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Comazaphilones A−F, Azaphilone Derivatives from the Marine Sediment-Derived Fungus *Penicillium commune* QSD-17

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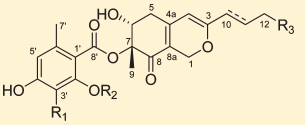
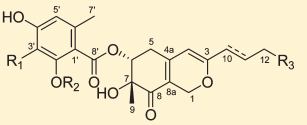
Supporting Information

ABSTRACT: Chemical investigation of *Penicillium commune* QSD-17, a fungus isolated from a marine sediment sample collected in the southern China Sea, yielded six new azaphilone derivatives, namely, comazaphilones A−F (**1**−**6**). The structures of these compounds were established on the basis of spectroscopic analysis. Attempts to define the absolute configuration of these azaphilones through investigation of Mosher's esters failed, possibly due to steric crowding at C-6 and C-7 and due to the degradation of these azaphilone derivatives under the reaction conditions. The inhibitory activities of the six azaphilones against four bacteria, one pathogenic fungus, and seven tumor cell lines were evaluated. Compounds **3**−**5** displayed potent inhibitory activity against several of these bacteria, while compounds **4**−**6** showed cytotoxic activity against human pancreatic tumor cell line SW1990. The preliminary SAR results indicated that the double bond at C-10 and the location of the orsellinic acid unit at C-6 in these azaphilones are important for the antibacterial activity and cytotoxicity, respectively. This is the first report of the isolation of azaphilone derivatives from a marine sediment-derived fungus.

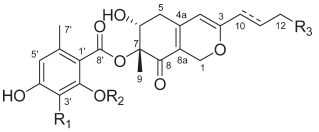


1 R_1=R_3=H, R_2=Me
2 R_1=OH, R_2=Me, R_3=H
3 R_1=OH, R_2=Me, R_3=H, Δ^{10}

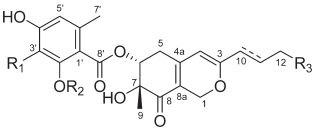


4 R_1=R_2=R_3=H, Δ^{10}
5 R_1=OH, R_2=Me, R_3=H, Δ^{10}
6 R_1=OH, R_2=Me, R_3=H

Azaphilone derivatives, secondary metabolites mainly characterized from fungal genera including *Aspergillus*, *Monascus*, and *Penicillium* as well as from the higher fungi of the genus *Hypoxylon* (Xylariaceae), are structurally diverse pigments, with most of them possessing highly oxygenated bicyclic hexatomic rings.[1−6] These compounds display various biologically beneficiary properties including radical scavenging,[4] antibacterial,[5,6] and lipoxygenase inhibitory activities.[4] As part of our recently initiated program to assess the chemical and biological diversity of marine-derived fungi from the China Sea region,[7−17] six new azaphilone derivatives, comazaphilones A−F (**1**−**6**), were obtained from the culture extracts of *Penicillium commune* QSD-17, a fungus isolated from a marine sediment sample collected in the southern China Sea. In addition, four known steroids and one known sphingolipid were also isolated and identified (Scheme S1, Supporting Information). The inhibitory activities of six azaphilones against four bacteria, one pathogenic fungus, and seven tumor cell lines were evaluated. The isolation, structure determination, and antibacterial and cytotoxic activity as well as the preliminary SAR of these azaphilone derivatives are described in this paper. This is the first report of the isolation of azaphilone derivatives from the marine sediment-derived fungus.



1 R_1=R_3=H, R_2=Me
2 R_1=OH, R_2=Me, R_3=H
3 R_1=OH, R_2=Me, R_3=H, Δ^{10}
7 R_1=R_2=H, R_3=OH, Δ^{10}



4 R_1=R_2=R_3=H, Δ^{10}
5 R_1=OH, R_2=Me, R_3=H, Δ^{10}
6 R_1=OH, R_2=Me, R_3=H
8 R_1=R_2=H, R_3=OAc, Δ^{10}

RESULTS AND DISCUSSION

The mycelia and culture broth of *P. commune* QSD-17 were separated by filtration and exhaustively extracted with MeOH and EtOAc, respectively. The combined extracts were further purified by a combination of column chromatography (CC) including silica gel, Sephadex LH-20, Lobar LiChroprep RP-18,

