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Peniciketals A–C, New Spiroketals from Saline Soil Derived *Penicillium raistrichii*

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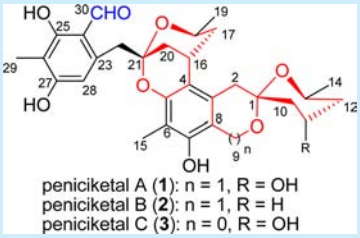
Supporting Information

ABSTRACT: Peniciketals A–C (**1**–**3**), three new spiroketals with a benzo-fused 2,8-dioxabicyclo[3.3.1]nonane moiety, were isolated from the saline soil derived fungus *Penicillium raistrichii*. Their structures including absolute configurations were established by NMR, X-ray diffraction, and ECD calculations. Their cytotoxicities were tested against A549, HL-60, and K562 cell lines, and **1**–**3** showed the selective effects on HL-60 cells with IC_{50} values of 3.2, 6.7, and 4.5 μM, respectively.



Spiroketals have been found from various sources such as plants, microorganisms, insects, and marine organisms, with spiroketal moieties conjugated to other building blocks to form diverse structurally intriguing and biologically active natural compounds.[1] Among the reported compounds in this family, benzannulated spiroketals are relatively rare,[2] especially those with the benzannulated 6,6-spiroring systems.[3] Attracted by their challenging frameworks and extensive biological activities, spiroketals have been the focus of considerable attention in both chemical and biological communities.[4]

During our continuing search for novel bioactive natural products from saline soil derived fungi,[5] *Penicillium raistrickii*[6] was obtained from the sample collected along the coast of Bohai Bay in Zhanhua, China. Previous study of this fungus resulted in the isolation of three new spiroketals, one new isocoumarin and two known quinolinone alkaloids.[7] Further investigation led to the discovery of three new spiroketals incorporated with a benzo-fused 2,8-dioxabicyclo[3.3.1]nonane moiety, named peniciketals A–C (**1**–**3**). On the basis of the their bioactivities, Chinese patent protection has been sought.[8] Details of the isolation, structure elucidation, cytotoxicity, and hypothetical biogenesis of **1**–**3** are presented here.

The working fungus *P. raistrickii* was fermented in seawater-based culture medium on a rotatory shaker at 28 °C for 10 days. The ethyl acetate extract (42 g) was subjected to silica gel column chromatography followed by Sephadex LH-20 and semipreparative HPLC to give compounds **1**, **2**, and **3** (65, 1.5, and 2.8 mg, respectively).

Peniciketal A (**1**)[9] was isolated as colorless blocks. Its molecular formula was determined to be $C_{30}H_{36}O_9$ on the basis of HRESIMS at m/z 539.2279 $[M - H]^-$ (calcd for $C_{30}H_{35}O_9$, 539.2276). The ^1H and ^{13}C NMR spectra displayed resonances for an aldehyde group, 12 sp^2 carbons including one protonated and four oxygenated ones (which suggested two phenyl rings: one pentasubstituted, the other hexasubstituted), two doubly oxygenated sp^3 quaternary carbons,[10] four sp^3 methines (three oxygen-bearing), seven sp^3 methylenes with one oxygenated, and two aromatic and two secondary methyl groups (Table S1, Supporting Information). The above information accounted for nine out of the 13 unsaturation degrees according to the molecular formula, indicating **1** contained four additional rings.

