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Anti-phytopathogenic sesquiterpenoid-xanthone adducts from potato endophytic fungus *Bipolaris eleusines*†

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A bioguided separation on the cultures of the potato endophytic fungus *Bipolaris eleusines* led to the isolation of two anti-phytopathogenic (*Alternaria solani*) sesquiterpenoid-xanthone adducts, namely bipolins I and J (**1** and **2**). Their structures were established *via* extensive spectroscopic analysis. Compounds **1** and **2** exhibit potent inhibitory activity against *A. solani* with MIC values of 8 and 16 μg mL^{-1}, respectively.

Introduction

Sativene sesquiterpenoids usually feature an unusual 5/6 or 5/7 bridged ring system,[1–4] which attracted great interest in total synthesis[5–8] and biological studies.[2,8–10] Our previous investigations on the potato endophytic fungus *Bipolaris eleusines* reported bipolenins A–H,[11–13] and indicated that the fungus is a good resource for sativene sesquiterpenoids. Some of them show cytotoxicities to human cancer cell lines and inhibitory activity on nitric oxide production. Our recent studies found that the potato endophytic fungus *B. eleusines* can inhibit the growth of potato pathogen *Alternaria solani* (early blight) on PDA (Fig. 1), which suggested the metabolites from the fungus may possess good inhibitions on the plant pathogen. This discovery prompted us to search for anti-pathogenic agents from the endophytic fungus *B. eleusines*. Therefore, a bioguided isolation on the cultures of the fungus *B. eleusines* was carried out. As a result, two novel sativene sesquiterpenoid-xanthone adducts, namely bipolenins I and J (**1** and **2**, Fig. 2), were obtained from liquid fermentation of the fungus. Their structures were established *via* extensive spectroscopic analyses. Pure compounds **1** and **2** were evaluated for their inhibitory activities on *A. solani*. Herein the isolation, structural elucidation, and bioactivity of compounds **1** and **2** are reported.

Results and discussion

Bipolenin I (**1**) had a molecular formula $C_{31}H_{36}O_9$ as determined on the basis of the positive HRESIMS, which showed a molecular ion peak at *m/z* 575.2250 $[M + Na]^+$ (calcd for $C_{31}H_{36}O_9Na$, 575.2252), corresponding to seven degrees of unsaturation. The UV data at 329 nm indicated a highly conjugated system, while the IR absorption bands at 3440 and 1745 cm^{-1} suggested the existence of hydroxy and carbonyl, respectively. The ^{1}H and ^{13}C NMR spectra, in association with the HSQC spectrum, revealed six methyl (one methoxy), four methene, nine methine, and twelve quaternary carbons (Table 1). Preliminary analysis on the NMR data, a possible moiety of sativene sesquiterpenoid was detected by comparison the data with those our reported previously.[1,2,4,11–13] As shown in unit A of Fig. 3, a long spin system was obtained by analysis of the ^{1}H–^{1}H

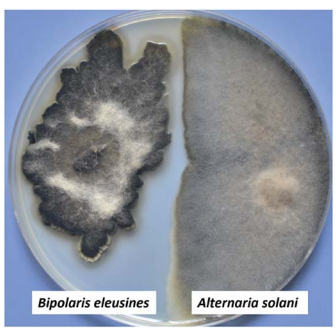

Fig. 1 The growth inhibition of potato endophytic fungus *B. eleusines* against potato pathogen *Alternaria solani* on PDA.

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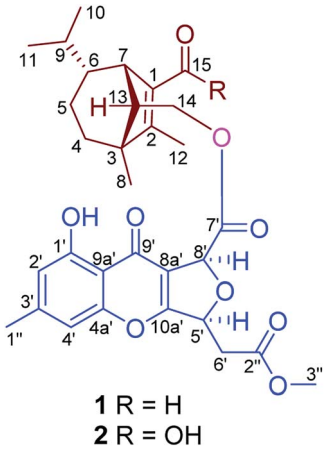

Fig. 2 Structures of bipolenins I and J (**1** and **2**) from cultures of the fungus *Bipolaris eleusines*.

