Fitoterapia 83 (2012) 209–214

Contents lists available at SciVerse ScienceDirect

Fitoterapia

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

Polyketides with antimicrobial activity from the solid culture of an endolichenic fungus *Ulocladium* sp.

Quan-Xin Wang [a,b,c,1], Li Bao [b,1], Xiao-Li Yang [b], Hui Guo [c], Rui-Nan Yang [b], Biao Ren [c], Li-Xin Zhang [a,c,*], Huan-Qin Dai [c], Liang-Dong Guo [b], Hong-Wei Liu [b,*]

[a] *School of Life Science, University of Science and Technology of China, No. 96 Jinzhai-Road, Hefei 230026, PR China*
[b] *Key Lab of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, No.9 Beiertiao, Zhongguancun, Haidian District, Beijing 100090, PR China*
[c] *CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, No.9 Beiertiao, Zhongguancun, Haidian District, Beijing 100090, PR China*

ARTICLE INFO

Article history:
Received 15 August 2011
Accepted 20 October 2011
Available online 28 October 2011

Keywords:
Polyketides
Endolichenic fungus
Ulocladium sp.
Radical scavenging activities
Antimicrobial

ABSTRACT

Two new polyketides, 7-hydroxy-3, 5-dimethyl-isochromen-1-one (**1**) and 6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3H-furo[3,2-c]isochromene-2,5-dione (**2**), along with eleven known compounds, 5′-methoxy-6-methyl-biphenyl-3,4,3′-triol (**3**), 7-hydroxy-3-(2-hydroxy-propyl)-5-methyl-isochromen-1-one (**4**), rubralactone (**5**), isoaltenuene (**6**), altenuene (**7**), dihydroaltenuenes A (**8**), altenusin (**9**), alterlactone (**10**), 6-O-methylnorlichexanthone (**11**), norlichexanthone (**12**), and griseoxanthone C (**13**) were isolated from the culture of the endolichenic fungus *Ulocladium* sp. Compound **2** was obtained as a racemate with an unprecedented chemical skeleton. The NMR data assignments for **3** and **4** were achieved for the first time. Compounds **1**–**13** were screened for their antimicrobial and radical scavenging activities. Compound **1** showed some antifungal activity against *Candida albicans* SC 5314 with IC_{50} of 97.93 ± 1.12 μM. Compounds **11**–**13** showed strong activity against *Bacillus subtilis* with IC_{50} in the range of 1–5 μM. Compound **12** significantly inhibited the growth of methicillin-resistant *Staphylococcus aureus* with IC_{50} of 20.95 ± 1.56 μM. Compounds **9** and **10** showed strong radical scavenging activity in comparison with vitamin C. The plausible biosynthetic pathways for compounds **1**, **2**, and **4**–**8** were discussed.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Although fungi are well-known producers of secondary metabolites possessing a variety of biological activities, several ecological groups of fungi remain underexplored as potential sources of new bioactive natural products. One such group is the endolichenic fungi that specialize in the thalli of lichens. Endolichenic fungi are analogous to the plant endophytes inhabiting the intercellular spaces of the hosts. A limited chemical investigation has been conducted on metabolites of endolichenic fungi, but they have showed great potential to be new source for novel bioactive natural products [1–4]. In our searching for new antimicrobial agents from endolichenic fungi, the EtOAc extract of an endolichenic fungus *Ulocladium* sp. showed strong antibacterial activity against *Bacillus subtilis* (inhibition rate (IR), 93.7%), and antifungal activity against *Candida albicans* SC5314 (IR, 70.5%) and *Aspergillus fumigatus* (IR, 95.4%) at the concentration of 200 μg/mL. Chemical investigation on its extract led to the isolation of thirteen secondary metabolites

* Corresponding author. Tel.: +86 10 62566577; fax: +86 10 62566511.
E-mail addresses: liuhw@im.ac.cn, lzhang03@gmail.com (H.-W. Liu).
[1] Authors contributed equally to this work.

0367-326X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2011.10.013

including two new polyketides (**1** and **2**) (Fig. 1). Details of the isolation, structure elucidation, and bioactivity screening of secondary metabolites isolated are presented here.

2. Experimental

2.1. General

Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter using MeOH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ESI-MS was conducted using a Bruker esquire 2000 mass spectrometer. HRTOFMS spectra were measured on a Bruker micrOTOF-Q instrument. ^{1}H- and ^{13}C-NMR, along with 2D-NMR spectra were obtained on a Varian Mercury 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) NMR spectrometer, using TMS as an internal standard. Chemical shifts were expressed in δ (ppm) and coupling constants (*J*) were reported in Hertz (Hz). TLC was carried out on Silica gel $60F_{254}$ and the spots were visualized by spraying with 10% H_2SO_4 and heating. LH-20 (Amersham Biosciences) and ODS (YMC) were used for column chromatography. Preparative HPLC HPLC was performed on Agilent 1200 system equipped with a diode array detector, using an RP-18 column (250 × 10 mm, 5 μm, YMC Pak; detector set at 220 nm and 254 nm) with a flowrate of 3.0 mL/min.

