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Structures and Absolute Configurations of Penicillactones A–C from an Endophytic Microorganism, *Penicillium dangeardii* Pitt

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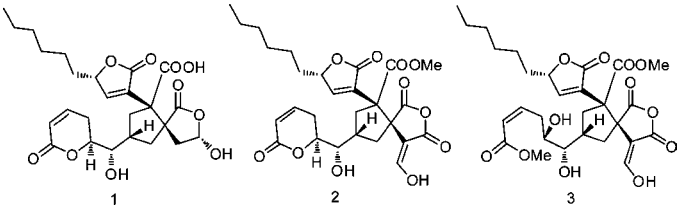
ABSTRACT



Penicillactones A–C (1–3) are structurally related natural products with a spirocyclic anhydride structure and were isolated from the endophytic fungus *Penicillium dangeardii* Pitt. Penicillactones B and C showed inhibition of the release of β-glucuronidase from polymorphonuclear leukocytes with ED_{50} values of 2.58 and 1.57 μM. A 2D INADEQUATE experiment of 1 was performed at natural abundance to confirm the arrangement of its carbon skeleton. The configurations of 1–3 were established through extensive NMR spectroscopic analysis, selective structural modifications, and CD analysis.

Among secondary metabolites, molecules with anhydride moieties have special biological activities including antibiotic activity and enzymatic inhibition.[1] While surveying endophytic fungi for new biologically active secondary metabolites, a family of structurally related anhydrides, penicillactones **A**–**C** (**1**–**3**), was isolated from the *Penicillium dangeardii* Pitt residing in a toxic plant, *Lysidice rhodostegia* (Leguminosae sp.).[2] Structurally, compounds **2** and **3** represent a new structural class of endophytic fungal isolates, which are characterized by the presence of a spirocyclic anhydride moiety.[1] In our study, the structures and absolute configuration were determined via extensive NMR spectroscopic analysis, conformational analysis, selective structural modifications, and CD analysis.

The HRESIMS of penicillactone **A** ($[M + H]^+$, m/z 493.2089) combined with the ^{13}C and ^1H NMR data (Table S1, Supporting Information) indicated a molecular formula of $C_{25}H_{32}O_{10}$ with 10 degrees of unsaturation. The ^{13}C NMR resonances were attributed to four carbonyl groups (δ 180.2, 172.4, 172.4, and 164.3) and four sp^2 carbons (δ 134.6, 154.1, 121.0, and 147.3), which account for 6 of the 10 degrees of unsaturation and therefore reveal the presence of four rings in the structure. Interpretation of the $^1H-^1H$ COSY and HSQC spectra led to the assignment of three isolated spin systems [I: C3–C4; II: C4′-C11′; III (III-1: C3″-C7″-C8 and III-2: C7–C9)] in **1** (Figure 1a). The 2D INADEQUATE spectrum of **1** at its natural abundance facilitated the unambiguous identification of three fragments (F1–F3) with contiguous carbon skeletons [F1: C3–C4, F2: C1–C5–C6(C6a)-C3′(C2′)-C10′, and F3: C2″-C7″-C8(C7)-C9] (Figures 1b and S4, Supporting Information) in the molecule, which accounted for all but three bonds—C4–C5, C6–C7, and C5–C9; these

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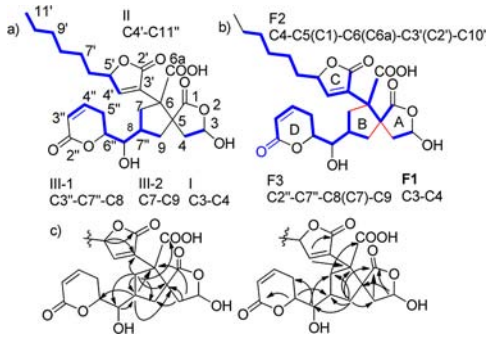
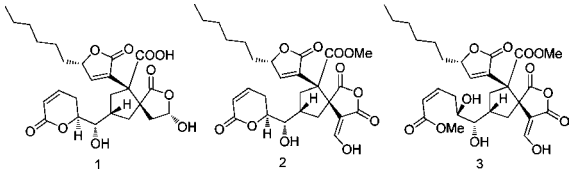


Figure 1. (a) Isolated ^{1}H spin systems (I−III); (b) 2D INADEQUATE ^{13}C−^{13}C NMR correlations (F1−F3); (c) key HMBC correlations of penicillactone **A**.

bonds could be easily deduced from the HMBC correlations (Figure 1c).



