Oleanane-Type Triterpenoids from *Aceriphyllum rossii* and Their Cytotoxic Activity

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The hexane-soluble fraction of the roots of *Aceriphyllum rossii* was used to isolate seven new oleanane-type triterpenoids, aceriphyllic acids C−I (**1**−**7**), together with seven known triterpenoids. The structures of aceriphyllic acids C−I were determined as 3α-hydroxyolean-12-en-23,29-dioic acid (**1**), 3*β*-hydroxyolean-12-en-23,29-dioic acid (**2**), 3*β*,23-dihydroxyolean-12-en-29-oic acid (**3**), 3α-*O*-acetylolean-12-en-23,27-dioic acid (**4**), 3α-*O*-caffeoylolean-12-en-27-oic acid (**5**), 3α-*O*-acetylolean-12-en-23,29-dioic acid (**6**), and 3α-hydroxyolean-12-en-23-al-27-oic acid (**7**) by spectroscopic analyses. In the evaluation of the *in vitro* cytotoxicity of these compounds against the MCF-7 and LLC cancer cell lines, compounds **10** and **13** exhibited cytotoxic activity against the LLC cancer cell line with IC_{50} values of 7.63 and 6.56 μM, respectively.

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* have been used as a nutritious food in Korea.[1] Previous studies of this plant have reported the isolation of several triterpenes and flavonol glycosides, together with their acyl-CoA: cholesterol acyltransferase inhibitory and antioxidant activities.[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 HL-60 and K562[5] and anticomplementary activity on the classical pathway.[6] In a further phytochemical study of this plant, the hexane-soluble fraction of the root showed cytotoxic activity against MCF-7 and LLC cancer cell lines, and assay-guided fractionation led to the isolation of seven new oleanane-type triterpenoids (**1**−**7**), together with seven known compounds (**8**−**14**). The present paper reports the isolation and structural elucidation of these triterpenes and their cytotoxic activity against MCF-7 and LLC cancer cell lines.

Results and Discussion

The MeOH extract of *A. rossii* was partitioned into *n*-hexane-, EtOAc-, and BuOH-soluble fractions. Chromatographic purification of the *n*-hexane-soluble fraction led to the isolation of 14 oleanane-type triterpenes (**1**−**14**). The structures of the seven known compounds were determined as 3*β*-hydroxyolean-12-en-29-oic acid (**8**),[7] oleanolic acid (**9**),[8] *β*-peltoboykinolic acid (**10**),[6,8] aceriphyllic acid A (**11**),[2] 3-oxoolean-12-en-27-oic acid (**12**),[8] 23-hydroxy-3-oxoolean-12-en-27-oic acid (**13**),[5,6] and 3α,23-isopropylidenedioxy-olean-12-en-27-oic acid (**14**)[5] by comparing their physicochemical (mp, $[\alpha]_D$) and spectroscopic data (infrared [IR], mass spectroscopy [MS], ^{1}H and ^{13}C nuclear magnetic spectroscopy [NMR]) with those of reported values.

