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Structure-Activity Relationships of Polyhydroxyursane-type Triterpenoids on the Cytoprotective and Anti-inflammatory Effects

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Abstract – Eleven polyhydroxyursane triterpenoids (PHUTs) were tested to determine their cytoprotective, immunosuppressive and anti-inflammatory effects. To compare the bioactivities of 19α-hydroxyursane-type triterpenoids {23-hydroxytormentic acid (**6**), its methyl ester (**7**), tormentic acid (**8**), niga-ichigoside F_1 (**9**), euscaphic acid (**10**) and kaji-ichigoside F_1 (**11**)} of the Rosaceae crude drugs (Rubi Fructus and Rosa rugosae Radix) with PHUTs possessing no 19α-hydroxyl of *Centella asiatica* (Umbelliferae), the four PHUTs, asiaticoside (**1**), madecassoside (**2**), asiatic acid (**3**), and madecassic acid (**4**) were isolated from *C. asiatica* and 23-hydroxyursolic acid (**5**) from *Cussonia bancoensis*. Cytoprotective effects were assessed by measuring cell viabilities against cisplatin-induced cytotoxocity in LLC-PK_1 cells (proximal tubule, pig kidney) to determine whether these agents have protective effects against nephrotoxicity caused by cisplatin. The inhibitory effect of 11 PHUTs on nitric oxide (NO) and prostaglandin E_2 (PGE_2) were evaluated by measuring nitrite accumulation in lipopolysaccharide (LPS)-induced macrophage RAW 264.7 cells, and their anti-inflammatory effects were tested in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema model. Six MHUTs (compounds **1**, **2**, **4**, **6**, **10**, and **11**) exhibited higher cell viabilities during cisplatin-induced cytotoxicity testing even at a concentration of 200 μg/ml than cisplatin only-treated group, suggesting that these compounds have the potent cytoprotective effects. Compounds **1** and **3** of the *C. asiatica* and niga-ichigoside F_1 exhibited no inhibitory effect on NO and/or PGE_2 production whereas other PHUTs produced mild to significant NO and/or PGE_2 production. The four compounds (**2**, **5**, **9**, and **10**) potently inhibited mouse ear edema induced by TPA whereas two compounds (**1** and **3**) had no activity in this test. These results suggest that many PHUTs are potent chemopreventives. Structure-activity relationship (SAR) was also discussed in each assay with regard to the significant role of OHs at the position of 2, 3, 6, 19, and 23 and to the glycoside linkage at the 28-carboxyl.
Keywords – polyhydroxyursane-type triterpenoid, madecassic acid, madecassoside, cytoprotective, immunosuppressive, inflammation

Introduction

We previsously reported on the antinociceptive and anti-inflammatory effects of polyhydroxylated ursane-type triterpenoids (PHUTs) isolated from the Rosaceae crude drugs, Rubi Fructus and Rosa rugosae Radix (Choi *et al.*, 1995; Jung *et al.*, 2005). These compounds have been revealed to be the active principles, e.g., euscaphic acid, tormentic acid, kaji-ichigoside F_1 and rosamultin from the roots of *Rosa rugosa* (Rosaceae) (Jung *et al.*, 2005) and 23-hydroxytormentic acid and niga-ichigoside F_1 from the unripe fruits of *Rubus coreanus* (Rosaceae) (Choi *et al.*, 2003), which were mainly found to have antinociceptive and anti-inflammatory effects. These compounds shared the common feature of possessing 19α-hydroxyl group of PHUTs, as shown in Fig. 1, and usually have the hydroxyls at C-2, 3, 19 and 23 or a D-glucose attached to the C_{28}-carboxyl. Many researchers have described the anti-inflammatory effect of triterpenoids (Recio *et al.*, 1995; Safahi *et al.*, 1997; Murakami *et al.*, 2002). 19α-Hydroxyursane-type triterpenoids of PHUTs have been known as the active principles in the Rosaceae crude drugs. To determine the significance of the 19α-