The connectivity of C4 and C5 were confirmed by the HMBC correlations between H4 and C1/C5/C6/C9. The INADEQUATE correlations of C3−C4 and C5−C1, the spin system I, and the HMBC correlations from H-3 to C1/C5 confirmed that C1 and C3 were connected through an oxygen atom to form a five-membered ring (ring A). Ring B was deduced from the INADEQUATE correlations of C5−C6 and C7−C8−C9 and the HMBC correlations from H7 to C5, H9 to C6, and H-8 to C6 and C5. The INADEQUATE correlations of C2′−C3′−C4′−C5′, the spin system of C4′−C5′, and the HMBC correlations from H5′ to C2′ indicated that one unsaturated γ-lactone unit (ring C) was incorporated into the structure. The unsaturated δ-lactone unit (ring D) was assigned by the spin system C3″−C6″, the INADEQUATE correlations C2″−C6″, and the HMBC correlation from H6″ to C2″. The HMBC correlations from H9 to C1/C4/C5/C6 and from H4 to C1/C5/C6/C9 indicated that rings A and B were fused through C5 to form a spirocyclic ring system. The connection of rings B and C through the C6−C3′ bond was verified by the HMBC correlations from H7 to C3′ and from H4′ to C6. The 2D INADEQUATE correlations (F3) and the COSY relationships between H6″/H7″ and H7″/H8 clearly indicated that the connectivity between rings B and D occurred through C7″, a finding that was further supported by the HMBC correlations between H6″/H7″ and C8, and between H8 and C6″. Therefore, the planar structure of compound **1** was identified and named penicillactone **A**. The structure of **1** features a hemiacetal at C3, a spirocyclic system (rings A and B), unsaturated γ- and δ-lactones (rings C and D), and alkyl chains; this structure contains seven stereocenters including C3, C5, C6, C8, C5′, C6″, and C7″.

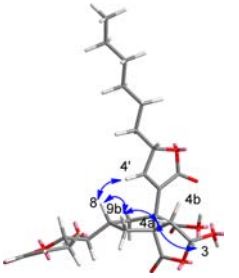


Figure 2. Key NOE correlations of penicillactone **A**.

The relative configurations were determined using the coupling constants, NOE correlations, and conformational analysis. Spin decoupling experiments facilitated the rapid identification of the vicinal coupling constants of H3/H4 ($^{3}J_{H3,H4a}$ = 6.0 Hz, $^{3}J_{H3,H4b}$ = 0 Hz) by removing the couplings to the irradiated spins (Figure S8, Supporting Information). Minimizing the molecular energy of **1** in ChemBio 3D elucidated the dihedral angles (φH3,H4a = 18° and φH3,H4b = 98°), which were in good agreement with the *J*-values and strong NOE correlations of H3/H4a (Figure 2). The NOE correlations of H8/H4′ indicated that ring C and H8 resided on the same face of ring B. The NOE correlations of H9b/H4a and H9b/H8 suggested that the spirocyclic system (rings A and B) should be fused in the arrangement shown in Figure 2. The combination of homonuclear coupling constants ($^{3}J_{H,H}$) and NOE correlations enabled the unambiguous identification of just one of the six possible staggered conformations of the flexible fragment of C6″−C7″, which were A1, A2, A3 and B1, B2, B3 (Figure 3, Figures S9 and S10, Supporting Information).[3] The vicinal coupling constant of H6″/H7″ ($^{3}J_{H6'',H7''}$ = 0 Hz) was assigned by removing all of the couplings to H8. The small coupling constant of H6″/H7″, which was indicative of a gauche orientation between these two protons, reduced the possible dominant rotamers by eliminating two out of the initial six, A1 and B1. The NOE correlations of H7″/H5″(both H5″a and H5″b), eliminated A3 and B2. The lack of NOE correlations between H8 and H5″ (H5″a and H5″b) also supported the exclusion of the A3 and B2 rotamers. The A2 rotamer was ruled out due to the NOE correlations of H6″/H7 (both H7a and H7b). The remaining rotamer, B3, matches the observed value of $^{3}J_{H6'',H7''}$ and the NOE correlations of H6″/H7″, H6″/5″b, H5″a/H7″, H5″b/H7″, and H6″/H8.

