Three New Megastigmane Glucopyranosides from the *Cardamine komarovii*

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Three new megastigmane glucopyranosides, komaroveside A [(3*S*,4*R*,5*Z*,7*E*)-3,4-dihydroxy-5,7-megastigmadien-9-one-3-*O*-*β*-D-glucopyranoside] (1), komaroveside B [(3*S*,4*S*,5*S*,6*R*,7*E*)-5,6-epoxy-3,4-dihydroxy-7-megastigmen-9-one-3-*O*-*β*-D-glucopyranoside] (2) and komaroveside C [(3*S*,4*S*,5*S*,6*R*,7*E*,9*S*)-5,6-epoxy-3,4,9-trihydroxy-7-megastigmen-3-*O*-*β*-D-glucopyranoside] (3) were isolated, together with eight known compounds, from *Cardamine komarovii*. The identification of these compounds and the elucidation of their structures were based on 1D- and 2D-NMR spectral data analysis. The isolated compounds were tested for their cytotoxicity against four human tumor cell lines (A549, SK-OV-3, SK-MEL-2, HCT15) *in vitro* using the sulforhodamine B bioassay.

Key words Cruciferae; megastigmane; komaroveside; cytotoxicity; *Cardamine komarovii*

Cardamine komarovii (Nakai) is a plant that is widely distributed throughout Korea. In Korea, the aerial parts of *C. komarovii* are considered edible.[1)] This indigenous herb is used in Chinese medicine as a treatment of hemostasis, depressed blood pressure, and sedation.[2)] Phytochemical studies of this plant have not yet been reported in the literature. Thus, we investigated the aerial part of *C. komarovii* as a part of our continuous study of the family of Cruciferae. Column chromatography based purification of $CHCl_3$ and *n*-BuOH-soluble fractions of the MeOH extract of this plant led to the isolation of three megastigmane glucopyranosides (**1**—**3**), together with eight known megastigmane compounds (**4**—**11**). The structures of the known compounds were determined to be (6*R*,9*S*)-3-oxo-*α*-ionol-9-*O*-*β*-D-glucopyranoside (**4**),[3)] (6*S*,9*S*)-roseoside (**5**),[4)] sammangaoside A (**6**),[5)] corchoionoside A (**7**),[6)] icariside B_2 (**8**),[7)] (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetrol-3-*O*-*β*-D-glucopyranoside (**9**),[8)] staphylionoside E (**10**),[9)] and annuionene D (**11**)[10)] by comparing their spectroscopic data with data in the literature (Fig 1).

Results and Discussion

Komaroveside A (**1**), was obtained as a colorless gum. The molecular formula of **1** was deduced to be $C_{19}H_{30}O_8$ by positive mode high resolution electrospray ionization mass spectrometry (HR-ESI-MS) data at *m*/*z* 409.1831 $[M+Na]^+$ (Calcd for $C_{19}H_{30}O_8Na$: 409.1838). The IR spectrum of **1** showed absorption bands at 3382 and 1660 cm^{-1} ascribable to hydroxyl and ketone functions, respectively. The ^{1}H-NMR spectrum (Table 1) of **1** displayed signals for four methyl groups at δ_H 2.31, 1.88, 1.14 and 1.09, two oxymethine proton signals at δ_H 4.11 (d, J=3.5 Hz) and 4.01 (dt, J=12.3, 3.5 Hz) and one methylene proton signals at δ_H 1.92 (br t, J=12.3 Hz) and 1.64 (dd, J=12.3, 3.5 Hz), and a disubstituted *trans* double bond at δ_H 7.26 (d, J=16.4 Hz) and 6.14 (d, J=16.4 Hz). In the ^{13}C-NMR spectrum, 13 carbon signals appeared besides those of the sugar unit, which included one carbonyl carbon at δ_C 199.5, four methyl carbons at δ_C 28.8,

