Phytochemistry Letters 14 (2015) 260–264

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Two new diketopiperazines and a new glucosyl sesterterpene from *Alternaria alternata*, an endophytic fungi from *Ceratostigma griffithii*

Da-Le Guo[a,c], Min Zhao[a,c], Shi-Ji Xiao[b], Bing Xia[a], Bo Wan[a], Yu-Cheng Gu[d], Li-Sheng Ding[a], Yan Zhou[a,*]

[a] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China
[b] Pharmacy School, Zunyi Medical College, Zunyi 563000, PR China
[c] University of Chinese Academy of Sciences, Yuquan Road 19A, Beijing 100049, PR China
[d] Syngenta Jealott's Hill International Research Centre, Berkshire RG42 6EY, UK

ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form 24 October 2015
Accepted 27 October 2015
Available online 10 November 2015

Keywords:
Ceratostigma griffithii
Alternaria alternata
Diketopiperazine
24-α-D-glucosyl-(−)-terpestacin

ABSTRACT

Two diketopiperazine derivatives, altenarizines A (**1**) and B (**2**), and a new glucosyl sesterterpene, 24-α-D-glucosyl-(−)-terpestacin (**3**), together with two known phytotoxic sesterterpenes, (−)-terpestacin (**4**) and fusaproliferin (**5**), were isolated from the fermentation broth of an endophytic fungus *Alternaria alternata*, which was obtained from the fresh root of *Ceratostigma griffithii*. Structures of all the isolates were identified by spectroscopic data.

1. Introduction

Alternaria species belong to Deuteromycetes, have been identified as a prolific fungal source of secondary metabolites such as steroids, terpenoids, pyrones, quinones, phenolics and nitrogen-containing compounds, which exhibit a variety of biological activities (Lou et al., 2013). For instance, alternariols and altenuenes, which were typical components from the genus *Alternaria*, were examined to have cytotoxicities (Aly et al., 2008) and antimicrobial (Jiao et al., 2006) properties. In the course of our ongoing research on the unique compounds from plant endophytes, *Alternatia alternata* as an endophytic fungi has been isolated and identified from *Ceratostigma griffithii*. The chemical investigation of its fermentation broth led to the isolation of two new diketopiperazine derivatives namely altenarizine A (**1**), altenarizine B (**2**) and a new glucosyl sesterterpene 24-α-D-glucosyl-(−)-terpestacin (**3**), along with two known phytotoxic sesterterpene, (−)-terpestacin (**4**), fusaproliferin (**5**). Details of the isolation, structure elucidation, and antibacterial activity of these compounds are discussed below.

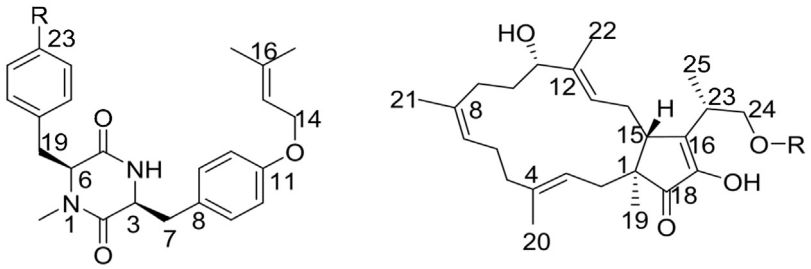
It is well known that *Alternaria* sp. has the ability to produce various of terpenoids, including diterpenoids (Visconti et al., 1992), sesquiterpenoids (Gamboa-Angulo et al., 2002), triterpenoids (Gamboa-Angulo et al., 1997) and mixed terpenoids which have a multiple biogenesis (Ichihara et al., 1983; Kjer et al., 2009; Pero et al., 1973; Thomas, 1961). However, to the best of our knowledge, there are rare reports on sesterterpeniods from *Alternaria* sp. This phenomenon makes our group take the strain's habitat into account. Qinghai-Tibet Plateau owns a special environment with high altitude, strong radiation, and large temperature difference between day and night. Thus, plants and endophytes which occupy this area should have special survival strategies including production of unique secondary metabolites (Fig. 1).

