Halawanones A−D, New Polycyclic Quinones from a Marine-Derived Streptomycete

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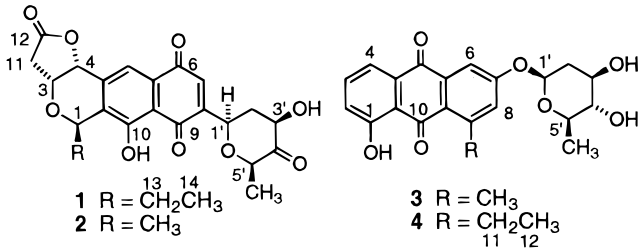
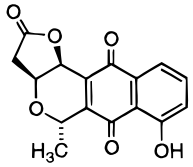
Four new quinone-containing metabolites, halawanones A−D (**1**−**4**), have been isolated along with the known compound nanaomycin D (**5**) from liquid cultures of a streptomycete obtained from a sediment sample collected in the estuarine environment at the mouth of Halawa stream, Oahu. The structures of the new compounds were determined through the interpretation of spectral data. The absolute stereochemistries were determined using a combination of CD and NMR.

Marine microorganisms have been the topic of an increasing number of natural products investigations.[1,2] Marine bacteria, in particular, have received increased attention as potential sources of biologically active metabolites.[3,4] Although bacteria have been isolated from a variety of marine sources, sediments continue to receive significant attention, perhaps because of their similarity to terrestrial soils and because they have been shown to be a good source of Gram-positive organisms, including actinomycetes.[5] Recent results include the isolation of caprolactins A and B,[6] γ-indomycinone,[7] the wailupemycins,[8] and the bioxalomycins,[9] which were all produced by sediment-derived Gram-positive bacteria. We now report the isolation and structure determination of halawanones A−D (**1**−**4**), quinone-containing metabolites that were obtained along with the known compound nanaomycin D (**5**)[10] from a streptomycete cultured from shallow-water estuarine sediments. Halawanones A (**1**) and B (**2**) incorporate naphthoquinone ring systems, while halawanones C (**3**) and D (**4**) incorporate anthraquinone moieties.





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Results and Discussion

Isolate BD-18T(41), which was cultured from sediments collected at the mouth of Halawa stream on the northeastern shore of Oahu, Hawaii, was determined to belong to the genus *Streptomyces* on the basis of morphological and chemotaxonomic analyses. Specifically, the organism produced abundant white aerial mycelium and yellowish brown substrate mycelium, which transformed into chains of smooth, cylindrical arthrospores, as shown by scanning-electron microscopy. Isolate BD-18T(41) demonstrated the ability to metabolize casein, tyrosine, and xanthine; it produced no characteristic sugars; and an analysis of the whole-cell hydrolysate revealed the presence of only the *ll*-isomer of diaminopimelic acid. The above properties are consistent with the genus *Streptomyces*, an assignment further supported by fatty acid methyl ester (FAME) analysis. The fatty acid pattern, consisting primarily of saturated iso and anteiso fatty acids, strongly matched that of *Streptomyces halstedii olivaceous*.

Isolate BD18T(41) was fermented for 5 days in a seawater-based medium, after which time the culture broth was filtered through Celite. Methanol extraction of the mycelium, followed by solvent partition provided a crude chloroform extract that, after Si gel flash chromatography, yielded a single compound, nanaomycin D (**5**). The culture filtrate was passed through a column of Diaion HP-20 resin. The column was flushed first with water and then with methanol. The methanol eluent was concentrated and subjected to a solvent partition scheme, yielding three lipophilic fractions (hexane, $CHCl_3$, and CCl_4) and one hydrophilic fraction (H_2O−MeOH). The CCl_4- and $CHCl_3$-soluble materials exhibited antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* and were combined on the basis of their ^{1}H NMR spectra. Although significant streaking was observed using a variety of chromatographic media and solvent systems, the combined CCl_4−$CHCl_3$ fraction was separated into two fractions, designated **A** and **B**, using repeated Si gel column chromatography. Fraction **A** was found to contain two new naphthoquinone metabolites as an inseparable mixture of ethyl and methyl homologues, **1** and **2**, respectively. Fraction **B** contained a 2:1 mixture of methyl and ethyl homologues, which were shown to be the new anthraquinone metabolites **3** and **4**, respectively.

