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Three Metabolites from the Mangrove Endophytic Fungus *Sporothrix* sp. (#4335) from the South China Sea

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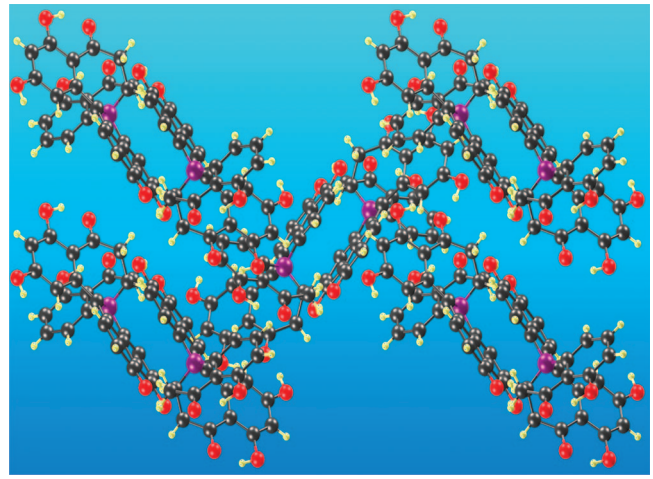

Three metabolites, sporothrins A, B, and C (**1**−**3**), were isolated from the mangrove endophytic fungus *Sporothrix* sp. (#4335). Their structures were identified by the spectral data and X-ray diffractive techniques, with compound **1** showing strong inhibition of acetylcholine esterase. 1,3,6,8-Tetrahydroxynaphthalene (T4HN) was deduced as one of the precursors in the biosynthesis of **1** and **2**. In a primary biosynthesis gene study, the partial gene fragment obtained with the LC1-Im/2c-Im primer pair was shown to be closely related to genes encoding T4HN synthase. The deduced protein sequences were highly homologous to the ketosynthase domains of other fungal PKS genes involved in T4HN biosynthesis.

Introduction

Marine microorganisms have been a focus of intense research over the past decade in the search for new bioactive compounds. This has resulted in the discovery of a wide range of new bioactive metabolites: aigalomycins A−E, xyloketals A−H, and Acremoxanthones A and B.[1] More recently, biomolecular techniques play an increasing role in the research of metabolites from marine microorganisms. [2] However, most of the studies on biosynthesis genes and their regulation of the polyketide metabolites have concentrated on bacterial symbionts in marine organisms, especially those recovered from sponges, bryozoans, and tunicates. [3] There have been few studies of the biosynthesis genes of marine fungal polyketides.[4]

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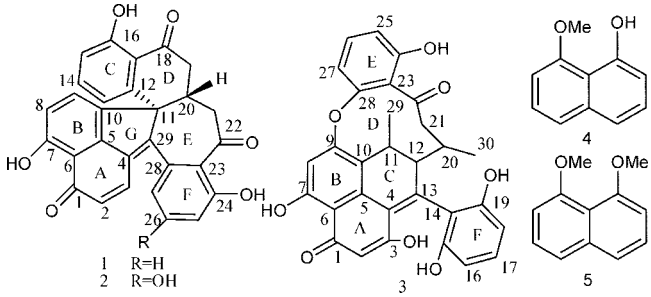

FIGURE 1. Sporothrins A (**1**), B (**2**), C (**3**), **4**, and **5**.

We have recently embarked on the study of marine fungal metabolites isolated from substrata collected in the South China Sea, especially mangrove fungi. These studies have yielded more than 42 new compounds and many significant known compounds.[1] We also initiated a project to study the biosynthesis of marine fungal metabolites, using biomolecular techniques.