2. Results and discussion

Alternarizine A (**1**) was isolated as a yellow gum. Its molecular formula was assigned as $C_{24}H_{28}N_2O_3$ on the basis of the HRESIMS ($[M+H]^+$ at *m/z* 393.2171, calcd. for 393.2173). The IR spectrum showed the presence of two amide carbonyl signals at 1675 and 1670 cm^{-1}; characteristic IR absorption bands indicated methylene (2934 cm^{-1}) and benzene (1634 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Table 1) in combination with ^1H-^1H COSY and HSQC recorded for **1** indicated the presence of a *N*-methylated phenylalanine (Phe) residue [δ_H 7.40 (2H, t, J = 7.4 Hz), 7.31 (1H, t, J = 7.4 Hz),

* Corresponding author. Fax: +86 028 828 908 10.
E-mail address: zhouyan@cib.ac.cn (Y. Zhou).

http://dx.doi.org/10.1016/j.phytol.2015.10.024
1874-3900/



	R
1	H
2	OMe

	R
3	α-D-Glc
4	H
5	OAc

Fig. 1. New compounds **1**–**3**, and known compounds **4**–**5** isolated from *Alternaria alternata*.

Table 1
NMR data of aternarizine A (**1**) and B (**2**).

Position	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	–	–	–	–
2	–	165.8	–	166.0
3	3.78 dd (11.7, 2.9)	57.1	3.83 d (11.6)	56.9
4	5.55 s	–	5.71 s	–
5	–	165.6	–	165.5
6	4.22 dd (3.5, 4.5)	63.2	4.17 t (3.7)	63.5
7	2.82 dd (13.6, 2.9) 0.66 dd (13.5, 11.7)	39.8	2.90dd (13.6, 2.9) 0.85 dd (13.5, 11.6)	39.7
8	–	128.0	–	127.8
9, 13	6.78 m	130.3	6.83 m	130.1
10, 12	6.78 m	115.3	6.83m	115.1
11	–	158.1	–	158.1
14	4.44 d (6.7)	64.8	4.46 d (6.7)	64.8
15	5.46 m	119.6	5.46 t (6.6)	119.5
16	–	138.2	–	138.4
17	1.79 s	26.1	1.79 s	26.0
18	1.73 s	18.1	1.73 s	18.4
19	3.31 dd (14.0, 3.5) 3.17 dd (14.0, 4.5)	36.7	3.22 dd (14.0, 3.5) 3.11 dd (14.2, 4.5)	35.8
20	–	134.8	–	126.7
21, 25	7.19 d (7.4)	130.6	6.92 d (8.5)	130.7
22, 24	7.40 t (7.4)	129.2	7.09 t (8.5)	114.5
23	7.31 t (7.4)	127.9	–	159.2
N-Me	3.12 s	33.2	3.10 s	33.5
23-OMe			3.74 s	55.4

400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$.

7.19 (2H, d, *J* = 7.4 Hz), 4.22 (1H, dd, *J* = 3.5,4.5 Hz), 3.31 (1H, dd, *J* = 14.0, 3.5 Hz), 3.17 (1H, dd, *J* = 14.0, 4.5 Hz), 3.12 (3H, *s*); δ_C 165.8, 134.8, 130.6, 129.2, 127.9, 63.2, 36.7, 33.2]; a tyrosine (Tyr) unit [δ_H 6.78 (4H, m), 5.55 (1H, br. s), 3.78 (1H, dd, *J* = 11.7, 2.9 Hz), 2.82 (1H, dd, *J* = 13.6, 2.9 Hz), 0.66 (1H, dd, *J* = 13.5, 11.7 Hz); δ_C 158.1, 130.3, 128.0, 115.3, 57.1, 39.8] and a isopentenyl group [δ_H 5.46 (1H, m), 4.44 (2H, d, *J* = 6.7 Hz), 1.79(3H, s), 1.73(3H, s); δ_C 138.2, 119.6, 64.8, 26.1, 18.1]. The HMBC cross peaks were observed between the *N*- methyl proton signal (δ_H 3.12) and the carbon signals of C-6 (δ_C 63.2) and C-2 (δ_C 165.8), which indicated the methyl group was at N-1. The correlations from the H-6 (δ_H 4.22) to C-5 (δ_C 165.6) and *N*-metheyl (δ_C 33.2) and the correlation from the H-3 (δ_H 3.78) to C-2 (δ_C 165.8) confirmed the dikepiprezine core skeleton. The proton H-14 (δ_H 4.44) showed correlation peaks with the carbon singals of C-11 (δ_C 158.1) and C-10, 12 (δ_C 115.3), suggesting the connection of the isopentenyl group to C-11 via a oxygen atom. Thus, the primary structure of **1** was established as shown in Fig. 2. The relative stereochemistry of **1** was established by NOESY correlations of

