Notes

Yanuthones: Novel Metabolites from a Marine Isolate of *Aspergillus niger*

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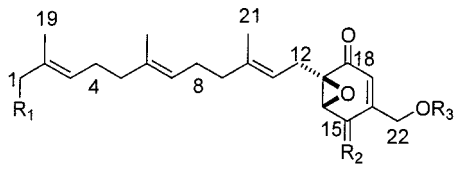
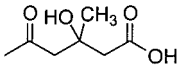
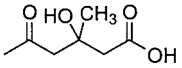
Fungi have provided a wealth of pharmaceutically important compounds. Terrestrial fungi, in particular, have been studied extensively, and a broad range of bioactive metabolites isolated and characterized. Conversely, fungi from marine sources have not been studied as extensively. Nonetheless, marine fungi have been shown to produce a wide structural range of biologically interesting secondary metabolites, and some studies indicate fungi from marine environments exhibit increased antimicrobial activity compared to terrestrial isolates.[1] More specifically, marine isolates of *Aspergillus* sp. have yielded a variety of novel compounds including chloropolyketides,[2] sesquiterpenoid nitrobenzoyl esters,[3] tripeptides,[4] and benzodiazepine analogues.[5] This paper describes the isolation and structure elucidation of eight novel bioactive farnesylated epoxy cyclohexenones from an *Aspergillus niger* isolate obtained from tissue homogenates of an orange *Aplidium* sp. ascidian. The compounds were given the following trivial names yanuthone A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), 1-hydroxyyanuthone A (**6**), 1-hydroxyyanuthone C (**7**), and 22-deacetylyanuthone A (**8**).

Isoprenoid epoxycyclohexenones have been reported from terrestrial fungal sources and associated with many bioactivities, including mitotic arresting activity.[6] A closely related class of compounds, the oligosporons, have the same carbon skeleton as the yanuthones and exhibit weak antibacterial, antiyeast, cytotoxic, and hemolytic effects.[7] The drimane epoxycyclohexenone macrophorins were shown to have phytotoxic, cytotoxic, and antimicrobial activities.[8−10] Interestingly, cyclofarnesylated quinones, the longithorones, were isolated from the ascidian *Aplidium longithorax*.[11−13] It has been hypothesized that microbes are responsible for several secondary metabolites isolated from some marine animals. The possibility exists that the quinone moiety could be a microbial product derived from shikimate, a biosynthetic pathway supposedly lacking in animals.



Compound	R_1	R_2	R_3
1	H	⁻OH,‴H	Ac
2	H	O	Ac
3	H	⁻OAc,‴H	H
4	H	O	
5	H	⁻OH,‴H	
6	OH	⁻OH,‴H	Ac
7	OH	⁻OAc,‴H	H
8	H	⁻OH,‴H	H

Fungal isolate F97S11 was grown in a 1 L culture and exhaustively extracted with ethyl acetate. The crude extract was partitioned between hexane, $CHCl_3$, and aqueous MeOH. Both the hexane-soluble material and the $CHCl_3$-soluble material was chromatographed using Sephadex LH-20 followed by flash SiO_2 and centrifugal countercurrent chromatography ($CHCl_3$ partition). Further purification by SiO_2 HPLC using ethyl acetate/hexane yielded eight related compounds that were isolated using antimicrobial activity guided fractionation.

Yanuthone A (**1**) was isolated as a pale yellow oil. A molecular formula of $C_{24}H_{34}O_5$, which gave eight double bond equivalents, was established by HRCIMS. The ^1H NMR spectrum showed five methyl singlets, two oxygenated methines, one oxygenated methylene, four olefinic methines, one diastereotopic methylene system, and four

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overlapping methylenes. On the basis of ^{1}H NMR chemical shifts (δ 1.57, 1.58, 1.61, 1.66), four of the five methyls were attached to olefins. Ten sp^2 carbons were observed in the ^{13}C NMR spectrum. Two carbon shifts, δ 170.4 and 193.1, were assigned to an ester and an α,β unsaturated ketone, which were subsequently confirmed by IR stretches at 1743, 1684 cm^{-1}. The remaining eight sp^2 carbons were assigned to olefins, two of which were part of the enone system. From the ^{1}H and ^{13}C NMR spectra, the remaining sp^2 carbons were assigned to three trisubstituted olefins. Analysis of the methyl shifts in conjunction with the trisubstitued olefins suggested a terpenoid system. In comparing the ^{13}C NMR data of yanuthone A (**1**) to those of farnesol,[14] 14 of 15 carbon shifts were nearly identical. HMBC data were also consistent with a farnesyl chain, which terminated in a diastereotopic methylene (δ 2.50, 2.78) indicating attachment to a chiral center. Since six of the eight units of unsaturation were attributed to the olefin and carbonyl moieties, yanuthone A (**1**) must have at least one ring. Assignment of the farnesyl chain and evidence of an acetyl leaves seven carbons unassigned, consistent with a six- or seven-membered ring.

