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Article

Cytotoxic Triterpenoid from *Rubus coreanus* Miq.

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Dried unripe fruits of *Rubus coreanus* Miq. were extracted with 80% aqueous MeOH and the concentrated extract was partitioned with EtOAc and H_2O. From the EtOAc fraction, four triterpenoids were isolated through repeated silica gel, ODS and Sephadex LH-20 column chromatographies. From the result of physico-chemical data including NMR, MS and IR, the chemical structures of the compounds were determined as tormentic acid (1), myrianthic acid (2), hovenic acid (3) and 2α,3β,19β,23-tetrahydroxylolean-12-en-28-oic acid (4). Compounds 3 and 4 were isolated for the first time from this plant. All isolated compounds were evaluated for cytotoxic activity against human colon carcinoma cells using *in vitro* three-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, compound 3 showed a higher cytotoxicity (IC_{50} = 7.8 μM) than doxorubicin (IC_{50} = 50 μM).

Key words: *cytotoxicity, HCT-116, hovenic acid, myrianthic acid,* Rubus coreanus, *2α,3β,19β,23-tetrahydroxylolean-12-en-28-oic acid, tormentic acid*

Rubus species have been cultivated for centuries to obtain their fruits, which have been used traditionally for therapeutic purposes as an aphrodisiac, astringent, ophthalmic and restorative [Jeung *et al.*, 1990]. *R. coreanus* Miq. (Rosaceae) is a perennial shrub distributed throughout southern Korea, where thickets on slopes and montane valleys at elevations of 100-1000 meters and growing to 3 m by 3 m. It is in flower from May to June, and the seeds ripen from July to August. The fruits are dark red or purplish black and 5-8 mm in diameter [Lee, 2003].

The dried unripe fruits of *R. coreanus*, well known as "Bok-bun-ja" in Korea, have been used as traditional herbal medicine for the treatment of impotence, spermatorrhea, enuresis, asthma and allergies, as well as a stomachic and tonic in Korea [Perry *et al.*, 1980; Kim *et al.*, 1996]. The fruit is also eaten fresh or as a processed food, such as bread, wine and health drinks [Kwon *et al.*, 2006; Kwon *et al.*, 2004].

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It has been reported that the functional constituents are niga-ichigoside F1, 23-hydroxytormentic acid, polyphenols, gallic acid and sanguine, which have anti-carcinogenic, anti-nociceptive, and anti-inflammatory effects [Cha *et al.*, 2001; Choi *et al.*, 2003; Pang *et al.*, 1996]. However, cytotoxicitic compounds against human colon and liver cancer cells has not yet been reported, though alcohol extracts showed cytotoxic activity against these cells. Currently, colon cancer and liver cancer are the most prevalent and deadly cancers in Western society and the incidence of these types of cancer are increasing in Asian countries. Therefore, this paper describes the isolation and identification of four triterpenoids as major components of the fruits of *R. coreanus*. The structure of the triterpenoids was characterized by spectroscopic methods. Among the identified compounds, two were isolated for the first time from this plant. The isolated compounds were tested for cytotoxicity against a human colon carcinoma (HCT-116) cell line, using *in vitro* MTT assay.

Materials and Methods

Plant materials. The dried unripe fruits of *R. coreanus*

were purchased at a herbal drug store in Daegu and identified by Prof. Dae-Keun Kim, College of Pharmacy, Woo Suk University, Jeonju, Korea. A voucher specimen (KHU060809) is reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Suwon, Korea.

Instruments and reagents. Optical rotations were measured on a JASCO P-1010 digital polarimeter (Tokyo, Japan). EI-MS data were recorded on a JEOL JMSAX 505-WA (Tokyo, Japan). FAB-MS data were recorded on a JEOL JMS-700 (Tokyo, Japan). IR spectra were run on a Perkin Elmer spectrum One FT-IR spectrometer (Buckinghamshire, England). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (Palo Alto, CA). RPMI medium 1640, Dulbecco's modified eagle medium (GIBCO BRL, Life Technologies Inc., NY) and penicillin-streptomycin were purchased from GIBOCO (Grand Island, NY). Fetal bovine serum was obtained from Hyclone (Logan, UT). MTT and DMSO were purchased from Sigma (St. Louis, MO).

