JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Olean-27-carboxylic Acid-Type Triterpenes with Potent Antibacterial Activity from *Aceriphyllum rossii*

CHANG-JI ZHENG, MI-JIN SOHN, KI-YOUNG KIM, HYUNG-EUN YU, AND WON-GON KIM*

Functional Metabolites Research Center, Korea Research Institute of Bioscience and Biotechnology, Post Office Box 115, Yusong, Daejeon 305-806, Korea

Aceriphyllum rossii Engler (Saxifragaceae) have been used as a nutritious food in Korea. We found that the methanol extract of the root portion of *A. rossii* potently inhibited the growth of *Staphylococcus aureus*, with a minimal inhibitory concentration (MIC) value of 8 μg/mL. Using mass spectrometry and nuclear magnetic resonance (NMR) studies, four active constituents were isolated and identified: aceriphyllic acid A, 3-oxoolean-12-en-27-oic acid, 3α-hydroxyolean-12-en-27-oic acid, and 3β-hydroxyolean-12-en-27-oic acid. Aceriphyllic acid A and 3-oxoolean-12-en-27-oic acid showed a potent antibacterial activity against several strains of *S. aureus*, including methicillin-resistant *S. aureus* and quinolone-resistant *S. aureus*, with MIC values of 2−8 μg/mL, while 3α-hydroxyolean-12-en-27-oic acid and 3β-hydroxyolean-12-en-27-oic acid exhibited a very weak activity, with MIC values of 128 μg/mL. The methyl ester derivative of aceriphyllic acid A lost its antibacterial activity. The time-kill study against *S. aureus* indicated that aceriphyllic acid A had rapid bactericidal activity. These results indicated that aceriphyllic acid A and 3-oxoolean-12-en-27-oic acid are the most active principles, and both the carboxylic group at C-27 and the hydroxyl group at C-24 in aceriphyllic acid A are critical for the rapid bactericidal activity.

KEYWORDS: *Aceriphyllum rossii*; antibacterial activity; aceriphyllic acid A; 3-oxo-12-oleanen-27-oic acid; bactericidal

INTRODUCTION

Aceriphyllum rossii Engler (Saxifragaceae) is a perennial herb that grows on damp rocks along valleys and is distributed in the midnorthern area of Korea and China. The young leaflets and stems have been used as a nutritious food in Korea (*1*−*3*). The other *Aceriphyllum* species, *Aceriphyllum acanthifolium* (Nakai) T. Lee, is also used as side dishes for rice (*3*). *Mukdenia rossii* (Oliv.) Koidz, which is another name of *A. rossii*, has been taken internally as a heart stimulant or a diuretic in China. The leaves of other greens of the same family Saxifragaceae, such as *Saxifraga stolonifera* Meerb, have been supplied for food in Japan and China (*4*). Only a few studies, however, have been reported on the constituents and biological activity of *A. rossii* (*5*−*7*). Recently, several triterpenes and flavonol glycosides have been reported as constituents of this plant. The triterpenes, such as aceriphyllic acids and 3-hydroxyolean-12-en-27-oic acid, have been reported to inhibit acyl-CoA: cholesterol acyltransferase (ACAT) (*5*). 3-Oxoolean-12-en-27-oic acid, 3α-hydroxyolean-12-en-27-oic acid, and 3α,23-diacetoxyolean-12-en-27-oic acid have been found to show significant anticomplementary activity on the classical pathway (*6*). The flavonol glycosides, such as kaempferol 3-*O*-β-D-glucopyranoside and quercetin 3-*O*-β-D-glucopyranoside, have been found to exhibit antioxidant activity (*7*). Antibacterial activity of *A. rossii*, however, has not been reported yet.

We have found that the methanol extract of the root portion of *A. rossii* potently inhibited the growth of *Staphylococcus aureus* with a minimal inhibitory concentration (MIC) value of 8 μg/mL. The aims of this study were to isolate and identify the antibacterial components in *A. rossii* and to examine their antibacterial activity.