The ^{1}H NMR spectrum of compound **5** showed signals that could be assigned to a hydrogen-bonded phenolic hydroxyl proton (11.83 ppm) and to the protons of a 1,2,3-trisubstituted aromatic ring (7.69, 7.66, and 7.29 ppm), characteristic of a β-hydroxyjuglone moiety. Further inspection of the ^{1}H and ^{13}C NMR data indicated a match

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Table 1. ^{1}H and ^{13}C NMR Data for Halawanones A (**1**) and C (**3**)

	1			**3**		
atom	^{13}C	^{1}H (mult., *J*)	HMBC (^{13}C → ^{1}H)	^{13}C	^{13}H (mult., *J*)	HMBC (^{13}C → ^{1}H)
1	72.2	4.99 (dd, 11.3, 3.1)	H-14, H-13B	162.8	12.97 (OH)	H-3, C-1OH
2				124.7	7.28 (dd, 8.3, 1.2)	H-4, C-1OH
3	65.6	4.74 (dd, 5.6, 3.0)	H-1, H-11A	136.0	7.62 (dd, 8.3, 7.5)	
4	73.8	5.15 (d, 2.9)	H-5, H-11A	118.9	7.74 (dd, 7.5, 1.2)	H-2
4a	135.6		H-1, H-4, H-5	133.5		H-3
5	120.8	7.76 (s)	H-4	183.1		H-4, H-6
5a	130.4		H-6	124.5		
6	183.1		H-5	113.1	7.83 (d, 2.2)	H-8
7	135.2	7.11 (d, 1.2)	H-1′	160.8		
8	148.8		H-1′, H-2′B, H-7	125.7	7.23 (d, 2.2)	H-11
9	189.2		H-7	146.0		
9a	114.3		H-5, C-10OH	105.6		H-6, H-11
10	157.5	12.28 (OH)	C-10OH	190.6		
10a	135.7		H-1, H-5, C-10OH	117.4		H-2, H-4, C-1OH
11	37.2	A 3.01 (dd, 17.9, 5.6) B 2.76 (d, 17.9)		24.1	2.83 (s)	
12	174.0		H-3, H-11A, H-11B			
13	24.4	A 1.96 (m) B 1.71 (m)	H-1, H-14			
14	10.7	1.15 (t, 7.4)	H-13A			
1′	70.0	5.13 (ddd, 11.9, 1.9, 1.2)	H-2′B, H-7	96.2	5.83 (br s)	H-2′A
2′	43.3	A 3.02 (ddd, 12.7, 7.0, 1.9) B 1.81 (ddd, 12.7, 12.3, 11.9)	H-1′, H-3′	37.6	A 2.38 (dd, 13.4, 4.7) B 1.91 (br t, 12.0)	
3′	72.4	4.55 (dd, 12.3, 7.4)	H-2′A, H-2′B	69.1	4.10 (m)	H-1′, H-2′A, H-4′
4′	206.4		H-2′A, H-3′, H-6′	78.0	3.21 (t, 9.1)	H-2′A, H-6′
5′	77.2	4.30 (q, 6.1)	H-1′, H-6′	69.5	3.67 (m)	H-1′, H-4′, H-6′
6′	14.1	1.43 (d, 6.1)	H-5′	17.8	1.23 (d, 6.3)	

with the NMR data reported for the enantiomeric microbial metabolites kalafungin[11,12] and nanaomycin D.[10,13] The specific rotation for compound **5** {$[\alpha]_D$ −273° (*c* 0.67, MeOH)} closely matched the value reported for nanaomycin D {$[\alpha]_D$ −278° (*c* 0.44, MeOH)},[10] allowing compound **5** to be identified as nanaomycin D.

Based on the ^{1}H NMR data, it was determined that fraction **A** was approximately a 3:1 mixture of homologues **1** and **2**. This result was consistent with the MS data; $[M + 3H]^+$ ions were observed at *m/z* 445 and 431 in the FAB spectrum,[14] and M^- ions were observed at *m/z* 442 and 428 in the negative ion CI spectrum.[15] Fortunately, the distinguishing NMR signals corresponding to each compound could be easily assigned. The NMR data for compound **1** (see Table 1) included signals for 23 carbons and 21 protons that together with the mass spectral data supported a molecular formula of $C_{23}H_{22}O_9$. The ^{13}C NMR data included two signals assignable to quinone carbonyls (183.1 and 189.2 ppm) along with eight signals in the region between δ 100−165, suggesting a substituted naphthoquinone ring system.

The ^{1}H NMR spectral data included signals characteristic of a peri-OH at δ 12.28, two aromatic protons [7.11 (s) and 7.76 (s) ppm], and four aliphatic protons that matched those assigned to the γ-lactone in nanaomycin D (**5**) [5.15 (d), 4.74 (m), 3.01 (dd), and 2.76 (d) ppm]. The HMBC data, however, indicated that compound **1** bore an ethyl group on C-1, instead of the more common methyl group at this position. The benzoisochromane portion of the molecule accounted for all but $C_6H_8O_3$, which could be assigned to a C-glycoside connected to C-8 on the basis of a ROESY correlation between H-7 and H-1′. Also supporting this assignment were HMBC correlations between C-8 and both H-1′ and H-2′B and between H-7 and both C-1′ and quinone carbonyl C-9. The regiochemistry of the molecule was then established based on an HMBC correlation from the other quinone carbonyl (C-6) to H-5.