The pentasubstituted phenyl ring was deduced on the basis of the HMBC correlations from H-29 to C-25/C-26/C-27, from OH-25 to C-24/C-25/C-26/C-30, from H-30 to C-23/C-24/C-25, from H-22 to C-23/C-24/C-28, and from H-28 to C-22/C-24/C-26/C-27 (Figure 1). The hexasubstituted phenyl ring was confirmed by the HMBC correlations from H-2 to C-3/C-4/C-8, from H-9 to C-3/C-7/C-8, and from H-15 to C-5/C-6/C-7. The connection of C-22 with C-20 through the doubly oxygenated carbon (C-21) was determined by analysis of the HMBC correlations from H-22 to C-21/C-20, from H-28 to C-21, and from H-20 to C-21. Analysis of the COSY and HSQC spectra led to the identification of two discrete proton spin-systems corresponding to H-10 - H-14 and H-20 - H-16 - H-19 (Figure 1). The HMBC correlations from H-20 to C-4, and from H-16 to C-3/C-4/C-5, suggested that C-16 was anchored to C-4. And the linkage between C-1 and C-10 was revealed by the HMBC correlations from H-2 to C-10, and from H-10 to C-2. The further HMBC correlations from both H-2 and H-9 to C-1 constructed the dihydropyran moiety. In view of the doubly oxygenated nature of C-1 (δ_C 98.1) and C-21 (δ_C 98.6), and the chemical shifts for C-13 (δ_C 61.9), C-18 (δ_C 66.5) and C-5 (δ_C 153.1), the connections between C-1 and C-13, between C-21 and C-18, and between C-21 and C-5, all through oxygen atoms, were arranged to satisfy the

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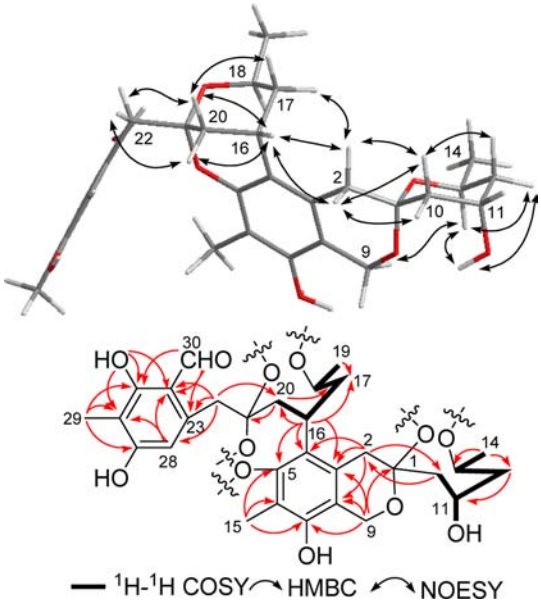

Figure 1. Selected 2D NMR correlations for compound **1**.

unsaturation degrees required by its molecular formula and the requirement of their chemical shifts.[7a]

In the NOESY experiment, the NOE correlations of H_{ax}-13 with OH-11 indicated H_{ax}-13 and OH-11 in axial positions, which were confirmed by the NOE correlations of both H_{eq}-11 and H_3-14 with H_{eq}-12 (δ_H 1.71) and H_{ax}-12 (δ_H 1.43). The NOE correlation between H_{ax}-10 (δ_H 1.84) and H_{ax}-12 suggested they were in the opposite axial orientations to H_{ax}-13. In addition, the NOE correlations between H_{eq}-10 (δ_H 1.92) and H_a-2 (δ_H 2.72), and between H_{ax}-13 and H_a-9 (δ_H 4.75), revealed the relative configuration of the spirocarbon (C-1) as shown in Figure 1. The relative configuration of C-16 was deduced from the NOE correlations of H_b-2 (δ_H 2.50) with H_2-17, and of H_a-2 with H_{eq}-10 and H_{ax}-10. The small coupling constants between H_{eq}-16 and H_{ax}-20 (δ_H 1.98, J = 2.7 Hz) and H_{eq}-20 (δ_H 1.63, J = 3.2 Hz), together with the NOE correlation of H_{ax}-20 with H_2-17, placed H_{eq}-16 equatorial, with C-4 and the oxygen atom which linked C-5 and C-21 in axial orientations, thus defining the configuration of C-21. Unfortunately, the relative stereochemistry of C-18 could not be confirmed from the NOE spectrum. Finally, the absolute configuration of **1** has been established by a single-crystal X-ray diffraction analysis (Figure 2).[11] The final refinement on the Cu Kα data resulted in a Flack parameter of −0.01, allowing an unambiguous assignment of the absolute configurations to be 1*R*,11*S*,13*S*,16*S*,18*S*,21*R*.