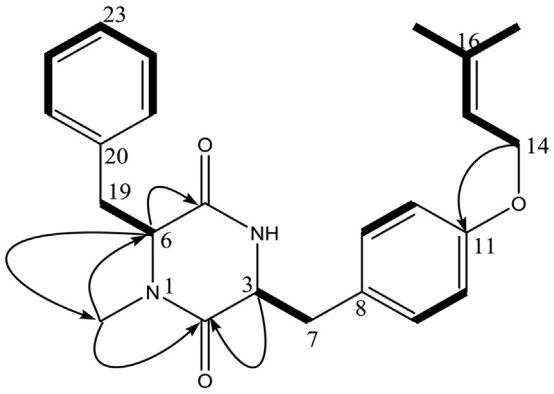


Fig. 2. The ^{1}H-^{1}H COSY (bold lines) and key HMBC (H → C) correlations of **1**.

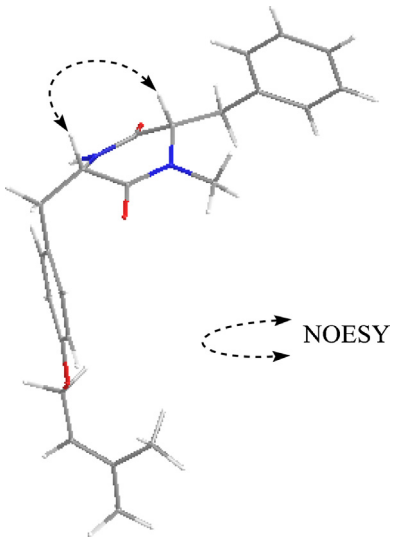


Fig. 3. The key NOESY correlations of **1**.

relevant protons as shown in Fig. 3. The presence of the NOE interaction between H-3 (δ_H 3.78) and H-6 (δ_H 4.22) indicated a boat form of the piperazine ring and the *cis* relationship between H-3 and H-6. The value of its negative optical rotation was close to those of (3S,6S)-3-benzyl-6-(4-hydroxybenzyl)-2,5-piperazinedione (Zeng et al., 2005), which suggested the 3S and 6S configuration of **1**. A Marfey's analysis had been done and the absolute configuration of

N-methyl-phenylalanine residue was confirmed as L (Marfey, 1984). Therefore, the absolute configuration of **1** was established as (3S,6S)-3-benzyl-6-(4-isoprenyloxybenzyl)-2,5-piperazinedione, and it was named alternarizine A (**1**).

Alternarizine B (**2**) was obtained as a yellow gum, and its molecular formula was determined as $C_{25}H_{30}N_2O_4$ by the HRESIMS ($[M+H]^+$ at *m/z* 423.2283, calcd. 423.2278) with 12 degrees of unsaturation. IR absorptions implied the presence of aromatic ring (1300, 827 cm^{-1}) and amide carbonyl (1676, 1663 cm^{-1}). The NMR data was similar to alternarizine A (**1**), and the differences were the methoxyl instead of a proton at C-23 which created a group of typical para-substituted benzene signals [δ_H 6.92 (2H, d, J = 8.5 Hz), 7.09 (2H, t, J = 8.5 Hz); δ_C 114.5, 126.7, 130.7, 159.2] and a oxymethyl signal [δ_H 3.74 (3H,s); δ_C 55.4]. The final structure was established by 2D NMR experiments including HSQC and HMBC. The absolute configuration of **2** was established as 3S, 6S by the similarity of optical rotation of **1** and **2**'s.

