Evaluation of New Sesquiterpene Quinones from Two *Dysidea* Sponge Species as Inhibitors of Protein Tyrosine Kinase

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Received June 15, 1992

Our program to discover inhibitors of enzymes thought to be important in tumorigenesis motivated a study of marine sponge-derived sesquiterpene quinones (hydroquinones) against pp60$^{v\text{-}src}$ protein tyrosine kinase (PTK). Five new metabolites were isolated from the sponge *Dysidea avara* including melemeleone A (**3**), melemeleone B (**4**), 18-methoxyavarone (**6a**), popolohuanone-C (**8**), and popolohuanone-D (**9**). These were accompanied by two known compounds, (+)-avarol (**2a**) and (+)-19-methoxyavarone (**7**). All of these compounds possess a 4,9-friedodrimane array linked to a quinone moiety containing additional heteroatoms. The new structures were determined from analysis of spectroscopic data, and all compounds were tested for inhibitory activity against pp60$^{v\text{-}src}$ PTK. Compound **4** had an IC_{50} of $\approx$28 μM and was considered to be moderately active against this enzyme. The other compounds were all inactive.

Introduction

Finding new inhibitors of enzymes important in the signaling pathways which regulate oncogenesis or cell proliferation should offer a way to discover unique classes of anticancer natural products. We have chosen the protein tyrosine kinase (PTK) family for study because many oncogene products and growth factor receptors are kinases which phosphorylate substrate proteins on tyrosine residues.[1,2] The list of low molecular weight natural products of microbial, plant, and marine origin known to inhibit PTK is small. They can be divided into compounds which (a) bind at the ATP site (e.g., lavendustins,[3] quercetin,[4] and genistein[5,6]), (b) bind at the peptide substrate site (e.g., erbstatin and piceatannol),[7] (c) are irreversible inhibitors (Michael acceptors) covalently binding to cysteine residues (e.g., herbimycin A),[8] or (d) operate by unknown mechanisms (e.g., doxorubicin,[2a] staurosporine,[2a] adriamycin,[9] and aeroplysinin[10]). We have recently examined halenaquinone and related analogs from *Xestospongia ?carbonaria* along with simple synthetic quinone models and found that halenaquinone (**1**) is a potent inhibitor of both the pp60$^{v\text{-}src}$ PTK (IC_{50} = 1.5 μM) and the epidermal growth factor (EGF) receptor (IC_{50} = 19 μM).[11] We now extend the scope of these initial studies by describing a series of sesquiterpene polyketide quinones and hydroquinones from two Solomon Island species of *Dysidea*. The new metabolites include **3**, **4**, **6a**, **8**, and **9** accompanied by known compounds **2a** and **7**, and all of these compounds were isolated from both collections of sponges.

Results and Discussion

We decided to investigate whether the striking pp60$^{v\text{-}src}$ PTK inhibitory activity of halenaquinone noted above could be duplicated or even exceeded in structurally different sponge-derived quinones. Dictyoceratida sponges, including those in nine different genera of three different families, are rich in terpenoid quinones.[12] These were immediately targeted for study since eight different Indo-Pacific *Dysidea* species (Order Dictyoceratida) were present in our repository. Two morphologically different *Dysidea* sponges (coll. no. 89110 and coll. no. 89113) were pursued in parallel because the ^{13}C NMR spectra of their respective crude extracts showed prominent peaks at δ 187 and 182, along with several peaks between δ 107 and 102, all ascribable to substituted quinone moieties. Despite intensive taxonomic examination, it was not possible to identify either sponge to the species level. One specimen (coll. no. 89110) was concluded to be *Dysidea ?avara* and the other (coll. no. 89113) was designated as *D. ?arenaria*. Eventually, two known sesquiterpenes, respectively (+)-avarol (**2a**) from *D. avara*[13] and (+)-19-methoxyavarone (**7**)[14] from *Dysidea* sp., were obtained from both our sponges. Not unexpectedly, extensive chromatographic operations were required to separate **2a** and **7** from some five additional new zoochromic compounds including two metabolites, **8** and **9**, which are related to the popolohuanones from a *Dysidea* sp. sponge reported by Scheuer.[15]

