

## SUPPLEMENTARY MATERIAL

### An Endophytic Fungus from *Trichoderma harzianum* SWUKD3.1610 That Produces Nigranoic acid and Its Analogues

MeiJun Han<sup>1</sup>, Dan Qin<sup>1</sup>, TianTian Ye<sup>2</sup>, Xiao Yan<sup>1</sup>, JunQi Wang<sup>1</sup>, XiaoXiang Duan<sup>1</sup>, JinYan Dong<sup>1</sup> \*

<sup>1</sup> Key Laboratory of Eco-environments in Three Gorges Reservoir Region (Ministry of Education),  
School of Life Science, Southwest University, Chongqing 400715, PR China

<sup>2</sup> Department of pulmonary disease, Chongqing Hospital of traditional Chinese medicine, Beibei  
District, 380 general road, Beibei District, Chongqing 400715, PR China

**Abstract:** The objective of this study was to determine whether endophytic fungi, isolated from *Kadsura angustifolia* produce nigranoic acid and its highly oxygenated derivatives. From the 426 endophytic fungi screened, *Trichoderma harzianum* SWUKD3.1610 was detected to have a component with the same TLC  $R_f$  value and HPLC retention time as authentic nigranoic acid. This component was further confirmed as nigranoic acid by investigating the chemical composition of the fungal extracts. Besides (1), one new triterpenoid, 7 $\beta$ -schinalactone C (2), and two known minor compounds were isolated and characterized by HRESIMS, 1D and 2D NMR spectroscopic methods. Our study indicates that endophytic fungus may play an important role in increasing the quality of the crude drugs from Chinese medicinal plant *K. angustifolia*. This study is the first to isolate, characterize, and identify schitriterpenes-producing *Trichoderma* spp.

**Keywords** Endophytic fungus; *Kadsura angustifolia*; Nigranoic acid; *Trichoderma harzianum*

### List of Supplementary Material

Figure S1. Thin layer chromatography (TLC) analysis of fungal nigranoic acid.

Figure S2. HPLC retention times of the authentic nigranoic acid (1), fungal extract (3) as well as mixtures of authentic nigranoic acid and fungal extract (2)

Figure S3. The correlation of HMBC, COSY and ROESY of compound 2.

Figure S4. Morphological characteristics of strain grow on PDA.

Figure S5. The morphological characteristics of hyphae and conidiophores by light microscope.

Figure S6. Phylogenetic tree of the endophytic fungus strain based on 5.8S and ITS region sequence.

Figure S7. HR-ESI-MS spectrum of compound 2.

Figure S8.  $^1\text{H}$  NRE spectrum of compound 2.

Figure S9.  $^{13}\text{C}$  NMR spectrum of compound 2.

Table S1. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectruml data of compounds 2 in  $\text{MeOH}_3$ .



Figure S1. Thin layer chromatography (TLC) analysis of fungal nigranoic acid.

Sample was subjected to TLC on a silica (Silica 60 GF254) plate. The chromatogram was developed using solvents  $\text{CH}_2\text{Cl}_2$ -EtoAC-Acetic acid (7:3:0.1%, v/v/v) and sprayed with 95%:5% (v/v) ethyl alcohol-sulfuric acid reagent to detect nigranoic acid.

A – Standard nigranoic acid

B – Mixtures of standard nigranoic acid and crude extract of *T. harzianum*

C - Crude extract of *T. harzianum*

Figure S2. HPLC retention times of the authentic nigranoic acid (1), fungal extract (3) as well as mixtures of authentic nigranoic acid and fungal extract (2)

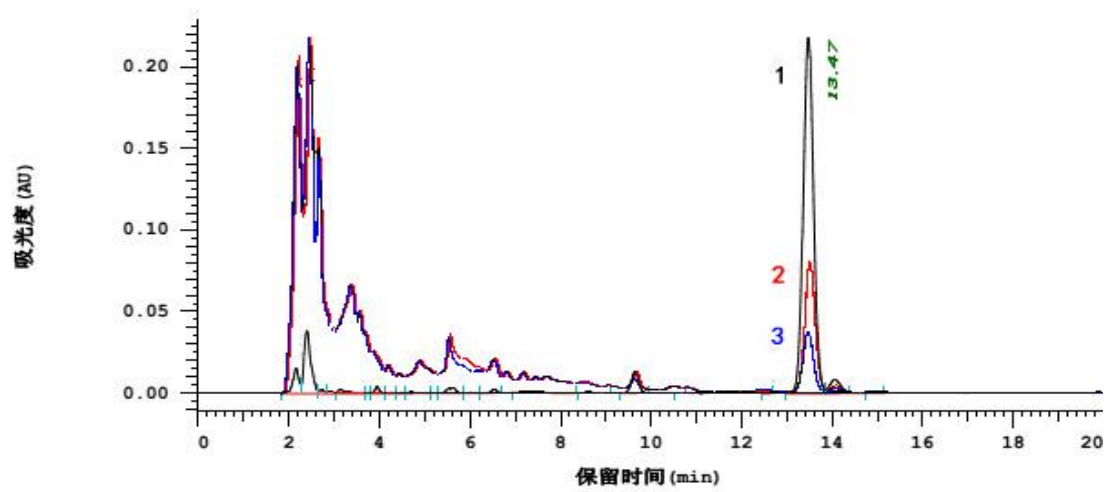


Figure S3. The correlation of HMBC, COSY and ROESY of compound 2.