2.2. Fungal material

The culture of *Ulocladium* sp. (Pleosporaceae) was isolated by one of the authors (L.G.) from the lichen *Everniastrum sp.* collected from Zixi Mountain, Yunnan, People's Republic of China, in November 2006. The fungus was identified by L.G. and assigned the accession no.65-11-81 in L.G.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar at 25 °C for 10 d. Agar plugs were inoculated in 500 mL Erlenmeyer flask containing 120 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization, and incubated at 25 °C on a rotary shaker at 170 rpm for 7 d. Large scale cultivation was carried out in thirty 500 mL Fernbach flasks each containing 80 g of rice and 120 mL of distilled H_2O. Each flask was inoculated with 5.0 mL of the culture medium and incubated at 25 °C for 40 d.

2.3. Extraction and isolation

The fermented rice substrate was extracted repeatedly with EtOAc (3 × 4 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (6.78 g). The EtOAc extracts were subjected to silica gel column chromatography using *n*-hexane/EtOAc gradient elution (1:0, 50:1, 20:1, 10:1, 9:1, 8:2, 7:3, 6:4 v/v) and CH_2Cl_2/MeOH gradient elution (50:1, 20:1, 10:1, 9:1, 8:2, 7:3, 6:4,v/v). Fractions were analyzed by TLC, and grouped into 16 fractions (A–P). Fr. G (433.5 mg) was separated by ODS column chromatography using a gradient of MeOH in H_2O (20%, 30%, 50%, 70%, 90%, 100%) to give 10 subfrations (G1–G10). Fr. G2 (65.2 mg) was purified by RP-HPLC (45% MeOH in H_2O) to afford **2** (2.2 mg, t_R 16.9 min), **3**(3 mg, t_R 14.8 min), **9** (60 mg, t_R 28.4 min), and the mixture of **1** and **10** (12 mg) that were further purified by RP-HPLC (40% MeOH in water) to yield **1** (3.7 mg, t_R 18.5 min) and **10** (6.9 mg, t_R 22.4 min). Fr. J (700 mg) was first separated by ODS column chromatography using MeOH/H_2O gradient elution (20%,

