Aceriphyllic Acid A, A New ACAT Inhibitory Triterpenoid, from *Aceriphyllum rossii*

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Abstract

Two new triterpenoids along with three known ones, 3-oxo-olean-12-en-27-oic acid (**1**), 3α-hydroxyolean-12-en-27-oic acid (**2**) and 3β-hydroxyolean-12-en-27-oic acid (**3**), were isolated from *Aceriphyllum rossii*. The structures of the new compounds were determined to be a 3α,23-dihydroxyolean-12-en-27-oic acid (**4**) and a 3α,23-dihydroxyolean-12-en-29-oic acid (**5**) by spectroscopic and chemical methods; they were designated aceriphyllic acids A and B, respectively. Compounds **2**, **3** and **4** remarkably inhibited the activity of ACAT.

Aceriphyllum rossii Engler (Saxifragaceae), a perennial herb indigenous to Korea, grows in scant amounts of soil on damp rocks along the valley in the mid-northern area of Korea. The young leaflets and stems are preferably ingested in a Korean diet. Because no study on the constituents of the genus *Aceriphyllum* including *A. rossii* has been found in the literature, research on biologically active secondary metabolites of this plant was initiated.

The methanolic extract obtained from the *A. rossii* was fractionated through solvent fractionation and column chromatography was repeated to supply five pentacyclic triterpenoids **1 – 5**, with the yields of 0.016, 0.015, 0.007, 0.095 and 0.004%, respectively.

Compounds **1 – 3** were identified by interpretation of spectral data and comparison with those of compounds described in the literature [1], [2] as 3-oxoolean-12-en-27-oic acid, 3α-hydroxyolean-12-en-27-oic acid and 3β-hydroxyolean-12-en-27-oic acid (peltoboykinolic acid), respectively. The simultaneous occurrence of 3α-hydroxy, 3β-hydroxy and 3-oxo derivatives of oleanane-skeleton triterpenoid in the same plant has rarely been reported.

NMR data of compound **4** were almost identical with those of compound **2** with the exception of signals of some atoms of ring A and B. ^{1}H- and ^{13}C-NMR data (Table **1**) of the methyl ester of compound **4** (compound **4a**) were assigned on the basis of the COSY, HMQC and HMBC spectra of **4a**. From these data it is evi-

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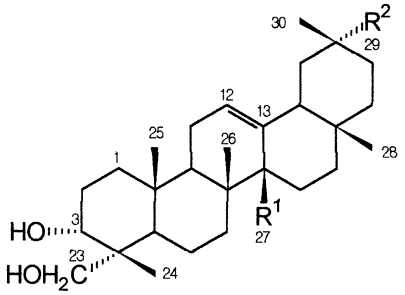

Aceriphyllic acid A (**4**) : $R^1 = COOH$, $R^2 = CH_3$
Aceriphyllic acid B (**5**) : $R^1 = CH_3$, $R^2 = COOH$

dent that C-23 is present as a hydroxymethylene group in **4** and **4a**. The small coupling constant of H-3 indicated an axial position of the hydroxy group at C-3 [3]. Thus, compound **4** was determined to be the 3α,23-dihydroxyolean-12-en-27-oic acid, a new compound, and was named aceriphyllic acid A.

NMR data of compound **5** were similar to those of aceriphyllic acid A (**4**), with the exception of the resonances of some atoms around C-27 and E-ring. ^{1}H- and ^{13}C-NMR data (Table **1**) of the methyl ester of compound **5** (compound **5a**) were assigned on the basis of the COSY, HMQC and HMBC spectra of **5a**. From these data it is evident that the carboxyl group is located at C-29 in **5** and **5a**. Thus, compound **5** was determined to be the 3α,23-dihydroxyolean-12-en-29-oic acid, a new compound, and was named aceriphyllic acid B.

Table **1** ^{13}C-NMR (100 MHz) data of compounds **4, 4a, 5a** ($CDCl_3$) and **5** (pyridine-d_5)