The endophytic fungus *Sporothrix* sp. (#4335) was isolated from the bark of the inshore mangrove tree *Kandelia candel*. *Sporothrix* is an anamorph of the genus *Ophiostoma*, which includes the human pathogen *Sporothrix schenckii* and various species that are associated with insects and cause sap stain of wood.[5] A few metabolites have been documented for *Sporothrix* species; for example, Carlos reported a lipid compound from the cell wall of *S. schenchii* that induces high liberation of NO and TNF-α in macrophage cultures.[6] Takamatsu isolated a cytocidal antibiotic, Chlovalicin, produced by a *Sporothrix* sp. FO-4649.[7] Two antibiotics, 4-methyl-7,11-heptadecadienal and 4-methyl-7,11-heptadecadienoic acid, have been reported from *Sporothrix flocculosa* and *Sporothrix rugulosa*.[8]

Here, we report on the isolation, structural determination, and biosynthesis of three compounds (**1**, **2**, and **3**) from *Sporothrix* sp. (#4335). In the primary biosynthesis gene study, a partial gene fragment was obtained with the LC1-Im/2c-Im primer pair, which was closely related to genes encoding 1,3,6,8-tetrahydroxynaphthalene (T4HN) synthase (T4HNS). The deduced protein sequences were highly homologous to the ketosynthase domains of other fungal PKS genes involved in T4HN biosynthesis by phylogenetic analysis of the sequence of the partial gene encoding T4HNS. T4HN was deduced as one of the precursors in the biosynthesis of compounds **1** and **2**.

Results and Discussion

Three compounds (**1**, **2**, and **3**), named sporothrins A, B, and C, respectively, were isolated together with two substituted naphthalenes (**4** and **5**) from the endophyte *Sporothrix* sp. (#4335). Compounds **1**, **2**, and **3** possess unique ring systems (Figure 1). Compound **1** exhibited strong inhibition of acetylcholine esterase.

Methanol extraction of the mycelium (dried weight 465 g) was carried out. Fractionation of the crude extract on silica gel columns led to the isolation of **1**−**5** (Figure 1).

Compound **1** consisted of red needle-shaped crystals. The HREIMS analysis provided the molecular formula $C_{29}H_{18}O_6$. (obsd *m*/*z* 462.1098 $[M]^+$, calcd 462.1083). Its ultraviolet spectrum showed the absorption bands of the aromatic rings at λ_{max} 337, 357, and 459 nm. In a weak base, **1** could complex with $AlCl_3$, resulting in a color change from light red to purple and then to deep blue. The ^1H NMR spectrum of the complex showed that aromatic proton signals shifted to low field. Crystallization of **1** from acetone/ethyl acetate resulted in cubic crystals. The structure of **1** was first elucidated by analysis of spectra and then confirmed by X-ray crystal diffraction technique. The compound crystallized in the centro-symmetric space group *P*2(1)/*c*, indicating that it is a racemic compound. The relative configurations of two chiral centers were (11*S**,20*R**) (Figure 2).

In the HNMR spectra of **1**, there were signals of two groups of aromatic protons of *ortho*-protons (δ 7.28/H-9 and 6.85/H-8; δ 7.84/H-3 and 6.80/H-2), two groups of three *ortho*-protons (δ 5.86/H-15, 7.04/H-14, and 6.71/H-13; δ 6.97/H-27, 7.45/H-26, and 6.94/H-25), one sp^3 methine group (δ 2.99), two methylene groups (δ 3.30 and 2.90; δ 3.28 and 2.84), and three chelated hydroxyl groups (δ 10.70, 11.99, and 12.13). The ^{13}C NMR and DEPT spectra indicated the presence of three carbonyl signals (δ 204.5, 201.0, and 188.6), and 22 olefinic carbons. The signals of these carbons and protons were assigned by 2D NMR spectra and chemical shift considerations. In the HMBC spectrum, the correlations of H-2 to C-4, of H-3 to C-1 and C-5, of H-8 to C-6, C-7, and C-10, and of H-9 to C-5 and C-7 allowed unambiguous assignments of C and H of aromatic ring A and B. Similarly, those of aromatic ring C and F were also determined. The correlations from H-19 to C-18, 11, and 20, and from H-21 to C-22, 23 and 20 established that the methylene CH_2-19 was positioned *ortho* to C-18 and CH_2-21 *ortho* to C-22. They were all *ortho* to C-20, respectively.

