DOI 10.5012/bkcs.2010.31.7.2051

Anti-melanogenic Fatty Acid Derivatives from the Tuber-barks of *Colocasia antiquorum* var. *esculenta*

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Received April 6, 2010, Accepted May 4, 2010

Key Words: *Colocasia antiquorum* var. *esculenta*, Araceae, Monoglyceride, Melan-a cells, Anti-melanogenic activity

As part of our continuing search for biologically active compounds from natural medicinal plant sources,[1-3] we investigated the anti-melanogenic constituents from thetuber-barks of *Colocasia antiquorum* var. *esculenta*, since the methanolic extract of tuber-barks of *C. antiquorum* var. *esculenta* showed inhibitory effects on melanin production in melan-a cells. *C. antiquorum* var. *esculenta* (Araceae) is widely spread in Korea, and is a variant of *C. antiquorum*, commonly known as 'Imperial Taro', exhibiting antifungal activity.[4] We have recently reported the lignan derivatives with anti-melanogenic activity from this source.[5] In a continuing study on this source, we have further isolated a new monoglyceride, (2'*S*)-1-*O*-(9-oxo-10(*E*),12(*E*)-octadecadienoyl) glycerol (**1**) (Figure 1), as well as nine fatty acid derivatives (**2-10**) from the EtOAc soluble fraction of its MeOH extract using a bioassay-guided column chromatographic separation (Figure 2). Here, we describe the isolation and structural elucidation of **1** as well as the anti-melanogenic activities of the isolated compounds (**1-10**).

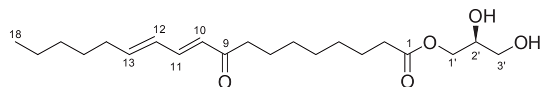

Figure 1. Chemical structure of **1**.

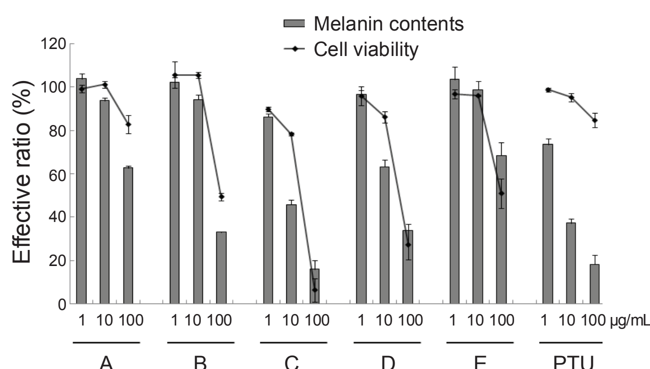

Figure 2. The effect of fractions A, B, C, D and E from the EtOAC soluble fraction of the methanolic extract of tuber-barks of *C. antiquorum* var. *esculenta* on melanogenesis in melan-a cells. Test samples were tested at three concentrations of 1, 10 and 100 μg/mL. PTU (1-phenyl-2-thiourea) was used as positive control. Test sample and medium were renewed daily. The cell viabilities and the melanin contents of melan-a cells were determined after 3 days. Inhibitory activity of melanogenesis and the effect of cell viability were expressed as a percentage of the control. The data shown represent the means ± SD of three independent experiments performed in duplicate.

