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海洋来源真菌 *Eutypella* sp. F0219 次级代谢产物研究

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摘要 弯孢聚壳属真菌能产生结构丰富多样的活性化合物. 对一株来自海洋沉积物的真菌 *Eutypella* sp. F0219 代谢产物研究中, 分离得到一个新的异戊烯基取代二氢异香豆素和一个新的色烯酰胺衍生物, 分别命名为 eutypellarins A 和 B (**1** 和 **2**), 以及两个已知的异戊二烯取代苯甲醛衍生物(**3** 和 **4**). 通过高分辨质谱、核磁共振波谱以及量子化学计算对新化合物的结构进行确定. 首次从弯孢聚壳属真菌中发现异戊二烯取代二氢异香豆素和色烯酰胺衍生物. 采用 2,2'-联氮-双-3-乙基苯并噻唑啉-6-磺酸(ABTS)自由基清除法和铁离子还原(FRAP)法对所有化合物进行总抗氧化能力的测定. 结果显示化合物 **1** 和 **4** 具有中等的抗氧化效果, 其 Trolox 等效抗氧化能力分别为(0.51±0.016)和(0.76±0.007).
关键词 海洋真菌; 弯孢聚壳属; 异戊二烯取代二氢异香豆素; 色烯酰胺衍生物; 总抗氧化能力测试

Study on Secondary Metabolites of Marine-Derived Fungus *Eutypella* sp. F0219

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Abstract The fungus *Eutypella* sp. is well known for producing bioactive compounds with diverse structures. Chemical investigation of the fungi *Eutypella* sp. F0219 isolated from marine sediment, yielded a new prenylated dihydroisocoumarin and a new chromene amide derivative, named eutypellarins A and B (**1** and **2**), together with two known prenylated benzaldehyde derivatives (**3** and **4**). The structures of these compounds were elucidated based on HR-ESIMS, extensive nucler magneti resonance (NMR) experiments, and quantum-chemical electronic circular dichroism (ECD) calculations. Prenylated dihydroisocoumarin and chromene amide derivatives are reported from the genus *Eutypella* for the first time. The total antioxidant capacities of all compounds were measured through 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and ferric-reducing antioxidant power (FRAP) assay. The results showed that compounds **1** and **4** exhibited moderate antioxidant properities with Trolox equivalent antioxidant capacity (TEAC) values (0.51±0.016) and (0.76±0.007), respectively.
Keywords marine-derived fungi; *Eutypella* sp.; prenylated dihydroisocoumarins; chromene amide derivatives; total antioxidant capacity assays

1 Introduction

Marine-derived fungi continue to attract considerable attention as an important spring of novel secondary metabolites displaying promising biological and pharmacological properties.[1] The *Eutypella* species are mainly reported to be distributed in the South China Sea, the soil of Antarctica and Arctic, and tropical forests of Australia and Thailand.[2] So far, a variety of secondary metabolites have been isolated from the genus of *Eutypella*, such as γ-lactones, sesquiterpenoids,[3-5] cytochalasin derivatives,[6] pimarane diterpenoids,[7-14] thiodiketopiperazines,[15-16] and cytosporin-related compounds,[17] many of which indicated antitumor, anti-inflammatory, anti-human immunodefi-

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ciency virus (HIV), immunosuppressive, and antimicrobial activities.[3-18] However, the prenylated dihydroisocoumarin and chromene amide derivatives have not been reported from this species.

In the course of our systematic investigation of fungus *Eutypella* sp. F0219 isolated from the sediment and collected in the northern part of the South China Sea, we firstly found a new prenylated dihydroisocoumarin and a new chromene amide derivative, named eutypellarins A (**1**) and B (**2**), together with two known prenylated benzaldehyde derivatives (**3** and **4**) (Figure 1). Compounds **1** and **4** displayed moderate antioxidant properties, whose Trolox equivalent antioxidant capacity (TEAC) values were found to be (0.51 ± 0.016) and (0.76 ± 0.007), respectively. Herein, we report the isolation, structural elucidation, and antioxidant activities of these compounds.

