Lignans from the Fruits of *Cornus kousa* Burg. and Their Cytotoxic Effects on Human Cancer Cell Lines

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The fruits of *Cornus kousa* Burg, were extracted with 80% aqueous MeOH, and the concentrated extract partitioned with EtOAc, n-BuOH and H2O. Six lignans were isolated from the EtOAc fraction through repeated silica gel, ODS and Sephadex LH-20 column chromatographies. From the physico-chemical data, including NMR, MS and IR, the chemical structures of the compounds were determined to be (+)-pinoreinol (1), (-)-balanophonin (2), (+)-laricresinol (3), erythro-guaiacylglycerol-β-coniferyl aldehyde ether (4), threo-guaiacylglycerol-β-coniferyl aldehyde ether (5) and dihydrodehydrodiconiferyl alcohol (6), which were isolated for the first time from this plant. Most of these compounds showed cytotoxicity against human colon carcinoma (HCT-116) and human hepatocellular carcinoma (HepG2) cell lines in vitro, with IC50 values ranging from 19.1 to 71.3 µg/mL.

Key words: *Cornus kousa*, Lignan, MTT assay, Cytotoxicity, Human colon carcinoma (HCT-116), Human hepatocellular carcinoma (HepG2)

INTRODUCTION

*Cornus kousa* Burg. (Cornaceae) is a tree distributed in the mountains of South Korea, China and Japan. The fruit of this plant has been used as a hemostatic agent and for the treatment of diarrhea in Korean traditional medicine (Lee, 2003), and their extracts have been reported to have immuno-regulatory properties (Kim et al., 1984). Some chemical constituents have also been reported from the leaves of *C. kousa*, such as isoquercitrin, gallic acid, tannin (Ryu et al., 1971), phenolics and flavonoids (Shaiju et al., 2006). However, isolation of the chemical components from the fruits of *C. kousa* remains to be reported. Therefore, in this paper, the isolation and identification of six lignans from the fruits of *C. kousa* are reported, and their structures characterized by spectroscopic methods. The isolated compounds were tested for cytotoxicity against human colon carcinoma (HCT-116) and human hepatocellular carcinoma (HepG2) cell lines in vitro using the MTT assay.

MATERIALS AND METHODS

Plant materials

The fruits of *Cornus kousa* Burg. (Cornaceae) were collected from the experimental farm in KyungHee University during August, 2005. A voucher specimen (KHU050914) has been reserved at the Laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea.

Instruments and regents

Optical rotations were measured on a JASCO P-1010 digital polarimeter (Tokyo, Japan). EI-MS were recorded on a JEOL JMSAX 505-WA (Tokyo, Japan). IR spectra were run on a Perkin Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). 'H-NMR (400 MHz) and 13C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (Lake forest, U.S.A.). RPMI Medium 1640, Dulbecco's Modified Eagle Medium (GIBCO BRL, Life Technologies Inc., NY) and...
Penicillin-Streptomycin were purchased from Gibco (Grand Island, NY). Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT). MTT (3[4, 5-Dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO).

**Extraction and isolation of lignans**

The dried and chopped fruits of *C. kousa* (1 kg) were extracted three times with 80% aqueous MeOH (3 L x 3) at room temperature. The extract was successively partitioned with water (1 L), EtOAc (1 L x 3) and n-BuOH (0.8 L x 3). The EtOAc extract (4 g) was subjected to silica gel chromatography (TLC), which gave two fractions (CKFE1 to CKFE21). Fraction CKFE1 was subjected to ODS c.c. (2.5 x 20 cm), eluted with MeOH:H₂O (2:1). Fraction CKFE10-1-8+9 (18 mg, VeNt 0.60-0.85, TLC (RP-18 F₂₅₄) Rf 0.55, MeOH:H₂O = 2:1) was subjected to TLC (RP-18 F₂₅₄) Rf 0.5, MeOH:H₂O = 1:1, to afford nine fractions (CKFE10-1 to CKFE10-9). Fraction CKFE10-1 [202 mg, VeNt 0.01-0.30 in MeOH:H₂O (5:1)] was subjected to TLC (SiO₂ F₂₅₄) Rf 0.80, CHCl₃:MeOH = 30:1. CKFE10-1-6+7 [45 mg, VeNt 0.50-0.70 in CHCl₃:MeOH (30:1)] were subjected to ODS c.c. (2 x 20 cm), eluted with MeOH:H₂O (1:1, 800 mL), to ultimately produce compound 2 [12 mg, VeNt 0.60-0.85, TLC (RP-18 F₂₅₄) Rₚ 0.55, MeOH:H₂O = 3:1]. CKFE10-1-8+9 [37 mg, VeNt 0.20-0.40 in CHCl₃ : MeOH (20 : 1)] was subjected to ODS c.c. (2 x 20 cm), eluted with MeOH:H₂O (1:2, 500 mL), to give a mixture of compounds 4 and 5 (12 mg, VeNt 0.25-0.30, TLC (RP-18 F₂₅₄) Rᵢ 0.5, MeOH:H₂O : 2:1). Fraction CKFE11 [78 mg, VeNt 0.15-0.18 in CHCl₃:MeOH (13:1)] was subjected to ODS c.c. (2.5 x 20 cm), eluted with MeOH:H₂O (1:2 -> 1:1 (300 mL of each), to ultimately yield fraction 6 [9 mg, VeNt 0.25-0.44 in MeOH:H₂O (1:2), TLC (RP-18 F₂₅₄) Rf 0.5, MeOH:H₂O = 1:1].

