# New Sesquiterpene Hydroquinones from a Taiwanese Marine Sponge *Polyfibrospongia australis*

Ya-Ching Shen* and Pei-Wen Hsieh

*Institute of Marine Resources, National Sun Yat-sen University, 70 Lien-Hai Rd., Kaohsiung, Taiwan 80424, Republic of China*

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Two new sesquiterpene hydroquinones, polyfibrospongols A (**1**) and B (**2**), were isolated from the Taiwanese marine sponge *Polyfibrospongia australis* Lendenfeld (Spongiidae) in addition to the known metabolites, dictyoceratin A (**3**), ilimaquinone (**4**), and 5-*epi*-ilimaquinone (**9**). The structures of these compounds have been determined mainly on the basis of spectral and chemical methods. Biological testing revealed that compounds **1** and **2** exhibited significant cytotoxicity against human KB-16, A-549, and murine P-388 tumor cell lines.

Sesquiterpene quinones and hydroquinones represent a group of still expanding $C_{15}-C_6$ metabolites with notable medicinal applications such as antitumor, antibacterial, and anti-HIV activities.[1−4] Of particular interest are two antimitotic agents called avarol and avarone, which were recently shown to inhibit HTLV-III replication in human H9 cells as measured by determination of reverse transcriptase.[5] This result suggested that sesquiterpene quinones and hydroquinones may be useful in the treatment of AIDS. Due to their potential antitumor and anti-HIV activities as well as the novelty of their structures, the exploration of natural sesquiterpene quinones has continued to grow in the past decade. Several bioactive metabolites such as ilimaquinone (**4**), peyssonols, and hyatellaquinone from various species of sponges and algae have been recently isolated and characterized.[6−10] A comprehensive review dealing with the chemistry and biological properties of sesquiterpene−shikimate derivatives has been published.[11]

In a program searching for bioactive substances from organisms of coral reefs, we have focused on Taiwanese marine sponges.[12,13] An EtOH extract of the sponge *Polyfibrospongia australis* Lendenfeld (Spongiidae), collected at Nan-wan (at a depth of 25 m), exhibited significant inhibition against human KB-16 (5.2 $\mu$g/mL) and murine P-388 (2.3 $\mu$g/mL) tumor cells. After extensive fractionation by chromatography, a series of sesquiterpene quinones, including ilimaquinone (**4**), 5-*epi*-ilimaquinone (**9**), and dictyoceratin A (**3**), and two new compounds, which we have named polyfibrospongols A (**1**) and B (**2**), were isolated. In this paper we report the isolation, structure elucidation, and antitumor cytotoxicity of these new sesquiterpene hydroquinones.

## Results and Discussion

The $CHCl_3$-soluble fraction from the alcoholic extract of the sponge *P. australis* was chromatographed on a Si gel column using eluents of increasing polarity of $CHCl_3$ and MeOH to yield a mixture of ilimaquinone and 5-*epi*-ilimaquinone (**4** and **9**, 125 mg) and polyfibrospongol A (**1**, 7 mg, 0.003% wet wt). Further separation by LC and preparative TLC on Si gel ($CHCl_3$/$Me_2CO$, 10:1) yielded an additional mixture of compounds **4** and **9** (95 mg), dictyoceratin A (**3**, 6 mg), and polyfibrospongol B (**2**, 5 mg, 0.002% wet wt). Chromatographic fractionation of an EtOH extract from an unidentified sponge (SP 137) furnished ilimaquinone (**4**). Ilimaquinone (**4**), 5-*epi*-ilimaquinone (**9**), and dictyoceratin A (**3**) have been previously isolated from *Hippospongia* sp. and other sponges.[6,8] The structures of compounds **3**, **4**, and **9** were identified by comparison of their EIMS, IR, [α], $^1$H-, and $^{13}$C-NMR spectral data with those of the previously reported compounds.[6] The structure of compound **3** was also confirmed by preparation of its diacetate (**7**), which had spectroscopic data identical to those of dictyoceratin A diacetate.[6]