Extraction and isolation. The dried and chopped fruits of *R. coreanus* (18 kg) were processed with 80% aqueous MeOH (40 L) three times at room temperature. The extracts were partitioned with H_2O (4 L) and EtOAc (4 L × 2). The EtOAc extract (144 g) was applied to silica gel column (10 × 60 cm) chromatography and eluted with $CHCl_3$-MeOH (12 : 1 → 10 : 1 → 7 : 1, 2 L of each) monitoring by TLC to produce thirteen fractions (RCE1 to RCE13). Fraction RCE8 [9.2 g, V_e/V_t (elution volume/ total volume) 0.53-0.60] was subjected to silica gel c.c. (5 × 45 cm) and eluted with $CHCl_3$-MeOH (15 : 1, 3.2 L) to produce seven subfractions (RCE8-1 to RCE8-7). Subfraction RCE8-2 (566 mg, V_e/V_t 0.23-0.30) was applied to ODS (3.5 × 50 cm) and eluted with MeOH-H_2O (3 : 1, 1.6 L) to afford seven subfractions (RCE8-2-1 to RCE8-2-7) and yielded compound **1** [16 mg, V_e/V_t 0.53-0.60, TLC (RP-18 F_{254}) R_f 0.55 in MeOH-H_2O=5:1]. Subfraction RCE8-5 (402 mg, V_e/V_t 0.78-0.82) was applied to the ODS (3 × 40 cm) and eluted with MeOH-H_2O (2 : 1, 1 L) to afford eight subfractions and yield compound **2** [25 mg, V_e/V_t 0.30-0.35, TLC (RP-18 F_{254}) R_f 0.65 in MeOH-H_2O = 3 : 1]. Subfraction RCE8-5-6 (120 mg, V_e/V_t 0.80-0.85) was subjected to Sephadex LH-20 (2 × 40 cm, 80% MeOH, 500 mL) to ultimately produce compound **3** [45 mg, V_e/V_t 0.60-0.85, TLC (RP-18 F_{254}) R_f 0.45 in MeOH-H_2O = 3 : 1]. Fraction RCE11 (820 mg, V_e/V_t 0.83-0.90) was applied to ODS (3.5 × 50 cm) and eluted with MeOH-H_2O (2 : 1, 1.5 L) to yield compound **4** [144 mg, V_e/V_t 0.70-0.80, TLC (RP-18 F_{254}) R_f 0.5 in MeOH-H_2O = 4 : 1].

Compound **1**: White powder (MeOH); mp 272°C; $[\alpha]_D^{20}$ = +29.1° (*c* = 0.10, MeOH); IR_υ (KBr, cm^{-1}) 3447, 1754, 1696; EI-MS *m/z*: 488 $[M]^+$, 470, 370, 264, 248, 146; ^{1}H-NMR (400 MHz, pyridine-d_5) δ 5.58 (1H, br. s, H-12), δ 4.09 (1H, ddd, *J* = 10.0, 9.4, 3.6 Hz, H-2), δ 3.37 (1H, d, *J* = 9.4 Hz, H-3), δ 3.04 (1H, s, H-18), δ 1.70 (3H, s, H-27), δ 1.42 (3H, s, H-29), δ 1.26 (3H, s, H-23), δ 1.11 (3H, d, *J* = 5.7 Hz, H-30), δ 1.07 (3H, s, H-24), δ 1.00 (3H, s, H-25), δ 0.98 (3H, s, H-26); ^{13}C-NMR (100 MHz, pyridine-d_5), see Table 1.

Compound **2**: White powder (MeOH); mp > 300°C; $[\alpha]_D^{20}$ = +33.0° (*c* = 0.05, MeOH); IR_υ (KBr, cm^{-1}) 3451, 1705, 1640; EI-MS *m/z*: 504 $[M]^+$, 458, 442, 386, 264, 248, 55; ^{1}H-NMR (400 MHz, pyridine-d_5) δ 5.57 (1H, br. s, H-12), δ 4.26 (1H, m, H-2), δ 4.12 (1H, d, *J* = 2.4 Hz, H-3), δ 3.90 (1H, d, *J* = 10.5 Hz, H-23a), δ 3.73 (1H, d, *J* = 10.5 Hz, H-23b), δ 3.02 (1H, br. s, H-18), δ 1.65 (3H, s, H-27), 1.39 (3H, s, H-29), δ 1.11 (3H, s, H-24), δ 1.08 (3H, d, *J* = 4.4 Hz, H-30), δ 1.00 (3H, s, H-25), δ 0.84 (3H, s, H-26); ^{13}C-NMR (100 MHz, pyridine-d_5) see Table 1.

