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# Anti-Inflammatory Activity of Constituents Isolated from *Ulmus davidiana* var. *japonica*

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**Abstract** − Twenty six compounds (1-26) were isolated from the root barks of *Ulmus davidiana* var. *japonica*. The anti-inflammatory activity of the isolated compounds were evaluated against the generation of inflammatory chemical mediators in bone marrow-derived mast cells. Among them, compounds 10, 11, 13, 15 and 19 inhibited not only cyclooxygenase-2 dependent prostaglandin $D_2$ generation but also 5-lipoxygenase dependent leukotrien $C_4$ generation in a concentration-dependent manner. In addition, compounds 11, 12, 13, 15 and 19 also inhibited β-hexosaminidase release, a marker of mast cell degranulation reaction, from bone marrow-derived mast cell. These results suggest that the anti-inflammatory activity of *U. davidiana* might in part occur by both the inhibition of eicosanoid generations and the degranulation reaction of mast cells.

**Keywords:** *Ulmus davidiana* var. *japonica*, Bone marrow-derived mast cells, Cyclooxygenase-2, 5-Lipoxygenase, β-hexosaminidase, Anti-inflammatory activity

## INTRODUCTION

Eicosanoids such as prostaglandins (PGs) and leukotriens (LTs) are major inflammatory lipid mediators (Gullikkson *et al*., 2006). These mediators are biosynthesized by cyclooxygenases (COX) and lipoxygenases (LOX) in many cell types and deeply associated with many inflammatory disorders. The enzyme responsible for PG synthesis exists as two isoforms, COX-1 (constitutive isoform) and COX-2 (inducible form) (Mitchell and Warner, 2006). Several COX-2 inhibitors have been developed and clinically prescribed showing less side effects (Rouzer and Marnett, 2009). Arachidonic acid can also be converted to leukotrienes (LTs) by the action of 5-lipoxygenase (5-LOX). Therefore, the development of dual inhibitors that can simultaneously inhibit COX-2 and 5-LOX might enhance their individual anti-inflammatory effects and reduce the undesirable side effects that are associated with non-steroidal anti-inflammatory drugs (NSAIDs) (Martel-Pelletier *et al*., 2003; Theoharides *et al*., 2007). Histamine released from mast cells is stored in secretory granules. The release of histamine and other pharmacological mediator from mast cells is a prominent feature of acute inflammatory processes including the immediate type anaphylactic reactions. There are various agents to induce the mast cell degranulation, which are commonly associated with the anaphylactic shocks in human and other mammals (Jippo *et al*., 2009; Metcalfe *et al*., 2009; Ono *et al*., 2009).

*U. davidiana* var. *japonica* (Ulmaceae) is a deciduous tree that is widely distributed in Korea, China and Japan. The barks of the stem and root of this plant have been used in the treatment of oedema, mastitis, gastric cancer, and inflammation (Jun *et al*., 1998). Anticancer, antiviral, antibacterial, and anti-inflammatory properties have also been reported (Jun *et al*., 1998; Jin *et al*., 2006, 2008; Kang *et al*., 2006; Suh *et al*., 2007). The solvent extract of *U. davidiana* has been reported to have anti-inflammatory activity on collagen-induced inflammation in rats and cyclooxygenase-2 (COX-2) (Song *et al*, 2006; Jin *et al*., 2008).

In the course of searching for anti-inflammatory compounds from plant sources, we found that the methanol (MeOH) extract of the root barks of *U. davidiana* var. *japonica* displays potent inhibitory effects on COX-2, 5-lipoxygenase (5-LOX) and degranulation. This paper de-

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scribes inhibitory effects of the isolated compounds on not only generations of both COX-2 dependent $PGD_2$ and 5-LOX dependent $LTC_4$ but also β-hexosaminidase release, a marker of mast cell degranulation reaction, from bone marrow-derived mast cell.

