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Phenolic Compounds from *Clinopodium urticifolium* and Their Antivirus Activities

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A new phenolic compound, clinopodphenol B (**1**), together with six known phenols, were isolated from the whole plant of *Clinopodium urticifolium*. The structure of **1** was elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compound **1** was also tested for their anti HIV-1 activity and antitobacco mosaic virus activity. The results showed that compound **1** have modest anti HIV-1 activity and anti-tobacco mosaic virus activity, respectively.

Key Words: *Clinopodium urticifolium*, Phenolic compounds, Anti HIV-1 activity, Antitobacco mosaic virus activities.

INTRODUCTION

Clinopodium genus species are popular traditional Chinese medicinal herbs used to treat bruises and swelling and are also purported to improve blood circulation[1,2]. In recent years, several papers have described phytochemistry investigations of various species of Clinopodium and it was found to be rich in saponins[3,4], flavones[5,6], polyphenols[7,8], terpenes[9,10], *etc*.

C. urticifolium belongs to the Clinopodium genus, which widely distributed in Shanxi, Gansu and Ningxia, northwest of China. In previous work, some bioactive compounds were isolated from this plant[9,11,12]. Motivated by search for bioactive metabolites from this plant, a reinvestigation the chemical constituents of the whole plant of *C. urticifolium* were carried out. As a result, a new phenolic compound, clinopodphenol B, together with six known phenols, were isolated from this plant. In addition, the anti HIV-1 activity and antitobacco mosaic virus activity of compound **1** was evaluated. This article deals with the isolation, structural elucidation and biological activities of the new compound.

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 × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 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.

Plant material: The whole plant of *C. urticifolium* was collected in Qudang prefecture of Ningxia Province, People's Republic of China, in September 2010. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-2010-09-22) has been deposited in our Laboratory.

Extraction and isolation: The air-dried and powdered of whole plant of *C. urticifolium* (3.5 kg) were extracted three times with 70 % aqueous MeOH (3.0 L × 4.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (320 g). This crude extract was applied to Si gel (200-300 mesh) column chromatography eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. The separation of fraction B (18.4 g) by Si gel column chromatography eluted with $CHCl_3$-acetone (20:1-1:2) yielded mixtures B1-B6. Fraction B2 (5.23 g) was

subjected to Si gel column chromatography using petroleum ether-acetone and preparative HPLC (60 % MeOH-H_2O, flow rate 12 mL/min) to give compounds **1** (11.4 mg), **4** (15.7 mg) and **5** (28.4 mg). Fraction B3 (3.15 g) was subjected to Si gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (40 % MeOH-H_2O, flow rate 12 mL/min) to yield compounds **2** (56.4 mg), **3** (27.8 mg). Fraction B5 (6.28 g) was subjected to Si gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (25 % MeOH-H_2O, flow rate 12 mL/min) to yield compounds **6** (56.4 mg), **7** (31.2 mg).

Anti TMV assays: The anti TMV activity was tested using the half-leaf method[21]. The inhibitory activities of the new 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 compound in the systemic infection host *N. tabacum* cv. K326. Ningnanmycin (20 μM), a commercial product for plant disease in China, 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})[22].

Clinopodphenol B: It was obtained as a pale yellow gum; $[\alpha]^{24.0}_D$ + 18.2 (c 0.22, MeOH); UV (MeOH), λ_{max} (log ε) 280 (2.26), 235 (3.59), 205 (4.08) nm; IR (KBr, ν_{max}, cm^{-1}): 3432, 2920, 1764, 1616, 1510, 1432, 1273, 1034, 956, 849, 758; ^1H and ^{13}C NMR data (CD_3COCD_3, 500 and 150 MHz, respectively), Table-1; ESIMS (positive ion mode) m/z 289 $[M + Na]^+$; HRESIMS (positive ion mode) m/z 289.1058 $[M + Na]^+$ [calcd. (%) 289.1052 for $C_{14}H_{18}O_5$].