Compound **3**: White powder (MeOH); mp 180-182°C; $[\alpha]_D^{25}$ = −35° (*c* = 0.02, MeOH); IR_υ (CaF_2 window in MeOH, cm^{-1}) 3400, 2927, 1715, 1687; EI-MS *m/z*: 488 $[M]^+$, 470, 370, 264, 248, 146; ^{1}H-NMR (400 MHz, pyridine-d_5) δ 4.92 (1H, br. d, *J* = 2.0 Hz, H-29b), δ 4.75 (1H, br. s, H-29a), δ 4.24 (1H, ddd, *J* = 11.4, 10.0, 4.5 Hz, H-2), δ 4.21 (1H, d, *J* = 10.0 Hz, H-3), δ 4.18 (1H, d, *J* = 11.2 Hz, H-23a), δ 3.71 (1H, d, *J* = 11.2 Hz, H-23b), δ 3.52 (1H, m, H-19), δ 2.72 (1H, ddd, *J* = 12.0, 12.0, 4.5 Hz, H-13), δ 1.76 (3H, s, H-30), δ 1.71 (1H, dd, *J* = 12.0, 12.0 Hz, H-18), δ 1.04 (3H, s, H-24), δ 0.98 (3H, s, H-25), δ 0.95 (3H, s, H-26), δ 0.92 (3H, s, H-27); ^{13}C-NMR (100 MHz, pyridine-d_5), see Table 1.

Compound **4**: White powder (MeOH); mp 280; $[\alpha]_D^{25}$ = +42° (*c* = 0.02, MeOH); IR (CaF_2 window in MeOH, cm^{-1}) ν_{max} 3367, 2917, 1751, 1690, 1451; Positive FABMS *m/z*: 527.3 $[M+Na]^+$; 505 $[M+H]^+$; ^{1}H-NMR (400 MHz, methanol-d_4): δ 5.31 (1H, t-like, *J* = 3.5 Hz, H-12), 3.69 (1H, ddd, *J* = 11.5, 10.0, 4.5 Hz, H-2), 3.50 (1H, d, *J* = 11.5 Hz, H-23a), 3.35 (d, *J* = 10.0 Hz, H-3), 3.25 (1H, *J* = 4.0 Hz, H-19), 3.27 (1H, d, *J* = 11.5, H-23b), 3.04 (1H, br. s, H-18), 1.31 (3H, s, H-27), 1.02 (3H, s, H-25), 0.96 (3H, s, H-30), 0.93 (3H, s, H-29), 0.76 (3H, s, H-26), 0.70 (3H, s, H-24); ^{13}C-NMR (100 MHz, methanol-d_4), see Table 1.

Cell culture. HCT-116 cells spontaneously originated from the human colon were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). They were maintained in RPMI medium 1640 and DMEM supplemented with 10% heat-inactivated FBS, 100 unit/mL penicillin-streptomycin and sodium bicarbonate, and incubated in a humidified atmosphere of 5% CO_2 at 37°C. They were

Table 1. ^{13}C NMR data of compounds 1, 2, 3 (in pyridine-d_5) and 4 (in methanol-d_4) from the fruits of *R. coreanus*

