NOTE

A new antifungal yellow pigment from *Aspergillus nishimurae*

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The incidence of life-threatening fungal infections has steadily increased in immunocompromised hosts such as HIV-infected persons and cancer and transplant patients.¹ Invasive pulmonary aspergillosis and *Pneumocystis carinii* pneumonia are leading causes of death in bone marrow transplant recipients and in HIV-infected patients, respectively. Moreover, resistance to the azoles, which are the most widely used anti-fungal medicines today, is attracting much attention. Therefore, there is a continuing need for new antifungal agents to overcome these fungal diseases. Screening for new antifungal substances from fungal sources was carried out using pathogenic filamentous fungi, *Aspergillus fumigatus* FRESSENIUS IFM41362 and *A. niger* VAN TIEGHEM IFM41398, and pathogenic yeasts, *Candida albicans* (ROBIN) BERKHOUT ATCC90028 and *Cryptococcus neoformans* (SANFELICE) VUILLEMIN ATCC90112. The MeOH extract of freshly isolated *A. nishimurae* IFM58441, cultivated on rice for 21 days at 25 °C, showed antifungal activities against the above four test organisms. The purification of this extract led to the isolation of a new yellow pigment, anishidiol (**1**) as the antifungal substance, along with 4-hydroxybenzaldehyde, 4-methylbenzylalcohol and monochaetin (**2**).² The structure elucidation, antifungal activities and cytotoxic activities of **1** are described in this paper.

Polished rice (480 g) was soaked in water for 30 min and then sterilized with an autoclave. *A. nishimurae* IFM58441 was cultivated for 14 days in Roux flasks, each containing 140 g of moist rice. The cultivated rice was extracted with MeOH, and the extract was concentrated *in vacuo*. The residue was suspended in water and extracted with ethyl acetate. The EtOAc extract (3.6 g) was partitioned between *n*-hexane and MeCN to yield an MeCN-soluble mixture. The MeCN extract (3.0 g) was chromatographed using a Sephadex LH-20 column (GE Healthcare UK Ltd., Buckinghamshire, UK; 280×35 mm (initial condition), solvent system: *n*-hexane–CHCl₃ (1:4) (180 ml), CHCl₃–acetone (3:2) (220 ml), (1:4) (200 ml), acetone (200 ml) and MeOH (500 ml)) to yield 11 fractions. CHCl₃–acetone (3:2) eluate was

rechromatographed using MPLC with a silica gel (URTRA PACK SI-40B (300×26 mm, YAMAZEN Corp., Osaka, Japan), *n*-hexane–acetone (20:1) 450 ml, followed by acetone 150 ml elute; 1 fraction=15 ml) to give monochaetin (**2**: 9 mg) from fractions 19–20. Acetone eluate was rechromatographed using HPLC with a silica gel (Inertsil SIL 100A (250×10 mm, GL Sciences Inc., Tokyo, Japan), CHCl₃–acetone (60:1)) to give the yellow compound, anishidiol (**1**: 3 mg). 4-Hydroxybenzaldehyde (4 mg) and 4-methylbenzylalcohol (16 mg) were obtained from MeOH eluate. The spectral data of **2** were identical to those from reports in the literature.²

The molecular formula of **1** (Figure 1) was confirmed as C₁₂H₁₂O₄ (seven degrees of unsaturation) by HR EI-MS. Detailed analysis of the ¹H- and ¹³C-NMR spectrum and heteronuclear single-quantum correlations of **1** revealed the presence of two exchangeable protons (δ 5.56 and δ 12.58), two methyl units (δ 2.00 and δ 2.11), a methylene unit (δ 5.12), a methine unit (δ 6.09), a formyl unit (δ 10.11) and seven quaternary carbons (three of which are oxygenated). These data accounted for the ¹H- and ¹³C-NMR resonances and required compound **1** to be bicyclic. The HMBC spectrum of **1** was measured for the connectivity of partial units. HMBC correlations from H₃-11 to C-3, C-4, from H₃-12 to C-6, C-7 and C-8, from H₂-1 to C-3, C-8, C-9 and C-10, from H-4 to C-3, C-10 and C-11, from H-13 to C-5 and C-6, and from 6-OH to C-5, C-6

Figure 1 Structures of compounds **1**–**3**.

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and C-7 enabled construction of the structure in **1** (Figure 2). From the above results, it was determined that the chemical structure of **1** is 6,8-dihydroxy-3,7-dimethyl-1*H*-isochromene-5-carbaldehyde, as shown in Figure 1.

It is likely that compound **1** is produced by a reductive aromatization of austdiol (**3**), which was isolated from *A. ustus*^{4,5} as a gastrointestinal toxin.

The strain IFM58441 was isolated from soil in Cairns, Australia. Colonies on malt extract agar grew fast and abundantly produced grayish green conidiogeneses. The aspergilla were uniseriate and flask-shaped, and the phialides covered the upper half of the vesicle. The conidia were ellipsoidal, almost smooth and $3.5\text{--}4 \times 2\text{--}3\text{ }\mu\text{m}$. The sequence of β -tubulin gene was equal to that of *Neosartorya nishimurae* as observed by Takada *et al.*⁶ However, the strain did not produce ascospores and produced only conidia. Therefore it was identified as *A. nishimurae*.

CONFLICT OF INTEREST

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