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NOTE

New 12,8-Eudesmanolides from *Eutypella* sp. 1–15

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Four new 12,8-Eudesmanolides (1 – 4) and one known compound 5 named 13-Hydroxy-3,7(11)-eudesmadien-12,8-olide, were isolated from a mangrove rhizosphere-derived fungus *Eutypella* sp. 1–15. Their structures with absolute stereochemistry were determined by the comprehensive spectroscopic data, experimental and calculated ECD analysis. Compound 1 exhibited potent anticancer activity against JEKO-1 and HepG2 with IC_{50} values of 8.4 and 28.5 μM, respectively. Additionally, compound 1 also showed moderate antimicrobial activity.
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Eudesmanolides, a group of well-known natural sesquiterpenoids, have diverse bioactivities, including antituberculosis,[1] anticancer[2] and anti-inflammatory activity.[3] These compounds have been classified into two subgroups: 12,6-eudesmanolides such as ludovicin A[4] and 12,8-eudesmanolides such as alantolactone.[5–7] As part of our continuous screening for bioactive metabolite from microbial sources, a fungal strain *Eutypella* sp. 1–15 isolated from the mangrove rhizosphere was selected for its excellent cytotoxic and antimicrobial activity, previous chemical investigations of this strain afforded series of cytochalasans,[8–10] a famous group of cytotoxic agent. We now have reinvestigated the strain for other bioactive constituents. In this study, we describe the fermentation, isolation, structure elucidation of four new 12,8-Eudesmanolides (**1–4**) along with one reported compound (**5**) 13-Hydroxy-3,7(11)-eudesmadien-12,8-olide,[11] as well as their cytotoxic and antibacterial activity.

The strain *Eutypella* sp. 1-15, isolated from the soil of mangrove rhizosphere in Jimei, Fujian Province, China, was identified as *Eutypella* sp.[10] according to its ITS sequence. The strain was inoculated on rice medium containing rice 1000 g, NaCl 100 g in 1.0 l water and cultured at 28 °C for 27 days. The final 2 l fermented culture were extracted with mixed solvent of EtOAc-MeOH-AcOH (80:15:5, v/v/v) to obtain a crude extract. The crude extract was partitioned between EtOAc and H_2O, and subsquently EtOAc extract was partitioned with MeOH and petroleum ether to afford the MeOH extract. Then, the MeOH extract was subjected to medium-pressure reverse-phase liquid chromatography, Sephadex LH-20, and finally on silica column to afford compounds **1–5** (Figure 1), respectively.

Compound **1** was obtained as colorless oil with $[\alpha]_D^{20}$+10.4 (*c* 0.87, MeOH), UV (MeOH) λ_{max} (log ε) 200 (3.47), 281 (2.78) nm, and IR (KBr) ν_{max} = 3420, 2927, 1762, 1435, 1001, 790 cm^{-1}. The molecular formula was deduced as $C_{15}H_{18}O_3$ according to HR-ESI-MS at *m/z* = 247.1329 $[M+H]^+$ (calcd 247.1329). The ^1H, ^{13}C NMR and HSQC spectra (Acetone-d_6) of **1** display signals for two methyls, four methylenes, three methines, and six non-protonated carbons (Table 1). The HMBC correlations from H_3-14 (δ_H 1.05) to C-1 (δ_C 34.9), C-5 (δ_C 45.2), C-9 (δ_C 120.7), C-10 (δ_C 34.6) and H_3-15 (δ_H 1.74) to C-3 (δ_C 128.1), C-4 (δ_C 132.9), C-5, as well as ^1H-^1H COSY correlations between H_2-1 (δ_H 1.70) and H_2-2 (δ_H 2.17, 2.10), H_2-2 and H-3 (δ_H 5.43) resulted in a six-membered ring (Figure 2a). HMBC correlations from H_2-6 (δ_H 3.35, 2.53) to C-4, C-5, C-8 (δ_C 148.4), C-10, C-11 (δ_C 123.7) and ^1H-^1H COSY correlations between H-5 (δ_H 2.54) and H_2-6 joined previously deduced fragments to form a 6/6 AB ring junction system, which was further supported by the HMBC correlations from H_2-13 (δ_H 4.41) to C-7 (δ_C 150.8), C-11, C-12 (δ_C 169.2). Moreover, the downfield C-7 (δ_C 150.8) and C-8 (δ_C 148.4), upfield C-11 (δ_C 123.7) and C-9 (δ_C 120.7) as well as a carbonyl signal C-12 (δ_C 169.2) suggested a α,β-unsaturated γ-lactone (Figure 2a). Finally, plus the oxygenated methylene C-13 ((δ_C 54.2), H_2-13 (δ_H 4.41)), the planar structure of **1** was elucidated as shown in Figure 1 named 13-Hydroxy-3,8,7(11)-eudesmatrien-12,8-olide. The stereochemistry of **1** was assigned as discussion below.