Carbon No.	Compound 1	Compound 2	[a]Compound 3	[a]Compound 4
1	47.22	42.44	47.61	46.59
2	68.59	66.24	68.66	69.57
3	83.85	78.88	77.61	78.15
4	39.50	42.12	43.22	42.63
5	55.05	43.55	47.30	47.61
6	18.28	18.34	18.07	19.26
7	32.90	33.19	33.97	33.28
8	40.44	40.48	40.70	40.66
9	47.28	47.72	50.51	48.25
10	38.76	38.31	38.12	39.12
11	24.03	24.09	20.95	24.88
12	126.23	127.91	25.60	124.51
13	139.65	139.97	38.12	144.48
14	42.11	41.86	42.41	41.46
15	30.88	29.95	29.77	29.45
16	26.95	26.33	32.38	28.54
17	48.23	48.24	56.09	44.07
18	54.42	54.56	49.22	45.07
19	72.68	72.64	47.66	82.28
20	41.87	42.01	150.63	35.99
21	26.97	26.90	30.72	29.40
22	38.79	38.38	37.11	33.97
23	29.33	71.20	65.83	66.27
24	17.65	16.76	13.95	13.87
25	16.87	17.69	17.73	17.82
26	17.24	17.29	16.05	17.46
27	24.68	24.66	14.44	24.68
28	179.55	180.67	178.19	182.09
29	27.09	27.04	109.44	28.72
30	16.77	16.99	19.00	24.88

[a]Assignments are carried out based on 2D NMR (COSY, HSQC, HMBC) experiments.

harvested by incubating in 0.25% trypsin for 2 min. The cells were seeded for 24 h and used for the experiments.

Cytotoxicity assay. The cytotoxicity of terpenoids isolated from *R. coreanus* was measured by MTT colorimetric assay. Compounds were dissolved in DMSO. The cells were seeded onto 96-well microplates at a density of 1×10^4 cells per well in 100 μL of medium each. After incubation at 37°C in a humidified incubator for 24 h, the cells were treated with various concentrations of each compound in serum-free medium for 24 h. After incubation, 50 μL of MTT (5 mg/mL in PBS) was added to each well of the plate. The cells were incubated at 37°C for 2 h. After removal from the medium, the cells were treated with 100 μL DMSO for 5 min and the optical density measured using a microplate reader (BIO-TEK Inc., Seoul, Korea) at 550 nm. Cell viability was calculated as a percentage of viable cells in the compound-treated group versus the control group by the following equation: Cell viability (%) = [OD (compound) – OD (Blank)/OD (Control) – OD (Blank)] × 100.

Results and Discussion

The MeOH extract of *R. coreanus* was found to be cytotoxic to some human cancer cells in a preliminary experiment. The MeOH extract was pooled in H_2O and extracted with EtOAc. The repeated silica gel, ODS and Sephadex LH-20 column chromatographies of the EtOAc fractions supplied four triterpenoids; compounds **1-4**.

Compound **1** was obtained as a white powder. The IR spectrum showed the presence of a hydroxyl group at 3447 cm^{-1}, an acid carbonyl group at 1754 cm^{-1} and a double bond at 1696 cm^{-1}. The EI-MS spectrum showed a molecular ion peak at *m/z* 488. The ^1H-NMR spectrum (400 MHz, pyridine-d_5) showed six tertiary singlet methyls at δ 1.70 (H-27), 1.42 (H-29), 1.26 (H-23), 1.10 (H-26), 1.07 (H-24), 1.00 (H-25), one doublet methyl at δ 1.11 (J = 5.7 Hz, H-30), an olefine proton at δ 5.58 (br. s, H-12) and one singlet proton at δ 3.04 (H-18), suggesting the presence of the urs-12-en type structure. Also, two oxygenated methine protons at δ 4.09 (ddd, J = 10.0, 9.4, 3.6 Hz, H-2) and δ 3.37 (d, J = 9.4 Hz, H-3) were suggestive of 2α and 3β dihydroxy structure. The ^{13}C-NMR spectrum (100 MHz, pyridine-d_5) suggested a trihydroxylated ursenoic acid structure with the presence of one carboxyl carbon signal at δ 179.55 (C-28), one olefine quaternary carbon signal at δ 139.65 (C-13), one olefine methine carbon signal at δ 126.23 (C-12), two oxygenated methine carbon signals at δ 68.59 (C-2) and 83.85 (C-3) and one oxygenated quaternary carbon signal at δ 72.68 (C-19). In the high magnet field region, seven methyl signals at δ 29.33 (C-23), 27.09 (C-29), 24.68 (C-27), 17.65 (C-24), 17.24 (C-26), 16.87 (C-25) and 16.77 (C-30) were observed. The structure of compound **1** (2α,3β,19α-trihydroxyurs-12-en-28-oic acid) was confirmed as tormentic acid by comparison of previous spectral data [Kitajma *et al.*, 1993].

