http://dx.doi.org/10.5012/bkcs.2011.32.10.3813

Three New Megastigmane Glycosides from *Hylomecon vernalis*

Seung Young Lee, Sang Un Choi,† and Kang Ro Lee*

Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
**E-mail: krlee@skku.ac.kr*
†Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea
Received July 25, 2011, Accepted August 30, 2011

Key Words : *Hylomecon vernalis*, Papaveraceae, Megastigmane glycoside, Cytotoxicity

Only two *Hylomecon* species, *H. hylomeconoides* and *H. vernalis*, grow in Korea. *H. vernalis* is widely distributed in mountainous regions of Korea, and China.[1,2] *H. vernalis* has been used as Chinese folk medicine for the treatment of arthritis, neuralgia, and eczema.[3] Previous phytochemical and pharmacological studies on this plant reported the isolation of several alkaloids and reported them to have *anti*-inflammatory, antispasmodic, antimicrobial, and *anti*-tumoral activities.[3,4] Column chromatographic purification of the BuOH-soluble fraction of the MeOH extract of the aerial parts of *H. vernalis* led to the isolation of three new megastigmane glycosides (**1-3**), together with four known compounds (**4-7**). The structures of these new compounds were elucidated on the basis of 1D and 2D NMR spectral analyses. The structures of the known compounds were identified to be (6*R*, 9*R*)-3-oxo-*α*-ionyl-9-*O*-*α*-L-rhamnopyranosyl-(1''→2')-*β*-D-glucopyranoside (**4**),[5] (6*R*, 9*R*)-9-hydroxy-megastigman-4-en-3-one 9-*O*-*α*-L-rhamnopyranosyl-(1''→2')-*β*-D-glucopyranoside (**5**),[5] 3-hydroxy-5,6-epoxy-*β*-ionol-9-*O*-*β*-D-glucopyranoside (**6**),[6] and megastigmane-7-ene-3,5,6,9-tetraol-9-*O*-*β*-D-glucopyranoside (**7**)[7] by comparing their spectroscopic data with data in the literature. The isolated compounds (**1-7**) were tested for cytotoxicity against four human tumor cells *in vitro* using a sulforhodamin B bioassay.

Compound **1** was obtained as a colorless gum. The molecular formula was determined to be $C_{19}H_{32}O_8$ from the $[M + Na]^+$ peak at *m/z* 411.1985 (calcd. for $C_{19}H_{32}O_8Na$: 411.1989) on HR-ESI-MS spectrum. The ^{1}H-NMR spectrum (Table 1) of **1** displayed signals for four methyl groups at δ_H = 1.40 (3H, s), 1.30 (3H, d, *J* = 6.4 Hz), 1.18 (3H, s), and 0.85 (3H, s), two oxymethine proton signals at δ_H = 4.41 (1H, m), and 4.33 (1H, m), and two olefinic proton signals at δ_H = 5.79 (2H, m). In the ^{13}C-NMR spectrum, 13 carbon signals appeared, including four methyl carbons at δ_C = 31.6, 30.3, 24.7, and 20.2, two methylene carbons at δ_C = 48.5, and 47.8, two oxygenated methine carbons at δ_C = 76.9, and 75.6, two olefinic carbons δ_C = 133.1, and 125.4, two oxygenated quaternary carbons at δ_C = 91.5, and 81.0, and one quaternary carbon at δ_C = 43.3. The ^{1}H-^{1}H COSY spectrum of **1** showed correlations at δ_H = 4.33 (H-3)/1.95 (H-2) and 1.75 (H-4), 5.75 (H-8)/5.75 (H-7) and 4.41 (H-9), 4.41 (H-9)/5.75 (H-8) and 1.30 (H-10), indicating the presence of partial segments (see bold lines in Figure 2). In the HMBC spectrum, long-range correlations were observed between the following protons and carbons: H-2 and C-1, C-3; H-3 and C-1, C-5, C-6; H-4 and C-2, C-6; H-7 and C-1, C-5, C-9; H-8 and C-6, C-10; H-9 and C-7, C-1'; H-10 and C-8, C-9; H-11, H-12 and C-1, C-2, C-6; H-13 and C-4, C-5, C-6 (Figure 2). These spectral data led us to conclude that the aglycone structure of **1** is 3,6-epoxy-7-megastigmene-5,9-diol, which was isolated from tobacco.[8,9] Also, the sugar moiety appeared at δ_H = 4.38 (1H, d, *J* = 7.5 Hz), 3.82 (1H,

