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PYRROLIZIDINE ALKALOIDS FROM THE ROOTS OF *PARIS VERTICILLATA*

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Abstract – Three new pyrrolizidine alkaloids, verticillatins A (**1**), B (**2**), and C (**3**), together with seven known pyrrolizidine alkaloids (**4**-**10**), were isolated from the roots of *Paris verticillata* (Liliaceae). Their structures were determined by spectroscopic data and chemical evidences. Verticillatin A (**1**), verticillatin B (**2**), verticillatin C (**3**), heliovinine *N*-oxide (**4**), and indicine *N*-oxide (**5**) showed cytotoxicity against the HCT15 cell line, with IC_{50} values of 67.6, 92.4, 35.7, 36.2, and 74.7 μM, respectively.

INTRODUCTION

Pyrrolizidine alkaloids (PAs) are deterrent and toxic to most vertebrates and insects and provide plants with defensive chemicals against herbivores.[1,2] Their toxicity is based primarily on metabolism by means of cytochrome P-450 oxidases. This metabolism results in bioactivation via the formation of highly reactive pyrrole intermediates, causing cytotoxicity, mutagenicity, and genotoxicity.[3,4] Individual PA patterns may be genetically controlled as a result of evolution under selective pressure.[5] Because of this evolutionary advantage for the structural diversity of PAs, it is worthwhile to study PAs from Korean traditional plants used in folk medicine.

We have performed cytotoxic activity screening on a methanol extract from the roots of *Paris veriticillata* BIEB. (Liliaceae) that showed cytotoxicity against A549 and HCT15 cell lines. The perennial herb *P. veriticillata* is distributed in Korean valleys and its root have been used as a Korean traditional medicine against asthma, boils, and chronic bronchitis.[6] Several compounds such as saponins, sterols, and flavonoids were reported from the aerial parts of *P. verticillata*,[7] and we have reported a phenolic amide and phenolic constituents from the roots of *P. verticillata*.[8] In continuing investigation of chemical

constituents from the roots of *P. verticillata*, we further isolated three new pyrrolizidine alkaloids, verticillatins A (**1**), B (**2**), and C (**3**), together with seven known pyrrolizidine alkaloids (**4**-**10**). We then examined the cytotoxic activity of these isolated compounds (**1**-**10**) against four human tumor cells, A549, SK-OV-3, SK-MEL-2, and HCT15. We describe herein the isolation, structure assignment, and bioactivity of these compounds.

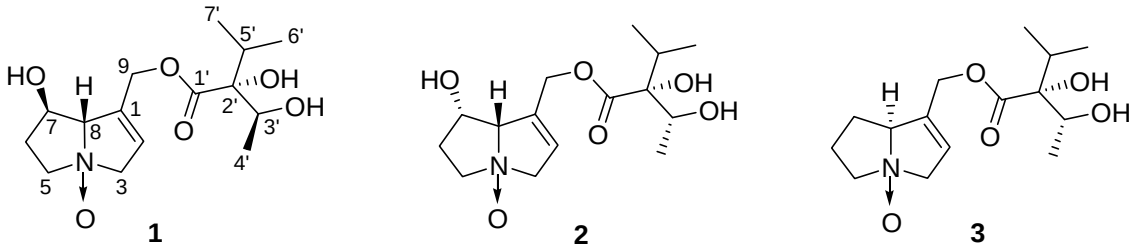

Figure 1. The structures of the isolated compounds (**1**–**3**) from *P. verticillata*

RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless gum. The molecular formula of **1** was determined to be $C_{15}H_{25}NO_6$ by the molecular ion peak $[M + H]^+$ at *m/z* 316.1760 (calcd. for $C_{15}H_{26}NO_6$: 316.1760) in HRFABMS. Its IR spectrum showed bands at 3388 cm^{-1} (OH) and 1720 cm^{-1} (C=O). The ^1H NMR spectrum of **1** (Table 1) displayed two methyl signals for an isopropyl group at δ_H 0.94 (d, *J* = 7.0 Hz, H-7′) and 0.96 (d, *J* = 7.0 Hz, H-6′), two methine signals at δ_H 1.99 (m, H-5′) and 4.08 (q, *J* = 6.5 Hz, H-3′), and one methyl signal at δ_H 1.18 (d, *J* = 6.5 Hz, H-4′), which were correlated with carbon signals at δ_C 16.5 (C-7′), 16.1 (C-6′), 33.0 (C-5′), 69.3 (C-3′), and 16.0 (C-4′) in the HMQC experiment, respectively, indicating that **1** has a trachelanthate ester unit (ΔδC-6′/C-7′ = 0.4).[9,10] Three methine protons were observed at δ_H 4.73 (br s, H-7, and H-8) and 5.94 (br s, H-2), which were correlated with carbon signals at δ_C 69.6 (C-7), 95.8 (C-8), and 122.4 (C-2) in the HMQC experiment. The downfield signal at δ_H 5.94 (H-2) in the ^1H NMR spectrum and the signals at δ_C 77.6 (C-3), 68.7 (C-5), 34.4 (C-6), 69.6 (C-7), and 95.8 (C-8) in the ^{13}C NMR spectrum suggest that the pyrrolizidine base is to be unsaturated pyrrolizidine *N*-oxide.[10] These assignments were also confirmed by further analyses of HMQC, HMBC, and ^1H-^1H COSY experiments (Figure 2). Specifically, HMBC correlation from H-9 to C-1′ indicated the position of the trachelanthic acid at C-9.

Acidic hydrolysis of **1** gave trachelanthic acid and 1-hydroxymethyl-7-hydroxypyrrolizidine *N*-oxide. The absolute streochemistry of **1b**, trachelanthic acid, was determined to be 2′*R*, 3′*S* by comparison of NMR data and negative optical rotation value; $[\alpha]_D^{25}$ -4.5 (*c* 0.05, MeOH) to those of its synthetic stereoisomer.[9] The pyrrolizidine base **1a** was identified as (-)-heliotridine *N*-oxide based on the NOESY experiment and

a convenient Mosher ester procedure carried out in an NMR tube.[11-14] Analysis of the chemical shift differences between MTPA ester derivatives of **1** (Figure 3) revealed *R*-configured C-7 and *S*- configured C-3′.

Table 1. ^{1}H and ^{13}C NMR data of **1**, **2**, and **3** in CD_3OD

No	**1**		**2**		**3**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		132.4		132.5		136.3
2	5.94 br s	122.4	5.92 br s	122.2	5.86 br s	121.3
3*α*	4.63 br d (16.5)	77.6	4.61 br d (16.5)	77.7	4.57 br d (16.5)	75.7
3*β*	4.35 br d (16.5)		4.35 br d (16.5)		4.32 br d (16.5)	
5*α*	3.83 m	68.7	3.83 m	68.7	3.58-3.62 m	70.2
5*β*	3.72 m		3.70 m			
6*α*	2.58 m	34.4	2.59 m	34.4	2.34 m	24.1
6*β*	2.07 m		2.07 m		2.00 m	
7	4.73 br s	69.6	4.71 br s	69.6	2.00 m	27.3
					2.44 m	
8	4.73 br s	95.8	4.70 br s	95.8	4.66 br s	88.9
9	4.77 d (14.0)	61.1	4.77 d (14.0)	61.1	4.77 d (14.0)	60.1
	4.89 d (14.0)		4.86 d (14.0)		4.86 d (14.0)	
1′		174.4		173.7		173.6
2′		83.4		83.7		83.7
3′	4.08 q (6.5)	69.3	3.97 q (6.5)	70.8	3.97 q (6.5)	70.7
4′	1.18 d (6.5)	16.0	1.24 d (6.5)	16.8	1.24 d (6.5)	16.8
5′	1.99 m	33.0	2.18 sept (7.0)	32.6	2.18 sept (7.0)	32.6
6′	0.96 d (7.0)	16.1	0.90 d (7.0)	17.1	0.90 d (7.0)	17.0
7′	0.94 d (7.0)	16.5	0.93 d (7.0)	15.3	0.93 d (7.0)	15.3

*NMR data were obtained at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

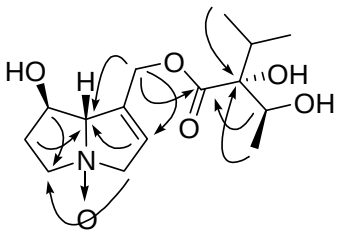

Figure 2. Key HMBC (↷) correlations of **1**

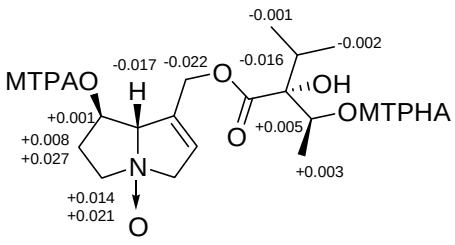

Figure 3. Values of δ_S- δ_R (data obtained in pyridine-d_5) of the MTPA esters of **1**