Similarly, the relative configuration of the flexible C7″−C8 fragment was identified by analyzing the coupling constant ($^{3}J_{H7'',H8}$ = 9.0 Hz, Figure S9, Supporting Information) and NOE correlations. The large coupling constant of H7″/H8 indicated that these two protons had an antiplanar conformation (C1 or D1). However,

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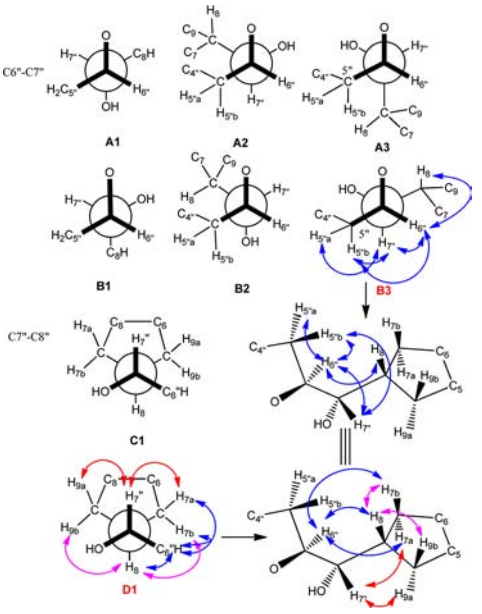


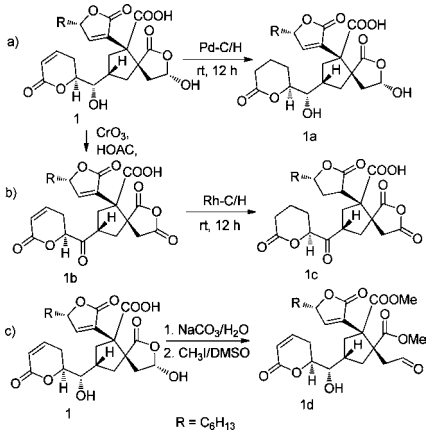
Figure 3. Assignment of the relative configurations of the C6″−C7″ and C7″−C8 fragments.

the dihedral angle between H7″/H8 varied from 180° due to the weak NOE correlations between these two protons. The NOE correlations (Figure S10, Supporting Information) of H6″/H7a, H6″/H7b, H8/H7b, H8/H9b, H7″/H7a, and H7″/H9a clearly demonstrated that the relative configuration of C7″−C8 was D1.

After establishing the relative configuration, our attention turned to the absolute configuration. What did first was to remove the Cotton effect interference between two α,β-unsaturated lactones. Compound **1** was selectively reduced to yield **1a**, with only one α,β-unsaturated γ-lactone (ring C, Scheme 1).

