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Lignans from the Tuber-barks of *Colocasia antiquorum* var. *esculenta* and Their Antimelanogenic Activity

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Colocasia antiquorum var. *esculenta*, a variant of *C. antiquorum*, commonly known as "Imperial Taro", is an edible vegetable in many tropical and subtropical regions of the world. This study with the aim of evaluating the potential of *C. antiquorum* var. *esculenta* as a functional food with a depigmenting effect resulted in the identification of a new sesquilignan, named colocasinol A (**1**), and a new acyclic phenylpropane lignanamide, named *cis*-grossamide K (**2**), together with 10 known compounds (**3**−**12**). The identification and structural elucidation of these compounds were based on 1D and 2D nuclear magnetic resonance (NMR) spectroscopic data analysis as well as high-resolution fast atom bombardment mass spectrometry (FABMS) and electron impact mass spectrometry (EIMS). Quantitation of the melanin contents and cell viability in murine melanocyte melan-a cells was used to assess the antimelanogenic activities of the isolated compounds. Among them, *cis*-grossamide K (**2**), isoamericanol A (**3**), americanol A (**4**), 2-hydroxy-3,2′-dimethoxy-4′-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)biphenyl (**5**), and (−)-pinoresinol (**6**) showed inhibitory effects on melanin production. Compounds **2**, **5**, and **6** exerted a particularly strong antimelanogenic activity on the cells without high cell toxicity (IC_{50} = 54.24, 53.49, and 56.26 μM, and LD_{50} = 163.60, 110.23, and >500 μM, respectively).

KEYWORDS: *Colocasia antiquorum* var. *esculenta*; Araceae; lignan; sesquilignan; lignanamide; antimelanogenic activity

INTRODUCTION

Skin is a complex structure providing important functions, particularly as an essential barrier against mechanical, chemical, and microbial factors that may affect the physiological status of the body. Melanocytes in the dermis or the basal layer of the epidermis are key players in this process because they produce and distribute melanins, which are the skin pigments in humans and protect the skin from UV light by the absorption of free radicals from the cytoplasm (*1*, *2*). However, the overproduction and accumulation of melanins in skin can cause many serious skin disorders, such as freckles, chloasoma dermatitis, and geriatric pigment spots (*3*). Moreover, various cosmetic problems can be induced because of hyperpigmentation. To control melanogenesis, some whitening agents have previously been developed. However, their effects on the inhibition of melanin biosynthesis are unsatisfactory and not safe (*4*, *5*). Recently, research has focused on attaining natural sources of whitening agents that are safer to use and more effective to bring about the decrease of melanogenesis in the human skin.

Colocasia antiquorum var. *esculenta* is an edible vegetable in many tropical and subtropical regions of the world. This species belongs to the Araceae family. This plant is a taro that is widely distributed in Korea and is a variant of *C. antiquorum*, commonly known as "Imperial Taro". It is called "Toran" in Korea, and its corm is edible as a nourishing meal and is made into a Korean traditional soup. Some fatty acids, sterols, and flavonoids have been reported as chemical constituents of *C. antiquorum* (*6*, *7*). Constituents of taro exhibited biological activities, such as antifungal activity (*7*) and inhibition of human lanosterol synthase (*8*). However, not many phytochemical and biological investigations on *C. antiquorum* var. *esculenta* have previously been conducted. In our screening procedures, the MeOH extract of tuber-barks of *C. antiquorum* var. *esculenta* showed inhibitory effects on melanin production in melan-a cells. Therefore, as part of a continuing search for bioactive constituents from Korean medicinal plant sources (*9*−*12*), we attempted to investigate the active constituents of this herb for antimelanin biosynthesis.

In this investigation, with the aim of evaluating the potential of *C. antiquorum* var. *esculenta* as a functional food with the depigmenting effect, we investigated the constituents of *C. antiquorum* var. *esculenta* and evaluated their inhibitory effects on melanogenesis by determination of melanin contents and cell viability in cultured murine melanocyte melan-a cells.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco, Easton, MD). IR spectra were recorded on a Bruker IFS-66/S FTIR spectrometer (Bruker, Karlsruhe, Germany). Circular dichroism (CD) spectra were measured on a Jasco