The NOESY spectrum of **1** showed correlations between H-8 and H-6*β* or H_3-6′, H_3-7′, and between H-7 and H-5*α*, which indicated *S*-configured C-8 (Figure 4). Thus, the base was identified as (-)-heliotridine *N*-oxide.[15] Furthermore, the base **1a** was reduced with zinc in dilute H_2SO_4 to give (-)-heliotridine.[10] A negative optical rotation value of $[\alpha]_D^{25}$ -10.5 (*c* 0.01 in MeOH) and the ^{1}H NMR data were in agreement with those of synthetic (-)-heliotridine.[15]

In the NOE difference spectra, a NOE enhancement between H-7, H-6*α*, and H-6*β* protons differed in magnitude, suggesting the conformation of pyrrolizidine base is an *endo*-buckled form in which H-7 and H-6*β* protons approximate a *trans*-configuration, resulting in different NOEs.[16,17] The new pyrrolizidine alkaloid, named verticillatin A, is thus 9-(-)-trachelanthyl-(-)-heliotridine *N*-oxide.

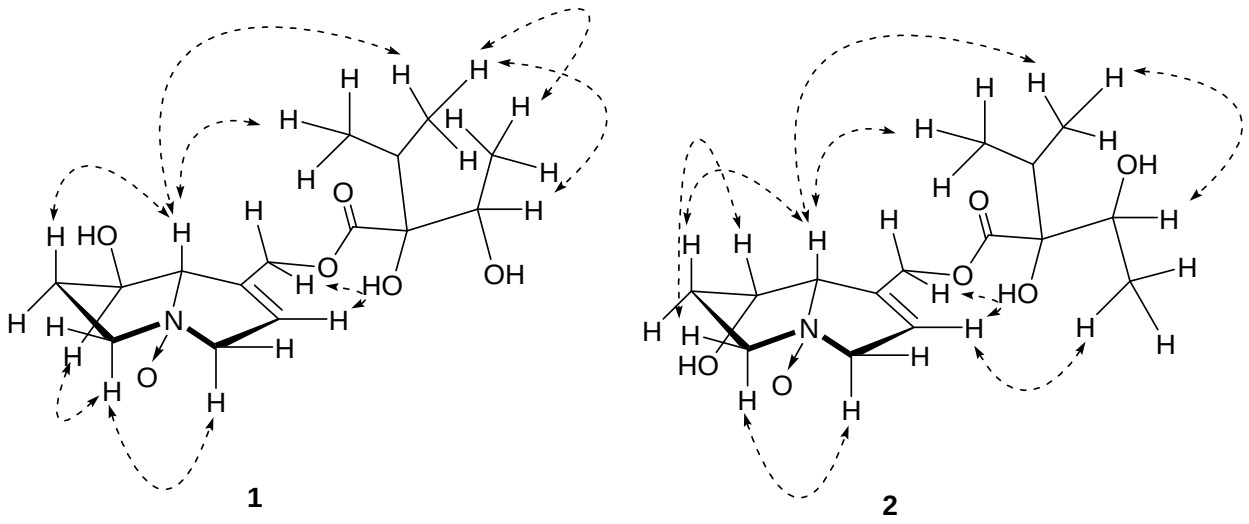

Figure 4. Key NOESY correlations of **1** and **2**

Compound **2** was obtained as a colorless gum, whose molecular formula was determined as $C_{15}H_{25}NO_6$ from the molecular ion peak $[M + H]^+$ at *m/z* 316.1759 in HRFABMS. IR, MS, and NMR data of compound **2** were similar to those of compound **1**. NMR spectral analyses, including HMQC and HMBC experiments, led to the chemical structure of **2**, implying that compounds **1** and **2** were mutual stereoisomer. This was confirmed by a convenient Mosher ester procedure[11-14] (Figure 5) and the NOESY correlations between H-7 and H-5*β* and between H-8 and H-6*β* or H_3-6′, H_3-7′ (Figure 4), which indicated *S*-configured C-7 and *S*-configured C-8. Acidic hydrolysis of **2** afforded side residue **2b**, viridifloric acid. The absolute stereochemistry of viridifloric acid was determined to be 2′*R* and 3′*R* by comparison of NMR data and positive optical rotation value; $[\alpha]_D^{25}$ +3.5 (*c* 0.05, MeOH).[9] Thus, compound **2** was determined to be 9-(+)-viridifloryl-(-)-retronecine *N*-oxide, named verticillatin B.

