CYTOTOXIC NAPHTHOQUINONES FROM *ULMUS DAVIDIANA*

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Summary

Seven cytotoxic sesquiterpene *ortho*-naphthoquinones, of which three are new compounds, were isolated from the methanolic extract of the root bark of *Ulmus davidiana*. The structures of these compounds were determined by the analyses of their physico-chemical properties and various spectral data including pulse field gradient two-dimensional NMR spectroscopy. The structures of three new compounds, davidianones A, B and C, were determined as 3-hydroxymethyl-6,9-dimethylnaphtho(1,8-*bc*)pyran-7,8-dione, 6-methoxycarbonyl-3,9-dimethylnaphtho(1,8-*bc*)pyran-7,8-dione and 6-dimethoxymethyl-3,9-dimethylnaphtho(1,8-*bc*)pyran-7,8-dione, respectively. The other four compounds were revealed as known mansonones E, F, H and I, which were isolated for the first time from *Ulmus davidiana*. All these compounds except mansonone H showed strong cytotoxicity against various human cancer cell lines with ED_{50} values of 0.1 - 2.0 μg/ml. They also showed antioxidative activities against rat liver microsomal lipid peroxidation with mansonone F showing the greatest activity.

Introduction

Ulmus davidiana Planch is a deciduous tree which is widely distributed in Korea. The stem and root bark of this species have been used in oriental traditional medicine for the treatment of edema, mastitis, gastric cancer and inflammation [1,2]. As a part of our search for new biologically active substances form traditional medicines [3,4], we isolated seven cytotoxic *ortho*-naphthoquinones from the 80% methanolic extract of the root bark of *Ulmus davidiana*. We report the isolation, physico-chemical properties, structures and biological activities of these compounds.

Materials and Methods

Plant material : The root bark of *Ulmus davidiana* was collected from Mt. Wonhyo, Kyungnam Province, Korea in May 1994, and identified by Prof. Jong-Hee Park, College of Pharmacy, Pusan National University, Korea. Fresh root bark was dried in a dark, well ventilated place. The voucher specimen is deposited in the Herbarium of this college.

Instrumental analysis : ^{1}H and ^{13}C NMR spectra were recorded on a JEOL α-600 spectrometer. The 2-D NMR spectroscopy were carried out at 600.05/150.8 MHz with $^{n}J_{CH}$ = 8.3 Hz for PFG-HMBC and $^{1}J_{CH}$ = 145 Hz for PFG-HMQC, using a pulse field gradient (PFG). EI-MS and HREI-MS spectra were obtained on a Hewlett Packard HP 5989A and a JEOL JMS-HX 110/110A mass spectrometer, respectively. UV-visible (MeOH) and IR spectra (KBr) were recorded on a Shimadzu UV-260 and a Laser Precision Analect RFX-65 spectrometer, respectively.

Cytotoxicity Assay : Seventeen human cancer cell lines and a normal fibroblast cell line were used for the assay of cytotoxicities of the compounds against human cancer cell lines. Cytotoxicity was measured by the sulforhodamine B (SRB) method [5] and the ED_{50} value was calculated by the 50% inhibition of cell growth.

Cytotoxicities of the compounds on primary cultured rat hepatocytes was calculated by the effect of compounds on the DNA synthesis of rat hepatocytes. Rat hepatocytes were isolated by collagenase perfusion method [6]. Hepatocytes were cultured on modified Waymouth AB medium for 3 days at 5% CO_2, 37°C. Inoculum densities were $3x10^5$ cells/ml. Total DNA synthesis was estimated by the radioactivity of incorporated ^3H-thymidine.

Antioxidative activities : Antioxidative activities were evaluated by the inhibitory activities of compounds against lipid peroxidation in rat liver microsomes according to the method of Ohkawa *et al* [7] with minor modifications. Rat liver microsomes were prepared by differential centrifugation method [8]. Reaction was initiated by the addition of 100 μM $FeSO_4 \cdot 7H_2O$ into a mixture of ascorbic acid (0.2 mM) and microsomal suspension (0.5 μg protein/ml). Lipid peroxidation was assessed by measuring the thiobarbituric acid reactive products at 532 nm.

