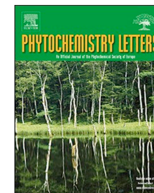
Phytochemistry Letters 31 (2019) 1–4

Contents lists available at ScienceDirect

Phytochemistry Letters

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



Four meroterpenoids from *Alternaria alternata* isolated from *Paeonia lactiflora*

Ju-Tao Wang[a,b], Zong-Hui Ma[a], Guo-Kai Wang[a,b], Feng-Qing Xu[a,b], Lin Chen[c], Yang Yu[d,e], Gang Wang[a,b], Jin-Song Liu[a,b,*]

[a] School of Pharmacy, Anhui University of Chinese Medicine, Anhui Key Laboratory of Modern Chinese Materia Medica, Hefei 230012, China
[b] Synergetic Innovation Center of Anhui Authentic Chinese Medicine Quality Improvement, Hefei 230012, China
[c] School of Pharmaceutical Sciences, South-Central University for Nationalities, Wuhan 430074, China
[d] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[e] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Keywords:
Alternaria alternata
Meroterpenoids
TCAs
Cytotoxic activity

ABSTRACT

Four new natural meroterpenoids, tricycloalternarenes (TCAs)15b–18b (**1–4**) along with four known TCAs (**5–8**) have been isolated from the fermentation broth of *Alternaria alternata* derived from *Paeonia lactiflora*. The structures of compounds **1–4** were elucidated by in-depth analyses of NMR spectra, HRESIMS and optical rotation experiments. Cytotoxic activities of **1–4** against five selected tumor cell lines were evaluated.

1. Introduction

Endophytic fungi, which are proven to have significant impacts on the growth of host plants (Alurappa et al., 2018), are a reservoir that produce various bioactive metabolites. Considering this capability, the importance of chemical investigation of endophytes cannot be underestimated in the era of energy shortage. Among endophytes, a striking case is the genus *Alternaria*, which is a fungus capable of producing diverse fungal second metabolites, including nitrogen-containing constituents, terpenoids, quinones and especially toxins (Dang et al., 2015; Fang et al., 2018; Guo et al., 2015).

Through our ongoing search for bioactive natural products from plant-derived endophytes (Wang et al., 2018; Zhang et al., 2018b), *Alternaria alternata* has been isolated from *Paeonia lactiflora*, which is abundant in Bozhou City, Anhui Province. Four new tricycloalternarenes (**1–4**), which are meroterpenoids structurally related to ACTG-toxins, along with four known congeners (**5–8**) were isolated from the fermentation broth of *A. alternata*. According to the literature, most tricycloalternarenes (TCAs) isolated from endophytes (Shi et al., 2017), exhibit cytotoxicity against tumor cell lines (Fang et al., 2018). Therefore, all novel TCAs obtained from *A. alternata* were tested in five selected tumor cell lines. Herein, the isolation, structural elucidation and bioactivity assays of four novel natural products are reported.