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Table 1. ^{1}H NMR Data for Compounds 1−6 in $CDCl_3$ (500 MHz, *J* in Hz)

position	1	2	3	4	5	6
1	5.03, d (12.6)	4.99, d (12.7)	5.01, d (12.5)	5.09, d (12.6)	5.01, d (12.3)	4.97, d (12.5)
	4.86, d (12.6)	4.86, d (12.7)	4.87, d (12.5)	4.77, d (12.6)	4.75, d (12.3)	4.78, d (12.5)
4	5.22, s	5.20, s	5.27, s	5.23, s	5.22, s	5.15, s
5	2.79, dd (18.9, 3.7)	2.74, dd (18.7, 4.1)	2.78, dd (18.8, 4.1)	2.96, dd (19.2, 3.8)	2.96, br d (19.2)	2.94, br d (19.8)
	2.70, dd (18.9, 3.7)	2.68, dd (18.7, 4.1)	2.69, dd (18.8, 4.1)	2.78, dd (19.2, 3.8)	2.79, br d (19.2)	2.75, br d (19.8)
6	4.88, t (3.7)	4.50, t (4.1)	4.49, t (4.1)	5.63, t (3.8)	5.52, br s	5.52, br s
9	1.86, s	1.79, s	1.79, s	1.46, s	1.45, s	1.45, s
10	2.19, t (7.5)	2.18, t (7.3)	5.91, d (15.3)	5.89, d (14.5)	5.89, d (14.9)	2.17, t (7.0)
11	1.58, m	1.57, m	6.48, m	6.51, dq (14.5, 7.1)	6.48, m	1.57, m
12	0.96, t (7.4)	0.95, t (7.4)	1.87, d (7.2)	1.87, d (7.1)	1.86, d (6.8)	0.94, t (7.3)
3′	5.99, d (1.9)			6.21, d (2.0)		
5′	6.06, d (1.9)	6.48, s	6.48, s	6.10, d (2.0)	6.48, s	6.51, s
7′	2.21, s	2.26, s	2.26, s	2.13, s	2.15, s	2.17, s
OH				11.50, s		
OCH_3	3.61, s	3.81, s	3.80, s		3.76, s	3.77, s

Table 2. ^{13}C NMR Data for Compounds 1−6 in $CDCl_3$ (125 MHz)

position	1	2	3	4	5	6
1	64.3, CH_2	64.3, CH_2	64.1, CH_2	63.8, CH_2	63.9, CH_2	64.1, CH_2
3	169.6, C	167.5, C	160.5, C	161.5, C	161.1, C	169.4, C
4	101.4, CH	101.3, CH	103.3, CH	103.1, CH	102.9, CH	101.0, CH
4a	148.8, C	147.9, C	147.9, C	148.5, C	147.8, C	147.7, C
5	33.3, CH_2	34.4, CH_2	34.4, CH_2	32.2, CH_2	32.2, CH_2	32.2, CH_2
6	70.9, CH	72.6, CH	72.7, CH	76.2, CH	76.4, CH	76.4, CH
7	86.2, C	86.6, C	86.7, C	74.3, C	74.3, C	74.2, C
8	191.6, C	189.4, C	189.2, C	195.5, C	195.5, C	195.7, C
8a	111.8, C	112.9, C	114.4, C	112.7, C	113.3, C	111.9, C
9	19.7, CH_3	19.5, CH_3	19.5, CH_3	24.2, CH_3	23.8, CH_3	23.8, CH_3
10	36.1, CH_2	36.0, CH_2	124.7, CH	124.5, CH	124.6, CH	36.0, CH_2
11	20.2, CH_2	20.2, CH_2	134.6, CH	135.6, CH	135.1, CH	20.2, CH_2
12	13.6, CH_3	13.6, CH_3	18.4, CH_3	18.4, CH_3	18.4, CH_3	13.6, CH_3
1′	114.8, C	118.7, C	118.7, C	105.2, C	118.3, C	118.3, C
2′	157.0, C	145.8, C	145.9, C	165.6, C	145.8, C	145.8, C
3′	96.6, CH	134.2, C	134.2, C	101.3, CH	134.3, C	134.2, C
4′	158.8, C	146.7, C	146.7, C	160.9, C	146.9, C	147.0, C
5′	109.9, CH	113.4, CH	113.3, CH	111.5, CH	113.5, CH	113.4, CH
6′	138.2, C	129.7, C	129.7, C	144.0, C	129.4, C	129.5, C
7′	19.2, CH_3	19.1, CH_3	19.0, CH_3	24.2, CH_3	19.6, CH_3	19.6, CH_3
8′	167.9, C	168.7, C	167.5, C	170.7, C	166.8, C	166.8, C
OCH_3	55.8, CH_3	62.4, CH_3	62.4, CH_3		62.2, CH_3	62.3, CH_3

