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

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Abstract: Chemical investigation of the EtOAc extract of an insect-associated fungus *Letendraea* 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; *Letendraea* sp.

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 *Letendraea* 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

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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 *Letendraea* sp. was isolated from the gut of a crab found on Zhairuoshan Island (N20.2920, E122.5), Zhejiang Province, China. The fungus was determined as *Letendraea* 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 ºC 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 silica gel column chromatography (CC) eluted in a gradient petroleum ether-EtOAc (20:1-1:1) to yield 10 fractions (Frs.1-10) based on TLC analysis. Fr.7 was further separated via preparative HPLC eluting with MeOH/H_2O (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/H_2O 30:70, 4 mL/min) and yielded **2** (2.55 mg). Fr.8 was initially separated by CC over silica gel with CH_2Cl_2-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/H_2O (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 CH_3CN/H_2O (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 CH_2Cl_2-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/H_2O (15:85, v/v) at 10 mL/min to obtain **1** (148 mg).

2.3. Spectral Data

*Letendronol D (**1**)*: White amorphous powder; molecular formula $C_{12}H_{20}O_4$; $[\alpha]^{20}_D$ -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, 2864, 1648, 1450, 1418, 1379, 1341, 1275, 1236,1186, 1119, 1030, 942, 889, 839 cm^{-1}; ^1H NMR data (500 MHz, in CD_3OD) and ^{13}C NMR data (125 MHz, in CD_3OD), see Table 1; HRESIMS *m/z* $[M-H]^-$ 227.1292 (calcd for $C_{12}H_{19}O_4$, 227.1283).

*Phomopsiketone H (**2**)*: Colorless crystal in methanol; mp 116-116.5 ºC; molecular formula $C_{12}H_{18}O_4$; $[\alpha]^{20}_D$ + 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 CD_3OD) and ^{13}C NMR data (125 MHz, in CD_3OD), see Table 1; HRESIMS *m/z* $[M+Na]^+$ 249.1100 (calcd for $C_{12}H_{18}O_4Na$, 249.1103).

*Phomopsiketone I (**3**)*: White amorphous powder; molecular formula $C_{12}H_{18}O_4$; $[\alpha]^{20}_D$ + 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 CD_3OD) and ^{13}C NMR data (150 MHz, in CD_3OD), see Table 1; HRESIMS *m/z* $[M+Na]^+$ 249.1103 (calcd for $C_{12}H_{18}O_4Na$, 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 4*R*, 7*S*, 10*R*-**3** and 2 (C3, C4) for 4*R*, 7*S*, 10*S*-**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, $\sigma = 0.2$). 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 (ΔG). Theoretical ECD spectra of the corresponding enantiomers (4*S*, 7*R*, 10*S*-**3** and 4*S*, 7*R*, 10*R*-**3**) were obtained by directly inverse of the ECD spectrum of 4*R*, 7*S*, 10*R*-**3** and 4*R*, 7*S*, 10*S*-**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***: $C_{12}H_{18}O_4$ (*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) Å^3, *Z* = 4, *T* = 170.0 K, μ (CuKα) = 0.783 mm^{-1}, *Dcalc* = 1.276 g/cm^3, 9022 reflections measured ($19.152° \leq 2\Theta \leq 136.74°$), 2097 unique ($R_{int}$ = 0.0175, R_{sigma} = 0.0151) which were used in all calculations. F (000) = 488.0. The final R_1 was 0.0281 ($I > 2\sigma(I)$) and wR_2 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 $C_{12}H_{20}O_4$ (with 3 degrees of unsaturation) deduced from its HRESIMS (*m/z* 227.1292 for $[M-H]^-$) and NMR data. ^{1}H NMR (Table 1) of **1** displayed one methyl (δ_H 0.95, t, *J* =7.0 Hz). The analysis of ^{13}C NMR and DEPT revealed 12 carbon signals, including two olefinic carbons (δ_C 138.4, 139.9), four oxygenated methine (δ_C 65.1, 65.5, 74.3, 90.7), one oxygenated methylene (δ_C 76.2), four methylene (δ_C 20.1, 31.7, 32.1, 34.8) and one methyl (δ_C 14.4). These signals were similar to those of letendronol A [9]. The same cyclohexene moiety was derived from the ^{1}H-^{1}H COSY correlations between H-4 (δ_H 4.27)/H$_2$-5 (δ_H 1.57, 2.09)/H$_2$-6 (δ_H 1.55, 2.11)/H-7 (δ_H 4.30), coupled with the HMBC correlations from H$_2$-5 to C-3 (δ_C 139.9) and H$_2$-6 to C-8 (δ_C 138.4) (Figure 2). The similar CH$_3$ (13)-CH$_2$ (12)-CH$_2$ (11)-CHO (10)-CHO (9)- aliphatic chain, derived from ^{1}H-^{1}H COSY correlations of H$_3$-13 (δ_H 0.95)/H$_2$-12 (δ_H 1.38, 1.60)/H$_2$-11 (δ_H 1.48, 1.51) /H-10 (δ_H 3.71)/ H-9 (δ_H 4.91), was positioned 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 H$_2$-2 (δ_H 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

letendronol A. Thus, compound **1** was determined as a new polyketone and named as letendronol D (Figure 1).

