Penifulvins B–E and a Silphinene Analogue: Sesquiterpenoids from a Fungicolous Isolate of *Penicillium griseofulvum*

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Penifulvins B–E (2–5), four new sesquiterpenoids with a dioxa[5.5.5.6]fenestrane ring system, have been isolated from cultures of an isolate of *Penicillium griseofulvum* (NRRL 35584), together with a new silphinene derivative, 12-hydroxysilphinene-15-oic acid (6). Penifulvins B–E (2–5) are oxidized analogues of penifulvin A (1) and were identified by analysis of NMR and MS data. 12-Hydroxysilphinene-15-oic acid (6) is biogenetically similar, and penifulvins A–E are presumed to be derived from a silphinene precursor. The structures of 2–6, including absolute configuration, were assigned by analysis of NMR data and application of chemical methods.

Mycoresistant and fungicidal fungi are those that parasitize or colonize the hyphae or survival structures of other species.¹² Our studies of mycoresistant and fungicidal fungi have led to the isolation of a variety of new bioactive secondary metabolites.³–⁵ In the course of this project, a Hawaiian isolate of *Penicillium griseofulvum* Dierckx (MYC-1728 = NRRL 35584) was subjected to chemical investigation. An organic extract from cultures of *P. griseofulvum* NRRL 35584 showed potent antifungal and antiinsect activity in preliminary assays. A sesquiterpenoid with a novel ring system (penifulvin A; 1) was initially isolated as the major component, together with the known antifungal compound mycofenolic acid.³ Continued studies of this extract have led to the identification of four additional analogues (penifulvins B–E; 2–5) and a new silphinene derivative (12-hydroxysilphinene-15-oic acid; 6), together with the known *Penicillium* metabolites asperphenamate, brevinamamide A, breviarnamide E, 1-deoxypebrolide, and desacetylpebrolide. Penifulvins B–E (2–5) are oxidized analogues of 1, while 12-hydroxysilphinene-15-oic acid (6) is a biogenetically related metabolite with a different ring system. This report describes the isolation and structure elucidation of compounds 2–6.

**Results and Discussion**

Penifulvins A–E (1–5) and 12-hydroxysilphinene-15-oic acid (6) were all obtained by processes involving silica gel column chromatography followed by reversed-phase HPLC. HRESIMS and NMR data established the molecular formula of penifulvin B (2) as C₁₅H₂₀O₅. This formula contains one more oxygen atom than that of penifulvin A (1). Analysis of ¹H and ¹³C NMR data for 2 (Tables 1 and 2) revealed considerable structural similarities to penifulvin A (1), which facilitated the structure elucidation of this metabolite. Characteristic resonances for the dioxa[5.5.5.6]fenestrane system found in 1, including the diagnostic acetal (δH 5.97; δC 103.9) and central quaternary carbon (δC 66.7) signals, were observed. ¹H, ¹³C, and DEPT NMR data indicated that 2 has two methyl groups, rather than three as in penifulvin A, and has an oxygenated methylene unit instead (δH 3.45 and 3.48; δC 56.2), suggesting that one of the three methyl groups in 1 is oxidized to a primary alcohol in 2. This conclusion was confirmed and the OH group was located by analysis of HMBC and NOESY data. The oxygenmethylene proton resonances showed HMBC correlations with one methyl carbon at δC 22.8, a quaternary carbon at δC 45.9, a methylene carbon at δC 53.9, and a methine carbon at δC 56.2, indicating that one of the geminal methyls at C-6 in 1 is replaced by a CH₂OH group in 2. Chemical shift changes at C-5, C-6, C-7, and C-13 relative to the corresponding data for 1 were consistent with addition of an OH group at this position. NOESY correlations of the oxymethylene signals with H-7 (δH 2.59) and H-5α (δH 2.07) indicated that the hydroxymethylene unit has the α-orientation. Thus, the structure of penifulvin B was determined as shown in 2.