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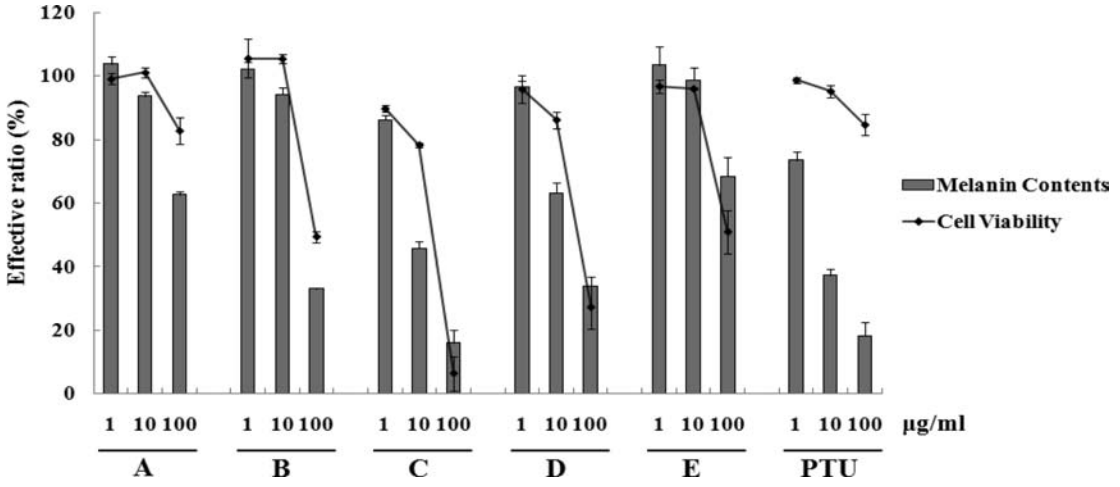

Figure 1. Effect of fractions A, B, C, D, and E from the EtOAC-soluble fraction of the methanolic extract of tuber-barks of *C. antiquorum* var. *esculenta* on melanogenesis in melan-a cells. The inhibitory activity of melanogenesis and the effect of cell viability were expressed as a percentage of the control. The data shown represent the means ± standard deviation (SD) of three independent experiments performed in duplicate.

J-715 spectropolarimeter (Jasco, Easton, MD). UV spectra were recorded with a Shimadzu UV-1601 UV−vis spectrophotometer (Shimadzu, Tokyo, Japan). Electron impact (EI) mass spectra were recorded on a JEOL SX102 mass spectrometer (JEOL, Peabody, MA). Fast atom bombardment (FAB) and high-resolution (HR)-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer (JEOL, Peabody, MA). Nuclear magnetic resonance (NMR) spectra, including $^1H-^1H$ correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC) experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer (Varian, Palo Alto, CA) operating at 500 MHz (^1H) and 125 MHz (^{13}C), with chemical shifts given in parts per million (ppm) (δ) using tetramethylsilane (TMS) as an internal standard. Preparative high-performance liquid chromatography (HPLC) used a Gilson 306 pump (Gilson, Middleton, WI) with a Shodex refractive index detector (Shodex, New York, NY). Low-pressure liquid chromatography was carried out over a LiChroprep Lobar-A Si 60 column (240 ×10 mm inner diameter) (Merck, Darmstadt, Germany) or a LiChroprep Lobar-A RP-18 column (240 ×10 mm inner diameter) (Merck, Darmstadt, Germany) with a FMI QSY-0 pump (Teledyne Isco, Lincoln, NE). Silica gel 60 (Merck, 70−230 and 230−400 mesh) and RP-C_{18} silica gel (Merck, 230−400 mesh) were used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates (Merck, Darmstadt, Germany) were used for thin-layer chromatography (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).