MATERIALS AND METHODS

Chemicals and Microorganisms. All chemicals used in the study, such as methanol (MeOH), ethyl acetate (EtOAc), chloroform ($CHCl_3$), hexane, and dimethylsulfoxide (DMSO), were analytical-grade. Vancomycin, oxacillin, norfloxacin, and methylthiazolyldiphenyl-tatrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Life Technologies (Gaithersburg, MD).

S. aureus (KCTC 1916), *Bacillus subtilis* (KCTC 1021), *Acinetobacter calcoaceticus* (KCTC 2357), *Micrococcus luteus* (KCTC 1056), *Escherichia coli* (KCTC 1358 and KCTC 1661), *Klebsiella aerogenes* (KCTC 2618), *Salmonella typhimurium* (KCTC 2421), and *Candida albicans* (KCTC 7535) were purchased from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). Two methicillin-resistant *S. aureus* (MRSA) strains (CCARM 3167 and CCARM 3506) and two

* To whom correspondence should be addressed. Telephone: +81-42-860-4298. Fax: +81-42-860-4595. E-mail: wgkim@kribb.re.kr.

10.1021/jf802832w CCC: $40.75
Published on Web 11/20/2008

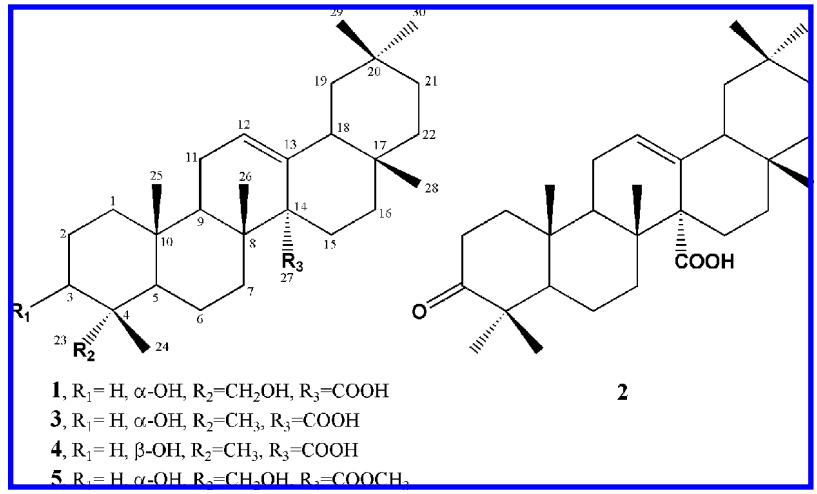

Figure 1. Structures of aceriphyllic acid A (**1**), 3-oxoolean-12-en-27-oic acid (**2**), 3α-hydroxyolean-12-en-27-oic acid (**3**), 3β-hydroxyolean-12-en-27-oic acid (**4**), and methyl ester of aceriphyllic acid A (**5**).

quinolone-resistant *S. aureus* (QRSA) strains (CCARM 3505 and CCARM 3519) were obtained from the Culture Collection of Antimicrobial Resistant Microbes (CCARM, Seoul, Korea).

Plants Material. *A. rossi* was collected in April 2002, in Danyang, Chungcheongnamdo Province, Korea, and identified by a staff at Plant Extract Bank, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, where a voucher specimen (PB3043.2) has been deposited. They were immediately transferred to the laboratory, washed in tap water, and finally rinsed with distilled water. The whole plants, root portion, and aerial portion were dried for 1 week in the shade and grinded.

