Antibiotic Terpenoid Chloro-Dihydroquinones from a New Marine Actinomycete

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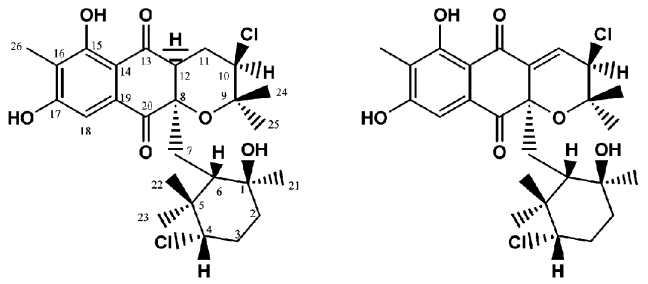
As part of our continuing interest in exploring the chemistry of actinomycete bacteria uniquely adapted for survival in ocean sediments, we encountered several new strains, which by 16S rDNA sequence-based phylogenetic analysis were recognized as members of a new genus (tentatively called MAR4) within the family Streptomycetaceae. We report here the isolation and structure elucidation of three new chlorinated dihydroquinones (**1**−**3**) and one previously reported analogue, **4**, from our strain CNQ-525, isolated from ocean sediments collected at a depth of 152 m near La Jolla, California. The compounds formally possess new carbon skeletons, but are related to several previously reported metabolites of the napyradiomycin class. The structures of the new molecules, which possess significant antibiotic properties and cancer cell cytotoxicities, were assigned by comprehensive spectral measurements and by comparison with NMR and other spectral data from the antibiotic A80915C (**5**), the full stereostructure of which was recently assigned by X-ray diffraction methods.

Of all known microorganisms, the filamentous bacteria of the order Actinomycetales (the "actinomycetes") are the most prolific source of structurally diverse secondary metabolites known.[1] More than 15 000 bioactive molecules are produced by these microbes including many that are used as drugs today. For more than 50 years, academic and pharmaceutical researchers focused their extensive studies mainly on soil-derived actinomycetes and, for numerous reasons, avoided the marine environment. This came in part from the belief that actinomycetes isolated from marine sources were largely of terrestrial origin and existed in the ocean as metabolically inactive spores.[2] Within the past three years, we have learned that true marine actinomycetes are found in the ocean, and a better picture of their diversity and secondary metabolites they produce has begun to emerge. Phylogenetic analyses of their 16S rRNA genes indicate that many of these strains belong to new taxa, including some that appear to be unique at the genus level.[3] Using this approach, we examined a population of actinomycete bacteria cultured from a sediment sample retrieved from a depth of 152 m near La Jolla, California. One strain, our culture number CNQ-525, belongs to what appears to be a new genus that we have tentatively called MAR4. By phylogenetic analysis, this strain was clearly a member of the family Streptomycetaceae but fell outside of the common terrestrial genus *Streptomyces*.[4] In saline culture, strain CNQ-525 produced a series of three new (**1**−**3**) and two previously reported (**4**, **5**)[5,6] chlorine-containing terpenoid dihydroquinones. These compounds possess significant antibacterial and cancer cell cytotoxic activities in in vitro bioassays.



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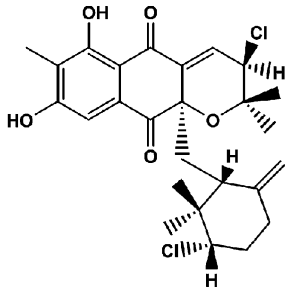

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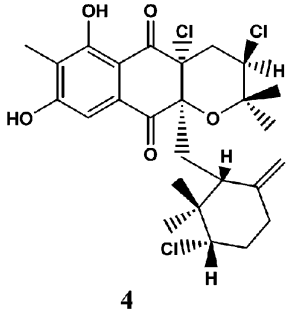

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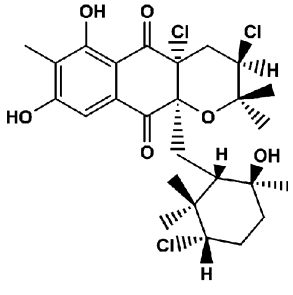

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Results and Discussion

Actinomycete strain CNQ-525 was cultured at 30 °C for 9 days by rotary shaking in multiple 2.8 L Fernbach flasks containing 1 L of a saltwater-based nutrient medium. The whole cultures were extracted using Amberlite XAD-7 resin, and the resin was collected by filtration and then extracted with acetone to obtain a crude extract after solvent removal. The extract was then fractionated by C-18 column chromatography and reversed-phase C-18 HPLC