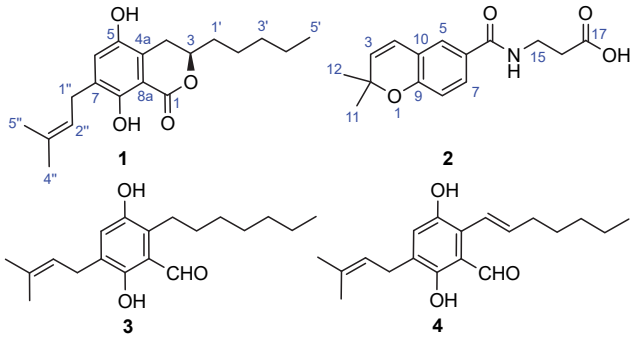

Figure 1 Structures of eutypellarins A and B (**1** and **2**) and two known benzaldehyde derivatives

2 Results and discussion

Compound **1** was obtained as pale yellow powder. Its negative HR-ESIMS measurements suggested a molecular formula of $C_{19}H_{26}O_4$ (*m/z* 317.1739, calcd for $[M-H]^-$ 317.1747), indicating that **1** had seven degrees of unsaturation. Based on the 1D-NMR spectroscopic data for **1** (Table 1), five degrees of unsaturation were due to eight olefinic carbons and a lactone group. Thus, compound **1** was to be bicyclic.

Detailed analysis of the 1D-NMR data revealed that **1** was a dihydroisocoumarin derivative, similarly to eurotiumide A.[19] The presence of 5,8-dihydroxyisochroman-1-one nucleus was established by an aromatic protons [δ_H 6.86 (s, H-6)], an oxygenated methine proton [δ_H 4.49～4.55 (m, H-3)], two methylene protons [δ_H 2.66 (dd, J= 16.8, 11.6 Hz), 3.08 (dd, J=16.8, 3.2 Hz), H_2-4], and six olefinic carbons signals [δ_C 121.4 (C-4a), 143.2 (C-5), 123.8 (C-6), 129.1 (C-7), 154.5 (C-8), and 107.9 (C-8a)], one ester carbonyl carbon signal [δ_C 170.4 (C-1)], one oxygenated methine carbon signal [δ_C 79.8 (C-3)], and one methylene carbon signal [δ_C 26.7 (C-4)], along with ^{1}H-^{1}H COSY correlation between H-3 and H_2-4, and HMBC correlations between H-6/C-4a, H-6/C-5, H-6/C-8, H_2-4/C-4a, H_2-4/C-5, H_2-4/C-8a, and H-3/C-4a (Figure 2). The hydroxy group placed at C-8 was established by HMBC correlations from the proton of a hydroxy function (δ_H 10.59, s) to C-7, C-8, and C-8a (Figure 2), whereas the other hydroxy function assigned at C-5 was supported by the downshifted C-5. Furthermore, a 3-methyl-2-butenyl group connected to C-7 was deduced from ^{1}H-^{1}H COSY correlation between H-1" (δ_H 3.32, br d, J=7.2 Hz) and H-2" (δ_H 5.28, br t, J=7.2 Hz) as well as HMBC correlations between H-1"/C-6, H-1"/C-7, H-1"/C-8, H-2"/C-4", H-2"/C-5", H-5"/C-2", H-5"/C-3", and H-5"/C-4" (Figure 2). The existence of a pentyl group located at C-3 was supported by the H-3−H_2-1'−H_2-2'−H_2-3'−H_2-4'−H_3-5' proton spin system, which was determined by ^{1}H-^{1}H COSY correlations (Figure 2), along with HMBC cross-peaks between H-3/C-2', H_2-1'/C-3', H_2-4'/C-2', and H_3-5'/C-3' (Figure 2). Therefore, the planar structure of **1** was established as depicted.