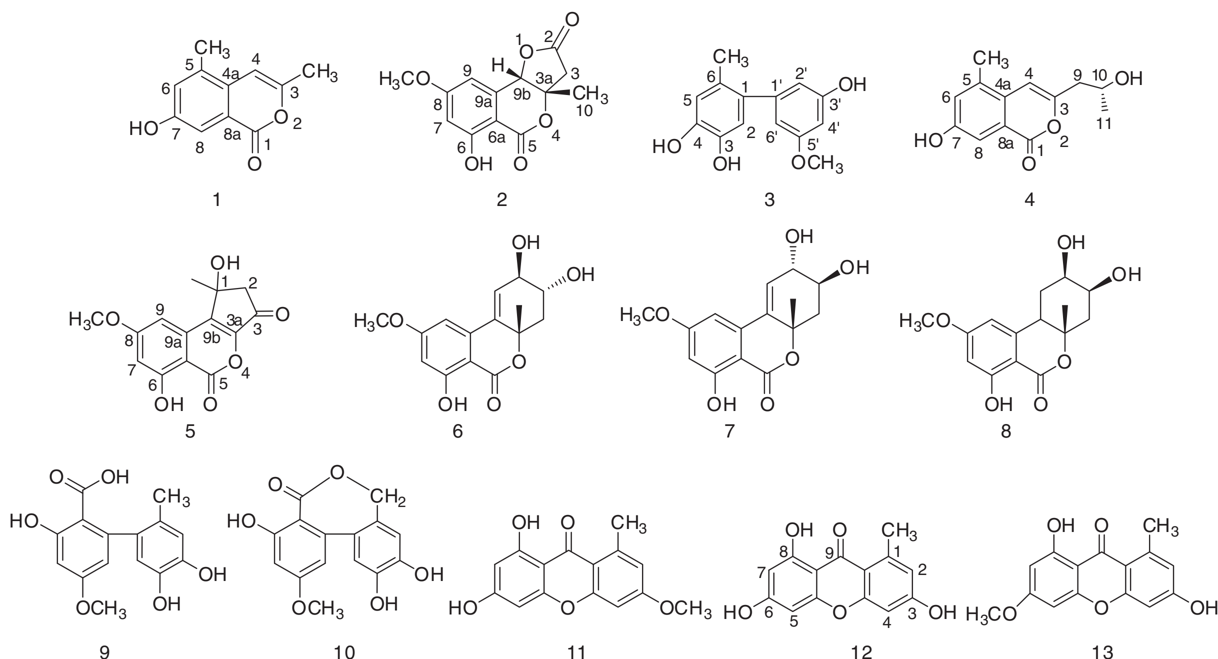


Fig. 1. Structures of isolated compounds **1–13.**

30%, 50%, 70%, 90%, 100%) to give 14 subfrations (J1–J14). **4** (2.2 mg, t_R 23.9 min) and **8** (3.6 mg, t_R 44.65 min) were obtained from Fr. J2 (112 mg) by RP-HPLC (20% CH_3CN in 0.04% TFA water). Fr. E (364 mg) and Fr. I (542 mg) were isolated in the same manner as described for Fr. J to give 13 subfrations (E1–E13) and 11 subfractions (I1–I11), respectively. **5** (6 mg, t_R 20.1 min) was purified from the Fr. E5 by RP-HPLC (55% MeOH in 0.04% TFA water). **6** (3.1 mg, t_R 31.0 min) and **7** (16 mg, t_R 28.2 min) were isolated from the Fr. I4 by RP-HPLC (18% CH_3CN in 0.04% TFA water). Fr. F was separated by Sephadex LH-20 column chromatography using 1:1 CHCl3/MeOH as eluents to yield **11**(5.7 mg) and **12**(6 mg). Compound **13** was obtained from Fr. D by RP-HPLC (70% MeOH in 0.04% TFA water; 8.6 mg, t_R 31.72 min).

2.3.1. Compound **1**

White powder, UV (MeOH) λ_{max} nm (log ε): 224(2.90), 251(2.89), 294 (2.68) ; IR(ν_{max}): 2968, 2921, 2685, 1655, 1621, 1550, 1441, 1350, 1270, 1160, 937, 852, 723 cm^{-1}; Positive ESI-MS: *m/z* 213 $[M+Na]^+$; Negative ESI-MS: *m/z* 189 $[M\text{-}H]^-$; Positive HRTOFMS: *m/z* $[M+H]^+$ 191.0704 (calcd. for $C_{11}H_{11}O_3$, 191.0703); for ^1H and ^{13}C-NMR spectroscopic data see Table 1.

2.3.2. Compound **2**

White powder, [α]25 D + 1.0 (c 0.1, MeOH); UV (MeOH) λ_{max} nm (log ε): 215(4.07), 232(4.05), 268(3.94), 307 (3.84); IR(ν_{max}): 3157, 2984, 1660, 1586, 1509, 1442, 1299, 1199, 973, 842, 721 cm^{-1} ; Positive ESI-MS: *m/z* 287 $[M+Na]^+$; Negative ESI-MS: *m/z* 263 $[M\text{-}H]^-$; Positive HRTOFMS: *m/z* $[M+H]^+$ 265.0708 (calcd. for $C_{13}H_{13}O_6$, 265.0707); for ^1H and ^{13}C-NMR spectroscopic data see Table 2.

Table 1
NMR spectral data for **1** and **4** in DMSO (500 MHz).

Position	**1**			**4**		
	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC
1	178.7			178.2		
3	164.3			164.8		
4	111.2	5.97 (*s*)	3, 3-CH_3, 4a, 5	111.5	5.95 (*s*)	3, 4a, 5, 9
4a	114.7			114.5		
5	141.9			141.4		
6	117.0	6.59–6.61 (*d*, 2.0)	5, 5-CH_3, 7, 8	116.4	6.59–6.61 (*d*, 2.0)	5, 5-CH_3, 7, 8
7	161.4			160.8		
8	101.0	6.62–6.63 (*d*, 2.0)	1, 4a, 6, 7, 8a	100.6	6.61–6.63 (*d*, 2.0)	4a, 6, 7, 8a
8a	159.6			159.2		
9				42.8	2.54–2.57 (*dd*, 14.0, 7.5) 2.57–2.61 (*dd*, 14.0, 5.0)	3, 4, 10, 11
10				64.0	3.99–4.05 (*m*)	3
11				23.4	1.12–1.16 (*d*, 6.5)	9, 10
7-OH		10.6 (*s*)	6, 7, 8		10.5 (*s*)	6, 7, 8
10-OH					4.81–4.85 (*d*, 5.0)	9, 10, 11
3-CH_3	19.8	2.26 (*s*)	3, 4			
5-CH_3	22.9	2.64 (*s*)	4a, 5, 6	22.4	2.64 (*s*)	4a, 5, 6

Table 2
NMR spectral data for **2** in DMSO (500 MHz).