Compound **1** was obtained as an amorphous gum with optical rotation $[\alpha]_D^{25}$ +4.28° (*c* 0.12, MeOH). The molecular formula was determined to be $C_{21}H_{36}O_5$ by HREIMS: observed, 368.2549 (calcd. for $C_{21}H_{36}O_5$, 368.2563). The ^{1}H NMR spectrum of **1** displayed signals for the presence of two conjugated *E*,*E*-form enone system at δ 7.24 (dd, J = 11.5, 15.5 Hz), 6.28 (dd, J = 11.5, 15.0 Hz), 6.27 (dd, J = 5.5, 15.0 Hz), and 6.13 (d, J = 15.5 Hz). Furthermore, the UV spectrum of **1** displayed a λ_{max} at 276 nm. The ^{1}H NMR spectrum also showed a triplet peak at δ 0.92 (H-18), a multiplet signal at δ 1.32-1.35 (H-4, 5, 6, 16, 17), and two triplets at δ 2.36 (H-2) and 2.60 (H-8). The ^{13}C NMR spectrum (including DEPT and HMQC) exhibited 21 carbon signals, including two carbonyl carbons at δ 202.7 (C-9) and 174.3 (C-1), four olefinic carbons at δ 146.2 (C-13), 144.1 (C-11), 129.0 (C-12), and 127.6 (C-10). The NMR spectral data of **1** were very similar to those of 9-oxo-10(*E*),12(*E*)-octadecadienoic acid.[6] Besides, an additional presence of a glycerol moiety [δ_H 4.16 (1H, dd, J = 11.6, 4.6 Hz), 4.08 (1H, dd, J = 11.6, 6.3 Hz), 3.82 (1H, m), 3.57 (1H, dd, J = 11.3, 5.3 Hz), 3.55 (1H, dd, J = 11.3, 5.7 Hz); δ_C 70.0 (C-2'), 65.3 (C-1'), 62.9 (C-3')] was observed.[7] The connectivity of **1** was confirmed by the HMBC spectrum, in which cross-peaks between H-1'/C-1, H-7/C-9, H-11/C-9, H-15/C-13, and H-18/C-16 were observed (Figure 3). The position of the dienone moiety was further confirmed by analysis of the mass fragmentation in the HREIMS data (Figure 3). Based on these data, the planar structure of **1**

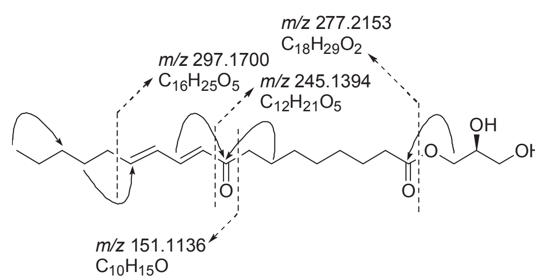

Figure 3. Key HMBC connectivities and HREIMS fragmentation of **1**.

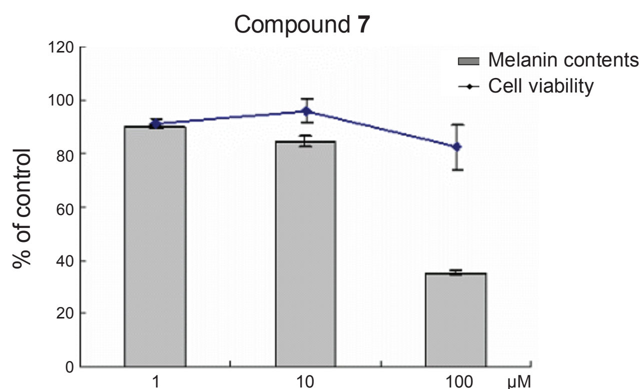

Figure 4. The effect of compound **7** on melanogenesis in melan-a cells. Compound **7** was tested at three concentrations of 1, 10 and 100 μM. Compound **7** and medium were renewed daily. The cell viabilities and the melanin contents of melan-a cells were determined after 3 days. Inhibitory activity of melanogenesis and the effect of cell viability were expressed as a percentage of the control. The data shown represent the means ± SD of three independent experiments performed in duplicate.

was determined to be 1-*O*-(9-oxo-10,12-octadecadienoyl) glycerol. The stereochemistries at C-10/11 and C-12/13 were determined to be 10(*E*) and 12(*E*), respectively, on the basis of the *J* value and comparison of chemical shift pattern at olefinic protons of related unsaturated fatty acids.[6,8] The absolute configuration at C-2' was determined using a dibenzoate chirality method.[9] The hydroxyl groups at C-2' and C-3' of **1** were esterified with benzoyl chloride in dry pyridine to obtain **1a**. The CD spectrum of **1a** exhibited a positive exciton couplet CD peak at 235 nm to reflect its 2'*S* configuration.[9] The optical rotation value of **1** ($[\alpha]_D^{25}$ +4.28°) was also in agreement with that of a synthetic monoglyceride in the *S*-form.[7] Thus, the structure of **1** was determined to be (2'*S*)-1-*O*-(9-oxo-10(*E*), 12(*E*)-octadecadienoyl) glycerol. Although the free fatty acid moiety of **1** has already been reported,[6] this is the first isolation of the monoglyceride (**1**) from natural sources.