Invaluable reference NMR data were provided by such compounds as avarol (**2a**),[13] 19-methoxyavarone (**7**),[14] 18-hydroxyavarone (**6b**),[16] and 18-methoxyavarone (**6a**). In 1974, Minale[13] proposed the structure of avarol based on

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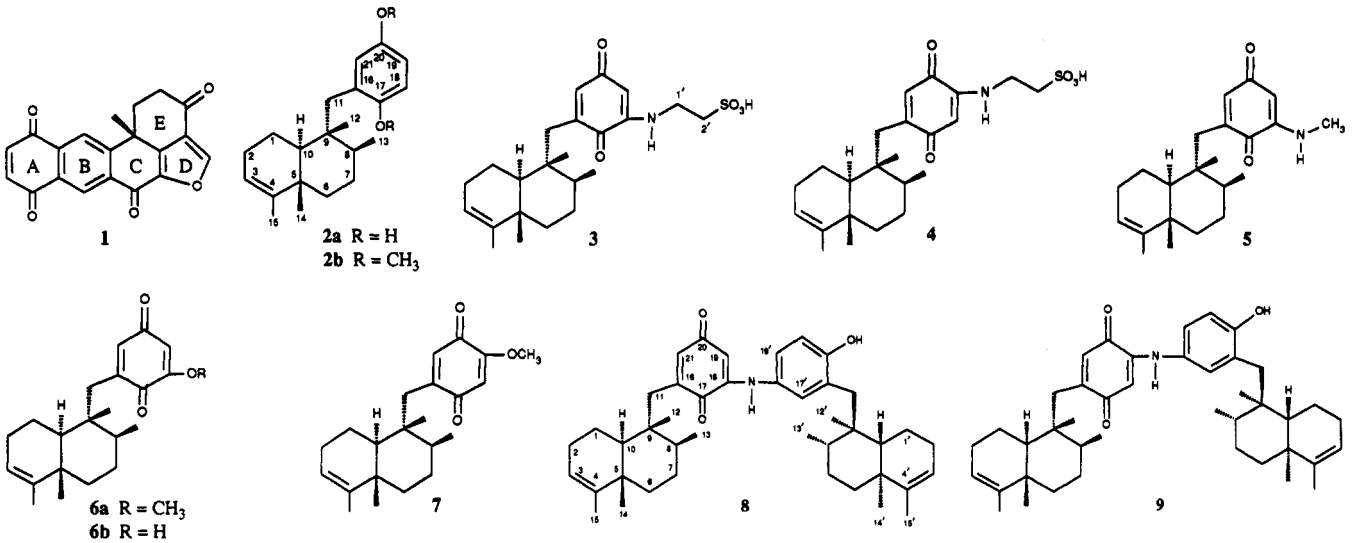
0022-3263/92/1957-6604$03.00/0

Chart I



spectral data and degradation reactions, and Sarma's total synthesis of (±)-**2a** contributed additional confirmation of its structure.[17] In a second publication, Minale proposed a 5*S*,8*S*,9*R*,10*S* absolute stereochemistry for **2a** based on CD measurements of an enone oxidation product of avarol dimethyl ether (**2b**).[18] A parallel 8*S*,9*R*,10*S* absolute stereochemistry has been assigned for several additional 4,9-friedodrimane-containing sesquiterpenes from Dictyoceratida sponges.[19] The ^{1}H NMR data of avarol (**2a**) has been published[14] whereas its ^{13}C NMR values are shown for the first time in Table I accompanied by data for avarol dimethyl ether (**2b**).[20] The ^{1}H NMR coupling pattern of the quinone ring protons of avarone[14] (H-18, d, J = 10 Hz; H-19, dd, J = 10, 2 Hz) indicates that the regiochemistry of an additional substituent appended to the avarone ring at either C-18, C-19, or C-21 can be rapidly deduced from the changes in multiplet patterns of the quinone ring protons.

