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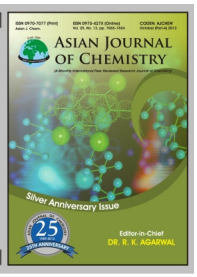

Pterocarpan Derivatives from *Clinopodium urticifolium* and Their Cytotoxicity

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A new pterocarpan, 3(S),4(S)-3'-methoxy-4'-hydroxy-7,8,-methylenedioxylpterocarpan (**1**), together with four known pterocarpan derivatives (**2-5**), were isolated from the whole plant of *Clinopodium urticifolium*. The structure of **1-5** was elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compound **1** was also evaluated for its cytotoxicity against five human tumor cell lines. The results revealed that compound **1** showed high cytotoxicity against PC3 cell with IC_{50} values of 3.5 μM and moderate cytotoxicities with IC_{50} valves 5-10 μM for other four tested cell lines.

Key Words: *Clinopodium urticifolium*, Pterocarpan derivatives, Cytotoxicity.

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*.

Clinopodium urticifolium belongs to the *Clinopodium* genus, which widely distributed in Gansu, shaanxi, ningxia, in northwest China. In previous work, some bioactive compounds were isolated from this plant[8,11,12]. Motivated by a search for bioactive metabolites from this plant, a reinvestigation for the chemical constituents of the whole plant of *C. urticifolium* were carried out. As a result, a new pterocarpan (**1**), together with four known pterocarpan derivatives (**2-5**), were isolated from this plant. In addition, the cytotoxicity of compound **1** was evaluated. This work 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. CD spectra were measured on a JASCO J-810 spectropolarimeter. 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), Sephadex LH-20 (Sigma-Aldrich, Inc, USA) 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 whole plant of *C. urticifolium* was collected in Gansu Province, People's Republic of China, in September 2010. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-10-09-34) has been deposited in our Laboratory.

Extraction and isolation: The air-dried and powdered whole plant of *C. urticifolium* (3.8 kg) was extracted four times with 70 % aqueous methanol (3.0 L × 4.5 L) at room temperature and filtered. The crude extract (182 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 separation of fraction

C (8:2, 14.6 g) by silica gel column chromatography, eluted with chloroform-methanol and preparative HPLC (40 % methanol, flow rate 12 mL/min) to give **1** (22.8 mg) and **2** (15.6 mg). The further separation of fraction D (7:3, 13.8 g) by silica gel column chromatography, eluted with chloroform-methanol and preparative HPLC (36 % methanol, flow rate 12 mL/min) to give **3** (18.8 mg), **4** (21.5 mg) and **5** (26.4 mg). 3(S),4(S)-3'-Methoxy-4'-hydroxy-7,8,-methylenedioxylpterocarpan (**1**): Obtained as pale yellow gum; $[\alpha]^{D}_{24.8}$ + 247 (c 0.020, MeOH); CD (c 0.02, MeOH), nm (Δε), 285 (-3.97), 250 (+18.4), 232 (-22.8); UV (MeOH), λ_{max} (log ε) 345 (2.22), 296 (3.77), 210 (4.28) nm; IR (KBr, ν_{max}, cm^{-1}): 3387, 2953, 2888, 1612, 1535, 1483, 1428, 1355, 1252, 1153, 1034, 862; ^1H and ^{13}C NMR data (CD_3OD, 500 and 125 MHz), Table-1; ESI-MS (positive ion mode) m/z 337 $[M + Na]^+$; HR-ESI-MS (positive ion mode) m/z 337.0693 $[M + Na]^+$ (calcd. (%) 337.0688 for $C_{17}H_{14}NaO_6$).

RESULTS AND DISCUSSION

The air-dried and powdered whole plant of *C. urticifolium* (3.8 kg) was extracted with 70 % aqueous methanol (3 × 4.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtain a crude extract (182 g). This crude extract was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford a new pterocarpan, 3(S),4(S)-3'-methoxy-4'-hydroxy-7,8,-methylenedioxylpterocarpan (**1**), together with four known pterocarpan derivatives (**2-5**). 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, were identified as: (-)-pterocarpan (**2**)[13], (-)-10-methoxy-medicarpin (**3**)[14] medicarpin (**4**)[15], 3-hydroxy-9- methycoumestan (**5**)[13].