2. Results and discussion

Compound **1** was isolated as a yellow oil with IR signals of hydroxyl (3455 cm^{-1}), carbonyl (1713 cm^{-1}) and carbon-carbon double bond (1619 cm^{-1}, 3055 cm^{-1}). Its molecular formula was established as $C_{22}H_{30}O_5$ from its quasi-molecule ion peak at *m/z* 397.1985 $[M + Na]^+$ (calcd. for $C_{22}H_{30}O_5Na$, 397.1985) in the positive HR-ESI-MS. The ^{13}C NMR spectrum of compound **1**showed 22 carbon signals, including seven quaternary carbons (two carbonyls at δ_{C-1} 170.2 and δ_{C-18} 199.6 and four olefinic carbons at δ_{C-2} 128.5, δ_{C-7} 150.7, δ_{C-13} 106.6 and δ_{C-14} 174.0), five methines (two olefinic carbons at δ_{C-3} 143.8 and δ_{C-8} 121.8), six methylenes and four methyls (one methoxy at δ_C 52.3). The ^1H NMR data presented a methoxy at δ_H 3.70 (3H, s); three methyls at δ_H 1.76 (3H, s, H-2'), 1.01 (3H, d, J =7.0 Hz, H-6') and δ_H 1.42 (3H, s, H-10'); and two olefinic protons at δ_H 6.68 (1H, t, J =7.6 Hz, H-3)and δ_H 5.39 (1H, s, H-8). The HMBC correlations from H-11 (δ_H 2.86, br d, J =6.5 Hz) to C-7, C-8, C-12 (δ_C 16.0) and C-13, from H_3-10' to C-9 (δ_C 45.9), C-10 (δ_C 89.8) and C-11 (δ_C 47.5), from H_2-12 (δ_H 2.65, dd, J = 17.0, 1.3 Hz, H-12a; 2.17, overlapped, H-12b) to C-14, from H_2-15 (δ_H 2.54, m, H-15a; 2.34, m, H-15b) to C-13, C-14, C-16 (δ_C 30.9) and C-17 (δ_C 72.2), and from H-17 (δ_H 4.05) to C-18 (δ_C 199.6) constructed the A–C rings in compound **1** in combination with the COSY correlations of H-8/H_2-9 (δ_H 2.57, dd, J = 16.4, 2.1 Hz, H-9a; 2.48, J = 16.4, 1.4 Hz, H-9b), H-11/H_2-12 and H_2-15/H_2-16 (δ_H 2.20, overlapped, H-

* Corresponding author at: School of Pharmacy, Anhui University of Chinese Medicine, Anhui Key Laboratory of Modern Chinese Materia Medica, Hefei 230012, China; Synergetic Innovation Center of Anhui Authentic Chinese Medicine Quality Improvement, Hefei 230012, China

E-mail address: jinsongliu@ahtcm.edu.cn (J.-S. Liu).

https://doi.org/10.1016/j.phytol.2019.03.001
Received 17 November 2018; Received in revised form 27 January 2019; Accepted 4 March 2019
Available online 08 March 2019
1874-3900/ © 2019 Phytochemical Society of Europe. Published by Elsevier Ltd. All rights reserved.

16a; 1.67, dd, J = 12.4, 5.2 Hz, H-16b)/H-17. Furthermore, the HMBC correlations from H_3-6' to C-5 (δ_C 34.8), C-6 (δ_C 33.7) and C-7, from H_2-4 (δ_H 2.10, m) to C-2, C-3 and C-5 (δ_C 34.8), from H_3-2' to C-1, C-2 and C-3 and from H-methoxy to C-1 established the side chain linked to ring A together with the COSY correlation of H-3/H_2-4/H_2-5 (δ_H 1.59, m, H-5a; 1.45, m, H-5b) /H-6 (δ_H 2.07, m). Thorough analyses of the above data and correlations led us to realize that compound **1** is the exact methyl esterification product from tricycloalternarene N (Li et al., 2018). The relative configuration of compound **1** was deduced from the analyses of ROESY data. The ROESY correlation from H-11 to H_3-10' indicated the cis-fused stereochemistry between ring A and B in **1**. The *E* geometry of the $\Delta^{2,3}$double bond was assigned by the ROESY correlation between H_3-2' and H_2-4. Given that the chemical shifts of C-17 (δ_C 72.2) and H-17 (δ_H 4.05, dd, J = 12.5, 5.2 Hz) were nearly identical to those of tricycloalternarene 2b (Liebermann et al., 1997) (δ_H 4.00, J = 12.9 and 5.3 Hz; δ_C 72.1), the configuration of C-17 (*R'*) was therefore the same as that of tricycloalternarene 2b, while the *S'* configuration of C-17 showed subtly varied coupling constants (H-15, J = 4.2, 10.2 Hz in TCA A; H-17, J = 4.3, 9.8 Hz in 17-*O*-Methyl-tricycloalternarene D) (Shi et al., 2018; Yuan et al., 2018). Hence, the structure of compound **1** was established as TCA 15b.