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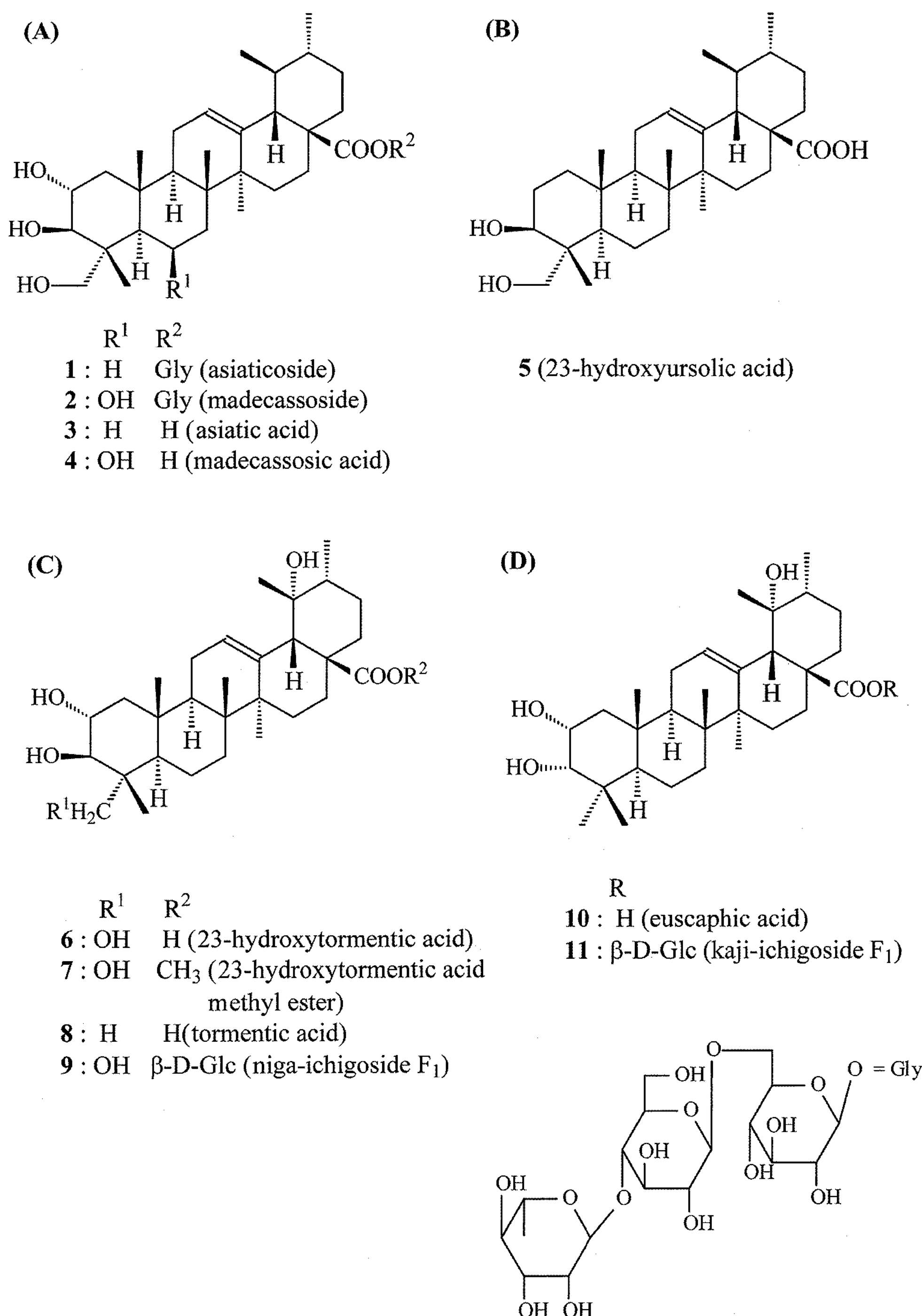

Fig. 1. Structure of the multihydroxyursane-type triterpenoids (MHUTs, **1-11**) tested.
The compounds in (A) and (B) were isolated from *Centella asiatica* and *Cussonia bancoensis*, respectively. The compounds in (C) and (D) were isolated from Rosaceaous plants or a derivatized compound (7), but (A) is the compounds with 3β-OH functional group and (B) is the compound with 3α-OH.

hydroxyl group of PHUTs with respect to their bioactivity and structure-activity relationships on the PHUTs, we isolated the four PHUTs without a 19α-hydroxyl group, namely asiatic acid, asiaticoside, madecassic acid and madecassoside from *Centella asiatica* (Umbelliferae), and undertook experiments to compare the bioactivities of several PHUTs under identical assay conditions. Several of the PHUTs examined showed significant bioactivities, whereas others exhibited no activity. Differential activities were attributed to molecular structural differences.