Compound **2** was obtained as red needle-shaped crystals and had a molecular formula of $C_{29}H_{18}O_7$ based on NMR and HREIMS data (obsd *m*/*z* 478.1047 $[M]^+$, calcd 478.1045), with an oxygen atom more than that of **1**. ^1H and ^{13}C NMR of **2** were very similar to those of **1**, except for the absence of the triplet of an aromatic proton but with the addition of the signal for a phenolic hydroxyl proton and two doublets of the aromatic protons changed to two signlets. The results suggested that **2** had an OH in ring C or ring F. The HMBC correlations of compound **2** were also similar to those of **1** but provided more information on the location of four hydroxyl groups (Figure 3). Finally, X-ray crystal structure analysis confirmed the structure of **2**. The crystal space group *P*2(1)/*c* also indicated it to be a racemic compound. The relative configuration of two chiral centers of **2** is the same as that of **1** (11*S**,20*R**).

Compound **3** was a red solid with a molecular formula of $C_{30}H_{24}O_8$, established by HREIMS (obsd *m*/*z* 512.1506 $[M]^+$, calcd 512.1466), requiring 19 degrees of unsaturation. Its ultraviolet absorption bands of polysubstituted aromatic rings were at λ_{max} 313, 326, and 482 nm. The infrared spectrum of **3** showed the characteristic bands of hydroxyl (3403 cm^{-1}) and carbonyl groups (1721 cm^{-1}). Probably, compound **3** was also a polysubstituted aromatic compound such as **1** and **2**. However, the ^1H and ^{13}C NMR spectrum of **3** had many differences from those of **1** and **2**, especially the addition of the signals of three

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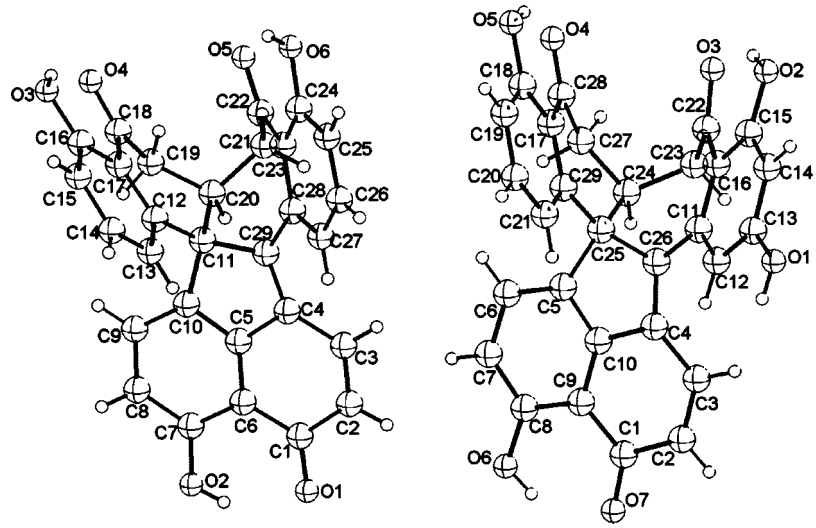

FIGURE 2. Stereoview of the crystal structures of **1** and **2**.

TABLE 1. NMR Data of 1 ($CDCl_3$) and 2 (Acetone-d_6), Measured at 500 MHz (^1H) and 125 MHz (^{13}C)