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Table 1. Assignments of ^{13}C and ^{1}H NMR Data for Compound **1**[a]

carbon #	δ_C (ppm)	δ_H ppm (*J*, Hz)	Me-HSQC	COSY	$^{1}H-^{13}C$ HMBC	ROESY
1	72.2					
2	42.5	1.76−1.82 m[b]	CH_2		C-4, C-6	
		1.52−1.56 m[c]				
3	31.9	1.75−1.79 m[b]	CH_2		C-1, C-5, C-6	
		1.79−1.85 m				
4	72.1	3.67 dd (12.0, 4.0)	CH	H-3−H-4	C-23	H-3b, H-6, H-22
5	41.8					
6	51.9	1.55 d (6.5)[c]	CH		C-1, C-5, C-8, C-21,C-23	H-4, H-2b, H-7b, H-22
7a	41.3	1.82 d (16.0)	CH_2		C-1, C-5	
7b		2.08 dd (16.0, 6.5)			C-1, C-8, C-20	
8	81.6					
9	81.2					
10	62.5	4.09 dd (12.0, 4.1)	CH	H-10−H-11	C-9, C-11, C-24, C-25	H-12, H-11a, H-25
11a	35.3	2.37 dt (12.0, 4.0, 4.1)	CH_2		C-8, C-10, C-12, C-13	
11b		2.06 q (12.0, 13, 13)			C-9, C-10, C-12	H-10, H-11a, H-12
12	58.4	3.36 dd (12.0, 4.0)	CH	H-12−H-11	C-7, C-8, C-10, C-11, C-13, C-14, C-20	H-10, H-11a, H-11b, H-7a
13	196.1					
14	109.6					
15	162.6					
16	120.2					
17	164.7					
18	108.0	7.08 s	CH		C-14, C-16, C-20	
19	133.4					
20	196.2					
21	24.7	1.15 s	CH_3		C-1, C-2, C-6	H-2a, H-7b, H-23
22	29.5	0.44 s	CH_3		C-4, C-5, C-6, C-23	H-4, H-6, H-7a, H-18, H-23
23	16.6	0.68 s	CH_3		C-4, C-5, C-6, C-22	H-3a, H-2a, H-7b, H-21, H-22
24	21.4	1.23 s	CH_3		C-9, C-10, C-25	H-11b, H-25
25	29.3	1.44 s	CH_3		C-9, C-10, C-24	H-10, H-24
26	8.4	2.13 s	CH_3		C-14, C-15, C-16, C-17, C-18	

[a] Spectra were recorded in deuteriomethanol at 300 MHz (^{1}H) and 75 MHz (^{13}C). Assignments were made on the basis of DEPT, HSQC, and HMBC sequence experiments. [b,c] Overlapping signals.

methods to yield compounds **1**−**5** in a total yield of approximately 12 mg/L.

With the molecular formulas and general spectral characteristics for **1**−**5** in hand, a literature search of the microbial metabolite database AntiBase revealed that these compounds were related to a class of antibiotics called the napyradiomycins isolated in the late 1980s by Shiomi and co-workers.[8,9] Comparison of the specific NMR data for compounds **4** and **5** showed that these compounds were identical to the more recently reported antibiotics A80915A and A80915C, the structures of which were incompletely reported by Fukuda and co-workers in a patent and strain isolation paper in 1990.[5,6] Unfortunately, details of how the structures were assigned, essential spectral data, and the absolute stereochemistry of compounds **4** and **5** were never reported. As a consequence, we recently determined the full structure, including absolute stereochemistry, of **5** by X-ray crystallographic methods, and details of this work have been reported elsewhere.[9] The overall spectral data for **4** are included in this report to allow comparisons with the related metabolites **1**−**3**. Spectral data for **5** were included in our previous communication.[9]

Compound **1** was isolated as pale yellow crystals, mp 198 °C, which showed $[\alpha]^{25}_{D}$ −24.3°. The complex molecular ion cluster in the ESI mass spectrum of **1** clearly showed the presence of two chlorine atoms in the molecule. High-resolution MALDI-FTMS analysis suggested the molecular formula $C_{26}H_{34}{}^{35}Cl_2O_6$ ($[M + H]^+$ *m/z* (obsd) 513.1805), indicating 9° of unsaturation. Compound **1** showed strong UV absorptions at 258, 300, and 356 nm, consistent with a highly conjugated aromatic or phenolic compound. The IR spectrum of **1** showed broad absorptions for multiple hydroxyl groups (3370 cm^{-1}) and characteristic conjugated carbonyl groups (1680 cm^{-1}).