24-α-D-Glucosyl-(−)-terpestacin (**3**) was isolated as a yellow gum. The molecular formula $C_{31}H_{48}O_9$ was confirmed by HRESIMS ($[M+Na]^+$ at *m/z* 587.3196, calcd. for 587.3191) and indicated 8 units of unsaturation. The IR spectrum showed absorption band at 3429 cm^{-1}, which suggested the presence of hydroxyl group in the molecule. Its ^{1}H NMR spectrum (Table 2) exhibited signals due to three olefinic protons [δ 5.43 (1H, m), 5.34 (1H, m), 5.18 (1H, m)], a set of typical α-glucosyl signals [δ 4.78 (1H, d, 3.7), 3.58 (1H, m), 3.63 (1H, m), 3.37 (1H, m), 3.29 (1H, m), 3.67 (1H, m), 3.80 (1H, dd, 11.8, 2.3)], three vinylic methyl singlets [δ 1.67 (6H, s), 1.55 (3H, s)], and two methyl singlets [δ 1.32 (3H, d, 7.0), 0.95 (3H, s)]. The ^{13}C NMR spectrum (Table 2), displayed 31 carbon signals, and HMQC data indicated the presence of five CH_3 (including three vinylic methyl carbons at δ 10.6, 15.4, 15.5), eight CH_2 (including a α-glucosyl carbons at δ 62.7), eleven CH (including three olefinic carbons at δ 123.5, 126.1, 130.1 and five α-glucosyl carbons at δ 71.9, 73.7, 74.2, 75.3, 100.5), and seven quaternary carbons (including five olefinic carbons at δ 133.9, 137.4, 138.7, 149.3, 151.8 and a keto carbon at δ 210.5). These data suggested that compound **3** is a bicyclic sesterterpenoid with a 2-hydroxyacryl ketone (three carbons at δ 151.8, 149.3, 210.5). ^{1}H-^{1}H COSY spectra suggested the presence of the spin systems —CH_2—CH=C(CH_3)-(**A**) [δ_H 5.34 (1H, m), 1.77 (1H, m), 2.34 (1H, m), 1.67 (3H, s); δ_C 138.7, 123.5, 40.4, 15.5], —CH_2—CH_2—CH=C(CH_3)-(**B**) [δ_H 5.18 (1H, m), 2.31 (1H, m), 2.29 (1H, m), 2.10 (1H, m), 2.04 (1H, m), 1.67 (3H, s); δ_C 133.9, 126.1, 41.6, 25.0, 15.4], —CH_2—CH_2—CH(OH)-(**C**) [δ_H 3.99 (1H, m), 2.06 (1H, m), 1.77 (2H, m), 1.64 (1H, m); δ_C 77.1, 36.1, 30.9], C(CH_3)=CH—CH_2—CH-(**D**) [δ_H 5.43 (1H, m), 2.81(1H, dd, 14.4, 7.0), 2.40 (1H, m), 1.90 (1H, m), 1.55 (3H, s); δ_C 137.4, 130.1, 50.4, 30.3, 10.6], —CH—CH_2-(**E**) [δ_H 3.89 (1H, dd 9.5, 3.7), 3.73 (1H, dd 9.5, 6.8), 2.84 (1H, m); δ_C 71.4, 36.3] and a set of typical α-glucosyl signals (**F**). These were linked together with the help of HMBC data (Fig. 4). The correlations between H-5 (δ_H 2.29, 2.04) and C-4 (δ_C 138.7), between H-9 (δ_H 2.06, 1.77) and C-8 (δ_C 133.9), between H-11 (δ_H 3.99) and C-12 (δ_C 137.4), and between H-15 (δ_H 2.81) and C-1 (δ_C 50.2) linked and cyclized spin systems **A**,**B**, **C**, and **D**, in sequence. Both HMBC cross peaks between H-15 (δ_H 2.81) and C-16 (δ_C 151.8) and between H-19 (δ_H 0.95) and C-18 (δ_C 210.5) connected spin system **D**, **A,** and 2-hydroxyacryl ketone and created another 5-membered carbocyclic ring system. Those correlations between H-23 and C-16 connected spin system **E** and 2-hydroxyacryl ketone and finally generated a terpestacin-type sesterterpenoid. The HMBC correlation between the H-24 (δ_H 3.89, 3.73) and the terminal carbon (δ_C 100.5) confirmed the location of the glycosyl. The absence of the NOE interaction between H-15 (δ_H 2.81) and H-19 (δ_H 0.95) indicated that they were not in the same orientation. The sugar unit and aglycone component was determined to be D-glucopyranosyl and (−)-terpestacin by TLC analysis of acid hydrolyzed **3** (Wu et al., 2015). Thus, the absolute configuration of **3** was deduced as 24-α-D-glucosyl-(−)-terpestacin and was reinforced on the positive sign of the specific rotation $[\alpha]_D^{20}$ = +38 for 3 and $[\alpha]_D^{20}$ = −21.1 for (−)-terpestacin (Jin and Qiu, 2012).

