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 sesquiglignan, named colosocasinol 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 (IC50 = 54.24, 53.49, and 56.26 μM, and LD50 = 163.60, 110.23, and >500 μM, respectively).

**KEYWORDS:** *Colocasia antiquorum* var. *esculenta*; Araceae; lignan; sesquiglignan; 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 anti-fungal 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.
was subjected to reversed-phase (RP)-C18 silica gel (230–400 mesh) and then partitioned with EtOAc at room temperature to give an EtOAc extract. The resultant MeOH extract (350 g) was suspended in distilled water (15 L) and then subjected to LPLC on a 240 μm column (Alltech, Nicholasville, KY) with a solvent system of MeOH/H2O (1:1; flow rate, 2 mL/min) as the eluent to yield four fractions (fractions ADB1–ADB6). Fraction ADB3 (150 mg) was purified by preparative RP HPLC using a solvent system of MeCN/H2O (3:7) as the eluent to yield compound 5 (4 mg, tR = 15.5 min) and 8 (7 mg, tR = 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 MeOH/H2O (1:1; flow rate, 2 mL/min) as the eluent to afford four fractions (fractions ADB1–ADB4). Fraction ADB2 (60 mg) was purified further by preparative RP HPLC using a solvent system of MeCN/H2O (3:7) as the eluent to obtain five fractions (7 mg, tR = 17.0 min and 9 mg, tR = 18.5 min). Fraction ADB3 (90 mg) was subjected to 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/H2O (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/H2O (3:7) as the eluent to obtain compounds 5 (7 mg, tR = 17.0 min) and 8 (7 mg, tR = 18.5 min). Fraction ADC3 (90 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 MeCN/H2O (3:7) as the eluent to furnish four fractions (fractions ADC1–ADC4). Fraction ADC2 (60 mg) was purified further by preparative RP HPLC using a solvent system of MeCN/H2O (3:7) as the eluent to obtain compounds 5 (7 mg, tR = 17.0 min) and 8 (7 mg, tR = 18.5 min). Fraction ADC3 (90 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 MeCN/H2O (3:7) as the eluent to furnish two subfractions. The subfractions were purified further by preparative RP HPLC using a solvent system of MeCN/H2O (4:6) as the eluent to give compound 1 (6 mg, tR = 14.0 min) and purified further by preparative RP HPLC using a solvent system of MeCN/H2O (3:7) as the eluent to yield compounds 2 (7 mg, tR = 15.5 min) and 12 (8 mg, tR = 17.0 min), respectively. Fraction ADC4 (40 mg) was purified by preparative RP HPLC using a solvent system of MeCN/H2O (3:7) as the eluent to furnish compounds 6 (8 mg, tR = 16.5 min) and 10 (6 mg, tR = 18.5 min). Final purification was performed on preparative RP HPLC using a solvent system of MeOH/H2O (1:1) as the eluent to obtain compounds 3 (18 mg, tR = 17.5 min) and 4 (11 mg, tR = 18.0 min) from fraction ADE (70 mg).

Colocasinol A (1). Yellowish gum. [α]D 20 +12.4 (c 0.10, MeOH). IR (KBr) νmax cm⁻¹: 3399 (OH), 1648 (C=C), 1454 (aromatic rings). UV (MeOH) λmax (log ε): 204 (4.3), 230 (3.7), 281 nm (3.3). CD (MeOH): [θ]208 +59 400, [θ]222 −6600, [θ]234 +15 300, [θ]277 +5100. 1H and 13C 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: 5992472 [M + H]+ (Calcd for C14H26O12, 5992492).

cis-Grossamidine K (2). Amorphous gum. [α]D 20 +91.5 (c 0.30, CHCl3). IR (KBr) νmax cm⁻¹: 3356 (N–H), 2936, 1650 (C=O), 1598, 1510 (C=C), 1026 cm⁻¹. UV (MeOH) λmax (log ε): 225.3 (3.2), 284.2 (1.8), 310.0 nm (1.7). CD (EtOH): [θ]213 +3600, [θ]234 −12300, [θ]255 +4500. 1H NMR...
pellets were dissolved in 1 mL of 1 N NaOH. The absorbance of melanin and 10 modification of the methods as described previously (1).

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 (Figure 2), along with four known compounds (9−12).

