Three New Polyketides from the Insect-Associated Fungus *Letendrea sp.* 5XNZ4-2

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Abstract: Chemical investigation of the EtOAc extract of an insect-associated fungus *Letendrea sp.* 5XNZ4-2 cultured in Potato Dextrose Broth (1/2 PDB) medium lead to the isolation of three new polyketides, named letendronol D (1), phomopsiketones H-I (2-3). The structures of new compounds were elucidated by the analysis of HRESIMS and NMR spectroscopic data, and the absolute configurations were determined by modified Mosher’s method, ECD calculation and single-crystal X-ray diffraction. Cytotoxicity and antibacterial activities of 1 were assayed and regrettably 1 didn’t display any cytotoxicity and antibacterial activity. 3 was the first phomopsiketone derivative obtaining the lactone.

Keywords: polyketides; insect-associated fungus; *Letendrea sp.* © 2020 ACG Publications. All rights reserved.

1. Introduction

Insect-associated fungi, which develop symbiotic relationships with their hosts [1], can provide biologically active and structurally interesting natural products [2], such as macrodiolides [3, 4], alkaloids [5], polyketides [6] and so on that likely protect insect hosts from infestation [7].

During the course of our efforts toward searching for structurally new and bioactive secondary metabolites from insect-associated fungi [8], nine polyketides have been isolated from the 1/2 PDB culture broth of endophytic fungus *Letendrea sp.* 5XNZ4-2 [9, 10], indicating that its metabolic pathway was unique. More studies were carried out for this strain to explore its metabolic potential. As a result, three new polyketides, letendronol D (1), phomopsiketone H (2) and phomopsiketone I (3), were isolated. Herein, we describe the isolation, structure identification, and bioactivity evaluation of the new compounds.

2. Materials and Methods

2.1. Materials and Instruments [9]

Optical rotations were recorded on Rudolph research analytical AUTOPOL I. The ultraviolet and Electronic circular dichroism (ECD) spectra were measured on Shimadzu UV-1800 spectrophotometer and JASCO J-1500 circular dichroism, respectively. The infrared (IR) spectra were
acquired from a Thermo Nicolet iS10. 1D and 2D NMR spectra were recorded on Bruker AVIII 500 MHz and JEOL 600Hz, both using TMS as the internal standard. HR-ESI-MS data were obtained from an Agilent 6224 TOF LC-MS. Analytical and preparative liquid chromatography were performed on Agilent 1260 and Agilent Technologies ProStar system, while C18 (Cosmosil, 5 μm, 4.6 × 250 mm) packing column was used for HPLC analysis. The column chromatography (CC) was performed on Silica gel (200–300 mesh, Qing Dao Hai Yang Chemical Group Co.).

The Letendreae sp. was isolated from the gut of a crab found on Zhairoushan Island (N20.2920, E122.5), Zhejiang Province, China. The fungus was determined as Letendreae sp. by 26s rDNA sequence analysis (GenBank accession no. MK743951).

2.2. Fermentation and Isolation

The strain was static cultured in 500 mL Erlenmeyer-flasks each containing 200 mL of 1/2 PDB media (100 g potato extraction; 17 g artificial sea salt and 10 g dextrose of 1 L pure water) at 28 °C for 30 days. The total culture broth was 20 L.