Compound **1**, obtained as colorless crystals in chloroform, $[\alpha]_D^{22}$ +50.4 (*c* 0.1, MeOH), gave a positive red coloration in the Liebermann−Burchard reaction. Its IR spectrum showed absorption bands for hydroxy and carbonyl groups at ν_{max} 3280 and 1705 cm^{-1}, respectively. In addition, the molecular formula was determined as $C_{30}H_{46}O_5$ from a molecular ion peak at *m*/*z* 486.3317 $[M]^-$ in the HRESIMS, suggesting that **1** is a triterpenoid. The ^{1}H NMR spectrum of **1** indicated the presence of six methyl groups at δ 1.49 (3H, s, H-24), 1.45 (3H, s, H-30), 1.11 (3H, s, H-27), 1.06 (3H, s, H-25), 1.04 (3H, s, H-26), and 0.93 (3H, s, H-28) and one olefinic group at δ 5.29 (1H, brs, H-12). A proton signal at δ 4.31 (1H, brs, H-3) was also observed and assigned to a methine proton on an oxygenated carbon, which also indicated an axial position of the hydroxy group at C-3, similar to aceriphyllic acid B.[2,7] The ^{13}C NMR and DEPT spectra showed 30 carbon signals, including two carboxylic carbons at δ 181.5 (C-29) and 179.8 (C-23), two olefinic carbons at δ 145.0 (C-13) and 123.6 (C-12), one oxygenated carbon at δ 73.1 (C-3), and six tertiary methyl carbons at δ 28.3 (C-28), 26.2 (C-27), 20.2 (C-30), 18.4 (C-24), 17.5 (C-25), and 16.3 (C-26). The other carbon signals were observed and assigned to nine methylene, three methine, and six quaternary carbons. These NMR spectra revealed features of an oleanane-type triterpene.[8] The full NMR assignments and connectivities were determined by HMQC, HMBC, and COSY data analyses (Table 1). These spectroscopic data were closely similar to those of aceriphyllic acid B, which was also isolated and reported previously from this plant,[2] except for the carboxylic carbon at C-23 of the oleanane-type skeleton. In the HMBC spectrum, the correlations from H-3 (δ 4.31, brs) to the carbonyl carbon at δ 179.8 and from the methyl protons at δ 1.52 (3H, s) to this carbonyl carbon, to C-3 (δ 73.1), and to C-5 (δ 44.9) indicated that the carboxylic group is located at C-23 or C-24 (see the Supporting Information). However, the upfield chemical shift of the methyl carbon at δ 18.6 suggested that this is C-24, and the carboxylic group is placed at C-23. These assignments were confirmed by NOESY correlations from δ 1.49 (3H, H-24) to δ 1.06 (3H, H-25). Therefore, the structure of **1** was determined as 3α-hydroxyolean-12-en-23,29-dioic acid and was named aceriphyllic acid C.

Compound **2** was obtained as white crystals in MeOH. It showed an HRESIMS fragmentation pattern superimposable on that of **2**. The comparisons of ^{1}H, ^{13}C, and 2D NMR spectra of the two compounds indicated their structural similarity. The main differ-

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Table 1. ^{1}H NMR (400 MHz) Spectroscopic Data (δ_H, *J*, Hz) of Compounds **1**−**7** in Pyridine-d_5

