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Phenylpropanoids from the Roots of *Codonopsis cordifolioidea* and Their Biological Activities

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The genus *Codonopsis* (Campanulaceae) is represented in China by 39 species. Some of *Codonopsis* species such as *C. pilosula* and *C. tangshen* are commonly used as herbal remedies due to their tonic effects.[1] In addition, the roots of some *Codonopsis* species including *C. cordifolioidea*, *C. bulleyana*, *C. micrantha*, and *C. subglobosa* are well-known vegetables in southwest China.[2,3] *C. cordifolioidea* Tsoong is a herbaceous plant spread in Yunnan, Tibet, and Sichuan Provinces. Its roots, locally known as Choushen, have been used as a food in Yunnan Province since ancient times. Meanwhile, this species has become an important economic plant widely cultivated in several areas of Yunnan Province.[4,5] The previous phytochemical researches on *C. cordifolioidea* has revealed that phenylpropanoids, lignans, as well as flavonoids are major components isolated from this plant.[5,6]

Motivated by search for bioactive metabolites from this plant, the phytochemical investigation on *C. cordifolioidea* was carried out. As a result, two new phenylpropanoids, together with nine known phenylpropanoids, were isolated from this plant. In addition, the *Anti*-HIV-1 activities and cytotoxicities of compounds **1-2** were evaluated. This article deals with the isolation, structural elucidation and biological activities of the new compounds.

Results and Discussion

A 70% aq. methanol extract prepared from the roots of *C. cordifolioidea* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1-11**, including two new phenylpropanoids, named cordifoliketones A-B (**1-2**), together with nine known phenylpropanoids, sinapinaldehyde (**3**),[7] coniferaldehyde (**4**),[7] coniferoside (**5**),[8] isoconiferin (**6**),[9] nervolan B (**7**),[10] nervolan C (**8**),[10] dillapiole (**9**),[11] 1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene (**10**),[12] 4-allyl-2-(3-methylbut-2-en-1-yl)phenol (**11**).[13] The structures of the compounds **1-11** were as shown in Figure 1.

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $C_{11}H_{14}O_5$ by HR-ESI-MS *m/z* 249.0746 $[M+Na]^+$ (calcd 249.0739). Its ^1H and ^{13}C NMR spectra (Table 1) showed signals to 14 hydrogens and 11 carbons, respectively, corresponding to one aromatic ring (δ_C 102.0, 111.0, 111.6, 142.8, 155.1, 158.5) with two aromatic protons (δ_H 6.71 s, 7.49 s), one methylene group (δ_C 42.7), one oxidated methylene group (δ_C 58.8), one carbonyl group (δ_C 198.2), two methoxyl groups (δ_C 55.8, 56.0), a phenolic hydroxyl group (δ_H 10.27), and a hydroxyl group (δ_H 4.95). Strong absorption bands accounting for hydroxyl (3376 cm^{-1}), carbonyl (1713 cm^{-1}) and aromatic group (1638, 1512, 1450 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 288 nm confirmed the existence of the aromatic function. The ^1H-^1H COSY of H-8/H-9; together with HMBC correlations (Fig. 2) of H-6 (δ_H 7.49) with C-7 (δ_C 198.2), of H-8 (δ_H 3.24) with C-1 (δ_C 111.0), of H-9 (δ_H 4.35) with C-7 (δ_C 198.2) and C-8 (δ_C 42.7) suggested that **1** is a 3-hydroxyl-1-phenyl-1-propanone (Ar-CO-CH_2-CH_2OH), and possess two methoxyl groups and a phenolic hydroxyl group on the aromatic ring. The HMBC correlations of aromatic hydroxyl proton signal (δ_H 10.27) with C-1 (δ_C 110.1), C-2 (δ_C 158.5) and C-3 (δ_C 102.0) indicated that the hydroxyl group should be located at C-2. The HMBC correlations of two methoxyl proton signals (δ_H 3.75, 3.79) with C-4 (δ_C 155.1), C-5 (δ_C 142.8) indicated two methoxyl groups should be located at C-4, and C-5, respectively. Thus, the structure of **1** was established and given the name as cordifoliketone A.