HRESIMS and NMR data indicated that penifulvin C (3) is an isomer of penifulvin B (2). The close resemblance of the ¹H and ¹³C NMR data for 3 to those of 2 (Tables 1 and 2) indicated the presence of the same skeleton found in 1 and 2 and suggested that the only difference is that C-13 bears the OH group, rather than C-12, as in 3. This conclusion was confirmed by analysis of HMBC and NOESY data. HMBC correlations from the oxymethylene signals at δH 3.45 and 3.49 matched those of the oxymethylene signals in 2, but in this instance, the CH₂OH protons showed NOESY correlations to H₂-11, while the methyl singlet (CH₃-12) showed correlations to H-1, H-7, and H-3α.

The molecular formula of penifulvin D (4) was also established as C₁₅H₂₃O₂ on the basis of HRESIMS and NMR data and again showed clear similarities to 1–3. In this instance, a broad

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exchangeable proton resonance was observed at δH 2.55, and 13C and DEPT NMR data for 4 suggested that the C-9 methine carbon found in 1–3 was oxidized to a tertiary alcohol (δC 76.8) in 4. HMBC correlations from the hydroxyl group to C-9, C-10, and C-15 and from H2-10 to C-9, C-11, and C-15 confirmed attachment of the hydroxy group to C-9. The relative configuration was assigned by analysis of NOESY correlations, which matched well with those of penifulvins A–C (1–3), suggesting that the 9-OH group has the same (β) orientation as H-9 in 1–3. Placement of the hydroxyl group at C-9 in an α-orientation would introduce considerable ring strain and would be expected to result in a quite different set of NOESY data.

HRESIMS and DEPT data for penifulvin E (5) established it as another isomer of penifulvins B–D. Its 1H and 13C NMR data were again similar to those of 1–4. In this case, signals for an additional oxygenated methine (δH 4.66; δC 76.8) appeared in the NMR spectra for 5, implying that one of the methylene units present in 1 was oxidized to a secondary alcohol in 5. HMBC correlations from the oxygenated methine proton to C-7, C-8, and C-15 indicated that the hydroxy group is attached to C-10. The relative configuration of the C-10 secondary alcohol moiety was assigned on the basis of NOESY data. In contrast to the assignment for 2–4, the signal for H-11β, recognized by virtue of its NOESY correlation to H-13, was more upfield in the 1H NMR spectrum of 5 than H-11α. Correlation of H-10 with H-7 and observation of a much stronger correlation of H-10 with H-11α than with H-11β enabled assignment of a β-orientation for the OH group.

The presence of a secondary alcohol moiety in 5 suggested that it might be suitable for stereochemical analysis using Mosher’s method.9 Treatment of 5 with (S)-MPAOH (α-methoxycarbonylpyridine-3-carboxylic acid) or (R)-MPAOH in the presence of EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide] and DMAP afforded the (S)-MPA ester (5a) or (R)-MPA et (5b), respectively. Formation of the esters was confirmed in each case by a significant downfield shift of the H-10 resonance and the appearance of the expected aromatic and methoxy signals in the 1H NMR spectrum. 1H NMR signals for 5a and 5b were assigned by comparison with the data for 5 and were confirmed by analysis of NOESY correlations. Upon comparison of 1H NMR chemical shifts for 5a and 5b (Δδ values shown in Figure 1), with one minor exception, all of the Δδ values observed for 5a and 5b (Figure 1) were consistent with assignment of the S-configuration at C-10, leading to the proposal of the overall absolute configuration as shown. Although the signal for H-11α showed a slight negative Δδ value, those for H-11β and other protons on the same side of the MPA group plane showed strongly positive Δδ values, particularly those on the same face of the ring system as the MPA group. Penifulvins A–D (1–4) are assumed to possess analogous absolute configurations.