Analysis of ^{1}H and ^{13}C NMR 2D spectral data (Table 1) and comparison with the same data from compound **5**[9] allowed the planar structure of **1** to be assigned. The ^{1}H NMR spectrum of **1** illustrated a single aromatic proton (C-18, δ ^{1}H 7.08 s), one aromatic methyl group (C-26, δ ^{1}H 2.13 s), five quaternary aliphatic methyl groups (C-21−C-25), and two methine protons adjacent to chlorine (C-4, δ ^{1}H 3.67 dd, J = 12, 4 Hz; C-10 δ ^{1}H 4.09 dd, J = 12, 4 Hz). The classic proton coupling constants of 12 and 4 Hz for these protons suggested that the chlorine substituents were in the same six-membered rings as in **5**. Analysis of HMBC and MeHSQC NMR data confirmed these assignments and allowed other proton assignments to be made. Two low-field aromatic carbons, C-15 and C-17 (δ ^{13}C 162.6 and 164.7, respectively), suggested the presence of two phenolic hydroxyl groups. These observations suggested that **1** possessed the same terpenoid substituents and a regiochemistry identical to that of **5**. The molecular formula for **1**, however, showed that two rather than three chlorine atoms were present, suggesting that **1** was the dechloro derivative of **5**. Comprehensive analysis of the 2D NMR data for **1** showed that the C-12 chlorine substituent in **5** had been replaced by hydrogen in **1**. The C-12 methine proton was clearly oriented α (axial), as illustrated by its coupling constants (dd, J = 13, 4 Hz) to the adjacent methylene protons at C-11. Analysis of ^{1}H NMR data for the monoterpene unit (C-1 to C-7 and C-21, -22, -32) in **1** showed that it formed a typical chair cyclohexane ring. Analysis of NMR data showed this ring to be identical to that observed in the X-ray structure of **5**.[9]

The relative stereochemistry of compound **1** was assigned by interpretation of 2D ^{1}H NMR ROESY data (Figure 1). ROESY correlations between the C-22 methyl protons and the methine protons at C-4 and C-6 showed these protons were on the same face of the cyclohexane ring. Correlations

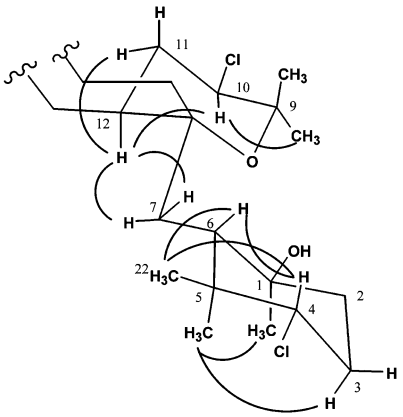

Figure 1. Solution conformation of compound **1** based upon 2D ROESY NMR analysis.

between the C-21 and C-23 methyl group protons showed that the orientation of the C-21 and C-23 methyls were α (axial) and on the bottom face of the ring. On the basis of strong correlations, H-6 and H-4 were positioned in a 1,3-diaxial position on the top face of the ring. In a similar fashion, the relative stereostructure of the tetrahydropyran ring was established. Key ROESY correlations between the methine proton at C-12 and one of the protons at C-7 showed that the tetrahydropyran ring was *cis*-fused to the dihydroquinone. Similar correlations between the C-12 proton and the methine proton at C-10 showed these were oriented on the bottom face (α) in axial positions on the tetrahydropyran ring. The classic axial−equatorial coupling constants from both H-12 (J = 13.0, 4.0 Hz) and H-10 (J = 12.0, 4.1 Hz) to the methylene proton pair at C-11 showed that the tetrahydropyran ring was in a chair form, identical to the configuration of this ring in the crystal structure of **5**. These same protons, H-10 and H-12, showed the expected ROESY correlations with H-11a and H-11b, respectively (Table 1). On the basis of these data, compound **1** was assigned as 3-chloro-10a-(3-chloro-6-hydroxy-2,2,6-trimethylcyclohexylmethyl)-6,8-dihydroxy-2,2,7-trimethyl-3,4,4a,10a-tetrahydro-2*H*-benzo[*g*]chromene-5,10-dione, with the relative stereochemistry shown.

The absolute stereochemistry of **5** was established in the previously reported X-ray structure elucidation.[9] Although we can only assign the absolute stereochemistry of **1** by analogy with **5**, we do note that the sodium D line rotations of **1**−**5** are all negative, providing evidence that all metabolites possess the same absolute configurations (for **5**: $[\alpha]^{25}_{D}$ −190° (*c* 0.031, $CHCl_3$)).[9]