Experiments were performed on eleven PHUTs shown in Fig. 1 to determine their protective effects against cisplatin-induced cytotoxicity in LLC-PK_1 cells (proximal tubule, pig kidney), the inhibitory effect on nitric oxide (NO) and prostaglandin E_2 (PGE_2) formation in lipopolysaccharide (LPS)-induced macrophage RAW 264.7 cells, and their anti-inflammatory effect on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema.

Experimental

Reagents – Dulbecco's modified Eagle's minimum essential medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were obtained from Life Technologies Inc (Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and *Escheichia coli* LPS were purchased from the Sigma Chemical Co. (St Louis, MO, USA), and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was initially supplied by Chemsyn Science Laboratory (Lenexa, KS) and later by Alex Biochemicals (San Diego, CA).

Isolation of 19α-hydroxyursane-type triterpenoids – The MHUTs, euscaphic acid, tormentic acid, kaji-ichigoside F_1, were isolated from *Rosa rugosa* roots and identified based on physicochemical data (mp, $[\alpha]_D$, NMR) comparisons *versus* authentic specimens as described previously (Jung *et al.*, 2005; Young *et al.*, 1987). The triterpenoids, niga-ichigoside F_1 and 23-hydroxytormentic acid, were isolated from the unripe fruits of *Rubus coreanus* as described in our previous article and further identified on the basis of physicochemical data (Choi *et al.*, 2003). To prepare 23-hydroxytormentic acid methyl ester, 23-hydroxytormentic acid was methylated with ethereal diazomethane by worked-up normally; Compound **7** (23-hydroxytormentic acid methyl ester): mp 170 °C, ^{1}H-NMR (C_5D_5N, 500 MHz): δ 3.70 (3H, s, $COOCH_3$); ^{13}C-NMR (C_5D_5N, 125 MHz); δ 178.2 ($\underline{C}OOCH_3$), 51.3 ($COO\underline{C}H_3$).

Extraction and fractionation from *Centella asiatica* – To obtain PHUTs lacking the 19-hydroxyl group, namely asiaticoside, madecassoside, asiatic acid and madecassic acid, the dried leaves of *Centella asiatica* (Umbelliferae; purchased from Sigma) were used. These were extracted three times with MeOH at 50 °C under reflux, and the MeOH extract (342 g) obtained concentrated on a rotatory evaporator under reduced pressure, then suspended in H_2O and then successively partitioned and dried to give a $CHCl_3$ fraction (79 g), and BuOH fraction (44 g) and a residual fraction (180 g).

Isolation of asiaticoside (1) and madecassoside (2) – The BuOH fraction was chromatographed on a silica gel column (280 g, 5.5 × 80 cm, Merck, Art 7734, Germany) using $CHCl_3$-MeOH-H_2O (7 : 3 : 1, lower phase) as eluent to give five fractions (Fr.1 - Fr. 5). Further chromatography of Fr. 2 (1.2 g) was performed on a reversed phase C_{18} column (200 g, 3.0 × 35.0 cm, ODS, YMC, AA12S75, Japan) and then recrystallized from MeOH to yield compound **1** (220 mg). ODS column chromatography of Fr. 4 (2 g) was performed using the same condition to yield compound **2** (500 mg). Compounds **1** and **2** were identified as asiaticosode and madecassoside, respectively, based on matches with previously reported physicochemical data (Sung *et al.*, 1992; Sahu *et al.*, 1989); Compound **1** (asiaticosode): mp 230 - 232 °C, $[\alpha]_D^{25}$ −15° (c = 0.65, MeOH), ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz); Compound **2** (madecassoside): mp 220 - 230 °C, $[\alpha]_D^{25}$ +12.0° (c = 0.65, MeOH), ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz).

Hydrolysis of the BuOH fraction and isolation of asiatic acid (3) and madecassic acid (4) – To obtain the aglycones of the PHUTs in *C. asiatica*, the BuOH fraction was hydrolyzed by refluxing it in 2% NaOH in H_2O-MeOH (8 : 2) solution (95 ml) for 4 h. The cooled reaction mixture was then acidified with diluted HCl and extracted with EtOAc, which was washed with water and dried to give the hydrolyzed fraction (5 g).