## MATERIALS AND METHODS

### Plant material

Root bark of *U. davidiana* var. *japonica* was purchased in February 2007 at a folk medicine market, "Yak-ryong-si", in Daegu, Republic of Korea. The preparations were confirmed taxonomically by Professor Ki-Hwan Bae, Chungnam National University, Daejeon, Republic of Korea. A voucher specimen (YNUD-2007) has been deposited at the College of Pharmacy, Yeungnam University.

### Instruments and reagents

Optical rotations were measured using a model DIP-1000 automatic digital polarimeter (Jasco, Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were recorded on a 250 MHz spectrometer (DMX 250, Bruker, Germany) using manufacturer's standard pulse program. Samples were dissolved in deuterated chloroform ($CDCl_3$-$d_1$), pyridine-$d_5$ or deuterated methanol ($CD_3OD$), with chemical shifts reported in ppm downfield from tetramethylsilane (TMS). Fast atom bombardment mass spectrometry (FABMS) was performed using a model JMS700 spectrometer (Jeol, Tokyo, Japan). The stationary phases used for column chromatography (Silica gel 60, 70-230 and 230-400 mesh, Lichroprep RP-18 gel, 40-63 μm, Sephadex$^{TM}$ LH-20) and thin layer chromatography (TLC) plates (Silica-gel 60 $F_{254}$ and RP-18 $F_{254}$, 0.25 mm) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under ultraviolet (UV) radiation and by spraying with 10% $H_2SO_4$, followed by heating. High pressure liquid chromatography was performed using a LC-20AD pump and SPD- 20A UV/VIS detector (both from Shimadzu, Tokyo, Japan). All other chemicals and solvents were analytical grade and used without further purification.

### Extraction and isolation

Preparations of *U. davidiana* dried root bark (10 kg) were extracted three times with 13 L of 70% MeOH by reflux. The dried MeOH extract (1.1 kg) was suspended in distilled 1.4 L water and the solution was successively partitioned with *n*-hexane (1.4 L×3), ethyl acetate (EtOAc, 1.4 L×3) and *n*-butanol (*n*-BuOH, 1.4 L×3). After drying, four solvent extracts were obtained: n-hexane (67.6 g), EtOAc (70.5 g), *n*-BuOH (320 g) and $H_2O$ (555 g). The *n*-hexane extract (67 g) was applied to a silica gel column (60×11 cm, No.9385, 230-400 mesh, Merck, Germany) and the column was eluted in a stepwise gradient mode with from 100% *n*-hexane to 100% EtOAc, and (from 100% EtOAc to 100% MeOH. Fractions were combined based on TLC analysis. Thirty-four fractions (UDH1-34) were obtained. Fractions UDH1, UDH3, UDH6, UDH9, UDH11, UDH22, UDH31 and UDH34 yielded compounds 1 (80 mg), 2 (120 mg), 3 (100 mg), 4 (20 mg), 5 (1 g), 6 (20 mg), 11 (33 mg) and 7 (1.5 g), respectively. Fraction UDH27 (1.0 g) was further separated into five fractions (UDH27-1-UDH27-5) by Sephadex LH-20 column (3×90 cm, 1 L) chromatography, and eluted with CHCl3:MeOH (4:6). Compound 10 (8 mg) was obtained from UDH27-2 using a LiChroprep RP-18 reverse-phase column (4×50 cm) with elution by MeOH-$H_2O$ (gradient from 70:30 to 100% MeOH). Fraction UDH23 (300 mg) was applied to a Sephadex LH-20 column (3×90 cm, 1 L) and eluted with 100% MeOH to give 8 (30 mg). Compound 9 (27 mg) was obtained from UDH30 by elution through a Sephadex LH-20 column (3×90 cm, 0.5 L) with MeOH and a LiChroprep RP-18 reverse-phase column (4×50 cm) with MeOH-$H_2O$ (gradient from 80:20 to 100% MeOH), successively. Fraction UDH18 (100 mg) was applied to a 4×50 cm LiChroprep RP-18 reverse-phase column with MeOH-$H_2O$ (gradient from 80:20 to 100% MeOH) to give 12 (14.5 mg).