Plant Material. The tuber-barks of *C. antiquorum* var. *esculenta* were collected at Girokdo, Goksung of the Jeonnam province, Korea, in November 2006. Samples of plant material were identified by one of the authors (K. R. Lee). A voucher specimen (SKKU 2006-11) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The dried tuber-barks of *C. antiquorum* var. *esculenta* (3.8 kg) were extracted with 85% MeOH using an ultrasonic apparatus for 1 h, and the combined extracts were concentrated *in vacuo*. The resultant MeOH extract (350 g) was suspended in distilled water (15 L) and then partitioned with EtOAc at room temperature to give an EtOAc extract (36 g) after removal of solvents. The EtOAc-soluble fraction (30 g) was subjected to reversed-phase (RP)-C_{18} silica gel (230−400 mesh, 500 g) column chromatography (CC), using a gradient solvent system of MeOH/H_2O (3:2−1:0) as the eluent. According to TLC analysis, five crude fractions (fractions A−E) were collected. All fractions were evaluated on antimelanogenic activities in melan-a cells (**Figure 1**). Fractions A (7 g) and D (7 g) showed significant inhibitory activities on melanin production. Fraction D had greater cell toxicity than fraction A; therefore, we used only fraction A in this study. Fraction A (7.0 g) was subjected to RP-C_{18} silica gel (230−400 mesh, 200 g) CC, using a gradient solvent system of MeOH/H_2O (2:3−1:0) as the eluent to afford four fractions (fractions AA−AD). Fraction AD (1.3 g) was further applied to a Sephadex LH-20 column, using a solvent system of 100% MeOH as the eluent to give five fractions (fractions ADA−ADE). Fraction ADB (400 mg) was subjected to further RP-C_{18} silica gel (230−400 mesh, 50 g) CC, using a solvent system of MeOH/H_2O (1:1) as the eluent to give six subfractions (fractions ADB1−ADB6). Fraction ADB3 (150 mg) was purified by preparative RP HPLC, using a 250 ×10 mm inner diameter, 10 μm, Econosil RP-18 column (Alltech, Nicholasville, KY) with a solvent system of MeOH/H_2O (1:1; flow rate, 2 mL/min) as the eluent to give compounds **7** (4 mg, t_R = 15.5 min) and **8** (7 mg, t_R = 16.5 min). Fraction ADB6 (20 mg) was purified by preparative normal-phase HPLC, using a 250 × 10 mm inner diameter, 5 μm, Apollo Silica column (Alltech, Nicholasville, KY) with a solvent system of *n*-hexane/EtOAc (2:1; flow rate, 2 mL/min) as the eluent to yield compound **11** (7 mg, t_R = 13.5 min). Fraction ADC (300 mg) was applied to low-pressure liquid chromatography (LPLC) on a 240 × 10 mm inner diameter, 40−63 μm, LiChroprep Lobar-A RP-18 column (Merck, Darmstadt, Germany) with a solvent system of MeOH/H_2O (1:1) as the eluent to give four fractions (fractions ADC1−ADC4). Fraction ADC2 (60 mg) was purified further by preparative RP HPLC, using a solvent system of MeCN/H_2O (3:7) as the eluent to obtain compounds **5** (7 mg, t_R = 17.0 min) and **9** (9 mg, t_R = 18.5 min). Fraction ADC3 (90 mg) was subjected to LPLC on a 240 × 10 mm inner diameter, 40−63 μm, LiChroprep Lobar-A Si 60 column (Merck, Darmstadt, Germany) with a solvent system of *n*-hexane/$CHCl_3$/MeOH (6:6:1) as the eluent to give two subfractions. The subfractions were purified further by preparative RP HPLC, using a solvent system of MeCN/H_2O (4:6) as the eluent to give compound **1** (6 mg, t_R = 14.0 min) and purified further by preparative RP HPLC, using a solvent system of MeCN/H_2O (3:7) as the eluent to yield compounds **2** (7 mg, t_R = 15.5 min) and **12** (8 mg, t_R = 17.0 min), respectively. Fraction ADC4 (40 mg) was purified by preparative RP HPLC, using a solvent system of MeCN/H_2O (3:7) as the eluent to furnish compounds **6** (8 mg, t_R = 16.5 min) and **10** (6 mg, t_R = 18.5 min). Final purification was performed on preparative RP HPLC, using a solvent system of MeOH/H_2O (1:1) as the eluent to obtain compounds **3** (18 mg, t_R = 17.5 min) and **4** (11 mg, t_R = 18.0 min) from fraction ADD (70 mg).

Colocasinol A (1). Yellowish gum. $[\alpha]_D^{25}$ −12.4 (*c* 0.10, MeOH). IR (KBr) ν_{max}: 3399 (OH), 1648 (C=C), 1454 cm^{-1} (aromatic rings). UV (MeOH) λ_{max} (log ε): 204 (4.3), 230 (3.7), 281 nm (3.3). CD (MeOH): $[\theta]_{208}$ +59 400, $[\theta]_{222}$ −6600, $[\theta]_{234}$ −15 300, $[\theta]_{277}$ +5100. ^1H and ^{13}C NMR spectra: see **Table 1**. Fast atom bombardment mass spectrometry (FABMS) (positive-ion mode) *m*/*z*: 599 $[M + H]^+$. HR-FABMS (positive-ion mode) *m*/*z*: 599.2472 $[M + H]^+$ (Calcd for $C_{32}H_{39}O_{11}$, 599.2492).

***cis*-Grossamide K (2).** Amorphous gum. $[\alpha]_D^{25}$ +91.5 (*c* 0.30, $CHCl_3$). IR (KBr) ν_{max}: 3356 (N−H), 2938, 1650 (C=O), 1598, 1510 (C=C), 1026 cm^{-1}. UV (MeOH) λ_{max} (log ε): 225.3 (3.2), 284.2 (1.8), 310.0 nm (1.7). CD (EtOH): $[\theta]_{213}$ +3600, $[\theta]_{234}$ −12 300, $[\theta]_{255}$ +4500. ^1H NMR

Table 1. ^{1}H and ^{13}C NMR Data for Compound **1** (δ in ppm, 500 MHz for ^{1}H and 125 MHz for ^{13}C, in CD_3OD)[a]