The two triterpenoids **3** and **4** in this hydrolyzed fraction had R_f values 0.44 and 0.29 on a silica gel-coated TLC (solvent, $CHCl_3$-MeOH-H_2O: 8 : 2 : 1, lower phase). Therefore, this hydrolyzed fraction was subjected to column chromatography using $CHCl_3$-MeOH-H_2O (90 : 7 : 1, lower phase) and gave 5 fractions (HFr. 1 - HFr. 5 fractions). Successive ODS column chromatography of HFr. 2 was performed using MeOH-H_2O (8 : 2) as eluent to yield compound **3**, and chromatography of HFr. 4 on a silica gel column was also performed using $CHCl_3$-MeOH-H_2O (8 : 2 : 1, lower phase) as eluent afforded compound **4**. Compounds **3** and **4** was identified as asiatic acid and medecassic acid, respectively, on the basis of physicochemical data; Compound **3** (asiatic acid): mp 301 - 305 °C, $[\alpha]_D^{25}$ +52° (c = 0.65, MeOH), ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz), which matched reported data (Ahmad *et al.*, 1994); Compound **4** (madecassic acid): mp 295 - 300 °C, $[\alpha]_D^{25}$ +31° (c = 1.5, MeOH), ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz), which was also identical to reported data (Sung *et al.*, 1992).

Protective activity against cisplatin-induced cytotoxicity – LLC-PK_1 cells (proximal tubule, pig kidney) were obtained from the American Type Culture Collection. LLC-PK_1 cells were maintained as monolayer cultures in

medium 199 supplemented with 5% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (complete culture medium). Two days before experiments, cells were seeded in plates at 2×10^4 cell/cm^2 of culture area. Cells were maintained in a 5% CO_2 humiditified atmosphere at 37 °C throughout. Eleven PHUTs were dissolved in dimethyl sulfoxide and diluted with complete culture medium resulting to produce a final dimethyl sulfoxide concentration of 0.1%.

Cell culture and sample treatment – RAW 264.7 murine macrophage cells were obtained from the Korean Cell Line Bank (Seoul), and grown at 37 °C in DMEM medium supplemented with 10% FBS, penicillin (100 units/mL) and streptomycin sulfate (100 μg/ml) in a humidified atmosphere of 5% CO_2 atmosphere. Cells were then incubated with the samples at different concentrations and stimulated with 1 μg/mL LPS.

Nitrite accumulation – Nitrite accumulated in culture medium was measured as an indicator of NO production using a Griess reaction based method. Briefly, 100 μL of cell culture medium was mixed with 100 μL of Griess reagent (equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamine-HCl), incubated at room temperature for 10 min, and then the absorbance at 550 nm was measured using a microplate reader. Fresh culture medium was used as a blank in all experiments. The amounts of nitrite in samples were read-off a sodium nitrite serial dilution standard curve.

PGE_2 Assay – PGE_2 assay levels in the macrophage culture media were quantified using EIA kits according to the manufacturer's instructions.

MTT assays of cell viability – Cell viabilities were measured in 96-well plates. RAW 264.7 cells were mechanically scraped and plated at 5×10^5 cells/well in 96-well plates containing 100 μL of DMEM with 10% FBS and incubated overnight. The samples were dissolved in DMSO ensuring that the same amounts of DMSO were added to all plates. After overnight incubation, test materials were added, and the plates were incubated for 24 h. Cells were washed once before adding 50 μL of FBS-free medium containing 5 mg/mL MTT. After 4 h of incubation at 37 °C, media were discarded and the formazan blue produced was dissolved in 100 μL DMSO. The optical densities were measured at 540 nm.

Animals – Female ICR mice (supplied by the Korean Laboratory Animal Co., Daejon, Korea), 6 weeks of age were, were used in this study. Animals were housed at 22 ± 2 °C under a 12 h lighting schedule with access to food and water *ad libitum*.