Extraction and Isolation. To isolate the antibacterial constituents of *A. rossi*, dried materials (1.5 g) of the root portion were extracted 3 times with 500 mL of methanol. After the methanol solution was concentrated *in vacuo*, the residual (196 mg) was suspended in water and successively partitioned using 100 mL of ethyl acetate 3 times. Subsequently, the activity was found in the ethyl acetate layer. After the ethyl acetate layer was concentrated *in vacuo*, the resultant residue (95 mg) was subjected to preparative silica gel thin-layer chromatography (TLC) with $CHCl_3$/MeOH (10:1) to give three active bands. The first band (19.6 mg) was further purified by preparative silica gel TLC with $CHCl_3$/MeOH (30:1) to give 3-oxoolean-12-en-27-oic acid (**2**) (11.3 mg). The second band (12.1 mg) was further purified by preparative silica gel TLC with *n*-hexane/EtOAc/EtOH (10:2:2) to afford 3α-hydroxyolean-12-en-27-oic acid (**3**) (1.0 mg) and 3β-hydroxyolean-12-en-27-oic acid (**4**) (2.3 mg). The third band (31.3 mg) was further purified by preparative silica gel TLC with $CHCl_3$/MeOH (15:1) to give aceriphyllic acid A (**1**) (18.3 mg). The purity of components **1**−**4** was determined to be over 99.9% at 210 nm by an analytical high-performance liquid chromatography (HPLC) column (4.6 × 250 mm, S-4 μm, YMC C_{18}) chromatography with methanol/water (45:55) at a flow rate of 0.4 mL/min, with retention times of 12.2, 18.9, 15.6, and 13.7 min, respectively (**Figure 1**).

Component 1: Aceriphyllic Acid A, 3α,23-Dihydrooxyolean-12-en-27-oic Acid ($C_{30}H_{48}O_4$). The ^1H and ^{13}C nuclear magnetic resonance (NMR), electrospray ionization−mass spectrometry (ESI−MS), and optical rotation value of **1** were identical to that reported for aceriphyllic acid A isolated from the whole plant of *A. rossii* (*5*).

Component 2: 3-Oxoolean-12-en-27-oic Acid ($C_{30}H_{46}O_3$). The ^1H and ^{13}C NMR, ESI−MS, and optical rotation value of **2** were identical to that reported for 3-oxoolean-12-en-27-oic acid isolated from leaves of the tropical tree *Cordia alliodora* (*8*).

Component 3: 3α-Hydroxyolean-12-en-27-oic Acid ($C_{30}H_{48}O_3$). The ^1H NMR, ESI−MS, and optical rotation value of **3** were identical to that reported for 3α-oxoolean-12-en-27-oic acid isolated from leaves of the tropical tree *C. alliodora* (*8*).

Component 4: 3β-Hydroxyolean-12-en-27-oic Acid ($C_{30}H_{48}O_3$). The ^1H NMR, ESI−MS, and optical rotation value of **4** were identical to that reported for 3β-oxoolean-12-en-27-oic acid isolated from the rhizomes of *Astilbe chinensis* (*9*).

Methylation. The MeOH solution of component **1** (5 mg) was treated with diazomethane in ether for 9 h to produce methyl ester and was subsequently purified by preparative silica gel TLC to yield the methyl ester of **1** (2.6 mg).

Compound 5: Methyl Ester of Aceriphyllic Acid A ($C_{31}H_{50}O_4$). The ^1H NMR, ESI−MS, and optical rotation value of **5** were identical to that reported for methyl ester of aceriphyllic acid A isolated from the whole plant of *A. rossii* (*5*).

HPLC Analysis of the Extracts. A total of 200 μg of methanol extracts of the root portion and aerial portion was suspended in 500 μL of water and partitioned with an equal volume of ethyl acetate 3 times, and then ethyl acetate layer was concentrated *in vacuo*. After the resultant residue was dissolved in 50 μL of methanol, 10 μL was subjected to reverse-phase HPLC column (4.6 × 250 mm, S-4 μm, YMC C_{18}) chromatography measured at 210 nm. The column was eluted with methanol−water (45:55) at a flow rate of 0.4 mL/min.

