Three Monoterpenoids Eucommiol from The Leaves of *Eucommia Ulmoides* Protect against Neurotoxicity in PC12 Cells

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Abstract—One new monoterpene compound 4'-acetyl-eucommiol (1) and two known compounds eucommiol(2), 1-deoxyeucommiol(3) were isolated from the leaves of *Eucommia ulmoides*. The structures of the new monoterpene eucommiol were elucidated by means of chemical evidence and 1D-and 2D-NMR (^1H , ^{13}C , HSQC, HMBC, ^1H - ^1H COSY and NOESY) spectroscopic analysis and HR-ESI-MS. We also investigated the protective effect of compounds 1-3 on the neurotoxicity of PC12 cells induced by amyloid-beta ($\text{A}\beta_{25-35}$), respectively. As a result, compounds 1 and 2 were seen to afford protection against $\text{A}\beta$ -induced toxicity in PC12 cells.

Keywords—leaves of *Eucommia ulmoides*; Cyclohexenone; Eucommiol; PC12

I. INTRODUCTION

The leaves of *Eucommia ulmoides* Oliv.(Eucommiaceae) are widely used as a traditional Chinese medicine in China for its anti-aging, antioxidant, lowering blood pressure, reducing blood lipids and regulate blood sugar level[1]. In the previous papers, the chemical constituents from the leaves of *Eucommia ulmoides* Oliv. are reported to be phenylpropanoids[2], flavonoids[3], iridoids[4], and lignans[5], et al. This paper deals with the isolation and structural elucidation of one new monoterpene compound 4'-acetyl-eucommiol (1) and two known compounds eucommiol(2), 1-deoxyeucommiol(3)[6], from the 60% ethanol elution parts of the leaves of *Eucommia ulmoides* Oliv. The PC12 cell line, derived from rat pheochromocytoma, displays phenotypic characteristics of sympathetic neurons. The cells were grown in the presence of various toxins mimicking the conditions taking place in neurodegenerative diseases, including amyloid-beta ($\text{A}\beta$, the peptide composing the amyloid plaques in brains of AD patients). In this paper, we also investigated the protective effect of compounds 1-3 on the neurotoxicity of PC12 cells induced by amyloid-beta ($\text{A}\beta_{25-35}$), respectively. As a result, compounds 1 and 2 were seen to afford protection against $\text{A}\beta$ -induced toxicity in PC12 cells. This study will contribute to revealing the chemical basis for the therapeutic effect of *Eucommia ulmoides* Oliv.

II. EXPERIMENTAL

A. General

HR-ESI-MS were recorded in a Finnigan-LC-QDECA. NMR spectra were recorded on Bruker AM-400. TLC

employed precoated silica gel plates (5-7 μm , Qingdao Haiyang). For column chromatography, silica gel (H, 100-200 and 200-300 mesh, Qingdao Haiyang). HPLC was performed on a waters 515 instrument equipped with a water-UV-2489 detector. A YMC-pack ODS-A (10 $\times$ 250 mm, i.d.) column was used for preparative purpose. Compounds were visualized either under UV light or by spraying with 10% H_2SO_4 followed by heating at 100 $^\circ\text{C}$ for 5-10 min. PC12 cells obtained from Institute of biochemistry and cell biology (Shanghai, China) were grown in Dulbecco's modified Eagle's medium (DMEM) (Hyclone, NRH0020), supplemented with 5% fetal bovine serum and 1% antibiotic mixture comprising penicillin-streptomycin, in a humidified atmosphere at 37 $^\circ\text{C}$ with 5% CO_2 in CO_2 incubator (SANYO, Electric Co., Ltd. Japan). Microplate reader (Biotek, ELx800, America) was used to determine the cell viability.

B. Plant Material

The leaves of *Eucommia ulmoides* Oliv were collected from Zhangshu City of Jiangxi province, People's Republic of China in April 2014 and identified by prof. Yueming Zuo of Jiangxi University of Traditional Chinese Medicines. The voucher specimen (JZ20140406) was deposited at College of Pharmacy, Jiangxi University of Traditional Chinese Medicines, China.

C. Extraction and Isolation

The leaves (26kg) of *Eucommia ulmoides* Oliv were successively extracted 4 times with 70% ethanol (each 208L $\times$ 4) under reflux for 2 h. The ethanol extracts were combined and evaporated under reduced pressure to afford crude extracts (4.704kg) and the residue (3.677kg) was scattered and adsorbed by AB-8 macroporous adsorption resin. In accordance with the water elution, 30% ethanol elution, 60% ethanol elution, 95% ethanol elution to give the corresponding water elution parts (1778g), 30% ethanol elution parts (390g), 60% ethanol elution parts (570g) and 95% ethanol elution parts (320g). The 60% ethanol elution parts (470g) was applied to a silica gel column using a CHCl_3 -MeOH (100:1 to 0:1) gradient solvent system to give 48 fractions (Fr.1-48). Fr.6 was separated by CC (silica gel; CHCl_3 /MeOH 20:1 to 8:1) and preparation of high performance liquid chromatography (MeOH/ H_2O 1:1) to afford compounds 1 (5 mg), 2(15 mg), 3(10mg).

(1) 4'-acetyl-eucommiol

a colorless oil.

^1H and ^{13}C NMR (DMSO- d_6): Table I.

Positive EIS-MS: m/z 253 $[\text{M}+\text{Na}]^+$, HR-ESI-MS: m/z $[\text{M}+\text{Na}]^+$ 253.0632($\text{C}_{11}\text{H}_{18}\text{O}_5$).