	1			**2**		
no.	δ_C, mult	δ_H (J in Hz)	HMBC	δ_C, mult	δ_H (J in Hz)	HMBC
1	188.6 (C)			190.8 (C)		
2	133.2 (CH)	6.80(d, 1H, 10.0)	C-4	135.4 (CH)	6.78(d, 1H, 10.0)	C-4
3	133.5 (CH)	7.84(d, 1H, 10.0)	C-1, 5, 29	136.4 (CH)	8.04(d, 1H, 10.0)	C-1, 4, 5, 29
4	132.1 (C)			133.9 (C)		
5	143.6 (C)			145.9 (C)		
6	141.4 (C)			144.2 (C)		
7	159.3 (C)			160.9 (C)		
8	115.2 (CH)	6.85(d, 1H, 8.4)	C-6, 7,10	116.3 (CH)	6.82(d, 1H, 8.4)	C-6, 7, 9, 10
9	127.3 (CH)	7.28(d, 1H, 8.4)	C-5, 7	129.7 (CH)	7.64(d, 1H, 8.4)	C-5, 7, 11
10	113.6 (C)			115.2 (C)		
11	64.2 (C)			66.0 (C)		
12	140.1 (C)			143.1 (C)		
13	117.2 (CH)	6.71(d, 1H, 8.4)	C-12, 15	120.5 (CH)	6.02(d, 1H, 7.8)	C-11, 15
14	137.2 (CH)	7.04(dd, 1H, 8.4, 7.6)	C-16	138.8 (CH)	7.13(dd, 1H, 8.1, 7.8)	C-12, 16
15	118.5 (CH)	5.86(d, 1H, 7.6)	C-11, 17	118.0 (CH)	6.65(d, 1H, 8.1)	C-13, 16, 17
16	162.0 (C)			163.7 (C)		
17	117.0 (C)			119.3 (C)		
18	201.0 (C)			204.0 (C)		
19	42.3 (CH_2)	a. 3.30(dd, 1H, 16.0, 6.0) b. 2.90(dd, 1H, 16.0, 5.0)	C-18, 20, 21	43.8 (CH_2)	a. 3.49(dd, 1H, 17, 6.6) b. 2.90(m, 1H)	C-11, 18, 20, 21
20	36.6 (CH)	2.99(dddd, 1H, 7.0, 6.0, 5.0, 3.0)	C-19, 21	38.2 (CH)	3.15(m, 1H)	
21	50.0 (CH_2)	a. (3.28, dd, 1H, 13.0, 7.0) b. (2.84, dd, 1H, 13.0, 3.0)	C-11, 19, 20, 22, 23	51.4 (CH_2)	a. 3.39(dd, 1H, 17, 6.6) b. 2.76(m, 1H)	C-11, 19, 20, 22, 23
22	204.5 (C)			205.3 (C)		
23	118.4 (C)			114.3 (C)		
24	162.7 (C)			167.5 (C)		
25	120.3 (CH)	6.94(d, 1H, 8.4)		105.6 (CH)	6.27(s, 1H)	C-23, 24, 26, 27
26	135.4 (CH)	7.45(dd, 1H, 8.4, 7.6)	C-24, 27, 28	165.2 (C)	9.78(brs, 1H,OH)	
27	124.0 (CH)	6.97(d, 1H, 7.6)	C-23, 25	115.7 (CH)	6.66(s, 1H)	C-23, 25
28	136.2 (C)			134.3 (C)		
29	133.2 (C)			133.9 (C)		
OH-7		10.70(brs, 1H)			10.77(s, 1H)	C-7, 8
OH-16		11.99(brs, 1H)			12.24(s, 1H)	
OH-24		12.13(brs, 1H)			12.58(s, 1H)	

methyl groups. The ^1H NMR spectrum of **3** showed the presence of eight aromatic protons: two groups of three *ortho*-protons (δ 6.20, 7.10, and 6.20; δ 6.95, 7.47, and 6.97), two single protons (5.93 and 6.45), and three sp^3 methine groups (δ3.69, 4.47, and 2.74), one methylene groups (δ 3.10 and 2.66), and five downfield hydroxyl groups (δ 13.94, 13.28, 12.90, 11.69, and 11.08). The ^{13}C NMR and DEPT spectra supported the above analysis and revealed the presence of two carbonyl signals (δ 189.6 and 209.1) and 22 olefinic carbon signals, accounting for 13 of 19 degrees of unsaturation required by the molecular formula. It suggested that **3** was a six-ring compound.

The HMBC spectrum enabled the determination of the overall structure of **3**, and especially the multiple correlations from H-11 to C-5, 9, 10, 12, 13, 20, and 29, from H-12 to C-10, 11, 13, 20, 21, and 29, and from H-21 to C-20 and 22 established the structural part from B, C, to D (Figure 2). The chelated hydroxyl group at δ13.94, and the correlations from H-2 to C-3, 4, and 6 and from H-8 to C-6, 7, 9, and 10 determined the connection

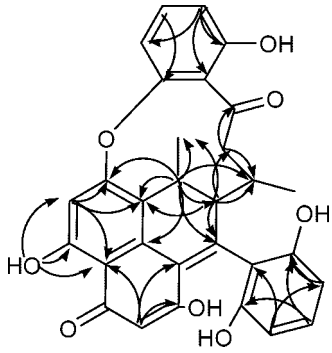

FIGURE 3. Key HMBC correlations of **3**.

