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Three new xanthone derivatives from an algicolous isolate of *Aspergillus wentii*

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Three new xanthone derivatives, yicathin A (1), yicathin B (2), and yicathin C (3), and three known anthraquinone derivatives, alatinone (4), 1,5-dihydroxy-3-methoxy-7-methylanthraquinone (5), and 5-hydroxy-1,3-dimethoxy-7-methylanthraquinone (6), were isolated from the cultures of *Aspergillus wentii* pt-1, an endophytic fungus isolated from the marine red alga *Gymnogongrus flabelliformis*. Their structures were unambiguously elucidated by NMR and mass spectroscopic methods as well as quantum chemical calculations. Compound 2 was active against *Escherichia coli*, and 3 could inhibit *E. coli*, *Staphylococcus aureus*, and *Colletotrichum lagenarium*.

Supporting information may be found in the online version of this article.

Keywords: NMR; ^{1}H NMR; ^{13}C NMR; two-dimensional NMR; yicathin A; yicathin B; yicathin C; *Aspergillus wentii*

Introduction

Natural xanthones and anthraquinones are typical polyketides, which represent a large family of secondary metabolites from plants and fungi and exhibit diverse bioactivities.[1–4] They possess higher ratios of quaternary carbons to protonated carbons, which often make trouble for the structure elucidation of these molecules using 1D and 2D NMR techniques. In our ongoing program to discover new bioactive compounds from algicolous fungi,[5–7] a fungus *Aspergillus wentii* obtained from the inner tissue of the marine red alga *Gymnogongrus flabelliformis* was examined. As a result, three new xanthone derivatives, yicathin A (**1**), yicathin B (**2**), and yicathin C (**3**), and three known anthraquinone derivatives, alatinone (**4**),[8] 1,5-dihydroxy-3-methoxy-7-methylanthraquinone (**5**),[9] and 5-hydroxy-1,3-dimethoxy-7-methylanthraquinone (**6**),[10] were isolated and identified (Fig. 1). The main subjects of this paper are the isolation, structure elucidation, and bioactivity of new compounds **1–3**.

Results and Discussion

Compound **1** was obtained as yellowish crystals. A molecular formula of $C_{17}H_{14}O_6$ was established by HREIMS (*m/z* 314.0786 $[M]^+$, calcd for $C_{17}H_{14}O_6$, 314.0790), requiring 11 degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) displayed one methyl singlet, two methoxyl singlets, two singlets and two doublets ascribed to four aromatic protons and one singlet attributed to an exchangeable proton. The ^{13}C NMR spectrum (Table 1) along with DEPT and HSQC data revealed the presence of three methyls, four methines, and ten unprotonated carbons. A detailed NMR data comparison with those reported for monodictyxanthone revealed the similarity of them.[11] However, replacing a proton at C-6 in monodictyxanthone, a methoxyl group was assigned to C-6 in **1** based on HMBC correlations from CH_3O-6, H-5, and H-7 to C-6. Additionally, the remaining methoxyl group was attached to C-12 by HMBC correlations from CH_3O-12 and H-2 to C-12. The other HMBC and ^{1}H–^{1}H COSY correlations (Fig. 2) further verified the structure of **1**, trivially named yicathin A.

Compound **2** was obtained as yellowish crystals. A molecular formula of $C_{17}H_{14}O_6$ was assigned based on HREIMS (*m/z* 314.0789 $[M]^+$, calcd for $C_{17}H_{14}O_6$, 314.0790), implying 11 degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) exhibited one methyl singlet, two methoxyl singlets, two singlets and two doublets attributable to four aromatic protons, and one singlet ascribable to an exchangeable proton. The ^{13}C NMR spectrum (Table 1) showed 17 resonances, which were classified into three methyls, four methines, and ten quaternary carbons by DEPT and HSQC experiments. A detailed analysis of the above data revealed that **2** should be an analogue of **1**. C-6 (δ_C 135.5) was deduced to be bonded to a carbomethoxy group according to its ^{13}C NMR value,[11] which was flanked by C-5 and C-7 based on HMBC correlations from H-5 and H-7 to C-12. C-8 was proposed to be methoxylated by HMBC correlations from CH_3O-8 to C-8, from H-7 to C-8 and C-8a, and from H-5 to C-8a and C-10a. Then, a hydrogen-bonded hydroxyl group was suggested to be at C-1 by the presence of a deshielding exchangeable proton (δ_H 12.35), and the connectivity of ring C was established by HMBC correlations from OH-1 to C-1, C-2, and C-9a, H-2 to C-1 and C-9a, from H-4 to C-4a, C-9, and C-9a, and from H-11 to C-2, C-3, and C-4. The other HMBC and ^{1}H–^{1}H COSY correlations (Fig. 2) further verified the structure of **2**, trivially named yicathin B.