The total culture broth (20 L) was filtered and extracted with an equal volume of EtOAc for 3 times to obtain 2.79 g metabolites extract. The extract was fractionated by column chromatography (CC) eluted in a gradient petroleum ether-EtOAc (20:1-1:1) to yield 10 fractions (Fr.s.1-10) based on TLC analysis. Fr.7 was further separated via preparative HPLC eluting with MeOH/H2O (40/60, v/v) at 8 mL/min to obtain four sub-fractions Fr.7.1-7.4. Sub-fraction Fr.7.3 (7 mg) was purified with semi-preparative HPLC (MeOH/H2O 30:70, 4 mL/min) and yielded 2 (2.55 mg). Fr.8 was initially separated by CC over silica gel with CH3Cl2-MeOH gradient from 80:1-5:1 based on TLC analysis to afford 10 sub-fractions Fr.8.1-8.10. Sub-fraction Fr.8.8 was purified by semi-preparative HPLC at 4 mL/min using MeOH/H2O (25/75, v/v) as the eluting solvents and got Fr.8.8.4 (23 mg). Sub-fraction Fr.8.8.4 was further purified by semi-preparative HPLC at 4 mL/min using CH3CN/H2O (15/85, v/v) as the eluting solvents and yielded compound 3 (4.6 mg). Fr.10 was purified by CC over silica gel using a gradient of CH3Cl2-MeOH (50:1-1:1) as a mobile phase to provide five fractions (Fr.11 to 15). Fr.13 was separated via preparative HPLC eluting with MeOH/H2O (15:85, v/v) at 10 mL/min to obtain 4 (148 mg).

2.3. Spectral Data

Letendronol D (1): White amorphous powder; molecular formula C12H20O4; [α]20 D -6 (c 0.1, MeOH); ECD (0.50 mg/mL, MeOH) λmax (Δ ε) 209 (-53.97) nm; UV (MeOH) λmax (log ε) 259 (2.98) nm; IR (λmax) 3316, 2954, 2935, 1648, 1450, 1379, 1341, 1275, 1236, 1186 cm⁻¹; 1H NMR data (500 MHz, in CD3OD) and 13C NMR data (125 MHz, in CD3OD), see Table 1; HRESIMS m/z [M-H] 227.1292 (calcd for C12H19O4, 227.1283).

Phomopsiketone H (2): Colorless crystal in methanol; mp 116-116.5 °C; molecular formula C12H20O4; [α]20 D +1.83 (c 0.5, MeOH); ECD (0.50 mg/mL, MeOH) λmax (Δ ε) 399 (-0.35), 338 (+4.41), 256 (-53.48), 224 (+50.72) nm; UV (MeOH) λmax (log ε) 234 (3.95) nm; IR (λmax) 3329, 2947, 2835, 1661, 1450, 1398, 1107 cm⁻¹; 1H NMR data (500 MHz, in CD3OD) and 13C NMR data (125 MHz, in CD3OD), see Table 1; HRESIMS m/z [M+Na]+ 249.1100 (calcd for C12H18O4Na, 249.1103).

Phomopsiketone I (3): White amorphous powder; molecular formula C12H18O4; [α]20 D + 66.18 (c 0.5, MeOH); ECD (0.50 mg/mL, MeOH) λmax (Δ ε) 399 (-0.35), 338 (+4.41), 256 (-53.48), 224 (+50.72) nm; UV (MeOH) λmax (log ε) 215 (3.86) nm; IR (λmax) 3334, 2960, 1646, 1403, 1260, 1209, 1170, 1089, 973 cm⁻¹; 1H NMR data (600 MHz, in CD3OD) and 13C NMR data (150 MHz, in CD3OD), see Table 1; HRESIMS m/z [M+Na]+ 249.1103 (calcd for C12H18O4Na, 249.1103).

2.4. Preparation of MTPA esters of Compounds 1

Two parts of compound 1 (4.5 mg) were dissolved with 0.5 mL anhydrous pyridine and then react with (R)- or (S)-MTPA chloride (50 μL), respectively. Each reaction mixture was stirred at
ambient temperature for 4 h and was terminated by adding 1 mL methanol. HPLC was also used for the isolation of 4, 7, 10-tri-S-MTPA ester and 4, 7, 10-tri-R-MTPA ester of 1.