	1	
position	δ_H	δ_C
1	3.15 m	54.1
2	3.90 dd (9.0, 4.0), 4.28 m	71.5
3		
4	4.75 d (4.0)	86.3
5	3.15 m	54.5
6	3.90 dd (9.0, 4.0), 4.28 m	71.7
7		
8	4.73 d (4.0)	86.1
1′		135.0
2′	6.69 s	103.1
3′		153.3
4′		137.7
5′		153.3
6′	6.69 s	103.1
1″		137.8
2″	6.79 d (1.5)	118.9
3″		147.9
4″		146.2
5″	6.95 d (8.0)	109.8
6″	6.84 dd (8.0, 1.5)	114.4
1‴		132.6
2‴	6.98 d (1.5)	110.3
3‴		147.4
4‴		145.7
5‴	6.80 d (8.0)	114.9
6‴	6.77 dd (8.0, 1.5)	119.5
7‴	4.91 d (3.3)	72.9
8‴	4.26 m	86.3
9‴	3.62 m, 3.89 m	60.5
4′-OCH_3	3.88 s	60.6
3′,5′-OCH_3	3.84 s	55.5
3″-OCH_3	3.88 s	55.2
3‴-OCH_3	3.84 s	55.1

[a] *J* values are in parentheses and reported in Hz. The assignments were based on ^{1}H−^{1}H COSY, HSQC, and HMBC experiments.

(500 MHz, CD_3OD) δ: 7.21 (1H, s, H-4), 7.05 (1H, s, H-6), 6.96 (2H, d, J = 8.0 Hz, H-2‴″, 6‴″), 6.92 (1H, d, J = 2.0 Hz, H-2′), 6.82 (1H, dd, J = 7.7, 2.0 Hz, H-6′), 6.75 (1H, d, J = 7.7 Hz, H-5′), 6.68 (2H, d, J = 8.0 Hz, H-3‴″, 5‴″), 6.65 (1H, d, J = 12.5 Hz, H-1‴), 5.86 (1H, d, J = 12.5 Hz, H-2‴), 5.57 (1H, d, J = 6.4 Hz, H-2), 3.86 (3H, s, 7-OCH_3), 3.89−3.79 (2H, m, H-1″), 3.78 (3H, s, 3′-OCH_3), 3.52 (1H, q, J = 6.4 Hz, H-3), 3.48 (2H, m, H-1‴′), 2.68 (2H, t, J = 7.0 Hz, H-2‴′). ^{13}C NMR (125 MHz, CD_3OD) δ: 170.3 (CONR), 157.0 (C-4‴″), 150.1 (C-8), 149.2 (C-3′), 147.4 (C-4′), 145.2 (C-7), 138.4 (C-1‴), 134.5 (C-1′), 131.3 (C-1‴″), 130.8 (C-9), 130.5 (C-2‴″, 6‴″), 129.7 (C-5), 122.4 (C-2‴), 120.2 (C-6), 119.8 (C-6′), 116.4 (C-3‴″, 5‴″), 116.3 (C-5′), 115.5 (C-4), 110.7 (C-2′), 89.6 (C-2), 65.0 (C-1″), 56.9 (7-OCH_3), 56.5 (3′-OCH_3), 55.2 (C-3), 42.5 (C-1‴′), 35.7 (C-2‴′). FABMS (positive-ion mode) m/z: 514 $[M + Na]^+$. HR-FABMS (positive-ion mode) m/z: 514.1847 $[M + Na]^+$ (Calcd for $C_{28}H_{29}NNaO_7$, 514.1842).

Biological Activity. The inhibitory effect of each compound on melanogenesis was evaluated by quantitating the amounts of melanin production and cell viability in murine melanocyte melan-a cells after treatment with various concentrations of each test compound. The melan-a cells were cultured in RPMI1640 medium with 10% fetal bovine serum, 100 μg/mL streptomycin−penicillin, and 200 nM phorbol 12-myristate 13-acetate conditions at 37 °C in an atmosphere containing 5% CO_2. The melan-a cells were seeded with 1×10^5 cells/well in the 24-well plate. After 1 day, the medium in each well was changed with 990 μL of fresh medium and 10 μL of various concentrations of each compound. Treatment was continued for 3 days at every same time, and media and test samples were renewed every day. The melanin contents were measured using a modification of the methods as described previously (*13*). Briefly, cell pellets were dissolved in 1 mL of 1 N NaOH. The absorbance of melanin was measured at 405 nm using a microplate reader. In this study, phenylthiourea (PTU) was used as the positive control (*14*). PTU inhibits first two rate-limiting steps in the melanogenesis: the hydrolysis of L-tyrosine (metabolic precursor of melanin) and the oxidation of L-dihydroxyphenylalanine (L-DOPA) by tyrosinase. The percentage of viable cells was determined using a crystal violet assay. The cells were stained with 0.1% crystal violet in 10% EtOH. After 5 min of incubation at room temperature, the cells were rinsed 3 times with water. Then, 1 mL of 95% EtOH was added, and the cells were agitated at room temperature for 30 min. The absorption of crystal violet was measured at 570 nm using a microplate reader. The inhibitory activity of melanogenesis was expressed as a percentage of the control (vehicle treatment group).