Determination of Antibacterial Activity. Test organisms were grown to mid-log phase in Mueller−Hinton broth (MHB) and diluted 1000-fold in the same medium. Cells (10^5/mL) were inoculated into MHB and dispensed at 0.2 mL/well in a 96-well microtiter plate. MICs were determined in triplicate by serial dilution of test compounds. As positive controls, vancomycin, oxacillin, and norfloxacin were used. Test compounds were prepared in DMSO, the final concentration of which did not exceed 0.05%. Cells were treated with 0.05% DMSO as a vehicle control. The MIC was defined as the concentration of a test compound that completely inhibited bacterial growth during a 24 h incubation at 37 °C. Bacterial growth was determined by measuring the absorption at 650 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader.

Cytotoxicity. The human hepatic HepG2 cell line were maintained in DMEM supplemented with 10% FBS, 50 units/mL penicillin, and 50 μg/mL streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxicity was measured using a modified MTT assay (*10*). HepG2 cells were cultured at an initial cell density of 5 × 10^4 cells/well in 96-well plates. After 24 h, the medium was replaced with DMEM supplemented with 10% FBS containing various concentrations of test compounds. A total of 10 μL of MTT (5 mg/mL in phosphate-buffered saline) was added to the each well 24 h after treatment. After incubation for 3 h, the medium was removed and the resulting formazan crystals were dissolved with 100 μL of DMSO. After shaking for 5 min, the optical density was measured at 570 nm using a microtiter ELISA reader. The IC_{50} value was defined as the concentration of sample that reduced absorbance by 50% relative to the vehicle-treated control.

Time-Kill Study. *S. aureus* incubated in MHB for 15 h at 37 °C was diluted with fresh MHB to approximately to 10^5 CFU/mL, and the diluted cultures were preincubated for 2 h. The antibacterial component was added to the culture at concentrations of 1×, 2×, and 4× MIC. Aliquots (0.1 mL) of the culture were removed at 0, 2, 4, 6, and 24 h of incubation, and serial 10-fold dilutions were prepared in

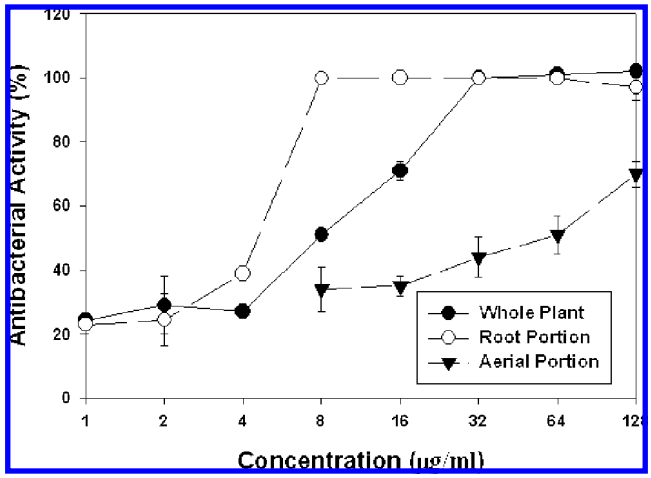

Figure 2. Antibacterial activity of the methanol extracts of the whole plant, root portion, and aerial portion of *A. rossi* against *S. aureus*. The values were represented as the mean ± standard deviation (SD) of experiments performed in triplicate.

saline as needed. The numbers of viable cells were determined on a drug-free MHA plate after 24 h incubation. Colony counts were performed on plates, yielding 30−300 colonies. The lower limit of sensitivity of colony counts was 100 CFU/mL. Antimicrobials were considered a bactericidal at the lowest concentration that reduced the original inoculum by 3 $\log_{10}$ CFU/mL (99.9%) at each of the time periods and bacteriostatic if the inoculum was reduced by 0−3 $\log_{10}$ CFU/mL.

