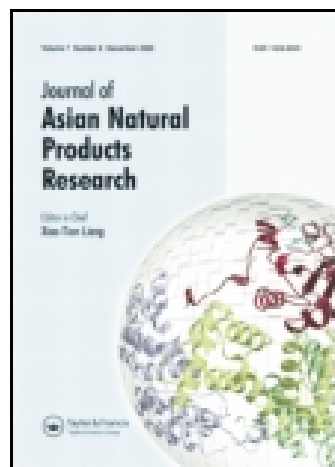


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## Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/ganp20>

### Two new compounds from an endophytic fungus *Alternaria solani*

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Published online: 29 Oct 2012.

To cite this article: Hong-Lian Ai, Li-Mei Zhang, Yan-Ping Chen, Shu-Hui Zi, Hong Xiang, Da-Ke Zhao & Yong Shen (2012) Two new compounds from an endophytic fungus *Alternaria solani*, Journal of Asian Natural Products Research, 14:12, 1144-1148, DOI: [10.1080/10286020.2012.733701](https://doi.org/10.1080/10286020.2012.733701)

To link to this article: <http://dx.doi.org/10.1080/10286020.2012.733701>

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## Two new compounds from an endophytic fungus *Alternaria solani*

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(Received 22 April 2012; final version received 21 September 2012)

Two new secondary metabolites, named 7-dehydroxyl-zinniol (**1**) and 20-hydroxyl-ergosta-4,6,8(14),22-tetraen-3-one (**2**), were isolated from the culture of *Alternaria solani*, an endophytic fungal strain residing in the roots of *Aconitum transsectum*. Their structures were elucidated on the basis of comprehensive spectroscopic analyses including IR, ESI-MS, HR-ESI-MS, 1D and 2D NMR. Biological activity tests indicated that compound **1** showed moderate anti-HBV activity.

**Keywords:** *Alternaria solani*; *Aconitum transsectum*; 7-dehydroxyl-zinniol; 20-hydroxyl-ergosta-4,6,8(14),22-tetraen-3-one; anti-HBV activity

### 1. Introduction

Fungal endophytes normally colonize in living internal tissues of plants without causing any obvious negative effects or external symptoms [1]. It was accepted as an important source of novel bioactive constituents because endophytes could produce various metabolites and play a great role in new drug development [2–5]. *Aconitum transsectum* Diels. (Ranunculaceae), a perennial herb distributing in northwestern of Yunnan province in China, had long been used as a folk medicine to treat rheumatism and pains [6]. Previous phytochemical investigations on this plant revealed that C<sub>19</sub>-diterpenoid alkaloids were the main constituents which displayed high toxicity [7–11]. To find more biologically active substances from fungal endophytes, we undertook the investigation on the fungal endophyte *Alternaria alternata* derived from the

fresh roots of *A. transsectum* to furnish two new metabolites named 7-dehydroxyl-zinniol (**1**) and 20-hydroxyl-ergosta-4,6,8(14),22-tetraen-3-one (**2**). Herein, we describe the isolation and structural elucidation of the two new derivatives (Figure 1) and their anti-hepatitis B virus (anti-HBV) activity.

### 2. Results and discussion

Compound **1** was isolated as a colorless powder. Its molecular formula was determined to be C<sub>15</sub>H<sub>22</sub>O<sub>3</sub> based on ESI-MS ([M + Na]<sup>+</sup>; *m/z* 273) and HR-ESI-MS (*m/z* 273.1437; [M + Na]<sup>+</sup>, calcd for 273.1461) analyses, indicating five degrees of unsaturation. The IR spectrum showed the absorption for hydroxyl (3378 cm<sup>-1</sup>) and aromatic ring (1607, 1584 and 1446 cm<sup>-1</sup>) functions. In the 1D NMR spectrum, an olefinic signal (δ<sub>H</sub> 5.49,