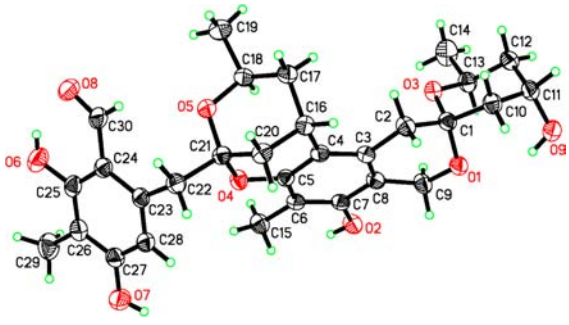

Figure 2. ORTEP representation of peniciketal A (**1**).

Peniciketal B (**2**)[12] was obtained as colorless solid; its molecular formula was established as $C_{30}H_{36}O_8$ from the HRESIMS data with one less oxygen atom than that of **1**. The molecular formula and the similar UV and IR data suggested that **2** was a deoxygenated analogue of **1**. Consistent with this inference, the NMR data of **2** showed the presence of an additional methylene and the disappearance of a oxymethine relative to that of **1**. Its planar structure was proved by the COSY and HMBC data (Figure S1, Supporting Information).

The same relative stereostructure for both compounds was deduced from their similar NOESY correlations (Figure S2, Supporting Information) and the almost identical ^{1}H and ^{13}C NMR data concerned. Since the splitting patterns of H_{ax}-13 and H_{ax}-18 in **2** were similar to those of the corresponding hydrogen signals in **1** (Figure S3, Supporting Information), the orientations of H_{ax}-13 and H_{ax}-18 were identical in the both compounds, respectively. Considering that **1** and **2** were produced by the same strain of *P. raistrickii* and exhibited almost the same CD absorptions (Figure S26, Supporting Information), the absolute configurations of **2** were assigned as 1*S*,13*S*,16*S*,18*S*,21*R*.

Peniciketal C (**3**)[13] was assigned the molecular formula $C_{29}H_{34}O_9$ on the basis of HRESIMS analysis, which was smaller than that of **1** by a CH_2. The ^{13}C NMR and DEPT spectra displayed that **3** was short of a oxygenated methylene relative to **1**, which was supported by the disappearance of two doublets assigned to a diastereotopic oxymethylene in the ^{1}H NMR spectrum of **1**. Since a doubly oxygenated quaternary carbon atom in **3** resonated downfiled (δ_c 112.0 ppm) from the usual chemical shift ($\sim\delta_c$ 98 ppm),[14] the spiro-carbon atom was anchored at the phenyl ring through an oxygen atom to form a benzodihydrofuran moiety. As a result, the chemical shifts from C-1 to C-8 in **3** and **1** were obviously different (Table S1, Supporting Information). The proton and carbon shift assignments were made by analysis of HMBC, HSQC and COSY data.

The NOE correlations of H_{eq}-11 with H_{eq}-10 (δ_H 2.12), H_{ax}-10 (δ_H 2.05), H_{eq}-12 (δ_H 1.74), and H_{ax}-12 (δ_H 1.50), of H_3-14 with H_{eq}-12 and H_{ax}-12, and of H_{ax}-10 with H_{ax}-12 suggested that H_{ax}-10 and H_{ax}-12 were at one axial orientation, while H_{ax}-13 and OH-11 were at another. The relative configuration of C-1 was deduced from the correlations of H_a-2 (δ_H 3.08) with H_{eq}-10 and H_{ax}-10, and of H_b-2 (δ_H 2.98) with H_2-17 (δ_H 1.57) and H_{ax}-10. Other NOE correlations (Figure 3), NMR data, and the splitting pattern of H_{ax}-13 and H_{ax}-18 (Figure S3, Supporting Information) were almost identical with those in **1** and **2**, so the relative configurations of **3** were established. The same CD absorptions (Figure S26, Supporting Information) of **1** and **3** suggested the absolute configurations of **3** to be 1*S*,11*S*,13*S*,16*S*,18*S*,21*R*.

