Study on the Constituents of Roots of *Aceriphyllum rossii*

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A new stereoisomer of a tetrahydrofuranoid lignan, acerifuranoid A (**1**), and two new oleanane-type triterpenoids, aceriphyllic acids J and K (**2** and **3**), were isolated from the roots of *Aceriphyllum rossii*. Their structures were elucidated on the basis of spectroscopic analyses and chemical evidence. These isolated compounds exhibited weak cytotoxic activity against various cancer cell lines with $IC_{50} > 150$ μM.

Introduction. – *Aceriphyllum rossii* ENGLER. (Saxifragaceae), an endemic species in Korea, is a perennial herb that grows on damp rocks along valleys in the central northern part of Korea. The fresh young leaves and stems of *A. rossii* are being used as a nutritious food in Korea [1]. Previous studies on the air-parts of this plant have reported the isolation of several triterpenes and flavonol glycosides, together with their acyl-CoA : cholesterol acyltransferase inhibitory and antioxidant properties [2][3]. Recently, the MeOH extract of *A. rossii* and some oleanane-type triterpenoid compounds were reported to potently inhibit the growth of *Staphylococcus aureus* bacteria [4]. In our previous studies on this plant, several triterpenoids were isolated and evaluated for cytotoxic activity against various cancer cell lines [5][6], and anticomplementary activity on the classical pathway [7]. In the present phytochemical study, extraction and isolation of the hexane-soluble fraction led to the isolation of one new lignan, **1**, and two new oleanane-type triterpenoids, **2** and **3**. This article reports the isolation and structural elucidation of these new components, as well as an evaluation of their cytotoxic activity against three human cancer cell lines, namely MCF-7, LLC, and A549.

Results and Discussion. – The roots of *A. rossii* were extracted with MeOH at room temperature. The MeOH extract was partitioned with hexane, AcOEt, and BuOH to obtain the corresponding fractions. The hexane-soluble fraction was chromatographed repeatedly to furnish the three compounds **1**–**3** (*Fig. 1*)[1]).

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

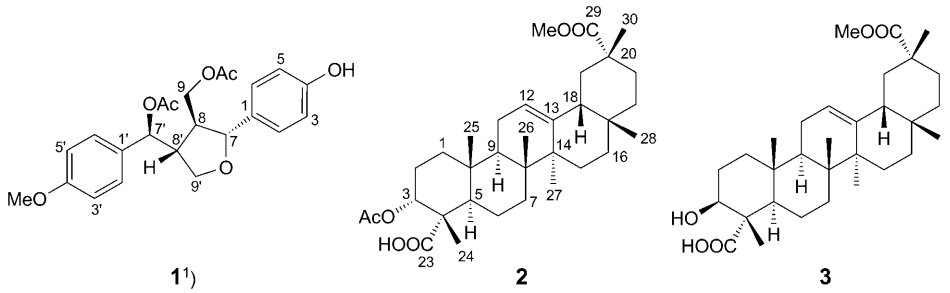
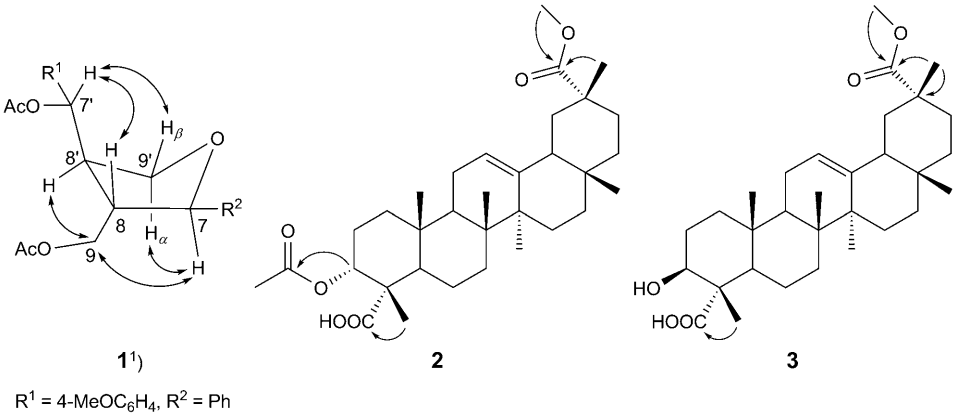