The molecular formula of compound **2** was determined as $C_{22}H_{34}O_4$ by HRESIMS (m/z 385.2347 $[M + Na]^+$) analysis and NMR data, requiring six degrees of unsaturation. Comparing the IR, UV and NMR data of compound **2** with those of compound **1**, it is not difficult to see that compound **2** has a similar structure to compound **1** except for different data from C-1 to C-6. Further analysis of the 2D NMR spectra in compound **2** led to the determination that this compound is the methylated product of TCA 14b (Zhang et al., 2018a). The hydroxyl at C-2 in TCA 14b was methylated to be methoxy group at C-2 in compound **2**, which was confirmed by the HMBC correlation from 2-OCH_3 (δ_H 3.13, s) to C-2 (δ_C 76.2). The same ROESY correlations in compound **2** as those in compound **1** suggested compound **2** to be the structure shown in Fig. 1 that was named TCA 16b. Fig. 2

Compound **3** was obtained as a yellow oil with an optical rotation of +185.8. Its HRESIMS spectrum showed a deduced molecular formula of $C_{22}H_{32}O_5$, with two hydrogen atoms more than compound **1**. Comparing the two groups of data between compounds **1** and **3**, one less double bond was found in compound **3** than in **1**, suggesting that compound **3** has a similar structure to compound **1**. Analysis of the 2D NMR data of compound **3** led to the determination that this compound is the reduction product of compound **1**, as deduced from the COSY correlations of H_3-2' (δ_H 1.09, d, J =7.0 Hz) /H-2 (δ_H 2.38, m) /H_2-3 (δ_H 1.53, m; 1.34, m) /H_2-4 (δ_H 1.18, m) /H_2-5 (δ_H 1.45, m; 1.28, m) /H-6 (δ_H 1.96, m) and the HMBC correlations from H_3-2' to C-1 (δ_C 178.9), from H-2 to C-1 and from H_2-3 to C-1. The ROESY data of compound **3** was similar to those of compound **1**, indicating that

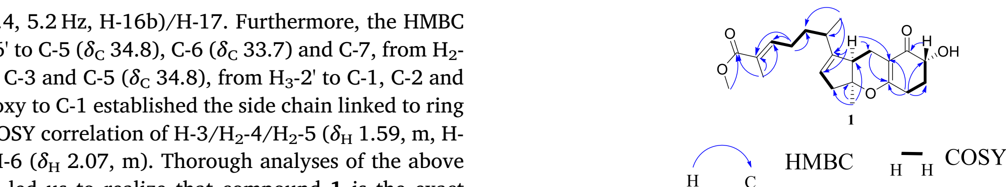


Fig. 2. Characteristic COSY, HMBC correlations of compound **1**.

compound **3** had the same relative configuration as compound **1**. Thus compound **3** was determined to be TCA 17b.

Compound **4**, a yellow oil, was elucidated to have the molecular formula of $C_{19}H_{26}O_5$ on the basis of the HRESIMS spectrum with a pseudomolecular ion peak at m/z 357.1678 $[M + Na]^+$ (calcd. for $C_{19}H_{26}O_5Na$, 357.1672). The NMR data of compound **4** featured a similar skeleton of an A/B/C fused ring, only with a different side chain in **4** from the above three compounds. Referring to the literature, compound **4** was eventually verified to be similar to TCA A (Yuan et al., 2008), and from the planar view, compound **4** was the exact methylated product of the hydroxyl at C-1 in TCA A, in association with the HMBC correlation from the proton (δ_H3.6, s) at methoxy to C-1 (δ_C175.8). Accordingly, compound **4** was assigned as TCA 18b with similar ROESY correlations with the aforementioned compounds.

In this paper, four known compounds (**5**–**8**) were also obtained from *A. alternata* and were respectively elucidated as methyl nor-tricycloalternarate, TCA 3a (Liebermann et al., 1997), TCA 3b (Liebermann et al., 1997) and TCA A according to the literature investigation.

