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Synthesis and melanin biosynthesis inhibitory activity of (±)-terrein produced by *Penicillium* sp. 20135

Sangku Lee,[a] Won-Gon Kim,[a] Eungsoo Kim,[a] In-Ja Ryoo,[a] Hyeong Kyu Lee,[a] Jae Nyoung Kim,[b] Sang-Hun Jung[c] and Ick-Dong Yoo[a,*]

[a]*Korea Research Institute of Bioscience and Biotechnology, 52 Oun, Yusong, Taejon 305-333, Republic of Korea*
[b]*Department of Chemistry and Institute of Basic Science, Chonnam National University, Gwangju 500-757, Republic of Korea*
[c]*College of Pharmacy, Chungnam National University, Taejon 305-764, Republic of Korea*

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Abstract—Terrein was isolated from *Penicillium* sp. 20135, prepared by a practical synthetic way, and evaluated first time for its melanin biosynthesis inhibitory activity.

Melanin pigments are known to be biosynthesized in the melanosomes of melanocytes, moved to kerotinocytes, and stored in the epidermis.[1] Hyperpigmentation such as chloasma, colouration or freckles increased abnormally the amounts of melanin in the epidermis. Melanogenesis consists of two rate-limiting reactions catalyzed by tyrosinase, that is, the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the subsequent oxidation of DOPA to DOPA-quinone.[2] Thus melanin biosynthesis inhibitors, such as inhibitors of tyrosinase or regulators of tyrosinase expression and activation, may be useful as a skin-whitening agent in cosmetics.

Fungi are a source of many important biomedical substances. Kojic acid currently used as a cosmetic agent for the purpose of skin whitening was isolated from *Aspergillus* oryzae.[3] Stronger and safer skin whitening agents, however, are needed because of a carcinogenic potential as well as a weak whitening effect of kojic acid.[4] For exploring a new class of cosmetic agents, we have screened melanin biosynthesis inhibitors from fungal metabolites and reported strong tyrosinase inhibitors, melanocins A–C, produced by *Eupenicillium shearii*.[5] In the course of our continuous screening for melanin biosynthesis inhibitors using melanocyte cells Mel-Ab, terrein (**1**) have been newly isolated from *Penicillium* sp. 20135 as a racemic form with an optical rotation of zero (*c* 0.1, MeOH)[6] (Fig. 1). Although terrein was discovered nearly seventy years ago,[7] there have been only a few reports about its biological activities.[8,9] Terrein was reported to show plant growth inhibition[8] and antibacterial activities.[9] We found that terrein inhibited effectively melanin formation in melanocyte Mel-Ab cells. As we have been interested in further biological studies including in vivo activities, we needed a practical synthetic approach to terrein, thereby also enabling access to terrein analogues for examining structure–activity relationships. Herein we describe a practical synthesis of terrein and its melanin biosynthesis inhibitory activity.

The synthesis of terrein was reported by several research groups. Auerbach and Weinreb synthesized racemic terrein from *cis*-1,4-bisbenzyloxy-2,3-epoxycyclopentane in a multistep route and in low overall yield.[10] Barton and Hulshoff prepared (±)-terrein by performing photochemical ring contraction of 5-hydroxy-4-pyrone.[11]

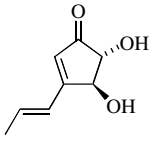

Figure 1. Structure of terrein (**1**).

Keywords: Fungal metabolite; Inhibition; Melanin; Cosmetics.
* Corresponding author. Tel.: +82 42 860 4330; fax: +82 42 860 4595; e-mail: idyoo@kribb.re.kr