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		43.3		43.3		43.4
2	1.58, d (11.7) 1.75, m	48.5	1.58 , d (11.7) 1.75, m	48.5	1.58, d (11.7) 1.75, m	48.5
3	4.33 m	75.6	4.38, m	75.7	4.38, m	75.6
4	1.65, d (11.7) 1.95, m	47.8	1.65, d (11.7) 1.95, m	47.8	1.65, d (11.7) 1.95, m	47.8
5		81.0		81.0		81.0
6		91.5		91.7		91.7
7	5.79, m	125.4	5.76, m	125.7	5.76, m	125.7
8		133.1		132.9		132.9
9	4.41, m	76.9	4.38, m	77.5	4.38, m	77.5
10	1.30, d (6.4)	20.2	1.30, d (6.4)	20.4	1.29, d (6.4)	20.5
11	1.40, s	24.7	1.40, s	24.7	1.40, s	24.7
12	0.85, s	30.3	0.85, s	30.5	0.85, s	30.5
13	1.18, s	31.6	1.18, s	31.6	1.19, s	31.6
1'	4.38, d (7.5)	101.6	4.32, d (7.5)	101.3	4.32, d (8.0)	101.3
2'	3.18, m	74.2	3.18, m	74.1	3.19, m	74.1
3'	3.21, m	76.9	3.21, m	76.8	3.21, m	76.8
4'	3.28, m	70.3	3.30, m	70.4	3.35, m	70.4
5'	3.35, m	76.7	3.40, m	75.5	3.42, m	75.5
6'	3.66, dd (12.0, 5.0) 3.82, dd (12.0, 3.0)	61.5	3.62, m 4.10, dd (12.0, 3.0)	68.8	3.61, m 4.12, dd (12.0, 3.0)	68.5
1''			4.25, d (7.5)	104.3	4.26, d (6.5)	104.4
2''			3.28, m	73.7	3.58, m	72.8
3''			3.34, m	76.4	3.54, m	71.2
4''			3.48, m	70.0	3.80, m	68.3
5''			3.19, m 3.85, m	65.7	3.54, m 3.85, m	65.6

[a]Chemical shifts in ppm relative to TMS; coupling constants (*J*) in Hz.

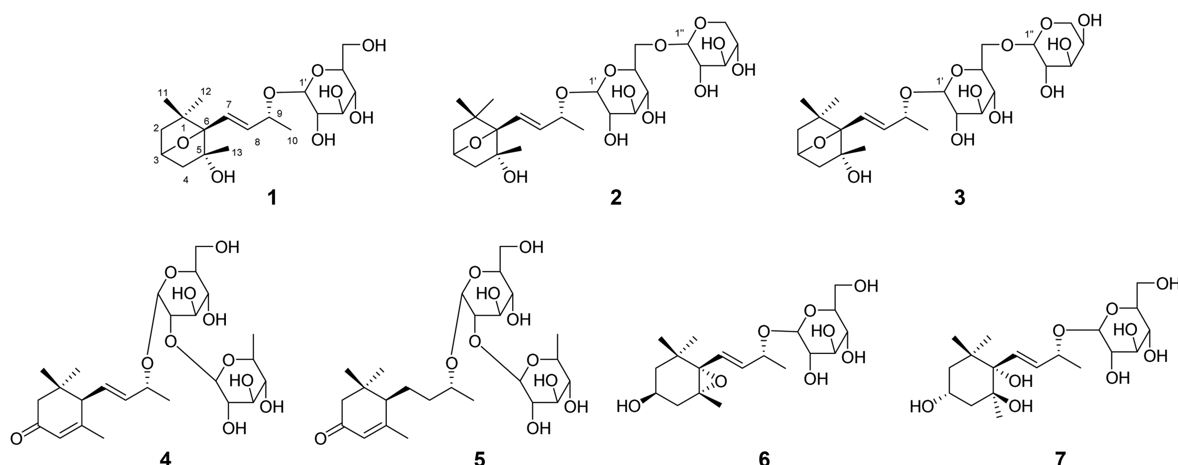

Figure 1. The structures of the isolated compounds **1**-**7** from *H. vernalis.*