Induction of edema – To assess the anti-inflammatory effect of triterpenoids, mice (three per group) were treated on the inner side of the right ear with 50 μl vehicle (acetone : DMSO = 1 : 1) containing each compound (10 μmol) or the same volume of the solvent alone 30 min prior to the application of 5 nmol TPA in 50 μl acetone. Control animals were treated with acetone in lieu of TPA. Mice were sacrificed 4 h after TPA application and both ears were removed. Circular sections (6 mm diameter) were taken using a cork borer with a diameter of 6 mm and weighed. Degrees of ear edema were calculated by subtracting the weight of the left ear sections from those of the right ear sections. Inhibition percentages were shown in Table 3.

Results and discussion

Cytoprotective effect – The cytoprotective effects of PHUTs are shown in Table 1. Cisplatin only-induced LLC-PK_1 cells showed 44.4% viability against the untreated control cells whereas 10 PHUTs (except niga-ichigoside F_1) exhibited marked cytoprotection at concentrations of less than 25 μg/ml. The antioxidant positive controls, ferulic acid and deferoxamine, showed potent cytoprotective; the former one exhibited concentration-dependent effects, but the latter one showed more than 78.2% cytoprotection throughout the concentrations. A few PHUTs reduced in cell viability at concentrations of more than 200 μg/ml, but the six compounds, asiaticoside (**1**), madecassoside (**2**), 23-hydroxytormentic acid (**6**), euscaphic acid (**10**), madecassic acid (**4**) and kaji-ichigoside F_1 (**11**) showed very high cell viabilities even at the highest concentration (200 μg/ml) examined. The PHUTs that were cytotoxic at high concentrations were; 23-hydroxyursolic acid (**5**), asiatic acid (**3**), 23-hydroxytormentic acid methyls ester (**6**), tormentic acid (**8**) and niga-ichigoside F_1 (**9**). Moreover, although 23-hydroxtormentic acid (**6**) showed a potent cytoprotective effect, its 28-*O*-glucoside (**9**) did not, which suggests that a sugar linkage to the triterpene acid enormously changes the cytoprotective activity at least according to this assay system.

Inhibition of LPS – induced NO in macrophage cells- The inhibitory effect of PHUTs on the nitric oxide production were measured in LPS-induced macrophage 264.7 cells by nitrite assay, and cytotoxicities was measured using MTT assays. IC_{50} data are shown in Table 2. The PHUTs with the inhibitory effect on NO production were; 23-hydroxytormentic acid (**6**), asiatic acid (**3**), euscaphic acid (**10**), tormentic acid (**8**), madecassic acid (**4**), and kaji-ichigoside F_1 (**11**).

Table 1. Cytoprotective effect of PHUTs (**1-11**) on cisplatin-induced cytotoxicity in the LLC-PK_1 cells (proximal tubule, pig kidney)

Treatment	Control	Cisplatin	Concentration (mg/ml)					
			6.25	12.5	25	50	100	200
1	100.0	44.4	70.7 [a]	73.9	61.4	61.8	58.1	56.8
2	100.0	44.4	72.6	80.1	67.8	68.5	74.1	81.2
3	100.0	44.4	82.0	77.0	82.2	67.6	25.2	10.4
4	100.0	44.4	49.0	52.0	55.1	67.9	85.2	92.5
5	100.0	44.4	82.8	98.6	61.8	23.4	10.7	16.4
6	100.0	44.4	85.4	75.5	76.2	81.8	87.6	93.1
7	100.0	44.4	75.4	74.1	74.8	85.3	43.1	10.9
8	100.0	44.4	84.0	77.9	83.6	114.7	60.6	10.4
9	100.0	44.4	37.1	31.9	11.3	12.0	11.1	12.1
10	100.0	44.4	76.5	78.2	81.1	99.3	121.8	177.1
11	100.0	44.4	50.5	55.4	61.1	67.9	79.6	96.0
Ferulic	100.0	44.4	48.9	54.0	59.5	65.6	77.6	80.3
Defer.	100.0	44.4	83.3	83.0	81.1	85.9	78.2	76.2

Abbreviation: Ferulic (ferulic acid), defer. (deferoxamine).
[a] The values represent the means of three independent experiments.