The diastereotopic protons at position 12 showed HMBC correlations to carbons at δ 140.1 (C-10), 115.8 (C-11), 61.3 (C-13), 58.9 (C-14), and 193.1 (C-18). These key correlations define connection between the farnesyl chain and the ring. C-14 was deduced to be part of an epoxide system on the basis of the J_{C-H} value of 180 Hz. Furthermore, the H-14 chemical shift (δ 3.70), considerably upfield for an oxygenated methine, is consistent with an epoxide. Only three oxygenated carbons remain unassigned: one methylene, one methine, and one quaternary carbon. The oxygenated methine was shown to bear a hydroxyl group by acetylation with pyridine and acetic anhydride. Therefore, the oxygenated quaternary carbon (C-13, δ 61.3) was assigned to the epoxide and is consistent with the observed upfield ^{13}C shift.

HMBC signals from H-14 and H-15 to C-16 suggested that the remaining olefin must be connected to C-15. The ^{13}C chemical shifts of C-16 (δ 151.7) and C-17 (δ 122.4) fit well with an α,β unsaturated ketone spin system.[15] HMBC signals between H-12 and the α,β unsaturated ketone showed that the ketone must be bonded to C-13 and confirmed assignment of the epoxycyclohexenone ring system.

By analyzing the remaining NMR data, the C22−C24 fragment was elaborated. H-24 (δ 2.11) was assigned as an acetyl methyl. The HMBC signals from H-22 (δ 4.76 and 4.85, J = 15.5 Hz) to C-15, C-16, C-17, C-18, and C-23 supported the assigned position of the geminally coupled methylene and its connection to the primary acetate.

The absolute stereochemistry of the yanuthones was investigated using CD. The CD spectrum of yanuthone A (**1**) was similar to that of the macrophorins and showed a positive Cotton effect at 340 nm and a negative Cotton effect at 240 nm. The *R*-stereochemistry of the macrophorins was determined by direct comparison of the CD spectra with the data reported for (+)-epoxydon.[16,17] Due to discrepancies in the literature[10,18] concerning the absolute stereochemistry of the macrophorins with identical CD spectra, we have not assigned absolute stereochemistry. The relative stereochemistry was determined by comparing coupling constants with values reported for similar epoxycyclohexenones. A cis relationship between the epoxide and the hydroxyl at position 15 results in J-values of 2.5 Hz for (+)-epoxydon and 3.0 Hz for (+)-isopanepoxydon.[16] The $J_{H14-H15}$ of 2.5 Hz for yanuthone A (**1**) is consistent with the cis relationship. These values are also consistent with the macrophorins, which have the same relative stereochemistry.[8−10,18] An NOE was observed between H-14 and H-15, also suggesting a cis relationship. Interproton distances were investigated using HyperChem.[19] All possible stereoisomers were constructed and the energy minimized using a combination of steepest descent and conjugate gradient methods. Analysis of the minimized structures shows that interproton distances vary only slightly between the cis and trans isomers. All structures had H-14, H-15 interproton distances of 3 Å or less, which is due in part to the structural constraints imposed by the epoxide. All yanuthones described here have the same stereochemistry based on CD spectra.

The ^{1}H NMR spectrum of yanuthone B (**2**) differed from that of yanuthone A (**1**) in two respects. First, the broad singlet at δ 4.59 (H-15) of yanuthone A (**1**) was not observed for yanuthone B (**2**). Second, the olefinic singlet (H-17) had shifted from δ 5.90 for yanuthone A (**1**) to δ 6.51 for yanuthone B (**2**). Mass spectral data showed that the two compounds differed by two mass units. The combined data suggested oxidation of the hydroxyl moiety of yanuthone A (**1**) to a ketone in yanuthone B (**2**). HMBC, ^{13}C, and DEPT NMR data supported the assignment of an epoxyquinone system (see Table 2). Mass spectral analysis (EI) showed a molecular ion at *m*/*z* 400, but high-resolution data was not obtained on the natural product. After bioassay and collection of spectral data, approximately 100 μg of yanuthone B was left. The sample was submitted for mass spectral analysis, but the results were marginal for unknown reasons. To confirm the structure of **2**, yanuthone A was oxidized using tetrapropylammonium perruthenate (TPAP) and 4-methylmorpholine *N*-oxide (NMO). The oxidized product was identical to yanuthone B by ^{1}H NMR, UV, and CD. The CI mass spectrum of the product was also consistent with the EI mass spectrum of **2**,with major masses shifted by one mass unit. HRCIMS for $[M + H]^+$ gave a molecular formula of $C_{24}H_{33}O_5$.

The ^{1}H NMR spectrum of yanuthone C (**3**) contained two signals at δ 5.88 and 6.12 that were similar to those of acetylated yanuthone A (**1**). This suggested that position 15 was an acetyl ester rather than an alcohol as in **1**. Furthermore, the geminally coupled methylene signals of yanuthone C (**3**) (H-22a, H-22b) were shifted upfield to around δ 4.22, and the difference in frequencies ($\Delta\nu$) was smaller, supporting a primary alcohol at position 22. HRCIMS showed that yanuthone C (**3**) had the same molecular formula as yanuthone A (**1**), $C_{24}H_{34}O_5$, confirming the structure. HMBC and ^{13}C NMR data were not collected due to insufficient material.

