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# Antimicrobial meroterpenoids from the endophytic fungus *Penicillium* sp. T2-8 associated with *Gastrodia elata*

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ABSTRACT

A new meroterpenoid preaustinoid D (**1**) and a new neogrifolin derivative dihydroxyneogrifolic acid (**2**), together with other five previously reported meroterpenoids (**3**-**7**) were isolated from the endophytic fungus *Penicillium* sp. T2-8 associated with *Gastrodia elata*. The structures were elucidated on the basis of spectroscopic data analyses. It was the first time that neogrifolin and its derivatives were isolated from microorganism. Some of these meroterpenoids exhibited obvious antimicrobial activities in the microdilution antimicrobial assays.



## 1. Introduction

The secondary metabolites produced by endophytic fungi are a valuable repository of natural bioactive compounds, many of which have been identified as useful research reagents and potential drug candidates (Ibrahim et al., 2015; Meinwald and Eisner, 2008). Novel secondary metabolites produced by filamentous endophytic fungi such as *Penicillium* and *Aspergillus* have been continuously discovered (Bode et al., 2002; Frisvad et al., 2004; Ibrahim et al., 2015). The meroterpenoids are a class of natural products from fungi which were formed from polyketides and terpenoid precursors. They also display a wide range of structurally diverse natural products with a lot of important biological activities (Geris and Simpson, 2009). In the meantime, meroterpenoids have been broadly discovered from both species of *Penicillium* and *Aspergillus* (Stierle et al., 2011; Lo et al., 2012).

As a part of our continuing interest in exploring bioactive metabolites from endophytic fungal source, we chemically investigated the secondary metabolites produced by the endophytic fungus *Penicillium* sp. T2-8 which was isolated from the fresh rhizomes of *Gastrodia elata* collected from Zhaotong, Yunnan Province, China. Chromatographic separation of the strain *Penicillium* sp. T2-8 yielded seven meroterpenoids. Among them, preaustinoid D (**1**) and dihydroxyneogrifolic acid (**2**) were two new compounds. Preaustinoid D (**1**) was an analogue of austin with the opened A ring, and dihydroxyneogrifolic acid (**2**) was a new neogrifolin derivative while it was the first time that neogrifolin and its derivatives were isolated from microorganism. Their structures were established by spectroscopic data analyses, and the known compounds were identified as preaustinoid A1 (**3**) (Santos and Rodrigues-Fo, 2003), dehydroaustinol (**4**), austin (**5**) (Hayashi et al., 1994), (*S*)-18,19-dihydroxyneogrifolin (**6**) (Liu et al., 2014) and neogrifolin (**7**) (Hellwig et al., 2003) by comparing the spectroscopic data with published literatures (Fig. 1). Furthermore, compounds **3**, **5** and **6** showed obvious antimicrobial activities in antimicrobial assays.

## 2. Results and discussion

Compound **1** was isolated as colorless needle crystal. The UV absorption maxima of purified compound **1** occurred at 217 nm. Its IR spectrum exhibited strong absorptions at 3444, 1723, 1714, and 1633 $cm^{-1}$, indicating the existence of hydroxy groups and carbonyl groups. The molecular formula $C_{27}H_{40}O_8$ with eight degrees of unsaturation was determined from the prominent ion peak at *m*/*z* 515.2613 $[M+Na]^+$ (calcd for $C_{27}H_{40}O_8Na$ 515.2621) observed in the HR-ESIMS spectrum and confirmed by $^{13}C$ NMR spectra. The $^{13}C$ NMR and DEPT spectra of compound **1** (Table 1) revealed eleven quaternary carbons (containing four carbonyl carbons), two methines, six methylenes (containing one vinyl

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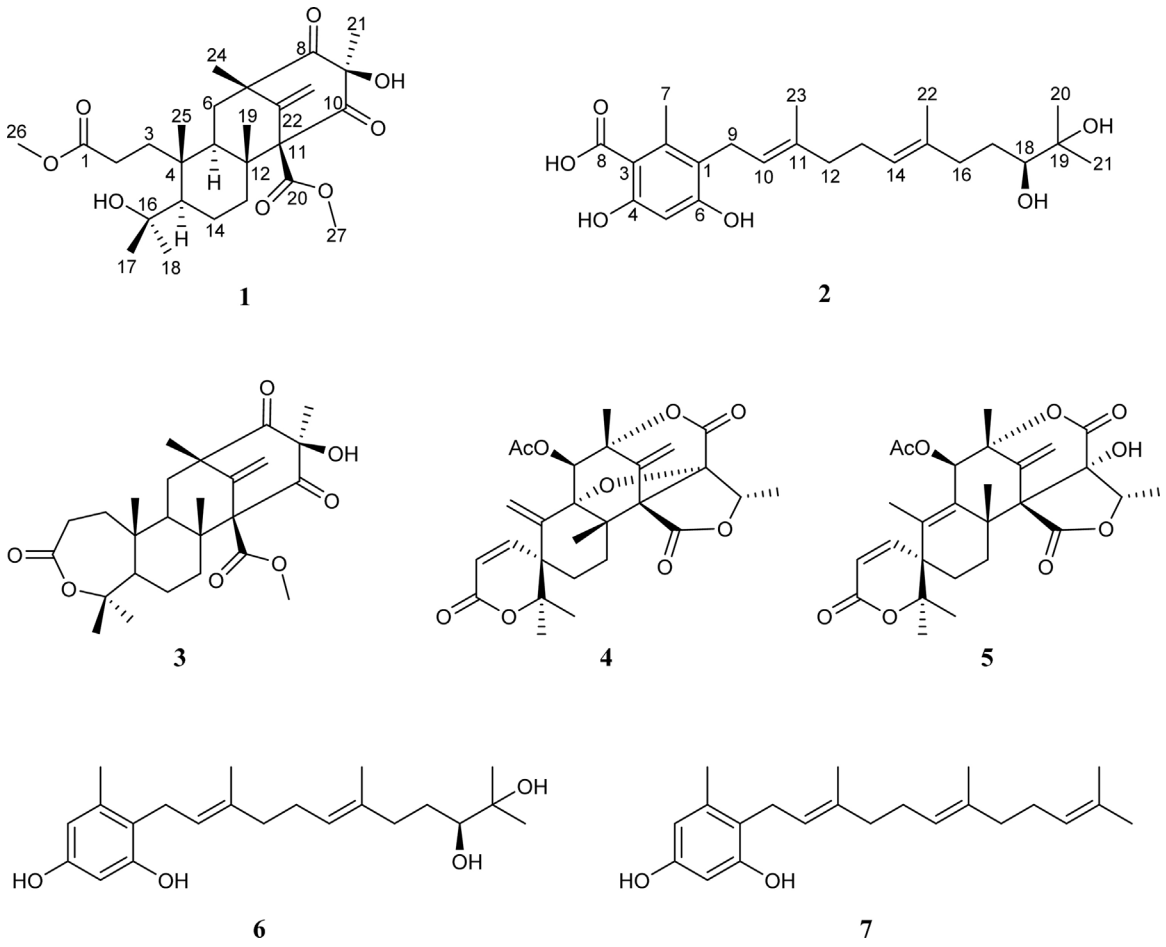