Compound **2** was isolated as a pale yellow oil that analyzed for the molecular formula $C_{26}H_{32}{}^{35}Cl_2O_6$ by HRESI-TOFMS analysis (*m/z* $[M + Na]^+$ (obsd) 533.1470). This formula required 10 degrees of unsaturation, one more than compound **1**. Analysis of ^1H and ^{13}C NMR spectral data (Table 2) showed chemical shifts similar to those of **1**, but illustrated the presence of a new olefinic bond at C-11−C-12. This double bond was easily assigned since the signal for H-12 and the methylene pair H_2-11 were lost and replaced by an alkene one-proton signal at δ 6.94 d (J = 1.8 Hz). In a consistent fashion, the ^{13}C NMR spectrum showed two new olefinic carbons for C-11 and C-12 at δ 135.0 (CH) and 133.4 (C). As in **1**, comprehensive analysis of 2D NMR data (Table 2) allowed the full planar structure of **2** to be assigned. ROESY NMR analysis (Figure 2) showed NOE correlations that were analogous to those from compound **1**. The new olefinic proton at C-11 showed a correlation to the methine proton at C-10. Analogous correlations from protons on the terpenoid cyclohexane ring confirmed that **2** has the same configuration as **1** at C-1, C-4, and C-6. Interestingly, additional ROESY correlations were observed from the C-1 hydroxyl proton to the *gem*-dimethyl groups at C-9. This correlation showed that, in solution, the cyclohexane ring adopts a rotational position beneath the tetrahydropyran ring, as opposed to being oriented beneath the dihydronaphthoquinone ring. On the basis of these comparisons, and comprehensive NMR analysis which allowed all protons and carbons to be assigned, compound **2** was assigned as 3-chloro-10a-(3-chloro-6-hydroxy-2,2,6-trimethylcyclohexylmethyl)-6,8-dihydroxy-2,2,7-trimethyl-3,10a-dihydro-2*H*-benzo[*g*]chromene-5,10-dione. Here too, the absolute stereochemistry is assumed to be identical to **5** on the basis of comparable $[\alpha]_D$ values. Compound **2** is closely related to napyradiomycin B4,[10] which is identical but lacks the methyl group at C-16. The observation of a C-11−C-12 olefin in **2** raises the question of whether this unsaturation is derived from the possible precursor **5**. While this possibility cannot be rigorously excluded, we see no evidence of elimination reactions (loss of HCl) during diverse chromatographic steps in the purification of compound **5**.

Compound **3** was isolated as white crystals, mp 179−181 °C, that analyzed for the molecular formula $C_{26}H_{30}{}^{35}Cl_2O_5$ by HRESI-TOFMS analysis (*m/z* $[M - H]^+$ (obsd) 491.1395, 11 degrees of unsaturation). In analogy with **1** and **2**, compound **3** showed UV absorptions at 272 and 327 nm and IR absorption for multiple hydroxyl (3320 cm^{-1}) and carbonyl functionalities (1720, 1650 cm^{-1}). Analysis of combined ^1H and ^{13}C NMR spectral data (Table 3) showed signals similar to those of **1** and **2**, but also revealed that the methyl tertiary alcohol at C-1 had been converted, via dehydration, to the corresponding exocyclic olefin. This new double bond was easily assigned since the signals for the methyl group (C-21) and the OH (C-1) were lost and replaced by two new proton signals at δ 4.77 (bs) and 4.33 (bs). As in **1** and **2**, interpretation of 2D HMQC, MeHSQC, and HMBC NMR data allowed all the protons and carbons to be assigned.

The relative stereochemistry of **3** was also assigned by analysis of ROESY NMR data (Table 3). Typical correlations of the C-22 methyl protons with H-6 and H-4 defined the cyclohexane ring. Likewise, correlations of the protons on the dihydropyran ring were identical to those observed in the ROESY experiment for **2**. These favorable comparisons allowed compound **3** to be assigned as 3-chloro-10a-(3-chloro-6-hydroxy-2,2,6-trimethylcyclohexylmethyl)-6,8-dihydroxy-2,2,7-trimethyl-3,4,4a,10a-tetrahydro-2*H*-benzo[*g*]-chromene-5,10-dione. This compound is very similar to napyradiomycin B2, the C-16 demethyl derivative reported by Shiomi and co-workers.[8]

Like metabolite **2**, there is also the possibility that the unsaturation at C-11−C-12 arises from an elimination of HCl from the potential precursor **4**. Again, during the purification of **4** there is no evidence that dehydrohalogenations occur in any steps.

