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

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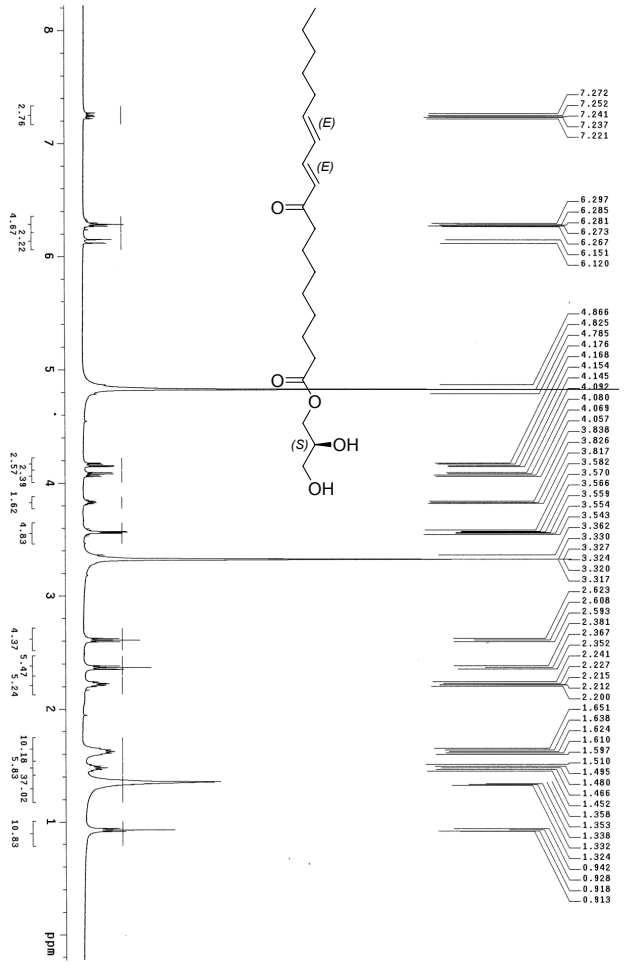
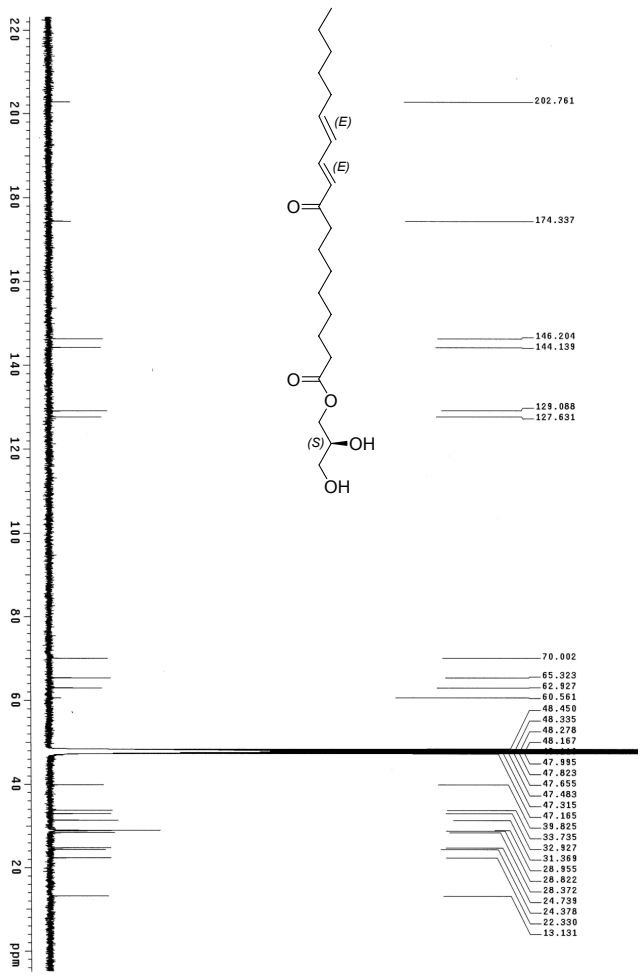
Extraction and Isolation

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. A (7 g) and Fr. D (7 g) showed significant inhibitory activities on melanin



The ^{1}H NMR data of **1** (CD_3OD, 500 MHz)



The ^{13}C NMR data of **1** (CD_3OD, 125 MHz)

Figure S1. The structures of compounds **1**-**10** isolated from *C. antiquorum* var. *esculenta*.

