Isolation of Chalcones from the Root of *Codonopsis cordifolioidea* and Their Antitobacco Mosaic Virus Activities

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A new chalcone, 4'-acetoxy-5-hydroxy-6-methoxy-chalcone (**1**) and four known chalcones (**2-5**) were isolated from the roots of *Codonopsis cordifolioidea*. Their structures were elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compounds **1-5** were tested for their antitobacco mosaic virus activities. Compounds **1** (20 μ M) showed moderate anti tobacco mosaic virus activity with inhibition rates of 18.2 %. Compounds **2-5** showed low anti tobacco mosaic virus activities with inhibitory rates below 10 %.

Key Words: Chalcone, *Codonopsis cordifolioidea*, Antitobacco mosaic virus activities.

INTRODUCTION

Some of *Codonopsis* species such as *C. pilosula* and *C. tangshen* are commonly used as herbal remedies due to their tonic effects¹. In addition, the roots of some *Codonopsis* species, such as *Codonopsis cordifolioidea*, are locally known as Choushen and have been used as a food in Yunnan, Tibet and Sichuan Provinces since ancient times^{2,3}. Meanwhile, as an important economic plant, this species has been widely cultivated in several areas of Yunnan province^{4,5}. The previous phytochemical studies on *C. cordifolioidea* have revealed that phenylpropanoids, lignans, as well as flavonoids are major components isolated from this plant⁵⁻⁹.

Motivated by searching for bioactive metabolites from this plant, the phytochemical investigation on *C. cordifolioidea* was carried out. As a result, a new chalcone (**1**) and four known chalcones (**2-5**) were isolated from this plant. In addition, the antitobacco mosaic virus (anti TMV) activities of compounds **1-5** were evaluated. This article deals with the isolation, structural elucidation and biological activities of these compounds.

EXPERIMENTAL

General methods: UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfrared spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ¹H, ¹³C and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or

on silica gel H (10-40 μ m, Qingdao Marine Chemical Inc., China). Preparative HPLC was used an Agilent 1100 HPLC equipped with ZORBAX-C₁₈ (21.2 mm $\times$ 250 mm, 7.0 μ m) column and DAD detector.

The roots of *C. cordifolioidea* were collected in Cuxiong Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Prof. Chen Y.J. (Yunnan University of Nationalities). A voucher specimen (YNNI 09-9-22) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered roots of *C. cordifolioidea* (2.8 kg) were extracted four times with 70 % methanol (4 L $\times$ 3 L) at room temperature and filtered. The crude extract (147 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 C (8:2, 16.2 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures C1-C5. Fraction C1 (9:1, 3.4 g) was subjected to preparative HPLC (46 % methanol, flow rate 12 mL/min) to give **1** (14.2 mg), **4** (18.8 mg) and **5** (28.6 mg). Fraction C2 (8:2, 2.8 g) was subjected to preparative HPLC (40 % methanol, flow rate 12 mL/min) to give **2** (18.8 mg) and **3** (22.1 mg).

Anti tobacco mosaic virus assays: The anti tobacco mosaic virus activities were tested using the half-leaf method²⁰. Ningnanmycin (2 % water solution), a commercial product for plant disease in China, was used as a positive control.

4'-Acetoxy-5-hydroxy-6-methoxy-chalcone (1): Yellow gum; UV (MeOH), $\lambda_{\max}$ (log ϵ) 375 (3.98), 312 (3.74), 252 (3.69), 230 (4.22) nm; IR (KBr, $\nu_{\max}$, cm^{-1}): 3359, 2964, 2905, 1728, 1672, 1614, 1546, 1485, 1267, 1146, 1052, 872, 758; ^1H and ^{13}C NMR data (CDCl_3 , 500 and 125 MHz, respectively) see Table-1; ESIMS (positive ion mode) m/z 349 $[\text{M} + \text{Na}]^+$; HRESIMS (positive ion mode) m/z 349.0682 $[\text{M} + \text{Na}]^+$ (calcd. (%) 349.0688 for $\text{C}_{18}\text{H}_{14}\text{O}_6\text{Na}$).