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Table 1. ^{1}H NMR Data ($CDCl_3$) for Compounds 1−8

atom	1	2	3[b]	4	5	6	7[b]	8[b]
1	1.66 (s)	1.66 (s)	1.66 (s)	1.66 (s)	1.66 (s)	3.99 (s)	3.99 (s)	1.66 (s)
3	5.06 (t, 7.0)	5.05 (t, 7.0)	5.07 (t, 7.0)	5.07 (t, 7.0)	5.07 (t, 7.0)	5.37 (t, 7.0)	5.38 (t, 7.0)	5.07 (t, 7.0)
4	2.03[a] (m)	2.02[a] (m)		2.04[a] (m)	2.03[a] (m)	1.98[a] (m)		
5	2.00[a] (m)	1.93[a] (m)		1.92[a] (m)	1.95[a] (m)	2.01[a] (m)		
7	5.04 (t, 7.0)	5.04 (t, 7.0)	5.04 (t, 7.0)	5.04 (t, 7.0)	5.05 (t, 7.0)	5.05 (t, 7.0)	5.05 (t, 7.0)	5.04 (t, 7.0)
8	2.05[a] (m)	2.05[a] (m)		2.08[a] (m)	2.04[a] (m)	2.09[a] (m)		
9	1.95[a] (m)	2.00[a] (m)		1.99[a] (m)	1.99[a] (m)	1.99[a] (m)		
11	4.99 (t, 7.0)	4.97 (t, 7.0)	4.99 (t, 7.0)	4.96 (t, 7.0)	4.99 (t, 7.0)	4.99 (t, 8.0, 7.0)	4.99 (t, 7.0)	4.99 (t, 7.0)
12a	2.50 (dd, 15.5, 7.0)	2.62 (dd, 15.5, 7.0)	2.48 (dd, 15.5, 7.0)	2.62 (dd, 15.5, 7.0)	2.47 (dd, 15.5, 7.0)	2.53 (dd, 15.5, 7.0)	2.45 (dd, 15.5, 7.0)	2.49 (dd, 15.5, 7.0)
12b	2.78 (dd, 15.5, 7.0)	2.87 (dd, 15.5, 7.0)	2.81 (dd, 15.5, 7.0)	2.87 (dd, 15.5, 7.0)	2.74 (dd, 15.5, 7.0)	2.76 (dd, 15.5, 8.0)	2.83 (dd, 15.5, 7.0)	2.79 (dd, 15.5, 7.0)
14	3.70 (d, 2.5)	3.65 (s)	3.69 (d, 2.9)	3.66 (s)	3.75 (d, 3.0)	3.69 (d, 2.5)	3.73 (d, 2.5)	3.69 (d, 2.5)
15	4.59 (bs)		5.88 (bs)		4.64 (bs)	4.59 (bd, 2.5)	5.88 (bs)	4.67 (bs)
17	5.90 (s)	6.51 (bs)	6.12 (s)	6.56 (bs)	5.93 (s)	5.90 (bs)	6.13 (s)	5.94 (s)
19	1.58 (s)	1.57 (s)	1.58 (s)	1.58 (s)	1.58 (s)	1.64 (s)	1.64 (s)	1.58 (s)
20	1.57 (s)	1.52 (s)	1.57 (s)	1.57 (s)	1.57 (s)	1.58 (s)	1.58 (s)	1.57 (s)
21	1.61 (s)	1.62 (s)	1.62 (s)	1.62 (s)	1.61 (s)	1.61 (s)	1.61 (s)	1.61 (s)
22a	4.76 (d, 15.5)	4.79 (dd, 17.0, 2.0)	4.20 (d, 16.0)	4.85 (dd, 16.5, 2.0)	4.75 (d, 15.5)	4.76 (d, 15.5)	4.20 (d, 16.0)	4.43 (d, 16.0)
22b	4.85 (d, 15.5)	4.94 (dd, 17.0, 2.0)	4.25 (d, 16.0)	4.98 (dd, 16.5, 2.0)	4.95 (d, 15.5)	4.85 (d, 15.5)	4.24 (d, 16.0)	4.67 (d, 16.0)
24a	2.11 (s)	2.12 (s)	2.20 (s)	2.64 (d, 15.5)	2.64 (d, 15.5)	2.11 (s)	2.20 (s)	
24b				2.72 (d, 15.5)	2.75 (d, 15.5)			
26a				2.68 (d, 15.5)	2.63 (d, 15.5)			
26b				2.75 (d, 15.5)	2.71 (d, 15.5)			
28				1.39 (s)	1.39 (s)			

[a] These methylene shifts were estimated from HMQC data. [b] HMQC NMR data were not collected for these compounds, and the methylene proton shifts (1.91−2.09 ppm) were not assigned for positions 4, 5, 8, and 9.

