Two New Secoanthraquinone Derivatives from the Marine-Derived Endophytic Fungus *Aspergillus wentii* EN-48

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Two new secoanthraquinone derivatives, wentiquinones A (**1**) and B (**2**), together with the eight known compounds **3**–**10**, were isolated from the culture extracts of *Aspergillus wentii* EN-48, an endophytic fungus derived from an unidentified marine brown algal species of the genus *Sargassum*. The structures of these compounds were elucidated on the basis of extensive spectroscopic analysis.

Introduction. – Marine microorganisms have become an important source of pharmacologically active metabolites [1][2]. Published reviews indicate the importance of these organisms as potential sources of pharmaceutical leads [1][2]. More specifically, fungi from the marine environment have shown great potential as suggested by the diversity of secondary metabolites. Anthraquinone derivatives, a kind of secondary metabolites from a variety of fungal species, exhibit a wide range of biological activities [3–7] including antifungal [3][4], antiviral [3][4], cytotoxic [5][6], and radical-scavenging activity [7]. During our ongoing search for new bioactive metabolites from marine-derived endophytes [8–16], we obtained a fungal strain, *Aspergillus wentii* EN-48, that was isolated from an unidentified marine brown algal species of the genus *Sargassum*. Chemical investigation of this fungal strain resulted in the isolation and characterization of eight tetranorlabdane diterpenoids from the PDB (potato dextrose broth, at 28°) fermentation culture [8]. Further investigation of this strain, has now resulted in the identification of two new secoanthraquinone derivatives, namely, wentiquinones A and B[1]) (**1** and **2**), as well as the eight related known metabolites **3**–**10**. The structures of these compounds were elucidated by analysis of their spectroscopic data. Details of the isolation and structure elucidation of these derivatives are reported herein. It should be noted that the structure of 2-(2,6-dihydroxy-4-methoxybenzoyl)-3,5-dimethoxybenzoic acid (**10**) could be searched on the Internet and *SciFinder*®, but no further reference and spectral data was found for this compound [17]. Therefore, compound **10**[1]) seems to be the first isolation as a natural product, and the fully assigned NMR data of **10** are reported in the *Table* (see below).

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Results and Discussion. – The AcOEt extract derived from the PDB culture of the fungal strain *A. wentii* EN-48 was subjected to various separation procedures by column chromatography on silica gel, *Lobar LiChroprep RP-18*, and *Sephadex LH-20*, to afford the two new compounds **1** and **2** and the eight known derivatives **3**–**10** (*Fig. 1*). By detailed spectroscopic analysis, the structures for the known compounds were determined as 1,8-dihydroxy-10-methoxy-3-methyldibenzo[*b*,*e*]oxepin-6,11-dione (**3**) [18], emodin-6,8-dimethyl ether (**4**) [19], physcion-10,10′-bianthrone (**5**) [20], atropisomers **6** and **7** of 8,8′-dihydroxy-1,1′,3,3′-tetramethoxy-6,6′-dimethyl-10,10′-bianthrone [21], 3,4-dihydro-3,9-dihydroxy-6,8-dimethoxy-3-methylanthracen-1(2*H*)-one (**8**) [22], 5-*O*-methylsulochrine (**9**) [21], and 2-(2,6-dihydroxy-4-methylbenzoyl)-3,5-dimethoxybenzoic acid (**10**) [17].