COSY spectrum. A methyl substituted 1,2-double bond-15-aldehyde moiety was established by the ^{13}C NMR data at δ_C 137.2 (s, C-1), 164.9 (s, C-2), 187.9 (d, C-15), and 10.6 (q, C-12), and supported by HMBC correlations from δ_H 10.00 (s, H-15) and 2.01 (3H, s, H-12) to C-1 and C-2. This moiety was connected by bonds of C-1/C-7 and C-2/C-3 as deduced from HMBC correlations from δ_H 3.11 (1H, br s, H-7) to C-2, and from H-12 to an sp^3 quaternary carbon at δ_C 50.9 (s, C-3). In addition, a singlet at δ_H 1.05 (3H, s, H-8) for a methyl group showed HMBC correlations to C-3, C-2, and to other two carbon resonances at δ_C 33.8 (d, C-4) and 57.5 (d, C-13). These data, as well as other HMBC correlations (Fig. 3), suggested that unit A was a sativene sesquiterpenoid related to helminthosporol.[1,4] In unit B (Fig. 3), a four-substituted benzene ring was identified by two aromatic protons at δ_H 6.68 (1H, s, H-2′) and 6.74 (1H, s, H-4′). A methyl and a hydroxy as two substituents in the benzene ring were identified by the HMBC correlations as shown in Fig. 3. Analysis of UV data and the chemical shifts at δ_C 178.0 (s, C-9′), 114.5 (s, C-8a′), 167.5 (s, C-10a′) suggested the existence of a chromone core of unit B. Due to the existence of an intramolecular hydrogen bond, the proton of the hydroxy group appeared as a singlet at δ_H 12.09 (s, OH-1′), which further confirmed the existence of the chromone core. The ^{1}H–^{1}H COSY spectrum revealed a short spin system of CH(5′)–CH$_2$(6′), which

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** and **2** in CDCl$_3$ (δ in ppm, J in Hz)

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	137.2, s		125.5, s	
2	164.9, s		161.3, s	
3	50.9, s		50.5, s	
4	33.8, t	1.38, m	33.6, t	1.34, m
5a	25.0, t	1.72, m	24.9, t	1.71, m
5b		0.84, m		0.97, m
6	44.6, d	0.98, m	44.9, d	0.96, m
7	41.7, d	3.11, br s	43.6, d	3.10, br s
8	18.4, q	1.05, s	18.9, q	1.00, s
9	31.5, t	0.97, m	31.5, d	1.17, m
10	20.6, q	0.71, s	20.8, q	0.74, d (6.5)
11	21.3, q	0.95, s	21.4, q	0.93, d (6.5)
12	10.6, q	2.01, s	12.5, q	2.00, s
13	57.5, d	1.81, dd (8.6, 5.6)	58.0, d	1.79, dd (8.3, 5.5)
14a	65.8, t	4.21, dd (11.1, 5.6)	66.0, t	4.26, dd (11.2, 5.5)
14b		3.89, dd (11.1, 5.6)		3.94, dd (11.2, 8.3)
15	187.9, d	10.00, s	168.6, s	
1′	160.9, s		160.9, s	
2′	113.5, d	6.68, s	113.5, d	6.66, br s
3′	147.4, s		147.4, s	
4′	107.9, d	6.74, s	107.9, d	6.73, br s
4a′	157.2, s		157.2, s	
5′a	78.0, d	5.69, ddd (6.3, 4.3, 4.0)	78.0, d	5.70, ddd (6.4, 4.3, 4.0)
5′b				
6′a	37.6, t	2.97, dd (16.0, 4.3)	37.6, t	2.97, dd (16.1, 4.3)
6′b		2.82, dd (16.0, 6.3)		2.82, dd (16.1, 6.4)
7′	169.4, s		169.5, s	
8′	79.4, d	5.58, d (4.0)	79.4, d	5.60, d (4.0)
8a′	114.5, s		114.6, s	
9′	178.0, s		177.9, s	
9a′	108.9, s		108.9, s	
10a′	167.5, s		167.5, s	
1″	22.3, q	2.41, s	22.3, q	2.40, s
2″	169.3, s		169.3, s	
OMe	52.2, q	3.73, s	52.2, q	3.73, s
1′-OH		12.09, s		12.10, s

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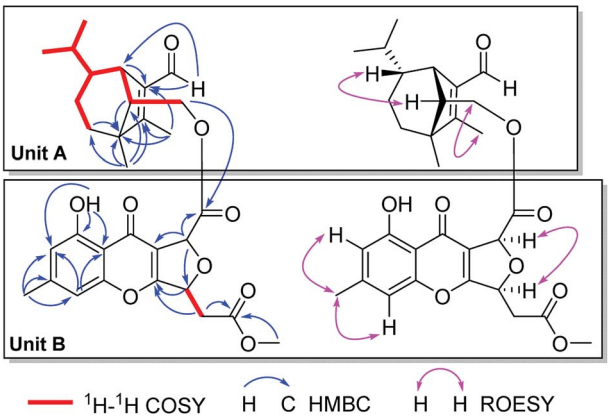

Fig. 3 Key 2D NMR correlations of **1**.