Compounds **1–5** were tested against *Candida albicans*, *Fusarium graminearum*, *Fusarium vasinfectum*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. However, the compounds were inactive even at the high concentrations 100 μg/mL.

Table 2
24-α-D-Glucosyl-(−)-terpestacin (**3**).

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1	–	50.2	17	–	149.3
2	1.77 m, 2.34 m	40.4	18	–	210.5
3	5.34 m	123.5	19	0.95 s	17.2
4	–	138.7	20	1.67 s	15.5
5	2.04, 2.29 m	41.6	21	1.67 s	15.4
6	2.10, 2.31 m	25.0	22	1.55 s	10.6
7	5.18 m	126.1	23	2.84 dd (11.3, 2.0)	36.3
8	–	133.9	24	3.89 dd (9.5, 3.7) 3.73 dd (9.5, 6.8)	71.4
9,	1.77, 2.06 m	36.1	25	1.32 d (7.0)	15.4
10	1.64, 1.77 m	30.9	1′	4.78 d (3.7)	100.5
11	3.99 dd (9.8, 3.5)	77.1	2′	3.58 m	74.2
12	–	137.4	3′	3.63 m	75.3
13	5.43 m	130.1	4′	3.37 m	73.7
14	1.90, 2.40 m	30.3	5′	3.29 m	71.9
15	2.81 dd (14.4, 7.0)	50.4	6′	3.80 dd (11.8, 2.3) 3.67 m	62.7
16	–	151.8			

400 MHz for ^{1}H and 100 MHz for ^{13}C, in CD_3OD

3. Experimental

3.1. General

Optical rotations were determined on a JASCO P-1020 polarimeter at room temperature. UV spectra were recorded on a PerkinElmer Lambda 35 UV–vis spectrophotometer. IR spectra were measured by PerkinElmer one FT-IR spectrometer (KBr). 1D and 2D NMR were carried out on a Bruker-Ascend-400 MHz instrument at 300 K, with TMS as internal standard. HRESIMS was recorded on a Bruker MicrO TOF-Q II mass spectrometer. Preparative HPLC was performed on a Waters 2545 equipped with a Kromasil RP-C18 column (10 × 250 mm, 5 μm) using a Waters 2489 UV detector. Column chromatography (CC) was performed with silica gel and Sephadex LH-20. All the solvent used were of analytical grade.

3.2. Fungal material

The title strain was isolated from the root of *C. griffithii*, collected from the suburb of Lhasa. Tibet Autonomous Region, People's Republic of China. The culture was grown on potato dextrose agar (PDA) and distinguished morphologically as *Alternaria* sp., which was further reinforced by 18S rDNA sequence with a 99% identity to *A. alternata*. The strain (GenBank acccession No. KR632488) has been preserved at Chengdu Institute of Biology, Chinese Academy of Sciences, China.

