**SHORT COMMUNICATION**

**Terragines F–G produced by endophytic *Bacillus* sp. SH-1.2-ROOT-18 from *Dendrobium officinale***

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**ABSTRACT**

Two new terragine analogs (1–2) with special succinimide and aminopentane moieties were isolated from the fermentation broth of *Bacillus* sp. SH-1.2-ROOT-18, an endophyte previously discovered from the root of *Dendrobium officinale*. The structures were elucidated base on comprehensive 1D/2D NMR and MS data analysis. Complete NMR assignments for the first reported naturally occurring metabolite 3 was also provided.

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**KEYWORDS**

Terragine; natural product; endophyte; Bacillus sp; *Dendrobium officinale*

1. **Introduction**

Natural products still hold out the best options for finding novel structures that can lead to effective agents in a variety of human diseases (Newman and Cragg 2020). Medicinal plants are a prolific source of natural products with remarkable chemical and biological properties. The endophytes in medicinal plants, which participate in biochemical pathways of their hosts, have been reported to produce useful and/or interesting secondary metabolites as their hosts did (Stierle et al. 1993; Newman and Cragg 2015; Parthasarathy and Sathiyabama 2015; Schafhauser et al. 2019; Zhao et al. 2020c). The appreciation of their abilities to produce useful compounds via genetic and molecular interactions has paved the way for health and welfare of plants, human
and ecosystems. *Dendrobium officinale* is a well-known traditional Chinese medicine with reported wide modern pharmacological actions (Tang et al. 2017; Liang et al. 2019) and rich active endophytic secondary metabolites (Wu et al. 2015; Shi et al. 2020). As part of our ongoing natural product initiative to characterise new metabolites produced by *D. officinale* endophytes, we have adapted an LCMS-based metabolomics strategy to rapidly delineate unique metabolite signatures as a primary strain prioritisation strategy. This process has facilitated the recent discovery of a range of new structurally diverse bacterial metabolites including peptidendrocins and chartspiroton (Zhao et al. 2020a, 2020b). Here we report two new terragine analogs (1–2) from the fermentation broth of *Bacillus* sp. SH-1.2-ROOT-18, an endophytic strain reported previously from the root of *D. officinale* (Zhao et al. 2020a). Terragines with special succinimide and aminopentane moieties were previously obtained from constructed soil DNA libraries in a *Streptomycete* host (Wang et al. 2000), and this is the first time for terragine analogs isolated from *Bacillus* sp. The structure of terragine F (1) was elucidated on basis of comprehensive 1D/2D NMR and MS data analysis, to confirm the acetyl at N-22 position versus a phenylacetyl in terragine A (7). Terragine G (2) was a newly isolated precursor of this class. Complete NMR assignment for the first reported naturally occurring metabolite 3 was also provided.

2. Results and discussion

Nineteen endophytic strains were previously purified from second generation plates derived from the root of one-year old *D. officinale* (Zhao et al. 2020a). Preliminary LC-MS strategy (Shaaban et al. 2014; Wang et al. 2017; Wang et al. 2019) used for analysis of crude extract prepared from *Bacillus* sp. SH-1.2-ROOT-18 revealed potentially new secondary metabolites. 10 L fermentation of this strain, followed by extraction and series of chromatographic isolation (supporting information Scheme S1), yielded two new compounds: terragines F (1, yield: 0.36 mg/L) and G (2, yield: 1.61 mg/L), together with ten known compounds 3–12 (Figure 1).

Compound 1 was obtained as yellow oil. The HRESIMS of 1 displayed quasi-molecular ion at m/z 443.2503 [M + H]⁺ and m/z 441.2355 [M – H]⁻ in positive and negative modes, suggesting a molecular formula of C₂₀H₃₄N₄O₇, with six degrees of unsaturation. The analysis of ¹H, ¹³C and HSQC NMR data indicated the presence of one methyl (δH 2.09 and δC 20.3), fourteen methylenes and five carbonyls (δC 173.6, 174.6, 175.1, 180.2 × 2). Three COSY spin systems were established as CH₂-6/CH₂-7/CH₂-8/CH₂-9/CH₂-10, CH₂-13/CH₂-14 and CH₂-17/CH₂-18/CH₂-19/CH₂-20/CH₂-21. The key HMBC correlations from H-6 to C-1, C-4, C-7 and C-8, from H-2 to C-1, C-4, and from H-3 to C-1 and C-4, allowed the construction of the succinimide with aminopentane moieties. Further analysis of 1D/2D NMR data revealed a close relationship between 1 and terragine A (7) (Wang et al. 2000). Terragine A was one of the novel natural products from constructed soil DNA libraries in a *Streptomycete* host, also was a confirmed metabolite of *Bacillus* sp. SH-1.2-ROOT-18. Their significant differences observed in NMR spectra were the substituent at N-22 position (an acetyl in 1 versus a phenylacetyl in 7) (Figure 1), which was confirmed through the HMBC correlations from H-21 (δH 3.60, m) to C-19 (δC 24.9), C-20 (δC 27.4) and C-23 (δC 173.6). The remaining HMBC
Figure 1. Chemical structures of isolated compounds from *Bacillus* sp. SH-1.2-ROOT-18.
correlations (supporting information Figure S1) and HPLC-MS/MS data (supporting information Figure S2) were in full agreement with 1 as a new terragine analog and thereby designated terragine F.

The determined molecular formula of compound 2 (C_{11}H_{18}N_{2}O_{4}) and its NMR data (supporting information Figures S10–S14) revealed 2 shares a partial structure of 1 from C-1 to C-12. The acetyl at N-11 position was deduced from the HMBC correlations of H-13/C-12 and H-10/C-12. The structure of 2 was then established as shown in Figure 1 and named as terragine G. Compound 3 was identified to be a deacetylate of 2 via HRESIMS and 1D/2D NMR (supporting information Figures S17–S22). As a new naturally occurring metabolite, its complete NMR assignment was reported for the first time.

The other nine known compounds were identified as N’-[5-[[4-[[5-(acetetyl-hydroxyamino)pentyl]amino]-1,4-di-oxobutyl]hydroxyamino]pentyl]-N-(4-carboxybutyl)-N-hydroxybutanediamide (4) (Dionis et al. 1989), desferrioxamine B (5) (Winkelmann et al. 1999), DH1 (6) (Winkelmann et al. 1999), terragine A (7) (Wang et al. 2000), terragine B (8) (Wang et al. 2000), norcardamine (9) (Wang et al. 2000), futalosine (10) (Li et al. 2011), 6-amino-6-deoxyfutalosine (11) (Li et al. 2011), and TCC (12) (Péter et al. 1998) through comparison with data in the literatures.

3. Conclusion

In summary, metabolic profiling of the medicinal plant endophytic Bacillus sp. SH-1.2-ROOT-18 led to the discovery of a set of nice terragine analogues including two new terragines F and G (1 and 2). Desferrioxamine B (5), a drug used for transfusional iron overload (Propper et al. 1976), along with its previously reported human urinary metabolite 4 (Dionis et al. 1989), were also obtained from this strain. Although compounds 1-12 were inactive at or below 40 \mu M in 96-well cell viability assay or 96-well Bacterium turbidity assay, the newly isolated 1-3 expand the structural diversity of terragine-associated scaffolds from endophytic bacterium and set the stage for future biosynthetic interrogation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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