RESULTS AND DISCUSSION

Isolation and Structure Elucidation of Compounds. The MeOH extract of dried tuber-barks of *C. antiquorum* var. *esculenta* was suspended in distilled water and then partitioned with EtOAc. To identify the active ingredients responsible for antimelanogenic activity, the EtOAc extract was fractionated into five fractions (fractions A−E) by CC and then each fraction was evaluated for an inhibitory effect on melanogenesis and cell viability in melan-a cells (**Figure 1**). Melanin production was inhibited by PTU with effective ratios of 73.5 ± 2.9, 37.3 ± 1.8, and 18.3 ± 4.3% at concentrations of 1, 10, and 100 μg/mL, respectively, in comparison to the control without significant cell death. However, fractions B and C showed high cell toxicity, and fraction E had no inhibitory effect of melanogenesis. Fractions A and D reduced melanin contents, significantly. They revealed effective ratios of 93.6 ± 1.4 and 62.8 ± 0.8% and 63.3 ± 3.1 and 33.7 ± 3.0% at concentrations of 10 and 100 μg/mL, respectively. However, fraction D indicated lower cell viability (86.2 ± 2.6 and 27.2 ± 6.8%) than fraction A (101.1 ± 1.5 and 82.8 ± 4.2%). Therefore, we suggest that fraction A is the active fraction of the EtOAc-soluble fraction of the methanolic extract. The fraction A was separated on a silica gel and C-18 open-column chromatography, followed by preparative HPLC, to afford eight lignans (**1**−**8**), including a new sesquilignan, colocasinol A (**1**), and a new acyclic phenylpropane lignanamide, *cis*-grossamide K (**2**) (**Figure 2**), along with four known compounds (**9**−**12**).

The known compounds were identified as isoamericanol (**3**) (*15*), americanol (**4**) (*15*), 2-hydroxy-3,2′-dimethoxy-4′-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)biphenyl (**5**) (*16*), (−)-pinoresinol (**6**) (*17*), (+)-yangambin (**7**) (*18*), (−)-syringaresinol (**8**) (*19*), kaempferol 3,7-*O*-α-L-dirhamnopyranoside (**9**) (*20*), *trans*-cinnamic acid (**10**) (*21*), β-sitosterol (**11**) (*22*), and *N*-*trans*-feruloyltyramine (**12**) (*23*), by comparison of their spectroscopic data to previously reported values. The absolute configurations of compounds **6**, **7**, and **8** were established on the basis of their optical rotation values: $[\alpha]_D^{25}$ −65 (*c* 0.2, $CHCl_3$) for compound **6**, $[\alpha]_D^{25}$ +45 (*c* 0.1, $CHCl_3$) for compound **7**, and $[\alpha]_D^{25}$ −47 (*c* 0.2, $CHCl_3$) for compound **8**. To the best of our knowledge, the above known compounds (**3**−**12**) were isolated for the first time from *C. antiquorum* var. *esculenta*.

Colocasinol A (**1**) was obtained as an optically active, yellowish gum ($[\alpha]_D^{25}$ −12.4), whose molecular formula was determined to be $C_{32}H_{38}O_{11}$, deduced by the HR-FABMS experiment. The IR spectrum of compound **1** indicated the presence of a hydroxyl group (3399 cm^{-1}) and an aromatic system (1648 cm^{-1}). The ^{1}H NMR spectrum (**Table 1**) of compound **1** showed the presence of five methoxy groups and two sets of aromatic protons at δ 6.79 (1H, d, J = 1.5 Hz, H-2″), 6.84 (1H, dd, J = 8.0, 1.5 Hz, H-6″), and 6.95 (1H, d, J = 8.0 Hz, H-5″) and 6.77 (1H, dd, J = 8.0, 1.5 Hz, H-6‴), 6.80 (1H, d, J = 8.0 Hz, H-5‴), and 6.98 (1H, d, J = 1.5 Hz, H-2‴). Additionally, an aromatic proton at δ 6.69 (2H, s, H-2′) was observed in the ^{1}H NMR spectrum. This further proposed the possibility that compound **1** comprised two