Polyfibrospongol A (**1**), [α] +5.3° ($CHCl_3$), was isolated as an amorphous solid. The molecular formula $C_{24}H_{34}O_4$ was deduced from its molecular ion at *m*/*z* 386.2447 in its HREIMS and was confirmed by $^1$H, $^{13}$C, and DEPT NMR. Its UV and IR bands indicated the presence of phenyl (227, 269 nm, 1602 cm$^{-1}$), hydroxyl (3416 cm$^{-1}$), and ester (1714 cm$^{-1}$) groups. This finding was also supported by fragment ions at *m*/*z* 355 $[M - OMe]^+$ and *m*/*z* 196 ($ArCH_2^{\bullet+}$) in the EIMS spectrum of **1**. The presence of a rearranged drimane skeleton was inferred from the observations of a significant fragment ion at *m*/*z* 191 $(M - ArCH_2^{\bullet})^+$ and characteristic resonances, such as two methyl singlets ($\delta$ 1.06, 0.88), a methyl doublet ($\delta$ 1.03), and a pair of exocyclic methylene signals ($\delta$ 4.41, 4.36) as well as corresponding carbon signals (Table 1).[6] A carbomethoxy moiety was also observed at $\delta$ 3.87, 51.9, and 167.2. The assignment of each aliphatic proton in the drimane moiety of **1** was determined by a COSY experiment. In addition, a correlation via meta-coupling was observed between aromatic protons at $\delta$ 7.46 and 7.38, indicating the presence of a 1,2,3,5-tetrasubstituted benzene moiety. A comparison with literature data indicated that polyfibrospongol A (**1**) exhibited a molecular weight 14 mass units greater than that of dictyoceratin A (**3**).[6] The $^1$H- and $^{13}$C-NMR spectra of **1** were similar to those of dictyoceratin A except that an additional methoxyl singlet was observed at $\delta$ 3.93 in **1**. On acetylation compound **1** yielded a monoacetate (**5**), which showed a molecular ion at *m*/*z* 428 and characteristic fragment ions at *m*/*z* 191 $(M - ArCH^{\bullet})^+$ and *m*/*z* 238 ($ArCH_2^{\bullet+}$). Besides, an aromatic acetyl singlet ($\delta$ 2.36) in the $^1$H-NMR spectrum of **5** suggested that the hydroxy might

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**Table 1.** $^1$H- and $^{13}$C-NMR Data for Polyfibrospongol A (**1**)

| position | $^1$H[a] | COSY | $^{13}$C[b] |
|---|---|---|---|
| 1a | 2.10 m | H-2a, 1b, 2b, 10 | 23.1 (t) |
| 1b | 1.60 m | H-2a, 1a, 2b, 10 | |
| 2a | 1.92 m | H-3a, 2b | 27.9 (t) |
| 2b | 1.40 m | H-3b, 2a, 1a | |
| 3a | 2.34 (dd, 14.4, 5.4) | H-3b, 2a, 2b, 11 | 33.1 (t) |
| 3b | 2.10 m | H-3a, 2a | |
| 4 | | | 160.3 (s) |
| 5 | | | 40.2 (s) |
| 6 | 1.20−1.50 m | H-7 | 36.6 (t) |
| 7 | 1.20−1.50 m | H-6, 8 | 27.7 (t) |
| 8 | 1.30 m | H-13 | 36.4 (d) |
| 9 | | | 42.1 (s) |
| 10 | 0.95 (dd, 1.8, 11.7) | H-1a, 1b | 48.0 (d) |
| 11 | 4.41 (s) | H-3a | 102.6 (t) |
| | 4.36 (s) | | |
| 12 | 1.06 (s) | | 20.6 (q) |
| 13 | 1.03 (d, 6.3) | H-8 | 17.6 (q) |
| 14 | 0.88 (s) | | 17.7 (q) |
| 15 | 2.68 (s) | | 36.8 (t) |
| 16 | | | 124.4 (s) |
| 17 | | | 149.2 (s) |
| 18 | | | 145.8 (s) |
| 19 | 7.38 (d, 2.1) | H-21 | 109.0 (d) |
| 20 | | | 120.4 (s) |
| 21 | 7.46 (d, 2.1) | H-19 | 127.5 (d) |
| OMe | 3.93 (s) | | 56.1 (q) |
| COOMe | 3.87 (s) | | 167.2 (s) |
| | | | 51.9 (q) |

[a] Multiplicities and coupling constants in Hz are in parentheses. [b] s = C, d = CH, t = $CH_2$, q = $CH_3$. Multiplicities and assignments were made by DEPT.