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF COMPOUND **1** IN CD_3COCD_3 (125 AND 500 MHz)

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	129.0 s	–
2	107.7 d	6.74, d, *J* = 1.9
3	148.6 s	–
4	149.8 s	–
5	113.8 d	6.84, d, *J* = 8.1
6	119.3 d	6.61, dd, *J* = 1.8, 8.1
7	85.5 d	5.11, d, J=9.4
8	49.9 d	2.01 m
9	7.90 q	1.03, d, *J* = 6.6
7′	176.0 s	–
8′	74.0 s	–
9′	22.0 q	1.50 s
3-OMe	55.8 q	3.79 s
4-OMe	56.0 q	3.81 s

RESULTS AND DISCUSSION

The air-dried and powdered whole plant of *C. urticifolium* (3.5 kg) was extracted with 70 % aqueous methanol (3 L × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtained a crude extract (320 g). This crude extract was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1-7** (Fig. 1), including a new phenolic compound, clinopodphenol B (**1**), together with six known phenols, rosmarinic acid (**2**)[13], clinopodic acid B (**3**)[8], futoquinol (**4**)[14], wallichinine (**5**)[15], coniferoside (**6**)[16], isoconiferin (**7**)[17].

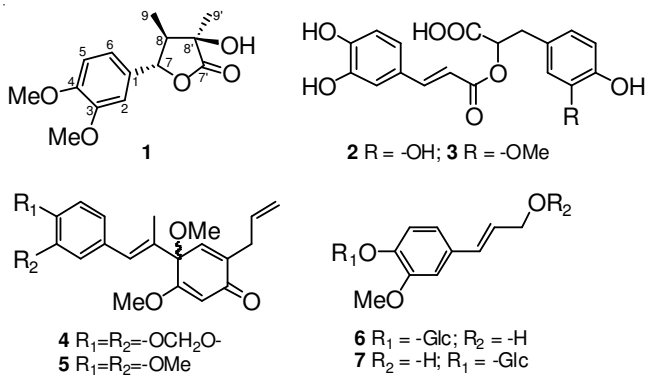

Fig. 1. Structure of phenolic compounds from *C. urticifolium*

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $C_{14}H_{18}O_5$ by HR-ESI-MS m/z 289.1058 $[M + Na]^+$ [calcd. (%) 289.1052]. Its ^1H and ^{13}C NMR spectral data (Table-1) showed signals to one 1,3,4-trisubstituted aromatic rings (δ_H 6.74, 6.84, 6.61), two methoxyl groups (δ_C 55.8, 56.0), two methyl groups (δ_C 7.90, 22.0), one methine group (δ_C 49.9), one oxidated methine group (δ_C 85.5), one oxidated quaternary carbon (δ_C 74.0) and one carbonyl group (δ_C 176.0). Strong absorption bands accounting for hydroxyl (3432 cm^{-1}), carbonyl group (1764 cm^{-1}) and aromatic group (1616, 1510, 1432 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 280, 236 nm also confirmed the existence of the aromatic function. On the basis of the molecular formula, one ring was needed to meet the required degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **1** were very similar to these of (+)-(7S,8R,8'R)-4,8'-dihydroxy-3-methoxy-1',2',3',4',5',6'-hexanorligna-7',7-lactone[18]. The obvious differences are the substitutents on aromatic rings. A phenolic hydroxyl group was replaced by a methoxyl group in **1**. The HMBC correlations (Fig. 2) of methoxyl groups proton signal (δ_H 3.79 s, 3.81 s) with C-3 (δ_C 145.8) and C-4 (δ_C 145.0) indicated that two methoxyl groups should be located at C-3 and C-4. The HMBC correlations observed from H-7 (δ_H 5.11) to C-1 (δ_C 129.0), C-2 (δ_C 107.7), C-6 (δ_C 119.3), C-7' (δ_C 176.0), C-8 (δ_C 49.9), C-8' (δ_C 74.0) and C-9 (δ_C 7.90); from H-8 (δ_H 2.01) to C-1 (δ_C 129.0), C-7' (δ_C 176.0), C-8' (δ_C 74.0), C-9 (δ_C 7.90), C-9' (δ_C 22.0); from CH_3-9 (δ_H 1.03) to C-7 (δ_C 85.5), C-8 (δ_C 49.9) and C-8' (δ_C 74.0); from CH_3-9' (δ_H 1.50) to C-7' (δ_C 176.0), C-8 (δ_C 49.9) and C-8' (δ_C 74.0) were also supporting the structure of compound **1**. The configurations of 7S, 8R, 8'R in **1** were deduced from the comparison of coupling constants and ROESY correlations (Fig. 3) with these of (+)-(7S,8R,8'R)-4,8'-dihydroxy-3-methoxy-1',2',3',4',5',6'-hexanorligna-7',7-lactone[18]. Thus, the structure of **1** was determined as shown and given the name as clinopodphenol B.