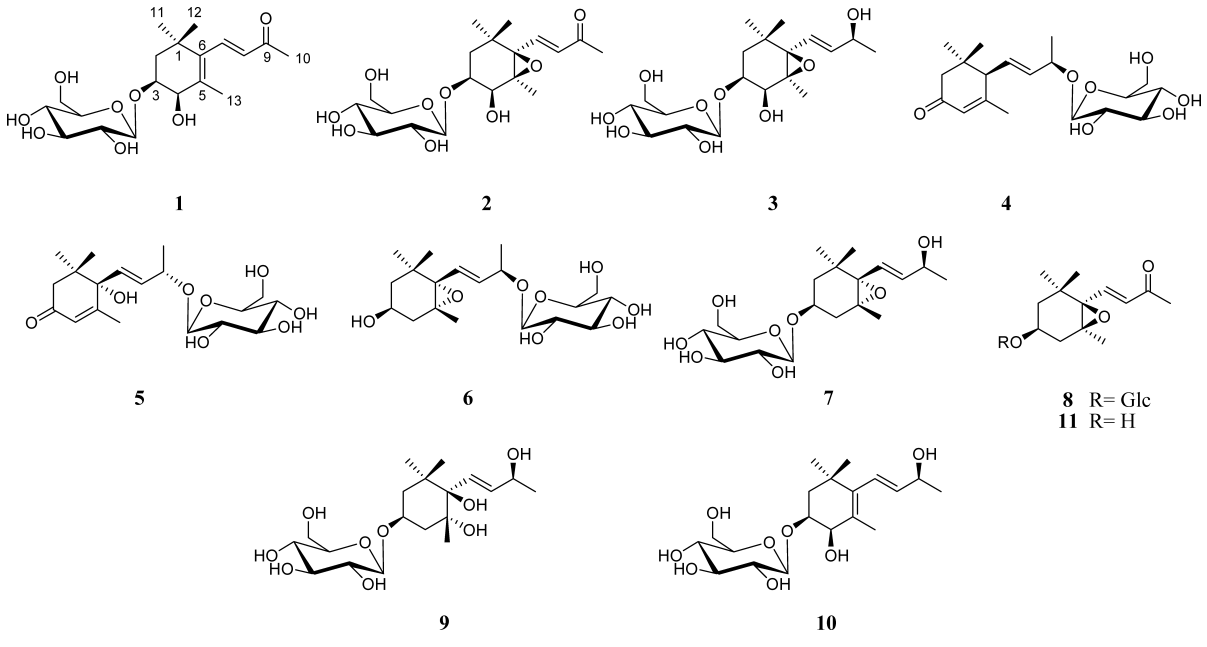

Fig. 1. The Structures of the Isolated Compounds **1**—**11** from *C. komarovii*

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Table 1. ^{1}H– ^{13}C-NMR Data of **1**, **2** and **3**

Position	**1**[a]		**2**[a]		**3**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		36.5		34.1		34.2
2α	1.64 (dd, 12.3, 3.5)	38.7	1.40 (dd, 12.5, 3.5)	37.2	1.35 (dd, 12.8, 3.5)	37.7
2β	1.92 (br t, 12.3)		1.84 (br t, 12.5)		1.76 (br t, 12.8)	
3	4.01 (dt, 12.3, 3.5)	74.3	3.97 (dt, 12.5, 3.5)	73.8	3.97 (dt, 12.8, 3.5)	74.2
4	4.11 (d, 3.5)	68.6	4.13 (d, 3.5)	69.2	4.12 (d, 3.5)	69.8
5		131.0		68.7		68.2
6		139.9		70.1		70.4
7	7.26 (d, 16.4)	142.8	7.11 (d, 15.8)	143.4	5.90 (d, 15.8)	124.8
8	6.14 (d, 16.4)	133.3	6.17 (d, 15.8)	132.6	5.66 (dd, 15.8, 6.1)	138.2
9		199.5		198.7	4.29 (dqd, 6.8, 6.1, 1.0)	67.4
10	2.31 (s)	26.4	2.29 (s)	26.0	1.22 (d, 6.7)	22.6
11	1.09 (s)	25.8	0.99 (s)	23.6	0.96 (s)	28.4
12	1.14 (s)	28.8	1.18 (s)	28.0	1.09 (s)	23.7
13	1.88 (s)	18.7	1.28 (s)	15.7	1.31 (s)	16.1
1′	4.50 (d, 7.6)	101.4	4.41 (d, 7.6)	101.5	4.40 (d, 7.5)	101.6
2′	3.22 (dd, 9.3, 7.6)	73.9	3.18 (dd, 9.3, 7.6)	73.8	3.17 (dd, 9.0, 7.5)	74.1
3′	3.38 (m)	76.6	3.35 (m)	76.5	3.36 (m)	76.8
4′	3.30 (m)	70.2	3.32 (m)	70.0	3.30 (m)	70.5
5′	3.26 (m)	76.7	3.28 (m)	76.6	3.30 (m)	76.8
6′	3.86 (dd, 12.6, 2.5)	61.3	3.87 (dd, 12.0, 2.5)	61.2	3.84 (dd, 12.2, 2.4)	61.5
	3.68 (dd, 12.6, 5.0)		3.66 (dd, 12.0, 5.0)		3.67 (dd, 12.2, 5.5)	

a) 500 MHz, CD_3OD; chemical shifts in ppm relative to TMS; coupling constants (*J*) in Hz.