position	**1**	**2**	**3**	**4**	**5**[a]	**6**[a]	**7**
1	1.37 m 1.40 m	1.66 m 1.70 m	1.60 m 1.75 m	1.39 m 2.21 m	1.35 m 1.90 m	1.23 m 1.38 m	1.49 m 1.83 m
2	1.85 m 2.02 m	1.75 m 1.96 m	1.92 m 2.05 m	1.81 m 1.92 m	1.68 m 1.96 m	1.72 m 1.87 m	1.68 m 1.93 m
3	4.31 brs	4.69 dd (6.8, 9.6)	4.18 dd (6.0, 10.8)	5.42 t (2.4)	4.70 brs	4.96 brs	3.98 brs
5	2.58 d (11.6)	2.00 m	1.50 m	2.46 d (10.8)	2.50 m	1.97 m	2.58 d (11.2)
6	1.08 m 1.62 m	0.88 m 1.23 m	0.81 m 1.71 m	1.51 m 1.92 m	1.50 m 2.02 m	0.90 m 1.44 m	1.50 m 1.92 m
7	1.33 m 1.55 dd (4.4,14.0)	1.26 m 1.60 m	1.26 m 1.58 m	1.43 m 1.98 m	1.35 m 1.96 m	1.26 m 1.88 m	1.17 m 1.94 m
9	2.00 m	1.80 m	1.71 m	3.05 dd (2.8, 11.2)	2.23 dd (5.6, 10.8)	1.78 m	3.10 dd (5.2, 11.6)
11	1.96 m 1.99 m	1.94 m 2.00 m	1.91 m 1.98 m	2.04 dd (2.4, 11.2) 2.10 t (4.8)	2.00 m 2.03 m	1.90 m 2.03 m	2.02 m 2.14 t (5.2)
12	5.29 brs	5.30 brs	5.29 m	5.82 dd (2.4, 4.8)	5.68 brs	5.24 brs	5.79 dd (2.0, 4.8)
15	0.89 m 1.60 m	1.03 m 1.65 m	1.10 m 1.61 m	1.39 m 1.89 m	1.40 m 1.89 m	1.26 m 1.70 m	1.37 m 1.82 m
16	0.79 m 1.96 m	0.86 m 1.95 m	1.03 m 1.96 m	0.97 m 2.41 m	0.95 m 2.47 m	0.86 m 1.94 m	0.97 m 2.45 m
18	2.14 m	2.18 dt (3.6, 14.0)	2.15 m	2.20 t (4.4)	2.01 m	1.97 m	2.20 m
19	1.68 m 2.48 t (13.6)	1.72 m 2.53 t (14.0)	1.69 m 2.52 (t, 13.6)	1.54 m 2.46 m	1.47 m 1.90 m	1.62 m 2.25 t (13.6)	1.37 m 1.80 m
21	1.32 m 1.70 m	0.82 m 1.63 m	0.89 m 1.55 m	1.09 m 1.25 m	1.09 brs 1.13 m	1.40 m 1.90 m	0.97 m 1.07 m
22	1.37 m 1.67 m	2.02 m 2.22 m	1.35 m 2.08 dd (3.4, 13.6)	1.37 m 1.48 m	1.24 m 1.35 m	1.37 m 1.52 m	1.25 m 1.45 dd (3.6, 12.8)
23			3.71 d (10.8) 4.17 d (10.8)		0.83 s		9.89 s
24	1.49 s	1.70 s	1.08 s	1.42 s	0.91 s	1.28 s	1.10 s
25	1.06 s	1.04 s	1.02 s	1.05 s	1.00 s	1.01 s	1.02 s
26	1.04 s	1.00 s	1.01 s	1.17 s	1.06 s	0.99 s	1.14 s
27	1.11 s	1.19 s	1.17 s			1.22 s	
28	0.93 s	0.93 s	0.93 s	1.03 s	0.87 s	0.87 s	1.01 s
29				0.79 s	0.72 s		0.75 s
30	1.45 s	1.47 s	1.46 s	0.91 s	0.82 s	1.23 s	0.88 s
3α-OCO*CH*$_3$				1.77 s		2.03 s	
2′					7.00 brs		
5′					6.83 d (8.0)		
6′					6.81 d (8.0)		
7′					7.44 d (16.0)		
8′					6.11 d (16.0)		

[a] In $CDC1_3$.

ences were the chemical shifts of H-3 (δ 4.69, dd, J = 6.8, 9.8 Hz in **2** versus δ 4.31, brs in **1**) in the ^{1}H NMR spectrum and C-3 (δ 75.8 in **2** versus δ 73.1 in **1**) in the ^{13}C NMR spectrum, which showed that the difference between the two should be confined to the stereochemistry at C-3. These assignments were confirmed by NOESY correlations between H-3 and H-5 (δ 2.00, 1H, m) and the lack of NOESY correlation between H-3 and H-25 (δ 1.04, 3H, s). The molecular formula, $C_{30}H_{46}O_5$, was observed from a molecular ion peak at *m*/z 486.3318 $[M]^-$ in the HRESIMS and supported this result. Hence, compound **2** was elucidated as the new triterpenoid 3β-hydroxyolean-12-en-23,29-dioic and was named aceriphyllic acid D.

Compound **3**, a white powder, $[\alpha]_D^{22}$ +48.01 (*c* 0.1, MeOH), was assigned a molecular formula of $C_{30}H_{48}O_4$ as determined by HRESIMS, which indicated an ion at *m*/*z* 472.3524 $[M]^-$. The UV, IR, and NMR data of **3** were closely related to those of aceriphyllic acid B. The only difference involved the 3β-hydroxy group indicated by the large coupling constant of H-3 [δ 4.18 (1H, dd, J = 6.0, 10.8 Hz)], instead of the 3α-hydroxy group in aceriphyllic acid B at δ 3.90 (1H, brs).[2] The relative configuration of **3** was established on the basis of the NOESY spectrum, which showed the connection between H-3 and H-5 (δ 1.50, 1H, m). Thus, the structure of **3** was determined as 3β,23-dihydroxyolean-12-en-29-oic acid and was named aceriphyllic acid E.