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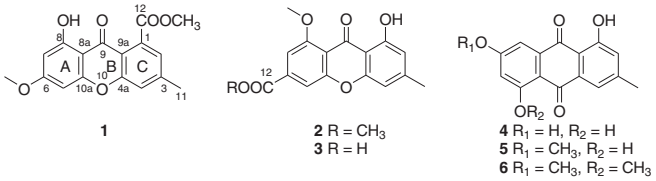

Figure 1. Structures of compounds **1–6**.

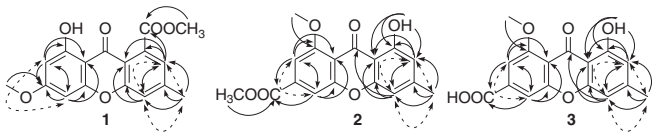

Figure 2. Key HMBC (bold arrows) and ^{1}H–^{1}H COSY (dashed arrows) correlations of **1–3**.

Compound **3** was obtained as yellowish crystals. A molecular formula of $C_{16}H_{12}O_6$ was determined by HREIMS (*m/z* 300.0632 $[M]^+$, calcd for $C_{16}H_{12}O_6$, 300.0634), consistent with 11 degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) displayed one methyl singlet, one methoxyl singlet, two singlets and two doublets assigned to four aromatic protons, and one singlet attributed to an exchangeable proton. The ^{13}C NMR spectrum (Table 1) along with DEPT and HSQC data exhibited the resonances for two methyls, four methines, and ten quaternary carbons. The above NMR data showed close similarity with those of **2** except for the lack of one methoxyl group. Then, **3** was deduced to be a deesterified derivative of **2** due to the presence of only one deshielding exchangeable proton (δ_H 12.40) and shifted ^{13}C NMR value (δ_C 163.8) of C-12, which was further supported by HMBC and ^{1}H–^{1}H COSY correlations as shown in Fig. 2. The above evidence established the structure of **3**, trivially named yicathin C.

The ^{13}C NMR data of compounds **1–3** were tentatively predicted by quantum chemical calculation using the gauge-independent atomic orbital (GIAO) method at the B3LYP/6-31 + G(d,p) level. The calculated ^{13}C NMR data (in supporting information) of **1–3** exhibited mean absolute deviations (MAD) of 3.1, 4.7, and 5.2 ppm, respectively, and most of the calculated values matched the experimental ones.

Compounds **1–3** were assayed for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against phytopathogens *Colletotrichum lagenarium* and *Fusarium oxysporum* using standard agar diffusion test at 10 μg/disk as well as brine shrimp toxicity (*Artemia salina*).[5] The results showed that **2** was active against *E. coli* (inhibition diameter 9 mm) and **3** could inhibit *E. coli* (12.0 mm), *S. aureus* (7.5 mm), and *C. lagenarium* (11.0 mm). Additionally, **1–3** exhibited weak brine shrimp toxicity with IC_{50}'s of 0.20, 0.22, and 0.30 μmol/mL, respectively.

In conclusion, three new xanthone derivatives, yicathin A–C (**1–3**), with antimicrobial activity and brine shrimp toxicity were isolated from the cultures of an algicolous *A. wentii* strain. Their structures were unequivocally identified by NMR and mass spectroscopic methods, and their ^{13}C NMR data were tentatively predicted by quantum chemical calculation with the GIAO method.

Experimental

General experimental procedures

Melting points were measured using an X-4 micro-melting point apparatus. High-resolution mass spectra were determined on an Autospec Premier P776 mass spectrometer. IR spectra were obtained on a JASCO FT/IR-4100 Fourier Transform InfraRed spectrometer. UV spectra were measured on a TU-1810 Spectrophotometer. Quantum chemical calculations were operated via Gaussian 09 software (IA32W-G09RevC.01). HPLC separation was carried out on an Agilent HPLC system (1260 infinity quaternary

Table 1. ^{1}H and ^{13}C NMR data for **1–3** (in acetone-d_6, δ in ppm, *J* in Hz)