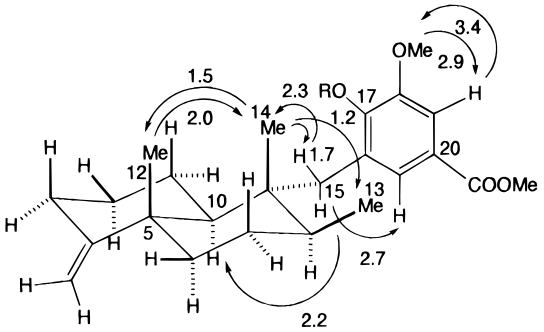



**Figure 1.** NOE studies of polyfibrospongol A (**1**, R=H) and polyfibrospongol A monoacetate (**5**, R=Ac).

be located at the C-17 or C-18 position. Detailed comparison of the resonances of H-19 and H-21 in the $^1$H-NMR spectrum of **1** and **5** with those of **3** and **7** suggested that the location of the hydroxyl group should be at C-17. This is because the chemical shifts of H-19 and H-21 in **5** remain unchanged after acetylation of **1**. The position of the acetyl group in **5** and the corresponding hydroxyl group in **1** should be located at the meta-direction of H-19 and H-21 of **5** and **1**, respectively. This was confirmed by an NOE study of **1**. Irradiation of the methoxy singlet at $\delta$ 3.93 causes enhancement of the signal of H-19 (2.9%), and vice versa (3.4%), confirming the hydroxy at C-17 in **1**. The stereochemistry of **1** was assigned by observation of NOE experiments and detailed comparison of the observed chemical shifts, coupling constants, and specific rotation with those of dictyoceratin A (**3**).

Assuming that **1** has the same absolute configuration at C-10 as other natural sesquiterpene hydroquinones,[11] selective NOE difference studies were performed on **1** and its monoacetate (**5**) (Figure 1) to determine the relative stereochemistry around other chiral centers.

**Table 2.** $^1$H- and $^{13}$C-NMR Data for Polyfibrospongol B (**2**)

| position | $^1$H[a] | COSY | $^{13}$C[b] |
|---|---|---|---|
| 1a | 2.10 m | H-1b, 2b | 24.1 (t) |
| 1b | 1.40 m | H-1a, 2b, 10 | |
| 2a | 1.88 m | H-3a, 2b | 28.3 (t) |
| 2b | 1.30 m | H-3a, 2a, 1a | |
| 3a | 2.30 m | H-3b, 2a, 2b, | 33.1 (t) |
| 3b | 2.08 m | H-3a | |
| 4 | | | 159.8 (s) |
| 5 | | | 40.1 (s) |
| 6 | 1.20−1.50 m | H-7 | 36.9 (t) |
| 7 | 1.30−1.50 m | H-6, 8 | 28.0 (t) |
| 8 | 1.38 m | H-13, 7 | 36.7 (d) |
| 9 | | | 46.6 (s) |
| 10 | 1.10 m | H-1b | 49.5 (d) |
| 11 | 4.44 (s) | | 103.2 (t) |
| | 4.40 (s) | | |
| 12 | 1.06 (s) | | 20.9 (q) |
| 13 | 1.11 (d, 6.3) | H-8 | 19.0 (q) |
| 14a | 3.81 (d, 11.6) | H-14b | 64.5 (q) |
| 14b | 3.92 (d, 11.6) | H-14a | |
| 15a | 3.09 (d, 14.4) | H-15b | 37.3 (t) |
| 15b | 2.85 (d, 14.4) | H-15a | |
| 16 | | | 124.3 (s) |
| 17 | | | 149.2 (s) |
| 18 | | | 146.0 (s) |
| 19 | 7.40 (d, 2.1) | H-21 | 109.3 (d) |
| 20 | | | 120.6 (s) |
| 21 | 7.50 (d, 2.1) | H-19 | 127.8 (d) |
| OMe | 3.94 (s) | | 56.1 (q) |
| COOMe | 3.88 (s) | | 167.1 (s) |
| | | | 51.2 (q) |

[a] Multiplicities and coupling constants in Hz are in parentheses. [b] s = C, d = CH, t = $CH_2$, q = $CH_3$. Multiplicities and assignments are made by DEPT.

The presence of enhancements among C12-Me, C13-Me, and C14-Me agreed with a cis relationship among these methyl protons. The absence of a NOE between H-10 and the methyl protons of C-12 and C-14 suggested that H-10 was trans to C-12 and C-14. The intensity of H-10 and H-21 was increased (2.2%, 2.7%) as the C-15 methylene singlet ($\delta$ 2.56, **5**) was irradiated, confirming a cis-disposition for H-10 and H-15, and a close relationship for H-15 and H-21 in space. A proposed model consistent with the results of NOE experiments for **1** is shown in Figure 1.