TABLE 2. NMR Data (Acetone-d_6) of 3, Measured at 500 MHz(^{1}H) and 125 MHz (^{13}C)

no.	δ_C, mult	δ_H(J in Hz)	HMBC
1	189.6 (C)		
2	102.4 (CH)	5.93(s, 1H)	C-3, 4, 6
3	160.8 (C)		
4	118.2 (C)		
5	130.2 (C)		
6	110.3 (C)		
7	163.6 (C)		
8	104.8 (CH)	6.45(s, 1H)	C-6, 7, 9, 10
9	159.8 (C)		
10	117.8 (C)		
11	26.2 (CH)	3.69(dq, 1H, 7.0, 0.5)	C-5, 9, 10, 12, 13, 20, 29
12	48.0 (CH)	4.47(dd, 1H, 3.5, 0.5)	C-10, 11, 13, 20, 21, 29
13	148.1 (C)		
14	112.0 (C)		
15	163.8 (C)		
16	109.1 (CH)	6.20(d, 1H, 8.0)	C-14, 15, 18
17	137.6 (CH)	7.10(t, 1H, 8.0)	C-15, 19
18	109.1 (CH)	6.20(d, 1H, 8.0)	C-14, 16, 19
19	163.8 (C)		
20	35.9 (CH)	2.74(dddq, 1H, 3.5, 3.5, 10, 7)	
21	48.4 (CH_2)	a.3.10(dd, 1H, 3.5, 17) b.2.66(dd, 1H, 17, 10)	C-20, 22
22	209.1 (C)		
23	112.1 (C)		
24	155.8 (C)		
25	114.0 (CH)	6.95(dd, 1H, 8.0, 1.0)	C-23, 24, 27
26	134.5 (CH)	7.47(t, 1H, 8.0)	C-24, 28
27	110.4 (CH)	6.97(dd, 1H, 8.0, 1.0)	C-23, 25
28	158.5 (C)		
29	23.1 (CH_3)	1.16(d, 3H, 7.0)	C-10, 11, 12
30	20.0 (CH_3)	1.16(d, 3H, 7.0)	C-12, 20, 21
OH-3		12.90(s, 1H)	
OH-7		13.94(brs, 1H)	C-6, 7, 8
OH-9		11.08(s, 1H)	
OH-15		11.69(s, 1H)	
OH-24		13.28(s, 1H)	

of the rings A and aromatic B. The correlations from H-25 to C-23, 24, and 27, and from H-26 to C-24 and 28 constructed the second aromatic ring E. Furthermore, the correlations of H-17 to C-15 and 19 and the concomitant correlations between H-16 and C-14 established the third aromatic ring F. Addition to CH-16 and CH-18 had equal ^{1}H and ^{13}C chemical shifts, and the ^{13}C shifts for C-15 and C-19 were also equal, indicating that the two hydroxys must symmetrically be located on ring F.

The amount of **3** was very small, so its sterochemistry has not been determined. However, the coupling constants between H-11 with H-12 and H-20 were 0.5 and 3.5 Hz, respectively, suggests that their dihedral angles were probably close to 90°. The three protons were tentatively deduced as *cis*.

Compounds **4** and **5** were naphthalene derivatives isolated from this fungus, and their structures were identified by spectral data and comparison with those reported in the literature.[9]

The structural shapes of **1** and **2** are peculiar, in which rings A, B, and G are on an approximate plane, while rings C and F are on another plane in different orientation, respectively. Rings A, B, C, and F fuse through the five-membered ring G and seven-membered ring E. The ring systems of sporothrins A and B, and the skeleton of sporothrin C were most unusual, and to our knowledge, they have not previously been encountered in nature or synthesized in the laboratory.

Sporothrins A (**1**), B (**2**), and C (**3**) and two naphthalene derivatives (**4**) and (**5**) biogenetically belong to polyketide compounds, a group of natural products whose diversity even exceeds those of isoprenoids. The biosynthesis pathway for sporothrins is outlined in Figure 4.