dd, J = 12.0, 3.0 Hz), 3.66 (1H, dd, J = 12.0, 5.0 Hz), 3.35 (1H, m), 3.28 (1H, m), 3.21 (1H, m), 3.18 (1H, m) in the ^{1}H-NMR spectrum and δ_C = 101.6, 76.9, 76.7, 74.2, 70.3, and 61.5 in the ^{13}C-NMR spectrum, which suggested the presence of D-glucopyranose moiety.[10] The coupling constant (J = 7.5 Hz) of the anomeric proton of D-glucose indicated to be the β-form.[10] The glycosidic position was established by an HMBC experiment, in which a long-range correlation was observed between the H-1' (δ_H = 4.38) of D-glucose and the C-9 (δ_C = 76.9) of the aglycone (Figure 2). The ^{1}H- and ^{13}C-NMR, HMQC, ^{1}H-^{1}H COSY, and HMBC spectra revealed that **1** had the same planar structure as crotalionoside C isolated from *Crotalaria zanzibarica*,[11] except for the optical rotation value. The optical rotation of **1** ($[\alpha]_D^{25}$: −13.0°) was almost of the same value but of opposite sign to that of crotalionoside C ($[\alpha]_D^{25}$: +15.8°), which suggested that compound **1** could be a stereoisomer of crotalionoside C. The relative stereochemistry of the aglycone moiety was characterized by a NOESY experiment, which showed NOE correlations between the following proton pairs (H-2a/H-4a, and H-12; H-2b/H-4b, and H-11; H-11/H-7 and H-3) as shown in Figure 3. The absolute configuration at C-9 was determined by application of a modified Mosher's method to be R (Figure 4).[11] But the absolute stereochemistries at C-3, C-5 and C-6 for the ring part could not be determined. Enzymatic hydrolysis of **1** with β-glucosidase (emulsin) yielded 3,6-epoxy-7-megastigmene-5,9-diol (**1a**), whose ^{1}H-NMR spectral data were in good agreement with values reported previously,[8,9] and D-glucose. Thus, the structure of **1** was determined to be megastigmane-7-en-3,6-epoxy-5,9-diol 9*R*-*O*-β-D-glucopyranoside.

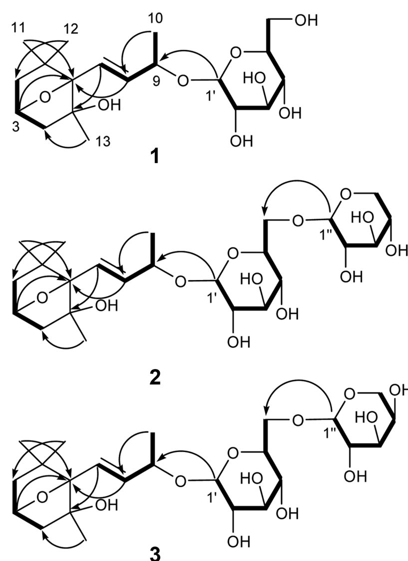

Figure 2. ^{1}H-^{1}H COSY (▬), and HMBC (⌒) correlations of **1**, **2** and **3**.

Compound **2** was obtained as a colorless gum. The molecular formula was determined to be $C_{24}H_{40}O_{12}$ from the [M + Na]$^+$ peak at *m/z* 543.2412 (calcd. for $C_{24}H_{40}O_{12}Na$: 543.2412)

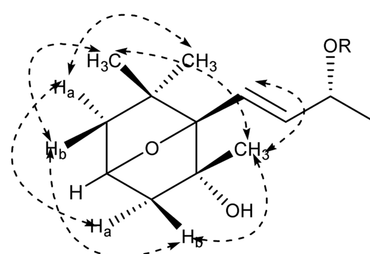

Figure 3. NOESY correlations of **1**, **2** and **3**.