No of C	*4*	*4a*	*5*	*5a*
1	36.48	36.36	36.48	35.87
2	25.38	26.15	26.43	26.25
3	77.82	76.16	75.52	76.52
4	39.82	40.16	40.20	39.93
5	47.57	47.18	47.82	45.99
6	17.43	17.86	18.33	18.02
7	32.71	33.05	32.59	32.16
8	35.90	36.84	40.64	40.29
9	49.49	49.09	46.53	47.39
10	38.58	39.86	37.13	36.76
11	22.96	22.70	23.86	23.47
12	123.07	125.42	122.67	122.66
13	140.49	137.52	144.15	144.15
14	58.10	56.05	41.95	41.75
15	22.89	22.29	26.34	25.93
16	28.08	27.59	27.21	26.91
17	31.09	30.88	32.73	32.39
18	41.49	42.85	43.48	42.75
19	42.11	43.85	41.44	40.49
20	33.66	32.91	42.75	42.71
21	34.90	34.30	29.82	29.02
22	36.72	36.51	33.48	32.80
23	68.75	71.08	71.20	71.33
24	17.43	17.93	18.17	17.95
25	16.29	16.46	17.09	16.79
26	19.45	18.10	15.96	15.58
27	184.23	176.24	26.04	26.10
28	28.29	28.23	28.41	28.18
29	33.42	33.21	181.17	179.37
30	23.39	23.51	19.97	19.32
MeO-		51.39		51.73

The triterpenoids possessing olean-12-en-27-oic acid units like compounds **1 – 4** have been widely distributed in the genera *Boykinia* [4], *Peltoboykinia* [4], *Astilbe* [2] and *Cordia* [1] of the family Saxifragaceae, and the ones possessing olean-12-en-29-oic acid unit like compound **5** have been found in the genus *Sandoricum* [5].

The inhibitory effect of each compound on ACAT (Acyl-CoA: cholesterol acyltransferase), an enzyme which catalyzes intracellular esterification of cholesterol [6], was evaluated. Compounds **2**, **3** and **4** showed higher inhibitory activity than baicalein (Table **2**), an ACAT inhibitory component of *Scutellaria baicalensis* [7], and the IC_{50} values were determined to be 65.0, 77.9 and 66.1 µM. The results indicated the necessitity of 3-hydroxy and 27-caboxyl in the oleanane skeleton for the exhibition of this activity.

ACAT is an enzyme primarily responsible for the esterification of cholesterol in all mammalian cells. Furthermore, since it has been designated as a key enzyme in cholesterol absorption with very low density lipoprotein secretion, along with a foam cell formation from macrophages and smooth muscle cell, the inhibition of ACAT activity in the arterial wall may prevent the formation of the macrophage-enriched fatty streak and the development of the clinically significant fibrous plaque [6]. Therefore, *A. rossii* could be utilized as a new type of medicine or functional food to treat arteriosclerosis.

Materials and Methods

Dried and powdered whole plant of *A. rossii* (2.5 kg), collected at Kapyung-Kun, Korea in July, 1998 (voucher specimen No. KHU980812), was extracted two times at r.t. with 80% aqueous MeOH (6 L×2). The extracts were partitioned with water (1500 ml), EtOAc (1.5 L×2) and *n*-BuOH (1 L×2). The EtOAc extract (41.7 g) was applied to silica gel column (5×12 cm) chromatography (c.c.) and eluted with *n*-hexane-EtOAc (3 : 1 and 1 : 1, each 900 mL), and $CHCl_3$-MeOH (20 : 1, 10 : 1, 7 : 1, 5 : 1 and 3 : 1, each 800 mL) monitoring by thin layer chromatography (TLC) to produce ten fractions (ARE-1 to ARE-10). The second fraction (ARE-2, 4.5 g) was further purified by c.c. on silica gel (5×13 cm) using *n*-hexane-EtOAc (4 : 1, 700 ml) as eluents to yield compound **1** (3-oxoolean-12-en-27-oic acid) {(411 mg, Rf: 0.50 on silica gel TLC in *n*-hexane-EtOAc (2 : 1)}; white powder (*n*-hexane-$CHCl_3$), m.p. 210 – 211 °C, $[\alpha]_D^{25}$: + 140°($CHCl_3$, *c* 1.32).

The third fraction (ARE-3, 6.0 g) was subjected on silica gel column (5×15 cm) eluted with *n*-hexane-EtOAc (5 : 2, 3 L) to afford five fractions (ARE-3-1 to ARE-3-5). The obtained fraction containing major components (ARE-3-2, 2.2 g) was applied to silica gel c.c. (4×15 cm) eluted with *n*-hexane-EtOAc (3 : 1, 2.5 L) to give four fractions (ARE-3-2-1 to ARE-3-2-4) and the third fraction (ARE-3-2-3, 920 mg) was subjected on silica gel column (3×13 cm) eluted with *n*-hexane-EtOAc-EtOH (10 : 2 : 10, 1.2 L) to

Table **2** The inhibitory effects of triterpenoids isolated from *Acriphyllum rossii* and baicalein on ACAT (Acyl-CoA: cholestrol acyltransferase)

Compounds *	*1*	*2*	*3*	*4*	*5*	*baicalein*
Inhibitory effect (%)**	68.5 ± 2.4	94.2 ± 3.2	89.3 ± 2.9	91.5 ± 2.8	28.8 ± 4.3	69.2 ± 3.7

* The concentration of each compound was 100 μg/ml.