Position	**2**		HMBC
	δ_C	δ_H (J in Hz)	
2	173.8		
3	42.7	2.93–2.99 (*d*, 16.5) 3.30–3.32 (*d*, 16.5)	2, 3a, 9b,10
3a	86.7		
5	166.5		
6	164.1		
6a	99.5		
7	103.4	6.71–6.72 (*d*, 2.5)	6, 6a, 8, 9
8	166.4		
9	110.8	6.83–6.85 (*d*, 2.5)	6a, 7, 8, 9b
9a	133.8		
9b	77.3	5.54 (*s*)	3a, 6a, 9, 9a, 10
10	20.4	1.53 (*s*)	3, 3a, 9a
6-OH		11.1 (*br. s*)	6, 6a, 7
8-OCH_3	56.6	3.86 (*s*)	8

2.3.3. Compound **3**

White powder, UV (MeOH) λ_{max} nm (log ε): 216(3.91), 232(3.91), 257(3.74), 388(3.58); IR(ν_{max}): 3223, 1680, 1594, 1434, 1281, 1202, 1147, 957, 843, 722 cm^{-1}; Positive ESI-MS: *m/z* 269 $[M+Na]^+$; Negative ESI-MS: *m/z* 245 $[M\text{-}H]^-$; Positive HRTOFMS: *m/z* $[M+H]^+$ 247.0968 (calcd. for $C_{14}H_{15}O_4$, 247.0965); for ^1H and ^{13}C-NMR spectroscopic data see Table 3.

2.3.4. Compound **4**

White powder, [α]25 D + 17.8 (c 0.3, MeOH); UV (MeOH) λ_{max} nm (log ε): 224(3.28), 251(3.21), 294(3.01); IR(ν_{max}): 3474, 2975, 2899, 2679, 1683, 1644, 1564, 1441, 1277, 1205, 953, 843, 722 cm^{-1}; Positive ESI-MS: *m/z* 257 $[M+Na]^+$; Negative ESI-MS: *m/z* 233 $[M\text{-}H]^-$; Positive HRTOFMS: *m/z* $[M+H]^+$ 235.0962 (calcd. for $C_{13}H_{15}O_4$, 235.0965); for ^1H and ^{13}C-NMR spectroscopic data see Table 1.

Table 3
NMR spectral data for **3** in DMSO (500 MHz).

Position	**3**		HMBC
	δ_C	δ_H (J in Hz)	
1	132.7		
2	117.1	6.54 (*s*)	1, 1′, 3, 4, 6
3	144.5[a]		
4	143.5[a]		
5	118.0	6.60 (*s*)	1, 3, 4, 6-CH_3
6	125.3		
1′	144.8		
2′	109.3	6.21 (*br.s*)	1, 1′, 3′, 4′, 6′
3′	158.5		
4′	99.7	6.25 (*br.s*)	2′, 3′, 5′, 6′
5′	160.4		
6′	106.2	6.20 (*br.s*)	
6-CH_3	19.8	2.05 (*s*)	1, 1′, 2′, 4′, 5′
3-OH		8.72 (*br.s*)	1, 5, 6
4-OH		8.78 (*br.s*)	2, 3, 4
3′-OH	55.4	9.42 (*br.s*)	3, 4, 5
5′-OCH_3		3.69 (*s*)	2′, 3′, 4′, 5′

2.4. Antimicrobial bioassay

Antimicrobial bioassays were conducted in triplicate by following National Center for Clinical Laboratory Standards (NCCLS) recommendations. *Escherichia coli* (ATCC 25922), *C. albicans* SC5314, *B. subtilis*(ATCC 6051), methicillin-resistant *Staphylococcus aureus* (MRSA, clinical isolates, Beijing Chaoyang Hospital), *Pseudomonas aeruginosa*(PAO1) and *Aspergilus fumigatus* (CGMCC 3.5835) were obtained from China General Microbial Culture Collection (CGMCC). *E. coli*, *P. aeruginosa*, *C. albicans* SC5314 and *B. subtilis* was grown in an agar plate using a LB medium consisting of 0.5% yeast extract, 1% peptone, 0.5%NaCl, 2% agar in deionized H_2O. *A. fumigatus* strain and MRSA were grown in the PDA medium. The assay was carried out in flat bottom 96-well microtiter plate, according to the method described in J. Riedlinger's report [5] with some modification. 2 μL of compounds dissolved in DMSO were transferred to each well. *B. subtilis*, *E. coli*, PAO1, and MRSA diluted by LB medium with the final concentration of 1×10^5 CFU/ml were added to 100 μL medium, and incubated at 28 °C for 24 h, and then the OD value was determined at 595 nm using a microplate reader. *C. albicans* SC5314 and *A. fumigatus* diluted by LB or PDB medium (final concentration 1×10^5 CFU/ml) were added to each well to achieve a final volume of 100 μL with alamar blue (2.5% solution), after incubation at 28 °C for 48 h, the fluorescence intensity was measured at Ex/Em = 544/590 nm using a fluorescent microplate reader. The inhibition rate was calculated and plotted versus test concentrations to afford the IC_{50}.