Results and Discussion

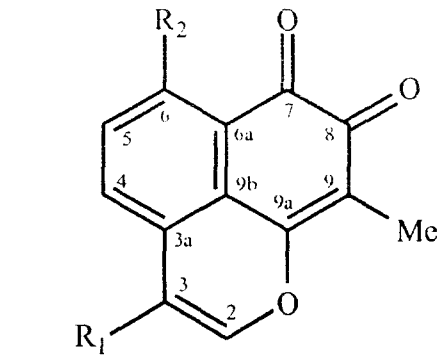
Isolation : The dried root bark (4 kg) was milled and extracted with 80% aqueous methanol at room temperature for 3 days. The extract was concentrated under reduced pressure. The residue (185 g) was subjected to successive extraction with *n*-hexane and $CHCl_3$. The $CHCl_3$ extract (6.4 g) was applied to a silica gel column eluted with $CHCl_3$-MeOH mixtures with

increasing proportion of MeOH. The active fractions were further purified by successive column chromatography on Sephadex LH-20 and C-18 column. By Silica gel prep. TLC and reverse phase HPLC, davidianones A (12.2 mg), B (1.9 mg), C (12.6 mg) and mansonones F (96.3 mg), H (10.3 mg), I (14.4 mg) were purified. From the *n*-hexane layer, mansonone E (183.0 mg) was purified by the combination of the above methods.

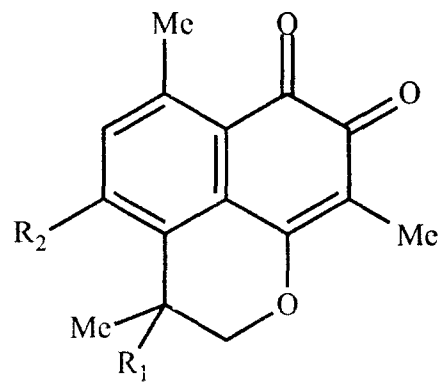
Physico-chemical properties : The physico-chemical properties of the davidianones A, B and C are very similar to each other. UV-visible spectra of these violet-colored compounds in MeOH are very similar to those of mansonone F, whose maximum absorptions are at 235, 255 (sh), 335 and 550 nm [7]. They show common IR absorptions at 1680 -1690 (C=O), 1620 - 1640 (olefinic C=C) and 1570 - 1610 cm^{-1} (aromatic C=C). The quinonoid nature of the compounds was indicated by disappearance of the colour from an alcoholic solution of the compounds on the addition of sodium dithionite and their IR spectra at 1680 - 1690 cm^{-1}. The EI-MS spectra were diagnostic, showing the relatively intense $[M+2]^+$ ion peaks that are characteristic of *ortho*-naphthoquinones [8], but not displayed by *para*-naphthoquinones. On the basis of HREI-MS and NMR data, The molecular formular of davidianones A, B and C were determined as $C_{15}H_{12}O_4$ (MW=256), $C_{16}H_{12}O_5$ (MW=284) and $C_{17}H_{16}O_5$ (MW=300), respectively.

Structure determination : The structures of the compounds were determined by the aid of spectroscopic methods of IR, MS, NMR including ^1H, ^{13}C, DEPT, NOE, DQF-COSY, PFG-HMQC and PFG-HMBC. As shown in Fig. 1, davidianones A, B and C were determined to be 3-hydroxymethyl-6,9-dimethylnaphtho(1,8-*bc*)pyran-7,8-dione, 6-methoxy-carbonyl-3,9-dimethylnaphtho(1,8-*bc*)pyran-7,8-dione, 6-dimethoxy-methyl-3,9-dimethylnaphtho(1,8-*bc*)pyran-7,8-dione, respectively. The

other four compounds were known to be mansonones E, F H and I, which were isolated for the first time from *Ulmus davidiana*.