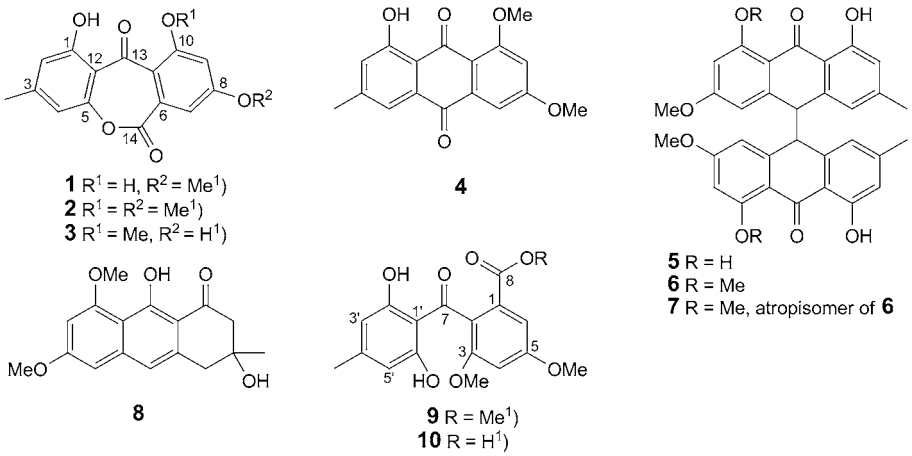

Fig. 1. *Compounds* **1**–**10**, *isolated from* Aspergillus wentii *EN-48*

Compound **1** was obtained as yellowish amorphous powder. The IR spectrum showed strong absorption bands for OH (3440 cm^{-1}), lactone (1739 cm^{-1}), α,β-unsaturated ketone (1652 cm^{-1}), and aromatic (1607 and 1569 cm^{-1}) functionalities in the molecule. Low-resolution ESI-MS displayed two quasi-molecular-ion peaks at *m/z* 301 ($[M+H]^+$) and 623 ($[2M+Na]^+$). The molecular formula was determined on the basis of the positive-mode HR-ESI-MS as $C_{16}H_{12}O_6$ (11 degrees of unsaturation), the same as that of its isomer, 1,8-dihydroxy-10-methoxy-3-methyldibenzo[*b*,*e*]oxepin-6,11-dione (**3**) [18]. The ^{1}H-NMR spectrum of **1** (*Table*) exhibited signals for two sets of *meta*-coupled aromatic H-atoms at δ(H) 6.66 (br. *d*, $J = 1.1$ Hz, H–C(2)) and 6.86 (br. *d*, $J = 1.0$ Hz, H–C(4)) and at δ(H) 7.18 (*d*, $J = 2.2$ Hz, H–C(7)) and 6.98 (*d*, $J = 2.2$ Hz, H–C(9)), one aromatic Me group at δ(H) 2.40 (Me–C(3)), one aromatic MeO group at δ(H) 3.95 (MeO–C(8)), and a chelated OH at δ(H) 12.32 (OH–C(1)). The ^{13}C-NMR (DEPT) data (*Table*) revealed the presence of 16 C-atoms including one Me, one MeO, and four aromatic CH groups, and ten quaternary C-atoms. Detailed comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of **3** [18] revealed that the structures of these two compounds are very similar, except for the MeO group at C(10)