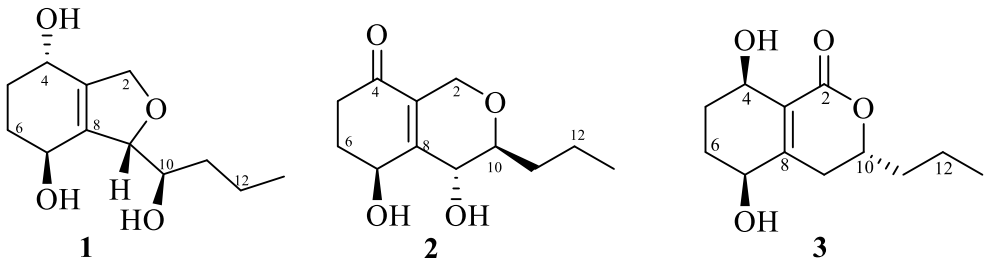


Figure 1. Chemical structures of compounds **1-3**

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 $\Delta\delta$ values ($\Delta\delta_{\mathbf{1a\text{-}1b}}$, Figure 3) between the MTPA adducts (**1a**/**1b**) showed noticeable differentiation around C-4 (negative values for H_2-5 and positive values for H_2-2), C-7 (negative values for H_2-6 and positive values for H-9) and C-10 (positive values for H_2-11, H_2-12 and H_3-13), confirming the 4*S*, 7*S*, and 10*R* 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 (4*S*,7*S*, 9*S*, 10*R*).

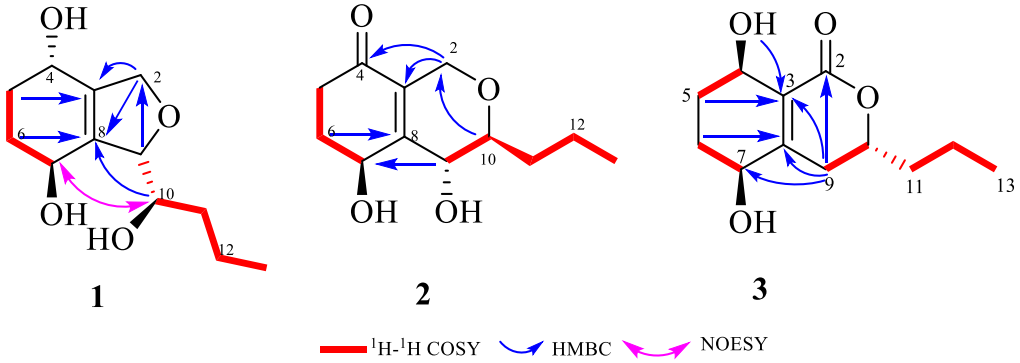
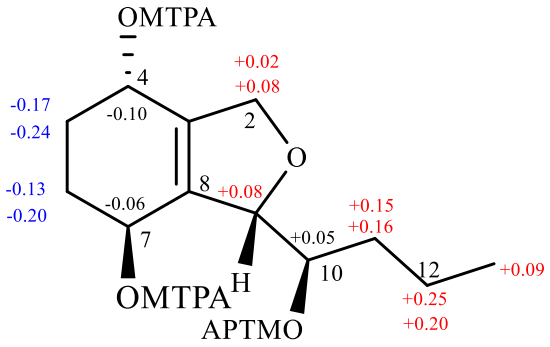


Figure 2. ^{1}H-^{1}H COSY, key HMBC and NOESY correlations of **1-3**



1a = 4,7, 10-*tri*-*S*-MTPA ester
1b = 4,7, 10-*tri*-*R*-MTPA ester

Figure 3. $\Delta\delta_{S\text{-}R}$ values for the MTPA esters (**1a**/**1b**)