The CD spectrum of **1a** indicated that ring C contributed a negative n−π* Cotton effect at 250 nm and a positive π−π* Cotton effect at 220 nm, revealing that the C6′−C5′−C4′═C3′ system possessed a right-handed helicity and that C5′ had an *S* configuration.[4] From the differential CD spectrum (**1**−**1a**) (Figure 4), one could infer that the α,β-unsaturated δ-lactone at ring D contributed a negative π−π* at 220 nm and a positive n−π* Cotton effect at 260 nm to the structure, indicative of the *S* absolute configuration at C6″ according to the octant rule.[5] Furthermore, to determine the configuration of the spiro carbon at C5, **1** was oxidized to generate **1b** before being reduced to form **1c** (Scheme 1),[6] which contained a succinic anhydride (ring A). The CD spectrum of **1c** revealed an n−π* electronic Cotton effect at 235 nm, which was attributed to the succinic anhydride moiety, and when this result was combined with the octant rule, the absolute configuration of C5 was determined to be *S* (Figure 4).[7] After elucidating the absolute configurations of C5, C5′, and C6″ via the CD data, the absolute configurations of the other stereocenters at C3, C6, C8, and C7″ were assigned according to their relative configurations. The absolute configurations of C7″ and C8 were both assigned as *S* based on the *S* configuration of C6″. The *S* configurations at C8 and C5 subsequently indicated that C3 and C6 had the *S* and *S* configurations, respectively. The absolute configuration of C7″ was further supported by the CD spectrum of the in situ-formed Rh-complex of **1d** (Figure S26, Supporting Information).[8] Finally, the absolute configuration of penicillactone **A** was elucidated as 3*S*,5*S*,6*S*,8*S*,5′*S*,6″*S*,7″*S*.

Scheme 1. (a) Reaction of Compound **1** with Pd−C/H and CrO_3 To Yield **1a** and **1b**, Respectively; (b) Reduction of **1b** To Yield **1c**; (c) Methylation of Penicillactone A



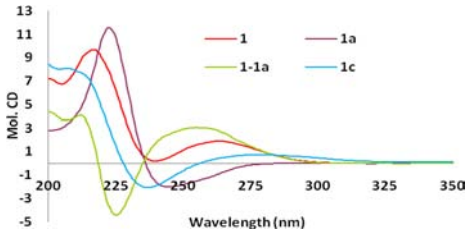


Figure 4. CD spectra of **1**, **1a**, **1**−**1a**, and **1c**.

Penicillactone **B** (**2**) was determined to have a molecular formula of $C_{27}H_{32}O_{11}$ based on the HRESIMS $[M + Na]^+$ mass ion peak at *m*/*z* 555.1853 and the NMR data. Given the similarities between the ^1H, and ^{13}C NMR spectra of **1** and **2**, one can conclude that **1** and **2** have similar carbon skeletons (Tables S1 and S2, Supporting Information). By interpreting the $^1H-^1H$ COSY and the HSQC spectra, two

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isolated spin systems [I: C4′-C-11′; II (II-1: C3″-C7″-C8 and II-2: C7−C9)] in **2** were assigned (Figure 5a). In addition to spin systems I and II, one singlet proton resonance at δ 7.74 ppm (H4a), one carbon signal at δ 157.1 ppm (C4a), and the corresponding HSQC experiment supported the presence of an additional oxygenized sp^2 carbon in the structure, which was determined to be a free enol group after two etherification reactions were performed yielding **2a** and **2b** (S1, Supporting Information).

The HMBC correlations (Figure 5b) from H4a to C3, C4, and C5 found that C4a on C4 was an olefinic bond. However, unlike **1**, the A ring system of **2** could not be assigned directly from the HMBC correlations due to a lack of protons at C1, C3, C4, and C5. In this case, the ring systems B−D were determined prior to the assignment of ring A. The HMBC correlations from H-7 to C-5, H-9 to C-6, H-8 to C6 and C5 demonstrated that C5 and C6 were directly connected with the spin system C7−C9 (II-2) to form one 5-membered ring (B). The HMBC correlations from H-4′/H-5′ to C-2′ indicated the presence of one unsaturated γ-lactone unit (ring C) in the structure. The HMBC correlations from H-4′ to C-6 and from H-7 to C-3′ placed ring C on C6 via C-3′. The identification of the unsaturated δ-lactone unit (ring D) was determined by the weak HMBC correlation from H-6″ to C-2″ and (−) HR-QTOF-ESI-MS^2 experiment (Figure S29, Supporting Information).