The first unusual metabolite isolated in this study was (−)-melemeleone A (**3**).[21] Its molecular formula $C_{23}H_{33}NSO_5$ was established by HRFABMS (m/z 436.2151, $[M + H]^+$). The spectral data revealed three substructures consisting of a sesquiterpene, a quinone, and a taurine. The NMR ^{13}C/^{1}H shifts of the sesquiterpene moiety in **3** exactly matched the ^{1}H NMR values in the literature[12,13] for **2a** and **2b** as well as the ^{13}C NMR values collected in Table I. Also, a difference NOE NMR experiment on **3** revealed NOE's from Me-12 to H-11, Me-13, and Me-14. Thus, both a structural and stereochemical analogy was justified for the terpenoid portion of **3** in comparison to that of **2a**. The NMR ^{13}C/^{1}H quinone ring signals of **3** at δ 5.45 (d, J = 2.4 Hz)/δ 97.3 and δ 6.32 (d, J = 2.4 Hz)/δ 140.3 were indicative of meta-oriented CH's with an NR substituent attached at C-18. As to be expected, these ^{13}C/^{1}H quinone ring chemical shifts were parallel to those of 18-(*N*-methylamino)avarone (**5**)[22] and of other quinone model compounds with NR substituents at C-18.[23] The remaining ^{1}H resonances at δ 3.05 (t, J = 6.6 Hz)/δ 39.5 (t) and 3.51 (t, J = 6.6 Hz)/48.3 (t) were recognized as belonging to a taurine moiety. Also in accord with the proposed structure was the negative ion FAB mass spectrum of **3** which featured diagnostic fragment ions at m/z 354 $(M - SO_3H)^-$ and 244/191 (cleavage at C-9/C-11).

A second melemeleone isomer (−)-**4** accompanied **3** in the CH_2Cl_2 partition fraction. The $C_{23}H_{33}NSO_5$ molecular formula of melemeleone B (**4**) was established from positive ion FAB mass spectrometry data, $[M + H]^+$ = 436.2152. The singular difference in the ^{13}C NMR reso-

Table I. ^{13}C NMR Data of Compounds 2–4, 6, and 7

C no.	2a[a]	2b[c]	3[a]	4[a]	6a[a]	6b[c]	7[b]
1	20.7	19.5	20.3	19.2	19.4	19.9	21.4
2	27.5	26.4	27.3	26.1	26.5	26.9	27.8
3	121.8	120.5	121.5	120.3	120.7	120.7	122.0
4	145.0	144.5	144.9	143.7	143.9	144.0	145.3
5	42.7	41.8	43.0	42.6	42.4	43.4	44.3
6	38.3	37.0	37.3	36.1	36.1	35.9	37.5
7	28.9	27.6	28.5	27.3	27.5	27.9	28.8
8	37.2	36.0	38.0	37.1	36.9	38.1	38.4
9	39.4	38.2	39.5	38.3	38.5	38.5	38.7
10	47.1	45.8	47.6	46.8	47.0	48.2	48.6
11	37.3	35.7	36.0	35.4	35.2	32.6	36.7
12	18.3	17.1	18.0	16.8	17.8	17.2	19.1
13	18.0	17.5	17.0	16.0	16.7	17.7	18.1
14	20.7	19.8	20.3	19.2	20.0	20.1	20.8
15	18.3	17.8	18.1	16.9	18.0	18.1	19.4
16	127.6	129.1	144.0	141.7	145.0	120.7	145.3
17	150.6	153.3	184.4	182.7	182.0	182.8	188.6
18	116.4*[d]	111.1	149.1	96.7	159.0	152.7	109.3
19	114.4*	111.0	97.3	150.8	106.9	139.9	159.5
20	150.0	153.1	187.2	185.7	187.1	187.7	183.4
21	120.4*	117.4	140.3	131.9	136.7	131.3	135.4
1′			38.5	38.0			
2′			48.3	48.5			
OCH_3					56.3		57.5

[a] In CD_3OD solvent. [b] In $CDCl_3$ solvent. [c] Reference 16. [d] Assignments marked with an asterisk (*) can be switched.