The known compounds were identified as (*R*)-(-)-9-hydroxydecenoic acid (**2**),[10,11] (*R*)-(-)-9-hydroxydecanoic acid (**3**),[11] (2*E*,4*S*)-4-hydroxy-2-nonenoic acid (**4**),[12] (*S*)-15,16-didehydrocoriolic acid (**5**),[13] 1-*O*-(octanoyl) glycerol (**6**),[14] 12,13-epoxyoctadec-9(*Z*)-enoic acid (**7**),[15,16] (9*S*,10*E*,12*Z*)-10,12-octadecadienoic acid methyl ester (**8**),[8] (9*S*,10*E*,12*Z*)-10,12-octadecadienoic acid (**9**),[8] and 12,13-epoxyoctadec-6(*Z*),9(*Z*)-dienoic acid (**10**),[17] respectively, by comparison of their physical, NMR and MS spectral data reported in previous literature. The compound **4**, (2*E*,4*S*)-4-hydroxy-2-nonenoic acid was isolated for the first time from natural sources, although previously synthesized.[12]

We evaluated the inhibitory effects of all the isolated compounds (**1**-**10**) on melanogenesis by examining their effects on melanin biosynthesis in melan-a cells. The melan-a cells were treated with various concentrations (1, 10, or 100 μM) of each test compound for 3 days. Compounds **2**, **3**, **4** and **9** did not affect melanogenesis in melan-a cells. But, compound **7** dose-dependently inhibited melanin content without significant cell toxicity. At a concentration of 100 μM, compound **7** strongly inhibited the melanin biosynthesis with an effective ratio of 35.43 ± 0.79% in comparison to the control (Figure 4). Monoglyceride (**6**) was also active in the inhibition of melanin content at a concentration of 10 μM, although it exhibited cell toxicity at higher concentrations (see the Supporting Information). The compound **10**, which contained an epoxy group, slightly reduced melanin contents but displayed cell toxicity at higher concentrations.

It was recently reported that 6-*O*-undecylenoyl *p*-hydroxyphenyl β-D-glucopyranoside, which was synthesized by transesterification of arbutin and undecylenic acid vinyl ester, more significantly suppressed melanin production in murine B16 melanoma cells than arbutin.[18] Several hydroquinone fatty acid esters have been investigated for the development of better depigmenting agents than hydroquinones.[19] The active fatty acids (**6**, **7**, and **10**) isolated from this source could be used as novel tools for the synthesis of effective depigmenting agents and also as good candidates for cosmetic development.

Experimental Section

General procedures. Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. CD spectra were measured on a Jasco J-715 spectropolarimeter. EI and HREI mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector. Low-pressure liquid chromatography was carried out over a Merck Lichroprep Lobar-A Si 60 (240 × 10 mm) with a FMI QSY-0 pump (ISCO). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) and RP-C_{18} silica gel (Merck, 230 - 400 mesh) were used for column chromatography (CC). Merck pre-coated Silica gel F_{254} plates and RP-18 F_{254s} plates were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (vol/vol).

Plant materials. The tuber-barks of *C. antiquorum* var. *esculenta* were collected at Girokdo, Goksung of 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. The resultant methanolic extract (350 g) was suspended in distilled water (15 L) and then successively partitioned with EtOAc to give an EtOAc extract (36 g). The EtOAc soluble fraction (36 g) was chromatographed on an RP-C_{18} silica gel (230 - 400 mesh, 500 g), eluting with gradient solvent system of MeOH/H_2O (3:2 and 1:0, vol/vol, 500 mL each). According to TLC analysis, five crude fractions (fr. A - E) were collected. All fractions were evaluated on anti-melanogenic activities in melan-a cells (Figure 2). Fr. C (3 g), which showed the most significant inhibitory activity among the five fractions tested, was applied to CC on a silica

Table 1. ^1H and ^{13}C NMR data of **1** (δ in ppm, 500 MHz for ^1H and 125 MHz for ^{13}C, in CD_3OD)