26.4, 25.8 and 18.7, one methylene carbon at δ_C 38.7, two oxygenated methine carbons at δ_C 74.3 and 68.6, four olefinic carbons δ_C 142.8, 139.9, 133.3 and 131.0 and one quaternary carbon at δ_C 36.5. The NMR data were very similar to those of saussureoside B, which was isolated from *Saussurea medusa*.[11] The differences were the chemical shifts in ^{13}C-NMR data ($\delta_{C\text{-}1'}$ 101.4, $\delta_{C\text{-}2}$ 38.7, $\delta_{C\text{-}3}$ 74.3, $\delta_{C\text{-}4}$ 68.6 in **1**, $\delta_{C\text{-}1'}$ 106.9, $\delta_{C\text{-}2}$ 43.0, $\delta_{C\text{-}3}$ 66.1, $\delta_{C\text{-}4}$ 84.3 in saussureoside B). The downfield shift at C-3 implied that **1** was glycosylated at C-3.[11] The signals from the sugar unit appeared at δ_H 4.50 (d, J=7.6 Hz), 3.86 (dd, J=12.6, 2.5 Hz), 3.68 (dd, J=12.6, 5.0 Hz), 3.38, 3.30, 3.26, 3.22 (dd, J=9.3, 7.6 Hz) and δ_C 101.4, 76.7, 76.6, 73.9, 70.2, 61.3 suggested the presence of D-glucopyranose.[12] The coupling constant (J=7.6 Hz) of the anomeric proton of D-glucose indicated it to be the β-form.[12] The glycosidic site was established by an heteronuclear multiple bond correlation (HMBC) experiment, in which long-range correlation was observed between the H-3 (δ_H 4.01, dt, J=12.3, 3.5 Hz) and the C-3 (δ_C 74.3) (Fig. 2). The glycosylated proton of H-3 at δ_H 4.01 (dt, J=12.3, 3.5 Hz) showed couplings in the ^{1}H–^{1}H correlation spectroscopy experiment (COSY) spectrum with H-2α, H-2β and H-4. In the HMBC experiment, long-range correlations were observed between the following protons and carbons (H-2, H-11, H-12 and C-1; H-2, H-4, H-1′ and C-3; H-4, H-13 and C-5; H-4, H-7, H-11, H-12 and C-6; H-7, H-8, H-10 and C-9). Thus, the structure of **1** was supposed to be 3,4-dihydroxy-5,7-megastigmadien-9-one-3-*O*-β-D-glucoside. The relative stereochemistry of the aglycone moiety in **1** was characterized by a nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs (H-2α and H-3; H-2β and H-12; H-3 and H-4; H-7 and H-12) as shown in Fig. 2. From the coupling constants at δ_H 4.11 (H-4, d, J=3.5 Hz), 4.01 (H-3, dt, J=12.3, 3.5 Hz), 1.92 (H-2β, t, J=12.3 Hz), 1.64 (H-2α, dd, J=12.3, 3.5 Hz), it is suggested

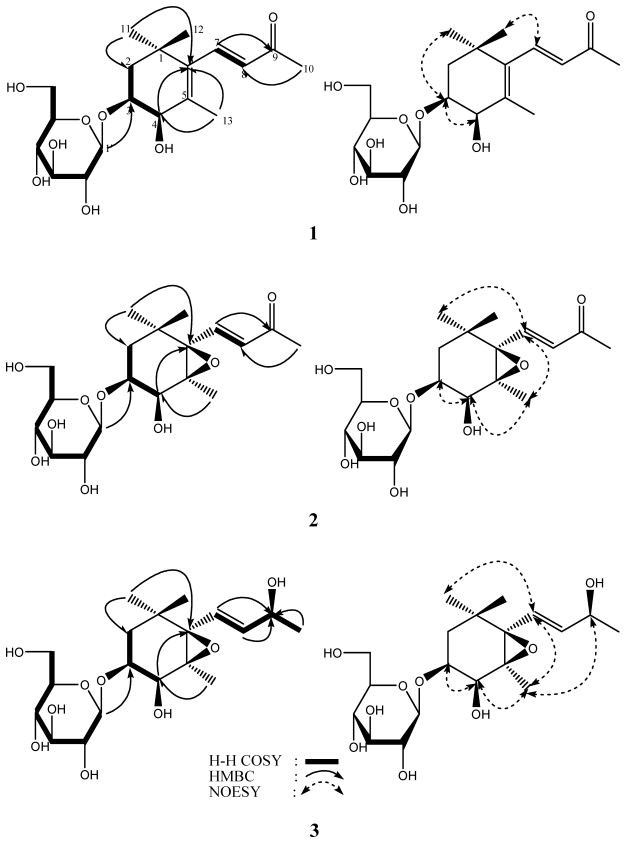

Fig. 2. ^{1}H–^{1}H COSY, HMBC, and NOESY Correlations of **1**, **2** and **3**

that two hydroxyl groups at C-3 and C-4 are in the *cis* configuration.[13,14] Enzymatic hydrolysis of **1** with β-glucosidase (emulsin) yielded 3,4-dihydroxy-5,7-megastigmadien-9-one (**1a**, $C_{13}H_{20}O_3$, $[\alpha]_D^{25}$ −68°), whose ^{1}H-NMR and MS spectra were in good agreement with values reported previously,[11,15] and D-glucose {$[\alpha]_D^{25}$ +50° (c=0.05, H_2O)}. The absolute

configuration at C-3 in **1a** was determined by a modified version of Mosher's method.[16] Based on further comparison of the NMR data with those in the literature, the absolute configuration at C-3 in **1** was determined to be the *S*-form.[11,15] Thus, the structure of **1** was determined to be (3*S*,4*R*,5*Z*,7*E*)-3,4-dihydroxy-5,7-megastigmadien-9-one-3-*O*-*β*-D-glucopyranoside, and was named komaroveside A.

