Lactone Derivatives from the Marine-Derived Fungus *Penicillium* sp. PSU-F44

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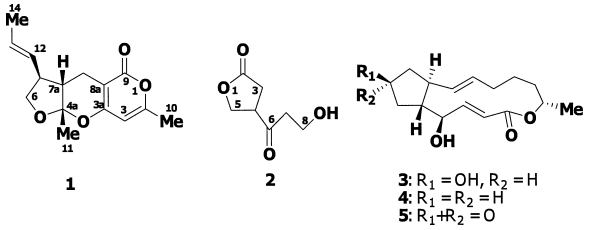
Two new fungal metabolites, penicipyrone (1), and penicilactone (2), were isolated from the marine-derived fungus *Penicillium* sp. PSU-F44 along with three known macrolides, (+)-brefeldin A (3), (+)-brefeldin C (4), and 7-oxobrefeldin A (5). Their antimicrobial activities against methicillin-resistant *Staphylococcus aureus* SK1 and *Microsporum gypseum* SH-MU-4 were examined.

Key words marine-derived fungus; *Penicillium* sp.; pyrone derivative; γ-lactone derivative; antibacterial activity; antifungal activity

The fungi in the genus *Penicillium* have produced a variety of compounds, for example, polyketide-terpenoid,[1)] alkaloid,[2)] lactone,[3)] and quinone derivatives.[4)] Some of these exhibit a wide range of biological and pharmacological activities, *e.g.*, anticancer penicidones,[2)] antifungal botryodiplodin,[5)] antibacterial rugulotrosins,[6)] and antioxidant pennicitrinone.[7)] Investigation of bioactive compounds from these fungi is therefore of interest. The EtOAc extract from the culture broth of the marine-derived fungus *Penicillium* sp. PSU-F44, isolated from a sea fan *Annella* sp., exhibited antibacterial activity against a clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) SK1 and a clinical isolate of *Microsporum gypseum* (MG) SH-MU-4 with the respective minimum inhibitory concentration (MIC) values of 320 and 160 μg/ml. We report herein the isolation of one new pyrone, penicipyrone (**1**), and one new γ-lactone, penicilactone (**2**), together with three known macrolides, (+)-brefeldin A (**3**),[8)] (+)-brefeldin C (**4**),[9)] and 7-oxobrefeldin A (**5**)[10)] from this extract. Their antimicrobial activities against MRSA SK1 and MG SH-MU-4 were evaluated.

Results and Discussion

Compounds **1**—**5** were isolated from the broth EtOAc extract of *Penicillium* sp. PSU-F44 using various chromatographic techniques. Their structures were elucidated by analysis of spectroscopic data, including IR, UV, NMR and MS. The relative configuration of **1** was assigned on the basis of nuclear Overhauser effect difference (NOEDIFF) data whereas the absolute configuration of the known compounds was determined by comparison of their optical rotations with those previously reported in the literature.