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(20) Kashman (ref 16) misassigned the resonances for the quinone carbonyls of **6b**. Extremely useful shielding effects of quinone ring substituents have been summarized by Neidlein (ref 23a) and include substituents ortho to the C=O Me (+0.5), *O*-alkyl (−5 to −6), and *N*-alkyl (−3) versus substituents meta to the C=O Me (−0.4 to +0.7), *O*-alkyl (0), and *N*-alkyl (−1 to −2).
(21) This compound is named in honor of the tradition established by Prof. Paul J. Scheuer (University of Hawaii). The exotic names of many natural products he has discovered have served to educate marine bioorganic scientists about the language, geography, and culture of traditional Hawaii. The name of compounds **3** and **4** are derived from the Manoa Valley neighborhood street on which P.J.S. resides!
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nances between this pair (see Table I) was at C-21; chemical shifts were respectively δ 140 (3) and δ 132 (4). The appearance of the ^1H NMR sharp singlets in 4 at δ 5.45 and 6.32 clearly indicated a para orientation of the quinone ring protons, and the ^{13}C shifts indicated that the *N*-methylamino group was attached at C-19. Most importantly, the parallel shifts of the remaining carbons of 3 and 4 indicated the identity of all additional structural and stereochemical features.

Two pairs of isomeric compounds, each containing a 4,9-friedodrimane sesquiterpene subunit, were isolated from the less polar solvent partition fractions. The hexane fraction yielded (+)-19-methoxyavarone (7), recently reported by Yamada,[14] accompanied by the 18-methoxy isomer **6a**. Both compounds displayed parallel LREIMS $M^+ = 342$ ($C_{21}H_{28}O_3$) while the NMR variations between this pair were confined to the quinone ring CH signals including ^{13}C resonances of **6a** δ 107/131 versus 7 δ 109/135 and ^1H resonances of **6a** δ 5.83 and 6.37 AB doublet J = 2.2 Hz versus 7 δ 5.90 and 6.45 both as sharp singlets. The CCl_4 solvent partition fraction afforded the remaining twosome, 8 and 9. The first indication that these were structurally related to popolohuanones A and B[15] was that resonances for eight methyls rather than four were present in each of their ^{13}C NMR spectra. Also, resonance doubling was apparent for most of the signals assignable to C-1 to C-11 of the sesquiterpene subunit. Comparison to the various NMR resonances summarized in Table I and in the literature[15] supported a straightforward assignment of these compounds as popolohuanones C (8) and D (9) as shown.

An absolute stereochemistry of 5*S*,8*S*,9*R*,10*S* has been assigned for (+)-avarol (**2a**), and the supporting arguments were discussed above. Assuming that a parallel biogenetic route produces the compounds isolated in this study, including **2a**, **3**, **4**, **6a**, and **7–9**, justifies the argument that all of these structures possess the same absolute configurations at C-5,5′/8,8′/9,9′/10,10′. An interesting additional biogenetic paradigm is indicated by the structures of the alkaloid quinone terpenes **3**, **4**, **8**, **9**. The melemeleones (**3** and **4**) combine a quinone, a 4,9-friedodrimane, and the amino acid taurine. A cyclized taurine residue has been observed in the other quinone-containing polyketide sponge metabolites of the halenaquinone family from an *Adocia* sponge reported by Schmitz[24] and recently isolated by our group from *Xestospongia? carbonaria*. A similar combination of a 4,9-friedodrimane/quinone/amino acid (decarboxylated) can be seen in the structures of sponge metabolites such as smenospongiarine and smenospongidine.[25] A condensation reaction between 18- or 19-aminoavarone and avarone followed by aromatization provides a simple explanation for the formation of the popolohuanones C (8) and D (9).

Compounds **2a**, **3**, **4**, **6a**, and **7–9** were tested for their inhibitory activity against $pp60^{v\text{-}src}$ PTK at 20 μg/mL, as described previously,[11] except that 20 mM 2-mercaptoethanol was included in the assay. Avarone, along with 18-aminoavarone and 19-aminoavarone which were all prepared from **2a**, were also tested. Only **4** was moderately active and exhibited an $IC_{50} \approx 28$ μM. These results, in comparison to the extreme potency of 1 against PTK, indicate that a quinone group plus additional functionalization is needed for effective activity. Bioassay-guided isolation work is in progress on non-Dictyoceratid sponge extracts which block $pp60^{v\text{-}src}$ PTK. These results will be communicated in due course and will extend knowledge of new marine natural product chemotypes with inhibitory activity against this enzyme.

