Isolation of 2-Pyridone Alkaloids from a New Zealand Marine-Derived *Penicillium* species[⊥]

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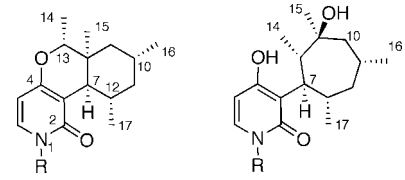
Fermentation of a *Penicillium* sp. isolated from a surface-sterilized thallus segment of the brown alga *Xiphophora gladiata*, collected from Macrocarpa Point, Otago, New Zealand, in half-strength potato dextrose broth led to the isolation and characterization of three alkaloids: the known *N*-hydroxy-2-pyridone, PF1140 (**1**), and two new 2-pyridones, **2** and **3**.

The definition of a marine fungus is open to conjecture, and for many applications the term "marine-derived" is perhaps more appropriate. The production of bioactive secondary metabolites from such marine-derived fungi is now well established, although numbers are low compared to those from other marine organisms.[1] Over 3000 compounds have now been isolated and characterized from the three major marine algal phyla, and these include numerous bioactive compounds.[2] However, algae are still a relatively underexplored source of fungi and fungal metabolites. Marine algae, as with most plants, harbor endophytes, and these, much the same as observed in their terrestrial counterparts, are potential sources of new metabolites.[3] As part of our ongoing program for the isolation of biologically active natural products from New Zealand fungi, we investigated a strain of a marine-derived *Penicillium* species (CANU MCPT14-1-5) isolated from the marine brown alga *Xiphophora gladiata*. The investigation led to the isolation and characterization of three alkaloids: the known *N*-hydroxy-2-pyridone, PF1140 (**1**),[4,5] and two new 2-pyridones, **2** and **3**. Compound **2** differs from **1** by the replacement of the *N*-OH group with *N*-H, while **3** is similarly related to the known 2-pyridone antibiotic akanthomycin (**4**).[6]

The *Penicillium* sp. was cultured for 64 days in half-strength potato dextrose broth ($^1/_2$ PDB) under static conditions. Preliminary reversed-phase analytical HPLC-DAD examination of the EtOAc extract from this fermentation showed the presence of three related compounds, **1**, **2**, and **3**, of which **1** was the major component. Following this initial analysis the three compounds were isolated by solvent/solvent partitioning, followed by size-exclusion chromatography, and semipreparative reversed-phase HPLC.

The molecular formula of **1** was determined as $C_{16}H_{23}NO_3$ on the basis of HRESIMS for the $[MNa]^+$ peak. Prominent features of the ^{1}H NMR spectrum of **1** in CD_3OD included four methyl groups at δ 0.69 (s), 0.88 (d), 0.90 (d), and 1.26 (d), an oxymethine at δ 4.70 (q), and two low-field signals at δ 5.90 and 7.56. A count of 22 protons indicated the presence of one exchangeable proton. The ^{13}C NMR spectrum exhibited 16 resonances consistent with the molecular formula, of which five were low-field sp^2 carbons (δ 100.2, 111.8, 134.5, 162.1, and 162.3) and provided insight into the functional nature of **1**. Analysis of the COSY, HSQC, and HMBC data (see Table 1) defined three spin systems and the linkages between them, which with the MS data established that **1** was identical with, or a stereoisomer of, the antibiotic PF1140 previously isolated from the *Eupenicillium* PF1140 strain.[4] PF1140 was reported initially with unspecified stereochemistry, but the absolute configuration of PF1140 was subsequently determined by X-ray crystallographic analysis of the *S*-2-methoxy-2-(1-naphthyl)-propionic ester derivative.[5] In neither report were NMR data for PF1140 reported, so the relative stereochemistry of **1** could not be assigned by a direct comparison of NMR data. Analysis of the NOESY data for **1** (Figure 1) established that **1** and PF1140 had the same relative configuration. The NOESY correlations observed were consistent with the X-ray crystallographic structure of PF1140,[5] where the cyclohexyl ring (C-7−C-12) had adopted a chair conformation with the three attached methyl groups in equatorial orientations with the C-13 oxymethine axially disposed. Appearance of H_α-9 as a triplet (J = 13.3 Hz) suggested that H-10 is axial (*trans* diaxial coupling to H_α-9), requiring an equatorial orientation for CH_3-16. In support of this, H_β-13 (δ 4.70) showed a strong correlation to the coincident axial H_β-10 and H_β-12 resonances at δ 1.63. This did not provide conclusive proof for, but was consistent with, the equatorial (α-) orientation of CH_3-16. The observed levorotation of **1** suggested that it had the same absolute configuration as PF1140 (also negative rotation),[4] which allowed the configuration at C-13 to be assigned as *R* by correlations in the NOESY spectrum between CH_3-14 (δ 1.26) and CH_3-15 (δ 0.69)/H_β-9 (δ 1.78).