	1		2		3	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		133.7 (C)		161.5 (C)		161.5 (C)
2	7.22 (s)	124.1 (CH)	6.64 (s)	111.4 (CH)	6.62 (s)	111.2 (CH)
3		147.2 (C)		149.1 (C)		148.9 (C)
4	7.48 (s)	118.7 (CH)	6.84 (s)	107.2 (CH)	6.82 (s)	107.1 (CH)
4a		156.0 (C)		155.9 (C)		155.8 (C)
5	6.57 (d, 2.2)	92.6 (CH)	7.15 (d, 2.4)	101.4 (CH)	6.98 (d, 1.6)	103.4 (CH)
6		167.2 (C)		135.5 (C)		135.9 (C)
7	6.37 (d, 2.2)	97.2 (CH)	6.98 (d, 2.4)	112.4 (CH)	6.90 (d, 1.6)	112.7 (CH)
8		163.4 (C)		168.2 (C)		168.4 (C)
8a		103.4 (C)		110.7 (C)		110.1 (C)
9		179.4 (C)		179.8 (C)		179.7 (C)
9a		114.8 (C)		106.3 (C)		106.2 (C)
10a		157.4 (C)		158.3 (C)		158.2 (C)
11	2.55 (s)	20.8 (CH_3)	2.44 (s)	21.5 (CH_3)	2.43 (s)	21.5 (CH_3)
12		168.7 (C)		165.2 (C)		163.8 (C)
CH_3O-6	3.97 (s)	55.7 (CH_3)				
CH_3O-8			3.92 (s)	52.1 (CH_3)	3.91 (s)	52.1 (CH_3)
CH_3O-12	3.92 (s)	52.0 (CH_3)	4.05 (s)	56.2 (CH_3)		
HO-1			12.35 (s)		12.40 (s)	
HO-8	12.55 (s)					

pump, 1260 infinity diode-array detector) using an Eclipse SB-C18 (5 μm, 9.4 × 250 mm) column. Column chromatography was performed with silica gel (100–200 and 200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China) and Sephadex LH-20 (Pharmacia). Precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co., Qingdao, China) were used for preparative TLC purification. All solvents were of analytical grade.

NMR spectra

The 1D and 2D NMR spectra were recorded on a Bruker Avance III 500 NMR spectrometer (equipped with 5-mm probe) in acetone-d_6 at 298 K. Chemical shifts (δ) in ppm are referenced to tetramethylsilane (TMS) at 0.00 ppm for ^{1}H and ^{13}C. Coupling constants (J) are given in Hertz. The pulse conditions were as follows: for ^{1}H, spectrometer frequency (SF) = 500.13 MHz, spectral width (SWH) 10330.58 Hz, pulse 90 width (P1) = 10.05 μs, fourier transform size (SI) = 65536, line broadening (LB) = 0.30 Hz, acquisition time (AQ) = 3.17 s, relaxation delay (D1) = 1.00 s, and number of dummy scans (DS) = 0; for ^{13}C, SF = 125.76 MHz, SWH = 29761.90 Hz, P1 = 9.60 μs, SI = 32768; LB = 1.00 Hz, AQ = 1.10 s, D1 = 2.0 s, DS = 4; for DEPT 90, SF = 125.76 MHz, SWH = 29761.90 Hz, SI = 32768; LB = 1.00 Hz, AQ = 1.10 s, D1 = 2.0 s, DS = 4; for DEPT 135, SF = 125.76 MHz, SWH = 29761.90 Hz, SI = 32768; LB = 1.00 Hz, AQ = 1.10 s, D1 = 2.0 s, DS = 4; for ^{1}H-^{1}H COSY, SF = 500.13 Hz, SWH = 4504.50 Hz, AQ = 0.23 s, D1 = 2.0 s, DS = 8; for HSQC, SF = 500.13 (^{1}H), 125.77 Hz (^{13}C), SWH = 4504.50 Hz, AQ = 0.11 s, D1 = 2.0 s, DS = 32; for HMBC, SF = 500.13 (^{1}H), 125.77 Hz (^{13}C), SWH = 4504.50 Hz, AQ = 0.45, D1 = 1.5 s, DS = 16.

Microorganism and fermentation

The fungal strain *A. wentii* pt-1 was isolated from the fresh tissue of surface-sterilized marine red alga *G. flabelliformis* that was collected from the coast of Pingtan Island, China, in May 2010. This fungus was identified by morphological observation and analysis of the ITS region of the rDNA, whose sequence data have been deposited at GenBank with the accession number JX436335. The strain was preserved at the Key Laboratory of Coastal Biology and Bioresource Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences.