Table 2. IC_{50} of PHUTs (**1-11**) on NO and PGE_2 production in LPS-activated macrophage RAW 264.7 cells and cytotoxicities

compound	IC_{50} (μg/ml)		
	NO	PGE_2	Cytotoxicity
1	69.3	71.6	63.5
2	100	95.4	> 150
3	52.7	88.1	85.3
4	85.4	70.4	153.8
5	14.7	3.1	19.2
6	44.3	98.9	150.3
7	36.2	39.1	50.0
8	15.8	11.4	43.7
9	75.2	114.3	114.3
10	29.0	51.4	130.9
11	83.3	> 150	142.7

[a] IC_{50} is defined as the concentration which results in a 50% decrease in NO and PGE_2 production and cell number as compared with that of the control cultures in the absence of an inhibitor. The values represent the mean of three independent experiments.

Inhibitory effect on the PGE_2 production in macrophage cells (in vitro anti-inflammatory) – The IC_{50} values of PHUTs on PGE_2 production by 1 μg/ml LPS-induced macrophage 264.7 cells are shown in Table 2. The IC_{50} value 3.1 μg/ml of 23-hydroxyursolic acid (**5**) suggest that this compound has a potent anti-inflammatory effect, whereas asiaticoside (**1**) and asiatic acid (**3**) of *C. asiatica* had higher IC_{50} values than those of the cytotoxicity, which suggest that these two compounds do not inhibit inflammation by inhibiting prostaglandin production. The two compounds madecassoside (**2**) and madecassic acid (**4**) inhibited PGE_2 production.

Table 3. Inhibitory effect of topical application of PHUTs derivative on TPA-induced edema of mouse ears

Treatment	Weight (mg)/punch	Inhibition (%)
Acetone/DMSO + acetone	0.583 ± 0.17	
Acetone/DMSO + TPA	3.840 ± 0.59	
TPA + asiaticoside (**1**)	5.067 ± 1.23	0
+ madecassoside (**2**)	1.167 ± 0.30*	82.1
+ asiatic acid (**3**)	4.700 ± 1.30	0
+ madecassic acid (**4**)	2.700 ± 1.20	35.0
+ 23-hydroxyursolic acid (**5**)	1.800 ± 0.32*	62.6
+ 23-HTA (**6**)	3.467 ± 0.55	11.5
+ 23-HTA methyl ester (**7**)	2.767 ± 1.04	33.0
+ tormentic acid (**8**)	3.100 ± 0.53	22.7
+ niga-ichigoside F_1 (**9**)	1.500 ± 0.17*	71.9
+ euscaphic acid (**10**)	2.033 ± 0.16*	55.5
+ kaji-ichigoside F_1 (**11**)	2.800 ± 0.40	31.9

Mouse ears were treated with acetone/DMSO (1 : 1) (50 μl), TPA (5 nmol) in acetone (50 ml) or TPA together with a MHUT in acetone/DMSO. Four hours later, animals were sacrificed and ear punches (diameter 6 mm) were weighed. The data represent the mean ± S.E. from 3 mice/group. * $p < 0.05$ statistically different versus animals treated with TPA alone. Abbreviation, HTA (hydroxyotrmentic acid).

Inhibitory effect on TPA-induced mouse ear edema – TPA is used to induce pathological animal models of inflammation or carcinogenesis. Fifty μl vehicle containing

10 μmole of each PHUT dissolved in acetone-DMSO (1 : 1) was applied topically to the inner side of the right ears 30 min prior to the application of 5 nmole TPA in 50 μl acetone. Inhibitory effects of the 11 agents (**1-11**) on TPA-induced mouse ear edema are shown in Table 3. Only asiaticoside (**1**) and asiatic acid (**3**) were inactive among the tested MHUTs. Moreover, four compounds, namely madecassoside (inhibitory percentage, 82.1%), 23-hydroxyursolic acid (62.6%), euscaphic acid (55.5%) and niga-ichigoside F_1 (71.9%) were obviously active. Of the four triterpenoids isolated from *C. asiatica*, the triteprenoids with a 6-OH, i.e., madecassic acid (**4**) and madecassoside (**2**) exhibited marked activities, whereas those without 6-OH lacked any activity. These findings suggest that the presence of 6-OH in the PHUTs of *C. asiatica* plays a key role in their bioactivities in terms of reducing TPA-induced mouse ear edema.