Compound **2** was obtained as a white powder. The IR spectrum was similar to that of compound **1**: a hydroxyl group at 3451 cm^{-1}, an acid carbonyl group at 1705 cm^{-1} and a double bond at 1640 cm^{-1}. The EI-MS spectrum showed a molecular ion peak at *m/z* 504. The ^1H-NMR spectrum (400 MHz, pyridine-d_5) showed five tertiary singlet methyl signals at δ 1.65 (H-27), 1.39 (H-29), 1.11 (H-24), 1.00 (H-25), 0.84 (H-26) and one doublet methyl

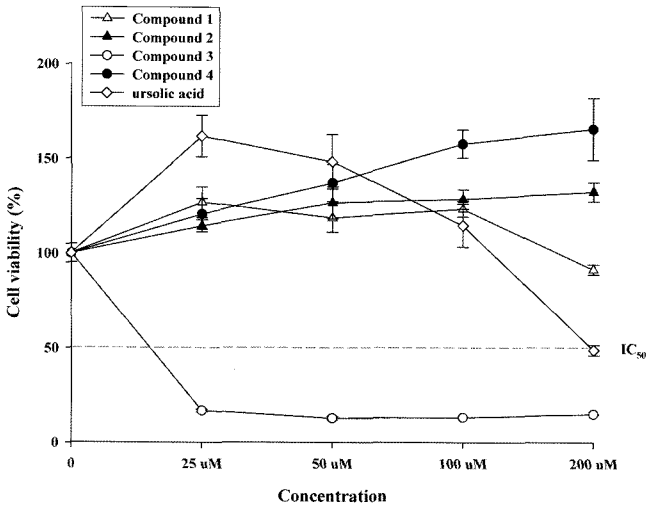

Fig. 1. Cytotoxicity of isolated triterpenoids from the fruits of *R. coreanus* against HCT-116 cells. The cells were treated with various concentrations of each compound for 24 h. Cell viability was determined by MTT assay. All data are represented as the mean ± S.D. of triplicates. IC_{50} reports the concentration of the compound inhibiting the growth of cells by 50%.

signal at δ 1.08 (J = 4.4 Hz, H-30). Furthermore, the presence of one singlet proton signal at δ 3.02 (H-18) and one olefine proton at δ 5.57 (H-12) indicated the presence of a similar urs-12-en type structure as found in compound **1**. Two oxygenated methine protons exhibited signals at ä 4.26 (m, H-2) and δ 4.12 (J = 2.4 Hz, H-3), assignable to 2β and 3β protons. Also, oxygenated methylene protons at δ 4.17 and δ 3.70 with J = 10.5 Hz were observed. The ^{13}C-NMR spectrum (100 MHz, pyridine-d_5) confirmed the presence of a pair of olefine carbons at δ 127.91 (C-12) and δ 139.97 (C-13), one carboxylic acid carbon at δ 180.67 (C-28), two oxygenated methine carbons at δ 66.24 (C-2) and 78.88 (C-3), an oxygenated quaternary carbon at δ 72.64 (C-19) and an oxygenated methylene carbon at δ 71.20 (C-23) on the ursane structure. In the high magnet field region, six methyl signals at δ 27.04 (C-29), 24.66 (C-27), 17.69 (C-25), 17.29 (C-26), 16.99 (C-30) and 16.76 (C-24) were observed. The data above suggests that the structure of compound **2** is myrianthic acid (2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid) [Hirai *et al.*, 2000].