Fig. 1. *Compounds* **1**–**3** *Isolated from* Aceriphyllum rossii

Compound **1** was obtained as optically active yellow oil. The molecular composition of **1** was determined as $C_{23}H_{26}O_7$ by the HR-ESI-MS (*m/z* 473.1803 ($[M+AcO]^-$)). The UV spectrum of **1** revealed characteristic absorption maxima of a tetrahydrofuranoid lignan at 239 and 275 nm [8]. The ^{1}H-NMR data revealed a 4-methoxyphenyl substituent, as indicated by four aromatic H-atoms (δ(H) 7.30 (*d*, $J=8.4$ Hz, 2 H) and 6.89 (*d*, $J=8.4$ Hz, 2 H)), a MeO signal (δ(H) 3.81 (*s*, 3 H)), and a 4-hydroxyphenyl group, as shown by four aromatic H-atoms (δ(H) 7.27 (*d*, $J=8.0$ Hz, 2 H) and 6.90 (*d*, $J=8.0$, 2 H)). Another salient feature of the ^{1}H-NMR spectrum indicated the presence of two Ac groups (δ(H) 2.03 (*s*, 3 H) and 1.96 (s, 3 H)), and of six CH−O moieties (δ(H) 5.75 (*d*, $J=9.6$ Hz, 1 H), 4.55 (*d*, $J=8.0$ Hz, 1 H), 4.36 (*dd*, $J=11.2$, 4.4 Hz, 1 H), 4.12 (*dd*, $J=11.2$, 6.8 Hz, 1 H), and 3.70–3.79 (*m*, 2 H)). The ^{13}C-NMR and DEPT spectra suggested that the skeleton consisted of 23 C-atoms including three primary, two secondary, twelve tertiary, and six quaternary C-atoms (including two carboxy C-atoms (δ(C) 170.4 and 171.1). In the HMBC spectrum, the correlations between the signal of an oxygenated methine H−C(7′) and C(1′), C(2′), C(6′), C(8), C(8′), and C(9′) were evident. An AcO group located at C(7′) was confirmed by the long-range correlations from H−C(7′) and MeCOO at δ(H) 2.04 to the C=O at δ(C) 170.4. The location of the other AcO group was assigned to C(9) by the correlations from CH_2(9) and MeCOO at δ(H) 1.96 to the C=O at δ(C) 171.1. In addition, long-range correlations between H−C(8), CH_2(9), H−C(8′), CH_2(9′), H−C(2), and H−C(6) and C(7) (δ(C) 85.2) were also observed. Therefore, the structure of **1** could be assigned as 4-hydroxy-4′-methoxy-7,9′-epoxylignane-7′,9-diyl diacetate. All-*trans* orientation between H−C(7), H−C(8), and H−C(8′) of **1** was determined by the cross-peaks H−C(7)/H−C(9), H−C(7)/H−C(8′), H−C(8)/H−C(7′), and H−C(8′)/H−C(9) in the ROESY plot (*Fig. 2*). The absolute configurations at the four chiral centers of **1** were determined by comparison of its optical rotation ($[\alpha]_D=-6.3$, $CHCl_3$) with that of the known analogous compound ($[\alpha]_D=-5.0$, $CHCl_3$) having (7*R*,8*S*,7′*S*,8′*R*)-configuration [8][9]. Thus, the structure of **1** was determined to be a new stereoisomer of the tetrahydrofuranoid lignan (7*R*,8*S*,7′*S*,8′*R*)-4-hydroxy-4′-methoxy-7,9′-epoxylignane-7′,9-diyl diacetate, named acerifuranoid A[1]).

Compound **2** was obtained as an amorphous powder, giving a positive red coloration in the *Liebermann–Burchard* reaction. The molecular formula of **2** was determined as $C_{33}H_{50}O_6$ from the molecular-ion peak at *m/z* 542.3551 (M^-) in the HR-ESI-MS. The ^{1}H-NMR spectrum of **2** indicated the presence of six Me (δ(H) 1.42, 1.27,



1[1]) **2** **3**

R^1 = 4-MeOC$_6$H$_4$, R^2 = Ph

Fig. 2. *Selected correlations in the ROESY* (H ↔ H) *of* **1** *and HMBC* (H → C) *of* **2** *and* **3**

