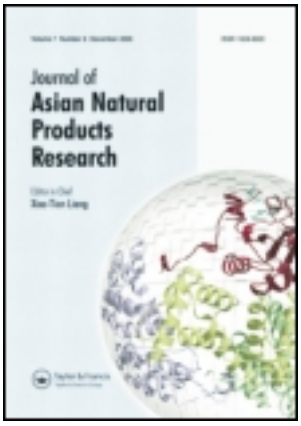

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Neuraminidase inhibitory terpenes from endophytic *Cochliobolus* sp.

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The chemical study of endophytic fungus of *Cochliobolus* led to the isolation of 10 terpenes (**1**–**10**), including one new compound named isocochlioquinone B (**1**). Their structures were elucidated on the basis of spectroscopic methods, including 2D NMR techniques. Compounds **5**–**7** showed significant neuraminidase inhibitory activity with IC_{50} values of 0.79–1.75 μM.

Keywords: *Cochliobolus*; isocochlioquinone; endophyte; neuraminidase inhibitor

1. Introduction

Endophytes are bacteria and fungi that reside in the tissues of the host plant without causing any plant disease. During the past two decades, the secondary metabolites of this kind of microorganisms have been studied and some of them have been shown to have novel structures and interesting biological activities [1].

Recent studies have shown that endophytes from medicinal plants of Poaceae are a promising resource of bioactive secondary metabolites [2–4]. *Phragmites australis* is a medicinal plant of Poaceae widely spread in China. However, little is known about the secondary metabolites of its endophytes. As a part of our continuous program to find bioactive and/or new compounds from the endophytes, one new terpene named isocochlioquinone B, together with nine known metabolites (Figure 1), was isolated from the culture broth of the strain *Cochliobolus* sp. derived from the leaves of *P. australis.*

2. Results and discussion

Isocochlioquinone B (**1**) was afforded as yellow solid, and its molecular formula was determined to be $C_{30}H_{42}O_8$ by analysis using HR-ESI-MS (*m/z* 553.2782 $[M + Na]^+$, calcd. for 553.2777). Its ^1H and ^{13}C NMR (Table 1), DEPT and HMQC spectra revealed the presence of eight methyl, three oxymethine, and two carbonyl carbons. The downfield shifted singlet at δ_H 10.77 indicated that the hydroxy group was hydrogen bonded to a carbonyl group. Comparison of the ^1H and ^{13}C NMR spectroscopic data of compound **1** with those of **2** showed that both structures were similar. The main difference observed in the ^1H NMR spectrum of compound **1** in relation to compound **2** was the presence of signals at δ_H 5.61 (H-2, q, $J = 7.0$ Hz), 1.62 (H-1, d, $J = 7.0$ Hz), and 1.63 (H-28, s). These different signals indicated that a double bond was located between C-2 and C-3, which was confirmed by the ^1H—^1H COSY correlation

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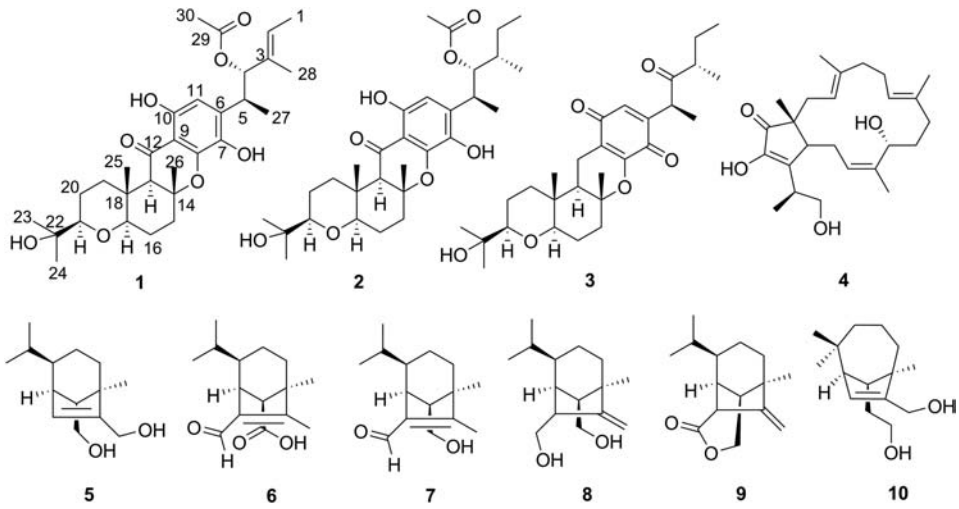