The relative configuration of the benzoisochromane portion of compound **1** was determined though analysis of the ^{1}H−^{1}H coupling constants, NOE difference experiments, and ROESY 2D NMR data. Specifically, the cis-ring junction of the γ-lactone was assigned based on a small coupling constant (J = 3 Hz) between the ring junction protons H-3 and H-4, and supported by a NOESY correlation between the same protons. Furthermore, a ROESY correlation between the C-3 methine proton at δ 4.74 and the H-13B methylene proton at δ 1.71 indicated these substituents were 1,3-diaxial. The relative configuration of the C-glycoside portion of the molecule was also easily deduced from ^{1}H−^{1}H coupling constants and a difference NOE experiment. The axial protons at C-1′, C-2′, and C-3′ all exhibited large diaxial ^{1}H−^{1}H coupling constants (ca. 12 Hz), and dipolar coupling was observed between H-3′ and H-5′, placing both methyl and hydroxyl groups in equatorial positions.

Assignment of the relative stereochemistry between the two asymmetric ends of compound **1** posed some difficulty. Because the large distance between the two ends of the molecule precludes any stereochemical interaction between the two chiral moieties, our approach was to determine the absolute stereochemistry of each end of the molecule independently and then compare the results. While the secondary alcohol at C-2′ of the C-glycoside was suitable for a modified Mosher's analysis,[16] the isochromane moiety was not amenable to direct derivatization with a chiral reagent and, in our hands, could not be converted to a derivative suitable for stereochemical evaluation. Therefore, we chose to utilize CD to assign the chirality of this end of the molecule.

The CD spectrum for fraction **A** exhibited a curve that was almost the mirror image of that obtained for nanaomycin D (**5**) (Figure 1), a benzoisochromane metabolite with known absolute configuration,[10] indicating that compound **1** has the same chirality as kalafungin (= enantiomer of **5**). Although compound **1** and nanaomycin D (**5**) have slightly different chromophores, presumably accounting for the variations in absorption maxima and CD curve shape, this comparative spectral technique for stereochemical

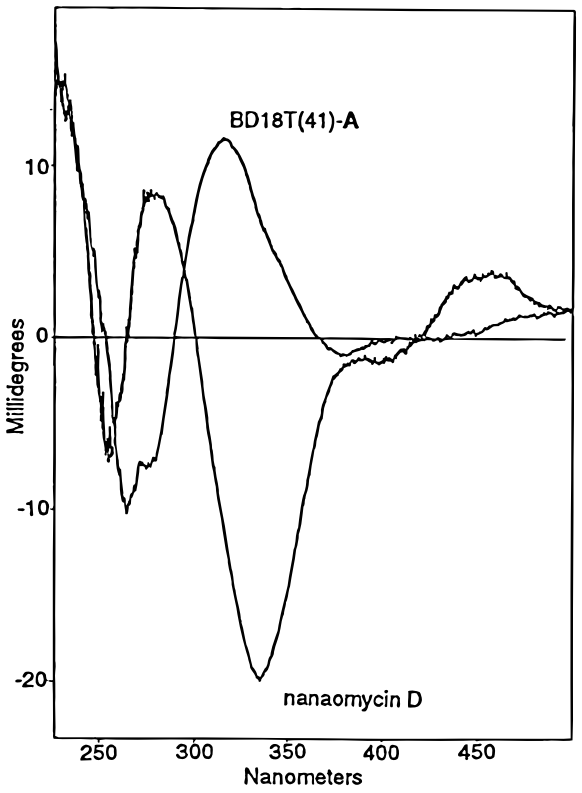

Figure 1. CD comparison of BD18T(41)-**A** (**1** + **2**) and nanaomycin D (**5**).

assignment has precedent in the determination of the absolute stereochemistry for griseusin A, a related benzoisochromane antibiotic.[17] The similarity of CD curves for griseusin A and actionorhodin−indazolquinone[18] was also used to assign griseusin A as having a substituted dihydropyran ring opposite in chirality to that of compound actinorhodin−indazolquinone. Likewise, a similar trend was observed in the CD spectrum of γ-naphthocyclinon-indazolquinone.[19]

The absolute stereochemistry of the C-glycoside portion of compound **1** was investigated using the modified Mosher's method.[16] Treatment of separate aliquots of fraction **A** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride in CH_2Cl_2 yielded the (*S*)- and (*R*)-MTPA esters, respectively. Subtraction of the ^{1}H NMR chemical shifts observed for the (*R*)-MTPA ester from those of the (*S*)-ester resulted in the $\delta\Delta$ values shown in Figure 2. Based on these results, the absolute stereochemistry was determined to be 1*R*,3*R*,4*R*,1′*R*,3′*R*,5′*R*.

