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Inhibitory Effect of Melanogenesis by 5-Pentyl-2-Furaldehyde Isolated from *Clitocybe* sp.

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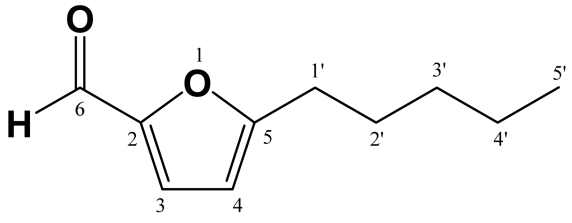
In the continued search for melanogenesis inhibitors from microbial metabolites, we found that the culture broth of *Clitocybe* sp. MKACC 53267 inhibited melanogenesis in B16F10 melanoma cells. The active component was purified by solvent extraction, silica gel chromatography, Sephadex LH-20 column chromatography, and finally by preparative HPLC. Its structure was determined as 5-pentyl-2-furaldehyde on the basis of the UV, NMR, and MS spectroscopic analysis. The 5-pentyl-2-furaldehyde potently inhibited melanogenesis in B16F10 cells with an IC_{50} value of 8.4 μg/ml, without cytotoxicity.

Keywords: B16F10 melanoma, *Clitocybe* sp., melanogenesis, 5-pentyl-2-furaldehyde

Melanin, a ubiquitous class of biological pigments that are responsible for the color of human skin, eyes, and hair, is produced by specialized pigment cells known as melanocytes and deposited within discrete membrane-bound organelles called melanosomes [11]. Melanogenesis is the process of melanin synthesis and distribution by a cascade of enzymatic and chemical reactions in melanocytes [5]. Melanin synthesis is principally responsible for skin color and plays a key role in the prevention of UV-induced skin damages. However, increased levels of epidermal melanin synthesis can darken the skin and produce various dermatologic disorders, such as melasma, age spots or liver spots, and actinic damage, resulting in the accumulation of excessive levels of epidermal and dermal pigmentations [2]. Melanin synthesis starts from the conversion of amino acid L-tyrosine into 3,4-dihydroxyphenylalanine (L-DOPA), and then the oxidation of L-DOPA by tyrosinase yields DOPA-quinone [9]. For this reason, melanin production is mainly controlled by the expression and activation of tyrosinase. Accordingly, tyrosinase inhibitors, such as kojic acid and arbutin, have been established as important constituents of cosmetic products and depigmenting agents against hyperpigmentation [3].

A number of melanogenesis inhibitors have already been reported and since used as cosmetic additives. However, many are of limited effectiveness, difficult to formulate, and even cause reactions or side effects after long-term usage. Thus, attention has recently been focused on the use of natural products in cosmetics [1, 7, 10]. In particular, mushrooms are ubiquitous in nature and produce various classes of secondary metabolites with interesting biological activities [6]. The medicinal mushrooms, such as *Phellinus linteus*, *P. baumii*, *Inonotus obliquus*, and *I. xeranticus*, have been used as traditional medicines for the treatment of various diseases, including stomach ailments, diabetes, gastroenteric disorders, lymphatic diseases, and cancers in Korea, China, Japan, and other Asian countries [13, 14]. Interestingly, the mycelial cultures of these medicinal fungi produce a yellowish antioxidant pigment that is composed of isoindolone class analogs [8]. In the course of our screening program for melanogenesis inhibitors, we isolated a known compound, 5-pentyl-2-furaldehyde (**1**, Fig. 1), from the culture broth of *Clitocybe* sp. Here, we report the isolation, structure elucidation, and biological activities of **1**. Moreover, its commercially available derivatives were investigated to evaluate structure–activity relationships.



Compound **1**

Fig. 1. Chemical structure of isolated compound **1.**

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The strain was obtained from the mushroom taxonomy laboratory at the National Academy of Agricultural Science (Suwon, Republic of Korea).