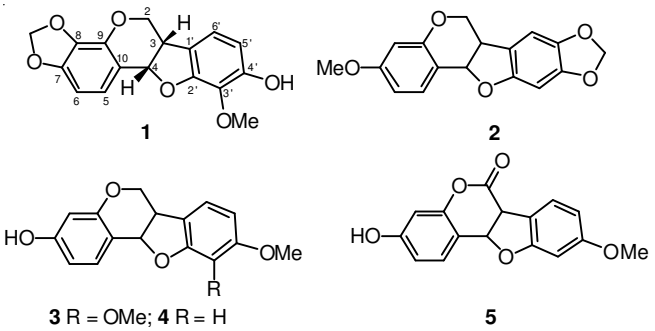

Fig. 1. Structures of pterocarpans from the *C. urticifolium*

Compound **1** was obtained as a pale yellow gum with $[\alpha]^{D}_{24.8}$ + 247 (c 0.020, MeOH). The absorption bands accounting for hydroxyl (3387 cm^{-1}) and aromatic groups (1612, 1535, 1483, 1428 cm^{-1}) could be observed in its IR (infrared) spectrum. The UV spectrum of **1** showed maximum absorption at 345, 296 and 210 nm which confirmed the existence of the aromatic functions. Its molecular formula was established by the negative mode HRESIMS (high-resolution electron spray ionization mass spectra) peak at m/z 337.0693 $[M + Na]^+$ (calcd. (%) 337.0688 for $C_{17}H_{14}NaO_6$). The ^1H NMR spectrum suggested a pterocarpan structure due to the splitting pattern of the protons at δ_H 4.31 (dd, J = 4.6, 10.5 Hz, H-4a), δ_H 3.55 (t, J = 10.5 Hz, H-2β), δ_H 3.48 (m, H-3) and δ_H 5.48 (d, J = 6.6 Hz, H-4), related to the protons of the heterocyclic ring B. This spectrum also allowed the identification of two pairs of *ortho* situated aromatic protons at δ_H 6.99 (d, J = 8.5 Hz, H-5), δ_H 6.61 (d, J = 8.5 Hz, H-6), δ_H 6.54 (d, J = 8.1 Hz, H-5') and δ_H 6.72 (d, J = 8.1 Hz, H-6') and gave a clear evidence of the 7,8,3',4'-substitution pattern of the pterocarpan moiety[16]. In addition, one methylenedioxy group signals (δ_H 5.89, 5.91 s) and one methoxy group signal (δ_H 3.80) was also observed. All these data were supported by the ^{13}C and DEPT spectrum that revealed 17 carbon atoms corresponding to one methyl, two methylene, six methines and eight non-hydrogenated carbons (Table-1). However, the confirmation of the above suggestion for **1** was supported by the HSQC and HMBC (Fig. 2) experiments, which allowed the unequivocal assignments of its ^{13}C and ^1H NMR data. The assignments of the position of the methoxy group at C-3' was defined in the HMBC spectrum that showed cross-peaks of the methoxyl proton at δ_H 3.80 (-OMe) with the carbon at δ_C 140.6 (C-4'). The methylenedioxy group located at C-7 and C-8 was supported by the HMBC correlations of methylenedioxyl proton at δ_H 5.89, 5.91 (-OCH_2O-) with the carbon at δ_C 146.9 (C-7) and δ_C 133.4 (C-8). Since the positions of the methoxy group and methylenedioxy group was determined, a hydroxy group should be located at C-4' to support the tetrassubstituted aromatic B-ring.

It is well known from the literature that, according to biogenetical regulations, the hydrogens (H-3 and H-4) at the B/C rings junction of all natural pterocarpans are always *cis*, either α, α or β, β, thus leading to only two enantiomeric forms. It is also known, through CD (circular dichroism) and/or ORD (optical rotatory dispersion) analyses, that (-) optical rotation can be associated with α,α positioning (3R, 4R), while the (+) optical rotation can be associated with the β,β-positioning

TABLE-1
^1H NMR AND ^{13}C NMR DATA OF COMPOUND **1** (OBTAINED IN CD_3OD)