Compound **2** was purified as colorless solid with $[\alpha]_D^{20}$+70.2 (*c* 1, MeOH). The ^1H and ^{13}C NMR spectra of **2** exhibited a pattern analogous to that of **1** (Table 1). The molecular formula was deduced as $C_{15}H_{16}O_3$ on the basis of HR-ESI-MS at *m/z* = 245.1172 $[M+H]^+$ (calcd 245.1172), suggesting that **2** was the dehydrogenated derivative of **1**. Further analysis of HSQC and HMBC spectra of **2** revealed the appearance of a pair of sp^2 carbons at C-5 (δ_C 155.1) and C-6 (δ_C 110.6) compared to **1**, which was indicated that the new generated

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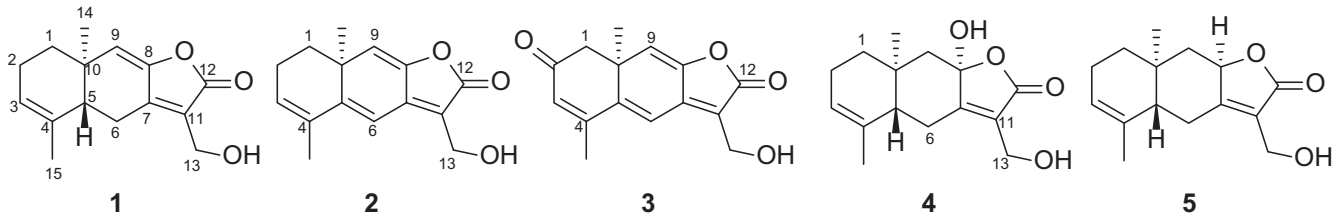

1 **2** **3** **4** **5**

Figure 1 Structures of compounds **1–5**.

Table 1 NMR data for 12,8-Eudesmanolides (1–4) in Acetone-d_6 (^{1}H at 600 MHz, ^{13}C at 150 MHz)

	1		*2*		*3*		*4*	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H, (*J* in Hz)
1a	34.9t	1.71 (m)	33.4t	1.91(dd, 6.1, 13.1)	48.5t	2.66 (d, 16.0)	37.3t	1.49 (m)
1b		1.70 (m)		1.64 (m)		2.55(d, 16.0)		1.37 (m)
2a	22.6t	2.17 (m)	23.0t	2.48 (m)	195.6s		22.5t	2.17 (m)
2b		2.10 (m)		2.28 (m)				2.02 (m)
3	128.1d	5.43 (br)	131.2d	5.97(br)	128.7d	6.11(s)	121.6d	5.40 (br)
4	132.9s		131.4s		151.4s		133.2s	
5	45.2d	2.54 (m)	155.1s		150.9s		48.8d	2.14 (m)
6a	22.5t	3.35 (m)	110.6d	6.93 (s)	117.1d	7.50(s)	23.6t	3.32 (dd, 3.1, 12.9)
6b		2.53 (m)						2.25 (m)
7	150.8s		144.2s		142.2s		163.9s	
8	148.4s		146.7s		146.5s		103.4s	
9	120.7d	5.78 (s)	116.7d	5.86 (s)	115.6d	6.01(s)	50.6t	2.23 (d, 13.4)
								1.46 (d, 13.4)
10	34.6s		39.4s		43.0s		33.0s	
11	123.7s		116.8s		120.6s		124.9s	
12	169.2s		170.1s		169.5s		170.3s	
13	54.2t	4.41 (d, 5.7)	54.3t	4.50 (s)	54.9t	4.59 (s)	53.8t	4.33 (s)
14	17.6q	1.05 (s)	24.9q	1.23 (s)	27.3q	1.36 (s)	15.3q	1.16 (s)
15	20.2q	1.74 (s)	18.9q	1.97 (s)	19.1q	2.28 (s)	20.5q	1.74 (s)
8-OH								6.22 (s)
13-OH		4.21 (t, 5.7)						

double bond was between C-5 and C-6, therefore, **2** was determined to be 13-Hydroxy-3,5,8,7(11)-eudesmatetraen-12,8-olide (Figure 2a).