The initial cultures were maintained on the potato dextrose agar (PDA) plates. Pieces of mycelia were cut into small segments and aseptically inoculated into 50 Erlenmeyer flasks (1 L), each containing 300-ml potato dextrose broth (PDB) culture media. Static fermentation was performed at room temperature (*ca.* 20 °C) for 30 days.

Extraction and isolation

The whole cultures (300 ml × 50 flasks) were filtered by cheesecloth to separate mycelia from broth. The broth was extracted with EtOAc to give a concentrated extract (10.6 g). The dried mycelia were homogenized and extracted with a mixture of $CHCl_3$ and MeOH (1:1, v/v), then the concentrated extract was partitioned between EtOAc and H_2O to yield an EtOAc-soluble extract (15.8 g). These two parts were combined for further separation based on their identical TLC profiles. The total EtOAc-soluble fraction (26.4 g) was subjected to step-gradient silica gel column chromatography (CC) with a solvent system consisting of 0%–100% petroleum ether (PE)–EtOAc to afford 16 fractions (Frs. 1-16) on the basis of TLC analysis. Fr. 7 eluted with PE/EtOAc(20:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and semi-preparative HPLC (MeOH/H_2O, 85:15) to yield **5** (35.2 mg). Fr. 12 eluted with PE/EtOAc (5:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel (PE/EtOAc, 5:1) and semi-preparative HPLC (MeOH/H_2O, 85:15) to give **4** (6.7 mg) and **6** (3.9 mg). Fr. 14 eluted with PE/EtOAc (2:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and semi-preparative HPLC (MeOH/H_2O, 80:20) to produce **3** (9.9 mg). Fr. 15 eluted with PE/EtOAc (2:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel (PE/EtOAc, 4:1), preparative TLC (PE/EtOAc, 4:1), and semi-preparative HPLC (MeOH/H_2O, 75:25) to afford **1** (3.1 mg) and **2** (3.4 mg).

Yicathin A, methyl 8-hydroxy-6-methoxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylate (**1**), yellowish crystals; mp 142–144 °C; UV ($CHCl_3$) λ_{max} (log ε) 242 (4.28), 268 (4.27), 303 (3.96), 365 (3.46) nm; IR (KBr) ν_{max} 3437, 2931, 2854, 1728, 1639, 1608, 1419, 1308, 1261, 1196, 1092, 1018, and 771 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 314.0786 [M]$^+$ (calcd for $C_{17}H_{14}O_6$, 314.0790).

Yicathin B, methyl 1-hydroxy-8-methoxy-3-methyl-9-oxo-9*H*-xanthene-6-carboxylate (**2**), yellowish crystals; mp 165–167 °C; UV ($CHCl_3$) λ_{max} (log ε) 240 (4.22), 260 (4.16), 311 (3.95), 365 (3.39) nm; IR (KBr) ν_{max} 3440, 2927, 2854, 1736, 1639, 1601, 1439, 1381, 1315, 1250, 1192, 1146, 1022, 825, and 764 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 314.0789 [M]$^+$ (calcd for $C_{17}H_{14}O_6$, 314.0790).

Yicathin C, 1-hydroxy-8-methoxy-3-methyl-9-oxo-9*H*-xanthene-6-carboxylic acid (**3**), yellowish crystals; mp 198–200 °C; UV ($CHCl_3$) λ_{max} (log ε) 239 (4.39), 256 (4.26), 307 (4.10), 366 (3.65) nm; IR (KBr) ν_{max} 3406, 2927, 1716, 1651, 1604, 1504, 1439, 1381, 1277, 1207, 1153, 1084, 1030, and 738 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 300.0632 [M]$^+$ (calcd for $C_{16}H_{12}O_6$, 300.0634).

Computational details

The energy-minimized conformers for compounds **1–3** were generated by the Dreiding force field in MarvinSketch (optimization limit = normal, diversity limit = 0.1) regardless of rotations of methyl and hydroxyl groups,[12] the geometries of which were further optimized using density function theory (DFT) at the B3LYP/6-31 G(d) level in acetone.[13] The optimized conformers were subjected to ^{13}C NMR calculations using the gauge-independent atomic orbital (GIAO) method at the B3LYP/6-31 + G(d,p) level in acetone with tetramethylsilane (TMS) as a reference. All the above calculations were performed with the integral equation formalism variant polarizable continuum model (IEF-PCM) as implemented in Gaussian 09.[14,15]

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