The EtOAc extract (65 g) was applied to a silica gel column (9×60 cm, NO. 9385, 230-400 mesh, Merck, Germany), and eluted with a gradient of methylene chloride ($CH_2Cl_2$)-MeOH (from 100% $CH_2Cl_2$ to 100% MeOH). The eluates were combined based on TLC, giving 29 fractions (UDE1-29). Compound 13 (30 mg) was obtained from UDE20 by Sephadex LH20 columns (3×90 cm, 1 L) with isocratic elution (100% MeOH). Fraction UDE26 was further chromatographed using Sephadex LH20 column (3×90 cm, 1 L) with isocratic elution (100% MeOH) to obtain 14 (500 mg) and fractions UDE26-1-UDE26-3. Fraction UDE26-3 purified by a 4×50 cm LiChroprep RP-18 reverse-phase column with MeOH-$H_2O$ gradient elution (from 10-100% MeOH) to yield compounds 15 (32 mg) and 16 (25 mg).

An *n*-BuOH extract (150 g) was applied into a column packed with silica gel (9×60 cm, No. 9385, 230-400 mesh, Merck, Germany) and eluted with $CH_2Cl_2$-MeOH (gradient from 100% $CH_2Cl_2$ to 100% MeOH) and 30 fractions (UDB1-UDB30) were acquired. Fraction UDB11 was further purified to give 17 (45 mg) by elution with a 4×50 cm LiChroprep RP-18 reverse-phase column with 10% MeOH. Compounds, 20 (20 mg), 24 (16 mg), 18 (11.6 mg) and 19 (5 mg) were obtained from UDB15 using repeated chromatography with a 4×50 cm LiChroprep RP-18 column. Fraction UDB16

was further separated into compounds 21 (8 mg, 55.2 min), 22 (39.8 mg, 44.4 min) and 23 (23 mg, 50.3 min) by HPLC (Inertsil® ODS-3 250×4.6 mm, 5 μm, GL Science, Japan) with isocratic elution with MeOH-$H_2O$ (33:67). Compounds 25 (17 mg) and 26 (26.5mg) were obtained from UDB23 by successive chromatography with a Sephadex LH20 column (3×90 cm, 1 L) with isocratic elution (100% MeOH), and a 4×50 cm LiChroprep RP-18 reverse-phase column with 10% MeOH.

**24-Ethylcholesta-5,22-dien-3β-ol-palmitic acid ester (1)**

Colorless needles; $[\alpha]_D^{25}$ −69° (*c* 0.1, $CHCl_3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Wang *et al*., 2006); FABMS *m/z* 673.6 $[M+Na]^+$.

**Friedelin (2)**

Colorless needles; $[\alpha]_D^{25}$ −59° (*c* 0.1, $CHCl_3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Ali *et al*., 1999); FABMS *m/z* 426.7 $[M]^+$.

**Epifriedelanol (3)**

White crystals; $[\alpha]_D^{25}$ 47° (*c* 0.1, $CHCl_3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Li *et al*., 2007); FABMS *m/z* 427 $[M-H]^-$.

**Eicosanoic acid (4)**

White powder; $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Chung *et al*., 2007); FABMS *m/z* 312.5 $[M]^+$.

**β-Sitosterol (5)**

White crystals; $[\alpha]_D^{25}$ −36° (*c* 0.1, $CHCl^3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Umlauf *et al*., 2004); FABMS *m/z* 414.4 $[M]^+$.

**Betulinic acid (6)**

White crystals; $[\alpha]_D^{25}$ +9° (*c* 0.1, $CHCl_3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Aguirre *et al*., 2006); FABMS *m/z* 456.7 $[M]^+$.

**Sitosterol-3-*O*-β-D-glucoside (7)**

Brown soild; $[\alpha]_D^{25}$ −51° (*c* 0.1, $CHCl_3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Sang *et al*., 2002); FABMS *m/z* 576.4 $[M]^+$.

