SUPPLEMENTARY MATERIAL

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.

Keywords: *Streptomyces*; adpA overexpression; O-cinnamoyl threonine derivative; structure elucidatio
**EXPERIMENTAL**

**General.** Optical rotation was measured on Perkin-Elmer 341 Polarimeter (Perkin-Elmer, Suzhou, China). IR spectrum in pressed KBr disk was obtained on a Nicolet Magna FT-IR 750 spectrometer (Nicolet, Tokyo, Japan) and UV spectrum was recorded on a Varian CARY 300 BIO spectrophotometer (Varian, Cary, NC, USA). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DRX-400 spectrometer (400 MHz for $^1$H and 100 MHz for $^{13}$C; Bruker, Rheinstetten, Germany). Chemical shifts are reported in p.p.m. ($\delta$), using CD$_3$OD ($\delta$H 3.31; $\delta$C 49.5) as an internal standard, and coupling constants ($J$) in Hz. The HRESIMS were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters Co, Milford, MA, USA). Column chromatography was carried out on silica gel (100–200 mesh; Qingdao Marine Chemical Group Co., Qingdao, Shandong, China) and Sephadex LH-20 (GE Healthcare, Glies, UK). Semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250×9.4 mm inner diameter; 1.5 ml/min; 220 nm; Agilent, Palo Alto, CA, USA) was further performed to obtain pure compound. Spots were detected on thin layer chromatography (TLC) under UV or by heating after spraying with sulfuric acid–ethanol (5:95, v/v).

**Acaricidal activity test.** The sample was prepared in acetone at a concentration of 1,000 mg/l and diluted to the required concentrations of 0.01, 0.005, 0.0025, 0.001, and 0.0005 mg/l with distilled water containing alkylphenol ethoxylates (~1/1000, vol/vol). The primary leaves of *Vicia faba* L. species were infected with *T. cinnabarinus*. At 2 hour after infection, 10 fourth instar mite larvae were dipped in the diluted solutions of related chemicals for 5 seconds before the superfluous liquid was removed, and the larvae were kept in a conditioned room. Three replicates were made for each concentration and a blank control. The mortality was evaluated 24 hour after treatment by checking the adult mites under a microscope to determine the living and dead individuals. The 50% lethal concentrations (LC$_{50}$) of compound 1 was calculated using the probit method.
Table S1. $^1$H and $^{13}$C NMR spectral data for 1 (in CD$_3$OD)

| position | $\delta_H$ (J in Hz)       | $\delta_C$ |
|----------|-----------------------------|------------|
| 1        | 133.5                       |            |
| 2        | 136.8                       |            |
| 3        | 7.24 (1H, d, 7.7)           | 124.8      |
| 4        | 7.31 (1H, dd, 7.7, 8.0)     | 129.4      |
| 5        | 7.29 (1H, dd, 7.6, 8.0)     | 127.7      |
| 6        | 7.64 (1H, d, 7.6)           | 125.9      |
| 7        | 7.95 (1H, d, 15.5)          | 136.9      |
| 8        | 6.79 (1H, d, 15.5)          | 122.9      |
| 9        |                             | 166.8      |
| 10       | 3.86 (1H, s)                | 56.8       |
| 11       | 2.91 (1H, m)                | 58.1       |
| 12       | 1.46 (3H, d, 4.8)           | 16.6       |
| 1'       |                             | 176.0      |
| 2'       | 4.56 (1H, br s)             | 59.1       |
| 3'       | 4.35 (1H, br s)             | 67.9       |
| 4'       | 1.25 (3H, d, 5.8)           | 18.5       |

Figure S2. Structure and key HMBC and $^1$H-$^1$H COSY correlations of 1
Figure S3. HPLC figure of the parental strain and gene *adpA* overexpression strain

*Streptomyces* sp. HS-NF-1222A

Figure S4. HRESIMS spectrum of compound 1
Figure S5. $^1$H-NMR spectrum of compound 1

Figure S6. $^{13}$C-NMR spectrum of compound 1
Figure S7. DEPT135 spectrum of compound 1

Figure S8. HMQC spectrum of compound 1
Figure S9. HMBC spectrum of compound 1

Figure S10. $^1$H-$^1$HCOSY spectrum of compound 1
Figure S11. UV spectrum of compound 1

Figure S12. IR spectrum of compound 1