RESULTS AND DISCUSSION

Isolation and Identification of the Active Components. The methanol extract of the whole plant of *A. rossii* inhibited concentration-dependently the growth of *S. aureus* (**Figure 2**). The methanol extract prevented the growth of *S. aureus* by 51 and 100% at concentrations of 8 and 32 μg/mL, respectively. To see which part of *A. rossii* is responsible for the antibacterial activity, the aerial and root portions were tested. Interestingly, the methanol extract of the root portion had a potent antibacterial activity, with a MIC of 8 μg/mL, while the aerial portion had almost no antibacterial activity, with a MIC over 128 μg/mL. Components **1**−**4** were isolated from the methanol extract of the root portion. The structures of components **1**−**4** were determined by mass and NMR studies. Components **1**−**4** were identified to be aceriphyllic acid A (*5*), 3-oxoolean-12-en-27-oic acid (*8*), 3α-hydroxyolean-12-en-27-oic acid (*8*), and 3β-hydroxyolean-12-en-27-oic acid (*9*), respectively, in which mass and NMR spectral data were identical to those of published values. The root and aerial portions were directly analyzed for components **1**−**4** by HPLC (**Figure 3**). Components **1**−**4** were detected in only the root portion as we expected, which is consistent with antibacterial activity of the root portion.

Table 1. Antibacterial Activity and Cytotoxicity for **1**−**5**

	test organisms	extract[a]	1	2	3	4	5
MIC (μg/mL)	*S. aureus* RN4220	8	4	4	128	128	>128
	MRSA CCARM 3506	8	4	4	128	128	>128
	QRSA CCARM 3505	16	4	8	128	128	>128
IC_{50} (μg/mL)	HepG2	42.9	69.1	25.0	28.9	22.3	41.8

[a] The methanol extract of the root portion.

Antibacterial Activity of the Active Components. Components **1** and **2** exhibited a potent antibacterial activity against *S. aureus*, with a MIC of 4 μg/mL, as shown in **Table 1**. They also showed the similar antibacterial potency against MRSA and QRSA. However, components **3** and **4**, dehydroxylated derivatives at C-23 of **1**, showed a very weak antibacterial activity against *S. aureus*, MRSA, and QRSA, with a MIC of 128 μg/mL. These data clearly showed that components **1** and **2** are the most active principles for the antibacterial activity of *A. rossii*, and the hydroxyl group at C-23 and the ketone at C-3 are important for the antibacterial activity of components **1** and **2**, respectively. To see whether the carboxylic group at C-27 affects the antibacterial activity, methyl ester of **1**, compound **5**, was prepared and then its antibacterial activity was evaluated (**Table 1**). Interestingly, compound **5** did not show any antibacterial activity, even at 128 μg/mL. Considering the almost no antibacterial activity of **3** and **4** with the carboxylic group at C-27 and no hydroxyl group at C-23, these data indicated that both the carboxylic group at C-27 and the hydroxyl group at C-23 are necessary for the antibacterial activity of component **1**. To see whether the antibacterial activity of **1** and **2** is selective, their cytotoxicity was evaluated (**Table 1**). Components **1** and **2** did not affect cell viability on the human hepatic cells, HepG2, at their MICs but showed cytotoxicity at much higher concentrations. Also, components **3**−**5** exhibited cytotoxicity with similar potency. The inconsistency of components **1**−**5** between their antibacterial activity and cytotoxicity suggests that there may be an antibacterial mechanism different from cytotoxicity.

The antibacterial activity of components **1** and **2** against other bacteria was evaluated together with standard antibiotics, such as vancomycin, oxacillin, and norfloxacin (**Table 2**). Compo-