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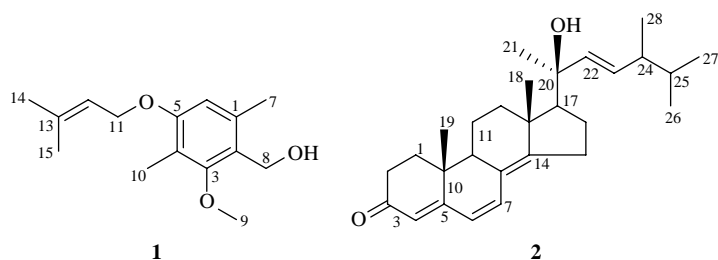


Figure 1. The structures of compounds **1** and **2**.

$^1\text{H}$ , m;  $\delta_{\text{C}}$  120.1, d; 137.3, s) was observed, together with one methoxyl group ( $\delta_{\text{H}}$  3.77, 3H, s;  $\delta_{\text{C}}$  61.5, q), four quaternary methyls ( $\delta_{\text{H}}$  1.74, 1.80, 2.14, 2.38, each 3H, s;  $\delta_{\text{C}}$  9.0, 18.2, 19.3, 25.8, q  $\times$  4), and an aromatic ring function ( $\delta_{\text{H}}$  6.50, 1H, s;  $\delta_{\text{C}}$  109.8, d; 117.3, s; 124.1, s; 135.7, s; 157.4, s; 158.1, s). Its  $^{13}\text{C}$  NMR (DEPT) spectrum revealed the presence of 15 carbon signals including 5 methyls, 2 methylenes, 2 methines, and 6 quaternary carbons. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of compound **1** were almost the same as those of zinniol [12] except for an additional methyl signal ( $\delta_{\text{H}}$  2.38, 3H, s;  $\delta_{\text{C}}$  19.3, q) in compound **1**. Considering that compound **1** contained one more quartered methyl than zinniol, it could be deduced that compound **1** might be a derivative of zinniol with a methyl substituted at C-1. This assumption was supported by the HMBC spectrum in which cross-peaks between H-7 ( $\delta_{\text{H}}$  2.38, 3H, s) and C-1 ( $\delta_{\text{C}}$  135.7, s), C-2 ( $\delta_{\text{C}}$  124.1, s), and

C-6 ( $\delta_{\text{C}}$  109.8, d) emerged (Figure 2). The full NMR data (Table 1) assignments of compound **1** were achieved according to the HSQC, HMBC,  $^1\text{H}$ - $^1\text{H}$  COSY, and ROESY spectral analyses. Consequently, compound **1** was elucidated as 7-dehydroxyl-zinniol.

Compound **2** was also obtained as a colorless powder and assigned the molecular formula  $\text{C}_{28}\text{H}_{40}\text{O}_2$  by analyses of ESI-MS ( $[\text{M} + \text{H}]^+ m/z$  409) and HR-ESI-MS ( $m/z$  409.3091;  $[\text{M} + \text{H}]^+$ , calcd 409.3101). IR spectrum displayed the absorption for hydroxyl ( $3433\text{ cm}^{-1}$ ) and a ketone group ( $1721\text{ cm}^{-1}$ ) function. The 1D NMR spectra of **2** displayed six methyls, four olefinic signals, and a ketone group. Comparing the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data (Table 1) of compound **2** with those of ergosta-4,6,8(14),22-tetraen-3-one [13] showed great similarity except that the chemical shift due to C-20 was down-shifted from  $\delta_{\text{C}}$  39.3 (d) to  $\delta_{\text{C}}$  74.6 (s) in compound **2**, suggesting that there is

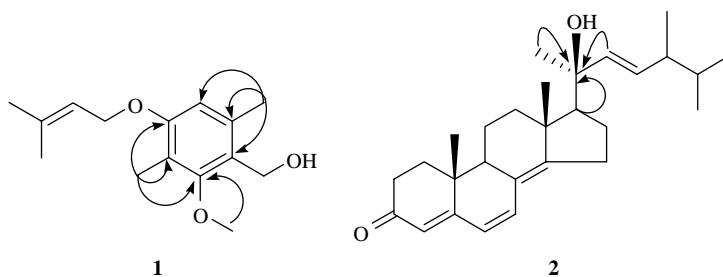


Figure 2. The key HMBC correlations of compounds **1** and **2**.