Compound **1** and **2** were probably produced through a series of transformations from three naphthalene derivatives, formed from pentaketide chains. This hypothesis is supported by the fact that two substituted naphthalenes (**4**) and (**5**) together with **1**−**3** were isolated from the same fungus. The twice phenol oxidation coupling reactions of three substituted naphthalenes resulted in the mediated product b, which was converted to c by the enol-keto equilibration of ring D and E. The product c underwent the oxidative cleavage of the ring E, the addition to the double bond of ring D, and dehydrogenation at C-4 and C-29 forming **1** (or **2**).

Compound **3** was also probably formed from three pentaketide chains as in **1** and **2**, but its route should have great difference. It was presumed that three pentaketide chains directly underwent reduction, aldol condensation, and cyclization, without the steps forming naphthalene rings, to give **3**.

Presumably, T4HN was one of precursors for assembling sporothins. We tried to obtain and sequence the partial gene encoding T4HN synthase that catalyzes the final step of T4HN biosynthesis.

PCR amplification was carried out using genomic DNA of *Sporothrix* sp. #4335 with the primers LC1-Im /LC2c-Im, specifically designed to amplify the ketosynthase (KS) domains of nonreducing PKS (polyketide synthase) genes. A 722-bp amplicon was produced and sequenced. The nucleotide sequences obtained in this study were submitted to the GenBank and assigned the accession number EU399182.

A blast search with the amplicon at GenBank returned a PKS sequence of a *Nodulisporium* sp. ATCC74245 (GenBank AAD38786) as the closest match (Score = 484 bits (1247), Expect = 2e-135, Identities = 235/240 (97%)). The product of pks1 enzyme of *Nodulisporium* sp. ATCC74245 is T4HN. The amino acid deduced from PCR product also showed high homology to other NR class of PKS T4HN, such as 92% similarity to the translated polypeptide of *Colletotrichum lagenarium* T4HN (E value 1e-126), 84% similarity to *Glarea lozoyensis* (E value 1e-17), 67% identity to *Exophiala dermatitidis* (E value 5e-91), and 58% identity to *Aspergillus fumigatus* (E value 5e-74). These results indicated that #4335 produces T4HN encoded by a NR PKS gene, which provides evidence for biosynthsis of sporothrin in *Sporothrix* sp. (#4335) strain.

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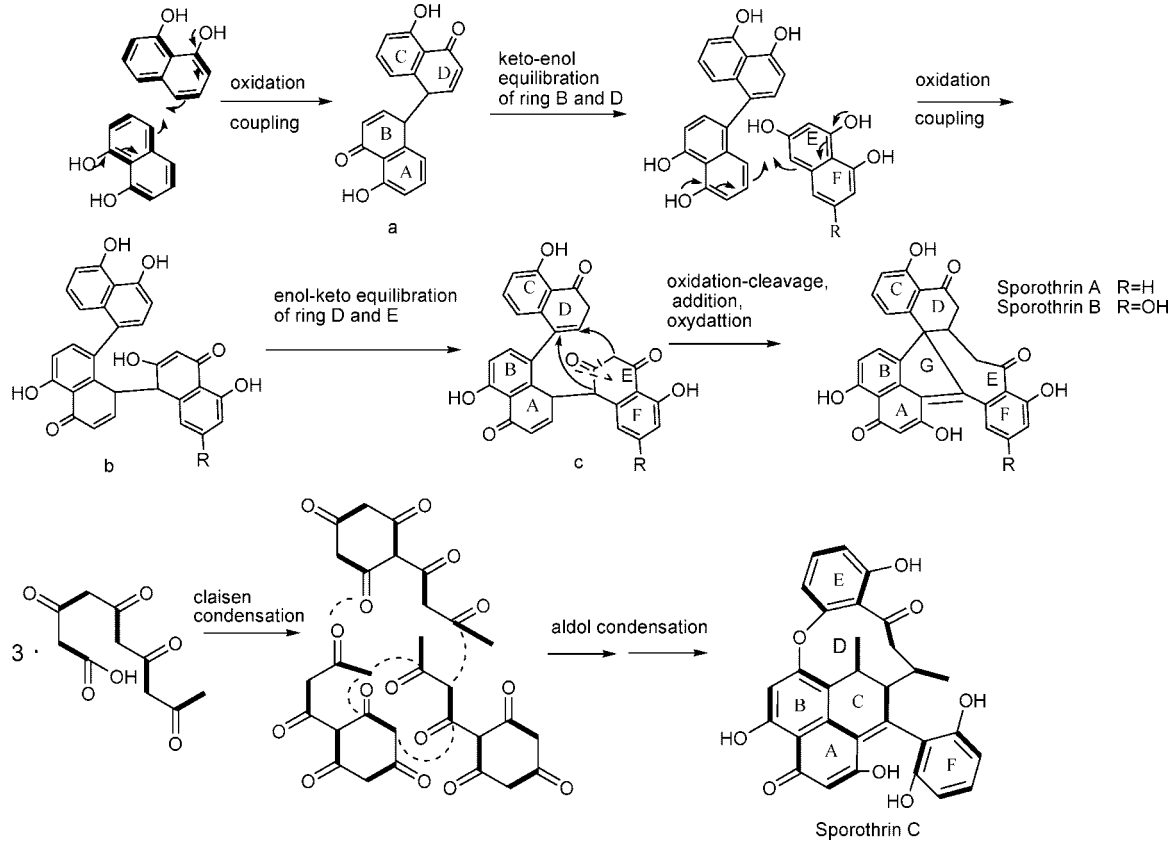

