HETEROCYCLES, Vol. 65, No. 3, 2005, pp. 633 - 636
Received, 14th October, 2004, Accepted, 27th January, 2005, Published online, 1st February, 2005

TWO NEW ALKALOIDS FROM *DENDROBIUM CHRYSANTHUM*

Li Yang, Chaofeng Zhang, Hong Yang, Mian Zhang, Zhengtao Wang,* and Luoshan Xu

Department of Pharmacognosy, China Pharmaceutical University, Naning 210038, P. R. China E-mail: wangzht@hotmail.com

Abstract –Two new alkaloids, *trans*- and *cis*-dendrochrysanines (**1** and **2**) were isolated from the stems of *Dendrobium chrysanthum* Wall. and their structures were identified as (2*S*)-*N*-*trans*-cinnamoyl-2-oxopropyrrolidine (**1**) and (2*S*)-*N*-*cis*-cinnamoyl-2-oxopropyrrolidine (**2**), respectively, on the basis of spectroscopic methods.

INTRODUCTION

The stems of *Dendrobium* species (Orchidaceae) are used in traditional Chinese medicine for antipyretic, eyes-benefiting, immunoregulatory etc.[1] Our previous phytochemical investigations of this genus including *D. candidum, D. fimbriatum, D. chrysotoxum, D. moniliforme, D. nobile.* and *D.thyrsiflorum* have shown the presence of alkaloids, bibenzyls, phenanthrenes, fluorenones and sesquiterpenoids and so on.[2] *D. chrysanthum* is an abundant species distributed in south China and recorded in Chinese Pharmacopoeia (2000 Edition). We have reported three bibenzyls, two fluorenones and other four compounds from the acetyl ester extracts of this plant.[2] The present note deals with the isolation and identification of two new pyrrolidine-type alkaloids (**1** and **2**) from the $CHCl_3$ soluble fraction of *D. chrysanthum* Wall.

RESULTS AND DISCUSSION

Compound (**1**) was obtained as viscous oil. The molecular formula of **1** was assigned as $C_{16}H_{19}NO_2$, from HREIMS spectrometry (m/z 257.1440, calcd 257.1462). In the ^{13}CNMR (DEPT) spectra of **1**, 16 carbon signals were observed as one methyl, four methylenes, eight methines and three quaternaries (**Table I**). In the ^{1}HNMR spectrum of **1**(**Table I**), the signals at δ 1.99 (m, 2H), 3.65 (m, 2H), 2.12 (m, 1H), 1.76 (m, 1H), 4.52 (m, 1H) indicated a 2-substituted pyrrolidine ring,[3] and the signals at δ 7.52 (m, 2H), 7.33(m,

3H), 7.68(d, 1H, J=15.4 Hz), 6.71(d, 1H, J=15.4 Hz), together with the carbon signals at δ_C 164.5 for carbonyl, δ_C 141.7 and 118.7 for *trans*-vinyl carbons, and δ_C 134.9, 127.6, 128.5, 129.4, 128.5 and 127.6 ppm for phenyl suggested a *trans*-cinnammoyl substitution in **1**,[3] which was also supported by the EI-MS peak at m/z 131. An acetyl can be also deduced from the signals at δ 2.19(s, 3H) and δ_C 206.9 ppm. In order to establish the linking position and to assign the structure unambiguously, NMR spectral experiments including ^{1}H-^{1}H COSY, HMQC and HMBC were performed. The 2-substituted pyrrolidine ring was more proved according to the correlation in its ^{1}H-^{1}H COSY spectrum between H-2 (δ 4.52) and H-3a, 3b (δ 2.12, 1.76); between H-4 (δ 1.99) and H-3a, 3b; between H-5 (δ 3.65) and H-4; between H-2 and H-1'"a, 1'"b(δ 3.26, 2.46)(**Figure 2**). In HMBC spectrum, the crosspeaks between C-1'" and H-2, H-3'" (s, 3H, 2.19), C-2'" (206.9) and H-1'" a, 1'" b indicated a 2-oxopropyl substitution at the second position of the pyrrolidine ring (**Figure 2**), which also can be proved by the EI-MS ion peak at m/z 126, 112, 84 and 70. Through extensive analysis of the HMBC spectrum, the correlations between C-1' (164.5 ppm) and H-2, H_2-5 suggested that the cinnammoyl connected to the nitrogen (**Figure 2**). As the result of above analysis, compound (**1)** was deduced as *E*-1-[2-(2-oxopropyl)pyrrolidin-1-yl]-3-phenylpropenone. The NMR spectral assignments of **1** were thoroughly carried out on the basis of 2D NMR (^{1}H-^{1}H COSY, HMQC and HMBC) experiments.