connected to a methyl ester group as established by the HMBC correlations from δ_H 2.97 (1H, dd, J = 16.0, 4.3 Hz, H-6′a), 2.82 (1H, dd, J = 16.0, 4.3 Hz, H-6′b) and 3.73 (3H, s, OMe) to δ_C 169.3 (s, C-2″). The spin system also connected to C-10a′ based on the HMBC correlation from δ_H 5.69 (1H, ddd, J = 6.3, 4.3, 4.0 Hz, H-5′) to C-10a′. So far, only two carbons including a methine (CH-8′) and a carbonyl carbon at δ_C 169.4 (s, C-7′) were left. Further analysis of HMBC spectrum, the correlations from δ_H 5.88 (1H, d, J = 4.0 Hz, H-8′) to C-7′, C-8a′, and C-5′ suggested that C-8′ was connected to C-8a′ and C-7′, respectively, and an ether bond was established between C-5′ and C-8′. The unit B was, therefore, identified as a seco-xanthone related to a synthetic product in the literature.[14] The connection by an ester bond between units A and B was revealed by an HMBC correlation from H-14 to C-7′, as well as analysis of the MS data. An ROESY experiment was carried out to establish the relative configuration of **1** (Fig. 3). In unit A, a cross peak between H-6 and H-13 suggested that H-6 and H-13 were in the same side. Another cross peak between H-12 and H-14 confirmed the relative configuration of C-13 as shown. In unit B, a key cross peak between H-5′ and H-8′ indicated that H-5′ and H-8′ were in the same side, while the cross peaks of H-1″/H-4′ and H-1″/H-2′ helped with the position identification of substituents in the benzene ring. The structure of 1 was, finally, established as depicted.

Compound **2** was isolated as a light yellow gum. Its molecular formula $C_{31}H_{36}O_{10}$ was determined by an HRESIMS ion peak at m/z 567.2240 $[M - H]^-$ (calcd for $C_{31}H_{35}O_{10}$, 567.2236), 16 unit more than that of **1**. All the spectroscopic data of **2** displayed almost the same patterns to those in **1**, except that a carboxyl signal at δ_C 168.6 (s, C-15) in the ^{13}C NMR of **2** replaced the aldehyde signal at δ_C 187.9 in that of **1**. It was supported by an HMBC correlation from δ_H 3.10 (1H, br s, H-7) to C-15, while the other parts of **2** were elucidated as the same to those of **1** by detailed analysis of 2D NMR data. The structure of **2** was established, and named as bipolenin J.

Compounds **1** and **2** were evaluated for their anti-phytopathogenic activities against four plant pathogens including *Phytophthora infestane*, *Alternaria solani*, *Rhizoctonia solani*, and *Fusarium oxysporum*. As a result (Table 2), compounds **1** and **2** all exhibited potent inhibitory activity against *A. solani* with MIC values of 8 and 16 μg mL^{-1}, respectively. While compound **1** also weakly inhibited *F. oxysporum* with an MIC values of 64 μg mL^{-1}.

Table 2 Inhibitions of **1** and **2** on four phytopathogens (μg mL^{-1})

Comps	*P. infestans*	*A. solani*	*R. solani*	*F. oxysporum*
1	NA	8	NA	64
2	NA	16	NA	NA
Hygromycin B[a]	8	<4	8	64

[a] Positive control; NA: no activity.

Conclusions

In summary, two novel sesquiterpenoid-xanthone adducts, bipolenins I and J, were obtained from potato endophytic fungus *Bipolaris eleusines* by a bioguided isolation. The potent inhibitory activities of *B. eleusines* and its metabolites on phytopathogens revealed a possible application prospect on biological pesticide for *B. eleusines*.

Experimental section

General experimental procedures

The melting points were tested by a Putiantongchuang WRX-5A apparatus. Optical rotations were measured on a Jasco-P-1020 polarimeter. IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with instrument of a Bruker DRX-500. HSESIMS was measured on an API QSTAR Pulsar spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) and spots were visualized by heating silica gel plates immersed in vanillin–H_2SO_4 in EtOH, in combination with Agilent 1200 series HPLC system (Eclipse XDB-C18 column, 5 μm, 4.6 × 150 mm). Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 μm, 9.4 × 150 mm) column.

Fungal material and cultivation conditions

The fungus *Bipolaris eleusines* was isolated from fresh potatoes sampled from the nursery of Yunnan Agricultural University at random in July 2012. The fungus was identified by observing the morphological characteristics and analysis of the internal transcribed spacer (ITS) regions (max identity: 99%; query cover: 98%; accession: KJ909768.1). The strain is deposited at South-Central University for Nationalities, China (No. PE20120728-SCUN-201603).

This fungal strain was cultured on potato dextrose agar (PDA) medium at 24 °C for 10 days. The agar plugs were inoculated in 500 mL Erlenmeyer flasks, each containing 100 mL of potato dextrose media. Flask cultures were incubated at 28 °C on a rotary shaker at 160 rpm for two days as seed culture. One hundred and twenty 500 mL Erlenmeyer flasks each containing

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150 mL of potato dextrose broth (PDB) were individually inoculated with 25 mL of seed culture, and were incubated at 25 °C on a rotary shaker at 160 rpm for 20 days.