3.3. Fungal culture and extraction

This fungus was cultivated on 4.8 L scale using 500 mL Erlenmeyer flasks containing 200 mL of the seed PDA medium for three days then on 60 L scale using 1 L Erlenmeyer flasks containing 10 mL seed PDA medium and 400 mL fermentation medium (soluble starch 0.8%, peptone 0.5%, NaCl 0.2%, $CaCO_3$ 0.2%,

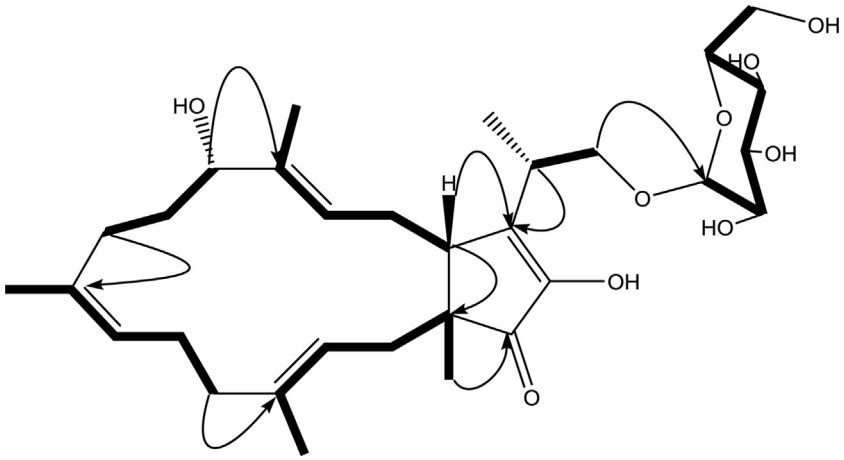


Fig. 4. The ^{1}H-^{1}H COSY (bold lines) and key HMBC (H → C) correlations of **3**.

$MgSO_4 \cdot 7H_2O$ 0.05%, K_2HPO_4 0.05%) for 14 days at 28 °C on a rotary shaker (250 rpm). The fermentation broth (60 L) of *A. alternata* was filtered. The filtrate was firstly extracted with petroleum and followed by EtOAc. The EtOAc solution was dried under vacuum and yielded 16 g extract.

3.4. Fractionation and isolation

The EtOAc residue (16 g) was separated into 4 fractions by CC on silica gel (300–400 mesh), eluting stepwise with $CHCl_3$/MeOH gradient ($CHCl_3$, $CHCl_3$/MeOH: 10:1(v/v), $CHCl_3$/MeOH: 3:1(v/v), MeOH). LC-MS analysis was performed on these fractions. The fourth fraction (eluted with MeOH) was slected. Sephadex LH-20 separation of this fraction ($CHCl_3$/MeOH: 1:1, v/v) afforded 3 subfractions (Fr.1–Fr.3). Fr.2 was further purified on a Waters preparative HPLC equipped with a Kromasil RP-C18 column (10 × 250 mm, ID × L; MeOH/H_2O: 70:30, v/v) to afford **1** (8.3 mg), **2** (2.5 mg), **3** (4.6 mg), **4** (10.3 mg), and **5** (4.7 mg).

3.4.1. Altenarizine A (*1*)

Yellow gum, $[\alpha]_D^{20} = -96$ ($c = 0.01$, MeOH); IR (KBr) 2934, 1675, 1670, 1456, 1250, 1033, 827 cm^{-1}; UV (MeOH) λ_{max} 227.3 (4.12), 276.3 (2.93); HRESIMS m/z $[M+H]^+$ 393.2171 (calcd. 393.2173); ^{1}H NMR and ^{13}C NMR are seen in Table 1.

3.4.2. Altenarizine B (*2*)

Yellow gum, $[\alpha]_D^{20} = -124$ ($c = 0.01$, MeOH); IR (KBr) 2935, 1675, 1662, 1456, 1249, 1032, 825 cm^{-1}; UV (MeOH) λ_{max} 226.9 (4.17), 275.7 (2.96); HRESIMS m/z $[M+H]^+$ 423.2283 (calcd. 423.2278); ^{1}H NMR and ^{13}C NMR are seen in Table 1.

3.4.3. 24-α-D-Glucosyl-(−)-terpestacin (*3*)

Yellow gum, $[\alpha]_D^{20} = +38$ ($c = 0.01$, MeOH); IR (KBr) 3429, 2922, 1632, 1384, 1026, 557 cm^{-1}; UV (MeOH) λ_{max} 262 (3.82); HRESIMS m/z $[M+Na]^+$ 587.3196 (calcd. 587.3191); ^{1}H NMR and ^{13}C NMR are seen in Table 2.