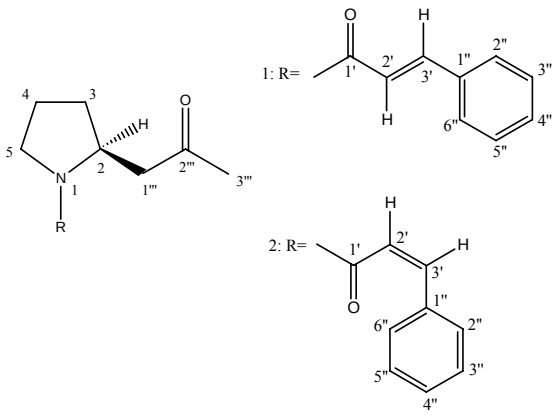

Figure 1 The structure of compounds (**1-2**)

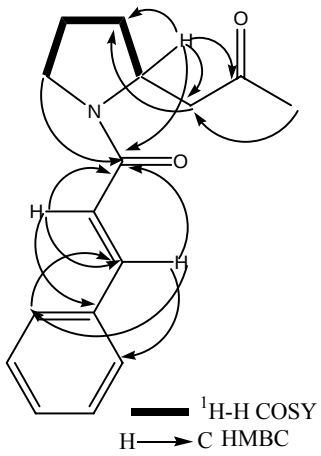

Figure 2 The key ^{1}H-^{1}H COSY and HMBC

Compound (**2**)**,** viscous oil, $[\alpha]_D^{20}$ –17.7°(c 3.5, chloroform). It had the same molecular formula $C_{16}H_{19}NO_2$ with **1**, from ESI-MS m/z 258, $[M+H]^-$ and gave very similar ^{1}HNMR and ^{13}CNMR spectra to **1**. The significant differences were the signals of *cis*-ethylene at δ 6.64 (d, 1H, J=12.5 Hz) and 6.02 (d, 1H, J=12.5 Hz) instead of the *trans*-ethylene at δ 7.68 (d, 1H, J=15.4 Hz) and 6.71 (d, 1H, J=15.4 Hz) of **1** in the ^{1}HNMR spectra (**Table 1**).

Table 1 ^{1}H and ^{13}C NMR spectral data for **1** and **2** in $CDCl_3$[a]

No.	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H
2	53.6 (d)	4.52 (m)	54.8 (d)	4.42 (m)
3	30.0 (t)		32.2 (t)	
3a		2.12 (m)		2.06 (m)
3b		1.76 (m)		1.57 (m)
4	23.7 (t)	1.99 (m, 2H)	25.5 (t)	1.72 (m, 2H)
5	46.7(t)	3.65 (m, 2H)	48.1 (t)	3.30 (m, 2H)
1'	164.5 (s)	/	168.6 (s)	/
2'	141.7 (d)	6.71 (d, 15.4)	135.4 (d)	6.02 (d, 12.5)
3'	118.7 (d)	7.68 (d, 15.4)	126.0 (d)	6.64 (d, 12.5)
1"	134.9 (s)		137.0 (s)	
2",6"	127.6 (d)	7.52 (m, 2H)	127.6 (d)	7.40 (m, 2H)
3",5"	128.5 (d)	7.33 (m, 2H)	130.1 (d)	7.30 (m, 2H)
4"	129.4 (d)	7.33 (m)	131.1 (d)	7.30 (m)
1'"	46.8 (t)		48.9 (t)	
1'"a		3.26 (dd, 14.6, 9.5)		3.11 (dd, 14.8, 9.6)
1'"b		2.46 (dd, 14.6, 6.0)		2.37 (dd, 14.8, 6.6)
2'"	206.9 (s)	/	208.5 (s)	/
3'"	29.9 (q)	2.19 (s, 3H)	31.9 (q)	2.17 (s, 3H)

a ^{1}H and ^{13}CNMR spectra were obtained at 300 and 75 MHz, respectively, at room temperature. Coupling constants were presented in Hz, unless otherwise indicated, all proton signals integrate to 1H.

The absolute configuration of **1** and **2** has been established by comparing their optical rotations, the CD curve and the NMR spectral data with those of known compounds, *N-cis*-cinnamoyl-L-prolinol and *N-cis*-cinnamoyl-L-2-methylpyrrolidine. [3] In the CD spectral investigation, **1** gave a positive signed Cotton effect at 298 nm (Δε+2.83) and a negative Cotton effect at 261 nm (Δε -8.80) , while **2** showed a positive signed Cotton effect at 300 nm (Δε +2.30) and a negative Cotton effect at 267 nm (Δε -8.27). It follows from the similarities of these data that the four compounds have the same absolute configuration. Thus, **1** was identified to be (2*S*)-*N-trans*-cinnamoyl-2-oxopropyrrolidine, and named as *trans*-dendrochrysanine, accordingly, **2** was identified as (2*S*)-*N-cis*-cinnamoyl-2-oxopropyrrolidine, and named as *cis*-dendrochrysanine.