Compound **4** was obtained as colorless crystals in MeOH, with $[\alpha]_D^{22}$ +52.0 (*c* 0.1, MeOH). Its IR spectrum exhibited absorption bands for the carbonyl groups (1710 cm^{-1}) and trisubstituted double bond (1665, 850 cm^{-1}). The ^{1}H NMR spectrum displayed the characteristic signals of an olefinic proton at δ 5.82 (1H, dd, J = 2.4, 4.8 Hz, H-12) and hydroxymethine proton at δ 5.42 (1H, t, J = 2.4 Hz, H-3). The ^{13}C NMR and DEPT spectra revealed a carbonyl carbon at δ 177.9, olefinic carbons at δ 139.2 and 125.7, a hydroxymethine carbon at δ 76.2, and six methyl carbons at δ_C 33.8 (C-29), 28.8 (C-28), 19.2 (C-26), 24.2 (C-30), 18.1 (C-24), and 17.0 (C-25), which are typical for the olean-12-en-27-oic acid series.[2,8] The full NMR assignments and connectivities were determined by HMQC, HMBC, and COSY spectroscopic data analyses. These spectroscopic data were very similar to those of aceriphyllic acid A, which was also isolated previously from this plant,[2] except for the addition of an *O*-acetyl group {[δ_H 1.77 (3H, s), δ_C 21.2], [δ_C 170.2]} instead of a hydroxy group at C-3 and a carboxyl carbon (δ_C 178.6) instead of a hydroxymethylene group at C-23 of oleanane-12-en-27-oic acid. The HMBC spectrum confirmed the connectivities of the acetyl and carboxylic groups by the correlations of the oxygenated proton (δ 5.42, t, J = 2.4, H-3) with the carbonyl carbon of the acetyl group (δ 170.2), and methyl protons (δ 1.42, s, H-24) with C-23 (δ 178.6). The molecular formula, $C_{32}H_{48}O_6$, was observed from a molecular ion peak at *m*/*z* 528.3401 $[M]^-$ in the HRESIMS. Thus, compound **4** was elucidated as 3α-*O*-acetylolean-12-en-23,27-dioic acid and was named aceriphyllic acid F.

Compound **5** was isolated as a white, amorphous powder, with $[\alpha]_D^{22}$ +80.0 (*c* 0.08, MeOH). The ^{1}H and ^{13}C NMR signals were similar to 3α-hydroxyolean-12-en-27-oic acid.[2,8] Additionally, in the ^{1}H NMR spectrum, a *trans*-caffeoyl moiety[9] was assigned and indicated by the alkene signals at δ 7.44 (1H, d, J = 16.0 Hz, H-7′)

Table 2. ^{13}C NMR (δ_C, 100 MHz) Spectroscopic Data of Compounds **1**−**7** in Pyridine-d_5