All the new compounds were assayed for cytotoxic activities against five selected tumor cell lines (A-549, MDA-MB-231, MCF-7, KB, and KB-VIN), but unfortunately, none of them showed activity against the tumor cell lines with an IC_{50} of over 40.0 μM.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). UV/Vis spectra were obtained using a Shimadzu UV2401PC spectrometer (Shimadzu, Kyoto, Japan). IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Inc., Billerica, MA) with KBr pellets. NMR spectra, both 1D and 2D, were measured on a Bruker Avance III 500 MHz spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany). HRESIMS were recorded on an Agilent 6200 Q-TOF MS system (Agilent Technologies, Santa Clara, CA, USA). Silica gel (200–300 mesh, Qingdao Haiyang

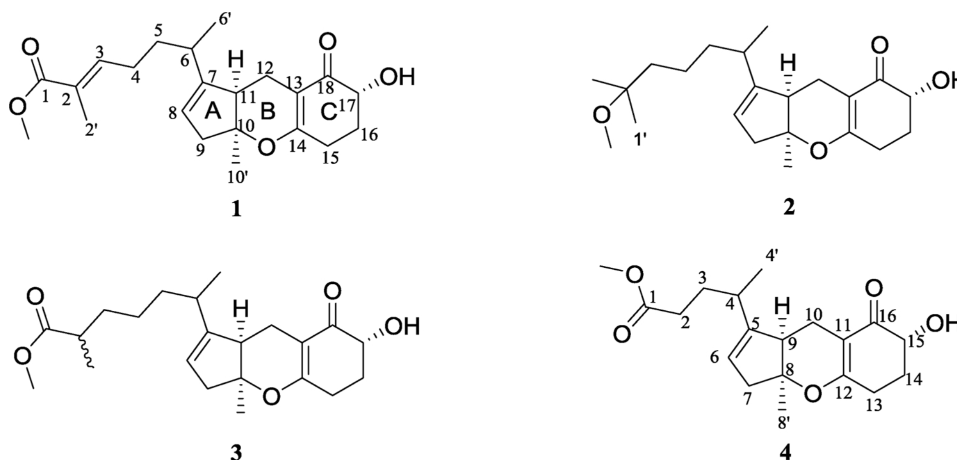


Fig. 1. Structures of compounds **1**–**4** from *Alternaria alternate*.

Chemical Co., Ltd, P. R. China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for CC. MPLC was performed on a Büchi Sepacore System equipped with pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Switzerland), and columns packed with Chromatorex C18 (40–75 mm, Fuji Silysia Chemical Ltd., Japan). Preparative HPLC was performed on an Agilent 1260 liquid chromatography system equipped with two types of Zorbax SB-C18 columns (9.4 mm × 150 mm and 21.2 mm × 150 mm, particle size 5 mm).

3.2. Fungal material

The *A. alternata* was isolated from the fresh *P. lactiflora* collected from the Chinese peony plantation in Qiaodong Town, Bozhou City, China and identified by Prof. C.-W. Fang at Anhui University of Chinese Medicine. The sequence data of the fungus is attached to the supplemental material, and a BLAST search result showed that this fungi is most similar (100%) to the sequence of *A. alternata* (GenBank accession NO. MK078593).

3.3. Fermentation, extraction, and isolation

An *A. alternata* agar slant was transferred onto a PDB plate and incubated for 7 days. The agar plugs were punched into small pieces (6 mm), then the strain was inoculated into Erlenmeyer flasks. Each Erlenmeyer flask contained 1 L PDB medium (potato 5.0 g; glucose 15.0 g; peptone 10.0 g; NaCl 5.0 g in 1 L deionized water). Fermentation was performed out in two rotary shakers at 28 °C and 150 rpm for 15 days.