and semipreparative HPLC to yield six new azaphilone derivatives (**1**−**6**) and five known compounds.

Compound **1** was obtained as a yellow, amorphous powder. The low-resolution ESIMS spectrum displayed ion peaks at *m*/*z* 403 $[M + H]^+$, 425 $[M + Na]^+$, and 827 $[2\ M + Na]^+$. The molecular formula was determined as $C_{22}H_{26}O_7$ by positive HRESIMS data. The IR spectrum showed the presence of hydroxy (3375 cm^{-1}), conjugated ester (1712 cm^{-1}), conjugated ketone (1651 cm^{-1}), and aromatic (1604, 1550, 841, and 756 cm^{-1}) functional groups. The ^{1}H NMR spectroscopic data (Table 1) displayed two *meta*-coupled aromatic proton signals at δ_H 5.99 (d, *J* = 1.9 Hz, H-3′) and 6.06 (d, *J* = 1.9 Hz, H-5′), together with one olefinic proton signal at δ_H 5.22 (s, H-4). Additionally, resonances for one methoxy group at δ_H 3.61 (2′-OCH_3), two methyl singlets at δ_H 1.86 (H-9) and 2.21 (H-7′), and one methyl triplet at δ_H 0.96 (*J* = 7.4 Hz, H-12) were also observed. The ^{13}C NMR and DEPT spectra (Table 2) revealed the presence of four methyls (with one methoxy), four methylenes (with one oxygenated), four methines (with one oxygenated, one olefinic, and two aromatic), and 10 quaternary (with one oxygenated, three olefinic, four aromatic, and two keto) carbon atoms in **1**. The general features of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) suggested the presence of azaphilone and methoxylated orsellinic acid moieties in **1**, which closely resembled those of rubiginosin B (**7**), an azaphilone derivative isolated from the inedible mushroom *Hypoxylon rubiginosum*.[18] However, the olefinic carbon signals for

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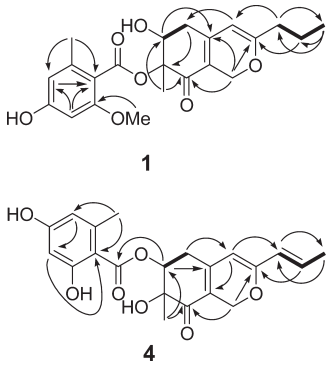

Figure 1. Key HMBC (arrows) and COSY (bold lines) correlations of compounds **1** and **4**.

C-10 (δ_C 123.4, CH) and C-11 (δ_C 138.1, CH) as well as the oxygenated methylene carbon signal for C-12 (δ_C 62.6, CH_2) in **7** disappeared in the ^{13}C NMR spectrum of **1**. Instead, two methylene carbon signals at δ_C 36.1 and 20.2 for C-10 and C-11, respectively, as well as a methyl carbon signal at δ_C 13.6 for C-12, were observed. Furthermore, an additional methoxy carbon signal at δ_C 55.8 was also detected in the ^{13}C NMR spectrum of **1** (Table 2). Accordingly, two olefinic proton signals at δ_H 6.21 (H-10) and 6.51 (H-11) as well as the oxygenated methylene signal at δ_H 4.21 (H-12) in the ^1H NMR spectrum of **7** were replaced by two methylene proton signals at δ_H 2.19 (H-10) and 1.58 (H-11) as well as by one methyl triplet at δ_H 0.96 (H-12) in **1**, respectively. In addition, a methoxy signal at δ_H 3.61 was also detected (Table 1). This evidence clearly indicated that the propenol side chain at C-3 and the OH group at C-2′ in **7** were replaced by propyl side chain and an OCH_3 groups in **1**, respectively. The observed $^1H-^1H$ COSY correlations from H-11 to H-10 and H-12 as well as the ^3J-HMBC cross-peaks from H-10 to C-4 and C-12, from H-11 to C-3, and from H-12 to C-10 confirmed the above deduction (Figure 1). The placement of a methoxy group at C-2′ of the orsellinic acid moiety was confirmed by the observed ^3J-HMBC correlation from the methoxy protons to C-2′ (Figure 1). The relative configurations of the two stereogenic centers (C-6 and C-7) were proposed to be *cis* by the observed NOESY correlations between H-6 and H_3-9. On the basis of the above evidence, the structure of compound **1** was determined, and the trivial name comazaphilone A was assigned to this compound.