**(-)-Balanophonin (2)**

Colorless oil (MeOH); [α]_D^25 = -68° (c=0.1, MeOH) {lit. Yuen et al., 1998, (-)-balanophonin, [α]_D^25 = -114° (c=0.34, CHCl₃)}. Colorless oil (MeOH); [α]_D^25 = -69.0 ~ (c=0.10, MeOH) {lit. Abe et al., 1998, (-)-balanophonin, [α]_D^25 = +118° (c=0.41, CHCl₃)}; El/MS m/z: 356 [M⁺], 327, 221, 205, 180, 163, 152, 151, 150, 137, 131, 124; IR (CHCl₃, cm⁻¹) 3420, 1680; 1H-NMR (400 MHz, pyridine-d₅, δ) 7.26 (2H, d, J = 8.0 Hz, H-5/5'), 7.24 (2H, d, J = 2.4 Hz, H-2/2'), 7.07 (2H, dd, J = 8.0, 2.4 Hz, H-6/6'), 4.80 (2H, d, J = 4.4 Hz, H-7/7'), 4.33 (2H, dd, J = 8.8, 6.8 Hz, H-9a/9a'), 4.01 (2H, dd, J = 8.8, 3.6 Hz, H-9b/9b'), 3.77 (6H, s, H-10/10'), 3.22 (2H, ddd, J = 3.6, 6.8, 4.4 Hz, H-8/8'); 13C-NMR (100 MHz, pyridine-d₅, δ) 148.7 (C-3/3'), 147.8 (C-4/4'), 133.1 (C-1/1'), 119.7 (C-6/6'), 116.4 (C-5/5'), 110.9 (C-2/2'), 86.5 (C-7/7'), 72.0 (C-9/9'), 56.4 (C-10/10'), 54.9 (C-8/8').

**(+)-Lariciresinol (3)**

Amorphous powder (MeOH); [α]_D^25 = +39° (c=0.10, CHCl₃) {lit. Li et al., 2003, (+)-lariciresinol, [α]_D^20 = +30° (c=0.10, MeOH)}. Amorphous powder (MeOH); [α]_D^20 = +32° (MeOH); El/MS m/z: 360 [M⁺], 236, 221, 219, 206, 205, 194, 191; IR (CHCl₃, cm⁻¹) 3432, 3011, 1709, 1506, 1488; 1H-NMR (400 MHz, pyridine-d₅, δ) 7.31 (1H, d, J = 2.0 Hz, H-5'), 7.25 (1H, d, J = 8.0 Hz, H-6'), 7.19 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 7.19 (1H, dd, J = 8.0 Hz, H-5), 6.99 (1H, J = 2.0 Hz, H-2'), 6.89 (1H, dd, J = 8.0, 2.0 Hz, H-6), 5.33 (1H, d, J = 6.8 Hz, H-9a), 4.25 (2H, d, J = 5.6 Hz, H-9), 3.99 (1H, td, J = 5.6, 6.8 Hz, H-8), 3.85 (3H, s, H-10), 3.66 (3H, s, H-10'); 13C-NMR (100 MHz, pyridine-d₅, δ) 193.7 (C-9), 153.9 (C-7), 152.2 (C-4), 149.1 (C-3'), 148.7 (C-4'), 145.4 (C-3), 133.1 (C-1'), 131.6 (C-5), 128.8 (C-1), 127.0 (C-8), 120.2 (C-2'), 120.0 (C-6), 116.9 (C-5'), 113.7 (C-2'), 111.2 (C-6'), 89.9 (C-7'), 64.2 (C-9'), 56.5 (C-10), 56.2 (C-10'), 54.5 (C-8').

**(+)-Pinoresinol (1)**

Amorphous powder (MeOH); [α]_D^25 = +72.0° (c=0.20, MeOH) {lit. Li et al., 2003, [α]_D^25 = +69.0° (c=0.10, MeOH). Amorphous powder (MeOH); [α]_D^20 = +71.1° (MeOH)}; El/MS m/z: 358 [M⁺], 327, 221, 205, 180, 163, 152, 151, 150, 137, 131, 124; IR (CHCl₃, cm⁻¹) 3420, 1680; 1H-NMR (400 MHz, pyridine-d₅, δ) 7.26 (2H, d, J = 8.0 Hz, H-5/5'), 7.24 (2H, d, J = 2.4 Hz, H-2/2'), 7.07 (2H, dd, J = 8.0, 2.4 Hz, H-6/6'), 4.80 (2H, d, J = 4.4 Hz, H-7/7'), 4.33 (2H, dd, J = 8.8, 6.8 Hz, H-9a/9a'), 4.01 (2H, dd, J = 8.8, 3.6 Hz, H-9b/9b'), 3.77 (6H, s, H-10/10'), 3.22 (2H, ddd, J = 3.6, 6.8, 4.4 Hz, H-8/8'); 13C-NMR (100 MHz, pyridine-d₅, δ) 148.7 (C-3/3'), 147.8 (C-4/4'), 133.1 (C-1/1'), 119.7 (C-6/6'), 116.4 (C-5/5'), 110.9 (C-2/2'), 86.5 (C-7/7'), 72.0 (C-9/9'), 56.4 (C-10/10'), 54.9 (C-8/8').

**Cytotoxic Lignans from the Fruits of Comus kousa**