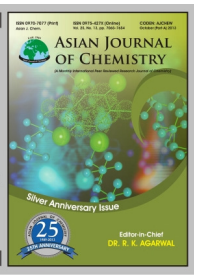
Asian Journal of Chemistry; Vol. 25, No. 14 (2013), 7888-7890

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14677



Phenylethanoids from the Roots of *Codonopsis cordifolioidea* and Their Anti HIV-1 Activities

YANQIONG SHEN[1,2], JIANHUA YAO[2], LIDAN SHU[1], LIYING YANG[1], XUEMEI GAO[1,*] and QIU-FEN HU[1,*]

[1]Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities, Kunming 650031, P.R. China
[2]Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China

*Corresponding authors: Fax: +86 871 5910017; Tel: +86 871 5910013; E-mail: gao_xuemei@hotmail.com; huqiufena@yahoo.com

(*Received*: 15 October 2012; *Accepted*: 22 July 2013) AJC-13834

A new phenylethanoid, 3,4-dihydroxyphenethyl-5-hydroxy-4-oxopentanoate (**1**), together with four known phenylethanoids (**2-6**), were isolated from the roots of *Codonopsis cordifolioidea*. Their structures were determined by means of HR ESI MS, extensive 1D and 2D NMR spectroscopic studies and chemical evidence. Compounds **1-6** were tested for their anti HIV-1 activities. Compounds **1** and **3** showed obvious anti HIV-1 activities with therapeutic index values above 50.

Key Words: *Codonopsis cordifolioidea*, Phenylethanoids, Anti HIV-1 activity.

INTRODUCTION

The genus *Codonopsis* (Campanulaceae) in China is represented by 39 species. Some of them, 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, such as *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 China 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[5-8]. Motivated by search for bioactive metabolites from this plant, the phytochemical investigation on the roots of *C. cordifolioidea* was carried out. As a result, a new phenylethanoid, together with five known phenylethanoids, were isolated from the roots of this plant. In addition, the anti HIV-1 activities of those compounds were evaluated. This article deals with the isolation, structural elucidation and biological activities of the compounds.

EXPERIMENTAL

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. HR ESI MS 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 m) column or a Venusil MP C18 (20 mm × 25 cm, 5.0 μm) 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 2010. The identification of the plant material was verified by Prof. Yuan Ning (Yunnan Nationalities University). A voucher specimen (YNNI 10-9-64) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered roots of *C. cordifolioidea* (3.5 kg) were extracted four times with 70 % methanol (4 × 3 L) at room temperature and filtered. The crude extract (138 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, 26.8 g) by silica gel column chromatography, eluted

with chloroform-methanol and preparative HPLC (35 % methanol, flow rate 12 mL/min) to give **1** (15.9 mg), **3** (22.6 mg) and **4** (28.8 mg). On the other hand, separation of fraction E (6:4, 21.2 g) by silica gel column chromatography and preparative HPLC (22 % methanol, flow rate 12 mL/min) led to the purification of **2** (19.3 mg), **5** (33.2 mg) and **6** (38.5 mg).

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})[17].

3,4-Dihydroxyphenethyl-5-hydroxy-4-oxopentanoate (1): Obtained as white powder; UV (MeOH) λ_{max} (log ε) 324 (2.52), 286 (3.88), 246 (3.01), 210 (4.18) nm; IR (KBr, ν_{max}, cm^{-1}): 3450, 2922, 2853, 1746, 1710, 1618, 1543, 1455, 1430, 1359, 1172, 1088, 975, 826; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table-1; negative ESI MS *m/z* 267 $[M-H]^-$; HR ESI MS *m/z* 267.0862 $[M-H]^-$ (calcd. (%) 267.0869 for $C_{13}H_{15}O_6$).

RESULTS AND DISCUSSION

A 70 % aqueous methanol extract prepared from the roots of *Codonopsis cordifolioidea* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford a new phenylethanoid, 3,4-dihydroxyphenethyl-5-hydroxy-4-oxopentanoate (**1**) and five known phenylethanoids (**2-6**). The structures of the compounds **1-6** were as shown in Fig. 1 and the ^1H and ^{13}C NMR data of the compound **1** were listed in Table-1. The known compounds, compared with literature data, were identified as. 2-(3-O-β-D-glucopyranosyl-4-hydroxyphenyl)ethanol (**2**)[9], 2-(3,4-dihydroxyphenyl)ethanol (**3**)[9], 4-(2-acetoxyethy1)-1,2-dihydroxybenzene (**4**)[10], 1'-O-β-D-(3,4-dihydroxypheny1)-ethyl-6'-O-vanilloyl-glucopyranoside (**5**)[11], 3,4-dihydroxyphenylethano1-8-O-[β-D-apiofuranosyl(1→2)]-β-D-glucopyranoside (**6**)[12].