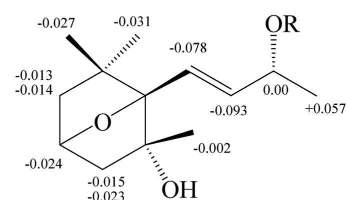

Figure 4. Values of δ_S-δ_R of the MTPA esters of **1a**.

on HR-ESI-MS spectrum. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**. The only differences were the signals from an additional sugar unit that appeared at δ_H = 4.25 (1H, d, J = 7.5 Hz, H-1''), 3.85 (1H, m), 3.48 (1H, m), 3.34 (1H, m), 3.19 (1H, m), 3.18 (1H, m) and δ_C = 104.3, 76.4, 73.7, 70.0, 65.7 in the ^{1}H- and ^{13}C-NMR spectra, indicating that **2** has an additional D-xylopyranose moiety.[12] The coupling constant (J = 7.5 Hz) of the anomeric proton of D-xylose indicated that it was in the *β*-form.[12] The HMBC spectrum showed correlations between H-1' (δ_H = 4.32) of the D-glucose moiety and C-9 (δ_C = 77.5) of the aglycone structure,[11] and between H-1'' (δ_H = 4.25) of D-xylose and C-6' (δ_C = 68.8) of D-glucose (Figure 2).[12] Enzymatic hydrolysis of **2** with hesperidinase yielded **2a**. The NMR data of **2a** were same to those of **1a.** The sugars were confirmed to be D-glucose and D-xylose by comparison of optical rotation and GC-MS analyses. Thus, the structure of **2** was determined to be megastigmane-7-en-3,6-epoxy-5,9-diol 9*R*-*O*-*β*-D-xylopyranosyl-(1''→6')-*β*-D-glucopyranoside.

Compound **3** was obtained as a colorless gum. The molecular formula was determined to be $C_{24}H_{40}O_{12}$ from the $[M + Na]^+$ peak at *m/z* 543.2412 (calcd. for $C_{24}H_{40}O_{12}Na$: 543.2419) in the HR-ESI-MS spectrum. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **3** were very similar to those of **2**. The major difference was the terminal sugar unit; signals from the sugar unit appeared at δ_H = 4.26 (1H, d, J = 6.5 Hz, H-1''), 3.85 (1H, m), 3.80 (1H, m), 3.58 (1H, m), 3.54 (2H, m) in the ^{1}H-NMR spectrum and δ_C = 104.4, 72.8, 71.2, 68.3, 65.6 in the ^{13}C-NMR spectrum, indicating that **3** has an L-arabinopyranose moiety instead of the D-xylopyranose moiety in **2**.[13] The coupling constant (J = 6.5 Hz) of the anomeric proton of L-arabinose indicated that it was in the *α*-form.[13] The positions of sugar residues in **3** were established by an HMBC experiment. The HMBC spectrum showed correlations between H-1' (δ_H = 4.32) of D-glucose and C-9 (δ_C = 77.5) of the aglycone,[11] and between H-1'' (δ_H = 4.26) of L-arabinose and C-6' (δ_C = 68.5) of D-glucose (Figure 2).[13] Enzymatic hydrolysis of **3** with hesperidinase yielded **3a**. The NMR data of **3a** were same to those of **1a**. The sugars were confirmed as D-glucose and L-arabinose by comparison of optical rotation and GC-MS analyses. Thus, the structure of **3** was determined to be megastigmane-7-en-3,6-epoxy-5,9-diol 9*R*-*O*-*α*-L-arabinopyranosyl-(1''→6')-*β*-D-glucopyranoside.

The cytotoxic activities of the isolated compounds (**1-7**) 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.[14] All the compounds showed little cytotoxicity against any tested cell line (IC_{50} > 30 μM).

Experimental Section

Plant Materials. The aerial parts of *H. vernalis* Maxim (Papaveraceae) (2.6 kg) were collected at Taebaek mountain in Gangwon-Do province, Korea in May 2009. A voucher specimen of the plant (SKK-09-002) was deposited at the School of Pharmacy in Sungkyunkwan University.

