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Pterocarine, a New Diarylheptanoid from *Pterocarya tonkinesis*, its Cell Cycle Inhibition at G_0/G_1 Phase and Induction of Apoptosis in HCT-15 and K562 Cells

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Abstract: Pterocarine (**1**), a new diarylheptanoidal compound, was isolated from *Pterocarya tonkinesis* (Franch.) Dode. together with a known diarylheptanoid, myricatomentogenin (**2**), through a bioassay-guided fractionation procedure. The structure of **1** was elucidated as (+)-3', 4''-epoxy-1-(4'-hydroxyphenyl)-7-(3''-hydroxyphenyl)-heptane-3-one by the spectroscopic methods. Pterocarine (**1**) inhibited the proliferation of tsFT210, HCT-15 and K562 cells with the inhibition rates of 20.2±2.4, 23.8±2.4 and 50.5±1.2% at 100 μg/mL, respectively. Flow cytometric analysis indicated that **1** could inhibit the cell cycle of tsFT210, HCT-15 and K562 cells at the G_0/G_1 phase and could also induce apoptosis in HCT-15 (19%) and K562 (11%) cells.

Keywords: Pterocarine, diarylheptanoid, phenolic compound, structure, cell cycle inhibitor, apoptosis inducer, *Pterocarya tonkinesis*, Juglandaceae.

Pterocarya tonkinesis (Franch.) Dode. (Juglandaceae) forms a typical community of riverside season rain forest in Xishuangbanna area of Yunnan province[1]. The same genus plant *P. stenoptera* C. DC., has long been used as a traditional Chinese herbal medicine[2] and *P. tonkinesis*, itself, is also used as a folk medicine for the treatment of certain cancers in partial area of China. However, no report had so far been seen on the chemical constituents and their biological activities of the title plant yet.

In the course of our screening for new anticancer agents from natural resources[3,4], we found that the alcoholic extract of *P. tonkinesis* significantly induced apoptosis in tsFT210 cells and inhibited the cell cycle at the G_0/G_1 phase. From the extract, we have isolated a new diarylheptanoidal compound, named pterocarine(**1**), together with a known diarylheptanoid, myricatomentogenin[5](**2**). In this communication, the isolation, structure determination and brief biological assay of **1** were described.

The following separation procedure was monitored by the same activity detected in the preliminary screening test. The air-dried stems and barks (3.5 kg) of *P. tonkinesis*, collected at Xishuangbanna area in Yunnan province, China, were extracted with 60%

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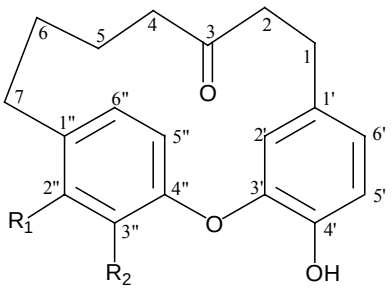
aqueous alcohol (10 L) at room temperature. The alcoholic solution was evaporated under the reduced pressure to remove alcohol. The remained water suspension (2 L) was extracted with the same volume of chloroform to obtain an active chloroform extract (10.4 g). The chloroform extract was successively chromatographed on silica gel and Sephadex LH-20 columns and then on preparative TLC plates to give **1** (5.2 mg) and **2** (2.0 mg).

Pterocarine **1**, white amorphous solid, $[\alpha]_D^{27}$ +60.5 (*c* 1.0, $CHCl_3$), showed positive color reaction with the ferric chloride reagent, indicating that **1** is a phenolic compound. It gave *quasi*-molecular ion $[M+H]^+$ peak at *m/z* 313 in the positive TOF-MS and its molecular formula, $C_{19}H_{20}O_4$, could be determined by HR-TOF-MS measurement (calcd. for $C_{19}H_{21}O_4$ $[M+H]^+$ 313.1440, found 313.1452). It showed maximum UV absorptions at 281.5 (log ε 3.42), 222.5 (sh, 3.95) and 204.5 nm (4.37, the end absorption), ascribed to substituted aromatic rings. The IR spectrum (KBr) suggested the presence of hydroxyl groups (3404 and 1269 cm^{-1}), carbonyl group (1708 cm^{-1}) and aromatic rings (3032, 1597, 1517 and 1503 cm^{-1}).

The ^1H and ^{13}C NMR spectra of **1** in $CDCl_3$ (**Table 1**) including PFG ^1H-^1H COSY, DEPT and PFG HMQC, indicated the presence of two hydroxyl (δ_H 5.75, s, 3"-OH; 5.81, s, 4'-OH), one ketocarbonyl (δ_C 210.47, s, C-3) and two linear carbon chain groups, $–CH_2$-CH_2-CH_2-$CH_2–$ and $–CH_2$-$CH_2–$, together with two 1,3,4-trisubstituted benzene rings in **1**. The two 1, 3, 4-trisubstituted benzene rings could be confirmed without any doubt by the HMBC spectrum (**Table 1**).