The HMBC correlations from H4a to C3 revealed the presence of a C3−C4−C4a unit. The HMBC correlations from H-9 to C4 and from H-4a to C5 indicated that C4 and C5 were connected. The HMBC correlation from H-9 to C1 also supported the linkage between C1 and C-5. The identified five carbonyl groups, three double bonds, and three rings (B-D) account for 11 of the 12 degrees of unsaturation, suggesting the presence of an additional ring in the structure; these data imply that the two carbonyl groups at C-1 and C-3 were members of an anhydride ring. The presence of the anhydride ring was further verified by its distinctive IR absorption at 1819 cm^{-1} (Figure S38, Supporting Information). Finally, the structure of **2** was determined to possess the same carbon skeleton as **1**, aside from the presence of a succinic anhydride moiety and an enol group on ring A.

The relative configurations of **2** was determined to be the same as **1** based on the NOE and conformational analyses. The NOE interactions between H8 and H4′ indicated that ring C and H8 resided on same face of ring B (Figure 5c). The NOE interactions of H4a/H9a and H9a/H8 also indicated that the C4−C4a bond and H8 were in the same orientation (Figure 5c). The relative configurations of C6″−C7″ and C7″−C8 were identified as staggered conformations of B3 and D1 by analyzing their homonuclear coupling constants ($^3J_{H,H}$) and NOE correlations as described in **1** (Figure S36, Supporting Information).

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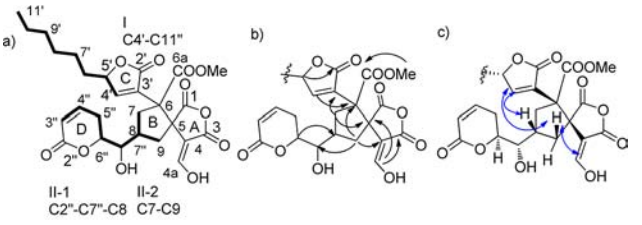

Figure 5. (a) Isolated ^1H spin systems; (b) key HMBC correlations; (c) key NOE correlations of penicillactone B.

The absolute configuration of **2** was determined via the same experiments as those performed for **1**. From the positive n−π* Cotton effect at 250 nm and the negative π−π* Cotton effect at 215 nm in the differential CD spectrum generated by subtracting **2c** from **2** (Figure S55, Supporting Information), one can conclude that unsaturated δ-lactone (ring D) contributes a positive Cotton effect to the structure. Therefore, the configuration at C-6″ must be *S* according to the Snatzke rule.[5] Based on the relative configuration, the *S* configuration at C6″ was translated to 5*R*,6*S*,8*S*,6″*S*,7″*S* on **2**. Based on the biogenesis, the C5′ in **2** was determined to be *S*, just as in **1**. The similar NMR resonance at C5′ also supported the assignment of the *S* configuration at C5.

The structural assignment of penicillactone **C** (**3**) was confirmed as an acyclic ester for spin system II at C2″−C7″ instead of a lactone (ring D), as observed in **2**. The configuration of penicillactone **C** was determined to be same as **2** based on its highly similar carbon skeleton and biogenesis (Table S3, Supporting Information).

Biologically, penicillactones **B** and **C** demonstrate inhibition of the release of β-glucuronidase from polymorphonuclear leukocytes induced by the platelet-activating factor (PAF);[9] the ED_{50} values (50% effective doses) for this inhibitory activity were 2.58 μM and 1.57 μM for penicillactones **B** and **C**, respectively. Therefore, these structures represent new potential leads in the development of the inhibitors of the release of β-glucuronidase. In the same assay, penicillactone **A** exhibited a weaker bioactivity ($ED_{50} > 50\,\mu$M), possibly due to the structural differences in the rings of A of **1**−**3**.

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Supporting Information Available. Experimental details, NMR data assignments, UV, IR, MS, 1D and 2D NMR, and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.