Table. *^1H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of* **1**, **2**, *and* **10**[1]). δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])		Position	**10**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)		δ(H)	δ(C)
C(1)	–	160.5 (*s*)	–	161.5 (*s*)	C(1)	–	129.4 (*s*)
H–C(2)	6.66 (br. *d*, $J=1.1$)	111.2 (*d*)	6.60 (br. *d*, $J=1.1$)	111.7 (*d*)	C(2)	–	127.8 (*s*)
C(3)	–	148.7 (*s*)	–	148.6 (*s*)	C(3)	–	156.6 (*s*)
H–C(4)	6.86 (br. *d*, $J=1.0$)	107.1 (*d*)	6.69 (br. *d*, $J=1.1$)	107.1 (*d*)	H–C(4)	6.82 (*d*, $J=2.2$)	102.5 (*d*)
C(5)	–	155.1 (*s*)	–	155.8 (*s*)	C(5)	–	159.6 (*s*)
C(6)	–	136.6 (*s*)	–	135.1 (*s*)	H–C(6)	7.00 (*d*, $J=2.2$)	105.3 (*d*)
H–C(7)	7.18 (*d*, $J=2.2$)	101.1 (*d*)	6.89 (*d*, $J=2.5$)	101.5 (*d*)	C(7)	–	199.6 (*s*)
C(8)	–	164.8 (*s*)	–	164.7 (*s*)	C(8)	–	166.5 (*s*)
H–C(9)	6.98 (*d*, $J=2.2$)	111.7 (*d*)	6.87 (*d*, $J=2.5$)	112.1 (*d*)	C(1′)	–	109.2 (*s*)
C(10)	–	168.8 (*s*)	–	169.2 (*s*)	C(2′), C(6′)	–	161.6 (*s*)
C(11)	–	109.7 (*s*)	–	111.4 (*s*)	H–C(3′),	6.08 (*s*)	107.5 (*d*)
C(12)	–	105.8 (*s*)	–	106.7 (*s*)	H–C(5′)		
C(12)	–	105.8 (*s*)	–	106.7 (*s*)	C(4′)	–	147.1 (*s*)
C(13)	–	179.2 (*s*)	–	179.7 (*s*)	C(4′)	2.15 (*s*)	147.1 (*s*)
C(14)	–	157.6 (*s*)	–	158.1 (*s*)	*Me*–C(4′)	2.15 (*s*)	21.5 (*q*)
Me–C(3)	2.40 (*s*)	21.8 (*q*)	2.41 (*s*)	22.5 (*q*)	*Me*O–C(3)	3.69 (*s*)	56.2 (*q*)
*Me*O–C(8)	3.95 (*s*)	52.5 (*q*)	4.01 (*s*)	53.1 (*q*)	*Me*O–C(5)	3.83 (*s*)	55.5 (*q*)
*Me*O–C(10)	–	–	3.97 (*s*)	56.1 (*q*)	OH–C(2′)[c])	11.43 (br. *s*)	–
OH–C(1)	12.32 (br. *s*)	–	12.27 (br. *s*)	–	OH–C(6′)[c])	11.45 (br. *s*)	–

[a]) Measured in (D_6)DMSO. [b]) Measured in $CDCl_3$. [c]) Signals interchangeable.

and OH group at C(8) of **3** being interchanged in **1**. This was supported by the observed ^3J correlations from MeO–C(8) to C(8) in the HMBC spectrum of **1**. HMBCs (*Fig. 2*) were also observed from H–C(2) to C(1), C(4), C(12), and *Me*–C(3), from H–C(4) to C(2), C(5), C(12), and *Me*–C(3), from H–C(7) to C(8), C(9), C(11), and C(14), from H–C(9) to C(7), C(8), C(10), and C(11), and from OH–C(1) to C(2) and C(12). Therefore, the structure of compound **1** was determined, and it was named wentiquinone A.

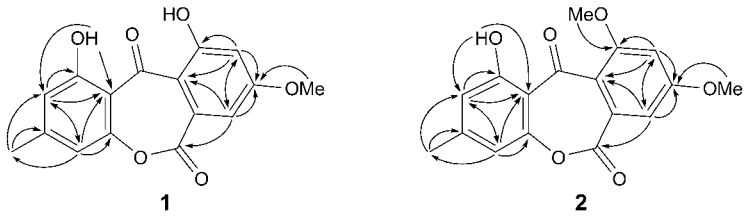

Fig. 2. *Key HMBCs of compounds* **1** *and* **2**

Compound **2** was also obtained as a yellowish amorphous powder. The IR spectrum displayed strong and broad absorptions for OH (3340 cm^{-1}), C=O (1734 cm^{-1}), and aromatic groups (1654, 1609, and 1575 cm^{-1}). The ESI-MS exhibited two quasi-molecular-ion peaks at m/z 337 ($[M+Na]^+$) and 651 ($[2M+Na]^+$). Its molecular

formula was determined on the basis of positive-mode HR-ESI-MS data as $C_{17}H_{14}O_6$, which indicated 14 mass units more than that of **1**, suggesting the presence of an additional MeO group in **2**. The general features of its ^{1}H- and ^{13}C-NMR data (*Table*) closely resembled those of **1**. However, one more MeO signal at δ(H) 3.97 (MeO–C(10)) and at δ(C) 56.1 (*Me*O–C(10)) in the NMR spectra was observed in **2**. Moreover, the observed HMBC cross-peak *Me*O–C(10)/C(10) indicated that the additional MeO group was attached to C(10). From the above evidence, the structure of compound **2** was deduced, and it was named wentiquinone B.