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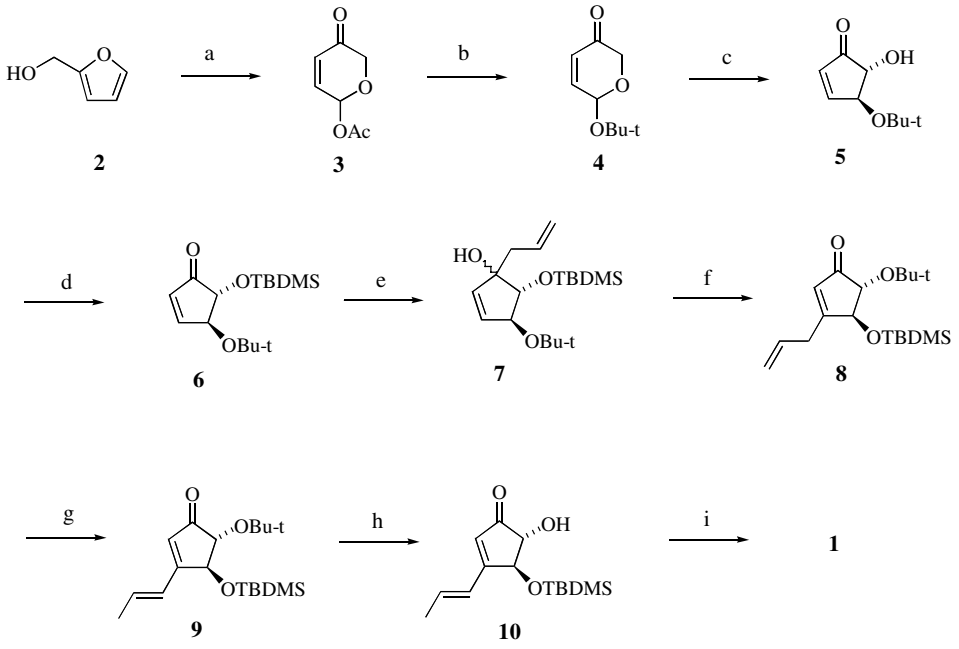

Scheme 1. Reagents and conditions: (a) NBS, $NaHCO_3$, Ac_2O, THF/H_2O, 0 °C to rt, 18 h, 55%; (b) *t*-BuOH, $SnCl_4$, CH_2Cl_2, rt, 4 h, 93%; (c) Et_3N, DMF, 70 °C, 24 h, 47%; (d) TBDMSCl, imidazole, CH_2Cl_2, rt, 2 h, 92%; (e) allylmagnesium bromide, Et_2O, −78 °C to rt, 16 h, 97%; (f) PCC, Celite, CH_2Cl_2, rt, 18 h, 60%; (g) NaOH, MeOH, rt, 15 min, 77%; (h) $TiCl_4$, CH_2Cl_2, 0 °C, 20 min, 92%; (i) HCl, $EtOH/H_2O$, rt, 2 h, 62%.

The approach has some drawbacks in the construction of the *E*-double bond and the *trans*-dihydroxy moieties to be nonstereoselective. Zwanenburg and co-workers completed the synthesis of (±)-terrein via flash vacuum pyrolysis of functionalized tricyclo[5.2.1.0]decenone epoxide to cyclopentadienone epoxide.[12] Recently, Kolb and Hoffmann prepared terrein utilizing the ring contraction of a pyranone to a cyclopentenone,[13] however their synthetic route involves expensive reagents such as 2-(trimethylsilyl)ethanol and *E*-1-propenyllithium.

Caddick et al. reported a base-mediated isomerization of pyranones to functionalized cyclopentenones.[14] We have envisioned that the application of the isomerization reaction of pyranone to a dihydroxylated cyclopentenone would provide a practical synthetic approach to terrein. To this end, acetoxy pyranone **3** was prepared from furfuryl alcohol (**2**) by treatment with *N*-bromosuccinimide and acetic anhydride in aqueous THF (Scheme 1).[15] Acetate **3** was converted to acetal **4** by treatment with *tert*-butyl alcohol in the presence of $SnCl_4$.[16] Isomerization of pyranone **4** with triethylamine in DMF at 70 °C yielded five-membered cyclopentenone **5** in a stereospecific fashion.[15b,17] Silylation of secondary alcohol **5** followed by addition of allyl magnesium bromide provided tertiary alcohol **7**. Oxidation of tertiary allylic alcohol **7** using PCC[18] afforded enone **8**, which upon exposure to a methanolic NaOH solution afforded fully conjugated cyclopentadienone **9**. Finally, cleavage of *tertiary*-butyl ether in **9** utilizing $TiCl_4$[19] and subsequent desilylation gave (±)-**1**. This synthetic pathway provided a practical route to be easily amenable to large scale and produced 10 g quantities of (±)-**1** starting from 115 g of furfuryl alcohol (**2**). The synthetic compound **1** was in good accord with a natural authentic sample in all aspects including IR, ^1H NMR, ^{13}C NMR and TLC in three different solvent systems. Also, the synthetic compound **1** showed identical melanin biosynthesis inhibitory activities to naturally occurring (±)-terrein.