The structure of the minor component of fraction **A** was assigned structure **2** based on the MS results mentioned above and on the NMR data. The ^{1}H NMR spectrum clearly shows a subset of signals, all associated with the benzoisochromane portion of molecule, which are approximately one-third the size of those attributed to compound **1**. From the COSY spectrum, the signals could be incorporated into two spin systems. One included signals for a methyl group at δ 1.58 (d), which is coupled to a methine at δ 5.30 (q), and the other contained signals assignable to one of the H-11 methylene protons (d, 2.76 ppm) and the H-3 methine proton (dd, 4.83 ppm). These data are consistent with the replacement of the ethyl group in compound **1** with a methyl group. Although no dipolar coupling was evident for the minor homologue in our NOE

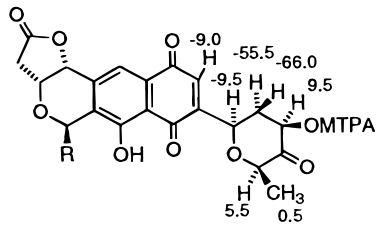

Figure 2. $\Delta\delta$ values (Hz) generated by subtracting $\delta_S - \delta_R$ for the (*S*)- and (*R*)-MTPA esters of fraction **A** (**1** + **2**).

experiments, the relative stereochemistry could be confirmed based on chemical shift comparisons to known isochromanquinones.[20−22]

Fraction **B** contained a mixture of homologues, as was evident from the FABMS $[M + H]^+$ ions at *m*/*z* 399 and 385, consistent with molecular formulas of $C_{22}H_{22}O_7$ and $C_{21}H_{20}O_7$ for compounds halawanones D (**4**) and C (**3**), respectively. Fortunately, as with compounds **1** and **2**, the distinguishing NMR signals for **3** and **4** could be easily assigned. For halawanone C (**3**), the ^{13}C NMR spectrum (Table 1) showed two signals for quinone carbonyls at δ 190.6 and 183.1, along with those for 12 additional sp^2 hybridized carbons, suggesting an anthraquinone ring system. The ^{1}H NMR spectrum showed signals for five aromatic protons that could be divided into two spin systems. The first system contained three signals, representative of a 1,2,3-trisubstituted benzene ring, at δ 7.74 (dd), 7.62 (dd), and 7.28 (dd). HMBC correlations between C-2 (124.7 ppm) and a hydroxyl proton at δ 12.97 and between the C-5 quinone carbonyl (183.1 ppm) and H-4 (7.74 ppm) placed the spin system on an aromatic ring bearing a peri-OH. On the basis of three-bond correlations observed in the HMBC data (see Table 1), the two remaining aromatic protons, H-6 [7.83 ppm (d)] and H-8 [7.23 ppm (d)], were assigned to the second spin system, a 1,2,3,5-tetrasubstituted aromatic ring bearing both a methyl group (2.83 ppm, s) and an oxygen atom. Specifically, H-6 coupled to both the quinone carbonyl C-5 and to C-8, which further coupled to the methyl group at δ 2.83 (H-11).

The anthraquinone ring system accounts for $C_{15}H_9O_4$, leaving $C_6H_{11}O_3$. Of the six remaining carbons, three could be assigned to mono-oxygenated carbons (78.0, 69.5, and 69.1 ppm) and one could be assigned to an acetal (96.2 ppm). The final two carbons corresponded to a methylene (37.6 ppm) and a methyl (17.8 ppm). All together, these data supported the presence of a dideoxyhexose unit connected to C-7 through an *O*-glycosidic linkage. Stepwise scalar coupling could clearly be observed in the COSY spectrum from H-1′ to H-6′.

The relative stereochemistry for halawanone C was assigned based on an analysis of the scalar coupling constants. For example, H-4′ exhibited large (9.1 Hz) couplings to both H-3′ and H-5′, placing all three protons in axial positions and, consequently, placing the two hydroxyls and the methyl group in equatorial positions. H-2′B showed a large (12 Hz) coupling to H-3′, allowing it to be assigned as the axial proton on C-2′, but a small coupling to H-1′, assigning the anomeric proton as equatorial. This coupling data is consistent with that reported for the C-glycosidic olivose sugar moiety in aquayamycin, a related benzanthracene metabolite from *Streptomyces misawanensis*.[23] The assignment of the anomeric proton (5.83 ppm) as equatorial is in accord with the study by Kasai et al., which demonstrated that the anomeric protons of α-D-mannosides and α-L-rhamnosides consistently appear at lower field (5.02−5.92 ppm) than those of corresponding β-anomers (4.55−4.93 ppm).[24]

