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Metabolites from the endophytic fungus *Cylindrocarpon* sp. isolated from tropical plant *Sapium ellipticum*

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ABSTRACT

Three new polyketides, cylindrocarpones A − C (**1–3**), two new pyridone alkaloids, cylindrocarpyridones A − B (**5–6**), a new pyrone cylindropyrone (**7**), together with seven know compounds were isolated from the endophytic fungus, *Cylindrocarpon* sp., obtained from the tropical plant *Sapium ellipticum*. The structures of the new compounds were elucidated by extensive analysis of their spectroscopic data (1D and 2D NMR, HRESIMS). The absolute configuration of 19-*O*-methyl-pyrrocidine B (**13**) was confirmed by X-ray analysis. All isolated compounds were screened for their cytotoxic and antibacterial activities. Pyrrocidine A (**12**) exhibited potent cytotoxicity against the human ovarian cancer cell line A2780 with an IC_{50} value of 1.7 μM. 19-*O*-Methyl-pyrrocidine B (**13**) showed moderate antibacterial activity against *S. aureus* ATCC25923 and ATCC700699 with MIC values of 50 and 25 μM, respectively.

1. Introduction

Endophytic fungi are known as an important source of polyketides [1,2]. This class of natural products exhibits a broad range of bioactivities, including antibiotic, anticancer, antifungal, antiparasitic and immunosuppressive properties, such as erythromycin, eribulin, bryostatin, and spongistatin [3–5]. Fungi of the genus *Cylindrocarpon* have been reported to produce structurally diverse secondary metabolites such as two inhibitors of pollen development in *Arabidopsis thaliana*, roridin A and verrucarin A [6], inhibitors of dihydroxynaphthalene-melanin biosynthesis, fusarins [7], a cytotoxic cyclopeptide, cylindrocyclin A [8], and an ascochlorin congener, cylindrol A_5 [9].

In our ongoing search for structurally novel and bioactive metabolites from endophytic fungi isolated from African tropical rain forest plants, cytotoxic penicolinate A, and two new *o*-aminobenzoic acid derivatives, bionectriamines A and B were isolated from the fungus *Bionectria* sp. [10], while a new cyclohexapeptide, penitropeptide and a new polyketide, penitropone were obtained from *Penicillium tropicum* [11]. We have now analyzed the endophytic fungus *Cylindrocarpon* sp. that was isolated from the tropical plant *Sapium ellipticum*.

We obtained three new polyketides, cylindrocarpones A − C (**1–3**), two new pyridone alkaloids, cylindrocarpyridone (**5–6**), a new pyrone cylindropyrone (**7**), as well as seven known compounds which included lamellicolic anhydride (**4**) [12], 5-chloro-6,8,10-trihydorxy-1-methoxy-3-methyl-9(10*H*)-anthracenone (**8**) [13], 1-*O*-methylemodin (**9**) [14], 5-chloro-1-*O*-methylemodin (**10**) [15], dihydroramulosin (**11**) [16], pyrrocidine A (**12**) [17,18], and 19-*O*-methyl-pyrrocidine B (**13**) [18] (Fig. 1). Here we report the structure elucidation of the new metabolites and the biological activities of all isolated compounds.

2. Results and discussion

Compound **1** was isolated as a yellow powder. The molecular formula $C_{14}H_{12}O_6$, indicating nine degrees of unsaturation, was deduced from the HREIMS data. The ^{13}C and ^{1}H NMR spectrum of **1** (Table 1) displayed signals of a methyl group at δ_C 25.8 (Me-11) and δ_H 2.76 (s, Me-11), of a methoxy group at δ_C 55.9 (OMe-1) and δ_H 3.91 (s, OMe-1), of two aromatic methines at δ_C 117.8 (C-7), 96.9 (C-2) and δ_H 6.66 (H-7) and 6.45 (H-2), of an oxygenated methine at δ_C 101.3 (C-10) and δ_H 6.47 (H-10), as well as the signal of a carbonyl carbon at δ_C 171.3 (C-9) in addition to signals of eight aromatic quaternary carbons. The UV and NMR data of **1** showed similarities to those of the co-isolated known