2.5. Scavenging ability on 1, 1-diphenyl-2-picrylhydrazyl radicals (DPPH) [6]

Each compound in DMSO (final concentration 200 μM, 100 μL) was mixed with 100 μL ethanolic solution containing DPPH (sigma) radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. Ascorbic acid was used as a positive control.

3. Results and discussion

The molecular formula of **1** was assigned as $C_{11}H_{10}O_3$ (seven degrees of unsaturation) on the basis of HRTOFMS at *m/z* 191.0704 (calcd. for $C_{11}H_{11}O_3$, 191.0703) and NMR data (Table 1). The ^{1}H NMR spectrum of **1** exhibited signals for a pair of meta-coupled aromatic protons at δ_H 6.60 (*d*, *J* = 2.0 Hz) and 6.62 (*d*, *J* = 2.0 Hz), an olefinic proton at δ_H 5.97 (*br s*), two methyl at δ_H 2.26 (s) and 2.64 (s), and one phenolic hydroxyl group at δ_H 10.6 (*s*). Its ^{13}C NMR spectrum showed signals due to two methyl carbons and nine sp^2 carbons including three CH = carbons assigned by HSQC spectrum and an ester carbonyl carbon at δ_C 178.7. These data accounted for all the ^{1}H and ^{13}C NMR resonances and required **1** to be a bicyclic compound. The HMBC correlations from H-C(4) to C(3), C(4), and C(5), from H-C(6) to C(4a), C(5), C(7), and C(8), from H-C(8) to C(1), C(6), C(7), C(8), C(4a), and C(8a), as well as the molecular formula of **1** established an isocoumarin skeleton. Two methyl groups and one hydroxyl group was attached at C(3), C(5), and C(7) by HMBC correlations from methyl protons at δ_H 2.64 to C(4a), C(5), and C(6), from methyl protons at δ_H 2.26 to C(3), and C(4), and from phenolic hydroxyl proton to C(6), C(7), and C(8). Based on the above analysis, **1** was determined to be 7-hydroxy-3, 5-dimethyl-isochromen-1-one.

The molecular formula of **2** was assigned as $C_{13}H_{12}O_6$ (eight degrees of unsaturation) on the basis of HRTOFMS at *m/z* 265.0708 (calcd. for $C_{13}H_{13}O_6$, 265.0707) and NMR data (Table 2). The ^{1}H and ^{13}C NMR spectra together with HSQC correlations of **2** showed resonances for one methoxyl group (δ_H/δ_C, 3.86 (*s*)/56.6), one phenolic hydroxyl group (δ_H, 11.1 (*br.s*)), one methyl group (δ_H/δ_C, 1.53 (*s*)/20.4), one oxymethine (δ_H/δ_C, 5.54 (*s*)/77.3), one methylene (δ_H/δ_C, 2.97 (*d*, *J* = 16.5 Hz); 3.33 (*d*, *J* = 16.5 Hz)/42.7), one oxygenated quaternary carbon (δ_C, 86.7), one tetrasubstituted benzene ring including two meta-coupled aromatic protons (δ_H, 6.72 (*d*, *J* = 2.5 Hz); 6.842 (*d*, *J* = 2.5 Hz)), and two carbonyl carbons. These data accounted for all the ^{1}H and ^{13}C NMR resonances and required **2** to be a tricyclic compound. The HMBC correlations from H-C(7) to C(6), C(6a), C(8), and C(9), from H-C(9) to C(6a), C(7), C(8), and C(9b), from methoxyl protons to C(8), from hydroxyl proton to C(6), C(6a), and C(7), from H-C(9b) to C(3a), C(6a), C(9), C(9a) and C(10), from H_3-C(10) to C(3), C(3a), and C(9b), and from H-C(3) to C(2), C(3a), C(9b), and C(10) established the partial structure for **2** (Fig. 2). Considering the chemical shifts for the remaining carbons (δ_C, 166.5 and 173.8) and the requirement of the unsaturation degree, **2** should have two lactone moieties. The ^{13}C NMR data for the benzene ring and the bigger chemical shift for HO-C(6) (δ_H, 11.1) in **2** was quite similar to that of **5**, which indicated an isocoumarin structural feature. The HMBC correlation from H-C(3) to C(2) and the four-bond *W*-type HMBC correlation from H-C(3) to C(5) further confirmed the gross structure of **2** to be 6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3H-furo [3,2-c]isochromene-2,5-dione. The relative configurations were determined by NOESY correlation between H-C(9b) and H_3-10. Since the optical rotation value of **2** was near the zero and its CD spectrum showed no Cotton effect, compound **2** was obtained as a racemate. The separation of **2** on a Chiralcel OD column (Hexane/2-propanol) failed to yield each isomer. Compound **2** possess a novel chemical skeleton.