Compound **4** gave a ^{1}H NMR spectrum very similar to that of yanuthone B (**2**). The ^{1}H NMR spectrum of yanuthone D (**4**) lacked the acetyl methyl but contained a methyl signal at δ 1.39. Four geminally coupled doublets (J = 15.5 Hz) were also observed for yanuthone D (**4**). The ^{13}C spectrum showed four signals that differed significantly from those of yanuthone B (**2**) (δ 27.4, 44.5, 44.7, 173.6). By comparing the carbon shifts with those reported for macrophorin-D, the difference between the two compounds was determined to be the replacement of the acetyl at position 22 with a hydroxymethyl glutarate (HMG).[8] The *R*-stereochemistry of the HMG had been determined for macrophorin-D using the method of Hirai and Koshimizu.[20] The absolute stereochemistry of the HMG moiety in yanuthone D (**4**) was not determined. A molecular ion was not observed in the EI, CI, or FAB mass spectrum. However, the isobutane CI spectrum yielded two peaks (*m*/*z* 359 and 145) due to fragmentation of the HMG ester linkage. The *m*/*z* 359 peak is consistent with cleavage between the oxygen attached to C-22 and the adjacent carbonyl plus the addition of one hydrogen radical and a proton. The *m*/*z* 145 peak is consistent with protonation of the carbonyl and subsequent alpha cleavage. HRCIMS analysis using isobutane yielded molecular formulas for *m*/*z* 145 ($C_5H_9O_4$), *m*/*z* 358 ($C_{22}H_{30}O_4$), and *m*/*z* 359 ($C_{22}H_{31}O_4$), all of which are consistent with the proposed structure.

Yanuthone E (**5**) also contained the HMG moiety based on ^{1}H and ^{13}C NMR data. The remaining ^{13}C/^{1}H signals were nearly identical to those of yanuthone A (**1**). The similarity of spectra between **1** and **5** combined with HMBC data showed that yanuthone E (**5**) differed from **1** only by the ester group. Yanuthone E (**5**) was converted to the methyl ester using diazomethane, and HRCIMS gave a molecular formula of $C_{29}H_{43}O_8$ for the $[M + H]^+$ ion, confirming both the presence of the acid and the structure.

The structure of 1-hydroxyyanuthone A (**6**) was determined largely by the appearance of an oxygenated methylene in the proton spectrum at δ 3.98. Further structural evidence came from the different ^{13}C NMR shifts of the farnesyl methyls as compared to those of compounds **1**−**5**. One methyl ^{13}C signal exhibited an upfield chemical shift of δ 13.8. Furthermore, the *E*-methyl resonance (C-1) observed for compounds **1**−**5** was missing, but a new signal appeared at δ 69.0, suggesting that C-1 was oxidized in 1-hydroxyyanuthone A (**6**). HMBC correlations from the oxygenated methylene (C-1) to C-21, C-2, and C-3 confirmed a farnesyl-terminating location for C-1. HRCIMS gave a molecular formula of $C_{24}H_{34}O_6$ and confirmed that the difference between yanuthone A (**1**) and 1-hydroxyyanuthone A (**6**) was the hydroxyl at C-1.

1-Hydroxyyanuthone C (**7**) was obtained as a nearly colorless oil that was shown to have the same molecular formula as 1-hydroxyyanuthone A (**6**) by HRCIMS. The ^{1}H NMR spectra of **6** and **7** indicated that the difference between the compounds was the position of the acetyl (see Table 1). Acetylation at position 15 was indicated by a significant downfield shift of H-15. The ^{1}H shift of the acetyl methyl was consistent with acetylation at C-15 and not at C-22. Neither ^{13}C nor HMBC NMR data were collected, due to the small quantities of **7**.

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Table 2. ^{13}C NMR Data ($CDCl_3$) for Compounds 1, 2, 4, 5, and 6

