Bioorganic & Medicinal Chemistry Letters 20 (2010) 5677–5680

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Indoloditerpenes from an algicolous isolate of *Aspergillus oryzae*

Ming-Feng Qiao [a,b], Nai-Yun Ji [a,*], Xiang-Hong Liu [a], Ke Li [c], Qing-Mei Zhu [a], Qin-Zhao Xue [a]

[a] *Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China*
[b] *Graduate School of Chinese Academy of Sciences, Beijing 100049, China*
[c] *Michigan State University, East Lansing, MI 48824, USA*

ARTICLE INFO

Article history:
Received 21 May 2010
Revised 4 August 2010
Accepted 5 August 2010
Available online 8 August 2010

Keywords:
Indoloditerpene
Aspergillus oryzae
Endophytic fungus
Heterosiphonia japonica
Red alga

ABSTRACT

Two new indoloditerpene derivatives asporyzin A (**1**) and asporyzin B (**2**), one new indoloditerpene asporyzin C (**3**), and three known related indoloditerpenes JBIR-03 (**4**), emindole SB (**5**), and emeniveol (**6**) were isolated from an endophytic fungus *Aspergillus oryzae*, isolated from the marine red alga *Heterosiphonia japonica*. Their structures were unambiguously established by spectroscopic techniques. In addition, all the isolates were evaluated preliminarily for insecticidal and antimicrobial activities in order to probe into their chemical defensive function. Compound **4** was more active against brine shrimp than the others, and **3** possessed potent activity against *Escherichia coli*.

Indoloditerpenes generally possess an indole nucleus connected to a partially or fully cyclized diterpene unit, and are produced by a diverse group of fungi including *Aspergillus*, *Penicillium*, *Nodulisporium*, *Emericella*, *Dichotomomyces*.[1–6] Some of these metabolites are known as tremorgens, and recently some of them exhibit potent insecticidal, antiinsectan, and antibiotic activities.[1–3,7] In our screening program for biologically active natural products from marine algae and their associated fungi, we have investigated an isolate of the fungus *Aspergillus oryzae* obtained from the marine red alga *Heterosiphonia japonica*. Two new indoloditerpene derivatives designated asporyzin A (**1**) and asporyzin B (**2**), one new indoloditerpene named asporyzin C (**3**), and three known indoloditerpenes JBIR-03 (**4**),[6] emindole SB (**5**),[5] and emeniveol (**6**)[4] were isolated and identified from the fungus (Fig. 1). Details of the isolation, structure elucidation, and bioactivity of compounds **1**–**6** are present here.

The EtOAc-soluble fraction derived from fermentation cultures of *A. oryzae* was purified by a combination of silica gel and Sephadex LH-20 column chromatography, as well as preparative TLC, to yield compounds **1**–**6**.

Asporyzin A (**1**) was obtained as a colorless oil. The molecular formula was determined to be $C_{28}H_{37}NO_3$ on the basis of HREIMS (*m/z* 435.2769 $[M]^+$, calcd for $C_{28}H_{37}NO_3$, 435.2773), indicating 11 degrees of unsaturation. The ^1H, ^{13}C, and DEPT NMR spectra (Table 1) along with HSQC data revealed signals characteristic of three methyl groups (C-24, C-25, and C-30), two vinyl methyl groups (C-28 and C-29), six sp^3 methylenes (C-5, C-6, C-10, C-13, C-14, and C-16), two sp^3 methines (C-12 and C-15), two oxygenated methines (C-7 and C-9), one olefinic methine (C-26), four aromatic methines (C-19, C-20, C-21, and C-22), three sp^3 quaternary carbons (C-3, C-4, and C-11), one olefinic quaternary carbon (C-27), two aromatic quaternary carbons (C-18 and C-23), and two carbonyl carbons (C-2 and C-17). A detailed NMR data comparison with those reported for JBIR-03 (**4**) revealed that **1** differed from **4** mainly at C-2, C-17, and their vicinal positions C-3, C-15, C-16, and C-18 (δ_C 151.9, 117.8, 53.9, 50.1, 28.3, and 126.0 for JBIR-03).[6] However, the chemical shifts of C-2, C-17, and their vicinal carbons were identical with those of 2,18-dioxo-2,18-secopaxilline (δ_C 176.0, 203.2, 57.4, 35.4, 47.8, and 134.7), which suggested the presence of an eight-membered keto-amine central ring in **1**.[8] Thus, **1** was established to be a dioxoindole derivative of **4**, presumably formed via oxidation of the C-2–C-17 bond of **4**. The connectivity of **1** was further verified by analysis of ^1H–^1H COSY and HMBC data as shown in Figure 2.