Compound **3** was determined to have the molecular formula of $C_{14}H_{14}O_4$ (eight degrees of unsaturation) on the basis of HRTOFMS at *m/z* 247.0968 (calcd. for $C_{14}H_{15}O_4$, 247.0965) and NMR data (Table 3). The ^{1}H and ^{13}C NMR spectra of **3** showed resonances for one methoxyl group (δ_H/δ_C, 3.69 (*s*)/55.4), three phenolic hydroxyl groups (δ_H, 8.72 (*br.s*), 8.78 (*br.s*), 9.42 (*br.s*)), one methyl group (δ_H/δ_C, 3.05 (*s*)/19.8), two benzenes including five aromatic protons (δ_H, 6.20(*br.s*), 6.21(*br.s*), 6.25(*br.s*), 6.54(*s*), and 6.60 (*s*)). The substitutions on two benzenes and signal assignments were achieved by HMBC correlation analysis (Table 3). Compound **3** has been reported as the decarboxylated product of altenusin and the reduction product of dehydroaltenusin [7]. It is the first time to report the isolation of **3** as a natural product and its structural assignment.

Compound **4** was determined to be have the molecular formula of $C_{13}H_{14}O_4$ (seven degrees of unsaturation) on the basis of HRTOFMS at m/z 235.0962 (calcd. for $C_{13}H_{15}O_4$, 235.0965) and NMR data (Table 1). Analysis of its ^{1}H and

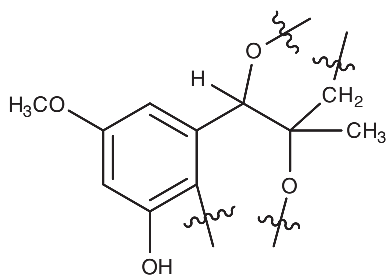


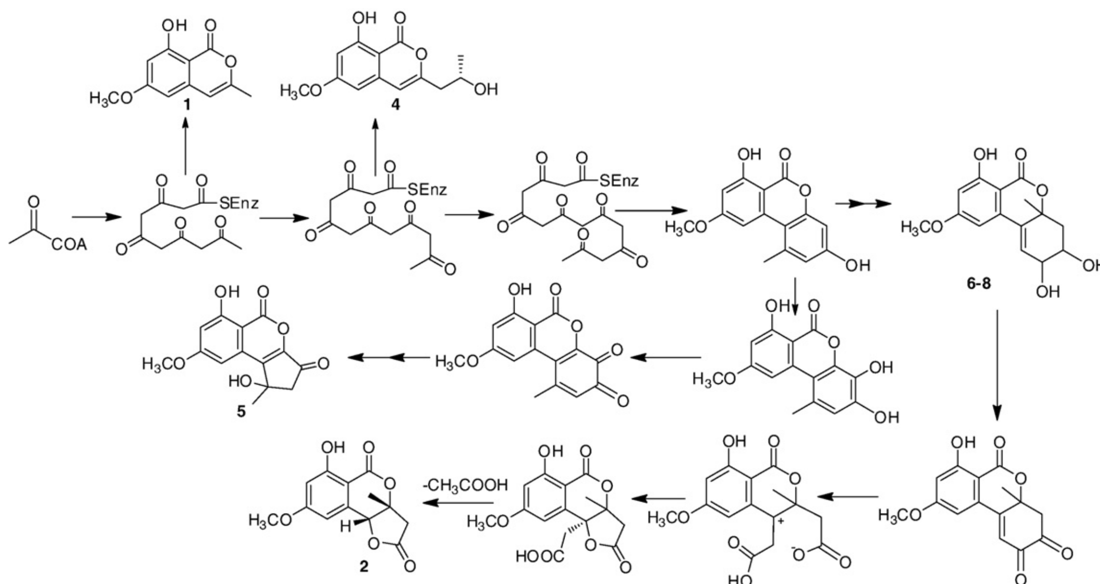
Fig. 2. Partial structure of **2**.