Komaroveside B (**2**), was obtained as a colorless gum. The molecular formula of **2** was deduced to be $C_{19}H_{30}O_9$ by positive mode high resolution electrospray ionization mass spectrometry (HR-ESI-MS) data at *m*/*z* 425.1781 $[M+Na]^+$ (Calcd for $C_{19}H_{30}O_9Na$: 425.1788). The IR spectrum of **2** showed absorption bands at 3383 and 1658 cm^{-1} ascribable to a hydroxyl and an unsaturated carbon, respectively. The NMR spectra of **2** were very similar to those of compound **1**. In the ^{13}C-NMR spectrum of **2**, two olefinic carbon signals observed at δ_C 139.9 and 131.0 in **1** were shifted upfield to δ_C 70.1 and 68.7 in **2**, which implied that **2** has an epoxide ring instead of the double bond in **1**. These observations suggested the structure of **2** to be 5,6-epoxy-3,4-dihydroxy-7-megastigmen-9-one-3-*O*-*β*-D-glucoside. The relative stereochemistry of the aglycone in **2** was characterized by a NOESY experiment, which showed NOE correlations between the following proton pairs (H-3 and H-4, 11; H-4 and H-13; H-7 and H-11, 13) as shown in Fig. 2. From the coupling constants at δ_H 4.13 (H-4, d, J=3.5 Hz), 3.97 (H-3, dt, J=12.5, 3.5 Hz), 1.84 (H-2β, t, J=12.5 Hz), 1.40 (H-2α, dd, J=12.5, 3.5 Hz), it is suggested that two hydroxyl groups at C-3 and C-4 are in the *cis* configuration.[13,14] Enzymatic hydrolysis of **2** with *β*-glucosidase (emulsin) yielded 5,6-epoxy-3,4-dihydroxy-7-megastigmen-9-one (**2a**, $C_{13}H_{20}O_3$, $[\alpha]_D^{25}$ −60.0°), whose ^1H-NMR and MS spectra were in good agreement with values reported previously,[11,17] and D-glucose {$[\alpha]_D^{25}$ +52° (c=0.05, H_2O)}. The absolute configuration at C-3 of **2a** was determined to be the *S*-form.[11,15] by a modified version of Mosher's method.[16] Thus, the structure of **2** was determined to be (3*S*,4*S*,5*S*,6*R*,7*E*)-5,6-epoxy-3,4-dihydroxy-7-megastigmen-9-one-3-*O*-*β*-D-glucopyranoside, and was named komaroveside B.

Komaroveside C (**3**), was obtained as a colorless gum. molecular formula of **2** was deduced to be $C_{19}H_{34}O_{10}$ by positive mode high resolution electrospray ionization mass spectrometry (HR-ESI-MS) data at *m*/*z* 427.1936 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_9Na$: 427.1944. The IR spectrum of **3** showed an absorption band at 3382 cm^{-1} ascribable to a hydroxyl function. The NMR spectra of **3** were very similar to those of compound **2**. In the NMR spectra of **3**, the signals at δ_H 4.29 (dqd, J=6.8, 6.1, 1.0 Hz) and δ_C 67.4 implied that **3** has a hydroxylated function at C-9 instead of the ketone group in **2**. The proton signal at δ_H 4.29 (H-9, dqd, J=6.8, 6.1, 1.0 Hz) showed coupling in the ^1H–^1H COSY spectrum with H-8 and H-10. Thus, the structure of **3** was supposed to be 5,6-epoxy-3,4,9-trihydroxy-7-megastigmen-3-O-*β*-D-glucoside. The relative stereochemistry of the aglycone in **3** was characterized by a NOESY experiment, which showed NOE correlations between the following proton pairs (H-3 and H-4, 11; H-4 and H-13; H-7 and H-11, 13; H-9 and H-13) as shown in Fig. 2. From the coupling constants at δ_H 4.12 (H-4, d, J=3.5 Hz), 3.97 (H-3, dt, J=12.8, 3.5 Hz), 1.76 (H-2β, t, J=12.8 Hz), and 1.35 (H-2α, dd, J=12.8, 3.5 Hz), it is suggested that two hydroxyl groups at C-3 and C-4 are in the *cis* configuration.[13,14] Enzymatic hydrolysis of **3** with *β*-glucosidase (emulsin) yielded 5,6-epoxy-3,4,9-trihydroxy-7-megastigmen (**3a**, $C_{13}H_{20}O_3$, $[\alpha]_D^{25}$ −77.0°), whose ^1H-NMR, ^{13}C-NMR and MS spectra were very similar to those of 5,6-epoxy-3,4,9-trihydroxy-7-megastigmen, which was isolated from *Staphylea bumalda*,[9] and D-glucose {$[\alpha]_D^{25}$ +51° (c=0.05, H_2O)}. Determination of the absolute configuration at C-3 and C-9 of **3** was done using Mosher's method.[16] Compound **3a**, which was obtained by enzymatic hydrolysis of **3**, was treated with (*S*)-(+), and (*R*)-(−)-*α*-methoxy-*α*-trifluoromethylphenyl acetic acid chloride (MTPA-Cl) to give (*R*)- and (*S*)-MTPA esters (**3b**, **c**, respectively). Consequently, the absolute configurations at C-3 and C-9 in **3** were the *S*-form. Thus, the structure of **3** was determined to be (3*S*,4*S*,5*S*,6*R*,7*E*,9*S*)-5,6-epoxy-3,4,9-trihydroxy-7-megastigmen-3-*O*-*β*-D-glucopyranoside, and was named komaroveside C.