1.08, 1.00, 0.98, and 0.89), an AcO (δ(H) 1.96), a MeO (δ(H) 3.65), and a CH=C group (δ(H) 5.31 (br. *s*, 1 H)). A H-atom signal at δ(H) 5.45 (*t*, *J* = 2.4 Hz, 1 H) was assigned to a CH–O moiety, *i.e.*, H–C(3), which also indicated an axial position of the AcO group at C(3), similar to aceriphyllic acid B (=(3α,4α,20α)-3,23-dihydroxyolean-12-en-29-oic acid) [5][6]. The ^{13}C-NMR and DEPT spectra showed 33 C-atom signals, including three COO groups (δ(C) 181.1, 178.1, and 170.3), two olefinic C-atoms (δ(C) 145.1, and 123.2), one oxygenated C-atom (δ(C) 76.3), a MeO group (δ(C) 52.0), an *Me*COO group (δ(C) 21.8), and six tertiary Me groups (δ(C) 28.4, 26.2, 19.8, 17.9, 17.5, and 15.9). The other C-atom signals were assigned to nine CH_2 and three CH groups, and six quaternary C-atoms which are known features of an oleanane-type triterpene [6][7]. The full NMR assignments and connectivities were determined by HSQC, HSBC, and COSY data analyses. In the HMBC spectrum (*Fig. 2*), the correlations from H–C(3) (δ(H) 5.45) to the C=O C-atoms (δ(C) 178.1 and 170.3), and from the Me group (δ(H) 1.42) to C=O (δ(C) 178.1), to C(3) (δ(C) 76.3), and to C(5) (δ(C) 45.8) indicated that the (C)OOH group is located at C(23) or C(24). However, the upfield chemical shift of the Me group at δ(C) 17.9 suggested that this is C(24), and that the (C)OOH group is placed at C(23) [7]. These assignments were confirmed by NOESY correlations from Me(24) (δ(H) 1.42) to Me(25) (δ(H) 0.98). In addition, the long-range correlation between the MeO group (δ(H) 3.65) and C(29) (δ(C) 181.1) indicated the location of the MeO group at C(29). Therefore, the structure of **2** was determined as (3α,4α,20α)-3-(acetyloxyolean-12-ene-23,29-dioic acid 29-methyl ester, named aceriphyllic acid J.

Compound **3** was obtained as an amorphous optically active powder. The ^{1}H- and ^{13}C-NMR spectra of **3** were closely similar to those of **2**. The difference involved a β-OH group at C(3), as indicated by the large coupling constant of H–C(3) (δ(H) 4.69 (*dd*, *J* = 6.8, 9.8 Hz, 1 H)), instead of the 3α-AcO group of **2**. The molecular formula $C_{31}H_{48}O_5$ was deduced from a molecular-ion peak at *m*/*z* 500.3455 (M^-) in the HR-ESI-MS. Hence, compound **3** was elucidated as the new triterpenoid (3β,4α,20α)-3-hydroxyolean-12-ene-23,29-dioic acid 29-methyl ester, named aceriphyllic acid K.

Pentacyclic triterpenoids have been known as the main components of *A. rossii*. However, this is the first time that the presence of a tetrahydrofuranoid lignan is reported. The isolates were evaluated *in vitro* for their cytotoxic activity against cancer cell lines MCF-7, LLC, and A549 with the MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide), assay method [7][10]. All of the isolated compounds exhibited weak activity against all tested cell lines with IC_{50} values >150 μM.

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Experimental Part

General. Solvents were purchased from *Samchun Chemical Co.* RPMI 1640, DMEM, FBS, PBS buffer, penicillin/streptomycin, and 10% trypsin/EDTA were purchased from *GIBCO.* MTT Reagent and DMSO were obtained from *Sigma–Aldrich.* HPLC: *Waters 400* workstation for purification and isolation, *YMC-ODS-80* prep. HPLC column. Column chromatography (CC): silica gel *60* (70–230 mesh and 230–400 mesh; *Merck*) or reversed-phase silica gel (*LiChroprep® RP-18*, 40–63 μm; *Merck*). TLC: *Merck* pre-coated silica gel *60* F_{254} and/or *RP-18* F_{254s} plates (0.25 mm); detection by spraying the dried plates with 10% H_2SO_4, followed by heating. Optical density (*OD*) values in the cytotoxic-activity determination by the MTT assays were read on a *Tecan-ELISA* microplate reader. Optical rotations: *Jasco-DIP-370* polarimeter; 100 mm glass cell. UV Spectra: *Thermo-9423AQA2200E* UV spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker-Equinox-55* FT-IR spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Varian* NMR spectrometer at 400 MHz; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *JMS-700-MStation* mass spectrometer; in m/z (rel. %).