^{13}C NMR data revealed an isocoumarin structural feature similar to those of **1**. The ^{1}H and ^{13}C NMR signals at δ_H/δ_C 2.55 (*dd*, *J* = 14.0, 7.5 Hz) and 2.59 (*dd*, *J* = 14.0, 5.0 Hz)/ 48.8, 4.01 (*m*)/64.0, 1.12 (*d*, *J* = 6.5 Hz)/23.4, and 4.81 (*d*, *J* = 5.0 Hz, an alcohol hydroxyl proton) together with HMBC correlations from H-C(11) to C(9) and C(10), from the hydroxyl proton at δ_H 4.81 to C(9) and C(10) confirmed the presence of 2-hydroxyl-propanyl group. The attachment position of 2-hydroxyl-propanyl group was determined to be at C(3) by HMBC correlations of H-C(9) to C(3) and C(4) and H-C(4) to C(9). The HSQC and HMBC spectral analyses established the gross structure of **4** to be 7-hydroxy-3-(2-hydroxy-propyl)-5-methyl-isochromen-1-one. The absolute configuration at C-10 was determined to be *R* by comparison of its optical rotation value with that of orthosporin (**4**: [α]25 D + 17.8 (c 0.3, MeOH); [α]22 D + 61.8, orthosporin) [8,9]. While **4** has been collected in the Aurora Screening Library (http://www.aurorafiinechemicals.com), the information about its isolation and structure assignments have not been published. Herein, we report the isolation and structure assignment of **4** for the first time.

The structures of rubralactone (**5**), isoaltenuene (**6**), altenuene (**7**), dihydroaltenuene A (**8**), altenusin (**9**), alterlactone (**10**), 6-O-methylnorlichexanthone (**11**), norlichexanthone (**12**), and griseoxanthone C (13) were confirmed by comparison of their NMR and MS data with literature values [10, 11–16]. Compounds **6**–**10** have been previously reported from isolates of the common fungal genus *Alternaria*, and found to show broad antimicrobial activity against bacterial and fungal strains [13,17–19]. Based on the biosynthetic studies of alternariol [20] and cephalosol [21], the biosynthetic pathways for **1**, **2**, **4**–**8** were proposed in Scheme 1. Compound **2** could be synthesized using altenuenes as intermediates.

In antibacterial assays (Table 4), compounds **11**–**13** showed strong activity against *B. subtilis* with IC_{50} in the range of 1–5 μM. The new compound **2** showed weak activity against *B. subtilis* with IC_{50} value of 94.4 ± 4.21 μM. These metabolites contributed to the strong activity against *B. subtilis* detected in culture extract. Norlichexanthone (**12**) significantly inhibited the growth of methicillin-resistant *S. aureus* (MRSA) with IC_{50} of 20.95 ± 1.56 μM. This is the first report of the anti-MRSA activity for **12**. None of the compounds showed activity against *E. coli* and *Pseudomonas aeruginosa* at a concentration up to 250 μM. Although the culture extract of the fungus *Ulocladium* sp. showed strong antifungal activity against *C. albicans* and *A. fumigatus*, compounds isolated did not present strong antifungal activity. Thus, the strong antifungal activity observed in its culture extract might be the combinational effects from these metabolites. Due to the phenolic hydroxyl feature in the structures, the radical scavenging activity of **1**–**13** was evaluated by DPPH bioassay. Compounds **3**, **9**, and **10** with two ortho phenolic hydroxyl groups in structures showed stronger radical scavenging activity than other metabolites (Table 4).

The mutual relationship between lichen and endolichenic fungi is complex, and remaining unclear. It is generally believed that endolichenic fungi might have similarly beneficial effects to the growth of host lichen as that of plant endophytes [22–24]. In this work, the secondary metabolites from the endolichenic fungus *Ulocladium* sp. were found to have some antioxidant and antimicrobial activities. These



Scheme 1. Plausible biosynthetic pathway for metabolites **1**, **2**, and **4–8**.

Table 4
Antimicrobial and antioxidant activities for compounds **1**–**13**.