position	**1**	**2**	**3**	**4**	**5**[a]	**6**[a]	**7**
1	33.1	39.5	39.2	33.7	34.2	32.8	33.6
2	24.1	26.8	28.0	23.6	34.5	29.4	26.9
3	73.1	75.8	73.8	76.2	78.2	75.3	73.2
4	52.0	54.8	43.2	50.3	37.0	50.4	52.6
5	44.9	52.2	48.9	46.0	50.2	44.8	44.4
6	22.0	22.0	18.9	22.1	22.3	22.4	21.4
7	33.07	33.1	32.9	37.4	36.5	32.2	37.3
8	40.9	40.8	40.7	40.7	40.6	40.5	40.7
9	48.2	48.5	48.3	48.1	47.5	47.6	47.6
10	37.3	37.1	37.4	37.3	37.2	36.7	37.1
11	24.1	24.3	24.2	23.4	23.0	23.6	23.6
12	123.6	123.3	124.3	125.7	126.3	122.8	125.6
13	145.0	145.0	144.9	139.2	144.5	144.3	130.9
14	42.2	42.2	42.2	57.0	56.3	41.9	56.9
15	26.6	26.8	26.7	22.9	22.9	26.3	23.2
16	27.4	27.5	27.5	29.0	27.9	27.1	28.6
17	33.02	37.1	33.1	37.4	33.0	32.6	33.7
18	46.7	46.8	46.9	50.4	49.3	46.0	50.3
19	41.6	41.8	41.8	44.7	44.7	40.3	44.7
20	43.0	43.2	43.1	31.6	31.2	43.1	31.5
21	30.0	30.2	30.2	35.1	34.5	29.4	35.0
22	36.7	36.8	36.8	33.7	36.8	36.0	37.1
23	179.8	181.0	68.3	178.6	28.1	182.6	210.1
24	18.4	12.7	13.4	18.1	22.0	17.3	15.1
25	17.5	16.5	16.5	17.0	16.3	15.9	16.9
26	16.3	17.3	17.4	19.2	18.2	17.1	18.9
27	26.2	26.4	26.4	177.9	179.2	26.4	178.6
28	28.8	28.7	28.7	28.8	28.5	28.3	28.9
29	181.5	181.6	181.6	33.8	33.4	186.1	33.7
30	20.2	20.4	20.4	24.2	23.8	18.9	24.2
3-O*C*OCH$_3$				170.2		170.4	
				21.2		21.5	
1′					127.2		
2′					113.6		
3′					147.1		
4′					144.3		
5′					115.4		
6′					122.6		
7′					144.6		
8′					116.0		
-*C*O-					167.1		

[a] In $CDC1_3$.

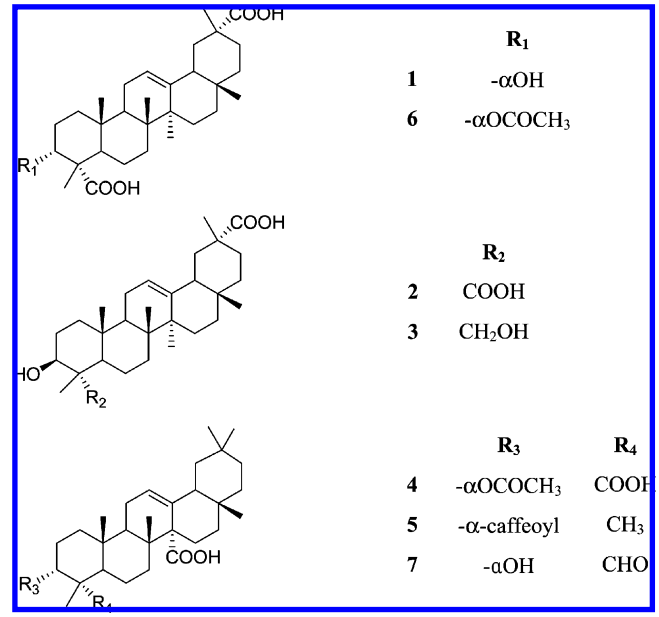
Table 3. Cytotoxicity of Isolated Compounds against MCF-7 and LLC Cancer Cell Lines

	IC_{50} (μM)	
compound	MCF-7	LLC
1	62.44	47.82
2	>150	>150
3	107.22	>150
4	58.44	>150
5	>150	>150
6	>150	>150
7	47.02	39.25
8	>150	16.62
9	>150	>150
10	>150	7.63
11	>150	8.36
12	53.57	>150
13	39.17	6.56
14	>150	>150
adriamycine[a]	0.65	0.23

[a] Used as positive control.

and 6.11 (1H, d, J = 16.0 Hz, H-8′), together with signals for a 1,3,4-trisubstituted benzene ring at δ 7.0 (1H, brs, H-2′), 6.83 (1H, d, J = 8.0 Hz, H-5′) and δ 6.81 (1H, d, J = 8.0 Hz, H-6′), which was also supported by ^{13}C and DEPT NMR spectra (Table 2). The molecular formula, $C_{39}H_{54}O_6$, was deduced from a molecular ion peak at *m/z* 618.3902 $[M]^-$ in the HRESIMS. The attachment of

Chart 1. Compounds from the *n*-Hexane Fraction of *Aceriphyllum rossii*



the caffeoyl group at C-3 (δ 78.2) was confirmed by long-range correlations from H-3 (δ 4.70, 1H, brs) to the C═O (δ 167.1) in the HMBC spectrum. Hence, compound **5** was elucidated as a new triterpenoid, 3α-*O*-caffeoylolean-12-en-27-oic acid and was named aceriphyllic acid G.