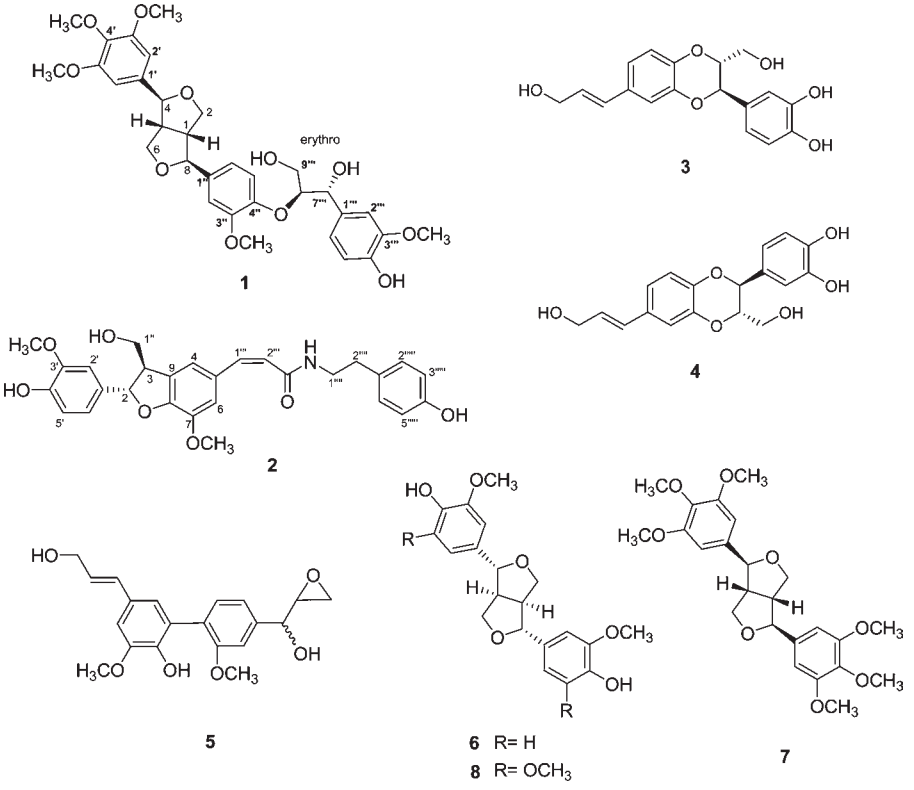

Figure 2. Structures of compounds **1**−**8**.

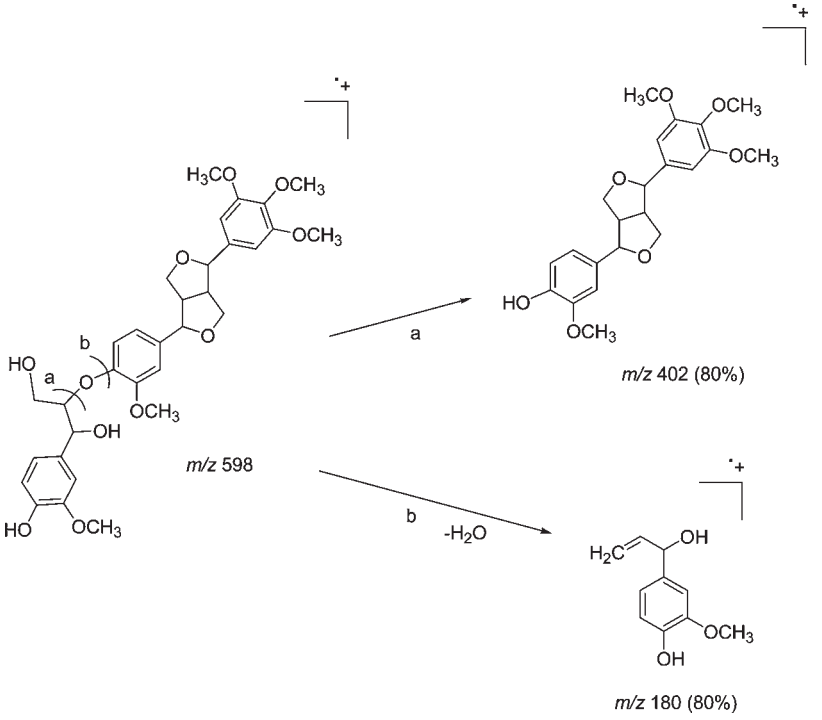

Figure 3. Proposed EIMS fragmentation of compound **1**.

coniferyl and one 3,4,5-trimethoxyl phenylpropanoid residues. The ^{13}C NMR signals at δ 54.1, 54.5, 71.5, 71.7, 86.1, and 86.3 were typical of the furofuran lignan with 2,6-diequatorial diaryl substitution containing different aryl groups (*24*, *25*). The ^{1}H and ^{13}C NMR (**Table 1**) signals at δ_H 4.91 (d, J = 3.3 Hz, H-7‴), 4.26 (m, H-8‴), 3.89 (m, H-9‴a), and 3.62 (m, H-9‴b) and δ_C 72.9 (C-7‴), 86.3 (C-8‴), and 60.5 (C-9‴) indicated the presence of the glyceryl moiety, which was very similar to that of 1-aryl glycerol with phenoxy linked at C-2, similar to buddlenol C (*26*) and carinatidiol (*27*). Overall, the NMR data of compound **1** was almost the same as those of ficusesquilignan A, except for addition of one methoxy group in compound **1** (*24*). The major fragment peaks at m/z 402 and 180 in the electron impact mass spectrometry (EIMS) of compound **1** suggested that compound **1** was composed of the furofuran lignan ($C_{22}H_{26}O_7$) and coniferyl alcohol ($C_{10}H_{12}O_3$), as shown in **Figure 3**. The above evidence