Extraction and Isolation. The half dried aerial parts of *H. vernalis* Maxim (Papaveraceae) (2.6 kg) were extracted with 80% MeOH three times at room temperature. The resultant MeOH extracts (240 g) were suspended in distilled water (800 mL × 3) and then successively partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH, yielding 40 g, 1 g, 3 g and 30 g, respectively. The *n*-BuOH soluble fraction (30 g) was chromatographed on a Diaion HP-20, eluting a gradient solvent system of 100% H_2O and 100% MeOH to give two fractions (Fraction A-B). Fraction B (8 g) was separated over a silica gel column (230-400 mesh, 360 g) with a solvent system of $CHCl_3$/MeOH/H_2O (15:4:0.5 – 6:4:1) as the eluent to give thirteen fractions (fr. B1-B13). Fr. B4 (240 mg) was purified by preparative reversed-phase HPLC, using a solvent system of 45% MeOH to obtain **6** (22 mg) and **1** (10 mg). Fr. B5 (180 mg) was purified by preparative normal-phase HPLC, using a solvent system of $CHCl_3$/MeOH (7:1) to give **4** (6 mg) and **5** (7 mg). Fr. B6 (280 mg) was purified by preparative reversed-phase HPLC, using a solvent system of 53% MeOH to obtain **2** (5 mg). Fr. B7 (980 mg) was purified by preparative reversed-phase HPLC, using a solvent system of 50% MeOH to furnish **3** (5 mg). Fr. B8 (500 mg) was purified by preparative normal-phase HPLC, using a solvent system of CH_2Cl_2/MeOH (4:1) to obtain **7** (6 mg).

Megastigmane-7-en-3,6-epoxy-5,9-diol 9*R*-*O*-*β*-D-glucopyranoside (1): Colorless gum. $[\alpha]_D^{25}$: −13.0° (c 0.04, MeOH); IR (KBr) ν_{max} cm^{-1}: 3402, 2965, 1642, 1530, 1024, 673; ^{1}H- NMR (CD_3OD, 500 MHz): see Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1; HR-ESI-MS *m/z* = 411.1985 $[M + Na]^+$ (calcd for: 411.1989).

Megastigmane-7-en-3,6-epoxy-5,9-diol 9*R*-*O*-*β*-D-xylopyranosyl-(1''→6')-*β*-D-glucopyranoside (2): Colorless gum. $[\alpha]_D^{25}$: −10.0° (c 0.06, MeOH); IR (KBr) ν_{max} cm^{-1}: 3402, 2963, 1648, 1529, 1026, 672; ^{1}H- NMR (CD_3OD, 500 MHz): see Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1; HR-ESI-MS *m/z* = 543.2412 $[M + Na]^+$ (calcd for: 543.2412).

Megastigmane-7-en-3,6-epoxy-5,9-diol 9*R*-*O*-*α*-L-arabinopyranosyl-(1''→6')-*β*-D-glucopyranoside (3): Colorless gum. $[\alpha]_D^{25}$: −16.0° (c 0.2, MeOH); IR (KBr) ν_{max} cm^{-1}: 3383, 2947, 2837, 1649, 1459, 1026, 672; ^{1}H-NMR (CD_3OD, 500 MHz): see Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1; HR-ESI-MS *m/z* = 543.2419 $[M + Na]^+$ (calcd for: 543.2412).

Enzymatic Hydrolysis of 1, 2 and 3 Compound **1** (1.0 mg) with 1 mL of H_2O and 4 mg of *β*-glucosidase (Emulsin) was stirred at 37 °C for 8 days, and then extracted with $CHCl_3$ three times, and the $CHCl_3$ extract was evaporated in *vacuo*. The $CHCl_3$ extract (0.5 mg) was purified using Silica HPLC ($CHCl_3$:MeOH = 10:1) to afford an aglycone **1a** as a colorless gum $[\alpha]_D^{25}$: 10.0° (c 0.03, MeOH), ^{1}H-NMR ($CDCl_3$, 500 MHz). The sugar in water layer was identified as D-glucose by co-TLC (EtOAc:MeOH:H_2O = 9:3:1, R_f value: 0.2) with a D-glucose standard (Aldrich Co., USA). Each compound **2-3** (each 1.0 mg) with 1 mL of 0.2 M citrate

buffer (pH 4) and hesperidinase (each 10 mg) was stirred at 42 °C for 5 days. After cooling, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ extract (2.0 mg) was purified using Silica HPLC ($CHCl_3$:MeOH = 10:1) to afford aglycones **2a** as a colorless gum $[\alpha]_D^{25}$: 8.0° (*c* 0.04, MeOH), ^{1}H-NMR ($CDCl_3$, 500 MHz) and **3a** as a colorless gutm $[\alpha]_D^{25}$: 16.0° (*c* 0.06, MeOH), ^{1}H-NMR ($CDCl_3$, 500 MHz).

