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# Indole diterpenoids from the endophytic fungus *Drechmeria* sp. as natural antimicrobial agents

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ABSTRACT

A fungal strain, *Drechmeria* sp., was isolated from the root of *Panax notoginseng*. Totally, seven new indole diterpenoids, drechmerins A-G (**1**–**7**), were isolated from the fermentation broth of *Drechmeria* sp. together with four known analogues (**8**–**11**). Their structures were determined on the basis of 1D and 2D NMR and electronic circular dichroism (ECD) spectroscopic analyses as well as theoretical calculations. All the isolated compounds were evaluated for their antimicrobial activities against *Candida albicans*, *Staphylococcus aureus*, *Bacillus cereus*, *B. subtillis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, respectively. Drechmerin B (**2**) displayed antimicrobial activity against *C. albicans* with an MIC value of 12.5 μg/mL. Molecular docking was used to investigate interactions of peptide deformylase with compounds **1**–**3**, **5**–**7**, **9**, and **10**.



## 1. Introduction

Endophytic fungi are important sources of bioactive metabolites (Wibowo et al., 2014, 2016; Senadeera et al., 2012), the indole diterpenoids, as secondary metabolites with a cyclic diterpene skeleton and an indole, which derive from geranylgeranyl diphosphate (GGPP) and indole-3-glycerol phosphate (Byrne et al., 2002; Kyriakidis et al., 1981), are mainly produced by filamentous fungi, especially *Neotyphodium*, *Aspergillus*, *Claviceps*, and *Penicillium* (Li, 2010; Yu and Li, 2012). The indole diterpenoids can be classified into two subtypes, including the paxilline-type indole diterpenoids and non-paxilline-type indole diterpenoids (Saikia et al., 2008). To data, more than 100 indole diterpenoids were isolated from the fungi (Fan et al., 2013; Gao et al., 2016; Saikia et al., 2008), such as paxilline, aflatrem, emindole DB, nodulisporic acid A, and 3-deoxo-4b-deoxypaxilline. Some of them exhibited a wide variety of pharmacological effects, including anti-H1N1 (Fan et al., 2013), antibiotic (Dowd et al., 1988; Shoop et al., 2001), antifungal (Calvo and Cary, 2015), and anti-insectan activities (Singh et al., 2004; Smith et al., 2007). The insecticidal underlying mechanism of the indole diterpenoids was ascribed to regulate glutamategated chloride ion channels of insects (Smith et al., 2000). Therefore, the indole diterpenoids have been recently paid more attention from synthetic chemists and pharmacists that mainly focused on the cloning and characterization of the genes and gene products (Ji and Wang, 2006; Lee et al., 2016; Lu et al., 2015; Sharpe and Johnson, 2015), due to their complex structures and remarkable bioactivities.

As part of our successive research on the discovery of antimicrobial agents from secondary metabolites of the fungi and plants (An et al., 2016; Mai et al., 2015; Sun et al., 2016, 2017a, 2017b, 2017c; Yu et al., 2017; Wang et al., 2017). The investigation of the fermentation broth of *Drechmeria* sp. has led to the isolation of seven new indole diterpenoids (**1**–**7**) and four known analogues (**8**–**11**) (Fig. 1). In this paper, their structural elucidations were

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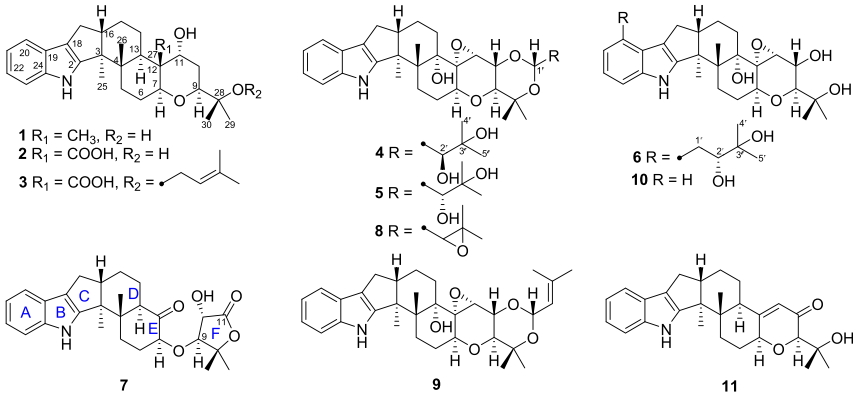


**Fig. 1.** Metabolites of *Drechmeria* sp.

determined by 1D and 2D NMR, electronic circular dichroism (ECD) analyses and quantum chemistry calculations. All the isolated compounds were bioassayed for antimicrobial activities against *Candida albicans*, *Staphylococcus aureus*, *Bacillus cereus*, *B. subtillis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*.