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of compounds **1** and **2** in CDCl<sub>3</sub>, δ in ppm, *J* in Hz.

No.	<b>1</b>		<b>2</b>	
	δ <sub>H</sub> <sup>a</sup>	δ <sub>C</sub> <sup>b</sup>	δ <sub>H</sub> <sup>a</sup>	δ <sub>C</sub> <sup>b</sup>
1		135.7 s	1.72 (1H, m), 2.56 (1H, m)	34.1 t
2		124.1 s	2.52 (1H, m), 2.54 (1H, m)	34.1 t
3		158.1 s		199.5 s
4		117.3 s	5.74 (1H, s)	123.0 d
5		157.4 s		124.6 s
6	6.50 (1H, s)	109.8 d	6.03 (1H, d, <i>J</i> = 9.5 Hz)	124.2 d
7	2.38 (3H, s)	19.3 q	6.61 (1H, d, <i>J</i> = 9.5 Hz)	133.8 d
8	4.69 (2H, s)	57.5 t		164.3 s
9	3.77 (3H, s)	61.5 q	1.62 (1H, m)	44.2 d
10	2.14 (3H, s)	9.0 q		36.7 s
11	4.51 (2H, d, <i>J</i> = 6.4 Hz)	65.2 t	1.75 (1H, m), 2.01 (1H, m)	18.7 t
12	5.49 (1H, m)	120.1 d	1.60 (1H, m), 1.95 (1H, m)	35.9 t
13		137.3 s		44.2 s
14	1.80 (3H, s)	25.8 q		155.4 s
15	1.74 (3H, s)	18.2 q	2.21 (1H, m), 2.47 (1H, m)	22.3 t
16			1.52 (1H, m), 1.88 (1H, m)	24.7 t
17			1.37 (1H, m)	58.9 d
18			0.98 (3H, s)	19.8 q
19			1.05 (3H, s)	16.6 q
20				74.6 s
21			1.28 (3H,s)	30.5 q
22			5.57 (1H, m)	136.6 d
23			5.57 (1H, m)	130.4 d
24			1.86 (1H, m)	42.5 d
25			1.48 (1H, m)	33.1 d
26			0.86 (3H,s)	20.4 q
27			0.88 (3H,s)	20.1 q
28			0.95 (3H,s)	17.2 q

<sup>a</sup>Recorded at 400 MHz; <sup>b</sup>Recorded at 100 MHz.

only one OH group locating at C-20. This was confirmed by the cross-peaks between H-17, H-21, and H-22, and C-20 (δ<sub>C</sub> 74.6, s) in the HMBC spectrum (Figure 2). Accordingly, compound **2** was established to be 20-hydroxyl-ergosta-4,6,8(14),22-tetraen-3-one.

Compounds **1** and **2** were tested for their anti-HBV activities *in vitro* on the HBV-transfected Hep G 2.2.15 cell line as reported previously [14,15]. Compound **1** showed moderate anti-HBV activity against the secretion of HBsAg with an IC<sub>50</sub> value of 0.38 mM (SI = 3.09); however, compound **2** showed no anti-HBV activity at the tested (highest) concentration of 2.57 mM.