Compounds **2** was obtained as pale yellow gum, and

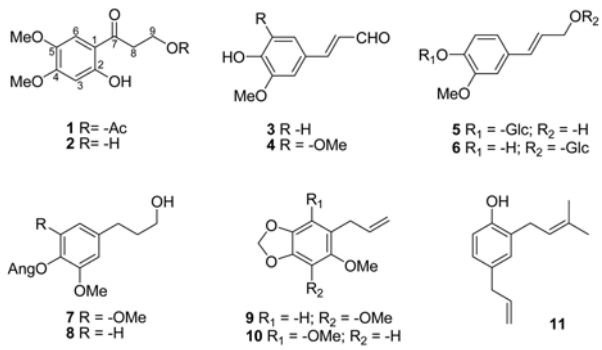

Figure 1. The structure of compounds **1-12**.

Table 1. ^{1}H NMR and ^{13}C NMR Data (in C_5D_5N) of compounds **1**-**2**

No.	Compound **1**		Compound **2**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	111.0 s		111.5 s	
2	158.5 s		158.8 s	
3	102.0 d	6.71 s	102.2 d	6.70 s
4	155.1 s		155.3 s	
5	142.8 s		142.8 s	
6	111.6 d	7.49 s	112.1 d	7.48 s
7	198.2 s		198.2 s	
8	42.7 t	3.24 t, *J* = 6.1	40.3 t	3.38 t, *J* = 6.1
9	58.8 t	4.35 t, *J* = 6.2	60.9 t	4.61 t, *J* = 6.2
OMe-4	55.8 q	3.79 s	55.7 q	3.78 s
OMe-5	56.0 q	3.75 s	56.0 q	3.74 s
Ar-OH		10.27 brs		10.25 brs
9-OH		4.95 brs		
1'-$COCH_3$			169.2 s	
2'-$COCH_3$			20.3 q	1.99 s

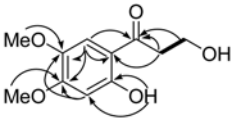

Figure 2. Selected HMBC (→) and ^{1}H-^{1}H COSY (–) correlations of compound **1**.

should sodiated molecular ions at *m/z* 291.0838 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 291.0845), corresponding to the molecular formula of $C_{13}H_{16}O_6$. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The only difference was a hydroxyl group in **1** was substituted by an acetoxyl group in **2**, which was supported by the disappearance of hydroxyl proton singal (δ_H 4.95 brs) and appearance of set of acetoxy group singal (δ_C 169.2 s, 20.3 q; δ_H 1.99) in **2**. The HMBC correlation of H-9 (δ_H 4.61) with C-2' (δ_C 169.2) indicated that the acetyl group is attached to C-9 position. Thus, the structure of **2** was established, and it has been accorded the trivial name of cordifoliketone B.

For *anti*-HIV-1 activity assay, the cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method and *anti*-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using AZT as a positive control (EC_{50} = 0.0045 μg/mL and CC_{50} > 200 μg/mL).[14] Compound **1** shows *anti*-HIV-1 activity with EC_{50} of 2.26 μg/mL, CC_{50} of 78.7 μg/mL, and TI (Therapeutic Index) of 34.8 with SD (n = 3) of 5.22. Compound **2** shows *anti*-HIV-1 activity with EC_{50} of 2.36 μg/mL, CC_{50} of 149.6 μg/mL, and TI of 83.6 with SD (n = 3) of 4.92. The results showed that compounds **1** and **2** have modest *anti*-HIV-1 bioctivities.

The cytotoxicity tests for the isolates were performed using a previously reported procedure.[15] All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic abilities against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with doxorubicin as the positive control) were shown in Table 2. The results showed that compounds **1**-2 have significant potential cytotoxic abilities.