Compound **6** was isolated as a white, amorphous powder, with $[\alpha]_D^{22}$ +41.4 (*c* 0.1, MeOH). The ^1H and ^{13}C NMR data of **6** were similar to those of **1**, except for the presence of a proton signal at δ 2.03 (3H, s) and carbon signals at δ 21.5 and 170.4 for an acetyl group and a downfield shift of the H-3 signal to δ 4.96, indicative of replacement of the 3-OH group of **1** by acetate. This conclusion was further verified by the HMBC correlations from H-3 (δ 4.96) to the carbonyl carbon at δ 170.4. The molecular formula of $C_{32}H_{48}O_6$ was confirmed from a molecular ion peak at *m/z* 528.3407 $[M]^-$ in the HRESIMS. Thus, compound **6** was elucidated as 3α-*O*-acetylolean-12-en-23,29-dioic acid and was named aceriphyllic acid H.

Compound **7** was isolated as a white, amorphous powder. The HRESIMS analysis indicated an ion peak at *m/z* 470.3355 $[M]^-$, which corresponded to the molecular formula $C_{30}H_{46}O_4$. The IR absorptions at 3320, 2995, 1725, and 1710 cm^{-1} showed the presence of a hydroxy, an aldehyde, and carboxylic groups. By comparison with compound **4**, the ^1H and ^{13}C NMR spectra of **7** indicated the presence of an aldehyde proton at δ_H 9.89 (1H, s) and carbon at δ_C 210.1. In addition, the proton signal at δ 3.98 (1H, brs, H-3) indicated an axial position of the hydroxy group at C-3, which was in agreement with the same position in aceryphyllic acid A.[2] In the HMBC spectrum, the correlations from H-3 (δ 3.98) to the aldehyde carbon at δ 210.1 and from the methyl proton at δ 1.10 (3H, s, H-24) to this carbon, and C-3 (δ 73.2), indicated that the aldehyde group is located at C-23. On the basis of the above analysis, the structure of compound **7** was elucidated as 3α-hydroxyolean-12-en-23-al-29-oic acid and was named aceriphyllic acid I.

The isolates were evaluated for *in vitro* cytotoxicity against the cancer cell lines MCF-7 and LLC using the MTT assay method.[10,11] Compounds **1**, **3**, **4**, **7**, **12**, and **13** exhibited weak cytotoxic activity against MCF-7 cell lines, with IC_{50} values ranging from 39.17 to 107.22 μM, while the other compounds were inactive. In the inhibition of LCC cell lines, compounds **10**, **11**, and **13** exhibited inhibitory activity, with IC_{50} values of 7.63, 8.36, and 6.56 μM, respectively. Compound **8** displayed moderate cytotoxic activity,

with an IC_{50} value of 16.6 μM. Our results indicated that the olean-12-en-27-oic acid showed stronger cytotoxic activity than olean-12-en-28-oic acid against MCF-7 and LLC cancer cell lines.

Experimental Section

General Experimental Procedures. 1D and 2D NMR experiments were performed on a Varian NMR spectrometer operating at 400 MHz. HRESIMS was measured on a JMS-700 Mstation mass spectrometer. Optical rotations were determined on a JASCO DIP-370 polarimeter using a 100 mm glass cell. UV spectra were measured on a Thermo 9423AQA2200E UV spectrometer. IR spectra (KBr) were recorded on a Bruker Equinox 55 FT-IR spectrometer. A Waters 400 HPLC was used for purification and isolation with an YMC ODS-80 preparative HPLC column. Open column chromatography was performed using silica gel (Kieselgel 60, 70−230 mesh and 230−400 mesh, Merck) or reversed-phase silica gel (LiChroprep RP-18, 40−63 μm, Merck). TLC tests were performed on Merck precoated silica gel 60 F_{254} and/or RP-18 F_{254s} plates (0.25 mm), and compounds were observed by spraying the dried plates with 10% H_2SO_4, followed by heating. Optical density (OD) values in the cytotoxic activity by MTT assays were read on a TECAN-ELISA microplate reader.

