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

HONGQIONG LENG^{1,2}, GUANGYU YANG², YONGKUAN CHEN², YADONG GUO^{1,*} and ZHANGYU CHEN^{1,3}

¹School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650500, P.R. China

²Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China

³China Tobacco Yunnan Industry Company (Ltd.), Kunming 650000, P.R. China

*Corresponding author: E-mail: yadong_guo@yahoo.com.cn; ygy1110@163.com

(Received: 20 December 2011;

Accepted: 10 October 2012)

AJC-12270

A new furanoflavone, cordifoliketone C (**1**), together with three known furanoflavones (**2-4**), were isolated from the roots of *Codonopsis cordifolioides*. Their structures were determined by means of HRESIMS, extensive ¹D and ²D 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 cordifolioides*, 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 effects¹. In addition, the roots of some *Codonopsis* species including *C. cordifolioides*, *C. bulleyana*, *C. micrantha* and *C. subglobosa* are well-known vegetables in southwest China^{2,3}. *C. cordifolioides* 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. cordifolioides* 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. cordifolioides* 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. cordifolioides*. 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. 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 $\times$ 25 cm, 7.0 mm) column or a Venusil MP C₁₈ (20 mm $\times$ 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 μ m, Merck, Darmstadt, Germany) and MCI gel (75-150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 5 % H₂SO₄ in EtOH.

The roots of *C. cordifolioides* 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. cordifolioides* (1.5 kg) were extracted four times with 70 % methanol (4 $\times$ 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 method¹². 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 μ M), 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 (CC₅₀) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC₅₀)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 ¹³C NMR data (C₅D₅N, 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 C₂₀H₁₆NaO₅).

RESULTS AND DISCUSSION

A 70 % aq. methanol extract prepared from the root of *C. cordifolioides* 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 furanoflavones, 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.

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

Compound **1** was isolated as a pale yellow gum. 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 C₂₀H₁₆O₅, corresponding to 14 ° 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 ¹³C 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-furane-flavone.

TABLE-1 ¹ H NMR AND ¹³ C NMR DATA (IN C ₅ D ₅ N) 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-methoxyfuran-(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 method¹² and anti-HIV activity according to literature¹³.

In anti-TMV activity test, the antiviral inhibition rates of the compounds at the concentration of 20 μ M 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 μ g/mL)²². Compound **1-4** showed modest anti-HIV-1 activities with EC_{50} values of 11.86, 2.47, 9.22, 13.50 μ g/mL, respectively and the all exerted minimal cytotoxicity against C8166 cells (CC_{50} > 200 μ g/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.

ACKNOWLEDGEMENTS

This project was supported financially by the Excellent Scientific and Technological Team of Yunnan (2009CI014) and the Excellent Scientific and Technological Team of Yunnan High School (2010CI08).

REFERENCES

1. D.Y. Hong, Y.S. Lian and L.D. Shen, *Flora of China*, Science Press, Beijing, Vol. 73, p. 32 (1983).
2. Yunnan Corporation of Materia Medica, *List of Chinese Herb Medicine Resources in Yunnan*, Science Publishing, p. 544 (1993).
3. Q.F. Duang, H. Zhao and Y.Q. Wang, *Chin. J. Yunnan. Med.*, **12**, 39 (2003).
4. Z.J. Chen, Q.H. Wei and J.Y. Zhou, *Yunnan. J. Tradit. Chin. Med. Mater.*, **27**, 49 (2006).
5. R.Q. Mei, Q. Lu, Y.F. Hu, H.Y. Liu, F.K. Bao, Y. Zhang and Y.X. Cheng, *Helv. Chim. Acta*, **91**, 90 (2008).
6. R.Q. Mei, Q. Lu, Y.F. Hu and Y.X. Cheng, *Chin. J. Nat. Prod. Res. Develop.*, **22**, 238 (2010).
7. A.M.C. Arriaga, G.A. Gomes and R. Braz-Filho, *Fitoterapia*, **71**, 211 (2000).
8. H. Liu, Y.J. Bai, Y.Y. Chen and Y.Y. Zhao, *China. J. Mat. Med.*, **32**, 1410 (2008).
9. W. Wang, Y.Y. Zhao and H. Liang, *J. Nat. Prod.*, **69**, 876 (2006).
10. F. Epifano, S. Genovese, L. Menghini and M. Curini, *Phytochemistry*, **68**, 939 (2007).
11. K. Hassan, T. Mahmud and A. Ather, *Biotech. Ann. Rev.*, **13**, 223 (2007).
12. X.H. Yan, J. Chen, Y.T. Di, X. Fang, J.H. Dong, P. Sang, Y.H. Wang, H.P. He, Z.K. Zhang and X.J. Hao, *J. Agric. Food. Chem.*, **58**, 1572 (2010).
13. P.L. Darke and J.R. Huff, *Adv. Pharmacol.*, **25**, 399 (1994).