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Experimental Section

The NMR spectra were recorded at 300 MHz for ^1H, and 75 MHz for ^{13}C. Multiplicities of ^{13}C NMR resonances were determined from APT data and COSY experiments. Both ^1H–^1H and ^{13}C–^1H COSY NMR data were used to assign resonances of compounds **3** and **8**. Low-resolution electron-impact mass spectrometry (LREIMS) data were obtained on a quadrupole instrument while high-resolution mass spectral data were obtained on a double-focusing instrument. HPLC was done using columns that included 10 μm of ODS or 10 μm of silica. All solvents were distilled and dried for HPLC use and were spectral grade for spectroscopy.

Identification. The sponges (Fam. Dysideidae, Order Dictyoceratida) including *Dysidea* sp.1 (coll. no. 89110) and *Dysidea* sp.2 (coll. no. 89113) were collected from the Solomon Islands and preserved in methanol, and voucher specimens and underwater photographs are available (from P.C.). Careful examination of these voucher specimens (M.C.D.) indicates that neither of these matched the descriptions or appearance of some five shallow water tropical *Dysidea* sponges reported from the west-central Pacific.[26] These sponges are closest in appearance to the description of *D. avara* (coll. no. 89110)[26] and *D. arenaria* (coll. no. 89113),[27] respectively. However, both sponges are clearly different in comparison to a specimen in our repository which has been tentatively identified as *D. avara* (coll. no. 91001).

Dysidea sp.1 was a massive flabellate to branching sponge, pink to tan in color alive. The surface was strongly conulose, and the texture was soft and compressible. The extosome was charged with foreign material, mainly sand. The choanosome was cavernous with abundant sand and broken spicules both in the internal cavities and skeleton. The choanosomal skeleton was a gibroreticle with cored fibers (sand and broken spicules), with abundant fibrofascicles. The overall constitution of the fibers was obscured by the amount of material coring them.

Dysidea sp.2 was a white-tan, ramose-branching sponge. The body consisted of long ramose tubes branching out in many directions with oscules that protruded from the surface. The texture was firm, stiff yet compressible. Small conules were regularly distributed all over the surface. The ectosomal skeleton was a regular reticulum formed by thicker primary fibers (that give rise to the conules) and thinner secondary fibers. The same pattern is found in the choanosomal skeleton; primaries were 300–400 μm in diameter and secondaries 20–50 μm in diameter, both cored by foreign material.

Extraction. All sponges were preserved by a unique procedure which is just as effective as freezing but avoids this cumbersome procedure in areas where cold storage is not available. The full details will be published elsewhere and consisted of soaking the freshly collected sponges for up to 24 h in a 1:1 EtOH/H_2O (seawater) solution which was then discarded. The damp organisms were placed in containers and returned at ambient temperatures to UC Santa Cruz for workup. This involved a soaking (48 h, rt) of all specimens in MeOH (anhydrous) three times. Parallel concentration of these extracts afforded viscous oils, and ^{13}C NMR spectra revealed that each contained a mixture of lipids and quinone terpenes. Combined extracts from *Dysidea* sp.2 were investigated first. The crude oil (20.96 g) was subjected to solvent partitioning with aqueous MeOH against hexane, CCl_4, and CH_2Cl_2 with the percent of H_2O adjusted to produce a biphasic solution and yielded hexane (16.78 g), CCl_4 (1.00 g), and CH_2Cl_2 (2.08 g). The CH_2Cl_2 fraction which was first chromatographed on Sephadex LH-20 and then subjected to HPLC (10-μm ODS column; solvent, MeOH–H_2O, 7.5:2.5) afforded avarol (**2a**), melemeleone A (**3**), and melemeleone B (**4**). The hexane soluble fraction afforded, after HPLC (regular phase, 10-μm silica column; solvent, hexanes–EtOAc, 8:2), 18-methoxyavarone (**6a**) and 19-methoxyavarone (**7**). The CCl_4 fraction was chromato-

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graphed on Sephadex LH-20 (CH_2Cl_2–MeOH) and afforded a deep violet fraction. Further purification was achieved by using a chromatotron (silica gel plate, hexane–EtOAc (8:2)) and afforded popolohuanone C (**8**) and popolohuanone D (**9**). The oil from sponge *Dysidea* sp.1 was processed in a parallel manner and yielded all of the above compounds.