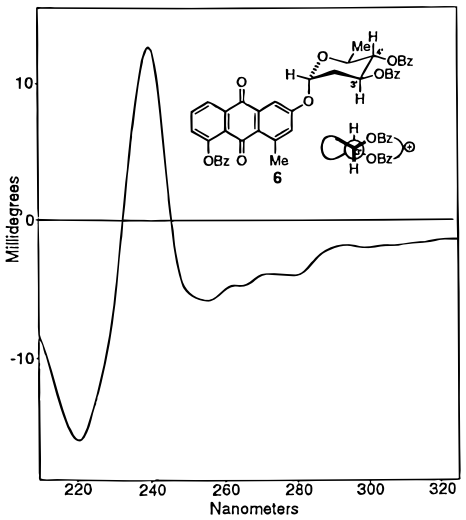

Figure 3. CD spectrum of halawanone C tribenzoate (**6**).

The structure of halawanone D was assigned structure **4** based on the MS results mentioned above, which indicate that it contained an additional CH_2 as compared to halawanone C, and on the NMR data. Although the ^1H NMR spectra for compounds **3** and **4** were largely coincident, particularly important were the signals that could be attributed to the minor homologue, observed at δ 3.28 (q) and 1.30 (t) and assigned to an ethyl group. HMBC correlations among all five protons of the ethyl group and carbons at δ 152.19 and 124.7 suggested that an ethyl group has replaced the C-11 methyl group of halawanone C.

The absolute configurations of halawanones C (**3**) and D (**4**) were determined using the CD exciton coupling method of Harada and Nakanishi.[25] Treatment of fraction **B** with benzoyl chloride gave, after chromatography, the tribenzoate derivative **6** containing trace amounts of the derivatized ethyl homologue. Analysis of the CD spectrum (Figure 3) indicates a bisignate Cotton effect ($\Delta\epsilon$ −16 220 nm; +13 237 nm) corresponding to positive helicity and a 1′*R*,3′*R*,4′*R*,5′*R* configuration for **3** and **4**. Unfortunately, we were unable to obtain MS data to corroborate the structures of tribenzoate derivative **6**; therefore, this assignment must be viewed as tentative.

Halawanones A (**1**) and B (**2**) are related to a general class of isochromane quinone antibiotics that includes granaticin,[26] kalafungin (= enantiomer of **5**),[11,12] nanaomycin D (**5**),[10] and griseusin A.[17] All are produced by cultures of actinomycetes of the genus *Streptomyces* and, in fact, granaticin, the metabolite most closely related to compounds **1** and **2**, was first isolated from a culture of *Streptomyces olivaceous*, the same species classification given to isolate BD18T(41). The fraction containing halawanones A and B was active in a disk diffusion assay against *B. subtilis* (26-mm zone of inhibition at 100 μg/disk) and *S. aureus* (26-mm zone at 100 μg/disk) but inactive against *Escherichia coli* (100 μg/disk). Likewise, granaticin is reported to be active against Gram-positive bacteria, but has little or no activity against Gram-negative bacteria, fungi, or yeasts. Insufficient sample prevented biological testing halawanones C and D.

Experimental Section

General Experimental Procedures. All NMR experiments were performed on a GE Omega 500 instrument at 500 and 125 MHz operating frequencies for ^1H and ^{13}C experiments, respectively. Chemical shifts are referenced to solvent peaks: 7.26 ppm (residual $CHCl_3$) and 77.0 ppm for $CDCl_3$. IR spectra were obtained using a Perkin-Elmer 1600 FTIR, and MS data were obtained on a VG-70SE mass spectrometer operating in the EI and FAB modes. Chemical ionization (CI) mass spectral analyses were performed by Dr. Alan Harmon at McCormick and Company, Inc., Hunt Valley, MD, using the negative ion mode with methane as reagent gas. CD spectra were obtained using either MeOH or 2-propanol as solvent. Bacterial cell wall fatty acid analysis was performed by Microbial ID, Inc., Newark, DE.

Culture Conditions. The bacterium, designated BD18T-(41), was obtained from a shallow-water sediment sample collected at the mouth of Halawa stream on the northeast shore of Oahu, Hawaii. Approximately 1 mL of sediment was placed in a sterile tube containing seawater and stored at 4 °C until use. Sediments were diluted with artificial seawater and shaken thoroughly. The supernatant from each sample (ca. 2 mL) was transferred into test tubes and heat treated at 55 °C for 6 min. A small aliquot (50 μL) of each sample was plated directly onto starch−casein media containing 5 μg/mL of the antibiotic rifampicin and 50 μg/mL cyclohexamide. After incubation at 23−28 °C for up to 30 days, single colonies were harvested and restreaked for purity. The bacterial isolate was then used to initiate liquid seed cultures into test tubes containing 10 mL of Difco marine broth supplemented with soluble starch. The tubes were incubated at 23−27 °C on a platform shaker at 200 rpm. After sufficient growth (typically 3−5 days) the tube cultures were used to seed 40 × 500 mL of liquid media in 2000-mL Erlenmeyer flasks, which were incubated for 4−5 days under conditions similar to the seed cultures.