** Values given indicates means ± SEM of three compounds.

ultimately produce purified compounds **2** {(370 mg, Rf: 0.34 on silica gel TLC in *n*-hexane-EtOAc-EtOH (10 : 2 : 2)} and **3** {(168 mg, Rf: 0.19 on silica gel TLC in *n*-hexane-EtOAc-EtOH (10 : 2 : 2)}. Compound **2** (3α-hydroxyolean-12-en-27-oic acid) [1]: Colorless plates (*n*-hexane-$CHCl_3$), m. p. 212 – 213 °C, $[\alpha]_D^{25}$: + 80°($CHCl_3$, *c* 1.11). Compound **3** (β-peltoboykinolic acid, 3β-hydroxyolean-12-en-27-oic acid) [2]: Colorless needles (*n*-hexane-$CHCl_3$), m. p. 226 – 227 °C, $[\alpha]_D^{25}$: + 106°($CHCl_3$, *c* 1.31).

The sixth fraction (ARE-6, 7.9 g) was applied to silica gel column (5 × 14 cm) eluting with *n*-hexane-$CHCl_3$-MeOH (7 : 13 : 2, lower phase, 1.7 L) to afford two fractions (ARE-6 – 1 and ARE-6-2). The first fraction of them (ARE-6-1) was purified by silica gel (5 × 12 cm) c. c. using *n*-hexane-EtOAc-EtOH (5 : 2 : 1, 1.5 L) as eluents to yield compound **4** {2.38 g, Rf: 0.45 on silica gel TLC in *n*-hexane-EtOAc (1 : 2)}; the second fraction (ARE-6-2) was purified by silica gel (4 × 12 cm) c. c. using *n*-hexane-EtOAc-EtOH (5 : 4 : 2, 1.2 L) as eluents to yield compound **5** {93.4 mg, Rf: 0.52 on silica gel TLC in *n*-hexane-EtOAc (1 : 2)}.

Compound **4** (aceriphyllic acid A, 3α,23-dihydroxyolean-12-en-27-oic acid): White powder (*n*-hexane-$CHCl_3$), m. p. 249 – 250 °C, $[\alpha]_D^{25}$: + 105°($CHCl_3$, *c* 1.09); IR (film): ν_{max} = 3434, 2950, 1712, 1634, 1385, 760 cm^{-1}; EIMS: *m*/*z* = 472 (M^+), 457, 454, 439, 426, 248, 233, 223, 205, 203. HR-MS: Found: 472.3558, Calcd for $C_{30}H_{48}O_4$: 472.3554; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.57 (1H, br, s, H-12), 3.47 (1H, br, s, H-3), 3.32 (1H, d, *J* = 10.7 Hz, H-23a), 2.82 (1H, d, *J* = 10.7 Hz, H-23b), 0.98 (3H, s, H-26), 0.96 (3H, s, H-25), 0.86 (3H, s), 0.83 (3H × 2, s), (H-28, H-29 and H-30), 0.50 (3H, s, H-24); ^{13}C-NMR: see Table **1**.

Compound **5** (aceriphyllic acid B, 3α,23-dihydroxyolean-12-en-29-oic acid): White powder (MeOH-pyridine), m. p. 220 – 221 °C, $[\alpha]_D^{25}$: + 82°(MeOH, *c* 1.09); IR (film): ν_{max} = 3442, 2964, 1714, 1635, 1382, 762 cm^{-1}; EIMS: *m*/*z* = 472 (M^+), 457, 454, 426, 248, 233, 223, 205, 133; HR-MS: Found: 472.3558, Calcd for $C_{30}H_{48}O_4$: 472.3554; ^{1}H-NMR (400 MHz, C_5D_5N): δ = 5.30 (1H, br, s, H-12), 3.90 (1H, br, s, H-3), 3.84 (1H, d, *J* = 10.7 Hz, H-23a), 3.64 (1H, d, *J* = 10.7 Hz, H-23b), 1.46 (3H, s, H-30), 1.02 (3H, s, H-25), 1.00 (3H, s, H-26), 1.14 (3H, s, H-27) 0.93 (3H, s, H-28) 0.79 (3H, s, H-24); ^{13}C-NMR: see Table **1**.