atom	1	2	4	5	6
1	25.7 (3, 19)[a]	25.7	25.8 (3, 19)	25.7 (3, 19)	69.0 (3, 19)
2	131.4 (1, 19)	131.4 (1, 19)	131.3 (1, 19)	131.4 (1, 19)	134.6 (1, 19)
3	124.3 (1, 19)	124.3 (1, 19)	124.2 (1, 19)	124.3 (1, 19)	126.1 (1, 19)
4	26.7 (3)	26.7	26.8 (3, 19)	26.7	26.0 (3)
5	39.7 (7, 20)	39.7	39.7 (3, 20)	39.7 (3, 20)	39.2 (20)
6	135.3 (20)	135.4 (20)	135.4 (20)	135.3 (20)	134.8 (20)
7	123.8 (20)	123.6 (20)	123.6 (20)	123.8 (20)	124.1 (20)
8	26.4	26.3	26.4 (7)	26.4 (7)	26.1 (7)
9	39.7 (11, 21)	39.7	39.7 (7, 11, 21)	39.7 (7, 11, 21)	39.6 (7, 11, 21)
10	140.1 (12a, 12b, 21)	141.1 (21)	141.1 (12a, 12b, 21)	140.1 (12a, 12b, 21)	139.8 (12a, 12b, 21)
11	115.8 (12a, 12b, 21)	114.7 (12a, 12b, 21)	114.5 (12a, 12b, 21)	115.8 (12a, 12b, 21)	116.0 (12a, 12b, 21)
12	25.9 (11, 14)	25.2	25.3 (11)	25.8 (11)	25.8 (11, 14)
13	61.3 (12a, 12b, 14)	62.4 (12a, 12b)	62.5 (12a, 12b, 14, 17)	61.3 (12a, 12b, 17, 21)	61.3 (12a, 12b, 14)
14	58.9 (12a, 12b)	57.5	57.5 (12a, 12b)	58.8 (12a, 12b)	58.9 (12a, 12b)
15	65.6 (14, 17, 22a, 22b)	192.0 (14)	191.8 (14, 17, 22a, 22b)	65.5 (14, 17, 22a, 22b)	65.6 (14, 17, 22a, 22b)
16	151.7 (14, 17, 22a, 22b)	143.1 (14, 22)	142.4 (14, 17, 22a, 22b)	151.5 (14, 15, 17, 22a, 22b)	152.0 (14, 17, 22a, 22b)
17	122.4 (22a, 22b)	132.3 (22)	132.6 (22a, 22b)	122.8 (15, 22a, 22b)	122.1 (22a, 22b)
18	193.1 (12a, 12b, 22a, 22b)	192.0 (14)	191. (12a, 12b, 22a, 22b)	193.4 (12a, 12b)	193.3 (12a, 12b)
19	17.7 (1)	17.7 (1)	17.8 (1)	17.7 (1)	13.8 (1, 3)
20	16.0 (7)	16.0 (7)	16.1 (7)	16.0 (7)	16.0 (7)
21	16.4 (11)	16.4 (11)	16.5 (11)	16.4 (11)	16.3 (11)
22	62.8 (14, 17, 24)	59.4 (14)	59.8 (14, 17)	63.6 (17)	62.8 (14, 17, 24)
23	170.4 (22a, 22b, 24)	169.8 (24)	170.7 (22a, 22b, 24a, 24b, 26a, 26b)	171.0 (22a, 22b, 24a, 24b, 26a, 26b)	170.5 (22a, 22b, 24)
24	20.7	20.6	44.47[b] (26, 28)	44.68[b] (26, 28)	20.7
25			69.7 (24a, 24b, 26a, 26b, 28)	70.0 (24a, 24b, 26a, 26b, 28)	
26			44.68[b] (24, 28)	44.79[b] (24, 28)	
27			174.7 (24, 26)	174.6 (24, 26)	
28			27.4 (24, 26)	27.4 (24, 26)	

[a] Numbers in parentheses are protons to which the carbon correlated in HMBC NMR experiments. [b] Assignments may be reversed.

Table 3. Antimicrobial Activity of the Yanuthones

compd	amount tested, μg	MS[a] *S. aureus*	MR[b] *S. aureus*	*Candida albicans*	vancomycin resistant *Enterococcus*	IMP mutation *E. coli*	WT *E. coli*
1	175	8[c]H[d]	-	-	-	-	-
2	250	10	10	10H	9	-	-
3	72	8	8	-	5	trace[e]	-
4	62	15	17	12	15	9	-
5	100	13	17	-	12	8	-
6	75	9	10	7H	-	9	-
7	75	10	15	-	12	9	-
8	72	8	9H	-	trace	trace	-

[a] Methicillin sensitive. [b] Methicillin resistant. [c] Zone of inhibition measured in mm. [d] Hazy zone. [e] Zone was too small to measure.

The ^1H NMR spectrum of 22-deacetylyanuthone A (**8**) clearly showed that the compound contained all of the same proton signals as **1,** except that of the acetyl methyl. The upfield shift of the geminally coupled methylene (H22a, H22b) is indicative of an hydroxy rather than an acetate. HRCIMS gave a molecular formula of $C_{22}H_{32}O_5$. The data were consistent with 22-deacetylyanuthone A (**8**) having hydroxyls at positions 15 and 22. Due to the small amount of 22-deacetylyanuthone A (**8**), ^{13}C and HMBC NMR data were not collected.