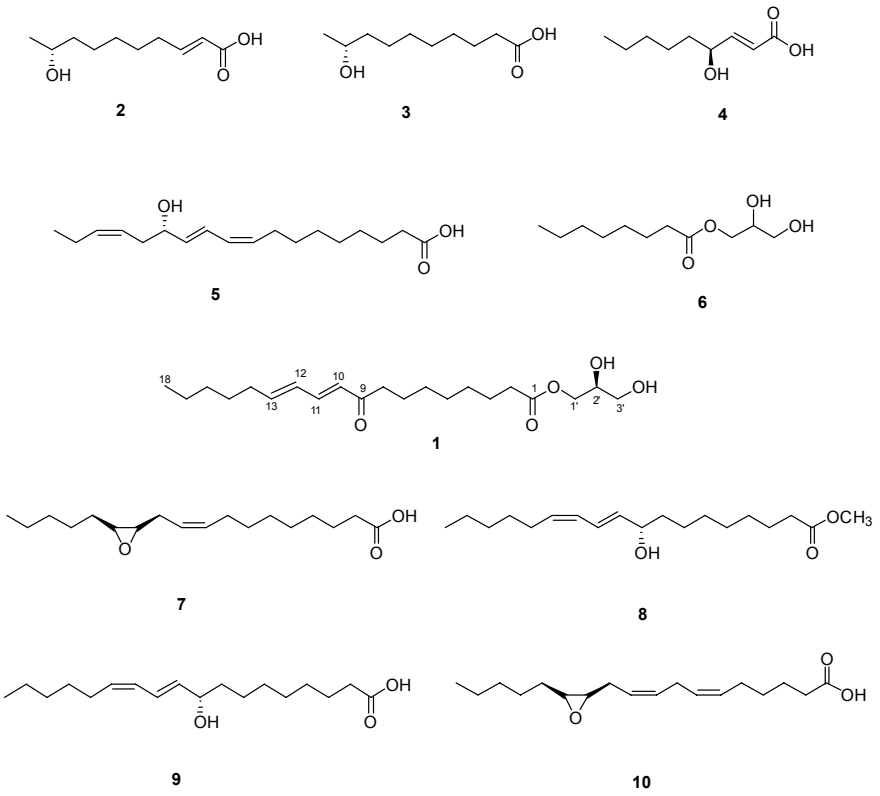


Figure S1. Continued.

production. But Fr. D had cell toxicity more than Fr. A, so we used only Fr. A in this study. Fr. A was applied to CC further on an RP-C_{18} silica gel (230 - 400 mesh, 100 g), eluting with solvent system of MeOH/H_2O (2:3 and 1:0, vol/vol, 500 mL each) to give four subfractions (fr. A1 - A4). Among four subfractions, fr. A4 (1 g) which showed the most significant inhibitory activities on melanin production was applied to CC on an RP-C_{18} silica gel (230 - 400 mesh, 50 g), eluting with solvent system of MeOH/H_2O (2:3 and 1:0, vol/vol, 300 mL each) to afford five subfractions (fr. A41 - A45). The active subfraction A42 (400 mg) was subjected to an RP-C_{18} silica gel CC using solvent system of MeOH/H_2O (1:1, vol/vol, 300 mL) to give six subfractions (A421 - A426). Fr. A422 (100 mg) was purified by reversed-phase MPLC on LiChroprep RP-18 column (250 × 10 mm; 40 - 63 μm particle size; 5 mL/min; eluted with 50% MeOH), and finally, purified by preparative reversed-phase HPLC, using a solvent system of 25% 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 afford compounds **2** (4 mg) and **3** (7 mg), respectively. Fr. A423 (70 mg) was purified by preparative reversed-phase HPLC, using a solvent system of 32% MeCN over 30 min as described above, to yield compound **4** (5 mg). Fr. C (3 g), which showed the most significant inhibitory activities among the five fractions tested (Figure 2), was applied to CC on a silica 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 activities, 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. C22 (100 mg) was separated by MPLC on LiChroprep Si 60 column (250 × 10 mm; 40 - 63 μm particle size; 5 mL/min; eluted with *n*-hexane/EtOAc, 1:1, vol/vol), and finally, purified by preparative reversed-phase HPLC, using a solvent system of 80% MeOH over 30 min as described above, to afford compound **10** (11 mg). Fr. C23 (200 mg) was further purified by MPLC on LiChroprep 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 as described above, to yield compounds **5** (12 mg), **6** (5 mg), and **1** (5 mg), respectively. Fr. C24 (250 mg) was separated by a Sephadex LH-20 CC, using a solvent system of

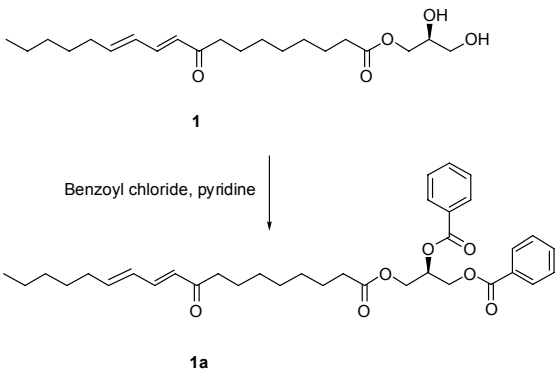


Figure 2S. Synthesis of **1a**

100% MeOH, and purified by preparative HPLC, using a solvent system of *n*-hexane/$CHCl_3$/MeOH (15:5:1, vol/vol/vol) over 30 min at a flow rate of 2.0 mL/min (Apollo Silica 5 μm column; 250 × 10 mm; Shodex refractive index detector) to yield compound **9** (24 mg). Fr. C25 (100 mg) was further purified by MPLC on LiChroprep 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 75% MeCN over 30 min as described above, to yield compounds **7** (10 mg) and **8** (8 mg), respectively.

