Synthesis and *In Vitro* Cytotoxicity of Cinnamaldehydes to Human Solid Tumor Cells

Byoung-Mog Kwon¹, Seung-Ho Lee¹, Sang Un Choi³, Sung Hee Park³, Chong Ock Lee³, Young-Kwon Cho², Nack-Do Sung² and Song-Hae Bok¹

¹Protein Regulator RU, Korea Research Institute of Bioscience & Biotechnology, P.O. Box 115 Yusong, Taejon, 305-600 Korea, ²Department of Agricultural Chemistry, Chonnam National University and ³Pharmaceutical Screening Lab., Korea Research Institute of Chemical Technology, P.O. Box 115 Yusong, Taejon 305-606 Korea

(Received September 19, 1997)

Cinnamaldehydes and related compounds were synthesized from various cinnamic acids based on the 2'-hydroxycinnamaldehyde isolated from the bark of *Cinnamomum cassia* Blume. The cytotoxicity to human solid tumor cells such as A549, SK-OV-3, SK-MEL-2, XF498 and HCT15 were measured. Cinnamic acid, cinnamates and cinnamyl alcohols did not show any cytotoxicity against the human tumor cells. Cinnamaldehydes and related compounds were resistant to A549 cell line up to 15 μg/ml. In contrast, HCT15 and SK-MEL-2 cells were much sensitive to these cinnamaldehyde analogues which showed ED₅₀ values 0.63~8.1 μg/ml. Cytotoxicity of the saturated aldehydes was much weak compared to their unsaturated aldehydes. From these studies, it was found that the key functional group of the cinnamaldehyde-related compounds in the antitumor activity is the propenal group.

**Key words**: Cinnamaldehyde, Human tumor cell, Cytotoxicity, *Cinnamomum cassia*

**INTRODUCTION**

A large number of aliphatic and aromatic aldehydes and their derivatives showed cytotoxicity against tumor cell lines (Piantadosi, *et al.*, 1964). Several of these aldehydes and their derivatives were also used clinically as antitumor drugs (Goldin, *et al.*, 1966). Cinnamaldehydes, one of aromatic aldehydes, are widely distributed in nature. It was reported that cinnamaldehydes have an antimutagenic effect in the cells mutagenized with 4-nitroquinoline-N-oxide (4NQO) (Feron, *et al.*, 1991) and aromatic aldehydes including cinnamaldehyde protect cells from inactivating effect of cis-diamminedichloroplatinum (II) (cis-DDP) (Domish, *et al.*, 1989). These previous studies showed the feasibility of a clinical application of cinnamaldehydes to enhance the therapeutic effect of cis-DDP.

Billman and Tonnis (1971) reported substituted aralkyl aldehydes and described an antitumor activity. However, they could not find an antitumor effect of the cinnamaldehydes because the aralkyl aldehydes were only screened against leukemia L-1210. Kwon, *et al.*, 1996 and 1997 reported the farnesyl transferase inhibitory and antiangiogenic activity of 2'-hydroxycinnamaldehyde and related compounds.

In this paper, we report cytotoxic activity studies of naturally occurring compounds and synthetic derivatives against human solid tumor cell lines such as A549, SK-OV-3, SK-MEL-2, XF498 and HCT15 *in vitro*. And we also discussed the relationship between the propanal group of cinnamaldehydes and its biological activities.

**MATERIALS AND METHODS**

**Reagents**

Chemical reagents were purchased from Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise mentioned.

2'-Hydroxycinnamaldehyde (4a) was isolated from stem bark of *Cinnamomum cassia* according to the reported procedure (Kwon *et al.*, 1996).

**Instruments**

UV and FT-IR spectra were recorded on a Shimadzu UV-265 and Bio-Rad Digible Division FTS-80 spectrophotometer, respectively. NMR spectra were measured in CDCl₃ or acetone-d₆ (Aldrich) on a Brucker AM 500 and Varian 300 MHz spectrometer. Exact mass
of the unknown compounds were measured on a VG ZAB-7070 by the University of California at Riverside Mass Spectrometry Facility using the electron impact (El) or the chemical ionization (CI) mode with NH3.