2.5. ECD Calculation of 3

Conformational analyses were carried out via random searching in the Sybyl-X 2.0 using the MMFF94S force field with an energy cutoff of 2.0 kcal/mol [11]. The results showed 2 (C1, C2) lowest energy conformers for 4R, 7S, 10R-3 and 2 (C3, C4) for 4R, 7S, 10S-3. Subsequently, the conformers were reoptimized using DFT at b3lyp/6-31+g (d,p) level in MeOH by the GAUSSIAN 09 program. The energies, oscillator strengths, and rotational strengths (velocity) of the first 30 electronic excitations were calculated using the TDDFT methodology at the cam-b3lyp/TZVP level using the polarizable continuum model in MeOH. The ECD spectrum were simulated by the overlapping Gaussian function (half the bandwidth at 1/e peak height, σ = 0.2). To get the final spectra, the simulated spectra of the conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (∆G). Theoretical ECD spectra of the corresponding enantiomers (4S, 7R, 10S-3 and 4S, 7R, 10R-3) were obtained by directly inverse of the ECD spectrum of 4R, 7S, 10R-3 and 4R, 7S, 10S-3, respectively.

2.6. X-ray Crystallographic Analysis of 2

Compound 2 was obtained as colorless crystals from methanol. X-ray single-crystal diffraction data of 2 was selected on a Bruker APEX-II CCD diffractometer at 170 K. Using Olex2 [12], the structure was solved with the ShelXT [13] structure solution program using Intrinsic Phasing and refined with the ShelXL [14] refinement package using Least Squares minimisation. Crystallographic data for 2 has been deposited in the Cambridge Crystallographic Data Centre database (CCDC Number: 2008387).

Crystal Data of 2: C13H18O4 (M =226.26 g/mol): monoclinic, space group C2 (no. 5), a = 20.2305 (12) Å, b = 7.6248 (5) Å, c = 8.3334 (5) Å, β = 113.6120(10)°, V = 1177.84 (13) Å3, Z = 4, T = 170.0 K, μ (CuKa) = 0.783 mm−1, Dealc = 1.276 g/cm3, 9022 reflections measured (19.152° ≤ 2Θ ≤ 136.74°), 2097 unique (RI = 0.0175, Rsigma = 0.0151) which were used in all calculations. F (000) = 488.0. The final R1 was 0.0281 (1 > 2σ(I)) and wR2 was 0.0800 (all data). Flack parameter = 0.13 (3).

3. Results and Discussion

3.1. Structure Elucidation

Compound 1 was obtained as a white amorphous powder, and has a molecular formula of C13H20O4 (with 3 degrees of unsaturation) deduced from its HRESIMS (m/z 227.1292 for [M-H]) and NMR data. 1H NMR (Table 1) of 1 displayed one methyl (δH 0.95, t, J =7.0 Hz). The analysis of 13C NMR and DEPT revealed 12 carbon signals, including two olefinic carbons (δC 138.4, 139.9), four oxygenated methine (δC 65.1, 65.5, 74.3, 90.7), one oxygenated methylene (δC 76.2), four methylene (δC 20.1, 31.7, 32.1, 34.8) and one methyl (δC 14.4). These signals were similar to those of letendronol A [9]. The same cyclohexene moiety was derived from the 1H-1H COSY correlations between H-4 (δH 4.27)/H-5 (δH 1.57, 2.09)/H-6 (δH 1.55, 2.11)/H-7 (δH 4.30), coupled with the HMBC correlations from H2-5 to C-3 (δC 139.9) and H2-6 to C-8 (δC 138.4) (Figure 2). The similar CH3 (13)-CH2 (11)-CHO (10)-CHO (9)- aliphatic chain, derived from 1H-1H COSY correlations of H3-13 (δH 0.95)/H2-12 (δH 1.38, 1.60)/H2-11 (δH 1.48, 1.51)/H-10 (δH 3.71)/H-9 (δH 4.91), was position at C-8 according to the HMBC correlation from H-10 to C-8. Meanwhile, C-2 was connected with C-3 because of the HMBC correlations from H2-2 (δH 4.55, 4.77) to C-8, C-3. A dihydrofuran ring was formed by the HMBC correlation from H-9 to C-2, which was different from the dihydropyran ring in
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letendronol A. Thus, compound 1 was determined as a new polyketone and named as letendronol D (Figure 1).

