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Isolation and Biological Activities of Furanoflavones from the Roots of *Codonopsis cordifolioidea*

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A new furanoflavone, cordifoliketone C (**1**), together with three known furanoflavones (**2-4**), were isolated from the roots of *Codonopsis cordifolioidea*. Their structures were determined by means of HRESIMS, extensive ^{1}D and ^{2}D NMR spectroscopic studies and chemical evidence. Compounds **1-4** were tested for their anti-HIV-1 activity and antitobacco mosaic virus activity, respectively. The results showed that compounds **1-4** have modest anti-HIV-1 activity and anti-tobacco mosaic virus activity, respectively.

Key Words: *Codonopsis cordifolioidea*, Furanoflavones, Anti-HIV-1 activity, Antitobacco mosaic virus activity.

INTRODUCTION

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, a new furanoflavone, cordifoliketone C (**1**), together with three known furanoflavones (**2-4**), were isolated from the roots of *C. cordifolioidea*. The anti-HIV-1 activity and antitobacco mosaic virus activity of compounds **1-4** were tested. This work deals with the isolation, structural elucidation and biological activities of the furanoflavones isolated.

EXPERIMENTAL

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. ^{1}D and ^{2}D 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 C_{18} (20 mm × 25 cm, 5.0 mm) column. Column chromatography was performed with Si gel (200-300 mesh, Qing-dao 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.

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. Y.J. Chen (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 C (9:1, 15.8 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, 3.15 g) was subjected to preparative HPLC (52 % methanol, flow rate 12 mL/min) to give **1** (22.5 mg) and **2** (28.2 mg). Fraction C2 (8:2, 2.94 g) was subjected to preparative HPLC (40 % methanol, flow rate 12 mL/min) to give 3 (35.8 mg) and 4 (24.0 mg).

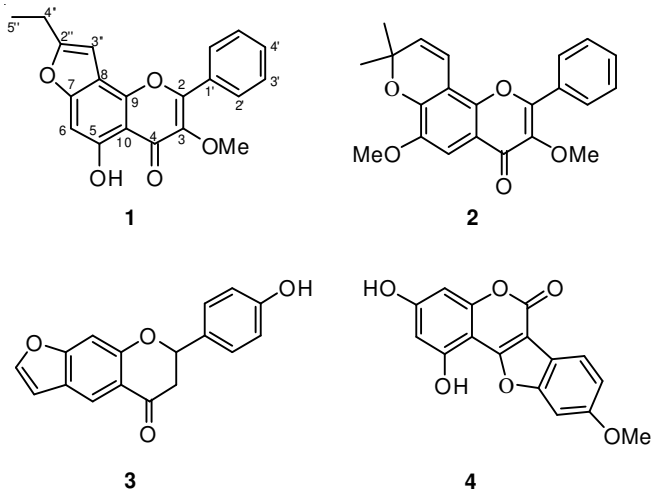
Anti-TMV assays: The Anti TMV activity was tested using the half-leaf method[12]. The inhibitory activities of the compounds against TMV replication were tested using two approaches. First, the half-leaf method was used to test the antiviral activity in the local lesion host *N. glutinosa in vivo*. Then, the leaf-disk method was used to evaluate the antiviral activity of the compounds in the systemic infection host *N. tabacum cv*. K326. Ningnanmycin (20 μM), a commercial product for plant disease in China, with inhibition rate of 36.5 %, was used as a positive control.

Anti-HIV1 assays: 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})[13].

Cordifoliketone C (1): It is obtained as pale yellow gum; UV (MeOH) λ_{max} (log ε) 362 (3.21), 276 (3.48), 242 (3.05), 210 (4.01) nm; IR (KBr, ν_{max}, cm^{-1}): 3422, 2929, 1655, 1600, 1512, 1451, 1354, 1222, 1155, 1001, 769, 684; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively) (Table-1); positive ESIMS *m/z* 359 $[M+Na]^+$; HRESIMS *m/z* 359.0890 $[M+Na]^+$ (calcd. 359.0895 for $C_{20}H_{16}NaO_5$).

RESULTS AND DISCUSSION

A 70 % aq. methanol extract prepared from the root of *C. cordifolioidea* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1-4**, including one new furanoflavone, named cordifoliketone C(**1**), together with three known furanoflavone, 3,6-dimethoxy-2′,2′-dimethyl-chromene-(3′,4′:7,8)-flavone (**2**)[7], furano(2″,3″,7,6)-4′- hydroxy-flavanone (1) (**3**)[8], hedysarimcoumestans B (**4**)[9]. The structures of the compounds **1-4** were as shown in Fig. 1.