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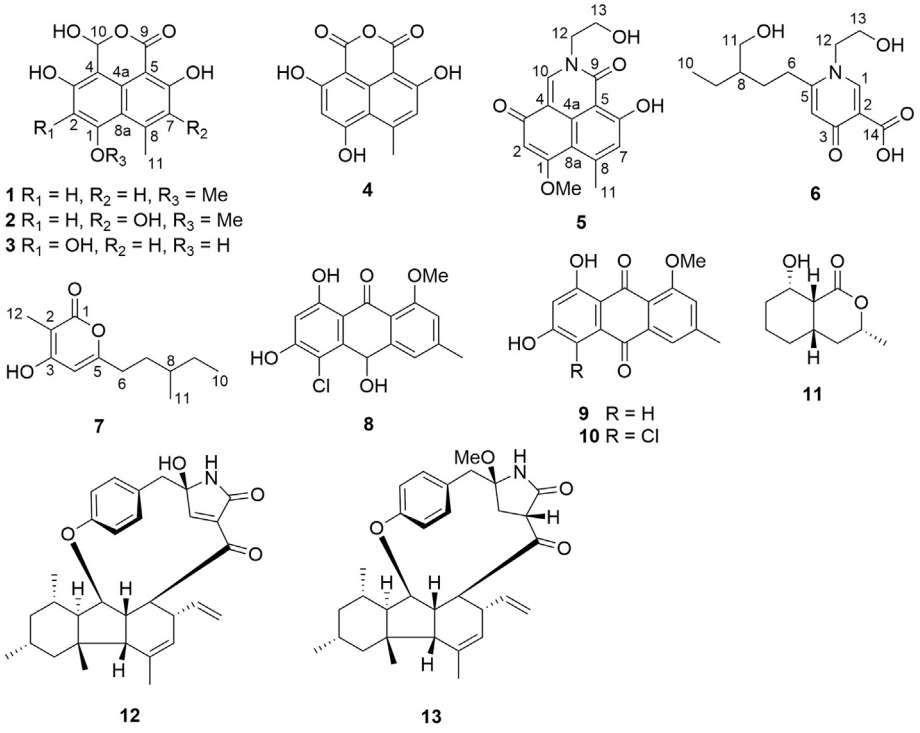

Fig. 1. Structures of isolated compounds.

Table 1
NMR data of compounds **1**–**3**.[a]

No.	1		2		3	
	δ_C, type	δ_H	δ_C, type	δ_H	δ_C, type	δ_H
1	162.3, C		161.4, C		160.3, C	
2	96.9, CH	6.45, s	97.7, CH	6.48, s	n.d.[b]	
3	157.6, C		154.9, C		157.3, C	
4	101.9, C		101.5, C		100.5, C	
4a	133.5, C		127.2, C		133.4, C	
5	97.7, C		97.6, C		97.0, C	
6	164.8, C		155.2, C		165.0, C	
7	117.8, CH	6.66, s	141.8, C		116.7, CH	6.63, s
8	149.4, C		130.4, C		150.0, C	
8a	113.4, C		113.8, C		112.5, C	
9	171.3, C		171.6, C		171.2, C	
10	101.3, CH	6.47, s	101.6, CH	6.50, s	101.3, CH	6.47, s
11	25.8, CH_3	2.76, s	15.4, CH_3	2.73, s	25.2, CH_3	2.82, s
OMe-1	55.9, CH_3	3.91, s	55.9, CH_3	3.90, s		

[a] Recorded at 600 MHz for ^1H and 150 MHz for ^{13}C in CD_3OD.
[b] Not detected.