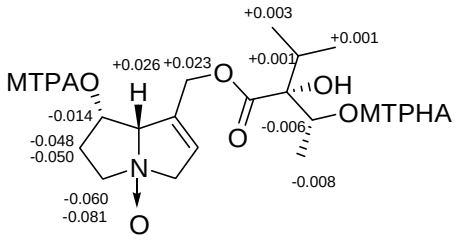

Figure 5. Values of δ_S- δ_R (data obtained in pyridine-d_5) of the MTPA esters of **2**

Compound **3** was isolated as a colorless gum, whose molecular formula was determined from the molecular ion peak $[M + H]^+$ at *m/z* 300.1811, corresponding to a molecular formula of $C_{15}H_{26}NO_5$ (calcd. for $C_{15}H_{26}NO_5$: 300.1811). The ^1H and ^{13}C NMR spectra were also similar to those of verticillatin A (**1**), except for values assigned to C-7 in the ^1H and ^{13}C NMR spectra, in which the methine (δ_H 4.73/δ_C 69.6) at C-7 of verticillatin A (**1**) was replaced by methylene (δ_H 2.00 and 2.44/δ_C 27.3) at C-7 of **3**. The ^1H and ^{13}C NMR assignments (Table 1) of **3** were confirmed by detailed analyses of 2D-NMR data, including HMQC, HMBC, and NOESY. Key HMBC showed correlations from H-8 to C-1, C-2, C-6, C-7, and C-9, and from H-9 to C-1′ (Figure 6). In NOESY spectrum of **3**, the key correlation of H-2 with H_3-4′ supported the proposed configuration at C-3′. Acidic hydrolysis of **3** afforded side residue, (+)-viridifloric acid, which was confirmed by co-TLC along with the produced **2b** and direct comparison of its ^1H NMR data and optical rotation value; $[\alpha]_D^{25}$ +5.2 (*c* 0.02, MeOH) with those of **2b**.[9] From the above evidence, compound **3** was identified as coromandalinine *N*-oxide, named verticillatin C.

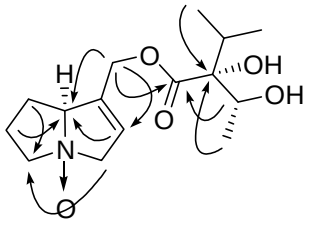

Figure 6. Key HMBC ( ) correlations of **3**

Isolated known compounds were identified as heliovinine *N*-oxide (**4**),[18] indicine *N*-oxide (**5**),[10] (+)-viridifloryl heliotridine *N*-oxide (**6**),[19] (-)-indicine *N*-oxide (**7**),[19] (+)-viridifloryl-(+)-retronecine *N*-oxide (**8**),[9] (7*S*)-indicine *N*-oxide (**9**),[18] and isatinecine (**10**)[9] by comparison with published data. This is the first report of pyrrolizidine alkaloids from this plant.

The cytotoxicities of the isolated compounds (**1**-**10**) against A549, SK-OV-3, SK-MEL-2, and HCT15 human tumor cell lines were evaluated using the SRB assay *in vitro*. Verticillatin A (**1**), verticillatin B (**2**), verticillatin C (**3**), heliovinine *N*-oxide (**4**), and indicine *N*-oxide (**5**) showed weak cytotoxicity against the

HCT15 cell line, with IC_{50} values of 67.6, 92.4, 35.7, 36.2, and 74.7 μM, respectively, but were inactive against other tested cell lines (IC_{50} > 100 μM). Compounds **6-10** showed little cytotoxicity against any tested cell line (IC_{50} > 100 μM). The roots of *P. verticillata* have often been used as Korean traditional medicine, but we suggest here that its application should be more carefully monitored due to the isolation of various toxic pyrrolizidine alkaloids.[3,4]

EXPERIMENTAL DETAILS

General. 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. FAB and HRFAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) with chemical shifts given in ppm (δ). Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector and Apollo Silica 5μ column (250 × 10 mm). Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh) was used for column chromatography. Merck precoated 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 EtOH (v/v).