1 **2** **3** **4**

Fig. 1. Furanoflavones from the roots of *C. cordifolioidea*

Compound **1** was isolated as a pale yellow gam. Its IR spectrum exhibited absorption bands at 3422 (hydroxyl), 1655 (carbonyl) and 1600 cm^{-1} (benzene ring) compatible with a flavonoid skeleton. Its HRESIMS spectrum, in the positive mode, revealed a peak at *m/z* 359.0890 $[M+Na]^+$ indicating the molecular formula of $C_{20}H_{16}O_5$, corresponding to 14 º of unsaturation. The ^1H NMR spectrum showed a signal at δ_H 12.60 (s) for a chelated hydroxyl group, two multiplets at δ_H 8.08 (m, H-2′/H-6′) and 7.52 (m, H-3′/H-4′/H-5′) appropriate for a monosubstituted benzene ring, two singlets at δ_H 6.85 (s, H-6) and 6.42 (s, H-3″) and a signal for one methoxyl group at δ_H 3.86 (s, 3-OMe). In addition, the ^1H NMR spectrum showed characteristic signals for an ethyl moiety at δ_H 2.52 (q, H-4″) and 1.32 (t, H-5″). Apart from the signals typical of a flavonoidic skeleton, the ^{13}C NMR spectrum of **1** showed the signals at δ_C 21.9 (C-4″) and 13.2 (C-5″), which were inferred to the ethyl moiety already suggested by the ^1H NMR data. The signals at δ_C 157.4 (C-2″) and 98.4 (C-3″), were associated with a furan ring while a signal at δ_C 60.8 with the methoxyl group located on C ring. The NMR spectral data (Table-1) of compound **1** combined with the molecular formula suggested a methoxy-furaneflavone.

TABLE-1
^1H NMR AND ^{13}C NMR DATA (IN C_5D_5N) OF COMPOUND **1**

No.	δ_C (mult)	δ_H (mult, *J*, Hz)
2	154.8 s	
3	141.3 s	
4	179.2 s	
5	161.5 s	
6	97.6 d	6.85 s
7	158.2 s	
8	112.3 s	
9	148.4 s	
10	109.5 s	
1′	130.2 s	
2′	128.0 d	8.08 m
3′	129.3 d	7.52 m
4′	131.5 s	7.52 m
5′	129.3 d	7.52 m
6′	128.0 d	8.08 m
2″	157.4 s	
3″	98.4 d	6.42 s
4″	21.9 t	2.52 q
5″	13.2 q	1.32 t
3-OMe	60.8 q	3.86 s
5-OH		12.60 s

In the HMBC spectrum (Fig. 2), the correlations of the hydrogens at δ_H 6.42 (H-3″), 1.32 (H-5″) and 2.52 (H-4″) with the carbon at δ_C 157.4 (C-2″) were in agreement with the presence of the ethyl moiety at C-2″, while the long range correlation between the proton signal at δ_H 6.42 (H-3″) with the carbon at δ_C 148.4 (C-9) confirmed the location of the furan ring at the C-7/C-8 position. In order to attend to the feature of a monosubstituted ring B in the structure of **1**, the methoxyl group was located in the C ring at the C-3 position in accordance with the carbon chemical shift at δ_C 141.3 (C-3) and the correlation between the signal at δ_H 3.86 (-OMe) with that carbon. Based on all spectroscopic evidences, the

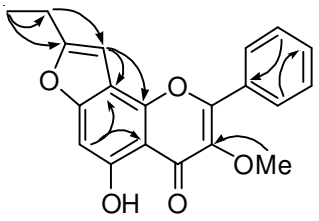


Fig. 2. Selected HMBC ( ) of compound **1**

structure of **1** was established as the 5-hydroxy-2″-ethyl-3-methoxyfurane-(2″,3″:7,8)-flavone and given the trivial name of cordifoliketone C.

Since some of the furanoflavones exhibited anti virus activities[10,11], compounds **1-4** were tested for the anti-TMV activity using the half-leaf method[12] and anti-HIV activity according to literature[13].

In anti-TMV activity test, the antiviral inhibition rates of the compounds at the concentration of 20 μM were tested by the half-leaf method. The results showed that the compound **1-4** exhibited inhibition rates of 25.4, 8.26, 11.8 and 6.35 %, respectively. The results revealed that compound **1** exhibited modest anti-TMV activity; its inhibition rate is close to that of positive control.

In anti-HIV1 activity test, the cytotoxicity assay against C8166 cells (CC^{50}) and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using azidothymidine (AZT) as a positive control (EC_{50} = 0.034 mg/mL and CC_{50} > 200 μg/mL)[22]. Compound 1-4 showed modest anti-HIV-1 activities with EC_{50} values of 11.86, 2.47, 9.22, 13.50 μg/mL, respectively and the all exerted minimal cytotoxicity against C8166 cells (CC_{50} > 200 μg/mL). The therapeutic index (TI) values (CC_{50}/EC_{50}) of **1-4** was more than 16.86, 80.97, 21.69, 14.81, respectively.

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