FIGURE 4. Hypothetical outline of the biosynthesis of sporothrins A, B, and C.

A phylogram was constructed for the predicted proteins encoded by the LC-primer PCR product of #4335, together with various other fungal PKS genes. This diagram indicates a possible evolutionary relationship between the different fungal PKS sequences and the deduced amino acid sequences of the corresponding unit of #4335-Sporothrin-PKS. Phylogenetic analyses provided further evidence that the deduced protein sequences were highly homologous to the ketosynthase domains of other fungal PKS genes involved in T4HN biosynthesis. Further work is in progress to determine the sporothrix gene cluster.

Compounds **1** and **2** were screened for cytotoxicity against hepG2 cell lines using the microculture tetrazolium (MTT) assay. The results showed that they exhibited modest antitumor activity (IC_{50} = 50 and 20 μg/mL, respectively). Sporothrin A inhibited acetylcholine esterase (AChE) activity in vitro with the modified method of Ellmen (IC_{50} was 1.05 μM.).[10] Compounds **2** and **3** were not tested due to lack sufficient material for the assay.

Experimental Section

Fungus Material and Culture Conditions. The strain was isolated as an endophyte from the bark of *Kandelia candel*, from a brackish water mangrove in the South China Sea. Axenic cultures are deposited in the Department of Applied Chemistry, Zhongshan University, Guangzhou, China and maintained on cornmeal seawater agar. Plugs of agar supporting mycelium growth were cut and transferred aseptically to a 250-mL Erlenmeyer flask containing 100 mL of liquid medium GYT (glucose 1%, peptone 0.1%, yeast extract 0.2%, sea salt 0.25%). The flask was incubated on a rotary shaker for 5−7 days, and then the mycelium was aseptically transferred to 500-mL Erlenmeyer flasks containing 250 mL of liquid medium. The flasks were incubated at 27 °C in standing culture for 30 days.

Isolation and Purification. The culture was filtered through cheesecloth, and the mycelium was air-dried (dried weigh 465 g) and extracted in methanol. The crude extract was concentrated and partitioned between ethyl acetate and water, and the organic fraction was purified by chromatography on silica gel columns, using a gradient from petroleum to ethyl acetate, then from acetate to methanol, to afford **1** (55 mg), **2** (5 mg), **3** (2 mg), **4** (5 mg), and **5** (6 mg).

Sporothrin A (**1**) was obtained from the fraction eluted with 30% ethyl acetate/petroleum ether (v/v) as red needle-shaped crystals. Mp 283−284 °C; $[\alpha]^{20}_D = 0°$ (*c* 0.1 in acetone). NMR data (500 MHz, $CDCl_3$, 25 °C, TMS): see Table 1. IR(KBr): ν_{max} = 3646, 2923, 1642, 1607, 1515, 1446, 1213, 1115 cm^{-1}. UV−vis (acetone): λ_{max} (ε) = 337 (4.13), 357(3.92), 459 nm (3.89). EIMS: 462 [M^+]. HREIMS: *m*/*z* 462.1098 [M^+], calcd for $C_{29}H_{18}O_6$, 462.1083.