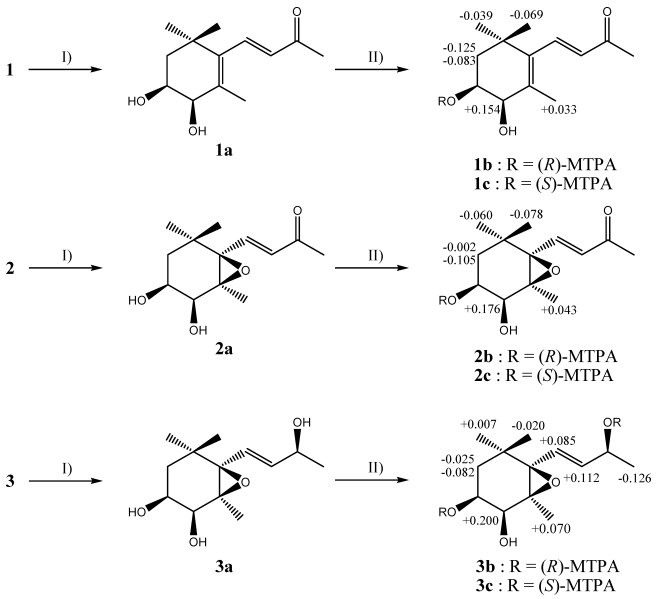

Fig. 3. Results of Hydrolysis and Mosher's Method of **1a**, **2a**, and **3a**
Reagents and Conditions
I) *β*-glucosidase/H_2O, 37°C. II) (*R*)-MTPA and (*S*)-MTPA, EDC·HCl, 4-DMAP/CH_2Cl_2, room temp.

The cytotoxic activities of the isolated compounds (**1**—**11**) were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the sulforhodamine B (SRB) assay.[17] All the compounds showed little cytotoxicity against any tested cell line (IC_{50}>100 μM).

Experimental

General Experimental Procedures Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Shimadzu UV-1601 UV–Visible spectrophotometer. ESI and HR-ESI mass spectra were obtained on a IT-TOF (Shimadzu, Japan) mass spectrometer. NMR spectra, including NOESY, COSY, heteronuclear multiple quantum coherence (HMQC) and HMBC experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ). Preparative high-performance liquid chromatography (HPLC) used a Gilson 306 pump with a Shodex refractive index detector. Silica gel 60 (Merck, 70—230 mesh and 230—400 mesh) and RP-C_{18} silica gel (Merck, 230—400 mesh) were used for column chromatography. Merck pre-coated Silica gel F_{254} plates and RP-18 F_{254s} plates were used for TLC. Spots were detected on TLC under UV

light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (v/v). The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co. U.S.A.). Low pressure liquid chromatography was carried out over a Merck Lichroprep Lobar®-A Si 60 (240×10 mm) or a Lichroprep Lobar®-A RP-18 (240×10 mm) column with a FMI QSY-0 pump (ISCO).

Plant Materials *Cardamine komarovii* (NAKAI) plants (1.7 kg) were collected at Goseong-gun in Gangwon-Do province, Korea in August 2008. A voucher specimen of the plant (SKK-08-011) was deposited at the School of Pharmacy in Sungkyunkwan University.