Atom no.	^1H NMR			^{13}C NMR
	δ	m	*J* (Hz)	δ (DEPT)
1				174.3 (C)
2	2.36	t	7.0	33.7 (CH_2)
3	1.62	m		24.7 (CH_2)
4	1.32-1.35	m		28.8[a] (CH_2)
5	1.32-1.35	m		28.8[a] (CH_2)
6	1.32-1.35	m		28.9[a] (CH_2)
7	1.62	m		24.3 (CH_2)
8	2.60	t	7.5	39.8 (CH_2)
9				202.7 (C)
10	6.13	d	15.5	127.6 (CH)
11	7.24	dd	11.5, 15.5	144.1 (CH)
12	6.28	dd	11.5,. 15.0	129.0(CH)
13	6.27	dd	5.5, 15.0	146.2 (CH)
14	2.21	m		32.9 (CH_2)
15	1.46	m		28.3 (CH_2)
16	1.32-1.35	m		31.3 (CH_2)
17	1.32-1.35	m		22.3 (CH_2)
18	0.92	t	7.0	13.1 (CH_3)
1'	4.16 4.08	dd dd	4.6, 11.6 6.3, 11.6	65.3 (CH_2)
2'	3.82	m		70.0 (CH)
3'	3.57 3.55	dd dd	5.3, 11.3 5.7, 11.3	62.9 (CH_2)

[a]Interchangeable signals.

gel (230 - 400 mesh, 80 g), eluting with solvent system of $CHCl_3$/MeOH (10:1, vol/vol, 500 mL) to give three subfractions (fr. C1 - C3). Among three subfractions, fr. C2 (1.3 g), which showed the most significant inhibitory activity, was applied to CC on an RP-C_{18} silica gel (230 - 400 mesh, 50 g), eluting with solvent system of MeOH/H_2O (4:1, vol/vol, 500 mL) to afford five subfractions (fr. C21 - C25). Fr. C23 (200 mg) was further purified by LPLC on Lichroprep Lobar-A Si 60 column (5 mL/min; eluted with *n*-hexane/EtOAc, 1:1, vol/vol), and preparative reversed-phase HPLC, using a solvent system of 63% MeCN over 30 min at a flow rate of 2.0 mL/min (Econosil RP-18 10 μm column; 250 × 10 mm; Shodex refractive index detector), to yield compound **1** (5 mg). Purification of the known compounds (**2-10**) was described in the Supporting Information.

(2'*S*)-1-*O*-(9-Oxo-10(*E*),12(*E*)-octadecadienoyl) glycerol (1). Amorphous gum, $[\alpha]_D^{25}$ +4.28° (*c* 0.12, MeOH) IR (KBr) ν_{max} 3382, 2950, 1658, 1414 cm^{-1}; UV (MeOH) λ_{max} (log ε), 276 (1.30); ^1H and ^{13}C NMR data see Table 1; EIMS *m/z* 368 $[M]^+$; HREIMS *m/z* (rel. int. %) 368.2549 $[M]^+$ (9, $C_{21}H_{36}O_5$, calcd. for 368.2563), 297.1700 (48), 277.2153 (52), 245.1394 (7), 223.1547 (23), 166.7544 (56), 151.1136 (94), 95.2257 (89), 91.3241 (100).

Synthesis of 1a (benzoyl ester of 1). To synthesize the benzoyl derivative of **1**, an ice-cooled solution of **1** (1.0 mg) in dry pyridine (0.75 mL) was mixed with benzoyl chloride (0.15 mL), and the mixture was stirred at room temperature for 20 h. Two drops of MeOH were added to the reaction mixture, stirred for 10 min, and then diluted with EtOAc and aqueous Na_2CO_3, and the layers were separated. The organic layer was washed with brine, and the combined aqueous layer was extracted with EtOAc. The combined organic extract was dried over $MgSO_4$ and concentrated. The residual dark-brown oil was purified by a silica gel Waters Sep-Pak Vac 6cc with *n*-hexane/EtOAc (4:1, vol/vol) to give **1a** (0.5 mg) as a colorless oil (see the Supporting Information).

Biological activity. The melanin contents were measured using a modification of the methods reported by Hosoi.[20] In this study, phenylthiourea (PTU) was used as the positive control.[21] (see the Supporting Information).

Acknowledgments. The authors would like to thank Mr. Do Kyun Kim, Dr. Eun Jung Bang and Dr. Jung Ju Seo at the Korea Basic Science Institute for the measurements of NMR and MS spectra. This work was supported by the R & D Institute, Miwon Commercial, Co., LTD in Korea.

Supporting Information. NMR data (^1H and ^{13}C NMR) of **1**, purification and chemical structures of known compounds (**2**-**10**), biological activity, and synthesis of **1a** are available on request from the correspondence author.

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