Chemicals and Reagents. Solvents were purchased from Samchun Chemicals Company. 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.

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

Extraction and Isolation. The roots of *A. rossii* (10 kg) were extracted with MeOH (× 3) at room temperature for one month, filtered, and concentrated to give an MeOH extract (1.43 kg). The MeOH extract (1.43 kg) was suspended in hot H_2O and then partitioned successively with *n*-hexane (4.0 L × 3), EtOAc (4.0 L × 3), and *n*-BuOH (4.0 L × 3) to afford hexane (230 g)-, EtOAc (208 g)-, and BuOH (180 g)-soluble fractions, respectively. The hexane-soluble fraction (230 g) was chromatographed over a silica gel column (40 × 15 cm) eluted with a gradient of *n*-hexane−EtOAc (10:1 to 0:1) to afford 12 fractions (H1−H12). Fraction H6 (230 mg) was repeatedly passed through a silica gel column (30 × 5 cm) using an *n*-hexane−acetone mixture as solvent, with a stepwise gradient (10:1 to 3:1) to give eight subfractions (H6.1−H6.8). Compound **12** (20.6 mg) was obtained as crystals in *n*-hexane from fraction H6.3. Repeated silica gel column chromatography (50 × 5.0 cm) of fraction H9 (2.5 g) using *n*-hexane−acetone (5:1, 3:1) gave four subfractions (H9.1−H9.4). Compounds **8** (3 mg), **9** (6 mg), and **10** (34.5 mg) were crystallized from subfractions H9.1, H9.2, and H9.3, respectively, with *n*-hexane. Fraction H9.4 was passed through a silica gel column using $CHCl_3$−MeOH (10:1−50:1), then purified by preparative HPLC with MeOH−H_2O (95:5) to give compound **5** (10.5 mg). Fraction H10 was eluted on a silica gel column (50 × 5.0 cm) with $CHCl_3$−acetone (5:1) to afford compound **7** (10.2 mg). Fraction H11 was passed through a silica gel column using $CHCl_3$−acetone (20:1 to 0:1) to give nine subfractions (H11.1−9). Fraction H11.6 was eluted on a silica gel column (50 × 5.0 cm) with $CHCl_3$−acetone (5:1 to 1:1) to afford compound **6** (12.3 mg). Subfraction H11.7 was subjected to C-18 column chromatography using an MeCN−H_2O gradient (50:1 to 100:1) to afford compounds **4** (13.4 mg), **11** (400 mg), and **13** (37.5 mg). Subfraction H11.8 was repeatedly passed through a silica gel column eluted with $CHCl_3$−acetone (10:1 to 1:1) to give compound **14** (90.7 mg). Fraction H12 was repeatedly chromatographed on a silica gel column eluted with $CHCl_3$−acetone (10:1 to 0:1) to give five subfractions (H12.1−5). Crystalline compound **1** (4.1 mg) was obtained by crystallization in $CHCl_3$ of the collected subfraction H12.2. Compound **3** (3.5 mg) was obtained by applying subfraction H12.3 on an RP-18 column eluting with a MeOH−H_2O gradient (10:1 to 100:0). Fraction H12.5 was repeatedly subjected to a silica gel chromatography column eluted with *n*-hexane−EtOAc (15:1 to 5:1), and the collected subfraction was further chromatographed on a C-18 column eluted with an MeCN−H_2O gradient (50:1 to 100:1) to give compound **2** (2 mg).

Aceriphyllic acid C (1): colorless crystals; mp 252 °C; $[\alpha]_D^{22}$ +50.4 (*c* 0.1, MeOH); IR ν_{max} (KBr) cm^{-1} 3280, 2980 (OH), 1705 (COOH), 1015, 1235 (OH); UV $[\lambda]_{max}^{MeOH}$ (nm) 203, 258; HRESIMS *m/z* 486.3317 $[M]^-$ (calcd for $C_{30}H_{46}O_5$, 486.3345); ^1H NMR see Table 1, and ^{13}C NMR see Table 2.