3. Experimental

3.1 General experimental procedures

Optical rotations were carried out on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). IR spectra were recorded on a Bio-Rad FTS-135 spectrometer (Bio-Rad Corporation, Hercules, CA, USA) with KBr pellets, ν in cm<sup>-1</sup>. UV spectra were measured on UV-210A spectrometer (Shimadzu, Kyoto, Japan); NMR spectra were measured on Bruker AV-400 or DRX-500 spectrometers (Bruker, Karlsruhe, Germany) with tetramethylsilane as internal standard; chemical shifts (δ) were expressed in ppm and coupling constants (*J*) in Hz. ESI-MS and HR-ESI-MS were recorded on

a API Qstar-Pulsar-1 mass spectrometer (Applied Biosystems/MDS Sciex, Ontario, Vaughan, Canada). Column chromatography (CC) separations were carried out on silica gel (200–300 mesh, Qingdao Meigao Chemical Co., Ltd, Qingdao, China),  $\text{Al}_2\text{O}_3$  (Shanghai Wusi Chemical Reagents Company, Shanghai, China), and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Fractions were monitored by thin layer chromatography and visualized by spraying with 10%  $\text{H}_2\text{SO}_4$  in EtOH followed by heating.

### 3.2 Fungal material

The fresh roots of *A. transsectum* were collected in Lijiang, Yunnan, China, and were authenticated by Prof. Dr Li-Gong Lei from Kunming Institute of Botany, Chinese Academy of Sciences. They were surface sterilized, cut, and inoculated onto Petri dishes containing potato dextrose agar to culture endophytic fungi. Endophytic fungal strains were isolated using routine microbiological methods. Identification was carried out using a molecular protocol by sequencing of the internal transcribed spacer (ITS) region. A BLASTN search of the ITS of the endophytic fungus revealed that the fungus used for the isolation and identification of secondary metabolites was highly similar to *Alternaria solani* (max identity: 99%; accessions: AY154716.1). The strain was preserved at the School of Agriculture and Biological Technic, Yunnan Agricultural University.

### 3.3 Cultivation

The culture medium consisted of potato (peeled) (200 g), glucose (20 g),  $\text{KH}_2\text{PO}_4$  (3 g),  $\text{MgSO}_4$  (1.5 g), citric acid (0.1 g), and thiamine hydrochloride (10 mg) in 1 liter of deionized  $\text{H}_2\text{O}$ . The fungus was grown in reagent bottles (500 ml; media of 300 ml). The pH was adjusted to 6.5 before autoclaving, and fermentation was carried

out on a shaker at 25°C and 150 rpm for 24 days.

### 3.4 Extraction and isolation

The whole culture broth of *A. solani* (30 liters) was filtered, and the filtrate was extracted three times with  $\text{CHCl}_3$ . The organic layer was concentrated *in vacuo* to give a crude extract (20.4 g). The extract was chromatographed over silica gel (200 g, 200–300 mesh) CC and eluted with  $\text{CHCl}_3$ –MeOH (100:1, 50:1, 25:1, 10:1, 5:1, v/v) to provide fractions A–E. Fraction B (5.8 g) was chromatographed on silica gel CC (petroleum ether–acetone, 30:1;  $\text{CHCl}_3$ –MeOH, 95:5), followed by Sephadex LH-20 ( $\text{CHCl}_3$ –MeOH, 1:1) to yield compound **1** (8.5 mg). Fraction C (6.7 g) was subjected to silica gel CC with an eluent of petroleum ether–acetone (25:1) and  $\text{CHCl}_3$ –MeOH (95:5), and further purified through Sephadex LH-20 ( $\text{CHCl}_3$ –MeOH, 1:1) to afford compound **2** (7.1 mg).

#### 3.4.1 7-Dehydroxyl-zinniol (**1**)

A colorless powder.  $[\alpha]_{\text{D}}^{15.7} - 1.40$  (c 0.56, MeOH). UV (MeOH)  $\lambda_{\text{max}}^{\text{MeOH}}$  (log  $\epsilon$ ): 207 (5.01) nm. IR (KBr)  $\nu_{\text{max}}$ : 3378, 2920, 1607, 1584, 1446, 1404, 1325, 1128, 980, and 831  $\text{cm}^{-1}$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral ( $\text{CDCl}_3$ ) data are listed in Table 1. ESI-MS (positive):  $m/z$  273  $[\text{M} + \text{Na}]^+$ . HR-ESI-MS (positive.):  $m/z$  273.1437  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{15}\text{H}_{22}\text{O}_3\text{Na}$ , 273.1461).