Extraction and Isolation The half dried aerial parts of *C. komarovii* (NAKAI) (1.7 kg) were extracted with 80% MeOH three times at room temperature. The resultant MeOH extracts (120 g) were suspended in distilled water (800 ml×3) and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH, yielding residues weighing 12 g, 13 g, 3 g and 26 g, respectively. The $CHCl_3$ soluble fraction (13 g) was chromatographed on a silica gel column, eluting with a gradient solvent system of $CHCl_3$/MeOH (40 : 1—1 : 1) as the eluant to yield seven fraction (fr. PC1—PC7). Fraction PC3 (1.0 g) was isolated by sephadex LH-20 chromatography (80% MeOH) and was purified with a silica gel preparatory HPLC with a solvent system of $CHCl_3$: MeOH (25 : 1) to yield **11** (5 mg). The *n*-BuOH soluble fraction (26 g) was chromatographed on a diaion HP-20 column, eluting with a gradient solvent system consisting of 100% water and 100% MeOH. This yielded two subfractions. Fraction B (48 g) was isolated using a silica gel column, eluting with a solvent system of $CHCl_3$/MeOH/water (10 : 4 : 0.5). According to TLC analysis, fourteen crude fractions (fr. PB1—PB14) were collected. Fr. B3 (0.2 g) purified using a Lobar®-A RP-18 (240×10 mm) column (40% MeOH), and purified further by preparative reverse-phase HPLC, using a solvent system of 45% MeOH and 25% MeCN, yielded **4** (28 mg) and **8** (14 mg). Fr. B4 (0.5 g) purified by a Lobar®-B RP-18 (310×25 mm) column (30% MeOH), and purified further by a preparative reverse-phase HPLC, using a solvent system of 30% MeOH and 20% MeCN, yielded **1** (13 mg), **2** (7 mg) and **5** (19 mg). Fr. PB5 (0.3 g) purified by a Lobar®-A RP-18 (240×10 mm) column (35% MeOH), and purified further by preparative reverse-phase HPLC, using a solvent system of 15% MeCN, yielded **6** (5 mg), **7** (10 mg) and **9** (15 mg). Fr. B7 (0.3 g), which was purified by a Lobar®-B RP-18 (310×25 mm) column (30% MeOH), and purified further by preparative normal-phase HPLC, using a solvent system of $CHCl_3$/MeOH (7 : 1, 3 : 1) yielded **3** (12 mg) and **10** (9 mg).

Komaroveside A (**1**): Colorless gum. $[\alpha]_D^{25}$ −36.8° (c=0.25, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.0), 279 (3.9) nm; IR (KBr) ν_{max} cm^{-1}: 3382, 2924, 1660, 1608, 1425, 1365, 1076; ^{1}H-NMR (CD_3OD, 500 MHz): see Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1; HR-ESI-MS (positive mode) *m/z*: 409.1831 $[M+Na]^+$ (Calcd for $C_{19}H_{30}O_8Na$: 409.1838).

Komaroveside B (**2**): Colorless gum. $[\alpha]_D^{25}$ −65.4° (c=0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.1) nm; IR (KBr) ν_{max} cm^{-1}: 3383, 2948, 1658, 1450, 1370; ^{1}H-NMR (CD_3OD, 500 MHz): see Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1; HR-ESI-MS (positive mode) *m/z*: 425.1781 $[M+Na]^+$ (Calcd for $C_{19}H_{30}O_9Na$: 425.1788).

Komaroveside C (**3**): Colorless gum. $[\alpha]_D^{25}$ −61.4° (c=0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3382, 2966, 1739, 1627, 1450, 137; positive; ^{1}H-NMR (CD_3OD, 500 MHz): see Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1; HR-ESI-MS (positive mode) *m/z*: 427.1936 $[M+Na]^+$ (Calcd for $C_{19}H_{32}O_9Na$:427.1944)

Enzymatic Hydrolysis of 1, 2 and 3 Compound **1** (3.0 mg) with 1 ml of H_2O and 3 mg of β-glucosidase (Emulsin) was shaken for 7 d at 37 °C. The H_2O solution was then extracted with $CHCl_3$ three times, and the $CHCl_3$ extract was evaporated *in vacuo*. The $CHCl_3$ extract (2.0 mg) was purified using reverse-phase HPLC (50% MeOH) to yield the aglycone **1a** as a colorless gum $[\alpha]_D^{25}$ −60.5° (c=0.1, $CHCl_3$), ^{1}H-NMR (pyridine-d_5, 500 MHz). Compound **2** (3.0 mg) was treated by the same method. The $CHCl_3$ extract (2.0 mg) was purified using reverse-phase HPLC (40% MeOH) to yield the aglycone **2a** as a colorless gum $[\alpha]_D^{25}$ −60.2° (c=0.1, $CHCl_3$), ^{1}H-NMR (pyridine-d_5, 500 MHz). Compound **3** (3.0 mg) was treated by the same method. The $CHCl_3$ extract (1.0 mg) was purified using reverse-phase HPLC (40% MeOH) to yield the aglycone **3a** as a colorless gum $[\alpha]_D^{25}$ −77.0° (c=0.1, $CHCl_3$), ^{1}H-NMR (pyridine-d_5, 500 MHz). The sugar in the distilled water layer was identified as D-glucose by co-TLC (EtOAc : MeOH : H_2O= 9 : 3 : 1, *Rf* value: 0.2, **1a**: 1.5 mg, **2a**: 1.5 mg, **3a**: 2.0 mg) with a D-glucose standard (Aldrich Co., U.S.A.).