1 R = OH
2 R = H

3 R = H
4 R = OH

The molecular formula of **2**, $C_{16}H_{23}NO_2$ from HRESIMS, contained one less oxygen atom than PF1140 (**1**). A comparison of ^{1}H and ^{13}C NMR data of **1** and **2** showed that the two compounds were closely related. The only notable differences in the NMR data came from the 2-pyridone moiety [H-6, δ 7.56 (**1**), δ 7.15 (**2**); C-2, δ 162.3 (**1**), δ 167.2 (**2**); and C-4, δ 162.1 (**1**), δ 164.9 (**2**)], while for the balance of the molecule the data were almost identical, suggesting that the regio- and stereochemistries were the same. On the basis of these observations and HMBC analysis (Table 1) the structure of **2** was determined to be *N*-deoxy-PF1140, in which the *N*-OH of **1** is replaced by *N*-H.

The molecular formula for compound **3** was established as $C_{16}H_{25}NO_3$ (five degrees of unsaturation) by HRESIMS ($[MNa]^+$). The ^{13}C and ^{1}H NMR spectra of **3** each comprised two sets of closely related resonances suggestive of atropisomerism. All

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Table 1. NMR Spectroscopic Data (500 MHz, CD_3OD) for PF1140 (**1**) and Deoxy-PF1140 (**2**)

		PF1140 (**1**)		deoxy-PF1140 (**2**)	
position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	δ_C, mult.	δ_H (J in Hz)
1	NOH			NH	
2	162.3, qC			167.2, qC	
3	111.8, qC			111.6, qC	
4	162.1, qC			164.9, qC	
5	100.2, CH	5.90, d (7.2)	3, 4, 6	102.6, CH	5.95, d (6.8)
6	134.5, CH	7.56, d (7.2)	2, 4, 5	133.7, CH	7.15, d (6.8)
7	46.9, CH	2.21, d (10.8)	2, 3, 4, 8, 12, 13, 15, 17	45.99	2.16, d (10.8)
8	35.2, qC			35.3, qC	
9	45.94, CH_2	1.78, brd (13.4) (13.8)		45.99, CH_2	1.78, brd (13.8)
		1.02, t (13.3)	8, 10, 13, 15, 16		1.02, t (13.3)
10	28.3, CH	1.63, m		28.4, CH	1.63, m
11	45.86, CH_2	1.70 (brd (13.4)		45.86, CH_2	1.70, brd (13.8)
		0.85, m			0.86, m
12	39.2, CH	1.63, m		39.2, CH	1.63, m
13	75.9, CH	4.70, q (6.1)	7,[b] 8, 14, 15	76.1, CH	4.72, q (6.4)
14	15.3, CH_3	1.26, d (6.1)	8, 13	15.3, CH_3	1.26, q (6.4)
15	22.3, CH_3	0.69, s	7,[b] 8, 10, 13	22.3, CH_3	0.69, s
16	23.5, CH_3	0.88, d (6.4)	10,	23.5, CH_3	0.88, d (6.4)
17	21.3, CH_3	0.90, d (6.4)	7,[b]12	21.3, CH_3	0.91, d (6.6)

[a] Unless noted, HMBC correlations are observed in both **1** and **2**. [b] Distinguishable only for **1**.

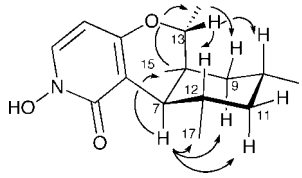
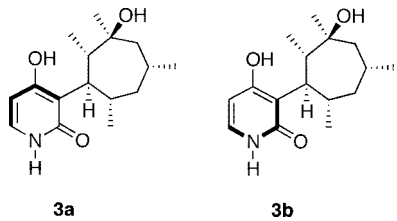


Figure 1. Relevant NOESY correlations observed for **1**.