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for **1** in $CDCl_3$

Position	δ_H	δ_C
1		170.4, qC
3	4.49～4.55, m	79.8, CH
4	2.66, dd (16.8, 11.6) 3.08, dd (16.8, 3.2)	26.7, CH_2
4a		121.4, qC
5		143.2, qC
6	6.86, s	123.8, CH
7		129.1, qC
8		154.5, qC
8a		107.9, qC
1'	1.69～1.77, m; 1.85～1.92, m	34.9, CH_2
2'	1.43～1.50, m; 1.52～1.59, m	24.6, CH_2
3'	1.28～1.38, m	31.6, CH_2
4'	1.31～1.37, m	22.5, CH_2
5'	0.91, t (6.4)	14.0, CH_3
1"	3.32, br d (7.2)	27.5, CH_2
2"	5.28, br t (7.2)	121.2, CH
3"		133.9, qC
4"	1.76, s	25.8, CH_3
5"	1.70, s	17.8, CH_3
8-OH	10.59, s	
5-OH	4.52[a]	

[a] Overlapped signals are reported without designating multiplicity.

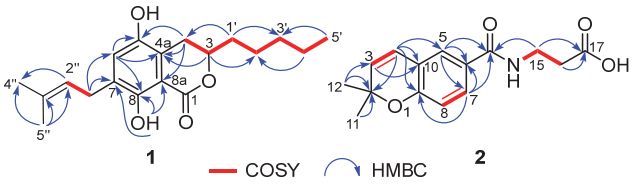

Figure 2 Key ^{1}H-^{1}H COSY and HMBC correlations of **1** and **2**

In order to determine the absolute configuration of **1**, quantum-chemical electronic circular dichroism (ECD) calculations were applied. The calculated ECD curve of 3*R*-**1** nicely matched with the measured one (Figure 3), and assigned the absolute configuration of **1** as given in Figure 1.

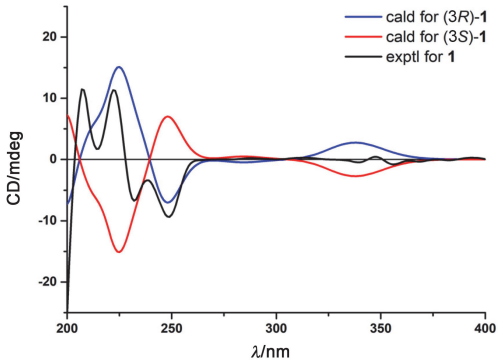

Figure 3 Comparison of the experimental ECD spectrum of **1** with those calculated for (3*R*)-**1** and its enantiomer

Compound **2** was obtained as yellow solid. The molecular formula of **2** was deduced from the negative HR- ESI-MS ion peak at *m*/*z* 274.1070 (calcd for $[M-H]^-$ 274.1074) to be $C_{15}H_{17}O_4N$, indicating eight degrees of unsaturation. The ^1H NMR and ^{13}C NMR spectroscopic data (Table 2) revealed the presence of two methyls [δ_H 1.41 (s, H_3-11 and H_3-12); δ_C 28.3 (C-11 and C-12)], two methenes [δ_H 3.59～3.64 (m, H_2-15), 2.64 (t, J=6.8 Hz, H_2-16); δ_C 36.5 (C-15, 34.5, C-16)], two olefinic methines [δ_H 5.78 (d, J=9.6 Hz, H-3), 6.42 (d, J=9.6 Hz, H-4); δ_C 132.2 (C-3, 122.5, C-4)], one oxygenated quaternary carbon [δ_C 77.7 (C-2)], two carbonyls [δ_C 167.2 (C-13), 173.6, (C-17)], an amino group [δ_H 7.72 (br s, 14-NH)], and a trisubtituted benzene ring [δ_H 7.59 (br s, H-5), 7.67 (br d, J=8.4 Hz, H-7), 6.75 (d, J=8.4 Hz, H-8); δ_C 126.5 (C-5), 128.1 (C-6), 129.2 (C-7), 116.6 (C-8), 156.4 (C-9), 121.6 (C-10)]. The above data suggested that **1** would be a chroman derivative, which was similar to *N*-butyl-2,2-dimethylchroman-6-carboxamide,[20] except for the presence of a $\Delta^{3,4}$ double bond and the replacement of the ethyl group at C-16 in *N*-butyl-2,2-dimethylchroman-6-carboxamide by a carboxylic group in **2**. ^1H-^1H COSY correlation between H-3 and H-4, and HMBC correlations between H-4/C-2, H-4/C-5, H-4/C-9, H-4/C-10, H-3/C-10, H_3-12/C-2, and H_3-12/C-3 demonstrated the existence of a $\Delta^{3,4}$ double bond (Figure 2). The absence of a methyl and a methene proton signals [δ_H 0.90～0.97 (m, H_3-4'), 1.25～1.43 (m, H_2-3')] and HMBC correlations from H_2-15 and H_2-16 to the carbonyl carbon [δ_C 173.6 (C-17)] in **2** indicated a carboxylic group placed at C-16 (Figure 2). Thus, the structure of **2** was assigned as depicted and named eutypellarin B.