Plant Material. The roots of *A. rossii* were collected at Jeongbong, Kangwondo, Korea, in June 2007 and identified by *H. K. L.* A voucher specimen (PB-1636) was deposited with the herbarium of the Korea Research Institute of Bioscience and Biotechnology, Korea.

Extractions and Isolation. The roots of *A. rossii* (20 kg) were extracted three times with MeOH at r.t. for a week. The MeOH extract was filtered and concentrated resulting in a residue (1.43 kg). The MeOH extract was suspended in hot H_2O and then partitioned successively with hexane (3 × 4.0 l), AcOEt (3 × 4.0 l), and BuOH (3 × 4.0 l) to afford fractions soluble in hexane (230 g), AcOEt (208 g), and BuOH (180 g). The hexane-soluble fraction (230 g) was subjected to CC (silica gel (40 × 15 cm), hexane/AcOEt 10:1 → 0:1): *Frs. H1–H12*. Repeated CC (silica gel (50 × 5.0 cm), hexane/acetone 5:1 → 3:1) of *Fr. H9* (2.5 g) gave *Frs. H9.1–H9.4*. Compound **1** (4.8 mg) was obtained by application of *Fr. H9.2* to CC (*RP-18*, MeOH/H_2O 10:1). *Fr. H11* was subjected to CC (silica gel, $CHCl_3$ acetone 20:1 → 0:1): *Frs. H11.1–H11.9*). *Fr. H11.5* was applied to CC (silica gel, $CHCl_3$/acetone 10:1 and 1:1): *Frs. H11.5.1–H11.5.5*. Compounds **2** (2.0 mg) and **3** (2.1 mg) were isolated from *Fr. H11.5.3* by CC (*RP-18*, MeOH/H_2O 20:1 → 30:1).

Acerifuranoid A (=*(7*R,*8*S,*7′*S,*8′*R*)-4-Hydroxy-4′-methoxy-7,9′-epoxylignane-7′,9-diyl Diacetate* = *(α*^{3}S,*3*R,*4*S,*5*R*)-Tetrahydro-5-(4-hydroxyphenyl)-α*^{3}*-(4-methoxyphenyl)furan-3,4-dimethanol 3,4-Diacetate*; **1**): Sticky oil. $[\alpha]_D^{22} = -6.30$ ($c = 0.5$, $CHCl_3$). UV ($CDCl_3$): 239, 275. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.96 (*s*, AcO–C(3)); 2.03 (*s*, AcO–C(7′)); 2.33–2.39 (*m*, H–C(8)); 2.67–2.75 (*m*, H–C(8′)); 3.70–3.79 (*m*, CH_2(9′)); 3.81 (*s*, MeO–C(4′)); 4.12 (*dd*, $J = 6.8, 11.2$, H_α–C(9)); 4.36 (*dd*, $J = 4.4, 11.2$, H_β–C(9)); 4.55 (*d*, $J = 8.0$, H–C(7)); 5.75 (*d*, $J = 9.6$, H–C(7′)); 6.89 (*d*, $J = 8.4$, H–C(3′,5′)); 6.90 (*d*, $J = 8.0$, H–C(3,5)); 7.27 (*d*, $J = 8.0$, H–C(2,6)); 7.30 (*d*, $J = 8.4$, H–C(2′,6′)). ^{13}C-NMR (100 MHz): 21.0 (*Me*COO–C(9)); 21.5 (*Me*COO–C(7′)); 47.9 (C(8′)); 50.5 (C(8)); 55.5 (*Me*O–C(4′)); 64.7 (C(9)); 69.7 (C(9′)); 77.9 (C(7′)); 85.5 (C(7)); 114.2 (C(3′,5′)); 114.3 (C(3,5)); 128.1 (C(2,6)); 128.8 (C(2′,6′)); 131.4 (C(1′)); 132.8 (C(1)); 159.6 (C(4)); 159.9 (C(4′)); 170.4 (Me*C*OO–C(7′)); 171.1 (Me*C*OO–C(9)). HR-ESI-MS: 473.1803 ($[M + AcO]^-$, $C_{23}H_{25}O_7^-$; calc. 473.1805).