Compound **1** was obtained as white powder. Its molecular formula was determined as $C_{13}H_{16}O_6$ by HR-ESI-MS *m/z* 267.0862 $[M-H]^-$ (calcd. (%) 267.0869). Its ^1H and ^{13}C NMR spectra (Table-1) showed signals to 16 hydrogens and 13 carbons, respectively, corresponding to one aromatic ring (δ_C 130.1, 115.3, 148.5, 146.3, 118.0, 120.8) with three aromatic protons (ν_H 7.11 d *J* = 1.8, 7.31 d *J* = 7.8, 6.77 dd *J* = 1.8 7.8), three methylene groups (δ_C 34.5, 28.3, 33.2), two oxidated methylene group (δ_C 65.8, 68.8), one ketone group (δ_C 209.2), an ester carbonyl group (δ_C 172.9) and two phenolic hydroxy proton signal (δ_H 10.86, 11.25). The ^1H-^1H COSY of H-7/H-8; together with HMBC correlations (Fig. 2) of H-6 (δ_H 6.77) with C-7 (δ_C 34.5), of H-8 (δ_H 4.32) with C-1 (δ_C 130.1) revealed

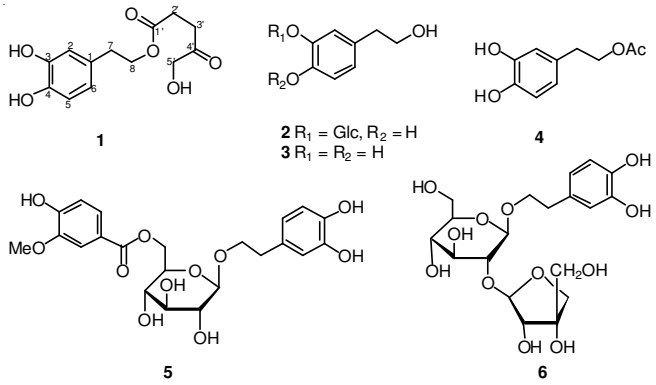


Fig. 1. Structures of phenylethanoids isolated from the roots of *C. cordifolioidea*

that the exist of a 3,4-substituted phenylethanoid structural unit[13]. In addition, the ^1H-^1H COSY of H-2'/H-3' together with HMBC correlations of H-5' (δ_H 4.52) with C-4' (δ_C 209.2), C-3' (δ_C 33.2), of H-3' (δ_H 2.87) with C-1' (δ_C 172.9), C-2' (δ_C 28.3), C-4' (δ_C 209.2), C-5' (δ_C 68.8), of H-2' (δ_H 2.71) with C-1' (δ_C 172.9), C-3' (δ_C 33.2), C-4' (δ_C 209.2) also suggested that the exist of a 5-hydroxy-4-oxoamylacyl group (-OC(O)-CH_2CH_2-C(O)CH_2OH)[13]. The HMBC of H-8 (δ_H 4.32) with C-1' (δ_C 172.9) indicated that the 5-hydroxy-4-oxoamylacyl group located at C-8. Thus, the structure of **1** was established as 3,4-dihydroxyphenethyl-5-hydroxy-4-oxopentanoate.

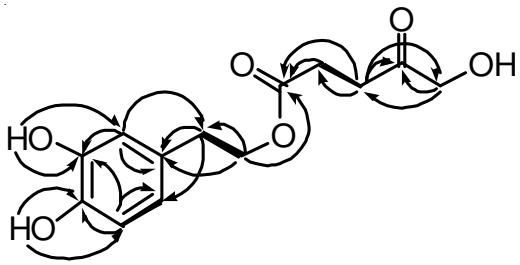


Fig. 2. Selected HMBC (↷) ^1H-^1H COSY (–) correlations of **1**

Since certain phenylethanoids exhibit potential anti HIV-1 activity[14-16], Compounds **1-6** were tested for their anti HIV-1 activity[17]. The cytotoxicity assay against C8166 cells (CC_{50}) and anti HIV-1 activity were 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 g/mL and CC_{50} > 200 g/mL). The results are shown in Table-2. The results show that compounds **1** and **3** showed obvious anti HIV-1 activities with therapeutic index (TI) values above 50, respectively.