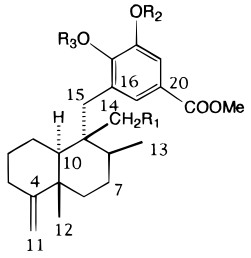
Polyfibrospongol B (**2**), [$\alpha$] +1.8° ($CHCl_3$), had the composition $C_{24}H_{34}O_5$ as derived from a molecular ion at *m/z* 402.2408 (calcd 402.2406) in the HREIMS of **2**, and also from FABMS (*m/z* 425, $[M + Na]^+$, *m/z* 403 $[M + H]^+$), and DEPT NMR. Its UV and IR spectra indicated the presence of hydroxyl (3420 $cm^{-1}$), ester (1710 $cm^{-1}$), and phenolic (227, 270 nm, 1645 $cm^{-1}$) groups. This was also supported by fragment ions at *m/z* 371 $[M - OMe]^+$, *m/z* 207 $(M - ArCH_2^{\bullet})^+$, and a base peak at *m/z* 196 ($ArCH_2^{\bullet +}$) in the EIMS spectrum. The presence of a carbomethoxy and two meta-coupling aromatic protons was verified by the observation of $^1$H- and $^{13}$C-NMR spectral data (Table 2). As in **1**, the characteristic resonances of a methyl singlet at $\delta$ 1.06 (12-Me), a methyl doublet at $\delta$ 1.11 (13-Me), and a group of aliphatic protons between $\delta$ 1.20 and 2.30 indicated that compound **2** is an analogue of **1**. A pair of broad singlets at $\delta$ 4.44 and 4.40 accounted for the C-11 exocyclic methylene protons of the drimane skeleton.[6] Although compounds **1** and **2** showed similar $^1$H- and $^{13}$C-NMR spectra, the major difference between them was in the chemical shifts and coupling pattern of the C-15 methylene protons. In compound **2** they appeared as an AB spin system at $\delta$ 3.09 ($J$ = 14.4 Hz) and $\delta$

**Table 3.** Cytotoxicities ($IC_{50}$, $\mu$g/mL) of Compounds **1**−**8** and **10**

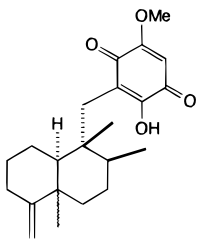
| compd/tumor cells | P-388 | KB-16 | A-549 |
|---|---|---|---|
| **1** | 0.7 | 1.4 | 0.6 |
| **2** | 1.0 | 2.0 | 1.0 |
| **3** | 1.4 | 1.4 | 0.7 |
| **4** | 0.2 | 0.7 | 0.4 |
| **5** | 3.4 | 2.1 | 3.1 |
| **6** | >50 | >50 | >50 |
| **7** | 1.9 | 1.8 | 1.5 |
| **8** | 0.7 | 1.9 | 2.7 |
| **10** | 0.6 | 2.1 | 1.2 |

[a] The concentration of compound inhibiting 50% ($IC_{50}$) of the growth of murine and human tumor cell lines, P-388 (murine leukemia), KB-16 (human nasopharyngeal carcinoma), and A-549 (human lung adenocarcinoma), after 3, 3, and 6 days of drug exposure according to a published method.[17]

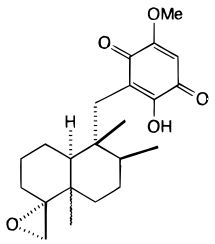
2.85 ($J$ = 14.4 Hz) but were at $\delta$ 2.68 ppm in compound **1**. Moreover, The C-14 methyl singlet ($\delta$ 0.88) in **1** was missing in **2**, and an oxygenated methylene AB quartet was observed instead ($\delta$ 3.92, 3.81, $J$ = 11.6 Hz). These findings clearly indicated that polyfibrospongol B (**2**) has an additional free hydroxyl group at C-14. The assigned structure was also supported by the $^{13}$C-NMR spectrum of **2**, which exhibited a primary hydroxylated carbon at $\delta$ 64.5. Upon acetylation compound **2** provided a diacetate (**6**), which showed the molecular ion at $m/z$ 486 in the mass spectrum and two acetyl singlets at $\delta$ 2.09 and 2.34 in the $^1$H-NMR spectrum. In addition, the chemical shift of the C-14 oxygenated methylene was shifted downfield to 4.24 ppm. The fragment ions at $m/z$ 249 $(M - ArCH_2^{\bullet})^+$ and $m/z$ 238 $(ArCH_2^{\bullet +})$ in the EIMS spectrum of **6** were also consistent with the assigned structure of **2**. Because the observed chemical shifts, coupling pattern, and specific rotation of **2** are similar to those of **1**, the stereochemistry of **2** is presumed to be identical to that of **1**.