Figure 1. Structures of compounds **1**–**10**.

between H-1 and H-2, and HMBC correlations (Figure 2) from H-1 to C-2 (δ_C 125.2), C-3 (δ_C 132.1); H-4 to C-2, C-3; and H-28 to C-2, C-3. Thus, the planar structure of **1** was determined.

The observed NOE correlation between H-2 and H-4 revealed the *E-geometry* for the double bond at C-2. The relative configuration of the tricyclic moiety was assigned by NOESY correlations (Figure 2), such as from H-25 to H_β-16, from H-21 to H-17, from H-26 to H_β-16, from H-17 to H-15, from H-17 to H-19, from H-17 to H-13, and from H-25 to H-26. The relative configurations of the chiral centers on the aliphatic side chain were assumed to be identical to those of isocochlioquinone A, whose structure has been assigned by X-ray crystallography [5].

The known compounds, including two cochlioquinones, one sesterterpene, and six sesquiterpenes, were identified as isocochlioquinone A (**2**), cochlioquinone B (**3**) [5], terpestacin (**4**) [6], drechslerine A (**5**) [7], helminthosporal acid (**6**), helminthosporol (**7**), dihydroprehelminthosporol (**8**), prehelminthosporol lactone (**9**) [8], and secolongifolene diol (**10**) [9] by comparison of their spectroscopic data with literature values.

Neuraminidase plays an important role in the infection process of influenza viral, and treatment of viral infections using neuraminidase inhibitors has proven to be effective [10]. Compounds **1**–**10** were subjected to *in vitro* neuraminidase inhibitory evaluations and compounds **5**–**7** showed significant neuraminidase inhibitory activity comparable with positive control, oseltamivir, a clinical drug for influenza viral infections (Table 2).

This was the first report in which three kinds of terpenes were isolated from the strain *Cochliobolus* sp. To date, cochlioquinone and its derivatives have been reported mainly from fungi belonging to genera *Cochliobolus* (anamorph: *Bipolaris*) [11]. Therefore, cochlioquinone and its related derivatives could be of some biomarker values. Compounds **6**–**7** were usually thought to be plant-growth regulators [12,13] and received less attention on other biological activities. Herein, we first reported the potential neuraminidase inhibitory activity of these compounds.

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** ($CDCl_3$, J in Hz).

Position	δ_C	δ_H
1	13.1 (q)	1.62 (d, $J = 7.0$)
2	125.2 (d)	5.61 (q, $J = 7.0$)
3	132.1 (s)	
4	82.1 (d)	5.38 (d, $J = 9.0$)
5	36.1 (d)	3.43 (m)
6	140.1 (s)	
7	135.2 (s)	
8	143.8 (s)	
9	106.9 (s)	
10	153.5 (s)	
11	107.3 (d)	6.33 (s)
12	198.2 (s)	
13	60.4 (d)	2.76 (s)
14	83.4 (s)	
15	37.6 (t)	2.01–2.10 (m)
16	24.9 (t)	1.79–1.83 (m)
17	83.7 (d)	3.15 (dd, $J = 12.0, 3.0$)
18	35.4 (s)	
19	37.3 (t)	1.21–1.30 (m), 2.73[a]
20	21.2 (t)	1.40–1.50[a], 1.72–1.75 (m)
21	85.3 (d)	3.26 (dd, $J = 12.0, 2.0$)
22	71.9 (s)	
23	25.9 (q)	1.18 (s)
24	23.7 (q)	1.18 (s)
25	12.1 (q)	1.12 (s)
26	21.9 (q)	1.46 (s)
27	16.7 (q)	1.09 (d, $J = 7.5$)
28	11.3 (q)	1.63 (s)
29	169.8 (s)	
30	21.0 (q)	1.83 (s)
7-OH		5.08 (br s)
10-OH		10.77 (s)

Note: [a]Signals overlapped.