Taxonomic Study. Bacterial cells from BD18T(41) were collected by centrifugation at 5000 rpm for 20 min after 4 days of fermentation. The pellet was washed with distilled H_2O and 95% EtOH and lyophilized to a dry powder and stored in the freezer until use.

Hydrolysis of the whole cell preceded diaminopimelic acid (DAP) analysis. Approximately 3 mg of the dried cells were placed in a small vial with 1 mL of 6 N HCl. The sealed vial was heated at 100 °C in an oil bath for 16 h. After cooling, the hydrolysate was vacuum filtered and the filtrate evaporated to dryness. The crude white solid was redissolved in 0.3 mL of H_2O, and approximately 1 μL was applied to the baseline of a TLC sheet (Eastman Kodak no. 13254 cellulose, 10 cm × 10 cm) together with a DAP standard (0.01 M DL-DAP, which contained both meso and L-DAP isomer). Ascending TLC was performed with the solvent system MeOH−H_2O−6 N HCl−pyridine (80:26:4:10). Spots were visualized with a solution of ninhydrin in EtOH followed by heating.

Extraction and Isolation. The liquid culture (20 L) was filtered through a Buchner funnel packed with Celite (frequent repacking of the funnel expedited this process). The solid mycelial/Celite filter cake was soaked in MeOH (ca. 1.5 L) overnight. The slurry was filtered, and the clear yellow filtrate was concentrated to yield a crude yellow solid (3.0 g). This crude material was dissolved in a minimal amount of MeOH (ca. 25 mL) and extracted with hexane (3 × 50 mL). The remaining MeOH fraction was diluted 10% by H_2O and extracted further with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ fractions were combined and concentrated to a yellow oil. Flash chromatography over Si gel with 95:5 $CHCl_3$−MeOH eluent provided 3.4 mg of compound **5**, identical in all respects the known compound nanaomycin D.

The supernatant from the initial filtration of the culture broth was passed through a column (3" × 6") containing an aqueous slurry of Diaion HP-20 resin at a rate of approximately 3−6 bed volumes per hour. Afterward, the resin was washed thoroughly with H_2O (ca. 10 L) and the organic residue removed from the resin by elution with MeOH (4 × 500 mL). The MeOH fractions were concentrated to a dark oily sludge, dissolved in a small amount of MeOH−H_2O, and subjected to solvent partition (hexane, CCl_4, $CHCl_3$). Concentration of the four resulting fractions yielded 1.2 g of hexane-soluble material, 70 mg of CCl_4-soluble material, 796 mg of

$CHCl_3$-soluble material, and 6.3 g of an aqueous MeOH residue. The CCl_4 and $CHCl_3$ fractions were combined and chromatographed over Si gel with 9:1 $CHCl_3$−MeOH as eluent. This procedure provided two fractions; (a) BD18T(41)-**A**, containing compounds **1** and **2** as a 3:1 mixture (430 mg as a yellow-brown glass), and (b) BD18T(41)-**B**, containing compounds **3** and **4** as a mixture (2:1) of methyl and ethyl homologues, respectively (1.6 mg as a yellow solid).

Halawanone A (1): UV (MeOH) λ_{max} 217 (ϵ 20 300), 255 (ϵ 6360), 325 (ϵ 2550), 428 (ϵ 2300); IR (film) υ_{max} 3454, 2976, 2938, 1741, 1667, 1639, 1608, 1392, 1112, 979, 912, 754, 733 cm^{-1}; ^1H and ^{13}C NMR see Table 1; FABMS (gly matrix) *m*/*z* (rel int) 445 (100), 431 (65), 413 (30), 369 (70); NCIMS (methane) *m*/*z* (rel int) 442 (100), 428 (50), 384 (8), 301 (5), 242 (8).

Halawanone B (2): ^1H NMR δ 12.28 (s, 1H, C-10−OH), 5.30 (q, 1H, J = 7.0, H-1), 4.83 (dd, 1H, J = 5.3, 3.3, H-3), 2.76 (d, 1H, J = 17.6, H-11B), 1.58 (d, 3H, J = 7.0, H-13); ^{13}C NMR δ 67.1 (C-3), 65.9 (C-1), 37.4 (C-11), 17.9 (C-13).