Avarol (2a): colorless needles (150 mg); mp 138–140 °C (lit.[13] mp 148–150 °C); $[\alpha]^{20}_D$ = +6.4° (*c* = 0.08, $CHCl_3$) (lit.[13] $[\alpha]^{20}_D$ = +6.1°). The ^1H and ^{13}C NMR data of **2a** are in good accordance with those in the literature.[13]

Melemeleone A (3): red amorphous solid (20 mg); mp 110–115 °C; $[\alpha]^{20}_D$ = −20.1° (*c* = 0.006, CH_2Cl_2); UV (MeOH) λ_{max} 484, 288, 214 nm; HRFABMS (positive) 436.2151 $[M + H]^+$ ($C_{23}H_{34}NSO_5$, Δ 0.6 mmu of calcd); LRFABMS (negative) *m/z* (rel int) 434 $[M - H]^-$ (20), 54 (2), 325 (2), 244 (20), 191 (2), 162 (40), 80 (75); ^1H NMR (CD_3OD) [atom number] δ [1] 1.88 (m, 2 H), [2] 1.94 (m, 2 H), [3] 5.11 (br s), [6_{eq}] 1.64 (dt, *J* = 12.6, 3.0 Hz), [6_{ax}] 1.02 (br t, *J* = 11.4 Hz), [7] 1.37 (m, 2 H), [8_{ax}] 1.17 (ddq, *J* = 11, 6, 6 Hz), [10_{ax}] 1.05 (bd, *J* = 10 Hz), [11] 2.58 and 2.46 (AB, 2 H, *J* = 13.8 Hz), [12] 0.83 (s, 3 H), [13] 0.92 (d, 3 H, *J* = 6.6 Hz), [14] 0.99 (s, 3 H), [15] 1.50 (br s, 3 H), [19] 5.45 (d, *J* = 2.4 Hz), [21] 6.32 (d, *J* = 2.4 Hz), [1′] 3.05 (t, 2 H, *J* = 6.6 Hz), [2′] 3.51 (t, 2 H, *J* = 6.6 Hz).

Melemeleone B (4): red amorphous solid (9 mg); mp 190–200 °C; $[\alpha]^{20}_D$ = −22.0° (*c* = 0.01, CH_2Cl_2); UV (MeOH) λ_{max} 483, 290, 214 nm; HRFABMS (positive) 436.2152 $[M + H]^+$ ($C_{23}H_{34}NSO_5$, Δ 7.5 mmu of calcd); LRFABMS (negative) *m/z* (rel int) 434 $[M - H]^-$ (18), 354 (1), 244 (20), 191 (3), 80 (76); ^1H NMR (CD_3OD) [atom number]: δ [1] 1.87 (m, 2 H), [2] 1.92 (m, 2 H), [3] 5.10 (br s), [6_{eq}] 1.64 (dt, *J* = 12.6, 3.0 Hz), [6_{ax}] 1.04 (br t, *J* = 10.8 Hz), [7] 1.37 (m, 2 H), [8_{ax}] 1.26 (ddq, *J* ≈ 11, 6, 6 Hz), [10_{ax}] 1.10 (d, *J* = 12.0 Hz), [11] 2.61 and 2.47 (AB, 2 H, *J* = 13.2 Hz), [12] 0.84 (s, 3 H), [13] 0.92 (d, 3 H, *J* = 6.6 Hz), [14] 0.99 (s, 3 H), [15] 1.50 (br s, 3 H), [18] 5.46 (s), [21] 6.33 (s), [1′] 3.05 (t, 2 H, *J* = 6.6 Hz), [2′] 3.50 (t, 2 H, *J* = 6.6 Hz).