**Oleanolic acid (8)**

White crystals; $[\alpha]_D^{25}$ +65° (*c* 0.1, $CHCl_3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Seebacher *et al*., 2003); FABMS *m/z* 438.3 $[M-OH]^+$.

**Maslinic acid (9)**

White powder; $[\alpha]_D^{25}$ +60° (*c* 0.1, $CHCl_3$); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Hisashi and Haruo, 1989); FABMS *m/z* 472.3 $[M]^+$.

**Stigmast-5-ene-3β,4α-diol (10)**

White powder; $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Yumiko *et al*., 1995; Siddiqui *et al*., 2006); FABMS *m/z* 430.3 $[M]^+$.

**3-*O*-(6-*O*-Palmitoryl)-β-D-glucopyranosyl stigmasterol (11)**

White powder; $[\alpha]_D^{25}$ −25.4° (*c* 0.1 pyridine); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Lavaud *et al*., 1994); FABMS *m/z* 835.6 $[M+Na]^+$.

**Acorusnol (12)**

Viscous oil; $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Nawamaki and Kuroyanagi, 1996); FABMS *m/z* 236.1 $[M]^+$.

**(−)-Catechin (13)**

Brown amorphous powder; $[\alpha]_D^{25}$: −20.5° (*c* 0.2 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Nahrstedt *et al*., 1987); FABMS *m/z* 290.1 $[M]^+$.

**Catechin-7-*O*-β-apiofuranoside (14)**

Colorless needles; $[\alpha]_D^{25}$: +31.6° (*c* 0.1 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Na *et al*., 2002); FABMS *m/z* 423.1 $[M+H]^+$.

**Catechin-7-*O*-α-L-rhamnopyranoside (15)**

Yellowish amorphous solid; $[\alpha]_D^{25}$: −96.1° (*c* 0.1 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Moon and Rim, 1995); FABMS *m/z* 436.1 $[M]^+$.

**Catechin-3-*O*-α-L-rhamnopyranoside (16)**

Colorless needles; $[\alpha]^{25}{}_D$: −56.4° (*c* 0.1 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Ishimaru *et al*., 1987); FABMS *m/z* 437.2 $[M+H]^+$.

**Butyl α-D-fructofuranoside (17)**

Amorphous powder; $[\alpha]_D^{25}$: +31.0° (*c* 0.2 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Zhang *et al*., 1996); FABMS *m/z* 259.1 $[M+Na]^+$.

**Ampelopsisionoside (18)**

White powder; $[\alpha]_D^{25}$ −32.5° (*c* 0.01, MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Pabst *et al*., 1992); FABMS *m/z* 411.2 $[M+Na]^+$.

***cis*-Roseoside (19)**

Colorless needles; $[\alpha]_D^{25}$: −60° (*c* 0.01 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Pabst *et al*., 1992); FABMS *m/z* 409.2 $[M+Na]^+$.

**(+)-5-Methoxyisolariciresinol-9-*O*-β-D-xylopyranoside (20)**

Yellowish amorphous powder; $[\alpha]_D^{25}$: +35.1° (*c* 0.01, MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Lee *et al*., 2001); FABMS *m/z* 522.2 $[M]^+$.

**(+)-Isolariciresinol-9'-*O*-β-D-xylopyranoside (21)**

Colorless needles: $[\alpha]_D^{25}$: +49.2° (*c* 0.01 MeOH). $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Lee *et al*., 2001); FABMS *m/z* 492.2 $[M]^+$.

**Lyoniside (22)**

Colorless needles; $[\alpha]_D^{25}$: +23.5° (*c* 0.2 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Inoshiri *et al*., 1987; Smite *et al*., 1995); FABMS *m/z* 552.3 $[M]^+$.

