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

Vatcharin RUKACHAISIRIKUL,*[,a] Juthanat KAEOBAMRUNG,[a] Wannarat PANWIRIYARAT,[a] Pimchanok SAITAI,[a] Yaowapa SUKPONDMA,[a] Souwalak PHONGPAICHIT,[b] and Jariya SAKAYAROJ[c]

[a] *Department of Chemistry, Faculty of Science, Prince of Songkla University;* [b] *Department of Microbiology, Faculty of Science, Prince of Songkla University; Hat Yai, Songkhla 90112, Thailand: and* [c] *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] antiinsectan indole diterpenoids,[2] and antibacterial polyoxygenated farnesylcyclohexenones.[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 acitvity 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] paxisterol[5] 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 $C_{11}H_{14}SO_4$ 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 ^{13}C-NMR and DEPT spectra demonstrated that **1** possessed the number and type of carbons identical to **3**. In addition, the ^{1}H-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_2-8 (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, s) 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_3-11)/C-4. However, in the UV spectrum, a strong absorption band at longer wavelenght than that in **3** (λ_{max} 285 nm) revealed the presence of a longer conjugated chromophore. Analysis of the ^{13}C-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 NOEDIFF experiment enhanced signal intensity of H-3 (δ 5.29, s) and H_3-12 (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_3-12/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. ^{1}H- and ^{13}C-NMR spectra were recorded on a 300 MHz Bruker FTNMR Ultra Shield^{TM} spectrometer. Mass spectra were obtained on a MAT 95 XL Mass Spectrometer (Thermofinnigan). 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 cm^2) of mycelial agar plugs were inoculated into 500 ml Erlen-

* To whom correspondence should be addressed. e-mail: vatcharin.r@psu.ac.th

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_2SO_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_2Cl_2 to give five subfractions (B1—B5). Subfraction B1 contained **2** (56.9 mg). Subfraction B2 (26.4 mg, 1—2% MeOH–CH_2Cl_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_2Cl_2) was then purified by precoated TLC using 2% MeOH–CH_2Cl_2 (7 runs) to give **1** (3.5 mg). Fraction C (10.2 mg), upon purification on precoated TLC using 0.5% MeOH–CH_2Cl_2 (5 runs), yielded paxilline (1.8 mg).

Penicillone (**1**): Colorless gum. ^{1}H-NMR ($CDCl_3$) δ: 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 ($CDCl_3$) δ: 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 λ_{max} (MeOH) nm (log ε): 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° (c=0.04, MeOH).

Antifungal Assay The hyphal extension-inhibition assay[9] was used. A modification of the NCCLS M38-A broth microdilution test[10] was performed against *M. gypseum* SH-MU-4. Equal volumes of a suspension of conidia (approximately 4×10^3 conidia/ml) were added to each test dilution to make final concentrations of 1—128 μg/ml in triplicate. Plates were incubated at 25 °C for 72 h. Miconazole, a standard antifungal agent, gave a MIC value of 1 μg/ml. The MICs were recorded for the lowest concentration that resulted in a reduction approximately 50% of the fungal growth.

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

1) Breinholt J., Jensen G. W., Nielsen R. I., Olsen C. E., Frisvad J. C., *J. Antibiot.*, **46**, 1101—1108 (1993).
2) Li C., Gloer J. B., Wicklow D. T., Dowd P. F., *Org. Lett.*, **4**, 3095—3098 (2002).
3) Li X., Choi H. D., Kang J. S., Lee C.-O., Son B. W., *J. Nat. Prod.*, **66**, 1499—1500 (2003).
4) Springer J. P., Clardy J., Wells J. M., Cole R. J., Kirksey J. W., *Tetrahedron Lett.*, **30**, 2531—2534 (1975).
5) Yasuzawa T., Yoshida M., Sano H., *J. Chem. Soc.*, *Perkin Trans. I*, **1990**, 3145—3149 (1990).
6) Munday-Finch S. C., Wilkins A. L., Miles C. O., *Phytochemistry*, **41**, 327—332 (1996).
7) Sato H., Konoma K., Sakamura S., Furusaki A., Matsumoto T., Matsuzaki T., *Agric. Biol. Chem.*, **45**, 795—797 (1981).
8) Sato H., Konoma K., Sakamura S., *Agric. Biol. Chem.*, **43**, 2409—2411 (1979).
9) Huang X., Xie W., Gong Z., *FEBS Lett.*, **478**, 123—126 (2000).
10) National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard. NCCLS documents M38-A. Wayne, Pa.: National Committee for Clinical Laboratory Standards, 2002.