![Chemical structures of compounds 1-3](image)

Figure 1. Chemical structures of compounds 1-3

In the NOESY experiment of 1, the correlation between H-7/H-10 suggested that H-7 and H-9 adopted different orientations with each other.

The absolute configuration of 1 was determined by a modified Mosher’s esterification method [15] and esters were purified with preparative HPLC. The adducts were determined as 4,7,10-tri-S-MTPA ester (1a), 4,7,10-tri-R-MTPA ester (1b), respectively, by HRESIMS (4,7,10-tri-S-MTPA ester m/z 899.2454; 4,7,10-tri-R-MTPA ester m/z 899.2445 for [M+Na]⁺, Figures S39 and S40). The Δδ values (Δδ₁a₁b, Figure 3) between the MTPA adducts (1a/1b) showed noticeable differentiation around C-4 (negative values for H-2 and positive values for H-3), C-7 (negative values for H-5 and positive values for H-8) and C-10 (positive values for H-4, H-5 and H-13), confirming the 4S, 7S, and 10R configurations. The NOESY correlation between H-7 and H-10 deduced the configuration of C-9 as S. Thus, the absolute configuration of 1 was determined as (4S,7S,9S,10R).

![NOESY correlations of 1-3](image)

Figure 2. ¹H-¹H COSY, key HMBC and NOESY correlations of 1-3

![Δδ values for the MTPA esters (1a/1b)](image)

Figure 3. Δδₛₗ values for the MTPA esters (1a/1b)
### Table 1. NMR data of compounds 1-3

| Position | 1a (in CD3OD) | 2b (in CD3OD) | 3b (in DMSO) |
|----------|---------------|---------------|-------------|
|          | δc, type      | δH, m (J in Hz) | δc, type      | δH, m (J in Hz) | δc, type      | δH, m (J in Hz) |
| 2        | 76.2 CH2      | 4.55, m       | 64.2 CH2      | 4.11, dt (16.4, 2.6) | 166.0 C      |
|          | 4.77, m       |               | 4.34, dt (16.4, 2.6) |               |             |
| 3        | 139.9 C       |               | 133.4 C       |               | 126.1 C      |
| 4        | 65.1 CH       | 4.27, m       | 199.5 C       |               | 60.9 CH      |
|          |               |               | 4.23, br s    |               | 1.74, m      |
| 5        | 31.7 CH2      | 1.57, m       | 35.1 CH2      | 1.99, m       | 26.8 CH2     |
|          | 2.09, m       |               | 2.24, m       |               | 1.74, m      |
| 6        | 32.1 CH2      | 1.55, m       | 32.2 CH2      | 1.99, m       | 26.8 CH2     |
|          | 2.11, m       |               | 2.24, m       |               | 1.79, m      |
| 7        | 65.5 CH       | 4.30, m       | 64.4 CH       | 4.71, m       | 68.7 CH      |
|          |               |               | 4.04, m       |               |             |
| 8        | 138.4 C       |               | 158.2 C       |               | 157.6 C      |
| 9        | 90.7 CH       | 4.91, m       | 79.6 CH       | 3.24, td (8.2, 2.7) | 30.5 CH2     |
|          |               |               | 2.38, overlapped |               |             |
| 10       | 74.3 CH       | 3.71, dt (8.6, 4.0) | 67.4 CH       | 4.17, d (8.1) | 77.2 CH      |
|          |               |               | 4.33, m       |               |             |
| 11       | 34.8 CH2      | 1.48, m       | 35.5 CH2      | 1.47, m       | 37.2 CH2     |
|          | 1.51, m       |               | 1.81, m       |               | 1.56, m      |
| 12       | 20.1 CH2      | 1.38, m       | 19.8 CH2      | 1.42, m       | 18.7 CH2     |
|          | 1.60, m       |               | 1.58, m       |               | 1.35, m      |
| 13       | 14.4 CH3      | 0.95, t (7.0) | 14.4 CH3      | 0.96, t (7.2) | 14.6 CH3     |
|          |               |               | 0.91, t (7.4) |               |             |
| C4-OH    |               |               | 4.60, d (4.3) |               |             |
| C7-OH    |               |               | 5.23, d (5.6) |               |             |

*a*Measured at 500 MHz NMR. *b*Measured at 600 MHz NMR.