**Nudiposide (23)**

Colorless needles;$[\alpha]_D^{25}$: −32.5° (*c* 0.2 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Inoshiri *et al*., 1987; Smite *et al*., 1995); FABMS *m/z* 552.3 $[M]^+$.

**Ssioriside (24)**

Yellow amorphous powder; $[\alpha]_D^{25}$ +20.5° (*c* 0.1, MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Yoshinari *et al*., 1989); FABMS *m/z* 554.2 $[M]^+$.

**Catechin-7-*O*-β-D-glucopyranoside (25)**

Colorless solid; $[\alpha]_D^{25}$: −86.5° (*c* 0.1 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Foo and Karchesy, 1989); FABMS *m/z* 452.1 $[M]^+$.

**Procyanidin B3 (26)**

Brown powder; $[\alpha]_D^{25}$: −174.5° (*c* 0.01 MeOH); $^1$H- and $^{13}$C-NMR data were consistent with the literature values (Kohler *et al*., 2008); FABMS *m/z* 601.1 $[M+Na]^+$.

**Preparation and activation of bone marrow-derived mast cells (BMMCs)**

Bone marrow cells from male Balb/cJ mice were cultured for up to 10 weeks in 50% enriched medium (RPMI 1,640 containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics and 10% fetal calf serum) and 50% WEHI-3 cell conditioned medium as a source of interleukin (IL)-3. After 3 weeks, >98% of the cells were BMMCs when checked as previously described (Murakami *et al*., 1994).

**Determination of prostaglandin $D_2$ ($PGD_2$)**

In order to measure the inhibitory activity on COX-2 by samples, cells were suspended in enriched medium at a cell density of $5\times10^5$ cells/ml and preincubated with aspirin (10 μg/ml) for 2 h to irreversibly inactivate any preexisting COX-1. After washing, the BMMCs were activated with *c-kit* ligand (KL, 100 ng/ml), IL-10 (100 U/ml) and lipopolysaccharide (LPS, 100 ng/ml) at 37°C for 8 h in the presence or absence of samples previously dissolved in dimethylsulfoxide (DMSO). All reactions were quenched by centrifugation at 120 g at 4°C for 5 min. The supernatant and cell pellets were frozen immediately in liquid $N_2$ and stored at −80°C until needed for further analysis. Concentrations of $PGD_2$ in the supernatant were measured using a $PGD_2$ assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Under these conditions, the COX-2-dependent phases of $PGD_2$ generation reached 1.6 ng/$10^6$ cells. The data is reported as the arithmetic mean of triplicate determinations.

**Determination of leukotriene $C_4$ ($LTC_4$)**

BMMCs suspended in the aforementioned enriched medium at a density of $1\times10^6$ cells/ml were pretreated with the samples for 15 min at 37°C and stimulated with KL (100 ng/ml). After 20 min, the supernatants were retrieved and analyzed by enzyme immunoassay (EIA). The $LTC_4$ level was determined using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Under these conditions, the $LTC_4$ reached 5 ng/$10^6$ cells. The data is reported as the arithmetic mean of triplicate determinations.

**Assay of β-HEX release**

β-HEX, a marker of mast cell degranulation, was quantified by spectrophotometric analysis of the hydrolysis of p-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside (PNP-GluNAc, Sigma-Aldrich, St. Louis, MO, USA). Briefly, after harvesting supernatant, cells were lysed in the same volume of medium by three cycles of freezing and thawing. Ten milliliters of the BMMC lysate or supernatant samples were mixed with 50 μl of β-HEX substrate solution (1.3 mg/ml PNP-GluNAc in 100 mM sodium citrate, pH 4.5) in each well of 96-well plates and then incubated at 37°C for 60 min. The reaction was stopped by adding 140 μl of 0.2 M Glycine-NaOH (pH 10.7). The absorbance at 410 nm was measured in a microplate reader. The percentage of

β-HEX released into the supernatant was calculated by the following formula: [S/(S + P)]×100, where S and P are the β-HEX contents of supernatant and cell pellet.

