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SHORT COMMUNICATION

A new *O*-cinnamoyl threonine derivative from gene *adpA* overexpression strain *Streptomyces* sp. HS-NF-1222A

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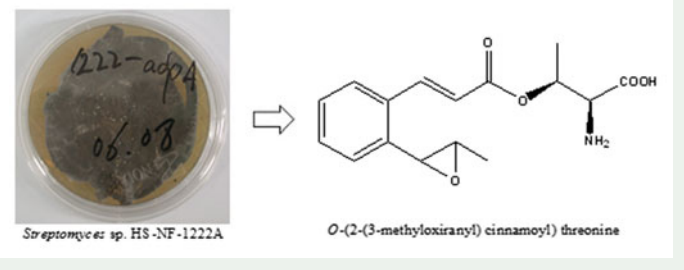
ABSTRACT

A new *O*-cinnamoyl threonine derivative, *O*-(2-(3-methyloxiranyl) cinnamoyl) threonine (**1**), was isolated from the gene *adpA* overexpression strain *Streptomyces* sp. HS-NF-1222A. The structure of **1** was determined based on HRESIMS and extensive NMR analysis.



Streptomyces sp. HS-NF-1222A

O-(2-(3-methyloxiranyl) cinnamoyl) threonine

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KEYWORDS
Streptomyces; adpA overexpression; *O*-cinnamoyl threonine derivative; structure elucidation

1. Introduction

Microorganisms have provided abundant sources of natural products which have been developed as commercial products for human medicine, animal health, and plant crop protection (Leonard and Richard 2016). Nevertheless, the growing number of sequenced microbial genomes has revealed a remarkably large number of natural product biosynthetic clusters for which the products are still unknown (Chiang et al. 2011). Investigators have uncovered numerous cryptic secondary metabolite biosynthetic pathways not associated with the production of known metabolites, suggesting that the majority of these gene clusters are silent or are expressed below detectable levels (Luisa et al. 2011; Gregory 2008). To activate such gene clusters, many strategies have been developed (Jin et al. 2006; Li et al. 2014, 2017). It has been reported that manipulation of regulatory genes could activate the expression of silent gene clusters,

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such as overexpression of global positive regulator gene *adpA* (Liu et al. 2013; Yasuo et al. 1999, 2005). During the course of searching for novel microbe-derived bioactive secondary metabolites, we obtained a hygromycin A producing strain *Streptomyces* sp. HS-NF-1222. In order to tap the potential of this strain, we tried to activate the silent gene clusters in *Streptomyces* sp. HS-NF-1222. As a result, while we over-expressed the gene *adpA* in *Streptomyces* sp. HS-NF-1222 by an integrative expression vector, a recombination strain HS-NF-1222A with an extra peak compared to parental strain in the HPLC chromatogram of the fermentation product was obtained. Further chemical investigation led to the isolation of a new *O*-cinnamoyl threonine derivative **1** corresponding to the extra peak. In this paper, we describe the fermentation, isolation and structure elucidation of **1**.