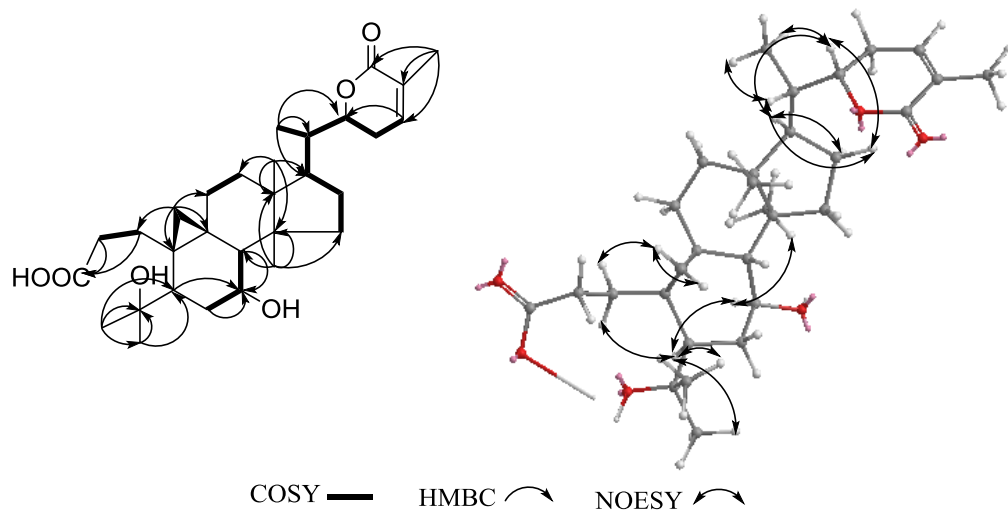


Figure S4. Morphological characteristics of strain grow on PDA.

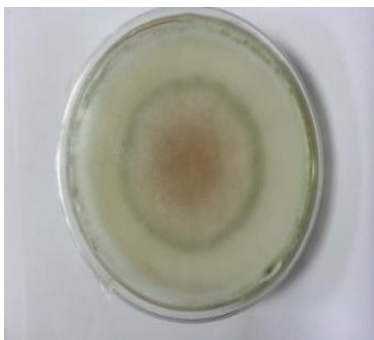


Figure S5. The morphological characteristics of hyphae and conidiophores by light microscope.



Figure S6. Phylogenetic tree of the endophytic fungus strain based on 5.8S and ITS region sequence.

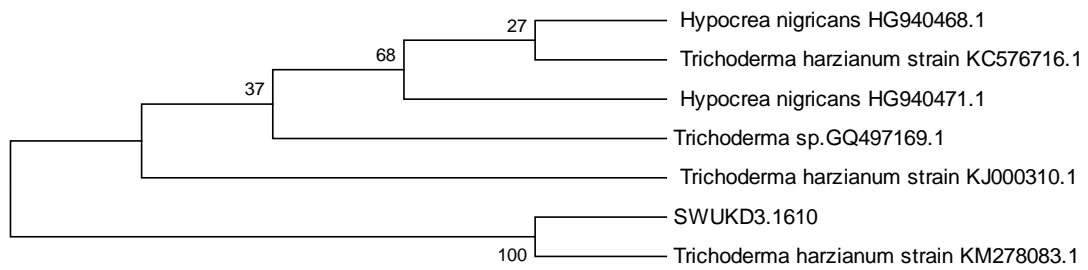


Figure S7. HR-ESI-MS spectrum of compound 2.

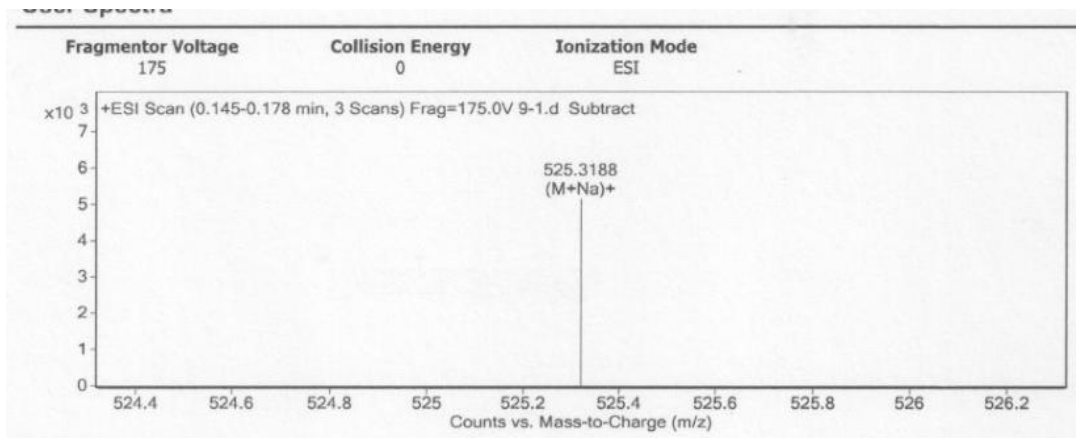


Figure S8. <sup>1</sup>H spectrum of compound 2.