Compound 2 was obtained as a colorless crystal in methanol. The molecular formula of 2 was determined as C12H18O4, according to its HRESIMS (m/z 249.1100 for [M+Na]+). 13C NMR (Table 1) of 2 displayed 12 carbon signals, including two olefinic carbons (δC 158.2, 133.4), one oxygenated methylene (δC 64.2), three oxygenated methine (δC 64.4, 67.4, 79.6), four methylene (δC 19.8, 32.2, 35.1, 35.5) and one methyl (δC 14.4), which were similar to those of phomopsiketone D [9]. 2 also had the same C5 aliphatic chain and cyclohexene moiety according to the 2D NMR (Figure 2). While a dihydropyran ring was formed by the HMBC correlation from H-10 (δH 4.17) to C-2 (δC 64.2), which was different from dihydrofuran in phomopsiketone D. Thus, 2 was also a new family member of phomopsiketones and named as phomopsiketone H (Figure 1).

![Figure 4. X-ray crystal structure of 2 (Flack parameter = 0.13(3))](image-url)
The vicinal coupling constant $J_{9H,10H}$ (8.1 Hz) indicated the trans relationship between H-9 and H-10 [16]. The configuration of 2 was unambiguously confirmed as (7S, 9R and 10S) by X-ray analysis (Figure 4).

Compound 3 was contained as white amorphous powder and has the same molecular formula of C_{12}H_{18}O_4 (with 4 degrees of unsaturation) as that of 2 according to its HRESIMS (m/z 249.1103 for [M+Na]⁺) and NMR data. The analysis of ¹³C NMR and HSQC revealed two olefinic carbons (δc 126.1, 157.6) and one ester (δc 166.0). Calculation of unsaturation revealed that compound 3 also contained bicyclic skeleton. Comparison of 1D NMR data between 3 and 2 revealed that the ketone carbonyl and oxygenated methylene in 2 was replaced by oxygenated methine (δc 60.9, C-4 in 3) and lactone (δc 166.0, C-2 in 3) (Table 1), which was confirmed by ¹H-¹H COSY correlations of H₂-5 (δH 1.52, 1.70)/H-4 (δH 4.23) (Figure 2) as well as the HMBC correlation from H₂-9 (δH 2.38) to C-2. The similar C₅ side chain as those in 1-2 was derived from ¹H-¹H COSY correlations between H₃-13 (δH 0.91)/H₂-12 (δH 1.35, 1.41)/H₂-11(δH 1.56, 1.65)/H-10 (δH 4.33)/H₂-9 (δH 2.38) and connected at C-8 (δc 157.6) according to the HMBC correlations from H-9 to C-8, C-3 (δc 126.1) and C-7 (δc 68.7). Different with 1 and 2, 3 was a new lactone and named as phomopsiketone I (Figure 1). 3 was the first phomopsiketone derivative obtaining the lactone.

The NOESY correlation between C4-OH and C7-OH suggested that H-4 and H-7 adopted same orientations (Figure S33).

The absolute configuration of 3 was established by the comparison between experimental ECD spectrum and the theoretically calculated values of four possible stereoisomers (4R, 7S, 10R)-3, (4R, 7S, 10S)-3, (4S, 7R, 10R)-3 and (4S, 7R, 10S)-3. The experimental ECD (Figure 5) of 3 showed a negative Cotton effect at 265 nm, a positive Cotton effect at 240 nm and a negative Cotton effect at 210 nm, which matched well with the calculated value of (4R, 7S, 10R)-3, and contributed to determine the absolute configuration of 3 as (4R, 7S, 10R)

![Figure 5. Comparison between calculated ECD spectra and experimental curves of 3](image)

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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