**Methyl 3-(2'-hydroxyphenyl)-2-propenolate (2)**

To a solution of 2'-hydroxycinnamic acid (1, 10 g) dissolved in 100 mL of CH3OH, diazomethane (CH2N2), which was prepared from Diazal, was slowly added at 0°C. The reaction was followed by TLC (Hexane: EtOAc=4:6, v/v) until the starting material was completely disappeared, then the addition of CH2N2 was stopped. After concentration in vacuo, the residue was chromatographed to give 9.2 g of 2 as a white solid.

**3-(Substituted phenyl)-2-propenolate (3)**

Methyl 2'-hydroxycinnamate (2, 5 g) dissolved in the solution of THF was placed in a 500-mL three-necked round-bottomed flask equipped with a magnetic stirrer and a thermometer in dry-ice-acetonitrile (-40°C), and then Diisobutyl-aluminium hydride (DIBAL-H) was slowly added via cannula into the solution until the starting material (2) was completely disappeared on TLC. After the addition was completed, the reaction mixture was quenched with saturated ammonium chloride (NH4Cl) solution. The reaction mixture was extracted with EtOAc (2× 200 mL) and the organic layer was separated with sodium sulfate. After concentration in vacuo, the residue was chromatographed on silica gel to give 4.8 g of 3a as a colorless solid.

**3-(2'-Hydroxyphenyl)-2-propenal (3a):** HRMS: [MH]+ 150.0673 (C9H9O2). Calcld. 150.0680. mp: 83~88°C. IR (KBr pellet, cm−1): 3400 cm−1 (H-bonded OH), 3460 cm−1 (free OH), 1630, 1490 cm−1 (ar, C=C), 1045 cm−1 (C-O bond). 1H NMR (300 MHz, acetone-d6) δ 7.62 (1H, dd, J=-7.2, 1.5 Hz), 7.37 (1H, dd, J=1.2, 1.8 Hz), 6.87 (1H, s), 6.70 (1H, dt, J=1.8, 8.1 Hz), 6.53 (1H, d, J=7.5 Hz, C6H4-CH=CH=CH=CH(OH)), 6.33 (1H, dt, J=7.5, 15.9 Hz, C6H4-CH=CH(OH)), 4.21 (2H, d, J=3.9 Hz, CH2OH).

**3-(3'-Hydroxyphenyl)-2-propenal (3b):** HRMS: [MH]+ 150.0674 (C9H9O2). Calcld. 150.0680. mp: 47~50°C. IR (KBr pellet, cm−1): 3000~3500 cm−1 (H-bonded OH), 1680 cm−1 (C=O, aldehyde), 1658 cm−1 (C=O, aldehyde), 1612, 1600, 1450 cm−1 (ar, C-'-C) and 1145 cm−1 (C-O bond). 1H NMR (300 MHz, acetone-d6) δ 9.68 (1H, d, J=-7.8 Hz, -CHO), 7.91 (1H, t, J=-7.2 Hz), 7.54 (1H, d, J=7.5 Hz), 7.48 (1H, dt, J=7.2, 1.8 Hz), 7.42 (1H, dt, J=7.2, 1.5 Hz), 6.80 (1H, dd, J=7.5, 15.9 Hz, C6H4-CH=CH(OH)).

**3-(2'-Hydroxyphenyl)-2-propenal (4a):** Compound 4a was isolated from the bark of Cinnamomum cassia Blume and synthesized from 2-hydroxycinnamyl alcohol (Kwon et al., 1996). 3-(2-Hydroxyphenyl)-2-propenal (3a, 1 g) dissolved in 100 mL of methylene chloride was placed in a round-bottomed flask equipped with a magnetic stirring bar and a reflux condenser, and then manganese oxide (MnO2, 2×2 g) was added into the reaction vessel (25°C). The reaction mixture was refluxed in sand bath for 4 hours. When 3a was completely disappeared on the TLC, manganese oxide was removed with a filter paper and washed with acetone (2×50 mL). After concentration in vacuo, the residue was chromatographed on silica gel to give 4a (320 mg, 32%) as a yellowish solid.