Halawanone C (3): IR (film) υ_{max} 3380, 2919, 2849, 1676, 1643, 1595, 1453, 1305, 1248, 1199 cm^{-1}; ^1H and ^{13}C NMR see Table 1; FABMS (MB matrix) *m*/*z* (rel int) 399 (35), 385 (30), 329 (15), 269 (95), 255 (100), 177 (50).

Halawanone D (4): ^1H NMR δ 12.99 (s, 1H, C-1−OH), 7.84 (d, 1H, J = 2.6, H-6), 7.74 (dd, 1H, J = 7.5, 1.2, H-4), 7.25 (d, 1H, J = 2.6, H-8), 3.28 (q, 2H, J = 7.4, H-11), 1.30 (t, 3H, J = 7.4, H-12); ^{13}C NMR δ 152.2 (C-9), 124.7 (C-8), 29.6 (t, C-11), 15.08 (q, C-12).

Preparation of BD18T(41)-A (*R*)- and (*S*)-MTPA Esters. In a screw-top pressure tube a solution of BD18T(41)-**A** (13 mg, 0.03 μmol), dry CH_2Cl_2 (5 mL), (*R*)-MTPA chloride (30 μL, 0.16 μmol), and a few crystals of DMAP were combined, and the solution was heated in an oil bath (100 °C) for 16 h. After this time, the solution was cooled to room temperature and concentrated. The resulting residue was subjected to flash chromatography over Si gel (98:2, $CHCl_3$−MeOH) affording the (*S*)-MTPA ester (8.3 mg, 43% yield): ^1H NMR ($CDCl_3$) δ 12.22 (s, 1H), 7.75 (s, 1H), 7.67 (m, 2H), 7.41 (m, 3H), 7.14 (s, 1H), 5.78 (dd, 1H, J = 12.3, 7.4 Hz), 5.20 (d, 1H, J = 11.0 Hz), 5.14 (d, 1H, J = 2.9 Hz), 4.99 (dd, 1H, J = 11.2, 3.0 Hz), 4.73 (dd, 1H, J = 5.5, 3.5 Hz), 4.35 (q, 1H, J = 12.3 Hz), 3.67 (s, 3H), 3.01 (dd, 1H, J = 18.0, 5 Hz), 2.86 (dd, 1H, J = 11.5, 7.5 Hz), 2.76 (d, 1H, J = 11.8 Hz), 2.08 (q, 1H, J = 12 Hz), 1.95 (ddd, 1H, J = 15.0, 10.5, 3.0 Hz), 1.68 (m, 1H), 1.44 (d, 3H, J = 6.2 Hz), 1.14 (t, 3H, J = 7.4 Hz).

The preparation of (*R*)-MTPA ester of BD18T(41)-**A** followed the same procedure as the preparation of (S)-MTPA ester, only (*S*)-MTPA chloride was utilized to form the (*R*)-ester. The same scale reaction yielded 6.3 mg (34%) of the (*R*)-MTPA ester of BD18T(41)-**A**: ^1H NMR ($CDCl_3$) δ 12.25 (s, 1H), 7.76 (s, 1H), 7.64 (m, 2H), 7.43 (m, 3H), 7.14 (s, 1H), 5.78 (dd, 1H, J = 13.0, 7.5 Hz), 5.22 (dd, 1H, J = 10.0, 3.5 Hz), 5.15 (d, 1H, J = 3.5 Hz), 5.00 (dd, 1H, J = 11.2, 3.0 Hz), 4.74 (dd, 1H, J = 5.5, 3.5 Hz), 4.35 (q, 1H, J = 12.3 Hz), 3.58 (s, 3H), 3.01 (dd, 1H, J = 18.0, 5.5 Hz), 2.99 (dd, 1H, J = 8.5, 6.0 Hz), 2.77 (d, 1H, J = 17.5 Hz), 2.20 (q, 1H, J = 12 Hz), 1.98 (ddd, 1H, J = 15.0, 10.5, 3.0 Hz), 1.70 (m, 1H), 1.43 (d, 3H, J = 6.2 Hz), 1.14 (t, 3H, J = 7.4 Hz).

Preparation of Tribenzoate Derivatives of Halawanones C and D. In a small vial, a small quantity of fraction B (0.5 mg) was dissolved in pyridine, and to the solution was added an excess of benzoyl chloride. After stirring at room temperature overnight, the pyridine was removed under reduced pressure and the residue was purified using preparative TLC to give a sample of tribenzoate **6**. Due to the small sample size, complete spectral assignments were not possible. Tentative assignments are as follows: ^1H NMR δ 5.72 (ddd, 1H, H-3′), 5.47 (br s, 1H, H-1′), 5.23 (t, 1H, H-4′), 4.36 (dq, 1H, H-5′), 2.53 (ddd, 1H, H-2′A), 2.32 (s, 3H, H-11), 1.97 (br t, 1H, H-2′B), 1.28 (d, 3H, H-6′).

