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Penifulvin A: A Sesquiterpenoid-Derived Metabolite Containing a Novel Dioxa[5,5,5,6]fenestrane Ring System from a Fungicolous Isolate of *Penicillium griseofulvum*

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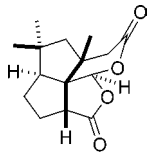
ABSTRACT



penifulvin A

Penifulvin A (1), a new fungal metabolite with a previously undescribed ring system, has been isolated from cultures of an isolate of *Penicillium griseofulvum* (NRRL 35584) obtained from a white mycelial growth on a dead hardwood branch collected in a Hawaiian forest. The structure was assigned by analysis of NMR data and confirmed by single-crystal X-ray diffraction analysis. Penifulvin A (1) shows significant activity in dietary assays against the fall armyworm *Spodoptera frugiperda*.

Our ongoing studies of mycoparasitic and fungicolous fungal isolates as sources of bioactive secondary metabolites have led to the discovery of a variety of new natural products.[1] The occurrence of antifungal metabolites is proving to be common among these fungi, as might be predicted on the basis of their tendency to cause damage to host species, but compounds with other bioactivities have also been encountered. In the course of this project, a Hawaiian isolate of *Penicillium griseofulvum* Dierckx (MYC-1728 = NRRL 35584)[2] was subjected to chemical investigation. *P. griseofulvum* (syn. *P. patulum* Bain.; *P. urticae* Bain.) is worldwide in its distribution. This fungus has been recorded from grassland, desert soil, decaying plants, cereal grains, and animal feed and is known to produce several important bioactive metabolites, including roquefortine C, cyclopiazonic acid, patulin, and griseofulvin.[3] An organic extract from cultures of *P. griseofulvum* NRRL 35584 showed potent antifungal and antiinsectan activity in preliminary assays. Studies of this extract have led to the discovery of a novel antiinsectan sesquiterpenoid that we named penifulvin A (**1**).

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(2) The culture employed in this work (MYC 1728 = NRRL 35584) was originally isolated by D.T.W. from a white mycelial growth on the undersurface of a dead hardwood branch that was collected in a montane dry forest (Ohi'a), Hue Hue Street, Kailua-Kona, Hawaii Co., HI, by D.T.W. in November 2002.

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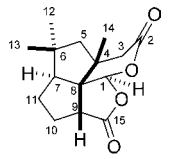
Table 1. NMR Data for Penifulvin A (**1**)[a]

position	δ_H[a] (mult, *J* in Hz)	δ_C[a]	NOESY	HMBC (H# → C#)
1	5.95 (s)	103.7	3α, 7, 12	7, 8, 9, 15
2		168.3		
3α	2.77 (d, 16)	43.9	1, 3*β*, 5α, 5*β*, 12	1,[b] 2, 4, 5, 8, 14
3*β*	2.43 (d, 16)		3α, 5α, 5*β*, 14	1,[b] 2, 4, 5, 8, 9,[b] 14
4		41.9		
5α	1.76 (d, 14)	55.4	5*β*, 12	1,[b] 2,[b] 3, 4, 6, 7, 8, 12, 13, 14
5*β*	1.71 (d, 14)		5α, 13, 14	1,[b] 2,[b] 3, 4, 6, 7, 8, 12, 13, 14
6		40.2		
7	2.30 (dd, 5.1, 9.8)	60.7	1, 11α, 12	1, 5, 6, 8, 10, 11, 12
8		66.7		
9	2.95 (dd, 1.2, 8.0)	46.2	10*β*, 11*β*, 14	1, 4, 7, 8, 10, 11, 15
10α	2.20 (dddd, 2.0, 3.4, 8.0, 14)	29.9	9, 10*β*, 11α, 11*β*	7, 8, 9, 11, 15
10*β*	1.90 (ddt, 8.0, 8.0, 14)		9, 10α, 11α, 11*β*, 13, 14	7, 8, 9, 11, 15
11α	1.71 (m)	28.0	7, 10α, 11*β*	6, 7, 8, 9, 10
11*β*	1.83 (m)		9, 10α, 10*β*, 11α, 13	6, 7, 8, 9, 10
12	1.22 (s)	32.6	1, 3α, 5α, 7, 13	5, 6, 7, 13
13	1.00 (s)	27.4	5*β*, 11*β*, 12, 14	5, 6, 7, 12
14	1.07 (s)	27.2	3*β*, 5*β*, 9, 10*β*, 13	3, 4, 5, 8
15		177.8		

[a] Data were recorded in $CDCl_3$ at 600 MHz (^1H, COSY, HMQC, HMBC) and 100 MHz (^{13}C). [b] Four-bond correlations, most of which were of relatively low intensity.

Details of the isolation and structure determination of **1** are presented here.