18-Methoxyavarone (6): yellow solid (34 mg); mp 120–125 °C; $[\alpha]^{20}_D$ = −14.5° (*c* = 0.02, CH_2Cl_2); UV (MeOH) λ_{max} 270 nm; LREIMS *m/z* (rel int) 342 $[M]^+$ (1), 246 (2), 222 (2), 191 (30), 189 (75), 151 (3), 95 (100); ^1H NMR ($CDCl_3$) [atom number] δ [1] 1.79 (m, 2 H), [2] 1.86 (m, 2 H), [3] 5.09 (br s), [6_{eq}] 1.59 (dt, *J* = 9.6, 6.6 Hz), [6_{ax} and 10_{ax}] 0.98 (m, 2 H), [7] 1.37 (m, 2 H), [8_{ax}] 1.16 (m), [11] 2.59 and 2.42 (AB, 2 H, *J* = 13.5 Hz), [12] 0.80 (s, 3 H), [13] 0.89 (d, 3 H, *J* = 6.6 Hz), [14] 0.95 (s, 3 H), [15] 1.48 (br s, 3 H), [19] 5.83 (d, *J* = 2.2 Hz), [21] 6.37 (d, *J* = 2.2 Hz), [OCH_3] 3.77 (s, 3 H).

19-Methoxyavarone (7): yellow solid (24 mg); mp 149–151 °C (lit.[14] mp 150–152 °C); $[\alpha]^{20}_D$ = +13° (*c* = 0.02, CH_2Cl_2) (lit.[14] $[\alpha]^{20}_D$ = +16.4°). The ^1H NMR and LREIMS data of **7** are in good accordance with those of the literature.[14] The ^{13}C NMR data are listed in Table I.

Popolohuanone C (8): dark violet amorphous solid (21 mg); mp 125–135 °C; $[\alpha]^{20}_D$ = −9° (*c* = 0.08, CH_2Cl_2); UV (MeOH) λ_{max} 532, 275, 232 nm; LREIMS *m/z* (rel int) 623 M^+ (1), 433 (8), 418 (2), 241 (14), 191 (26), 175 (31), 95 (100); HREIMS 623.4329 M^+ ($C_{42}H_{57}NO_3$, Δ 0.9 mmu of calcd); ^1H NMR ($CDCl_3$) [atom number] assignments are made by analogy to arguments presented by Scheuer:[23] δ [1] 1.89 (m, 2 H), [1′, 2, 2′] 2.00 (m, 6 H), [3] 5.09 (br s), [3′] 5.15 (br s), [6_{eq} and $6'_{eq}$] 1.58 (m, 2 H), [$6'_{ax}$] 1.14 (t, *J* = 11.1 Hz), [6_{ax}] 1.02 (t, *J* = 10.2 Hz), [7 and 7′] 1.39 (m, 4 H), [8 and 8′] 1.27 (m, 2 H), [10] 1.16 (d, *J* = 12 Hz), [10′] 1.08 (d, *J* = 12 Hz), [11] 2.43 and 2.67 (AB, 2 H, *J* = 13.8 Hz), [11′] 2.54 and 2.74 (AB, 2 H, *J* = 14.1 Hz), [12] 0.86 (s, 3 H), [12′] 0.85 (s, 3 H), [13] 0.96 (d, 3 H, *J* = 6.0 Hz), [13′] 0.99 (d, 3 H, *J* = 6.0 Hz), [14] 1.01 (s, 3 H), [14′] 1.00 (s, 3 H), [15/15′] 1.50/1.53 (s, 3 H/3 H), [17′] 6.86 (br s), [19] 5.83 (d, *J* = 1.6 Hz), [19′] 6.89 (br d, *J* = 8.4 Hz), [20′] 6.73 (d, *J* = 8.3 Hz), [21] 6.41 (d, *J* = 1.6 Hz); ^{13}C NMR ($CDCl_3$) [1] 20.8, [1′] 21.1, [2 & 2′] 28.0, [3] 121.7, [3′] 122.0, [4] 145.3, [4′] 145.7, [5] 43.2, [5′] 43.6, [6] 37.4, [6′] 37.6, [7] 28.8, [7′] 29.1, [8] 38.1, [8′] 38.7, [9] 43.2, [9′] 43.6, [10] 47.5, [10′] 48.3, [11] 36.6, [11′] 39.9, [12 & 12′] 19.1, [13] 18.1, [13′] 18.8, [14 & 14′] 21.4, [15 & 15′] 19.5, [16] 143.8, [16′] 128.5, [17] 185.5, [17′] 129.9, [18] 146.2, [18′] 130.3, [19] 100.5, [19′] 123.6, [20] 187.7, [20′] 117.4, [21] 140.9, [21′] 154.9.