The whole culture was extracted exhaustively with EtOAc after the incubation. The crude extract (63 g) was fractionated by MPLC with a stepwise gradient of MeOH/H_2O (v/v 0:100 – 100:0) to give ten fractions (Fr.A–J). Fr.G was subjected to normal-phase silica gel column chromatography with an isocratic elution of petroleum/acetone (10:1, v/v) to obtain eight subfractions (G1–G8) based on TLC analyses. Compound **4** (6.1 mg) was obtained from Fr. G4 by Sephadex LH-20 (MeOH) and then Sephadex LH-20 (Acetone). Fr.G5 was subjected to prep-HPLC using a gradient elution (MeCN-H_2O 30%–45%, 7 mL·min^{-1}, 35 min) and Sephadex LH-20 (acetone) to yield compound **5** (10.0 mg). Fr.G6 was subjected to Sephadex LH-20 (MeOH) and prep-HPLC using a gradient elution (MeCN-H_2O 30%–48%, 7 mL·min^{-1}, 20 min) to yield compound **8** (5.6 mg). Fr.I was subjected to normal-phase silica gel column with an isocratic elution (petroleum-acetone, 10:1) and was further filtered to remove the deposition. The filtrate was purified with a normal-phase silica gel column with an isocratic elution (petroleum-acetone, 15:1) then subjected to prep-HPLC using gradient elution (MeCN-H_2O 40%–50%, 7 mL·min^{-1}, 20 min) and Sephadex LH-20 (acetone) to obtain compound **2** (3.5 mg). Fr.J was partitioned through a normal-phase silica gel column with an isocratic elution (petroleum-acetone, 8:1) to obtain four subfractions (J1–J4). Compounds **10** (3.1 mg), **3** (5.4 mg) and **7** (1.5 mg) were produced from Fr.J3 by prep-HPLC (MeCN-H_2O 43%–60%, 7 mL·min^{-1}, 30 min). Compound **6** (2.3 mg) was yielded from Fr.J4 by Sephadex LH-20 (chloroform-methanol, 1:1) and prep-HPLC using a gradient elution (MeCN-H_2O 25%–55%, 7 mL·min^{-1}, 30 min).

3.4. Spectroscopic data

3.4.1. TCA 15b (*1*)

Yellow oil; [α]23.7 D +159.5° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.11), 213 (4.10), 264 (4.13) nm; IR (KBr) υ_{max}3455, 2932, 1713, 1381, 1268, 1082 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS: *m/z*397.1985 [M + Na]$^+$ (calcd for $C_{22}H_{30}O_5Na$, 397.1985).

3.4.2. TCA 16b (*2*)

Yellow oil; [α]23.7 D +103.7° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 263 (3.99), 201 (3.77) nm; IR (KBr) υ_{max}3453, 2936, 1620, 1382, 1081 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS: *m/z*385.2347 [M + Na]$^+$ (calcd for $C_{22}H_{34}O_4Na$, 385.2349).

Table 1
^1H and ^{13}C NMR Spectroscopic Data of **1–2** (δ in ppm).

No.	1		2	
	δ_C[a]	δ_H[b] (*J* in Hz)	δ_C[a]	δ_H[b] (*J* in Hz)
1	170.2, C		25.3, CH_3	1.10 s
2	128.5, C		76.2, C	
3	143.8, CH	6.68 t (7.6)	40.8, CH_2	1.39 m 1.23 m
4	27.7, CH_2	2.10 m	36.6, CH_2	1.46 m 1.27 m
5	34.8, CH_2	1.59 m 1.45 m	22.8, CH_2	1.23 m
6	33.7, CH	2.07 m	34.0, CH	1.99 m
7	150.7, C		151.3, C	
8	121.8, CH	5.39 s	121.2, CH	5.34 s
9	45.9, CH_2	2.57 dd (16.4, 2.1) 2.48 dd (16.4, 1.4)	45.9, CH_2	2.55 m 2.47 m
10	89.8, C		89.8, C	
11	47.5, CH	2.86 br d (6.5)	47.5, CH	2.82 m
12	16.0, CH_2	2.65 dd (17.0, 1.3) 2.17 overlapped	16.1, CH_2	2.65 br d (16.9) 2.17 br d (16.9)
13	106.6, C		106.7, C	
14	174.0, C		174.0, C	
15	28.8, CH_2	2.54 m 2.34 m	28.8, CH_2	2.55 m 2.34 m
16	30.9, CH_2	2.20 overlapped 1.67 dd (12.4, 5.2)	30.9, CH_2	2.20 m 1.76 m
17	72.2, CH	4.05 dd (12.5, 5.2)	72.2, CH	4.04 dd (12.6, 5.2)
18	199.6, C		199.6, C	
2'	12.5, CH_3	1.76 s	25.3, CH_3	1.10 s
6'	20.7, CH_3	1.01 d (7.0)	20.7, CH_3	0.98 d (6.9)
10'	23.2, CH_3	1.42 s	23.3, CH_3	1.43 s
OCH_3	52.3, CH_3	3.70 s	49.2, CH_3	3.13 s

[a]Measured in methanol-d_4 at 125 MHz, [b]Measuredin methanol-d_4 at 500 MHz.