Inhibitory activities of terrein on melanin formation were evaluated by measurement of the amounts of melanin produced by melanocyte Mel-Ab cells.[20] After terrein was treated at the concentrations of 5–50 μM for 4 days, terrein-treated cells were much less pigmented than the untreated cells (Fig. 2). In agreement with the microscopic observations, melanin levels were strongly reduced in a dose-dependent manner with an IC_{50} value of 18.8 μM. Terrein showed above 10 times stronger activity than kojic acid, as a control, which exhibited

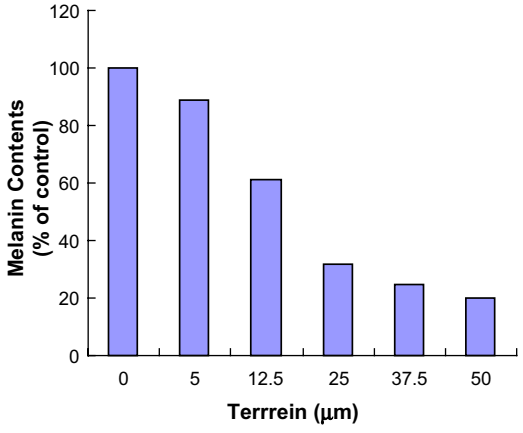

Figure 2. Inhibitory activity of terrein (**1**) on melanin biosynthesis.

inhibition of melanin synthesis by 20% at 100 μM. Interestingly, terrein showed no inhibitory activity against tyrosinase even at 200 μM concentration.

In conclusion, terrein, isolated from *Penicillium* sp. 20135, was prepared by a practical synthetic way that would bring a large quantity of **1** and its melanin biosynthesis inhibitory activity was first evaluated. Further studies on mechanistic aspects for melanin formation inhibition of terrein are under investigation.

Acknowledgements

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6. Isolation of terrein from the *Penicillium* culture: The culture broth (1 L) was extracted with 80% acetone and the extract was concentrated in vacuo to an aqueous solution, which was then extracted with an equal volume of EtOAc three times. EtOAc extract was concentrated in vacuo to dryness. The crude extract was subjected to SiO_2 (Merck Art No. 7734.9025) column chromatography followed by stepwise elution with $CHCl_3$–MeOH (50:1, 20:1, 10:1). The active fractions eluted with $CHCl_3$–MeOH (20:1) were pooled and concentrated in vacuo. The residue was finally applied to a Sephadex LH-20 and then eluted with MeOH to afford the purified compound (15 mg). The purity of the compound was checked to be over than 99% by high-performance liquid chromatography with a ODS column (YMC C_{18}) eluted with MeOH–H_2O (30:70).
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20. Melanin synthesis inhibition assay: The Mel-Ab is a mouse-derived spontaneously immortalized melanocyte cell line that produces large amounts of melanin.[21] Mel-Ab cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 nM TPA, 1 nM CT, 50 μg/mL streptomycin and 50 U/mL penicillin at 37 °C in 5% CO_2. Melanin contents were measured as described previously[22] with slight modification. Briefly, cells were treated with terrein at various concentrations for 4 days. Cell pellets were dissolved in 1 mL of 1 N NaOH at 100 °C for 30 min and centrifuged for 20 min at 16,000*g*. Optical densities (OD) of the supernatants were measured at 400 nm using an ELISA reader.
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