The relative configuration of **1** was established to be the same as for **4** by analysis of NOESY correlations. In the NOESY spectrum, the observed correlations of H-12 with H-7 and H-24 indicated H-12, H-7, and C-24 to be located on the same face of the molecule. Likewise, the correlations between H-25/H-15, H-30 and between H-9/H-30 allowed H-9, H-15, C-25, and C-30 to be the same orientation. C-24 and C-25 were opposite based on the NOESY correlation between H-24/H-5b and between H-25/H-5a. So, H-15 and C-24 were also located on the opposite face. These configurations matched those found in **4** and were confirmed by the identical chemical shifts of C-4, C-7, C-9, C-11, C-12, and C-15 in **1** and **4**.

* Corresponding author. Tel.: +86 535 2109176; fax: +86 535 2109000.
E-mail address: nyji@yic.ac.cn (N.-Y. Ji).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.08.024

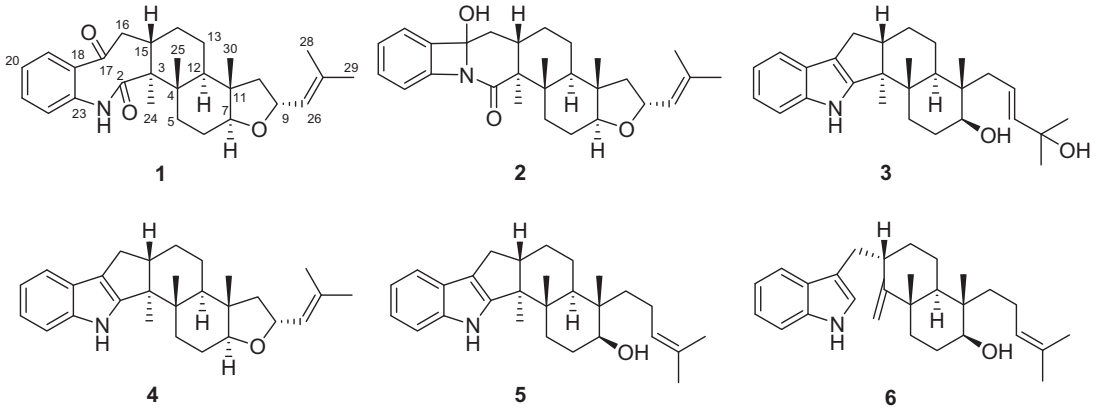

Figure 1. Structures of compounds **1**–**6**.

Table 1
^{1}H and ^{13}C NMR data of asporyzins A–C (**1**–**3**)