(**1**) $R_1 = R_3 = H$, $R_2$ = Me
(**2**) $R_1$ = OH, $R_2$ = Me, $R_3 = H$
(**3**) $R_1 = R_2 = R_3 = H$
(**5**) $R_1 = H$, $R_2$ = Me, $R_3$ = Ac
(**6**) $R_1$ = OAc, $R_2$ = Me, $R_3$ = Ac
(**7**) $R_1 = H$, $R_2 = R_3$ = Ac

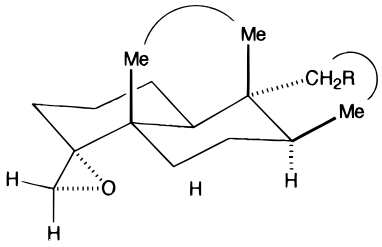



(**4**) 5β-Me
(**9**) 5α-Me

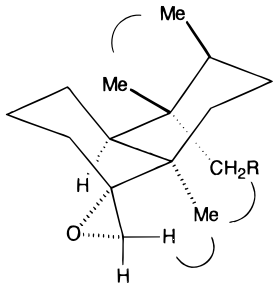



(**8**) 5β-Me
(**10**) 5α-Me

The cytotoxicities of the new sesquiterpene hydroquinones **1** and **2** and their derivatives were evaluated in vitro against human and murine tumor cell lines. As shown in Table 3, compounds **1**−**5**, **7**, **8**, and **10** exhibited significant cytotoxicities against human lung (A-549) and nasopharyngeal (KB-16) cancers and against murine lymphocytic leukemia (P-388) cells. Polyfibrospongols A (**1**) and B (**2**) and dictyoceratin A (**3**) showed




**8**




**10**

**Figure 2.** Selective NOESY studies of compounds **8** and **10**.

similar and promising activity against three tumor cell lines. However, their acetates (compounds **5**−**7**) were less active. The most active of the compounds isolated was ilimaquinone (**4**). In order to investigate structural effects on its activity, its 4(11) epoxide (**8**) and the stereoisomeric epoxide (**10**) were prepared from **4** and a mixture of **4** and **9**, respectively. The structures of both **8** and **10** were determined by spectral analysis and confirmed by the NOESY experiments. Oxygenation of the 4(11) double bonds of **4** and **9** occurred primarily from the less-hindered *Re* face in preference to the hindered *Si* face.[15] The proposed configurations of **8** and **10** were consistent with the results of NOESY studies as shown in Figure 2. The biological activities of **8** and **10** were slightly less than the activity of **4** in each cell line tested, indicating that the 4(11) double bond does not play a major role in the bioactivity of compound **4**.

## Experimental Section

**General Experimental Procedures.** Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi V-3210 spectrophotometers, respectively. EI, FAB, and HREIMS spectra were recorded on a VG Quattro 5022 and JEOL JMS-HX 110 mass spectrometers. The $^1$H-, $^{13}$C-NMR, COSY and NOE, and NOESY spectra were recorded on a Varian FT-300, a Varian FT-400, or a Bruker AMX 400 spectrometer. The chemical shifts are given in $\delta$ (ppm) and coupling constants in Hz. Sephadex LH-20 (Pharmacia) and Si gel 60 (Merck) were used for column chromatography, and precoated Si gel plates (Merck, Kieselgel 60 $F_{254}$, 1 mm) were used for preparative TLC.

**Animal Material.** *P. australis* Lendenfeld (Spongiidae) was collected from Nan-Wan, Taiwan, in October 1993, and stored at −20 °C before extraction. The sponge has an irregular shape and exhibited a brown massive architecture. The soft thorny-looking tissue was surrounded by numerous small ostia and exhalent pores rising to the surface of the sponge. In order to observe the spicules, the sponge was treated with a mixture of $HNO_3$ (12 N, 3 mL) and $H_2SO_4$ (18 N, 1 mL),

and heated for 2 h. Its needle-like spicules were observed under a microscope.[14] A voucher specimen (sp 125), identified by Dr. G. H. Lee, has been deposited in the Department of Marine Resources, National Sun Yat-sen University.

**Extraction and Isolation.** The fresh sponge (230 g, wet wt) was crushed in a blender in 95% EtOH and extracted with the same solvent (2 L × 3). The combined filtrate was concentrated under vacuum to give a suspension, which was adjusted to 400 mL by the addition of $H_2O$. The suspension was extracted exhaustively with $CHCl_3$ (400 mL). The $CHCl_3$ layer was concentrated to give a residue (0.4 g), which was applied to a Si gel (20 g) column eluted with $CHCl_3$ and $CHCl_3$−MeOH (10:1) to afford fractions A−D. Collection of fraction A ($CHCl_3$, 500 mL) yielded polyfibrospongol A (**1**, 7 mg). A mixture (125 mg) of ilimaquinone (**4**) and 5-*epi*-ilimaquinone (**9**) was obtained from fraction B ($CHCl_3$−MeOH,10:1, 500 mL). Fraction D (130 mg) was chromatographed on a Si gel column eluted with $CHCl_3$−$Me_2CO$ (10:1, 440 mL) to furnish an additional mixture (95 mg) of compounds **4** and **9**, compound **3** (6 mg), and an unknown fraction, which was purified by a preparative TLC plate (Si gel, $CHCl_3$−$Me_2CO$, 10:1) to yield polyfibrospongol B (**2**, 5 mg).