Nanaomycin D (5): ^1H NMR ($CDCl_3$) δ 11.83 (s), 7.69 (dd, 1H), 7.66 (t, 1H), 7.29 (dd, 1H), 5.24 (d, J = 2.8), 5.07 (q, 1H, J = 6.9), 4.71 (dd, 1H), 2.95 (dd, 1H, J = 17.9, 5.2), 2.69 (d, 1H, J = 17.8), 1.55 (d, 3H, J = 6.9).

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References and Notes

(1) Davidson, B. S. *Curr. Opin. Biotechnol.* **1995**, *6*, 284−290.
(2) Fenical, W.; Jensen, P. R. In *Marine Biotechnology. Pharmaceutical and Bioactive Natural Products*; Attaway, D. H., Zaborsky, O. R., Eds.; Plenum: New York, 1993; pp 419−457.
(3) Jensen, P. R.; Fenical, W. *Annu. Rev. Microbiol.* **1994**, *48*, 559−584.
(4) Fenical, W. *Chem. Rev.* **1993**, *93*, 1673−1683.
(5) Jensen, P. R.; Fenical, W. *Microb. Ecol.* **1995**, *29*, 249−257.
(6) Davidson, B. S.; Schumacher, R. W. *Tetrahedron* **1993**, *49*, 6569−6574.
(7) Schumacher, R. W.; Davidson, B. S.; Montenegro, D. A.; Bernan, V. S. *J. Nat. Prod.* **1995**, *58*, 613−617.
(8) Sitachitta, N.; Gadepalli, M.; Davidson, B. S. *Tetrahedron* **1996**, *52*, 8073−8080.
(9) Zaccardi, J.; Alluri, M.; Ashcroft, J.; Bernan, V.; Korshalla, J. D.; Morton, G. O.; Siegle, M.; Tsao, R.; Williams, D. R.; Maiese, W.; Ellestad, G. A. *J. Org. Chem.* **1994**, *59*, 4045−4047.
(10) Omura, S.; Tanaka, H.; Okada, Y.; Marumo, H. *J. Chem. Soc., Chem. Commun.* **1976**, 320−321.
(11) Bergy, M. E. *J. Antibiotics* **1976**, *29*, 454−457.
(12) Kraus, G. A.; Li, J.; Gordon, M. S.; Jensen, J. H. *J. Org. Chem.* **1995**, *60*, 1154−1159.
(13) Tatsuta, K.; Akimoto, K.; Annaka, M.; Ohno, Y.; Kinoshita, M. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1699−1706.
(14) $[M + 3H]^+$ ions can be assigned to protonated hydroquinone reduction products and are quite common for quinone containing compounds. See Cooper, R.; Unger, S. *J. Antibiotics* **1985**, *38*, 24−30.
(15) Lambert, J. B.; Shurvell, H. F.; Lightner, D. A.; Cooks, R. G. *Organic Structural Spectroscopy*; Prentice-Hall: Upper Saddle River, NJ, 1998; pp 358−360.
(16) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(17) Tsuji, N.; Kobayashi, M.; Terui, Y.; Tori, K. *Tetrahedron* **1976**, *32*, 2207−2210.
(18) Zeeck, A.; Mardin, M. *Liebigs Ann. Chem.* **1974**, 1063−1099.
(19) Zeeck, A.; Zahner, H.; Mardin, M. *Liebigs Ann. Chem.* **1974**, 1100−1125.
(20) Krone, B.; Zeeck, A. *Liebigs Ann. Chem.* **1987**, 751−758.
(21) Kraus, G. A.; Roth, B. *J. Org. Chem.* **1978**, *43*, 4923−4924.
(22) Hoffman, B.; Lackner, H. *Liebigs Ann.* **1995**, 87−94.
(23) Sezaki, M.; Kondo, S.; Maeda, K.; Umezawa, H. *Tetrahedron* **1970**, *26*, 5171−5190.
(24) Kasai, R.; Okihara, M.; Asakawa, J.; Mitzutani, K.; Tanaka, O. *Tetrahedron* **1979**, *35*, 1427−1432.
(25) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983; pp 460−465.
(26) Corbaz, R.; Ettlinger, L.; Gaumann, E.; Kalvoda, J.; Keller-Schierlein, W.; Kraddofer, F.; Manukian, B. K.; Neipp, L.; Prelog, V.; Reusser, P.; Zahner, H. *Helv. Chim. Acta* **1957**, *40*, 1262−1269.

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