3.4.3. TCA 17b (*3*)

Yellow oil; [α]23.7 D +185.8° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 262 (4.20), 202 (3.90) nm; IR (KBr) υ_{max}3453, 2935, 1735, 1617, 1379, 1080 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS: *m/z*399.2143 [M + Na]$^+$ (calcd for $C_{22}H_{32}O_5Na$, 399.2142).

3.4.4. TCA 18b (*4*)

Yellow oil; [α]23.5 D +180.9° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 263(4.14), 204 (3.76) nm; IR (KBr) υ_{max} 3450, 2955, 1737, 1619, 1381, 1267, 1150 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS: *m/z*357.1678 [M + Na]$^+$ (calcd for $C_{19}H_{26}O_5Na$, 357.1672).

3.5. In vitro cytotoxicity assay

The human tumor cell lines used in the cytotoxic assay were A-549, MDA-MB-231, MCF-7, KB and KB-VIN the assay was performed according to the SRB method (Skehan et al., 1990). They were cultured in RMPI-1640 medium supplemented with 10% fetal bovine serum (Biological Industries) at 37 °C with 5% CO_2 and then seeded evenly into each well of a 96-well cell culture plate with 100 μL of cell suspension at optimum density. After incubation for 24 h, the test compound (50 μM) was added. After incubation for 2 days, cells were fixed with ice-cold TCA for 1 h at 4 °C. Then, the plates were washed with distilled water five times. After the plates were air dried, 100 μL of 4% SRB solution was added to each well for 30 min at room temperature. Acetic acid (1% v/v) was used to wash the plates five times, and the bound SRB was solubilized by 100 μL of 10 mM Tris Base (pH = 10.5). The plates were then put on a shaker platform for 5 min, and finally, the reading was taken with Spectra-Max-190 (Molecular Devices,

Table 2
^{1}H and ^{13}C NMR Spectroscopic Data of **3–4** (δ in ppm).

No.	**3**		No.	**4**	
	δ_C[a]	δ_H[b] (J in Hz)		δ_C[a]	δ_H[b] (J in Hz)
1	178.9, C		1	175.8, C	
2	40.7, CH	2.38 m	2	32.8, CH_2	2.21 m
3	35.0, CH_2	1.53 m	3	28.8, CH_2	2,35 m
		1.34 m	4	33.3, CH	2.00 m
4	26.2, CH_2	1.18 m	5	150.3, C	
			6	121.9, CH	5.38 s
5	35.8, CH_2	1.45m	7	45.9, CH_3	2.57 m
		1.28 m			2.48 m
6	33.7, CH	1.96 m	8	89.8, C	
7	151.1, C		9	47.4, CH	2.86 m
8	121.3, CH	5.33 s	10	15.8, CH_2	2.64 m
9	45.9, CH_2	2.56 m	11	106.5, C	
		2.45 m	12	174.0, C	
10	89.8, C		13	30.9, CH_2	1.75 m
11	47.4, CH	2.83 m	14	30.8, CH_2	2.19 m
12	16.0, CH_2	2.66 m			1.63 m
		2.16 m	15	72.2, CH	4.02 dd (12.6, 5.1)
13	106.6, C		16	199.6, C	
14	174.0, C		4'	20.7, CH_3	0.98 d (6.9)
15	28.8, CH_2	2.55 m	8'	23.1, CH_3	1.43 s
		2.35 m	1-OCH_3	52.1, CH_3	3.60 s
16	31.0, CH_2	2.19 m			
		1.72 m			
17	72.2, CH	4.01 dd (12.7, 5.2)			
18	199.6, C				
2'	17.6, CH_3	1.09 d (7.0)			
6'	20.7, CH_3	0.96 d (7.0)			
10'	23.2, CH_3	1.42 s			
1-OCH_3	52.1, CH_3	3.63 s			

[a]Measured in methanol-d_4 at 125 MHz, [b]Measuredin methanol-d_4 at 500 MHz.