Since some of the lignans exhibited anti virus activities[19,20], compound **1** were tested for the anti TMV activity using the half-leaf method[21] and anti HIV activity according to literature[22].

HMBC ( ) ^{1}H-^{1}H COSY ( )

Fig. 2. Key HMBC and ^{1}H-^{1}H COSY correlations of **1**

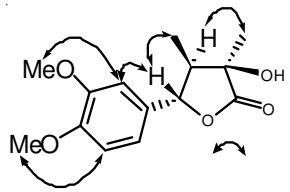

Fig. 3. Key ROESY ( ) correlations of **1**

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** exhibited inhibition rates of 25.8 %, respectively. The results showed 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 and CC_{50} > 200 μg/mL)[22]. Compound **1** showed modest anti HIV-1 activities with EC_{50} values of 1.86 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** was more than 107.5.

REFERENCES

1. H.D. Chi and J.C. Lu, *J. Shenyang. Pharm. Univ*., **23**, 123 (2006).
2. J.L. Trusty, R.G. Olmstead, D.J. Bogler and J. Francisco-Ortega, *Syst. Bot*., **29**, 702 (2004).
3. A. Yamamoto, T. Miyase, A. Ueno amd T. Maeda, *Phytochemistry*, **34**, 485 (1993).
4. Z. Liu, D. Li, N.L. Owen, D.M. Grant, R.G. Cates and Z. Jia, *J. Nat. Prod*., **58**, 1600 (1995).
5. Y.H. Kuo, H.M.S. Lee and J.S.H. Lai, *J. Chin. Chem. Soc (Taipei)*, **47**, 241 (2000).
6. Y.H. Kuo, H.M.S. Lee and J.S.H. Lai, *Chin. Pharm. J. (Taipei)*, **52**, 27 (2000).
7. P. Pripdeevech, E. Chukeatirote and Kachai, *Food. Chem. Toxicol*., **48**, 2754 (2010).
8. T. Murata, K. Sasaki, K. Sato, F. Yoshizaki, H. Yamada and H. Mutoh, *J. Nat. Prod*., **72**, 1379 (2009).
9. X.M. Wei, J.K. Cheng, D.L. Cheng and L.M. Gao, *J. Chin. Chem. Soc. (Taipei)*, **51**, 1043 (2004).
10. F. Mori, T. Miyase and A. Ueno, *Phytochemistry*, **36**, 1485 (1994).
11. S.H.M. Lee, J.S.H. Lai and Y.H. Kuo, *J. Chin. Chem. Soc. (Taipei)*, **40**, 87 (1993).
12. L.M. Gao, X.M. Wei and D.L. Cheng, *Chin. Chem. Lett*., **14**, 1041 (2003).
13. M. Petersen, *Phytochemistry*, **45**, 1165 (1997).
14. O.D. Tyagi, S. Hensen, P.M. Boll, N.K. Sharma, K.S. Bisht and S. Parmar, *Phytochemistry*, **32**, 445 (1993).
15. G.Q. Han, L.H. Wei, C.L. Li, L. Qiao, Y.Z. Jia and Q.T. Zheng, *Acta Pharm. Sin*., **24**, 438 (1989).
16. A. Tolonen, M. Pakonen, A. Hohtola and J. Jalonen, *Chem. Pharm. Bull*., **51**, 467 (2003).
17. T. Nakanishi, N. Iida, Y. Inatomi, H. Murata, A. Inada, J. Murata, F.A. Lang, M. Iinuma and T. Tanaka, *Heterocycles*, **63**, 2573 (2004).
18. W. Cheng, C.G. Zhu, W.D. Xu, X.N. Fan, Y.C. Yang, Y. Li, X.G. Chen, W.J. Wang and J.G. Shi, *J. Nat. Prod*., **72**, 2145 (2009).
19. X.J. Zhang, G.Y. Yang, R.R. Wang, J.X. Pu, H.D. Sun, W.L. Xiao and Y.T. Zheng, *Chem. Biodiver*., **7**, 2692 (2010).
20. D. Chattopadhyay, M.C. Sarkar, T. Chatterjee, D.R. Sharma, P. Bag, S. Chakraborti and M.T.H. Khan, *New Biotechnol*., **25**, 347 (2009).
21. 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).
22. P.L. Darke and J.R. Huff, *Adv. Pharmacol*., **25**, 399 (1994).