	R_1	R_2
1	CH_2OH	Me
2	Me	COOMe
3	Me	$CH(OMe)_2$
4	Me	Me



	R_1	R_2
5	H	H
6	H	OH
7	OH	H

Fig. 1. Structures of davidianones A(1), B(2), C(3) and mansonones E(5), F(4), H(6) and I(7)

Cytotoxicities : Cytotoxicities of the compounds against various human cancer cell lines are summarized in Table 1. The ED50 (mg/ml) values of the compounds indicated the potent cytotoxicities of the compounds against cancer cell lines. Although mansonone H is structurally very similar to the other compounds, it showed very low cytotoxicity.

When compared with an anticancer agent, adriamycin, davidianones and mansonones were less cytotoxic. However, they showed potent cytotoxicities against adriamycin resistance breast cancer cell line, MCF-7/ADR. The cytotoxicities of the compounds against mouse normal fibroblast cell line, NIH3T3, exhibited characteristic properties. The compounds with saturated heterocyclic ring (mansonones E and I) showed selectivity for cancer cell lines and did not show cytotoxicity against mouse normal fibroblast cell line. However, the compounds with unsaturated heterocyclic ring (mansonone F, davidianones A and C) were cytotoxic against both of human cancer cell lines and mouse normal fibroblast cell line with similar potency. Although these compounds were cytotoxic against human cancer cell lines, they did not show any cytotoxic effects against primary cultured rat hepatocytes.

Antioxidative activities : Antioxidative activities of the compounds were evaluated by inhibitory activities against rat liver microsomal lipid peroxidation. The IC_{50} values of davidianones A, B, C and mansonones E, F were 0.12, 6.90, 0.80, 0.03 and 0.04 μg/ml, respectively. When compared with a-tocopherol (IC_{50} 0.10 μg/ml), mansonones E and F exhibited greater antioxidative activities in this system.

Table 1. Cytotoxicities of the compounds against human cancer cell lines and normal fibroblast cell

Cell lines	ED_{50} (μg/ml)						
	Dav A	Dav C	Man E	Man F	Man H	Man I	ADR*
Leukemia							
MOLT-4F	>10.0	0.2	0.4	1.3	> 10.0	1.9	0.04
K562	< 0.1	< 0.1	< 0.1	0.6	8.9	0.4	0.28
RPMI8266	0.2	0.2	< 0.1	2.6	> 10.0	0.7	0.13
Renal							
ACHN	0.7	1.0	1.0	0.7	> 10.0	2.7	0.35
UO-31	1.4	2.8	1.0	6.0	> 10.0	2.9	0.38
CNS							
SF539	1.0	1.0	1.0	2.8	> 10.0	1.1	0.05
Skin							
G361	0.5	0.5	0.5	1.8	> 10.0	1.0	0.08
UACC62	1.2	0.9	1.0	2.4	> 10.0	1.1	0.10
Prostate							
PC-3	7.9	>10.0	>10.0	> 10.0	> 10.0	>10.0	0.43
Lung							
A549	1.1	2.5	1.8	3.0	> 10.0	4.2	0.36
NCI-H266	1.2	1.0	1.0	2.2	> 10.0	1.8	0.07
Ovary							
OVCAR-4	0.3	1.4	2.1	2.4	> 10.0	1.5	0.33
Colon							
HCT15	1.1	2.5	1.0	4.3	> 10.0	4.6	0.42
KM12	< 0.1	3.9	3.3	1.6	> 10.0	2.9	0.16
Colo#205	0.7	0.7	1.1	0.6	8.6	3.0	0.29
Breast							
MCF-7	0.5	1.2	1.0	3.1	> 10.0	2.0	0.39
MCF-7/ADR	0.2	0.6	0.5	< 0.1	> 10.0	< 0.1	> 10.0
Normal fibroblast							
NIH3T3	1.0	2.0	>10.0	2.3	> 10.0	>10.0	0.10

* Adriamycin

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