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
2α	68.0 t	4.31, dd, *J* = 4.6, 10.5	10	114.0 s	–
2β	68.0 t	3.55, t, *J* = 10.5	1'	122.9 s	–
3	42.0 d	3.48 m	2'	147.0 s	–
4	80.9 d	5.48, d, *J* = 6.6	3'	140.6 s	–
5	121.9 d	6.99, d, *J* = 8.5	4'	144.0 s	–
6	110.2 d	6.61, d, *J* = 8.5	5'	106.9 d	6.54, d, *J* = 8.1
7	146.9 s	–	6'	115.8 d	6.72, d, *J* = 8.1
8	133.4 s	–	-OCH_2O-	101.2 t	5.89, 5.91 s
9	145.8 s	–	-OMe-3'	61.2 q	3.80, s

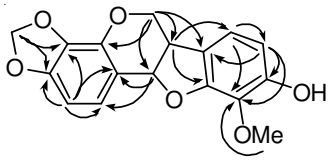

Fig. 2. Selected HMBC (↷) correlations of **1**

(3S, 4S) of both series[17,18]. From the (+) optical rotation of compound **1**, it could be assumed an (3S, 4S) absolute configuration for it. As expected, the CD spectrum of **1** should a similar profile of that from (+)-pterocarpin and almost a mirror image of (-)-maackiain, what is in agreement with the suggested (3S, 4S) absolute stereochemistry for compound **1**. Thus, the structure of **1** was determined as 3(S),4(S)-3'-methoxy-4'-hydroxy-7,8,-methylenedioxylpterocarpan.

Since certain of the phenolic compounds exhibit potential cytotoxicity[19-23], the compound **1** was tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously[24]. Taxol was used as the positive control. The results revealed that compound **1** showed high cytotoxicity against PC3 cell with IC_{50} values of 3.5 μM and moderate cytotoxicities with IC_{50} valves 5-10 μM for other four tested cell lines.

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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, A. Santos-Guerra and J. Francisco-Ortega, *Syst. Bot*., **29**, 702 (2004).
3. A. Yamamoto, H. Suzuki, T. Miyase, A. Ueno and 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 and E. Chukeatirote, *Food Chem. Toxicol.*, **48**, 2754 (2010).
8. Q.F. Hu, H.X. Mu, H.T. Huang, H.Y. Lv, S.D. Li and G.Y. Yang, *Chem. Nat. Compd*., **48**, 198 (2012).
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. D.Q. Yu and J.S. Yang, Handbook of Analytical Chemistry, Nuclear Magnetic Resonance spectroscopy, Chemical Industry Press, Beijing, edn 2, vol. 7, p. 323 (1999).
14. G.F. Spencer, B.E. Jones, R.D. Plattner, D.E. Barnekow, L.S. Brinen and J. Clardy, *Phytochemistry*, **30**, 4147 (1991).
15. A.A. Chalmers, G.J.H. Rall and M.E. Oberholzer, *Tetrahedron*, **33**, 1735 (1977).
16. X.Y. Zeng, W. Zhao, T. Zhang, L. Wang, G.Y. Yang, Y.K. Chen, Q.F. Hu and M.M. Miao, *Phytochem. Lett*., **5**, 238 (2013).
17. P.A. Veloso, A.T.A. Pimenta, S. Francisco M. de, M.J.C. Falcão, N.V. Gramosa, J.N. Silva Junior, E.R. Silveira and M.A.S. Lima, *J. Braz. Chem. Soc*., **23**, 1239 (2012).
18. T. Miyase, A. Ohtsubo, A. Ueno, T. Noro, M. Kuroyanagi and S. Fukushima, *Chem. Pharm. Bull. (Tokyo)*, **30**, 1986 (1982).
19. 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).
20. Y. Zhang, Q. Hu, X. Gao, L. Yang, L. Shu and Y. Shen, *Heterocycles*, **85**, 1925 (2012).
21. Q.F. Hu, X.S. Li, H.T. Huang, H.X. Mu, P.F. Tu and G.P. Li, *Helv. Chim. Acta*, **95**, 349 (2012).
22. 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. (Tokyo)*, **59**, 1421 (2011).
23. D.R. Mou, W. Zhao, T. Zhang, W. Liu, G.Y. Yang, Y.K. Chen, Q.F. Hu and M.M. Miao, *Asian J. Chem*., **25**, 6983 (2013).
24. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).