1a: $[\alpha]_D^{25}$ −60.5° (c=0.1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 7.31 (1H, d, J=16.4 Hz, H-7), 6.25 (1H, d, J=16.4, H-8), 4.23 (1H, br s, H-4), 4.10 (1H, dt, J=12.3, 3.0 Hz, H-3), 2.25 (3H, s, H-10), 2.19 (1H, br t, J= 12.3 Hz, H-2β), 1.99 (3H, s, H-13), 1.71 (1H, dd, J=12.3, 2.3 Hz, H-2α), 1.06 (3H, s, H-12), 0.98 (3H, s, H-11); HR-ESI-MS (positive mode) *m/z*: 247.1307 $[M+Na]^+$ (Calcd for $C_{13}H_{20}O_3Na$: 247.1310).

2a: $[\alpha]_D^{25}$ −60.2° (c=0.1, $CHCl_3$); ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 7.30 (1H, d, J=16.0 Hz, H-7), 6.54 (1H, d, J=16.0 Hz, H-8), 4.31 (1H, dt, J=12.3, 3.5 Hz, H-3), 2.28 (3H, s, H-10), 2.18 (1H, br t, J=12.3 Hz, H-2β), 1.54 (1H, dd, J=12.3, 3.0 Hz, H-2α), 1.51 (3H, s, H-13), 1.15 (3H, s, H-12), 1.06 (3H, s, H-11); HR-ESI-MS (positive mode) *m/z*: 263.1254 $[M+Na]^+$ (Calcd for $C_{13}H_{20}O_4Na$: 263.1259).

3a: $[\alpha]_D^{25}$ −77.0° (c=0.1, $CHCl_3$); positive-ion EI-MS *m/z*: 265.15 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.93 (1H, dd, J=15.5, 1.0 Hz, H-7), 5.77 (1H, dd, J=15.5, 5.5 Hz, H-8), 4.42 (1H, dqd, J=6.5, 5.5, 1.0 Hz, H-9), 4.03 (1H, d, J=3.0 Hz, H-4), 3.97 (1H, dt, J=12.2, 3.3 Hz, H-3), 1.65 (1H, br t, J=12.2 Hz, H-2β), 1.36 (3H, s, H-13), 1.34 (1H, m, H-2α), 1.30 (3H, d, J=6.5 Hz, H-10), 1.12 (3H, s, H-12), 0.99 (3H, s, H-11); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 138.1 (C-8), 124.7 (C-7), 72.1 (C-3), 70.1 (C-6), 68.2 (C-4), 68.0 (C-9), 65.9 (C-5), 39.6 (C-2), 34.2 (C-1), 29.3 (C-11), 24.4 (C-12), 23.7 (C-10), 16.8 (C-13); HR-ESI-MS (positive mode) *m/z*: 265.1412 $[M+Na]^+$ (Calcd for $C_{13}H_{22}O_4Na$: 265.1416).

Preparation of the (*R*)- and (*S*)-MTPA-Cl Ester Derivatives of 1a, 2a and 3a by a Convenient Mosher Ester A solution of **1a** (0.7 mg), **2a** (0.7 mg) and **3a** (1.0 mg) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-MTPA (5 μl) in the presence of 1-ethyl-3-(3-dimethoxylaminopropyl)-carbodiimide hydrochloride (EDC·HCl, 1.5 mg) and 4-methylaminopyridine (4-DMAP, 0.7 mg), and the mixture was stirred at room temperature for 7 h. After cooling, the reaction mixture was poured into water and the whole reaction mixture was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was separated by HPLC [MeOH : H_2O=45 : 55] to give **1b** (0.4 mg), **2b** (0.4 mg) and **3b** (0.5 mg), respectively. Using a similar procedure, (*S*)-MTPA esters **1c** (0.4 mg), **2c** (0.4 mg) and **3c** (0.6 mg) obtained from **1a**, **2a**, and **3a**.

1b: (500 MHz, pyridine-d_5) δ: 7.457—7.334 (1H, m, H-7), 6.258 (1H, d, J=16.5 Hz, H-8), 5.449 (dt, J=12.3, 3.5 Hz, H-3), 4.458 (1H, d, J=3.5 Hz, H-4), 2.410 (1H, d, J=12.3 Hz, H-2β), 2.293 (3H, s, H-10), 1.960 (3H, s, H-13), 1.842 (1H, dd, J=12.3, 3.5 Hz, H-2α), 1.139 (3H, s, H-12), 1.023 (3H, s, H-11); HR-ESI-MS (positive mode) *m/z*: 463.1702 $[M+Na]^+$ (Calcd for $C_{23}H_{27}O_5F_3Na$: 463.1708).