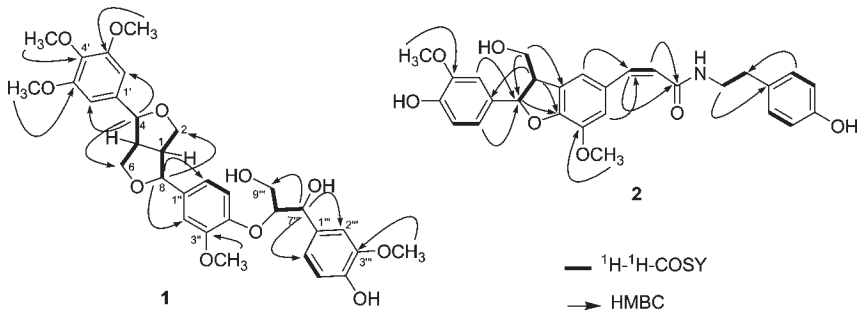

Figure 4. Key ^{1}H−^{1}H COSY and HMBC correlations of compounds **1** and **2**.

was reconfirmed by the HMBC spectrum (**Figure 4**). On the basis of the above consideration and analysis of 2D NMR experiments (^{1}H−^{1}H COSY, HSQC, and HMBC), the planar structure of compound **1** was established. Furthermore, the small coupling constants (J = 4.0 Hz) of H-4 (δ_H 4.75) and H-8 (δ_H 4.73) and the chemical shifts for bridge carbons (C-1/C-5, δ_C 54.1/54.5) indicated that two aryl substituents are equatorial in compound **1** (*24*, *25*, *28*). This was further confirmed by the cross-peaks from H_{ax}-4 (δ_H 4.75) to H_{ax}-2 and H_{ax}-6 (δ_H 3.90) and from H_{ax}-8 (δ_H 4.73) to H_{ax}-2 and H_{ax}-6 (δ_H 3.90) in the nuclear Overhauser effect spectrometry (NOESY) spectrum. The similarity between the characteristic CD curve (λ_{max} = 208 nm for the strong positive and 277 nm for the weak positive) of compound **1** and those of (+)-aschantin and (+)-yangambin (*29*) revealed that the absolute configuration of the furofuran unit was 1*R*,4*S*,5*R*,8*S*. The small coupling constant (J = 3.3 Hz) observed between H-7‴ and H-8‴ and the chemical shift of C-7‴ (δ_C 72.9) of the glycerol moiety indicated that it existed as the relative *erythro* configuration (*24*, *25*, *30*, *31*). The CD spectrum of compound **1** also showed negative Cotton effects at 234 nm, indicating that the absolute configurations at C-7‴ and C-8‴ of compound **1** were to be 7‴*S* and 8‴*R* form (*31*−*33*). Thus, the structure of compound **1** was determined as shown in **Figure 2** and named as colocasinol A.

cis-Grossamide K (**2**) was obtained as an amorphous gum. The molecular formula of compound **2** was determined to be $C_{28}H_{29}NO_7$, deduced by the HR-FABMS experiment. The ^{1}H and ^{13}C NMR spectrum of compound **2** indicated the presence of a tyramine moiety, tri-substituted aromatic group, and tetra-substituted aromatic group from the signals at δ_H 6.96 (2H, d, H-2‴′, 6‴′), 6.68 (2H, d, H-3‴′, 5‴′), 3.48 (2H, m, H-1‴′), 2.68 (2H, t, H-2‴′) and δ_C 157.0 (C-4‴′), 131.3 (C-1‴′), 130.5 (C-2‴′, 6‴′), 116.4 (C-3‴′, 5‴′), 42.5 (C-1‴′), 35.7 (C-2‴′), the signals at δ_H 6.92 (1H, d, H-2′), 6.82 (1H, dd, H-6′), 6.75 (1H, d, H-5′) and δ_C 149.2 (C-3′), 147.4 (C-4′), 134.5 (C-1′), 119.8 (C-6′), 116.3 (C-5′), 110.7 (C-2′), and the signals at δ_H 7.21 (1H, s, H-4), 7.05 (1H, s, H-6) and δ_C 150.1 (C-8), 145.2 (C-7), 130.8 (C-9), 129.7 (C-5), 120.2 (C-6), 115.5 (C-4), respectively. These ^{1}H and ^{13}C NMR spectra of compound **2** were very similar to those of grossamide K (*34*), except for the chemical shifts at C-1‴ (δ_C 138.4) and C-2‴ (δ_C 122.4) in compound **2**. The ^{1}H NMR spectrum of compound **2** showed signals of the olefinic protons at δ 6.65 (H-1‴) and 5.86 (H-2‴) and the coupling constant (J = 12.5 Hz) of the olefinic protons, which indicated that compound **2** possessed *cis*-olefinic protons, in comparison to the reported data for *trans*-olefinic ones (δ_H 6.48, 7.41; J = 15.7 Hz) (*35*). The structural proof was reconfirmed by the ^{1}H−^{1}H COSY and the HMBC spectrum (**Figure 4**). The chemical shifts at C-2 and C-3 (δ_H 5.57/δ_C 89.6 and δ_H 3.52/δ_C 55.3) and their relatively small coupling constant (J = 6.4 Hz) suggested that these protons possessed *trans* configuration (*34*−*36*). The similar CD data (λ_{max} = 213 nm for the first positive, 234 nm for the second negative, and 255 nm for the third positive) of compound **2** ($[\alpha]_D^{25}$ +91.5) as those of the known corresponding dihydrobenzo-[*b*]furans (*37*) revealed that the absolute configuration at C-2 and C-3 was 2*S*,3*R*. On the basis of these findings, the structure of compound **2** was assigned and named as *cis*-grossamide K. A survey of the literature revealed that compound **2** was the geometric isomer of lignanamide, isolated from *Hibiscus cannabinus* (*34*).