Compounds	*Bacillus subtilis*	methicillin-resistant *Staphylococcus aureus*	*Candida albicans*	*Aspergilus fumigatus*	DPPH
$IC_{50} \pm \mu M$					
1	>250	>250	97.9 ± 1.12	>250	>200
2	94.4 ± 4.21	>250	>250	>250	>200
3	>250	>250	>250	>250	148 ± 3.24
4	>250	>250	193 ± 2.98	>250	>200
5	>250	>250	198 ± 2.68	229 ± 8.26	>200
6	50.3 ± 3.49	>250	>250	>250	>200
7	>250	>250	>250	>250	>200
8	>250	>250	>250	>250	>200
9	39.1 ± 2.22	>250	>250	198 ± 3.69	52.8 ± 3.57
10	41.1 ± 3.05	>250	>250	>250	99.0 ± 1.67
11	1.43 ± 0.18	>250	>250	>250	>200
12	2.25 ± 0.09	20.9 ± 1.56	>250	169 ± 3.23	>200
13	1.29 ± 0.45	>250	149 ± 2.75	>250	>200
Gentamicin	<0.1	NT	NT[a]	NT	NT
Vanomycin	NT	<0.71	NT	NT	NT
Amphotericin B	NT	NT	1.11 ± 0.18	0.80 ± 0.09	NT
Vitamin C	NT	NT	NT	NT	51.7 ± 2.74

[a] Not tested in bioassay.

bioactive metabolites could protect the host lichen against oxidation stress and pathogenic microorganism in nature.

Acknowledgements

This work was supported by National Key Basic Research Project of China (2009CB522300) and MOST (2007DFB31620), Chinese National 863 High Technology (2007AA09Z443), NSFC (21072219, 31000036, 3091112048, 30911120484, 30901849), and grants from Chinese Academy Science (KSCX2-YW-G-074-04).

References

[1] Wang Y, Zheng Z, Liu S, Zhang H, Li E, Guo L, Che Y. J Nat Prod 2010;73:920.
[2] Paranagama PA, Wijeratne EMK, Burns AM, Marron MT, Gunatilaka MK, Arnold AE, Gunatilaka AAL. J Nat Prod 2007;70:1700.
[3] Ding G, Li Y, Fu S, Liu S, Wei J, Che Y. J Nat Prod 2009;72:182.
[4] Zhang F, Liu S, Lu X, Guo L, Zhang H, Che Y. J Nat Prod 2009;72:1782.
[5] Riedlinger J, Reicke A, Zahner H, Krismer B, Bull AT, Maldonado LA. J Antibiot 2004;57:271.
[6] Hatano T, Kagawa H, Yasuhara T, Okuta T. Chem Pharm Bull 1988;36:1090.
[7] Kameda K, Aoki H, Namiki M. Tetra Lett 1974;15:103.
[8] Hallock YF, Clardy J, Kenfield DS, Strobel G. Phytochemistry 1988;27:3123.
[9] Ichihara A, Hashimoto M, Hirai T. Chem Lett 1989;18:1495.
[10] Kimura Y, Yoshinari T, Koshino H, Fujioka S, Okada K, Shimada A. Biosci Biotechnol Biochem 2007;71:1896.
[11] Bradburn N, Coker RD, Blunden G, Turner CH, Crabb TA. Phytochemistry 1994;35:665.
[12] Visconti A, Botalico A, Solfrizzo M, Palmisano F. Mycotoxin Res 1989;5:69.
[13] Jiao P, Gloer JB, Campbell J, Shearer CA. J Nat Prod 2006;69:612.
[14] Coombe RG, Jacobs JJ, Watson TR. Aust J Chem 1970;23:2343.
[15] Aly AH, Edrada-Ebel R, Indriani ID, Wray V, Mu¨ller WEG, Totzke F, Zirrgiebel U, Scha¨chtele C, Kubbutat MHG, Lin WH, Proksch P, Ebel R. J Nat Prod 2008;71:972.
[16] Broadbent D, Mabelis RP, Sperncer H. Phytochemistry 1975;14:2082.
[17] King Jr AD, Schade JE. J Food Prot 1984;38:267.
[18] Schade JE, King AD. J Food Prot 1984;47:978.
[19] Kjer J, Wray V, Edrada-Ebel R, Ebel R, Pretsch A, Lin W, Proksch P. J Nat Prod 2009;72:2053.
[20] Stinson EE, Wise WB, Moreau RA, Jurewicz AJ, Pfeffer PE. Can J Chem 1986;64:1590.
[21] Zhang HW, Huang WY, Chen JR, Yan WZ, Xie DQ, Tan RX. Chemistry 2008;14:10670.
[22] Strobel GA. Can J Plant Pathol 2002;24:14.
[23] Zhang HW, Song YC, Tan RX. Nat Prod Rep 2006;23:753.
[24] Yang XS, Strobel G, Stierle A, Hess WM, Lee, Clardy J. Plant Sci 1994;102:1.