Plant material. The roots of *P. verticillata* were collected at Mt. O-Dae, Gangwon Province in August, 2002, and the plants were identified by one of the authors (K.R.L.). A voucher specimen (SKKU 2002-08) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation. The roots of *P. verticillata* (1.5 kg) were extracted using 80% MeOH at room temperature over a period of 3 days three times. The solvent was evaporated under reduced pressure to give a crude extract (180 g). The systematic solvent partitioning of the MeOH extract afforded *n*-hexane soluble (5.2 g), methylene chloride soluble (3.5 g), EtOAc soluble (4.4 g), and *n*-BuOH soluble (20 g) fractions. The *n*-BuOH soluble fraction (10 g) was subjected to column chromatography over HP-20 resin with 100% H_2O and 100% MeOH to give two fractions (B1 - 100% H_2O and B2 - 100% MeOH). The B2 fraction (3 g) was subjected to normal phase column chromatography over a silica gel ($CHCl_3$-MeOH-H_2O = 10:10:0.5) to give six fractions (B21 to B26). B24 fraction (1.2 g) was further separated over a silica gel column ($CHCl_3$-MeOH-H_2O = 10:4:0.5) to give two subfractions (B241 to B242). The B241 fraction (600 mg) was purified by semi-preparative HPLC, using a solvent system of $CHCl_3$-EtOAc-MeOH-H_2O-NH_4OH (10:2:4:0.5:0.2) for 30 min at a flow rate of 2.0 mL/min (Apollo Silica 5μ column; 250 × 10 mm; 5 μm particle size; Shodex refractive index detector) to obtain **2** (20 mg,

t_R = 16.5 min), **3** (7 mg, t_R = 14.9 min), **4** (6 mg, t_R = 14.5 min), **6** (8 mg, t_R = 17.3 min), **8** (8 mg, t_R = 18.5 min), and **9** (10 mg, t_R = 18.0 min). The B242 fraction (800 mg) was also purified by semi-preparative HPLC as above, using a solvent system of $CHCl_3$-EtOAc-MeOH-H_2O-NH_4OH (10:2:4:0.5:0.2) to yield **1** (10 mg, t_R = 16.2 min), **7** (4 mg, t_R = 15.5 min) and **10** (5 mg, t_R = 17.5 min). The B25 fraction (50 mg) was purified by semi-preparative HPLC as above, using a solvent system of $CHCl_3$-MeOH-H_2O (9:3.5:0.5) to afford **5** (5 mg, t_R = 18.0 min).

Verticillatin A (1). colorless gum; $[\alpha]_D^{25}$ +2.5 (*c* 0.20, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 204 (+38.5), 213 (+25.1), 223 (+12.3) nm; IR (KBr) ν_{max} 3388 (OH), 2948 (CH), 1720 (C=O), 1667 (C=C), 1453 (CH_3), 1230 (C-O) cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; FABMS *m/z* 316 $[M + H]^+$; HR-FABMS *m/z* 316.1760 $[M + H]^+$ (calcd for $C_{15}H_{26}NO_6$, 316.1760).

Verticillatin B (2). colorless gum; $[\alpha]_D^{25}$ +4.0 (*c* 0.60, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (+31.2), 212 (+17.5), 223 (+5.1) nm; IR (KBr) ν_{max} 3388 (OH), 2947 (CH), 1720 (C=O), 1667 (C=C), 1452 (CH_3), 1230 (C-O) cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; FABMS *m/z* 316 $[M + H]^+$; HR-FABMS *m/z* 316.1759 $[M + H]^+$ (calcd for $C_{15}H_{26}NO_6$, 316.1760).

Verticillatin C (3). colorless gum; $[\alpha]_D^{25}$ -2.8 (*c* 0.10, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+33.1), 213 (+22.0), 223 (+22.8) nm; IR (KBr) ν_{max} 3387 (OH), 2948 (CH), 1721 (C=O), 1666 (C=C), 1450 (CH_3), 1231 (C-O) cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; FABMS *m/z* 300 $[M + H]^+$; HR-FABMS *m/z* 300.1811 $[M + H]^+$ (calcd for $C_{15}H_{26}NO_5$, 300.1811).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 1 and 2 by a Convenient Mosher Ester Procedure. Compound **1** (1.8 mg) in deuterated pyridine (0.75 mL) was transferred into a clean NMR tube. (*S*)-(+)-*α*-Methoxy-*α*-(trifluoromethyl)phenylacetyl chloride, (*S*)-MTPA-Cl (5 *μ*L) was added immediately into the NMR tube under a stream of N_2 gas and the NMR tube was shaken carefully to mix the sample and (*S*)-MTPA-Cl evenly. The reaction NMR tube stood at room temperature overnight. The completed reaction afforded the (*R*)-MTPA ester derivative (**1r**) of **1.** The (*S*)-MTPA ester derivative of **1** (**1s**) and the (*R*)- and (*S*)-MTPA ester derivatives of **2** (**2r** and **2s**) were obtained in the same manner as described for **1r**. The ^1H NMR spectra of **1r**, **1s**, **2r** and **2s** were directly measured with the reaction NMR tubes.