Compound **3** was also obtained as a white powder. The IR spectrum indicated the absorption for the hydroxyl group was 3400 cm^{-1}, with a terminal double bond (2927, 1687 cm^{-1}) and a carbonyl group at 1715 cm^{-1}. The ^{1}H-NMR spectrum (400 MHz, pyridine-d_5) of compound **3** revealed signals for four tertiary methyl signals at δ 0.92 (H-27), 0.95 (H-26), 0.98 (H-25) and 1.04 (H-24). Two oxygenated methine protons at δ 4.24 (J = 11.4, 10.0, 4.5 Hz, H-2) and δ 4.21 (J = 10.0 Hz, H-3) were suggestive of α-OH at the H-2 position and β-OH at the H-3 position. Also, oxygenated methylene protons at δ 4.18 and 3.71 with germinal coupling (J = 11.2 Hz) were observed. An isopropenyl moiety at δ 1.76 (H-30), 4.92 (d, J = 2.0 Hz, H-29b) and 4.75 (d, J = 2.0, H-29a) were also observed. The ^{13}C-NMR spectrum (100 MHz, pyridine-d_5) exhibited thirty carbon signals, including one carboxyl carbon at δ 178.19 (C-28), two oxygenated methine carbons at δ 68.66 (C-2) and 77.61 (C-3), one oxygenated methylene carbon at δ 65.83 (C-23), a pair of one olefine carbons at δ 109.44 (C-29) and δ 150.63 (C-20) and five methyl carbons at δ 19.00 (C-30), 17.73 (C-25), 16.05 (C-26), 14.44 (C-27) and 13.95 (C-24). The HMBC spectrum also showed correlations between a methyl proton at δ 1.76 (H-30) and an olefine quaternary carbon at δ 150.63 (C-20), as well as between a methine proton at δ 1.71 (H-18) and a carboxyl carbon at δ 178.19 (C-28). Thus, compound **3** was identified as a lupane-triterpene, hovenic acid [2α,3β,23-trihydroxylup-20(29)-en-28-oic acid] and confirmed by comparison of reported spectral data [Yoshikawa *et al.*, 1998].

Table 2. The cytotoxicity of compound 3 from the fruits of *R. coreanus* against HCT-116 cell

Time	[a]IC_{50} values (μM)	
	Compounds	
	Compound **3**	Doxorubicin
24 hr	7.8 ± 0.02	50 ± 0.09

[a]IC_{50} value reports the concentration of the compound inhibiting cell growth by 50%. The cells were treated with various concentrations of each compound for 24 h and cell viability was determined by MTT assay. All data were represented as the mean ± S.D. of triplicates.

Compound **4** was obtained as a white powder. The IR spectrum was similar to compound **1**: a hydroxyl group at 3367 cm^{-1}, an acid carbonyl group at 1751 cm^{-1} and a double bond at 1690 cm^{-1}. The positive FAB-MS spectrum showed a pseudomolecular ion peak at *m/z* 505.5 $[M+H]^+$. The ^{1}H-NMR spectrum (400 MHz methanol-d_4) showed six integrated singlets for three protons each at δ 1.31 (H-27), 1.02 (H-25), 0.96 (H-30), 0.93 (H-29), 0.76 (H-26) and 0.70 (H-24), suggesting six quaternary methyl protons. Three oxygenated methine protons at δ 3.69 (ddd, J = 11.5, 10.0, 4.5 Hz), 3.35 (d, J = 10.0 Hz) and 3.25 (1H, J = 4.0 Hz) suggested α-OH at the H-2 position and β-OH at the H-3 position. Another oxygenated methine proton signal at δ 3.25 (d) with an axial-equatorial coupling of 4.0 Hz observed between H-19 and H-18, was indicative that H-19 is α-positioned and thus