The two known prenylated benzaldehyde derivatives (**3** and **4**) were elucidated as flavoglaucin[21] and tetrahydroauroglaucin[21], respectively, by comparing their spectroscopic data with those reported in literatures.

In addition, the free-radical-scavenging activity of compounds **1**～**4** was determined through the ABTS and the FRAP assays. The results showed that compounds **1** and **4** exhibited moderate antioxidant capacities, and corresponding TEAC values were (0.51±0.016) and (0.76±0.007), respectively. However, compound **3**, being 1',2'-dihydrogen analog of **4**, showed weak antioxidant capacity, suggesting that the $\Delta^{1',2'}$ double bond is pivotal for the antioxidant capacity of **4**. But the mechanisms still need to be further investigated. Whereas, $FeSO_4$ equivalent antioxidant capacity (FEAC) values of all compounds were below 0.5.

Table 2 ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data for **2** in acetone-d_6

Position	δ_H	δ_C
1		
2		77.7, qC
3	5.78, d (9.6)	132.2, CH
4	6.42, d (9.6)	122.5, CH
5	7.59, br s	126.5, CH
6		128.1, qC
7	7.67, br d (8.4)	129.2, CH
8	6.75, d (8.4)	116.6, CH
9		156.4, qC
10		121.6, qC
11	1.41, s	28.3, CH_3
12	1.41, s	28.3, CH_3
13		167.2, qC
14-NH	7.72, br s	
15	3.59～3.64, m	36.5, CH_2
16	2.64, t (6.8)	34.5, CH_2
17		173.6, qC

3 Conclusions

In conclusion, one new prenylated dihydroisocoumarin, eutypellarin A (**1**), and one new chromene amide derivative, eutypellarin B (**2**), as well as two known prenylated benzaldehyde derivatives (**3** and **4**) were obtained from the marine-derived fungi *Eutypella* sp. F0219. The structures of new compounds were extensively determined by HR-ESIMS, 1D- and 2D-NMR. The absolute configuration of compound **1** was elucidated by the ECD calculation method. Eutypellarins A and B are the first examples of prenylated dihydroisocoumarin derivative and chromene amide derivative found from the genus *Eutypella* sp., respectively. Compounds **1** and **4** exhibited moderate antioxidant capacities, and their TEAC values were (0.51±0.016) and (0.76±0.007), respectively.

4 Experimental section

4.1 Instruments and reagents

HR-ESIMS were obtained on an Agilent G3250AA LC/MSDTOF mass spectrometer. 1D- and 2D-NMR spectra were measured on a Bruker AV-400 MHz NMR spectrometer. Optical rotations were determined on an MCP 500 modular circular polarimeter (Anton Paar GmbH) with a 1.0 cm cell at 25 ℃. Preparative HPLC was performed on a Waters 2545 pump equipped with a YMC C18 reversed-phase column (250 mm×10 mm i.d., 5 μm) and a 2998 photodiode array detector. For column chromatography, C18 reversed-phase silica gel (ODS-A-HG 12 nm, 50 μm,

YMC, Japan) was employed. The solvents used for column chromatography were purchased from Xilong Chemical Reagent Factory (Guangzhou, China) and the solvents used for preparative HPLC were purchased from Fisher Scientific (America).