2. Results and discussion

2.1. Structure determination

Compound **1** was isolated as white amorphous powder. The molecular formula of **1** was determined to be $C_{16}H_{19}NO_5$ on the basis of HRESIMS at *m/z* 306.1337 $[M + H]^+$ (calcd for $C_{16}H_{20}NO_5$, 306.1336) in combination with NMR data (Table S1), suggesting eight degrees of unsaturation. The IR spectrum displayed absorption band for carbonyl group (1680 cm^{-1}). The ^1H NMR spectrum (Table S1) of **1** showed the signals of a 1,2-disubstituted benzene ring at δ_H 7.24 (1H, d, J = 7.7 Hz), 7.29 (1H, dd, J = 7.6, 8.0 Hz), 7.31 (1H, dd, J = 7.7, 8.0 Hz), 7.64 (1H, d, J = 7.6 Hz), a trans double-bond at δ_H 6.79 (1H, d, J = 15.5 Hz), 7.95 (1H, d, J = 15.5 Hz), two aliphatic doublet methyls at δ_H 1.25 (3H, d, J = 5.8 Hz), 1.46 (3H, d, J = 4.8 Hz), four sp^3 methine proton signals at δ_H 2.91 (1H, m), 3.86 (1H, s), 4.35 (1H, br s), 4.56 (1H, br s). The ^{13}C NMR spectrum along with DEPT135 revealed the presence of two carbonyl carbons at δ_C 176.0, 166.8, six sp^2 methines at δ_C 122.9, 124.8, 125.9, 127.7, 129.4, 136.9, two sp^2 quaternary carbons at δ_C 133.5, 136.8, four sp^3 methines at δ_C 56.8, 58.1, 59.1, 67.9 and two methyls at δ_C 16.6, 18.5. The complete assignment of all the ^1H and ^{13}C NMR spectral data of **1** was subsequently accomplished by the ^1H-^1H COSY, HMQC and HMBC spectra. The ^1H-^1H COSY correlation (Figure S2) of H-7/H-8 and the observed HMBC correlations (Figure S2) from H-8 to C-1 and from H-7 to C-9 indicated the presence of a cinnamic acid structure unit. The correlations of H-2′/H-3′/H-4′ in the ^1H-^1H COSY spectrum and the long-range correlations from H-4′ to C-2′ and from H-3′ to C-1′ in conjunction with the ^1H and ^{13}C NMR data of C-2′ revealed the presence of a threonine moiety. The ^1H-^1H COSY correlations of H-10/H-11/H-12 and the ^1H and ^{13}C NMR chemical shifts of C-10 and C-11 supported a 3-methyl oxiran structural unit in **1**. The connectivity of the 3-methyl oxiran structural unit with C-2 was evidenced by the HMBC correlation from H-10 to C-2. Finally, the connection of C-9 to C-3′ via an oxygen atom was confirmed by the HMBC correlation between H-3′ and C-9. Considered the formula $C_{16}H_{19}NO_5$ of **1**, the gross structure of **1** was established as shown in Figure 1. Previously, a synthetic analogue, *O*-cinnamoyl threonine, was reported as a deodorant composition (Laney 1998). Difference between **1** and O-cinnamoyl threonine was that a 3-methyl oxiran moiety was present in **1**. The configuration of threonine residue in **1** was investigated using the advanced Marfey's method after acidic hydrolysis (Peter 1984; Soumini et al.

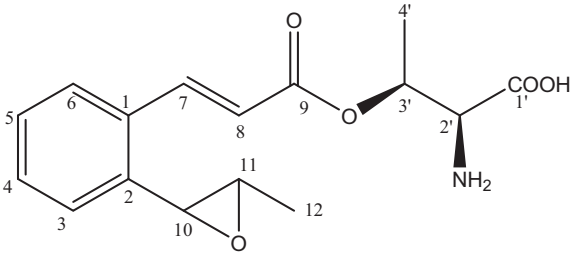

Figure 1. The chemical structure of compound **1**.

2016) and the configuration of threonine was determined as L. The configurations at C-10 and C-11 remained unassigned.

2.2. Acaricidal activity

The larvicidal capacity of compound **1** against *Tetranychus cinnabarinus* reared in the laboratory was evaluated. The results showed that compound **1** exhibited lethality against female *Tetranychus cinnabarinus* with LC_{50} value of 48.5 mg/L.

3. Experimental

3.1. Organism and fermentation

The parental strain *Streptomyces* sp. HS-NF-1222 was isolated from soil sample collected from the Jiujiang, Jiangxi province, China. The strain was identified as the genus *Streptomyces* because its 16s rDNA sequence (accession No.: MH727816) exhibited a high sequence similarity of 99% with that of *Streptomyces endus* strain NRRL 2339 (accession No.: NR_043379.1). The strain was grown and maintained on GYM agar plate containing malt extract 1%, yeast extract 0.4%, glucose 0.4%, and agar 2.0% at pH 7.0. The global positive regulator gene *adpA* was generated from *Streptomyces coelicolor*. A3(2) by PCR and harbored by an integrative expression vector (Figure S1). *Escherichia coli* DH5α was used for cloning purpose, and *E. coli* ET12567 (pUZ8002) was used for conjugation. Both of the *E. coli* strains were cultured in LB medium. After the vector transformed into the parental strain, the recombination strains obtained and each recombination strain was fermented by shake flask with 25 mL medium consisted of malt extract 1%, yeast extract 0.4%, glucose 0.4%, soluble starch 4.0% and $CaCO_3$ 0.2% at pH 7.2 on a rotary shaker (250 rpm, 28 °C) for 7 days. The metabolic profiles of the fermentation product were assayed by HPLC. Then, the chromatogram of a recombination strain exhibited an extra peak (Figure S3) which absent in the chromatogram of the parental strain and the recombination strain was named *Streptomyces* sp. HS-NF-1222A.