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

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	130.1 s	–	1'	172.9 s	–
2	115.3 d	7.11, d, *J*=1.8	2'	28.3 t	2.71, t, *J* = 6.4
3	148.5 s	–	3'	33.2 t	2.87, t, *J* = 6.4
4	146.3 s	–	4'	209.2 s	–
5	118.0 d	7.31, d, *J* = 7.8	5'	68.8 t	4.52, s
6	120.8 d	6.77, dd, *J* = 1.8, 7.8	Ar-OH-4	–	11.25
7	34.5 t	2.83, t, *J* = 7.1	Ar-OH-3	–	10.86
8	65.8 t	4.32, t, *J* = 7.1	–	–	–

TABLE-2
ANTI HIV ACTIVITIES OF COMPOUNDS **1-6**

Compounds	CC_{50} (μg/mL)	EC_{50} (μg/mL)	TI[a]
1	>200 ± 4.8	2.26 ± 0.11	>88.5
2	132.5 ± 3.6	3.05 ± 0.12	43.4
3	>200 ± 5.1	2.58 ± 0.17	>77.5
4	115.0 ± 4.5	6.31 ± 0.25	18.2
5	164.3 ± 4.2	8.11 ± 0.24	20.3
6	102.7 ± 3.8	2.96 ± 1.8	34.7
AZT	>200 ± 4.0	0.034 ± 0.02	>5881

[a]TI = Therapeutic index EC_{50}/CC_{50}, n = 3 for all groups.

ACKNOWLEDGEMENTS

This project was supported financially by the Excellent Scientific and Technological Team of Yunnan High School (2010CI08) and the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008) and Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities) (2010XY08).

REFERENCES

1. D.Y. Hong, Y.S. Lian and L.D. Shen, Flora of China, Chinese Science Press: Beijing, 73, 32 (1983).
2. Yunnan Corporation of Materia Medica, List of Chinese Herb Medicine Resources in Yunnan, 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 Zhou J.Y. 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. H.Q. Leng, G.Y. Yang, Y.K. Chen, Y.D. Guo and Z.Y. Chen, *Asian J. Chem*., **25**, 2024 (2013).
7. Q.F. Hu, X.S. Li, H.T. Huang, H.X. Mu and G.P. Li, *Bull. Korean Chem. Soc*., **33**, 278 (2012).
8. X.M. Gao, H.X. Mu, X.S. Li, G.Y. Yang, G.P. Li and Q.F. Hu, *J. Chin. Chem. Soc*., **59**, 440 (2012).
9. A. Bianco, R.A. Mazzei, C. Melchioni, G. Romeo, M.L. Scarpati, A. Soriero and N. Uccella, *Food Chem*., **63**, 461 (1998).
10. M. Kikuchi and Y. Yamauchi, *Yakugaku Zasshi*, **103**, 360 (1983).
11. X.K. Zheng, Y.B. Liu, J. Li and W.S. Feng, *Acta Pharm. Sin.*, **39**, 716 (2004).
12. X.K. Zheng, J. Li, W.S. Feng, Y.F. Bi and C.R. Ji, *Acta Pharm. Sin*., **38**, 268 (2003).
13. X.M. Gao, L.Y. Yang, Y.Q. Shen, L.D. Shu, X.M. Li and Q.F. Hu, *Bull. Korean Chem. Soc*., **33**, 278 (2012).
14. Y.L. Lu, X.S. Li, H.X. Mu, H.T. Huang, G.P. Li and Q.F. Hu, *J. Braz. Chem. Soc*., **23**, 656 (2012).
15. Q.F. Hu, H.X. Mu, H.T. Huang, H.Y. Lv, S.L. Li, P.F. Tu and G.P. Li, *Chem. Pharm. Bull*., **59**, 1421 (2011).
16. Q.F. Hu, X.S. Li, H.T. Huang, H.X. Mu and P.F. Tu, *Helv. Chim. Acta*, **95**, 349 (2012).
17. X.J. Zhang, G.Y. Yang, R.R. Wang, J.X. Pu, H.D. Sun, W.L. Xiao and Y.T. Zheng, *Chem. Biodivers*., **7**, 2692 (2010).