The molecular formula for 6 was established as C15H20O5 on the basis of HRESIMS, 13C NMR, and DEPT data. Although several similar structural features were evident, the pattern of NMR signals for 6 was quite different from those of 1–5. Most notably, signals for a 1,2-disubstituted olefin and a single ester or acid carbonyl signal were present, while the acetal signals characteristic of 1–5 were not observed. The presence of one olefin unit and one carbonyl group indicated that 6 is tricyclic. The DEPT data in conjunction with the molecular formula indicated the presence of two exchangeable protons, which must be accounted for by hydroxy and carboxylic acid groups. 1H and 13C NMR signals clearly indicated that the hydroxy group was part of an isolated hydroxymethylene unit. Analysis of COSY data revealed the same set of spin systems
found in 2 except that the C-3 methylene protons were coupled to those of the olefin unit. These data were strongly suggestive of a silphinen skeleton.10,11

The structure of compound 6 was established by interpretation of HMBC data (Table 3). HMBC correlations from H-9 to C-1, C-4, C-8, and carboxylic acid carbon C-15, together with those from the oxymethylene protons to C-5, C-6, C-7, and C-13 and from H-14 to C-3, C-4, C-5, and C-8 indicated that 6 is 12-hydroxysilphinen-15-oic acid. Other correlations observed were fully consistent with this assignment. The relative configuration was as described above support this relationship. The absolute configurations for 1 and 5 were analogous to those of previously reported silphinenes,10,11 as described above support this proposed biosynthetic relationship. The absolute configurations assigned for 1–6 were analogous to those of previously reported silphinenes,10,11 although no absolute configuration has been previously reported for a fungal silphinen.

Determination of the absolute configuration of 6 was attempted using a method developed for chiral a,α-disubstituted propionic acid derivatives.14 Treatment of compound 6 with (R)- and (S)-phenylglycine methyl ester (PGME) under suitable amidation conditions produced the corresponding amide derivatives, which were purified by HPLC and analyzed by 1H NMR. The 1H NMR chemical shift differences (Δδ = δR – δS) between signals for the diastereomeric (R)- and (S)-PGME amide derivatives are shown in Figure 2. Negative Δδ values are expected for protons that reside on the same side of the PGME amide bond plane as the phenyl group in a given PGME derivative, and positive Δδ values are expected for protons on the opposite side.14 Interpretation of the results on this basis led to assignment of the R-configuration at C-9, and the overall absolute configuration of 6 was therefore assigned as shown.

As noted in the earlier report describing lead compound 1, penifulvins appear to be biogenetically related to silphinenes. Although silphinenes have been isolated primarily from plants such as Silphium perfoliatum,15,16 silphinenes analogues have been previously reported from one other fungal source (phomalairdenones and phomalairdenols; phytotoxins from the blackleg fungus Phoma lingam).12,13 Penifulvins are proposed to be biosynthesized from farnesyl cation via caryophyllene15 and silphinen intermediates. For example, as depicted in Scheme 1, oxidative cleavage of the C-1–C-2 olefin unit of 6 with appropriate adjustment of oxidation state at C-1 and subsequent bis-lactonization could afford penifulvin B (2). Isolation of new silphinen analogue 6 from the same source organism and independent determination of analogous absolute configurations for 5 and 6 as described above support this proposed biosynthetic relationship. The absolute configurations assigned for 1–6 are analogous to those of previously reported silphinenes,10,11 although no absolute configuration has been previously reported for a fungal silphinen.

The two most abundant analogues described here (penifulvin B, 2; 12-hydroxysilphinen-15-oic acid, 6) were tested in standard agar disk diffusion assays at 100 μg/disk against Aspergillus flavus

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**Table 3. HMBC Data for Penifulvins B–E (2–5) and 12-Hydroxysilphinen-15-oic Acid (6)**

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* Data were recorded using CDCl3 solutions at 600 MHz. 4Four-bond correlations, most of which were of relatively low intensity.

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**Figure 2.** Observed chemical shift differences (Δδ = δR – δS, 400 MHz) for the R- and S-PGME-amides of 12-hydroxysilphinen-15-oic acid (6).