## RESULTS AND DISCUSSION

During our search for biologically active compounds derived from endemic species in Korea, MeOH extracts of the roots of *U. davidiana* were demonstrated to possess COX-2 and 5-LOX dual inhibitory activities by assessing their effects on the production of the $PGD_2$ and $LTC_4$ in mouse BMMCs. Repeated normal-phase silica gel, reverse-phase, Sephadex LH-20 column chromatography and HPLC lead to the isolation of one fatty acid (4) and eleven terpens (1-3, 5-12) from the n-hexane extract; four flavonoids (13-16) from the EtOAc extract; and two flavonoids (25, 26), five lignans (20-24), two butenyl clohexnone glycosides (18, 19) and one fructofuranoside (17) from the n-BuOH extract (Fig. 1). Chemical structures of the isolated compounds were determined by comparison of optical rotation values, $^{1}H$- and $^{13}C$-NMR, and mass spectral data of each compound with those published.

The use of BMMCs as a model appears to be suitable for screening of selective COX-1/COX-2 or 5-LOX and COX-2/5-LOX dual inhibitors and degranulation inhibitors from various sources (Moon *et al*., 1999; Lee *et al*., 2004; Son *et al*., 2005; Jin *et al*., 2009). The generation of COX-2 dependent $PGD_2$ and 5-LOX dependent $LTC_4$ were tested after activation of the BMMCs with a combination of KL, interleukin and LPS in the presence or absence of each compound. Compounds 10, 11, 13, 15 and 19 inhibited COX-2-dependent $PGD_2$ generation with $IC_{50}$ values of 30.8, 12.6, 4.7, 17.0 and 30.9 μM, respectively, and the generation of $LTC_4$ in the 5-LOX dependent phase with $IC_{50}$ values of 29.6, 38.9, 0.8, 8.0 and 10.3 μM, respectively (Table I). Compound 12 showed no inhibiton of COX-2-dependent $PGD_2$ generation, but inhibited 5-LOX with an $IC_{50}$ value of 11.8 μM. Licofelone, a dual inhibitor of