Compounds **2** and **3** were also obtained as yellow, amorphous powders. The structure elucidation of these two compounds was straightforward due to their close relationships with compound **1**. Compound **2** was assigned the molecular formula $C_{22}H_{26}O_8$, having one oxygen atom more than **1**, on the basis of positive HRESIMS data. Its NMR spectroscopic data were consistent with the presence of one more hydroxy group in **2**. The signals at δ_H 5.99 (d, J = 1.9 Hz) for H-3′ and δ_C 96.6 (CH) for C-3′ in **1** were missing in the NMR spectrum of **2**. Instead, an oxygenated quaternary carbon signal at δ_C 134.2 (C-3′) was observed in the ^{13}C NMR spectrum of **2**. Accordingly, the doublet signal at δ_H 6.06 (d, J = 1.9 Hz) for H-5′ in **1** was replaced by a singlet signal at δ 6.48 (s) for H-5′ in **2**. These observations indicated that the additional hydroxy group was present at C-3′. The HMBC correlations from H-5′ to C-1′ and C-3′ also supported this deduction. The relative configuration at C-6 and C-7 of **2** was also deduced to be *cis* by the observed NOE correlation between H-6 and H_3-9. Thus, the structure of **2** was assigned, and it was named comazaphilone B.

The molecular formula of compound **3** was determined to be $C_{22}H_{24}O_8$, two hydrogen atoms less than that of **2**, based on the positive HRESIMS data. The main difference between **3** and **2** was observed with regard to the chemical shifts at positions C-10 and C-11. The two methylene signals at δ_C 36.0 (C-10) and 20.2 (C-11) in the ^{13}C NMR spectrum of **2** were replaced by two olefinic methine signals at δ_C 124.7 (C-10) and 134.6 (C-11), respectively, in **3** (Table 2). This assumption was strongly supported by the fact that the two methylene signals, with one triplet at δ_H 2.18 (J = 7.3 Hz) for H-10 and one multiplet at δ_H 1.57 for H-11 in **2**, disappeared in the ^1H NMR spectrum of **3**. Instead, two olefinic proton signals, with one doublet at δ_H 5.91 (J = 15.3 Hz) for H-10 and one multiplet at δ_H 6.48 for H-11, were observed in the ^1H NMR spectrum of **3**. The $^1H-^1H$ COSY correlations from H-11 to H-10 and H-12 as well as the HMBC correlations from H-10 to C-3 and from H-12 to C-10 and C-11 supported the above deduction. The large coupling constant (J = 15.3 Hz) for the olefinic protons H-10 and H-11 as well as the observed NOE correlation between H-4 and H-10 indicated the *E*-geometry for the double bond at C-10. The *cis*-configuration of C-6 and C-7 was assigned on the basis of the observed NOE correlation from H-6 to H_3-9 in the NOESY spectrum. The structure of compound **3** was thus assigned, and this compound was named comazaphilone C.

Comazaphilones D−F (**4**−**6**) were also obtained as yellow, amorphous powders. Detailed analysis of their NMR (Tables 1 and 2) and MS data as well as comparison with literature reports revealed that all of them possessed azaphilone and orsellinic acid units like comazaphilones A−C (**1**−**3**). However, the ester linkage between the orsellinic acid and the azaphilone moiety is at C-6 in comazaphilones D−F (**4**−**6**), instead of at C-7 as in comazaphilones A−C (**1**−**3**).