All compounds were tested against a panel of microorganisms using standard agar diffusion methods with one measurement. Compound **1** displayed minimal activity against methicillin sensitive (ms) *Staphylococcus aureus* (see Table 3). The most active compounds contained an HMG at position 22, which indicates that the group at this position affects the observed bioactivity. The increased activity of compounds **4** and **5** may be a simple function of polarity leading to increased diffusion and larger zones of inhibition. Interestingly, a similar trend is observed for the self-growth inhibiting activity of the macrophorins.[8]

Conclusion

The study of fungal secondary metabolites continues to yield novel bioactive natural products. Fungal secondary metabolites are quite often derived from mixed biosynthetic pathways, as are the yanuthones, which lead to incredible structural diversity. The structural similarities between the yanuthones and the longithorones raise some question about the origin of the longithorones or at least the origin of the quinone portion of the longithorones. Furthermore, quinones are derived from the shikimic acid pathway, a pathway that should not be present in ascidians. However unlikely, the possibility exists that the prenylated quinone portion of the longithorones is derived from endogenous quinones used for electron transport. Since fungi do have the shikimic acid pathway and produce a wide variety of quinone type compounds, there is a high probability that part of the longithorones may be of fungal origin. Many groups are currently studying microbes living symbiotically or epibiotically with marine animals to investigate structural relations with compounds attributed to the animal. The study of fungi from marine animals may provide additional insights about the origin of some marine natural products.

Experimental Section

General Experimental Procedures. The ^1H and ^{13}C spectra were obtained at 500 and 125 MHz. Proton shifts are reported in parts per million relative to residual nondeuterated chloroform (δ 7.24 for ^1H and δ 77.0 for ^{13}C). HPLC was conducted using a Rainin Dynamax-60 Å SiO_2 column (10 × 250 mm, 8 μm) at a flow rate of 3.0 mL/min with photodiode array detection. Sephadex LH-20 chromatography gel (25−200 μm bead size) was purchased from Sigma.

Biological Material. Strain F97S11 was isolated from an orange *Aplidium* species ascidian collected in the waters surrounding Caesar's Rock in Benga, Fiji, at a depth of 50 ft. The fungus was isolated from a core sample of ascidian to try and eliminate surface contamination. Results from the taxonomic characterization of strain F97S11 identified the culture as *Aspergillus niger*. Strain F97S11 has been deposited in the Wyeth-Ayerst Culture Collection in Pearl River, NY.

Fermentation, Extraction, and Isolation. Isolate F97S11 grown on Bennett's agar [10 g/L dextrose, 1 g/L beef extract (Difco), 1 g/L yeast extract (Difco), 2 g/L N−Z-Amine A (Quest International), 15 g/L agar] was inoculated into 10 mL of potato dextrose broth (PDB, Difco) at pH 7 in a 25 • 150 mm test tube and incubated at 22° C, 160 rpm for 7 days. A second stage seed was prepared by transferring the 10 mL culture to a 250 mL Erlenmeyer flask containing 50 mL of PDB, which was incubated at 22 °C, 200 rpm for 4 days.

Production fermentation was performed in a 2.8 L Fernback flask containing 1 L Czapek-Dox medium (Difco). Each flask was inoculated with 50 mL of the second stage seed and incubated at 22 °C and 200 rpm for 7 days. After 7 days the fermentation broth was combined with an equal volume of ethyl acetate and shaken at 200 rpm for 1 h. The aqueous and ethyl acetate phases were separated by centrifugation and the ethyl acetate extract was dried by rotary evaporation.

The crude extract was tested against the following microorganisms: *Staphylococcus aureus* (methicillin resistant and methicillin sensitive), *Escherichia coli*, *Enterococcus* (vancomycin resistant), and *Candida albicans*. Using standard diffusion assays, zones of growth inhibition were measured. Bioactivity was used as a guide for the purification of all compounds.

The ethyl acetate extract (5.09 g) was dissolved in 110 mL of 10% H_2O/MeOH and extracted three times with 200 mL of hexanes. The hexane layers were combined, and the solvent was removed by rotary evaporation to yield 192.8 mg of hexane-soluble material. Water was then added to the aqueous MeOH portion until the water content was 30%. The aqueous layer was extracted two times with 200 mL of $CHCl_3$. The solvent was removed by rotary evaporation to give 588.1 mg of $CHCl_3$ solubles. A portion (80 mg) of hexane-soluble material was chromatographed on LH-20, (2.5 cm by 40 cm, 1:1 $CHCl_3$/MeOH) to remove fats. A yellow band eluted with 25 mL, was collected, and chromatographed using silica gel (2.5 cm by 30 cm, hexane/ethyl acetate 95:5 to 100:5 ethyl acetate/MeOH). The most active fraction eluted with 25:75 ethyl acetate/hexane. A more polar fraction eluted with 50:50:1 hexane/ethyl acetate/MeOH but had minimal bioactivity. However, comparison of the ^1H NMR spectra indicated that the two fractions contained related compounds, as did the last fraction to elute from the column. Each of the three fractions was purified by gradient HPLC using 5−80% ethyl acetate/hexanes. Yanuthone A (**1**) eluted with 50% ethyl acetate to yield 4.4 mg of a pale yellow oil. Yanuthone B (**2**) eluted with 25% ethyl acetate to yield 1.3 mg of a pale yellow oil. Yanuthone C (**3**) eluted with 64% ethyl acetate to give 1.7 mg of a pale oil.