#### 3.4.2 20-Hydroxyl-ergosta-4,6,8(14),22-tetraen-3-one (**2**)

A colorless powder.  $[\alpha]_{\text{D}}^{16.2} + 119.45$  (c 0.40, MeOH). UV (MeOH)  $\lambda_{\text{max}}^{\text{MeOH}}$  (log  $\epsilon$ ): 345 (3.75) nm. IR (KBr)  $\nu_{\text{max}}$ : 3433, 2962, 1721, 1639, and 1587  $\text{cm}^{-1}$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral ( $\text{CDCl}_3$ ) data are listed in

Table 1. ESI-MS (positive):  $m/z$  409  $[M + H]^+$ . HR-ESI-MS (positive.):  $m/z$  409.3091  $[M + H]^+$  (calcd for  $C_{28}H_{41}O_2$ , 409.3101).

### Acknowledgment

This study was supported by the State Key Laboratory of Phytochemistry and Plant Resources in West China, KIB, CAS (No. P2012-KF04).

### References

- [1] C.W. Bacon and J.F. White, *Microbial Endophytes* (Marcel Dekker Inc, New York, 2000), pp. 4–5.
- [2] G.A. Strobel, *Crit. Rev. Biotechnol.* **22**, 315 (2002).
- [3] G.A. Strobel, *Microbes Infect.* **5**, 535 (2003).
- [4] R.X. Tan and W.X. Zou, *Nat. Prod. Rep.* **18**, 448 (2001).
- [5] H.W. Zhang, Y.C. Song, and R.X. Tan, *Nat. Prod. Rep.* **23**, 753 (2006).
- [6] Jiangsu New Medical College (ed.), *Zhong-yao-da-ci-dian* (The Dictionary of Chinese Crude Drugs) (Shanghai Science and Technology Press, Shanghai, China, 1977), pp. 2089–2090.
- [7] S.Z. Zheng, L.M. Gao, X.J. Hao, X.X. Wang, and X.W. Shen, *Phytochemistry* **46**, 951 (1997).
- [8] D.L. Chen, X.X. Jian, Q.H. Chen, and F.P. Wang, *Acta Chim. Sin.* **61**, 901 (2003).
- [9] D.L. Chen, X.X. Jian, Q.H. Chen, and F.P. Wang, *West China J. Pharm. Sci.* **17**, 326 (2002).
- [10] Y. Shen, H.L. Ai, T.W. Cao, J.J. Wang, S.H. Zi, X.M. Zhang, and J.J. Chen, *Helv. Chim. Acta* **95**, 509 (2012).
- [11] Y. Shen, H.L. Ai, S.H. Zi, J.J. Wang, X.M. Zhang, and J.J. Chen, *J. Asian Nat. Prod. Res.* **14**, 244 (2012).
- [12] M.M. Gamboa-Angulo, F. Alejos-González, F. Escalante-Erosa, K. García-Sosa, G. Delgado-Lamas, and L.M. Peña-Rodríguez, *J. Nat. Prod.* **63**, 1117 (2000).
- [13] H. Fujimoto, E. Nakamura, E. Okuyama, and M. Ishibashi, *Chem. Pharm. Bull.* **52**, 1005 (2004).
- [14] Z.Y. Jiang, X.M. Zhang, F.X. Zhang, N. Liu, F. Zhao, J. Zhou, and J.J. Chen, *Planta Med.* **72**, 951 (2006).
- [15] J. Lupberger, A. Mund, J. Kock, and E.J. Hildt, *Hepatology* **45**, 547 (2006).