1a. $[\alpha]_D^{25}$: 10.0° (*c* 0.03, MeOH); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.30 (1H, dd, *J* = 16.0, 5 Hz, H-7), 6.20 (1H, d, *J* = 16.0, H-8), 4.65 (1H, m, H-9), 4.40 (1H, m, H-3), 2.06 (1H, m, H-4β), 1.86 (1H, d, *J* = 11.5 Hz, H-2β), 1.81 (1H, m, H-4α), 1.71 (3H, s, H-11), 1.66 (1H, d, *J* = 11.5 Hz, H-2α), 1.51 (3H, s, H-13), 1.45 (3H, d, *J* = 6.5 Hz, H-10), 1.06 (3H, s, H-12).

2a. $[\alpha]_D^{25}$: 8.0° (*c* 0.04, MeOH); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.25 (1H, dd, *J* = 16.0, 5 Hz, H-7), 6.17 (1H, d, *J* = 16.0, H-8), 4.61 (1H, m, H-9), 4.36 (1H, m, H-3), 2.06 (1H, m, H-4β), 1.82 (1H, d, *J* = 11.5 Hz, H-2β), 1.75 (1H, m, H-4α), 1.67 (3H, s, H-11), 1.62 (1H, d, *J* = 11.5 Hz, H-2α), 1.47 (3H, s, H-13), 1.41 (3H, d, *J* = 6.5 Hz, H-10), 1.02 (3H, s, H-12).

3a. $[\alpha]_D^{25}$: 16.0° (*c* 0.06, MeOH); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.29 (1H, dd, *J* = 16.0, 5 Hz, H-7), 6.20 (1H, d, *J* = 16.0, H-8), 4.65 (1H, m, H-9), 4.39 (1H, m, H-3), 2.08 (1H, m, H-4β), 1.86 (1H, d, *J* = 11.5 Hz, H-2β), 1.81 (1H, m, H-4α), 1.71 (3H, s, H-11), 1.66 (1H, d, *J* = 11.5 Hz, H-2α), 1.51 (3H, s, H-13), 1.45 (3H, d, *J* = 6.5 Hz, H-10), 1.00 (3H, s, H-12).

Determination of the Sugars of Compounds 2-3 Each sugar (each *ca.* 0.5 mg) obtained from the hydrolysis of **2-3** was dissolved in anhydrous pyridine (0.1 mL) and L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was stirred at 60 °C for 1.5 h. After the reaction mixture was dried in *vacuo*, the residue was trimethyl-silylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_20 (0.3 mL each), and the organic layer (1 μL) was analyzed by GC-MS.[15] Identification of D-glucose, D-xylose, and L-arabinose for **2** and **3** were detected in each case by co-injection of the hydrolysate with standard silylated samples, giving single peaks at D-glucose (10.11 min), and D-xylose (5.54 min) of **2**, and D-glucose (10.19 min), and L-arabinose (5.39 min) of **3**. Retention times of authentic samples treated in the same way with 1-trimethylsilylimidazole in pyridine, were D-glucose (10.04 min), D-xylose (5.55 min), and L-arabinose (5.41 min).

Preparation of the (*R*)-MTPA Ester and the (*S*)-MTPA Ester from 1a Compound **1a** (1.0 mg), in deuterated pyridine (0.2 mL), was transferred to a clean NMR tube. (*S*)-(+)-α-(trifluoromethyl)phenylacetyl chloride (7 μL) was immediately added under a N_2 gas stream, and the NMR tube was permitted to stand at room temperature overnight. When the reaction was completed, it afforded the (*R*)-MTPA ester derivative (**1b**) of **1a**. In the same manner as described for **1b**, the (*S*)-MTPA ester derivative (**1c**) of **1a** was obtained. The ^{1}H-NMR spectra of **1b**, and **1c** were measured in the NMR reaction tubes.