In another batch of unidentified sponge (sp-137), the fresh body (100 g) was crushed with EtOH (200 mL × 2). After removal of solvent *in vacuo*, the residue (7 g) was partitioned between equal volumes of $CHCl_3$ and $H_2O$ (each, 300 mL). The $CHCl_3$-soluble fraction (180 mg) was subjected to a Sephadex LH-20 column (30 g) and eluted with MeOH to yield a residue. Purification of the residue by a Si gel column (15 g) using $CHCl_3$ as solvent gave compound **4** (15 mg).

**Polyfibrospongol A** (**1**): amorphous solid; $[\alpha]^{25}_D$ + 5.3° (*c* 0.3, $CHCl_3$); UV (MeOH) $\lambda$ max (log $\epsilon$) 227 (4.51), 269 (4.22), 297 (3.88) nm; IR (neat) $\nu$ max 3416 (OH), 1714 (O−C═O, conjugated ester), 1602, 1496, 1464, 1432, 1304, 1220, 1110, 1080, 948, 894, 766 $cm^{-1}$; $^1$H- and $^{13}$C-NMR data, see Table 1; EIMS (70 eV) *m*/*z* $[M]^+$ 386 (0.9), 355 (1.9), 196 (83.1), 191 (61.4), 175 (22.1), 135 (22.1), 121 (35.8), 95 (100); HREIMS (70 eV) *m*/*z* 386.2447, calcd for $C_{24}H_{34}O_4$ 386.2457.

**Polyfibrospongol B** (**2**): amorphous solid; $[\alpha]^{25}_D$ +1.8° (*c* 0.18, $CHCl_3$); UV (MeOH) $\lambda$ max (log $\epsilon$) 227 (4.58), 270 (4.23), 301 (3.85) nm; IR (neat) $\nu$ max 3420, 2940, 1710, 1645, 1440, 1310, 1220, 1025, 900, 815, 760 $cm^{-1}$; $^1$H- and $^{13}$C-NMR data, see Table 2; EIMS *m*/*z* $[M]^+$ 402 (7.2), 371 (2.7), 207 (3.8), 197 (25.8), 196 (100), 195 (39.0), 189 (15), 164 (20.5), 149 (11.5), 137 (30.1), 119 (15.9), 107 (23.7), 95 (67.9), 81 (51.0), 69 (34.2), 55(36.8); FABMS *m*/*z* $[M + Na]^+$ 425 (2.0), $[M + 1]^+$ 403 (4.4), $[M]^+$ 402 (12.4); HREIMS (70 eV) *m*/*z* 402.2408, calcd for $C_{24}H_{34}O_5$ 402.2406.

**Compound 3**: isolated as an amorphous solid; $[\alpha]^{25}_D$ +4.9° (*c* 0.17, $CHCl_3$); $^{13}$C NMR ($CDCl_3$, 75.4 MHz) $\delta$ 23.2 (t, C-1), 27.9 (t, C-2), 33.0 (t, C-3), 160.1 (s, C-4), 40.2 (t, C-5), 36.6 (t, C-6), 27.7 (t, C-7), 36.3 (d, C-8), 42.1 (s, C-9), 48.0 (d, C-10), 102.7 (t, C-11), 20.6 (q, C-12), 17.6 (q, C-13), 17.7 (q, C-14), 36.9 (t, C-15), 125.1 (s, C-16), 127.3 (s, C-17), 120.4 (s, C-18), 113.9 (d, C-19), 142.4 (s, C-20), 148.7 (s, C-21), 167.5 (s, *C*OOCH$_3$), 52.0 (q, COO*C*H$_3$); the UV, IR, $^1$H-NMR, and MS data of **3** were identical with those reported for dictyoceratin A.[6]