The *Clitocybe* sp. strain was maintained in solid potato dextrose agar medium. A piece of the *Clitocybe* sp. strain from a mature plate culture was added to a 500 ml Erlenmeyer flask containing 100 ml of medium composed of 2% glucose, 0.5% polypeptone, 0.2% yeast extract, 0.1% KH_2PO_4, and 0.05% $MgSO_4 \cdot 7H_2O$ (pH 5.7). The strain was cultured on a rotary shaker (140 rpm) at 28°C for 7 days. After 7 days of culture, the entire 3 L culture was harvested, and the culture broth was then separated from the mycelium by filtration. The mycelium was extracted with 80% aqueous acetone. The culture broth and the evaporated mycelium extract were then combined and the extract was concentrated *in vacuo* to provide an aqueous solution, which was then extracted three times with equal volumes of ethyl acetate. The concentrated ethyl acetate extract (2.73 g) was then applied to a column of silica gel and eluted with methanol–chloroform [1:100–1:10 (v/v)]. Next, the active fractions were combined and concentrated *in vacuo* to give an oily residue. The residue dissolved in methanol was then further purified by Sephadex LH-20 column chromatography and eluted with methanol. An active fraction was then followed by preparative HPLC using a YMC-pack ODS-A column (10 mm i.d. × 150 mm) eluted with 80% aqueous methanol with a retention time of 12.4 min to give **1** (50.4 mg). Compound **1** was determined to be 5-pentyl-2-furaldehyde on the basis of 1D and 2D NMRs and MS spectroscopic analysis (Fig. 1).

5-Pentyl-2-furaldehyde (1): yellow oil; UV (CH_3OH) λmax: 225, 287 nm; EI-MS *m/z* 166 [M^+]; ^{1}H NMR (CD_3OD, 400 MHz): δ 9.51 (1H, s, H-6), 7.17 (1H, d, *J* = 3.6 Hz, H-3), 6.23 (1H, d, *J* = 3.6 Hz, H-4), 2.71 (2H, t, *J* = 6.8 Hz, H-1'), 1.70 (2H, m, H-2'), 1.34 (2H, m, H-3'), 1.31 (2H, m, H-4'), 0.90 (3H, t, *J* = 6.6 Hz, H-5'); ^{13}C NMR (CD_3OD, 100 MHz): δ 177.2 (C-6), 164.5 (C-5), 151.9 (C-2), 124.5 (C-3), 108.8 (C-4), 31.5 (C-3'), 28.6 (C-1'), 27.4 (C-2'), 22.5 (C-4'), 14.1 (C-5').

5-Pentyl-2-furaldehyde derivatives, 2-pentyl-furan, 5-ethyl-2-furaldehyde, 5-methyl-2-furaldehyde, 5-methyl-2-acetylfuran, and 5-methyl-2-furoate were purchased from Fluka (Buchs, Switzerland) or Aldrich (Milwaukee, WI, USA). Arbutin was purchased from Sigma (St. Louis, MO, USA).

Melanogenesis inhibitory activity was evaluated according to the method previously reported with minor modifications [12]. The B16F10 murine melanoma cell line, strain KCLB 80008, was purchased from the Korean Cell Line Bank (Seoul, Republic of Korea) and maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The cells were cultured in 6-well plates at a density of 1×10^5 cells/ml and were maintained in 2 ml of DMEM containing 10% (v/v) FBS for 24 h, and then the cells were washed twice with 2 ml of phosphate-buffered saline (PBS), fed 2 ml of fresh medium (phenol-red-free DMEM), distributed as 2 μl samples to the dishes in a dilution series, and treated with α-MSH (Sigma, St. Louis, MO, USA) at 100 nM. After incubation for 48 h, the supernatant was added to a 96-well plate, and the amounts of melanin in the medium were measured at 405 nm with the ELISA microplate reader.

Cell viability was determined using MTT assays. Briefly, after treatment of the cells with or without chemicals for 24 h, 5 mg/ml MTT in PBS was added to each well. Cells were incubated at 37°C for 3 h and DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm with a microplate reader.

The molecular formula of **1** was established as $C_{10}H_{14}O_2$ from electron ionization (EI) mass and NMR spectral data. The positive EI mass of **1** provided ion peaks at *m/z* 166.0476 [$M]^+$, suggesting a molecular mass of 166. The UV spectrum of **1** showed an absorption maximum at 225 and 287 nm, and the IR spectrum indicated the presence of a furfural chromophore at 1,682 cm^{-1}. The ^{1}H NMR spectrum showed the presence of a methyl group at δ 0.90 (3H, t, *J* = 6.6 Hz), four methylene groups at δ 1.31~1.34 (4H, m), 1.70 (2H, m), and 2.71 (2H, t, *J* = 6.8 Hz), two aromatic protons at δ 6.23 (1H, d, *J* = 3.6) and 7.17 (1H, d, *J* = 3.6 Hz), and an aldehyde group at δ 9.51 (1H, s). The chemical shifts and coupling constants of the aromatic protons indicated the presence of a 5-substituted furfural structure in the molecule. Its ^{13}C NMR spectrum in CD_3OD showed ten peaks and exhibited the presence of four methylene carbons (δ 22.5, 27.4, 28.6, and 31.5), a methyl carbon (δ 14.1), two unsubstituted aromatic carbons (δ 108.8 and 124.5), two substituted aromatic carbons (δ 151.9 and 164.5), and an aldehyde group (δ 177.2). The long-range correlations from the aromatic proton at δ 7.17 to C-2 (δ 151.9) and C-6 (δ 177.2), and from the methylene

Table 1. Melanogenesis inhibitory activities of 5-pentyl-2-furaldehyde (**1**) and its derivatives in B16F10 melanoma cells.