Aceriphyllic acid D (2): white crystals; mp >300 °C; $[\alpha]_D^{22}$ +11.0 (*c* 0.08, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3340, 2990 (OH), 1710 (COOH), 1110, 1250 (OH); UV $[\lambda]_{max}^{MeOH}$ (nm) 202, 259; HRESIMS *m/z* 486.3318 $[M]^-$ (calcd for $C_{30}H_{46}O_5$, 486.3345); ^1H NMR see Table 1, and ^{13}C NMR see Table 2.

Aceriphyllic acid E (3): white powder; mp >300 °C; $[\alpha]_D^{22}$ +48.01 (*c* 0.08, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3332, 2990 (OH), 1720 (COOH), 1200, 1250 (OH); UV $[\lambda]_{max}^{MeOH}$ (nm) 202, 260; HRESIMS *m/z* $[M]^-$ 472.3524 $[M]^-$ (calcd for $C_{30}H_{48}O_4$, 472.3553); ^1H NMR see Table 1, and ^{13}C NMR see Table 2.

Aceriphyllic acid F (4): colorless crystals; mp 260 °C; $[\alpha]_D^{22}$ +52.0 (*c* 0.35, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3200, 2980 (OH), 1736 (O═C−O), 1713 (COOH), 1200, 1253 (OH); UV $[\lambda]_{max}^{MeOH}$ (nm) 205, 251, 257.5, 263.5; HRESIMS *m/z* $[M]^-$ 528.3401 $[M]^-$ (calcd for $C_{32}H_{48}O_6$, 528.3451); ^1H NMR see Table 1, and ^{13}C NMR see Table 2.

Aceriphyllic acid G (5): colorless, amorphous powder; $[\alpha]_D^{22}$ +80.0 (*c* 0.08, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3332, 2990 (OH), 1735, 1730 (COOH, O═C−O), 1225, 1253 (OH), 1597, 1446 (benzene ring); UV $[\lambda]_{max}^{MeOH}$ (nm) 201.5, 216, 245, 329; HRESIMS *m/z* $[M]^-$ 618.3902 $[M]^-$ (calcd for $C_{39}H_{54}O_6$, 618.3920); ^1H NMR see Table 1, and ^{13}C NMR see Table 2.

Aceriphyllic acid H (6): white, amorphous powder; $[\alpha]_D^{22}$ +41.4 (*c* 0.1, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3300, 2985 (OH), 1736 (COO), 1710 (COOH), 1100, 1262 (OH); UV $[\lambda]_{max}^{MeOH}$ (nm) 203, 206, 251, 258, 263; HRESIMS *m/z* $[M]^-$ 528.3407 $[M]^-$ (calcd for $C_{32}H_{48}O_6$, 528.3451); ^1H NMR see Table 1, and ^{13}C NMR see Table 2.

Aceriphyllum acid I (7): white amorphous; $[\alpha]_D^{22}$ +15.01 (*c* 0.1, MeOH); IR (KBr) ν_{max} (cm^{-1}) 3320, 2995 (OH), 1725 (CHO), 1710 (COOH), 1120, 1200 (OH); UV $[\lambda]_{max}^{MeOH}$ (nm) 203, 251.5, 257.5, 263; HRESIMS *m/z* $[M]^-$ 470.3355 (calcd for $C_{30}H_{46}O_4$, 470.3397); ^1H NMR see Table 1, and ^{13}C NMR see Table 2.

Cytotoxicity Assay. The cancer cell lines (MCF-7 and LLC) were maintained in RPMI 1640, which included L-glutamine with 10% FBS and 2% penicillin−streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxicity was measured using a modified MTT assay.[10,11] Viable cells were seeded in the growth medium (100 μL) into 96-well microtiter plates (1×10^4 cells per well) and incubated at 37 °C 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. On removing medium after 48 h of the test sample treatment, MTT (10 μL) was also added to the each well (final concentration, 5 mg/mL). After 4 h incubation, the plates were removed, and the resulting formazan crystals were dissolved in DMSO (150 mL). The OD was measured at 570 nm. The IC_{50} value was defined as the concentration of sample that reduced absorbance by 50% relative to the vehicle-treated control.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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