Compounds (**1**) and (**2**) were tested for immunoregulatory activity and showed no significant activity on mRNA expression of TNFα, Il8, IL10, NOS1 and phosphorylation of P38 MAPK in abdominal macrophage of mice.

EXPERIMENTAL

General Experimental Procedures. Column chromatography (CC): silica gel, 200-300 mesh. TLC: Precoated silica GF_{254} plates: detection at 254 nm, and by a modified Dragendorff reagent. Optical rotations were determined on Horiba SEPA-300 polarimeter. NMR spectra were recorded on a Bruker ACF-300 instrument with $CDCl_3$ as solvent. The EIMS and HREIMS were carried out on a HP 5989A

spectrometer. The ESIMS were detected on a Agilent 1100 MSD, with a negative ion mode.

Plant material. *D. chrysanthum* was collected in Yunnan province, P. R. China in August 2002, and authenticated by Prof. Luoshan Xu, Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. DC-YN0208-1) is deposited at Herbarium of China Pharmaceutical University.

Extraction and Isolation. The air-dried stems of *Dendrobium chrysanthum* (10 kg) were cut into small pieces and extracted with 95 % EtOH under reflux (3×50 L) for 3 h each. After removal of solvent *in vacuo*, the extract (500 g) was suspended in 0.1 mol/L HCl and extracted with EtOAc and n-BuOH successively to remove the non-alkaloids. Then after adding NaOH to the residue (120 g) until pH 10, the solution was partitioned with $CHCl_3$. The $CHCl_3$ extract (45 g) was washed with H_2O to pH 7 and subjected to repeated silica gel column chromatography eluting with CH_2Cl_2- EtOAc (100:1 to 0:1, each 500 mL). The fractions (3.6 g) elucidated with CH_2Cl_2-EtOAc (8:2) were further purified by column chromatography with CH_2Cl_2- EtOAc (85:15) to give compounds (**1)** (56 mg) and (**2)** (20 mg).

(2*S*)-*N*-*trans*-Cinnamoyl-2-oxopropyrrolidine (**1**), viscous oil, CD λ_{max} (MeOH) nm ($\Delta\varepsilon$), 298.5 (+2.84), 261.3 (-8.80); $[\alpha]_D^{20}$ -19.2°(c 3.4, chloroform). ESIMS m/z 258, $[M+H]^-$; EIMS *m/z* (%): 257 $[M]^+$ (4), 214 (3), 149 (2), 131 (100), 126 (30), 112(1), 103 (48), 84 (33), 77 (28), 70 (14), 43 (27); HREIMS m/z 257.1440 (calcd for $C_{16}H_{19}NO_2$ 257.1462), ^{1}HNMR and ^{13}CNMR spectral data see **Table 1**.

(2*S*)-*N*-*cis*-Cinnamoyl-2-oxopropyrrolidine (**2**), viscous oil, CD λ_{max} (MeOH) nm ($\Delta\varepsilon$), 300 (+2.30), 267.2 (-8.27); $[\alpha]_D^{20}$-17.7° (c 3.5, chloroform); ESIMS m/z 258,$[M+H]^-$. ^{1}HNMR and ^{13}CNMR spectral data see **Table 1**. The purities of **1** and **2** (>98%) were tested by HPLC.

ACKNOWLEDGEMENTS

This research was financially supported by the Natural Science Foundation of China (NSFC) for Prof. Luoshan Xu (No.30171144).

REFERENCES (AND NOTES)

1. The State Pharmacopoeia Commission of the People's Republic of China. 'Pharmacopoeia of the People's Republic of China', Vol I, ed. by Chemical Industrial Press, Peking, 2000, p.104.
2. Z. M. Bi, L. Yang, Z. T. Wang, L. S. Xu, and G. J. Xu, *Chin. Chem. Lett.*, 2002, **13**, 535; Z. M. Bi, Z. T. Wang, L. S. Xu, and G. J. Xu, *Acta Pharm. Sin.,* 2003, **38**, 526; Z. M. Bi, Z. T. Wang, and L. S. Xu, *Acta Bot. Sin.*, 2004, **46**, 124; H. Yang, G. X. Chou, Z. T. Wang, Z. B. Hu, and L. S. Xu, *J. Asian Nat. Prod. Res.,* 2004, **6**, 35; L. Yang, Y. Wang, Z. M. Bi, P. Lin, Z. T. Wang, and L. S. Xu, *Chin. J. Nat. Med.,* 2004, **2**, 280.
3. U. Ekevåg, M. Elander, L. Gawell, K. Leander, and B. Lüning, *Acta Chem. Scand.,* 1973, **27**, 1982.
4. L. Witte, K. Müller, and H. A. Arfmann. Planta Medica, 1987, **53**,1 92