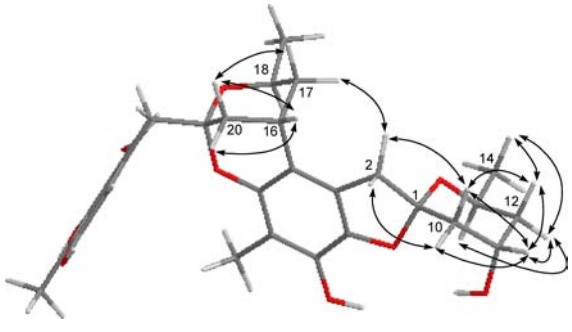

Figure 3. Selected NOESY correlations for compound **3**.

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Theoretical ECD calculations with the time-dependent density functional theory (TDDFT) provide a powerful and reliable method for determining the absolute configuration of complex natural products.[15] In order to further confirm our assignment of the absolute configuration of compound **3**, a comparison was made between the experimental and predicted ECD spectra. As shown in Figure 4, the calculated ECD curve matched very well with the experimental ECD, indicating that the absolute configuration of **3** was assigned unambiguously.

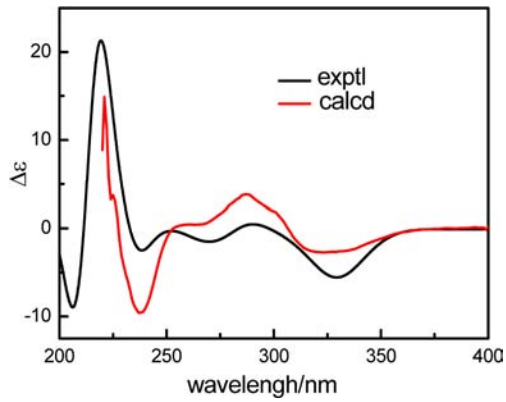

Figure 4. Experimental (black) and suitable calculated ECD (red) spectra of peniciketal C (**3**).

A plausible biogenetic pathway of **1**−**3** is proposed as shown in Scheme 1. The important presumed intermediate **a** is synthesized via the acetyl malonyl pathway. Intermediates **c**, **d**, and **e**, which are derived from **a**, produced the intermediates **f**, **g**, and **h** through intramolecular ketal reaction. Then **f**, **g**, and **h** combine with **b**, respectively, through electrophilic aromatic substitution and ketal formation to obtain **1**, **2** and **3**.

Compounds **1**−**3** were evaluated for their cytotoxic effects on A549, HL-60, and K562 cancer cell lines using the MTT method[16] with doxorubicin as a positive control (IC_{50}s: 0.31, 0.085, and 0.23 μM, respectively). Compounds **1**−**3** showed selective activities against HL-60 cells with IC_{50} values of 3.2, 6.7, and 4.5 μM, respectively, while were not active on other cells (IC_{50} > 10 μM).

In conclusion, we isolated and characterized three novel spiroketals from saline soil derived *P. raistrickii*. Their most intriguing feature is one phenyl ring fused not only to a 6,6- or 5,6-spiroring but also to a 2,8-dioxabicyclo[3.3.1]nonane moiety. In addition, there is an aldehyde group in each of them, which may be used to modify their structures for the sake of improving their bioactivities. To the best of our knowledge, this structural framework has no counterpart in the literature. The discovery of **1**−**3** presents a new challenge to organic synthesis chemists. And the postulated biosynthetic route might be helpful in the total syntheses of these unique spiroketals.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, ^{1}H, ^{13}C, and 2D NMR data, HRESIMS, IR, UV, and CD spectra, and details of the quantum chemical ECD calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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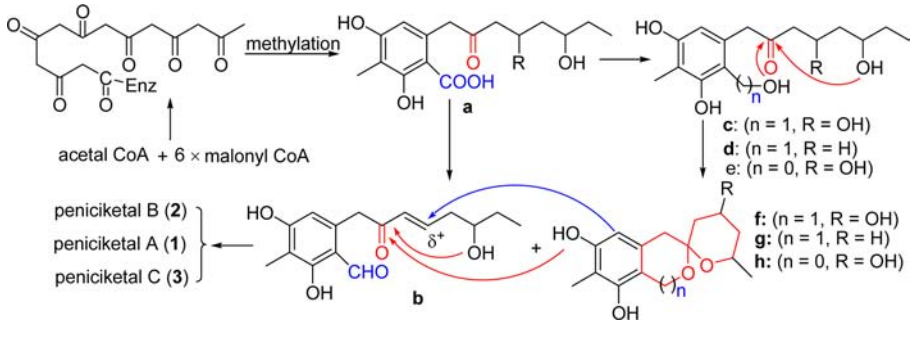
Scheme 1. Hypothetical Biosynthetic Pathways for 1−3