The fermentation for the *Streptomyces* sp. HS-NF-1222A was carried out in a 50 L fermenter containing 30 L of production medium consisted of malt extract 1%, yeast extract 0.4%, glucose 0.4%, soluble starch 4.0% and $CaCO_3$ 0.2% at pH 7.2. The seed medium consisted of glucose 0.4%, malt extract 1%, yeast extract 0.4% and $CaCO_3$ 0.2% at pH 7.2. A total of 1 liter seed was incubated by 1 liter flasks containing 250

mL of the seed medium at 28 °C for 24 h, shaken at 250 rpm. The fermentation was carried out at 28 °C for 7 days, stirred at 200 r.p.m. with the aeration rate of 1000 L of air per hour, tank pressure control at 0.05 MPa.

3.3. Extraction and isolation

The completed fermentation broth (30 L) was separated into supernatant and mycelia by centrifugation. The mycelia was washed with water (3 L) and subsequently extracted with MeOH (3 L). The supernatant and the wash water were subjected to a Diaion HP-20 resin column eluting with 95% EtOH. The MeOH extract and the EtOH eluents were combined and evaporated in vacuo to yield the crude extract. The mixture was chromatographed on a silica gel column and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (95:5-40:60, vol/vol) to give six fractions (Fr.1-Fr.6) based on the TLC profiles. TLC was performed on silica-gel plates with a solvent system of $CHCl_3$/MeOH (9:1) and the developed TLC plates were observed under a UV lamp at 254 nm or by heating after spraying with sulfuric acid-ethanol, 5:95 (vol/vol). The Fr.4 was subjected to a Sephadex LH-20 column eluted with $CHCl_3$/MeOH (1:1, vol/vol) and detected by TLC to give three subtractions (Fr.4-1–Fr.4-3). The Fr.4-2 was further purified by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm inner diameter; 1.5 mL min^{-1}; 220 nm; Agilent, Palo Alto, CA, USA) eluting with CH_3CN/H_2O (13:87, vol/vol) to give compound **1** (t_R 18.6 min, 35 mg).

Compound **1**. White amorphous powder; $[\alpha]_D^{25}$ -87 (*c* 0.05, EtOH); UV (EtOH) λ_{max} nm (log ε): 221 (4.51), 277 (4.57) nm; IR (KBr) ν_{max}: 3339, 2972, 2922, 1680, 1612, 1421, 1218, 1085, 1048, 976, 878, 765 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table S1; HRESIMS: *m/z* 306.1337 $[M + H]^+$ (calcd for 306.1336).

3.4. Acid hydrolysis and Marfey's method

Compound **1** (1 mg) in 2 M HCl (1 mL) was heated at 100 °C in a sealed vial for 2 ∼ 3 h, after which the hydrolysate was concentrated to dryness under a stream of dry N_2. The hydrolysate was then treated with 1 M $NaHCO_3$ (1 mL) and $_D$-FDAA (10% solution in acetone, 2 mL) at 40 °C for 1 h, after which the reaction was neutralized with 1 M HCl, filtered (0.45 μm PTFE) prior to HPLC analysis. An aliquot (5 μL) of the analyte was injected into an Agilent Eclipse XDB-C18 column, 5 μm, 9.4 × 250 mm, 30 °C, with a 1 mL/min, 30 min linear gradient elution from 30% to 70% MeCN/H_2O with 0.1% trifluoroacetic acid in H_2O. The threonine content in analytes was assessed by UV (340 nm), with comparison to authentic standard.

3.5. Acaricidal activity test

The acaricidal capacity of compound **1** against *Tetranychus cinnabarinus* reared in the laboratory was evaluated according to our reported paper (Huang et al. 2015).

4. Conclusion

In this study, a new *O*-cinnamoyl threonine derivative, *O*-(2-(3-methyloxiranyl) cinnamoyl) threonine (**1**), was obtained from the fermentation broth of the gene *adpA* overexpression strain *Streptomyces* sp. HS-NF-1222A. To the best of our knowledge, compound **1** represent a new kind of natural *O*-cinnamoyl threonine derivatives and the study may provide a viable option for screening novel secondary metabolites from known antibiotic-producing streptomycetes. In addition, studies on the *O*-cinnamoyl threonine derivative with diverse activities are currently underway.

Disclosure statement

No potential conflict of interest was reported by the authors.

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