1b. ^{1}H-NMR (Pyridine-d_5, 500 MHz): δ 0.968 (3H, s, H-12), 1.288 (3H, d, *J* = 6.5, H-10), 1.465 (3H, s, H-13), 1.639 (1H, d, *J* = 11.5, H-2a), 1.693 (3H, s, H-11), 1.781 (1H, m, H-4a), 1.849 (1H, d, *J* = 11.0, H-2b), 2.047 (1H, m, H-4b), 4.384 (1H, m, H-3), 5.776 (1H, m, H-9), 6.057 (1H, dd, *J* = 15.8, 7.6, H-8), 6.259 (1H, d, *J* = 7.6, H-7).

1c. ^{1}H-NMR (Pyridine-d_5, 500 MHz): δ 0.937 (3H, s, H-12), 1.345 (3H, d, *J* = 6.5, H-10), 1.463 (3H, s, H-13), 1.625 (1H, d, *J* = 11.5, H-2a), 1.666 (3H, s, H-11), 1.758 (1H, m, H-4a), 1.836 (1H, d, *J* = 11.0, H-2b), 2.032 (1H, m, H-4b), 4.360 (1H, m, H-3), 5.776 (1H, m, H-9), 5.964 (1H, dd, *J* = 15.8, 7.6, H-8), 6.181 (1H, d, *J* = 7.6, H-7).

Acknowledgments. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20100029358). We thank Drs. E. J. Bang, S. G. Kim, and J. J. Seo at the Korea Basic Science Institute for their aid in obtaining the NMR and mass spectra.

Supporting Information. The spectral data of compounds **1-3**, the general experimental procedures, and bioassays protocols are available on request from the correspondence author.

References

1. Lee, Y. N. *Flora of Korea*; Kyo-Hak Publishing Co., Seoul, 1996; p 237.
2. Bae, K. H. *The Medicinal Plant of Korea*; Kyo-Hak Publishing Co., Seoul, 2000; p 268.
3. Kim, S. W.; In, D. S.; Kim, T. J.; Liu, J. R. *Plant Cell, Tissue Organ Cult*. **2003**, 74.
4. Kang, J. S.; Long, P. H.; Lim, H. M.; Kim, Y. H.; Blaschke, G. *Arch. Pharm. Res.* **2003**, *26*, 114.
5. Yu, L. L.; Hu, W. C.; Ding, G.; Li, R. T.; Wei, J. H.; Zou, Z. M.; Wang, M. H. *J. Nat. Prod.* **2011**, *74*, 1009.
6. Sudo, H.; Ide, T.; Otsuka, H.; Hirata, E.; Takushi, A.; Shinzato, T.; Takeda, Y. *Chem. Pharm. Bull.* **2000**, *48*, 542.
7. Sueyoshi, E.; Liu, H.; Matsunami, K.; Otsuka, H.; Shinzato, T.; Aramoto, M.; Takedam, Y. *Phytochemistry* **2006**, *67*, 2483.
8. Behr, D.; Wahlberg, I.; Nishida, T.; Enzell, C. R. *Acta Chem. Scand.* **1979**, *33*, 701.
9. Takagi, Y.; Fujimori, T.; Hata, T.; Kaneko, H.; Kato, K. *Agric. Biol. Chem.* **1980**, *44*, 705.
10. Perkins, S. J.; Johnson, L. N.; Phillips, D. C. *Carbohydr. Res.* **1977**, *59*, 19.
11. Shitamoto, J.; Matsunami, K.; Otsuka, H.; Shinzato, T.; Takeda, Y. *Chem. Pharm. Bull.* **2010**, *58*, 1026.
12. Ito, H.; Kobayashi, E.; Li, S. H.; Hatano, T.; Sugita, D.; Kubo, N.; Shimura, S.; Itoh, Y.; Yoshida, T. *J. Nat. Prod.* **2001**, *64*, 737.
13. Nakamura, S.; Zhang, Y.; Pongpiriyadacha, Y.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Heterocycles* **2008**, *75*, 131.
14. Skehan, P.; Stroreng, R.; Scudiero, D.; Monks, A.; Mcmahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107.
15. Jiang, W.; Li, W.; Han, L.; Liu, L.; Zhang, Q.; Zhang, S.; Nikaido, T.; Koike, K. *J. Nat. Prod.* **2006**, *69*, 1577.