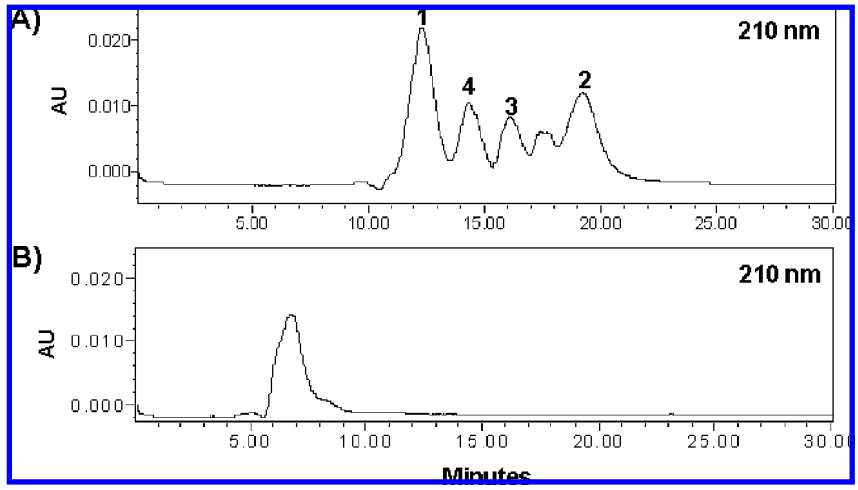

Figure 3. HPLC profile for ethyl acetate extracts of the (A) root portion and (B) aerial portion. The chromatograms were measured at 210 nm.

Table 2. Antibacterial Activity for **1** and **2** (MIC, μg/mL)

test organisms	extract[a]	1	2	vancomycin	oxacillin	norfloxacin
S. aureus 503	4	2	2	0.25	0.25	1
S. aureus KCTC 1916	8	4	2	0.25	0.25	0.25
S. aureus RN4220	8	4	4	1	0.25	1
MRSA CCARM 3167	8	4	4	1	500	8
MRSA CCARM 3506	8	4	8	0.25	500	1
QRSA CCARM 3505	16	4	8	1	0.5	250
QRSA CCARM 3519	16	4	8	0.5	0.5	125
B. subtilis KCTC 1021	8	1	2	0.125	0.25	0.25
A. calcoaceticus KCTC 2357	4	2	2	1	0.125	0.25
M. luteus KCTC 1056	8	2	2	0.06	8	2
E. coli KCTC 1358	>64	>64	>64	>64	>64	16
E. coli KCTC 1662	>64	>64	>64	>64	>64	>64
K. aerogenes KCTC 2618	>64	>64	>64	>64	>64	0.25
S. typhimurium KCTC 2421	>64	>64	>64	>64	>64	2
C. albicans KCTC 7535	>64	>64	>64	32	16	8

[a] The methanol extract of the root portion.

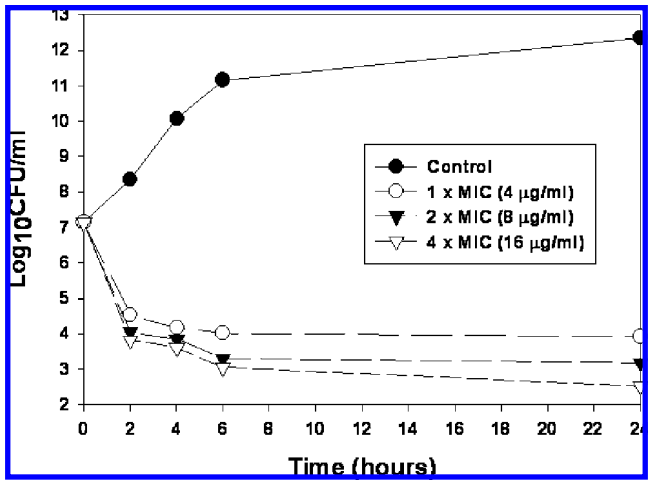

Figure 4. Time-kill experiment of aceriphyllic acid A against *S. aureus*.

nents **1** and **2** also exhibited a potent antibacterial activity against several Gram-(+) bacteria, including *B. subtilis*, *A. calcoaceticus*, and *M. luteus*, but did not show any antibacterial activity against Gram-(−) bacteria, including *E. coli*, *K. aerogenes*, and *S. typhimurium*, as well as against yeast, such as *C. albicans*, even at 128 μg/mL. Consistently with the antibacterial spectrum of components **1** and **2**, the methanol extract of the root portion inhibited the growth of the Gram-(+) bacteria, which did not affect the growth of Gram-(−) bacteria (**Table 2**).