3. Experimental

3.1 General experimental procedures

HR-ESI-MS spectra were recorded on Agilent 6210 TOF LC/MS. IR spectra were obtained on Nexus 870 FT-IR spectrometer. NMR spectra were recorded on Bruker DPX-300 and DRX-500 NMR spectrometers. Silica gel (SiO_2, 200–300 mesh) for column chromatography (CC) and silica GF_{254} (10–20 mm) for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was obtained from Pharmacia Biotech, Uppsala, Sweden. HPLC was performed with a Hitachi L-7110 pump, and UV detector L-7400 equipped with an Apollo C18 column (5 μm, 250 × 4.6 mm; Alltech Associates, Inc. Chicago, IL, USA).

3.2 Extraction and isolation

The culture was filtered to separate broth and mycelia. The culture broth was extracted three times with EtOAc (3 × 20 l). The organic layer was then concentrated under reduced pressure to obtain a crude extract (1.5 g). The crude extract was separated by SiO_2 CC (200–300 mesh) and eluted with a gradient of chloroform:methanol (v:v = 100:0, 100:1, 100:2, 100:4, 100:8) to afford five fractions, F-1–F-5. F-2 (23.1 mg) was purified by HPLC (MeOH:H_2O; v:v = 80:20; 2.0 ml/min) to provide compound **9** (10.0 mg; 21.2–22.3 min). F-3 (200.3 mg)

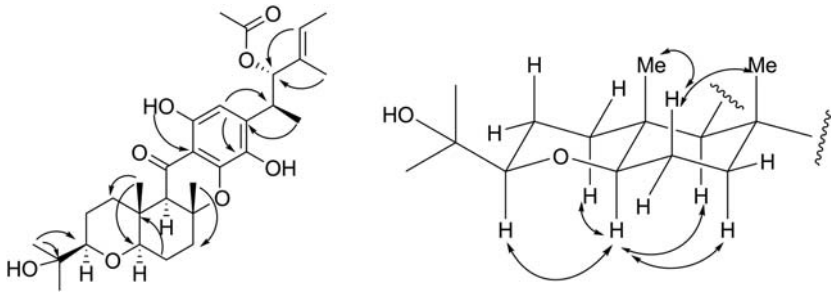

Figure 2. Key HMBC and NOESY correlations of **1**.

Table 2. IC_{50} values of compounds **1**–**10** tested *in vitro* for neuraminidase inhibitory activity.

Compound	IC_{50} (μM)
1	>40.0
2	>40.0
3	>40.0
4	>40.0
5	0.79 ± 0.07
6	1.75 ± 0.35
7	1.08 ± 0.27
8	>40.0
9	>40.0
10	>40.0
Oseltamivir	0.14 ± 0.09

was separated by ODS CC with MeOH:H_2O (v:v = 75:25) to afford six fractions, F-3-1–F-3-6. F-3-5 (61.5 mg) was further separated by HPLC (MeOH:H_2O; v:v = 85:15; 2.0 ml/min) to give **1** (10.2 mg; 39.1–40.2 min), **2** (30.3 mg; 36.1–36.9 min), and **3** (12.1 mg; 15.2–15.7 min). F-3-4 (15.3 mg) was purified by HPLC (MeOH:H_2O; v:v = 75:25; 2.0 ml/min) to afford **7** (10.7 mg, 25.3–26.0 min). F-4 (102.4 mg) was separated by ODS CC with a gradient system of MeOH:H_2O (v:v = 30:70, 50:50, 65:35, 70:30) to yield four fractions, F-4-1–F-4-4. F-4-2 (20.8 mg) was subjected to Sephadex LH-20 eluted with methanol to afford **4** (13.1 mg). F-4-3 (58.1 mg) was purified by Sephadex LH-20 using methanol, followed by HPLC (MeOH:H_2O; v:v = 78:22; 2.0 ml/min) to give **5** (7.1 mg, 21.3–22.0 min), **6** (5.3 mg, 23.1–23.5 min), and **8** (13.5 mg, 24.2–25.0 min). F-4-4 (20.3 mg) was purified by HPLC (MeOH/H_2O; v:v = 78:22; 2.0 ml/min) to yield **10** (11.2 mg, 26.1–26.7 min).

