A New Pyrone Derivative from the Endophytic Fungus *Penicillium paxilli* PSU-A71

Vatcharin RUKCHACHAISIRIKUL,*,| Juthanat KAOEBAMRUNG,| Wannarat PANWIRIYARAT,| Pimchanok SAITAI,| Yaowapa SUKPONDMA,| Souwalak PHONPAICHIT,| and Jariya SAKAYAROY |

*Department of Chemistry, Faculty of Songklanakarin University; †Department of Microbiology, Faculty of Science, Prince of Songkla University; Hat Yai, Songkhla 90112, Thailand; and ‡National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand.

Received February 17, 2007; accepted June 29, 2007

One new pyrone derivative, named penicillone (1), together with paxilline, pyrenocines A (2) and B (3) were isolated from the endophytic fungus *Penicillium paxilli* PSU-A71. The structures were elucidated by spectroscopic methods. Pyrenocine B (3) mildly inhibited the growth of *Microsporum gypseum* SH-MU-4 with a MIC value of 32 µg/ml.

Key words  *Penicillium paxilli*; pyrone; antifungal activity

The genus *Penicillium* is a rich source of biologically active secondary metabolites including antifungal macrocyclic polyacetones,1) antitumor indole diterpenoids,2) and antibacterial polyoxynogenated farnesylcholholoxenones.3) In our continuing search for antimicrobial substances from endophytic fungi isolated from the leaves of *Garcinia* plants, the crude extract of *Penicillium paxilli* PSU-A71 exhibited interesting antifungal activity against *Microsporum gypseum* SH-MU-4 and showed no activity against pathogenic bacteria (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) and other fungi (*Candida albicans* and *Cryptococcus neoformans*). *M. gypseum*, a human pathogenic fungus, causes infections of the skin and scalp (ringworm or tinea or dermatophytosis) and disseminated infections in AIDS patients. Previous investigation of *P. paxilli* led to the isolation of paxilline,4) paxisterol5) and paspaline B.6) We describe herein the isolation and structural elucidation of one new pyrone derivative, penicillone (1), paxilline,4) pyrenocines A (2)7) and B (3)7,8) from the broth EtOAc extract of *P. paxilli* PSU-A71. All isolates were tested for antifungal activity against *M. gypseum* SH-MU-4.

Penicillone (1) had the molecular formula C_{14}H_{14}SO_{4} by HR-MS. The IR spectrum showed absorption bands for conjugated ester carbonyl (1721 cm⁻¹) and conjugated ketone carbonyl (1668 cm⁻¹) groups. The ¹³C-NMR and DEPT spectra demonstrated that 1 possessed the number and type of carbons identical to 3. In addition, the ¹H-NMR data of 1 were closely related to those of 3 except for the major difference in the chemical shift of H-9 (δ 3.62 in 1 and δ 4.30 in 3). These suggested that 1 had a pyrone structure with the presence of a SH group, not an OH group, at C-9. This assignment was supported by the carbon resonance of C-9 at δ 36.5 and HMBC correlations of H-8 (δ 2.83, dd, J=16.0, 3.0 Hz) and δ 2.55, dd, J=16.0, 11.5 Hz)/C-7 (δ 191.7), C-9 and C-10 (δ 19.7). The location of an olefinic proton (δ 5.29, 3.62 in 1) and a methoxyl group (δ 3.76, s) at C-3 and C-4 of the pyrone unit, respectively, was confirmed on the basis of HMBC correlations of H-3/C-2 (δ 168.7), C-4 (δ 168.7) and C-5 (δ 128.9) and that of the methoxy protons (H-11)/C-4. However, in the UV spectrum, a strong absorption band at longer wavelength than that in 3 (λ_{max} 285 nm) revealed the presence of a longer conjugated chromophore. Analysis of the ¹³C-NMR spectrum revealed that C-2, C-3 (δ 94.5) and C-5 in 1 resonated at lower field than those in 3. In contrast, C-6 (δ 156.6) in 1 appeared at higher field. Irradiation of the methoxy protons in the NOEDIF experiment enhanced signal intensity of H-3 (δ 5.29, s) and H-12 (δ 2.04, s), thus indicating the attachment of the methyl group in 1 at C-5, not at C-6 as found in 3. The HMBC correlations of H-12/C-4, C-5 and C-6 confirmed the assigned location. The remaining 1-oxobutyl group was then located at C-6. This would affect the chemical shift of carbons in the pyrone skeleton as well as the UV absorption band due to the resonance effect and extended conjugation. Consequently, 1 was identified as a new thiol.