4.2 Fungal material

The fungus stain, *Eutypella* sp. F0219, was isolated from a marine sediment sample collected in the northern part of the South China Sea (GPS 114.6609° E, 21.5942° N) at a water depth of 75 m in July 2020. This fungus was identified as *Eutypella* genus based on the ITS region sequence deposited at GenBank (accession no. OL825643). The voucher strain is preserved at the Key Laboratory of Tropical Medicinal Resource Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, P. R. China.

4.3 Fermentation, extraction and isolation

Two pieces of agar plugs (5 mm i.d.) of the fungal stain were inoculated into 1000 mL Erlenmeyer flasks containing 400 mL of potato dextrose broth (PDB; 20% potato, 2% glucose, 0.3% sea salt). Subsequently, flasks with the inoculated fungal strain were incubated on a rotary shaker (180 r/min) at 28 ℃ for 2 d to obtain the seed culture. Large fermentation was performed on solid rice medium in 50 Erlenmeyer flasks (80 g of rice, 0.36 g of sea salt, and 120 mL of distilled water per flask, autoclaved at 121 ℃ for 21 min before inoculation). Each flask was inoculated with 5.0 mL of the spore inoculum and incubated at room temperature for 28 d under static conditions.

The fermentation material was extracted with EtOAc at room temperature for five times to yield an extract (29.8 g), which was subjected to a C18 reversed-phase column (80 cm×6 cm, i.d.), eluted with a gradient mixture of MeOH/H_2O (V∶V=10∶90 to 100∶0) to afford 45 fractions. Fractions 24～28 (4.5 g) were combined and further separated on another C18 reversed-phase column (60 cm×2 cm, i.d.), eluted with a gradient mixture of acetone/H_2O (V∶V=20∶80～10∶0) to yield 75 subfractions. The subfraction 51 was purified by semi-preparative HPLC (YMC-Pack ODS-5-A, 250 mm×10 mm i.d., MeCN/H_2O, V∶V=60∶40) to afford compound **1** (16.3 mg, t_R 38.7 min). The subfraction 7 was subjected to preparative HPLC (ODS-5-A, 250 mm×10 mm i.d., MeCN/H_2O, V∶V=70∶30) to yield compound **2** (4.1 mg, t_R 27.8 min).

Eutypellarin A (**1**): Pale yellow amorphous powder; $[\alpha]_D^{25}$ −8 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max}: 196.4, 255.4, 349.5 nm; ECD (0.7 mmol/L, MeOH) λ_{max} ($\Delta\varepsilon$): 190 (−3.9), 206 (+2.4), 223 (+2.5), 231 (−1.4), 250 (−1.8) nm; IR (MeOH) ν_{max}: 3367.7, 1645.3, 1456.3, 1303.9, 1180.4, 667.4, 607.6 cm^{-1}; HR-ESIMS calcd for $C_{19}H_{25}O_4$ $[M-H]^-$ 317.1747, found 317.1739; ^1H NMR and ^{13}C NMR spectroscopic data see Table 1.

Eutypellarin B (**2**): Yellow amorphous powder; $[\alpha]_D^{25}$ +26 (*c* 0.16, CH_3OH); UV (CH_3OH) λ_{max}: 238.8 nm; IR (MeOH) ν_{max}: 3390.9, 1716.7, 1604.8, 1541.1, 1487.1, 1367.5, 1273.0, 1203.6, 1128.4, 960.6, 902.7, 835.2, 765.7 cm^{-1}; HR-ESIMS calcd for $C_{15}H_{16}O_4N$ $[M-H]^-$ 274.1074, found 274.1070; ^1H NMR and ^{13}C NMR spectroscopic data see Table 2.

4.4 Antioxidant capacity assay

The antioxidant capacities of all compounds were determined through accessing the scavenging abilities of free radicals of ABTS and FRAP, in which results were respectively expressed as Trolox equivalent antioxidant capacity and $FeSO_4$ equivalent antioxidant capacity.

Supporting Information HR-ESIMS, UV, 1D, and 2D NMR spectra of compounds **1** and **2**, and the total antioxidant activity methods were reported in supporting information. The supporting information is available free of charge via the Internet at http://sioc-journal.cn/.

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