**Polyfibrospongol A Monoacetate (5).** Acetylation ($Ac_2O$−Py, each 0.2 mL, room temperature) of **1** (3 mg) gave after workup **5** (3.1 mg) as a solid: $[\alpha]^{25}_D$ + 6.5° (*c* 0.18, $CHCl_3$); UV (MeOH) $\lambda$ max (log $\epsilon$) 227 (4.55), 245 (4.49), 289 (4.11) nm; IR (neat) $\nu$ max 2932, 1768, 1724, 1636, 1596, 1464, 1438, 1422, 1344, 1302, 1244, 1168, 1080, 892, 770 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.9−2.4 (12H, m, H-1−H-10), 4.43 (1H, s, H-11a), 4.37 (1H, s, H-11b), 1.07 (3H, s, H-12), 1.00 (3H, d, $J$ = 6.3 Hz, H-13), 0.86 (3H, s, H-14), 2.56 (2H, s, H-15), 7.49 (1H, d, $J$ = 1.5 Hz, H-17), 7.47 (1H, d, $J$ = 1.8 Hz, H-19), 3.90 (3H, s, OMe), 3.86 (3H, $COOCH_3$), 2.36 (3H, s, OAc); EIMS (70 eV) *m*/*z* $[M]^+$ 428 (0.4), 369 (0.3), 238 (4.5), 196 (20.9), 191 (42.4), 177 (17.6), 135 (19.8), 121 (27.0), 109 (29.2), 95 (100).

**Polyfibrospongol B Diacetate (6).** Acetylation ($Ac_2O$−Py, each 0.1 mL, room temperature) of **2** (1.5 mg) gave after workup **6** (1.8 mg) as a solid: $[\alpha]^{25}_D$ +1.5° (*c* 0.18, $CHCl_3$); UV (MeOH) $\lambda$ max (log $\epsilon$) 228 (4.72), 278 (4.04) nm; IR (neat) $\nu$ max 2940, 1775, 1730, 1650, 1465, 1345, 1305, 1240, 1200, 1170, 910, 810, 760 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.7−2.4 (12H, m, H-1−H-10), 4.44 (1H, s, H-11a), 4.39 (1H, s, H-11b), 1.07 (3H, s, H-12), 1.09 (3H, d, $J$ = 6.3 Hz, H-13), 4.24 (2H, s, H-14), 2.73 (1H, d, $J$ = 14.4 Hz, H-15a), 2.90 (1H, d, $J$ = 14.4 Hz, H-15b), 7.58 (1H, d, $J$ = 1.8 Hz, H-17), 7.50 (1H, d, $J$ =1.8 Hz, H-19), 3.90 (3H, s, OMe), 3.87 (3H, $COOCH_3$), 2.34 (3H, s, OAc), 2.09 (3H, s, OAc); EIMS (70 eV) *m*/*z* $[M]^+$ 486 (0.7), 445 (0.9), 444 (2.9), 413 (1.3), 377 (2.0), 362 (2.4), 249 (62.2), 238 (11.6), 196 (38.3), 189 (100), 161 (12.8), 147 (20.7), 133 (34.3), 119 (25), 107 (32.2), 95 (67.1), 81 (53.1), 69 (38.0), 55 (36.3), 43 (78.1); FABMS *m*/*z* $[M + Na]^+$ 509 (2.0), $[M + H]^+$ 487 (3.4).

**Compound 7.** Acetylation ($Ac_2O$−Py, each 0.1 mL, room temperature) of **3** (1.5 mg) gave after workup **7** (1.8 mg) as a solid: $[\alpha]^{25}_D$ +0.1° (*c* 0.04, $CHCl_3$); IR (neat) $\nu$ max 2932, 1778, 1728, 1438, 1374, 1328, 1308, 1234, 1200, 1164, 1032, 895, 756 $cm^{-1}$; EIMS (70 eV) *m*/*z* $[M]^+$ 456 (1.5), 425 (2.0), 413 (1.2), 383 (1.6), 224 (20), 191 (88), 177 (36), 135 (34), 121 (54), 109 (58), 95 (100), 81 (56). The $^1$H-NMR spectrum was consistent with that reported for dictyoceratin A diacetate.[6]