1s. ^1H NMR (500 MHz, pyridine-d_5): δ 5.934 (1H, br s, H-7), 5.895 (1H, br s, H-2), 5.181 (1H, d, *J* = 14.0 Hz, H-9a), 5.178 (1H, d, *J* = 14.0 Hz, H-9b), 5.109 (1H, br s, H-8), 5.097 (1H, br d, *J* = 16.5 Hz, H-3a), 4.926 (1H, br d, *J* = 16.5 Hz, H-3b), 4.747 (1H, m, H-5a), 4.548 (1H, q, *J* = 6.5 Hz, H-3′), 4.193

(1H, m, H-5b), 2.752 (1H, m, H-6a), 2.282 (1H, m, H-5′), 2.168 (1H, m, H-6b), 1.487 (3H, d, *J* = 6.5 Hz, H-4′), 1.167 (3H, d, *J* = 7.0 Hz, H-6′), 1.001 (3H, d, *J* = 7.0 Hz, H-7′).

1r. ^{1}H NMR (500 MHz, pyridine-d_5): δ 5.933 (1H, br s, H-7), 5.895 (1H, br s, H-2), 5.203 (1H, d, *J* = 14.0 Hz, H-9a), 5.199 (1H, d, *J* = 14.0 Hz, H-9b), 5.126 (1H, br s, H-8), 5.098 (1H, br d, *J* = 16.5 Hz, H-3a), 4.927 (1H, br d, *J* = 16.5 Hz, H-3b), 4.726 (1H, m, H-5a), 4.543 (1H, q, *J* = 6.5 Hz, H-3′), 4.179 (1H, m, H-5b), 2.725 (1H, m, H-6a), 2.298 (1H, m, H-5′), 2.160 (1H, m, H-6b), 1.484 (3H, d, *J* = 6.5 Hz, H-4′), 1.168 (3H, d, *J* = 7.0 Hz, H-6′), 1.003 (3H, d, *J* = 7.0 Hz, H-7′).

2s. ^{1}H NMR (500 MHz, pyridine-d_5): δ 5.910 (1H, br s, H-7), 5.885 (1H, br s, H-2), 5.252 (1H, d, *J* = 14.0 Hz, H-9a), 5.245 (1H, d, *J* = 14.0 Hz, H-9b), 5.180 (1H, br s, H-8), 5.122 (1H, br d, *J* = 16.5 Hz, H-3a), 4.914 (1H, br d, *J* = 16.5 Hz, H-3b), 4.519 (1H, m, H-5a), 4.396 (1H, q, *J* = 6.5 Hz, H-3′), 4.135 (1H, m, H-5b), 2.650 (1H, m, H-6a), 2.459 (1H, m, H-5′), 2.113 (1H, m, H-6b), 1.544 (3H, d, *J* = 6.5 Hz, H-4′), 1.222 (3H, d, *J* = 7.0 Hz, H-6′), 1.081 (3H, d, *J* = 7.0 Hz, H-7′).

2r. ^{1}H NMR (500 MHz, pyridine-d_5): δ 5.924 (1H, br s, H-7), 5.885 (1H, br s, H-2), 5.229 (1H, d, *J* = 14.0 Hz, H-9a), 5.211 (1H, d, *J* = 14.0 Hz, H-9b), 5.154 (1H, br s, H-8), 5.119 (1H, br d, *J* = 16.5 Hz, H-3a), 4.910 (1H, br d, *J* = 16.5 Hz, H-3b), 4.600 (1H, m, H-5a), 4.402 (1H, q, *J* = 6.5 Hz, H-3′), 4.195 (1H, m, H-5b), 2.700 (1H, m, H-6a), 2.458 (1H, m, H-5′), 2.161 (1H, m, H-6b), 1.552 (3H, d, *J* = 6.5 Hz, H-4′), 1.119 (3H, d, *J* = 7.0 Hz, H-6′), 1.080 (3H, d, *J* = 7.0 Hz, H-7′).