1 **2** **3**: R_1 = OH, R_2 = H; **4**: R_1 = R_2 = H; **5**: R_1+R_2 = O

Penicipyrone (**1**) with the molecular formula $C_{15}H_{18}O_4$ from HR-electron ionization (EI)-MS, was obtained as a colorless gum. The IR spectrum displayed absorption bands at 1713 and 1653 cm^{-1} for ester carbonyl and double bond functional groups, respectively. The UV spectrum exhibited maximum absorption bands at λ_{max} 244 and 269 nm, indicating that **1** had a conjugated chromophore. The ^{1}H-NMR spectrum displayed signals for a *trans*-propenyl unit [δ 5.52 (1H, dq, J=15.0, 6.5 Hz), 5.22 (1H, ddq, J=15.0, 9.0, 1.5 Hz) and 1.67 (3H, dd, J=6.5, 1.5 Hz)], one olefinic proton of a trisubstituted double bond (δ 5.75, 1H, s), two nonequivalent oxymethylene protons [δ 4.16 (1H, t, J=9.0 Hz) and 3.71 (1H, t, J=9.0 Hz)], two nonequivalent methylene protons [δ 2.61 (1H, d, J=18.0 Hz) and 2.47 (1H, dd, J=18.0, 6.5 Hz)], two methine protons [δ 2.55 (1H, m) and 2.18 (1H, m)] and two methyl groups [δ 2.21 (3H, s) and 1.52 (3H, s)]. In the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum, the oxymethylene protons, H_{ab}-6 (δ 4.16, 3.71), showed cross peaks with the methine proton, H-7 (δ 2.55), which was further coupled with the other methine proton, H-7a (δ 2.18). Furthermore, H-7a was coupled with the nonequivalent methylene protons, H_{ab}-8 (δ 2.61, 2.47). A tetrahydrofuran unit having a methylene group attached at C-7a was then established on the basis of a ^3J heteronulear multiple bond correlation (HMBC) of H_{ab}-6 with C-4a (δ 109.2). The methyl protons, H_3-11 (δ 1.52), showed the HMBC cross peaks with C-4a and C-7a (δ 45.9), thus connecting the methyl group at C-4a. The *trans*-propenyl unit was attached at C-7 of the tetrahydrofuran ring according to the ^{1}H–^{1}H COSY correlation between H-12 (δ 5.22) and H-7 as well as the ^3J HMBC correlation of H-13 with C-7. Moreover, the ^{1}H- and ^{13}C-NMR spectra of **1** revealed the presence of a pyrone ring with the methyl and the oxysubstituent groups at C-2 (δ 160.6) and C-3a (δ 160.6), respectively. This conclusion was confirmed by following HMBC correlations: H-3/C-2, C-3a, C-8a (δ 94.1) and C-10 (δ 19.6); H_3-10/C-2 and C-3 (δ 100.5), as well as the chemical shift of C-3a. The bond connection between C-8 (δ 17.8) with C-8a of the pyrone ring was established according to the HMBC correlations of H_{ab}-8 with C-8a and C-9 (δ 163.1). The chemical shift of C-4a and

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Table 1. ^{1}H-, ^{13}C-NMR and HMBC Data of Compound **1** in $CDCl_3$

Position	^{1}H, δ (mult. J in Hz)	^{13}C , δ (mult.)	HMBC correlation
2		160.6, s	
3	5.75 (s)	100.5, d	C-2, C-3a, C-8a, C-10
3a		160.6, s	
4a		109.2, s	
6	a: 4.16 (t, 9.0)	72.5, t	C-4a, C-7, C-7a, C-12
	b: 3.71 (t, 9.0)		
7	2.55 (m)	44.7, d	
7a	2.18 (m)	45.9, d	C-7, C-8, C-8a, C-12
8	a: 2.61 (d, 18.0)	17.8, t	C-4a, C-7, C-7a, C-8a, C-9
	b: 2.47 (dd, 18.0, 6.5)		
8a		94.1, s	
9		163.1, s	
10	2.21 (s)	19.6, q	C-2, C-3
11	1.52 (s)	22.3, q	C-4a, C-7a
12	5.22 (ddq, 15.0, 9.0, 1.5)	128.5, d	C-14
13	5.52 (dq, 15.0, 6.5)	129.8, d	C-7, C-14
14	1.67 (dd, 6.5, 1.5)	17.8, q	C-12, C-13

the molecular formula constructed an ether linkage between C-3a and C-4a to form a hydropyran unit. The spatial arrangement of H-7a, H_3-11 and the propenyl moiety is all *cis* on the basis of the NOEDIFF enhancement of H_3-11 and H-12 upon irradiation of H-7a. Consequently, penicipyrone (**1**) was identified as a new tricyclic pyrone derivative.

Penicilactone (**2**) was obtained as a colorless gum whose molecular formula was assigned as $C_7H_{10}O_4$ by HR-EI-MS. The IR spectrum showed absorption bands for a hydroxyl (3414 cm^{-1}), a γ-lactone carbonyl (1769 cm^{-1}) and a ketone carbonyl (1715 cm^{-1}). The ^{1}H-NMR spectrum displayed signals for a 1-oxo-3-hydroxypropyl unit [δ 3.93 (2H, t, J=5.5 Hz) and 2.75 (2H, t, J=5.5 Hz)], one methine proton (δ 3.63, 1H, qn, J=8.0 Hz), two nonequivalent oxymethylene protons [δ 4.50 (1H, t, J=9.0 Hz) and 4.44 (1H, t, J=9.0 Hz)] and two nonequivalent methylene protons [δ 2.84 (1H, dd, J=17.5, 7.5 Hz) and 2.71 (1H, dd, J=17.5, 9.5 Hz)]. The methine proton, H-4 (δ 3.63), showed ^{1}H–^{1}H COSY cross peaks with the methylene protons, H_{ab}-3 (δ 2.84, 2.71) and the oxymethylene protons, H_{ab}-5 (δ 4.50, 4.44). Both H_{ab}-3 and H_{ab}-5 were correlated with the same lactone carbonyl carbon, C-2 (δ 174.8) in the HMBC spectrum, thus constructing a γ-lactone unit. The 1-oxo-3-hydroxypropyl unit was located at C-4 (δ 46.9) of the lactone ring on the basis of a HMBC correlation between H_2-7 (δ 2.75) of the 1-oxo-3-hydroxypropyl unit and C-4. Therefore, penicilactone (**2**) was identified as a new γ-lactone derivative.