No.	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	7.15 (br s)				7.70 (br s)	
2		176.6 (s)		175.7 (s)		150.8 (s)
3		59.7 (s)		53.7 (s)		53.0 (s)
4		42.1 (s)		41.4 (s)		39.2 (s)
5a	1.62 (m)	33.9 (t)	1.80 (m)	32.6 (t)	1.58 (m)	33.4 (t)
5b	1.78 (m)		2.34 (m)		1.90 (m)	
6a	1.59 (m)	22.3 (t)	1.78 (m)	22.4 (t)	1.82 (m)	27.4 (t)
6b	1.88 (m)		1.96 (m)		1.88 (m)	
7	3.17 (dd, 12.2, 3.2)	85.5 (d)	3.22 (dd, 12.1, 3.4)	86.1 (d)	3.52 (t, 7.7)	73.7 (d)
9	4.77 (m)	73.8 (d)	4.81 (m)	73.4 (d)	5.72 (m)	122.6 (d)
10a	1.14 (m)	48.9 (t)	1.23 (m)	48.7 (t)	2.00 (m)	40.2 (t)
10b	1.87 (m)		1.94 (m)		2.33 (m)	
11		45.0 (s)		45.1 (s)		42.4 (s)
12	1.50 (dd, 13.0, 2.6)	45.0 (d)	1.68 (m)	46.6 (d)	1.64 (dd, 12.5, 3.0)	40.6 (d)
13a	1.34 (m)	23.9 (t)	1.49 (m)	24.3 (t)	1.42 (m)	22.5 (t)
13b	1.43 (m)		1.80 (m)		1.73 (m)	
14a	1.44 (m)	30.0 (t)	1.53 (m)	24.9 (t)	1.58 (m)	25.0 (t)
14b	1.57 (m)		1.69 (m)		1.77 (m)	
15	2.84 (m)	34.9 (d)	2.60 (m)	37.3 (d)	2.75 (m)	48.8 (d)
16a	2.38 (dd, 18.0, 4.9)	48.7 (t)	1.98 (dd, 12.2, 6.2)	39.7 (t)	2.32 (dd, 13.2, 3.7)	27.4 (t)
16b	2.99 (dd, 18.0, 3.4)		2.14 (dd, 12.2, 5.3)		2.67 (dd, 13.2, 6.4)	
17		202.9 (s)		93.7 (s)		118.4 (s)
18		134.8 (s)		133.9 (s)		125.1 (s)
19	7.67 (dd, 7.6, 1.5)	129.3 (d)	7.42 (dd, 7.8, 1.6)	127.3 (d)	7.42 (d, 7.8)	118.4 (d)
20	7.37 (t, 7.6)	127.6 (d)	7.07 (td, 7.8, 1.3)	122.8 (d)	7.06 (t, 7.8)	119.6 (d)
21	7.48 (td, 7.6, 1.5)	132.7 (d)	7.17 (td, 7.8, 1.6)	127.9 (d)	7.08 (t, 7.8)	120.5 (d)
22	7.08 (d, 7.6)	127.2 (d)	7.03 (dd, 7.8, 1.3)	117.6 (d)	7.29 (d, 7.8)	111.4 (d)
23		136.9 (s)		143.8 (s)		139.9 (s)
24	1.37 (s)	15.3 (q)	1.14 (s)	16.6 (q)	0.97 (s)	14.5 (q)
25	1.01 (s)	19.6 (q)	1.20 (s)	20.8 (q)	1.10 (s)	19.0 (q)
26	5.31 (d, 9.0)	126.7 (d)	5.33 (d, 9.0)	126.9 (d)	5.72 (m)	141.5 (d)
27		134.9 (s)		134.9 (s)		70.8 (s)
28	1.66 (s)	18.0 (q)	1.70 (s)	18.0 (q)	1.34 (s)	30.0 (q)
29	1.72 (s)	25.9 (q)	1.74 (s)	25.9 (q)	1.35 (s)	30.3 (q)
30	0.79 (s)	15.5 (q)	0.92 (s)	15.2 (q)	0.86 (s)	15.7 (q)
OH			2.82 (br s)			

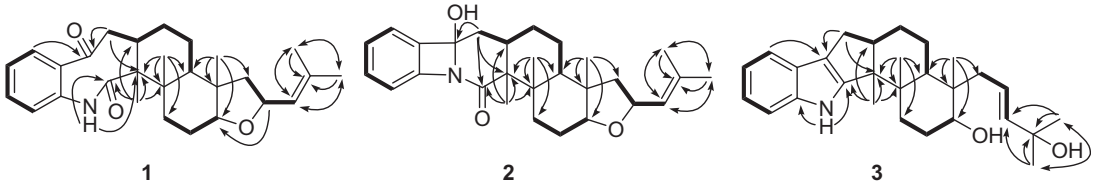

Figure 2. Key correlations in ^{1}H–^{1}H COSY (bold lines) and HMBC (arrows) spectra of **1**–**3**.

Asporyzin B (**2**) was obtained as a colorless oil. The molecular formula was determined as $C_{28}H_{37}NO_3$ based on HREIMS (*m/z* 435.2767 $[M]^+$, calcd for $C_{28}H_{37}NO_3$, 435.2773), suggesting 11 degrees of unsaturation. Compound **2** was deduced to be an analog of **1** on the basis of their identical elemental compositions. On the other hand, the ^1H, ^{13}C, and DEPT NMR spectra (Table 1) along with HSQC data also revealed signals characteristic of three methyl groups (C-24, C-25, and C-30), two vinyl methyl groups (C-28 and C-29), six sp^3 methylenes (C-5, C-6, C-10, C-13, C-14, and C-16), two sp^3 methines (C-12 and C-15), two oxygenated methines (C-7 and C-9), one olefinic methine (C-26), four aromatic methines (C-19, C-20, C-21, and C-22), three sp^3 quaternary carbons (C-3, C-4, and C-11), one olefinic quaternary carbon (C-27), two aromatic quaternary carbons (C-18 and C-23), and one carbonyl carbons (C-2), which were in accordance with those of **1**. However, replacing a carbonyl signal at δ_C 202.9 (C-17) in the ^{13}C NMR spectrum of **1**, a signal at δ_C 93.7 for oxygenated quaternary carbon atom emerged in the ^{13}C NMR spectrum of **2**. Moreover, a broad singlet at δ_H 2.82 appeared in the ^1H NMR spectrum of **2** and the broad singlet at δ_H 7.15 for NH in **1** disappeared. By analysis of the above NMR data, **2** could be a derivative of **1**, which was formed by attacking of the lone electron pair of the nitrogen atom on the carbonyl at C-17. The presence of a hydroxyl group was verified by the fragment ion $[M-OH]^+$ peak at *m/z* 418 in the EIMS. The gross structure of **2** was further supported by the ^1H–^1H COSY and HMBC correlations as shown in Figure 2.