TABLE-1
 ^1H AND ^{13}C NMR DATA OF COMPOUNDS 1
(DATA OBTAINED IN CDCl_3 , 500 AND 125 MHz)

No.	δ_{C} (m)	δ_{H} (m, J, Hz)	No.	δ_{C} (m)	δ_{H} (m, J, Hz)
2	151.2 s	—	1'	123.9 s	—
3	115.5 d	7.56, s	2',6'	129.8 d	7.85, d, $J=8.6$
4	106.2 d	7.13, s	3',5'	120.5 d	7.20, d, $J=8.6$
5	145.8 s	—	4'	153.9 s	—
6	148.2 s	—	OMe-6	55.9 q	3.87, s
7	101.0 d	6.88, s	Ar-OH-4'	—	9.18, brs
8	156.6 s	—	1''	169.2 s	—
9	126.2 s	—	2''	21.1 q	2.05, s
10	186.6 s	—	—	—	—

RESULTS AND DISCUSSION

A 70 % aq. methanol extract prepared from the roots of *C. cordifolioides* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford a new chalcone, 4'-acetoxy-5-hydroxy-6-methoxy-chalcone (**1**) and four known chalcones (**2-6**), the structures of the compounds **1-5** were as shown in Fig. 1 and the ^1H and ^{13}C NMR data of compound **1** were listed in Table-1. The known compounds, compared with literature, were identified as 4,2',4'-trihydroxy-chalcone (**2**)¹⁰, 4,2'-dihydroxy-4'-methoxy-chalcone (**3**)¹⁰, 4-hydroxylonchocarpin (**4**)¹¹ and crotmadine (**5**)¹².

Fig. 1. Structure of chalcones from the flowers of *C. cordifolioides*

The HRESIMS of compound **1** showed a quasi-molecular ion peak at m/z 349.0688 $[\text{M} + \text{Na}]^+$, corresponding to a molecular formula of $\text{C}_{18}\text{H}_{14}\text{O}_6$. The ^1H and ^{13}C NMR spectrum of **1** (Table-1) displayed 18 carbon and 14 proton signals, respectively, corresponding to two aromatic rings (C-4-C-9 and C-1'-C-6'), a carbonyl carbon (C-10), a pair of double bond (C-2 and C-3), a methoxy group (δ_{C} 55.9 and δ_{H} 3.87), an acetoxy group (δ_{C} 169.2, 21.2; δ_{H} 2.05) and a phenolic

hydroxy group (δ_{H} 9.18). Strong absorption bands accounting for hydroxy (3359 cm^{-1}), carbonyl (1728, 1672) and aromatic groups (1614, 1546, 1485, 1267 cm^{-1}) could be observed in its IR spectrum. The UV absorptions at 375, 312, 252 and 230 nm also suggested the presence of a conjugated aromatic ring system. The NMR data of C-2 (δ_{C} 151.2), C-3 (δ_{C} 115.5), C-10 (δ_{C} 186.6) and H-3 (δ_{H} 7.56, s), together with the HMBC correlations (Fig. 2) of H-3 with C-2/C-4/C-8/C-9/C-10, suggested that **1** should be a chalcone derivative fused with a furan ring at C-2 and C-8¹³. The signals for four coupled aromatic protons at δ_{H} 7.03 (d, $J = 8.6$ Hz, 2H) and 7.74 (d, $J = 8.6$, 2H), suggested a 4'-monosubstituted for C ring¹⁴ and the proton signals for two singlets at δ_{H} 7.29 (s, 1H) and δ_{H} 6.69 (s, 1H) also revealed that the substituents for B-ring should be located at C-5 and C-6¹⁵. The HMBC correlations (Fig. 2) between the methoxy proton signals (δ_{H} 3.87) and C-6 (δ_{C} 148.2) suggested the positions of the methoxy groups at C-6. On the other hand, the phenolic hydroxy group at C-5 was supported by the HMBC correlations observed between the hydroxy proton (δ_{H} 9.18) and C-4 (δ_{C} 106.2), C-5 (δ_{C} 145.8) and C-6 (δ_{C} 148.2). Since the positions of methoxy and hydroxy groups were determined, the acetoxy group should be located C-4' to support the 4'-monosubstitution for C ring. On the basis of the above observations, the structure of **1** was elucidated as 4'-acetoxy-5-hydroxy-6-methoxy-chalcone.

Fig. 2. Selected HMBC ($\curvearrowright$) correlations of compound **1**

Many polyphenols are known to exhibit anti tobacco mosaic virus activities¹⁶⁻¹⁹. Compounds **1-5** were therefore tested for anti tobacco mosaic virus activities using the half-leaf method²⁰. Ningnanmycin, a biochemical pesticide against virus diseases on tobacco, with inhibitory of 31.8 %, was used as the positive control. Compounds **1** (20 μM) showed moderate anti tobacco mosaic virus activity with inhibition rates of 18.2 %. Compounds **2-5** showed low anti tobacco mosaic virus activities with inhibitory rates below 10 %.

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