Extraction and isolation

A total of 20 L culture broth was extracted three times with EtOAc. The organic layer was evaporated to give a crude extract (15 g). The crude extract was subjected to silica gel CC (200–300 mesh) eluted with a $CHCl_3$–MeOH solvent system (a gradient from 1 : 0 to 0 : 1) to afford seven fractions A–G. Fraction C (1.2 g) was further separated by silica gel CC eluted with petroleum ether–Me_2CO (12 : 1) to afford subfractions C1–C6. Fraction C2 (90 mg) was isolated by Sephadex LH-20 CC (MeOH) and by HPLC (MeCN–H_2O, from 3 : 7 to 6 : 4 in 25 min, 10 mL min^{-1}) to give **1** (4.2 mg) and **2** (3.5 mg).

Bipolenin I (1). Yellow oil; $[\alpha]_D^{21.7}$ +81.3 (c = 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 203.0 (3.65), 250.5 (3.66), 329 (2.88) nm; IR (KBr) ν_{max}: 3440, 2924, 1745, 1661, 1635, 1619, 1462, 1383, 1208 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1, HRESIMS m/z 575.2250 $[M + Na]^+$ (calcd for $C_{31}H_{36}O_9Na$, 575.2252).

Bipolenin J (2). Light yellow gum; $[\alpha]_D^{21.5}$ +22.0 (c = 0.08, MeOH) UV (MeOH) λ_{max} (log ε) 203 (3.53), 246 (3.52), 329.5 (2.58), 382.5 (2.37) nm; IR (KBr) ν_{max} 3441, 2923, 1740, 1632, 1462, 1384, 1206, 1061 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1, HRESIMS m/z 567.2240 $[M - H]^-$ (calcd for $C_{31}H_{35}O_{10}$, 567.2236).

Anti-phytopathogenic assay

Compounds **1** and **2** were subjected to minimal inhibitory concentration (MIC) tests against four phytopathogenic fungi (*Phytophthora infestane*, *Alternaria solani*, *Rhizoctonia solani*, *Fusarium oxysporum*) on the PDA medium using a twofold serial dilution in the microplate wells over the range of 4–256 μg mL^{-1}. To this end, into each well of 96 well plate was placed 80 μL of PDA along with a 20 μL volume of an aqueous test sample solution. The test solutions held different concentrations of the sample being tested. The control well held 80 μL PDA and 20 μL of sterile water. Then, agar plugs (1 mm^3) with fresh phytopathogens were inoculated into each well. Observations of the plates were made after 24 h of incubation at 26 °C in order to acquire the MICs with no growth in the well taken as that value. Three replicates were maintained to confirm the antifungal activity.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 S. Tamura, A. Sakurai, K. Kainuma and M. Takai, *Agric. Biol. Chem.*, 1963, **27**, 738–739.
2 P. de Mayo and R. E. Williams, *J. Am. Chem. Soc.*, 1965, **87**, 3275.
3 F. Dorn and D. Arigoni, *Experientia*, 1974, **30**, 851–852.
4 C. Osterhage, G. M. König, U. Höller and A. D. Wright, *J. Nat. Prod.*, 2002, **65**, 306–313.
5 G. L. Hodgson, D. F. MacSweeney and T. Money, *J. Chem. Soc., Perkin Trans. 1*, 1973, **19**, 2113–2130.
6 P. Bakuzis, O. O. S. Campos and M. L. F. Bakuzis, *J. Org. Chem.*, 1976, **41**, 3261–3264.
7 S. Karimi, *J. Nat. Prod.*, 2001, **64**, 406–410.
8 A. Khanum Shaukath, S. Shashikanth, G. Sathyanarayana Syagadadu, S. Lokesh and A. Deepak Saligrama, *Pest Manage. Sci.*, 2009, **65**, 776–780.
9 H. Miyagawa, S. Nagai, T. Tsurushima, M. Sato, T. Ueno and H. Fukami, *Biosci., Biotechnol., Biochem.*, 1994, **58**, 1143–1145.
10 J. K. Park, K. Hasumi and A. Endo, *J. Antibiot.*, 1993, **46**, 1303–1305.
11 H. L. Ai, M. S. Yang, S. H. Zi and H. C. Guo, *J. Asian Nat. Prod. Res.*, 2015, **17**, 982–987.
12 M. S. Yang, X. Y. Cai, Y. Y. He, M. Y. Lu, S. Liu, W. X. Wang, Z. H. Li, H. L. Ai and T. Feng, *Nat. Prod. Bioprospect.*, 2017, **7**, 147–150.
13 Z.-H. Li, H.-L. Ai, M.-S. Yang, J. He and T. Feng, *Phytochem. Lett.*, 2018, **27**, 87–89.
14 T. Sassa, H. Kachi and M. Nukina, *J. Antibiot.*, 1985, **38**, 439–441.