III. RESULTS AND DISCUSSION

Compound 1, $\text{C}_{11}\text{H}_{18}\text{O}_5$ (M^+ at m/z $[\text{M}+\text{Na}]^+$ 253.0632), a colorless oil. The ^1H - and ^{13}C -NMR spectra of compound 1 revealed the presence of a double bond group [δ_{C} : 129.23(s, C-3) and 142.13(s, C-4)], four oxygenated methylenes [δ_{H} : 4.57(2H, d, $J=12.8$ Hz, H-4'), δ_{C} : 59.95(s, C-4'); δ_{H} : 4.08(1H, d, $J=13.2$ Hz, H-3'a)/3.89(1H, d, $J=13.2$ Hz, H-3'b), δ_{C} : 55.60(s, C-3'); 3.92(1H, m, H-1), δ_{C} : 74.33(s, C-1); 3.46(2H, m, H-2''), δ_{C} : 62.43(s, C-2'')], three methylenes [δ_{H} : 2.58(1H, m, H-5a)/2.14(1H, m, H-5b), δ_{C} : 42.42 (s, C-5); δ_{H} : 2.53(2H, m, H-2), δ_{C} : 52.63(s, C-2); δ_{H} : 1.76(1H, m, H-2'a)/1.27(1H, m, H-2'b), δ_{C} : 34.00(s, C-2')], a acetyl group [δ_{H} : 2.00(3H, s, CH_3CO -); δ_{C} : 20.65(s, CH_3CO -) and 170.28(s, CH_3CO -)]. In the ^1H - ^1H COSY, cross-peaks were observed between 1-H and 2-H, 2-H and 2'-H, 2'-H and 2''-H, 1-H and 5-H. These data suggested that 1 was eucommiol basic structure [6]. In the HMBC experiment, the signals of δ_{H} : 4.57(2H, d, $J=12.8$ Hz, H-4') appeared correlation with δ_{C} : 129.23(s, C-3), 142.13(s, C-4) and 170.28(s, CH_3CO -), prompting an acetyl connection with C-4'. On the basis of the above data, the structure of 1 was determined to be as 4'-acetyl-eucommiol and be as depicted in Table I.

Two known compounds eucommiol(2) and 1-deoxyeucommiol(3)[6], the ^{13}C -NMR spectra be as depicted in Table I.

The neuroprotective effects of compounds 1-3 against $\text{A}\beta_{25-35}$ induced cell death in PC12 cells were assessed using an established MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide) assay. $\text{A}\beta_{25-35}$ induced cytotoxicity($62.76\pm 1.71\%$) in the cells when it was added at a concentration of $20\mu\text{M}$ for 24h. When PC12 cells were preincubated with compounds 1-3, the toxicity of $\text{A}\beta_{25-35}$ was significantly alleviated by compounds 1-3 in a dose-dependent manner (Table II). While compounds I and II showed protective effects on the cell viability.

IV. CONCLUSIONS

We investigated the chemical constituents of *Eucommia ulmoides* Oliv. Based on its activity towards AD for the time and three monoterpenoids Eucommiol were obtained. Their structures were identified as 4'-acetyl-eucommiol (1), eucommiol(2), 1-deoxyeucommiol(3), respectively. As a result, compounds 1 and 2 were seen to afford protection against $\text{A}\beta$ -induced toxicity in PC12 cells. This study will contribute to revealing the chemical basis for the therapeutic effect of *Eucommia ulmoides* Oliv.

ACKNOWLEDGMENT

The authors acknowledge the financial support of the National Natural Science Foundation of China(NSFC) (No.81360632).

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Figure 1. Structures of compounds 1-3

TABLE I. ^1H -NMR(400MHZ) AND ^{13}C -NMR(100MHZ) SPECTROSCOPIC DATA OF COMPOUNDS 1-3(DMSO- D_6)

position.	1		2		3
	$\delta\text{C/pmm}$	$\delta\text{H (J)/pmm}$	$\delta\text{C/pmm}$	$\delta\text{C/pmm}$	$\delta\text{C/pmm}$
1	74.33	3.92, m	74.75		27.98
2	52.63	2.53, m	52.56		42.97
3	129.23		135.43		137.81
4	142.13		137.52		139.74
5	42.42	2.58, m	42.34		36.19
		2.14, m			
2'	34.00	1.76, m	34.24		32.72
		1.27, m			
2''	62.43	3.46, m	57.04		57.12
3'	55.60	4.08, d (13.2)	55.26		55.19
		3.89, d (13.2)			
4'	59.95	4.57, d (12.8)	59.79		59.66
CH ₃ CO-	20.65	2.00, s			
CH ₃ CO-	170.28				

TABLE II. NEUROPROTECTIVE EFFECTS OF COMPOUNDS 1-3 AGAINST $\text{A}\beta_{25-35}$ -INDUCED PC12 CELLS DEATH

Compound	$8\mu\text{M}$	Cell viability (%)	
		**16 μM	*24 μM
1	65.50 $\pm$ 3.34	71.38 $\pm$ 4.14	71.91 $\pm$ 1.90
2	64.71 $\pm$ 1.64	65.45 $\pm$ 3.55	72.40 $\pm$ 3.70
3	63.96 $\pm$ 1.99	66.53 $\pm$ 1.84	69.60 $\pm$ 2.63

Effects of the tested compounds on $\text{A}\beta$ -induced PC12 cell death. Cell viability was measured by MTT assay. Result are expressed as mean $\pm$ SD(n=6) of two independent experiments. The 100% value was obtained from untreated control cells. *Significant difference compared 24 μM with 8 and 16 μM of compounds 1-3(*p<0.01) , **Significant difference compared 16 μM with 8 μM of compounds 1-3(**p<0.05)