The Al-complex of **1**: ^{1}H NMR (300 MHz, acetone-d_6, 25 °C, TMS): δ 8.11(1H, d, 9.9 Hz, C-3), 7.68(1H, d, 8.4 Hz, C-9), 7.56(1H, dd, 8.1, 7.5 Hz, C-26), 7.23(1H, d, 8.1 Hz, C-27), 7.06(1H, dd, 8.4, 8.1 Hz, C-14), 6.89(1H, d, 8.4 Hz), 6.83(1H, d, 7.8 Hz), 6.80(1H, d, 9.6 Hz), 6.63(1H, d, 8.1 Hz), 5.95(1H, d, 7.5 Hz). UV (MeOH): λ_{max} (ε) = 348 (4.21), 363 (4.21), 581 (3.80).

Sporothrin B (**2**) was obtained from the fraction eluted with 5% methanol/chloroform (v/v) as red needle-shaped crystals. Mp 280−282 °C; $[\alpha]^{20}_D = 0°$ (*c* 0.1 in acetone). NMR data (500 MHz, acetone-d_6, 25 °C, TMS): see Table 1. IR (KBr): ν_{max} 3615, 2923, 1645, 1609, 1516, 1455, 1210, 1115 cm^{-1}. UV−vis (acetone): λ_{max} (ε) = 210 (3.88), 313 (3.79), 346 (4.07), 359 (3.94), 453 nm (3.76). EIMS: 478 [M^+]. HREIMS: *m*/*z* 478.1047 [M^+], calcd for $C_{29}H_{18}O_7$, 478.1045.

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Sporothrin C (**3**) was obtained from the fraction eluted with 15% methanol/chloroform (v/v) as red solid. Mp >280 °C; $[\alpha]^{20}_{D} = 0°$ (c 1 × 10^{-4} g mL^{-1}, acetone). NMR data (500 MHz, acetone-d_6, TMS): see Table 2. UV (acetone): λ_{max} (ε) 313 (2.83), 326 (2.90), 482 (2.68) nm. IR (KBr): ν_{max} 3403, 2922, 1721, 1599, 1458, 1232, 1172, 889, 719 cm^{-1}. EIMS: m/z 512 $[M]^+$. HREIMS: m/z 512.1506 $[M]^+$, calcd for $C_{29}H_{18}O_6$, 512.1466.

1-Hydroxy 8-methoxy-naphthalene (**4**) was obtained from the fraction eluted with 8% ethyl acetate/petroleum ether (v/v) as white plane, mp 130−132 °C.

1,8-Dimethoxy-naphthalene (**5**) was obtained from the fraction eluted with with 6% ethyl acetate /petroleum ether (v/v) as white solid, mp 158−160 °C.

Molecular Methods. Total genomic DNA was extracted using Fungal DNA Kit (Omega). PCR amplification was carried out using genomic DNA of *Sporothrix* sp. (#4335) and the primers LC1-Im/LC2c-Im. A 722-bp amplicon was produced and sequenced (Genebank accession number EU399182). Sequence analyses of were performed with the basic sequence alignment BLASTx program run against the database (National Center for Biotechnology Information Web site [http://www.ncbi.nlm.nih.gov]). An overall alignment was produced with ClustalX1.8, and the alignment of the corresponding modules in PKSs involved in the synthesis of T4HN and the deduced amino acid sequence of PCR product of *Sporothrix* sp. (#4335) is in Supporting Information. Phylogenetic analyses were performed with PHYLIP software package (J. Felsenstein, University of Washington). Phylogenetic trees were drawn using TREEVIEW (Page, 1996). A phylogram has been constructed for the predicted proteins encoded by the LC-primer PCR product of #4335, together with various other fungal PKS genes.[12]

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Supporting Information Available: Experimental details and characterization data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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