All isolated metabolites, except for **1** and **4** which were obtained in small amount, were tested for antimicrobial activities against MRSA SK1 and MG SH-MU-4. All of them displayed better antibacterial activity than the crude extract with the same MIC value of >200 μg/ml against MRSA SK1. For antifungal activity against MG SH-MU-4, compound **3** showed the best activity with the MIC value of 64 μg/ml whereas the remaining compounds were inactive (MIC >200 μg/ml). These results indicated that compound **3**, the major component, would involve in antifungal activity of the crude extract. Moreover, it is worth to note that the hydroxyl group on the cyclopentane moiety in **3** would play an important role in this activity.

Experimental

General Procedures Infrared (IR) spectra were recorded on a Perkin-Elmer 783 FTS 165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-160A spectrophotometer. Optical rotations were measured on a JASCO P-1020 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a 300 MHz Bruker FTNMR Ultra Shield spectrometer. Chemical shifts are expressed in δ (ppm) referring to the tetramethylsilane (TMS) peak. Mass spectra were obtained on a MAT 95 XL mass spectrometer (Thermofinnigan). Thin-layer Chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{254} (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (70—230 mesh ASTM), on Sephadex LH-20 or on reverse phase silica gel C-18.

Fungal Material The marine-derived fungus *Penicillium* sp. PSU-F44 was isolated from the sea fan *Annella* sp., collected near the Similan Islands, Phangnga Province, Thailand, in 2006. This fungus was deposited as PSU-F44 at the Department of Microbiology, Faculty of Science, Prince of Songkla University.

Fermentation, Extraction and Isolation The marine-derived fungus *Penicillium* sp. PSU-F44 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 Erlenmeyer flasks containing 300 ml of potato dextrose broth (PDB) at room temperature for 4 weeks. The culture (15 l) was filtered to give the filtrate and mycelia. The filtrate was extracted three times with EtOAc to afford a broth extract (1.8 g) as a brown gum after evaporation to dryness under reduced pressure. The crude extract was fractionated by CC over Sephadex-LH 20 with MeOH to give four fractions (A—D). Fraction B (700 mg) was purified by silica gel CC with a gradient of MeOH–CH_2Cl_2 (2%, 7%, 10%, 20%, 60% MeOH–CH_2Cl_2 and 100% MeOH) to afford four fractions (B1—B4). Fraction B1 (8.0 mg) was purified by PTLC using 50% CH_2Cl_2–light petroleum as a mobile phase to give **1** (1.2 mg). Fraction B2 (13.2 mg) was then separated by PTLC with 80% acetone–light petroleum to give **5** (4.6 mg). Compound **2** (6.0 mg) was present in fraction B3. Fraction C (300 mg) was further separated by CC with a gradient of EtOAc–light petroleum (20%, 40%, 70% EtOAc–light petroleum and 100% EtOAc) to give three fractions. Compound **4** (1.6 mg) was obtained from the second fraction (141.2 mg) after CC over silica gel using the same gradient of EtOAc–light petroleum as fraction C. Fraction D (286.2 mg) was separated using the same procedure as fraction B to give three fractions. The second fraction (212.3 mg) contained **3** (123.3 mg).

Penicipyrone (**1**): Colorless gum. ^{1}H- and ^{13}C-NMR ($CDCl_3$) data see Table 1. IR (neat) cm^{-1}: 1713, 1653. UV λ_{max} (MeOH) nm (log ε): 244 (3.51), 269 (3.50). HR-EI-MS m/z: 262.1176 (Calcd for $C_{15}H_{18}O_4$: 262.1205). EI-MS m/z: 262 (M^+), 223, 139, 115. $[\alpha]_D^{25}$ +51° (c=0.07, MeOH).