All pyrone isolates were tested for antifungal activity against *M. gypseum* SH-MU-4. Pyrenocine B (3) showed mild activity with a MIC value of 32 µg/ml while penicillone (1) and pyrenocine A (2) were much less active than 3 with MIC values of 64 and 128 µg/ml, respectively.

**Experimental**

**General Procedures** Melting points were measured on an electrothermal melting point apparatus (Electrothermal 9100). Infrared spectra (IR) were recorded on a Perkin Elmer 783 FTS165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-160A spectrophotometer. ¹H- and ¹³C-NMR spectra were recorded on a 300MHz Bruker FTNMR Ultra Shield™ spectrometer. Mass spectra were obtained on a MAT 95 XL Mass Spectrometer (Thermoquest). Optical rotations were measured in MeOH on a JASCO P-1020 polarimeter. Thin-layer chromatography (TLC) and precoated TLC were performed on silica gel GF_{254} (Merck). Column chromatography (CC) was carried out on Sephadex LH-20 or silica gel (Merck) type 100 (70—230 Mesh ASTM).

**Fungal Material** The endophytic fungus *P. paxilli* PSU-A71 was isolated from the leaves of *Garcinia atroviridis*, collected in Songkhla Province, Thailand in 2005. The fungus was deposited as PSU-A71 at the Department of Microbiology, Faculty of Science, Prince of Songkla University.

**Fermentation and Isolation** The endophytic fungus *P. paxilli* PSU-A71 was grown on potato dextrose agar (PDA) at 25°C for 5 d. Three pieces (0.5×0.5 cm²) of mycelial agar plugs were inoculated into 500 ml Erlen-
meyer flasks containing 300 ml potato dextrose broth (PDB) at room temperature for 4 weeks. The culture (5 l) was filtered to give the filtrate and mycelia. The filtrate was extracted three times with EtOAc. The EtOAc layer was then dried over anhydrous Na$_2$SO$_4$ and evaporated to dryness under reduced pressure to obtain a brown gum (213.8 mg) which was fractionated by column chromatography (CC) over Sephadex LH-20 using MeOH as eluent to afford 4 fractions (A—D). Fraction B (171.0 mg) was further purified by CC on silica gel with a gradient of MeOH–CH$_2$Cl$_2$ to give five subfractions (B1—B5). Subfraction B1 contained 2 ($56.9$ mg). Subfraction B2 (26.4 mg, 1—2% MeOH–CH$_2$Cl$_2$) was further purified by precoated TLC using 4% EtOAc–light petroleum (4 runs) to give 3 (9.5 mg). Subfraction B4 (17.2 mg, 8—10% MeOH–CH$_2$Cl$_2$) was then purified by precoated TLC using 2% MeOH–CH$_2$Cl$_2$ (7 runs) to give 1 (3.5 mg). Fraction C (10.2 mg), upon purification on precoated TLC using 0.5% MeOH–CH$_2$Cl$_2$ (5 runs), yielded paxilline (1.8 mg).

Penicillone (1): Colorless gum. $^1$H-NMR (CDCl$_3$): $\delta$: 5.29 (1H, s, H-3), 3.76 (3H, s, H$_3$-11), 3.62 (1H, m, H-9), 2.83 (1H, dd, $J=16.0$, 3.0 Hz, H-8a), 2.55 (1H, dd, $J=16.0$, 11.5 Hz, H-8b), 2.04 (3H, s, H$_3$-12), 1.38 (3H, d, $J=7.0$ Hz, H$_3$-10). $^{13}$C-NMR (CDCl$_3$): $\delta$: 191.7 (s, C-7), 168.7 (s, C-2, C-4), 156.6 (s, C-6), 128.9 (s, C-5), 94.5 (d, C-3), 56.4 (q, C-11), 45.4 (t, C-8), 36.5 (d, C-9), 21.6 (q, C-12), 19.7 (q, C-10). FT-IR (neat) cm$^{-1}$: 1721, 1668, 1610, 1564. UV $\lambda_{max}$ (MeOH) nm (log e): 310 (4.52). HR-EI-MS m/z: 242.0578 [M]+ (Calcd for C$_{11}$H$_{14}$SO$_4$ 242.0613). EI-MS m/z: 242 (55), 227 (33), 197 (45), 183 (90), 172 (100). $[\alpha]D^29$ $^134.0^\circ$ ($c=0.04$, MeOH).

**Acknowledgments** J.K., W.P. and P.S. thank the Development and Promotion of Science and Technology Talent Project for scholarships. The Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC) is gratefully acknowledged for partial support. Finally, S.P. is grateful to the Bioresources Network (BRN) for a research grant.

**References**