The previously reported compound **4** was also isolated as a yellow oil that showed $[\alpha]^{25}_{D}$ −71.6° (lit $[\alpha]^{25}_{D}$ −89.7°)[5] and analyzed for the molecular formula $C_{26}H_{31}{}^{35}Cl_3O_5$ by HRESI-TOFMS analysis (*m/z* $[M - H]^+$ (obsd) 527.1153, 10 degrees of unsaturation). The ^1H and ^{13}C NMR data for **4** were virtually identical to NMR data reported by Fukuda et al. for antibiotic A80915A in a 1990 patent.[6] On the basis of this favorable comparison, **4** was confirmed as 3,4a-dichloro-10a-(3-chloro-2,2-dimethyl-6-methylenecyclohexylmethyl)-6,8-dihydroxy-2,2,7-trimethyl-3,4,4a,10a-tetrahydro-2*H*-benzo[*g*]chromene-5,10-dione and assigned the same

Table 2. Assignments of ^{13}C and ^{1}H NMR Data for Compound **2**[a]

carbon #	δ_C (ppm)	δ_H ppm (*J*, Hz)	Me-HSQC	COSY	$^{1}H-^{13}C$ HMBC	ROESY
1	71.6		C			
2	40.9	1.95−2.0 m[b]	CH_2		C-4	
		1.34−1.40 m				
3	30.0	1.9−1.95 m[b]	CH_2		H-21, H-2a, H-3b	
		1.73−1.79 m			H-2b, H-22, H-23	
4	70.9	3.44 dd (12.0, 3.0)	CH	H-4−H-3		H-22, H-2b, H-3b
5	40.7		C			
6	52.7	1.4 m	CH		C-1, C-5, C-6	H-2b
7a	40.1	2.0−2.02 m[c]	CH_2		C-1, C-6, C-8, C-20	H-7b, H-21, H-23, H-10
7b		2.07−2.11 m[c]			C-1, C-5, C-6	H-7a, H-22, H-23, H-2b
8	82.8		C			
9	79.0		C			
10	58.7	4.56 d (1.8)	CH	H-10−H-11	C-9, C-11, C-12, C-24, C-25	H-11, H-25, H-24, H-7b
11	135.0	6.94 d (1.8)	CH		C-8, C-13	H-10, H-24, H-25
12	133.4		C			
13	187.4		C			
14	110.2		C			
15	163.0		C			
16	119.2		C			
17	163.4		C			
18	108.1	7.37 s	CH		C-14, C-16, C-20	H-6, H-22
19	138		C			
20	192.3		C			
21	23.9	1.25 s	CH_3		C-1, C-2, C-6	H-23, H-3a, H-3b, H-7b, HO(1)
22	28.5	0.48 s	CH_3		C-4, C-5, C-6, C-23	H-23, H-6, H-7a, H-4
23	15.6	0.68 s	CH_3		C-4, C-5, C-6, C-22	H-22, H-21, H-7b, H-7a, H-3a
24	20.5	1.23 s	CH_3		C-9, C-10, C-25	H-25, H-10, H-11
25	27.0	1.59 s	CH_3		C-9, C-10, C-24	H-24, H-10
26	8.2	2.22 s	CH_3		C-16, C-17	
OH(1)		5.65 bs	OH		C-2	H-25, H-24, H-2a
OH(15)		12.78 bs	OH		C-16, C-19	H-26

[a] Spectra were recorded in deuteriochloroform at 300 MHz (^{1}H) and 75 MHz (^{13}C). Assignments were made on the basis of DEPT, HSQC, and HMBC sequence experiments. [b,c] Overlapping signals.

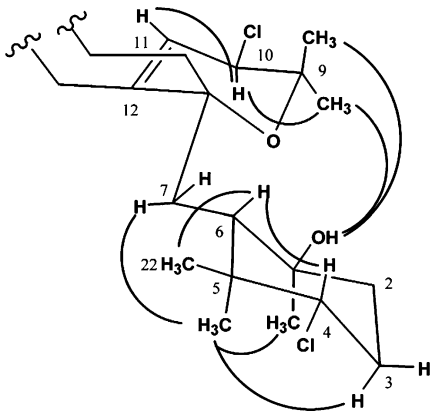

Figure 2. Solution conformation of compound **2** based upon 2D ROESY NMR analysis.

absolute stereochemistry on the basis of comparable $[\alpha]^{25}_{D}$ values to **1**−**3**.

All of the compounds reported here share the same structural feature of incorporating terpenoid substituents as part of the otherwise (apparently) polyketide-derived framework. The structure of the cyclohexane component of these compounds, as well as the formation of the chloro tetrahydropyran ring, appears to be the product of a halogen ("Cl^{+}") induced cyclization, in a fashion well-known for many cyclic bromoterpenoids produced by marine algae (Figure 3).[11] While our source for these compounds is a marine-derived actinomycete that, based on phylogenetic analyses, is distinct from the genus *Streptomyces*, the previously reported napyradiomycins and the antibiotics of the A-80915 complex were isolated from terrestrial-derived strains of *Streptomyces ruber* and *Streptomyces aculeolatus*, respectively. Given the remarkable similarity in the biosynthesis within these actinomycetes, it would appear reasonable to examine the taxonomic relationships of the two *Streptomyces* strains and our strain CNQ-525 using 16S rDNA sequence methods.