Time-kill study was performed to see whether component **1** is bactericidal or bacteriostatic against *S. aureus* (**Figure 4**). Component **1** approached the bactericidal threshold at 2 h, as the maximal reduction was 2.61 $\log_{10}$ CFU/mL at the concentration of MIC. The killing activity was sustained until terminal end points at 24 h, with the maximal reductions from baseline of 3.22 $\log_{10}$ CFU/mL. This rapid bactericidal phenomenon was more clearly observed at 2 and 4 times the MIC, as the maximal reductions were 3.1 and 3.3 $\log_{10}$ CFU/mL, respectively, at 2 h. These data indicated that component **1** had the rapid bactericidal activity.

Previous studies of *A. rossii* have reported that the methanol extract of the whole plant shows ACAT-inhibitory, anticomplementary, and cytotoxic activity (*5, 6, 9*). Aceriphyllic acid A, 3α-hydroxyolean-12-en-27-oic acid, and 3β-hydroxyolean-12-en-27-oic acid have been known to be the active components for the inhibition of ACAT, an enzyme which catalyzes intracellular esterification of cholesterol (*5*). 3-Oxoolean-12-en-27-oic acid, 3α-hydroxyolean-12-en-27-oic acid, and 3α,23-diacetoxyolean-12-en-27-oic acid were reported to have anticomplementary activity against the classical pathway, while 3β-hydroxyolean-12-en-27-oic acid and aceriphyllic acid A were inactive. Both the ketone at C-3 and the methyl at C-23 in the oleanane triterpenoids with a carboxyl group at C-27 has been reported to be important for the anticomplement activity (*6*). Aceriphyllic acid A, 3-oxoolean-12-en-27-oic acid, 3α-hydroxyolean-12-en-27-oic acid, 3β-hydroxyolean-12-en-27-oic acid, 3α,23-isopropylidenedioxyolean-12-en-27-oic acid, and 23-hydroxy-3-oxoolean-12en-27-oic acid have been reported to exhibit cytotoxicity against the human leukemia K562 and HL-60 cell lines with similar potency (*9*). Antibacterial activity of *A. rossii* and its components, however, was reported for the first time in this study.

Besides *A. rossii*, olean-27-carboxylic acid-type triterpenes are rarely found in nature and have only been isolated from *Astilbe chinensis* (*9*), *Astilbe thunbergii* (*12*), *Peltoboykinia watanabei* (*13*), *Boykinia lycoctonifolia* (*13*), *Chrysosplenium grayanum* (*14*), *Cordia alliodora* (*8*), and *Cornulaca monacantha* (*15*), but their biological activities have not been extensively reported. 3α-Hydroxyolean-12-en-27-oic acid from *C. alliodora* has repellent activity against the leafcutter ant (*8*). 3β-Hydroxyolean-12-en-27-oic acid from *A. chinensis* and *C. grayanum* have apoptosis-inducing activity in human cancer cells (*16*) and antitumor activity *in vivo* (*14*). To our knowledge, antibacterial activity of olean-27-carboxylic acid-type triterpenes was reported for the first time in this study.

In conclusion, the methanol extract of *A. rossii* had potent antibacterial activity against Gram-(+) bacteria, including *S. aureus*, MRSA, QRSA, and *B. subtilis*. Aceriphyllic acid A and 3-oxoolean-12-en-27-oic acid are the most active principles, and both the carboxylic group at C-27 and the hydroxyl group at C-24 in aceriphyllic acid A are critical for the antibacterial activity. Because *A. rossii* has been used as a nutritious food in Korea, the extract of *A. rossii* as well as its active components could have great potential for the control of Gram-(+) pathogens, including *S. aureus*, MRSA, and QRSA.

LITERATURE CITED

(1) Lee, C. B. *Illustrated Flora of Korea*; Hyangmunsa: Seoul, Korea, 1989; p 409.

(2) Lee, W. C. *Coloured Standard Illustrations of Korean Plants*; Academybook: Seoul, Korea, 1996; p 152.

(3) Lee, Y. N. *Flora of Korea*; Kyohak Publishing Co., Ltd.: Seoul, Korea, 2002; p 280.