The molecular formula of compound **4** was determined as $C_{21}H_{22}O_7$ by the HRESIMS data. Its ^1H and ^{13}C NMR chemical shift assignments (Tables 1 and 2) matched well with those of the corresponding signals for rubiginosin A (**8**), an azaphilone derivative also characterized from the inedible mushroom *H. rubiginosum*,[18] and revealed the same structural features present in **8** except for the absence of the C-12 acetoxy group, which was consistent with the difference in molecular formula. Correspondingly, the oxygenated methylene signals at δ_C 63.5 (C-12) and δ_H 4.71 (d, J = 5.8 Hz, H-12) and the acetoxy signals at δ_C 170.7 (CH_3CO-12) and 20.8 (CH_3CO-12) and δ_H 2.11 (CH_3CO-12) in the NMR spectra of **8** disappeared in those of **4**. Instead, a methyl signal resonating at δ_C 18.4 (C-12) and δ_H 1.87 (d, J = 7.1 Hz, H-12) was detected in the NMR spectra of **4** (Tables 1 and 2). The observed $^1H-^1H$ COSY correlation from H_3-12 to H-11 as well as the ^3J-HMBC correlations from H-10 to C-12 and from H-12 to C-10 confirmed the above deduction (Figure 1). The observed HMBC correlation from H-6 to C-8′ established the substitution of the orsellinic acid unit at C-6 of the azaphilone moiety in **4**. The observed NOE correlations from H-4 and H_3-12 to H-10 as well as the large coupling constant for H-10/H-11 (J = 14.5 Hz) indicated the *trans*-geometry for the double bond at C-10. The NOE correlation from H-6 to H_3-9 revealed a *cis*-configuration for the substituents at C-6 and C-7. On the basis of the above evidence, the structure of **4** was assigned and it was named comazaphilone D.

Comazaphilone E (**5**) was assigned the molecular formula $C_{22}H_{24}O_8$, having one oxygen and one CH_2 unit more than that of **4**, by positive HRESIMS data. The general features of its ^1H and ^{13}C NMR data (Tables 1 and 2) closely resembled those of **4**.

Table 3. MIC Values (μg/mL) of Compounds 1−6

	1	2	3	4	5	6	ampicillin[a]	nystatin[a]
methicillin-resistant *S. aureus*	>256	128	16	32	>256	128	8	nd
P. aeruginosa	>256	>256	>256	>256	>256	>256	2	nd
P. fluorescens	>256	128	64	16	32	64	4	nd
B. subtilis	256	64	32	>256	16	128	4	nd
C. albicans	>256	>256	>256	>256	>256	>256	nd	2

[a] Positive control. nd: not determined.

However, the methine signals of C-3′ at δ_C 101.3 and H-3′ at δ_H 6.21 in the NMR spectra of **4** were missing in that of **5**. Instead, a quaternary carbon signal resonating at δ_C 134.3 (C-3′) and a methoxy signal at δ_C 62.2 (CH_3O-2′) and δ_H 3.76 (CH_3O-2′) were observed in the NMR spectrum of **5**. The above evidence as well as comparison of NMR data with those of **2** and **3** indicated that the OH and methoxy groups were substituted at C-3′ and C-2′, respectively, in **5**. The relative configuration of the two stereogenic centers C-6 and C-7 and the geometry of the double bond at C-10 were deduced to be the same as those of **4**, according to the NOESY experiment and coupling constants. Thus, the structure of **5** was assigned and it was named comazaphilone E.

The ^{1}H and ^{13}C NMR and MS data revealed that **6** is an isomer of **2**. The primary difference in the ^{1}H NMR spectrum was that the H-6 resonance moved significantly downfield (from δ_H 4.50 in **2** to δ_H 5.52 in **6**). In addition, the carbon signals at δ_C 72.6 (d, C-6), 86.6 (s, C-7), and 189.4 (s, C-8) in the ^{13}C NMR spectrum of **2** shifted to δ_C 76.4 (d, C-6), 74.2 (s, C-7), and 195.7 (s, C-8), respectively, in **6**. These differences, along with HMBC correlation from H-6 to C-8′, revealed that the orsellinic acid unit was attached to C-6 of the azaphilone moiety in **6**. The chemical shifts for the other protons and carbons of **6** were nearly identical to those of **2**. Further analysis of the 2D NMR spectra (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY) confirmed the structure of **6**, which was named comazaphilone F.