Compounds **1**−**4** show significant antibacterial properties against the drug-resistant pathogens methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VREF). In addition, these compounds were found to be cytotoxic toward HCT-116 human colon carcinoma (Table 5). It is interesting that a reverse trend in potency is observed for compound **2** in these data. Compound **2** is only weakly active in the antibacterial assays (MIC >15 μg/mL) but is 15 times more potent in the HCT-116 colon carcinoma assay. Compounds **1**, **3**, and **4** are roughly equipotent in all assays.

Experimental Section

General Experimental Procedures. Optical rotations were measured in chloroform and/or methanol using a Rudolph Research Autopol III polarimeter with a path length of 10 cm at the sodium D line. UV spectra were obtained with a Beckman Coulter DU 640 spectrophotometer. IR spectra were obtained with a Perkin-Elmer 1600 series FTIR spectrophotometer. Proton and carbon NMR spectra were recorded on Varian Inova NMR spectrometers at 400 or 300 MHz for ^{1}H and 75 or 100 MHz for ^{13}C. The spectra were recorded in deuteriomethanol, deuteriochloroform, and deuterioacetone, as indicated in Tables 1−4. The chemical shifts were referenced to the solvent signals for deuteriomethanol (δ 4.78/49.0), deuteriochloroform (δ 7.24/77.0), and deuterioacetone (δ 2.04/206.0). All 1D and 2D ROESY, NOESY, $^{1}H-^{1}H$ COSY, $^{1}H-^{13}C$ HMBC, MeHSQC, and HMBC experiments were performed using the standard pulse sequences supplied with the instruments. High-resolution mass spectra were obtained using an Agilent ESI-TOF and by MALDI-FTMS methods. Column chromatography was performed using C-18, reversed-phase methods, while HPLC purifications were carried out

Table 3. Assignments of ^{13}C and ^{1}H NMR Data for Compound **3**[a]

carbon #	δ_H (ppm)	δ_H ppm (*J*, Hz)	Me-HSQC	COSY	$^{1}H-^{13}C$ HMBC	NOESY
1	147.0					
2	36.7	2.09 m	CH_2		C-1, C-6	
		2.26 m				
3	35.1	1.7 m	CH_2	H3−H2		H-4, H-6
4	71.4	4.2 dd (11.1, 4.8)	CH	H4−H3	C-4	H-3
5	42.7		C			
6	48.2	2.07 br s	CH		C-7	
7a	37.3	1.86 br s	CH_2		C-1, C-5, C-6, C-8, C-12	
7b		1.90 br s				H-23
8	83.2		C			
9	76.7		C			
10	60.4	4.97 d (1.5)	CH	H10−H11	C-9, C-11	
11	137.7	6.78 d (1.5)			C-8, C-13	
12	137.2		C			
13	189.4		C			
14	111.2					
15	163.8		C			
16	118.8		C			
17	164.1		C			
18	107.7	7.15 s	CH		C-14, C-16, C-17, C-19, C-20	
19	133.8		C			
20	194.7		C			
21a	109.4	4.77 br s	CH_2		C-1, C-2, C-6	
22b		4.33 br s			C-2, C-6	H-6
22	27.1	1.11 s	CH_3		C-4, C-6, C-23	
23	14.5	0.61 s	CH_3		C-4, C-5, C-6, C-22	
24	20.7	1.02 s	CH_3		C-9, C-10, C-25	
25	27.2	1.49 s	CH_3		C-9, C-10, C-24	H-21
26	8.2	2.14 s	CH_3		C-15, C-16	
OH(15)		12.98 bs	OH			

[a] Spectra were recorded in deuterioacetone at 300 MHz (^{1}H) and 75 MHz (^{13}C). Assignments were made on the basis of DEPT, HSQC, and HMBC sequence experiments.

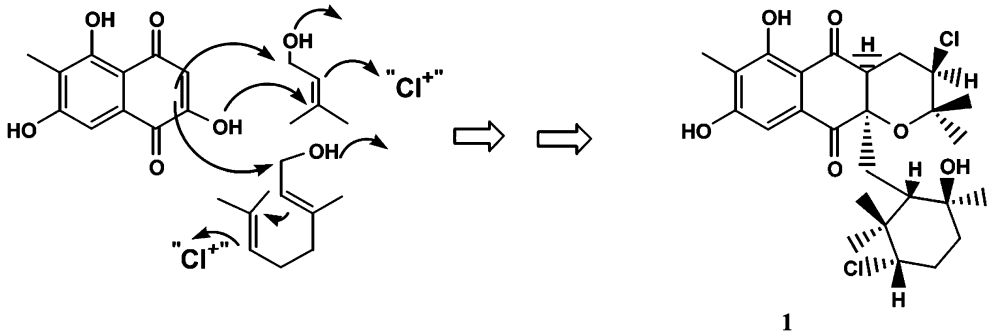

Figure 3. Proposed biosynthesis of compound **1** involving an electron-deficient chlorine-induced terpene cyclization (of isoprene and geraniol precursors) and alkylation of a polyketide precursor.