The relative configuration of **2** was also determined to be consistent with that of **4** based on NOESY data. The NOESY correlations between H-12/H-7, H-24 and between H-7/H-26 implied H-7, H-12, C-24, and C-26 to be located in the same direction. The same orientation for H-9, H-15, C-25, and C-30 was assigned according to the correlations between H-25/H-15, H-30 and between H-9/H-30. The only configuration that could not be independently assigned in **2** was that of C-17, due to a lack of relevant correlations.

Asporyzin C (**3**) was obtained as a colorless oil. The molecular formula $C_{28}H_{39}NO_2$ was established by HREIMS (*m/z* 421.2982 $[M]^+$, calcd for $C_{28}H_{39}NO_2$, 421.2981), implying 10 degrees of unsaturation. The ^1H NMR spectrum (Table 1) depicted five methyl singlets, one triplet characteristic of an oxygenated methine, one multiplet attributed to two olefinic protons, two triplets and two doublets assigned to four aromatic protons, and one broad singlet representative of a reactive proton. The ^{13}C and DEPT NMR spectra (Table 1) along with the HSQC experiment revealed the presence of 28 carbon atoms including five methyls, six methylenes, nine methines, and eight quaternary carbon atoms. A detailed comparison of the NMR data with those reported for emindole SB (**5**) revealed that **3** differed from **5** mainly at the side chain moiety.[5] However, the chemical shifts of this moiety were identical with those of 4-hydroxy-4-methylpent-2-enyl unit in polasol C and 3-bromo-2-chlorobisabol-9-en-7,11-diol.[9,10] The presence of this structural sequence was verified by the ^1H–^1H COSY correlation between H-9 and H-10 and the HMBC correlations from H-28 and H-29 to C-26 and C-27, which was supported by the fragment ion $[M-C_6H_{11}O]^+$ peak at *m/z* 322 in the EIMS. The planar structure of **3** was corroborated by the other correlations in ^1H–^1H COSY and HMBC spectra (Fig. 2).

The relative configuration of **3** was assigned by analysis of NOESY correlations and comparison with literature values. The same direction for H-7, H-12, C-10, and C-24 was supported by the NOESY correlations between H-12/H-7, H-24 and between H-7/H-10b. The correlations of H-25 with H-15, H-30 permitted H-15, C-25, and C-30 to be oriented on the same side. The double bond between C-9 and C-26 was assigned to be trans based on the identical NMR data with a corresponding unit.[9,10] The above spectral evidence established the structure of **3**.

Table 2
Lethal rates (lr) against brine shrimp (*Artemia salina*) and inhibitory rates (ir) against acetylcholinesterase at 100 μg/mL of **1**–**6**

	1	2	3	4	5	6	Huperzine A[a]
lr%	61.9	42.9	32.8	74.2	60.3	31.4	
ir%	13.7	4.0	7.3	−7.6	16.4	−11.4	98.0

[a] Positive control at 100 μg/mL in the assay for inhibitory rates against acetylcholinesterase.

Three known indoloditerpenes, JBIR-03 (**4**), emindole SB (**5**), and emeniveol (**6**), were also isolated from *Dichotomomyces cejpii*, *Emericella nivea*, *Emericella striata*, etc.[4–6] Identification of these compounds was accomplished by analysis of 1D and 2D NMR data and comparison with literature values.[4–6]