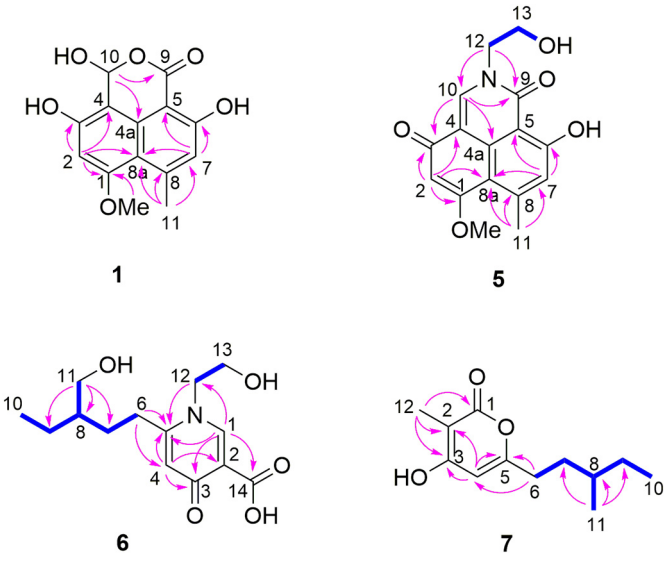

Fig. 2. COSY and key HMBC correlations of compounds **1**, **5**, **6** and **7**.

compound lamellicolic anhydride (**4**) [12], suggesting a similar skeletal structure. The HMBC correlations from H-2 to C-1 (δ_C 162.3), C-3 (δ_C 157.6), C-4 (δ_C 101.9) and C-8a (δ_C 113.4), from H-7 to C-5 (δ_C 97.7), C-6 (δ_C 164.8), C-8a and C-11 (δ_C 25.8), and from Me-11 to C-7 (δ_C 117.8), C-8 (δ_C 149.4) and C-8a established a naphthalene core structure with a methyl group at C-8 and two hydroxy groups at C-3 and C-6 (Fig. 2). In contrast to the known compound **4**, the HMBC correlations of **1** from OMe-1 to C-1 and from H-10 to C-9 (δ_C 171.3) and C-4a (δ_C 133.5) indicated the presence of a methoxy substituent at C-1 and of a hydroxy group at C-10 in the latter compound. Thus, the planar structure of **1** was elucidated as shown, for which the trivial name cylindrocarpone A is proposed.

The molecular formula of cylindrocarpone B (**2**) was determined as $C_{14}H_{12}O_7$ by HRESIMS thus containing an additional oxygen atom compared to **1**. The NMR data of **2** were similar to those of **1** except for the replacement of a methine by an aromatic quaternary carbon at δ_C 141.8 in **2**. The HMBC correlations from Me-11 (δ_H 2.73) to C-7 (δ_C 141.8), C-8 (δ_C 130.4) and C-8a (δ_C 113.8) indicated that an additional hydroxy group was attached at C-7. The remaining substructure of **2** was elucidated to be identical to that of **1** by detailed analysis of the 2D NMR of **2**.

Cylindrocarpone C (**3**) has the molecular formula $C_{13}H_{10}O_7$ as deduced from HRESIMS data, thus differing by the loss of a CH_2 moiety compared to **2**. The signals of the two methines at δ_H 6.63 (s) and 6.47 (s) were assigned to H-7 and H-10, respectively, based on the HMBC correlations from H-7 (δ_H 6.63, s) to C-5 (δ_C 97.0), C-6 (δ_C 165.0), C-8a (δ_C 112.5) and C-11 (δ_C 25.2), and from H-10 (δ_H 6.47, s) to C-3 (δ_C 157.3), C-4 (δ_C 100.5), C-9 (δ_C 171.2) and C-4a (δ_C 133.4). These data along with the disappearance of signals of the methoxy group suggested the presence of two hydroxy groups at C-1 and C-2 in compound **3**.