1

Fractionation of the crude EtOAc extract of solid-substrate fermentation cultures of MYC-1728 by silica gel column chromatography afforded penifulvin A (**1**),[4] together with the common fungal metabolite mycophenolic acid, as the major components. The molecular formula of penifulvin A (**1**) was determined to be $C_{15}H_{20}O_4$ (six unsaturations) by analysis of ^1H, ^{13}C, and DEPT NMR data and was verified by HRESIMS. All 20 protons were bound to carbon on the basis of DEPT results, so no exchangeable hydrogens were present. The NMR data (Table 1) revealed the presence of three singlet methyl groups, four methylene units (two of which were isolated), two aliphatic sp^3 methine units, one doubly oxygenated (acetal) sp^3 methine unit, three sp^3 quaternary carbons, and two ester groups. Accordingly, a tetracyclic structure was required for **1** to fulfill the unsaturation requirement.

An isolated $CHCH_2CH_2CH$ spin-system corresponding to the C7/C9−C11 unit in **1** was assigned on the basis of $^1H-^1H$ COSY data. Attachment of C-9 of this unit to an ester carbonyl carbon (C-15; δ_C 177.8) was evident from HMBC correlations (Figure 1) of H-9 and H_2-10 with C-15. An

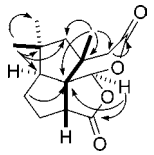


Figure 1. Key HMBC correlations observed for penifulvin A (**1**).

HMBC correlation of the acetal proton (H-1; δ_H 5.95) with C-15 led to attachment of acetal carbon C-1 to C-15 through an oxygen atom.

Two geminal methyl groups, C-12 and C-13, were connected to quaternary carbon C-6, which was, in turn, connected to C-7 and isolated methylene C-5 on the basis of HMBC correlations from H_3-12 and H_3-13 to C-5, C-6, and C-7. The remaining methyl singlet (H_3-14) showed HMBC correlations to quaternary carbons C-4 and C-8 as well as to isolated methylene carbons C-5 and C-3. This result enabled connection of quaternary carbons C-4 and C-8 and of C-3 and C-5 to one of these quaternary carbons, although at this point it was not clear which of the two

(4) General fermentation and extraction procedures used have been described elsewhere.[1e] The EtOAc extract (1.33 g) from solid-substrate fermentation cultures on rice (carried out in eight 500-mL Erlenmeyer flasks, each containing 50 g of rice) of *P. griseofulvum* was partitioned between CH_3CN and hexane. The CH_3CN-soluble portion (0.831 g) was subjected to silica gel column chromatography using a hexanes−EtOAc solvent gradient. The fraction eluted with 95:5 hexanes−EtOAc (120 mg) was crystallized from MeOH to yield penifulvin A (**1**, 55 mg). The fraction eluted with 90:10 hexanes−EtOAc (70 mg) was further separated by semipreparative reversed-phase HPLC (Alltech HS Hyperprep 100 BDS C18 column; 10 × 250 mm; flow rate, 2 mL/min; 30−100% CH_3CN in H_2O over 45 min) to afford an additional sample of penifulvin A (**1**, 9 mg, t_R = 21.6 min). Penifulvin A has the following properties: colorless needles; mp 153−155 °C.; $[\alpha]_D$ −3.5 (*c* 0.17 g/100 mL, MeOH); IR (CH_2Cl_2) ν_{max} 3035, 2988, 1804, 1774, 1551, 1422 cm^{-1}; ^1H NMR, ^{13}C NMR, HMBC, and NOESY data, see Table 1; HRESIMS obsd *m/z* 287.1256, calcd for $C_{15}H_{20}O_4$ + Na, 287.1259.

quaternary carbons is directly bonded to C-14, C-3, and C-5. Methylene carbon C-3 was further connected to the second ester carbonyl (C-2; δ_C 168.3) based on HMBC correlations from H_2-3 to C-2, as well as to C-4, C-14, and C-5.

HMBC correlations from H_2-11 to C-8 required C-8 to be connected to C-7, thereby enabling assignment of the locations of C-7 and C-8 as shown. At this point, all elements of the molecular formula had been accounted for, and a single ring remained to be assigned. Although H-1 did not show an HMBC correlation with the second ester carbon (C-2), the chemical shift values (δ_H 5.95; δ_C 103.7)[5] indicated that C-1 is doubly oxygenated, and combining this with the need for an additional ring and the absence of exchangeable protons led to closure of the second lactone ring to give gross structure **1** for penifulvin A.

NMR assignments for the two geminal methyl groups were made by analysis of NOESY data as shown in Table 1. Strong correlations between H_3-12 and H-1 and between H_3-13 and H_3-14 suggested their assignments as shown. Individual assignments for all methylene proton signals were made on the basis of NOESY correlations provided in Table 1.