The $CHCl_3$-soluble material was chromatographed on LH-20 (2.5 cm by 75 cm, 1:1 $CHCl_3$/MeOH). Two major bands were collected. The first band was light brown and contained mostly fats. Bioactivity was concentrated in the second, yellow band (eluted with 75 mL), which was separated using centrifugal countercurrent chromatography (CCCC) in normal-phase mode (1:2:2:1 hexane/$CHCl_3$/MeOH/H_2O, lower phase mobile, 800 rpm, 400 mL column, 2 mL/min). Two fractions appeared to contain compounds similar to 1−3 by ^1H NMR. The first fraction eluted from 15 to 20 min after the mobile phase came off the column. The second fraction eluted from 20 to 25 min after the mobile phase. Each of the two CCCC fractions was purified by gradient HPLC (SiO_2, hexane/ethyl acetate). HPLC (20−100% ethyl acetate/hexane) of CCCC fraction 1 yielded 11.9 mg of yanuthone D (**4**), which eluted with 48% ethyl acetate. HPLC (10−90% ethyl acetate/hexane) of CCCC fraction 2 gave three pure compounds (**5**−**7**). Yanuthone E (**5**) was isolated as a brown/yellow oil (16.4 mg) and eluted with 62% ethyl acetate. 1-Hydroxyyanuthone A (**6**) was isolated as colorless oil (6.3 mg) and eluted with 69% ethyl acetate. At 85% ethyl acetate, 1-hydroxyyanuthone C (**7**) eluted and yielded 0.9 mg. During the same HPLC, 22-deacetylyanuthone A (**8**) eluted as a mixture with 71% ethyl acetate. A final HPLC (SiO_2, 2−30% 2-propanol/hexane) gave 1.5 mg of **8**.

Yanuthone A (1). UV (MeOH) λ_{max} 232 (log ϵ 3.90), 310 (log ϵ 2.70); CD_{MeOH} $[\theta]_{240}$ −343, $[\theta]_{340}$ +224; IR 3460 (br), 2918, 2850, 1743, 1684 cm^{-1}; ^1H NMR data listed in Table 1; ^{13}C NMR and HMBC data listed in Table 2; 1D-difference NOE ($CDCl_3$) irrad. δ 4.59, enhanced δ 3.70 (5%), irrad. 3.70, enhanced δ 4.59 (4%), irrad. δ 2.78, enhanced δ 2.50 (11%); HRCIMS (isobutane) *m*/*z* 403.2476 ([M + H]$^+$, calcd for $C_{24}H_{35}O_5$, 403.2485).

Yanuthone B (2): UV (MeOH) λ_{max} 244 (log ϵ 3.65), 290 (log ϵ 3.29), 338 (log ϵ 2.92); CD_{MeOH} $[\theta]_{231}$ −30, $[\theta]_{243}$ +11, $[\theta]_{257}$ −11, $[\theta]_{292}$ +11; IR 2924, 2851, 1749, 1691 cm^{-1}; ^1H NMR data listed in Table 1; ^{13}C NMR and HMBC NMR data listed in Table 2; EIMS (150 eV) *m*/*z* 400 (0.32), 357 (0.28), 136 (25).

Yanuthone C (3): UV (MeOH) λ_{max} 232 (log ϵ 4.12), 280 (log ϵ 3.31), 322 (log ϵ 3.03); CD_{MeOH} $[\theta]_{233}$ −940, $[\theta]_{341}$ +435; IR 3444 (br), 2924, 2851, 1748, 1684 cm^{-1}; ^1H NMR data listed in Table 1; HRCIMS (isobutane) *m*/*z* 402.2398 (M$^+$, calcd for $C_{24}H_{34}O_5$, 402.2407).

Yanuthone D (4). UV (MeOH) λ_{max} 226 (log ϵ 3.90), 254 (log ϵ 3.57), 298 (log ϵ 3.06); CD_{MeOH} $[\theta]_{233}$ −932, $[\theta]_{241}$ +143, $[\theta]_{261}$ −574, $[\theta]_{303}$ +179; IR 3481 (br), 2972, 2872, 1737, 1690 cm^{-1}; ^1H NMR data listed in Table 1; ^{13}C NMR and HMBC data listed in Table 2; HRCIMS (isobutane) *m*/*z* 145.0499 (HMG fragment, calcd for $C_6H_9O_4$, 145.0500), 358.2140 ([M − HMG + H]$^+$, calcd for $C_{22}H_{30}O_4$, 358.2142), 359.2209 ([M − HMG + 2H]$^+$, calcd for $C_{22}H_{31}O_4$, 359.2220).

Yanuthone E (5). UV (MeOH) λ_{max} 234 (log ϵ 3.96), 280 (log ϵ 3.00), 340 (log ϵ 2.65); CD_{MeOH} $[\theta]_{241}$ −1389, $[\theta]_{342}$ +917; IR 3420 (br), 2969, 2848, 1732, 1684 cm^{-1}; ^1H NMR data listed in Table 1; ^{13}C NMR and HMBC data listed in Table 2; HRCIMS for the methyl ester (isobutane) *m*/*z* 519.2948 ([M + H]$^+$ calcd for $C_{29}H_{43}O_8$, 519.2946).