Aceriphyllic Acid J (*=(3α,4α,20α)-3-(Acetyloxy)olean-12-ene-23,29-dioic Acid 29-Methyl Ester*; **2**): White amorphous powder. $[\alpha]_D^{22} = +48.01$ ($c = 0.1$, MeOH). UV (MeOH): 205, 259. ^{1}H-NMR (400 MHz, (D_5)pyridine): 5.31 (br. *s*, H−C(12)); 5.45 (*t*, *J* = 2.4, H−C(3)); 3.65 (*s*, MeO); 1.96 (*s*, AcO−C(3)); 1.42 (*s*, Me(24)); 1.27 (*s*, Me(30)); 1.08 (*s*, Me(27)); 1.00 (*s*, Me(26)); 0.98 (*s*, Me(25)); 0.89 (*s*, Me(28)). ^{13}C-NMR (100 MHz, (D_5)pyridine)): 33.3 (C(1)); 27.2 (C(2)); 76.3 (C(3)); 50.2 (C(4)); 45.8 (C(5)); 21.4 (C(6)); 32.8 (C(7)); 40.7 (C(8)); 48.2 (C(9)); 37.0 (C(10)); 24.0 (C(11)); 123.2 (C(12)); 145.1 (C(13)); 42.1 (C(14)); 26.4 (C(15)); 27.2 (C(16)); 33.3 (C(17)); 46.4 (C(18)); 41.1 (C(19)); 43.0 (C(20)); 29.6 (C(21)); 37.2 (C(22)); 178.1 (C(23)); 17.9 (C(24)); 15.9 (C(25)); 17.5 (C(26)); 26.2 (C(27)); 28.4 (C(28)); 181.1 (C(29)); 19.8 (C(30)); 170.3 (Me*C*OO−C(3)); 21.8 (*Me*COO−C(3)); 52.0 (MeO). HR-ESI-MS: 542.3551 (M^-, $C_{32}H_{48}O_6^-$; calc. 542.3554).

Aceriphylic Acid K (*=(3β,4α,20α)-3-Hydroxyolean-12-ene-23,29-dioic Acid 29-Methyl Ester*; **3**): Colorless crystals. M.p. 282°. $[\alpha]_D^{22} = +47.4$ ($c = 0.1$, MeOH). UV (MeOH): 203, 258. ^{1}H-NMR: 5.28 (*t*, *J* = 3.6, H−C(12)); 4.68 (*dd*, *J* = 6.8, 9.6, H−C(3)); 3.65 (*s*, MeO); 1.69 (*s*, Me(24)); 1.29 (*s*, Me(30)); 1.14 (*s*, Me(27)); 1.05 (*s*, Me(25)); 0.99 (*s*, Me(26)); 0.90 (*s*, Me(28)). ^{13}C-NMR: 39.4 (C(1)); 28.1 (C(2)); 75.7 (C(3)); 54.7 (C(4)); 52.2 (C(5)); 21.9 (C(6)); 33.0 (C(7)); 40.7 (C(8)); 48.4 (C(9)); 37.0 (C(10)); 24.2 (C(11)); 123.3 (C(12)); 144.6 (C(13)); 42.1 (C(14)); 26.6 (C(15)); 27.3 (C(16)); 32.9 (C(17)); 46.5 (C(18)); 41.2 (C(19)); 43.2 (C(20)); 29.7 (C(21)); 36.4 (C(22)); 181.0 (C(23)); 12.6 (C(24)); 16.4 (C(25)); 17.2 (C(26)); 26.3 (C(27)); 28.5 (C(28)); 179.2 (C(29)); 19.9 (C(30)); 52.0 (MeO). HR-ESI-MS: 500.3455 (M^-, $C_{30}H_{46}O_5^-$; calc. 500.3459).

Cytotoxicity Assay. The cancer cell lines (MCF-7 and LLC) were maintained in RPMI 1640 that included L-glutamine with 10% FBS and 2% penicillin/streptomycin. Cells were cultured at 37° in a 5% CO_2 incubator. Cytotoxic activity was measured by using a modified MTT assay [7]. Viable cells were seeded in the growth medium (100 µl) into 96-well microtiter plates ($1 \cdot 10^4$ cells per well) and incubated at 37° in a 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations ranging from 5.0 to 150 µM by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to $< 0.1\%$. After standing for 24 h, 10 µl of the test sample was added to each well. The same volume of DMSO was added to the control wells. Removing medium after 48 h of the test-samples treatment, MTT (5 mg/ml; 10 µl) was also added to the each well. After 4 h in the incubator, the plates were removed, and the resulting formazan crystals were dissolved with DMSO (150 µl). The optical density was measured at 570 nm [10]. The IC_{50} value was defined as the concentration of sample which reduced absorbance by 50% rel. to the vehicle-treated control.

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