attempts to separate the two rotamers **3a** and **3b** by careful analytical HPLC failed, so structural elucidation was carried out on the mixture. Extensive analysis of NMR data (COSY, HSQC, HMBC (Table 2)) established that **3** is closely related to the *N*-hydroxy-2-pyridone antibiotic akanthomycin (**4**), previously isolated from the entomopathogenic fungus *Akanthomyces gracilis*.[6] The structure of akanthomycin, which exists as two atropisomers, was established by X-ray crystallography on one of the rotamers.[6] A comparison of NMR data showed that **3** differed from **4** by the replacement of the *N*-OH group of **4** by *N*-H. As with **1** and **2**, the only notable differences in NMR data were associated with the 2-pyridone moiety, while those arising from the seven-membered carbocyclic ring were identical and again suggested that the relative configurations of the two compounds were identical. This was confirmed from NOESY data with correlations from H-7 to Me-14, Me-15, and Me-17 in both **3a** and **3b** and consistent with their *cis* disposition and relative orientation (α-). H_β-13 in both rotamers (δ 2.62 in **3a** and δ 2.45 in **3b**) showed prominent NOESY correlations to the signal at δ 2.05. However, this did not provide unambiguous confirmation of the α-stereochemistry of CH_3-16 due to overlap (δ 2.05) of the H_β-8 and H_β-11 spin systems for each rotamer. Although two sets of NMR data could be recognized (Table 2) for the **3a** and **3b** rotamers, the configurations of the atropisomers have been arbitrarily assigned and may be reversed.



3a **3b**

Labeling experiments had established that PF1140 (**1**) was formed by a mixed biosynthetic pathway involving L-serine and a pentaketide moiety,[7] with one suggested route being via the carbocationic intermediate (**5**) (Figure 2). The co-occurrence of **1**, **2**, and **3** in this *Penicillium* sp. leads credence to the carbocationic pathway over the alternative, a Diels−Alder route, as intramolecular attack by the 4-hydroxyl group on carbocation (**5**) would lead to **1** and **2** (path a), while rearrangement of **5** to the tertiary carbocation **6** (path b) would ultimately lead to **3**.

PF1140 (**1**) was reported to be a wide-spectrum fungicide,[4] while akanthomycin (**4**) was active against *Staphylococcus aureus*.[6] In agar disk diffusion antimicrobial assays against *Bacillus subtilis* and *Candida albicans*, PF1140 (**1**) was active (30 μg/disk; inhibition zones of 8 and 11 mm, respectively), but both deoxy-PF1140 (**2**) and deoxyakanthomycin (**3**) were inactive under comparable conditions.[8] Comparable results were observed in the murine leukemia bioassay (P388) with PF1140 (**1**), found to be modestly active (IC_{50} 1.8 μg/mL), but deoxy-PF1140 (**2**) and deoxyakanthomycin (**3**) were inactive (IC_{50} >12.5 μg/mL).[8] The lack of activity of **2** and **3** suggests the *N*-OH functionality plays a key role in the antibacterial, antimalarial, and cytotoxic activities of *N*-hydroxy-2-pyridones. A similar structure−activity relationship has also been observed in previous studies.[6,9]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341. NMR spectra were recorded on a Varian (UNITY INOVA) AS-500 spectrometer (500 and 125 MHz for ^1H and ^{13}C NMR, respectively) using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 3.30 and δ_C 49.3 for CD_3OD). UV spectra were extracted from the diode array detector signal from a Dionex analytical HPLC system equipped with a UVD340U detector and connected to an Alltech ELSD 800; only relative intensities of the absorption maxima are given. IR spectra were recorded on a Shimadzu FTIR-8201 PC spectrometer. HRESIMS were acquired using a Micromass LCT TOF mass spectrometer. Preparative HPLC was performed on a Shimadzu LC-4A instrument equipped with a SPD-2AS UV spectrometric detector. Solvents used for extraction were distilled prior to use. Cytotoxicities against P388 murine leukemia cells and microbial activities were measured using a standard protocol.[8]

Isolation and Cultivation of the *Penicillium* sp. The fungus was isolated from a surface-sterilized thallus segment of the brown alga *Xiphophora gladiata* collected from the sea at Macrocarpa Point, Otago, New Zealand, and identified by A. L. J. Cole as a *Penicillium* sp. bearing symmetrically biverticillate penicilli. A voucher specimen of the fungus (CANU MCPT14-1-5) is deposited in the culture collection of the School of Biological Sciences, University of Canterbury.

Extraction and Isolation. For chemical investigation the fungus was cultured for 64 days in $^1/_2$ PDB (3 × 500 mL) at 26 °C under static conditions. The mycelium (9 g wet weight) was separated from the culture medium, macerated, and extracted with EtOAc (3 × 200

Table 2. NMR Spectroscopic Data (500 MHz, CD_3OD) for **3a** and **3b**[a]

