Paecilosetin, a New Bioactive Fungal Metabolite from a New Zealand Isolate of *Paecilomyces farinosus*

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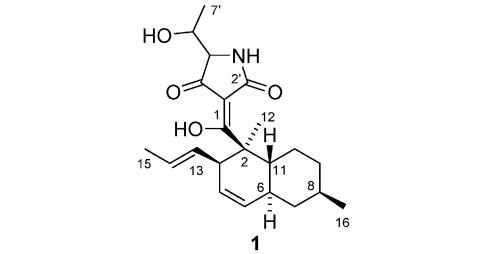
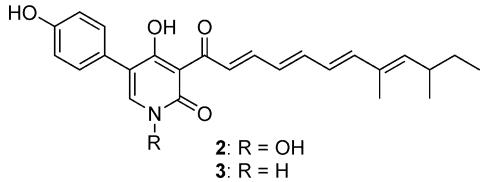
A new tetramic acid derivative, paecilosetin (**1**), along with a recently characterized *N*-hydroxypyridone, farinosone B (**2**), was isolated from the fungus *Paecilomyces farinosus*. Each compound showed activity against the P388 cell line with IC_{50} values of 3.1 and 1.1 μg/mL, respectively. Paecilosetin was also active against the microorganisms *Bacillus subtilis*, *Trichophyton mentagrophytes*, and *Cladosporium resinae*.

Insect pathogenic fungi belonging to the genus *Paecilomyces* have been the source of a wide range of biologically active natural products. For example, the highly cytotoxic and antifungal leucinostatin peptides[1] and a topoisomerase inhibiting quinone, saintopin,[2] have been reported. Within the scope of our continuing program, aimed at the identification of novel bioactive metabolites from New Zealand fungi, we investigated a strain of *Paecilomyces*. The extract of this strain had shown cytotoxic activity against the P388 cell line. Detailed investigation of the fungal extract led to the isolation of the new tetramic acid derivative paecilosetin (**1**), along with the recently reported *N*-hydroxy-2-pyridone farinosone B (**2**).[3]

The fungal strain was isolated from an infected insect larva from leaf litter collected from a suburban garden in Christchurch, New Zealand. By morphological criteria this fungus was identified as *Paecilomyces farinosus*. HPLC analysis of the combined ethyl acetate extracts of mycelium and culture filtrate showed the presence of two major metabolites, **1** and **2**, which were subsequently isolated by reversed-phase semipreparative HPLC on a C_{18} column.



1



2: R = OH
3: R = H

The molecular formula of compound **1** was established by HRESIMS to be $C_{22}H_{31}NO_4$. The UV spectrum, with a maximum at 286 nm as well as smaller maxima at 227 and 250 nm, was indicative of a tenuazonic acid derivative.[4] This assumption was corroborated by data from the one- and two-dimensional NMR experiments, which after analysis (COSY and HMBC NMR data) revealed the presence of the same bicyclic subunit (C-2 to C-16) as in the known fungal products equisetin[5] and trichosetin.[6] Compound **1** differs from these known compounds only in the substitution pattern of the tenuazonic acid moiety; at the 5′-position it carries a 1-hydroxyethyl group rather than the hydroxymethyl group of equisetin and trichosetin and the nitrogen is not methylated. As described before for this class of compounds,[5,6] the evaluation of the NMR spectra was complicated by the broad signals due to the tautomerization of the tenuazonic acid part of the molecule. A ^{13}C NMR spectrum with all signals detected could be obtained only after cooling the sample to −11 °C. NOESY correlations established that the relative configuration of the bicyclic subunit was identical to that in equisetin and trichosetin. As is the case for the known compounds in this class of natural products,[5,6] the negative circular dichroism at 280 nm suggested an *S*-configuration at C-5′. Since the specific optical rotation ($[\alpha]^{20}_D$ −398°) is very similar to that of equisetin and trichosetin, it has been tentatively assumed that the absolute configuration in the bicyclic part of the molecule is also the same as in equisetin and trichosetin. The only stereochemical detail that remains unclear is the configuration at C-6′. Compound **1** was named paecilosetin.

Compound **2** was a yellow pigment with a molecular formula of $C_{25}H_{27}NO_5$ (HRESIMS analysis in combination with ^{13}C NMR data). Extensive evaluation of the NMR data suggested that compound **2** was identical with farinosone B.[3] Farinosone B (**2**) and farinosone A (**3**)[3] are closely related to the known fungal metabolites bassianin and tenellin, isolated from strains of *Beauveria* and differing from **2** and **3** only in the length of the acyl chain.[7] Further related compounds are the militarinones from *Paecilomyces militaris*.[8,9]

Against the murine leukemic P388 cell line,[10,11] paecilosetin (**1**) and farinosone B (**2**) exhibited moderate to weak activity with IC_{50} values of 3.2 and 1.1 μg/mL, respectively. Like the other "-setins",[6,12] paecilosetin (**1**) possesses antimicrobial activity. In an agar diffusion assay[11] paecilosetin (**1**) caused considerable growth inhibition of *Bacillus subtilis* (3 mm zone at 5 μg/disk) and the fungi *Cladosporium resinae* (1 mm zone at 40 μg/disk) and *Trichophyton mentagrophytes* (3 mm zone at 40 μg/disk).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter and CD spectra with a Jasco J-20C spectropolarimeter. UV spectra

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were recorded on a GBC UV/vis 920 spectrometer and IR spectra on a Shimadzu FTIR-8201PC spectrometer. NMR spectra were recorded on a Varian (UNITY INOVA) AS-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references. HRESIMS were acquired using a Micromass LCT TOF mass spectrometer. Solvents used for extraction and isolation were distilled prior to use.