Knowing the absolute configuration of natural products is crucial because it provides essential information for both total synthesis and molecular mode of action of a bioactive metabolite. We have tried to determine the absolute configurations of compounds **1**−**3** by using the modified Mosher's method.[19] However, the reactions failed to yield the corresponding acylation products, possibly due to steric hindrance at C-6/C-7 and due to the degradation of these azaphilone derivatives under such reaction conditions. Attempts to remove the orsellinic acid group (relieving steric hindrance) by hydrolyzing compounds **1**−**3** under mild conditions (5% NaOH in EtOH)[20] did not yield a corresponding azaphilone core for Mosher ester formation due to the instability of these azaphilones under the hydrolytic conditions. So the absolute configurations of these new derivatives remain unknown.

In addition to the new comazaphilones A−F (**1**−**6**), four known steroids, (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one,[21] (22*E*,24*R*)-ergosta-7,22-diene-3,6-dione,[22] (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol,[23] and (22*E*,24*R*)-ergosta-5α,6α-epoxide-8,22-diene-3β,7α-diol,[23] and a known sphingolipid, aseramide B[14] (Scheme S1, Supporting Information), were also isolated and identified.

The biological activities of compounds **1**−**6** were evaluated by antimicrobial and cytotoxicity bioassays. In the initial antimicrobial screening, comazaphilones C (**3**), D (**4**), and E (**5**) displayed potent antibaterial activity (Table 3). Comazaphilone C (**3**) displayed activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas fluorescens*, and *Bacillus subtilis*, with MICs of 16, 64, and 32 μg/mL, respectively, while comazaphilone D (**4**) displayed activity against MRSA and *P. fluorescens* with MICs of 32 and 16 μg/mL, respectively. Comazaphilone E (**5**) also displayed activity against *P. fluorescens* and *B. subtilis* with MICs of 32 and 16 μg/mL, respectively. The other compounds (**1**, **2**, and **6**) showed only weak or no activity against certain bacteria (Table 3). The cytotoxicity against DU145, HepG2, HeLa, MCF-7, NCI-H460, SMMC-7721, and SW1990 tumor cell lines was also investigated, and comazaphilones D (**4**), E (**5**), and F (**6**) showed obvious selective activity against the SW1990 cell line with IC_{50} values of 51, 26, and 53 μM, respectively, which is stronger than that of the positive control, fluorouracil (with IC_{50} value of 120 μM). However, compounds **1**−**3** displayed weak or no appreciable activity against these tumor cell lines.

In the antibacterial screening, comazaphilones C (**3**), D (**4**), and E (**5**) showed better activity than comazaphilones A (**1**), B (**2**), and F (**6**), suggesting that the double bond at C-10 might be important for their activity against these bacterial targets. Furthermore, the significant differences of their ability to inhibit the SW1990 cell line indicated that the location of orsellinic acid being at C-6 of the azaphilone moiety is an essential structural feature for their cytotoxicity against the SW1990 cell line.

In summary, we described six new azaphilone derivatives, comazaphilones A−F (**1**−**6**), from the marine sediment-derived fungus *P. commune* QSD-17. Comazaphilones B (**2**), C (**3**), E (**5**), and F (**6**) are the first metabolites of this class reported to possess 2′-OCH_3 and 3′,4′-di-OH groups in their orsellinic acid unit. Furthermore, comazaphilones A (**1**), B (**2**), and F (**6**) possess a propyl side chain at C-3, which has been rarely characterized for azaphilone derivatives previously. The double bond at C-10 and the location of the orsellinic acid unit at C-6 were found to be important for their antibacterial activity and cytotoxicity, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were obtained on a PuXi TU-1810 UV−visible spectrophotometer. IR spectra were obtained on a Nicolet NEXUE 470 infrared spectrophotometer. 1D and 2D NMR spectra were recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance 500 MHz spectrometer with TMS as internal standard. Mass spectra were obtained on a VG Autospec 3000 mass spectrometer. Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Haiyang Chemical Co.), Lobar LiChroprep RP-18 (40−63 μm; Merck), and Sephadex LH-20 (18−110 μm, Merck). Semipreparative HPLC was performed using a HPLC (Dionex P680) system equipped with a Dionex P680 pump, ASI-100 automated sample injector, and UVD340U multiple wavelength detector controlled using Chromeleon software, version 6.80.