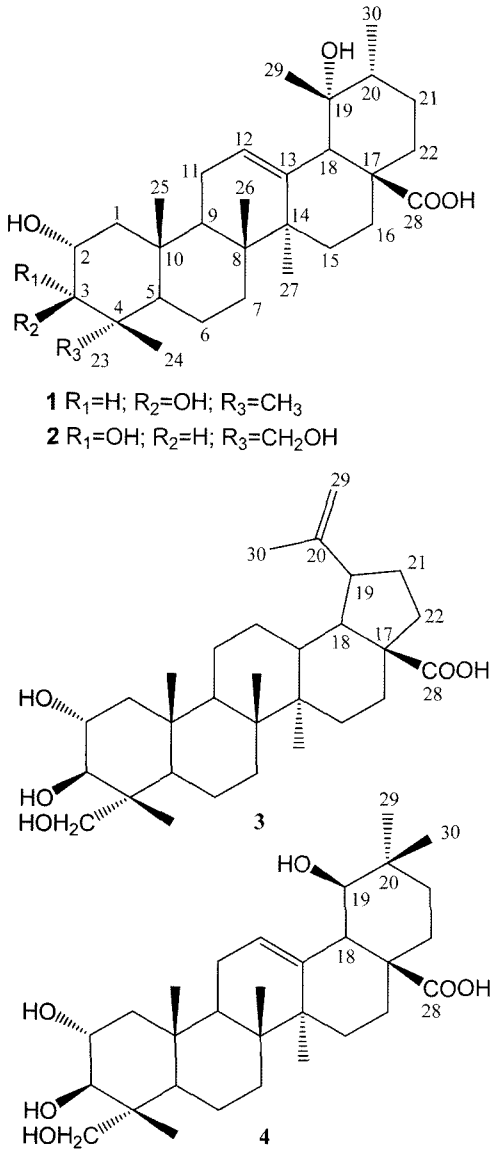

Fig. 2. Chemical structures of compounds 1-4 isolated from the fruits of *R. coreanus*.

confirmed β-OH at the H-19 position. Oxygenated methylene protons at δ 3.50 (d, J = 11.5 Hz) and δ 3.27 (d, J = 11.5 Hz) were assigned to the germinally coupled H-23a and H-23b. The olefine methine proton appeared at δ 5.31 (t-like, J = 3.5 Hz, H-12). The ^{13}C-NMR and DEPT (100 MHz, methanol-d_4) spectra of compound **4** also showed resonances for thirty carbons. A carboxyl carbon at δ 182.09 and olefine carbons at δ 124.51 (C-12) and δ 144.48 (C-13) were observed. Three oxygenated methine carbons at δ 69.57 (C-2), 78.15 (C-3) and 82.28 (C-19), as well as an oxygenated methylene carbon at δ 66.27 (C-23) were assigned. In the high magnetic field region, six methyl signals at δ 28.72 (C-29), 24.88 (C-30), 24.68 (C-27), 17.82 (C-25), 17.46 (C-26) and 13.87 (C-24) were observed. Therefore, the structure of compound **4** was identified as an oleanane triterpenoic acid, 2α,3β,19β, 23-tetrahydroxylolean-12-en-28-oic acid [Jossang *et al.*, 1996]. Furthermore, carbons data and chemical structures were summarized in Table 1 and Fig. 2.

During the search for cytotoxic compounds from natural sources, the MeOH extract of the fruits of *R. coreanus* were found to have a significant cytotoxic effect on the human cancer cell lines, and four compounds (**1-4**) were isolated as the major triterpenoids of the fruits of *R. coreanus*. Compounds **3** and **4** were isolated from this plant for the first time. All isolated compounds were evaluated for their cytotoxic activity against HCT-116 cancer cell *in vitro* using MTT assay method. The results are shown in Fig. 1. Compounds **2** and **4** showed no cytotoxicity against HCT-116 cancer cells at concentrations below 200 μM, while compound **1** had a mild cytotoxicity at 200 μM. These results might indicate the hydroxylation of oleanane- or ursane-triterpenoid at C-23 resulted in decreased cytotoxicity. Interestingly, compound **3** exhibited extremely high cytotoxicity against HCT-116 cells: the cytotoxicity of compound **3** (IC_{50} = 7.8 ± 0.02 μM) was much higher than doxorubicin (IC_{50} = 50 μM), a well known chemotherapeutic agent. (Table 2). Compound **3** is a lupane triterpenoid and has a cyclopentane structure of the E-ring, with an isopropenyl group at the C-19 position. The $\Delta^{20,29}$-functionality of isopropenyl group in this skeleton structure might be a key factor in cytotoxic activity. Some oleanane and ursane triterpenoids have been reported to show significant cytotoxicity against some tumor cell lines [Ma *et al.*, 2005; Tian *et al.*, 2006], whereas ursolic acid, the best known cytotoxic ursane triterpenoid, showed much lower cytotoxicity (IC_{50} = 200 μM) than compound **3** in this experiment (Fig. 1). Based on these results, it can be concluded that compound **3** may be a potential candidate for treatment of human colon cancer. In addition we are going on searching other active constituents from the active extracts.

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