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A PYRADINE-DERIVATIVE FROM THE MUSHROOM *ALBATRELLUS CONFLUENS*

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Key Word Index—*Albatrellus confluens*; Scutigeraceae; melanin synthesis; pyradine-derivative; 3,6-dibenzyl-2-hydroxy-5-methoxypyradine.

Abstract—A novel pyradine-derivative was isolated from the mushroom *Albatrellus confluens*. This compound promoted melanin synthesis by B16 melanoma cells.

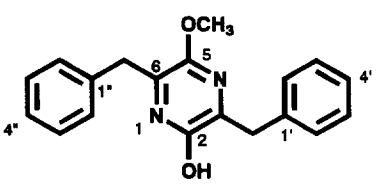
INTRODUCTION

In previous papers, we reported on the isolation of cholesterol-lowering compounds from the mushroom *Albatrellus confluens* (or *Polyporus confluens*; Ningyotake in Japanese) [1, 2]. We describe now a novel pyradine-derivative from the mushroom.

RESULTS AND DISCUSSION

Dried fruiting bodies of *A. confluens* were extracted with 85% EtOH, and the extract was concentrated and fractionated by solvent partition between EtOAc and water. Repeated silica gel chromatography and preparative TLC of the EtOAc extract gave **1** as white needles.

Compound **1** showed a $[M]^+$ at m/z 306 in its EI mass spectrum. It was assigned the molecular formula $C_{19}H_{18}N_2O_4$ by HRMS. The ^1H and ^{13}C NMR data in $CDCl_3$ are as follows: δ 3.96 (3H, *s*, OMe), 4.03 (2H, *s*, 6-CH_2), 4.11 (2H, *s*, 3-CH_2), 7.19–7.30 (6H, *m*, 3′-5′, 3″-5″), 7.41–7.43 (4H, *m*, 2′,6′, 2″, 6″); δ 36.1 (6-CH_2), 38.2 (3-CH_2), 54.0 (OMe), 126.2 (4′ or 4″), 126.4 (4′ or 4″), 128.3 (3″,5″ or 3′,5′), 128.4 (3′, 5′ or 3″ 5″), 128.9 (2″, 6″), 129.1 (2′, 6′), 134.6 (6), 138.1 (1″), 138.6 (1′), 140.5 (3), 151.3 (5), 153.2 (2). Finally, the structure was determined to be 3,6-dibenzyl-2-hydroxy-5-methoxypyradine by X-ray crystal analysis of the compound (Fig. 1).



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Compound **1** promoted melanin synthesis by B16 melanoma cells [3, 4]; the cells secreted 0.12 pg/cell^{-1} in the presence of **1** at 1.56 μM compared to 0.02 pg/cell^{-1} in the absence of **1**. This activity was stronger than that (0.08 pg/cell^{-1} at 1.85 μM) of a positive control compound (NH_4Cl).

EXPERIMENTAL

Mp: uncorr. ^1H NMR: 400 MHz, TMS as int. standard. ^{13}C NMR: 100 MHz.

Extraction and isolation. Dried fruiting bodies of *A. confluence* (84 g) were extracted with 80% EtOH (1 l × 3) and the solvent was concd under red. pres. and partitioned between EtOAc and H_2O. The residue (11.2 g) obtained after removing EtOAc was fractionated by repeated silica gel CC and prep. TLC to give **1** (23.3 mg, mp 159–161°) as white needles.

X-Ray crystal analysis. Crystal data: $C_{19}H_{18}N_2O_2$, monoclinic, space group C2/*c*, a = 36.459 (10), b = 4.697 (1), c = 24.227 (7) Å, ^{3}, z = 8, Dc = 1.287 g cm^{-3}, λ(MoKα) = 0.71073 Å, μ = 0.79 cm^{-1}, F(000) = 1296, R = 0.076 for 1181 unique reflections with $|F_0| > 5a(|F_0|)$. The structure was solved by the direct method using MULTAN 78. Hydrogen atoms were located by difference Fourier synthesis.

Melanin synthesis assay. B16 melanoma 4A5 cells (10×10^4 cells/ml) in 1.5 ml of Eagle's minimum essential medium (MEM) and 1.5 ml of each sample at various concentrations in DMSO were simultaneously

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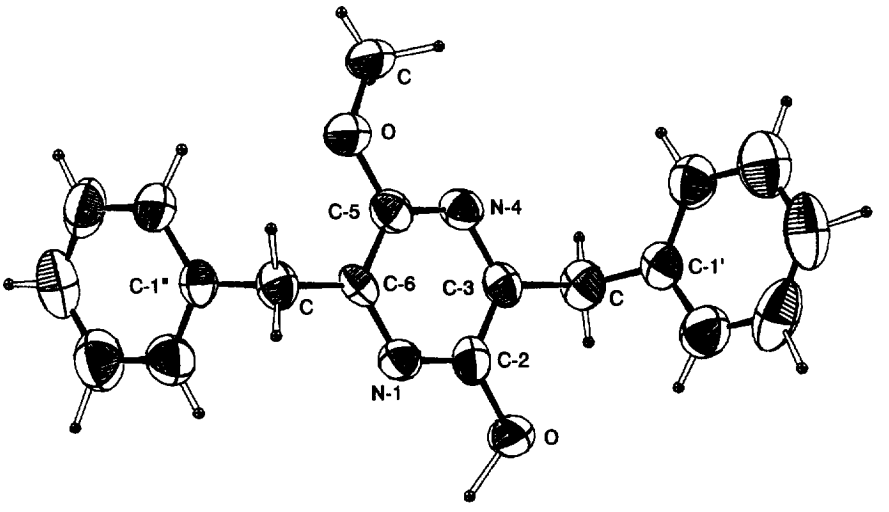

Fig. 1. An ORTEP drawing of compound **1**.

placed into a 6 well-plate and incubated for 4 days at 37° in a 5% CO_2/95% air atmosphere. After removing the medium, the cells were washed with phosphate-buffered saline, pH 7.4 (PBS), treated with 0.5 ml of 0.25% trypsin and placed ito a centrifugation tube. After removing the trypsin soln by centrifugation, the precipitated cells were cytolized by exposure to ultrasound in Solvable (Dupont). The content of melanin in the soln was determined by measurement of the absorbance at 400 nm.

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