Then, the connectivity of the above structural moieties in **1** could be determined by the HMBC correlations and NOE's (**Table 1**). The correlations between H-1, H-2 and H-4 and C-3 in the PFG HMBC spectrum established a linear seven-carbon chain, $–CH_2$-CH_2-CH_2-CH_2-CO-CH_2-$CH_2–$. The two benzene rings linked to two terminal carbons of the seven-carbon chain according to the HMBC correlations between C-1' and H-2 and between C-1" and H-6. The other related HMBC correlations are summarized in **Table 1**. Then the NOE correlations between H-2' and H-5" and between H-2' and 3"-OH in the PFG NOESY experiment, evidenced that the ether linked between C-3' and C-4" and showed the stereo-relationship between two benzene rings. Thus, the structure of **1** could be eventually determined as (+)-3', 4"-epoxy-1-(4'-hydroxyphenyl)-7-(3"-hydroxyphenyl)heptane-3-one[6] as shown in **Figure 1**.

Figure 1 The structures of compounds **1** and **2**



1 R_1=H, R_2=OH **2** R_1=OH, R_2=OCH_3

Table 1 600 MHz ^{1}H and 150 MHz ^{13}C NMR data for **1** in $CDCl_3$[a]

Positions	δ_H (J in Hz)	δ_C	^{1}H-^{1}H COSY	HMBC
1	2.82 AB type	27.26 t	H-1[b)]/H-2	H-1/C-2, 3, 1', 2', 6'
	2.87 AB type			
2	2.28 ddd (16.7, 8.3, 2.7)	41.12 t	H-2/H-1	H-2/C-1, 3, 1'
	2.36 ddd (16.7, 8.8, 2.8)			
3	——	210.47 s		
4	1.82 AB type	46.43 t	H-4[c)]/H-5	H-4/C-3, 5, 6
	1.89 AB type			
5	1.56 (2H) m	18.99 t	H-5/H-4, 6	H-5/C-4, 6, 7
6	1.64 m	27.22 t	H-6/H-5, 7	H-6/C-4, 5, 7, 1"
	1.68 m			
7	2.67 AB type	35.60 t	H-7[d)]/H-6	H-7/C-5, 6, 1", 2", 6"
	2.71 AB type			
1'	——	133.95 s		
2'	5.57 d (2.2)	112.51 d	H-2'/H-6'	H-2'/C-1, 1', 3', 4', 6'
3'	——	146.77 s		
4'	——	142.87 s		
5'	6.83 d (8.0)	115.57 d	H-5'/H-6'	H-5'/C-1', 3'
6'	6.64 dd (8.0, 2.2)	122.75 d	H-6'/H-2', 5'	H-6'/C-1, 2', 4', 5'
1"	——	140.58 s		
2"	6.94 d (1.9)	117.95 d	H-2"/H-6"	H-2"/C-7, 1", 3", 4", 6"
3"	——	148.79 s		
4"	——	140.61 s		
5"	6.89 d (8.0)	123.41 d	H-5"/H-6"	H-5"/C-1", 3"
6"	6.82 dd (8.0, 1.9)	122.88 d	H-6"/H-2", 5"	H-6"/C-7, 2", 4"
4'-O<u>H</u>	5.79 br s	———		
3"-O<u>H</u>	5.71 br s	———		

a) Signal assignments were based on the results of DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectroscopy. b) H-1 showed long-range correlations with H-2' and H-6' in the PFG ^{1}H-^{1}H COSY. c) H-4 showed W-form long-range correlation with H-6 (δ1.68) in the PFG ^{1}H-^{1}H COSY. d) H-7 showed long-range correlation with H-2" in the PFG ^{1}H-^{1}H COSY.

Pterocarine **1** inhibited the proliferation of tsFT210, HCT-15 and K562 cells with the inhibition rates of 20.2±2.4, 23.8±2.4 and 50.5±1.2% at the concentration of 100 μg/mL, respectively (by SRB method[7]). Flow cytometric analysis[3-4] indicated that **1** could inhibit the cell cycle of tsFT210, HCT-15, and K562 cells mainly at the G_0/G_1 phase and could also induce apoptosis in HCT-15 (19%) and K562 (11%) cells. Several diarylheptanoids and their biological activities have been reported[5, 8-10], however, **1** is a new member of this class of compounds and provides the first example of diarylheptanoids possessing the cell cycle inhibitory and apoptosis inducing activities.

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6. The ^{13}C NMR data of **1** closely resembled those of galeon, a 3"-monomethoxy derivative of **1**, except for the lack of a methoxy signal in **1** and the noticeable downfield shift of C-3" (+3.5 ppm) and upfield shifts of C-2" (-2.9 ppm) and C-4" (-2.4 ppm) in galeon (see reference 5), which coincided well with the methylation-induced shifts of the phenolic α and *ortho* carbons (see E. Breitmaier, W. Voelter, *Carbon-13 NMR Spectroscopy: High Resolution Methods and Application in Organic Chemistry and Biochemistry*, Third Completely Revised Edition, VCH Verlagsgesellschaft, Weiheim, Germany, **1987**, p451).
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