(2*E*,4*S*)-4-Hydroxy-2-nonenoic acid (4). Colorless gum; $[\alpha]_D^{25}$ +1.43° (*c* 0.10, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.03 (1H, dd, *J* = 15.9, 5.0 Hz), 6.03 (1H, dd, *J* = 15.9, 2.0 Hz), 4.33 (1H, ddt, *J* = 6.5, 5.0, 2.0 Hz), 1.56 (2H, m), 1.32 (6H, br s), 0.91 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 170.7, 151.4, 119.8, 70.4, 36.4, 31.7, 24.9, 22.4, 13.1; FAB-MS (positive mode) *m/z* 195 $[M + Na]^+$.

1a. colorless oil; CD (MeOH) $[\theta]_{219}$ -2300, $[\theta]_{235}$ +8200; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (3H, t, *J* = 7.0 Hz, H-18), 1.31-1.37 (10H, br s, H-4, 5, 6, 16, 17), 1.46 (2H, m, H-15), 1.63 (4H, m, H-3, 7), 2.23 (2H, m, H-14), 2.38 (2H, t, *J* = 7.5 Hz, H-2), 2.60 (2H, t, *J* = 7.5 Hz, H-8), 4.25 (1H, dd, *J* = 7.0, 12.0 Hz, H-1′a), 4.47 (1H, dd, *J* = 4.0, 12.0 Hz, H-1′b), 4.54-4.60 (2H, m, H-3′), 5.05 (1H, m, H-2′), 6.13 (1H, d, *J* = 15.5 Hz, H-10), 6.27-6.29 (2H, m, H-12, 13), 7.25 (1H, m, H-11), 7.49-7.52 (4H, m, Ar-H), 7.60-7.63 (2H, m, Ar-H), 8.01-8.02 (4H, m, Ar-H); FABMS *m/z* 576 $[M]^+$.

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 melanin contents were measured using a modification of the methods as described previously.[20] 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.[21] 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 three 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).

Table S1. The effect of compounds **1-10** isolated from the EtOAc fractions A and C from *C. antiquorum* var. *esculenta*on melanogenesis in melan-a cells. (Fr. A4 and C2 were subfarction of EtOAc fractions A and C, respectively)
Test samples were tested at three concentrations of 1, 10 and 100 μM or 1, 10 and 50 μM. 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.

Sample			Melanin Contents (% of control)	Cell Viability (% of control)
Fr.	Compound	μM		
A4	2	1	91.87 ± 0.37	91.13 ±0.66
		10	97.18 ± 5.56	96.00 ± 0.20
		100	99.36 ± 2.60	99.26 ± 1.25
	3	1	98.11 ± 2.73	104.98 ± 2.78
		10	97.43± 12.89	99.47 ± 0.55
		50	99.42 ± 4.93	114.52 ± 3.21
	4	1	109.57 ± 1.94	105.09 ± 1.95
		10	112.08 ± 0.93	107.89 ± 1.21
		100	97.42 ± 5.81	97.75 ± 8.86
C2	5	1	107.36 ± 3.06	103.44 ±0.44
		10	98.83 ± 3.57	101.00 ± 6.98
		100	53.18 ± 3.06	54.64 ± 2.23
	6	1	87.62 ± 0.99	111.85 ± 4.49
		10	79.91 ± 1.98	90.58 ± 1.38
		50	13.95 ± 4.93	19.43 ± 3.13
	1	1	107.32 ± 1.22	106.18 ± 14.06
		10	99.16 ± 4.13	93.34 ± 2.45
		50	46.22 ± 0.22	37.07 ± 12.31
	7	1	89.97 ± 0.39	91.36 ± 1.68
		10	84.49 ± 1.97	95.95 ± 4.37
		100	35.43 ± 0.79	82.23 ± 8.22
	8	1	96.65 ± 7.59	110.07 ± 1.22
		10	85.36 ± 5.60	99.90 ± 15.89
		50	64.05 ± 1.47	68.29 ± 2.17
	9	1	100.40 ± 1.55	103.57 ± 1.38
		10	99.60 ± 2.24	94.68 ± 3.05
		100	83.60 ± 1.03	88.78 ± 0.69
	10	1	94.77 ± 3.29	105.76 ± 4.28
		10	88.49 ± 10.53	102.55 ± 0.61
		50	40.54 ± 1.39	40.64 ± 5.79