Sunnydale, USA) at 490 nm (Houghton et al., 2007).

Acknowledgments

This work was financially supported by the Open Fund of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2017-KF03) and the special project to guide the Development of Local Science and Technology in Anhui Province (grant number 2017070702D173).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.03.001.

References

Alurappa, R., Chowdappa, S., Narayanaswamy, R., Sinniah, U.R., Mohanty, S.K., Swamy, M.K., 2018. Endophytic fungi and bioactive metabolites production: an update. In: Patra, J.K., Das, G., Shin, H.S. (Eds.), Microb. Biotechno. Springer, Singapore, pp. 455–482.

Dang, H.X., Pryor, B., Peever, T., Lawrence, C.B., 2015. The *Alternaria* genomes database: a comprehensive resource for a fungal genus comprised of saprophytes, plant pathogens, and allergenic species. BMC Genomics 16, 239–247.

Fang, S.T., Miao, F.P., Liu, X.H., Song, Y.P., Ji, N.Y., 2018. Two new tricycloalternarene acids from the marine-derived fungus *Alternaria alternata* ICD5-11. Phytochem. Lett. 23, 185–188.

Guo, D.L., Zhao, M., Xiao, S.J., Xia, B., Wan, B., Gu, Y.C., Ding, L.S., Zhou, Y., 2015. Two new diketopiperazines and a new glucosyl sesterterpene from *Alternaria alternata*, an endophytic fungi from *Ceratostigma griffithii*. Phytochem. Lett. 14, 260–264.

Houghton, P., Fang, R., Techatanawat, I., Steventon, G., Hylands, P.J., Lee, C.C., 2007. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods 42, 377–387.

Li, C., Hu, Z., Liu, Q., Wu, X., Cao, S., 2018. Two new tricycloalternarenes from Hawaiian endophytic fungus *Didymella* sp. FT433. Tetrahedron Lett. 59, 3381–3383.

Liebermann, B., Ellinger, R., Günther, W., Ihn, W., Gallander, H., 1997. Tricycloalternarenes produced by *Alternaria alternata* related to ACTG-toxins. Phytochemistry 46, 297–303.

Shi, Z.Z., Miao, F.P., Fang, S.T., Liu, X.H., Yin, X.L., Ji, N.Y., 2017. Sesteralterin and tricycloalterfurenes A–D: terpenes with rarely occurring frameworks from the marine-alga-epiphytic fungus *Alternaria alternata* k21-1. J. Nat. Prod. 80, 2524–2529.

Shi, Z.Z., Fang, S.T., Miao, F.P., Ji, N.Y., 2018. Two new tricycloalternarene esters from an alga-epiphytic isolate of Alternaria alternata. Nat. Prod. Res. 32, 2523–2528.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: J. Natl. Cancer Inst. 82, 1107–1112.

Wang, J.-T., Zhang, P.L., Liu, J.S., Wang, G.K., Xu, F.Q., Chen, L., Yu, Y., Wang, G., 2018. Aspergilates A to E, second metabolites from Aspergillus sp. isolated from Paeonia ostii. Fitoterapia 131, 204–208.

Yuan, L., Zhao, P.J., Ma, J., Li, G.H., Shen, Y.M., 2008. Tricycloalternarenes A – E: five new mixed terpenoids from the endophytic fungal strain *Alternaria alternata* Ly83. Helv. Chim. Acta 91, 1588–1594.

Zhang, H., Zhao, Z., Chen, J., Bai, X., Wang, H., 2018a. Tricycloalternarene analogs from a symbiotic fungus *Aspergillus* sp. D and their antimicrobial and cytotoxic effects. Molecules 23, 855–861.

Zhang, P.L., Wang, G., Liu, J.S., Xu, F.Q., Zhao, Z.Z., Wang, W.X., Wang, J.T., Wang, G.K., Wu, P.Y., 2018b. Three new metabolites from the endophytic fungus *Climacocystis montana* isolated from the root bark of *Paeonia ostia*. Phytochem. Lett. 26, 50–54.