**Fig. 1.** Chemical structures of compounds **1**–**7**.

methylene) and eight methyls (containing two methoxy groups). Comparing the $^{1}H$ and $^{13}C$ NMR spectra of compound **1** to those of preaustinoid A1 (Santos and Rodrigues-Fo, 2003) which was also produced by a *Penicillium* sp. indicated a close similarity between them and offered an efficient way in terms of establishing the structure of compound **1**. The B, C and D rings of them including the C-20 methyl ester were intact by detailed 1D NMR analysis (Table 1) and key 2D NMR correlations elucidation (Fig. 2) of **1**, which also accounted for seven degrees of unsaturation. As seen in the NMR spectra (C-1, $\delta_C$ 175.5), compound **1** contained one more carbonyl, and provided the last degree of unsaturation. All of these data indicated one additional degree of saturation of **1** than preaustinoid A1 (**3**), which could be accounted for by an opened A ring. The HMBC correlation from H-26 to C-1 together with all the other HMBC correlations (Fig. 2) confirmed the presence of the opened A ring with a methyl ester. The opened A ring of meroterpenoid was also discovered from the Pit Lake fungus *Penicillium rubrum* (Stierle et al., 2011). Therefore, the panel structure of compound **1** was deduced as shown in Fig. 1.

The relative stereochemistry of **1** was deduced from the ROESY correlations (Fig. 2). The ROESY correlations of H-5 with H-15 and H-21 indicated that these protons were located on the same side. The ROESY correlations of Ha-23 with H-19 and H-27, of H-24 with Hb-23 and H-27, and of H-19 with H-25 indicated these protons were located on the reverse side at the same time. It suggested that there were two possible isomers of compound **1**. The absolute configuration of **1** was elucidated by comparing its experimental ECD spectrum to the ECD spectra calculated for isomer-1 and -2 of compound **1** (Fig. 3). The ECD spectrum was simulated by electronic excitation energies and velocity rotational strengths. The results showed that the theoretical ECD data for isomer-1 of compound **1** was in good agreement with the experimental spectrum. Finally, the absolute configuration of **1** was assigned as shown in Fig. 1, and compound **1** was named as preaustinoid D.

Compound **2**, obtained as yellow oil, has a molecular formula of $C_{23}H_{34}O_6$ with seven degrees of unsaturation which was determined from the prominent ion peak at *m/z* 429.2248 $[M+Na]^+$ (calcd for $C_{23}H_{34}O_6Na$ 429.2253) observed in the HR-ESIMS spectrum. The IR spectrum displayed absorptions at 3445, 1663 and 1617 $cm^{-1}$, which gave the indication of the presence of hydroxy groups, carbonyl groups and double bond groups. The $^{1}H$ NMR spectrum of compound **2** (Table 1) showed signals for one aromatic proton ($\delta_H$ 6.19), two olefinic protons ($\delta_H$ 4.98 and 5.13), and five methyls ($\delta_H$ 2.41, 1.73, 1.57, 1.11 and 1.09). The data of NMR spectra were similar to those of (*S*)-18,19-dihydroxyneogrifolin (Liu et al., 2014), except for the 1,2,4,6-tetra-substituted phenyl ring which was replaced by a penta-substituted phenyl ring. In combination with the HMBC correlations from H-5 to C-1, C-3, C-4, C-6 and C-8, from H-7 to C-1, C-2, C-3 and C-8, from H-9 to C-1, C-2 and C-6 (Fig. 4), it suggested that there was substituted by a carboxyl group at C-3. The absolute configuration of C-18 in **2** was assigned to be *S* by comparing the optical rotation value of **2** ([α] 20*D* −4.0, MeOH) to that of (*S*)-18,19-dihydroxyneogrifolin ([α]21*D* −9.2, MeOH) (Liu et al., 2014), which also met the biogenetic point of view. As a result, the structure of **2** was established as shown in Fig. 1, named dihydroxyneogrifolic acid. Grifolin, neogrifolin and their derivatives were one kind of meroterpenoids with diverse biological activities, and they were mainly produced by the