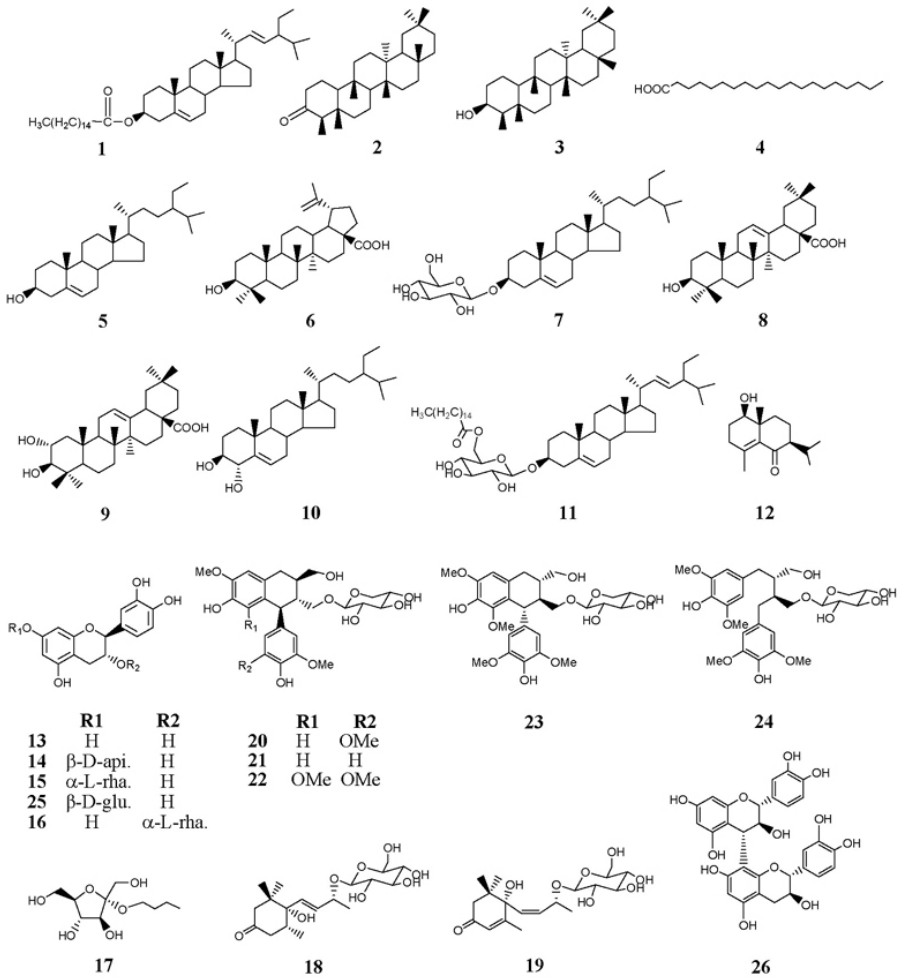


**Fig. 1.** Chemical structures of compounds 1-26 from *Ulmus davidiana* var. *japonica*.

**Table I.** Anti-inflammatory activity of isolated compounds from *Ulmus davidiana* var. *japonica*.

| Compound | Inhibition (%) | | | IC50 (μM) | | |
|---|---|---|---|---|---|---|
| | $PGD_2$ | $LTC_4$ | β-Hex | $PGD_2$ | $LTC_4$ | β-Hex |
| | 20 μM | 20 μM | 25 μM | | | |
| 1 | 13.2 | 1.6 | 26.5 | – | – | – |
| 2 | 15.1 | 0 | 31.3 | – | – | – |
| 3 | 39.6 | 11.7 | 1.3 | – | – | – |
| 4 | 9.0 | 0 | 30.6 | – | – | – |
| 5 | 11.4 | 0 | 8.7 | – | – | – |
| 6 | 0 | 0 | 0 | – | – | – |
| 7 | 7.7 | 8.2 | 18.5 | – | – | – |
| 8 | 9.3 | 35.7 | 0 | – | – | – |
| 9 | 23.9 | 74.7 | 25.7 | – | 38.70 | – |
| 10 | 60.6 | 63.6 | 0 | **30.8** | 29.59 | – |
| 11 | 73.6 | 63.5 | 69.9 | **12.62** | 38.88 | **28.72** |
| 12 | 31.7 | 81.4 | 78.9 | – | 11.84 | **82.38** |
| 13 | 68.6 | 99.8 | 76.4 | 4.7 | 0.78 | 1.4 |
| 14 | 3.4 | 0 | 0 | – | – | – |
| 15 | 67.7 | 94.5 | 64.0 | 17.0 | 8.04 | 16.8 |
| 16 | 0 | 12.5 | 46.3 | – | – | – |
| 17 | 14.4 | 0 | 0.03 | – | – | – |
| 18 | 0 | 0 | 3.4 | – | – | – |
| 19 | 59.4 | 60.6 | 63.3 | 30.9 | 10.32 | 16.6 |
| 20 | 1.8 | 0 | 0 | – | – | – |
| 21 | 0 | 0 | 0 | – | – | – |
| 22 | 0 | 0 | 0 | – | – | – |
| 23 | 0 | 0 | 0 | – | – | – |
| 24 | 0 | 0 | 0 | – | – | – |
| 25 | 4.8 | 0 | 9.2 | – | – | – |
| 26 | 0 | 0 | 0 | – | – | – |
| | $PGD_2$ positive control | | Licofelone | 0.025 | – | – |
| | $LTC_4$ positive control | | Licofelone | – | 0.86 | – |
| | β-Hex positive control | | DPT | – | – | 27.5 |