		3a		3b	
position	δ_C, mult.	δ_H (J in Hz)	HMBC[b]	δ_C, mult.	δ_H (J in Hz)
1	NH			NH	
2	166.1, qC			167.6, qC	
3	118.5, qC			118.4, qC	
4	166.5, qC			166.7, qC	
5	102.2, CH	6.08, d (7.2)	3, 4, 6	103.4, CH	6.01, d (7.0)
6	133.6, CH	7.16, d (7.2)	2, 4, 5	133.4, CH	7.14, d (7.0)
7	45.6, CH	2.76, dd (5.7, 10.6)	2, 3, 4, 8, 9, 13, 14, 17	44.7, CH	2.97, dd (5.7, 11.0)
8	48.3,[c] CH	2.05, m		48.5,[c] CH	2.05, m
9	76.9, qC			77.5, qC	
10	50.3, CH_2	1.60, m		50.8, CH_2	1.64, m
		1.57, m			1.60, m
11	29.8,[d] CH	2.05, m		29.7,[d] CH	2.05, m
12	51.2, CH_2	1.72, m		50.8, CH_2	1.72, m
		0.95, m			1.00, m
13	36.4, CH	2.62, m		37.7, CH	2.45, m
14	18.1, CH_3	0.89, d (8.3)	7, 8, 9	17.8, CH_3	0.87, d (7.7)
15	30.0, CH_3	1.09, s	8, 9	28.9, CH_3	1.15, s
16	25.6,[e] CH_3	0.90, d (6.6)	11	25.7,[e] CH_3	0.92, d (6.2)
17	23.7, CH_3	0.60, d (6.6)	7, 13	23.5, CH_3	0.63, d (6.6)

[a] The data sets for each of **3a** and **3b** may be reversed. [b] HMBC correlations are observed in both **3a** and **3b**. [c–e] Signals may be interchanged.

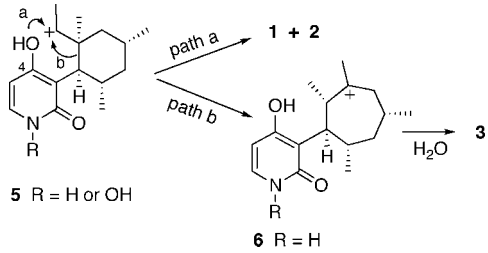


Figure 2. Proposed biosynthetic pathway for **1**, **2**, and **3**.

mL). The culture broth (3 L) was extracted with EtOAc (3 × 200 mL). The combined EtOAc extracts were concentrated under reduced pressure to yield a crude extract (737 mg), which was dissolved in a 19:1 mixture of MeOH and H_2O (200 mL) and extracted with petroleum ether (3 × 100 mL). The aqueous phase was concentrated and freeze-dried, and the resulting residue dissolved in EtOAc (200 mL) and extracted with H_2O (3 × 100 mL) to give the partially purified extract (283 mg). The EtOAc extract (283 mg) was subjected to size-exclusion chromatography on Sephadex LH-20 (2 cm × 60 cm) with MeOH. The fractions containing **1**, **2**, and **3** were combined and dried under reduced pressure. The resulting residue was chromatographed on a semipreparative HPLC column (Phenomenex Luna C_{18}, 10 × 250 mm, 5 μm) using a linear gradient with H_2O and MeCN (0 min 20% MeCN, 40 min 80% MeCN; 5 mL/min; UV detection 212 nm) to elute compounds **3**, as a mixture of atropisomers (4.2 mg), **2** (0.9 mg), and **1** (17.8 mg) at 12.6, 26.9, and 29.6 min, respectively.

PF1140 (1): white, amorphous solid; $[\alpha]^{20}_D$ −124 (*c* 0.26, MeOH); UV (MeCN/H_2O, 75:25) λ_{max} (rel int) 215.4 (100), 287.2 (20); IR (KBr disk) ν_{max} 2952, 2909, 2361, 2343, 1632, 1453, 1229 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 300.1576 (calcd for $C_{16}H_{23}NO_3Na$, 300.1576).

Deoxy-PF1140 (2): white, amorphous solid; $[\alpha]^{20}_D$ −76 (*c* 0.09, MeOH); UV (MeCN/H_2O, 75:25) λ_{max} (rel int) 212.8 (100), 283.5 (20); IR (KBr disk) ν_{max} 2926, 1652, 1457, 1234 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 262.1807 (calcd for $C_{16}H_{24}NO_2$, 262.1807).

Deoxyakanthomycin (3a and 3b): white, amorphous solid; $[\alpha]^{20}_D$ +0.8 (*c* 0.34, MeOH); UV (MeCN/H_2O, 65:35) λ_{max} (rel int) 215.4 (100), 287.2 (20); IR (KBr disk) ν_{max} 2961, 2924, 1614, 1464, 1281, 758 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 302.1718 (calcd for $C_{16}H_{25}NO_3Na$ 302.1732).

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Supporting Information Available: NMR spectra of **1**, **2**, and **3** are available free of charge via the Internet at http://pubs.acs.org.

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