using an Acuflow series II pump, a Waters differential refractometer R401 detector, and a Dynamax 60A C_{18} column (1.0 × 25 cm), at a flow rate of 2.0 mL/min. Normal-phase HPLC purifications were carried out using a Dynamax 60A Si column (1 × 25 cm), at a flow rate of 2.0 mL/min. TLC analyses were performed using 0.20 mm C-18 Whatman glass-backed plates and 0.20 mm Si gel 60 F_{254}Polygram plastic-backed plates.

Isolation and Cultivation of Bacterial Strain CNQ-525. Actinomycete strain CNQ-525 was isolated from a sediment sample collected at a depth of 152 m just west (ca. 2 miles) of the Scripps Institution of Oceanography in La Jolla, California. The bacterium was isolated using solid agar methods on a nutrient medium consisting of the following: 10 g of starch, 4 g of yeast extract, 2 g of bacto-peptone, 18 g of agar, and 1 L of seawater. The strain was cultured by shaking in a liquid medium composed of the same nutrients, at 30 °C for 9 days, and then extracted by stirring for 2 h with Amberlite XAD-7 resin (20−30 gm/L). The resin was filtered and extracted with acetone, and the solvent removed under vacuum to generate the crude extract.

Purification of Compounds 1−5. The crude acetone extract was fractionated by C-18 column chromatography using H_2O, 2:1 MeOH/H_2O, 1:1 MeOH/H_2O, 1:2 MeOH/H_2O, MeOH, EtOAc, and 100% CH_2Cl_2, to generate seven fractions, which were analyzed by TLC and NMR methods. The fraction eluted with 100% MeOH, which showed aromatic, olefin, and terpenoid signals in the ^{1}H NMR spectrum, was further triturated with CH_2Cl_2, and the CH_2Cl_2-soluble components were purified by HPLC on a C-18 column, eluting with 1:9 H_2O/MeCN at 2.0 mL/min flow. Compounds **1**−**4** were then isolated from several of the fractions by HPLC and crystallization. Compound **1** crystallized from 1:1 MeOH/CH_2Cl_2, as pure white crystals, while compound **2** was isolated as a pale yellow oil by HPLC. A mixture of compounds **3** and **4** was fractionated by normal-phase HPLC using a Dynamax 60A Si column (1 × 25 cm), at a flow rate of 2.0 mL/min. Compound **3** was isolated as a pale yellow solid, which was crystallized from Me_2CO to produce white, needle-shaped crystals. Compound **4** was also isolated as a yellow oil in the same HPLC experiment. The yields of **1**−**4** were as follows: **1** = 1.5 mg/L, **2** = 0.8 mg/L, **3** = 1.2 mg/L, and **4** = 3.0 mg/L.

Antibiotic and Cancer Cell Cytotoxicity Bioassays. Compounds **1**−**4** were tested against HCT-116 human colon carcinoma, methicillin-resistant *Staphylococcus aureus* (MRSA)-, and vancomycin-resistant *Enterococcus faecium* (VREF) using the following methods.

HCT-116 Assay. Human colon adenocarcinona (HCT-116) cells were incubated overnight at 37 °C in 5% CO_2/air in

Table 4. Assignments of ^{13}C and ^{1}H NMR Data for Compound **4**[a]

carbon #	δ_H (ppm)	δ_H ppm (*J*, Hz)	Me-HSQC	COSY	$^{1}H-^{13}C$ HMBC	NOESY
1	147.0					
2	35.0	2.03−2.06 m[b]	CH_2		C-6	
		2.27−2.3 m				
3	35.2	1.67−1.75 m	CH_2	H3−H2	C-1, C-5	
		1.98−2.03 m[b]				
4	71.7	3.90 dd (12.0, 4.0)	CH	H4−H3		H-2b, H-3a, H-7, H-22, H-23
5	43.3		C			
6	46.3	2.10−2.14 m	CH	H6−H7	C-1, C-5, C-7	
7	34.1	2.64−2.74 m	CH_2		C-1, C-6, C-8, C-12, C-20	H-6, H-25
8	84.9					
9	79.0					
10	60.1	4.47 dd (12.0, 4.0)	CH	H10−H11		H-11, H-7, H-24, H-25
11a	42.0	2.47−2.52 m[c]	CH_2	H11−H10	C-10	
11b		2.56−2.64 m[c]				
12	82.6					
13	193.5					
14	108.0					
15	163.8					
16	119.3					
17	164.1					
18	108.3	7.23 s	CH		C-14, C-16, C-20	
19	132.7					
20	194.3					
21	110.2	4.84 d (4.2)	CH_2	H-21−H-6	C-2, C-6	H-2a, H-2b, H-24, H-25
22	26.6	0.66 s	CH_3		C-4, C-5, C-6, C-23	
23	15.5	0.56 s	CH_3		C-4, C-5, C-6, C-22	
24	22.6	1.20 s	CH_3		C-9, C-10, C-25	
25	27.7	1.30 s	CH_3		C-9, C-10, C-24	H-21
26	8.4	2.14 s	CH_3		C-16, C-17	
OH(15)		12.39 bs	OH			