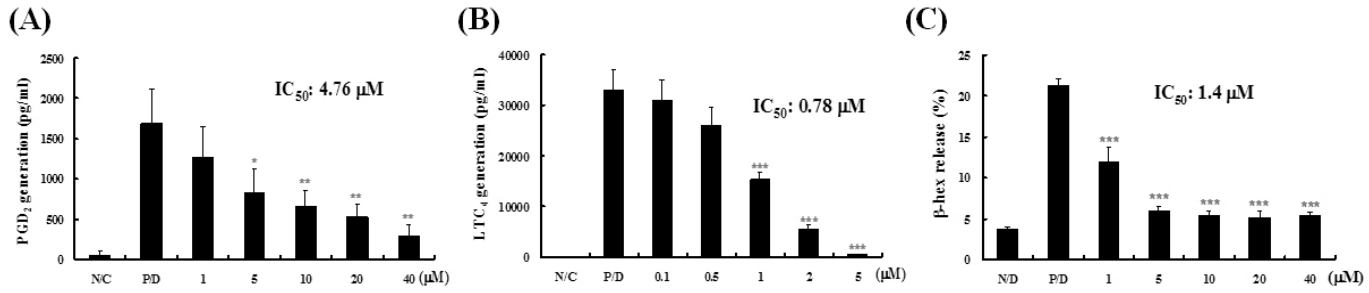


**Fig. 2.** Inhibitory effects of compound 13 on generations of $PGD_2$ (A) and $LTC_4$ (B), and degranulation reaction (C) from bone marrow-derived mast cell. (A) BMMC were pre-incubated for 30 min with the indicated concentration of 13 and then stimulated with KL (100 ng/ml), IL-10 (100 U/ml) and LPS (100 ng/ml) at 37°C for 8 h in the presence or absence of 13. $PGD_2$ released into the supernatant was quantified by EIA kit. (B) BMMC were pre-incubated for 30 min with the indicated concentrations of 13 and then stimulated with 100 ng/ml of KL for 15 min. $LTC_4$ released into the supernatant was quantified by EIA kit. (C) BMMC were pre-incubated for 30 min with the indicated concentrations of 13 and then stimulated with 100 ng/ml of KL for 15 min. β-HEX released into the supernatant cell lysate was measured. The data represent the mean ± S.D. of three different samples. $*p<0.05$, $**p<0.01$, $***p<0.001$ versus control.

COX-2 and 5-LOX, was used as positive control ($IC_{50}$ of 0.025 μM and 0.86 μM, respectively)(Boileau *et al*., 2002; Rotondo *et al*., 2002). These results clearly demonstrated that compounds 10, 11, 13, 15 and 19 have dual COX-2/5-LOX inhibitory activity. In addition, 11, 12, 13, 15 and 19 also inhibited β-HEX release in BMMCs in a concentration-dependent manner with an $IC_{50}$ of 28.7 μM, 82.4 mM, 1.4 mM, 16.8 mM and 16.6 mM, respectively, in comparison with the positive control deoxypodophyllotoxin (DPT) (Lee *et al*., 2004), which inhibited degranulation re-

action in a dose-dependent manner in BMMCs ($IC_{50}$: 27.5 μM). Among these compounds, 13 exhibited the strongest inhibitory effects not only on generation of $PGD_2$ and LTC4 but also on β-HEX release in BMMCs (Fig. 2). The inhibitory activity of 13 on COX-2 activity in RAW 264.7 cells but no inhibitory activities on 5-LOX or β-HEX release of 13 has been reported (Kim *et al*., 2004).

In conclusion, compounds 10, 11, 12, 13, 15 and 19 among those isolated from the roots of *U. davidiana* are principal compounds that inhibit COX-2-dependent $PGD_2$ generation, 5-LOX-dependent $LTC_4$ generation and β-HEX release in BMMCs. These results suggest that the anti-inflammatory activity of *U. davidiana* var. *japonica* might occur by both the inhibited generation of eicosanoids and obviated mast cell degranulation. Further studies are needed to investigate the mechanisms of action of the isolated compounds.

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