The known compounds were identified as isoamericanol (3) (15), americananol (4) (15), 2-hydroxy-3,2'-dimethoxy-4,4'- (3,4-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)bibenyl (5) (16), (−)-pinoselol (6) (17), (−)-yangambin (7) (18), (−)-syringaresinol (8) (19), kaempferol 3-O-Ar-dirhamnopyranoside (9) (20), trans-cinnamic acid (10) (21), β-sitosterol (11) (22), and N-trans-feruloyltartrazine (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: [α]D20 = −65 (c 0.2, CHCl3) for compound 6, [α]D20 = 45 (c 0.1, CHCl3) for compound 7, and [α]D20 = −47 (c 0.2, CHCl3) 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.

Colocasolin A (1) was obtained as an optically active, yellowish gum ([α]D20 = −12.4), whose molecular formula was determined to be C32H38O11, 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 1H 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-9′), 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 1H NMR spectrum. This further proposed the possibility that compound 1 comprised two
coniferyl and one 3,4,5-trimethoxy phenylpropanoid residues. The $^{13}$C NMR signals at $\delta$ 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 $\delta_H$ 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 $\delta_C$ 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 ficussequilignan 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
The small coupling constant (absolute configuration of the furofuran unit was 1H ax-4 (δ_H 4.75) and 13C NMR spectrum of compound 1 (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 Hax-4 (δ_H 4.75) to Hax-2 and Hax-6 (δ_H 3.90) and from Hax-8 (δ_H 4.73) to Hax-2 and Hax-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 1R,4S,5R,8S.

The small coupling constant (J = 3.3 Hz) observed between H-7′′′ and H-8′′′ and the chemical shift of C-7′′′ (δ_C 116.3 (C-5), 120.2 (C-6), 115.5 (C-4)), respectively. These signals at δ_C 116.3 (C-5), 120.2 (C-6), 115.5 (C-4) suggested the configuration (cis) of the 1H COSY and the chemical shift of C-7′′′ (δ_C 116.3 (C-5), 120.2 (C-6), 115.5 (C-4)) indicated the presence of -olefinic protons, in comparison to the reported data (1H) COSY and the HMBC spectra 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 colcosanol A.

cis-Grossamide K (2) was obtained as an amorphous gum. The molecular formula of compound 2 was determined to be C_{28}H_{33}NO_{5}, deduced by the HR-FABMS experiment. The 1H and 13C 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′′′′), 6.68 (2H, d, H-3′′′′), 6.70 (2H, d, H-4′′′′), 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 1H and 13C NMR spectra of compound 2 were very similar to those of grossamide K (34), except for the chemical shifts at C-1′′′′′′′ (δ_C 134.0) and C-2′′′′′′′ (δ_C 122.4) in compound 2. The 1H 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 1H-1H COSY and the HMBC spectrum (Figure 4).

The chemical shifts at C-2 and C-3 (δ_C 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 ((α)_D +91.5) as those of the known corresponding dihydrobenzo[θ]furans (37) revealed that the absolute configuration at C-2 and C-3 was 2S,3R. 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).

### 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

<table>
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<tr>
<th>Compound</th>
<th>Melanin Synthesis (IC_{50}, μM)</th>
<th>Cell Viability (LD_{50}, μM)</th>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>54.24</td>
<td>163.60</td>
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<tr>
<td>3</td>
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<td>12</td>
<td>215.72</td>
<td>334.91</td>
</tr>
<tr>
<td>PTU</td>
<td>49.08</td>
<td>&gt;500</td>
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</table>

*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.
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 presence of an additional methoxy group at C-5 of the phenyl group improves the 1-hydroxy-prop-2-enyl moiety at the phenyl group improves C. antiquorum the 1-hydroxy-prop-2-enyl moiety at the phenyl group improves C. antiquorum's antimelanogenic activity. This was further obtained antimelanogenic data suggested that the appearance of the 3,4-disubstituted phenyl group within lignan structures is important for its antimelanogenic activity. This was further obtained antimelanogenic data suggested that the appearance of the 3,4-disubstituted phenyl group also exhibited an inhibitory effect showed a big difference between them. The aromatic properties (1H and 13C NMR) and two-dimensional NMR (1H 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.

LITERATURE CITED


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