Compound	Melanogenesis
	IC_{50} (μg/ml)
2-Pentylfuran	150
5-Pentyl-2-furaldehyde (**1**)	8.4
5-Ethyl-2-furaldehyde	65
5-Methyl-2-furaldehyde	NA[b]
5-Methyl-2-acetylfuran	NA
5-Methyl-2-furoate	NA
Arbutin[a]	273.4

[a]Positive control.
[b]NA represents no inhibitory effect at a final concentration.

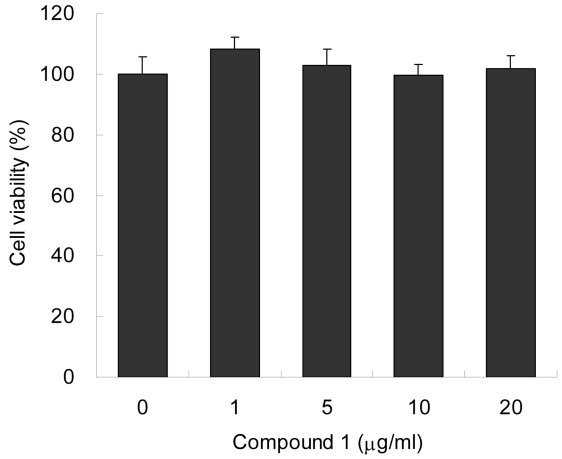

Fig. 2. Effect of compound 1 on B16F10 cell viability.
Cells were treated with 1–20 μg/ml of sample for 24 h. Cell viability was determined by MTT assays. Each determination was made in triplicate and data shown are means ± SD.

proton at δ 2.71 to C-4 (δ 108.8) and C-5 (δ 164.5), indicated that the aldehyde group and the methylene group were located closely at C-2 and C-5. Consequently, the structure of **1** was identified to 5-pentyl-2-furaldehyde by direct comparison of its spectral data with those reported in the literature [4].

The isolated compound **1** and its derivatives were evaluated for inhibitory effects on melanogenesis and the results are summarized in Table 1. As a result, compound **1** dose-dependently inhibited melanin synthesis with an IC_{50} value of 8.4 μg/ml without cytotoxicity (Fig. 2 and 3) in cultured B16F10 mouse melanoma cells. 5-Ethyl-2-furaldehyde exhibited 50% inhibitory effect at the concentration of 65 μg/ml, whereas 2-pentylfuran showed 50% inhibitory activity at the concentration of 150 μg/ml. The other compounds showed no inhibitory activities on melanogenesis. The compounds of the same substituent in the same position were found to have no inhibitory effects by the presence of other functional groups. These results indicate that the inhibitory effect of 5-pentyl-2-furaldehyde derivatives on melanogenesis was not related to their aldehyde. However, the carbon chain length and position in active compounds are considered as important factors for inhibitory activity on melanogenesis.

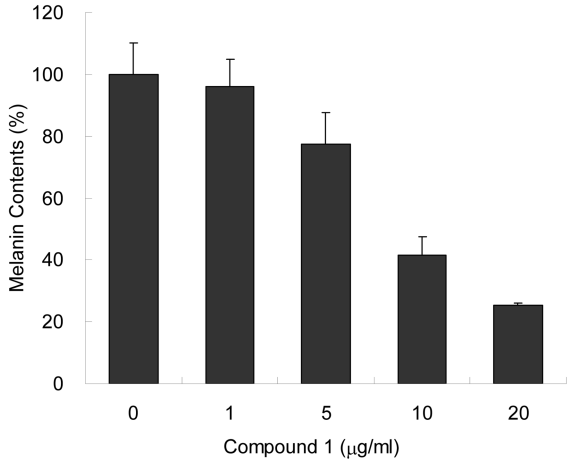

Fig. 3. Melanogenesis inhibitory effect of compound 1 in B16F10 melanoma cells.
Cells were cultured for 24 h in medium and treated with 1–20 μg/ml of sample and α-MSH for 24 h. After incubation for 48 h, the OD of the supernatants was measured at 405 nm using an ELISA reader.

Acknowledgments

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