Compound **3** was obtained as yellow powder with $[\alpha]_D^{20}$+162.1 (*c* 0.5, MeOH). The ^{1}H, ^{13}C and HSQC spectra of **3** revealed the absence of the methylene C-2 and the presence of a carbonyl group (δ_C 195.6). The downfield shift and doublet peak of H_2-1 (δ_H 2.66, 2.55, (d, J = 16.0 Hz)) indicated the methylene was replaced by a carbonyl group at C-2. The deduction was confirmed by the molecular formula of **3**, $C_{15}H_{14}O_4$, established by HR-ESI-MS at *m/z* = 259.0965 $[M+H]^+$ (calcd 259.0965). Therefore, **3** was assigned as 2-One-13-hydroxy-3,5,8,7(11)-eudesmatetraen-12,8-olide (Figure 1).

Compound **4** was obtained as colorless oil with $[\alpha]_D^{20}$-40.9 (*c* 0.6, MeOH). The molecular formula was deduced as $C_{15}H_{20}O_4$ according to HR-ESI-MS at *m/z* = 265.1435 $[M+H]^+$ (calcd 265.1434). The ^{1}H and ^{13}C NMR spectra of **4** exhibited a pattern analogous to that of **1** (Table 1). Detailed analysis of the 2D NMR spectra revealed the double bond between C-8 (δ_C 148.4) and C-9 (δ_C 120.7) in **1** was reduced. The doublet peak of H_2-9 (δ_H 2.23, 1.46, (d, J = 13.4 Hz)) and chemical shift of C-8 (δ_C 103.4) in **4** suggested the appearance of a hemiketal moiety at C-8 (Figure 1). Therefore, compound **4** was determined to be 8,13-Dihydroxy-3,7(11)-eudesmadien-12,8-olide. The NOESY cross-peaks at H_3-14/OH-8 indicated a *syn* orientation between 14-Me and 8-OH.

Compound **5** was found to be 13-Hydroxy-3,7(11)-eudesmadien-12,8-olide by comparing the NMR, MS and specific rotation data ($[\alpha]_D^{20}$-191.4) with the reported one ($[\alpha]_D^{25}$-37.3).[11] To determine the absolute configuration of these 12,8-Eudesmanolides, the calculated ECD spectra using time-dependent density functional theory (TDDFT) was applied. We found that calculated ECD spectrum of the conformers of **3** with 10*S* is in good accordance with the experimental spectrum (Figure 2b), also the calculated specific rotation of +531.9 further confirm the 10*S* conformer. Consequently, the absolute configuration of **3** was unambiguously assigned as shown in Figure 1. Considering the specific rotations of **2** and **3** were $[\alpha]_D^{20}$+70.2 and $[\alpha]_D^{20}$+162.1, respectively, we assume the absolute configuration of **2** was consistent with **3**. As for **1**, the good accordance of the calculated ECD spectrum of the conformers of **1** with 5*R*, 10*R* with the experimental ECD spectrum of **1** (Figure 2d) supported the stereochemistry of **1** as shown (Figure 1), the calculated specific rotation +101.5 also supported the conclusion. The *trans* orientation of 14-Me and 5-H of **1** was consistent with **5**, considering the same biogenetic origin and similar ECD curves of **1**, **4** and **5** (Figure 2c), we assume the absolute configuration of **1**, **4** and **5** was assigned as shown in Figure 1.

The compounds **1–5** were evaluated for antimicrobial activities against *Bacillus subtilis* CMCC63501, *Escherichia coli* CMCC44103,

