Supporting information). All quantum computations were performed using Gaussian 09 program package [27], on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College. In the 200–400 nm region, the theoretically calculated ECD spectrum of **1** was in good agreement with the experimental ECD (Fig. 4). This supported the assignment of the absolute configuration for **1**.

3. Results and discussion

Compound **1** was obtained as a white amorphous powder. The IR spectrum of **1** showed absorption bands for hydroxyl (3444 cm^{-1}) and aromatic ring (1612 and 1491 cm^{-1}) functionalities. The positive mode ESIMS of **1** exhibited quasimolecular ion peaks at *m/z* 385 $[M + H]^+$ and 407 $[M + Na]^+$. The molecular formula $C_{22}H_{24}O_6$, with 11 degrees of unsaturation, was determined by HR-ESIMS at *m/z* 385.1647 $[M + H]^+$ (calcd for $C_{22}H_{25}O_6$, 385.1646), combined with the NMR data (Table 1). The ^1H NMR spectrum of **1** in acetone-d_6 showed signals attributable to two penta-substituted benzene rings at δ_H 6.68 (s, H-6) and 6.27 (s, H-6′); four aromatic methoxy groups at δ_H 3.90 (s, MeO-3′), 3.84 (s, MeO-3), 3.74 (s, MeO-5), and 3.70 (s, MeO-5′); and two exchangeable phenolic hydroxyl groups at δ_H 7.02 (s, OH-4′) and 6.91 (s, OH-4), in addition to signals due to eight aliphatic methine and/or methylene protons (Table 1). Besides resonances corresponding to the two penta-substituted benzene rings and four aromatic methoxy groups, the ^{13}C NMR and DEPT spectra displayed six aliphatic carbon resonances including four methines and two methylnenes (Table 1). The presence of two penta-substituted benzene rings and six aliphatic methylene and methine units suggests that **1** is a lignanoid containing two C_6C_3 skeletal units with the phenolic hydroxyl and aromatic methoxy substituents. As compared with those of the lignanoids previously reported from the genus *Codonopsis* [9,28–30], to match requirement of the 11 degrees of unsaturation, the above spectroscopic