Compounds 4b and 4c were also prepared by the same way as that of the compound 4a from 3b and 3c, respectively.

**3-(2'-Hydroxyphenyl)-2-propenal (3b):** HRMS: [MH]+ 149.0594 (C9H8O2). Calcld. 149.0605. mp: 121~125°C. IR (KBr pellet, cm−1): 3000~3500 cm−1 (H-bonded OH), 1658 cm−1 (C=O, aldehyde), 1612, 1600, 1450 cm−1 (ar, C=C) and 1145 cm−1 (C-O bond). 1H NMR (300 MHz, acetone-d6) δ 7.40 (1H, d, J=-7.8 Hz, -CHO), 7.20 (1H, t, J=-8.7, 1.8 Hz), 6.70 (1H, dt, J=1.5, 8.1 Hz), 6.43 (1H, d, J=15.9 Hz, C6H4-CH=CH(OH)).

**3-(2'-Hydroxyphenyl)-2-propenal (4b):** HRMS: [MH]+ 149.0599 (C9H8O2). Calcld. 149.0605. mp: 52~54°C. 1H NMR (300 MHz, acetone-d6) δ 9.79 (1H, d, J=7.5 Hz, -CHO), 8.01 (1H, d, J=15.9 Hz, C6H4-CH=CH=CH=CH=CH(OH)). 1H NMR (300 MHz, acetone-d6) δ 9.68 (1H, d, J=-7.8 Hz, -CHO), 7.91 (1H, t, J=7.2 Hz), 7.54 (1H, d, J=7.5 Hz), 7.48 (1H, dt, J=7.2, 1.8 Hz), 7.42 (1H, dt, J=7.2, 1.5 Hz), 6.80 (1H, dd, J=7.5, 15.9 Hz, C6H4-CH=CH(OH)).

**3-(2'-Hydroxyphenyl)-2-propenal (4c):** HRMS: [MH]+ 167.0263 (C9H10O2). Calcld. 167.0263. mp: 118~120°C. IR (KBr pellet, cm−1): 3000~3500 cm−1 (H-bonded OH), 1680 cm−1 (C=O, aldehyde), 1600, 1470 cm−1 (ar, C=O) and 1170 cm−1 (C-O bond). 1H NMR (300 MHz, acetone-d6) δ 9.69 (1H, d, J=-7.8 Hz, -CHO), 7.60 (1H, d, J=15.9 Hz, C6H4-CH=CH=CH=CH=CH(OH)).

**3-(2'-Hydroxyphenyl)-2-propenal (3c):** HRMS: [MH]+ 149.0599 (C9H8O2). Calcld. 149.0605. mp: 121~125°C. IR (KBr pellet, cm−1): 3000~3500 cm−1 (H-bonded OH), 1658 cm−1 (C=O, aldehyde), 1612, 1600, 1450 cm−1 (ar, C=C) and 1145 cm−1 (C-O bond). 1H NMR (300 MHz, acetone-d6) δ 9.68 (1H, d, J=-7.8 Hz, -CHO), 7.60 (1H, d, J=15.9 Hz, C6H4-CH=CH=CH=CH=CH(OH)).

**3-(2'-Hydroxyphenyl)-2-propenal (4a):** Compound 4a was isolated from the bark of Cinnamomum cassia Blume and synthesized from 2-hydroxycinnamyl alcohol (Kwon et al., 1996). 3-(2-Hydroxyphenyl)-2-propenal (3a, 1 g) dissolved in 100 mL of methylene chloride was placed in a round-bottomed flask equipped with a magnetic stirring bar and a reflux condenser, and then manganese oxide (MnO2, 2×2 g) was added into the reaction vessel (25°C). The reaction mixture was refluxed in sand bath for 4 hours. When 3a was completely disappeared on the TLC, manganese oxide was removed with a filter paper and washed with acetone (2×50 mL). After concentration in vacuo, the residue was chromatographed on silica gel to give 4a (320 mg, 32%) as a yellowish solid.

Compounds 4b and 4c were also prepared by the same way as that of the compound 4a from 3b and 3c, respectively.