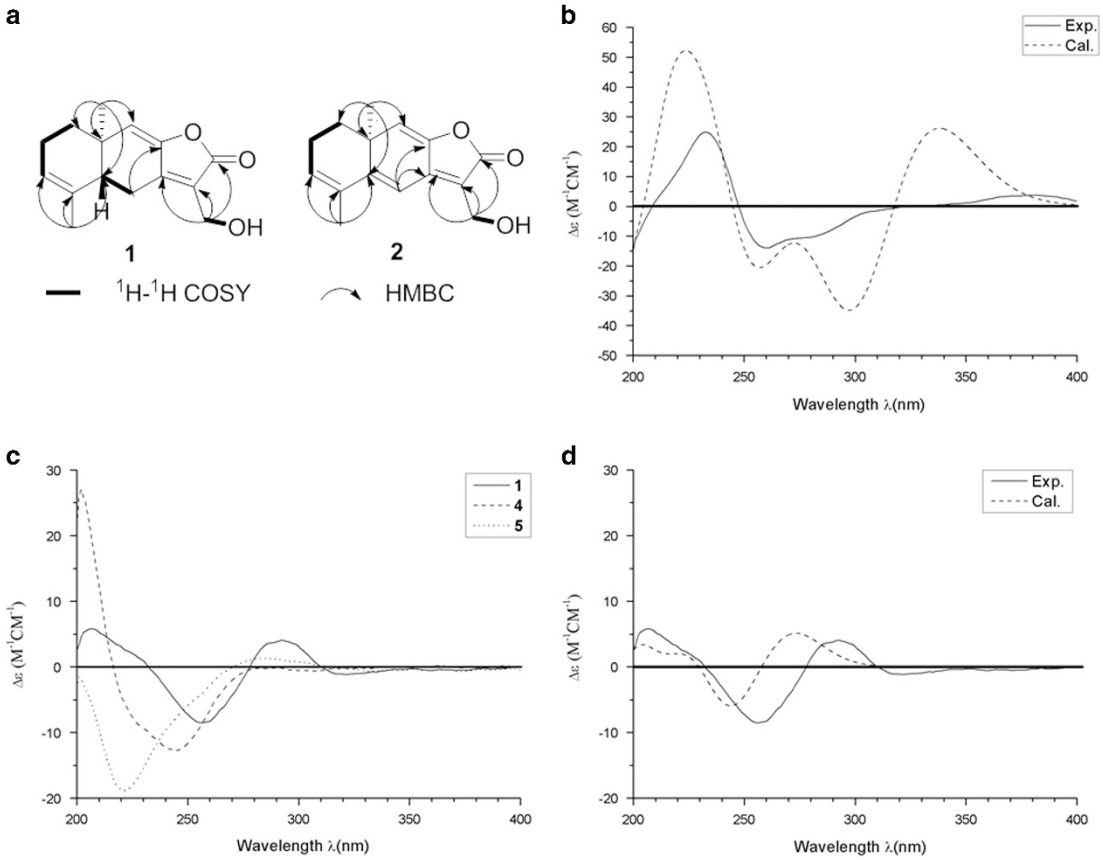

Figure 2 Structure elucidation of compounds **1–5**. (**a**) Key ^{1}H–^{1}H COSY and HMBC correlations of compounds **1** and **2**, (**b**) Experimental and calculated ECD spectra of compound **3**, (**c**) Experimental ECD spectra of **1**, **4** and **5**, (**d**) Experimental and calculated ECD spectra of compound **1**.

Table 2 Antimicrobial activity and cytotoxicity of compounds 1–5

	IC$_{50}$ (μM)			
compounds	*B. subtilis*	*B. pumilus*	*JEKO-1*	*HepG2*
1	18.1	23.8	8.4	28.5
2	>50	>50	>50	>50
3	>50	>50	>50	>50
4	>50	>50	>50	>50
5	>50	>50	>50	48.4

staphylococcus aureus CMCC26003, *Bacillus pumilus* CMCC63202 and *Micrococcus luteus* CMCC28001 using a plate assay.[12] Compound **1** exhibited inhibitory efficacy to *Bacillus subtilis* CMCC63501 and *Bacillus pumilus* CMCC63202 with IC_{50} value of 18.1 and 23.8 μM, respectively (Table 2). Compounds **2–5** did not show any activity (IC_{50} > 50 μM). Further, all isolates (**1–5**) were assessed for antiproliferative activities against a panel of cancer cells, including BGC823, SKGT4, MCF-7, A375, MDA-MB-231, HL-60, JEKO-1, HepG2 and Kyse450 using the MTT method.[13] Compound **1** displayed growth inhibitory effect against human Mantle cell lymphoma JEKO-1 and human Hepatoma carcinoma HepG2 with IC_{50} values of 8.4 and 28.5 μM, respectively (Table 2). However, at a concentration of 50 μM, no significant changes in cell viability were observed in the other tested cancer cells after exposure to **1** for 48 h. Compounds **2–4** show almost no cytotoxicity to all tested cancer cells. Compound **5** show weak cytotoxicity against HepG2 with IC_{50} value of 48.4 μM. These data suggested that compound **1** has moderate anticancer activity. The bioactivity of these new 12,8-Eudesmanolides was mediated by an elaborate network composed of functional group, configuration and conformation of the framework.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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