Popolohuanone D (9): dark violet amorphous solid (14 mg); mp 113–120 °C; $[\alpha]^{20}_D$ = −15° (*c* = 0.06, CH_2Cl_2); UV (MeOH) λ_{max} 533, 275, 234 nm; LREIMS *m/z* (rel int) 633 (5), 418 (2), 241 (16), 191 (20), 175 (20), 95 (100); ^1H NMR ($CDCl_3$) [atom number] δ [1] 1.85 (m, 2 H), [1′, 2, 2′] 2.00 (m, 6 H), [3] 5.09 (br s), [3′] 5.14 (br s), [6_{eq} and $6'_{eq}$] 1.60 (m, 2 H), [6_{ax} and $6'_{ax}$] 1.05 (m, 2 H), [7 and 7′] 1.39 (m, 4 H), [8 and 8′] 1.25 (m, 2 H), [10_{ax} and $10'_{ax}$] 1.14 (br d, 2 H, *J* = 12.6 Hz), [11] 2.50 and 2.65 (AB, 2 H, *J* = 13.2 Hz), [11′] 2.59 and 2.75 (AB, 2 H, *J* = 14.1 Hz), [12] 0.86 (s, 3 H), [12′] 0.85 (s, 3 H), [13] 0.94 (d, 3 H, *J* = 6.0 Hz), [13′] 0.98 (d, 3 H, *J* = 6.0 Hz), [14] 1.00 (s, 3 H), [14′] 1.01 (s, 3 H), [15/15′] 1.54/1.51 (br s, 3 H/3 H), [17′] 6.89 (br s), [18] 5.93 (s), [19′] 6.90 (dd, *J* = 8.6, 2.4 Hz), [20′] 6.72 (d, *J* = 8.4 Hz), [21] 6.44 (s); ^{13}C NMR (CD_3Cl) δ [1 and 1′] 20.2, [2] 26.6, [2′] 26.8, [3] 120.5, [3′] 120.8, [4] 144.1, [4′] 144.4, [5] 41.9, [5′] 43.3, [6] 35.8, [6′] 36.2, [7 and 7′] 27.7, [8] 38.4, [8′] 38.6, [9] 41.9, [9′] 43.3, [10] 46.1, [10′] 47.3, [11] 35.8, [11′] 37.4, [12, and 12′] 19.1, [13] 18.1, [13′] 18.2, [14 and 14′] 19.9, [15 & 15′] 19.9, [16] 143.5, [16′] 126.9, [17] 186.2, [17′] 127.8, [18] 100.1, [18′] 129.5, [19] 151.2, [19′] 121.8, [20] 183.8, [20′] 116.2, [21] 131.9, [21′] 152.8.

Acknowledgment. Partial support for this research was from NIH Grant CA52955. We thank Mr. Jim Loo (UCSC) for assistance with NMR and MS measurements. Thanks go to Ms. Lisa Hunter and Dr. Larry Manes for their participation in sponge collecting. The efforts of the crew of the Solomon Island vessel Bilikiki are also appreciated. We note support in processing incoming marine extracts and compounds at Syntex by Dr. Marshall Wallach and Ms. Amie Chuoung.

Supplementary Material Available: ^{13}C NMR spectra of **3**, **4**, and **7–9** (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.