Table 1. NMR data of compounds **1-3**

Position	1[a] (in CD_3OD)		2[b] (in CD_3OD)		3[b] (in DMSO)	
	δ_C, type	**δ_H, m (*J* in Hz)**	**δ_C, type**	**δ_H, m (*J* in Hz)**	**δ_C, type**	**δ_H, m (*J* in Hz)**
2	76.2 CH_2	4.55, m 4.77, m	64.2 CH_2	4.11, dt (16.4, 2.6) 4.34, dt (16.4, 2.6)	166.0 C	
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
5	31.7 CH_2	1.57, m 2.09, m	35.1 CH_2	2.35, dq (16.0, 4.6) 2.58, ddd (16.0, 7.4, 4.6)	29.7 CH_2	1.52, m 1.70, m
6	32.1 CH_2	1.55, m 2.11, m	32.2 CH_2	1.99, m 2.24, m	26.8 CH_2	1.74, 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 CH_2	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 CH_2	1.48, m 1.51, m	35.5 CH_2	1.47, m 1.81, m	37.2 CH_2	1.56, m 1.65, m
12	20.1 CH_2	1.38, m 1.60, m	19.8 CH_2	1.42, m 1.58, m	18.7 CH_2	1.35, m 1.41, m
13	14.4 CH_3	0.95, t (7.0)	14.4 CH_3	0.96, t (7.2)	14.6 CH_3	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 $C_{12}H_{18}O_4$, according to its HRESIMS (*m/z* 249.1100 for $[M+Na]^+$). ^{13}C NMR (Table 1) of **2** displayed 12 carbon signals, including two olefinic carbons (δ_C 158.2, 133.4), one oxygenated methylene (δ_C 64.2), three oxygenated methine (δ_C 64.4, 67.4, 79.6), four methylene (δ_C 19.8, 32.2, 35.1, 35.5) and one methyl (δ_C 14.4), which were similar to those of phomopsiketone D [9]. **2** also had the same C_5 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 (δ_H 4.17) to C-2 (δ_C 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).

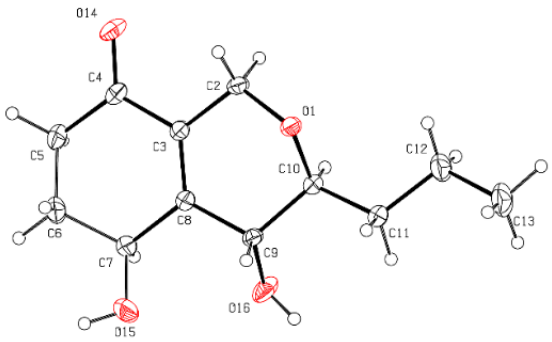


Figure 4. X-ray crystal structure of **2** (Flack parameter = 0.13(3))

The vicinal coupling constant $J_{H\text{-}9/H\text{-}10}$ (8.1 Hz) indicated the *trans* relationship between H-9 and H-10 [16]. The configuration of **2** was unambiguously confirmed as (7*S*, 9*R* and 10*S*) 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 ^{13}C NMR and HSQC revealed two olefinic carbons (δ_C 126.1, 157.6) and one ester (δ_C 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 (δ_C 60.9, C-4 in **3**) and lactone (δ_C 166.0, C-2 in **3**) (Table 1), which was confirmed by $^1H\text{-}^1H$ COSY correlations of H_2-5 (δ_H 1.52, 1.70)/H-4 (δ_H 4.23) (Figure 2) as well as the HMBC correlation from H_2-9 (δ_H 2.38) to C-2. The similar C_5 side chain as those in **1**-**2** was derived from $^1H\text{-}^1H$ COSY correlations between H_3-13 (δ_H 0.91)/H_2-12 (δ_H 1.35, 1.41)/H_2-11(δ_H 1.56, 1.65)/H-10 (δ_H 4.33)/H_2-9 (δ_H 2.38) and connected at C-8 (δ_C 157.6) according to the HMBC correlations from H-9 to C-8, C-3 (δ_C 126.1) and C-7 (δ_C 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 (4*R*, 7*S*, 10*R*)-**3**, (4*R*, 7*S*, 10*S*)-**3**, (4*S*, 7*R*, 10*R*)-**3** and (4*S*, 7*R*, 10*S*)-**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 (4*R*, 7*S*, 10*R*)-**3**, and contributed to determine the absolute configuration of **3** as (4*R*, 7*S*, *10R*)

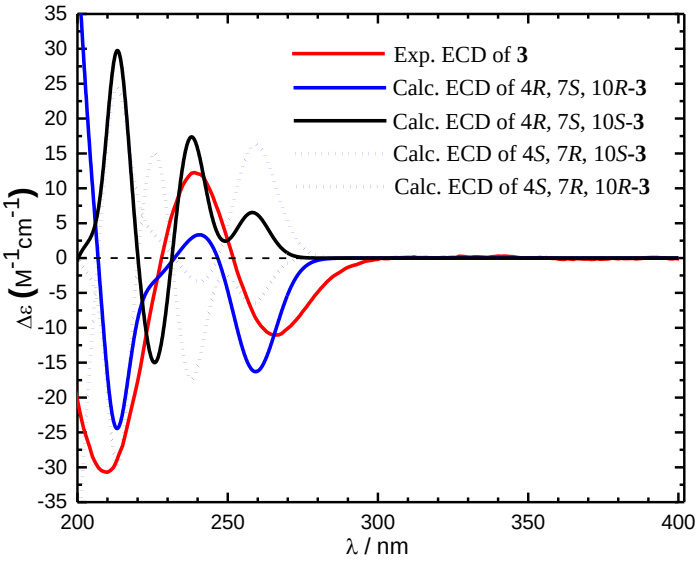


Figure 5. Comparison between calculated ECD spectra and experimental curves of **3**

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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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