This work was financially supported by programs of the *Ministry of Science and Technology* (2010CB833802 and 2011AA09070110), of the *Natural Science Foundation of China* (30910103914 and 30970293), and of the *RNAM Center for Marine Microbiology of the Chinese Academy of Sciences* (KSCX2-EW-G-12B).

Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2, 200–300 and 300–400 mesh; *Qingdao Haiyang Chemical Group Co.*), *Lobar LiChroprep RP-18* (40–63 μm; *Merck*), and *Sephadex LH-20* (*Pharmacia*). TLC: precoated silica gel plates *GF-254* (SiO_2; *Qingdao Haiyang Chemical Group Co.*). UV Spectra: *PuXi-TU-1810*-UV/VIS spectrophotometer; λ_{max} ($\Delta\varepsilon$) in nm. IR Spectra: *Jasco-FT/IR-4100* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-Avance-500* spectrometer; at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. Low- and high-resolution ESI-MS: *VG-Autospec-3000* mass spectrometer; in *m*/*z*.

Fungal Isolation and Cultivation. The isolation and identification of the fungal material were identical to those described in our previous report [8]. The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology of the Chinese Academy of Sciences, with accession number EN-48.

Extraction and Isolation. The procedures of fermentation and extraction were identical to those of our previous report [8]. The combined extract was subjected to CC (SiO_2, different solvents of increasing polarity from petroleum ether/AcOEt to MeOH): *Fractions 1–7* (TLC monitoring). *Fr. 2* (3.8 g) was further purified by CC (SiO_2, petroleum ether/AcOEt 50:1 → 10:1), then *Sephadex LH-20*, MeOH): **2** (6.3 mg), **4** (10.5 mg), and **5** (9.0 mg). *Fr. 3* (2.2 g) was further purified by CC (SiO_2, petroleum ether/AcOEt 10:1 → 2:1, then *Sephadex LH-20*, MeOH) and by prep. TLC (20 × 20 cm plate, petroleum ether/AcOEt 3:2): **3** (2.0 mg), **8** (2.0 mg), and **9** (80.5 mg). Further purification of *Fr. 4* (5.1 g) by CC (SiO_2, petroleum ether/AcOEt 2:1 → 1:1, then *Sephadex LH-20*, MeOH, and then *Lobar LiChroprep RP-18*, H_2O/MeOH 1:1 → 0:1) yielded **1** (10.5 mg) and **10** (3.5 mg). *Fr. 5* (1.8 g) was further purified by CC (SiO_2, petroleum ether/AcOEt 5:1 → 1:1, then *Sephadex LH-20*, MeOH/$CHCl_3$ 1:1) and by prep. TLC (20 × 20 cm plate, petroleum ether/AcOEt 1:1): **6** (5.0 mg) and **7** (6.0 mg).

Wentiquinone A (=*1,10-Dihydroxy-8-methoxy-3-methyldibenz*[b,e]*oxepin-6,11-dione*; **1**): Yellowish amorphous powder. UV (MeOH): 235 (4.62), 250 (4.51), 302 (4.37), 350 (3.95). IR (KBr): 3440, 1739, 1652, 1607, 1569, 1500, 1456, 1424, 1394, 1274, 1215, 1182, 1155, 1130. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 301 ($[M+H]^+$), 623 ($[2M+Na]^+$). HR-ESI-MS: 301.0705 ($[M+H]^+$, $C_{16}H_{13}O_6^+$; calc. 301.0712).

Wentiquinone B (=*1-Hydroxy-8,10-dimethoxy-3-methyldibenz*[b,e]*oxepin-6,11-dione*; **2**): Yellowish amorphous powder. UV (MeOH): 234 (4.63), 252 (4.50), 303 (4.40), 353 (3.96). IR (KBr): 3340, 1734, 1654, 1609, 1575, 1453, 1424, 1325, 1283, 1252, 1214, 1160, 1029. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 337 ($[M+Na]^+$), 651 ($[2M+Na]^+$). HR-ESI-MS: 337.0673 ($[M+Na]^+$, $C_{17}H_{14}O_6Na^+$; calc. 337.0688).

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Received April 18, 2012