Penicilactone (**2**): Colorless gum. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 4.50 (1H, t, J=9.0 Hz, H_a-5), 4.44 (1H, t, J=9.0 Hz, H_b-5), 3.93 (2H, t, J=5.5 Hz, H-8), 3.63 (1H, quintet, J=8.0 Hz, H-4), 2.84 (1H, dd, J=17.5, 7.5 Hz, H_a-3), 2.75 (2H, t, J=5.5 Hz, H-7), 2.71 (1H, dd, J=17.5, 9.5 Hz, H_b-3). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 206.9 (s, C-6), 174.8 (s, C-2), 68.0 (t, C-5), 57.5 (t, C-8), 46.9 (d, C-4), 44.0 (t, C-7), 30.0 (t, C-3). IR (neat) cm^{-1}: 3414, 1769, 1715. UV λ_{max} (MeOH) nm (log ε): 211 (2.23). HR-EI-MS m/z: 140.0473 (Calcd for $C_7H_8O_3$: 140.0473). EI-MS m/z: 140 ($M-H_2O^+$), 113, 86, 73. $[\alpha]_D^{25}$ +23° (c=0.12, MeOH).

Antibacterial Activity Testing MICs were determined by the agar microdilution method.[11] The test substances were dissolved in dimethyl sulfoxide (DMSO) (Merck, Germany). Serial 2-fold dilutions of the test substances were mixed with melted Mueller–Hinton agar (Difco) in the ratio of 1 : 100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar ranged from 0.03 to 200 μg/ml. SA and MRSA were used as test strains. Inoculum suspensions (10 μl) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin, a positive control drug, exhibited the MIC value of 1 μg/ml. Growth controls were performed on the agar containing DMSO.

Antifungal Activity Testing The hyphal extension-inhibition assay was used.[12] A modification of the NCCLS-M38-A broth microdilution test[13] was performed against MG. 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 of approximately 50% of the fungal growth.

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References

1) Stierle A. A., Stierle D. B., Patacini B., *J. Nat. Prod.*, **71**, 856—860 (2008).
2) Ge H. M., Shen Y., Zhu C. H., Tan S. H., Ding H., Song Y. C., Tan R. X., *Phytochemistry*, **69**, 571—576 (2008).
3) Li X., Yao Y., Zheng Y., Sattler I., Lin W., *Arch. Pharm. Res.*, **30**, 812—815 (2007).
4) Li X., Zheng Y., Sattler I., Lin W., *Arch. Pharm. Res.*, **29**, 942—945 (2006).
5) Cabedo N., Lopez-Gresa M. P., Primo J., Ciavatta M. L., Gonzales-Mas M. C., *J. Agric. Food Chem.*, **55**, 6977—6983 (2007).
6) Stewart M., Capon R. J., White J. M., Lacey E., Tennant S., Gill J. H., Shaddock M. P., *J. Nat. Prod.*, **67**, 728—730 (2004).
7) Lu Z.-Y., Lin Z.-J., Wang W.-L., Du L., Zhu T.-J., Fang Y.-C., Gu Q.-Q., Zhu W.-M., *J. Nat. Prod.*, **71**, 543—546 (2008).
8) Shibazaki M., Yamaguchi H., Sugawara T., Suzuki K., Yamamoto T., *J. Biosci. Bioeng.*, **96**, 344—348 (2003).
9) Archambaud S., Aphecetche-Julienne K., Guingant A., *Synlett*, **1**, 139—143 (2005).
10) Hutchinson C. R., Kurobane I., Cane D. E., Hasler H., McInnes A. G., *J. Am. Chem. Soc.*, **103**, 2477—2480 (1981).
11) Lorian V., "Antibiotics in Laboratory Medicine," 4th ed., William and Wilkins, Baltimore, 1996, pp. 28—32.
12) Huang X., Xie W.-J., Gong Z.-Z., *FEBS Lett.*, **478**, 123—126 (2000).
13) National Committee for Clinical Laboratory Standards, "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, Approved Standard, NCCLS documents M38-A," National Committee for Clinical Laboratory Standards, Wayne, PA, 2000.