Fungal Material. The fungus *Penicillium commune* QSD-17 was isolated from a marine sediment sample (depth 210 m) collected from the

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southern China Sea, in September 2008. The sediment sample was stored in a sterile plastic bag and transported to the laboratory immediately, where it was kept frozen until processed. The sample was diluted 10-, 100-, and 1000-fold using sterile seawater. One milliliter of each diluted sample was processed utilizing the spread plate method in PDA medium (200 g of sliced potato, 20 g of dextrose, 20 g of agar, and 1 L of seawater) plates. The plates were incubated at 28 °C for 7 days. After purifying the isolates several times, the final pure cultures were selected and deposited at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences. Fungal identification was carried out using a molecular biological protocol by DNA amplification and sequencing of the ITS region, as described in our previous report.[17] The sequence data derived from the fungal strain have been submitted to and deposited at GenBank with accession number HM366606. A BLAST search result showed that the sequence was the same (100%) as that of *Penicillium commune* (compared with FJ499451.1, GI215981677).

Fermentation, Extraction, and Isolation. Mass growth of the fungus for the isolation and identification of secondary metabolites was carried out in Erlenmeyer flasks (1 L each). The fungus was grown in liquid PDB medium (200 g sliced potato, 20 g dextrose, 5 g peptone, 3 g yeast extract, and seawater added up to 1000 mL, pH 6.5−7.0, adjusted with 10% NaOH or 36.5% HCl, liquid medium/flask = 300 mL, 100 flasks) at room temperature under static conditions for 30 days.

The mycelia and culture broth of *P. commune* QSD-17 were separated by filtration, the mycelia were homogenized using a Waring blender, and the mycelia and broth were exhaustively extracted with MeOH and EtOAc, respectively. Since the TLC and HPLC profiles of the two extracts were nearly identical, they were combined before further separation. The extract obtained was dried and partitioned between *n*-hexane and 90% MeOH. The 90% MeOH-soluble material (11 g) was subjected to column chromatography (CC) over silica gel, eluting with different solvents of increasing polarity from petroleum ether (PE) to MeOH to yield 10 fractions (Frs. 1−10) on the basis of TLC analysis. Fr. 4 (1.2 g) was further purified by CC on silica gel eluting with a $CHCl_3$−MeOH gradient (from 0:1 to 1:1), Sephadex LH-20 (MeOH), and Lobar LiChroprep RP-18 to afford (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one (30.1 mg), (22*E*,24*R*)-ergosta-7,22-diene-3,6-dione (25.2 mg), and (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (7.3 mg). Fr. 5 (1.9 g) was further purified by CC on silica gel eluting with a $CHCl_3$−MeOH gradient (from 80:1 to 1:1), Sephadex LH-20 (MeOH), and semipreparative HPLC using the gradient (MeOH/H_2O) 0−35 min, 65% MeOH; 35−36 min, 100% MeOH; 36−46 min, 100% MeOH, to yield **4** (6.1 mg), **5** (3.3 mg), and **6** (2.8 mg). Fr. 6 (1.1 g) was further purified by CC on silica gel eluting with a $CHCl_3$−MeOH gradient (from 80:1 to 1:1), Sephadex LH-20 (MeOH), and semipreparative HPLC using the gradient (MeOH/H_2O) 0−35 min, 70% MeOH; 35−36 min, 100% MeOH; 36−46 min, 100% MeOH, to give **1** (6.3 mg), **2** (3.8 mg), and **3** (2.5 mg). Fr. 7 (2.5 g) was further purified by CC on silica gel eluting with a $CHCl_3$−MeOH gradient (from 20:1 to 1:1) and Sephadex LH-20 (MeOH) to obtain (22*E*,24*R*)-ergosta-5α,6α-epoxide-8,22-diene-3β,7α-diol (5.6 mg) and asperamide B (15.2 mg).

Comazaphilone A (***1***): yellow, amorphous powder; $[\alpha]_D^{25}$ +90.7 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.7), 264 (3.1), 302 (2.8), 380 (3.5) nm; IR (KBr) ν_{max} 3375, 2962, 2938, 1712, 1651, 1604, 1550, 1462, 1435, 1335, 1269, 1165, 1088, 841, 756, 644 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 403 $[M + H]^+$, 425 $[M + Na]^+$, 827 $[2\ M + Na]^+$; positive HRESIMS *m/z* 403.1763 $[M + H]^+$ (calcd for $C_{22}H_{27}O_7$, 403.1756).