**Ilimaquinone Epoxide (8).** Ilimaquinone (**4**, 10 mg) was treated with *m*-CPBA (5 mg) at room temperature for 15 min. The reaction mixture after evaporation under vacuum was purified by a TLC plate (Si gel, $CHCl_3$) to yield ilimaquinone epoxide (**8**) (3 mg): $[\alpha]^{25}_D$ −9.7 (*c* 0.1, $CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.7−2.2 (12H, m, H-1−H-10), 2.74 (1H, d, $J$ = 4.5 Hz, H-11a), 2.30 (1H, d, $J$ = 4.5 Hz, H-11b), 1.10 (3H, s, H-12), 0.96 (3H, d, $J$ = 6.3 Hz, H-13), 0.82 (3H, s, H-14), 2.60 (1H, d, $J$ = 14.1 Hz, H-15a), 2.50 (1H, d, $J$ = 14.1 Hz, H-15b), 5.87 (1H, s, H-19), 3.87 (3H, s, OMe); $^{13}$C NMR ($CDCl_3$, 75.4 MHz) $\delta$ 22.7 (t, C-1), 27,1 (t, C-2), 29.7 (t, C-3), 65.4 (s, C-4), 37.9 (t, C-5), 30.8 (t, C-6), 30.0 (t, C-7), 37.3 (d, C-8), 42.9 (s, C-9), 46.8 (d, C-10), 49.9 (t, C-11), 19.7 (q, C-12), 17.8 (q, C-13), 17.2 (q, C-14), 32.0 (t, C-15), 117.1 (s, C-16), 153.5 (s, C-17), 182.3 (s, C-18), 102.1 (d, C-19), 161.8 (s, C-20), 182.2 (s, C-21), 56.9 (q, OMe); EIMS (70 eV) *m*/*z* $[M]^+$ 374 (1.8), 207 (87), 189 (30), 168 (100), 147 (14), 133 (19), 119 (20), 109 (24), 95 (47), 81 (26), 69 (26), 55 (37).

**5-*epi*-Ilimaquinone Epoxide (10).** A mixture (40 mg) of ilimaquinone (**4**) and 5-*epi*-ilimaquinone (**9**) was treated with *m*-CPBA (20 mg) at room temperature for 30 min. The reaction mixture after evaporation under

vacuum was chromatographed on a Sephadex LH-20 column eluted with MeOH−$CHCl_3$−*n*-hexane (2:1:1) to give ilimaquinone epoxide (**8**) and 5-*epi*-ilimaquinone epoxide (**10**, 20 mg). Compound **10**: $[\alpha]^{25}_D$ −34.6° (*c* 0.38, $CHCl_3$); UV (MeOH) $\lambda$ max (log $\epsilon$) 228 (4.46), 290 (4.57) nm; IR (neat) $\nu$ max 3324 (OH), 2936 (CH, aliphatic), 1646, 1610, 1538, 1462, 1448, 1380, 1324, 1230, 1038, 916, 754 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 0.7−2.4 (12H, m, H-1−H-10), 3.04 (1H, d, $J$ = 4.2 Hz, H-11a), 2.58 (1H, d, $J$ = 4.2 Hz, H-11b), 1.10 (3H, s, H-12), 0.96 (3H, d, $J$ = 6.3 Hz, H-13), 0.95 (3H, s, H-14), 2.66 (1H, d, $J$ = 13.8 Hz, H-15a), 2.55 (1H, d, $J$ = 13.8 Hz, H-15b), 5.88 (1H, s, H-19), 3.89 (3H, s, OMe); $^{13}C$ NMR ($CDCl_3$, 75.4 MHz) $\delta$ 22.5 (t, C-1), 23.8 (t, C-2), 30.0 (t, C-3), 60.9 (s, C-4), 36.3 (t, C-5), 32.1 (t, C-6), 31.2 (t, C-7), 38.1 (d, C-8), 44.3 (s, C-9), 48.3 (d, C-10), 55.9 (t, C-11), 29.6 (q, C-12), 19.2 (q, C-13), 18.2 (q, C-14), 32.6 (t, C-15), 117.4 (s, C-16), 153.4 (s, C-17), 182.4 (s, C-18), 102.0 (d, C-19), 161.8 (s, C-20), 182.0 (s, C-21), 56.9 (q, OMe); EIMS (70 eV) *m*/*z* $[M]^+$ 374 (0.5), 207 (37), 168 (100), 149 (22), 139 (18), 119 (27), 109 (35), 95 (66), 81 (53), 69 (76), 55 (73).

**Cytotoxicity Assays.** The cytotoxicity of compounds against P-388 (leukemia), KB-16 (nasopharynx), and A-549 (lung) tumor cells was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay with some modifications.[16] Each cell line was plated at 1000 cells/well in 96-well microtiter plates. Twofold serial dilutions of tested compounds were added to the cells, and P-388, KB-16, and A-549 cells were enumerated using MTT after 3, 3, and 6 days, respectively. MTT (50 $\mu$L, 1 mg/mL) was added to each well, and the plates were incubated at 37 °C for 5 h. Formazan crystals were redissolved in DMSO (Merck) for 10 min with shaking, and plates were then immediately read on a microtiter plate reader (Dynatech) at 540 nm. The $IC_{50}$ was defined as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay. Results are given in Table 3. As described in a previous paper mithramycin was used as a standard ($IC_{50}$ 0.06 $\mu$g/mL).[17]

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