In order to determine the chemical defensive function of this endophytic fungus *A. oryzae* for the marine red alga *H. japonica*, compounds **1**–**6** were examined for insecticidal and antimicrobial activities. Compound **4** was more active in the assay for insecticidal activity against brine shrimp (*Artemia salina*),[11] which was probably due to the presence of indole and tetrahydrofuran units by comparison with the other compounds (Table 2). It was interesting that the further oxidized structures **1** and **2** possessed lower insecticidal activity than their precursor **4**. This structure–activity relationship and susceptibility of indole unit offered new references for developing new indoloditerpene insecticides. In addition, in the assay for antibacterial activity against *Escherichia coli* and antifungal activity against plant pathogens *Colletotrichum lagenarium* and *Fusarium oxysporium* using standard agar diffusion tests at 30 μg/disk,[12] only **3** exhibited potent activities against *E. coli* with an inhibition diameter of 8.3 mm, and none of the compounds displayed any antifungal activity. The presence of 4-hydroxy-4-methylpent-2-enyl moiety in **3** was deduced to be necessary for the antibacterial activity against *E. coli*. These preliminary results, together with a previous Letter,[6] implied that endophytic fungus *A. oryzae* might play an important role to defend marine herbivores and bacteria for the red alga *H. japonica*.

Indoloditerpenes are known as potent tremorgenic mycotoxins, which arouse tremor in animals by affecting neurotransmission.[13] In order to further explore the insecticidal mechanism, compounds **1**–**6** were evaluated for acetylcholinesterase (AChE) inhibitory activity.[14] However, the results showed that **1**–**6** possessed low activity to modulate AChE (Table 2), which were not parallel with the insecticidal results yet. So, the insecticidal activity of **1**–**6** may be caused mainly by targeting other key receptors and ion channels,[15] aided by modulating AChE.

Acknowledgments

This work was financially supported by the Chinese Academy of Sciences for Key Topics in Innovation Engineering (KZCX2-YW-QN209, KZCX2-YW-225, KSCX2-YW-G-073), Shandong Province Natural Science Foundation (ZR2009BQ021), Yantai Municipal Sci-Tech Development Program (2009164), and Foundation of the Chinese Academy of Sciences for President's Scholarship (awarded to N.-Y.J.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.024.

References and notes

1. Gloer, J. B. *Acc. Chem. Res.* **1995**, *28*, 343.
2. Ondeyka, J. G.; Helms, G. L.; Hensens, O. D.; Goetz, M. A.; Zink, D. L.; Tsipouras, A.; Shoop, W. L.; Slayton, L.; Dombrowski, A. W.; Polishook, J. D.; Ostlind, D. A.; Tsou, N. N.; Ball, R. G.; Singh, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 8809.

3. Hensens, O. D.; Ondeyka, J. G.; Dombrowski, A. W.; Ostlind, D. A.; Zink, D. L. *Tetrahedron Lett.* **1999**, *40*, 5455.
4. Kimura, Y.; Nishibe, M.; Nakajima, H.; Hamasaki, T.; Shigemitsu, N.; Sugawara, F.; Stout, T. J.; Clardy, J. *Tetrahedron Lett.* **1992**, *33*, 6987.
5. Nozawa, K.; Nakajima, S.; Kawai, K. I.; Udagawa, S. I. *J. Chem. Soc., Perkin Trans. I* **1988**, 2607.
6. Ogata, M.; Ueda, J. Y.; Hoshi, M.; Hashimoto, J.; Nakashima, T.; Anzai, K.; Takagi, M.; Shin-ya, K. *J. Antibiot.* **2007**, *60*, 645.
7. Dowd, P. F.; Cole, R. J.; Vesonder, R. F. *J. Antibiot.* **1988**, *41*, 1868.
8. Mantle, P. G.; Burt, S. J.; MacGeorge, K. M.; Bilton, J. N.; Sheppard, R. N. *Xenobiotica* **1990**, *20*, 809.
9. Umeyama, A.; Nozaki, M.; Arihara, S. *J. Nat. Prod.* **1998**, *61*, 945.
10. Norte, M.; Fernández, J. J.; Padilla, A. *Phytochemistry* **1992**, *31*, 326.
11. Solis, P. N.; Wright, C. W.; Anderson, M. M.; Gupta, M. P.; Phillipson, J. D. *Planta Med.* **1993**, *59*, 250.
12. Schulz, B.; Sucker, J.; Aust, H. J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Döring, D. *Mycol. Res.* **1995**, *99*, 1007.
13. Bryden, W. L. *Proc. Nutr. Soc. Aust.* **1989**, *14*, 45.
14. López, S.; Bastida, J.; Viladomat, F.; Codina, C. *Life Sci.* **2002**, *71*, 2521.
15. Raymond-Delpech, V.; Matsuda, K.; Sattelle, B. M.; Rauh, J. J.; Sattelle, D. B. *Invert. Neurosci.* **2005**, *5*, 119.