Comazaphilone B (***2***): yellow, amorphous powder; $[\alpha]_D^{25}$ +95.7 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.3), 263 (2.7), 300 (2.3), 379 (3.2) nm; IR (KBr) ν_{max} 3494, 2931, 2870, 1720, 1651, 1550, 1458, 1416, 1277, 1165, 1076, 945, 756, 478 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 441 $[M + Na]^+$, 859 $[2\ M + Na]^+$; positive HRESIMS *m/z* 419.1717 $[M + H]^+$ (calcd for $C_{22}H_{27}O_8$, 419.1706).

Comazaphilone C (***3***): yellow, amorphous powder; $[\alpha]_D^{25}$ +205 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.8), 265 (3.1), 302 (2.6), 382 (3.6); IR (KBr) ν_{max} 3398, 2927, 2858, 1720, 1651, 1527, 1454, 1408, 1369, 1273, 1165, 1072, 949, 756, 582 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 439 $[M + Na]^+$, 855 $[2\ M + Na]^+$; positive HRESIMS *m/z* 417.1537 $[M + H]^+$ (calcd for $C_{22}H_{25}O_8$, 417.1549).

Comazaphilone D (***4***): yellow, amorphous powder; $[\alpha]_D^{25}$ +270 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 192 (5.75), 199 (5.67), 228 (6.49) nm; IR (KBr) ν_{max} 3382, 2974, 2931, 2861, 1643, 1523, 1450, 1307, 1257, 1200, 1165, 1107, 845, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 409 $[M + Na]^+$, 795 $[2\ M + Na]^+$; positive HRESIMS *m/z* 387.1455 $[M + H]^+$ (calcd for $C_{21}H_{23}O_7$, 387.1443).

Comazaphilone E (***5***): yellow, amorphous powder; $[\alpha]_D^{25}$ +54.5 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 192 (5.75), 199 (5.67), 228 (6.49) nm; IR (KBr) ν_{max} 3413, 2935, 2858, 1716, 1647, 1527, 1458, 1412, 1267, 1169, 1057, 1007, 945, 860, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 439 $[M + Na]^+$, 855 $[2\ M + Na]^+$; positive HRESIMS *m/z* 417.1537 $[M + H]^+$ (calcd for $C_{22}H_{25}O_8$, 417.1549).

Comazaphilone F (***6***): yellow, amorphous powder; $[\alpha]_D^{25}$ +30.8 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 192 (5.75), 199 (5.67), 228 (6.49) nm; IR (KBr) ν_{max} 3413, 2962, 2935, 2873, 1720, 1651, 1550, 1462, 1416, 1346, 1273, 1169, 1057, 1007, 937, 914, 856, 756, cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 441 $[M + Na]^+$, 859 $[2\ M + Na]^+$; positive HRESIMS *m/z* 441.1522 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_8Na$, 441.1525).

Cytotoxicity Assay. The cytotoxic activities against DU145 (human prostate carcinoma), HeLa (human epithelial carcinoma), HepG2 (human hepatocellular liver carcinoma), MCF-7 (human breast adenocarcinoma), NCI-H460 (human non-small-cell lung cancer), SMMC-7721 (human hepatoma), and SW1990 (human pancreatic cancer) cell lines were determined according to previously reported methods.[24] Fluorouracil was used as positive control.

Antimicrobial Activity. The MICs for the active components were determined by the broth microdilution method.[25] An inoculum level of 5×10^5 cfu/mL and a range of antibiotic concentrations (1024−1 μg/mL) were used. The MIC was determined after the microtiter plates were incubated for 18 h at 35 °C in an ambient air incubator. The test organisms were *Bacillus subtilis*, *Candida albicans*, *Pseudomonas aeruginosa*, *P. fluorescens*, and MRSA (methicillin-resistant *Staphylococcus aureus*). Ampicillin and nystatin were used as antibacterial and antifungal positive controls, respectively.

■ ASSOCIATED CONTENT

Supporting Information. Chemical structures of the new and known compounds (Scheme S1) as well as selected 1D and 2D NMR spectra of compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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