3.5. Absolute configuration of 1

An amino acid (*N*-methyl-L-phenylalmine or *N*-methyl-D-phenylalmine standard, 1 mg, Sigma, St. Louis, MO, USA) was dissolved in H_2O (30 μL) and treated with 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone (60 μL) and 6% Et_3N in 30 μL of H_2O at 40 °C for 1 h. After cooling to room temperature, the derivative was analyzed by UPLC with at UV absorption of 340 nm. A HSS T3 column (50 mm × 2.1 mm i.d., 1.8 μm particles, Waters) was eluted with a linear gradient of 50–98% (v/v) CH_3OH–H_2O containing 0.1% TFA for 8 min. The standards gave the following retention times: 4.71 min for *N*-methyl-L-phenylalmine, and 5.95 min for *N*-methyl-D-phenylalmine. Compound **1** (1.1 mg) was hydrolyzed in 6N HCl (1 mL) and heated at 120 °C in a sealed vial for 12 h to yield the corresponding amino acids. The cooled reaction mixture was evaporated to dryness under reduced pressure, and HCl was removed from the residual acid hydrolysate by repeated evaporation from frozen H_2O (1 mL). The amino acid mixture was then treated in the same manner as the standards above (1% FDAA and 6% Et_3N). The mixture of FDAA derivatives was filtered, and the filtrate was diluted with H_2O and analyzed by HPLC. The FDAA derivative of the amino acid liberated from **1** showed the peak at 4.71 min, matching the retention time of *N*-methyl-L-phenylalmine.

3.6. Acid hydrolysis

A solution of compound **3** (1.3 mg) in 2N aqueous HCl (5.0 mL) was refluxed at 80 °C for 2 h. The mixture was then diluted in water (10 mL) and extracted with EtOAc (3 × 3 mL). The combined water and EtOAc layers and evaporated to dryness to afford the glycoside and aglycone. The residue was analyzed by silica gel TLC by comparison with standard sugars and (−)-terpestacin. The solvent systems were *n*-BuOH–MeOH (4:1) for glycoside and $CHCl_3$–CH_3OH (10:1) for aglycone, and spots were visualized by spraying with H_2SO_4/EtOH (1:9), and then heated at 110 °C for 1 min. For the sugar of **3**, the Rf of glucose was 0.55, and the Rf of aglycone was 0.62 by TLC, respectively.

3.7. Antimicrobial assay

The antibacterial activity was evaluated according to the reported procedure with little modification (Shaaban et al., 2012). Activity was determined using the Oxford cup method with medium (dextrose 20.0 g/L, beef infusion 10 g/L, NaCl 5 g/L, agar 17 g/L) respectively, inoculated with strains of *Escherichia coli* 1.044 and *Bacillus subtilis* 1.079. To each cup was added 200 μL of sample (compounds **1**–**5**) dissolved in DMSO. Methicillin (5 μg/mL) and DMSO were used as positive and negative controls, respectively. The plates were incubated at 37 °C for 24 h. The antimicrobial activity was evaluated by measuring the diameter zone of growth inhibition against the test microorganism. MIC was detected complying with the method described by Kubo et al. (2004). All the test samples were dissolved in DMSO and the final concentration of DMSO was not over 5% (v/v). The final range of

test sample dilutions was between 50 and 0.19 μg/mL in the MHB broth. The final bacteria concentration in each dilution was 1×10^8 CFU/mL. The tubes were incubated at 37 °C for 24 h and then examined for evidence of the growth. MIC was determined as the lowest concentration of the antibacterial agents to inhibit bacterial growth. The antifungal activity was assayed by the Oxford cup method with medium [potato extract 20% (potato:water, w/v; boiling 20 min), dextrose 20.0 g/L, agar 17 g/L] inoculated with strains of *C. albicans*, *F. graminearum*, *F. vasinfectum*, *S. cerevisiae*, and *A. niger*. The plates were incubated at 31 °C for 72 h.

Acknowledgments

This work was financially supported a grant from the National Natural Sciences Foundation of China (21572219, 21302180), the State Key Laboratory of Phytochemistry and Plant Resources in West China (No. P2015-KF09) and a Syngenta Postgraduate Fellowship awarded to Da-Le Guo.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2015.10.024.