**Scheme 1.** Proposed Biosynthetic Formation of Penifulvin B (2) from Silphinen Analogue 6
Penifulvin E (5): amorphous powder; [α]D20 -100 (c 0.5 × 10-3 g/mL, CHCl3); 1H NMR, 13C NMR, and HMBC data, see Tables 1–3; NOESY correlations (CDCl3, H-# → H-9) H-1 ↔ H-3a, H-7 and H-12; H-3a ↔ H-1 and H-12; H-3b ↔ H-5a, H-5b, and H-12; 1H NMR, 13C NMR, and HMBC data, see Tables 1–3; NOESY correlations (CDCl3, H-# → H-9) H-1 ↔ H-3a, H-7 and H-12; H-3a ↔ H-1 and H-12; H-3b ↔ H-5a, H-5b, and H-12; H-5a ↔ H-7 and H-11b; H-10a ↔ H-7 and H-10b; H-11b ↔ H-9, H-10a, and H-11b; H-11a ↔ H-7, H-10a, and H-10b; H-11b ↔ H-9, H-10a, and H-11b; H-12 ↔ H-3a, H-5a, H-7, and H-12; H-13 ↔ H-5b and H-12; H-14 ↔ H-3b, H-5, and H-9; HRESIMS obsd m/z 303.1214 [M + Na]+, calecd for C19H20O16Na 303.1208.


(R)- and (S)-MPA Esters of Penifulvin E (5). A solution of 5.0 mg in distilled CHCl3 (500 μL) was treated with (S)-(+) or (R)-(−)-methoxypheylacetic acid [(S)-MPAOH, 1.0 mg, μmol], DMAP (one crystal), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.5 μmol). The mixture was stirred at 25° C for 5 h. One milliliter of H2O was added, and the mixture was extracted with CHCl3 (3 × 1.5 mL). The combined organic extracts were concentrated, filtered, and evaporated to give a white solid, which was then subjected to HPLC (30 to 100% CH3CN in H2O over 45 min) to afford (R)-MPA ester 5a (0.3 mg). Analogs of 5 (0.5 mg) using (R)-MPAOH afforded (R)-MPA ester 5b (0.2 mg).

(S)-MPA ester 5a: amorphous powder; [α]D20 5.86 (1H, s, H-1), 5.48 (d, d = 0.8, 2.2, 6.0 Hz, H-10), 3.05 (br s, H-9), 2.79 (d, d = 15 Hz, H-3a), 2.38 (d, d = 15 Hz, H-3b), 2.53 (dd, d = 14, 7 Hz, H-3a), 1.15 (m, H-10a), 0.85 (s, H-13), 0.80 (s, H-14); NOESY correlations (CDCl3, H-# → H-9) H-1 ↔ H-3a, H-3b, H-5, and H-7; H-2 ↔ H-3a, H-5, and H-7; H-7 ↔ H-11a, H-11b, and H-14; H-7 ↔ H-10a, H-11a, and H-12; H-9 ↔ H-10a and H-14; H-10a ↔ H-7, H-9, H-11a; H-11b ↔ H-7, H-9, and H-11a; H-11b ↔ H-7 and H-13; H-12 ↔ H-5a, H-7, and H-13; H-13 ↔ H-5b, H-11a, and H-12; H-14 ↔ H-3b, H-5, and H-9.

(S)-MPA ester 5b: amorphous powder; [α]D20 5.86 (1H, s, H-1), 5.48 (d, d = 0.8, 2.2, 6.0 Hz, H-10), 3.12 (br s, H-9), 2.79 (d, d = 15 Hz, H-3a), 2.38 (d, d = 15 Hz, H-3b), 2.53 (dd, d = 14, 7 Hz, H-3a), 1.15 (m, H-10a), 0.85 (s, H-13), 0.80 (s, H-14); NOESY correlations (CDCl3, H-# → H-9) H-1 ↔ H-3a, H-3b, H-5, and H-7; H-2 ↔ H-3a, H-5, and H-7; H-7 ↔ H-11a, H-11b, and H-14; H-7 ↔ H-10a, H-11a, and H-12; H-9 ↔ H-10a and H-14; H-10a ↔ H-7, H-9, H-11a; H-11b ↔ H-7, H-9, and H-11a; H-11b ↔ H-7 and H-13; H-12 ↔ H-5a, H-7, and H-13; H-13 ↔ H-5b, H-11a, and H-12; H-14 ↔ H-3b, H-5, and H-9.