Discussion

The results of the present study show that many PHUTs variably have the cytoprotective, in vitro or in vivo anti-inflammatory effects and suggest that PHUTs have potential use as chemopreventives. Although the bioactivity of PHUTs with 19-OH led to no general conclusion on structure-activity relationships, it was observed that the cytotoxicity of all triterpene acids possessing 19-OH were not strong. On the other hand, niga-ichigoside F_1 with a sugar linkage at C-23 in 23-hydroxytormentic acid displayed no significant *in vitro* activity.

Cisplatin is used to treat solid tumors such as those of testicular, ovarian, bladder, stomach, lung and cervical cancer and brains tumors (Rosenberg *et al.*, 1969; Connors *et al.*, 1972). Cisplatin inhibits the growth and/or prolifernation of cancer cells by preventing DNA replication by cross-linking DNA double strands (Carruthers *et al.*, 2002). Of the many adverse effects of cisplatin, the most prominent is a nephrotic function failure due to its high toxicity on the nephrotic tissues. Moreover, oxygen free radicals produced by stimulating immune cells cause lipid peroxidation in renal tissues (Sojuks *et al.*, 1991). Therefore, new cancer chemotherapies should have reduced toxicities, and, as shown in Table 1, the present study suggests that several PHUTs shown reduced toxicities in combination of cisplatin.

We previously reported the antinociceptive, anti-inflammatory and anti-gastropathic effects of 23-hydroxytormentic acid (**6**) and niga-ichigoside F_1 (**9**) (Nam *et al.*, 2002) and their *in vivo* protective effects versus cisplatin-induced nephrotoxicity (Kim, 2003). The present *in vitro* assays conducted in the present study using kidney cells, also demonstrate that the *in vivo* protective effect of niga-ichigoside F_1 (**9**) should be attributed to its aglycone, 23-hydroxytormentic acid (**6**), based on the lack of a cytoprotective effect of niga-ichigoside F_1. The potent effect of 23-hydroxytormentic acid (**6**) was also demonstrated by low protection level of its methyl ester (**7**), which indicates that the carboxyl of **6** is essential for the cytoprotective activity. More specifically, it appears that the glycoside (**9**) should be metabolized to the aglycone (**6**) to produce an effect *in vivo*.

The inhibitory effects of PHUTs on NO production were measured in LPS-induced macrophage 264.7 cells using nitrite assays. NO has a diverse physiological functions, e.g., it participates in defense mechanism against viruses, bacteria and parasites, although the excess NO production may cause arthritis, diabetes, stroke, septic shock, autoimmune disease, chronic inflammatory disease, or atherosclerosis (Bredt and Snyder, 1987). Therefore, it should be mentioned that PHUTs may have a variety of biological effects because of the inhibitory effects on NO production.

To determine whether the tested compounds inhibited the production of PGE_2, a pro-inflammatory mediator, macrophage RAW 264.7 cells were pre-incubated with individual PHUTs for 1 h, and then activated with 1 μg/ml LPS for 24 h. Only asiaticoside (**1**), asiatic acid (**3**) and niga-ichigoside F_1 had no inhibitory effect on PGE_2 production. On the other hand, it was interesting that madecassoside (**2**) and madecassic acid (**4**) (PHUTs from *C. asiatica*) which possess a 6-OH, had inhibited PGE_2 production, suggesting that 6-OH plays a critical role in this biological activities.

TPA is the reagent frequently used to induce of inflammation (Fujiki and Sugimura, 1987) or to promote tumor growth (Murakami *et al.*, 1978). Tumor promoter-induced inflammation is distinct from acute inflammation, which is exudative in nature and accompanied by fibroblast proliferation and granulation. Murakami *et al.* (2002) reported that the inhibitory effects of tormentic acid and euscaphic acid from *Rubus sieboldii* on TPA-induced inflammation are associated with their inhibition of the mammalian DNA polymerase activity, indicating that many PHUTs are chemopreventives and that they have anti-inflammatory effects.

In conclusion, the tested PHUTs variably exhibited their unique cytoprotective, *in vitro* or *in vivo* anti-inflammatory effects, and the attachment of hydroxyls or sugars at C_{28}-carboxyl appears to alter their potencies. It was noticeable that the PHUTs examined all protected

cells from the cytotoxic effect of cisplatin at relatively low concentrations. In particular, the present study demonstrates that 19α-Hydroxyursane-type tritepenoids in Rosaceae crude drugs find use in traditional medicine primarily because of their cytoprotective effects.

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