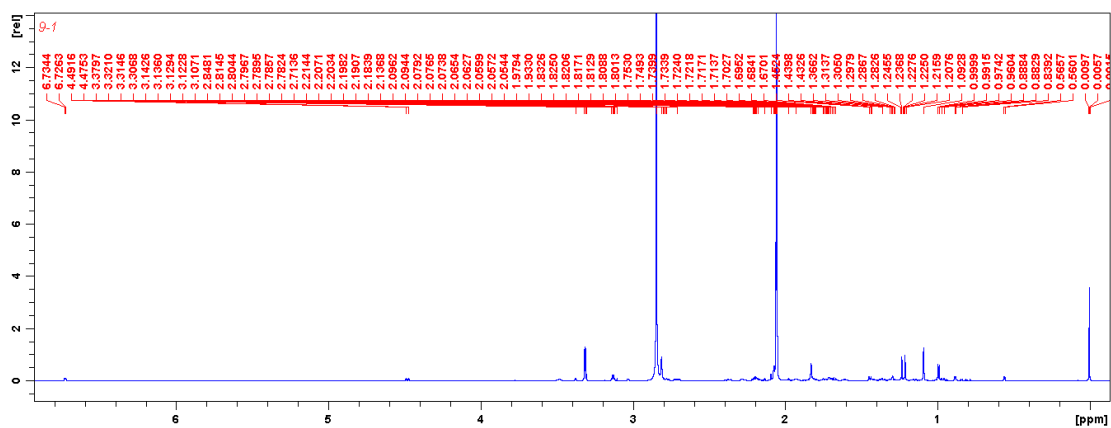


Figure S9.  $^{13}\text{C}$  NMR spectrum of compound **2**.

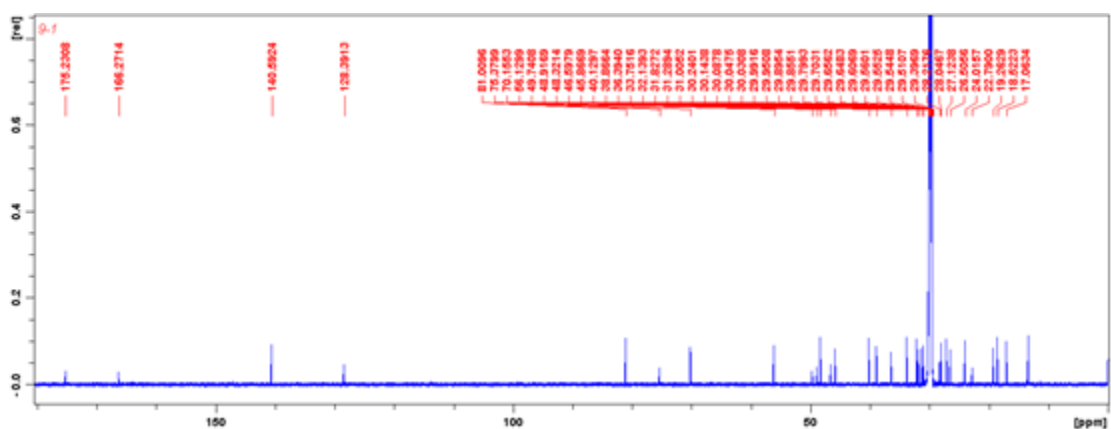


Table S1. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data of compounds **2** in  $\text{MeOH}_3$ .

No.	<b>2</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	2.78(m); 1.36(m)	31.2t
2	2.21(m); 2.70(m)	32.1t
3		175.2s
4		75.3s
5	2.21(dd, 4.3)	45.9d
6	1.81(m); 0.97(m)	39.3tt
7	3.48 (dt,4.0,7.6)	70.2d
8	1.45(m)	56.1d
9		22.8s
10		28.2s
11	1.29(m); 2.18(m)	27.1t
12	1.73(m); 1.68(m)	33.7t
13		46.5s
14		48.9s
15	1.71(m); 1.60(m)	38.8t
16	1.82(m); 1.42(m)	28.0t
17	1.68(d, 9.6)	48.3d
18	1.10(s)	19.2q

19	0.88(d,3.4,H <sub>a</sub> ); 0.56(d,3.4,H <sub>b</sub> )	31.0t
20	1.93(m)	40.1d
21	0.99(d,4.98)	13.3q
22	4.48(dt,2.6,5.4)	81.0d
23	2.36(m); 2.28(m)	24.0t
24	6.72(d,4.8)	140.6d
25		128.4s
26		166.3s
27	1.80(s)	17.0q
28	1.10(s)	18.5t
29	1.20(s)	26.5q
30	1.24(s)	31.8q

---