References

Aly, A.H., Edrada-Ebel, R., Indriani, I.D., Wray, V., Müller, W.E.G., Totzke, F., Zirrgiebel, U., Schächtele, C., Kubbutat, M.H.G., Lin, W.H., Proksch, P., Ebel, R., 2008. Cytotoxic metabolites from the fungal endophyte *Alternaria* sp. and their subsequent detection in its host plant *Polygonum senegalense*. J. Nat. Prod. 71, 972–980.

Gamboa-Angulo, M.M., Alejos-Gonzalez, F., Pena-Rodriguez, L.M., 1997. Homozinniol, a new phytotoxic metabolite from *Alternaria* solani. J. Agric. Food Chem. 45, 282–285.

Gamboa-Angulo, M.M., Escalante-Erosa, F., Garcia-Sosa, K., Alejos-Gonzalez, F., Delgado-Lamas, G., Pena-Rodriguez, L.M., 2002. Natural zinniol derivatives from *Alternaria* tagetica. Isolation, synthesis, and structure-activity correlation. J. Agric. Food Chem. 50, 1053–1058.

Ichihara, A., Tazaki, H., Sakamura, S., 1983. Solanapyrones A, B and C, phytotoxic metabolites from the fungus *Alternaria-solani*. Tetrahedron Lett. 24, 5373–5376.

Jiao, P., Gloer, J.B., Campbell, J., Shearer, C.A., 2006. Altenuene derivatives from an unidentified freshwater fungus in the family Tubeufiaceae. J. Nat. Prod. 69, 612–615.

Jin, Y., Qiu, F.G., 2012. A convergent stereocontrolled total synthesis of (−)-terpestacin. Org. Biomol. Chem. 10, 5452–5455.

Kjer, J., Wray, V., Edrada-Ebel, R., Ebel, R., Pretsch, A., Lin, W.H., Proksch, P., 2009. Xanalteric acids I and II and related phenolic compounds from an endophytic *Alternaria* sp isolated from the mangrove plant *Sonneratia alba*. J. Nat. Prod. 72, 2053–2057.

Kubo, I., Fujita, K.-, Kubo, A., Nihei, K.-, Ogura, T., 2004. Antibacterial activity of coriander volatile compounds against *Salmonella choleraesuis*. J. Agric. Food Chem. 52, 3329–3332.

Lou, J., Fu, L., Peng, Y., Zhou, L., 2013. Metabolites from *Alternaria* fungi and their bioactivities. Molecules 18, 5891–5935.

Marfey, P., 1984. Determination of -amino acids. II. Use of a bifunctional reagent,1,5-difluoro-2,4-dinitrobenzene. Carlsberg Res. Commun. 49, 591–596.

Pero, R.W., Harvan, D., Blois, M.C., 1973. Isolation of toxin, altenuisol, from fungus, *Alternaria-Tenuis* Auct. Tetrahedron Lett. 12, 948–1973.

Shaaban, K.A., Shepherd, M.D., Ahmed, T.A., Nybo, S.E., Leggas, M., Rohr, J., 2012. Pyramidamycins A–D and 3-hydroxyquinoline-2-carboxamide; cytotoxic benzamides from Streptomyces sp. DGC1. J. Antibiot. 65, 615–622.

Thomas, R., 1961. Studies in biosynthesis of fungal metabolites: 4. alternariol monomethyl ether and its relation to other phenolic metabolites of *Alternaria Tenuis*. Biochem. J. 80, 234–240.

Visconti, A., Sibilia, A., Sabia, C., 1992. Alternaria alternata from oilseed rape: mycotoxin production, and toxicity to *Artemia* salina larvae and rape seedlings. Mycotox. Res. 8, 9–16.

Wu, P., Gao, H., Li, Z.H., Liu, Z.Q., 2015. Two new triterpene saponins from the roots of Ilex pubescens. Phytochem. Lett. 12, 17–21.

Zeng, Y.B., Li, Q.S., Hanzlik, R.P., Aube, J., 2005. Synthesis of a small library of diketopiperazines as potential inhibitors of calpain. Bioorg. Med. Chem. Lett. 15, 3034–3038.