## 2. Results and discussion

Compound **1** was obtained as an amorphous powder, and its molecular formula was assigned as $C_{28}H_{39}NO_3$ by the positive HRESIMS (*m/z* 438.2998 $[M + H]^+$, calcd for $C_{28}H_{40}NO_3$, 438.3008) and $^{13}C$ NMR data. The $^1H$ NMR data (Table 1) of **1** displayed the characteristic signals of an indole unit at $\delta_H$ 7.28 (2H, d, $J = 7.8$ Hz, H-20 and H-23), 6.95 (1H, dd, $J = 7.8, 1.3$ Hz, H-22), and 6.92 (1H, dd, $J = 7.8, 1.3$ Hz, H-21), which was supported by the $^{13}C$ NMR data [$\delta_C$ 152.6 (C-2), 142.2 (C-25), 126.4 (C-19), 120.8 (C-22), 119.8 (C-21), 118.8 (C-20), 118.0 (C-18), and 112.8 (C-23)], and signals of five methyls at $\delta_H$ 1.18 (3H, s, Me-29), 1.17 (3H, s, Me-30), 1.16 (3H, s, Me-26), 1.06 (3H, s, Me-25), and 0.91 (3H, s, Me-27), indicating that **1** was an indole diterpenoid with five methyl groups (Fan et al., 2013; Munday-Finch et al., 1996; Springer and Clardy, 1980). The $^{13}C$ NMR data (Table 1) of **1** showed 28 carbon resonances, including the eight above-mentioned aromatic carbons, four oxygenated carbons [$\delta_C$ 80.6, 79.3, 73.0, and 71.1], six methylene carbons [$\delta_C$ 34.0, 31.0, 28.6, 26.5, 25.7, and 22.9], two methine carbons [$\delta_C$ 50.4 and 39.2], three quaternary carbons [$\delta_C$ 54.7, 42.0, and 41.1], and five methyl carbons [$\delta_C$ 25.9, 25.5, 20.4, 15.0, and 14.1]. A comparison of the NMR data of **1** with those of paspaline (Munday-Finch et al., 1996) revealed that a proton at C-11 was replaced by a hydroxy group in **1** since signals of a methylene [$\delta_H$ 1.85 (1H, m) and 1.14 (1H, m); $\delta_C$ 37.7] in paspaline were absent, and signals of an oxygenated methine [$\delta_H$ 3.77 (1H, br s) and $\delta_C$ 71.1] in **1** were present. The deduction was confirmed by HMBC correlations of H-10a with C-11/C-28, H-11 with C-9, and Me-27 with C-7/C-11/C-12 (Fig. 2). The relative configuration of **1** was determined based on NOESY correlations between H-7 and H-9/H-13, H-13 and Me-25, Me-26 and H-16/Me-27, and Me-27 and H-11 (Fig. 3), requiring that H-11, H-16, Me-26, and Me-27 were all $\beta$-oriented, and H-7, H-9, H-13, and Me-25 were all $\alpha$-oriented. The ECD spectrum of **1** displayed negative Cotton effects at 212 ($\Delta\varepsilon = -0.55$) and 243 ($\Delta\varepsilon = -0.04$) nm ascribed to $\pi - \pi^*$ transitions of the indole ring that showed negative Cotton effect at 210–250 nm (Fan et al., 2013; Gao et al., 2016; Su et al., 2014; Zhang et al., 2015), suggesting that the absolute configure of **1** was 3*S*,4*S*,7*S*,9*S*,11*R*,12*R*,13*R*,16*S*. And the X-ray diffraction of its analogue paspaline (Springer and Clardy, 1980) further confirmed the above conclusion. Accordingly, the structure of compound **1**, named as drechmerin A, was established.