Crystals of **1** were obtained from methanol, and X-ray crystallographic analysis[6] led to confirmation of the proposed structure, as well as establishment of the relative stereochemistry, as shown in Figure 2. Penifulvin A (**1**) has a unique tetracyclic ring system in which all four rings share a central quaternary carbon (C-8; 66.7). This feature makes **1** a member of an unusual and rare class of molecules generally referred to as fenestranes,[7] in which multiple rings share a central quaternary carbon in spiro fashion. While synthetic fenestranes are known, to our knowledge, the only naturally occurring fenestrane containing four rings that share a central quaternary carbon is laurenene.[8] Penifulvin A (**1**) is not a true fenestrane, as it is not entirely carbocyclic, but can be described as a dioxa[5,5,5,6]fenestrane. This ring system has not been previously described in the literature.

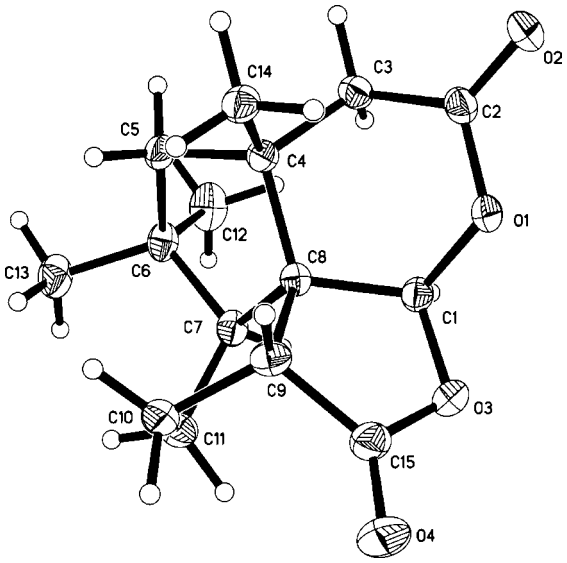
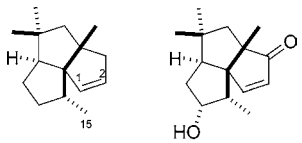


Figure 2. X-ray model of penifulvin A (**1**).

Penifulvin A (**1**) appears to be biogenetically related to the silphinenes, a small group of triquinane sesquiterpenoids that have been isolated primarily from plants, e.g., silphinene (**2**) from *Silphium perfoliatum*.[9] One compound with this skeleton has been encountered as a fungal metabolite (phomalairdenone, **3**; a host-selective phytotoxin from an isolate of the black leg fungus *Phoma lingam*).[10] Compound **1** is proposed to be biosynthesized from *E,E*-farnesyl cation via caryophyllene and silphinene-type intermediates. An oxidative cleavage of the C1−C2 olefin of a C15-oxidized analogue of silphinene (**2**), and subsequent bis-lactonization could afford **1**. The relative stereochemistry established for penifulvin A (**1**) is analogous to the relevant features of silphinene, which supports this proposed biogenetic origin.



2 **3**

Penifulvin A (**1**) showed no activity in standard agar disk diffusion assays at 100 μg/disk against *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Candida albicans* (ATCC 14053). Compound **1** was also inactive against *Aspergillus*

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(6) A colorless blade (0.48 × 0.18 × 0.04 mm) was isolated from the sample, mounted with grease on the tip of a glass capillary epoxied to a brass pin, and placed on the diffractometer with the long crystal dimension (unit cell *a*-axis) approximately parallel to the diffractometer φ axis. Data for penifulvin A (**1**) were collected on a Nonius KappaCCD diffractometer (Mo Kα radiation, graphite monochromator) at 190(2) K (cold N_2 gas stream) using standard CCD techniques yielding 21858 data. Lorentz, polarization, and a multiscan absorption corrections were applied (T_{max} = 0.9962, T_{min} = 0.9559). Equivalent data were averaged yielding 1760 unique data (R_{int} = 0.050, 1452$F > 4\sigma(F)$, Friedel pairs averaged for the last cycles of refinement). Based on a preliminary examination of the crystal, the space group $P2_12_12_1$ was assigned (no exceptions to the systematic absences: $h00$, h = odd, $0k0$, k = odd, $00l$, l = odd, were noted). The computer programs from the HKL package were used for data reduction. The preliminary model of the structure was obtained using XS, a direct methods program. Least-squares refining of the model vs the data was performed with XL computer program. Illustrations were made with the XP program and tables were made with the XCIF program. All of the programs are in the SHELXTL v6.1 package. Thermal ellipsoids shown in the illustrations are at the 35% level. All non-hydrogen atoms were refined with anisotropic thermal parameters. All H atoms were included with the riding model using the XL program default values. No further restraints or constraints were imposed on the refinement model. The final refinement gave R_1 = 0.0355 and wR_2 = 0.0793. Crystallographic data for penifulvin A (**1**) have been deposited with the Cambridge Crystallographic Data Centre (CCDC no. 284388).

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flavus (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457) at 200 μg/disk. The antifungal activity of the EtOAc extract was ascribed to the known compound mycophenolic acid. However, penifulvin A (**1**) showed significant antiinsectan activity in assays[11] against the fall armyworm *Spodoptera frugiperda*, causing 74% reduction in growth rate (RGR) relative to controls, when tested at a dietary level of 160 ppm.

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

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