1c: (500 MHz, pyridine-d_5) δ: 7.420—7.334 (1H, m, H-7), 6.261 (1H, d, J=16.5 Hz, H-8), 5.470 (dt, J=12.3, 3.5 Hz, H-3), 4.612 (1H, d, J=3.5 Hz, H-4), 2.327 (1H, d, J=12.3 Hz, H-2β), 2.303 (3H, s, H-10), 1.993 (3H, s, H-13), 1.717 (1H, dd, J=12.3, 3.5 Hz, H-2α), 1.100 (3H, s, H-12), 0.954 (3H, s, H-11); HR-ESI-MS (positive mode) *m/z*: 463.1689 $[M+Na]^+$ (Calcd for $C_{23}H_{27}O_5F_3Na$: 463.1708).

2b: (500 MHz, pyridine-d_5) δ: 7.304 (1H, J=16.0 Hz, H-7), 6.556 (1H, d, J=16.0 Hz, H-8), 5.705 (dt, J=12.3, 3.5 Hz, H-3), 4.547 (1H, d, J=3.5 Hz, H-4), 2.439 (1H, d, J=12.3 Hz, H-2β), 2.329 (3H, s, H-10), 1.567 (1H, dd, J=12.3, 3.0 Hz, H-2α), 1.507 (3H, s, H-13), 1.233 (3H, s, H-12), 1.167 (3H, s, H-11) HR-ESI-MS (positive mode) *m/z*: 479.4398 $[M+Na]^+$ (Calcd for $C_{23}H_{27}O_6F_3Na$: 479.4410).

2c: (500 MHz, pyridine-d_5) δ: 7.291 (1H, d, J=16.0 Hz, H-7), 6.535 (1H, d, J=16.0 Hz, H-8), 5.730 (dt, J=12.3, 3.5 Hz, H-3), 4.723 (1H, d, J=3.5 Hz, H-4), 2.334 (1H, d, J=12.3 Hz, H-2β), 2.311 (3H, s, H-10), 1.565 (1H, dd, J=12.3, 3.0 Hz, H-2α), 1.550 (3H, s, H-13), 1.155 (3H, s, H-12), 1.107 (3H, s, H-11); HR-ESI-MS (positive mode) *m/z*: 479.4401 $[M+Na]^+$ (Calcd for $C_{23}H_{27}O_6F_3Na$: 479.4410).

3b: (500 MHz, pyridine-d_5) δ: 6.202 (1H, d, J=15.8, H-7), 6.083 (1H, dd, J=15.8, 5.5 Hz, H-8), 5.851 (1H, m, H-9), 5.675 (1H, m, H-3), 4.486 (1H, d, J=3.5 Hz, H-4), 2.355 (1H, br t, J=12.8 Hz, H-2β), 1.484 (3H, s, H-13), 1.499 (1H, dd, J=12.8, 3.5 Hz, H-2α), 1.391 (3H, d, J=6.5 Hz, H-10), 1.118 (3H, s, H-12), 1.089 (3H, s, H-11); HR-ESI-MS (positive mode) *m/z*: 697.2198 $[M+Na]^+$ (Calcd for $C_{33}H_{36}O_8F_6Na$: 697.2212).

3c: (500 MHz, pyridine-d_5) δ: 6.287 (1H, d, J=15.8, H-7), 6.195 (1H, dd, J=15.8, 5.5 Hz, H-8), 5.879 (1H, m, H-9), 5.791 (1H, m, H-3), 4.689 (1H, d, J=3.5, H-4), 2.273 (1H, br t, J=12.8 Hz, H-2β), 1.554 (3H, s, H-13), 1.474 (1H, dd, J=12.8, 3.5 Hz, H-2α), 1.265 (3H, d, J=6.5 Hz, H-10), 1.125 (3H, s, H-12), 1.069 (3H, s, H-11); HR-ESI-MS (positive mode) *m/z*: 697.2215 $[M+Na]^+$ (Calcd for $C_{33}H_{36}O_8F_6Na$: 697.2212).

Cytotoxicity Assay A sulforhodamin B bioassay (SRB) was used to determine the cytotoxicity of the compounds. The cytotoxic activity of each compound against four cultured human tumor cells was examined *in vitro* at the Korea Research Institute of Chemical Technology. The tumor cell lines were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma) and HCT15 (colon cancer cells).[17]

Doxorubicin was used as the positive control. Regarding the cytotoxicity of doxorubicin, its IC_{50} values for toxicity to A549, SK-OV-3, SK-MEL-2, and HCT15 cells, respectively, were 0.0012, 0.0214, 0.0110 and 0.1322 μM.

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