Acidic Hydrolysis of Compounds 1-3. Compounds (**1**: 5.0 mg; **2**: 3.5 mg; and **3**: 3.0 mg) were hydrolyzed with 2N HCl (2 mL) at 40 °C for 3 h. Then H_2O (5 mL) was added and the mixture was extracted twice with EtOAc. The EtOAc layer was evaporated under reduced pressure to give an EtOAc extract. Each EtOAc extract was purified over a silica gel Waters Sep-Pak Vac 6cc [$CHCl_3$-MeOH, 10:1] to give pyrrolizidine bases **1a** (1.5 mg), **2a** (0.8 mg), and **3a** (0.8 mg). The aqueous layer of each reaction mixture was neutralized with dilute NH_4OH (monitored with indicator paper) and, then filtered to afford the side residues **1b** (0.8 mg) and **2b** (0.6 mg), respectively from reaction mixtures of **2** and **3**. The compounds, (-)-trachelanthic acid **1b** and (+)-viridifloric acid **2b** were identified by ^{1}H NMR, MS data and optical rotation values. The aqueous layer of the reaction mixture of **3** contained the side residue, (+)-viridifloric acid, which were separated by HPLC analysis ($CHCl_3$-MeOH-NH_4OH = 9:1:0.1) and confirmed by co-TLC and direct comparison of its ^{1}H NMR data and optical rotation value with those of **2b** obtained above. The optical rotation values of the pyrrolizidine bases **1a**, **2a**, and **3a** obtained from hydrolysis of **1**-**3** were $[\alpha]_D^{25}$ -20.1 (*c* 0.02, MeOH), $[\alpha]_D^{25}$ -9.5 (*c* 0.01, MeOH), and $[\alpha]_D^{25}$ -12.5 (*c* 0.01,

MeOH), respectively. The optical rotation value of the side residue, (+)-viridifloric acid obtained from hydrolysis of **3** was $[\alpha]_D^{25}$ +5.2 (*c* 0.02, MeOH) and its ^{1}H NMR and MS data were identical to those of **2b**.

1b. colorless gum; $[\alpha]_D^{25}$ -4.5 (*c* 0.05, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.12 (1H, q, *J* = 6.5 Hz, H-3), 2.05 (1H, m, H-5), 1.20 (3H, d, *J* = 6.5 Hz, H-4), 0.97 (3H, d, *J* = 7.0 Hz, H-6), 0.95 (3H, d, *J* = 7.0 Hz, H-7); FAB-MS *m/z* 163 $[M + H]^+$.

2b. colorless gum; $[\alpha]_D^{25}$ +3.5 (*c* 0.05, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.00 (1H, q, *J* = 6.5 Hz, H-3), 2.12 (1H, m, H-5), 1.27 (3H, d, *J* = 6.5 Hz, H-4), 0.92 (3H, d, *J* = 7.0 Hz, H-6), 0.90 (3H, d, *J* = 7.0 Hz, H-7); FAB-MS *m/z* 163 $[M + H]^+$.

Reduction of 1a. The pyrrolizidine base **1a** (1.5 mg) obtained from hydrolysis of **1** was reduced with zinc in dilute H_2SO_4, as described in previous paper.[10] The aqueous layer of the reaction mixture was then neutralized with dilute NH_4OH and extracted with $CHCl_3$ three times. The $CHCl_3$ extract was purified by HPLC analysis ($CHCl_3$-MeOH-NH_4OH, 5:1:0.1) to afford (-)-heliotridine (0.3 mg). The (-)-heliotridine was identified by its negative optical rotation value, $[\alpha]_D^{25}$ -10.5 (*c* 0.01, MeOH) and comparison of its ^{1}H NMR data with that of synthetic (-)-heliotridine.[15]

Cytotoxicity Assay. A sulforhodamin B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines. The assays were performed at the Korea Research Institute of Chemical Technology as described in previous paper.[20] The cell lines used were A549 (non-small cell lung carcinoma), SK-OV-3 (ovarian malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon adenocarcinoma). Doxorubicin was used as a positive control. Doxorubicin had IC_{50}s against A549, SK-OV-3, SK-MEL-2, and HCT15 of 0.007, 0.056, 0.017, and 0.024 μM, respectively.

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