Table 2. Cytotoxicities of compounds **1**-**3**

Compounds	Cell lines			
	HL-60	HepG2	KB	MDA-MB-231
1	2.26 (SD = 0.42)	4.27 (SD = 0.42)	1.28 (SD = 0.63)	2.69 (SD = 0.55)
3	1.47 (SD = 0.25)	2.85 (SD = 0.38)	6.42 (SD = 0.64)	5.21 (SD = 0.60)
doxorubicin	0.1	0.2	0.1	0.1

Data of IC_{50} values in μmol/L. For a compound to be deemed effective, an IC_{50} value < 100 μmol/L is required. doxorubicin was used as a positive control. HL-60, human acute promyelocytic leukemia; Hep-G2, human hepatocellular carcinoma; KB, human oropharyngeal epidermoid carcinoma; MDA-MB-231, human breast cancer cells. SD (n = 3).

Experimental Section

General Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7.0 mm) column or a Venusil MP C18 (20 mm × 25 cm, 5.0 mm) column. Column chromatography was performed with Si gel (200-300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The roots of *C. cordifolioidea* were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in September 2009. The identification of the plant material was verified by Prof. Chen Y. J (Yunnan Nationalities University). A voucher specimen (YNNI 09-9-13) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered roots of *C. cordifolioidea* (1.5 kg) were extracted four times with 70% methanol (4 × 2.0 L) at room temperature and filtered. The crude extract (102 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a chloroform-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. The further separation of fraction D (7:3, 19.6 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures D1-D5. Fraction D1 (9:1, 3.22 g) was

subjected to preparative HPLC (48% methanol, flow rate 12 mL/min) to give **9** (31.5 mg) with retention time 34.2 min, **10** (24.4 mg) with retention time 32.5 min, **11** (26.8 mg) with retention time 26.5 min. Fraction D2 (8:2, 2.55 g) was subjected to preparative HPLC (40% methanol, flow rate 12 mL/min) to give **1** (18.2 mg) with retention time 26.8 min, **2** (11 mg) with retention time 17.6 min, **3** (28.6 mg) with retention time 20.5 min, **4** (21.8 mg) with retention time 23.3 min, **7** (42.5 mg) with retention time 29.8 min, and **8** (4.58 mg) with retention time 27.3 min. Fraction D3 (7:3, 4.8 g) was subjected to preparative HPLC (25% methanol, flow rate 12 mL/min) to give **5** with retention time 15.5 min (31.6 mg), **6** (23.7 mg) with retention time 14.3 min.

***Anti*-HIV-1 Assay.** The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method and *anti*-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[14]

Cytotoxicity Assay. The cytotoxicity tests for the isolates were performed by against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with doxorubicin as the positive control).[15]

Cordifoliketone A (1): Obtained as pale yellow gum; UV (MeOH) λ_{max} (log ε) 325 (2.42), 288 (4.22), 248 (3.12), 210 (4.89) nm; IR (KBr) ν_{max} 3376, 2925, 2850, 1713, 1638, 1512, 1450, 1434, 1362, 1283, 1175, 1137, 1086, 1047, 971, 828 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; positive ESIMS *m/z* 249 $[M+Na]^+$; HRESIMS *m/z* 249.0746 $[M+Na]^+$ (calcd 249.0739 for $C_{11}H_{14}NaO_5$).

Cordifoliketone B (2): Obtained as pale yellow gum; UV (MeOH) λ_{max} (log ε) 324 (2.46), 288 (4.12), 248 (3.22), 210 (4.92) nm; IR (KBr) ν_{max} 3378, 2923, 2852, 1716, 1630, 1518, 1456, 1363, 1276, 1162, 1088, 975, 822 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; positive ESIMS *m/z* 249 $[M+Na]^+$; HRESIMS *m/z* 291.0838 $[M+Na]^+$ (calcd 291.0845 for $C_{13}H_{16}NaO_6$).

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