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Anticancer Compound of *Paulownia tomentosa*

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Abstract – A cytotoxic compound was purified from the flowers (46 g) of *Paulownia tomentosa* by normal column chromatography. As a result of the structure analysis by spectroscopic methods, the compound was identified as isoatriplicolide tiglate, which shows *in vitro* cytotoxicity.
Key words – *Paulownia tomentosa*, isoatriplicolide tiglate, *in vitro* cytotoxicity.

Introduction

Though *Paulownia tomentosa* is widely distributed in Korea (Lee, 1989), the flower of *P. tomentosa* is a wild herbal medicinal plant which has been used in traditional Chinese medicine for the treatment of bronchial diseases (Yuk, 1997). Research on the cytotoxic constituents of this plant has been insufficient. Present study was performed to investigate the cytotoxic compounds. It was found that cytotoxic activity of the methanol extracts was mainly concentrated in the chloroform-soluble fractions. The activity-guided fractionation, on the basis of the *in vitro* inhibitory activity upon the growth of tumor cell lines (Doyle, 1992; Ryu *et al.*, 1992) and repeated column chromatography afforded cytotoxic compound. One compound was isolated from *P. tomentosa* and identified as isoatriplicolide tiglate by the physicochemical and spectral properties.

Materials and Methods

General – ^{1}H- and ^{13}C-NMR spectra were run at 500 MHz and 125 MHz, respectively, and recorded by Bruker AMX-500 spectrometer. The EI/MS (70 eV) was determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). The UV spectrum was recorded on Shimadzu UV-Visible spectrophotometer. IR spectrum was measured on JASCO FT/IR-5300 Infrared spectrophotometer. Thin layer chromatography was carried out on Merck precoated silica gel F_{254} plates (Merck. 5715). All other chemicals and solvents were analytical grade and used without further purification.

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Plant material – The flowers (46 g) of *P. tomentosa* were collected in June 1998 in Munkyung. The voucher specimen (No. SKKU-9806-17), identified by the Division of Herbology at Kyunggi Pharmaceutical Research Center (KPRC) was preserved in the herbarium of the College of Pharmacy, Sung Kyun Kwan University, Suwon, Korea.

Extraction and isolation – The air-dried plant material (46 g) was extrated with methanol (2 *l*) for 15 days at room temperature and concentrated under pressure below 40°C. The resulting syrupy product (5 g) which was suspended in H_2O was partitioned successively with *n*-hexane (3 g), $CHCl_3$ (0.3 g), EtOAc (0.7 g) and BuOH (0.6 g). Evaluation of cytotoxicity of each fraction revealed that only the chloroform soluble fraction was active and was investigated extensively through serial fractionation by preparative column chromatography followed by the cytotoxicity monitoring, which finally led to the isolation of the following active constituents: The active constituents were subjected to column chromatography over SiO_2 with $CHCl_3$: MeOH (15:1) as solvents to give crystalline compound **1** (2 mg).

***In vitro* cytotoxicity test** – Sulforhodamine-B (SRB) bioassay was used as cytotoxicity screening method. (Boyd, 1989) Activities of fractions were monitored in several concentration level against five kinds of human tumor cell lines; A549, SK-OV-3, SK-Mel-2, XF498 and HCT15.

Results and Discussion

Compound **1** (mp 150°C), with a molecular formula of $C_{20}H_{22}O_6$ (*m/z* 358[M]$^+$) determined by high resolution mass measurement of the molecular ion, possessed an α-methylene-γ-lactone [IR: 1760 cm^{-1}; ^{13}C-NMR:

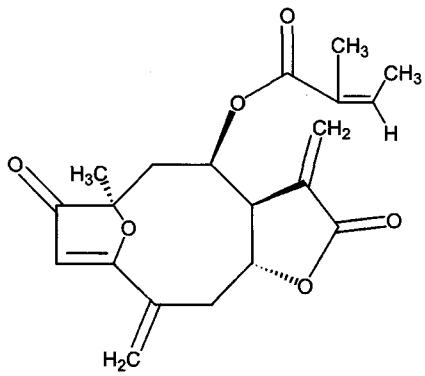

Fig 1. Structure of isoatriplicolide tiglate.

Table 1. ^{1}H- and ^{13}C-NMR chemical shift of compound **1**

Number	Compound **1**	
	^{1}H-NMR (500 mhz)	^{13}C-NMR (125mhz)
1		205.1
2	5.71(*s*)	103.7
3		184.4
4		136.4
5	3.13(*dddd J*=2, 2, 9.6, 14.5) 2.94(*br, d J*=1.5, 4.5)	41.8
6	4.31(*ddd J*=1.5, 5.5, 9.7)	77.3
7	3.58(*dddd J*=2, 2, 3.6, 5.5)	51.7
8	5.24(*ddd J*=2, 3, 5.5)	73.9
9	α 2.68(*dd J*=2, 2, 9.5, 14.7) β 2.27(*dd, J*=3, 5.4)	43.3
10		88.6
11		139.7
12		168.4
13	a 6.34(*d J*=3.5) b 5.68(*d J*=3)	122.1
14	1.48(*s*)	22.4
15	a 5.78(*d J*=2) b 5.63(*d J*=2)	121.0
1'		166.8
2'		127.4
3'	6.68(*qq J*=1.5, 7)	139.0
4'	1.77(*dq J*=1, 1.5)	14.4
5'	1.71(*br, s J*=1, 1.5)	11.7

δ166.8; ^{1}H-NMR: δ5.68 (*d*, *J*=3 Hz), δ6.34 (*d*, *J*=3.5 Hz)]. ^{1}H-NMR spin decoupling experiments located the signal for H-7 (3.58), which was coupled to complex doublet. The signal at δ5.24 (*ddd*, *J*=2, 3, 5.5 Hz) closely resembled the signal in isoatriplicolide tiglate assigned to H-8 (δ5.24, *ddd*, *J*=2, 3, 5.5 Hz). Irradiation at 5.24 in compound **1** simplified two methylene doublet of doublets at δ2.27 and 2.68 to an isolated AB pattern. The signal at δ4.31 was assigned to the proton at the site of lactone ring fusion. Irradiation of δ4.31 altered methylene signal at δ2.94 and 3.13. The proton at δ3.13 was spin coupled to two *exo*-methylene doublets at δ5.63 and 5.78. The structure of compound **1** was determined as isoatriplicolide tiglate on the basis of comparison of NMR spectral data and physical data with those reported in the previous paper. (Gershenzou *et al.*, 1984).

Isoatriplicolide tiglate was evaluated for *in vitro* cytotoxity in five cancer cell lines (A549, SK-OV-3, SK-Mel-2, XF498, HCT15). The *in vitro* cytotoxicity assay was in vestigated according to reference (Boyd, 1989). Isoatriplicolide tiglate showed cytotoxicity against five cancer cell lines: A549 (lung carcinoma, ED_{50} = 1.5 μg/ml), SK-OV-3 (adenocarcinoma, ED_{50} = 0.7 μg/ml), SK-Mel-2 (malignant melanoma, ED_{50} = 0.3 μg/ml), XF498 (central nerve system tumor, ED_{50} = 1.0 μg/ml), HCT15 (colon adenocarcinoma, ED_{50} = 0.2 μg/ml). These have comparative cytotoxic potential with reference adriamycin (ED_{50}=0.1~2.3 μg/ml).

Acknowledgements

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