The MeOH solutions of **4** (79.8 mg) and **5** (30.2 mg) were treated with diazomethane in ether for 15 h to produce methyl esters, and were subsequently purified by silica gel (70 g, *n*-hexane-EtOAc = 5 : 2, 1400 ml) c. c. to yield the methyl esters of **4** and **5**, **4a** {67.0 mg, Rf: 0.58 on silica gel TLC in *n*-hexane-EtOAc (1 : 2)} and **5a** {23.8 mg, Rf: 0.69 on silica gel TLC in *n*-hexane-EtOAc (1 : 2)}, respectively.

Compound **4a**: White powder (*n*-hexane-$CHCl_3$), m. p. 244 – 245 °C, $[\alpha]_D^{25}$: + 101°($CHCl_3$, c 1.22); IR (film): ν_{max} = 3346, 2944, 2861, 1724, 1432, 1211, 757 cm^{-1}; EIMS: *m*/*z* = 486 (M^+), 471, 468, 456, 427, 262, 223, 205, 203, 133. HR-MS: Found: 486.3714, Calcd for $C_{31}H_{50}O_4$: 486.3711; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.62 (1H, dd, *J* = 2.4 2.4 Hz, H-12), 3.66 (1H, br, s, H-3), 3.64 (3H, s, H-OMe), 3.60 (1H, d, *J* = 11.2 Hz, H-23a), 3.50 (1H, d, *J* = 11.2 Hz, H-23b), 2.17 (1H, dd, *J* = 5.4, 11.5 Hz, H-5), 2.04 (1H, m, H-15a), 2.00 (1H, dd, *J* = 4.0, 10.2 Hz, H-9a), 1.98 (1H, m, H-11a), 1.92 (1H, m, H-11b), 1.87 (1H, m, H-2a), 1.71 (1H, m, H-15b), 1.64 (1H, br. d, *J* = 13.2 Hz, H-1a), 1.57 (1H, dd, *J* = 1.7, 11.0 Hz, H-18), 1.48 (1H, m, H-2b), 1.45 ~ 1.24 (5H, m, H-6, H-7, H-22a), 1.21 (1H, br. d, *J* = 13.0 Hz, H-22a), 1.12 (1H, dd, *J* = 13.2, 5.5 Hz, H-1b), 1.07 (1H, br. d, *J* = 11.0 Hz, H-19a), 1.03 (2H, m, H-21), 1.02 (3H, s, H-26), 0.98 (3H, s, H-25), 0.93 (1H, m, H-19b), 0.85 (3H, s, H-28), 0.84 (3H, s, H-30), 0.82 (3H, s, H-29), 0.78 (2H, m, H-16), 0.68 (3H, s, H-24); ^{13}C-NMR: see Table **1**.

Compound **5a**: White powder (*n*-hexane-$CHCl_3$), m. p. 216 – 217 °C, $[\alpha]_D^{25}$: + 79°($CHCl_3$, *c* 1.27); IR (film): ν_{max} = 3382, 2952, 1720, 1430, 764 cm^{-1}; EIMS: *m*/*z* = 486 (M^+), 471, 468, 427, 262, 223, 205, 133. HR-MS: Found: 486.3715, Calcd for $C_{31}H_{50}O_4$: 486.3711; ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 5.23 (1H, dd, *J* = 3.4, 3.4 Hz, H-12), 3.69 (1H, dd, *J* = 2.7, 2.7 Hz, H-3), 3.66 (3H, s, H-OMe), 3.55 (1H, dd, *J* = 11.4 Hz, H-23a), 3.41 (1H, dd, *J* = 11.4 Hz, H-23b), 2.13 (1H, dd, *J* = 13.6, 13.6 Hz, H-19a), 2.03 (1H, m, H-16a), 1.97 (1H, br. d, *J* = 5.8 Hz, H-5), 1.92 (2H, m, H-11), 1.89 (1H, m, H-21a), 1.83 (1H, m, H-16b), 1.79 (1H, m, H-2a), 1.74 (1H, dd, *J* = 4.5, 10.1 Hz, H-9), 1.67 (1H, dd, *J* = 1.5, 13.6 Hz, H-18), 1.49 (1H, br. d, *J* = 2.9 Hz, H-2b), 1.44 (1H, m, H-21b), 1.39 (2H, m, H-6), 1.36 (2H, m, H-22), 1.33 (1H, m, H-19b), 1.30 (2H, m, H-1), 1.20 (3H, s, H-30), 1.18 (3H, s, H-27), 1.02 (2H, m, H-15), 0.98 (3H × 2, s, H-25 and H-26), 0.89 (2H, m, H-7), 0.86 (3H, s, H-28), 0.71 (3H, s, H-24); ^{13}C-NMR: see Table **1**.

ACAT activity was measured by the method as described in the literature [4] using a microsomal protein prepared from rat livers. Baicalein was used as a positive control.

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