Preparation of PGME Derivatives of 6. The PGME derivatives were prepared according to the method of Yabuuchi and Kusumi, using identical reagent concentrations and reaction conditions for the preparation of both sets of derivatives. A sample of 6 (0.7 mg) and 1.0
mg of (R or S)-PGME hydrochloride (Aldrich) were dissolved in 1 mL of dry DMF. The solution was cooled in an ice–water bath, and 2.0 mg of PyBOP, 1.0 mg of HOBT, and 50 µL of N-methylmorpholine were added. The mixture was stirred at room temperature for 7 h. EtOAc (5 mL) was added, and the organic layer was collected and extracted with H2O (2 × 5 mL). After removal of solvent, 1.3 mg of the crude (R)-PGME derivative and 1.7 mg of the crude (S)-PGME derivative were obtained in the two reactions. Purification by reversed-phase HPLC (C18, UV detection at 215 nm, 30 to 100% CH3CN in H2O over 45 min, 2 mL/min afforded 6a (0.3 mg) and 6b (0.5 mg).

12-Hydroxysilphinen-15-oic acid-(R)-PGME-amide (6a): amorphous powder; 1H NMR (400 MHz, CDCl3) δ 7.30 (5H, m, phenyl group protons), 6.39 (d, J = 6.9 Hz, NH), 5.61 (dt, J = 2.0, 5.8 Hz, H-1), 5.50 (d, J = 7.0 Hz, CHNH), 5.47 (dt, J = 2.2, 5.8 Hz, H-2), 3.68 (s, OCH3), 3.37 (d, J = 10.5 Hz, Ha-12), 3.31 (d, J = 10.5 Hz, Hb-12), 2.78 (t, J = 4.8 Hz, H-9), 2.38 (dt, J = 2.2, 17 Hz, H-3α), 2.29 (dt, J = 2.2, 17 Hz, H-3β), 2.11 (dd, J = 7.8, 11 Hz, H-7), 1.94 (m, H-10), 1.92 (d, J = 13 Hz, H-5α), 1.75 (m, H-11α), 1.56 (d, J = 13 Hz, H-5β), 1.38 (m, H-11β), 1.27 (s, H-14), 0.98 (s, H-13).

12-Hydroxysilphinen-15-oic acid-(S)-PGME-amide (6b): amorphous powder; 1H NMR (400 MHz, CDCl3) δ 7.29 (5H, m, phenyl group protons), 6.33 (d, J = 7.0 Hz, NH), 5.52 (d, J = 7.0 Hz, CHNH), 5.42 (dt, J = 2.2, 5.8 Hz, H-1), 5.29 (dt, J = 2.2, 5.8 Hz, H-2), 3.70 (s, OCH3), 3.28 (d, J = 0.5 Hz, Ha-12), 3.33 (d, J = 10.5 Hz, Hb-12), 2.79 (dd, J = 7.8, 9.0 Hz, H-9), 2.33 (dt, J = 2.2, 17 Hz, H-3α), 2.16 (dt, J = 2.2, 17 Hz, H-3β), 2.09 (dd, J = 8.0, 11 Hz, H-7), 1.98 (m, H-10), 1.88 (d, J = 13 Hz, H-5α), 1.76 (m, H-11α), 1.54 (d, J = 13 Hz, H-5β), 1.39 (m, H-11β), 1.23 (s, H-14), 0.97 (s, H-13).

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Supporting Information Available: 1H and 13C NMR spectra of 2–6. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


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