The molecular formula of compound **2** was assigned as $C_{28}H_{37}NO_5$ according to the quasi-molecular ion peak at *m/z* 490.2561 $[M + Na]^+$ (calcd for $C_{28}H_{37}NNaO_5$, 490.2569) in the HRESIMS spectrum. The $^1H$ and $^{13}C$ NMR data (Table 1) of **2** were closely resembling to those of **1**, with the exception that the chemical shift value of C-27 was deshielded from $\delta_C$ 14.1 in **1** to $\delta_C$ 178.2 in **2**, which indicated that the methyl group at C-27 was oxidized to be a carboxyl group. In the HMBC spectrum of **2**, long-range correlations from H-7 to COOH-27 and H-13 to COOH-27 (Fig. 2) confirmed the location of the carboxyl group. The cross-peaks of H-7 with H-9/H-13, H-11 with H-14a, H-13 with Me-25, and Me-26 with H-14a/H-16 (Fig. 3) in the NOESY spectrum of **2** established the same relative configuration as **1** in conjunction with the biosynthetic background of indole diterpenoids (Saikia et al., 2008). The absolute configuration of **2** was assigned as 3*S*,4*S*,7*S*,9*S*,11*R*,12*S*,13*R*,16*S* on a basis of negative Cotton effects at 222 ($\Delta\varepsilon = -0.60$) and 240 ($\Delta\varepsilon = -0.13$) nm in the ECD spectrum (Fan et al., 2013; Gao et al., 2016; Su et al., 2014; Zhang et al., 2015). Therefore, the structure of compound **2**, named as drechmerin B, was established.

HRESIMS (*m/z* 558.3196 $[M + Na]^+$, calcd for $C_{33}H_{45}NNaO_5$, 558.3195) data of compound **3** suggested its molecular formula of $C_{33}H_{45}NO_5$. Compared with **2**, **3** had five extra carbons of an isopentenyl unit [$\delta_C$ 136.3, 123.7, 60.1, 26.1, and 18.2], and the chemical shift value of C-28 was downfielded from $\delta_C$ 72.8 in **2** to $\delta_C$ 77.7 in **3**, indicating that the *O*-isopentenyl substituent was linked at C-28. This conclusion was confirmed through an HMBC spectrum, which showed long-range correlations of H-1′a/H-1′b with C-3′/C-28 and H-2′ with C-3′/C-4′/C-5′ (Fig. 2). Compound **3** possessed the same relative configuration as **1** and **2** according to cross-peaks of H-7 with H-9/H-13, H-13 with Me-25, H-11 with H-14a, H-13 with Me-25, and Me-26 with H-16/H-14a (Fig. 3) in the NOESY spectrum of **3**. The ECD spectrum suggested a (3*S*,4*S*,7*S*,9*S*,11*R*,12*S*,13*R*,16*S*)-configuration due to the negative Cotton effects at 221 ($\Delta\varepsilon = -0.10$) and 242 ($\Delta\varepsilon = -0.05$) nm similar to those of **2** (Fan et al., 2013; Gao et al., 2016; Su et al., 2014; Zhang et al., 2015). Thus, the structure of compound **3**, named as drechmerin C, was established.

Compounds **4** and **5** possessed the same molecular formula as $C_{32}H_{43}NO_7$ based on the positive HRESIMS (*m/z* 554.3115 $[M + H]^+$, calcd for $C_{32}H_{44}NO_7$, 554.3118 in **4**; *m/z* 554.3105 $[M + H]^+$, calcd for $C_{32}H_{44}NO_7$, 554.3118 in **5**) data. Comparison of $^1H$ and $^{13}C$ NMR data (Table 2) of **4**, **5**, and terpendole C (**9**) (Huang et al., 1995) revealed that **4** and **5** were the hydroxylated derivatives of the $\Delta^{2',3'}$ double bond of **9** since the chemical shift values of C-2′ and C-3′ were shielded from $\delta_C$ 122.5 and 137.5 in **9** to $\delta_C$ 77.5 and 77.9 in **4** and $\delta_C$ 77.6 and 77.8 in **5**, respectively, and the signal of an olefinic proton at $\delta_H$ 5.33 (1H, d, $J = 6.5$ Hz) in **9** was substituted by the signal of an