Table 2. Effect of Compounds **1**−**12** and PTU on Melanin Synthesis and Cell Viability in Melan-a Cells[a]

compound	melanin synthesis (IC_{50}, μM)	cell viability (LD_{50}, μM)
1	>500	>500
2	54.24	163.60
3	43.72	69.51
4	38.33	81.66
5	53.49	110.23
6	56.26	>500
7	>500	230.34
8	>500	>500
9	>500	>500
10	>500	499.71
11	362.04	374.86
12	215.72	334.91
PTU	49.08	>500

[a] PTU was used as the positive control in this study. The inhibitory activity of melanogenesis was expressed as an IC_{50} level, and the effect of each compound on cell viability was determined as an LD_{50} level.

Antimelanogenic Evaluation of Compounds. On the basis of the determination of melanin contents and cell viability, the antimelanogenic effects of compounds (**1**−**12**) isolated from *C. antiquorum* var. *esculenta* were tested in cultured murine melanocyte melan-a cells. PTU was used as a positive control in these studies because of its known inhibitory effect on melanin synthesis (*14*). The melanin contents were measured using a modification of the methods as described previously (*13*). As shown in **Table 2**, the antimelanogenic activity and the effect on cell viability were expressed as 50% inhibition concentration (IC_{50}) and 50% lethal dose (LD_{50}) levels. PTU showed a low IC_{50} level of 49.08 μM with high LD_{50} levels (> 500 μM). However, compounds **1** and **7**−**12** exhibited high IC_{50} levels. This result indicated that these compounds were unsuitable as depigmenting agents. Of the isolates, isoamericanol (**3**) and americanol (**4**) had lower IC_{50} levels (43.72 and 38.33 μM) than other compounds. However, they also showed low LD_{50} levels of 69.51 and 81.66 μM. *cis*-Grossamide K (**2**), 2-hydroxy-3,2′-dimethoxy-4′-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)-biphenyl (**5**), and (−)-pinoresinol (**6**) had IC_{50} levels of 54.24, 53.49, and 56.26 μM in comparison to their LD_{50} levels of 163.60, 110.23, and > 500 μM. Therefore, we suggest that

compounds **2**−**6**, especially compounds **2**, **5**, and **6**, which did not induce much cell death at high concentrations, possessed the potential to be used as depigmenting agents and could be candidates for the treatment of melanogenic skin diseases or whitening cosmetics improving hyperpigmentation.

Interestingly, although the structures of compounds **6** and **8** are quite similar, except of the position of the methoxy group, the inhibitory effect showed a big difference between them. The obtained antimelanogenic data suggested that the appearance of a 3,4-disubstituted phenyl group within lignan structures is important for its antimelanogenic activity. This was further supported from the observation that compounds **2**−**4** similarly possessing a 3,4-disubstituted phenyl group also exhibited a significant inhibitory effect. Taken together, it was indicated that the presence of an additional methoxy group at C-5 of the phenyl group might decrease the inhibitory activity, because compounds **1**, **7**, and **8** were inactive. In addition, it appears that the 1-hydroxy-prop-2-enyl moiety at the phenyl group improves antimelanogenic activity, because compounds **3**−**5** with the moiety were active compounds.

In conclusion, this study indicates that lignan derivatives are the main components of the tuber-barks of *C. antiquorum* var. *esculenta*. Additionally, a new sesquilignan, colocasinol A (**1**), and a new acyclic phenylpropane lignanamide, *cis*-grossamide K (**2**), were isolated from this plant source. Five lignans **2**−**6**, which significantly inhibited melanin production in melan-a cells, were also investigated. The present study thus indicates that these compounds would be good candidates for further research as skin-whitening agents and shows the potential of *C. antiquorum* var. *esculenta* as a functional food with a depigmenting effect.

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Supporting Information Available: One-dimensional (^{1}H and ^{13}C NMR) and two-dimensional NMR (^{1}H−^{1}H COSY, HSQC, and HMBC) data of compounds **1** and **2** and the structures of known compounds (**9**−**12**). This material is available free of charge via the Internet at http://pubs.acs.org.

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