Fungus. The fungal isolate was cultured from an infected insect in leaf litter from a suburban garden in Christchurch, New Zealand. The culture on potato dextrose agar produces floccose white mycelium with a bright yellow reverse and synnemata formation in older cultures. The conidiogenous structures consist of verticillate whorls of phialides with a swollen base tapering to a thin distinct neck. The conidia are borne in dry chains and are fusiform, measuring 2.0 × 1.0 μm. According to these characteristics the fungus was identified as *Paecilomyces farinosus*.[13] The strain has been deposited in the culture collection of the School of Biological Sciences, University of Canterbury (CANU TE108). For chemical investigation *Paecilomyces farinosus* was cultured for 21 days in half-strength Sabouraud dextrose yeast broth (2 × 500 mL) (SDY; as previously reported[14]) at 26 °C under static conditions.

Extraction. The mycelium was separated from the culture medium, macerated, and extracted first with EtOAc (200 mL) and then with MeOH (150 mL). The broth was extracted with EtOAc (3 × 250 mL). The combined extracts were dried to yield the crude extract (608 mg), which was partitioned between petroleum ether and MeOH−H_2O (9:1). The MeOH phase was dried and again partitioned between EtOAc and H_2O. The resulting EtOAc phase was concentrated (146 mg).

Isolation of 1 and 2. The extract was chromatographed on a semipreparative HPLC column (Phenomenex Luna C18, 10 × 250 mm, 5 μm) using isocratic conditions (92.5% MeCN, 7.5% H_2O + 0.05% TFA; 5 mL min^{-1}). Compounds **2** (15.7 mg) and **1** (36.6 mg) were eluted at 5.2 and 5.9 min, respectively.

Paecilosetin (1): pale yellow solid; $[\alpha]^{20}_D$ −398° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.95), 250 (3.91), 286 (4.08); CD (MeOH) λ_{max} ($\Delta\epsilon$) 280 (−27.2); IR (KBr) ν_{max} 3018, 2914, 2844, 1686, 1543, 1452, 1377, 1221, 968 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz, 23 °C) δ 5.55 (1H, m, H-5), 5.53 (1H, m, H-4), 5.37 (1H, m, H-14), 5.36 (1H, m, H-13), 4.17 (1H, m, H-6′), 3.78 (1H, br d, J = 2 Hz, H-5′), 3.58 (1H, br s, H-3), 2.14 (1H, m, H_a-10), 1.99 (1H, m, H-6), 1.95 (1H, m, H_a-7), 1.88 (1H, m, H_a-9), 1.80 (1H, br s, H-11), 1.66 (1H, m, H-8), 1.63 (3H, br d, J = 4.6 Hz, H-15), 1.56 (3H, br s, H-12), 1.42 (3H, d, J = 6.5 Hz, H-7′), 1.22 (1H, m, H_b-9), 1.20 (1H, m, H_b-10), 1.03 (3H, d, J = 6.5 Hz, H-16), 1.00 (1H, m, H_b-7); ^{13}C NMR (acetone-d_6, 125 MHz, −11 °C) δ 197.6 (C, C-1), 191.6 (C, C-4′), 179.5 (C, C-2′), 131.3 (CH, C-13), 130.0 (CH, C-5), 126.6 (CH, C-4), 126.6 (CH, C-14), 100.3 (C, C-3′), 66.5 (CH, C-5′), 66.5 (CH, C-6′), 48.3 (C, C-2), 44.6 (CH, C-3), 42.2 (CH_2, C-7), 39.9 (CH, C-11), 38.6 (CH, C-6), 35.7 (CH_2, C-9), 33.4 (CH, C-8), 28.2 (CH_2, C-10), 22.1 (CH_3, C-16), 20.1 (CH_3, C-7′), 17.5 (CH_3, C-15), 13.2 (CH_3, C-12); HRESIMS *m/z* 374.2345 $[M + H]^+$ (calcd for $C_{22}H_{32}NO_4$, 374.2331).

Farinosone B (2): yellow solid; $[\alpha]^{20}_D$ −18° (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ϵ) 254 (4.39), 414 (4.62); IR (KBr) ν_{max} 2963, 2924, 2856, 1715, 1643, 1591, 1516, 1429, 1371, 1325, 1223, 1007, 837 cm^{-1}; ^{1}H and ^{13}C NMR data and results from CIGAR, COSY, and NOESY were identical with, or consistent with, data reported in the literature;[3] HRESIMS *m/z* 422.1973 $[M + H]^+$ (calcd for $C_{25}H_{28}NO_5$, 422.1967).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of paecilosetin. This material is available free of charge via the Internet at http://pubs.acs.org.

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