3.2.1 *Isocochlioquinone B (**1**)*

Yellow solid; $[\alpha]_D^{25} + 390$ (*c* 0.027, MeOH); UV (MeOH) λ_{max}: 276 and 381 nm; IR (KBr) ν_{max}: 3443, 1728, 1646, 1383 cm^{-1}; ^1H and ^{13}C NMR spectral data: Table 1. HR-ESI-MS: *m/z* 553.2782 $[M + Na]^+$ (calcd. For $C_{30}H_{42}O_8Na$, 553.2777).

References

[1] A.A.L. Gunatilaka, *J. Nat. Prod.* **69**, 509 (2006).

[2] H.M. Ge, Y.C. Song, C.Y. Shan, Y.H. Ye, and R.X. Tan, *Planta Med.* **71**, 1063 (2005).

[3] G. Ding, Y.C. Song, J.R. Chen, C. Xu, H.M. Ge, X.T. Wang, and R.X. Tan, *J. Nat. Prod.* **69**, 302 (2006).

[4] J.Y. Liu, Y.C. Song, Z. Zhang, L. Wang, Z.J. Guo, W.X. Zou, and R.X. Tan, *J. Biotechnol*. **114**, 279 (2004).

[5] H. Miyagawa, S. Nagai, T. Sato, M. Tsurushima, T. Ueno, and H. Fukami, *Biosci. Biotechnol. Biochem.* **58**, 1143 (1994).

[6] M. Oka, S. Iimura, O. Tenmyo, Y. Sawada, M. Sugawara, N. Ohkusa, H. Yamamoto, K. Kawano, S.L. Hu, Y. Fukagawa, and T. Oki, *J. Antibiot.* **46**, 367 (1993).

[7] C. Osterhage, G.M. Konig, U. Holler, and A.D. Wright, *J. Nat. Prod.* **65**, 306 (2002).

[8] L.M. Penarodriguez, N.A. Armingeon, and W.S. Chilton, *J. Nat. Prod.* **51**, 821 (1988).

[9] F. Dorn and D. Arigoni, *Experientia* **30**, 851 (1974).

[10] H.M. Dobrovolny, R. Gieschke, B.E. Davies, N.L. Jumbe, and C.A.A. Beauchemin, *J. Theor. Biol.* **269**, 234 (2011).

[11] (a) P. Phuwapraisirisan, K. Sawang, P. Siripong, and S. Tip-Pyang, *Tetrahedron Lett*. **48**, 5193 (2007). (b) K. Yoganathan, L.K. Yang, C. Rossant, Y.C. Huang, S. Ng, M.S. Butler, and A.D. Buss, *J. Antibiot.* **57**, 59 (2004). (c) H.J. Jung, H.B. Lee, C.H. Lim, C.J. Kim, and H.J. Kwon, *Bioorg. Med. Chem.* **11**, 4743 (2003). (d) C.H. Lim, H. Miyagawa, M. Akamatsu, Y. Nakagawa, and T. Ueno, *J. Pestic. Sci.* **23**, 281 (1998). (e) C.H. Lim, H. Miyagawa, T. Tsurushima, T. Ueno, and M. Sato, *Biosci. Biotechnol. Biochem.* **60**, 724 (1996).

[12] Y. Kamiya, M. Kobayashi, S. Fujioka, H. Yamane, I. Nakayama, and A. Sakurai, *Plant Cell Physiol.* **32**, 1205 (1991).

[13] T. Hashimot and S. Tamura, *Plant Cell Physiol.* **8**, 197 (1967).