1-Hydroxyyanuthone A (6). UV (MeOH) λ_{max} 234 (log ϵ 3.93), 290 (log ϵ 2.34); CD_{MeOH} $[\theta]_{242}$ −1550, $[\theta]_{340}$ +947; IR 3399 (br), 2920, 2851, 1747, 1683 cm^{-1}; ^1H NMR data listed in Table 1; ^{13}C NMR and HMBC data listed in Table 2; HREIMS (isobutane) *m*/*z* 418.2388 (M$^+$, calcd for $C_{24}H_{34}O_6$, 418.2356), 400.2249 ([M − H_2O]$^+$, calcd for $C_{24}H_{32}O_5$, 400.2251).

1-Hydroxyyanuthone C (7). UV (MeOH) λ_{max} 232 (log ϵ 3.67), 282 (log ϵ 2.67); CD_{MeOH} $[\theta]_{242}$ −219, $[\theta]_{334}$ +139; IR 3399 (br), 2923, 2857, 1734, 1683 cm^{-1}; ^1H NMR data listed in Table 1; HRCIMS (isobutane) *m*/*z* 401.2290 ([M − H_2O]$^+$, calcd for $C_{24}H_{33}O_5$, 401.2329).

22-Deacetylyanuthone A (8). UV (MeOH) λ_{max} 238 (log ϵ 4.21), 302 (log ϵ 2.68); CD_{MeOH} $[\theta]_{240}$ −792, $[\theta]_{338}$ +458; IR 3373 (br), 2966, 2922, 2854, 1682 cm^{-1}; ^1H NMR data listed in Table 1; HRCIMS (isobutane) *m*/*z* 361.2362 ([M + H]$^+$, calcd for $C_{22}H_{33}O_4$, 361.2380).

Acetylation of 1. Compound **1** (5.7 mg) was dissolved in 100 μL of pyridine followed by the addition of 100 μL of acetic anhydride. The solution was stirred for 24 h at room temperature. The solution was dried in vacuo and purified by HPLC (SiO_2, hexane/$CHCl_3$) to yield 4.6 mg of colorless oil: UV (MeOH) λ_{max} 230 (log ϵ 4.01), 286 (log ϵ 2.52); CD_{MeOH} $[\theta]_{230}$ −1176, $[\theta]_{344}$ +547; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.03 (1H, d, J = 2.0 Hz, H-15), 5.87 (1H, s, H-17), 5.06 (1H, t, J = 7.0 Hz, H-3), 5.05 (1H, t, J = 7.0 Hz, H-7), 4.97 (1H, t, J = 7.0 Hz, H-11), 4.77 (1H, d, J = 15.5 Hz, H-22b), 4.58 (1H, d, J = 15.5 Hz, H-22a), 3.69 (1H, d, J = 3.0 Hz, H-14), 2.78 (1H, dd, J = 15.5, 7.5 Hz, H-12b), 2.49 (1H, dd, J = 16.0, 6.5 Hz, H-12a), 2.18 (3H, s, H_3-26), 2.07 (3H, s, H_3-24), 1.92−2.05 (8H, bm), 1.66 (3H, s, H_3-19), 1.61 (3H, s, H_3-21), 1.58 (3H, s, H_3-1), 1.57 (3H, s, H_3-20); 1D-difference NOE ($CDCl_3$) irrad. δ 5.87, enhanced δ 3.69 (8%), irrad. δ 3.69, enhanced δ 5.87 (9%), irrad. δ 2.78, enhanced δ 4.97 (7%) δ 2.49 (28%); HRCIMS (isobutane) *m*/*z* 444.2501 (M$^+$, calcd for $C_{26}H_{36}O_6$, 444.2413).

Oxidation of Yanuthone A (1). To a solution of yanuthone A (3.0 mg, 0.0075 mmol) in 400 μL of methylene chloride was added 2.0 mg (0.0171 mmol) of 4-methylmorpholine *N*-oxide (NMO) in 100 μL of methylene chloride. The mixture was stirred at room temperature for 3 min, followed by the addition of 4 Å molecular sieves and 0.8 mg (0.0023 mmol) of tetrapropylammonium perruthenate (TPAP) in one addition. The reaction mixture was stirred for 22 min, at which time TLC analysis on SiO_2 using 50:50 ethyl acetate/hexane showed no starting material. Flash SiO_2 chromatography using 50:50 ethyl acetate/hexane yielded 1.5 mg of product. The product was shown to be identical to yanuthone B (**2**) by ^{1}H NMR, CD, UV, and TLC (R_f 0.78, SiO_2; 1:1 ethyl acetate/hexane): HRCIMS (isobutane) *m*/*z* 401.2308 ([M + H]$^+$, calcd for $C_{24}H_{33}O_5$, 401.2319).

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Supporting Information Available: ^{1}H NMR spectra for compounds **1**−**8** and acetylated **1** and ^{13}C NMR spectra for compounds **1**, **2**, **4**, **5**, and **6** (S1−S16). This material is available free of charge via the Internet at http://pubs.acs.org.

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