[a] Spectra were recorded in deuterioacetone at 300 MHz (^{1}H) and 75 MHz (^{13}C). Assignments were made on the basis of DEPT, HSQC, and HMBC sequence experiments. [b,c] Overlapping signals.

Table 5. Bioactivities of Compounds **1**−**4**

compound	MRSA (MIC, μg/mL)	VREF (MIC, μg/mL)	HCT (IC_{50} μg/mL)
1	1.95	3.90	2.40
2	15.6	15.6	0.97
3	1.95	1.95	NT[a]
4	1.90	3.90	1.84

[a] NT = not tested.

microtiter plates. Test materials, etoposide (positive control), and DMSO (negative control) were added to the top row of a 96-well microtiter plate and serially diluted (1:4) downward. After a 72 h incubation, cell viability was determined colorimetrically, using a Molecular Devices Emax microplate reader (490 nm), recording the amount of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) reduced to formazan using the CellTiter 96 AQueous nonradioactive cell proliferation protocol (Promega). Minimum inhibitory concentration (IC_{50}) values were calculated using the program SOFTmax PRO (Molecular Devices).

Antibiotic Assay. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VREF) were grown overnight at 37 °C in GYT media (0.1% glucose, 0.25% yeast extract, 0.5% tryptone). The culture was diluted to 0.04−0.06 OD, further diluted 1:10, then added to the top row of a 96-well microtiter plate. Test materials were added to the top row, serially diluted (1:2) downward, and incubated for 16−18 h at 37 °C. Vancomycin (MRSA) and penicillin G (VREF) were the positive controls, and DMSO was the negative control. Optical density (OD) was measured at 600 nm using a Molecular Devices Emax microplate reader and the minimum inhibition concentration (MIC) determined using the program SOFTmax PRO.

Compound 1: pale yellow crystals; mp 197−198 °C; $[\alpha]^{25}_D$ −24.3° (*c* 0.14, MeOH); IR (film) $_{max}$ 3370 broad, 3110, 2900, 2860, 1680, 1600, 1260, 1100, 730 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; HRMALDIMS *m/z* 513.1805 (calc for $C_{26}H_{35}Cl_2O_6$, $[M + H]^+$ 513).

Compound 2: pale yellow oil; $[\alpha]^{25}_D$ −6.3° (*c* 0.13, MeOH); IR (film) $_{max}$ 3360 broad, 3110, 3030, 2920, 2860, 1750, 1630, 1280, 1120, 800−700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; HRESITOFMS *m/z* 533.1470 (calc for $C_{26}H_{32}Cl_2O_6Na$) $[M + Na]^+$ 533.

Compound 3: pale yellow solid; mp 179−181 °C; $[\alpha]^{25}_D$ −7.7° (*c* 0.39, MeOH); IR (film) $_{max}$ 3320 broad, 3110, 2930, 2870, 1720, 1650, 1280, 1100, 800−700 cm^{-1}; ^{1}H NMR (300 MHz, CD_3COCD_3) and ^{13}C NMR (125 MHz, CD_3COCD_3), see Table 1; HRESITOFMS *m/z* 491.1395 (calc for $C_{26}H_{29}Cl_2O_6$) $[M - H]^+$ 491.

Compound 4: yellow oil, $[\alpha]^{25}_D$ −71.6° (*c* 0.58, MeOH); IR (film) $_{max}$ 3360 broad, 3040, 2920, 2820, 1700, 1600, 1270, 1120, 750−670 cm^{-1}; ^{1}H NMR (300 MHz, CD_3COCD_3) and ^{13}C NMR (125 MHz, CD_3COCD_3), see Table 1; HRESITOFMS *m/z* 527.1153 (calc for $C_{26}H_{30}Cl_3O_5$) $[M - H]^+$ 527.

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Supporting Information Available: Spectral data for the new terpenoid dihydronaphthoquinine derivatives **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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