(4) Nakagiri, R.; Kamiya, T.; Suda, T.; Miki, I. Preventives or remedies for arthritis. US20040161478A1, 2004; p 2.

(5) Han, J.-T.; Kim, H.-Y.; Park, Y.-D.; Lee, Y.-H.; Lee, K.-R.; Kwon, B.-M.; Baek, N.-I. Aceriphyllic acid A, a new ACAT inhibitory triterpenoid, from *Aceriphyllum rossii*. *Planta Med.* **2002**, *68*, 558–561.

(6) Min, B.-S.; Lee, I. S.; Chang, M.-J.; Yoo, J. W.; Na, M. K.; Hung, T. M.; Thuong, P. T.; Lee, J. P.; Kim, J.-H.; Kim, J. C.; Woo, M. H.; Choi, J. S.; Lee, H.-K.; Bae, K.-H. Anticomplementary activity of triterpenoids from the whole plants of *Aceriphyllum rossii* against the classical pathway. *Planta Med.* **2008**, *74*, 726–729.

(7) Han, J. T.; Bang, M. H.; Chun, O. K.; Kim, D. O.; Lee, C. Y.; Baek, N. I. Flavonol glycosides from the aerial parts of *Aceriphyllum rossii* and their antioxidant activities. *Arch. Pharm. Res.* **2004**, *27*, 390–395.

(8) Chen, T. K.; Ales, D. C.; Baenziger, N. C.; Wiemer, D. F. Ant-repellent triterpenoids from *Cordia alliodora*. *J. Org. Chem.* **1983**, *48*, 3525–3531.

(9) Sun, H.; Zhang, J.; Ye, Y.; Pan, Y.; Shen, Y. Cytotoxic pentacyclic triterpenoids from the rhizome of *Astilbe chinensis*. *Helv. Chim. Acta* **2003**, *86*, 2414–2423.

(10) Mosmann, T. J. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63.

(11) Lee, I.; Yoo, J. K.; Na, M. K.; Min, B. S.; Lee, J. P.; Yun, B. S.; Jin, W. Y.; Kim, H. J.; Youn, U. J.; Chen, Q. C.; Song, K. S.; Seong, Y. H.; Bae, K. H. Cytotoxicity of triterpenes isolated from *Aceriphyllum rossii*. *Chem. Pharm. Bull.* **2007**, *55*, 1376–1378.

(12) Takahashi, K.; Kanayama, K.; Tanabe, Y.; Takani, M. Studies on constituents of medicinal plants. XI. Constituents of the roots of *Actilbe thunbergii* var. *congests* H. Boissieu. *Chem. Pharm. Bull.* **1972**, *20*, 2106−2111.

(13) Izawa, K.; Nagai, M.; Inoue, T. Triterpene acids and bergenin in *Peltoboykinia watanabei* and *Boykinia lycoctonifolia*. *Phytochemistry* **1973**, *12*, 1508.

(14) Arisawa, M.; Bai, H.; Shimizu, S.; Koshimura, S.; Tanaka, M.; Sasaki, T.; Morita, N. Isolation and identification of a cytotoxic principle from *Chrysosplenium grayanum* MAXIM. (Saxifragaceae) and its antitumor activities. *Chem. Pharm. Bull.* **1992**, *40*, 3274–3276.

(15) Dawidar, A.-A.; Reisch, J.; Amer, M. Structure of manevalic and azizic acids, two new triterpenes from *Cornulaca monacantha* DEL. *Chem. Pharm. Bull.* **1979**, *27*, 2938–2942.

(16) Tu, J.; Sun, H.-X.; Ye, Y.-P. 3β-Hydroxyolean-12-en-27-oic acid: A cytotoxic, apoptosis-inducing natural drug against COLO-205 cancer cells. *Chem. Biodiversity* **2006**, *3*, 69–78.

Received for review September 12, 2008. Revised manuscript received October 29, 2008. Accepted October 29, 2008. This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science and Technology (Grant MG08-0304-2-0), Republic of Korea, and a grant from KRIBB Research Initiative Program.

JF802832W