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(9) Peniciketal A (**1**): colorless blocks; mp 184−186 °C; $[\alpha]^{20}_{D}$ +23.7 (*c*, 0.53, acetone); UV (CH_3OH) λ_{max} (log ε) 292 (3.81), 206 (4.49) nm; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 323 (−0.36), 292 (+0.80), 239 (−0.43) nm; IR (ATR) ν_{max} 3362, 2976, 2926, 2871, 1672, 1604, 1495, 1429, 1376, 1350, 1296, 1254, 1172, 1162, 1121, 1100, 1048, 996, 970, 929, 871, 850, 792, 743 cm^{-1}; HRESIMS *m*/*z* 539.2279 $[M - H]^-$ (calcd for $C_{30}H_{35}O_9$ 539.2276).
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(11) Crystal data for peniciketal A (**1**): $C_{30}H_{36}O_9{\cdot}CH_3OH$; M_r = 572.63, monoclinic, space group $P2_1$, a = 8.3023(2) Å, b = 12.4556(4) Å, c = 13.7273(4) Å, β = 90.279(2)°, V = 1419.53(7) $Å^3$, Z = 2, T = 290(2) K, μ (Cu Kα) = 0.825 mm^{-1}, D_{calc} = 1.340 g/cm^3, F(000) = 612.0, 12163 reflections measured (6.44 ≤ 2θ ≤ 133.14), 4757 unique (R_{int} = 0.0120) which were used in all calculations. The final R_1 was 0.0252 [$I \geq 2\sigma(I)$] and wR_2 was 0.0701 (all data). The crystal structure of compound **1** was solved by direct methods SHELXS-97 and expanded using difference Fourier techniques, refined by the program SHLXL-97 and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 940798. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
(12) Peniciketal B (**2**): colorless solid; $[\alpha]^{20}_{D}$ +10.4 (*c*, 1.0, acetone); UV (CH_3OH) λ_{max} (log ε) 292 (4.18), 213 (4.44) nm; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 321 (−1.54), 292 (+3.61), 237 (−2.04) nm; IR (ATR) ν_{max} 3258, 2930, 2900, 1703, 1615, 1446, 1372, 1354, 1301, 1251, 1163, 1120, 1088, 1053, 996, 973, 925, 873, 755 cm^{-1}; HRESIMS *m*/*z* 523.2332 $[M - H]^-$ (calcd for $C_{30}H_{35}O_8$ 523.2326).
(13) Peniciketal C (**3**): colorless solid; $[\alpha]^{20}_{D}$ +9.1 (*c*, 1.9, acetone); UV (CH_3OH) λ_{max} (log ε) 292 (4.14), 210 (4.48) nm; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 323 (−2.73), 287 (+3.82), 237 (−9.61) nm; IR (ATR) ν_{max} 3236, 2925, 2900, 1700, 1615, 1493, 1436, 1300, 1250, 1224, 1170, 1120, 1093, 1051, 1027, 998, 983, 968, 940, 893, 839, 788, 757 cm^{-1}; HRESIMS *m*/*z* 525.2128 $[M - H]^-$ (calcd for $C_{29}H_{33}O_9$ 525.2119).
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