methylene chloride (35 mL) there was obtained 1.07 g (98%) of the epoxy sulfone **28** as a colorless solid with mp 77–80 °C: ^{1}H NMR ($CDCl_3$) δ 7.76 (m, 5 H), 6.76 (br m, 1 H), 5.91 (dq, J = 3.74, 10.55 Hz, 1 H), 4.39 (m, 1 H), 2.88 (m, 1 H), 2.48 (m, 1 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 1.31 (d, J = 4.84 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 168.55, 167.69, 161.99, 135.24, 134.69, 129.49, 129.28, 92.06, 82.31, 59.61, 58.91, 46.72, 45.80, 30.52, 30.19, 20.33, 17.08, 16.76; IR ($CDCl_3$) 3400, 1770, 1719, 1265, 1050, 1156, and 1326 cm^{-1}.

(B) From the acetoxy sulfone **12b** (0.91 g, 2.14 mmol) and *m*-chloroperoxybenzoic acid (0.46 g, 2.14 mmol) in methylene chloride (35 mL) there was obtained 0.92 g (97%) of the epoxy sulfone **28**.

(±)-Methyl 2,3,6-Trideoxy-3-(trichloroacetamido)-α-*ribo*-furanoside (30a). Sodium hydroxide (0.26 g, 3.24 mmol) was added to a solution of the acetoxysulfone **28** (1.43 g, 3.24 mmol) in methanol (25 mL) and water (2 mL). After 1 h, analysis by TLC indicated that the reaction was complete. Brine (10 mL) and methylene chloride (25 mL) were added, and the layers were separated. The aqueous phase was further extracted with methylene chloride (2 × 15 mL), and the combined organic solutions were dried ($MgSO_4$), filtered, and evaporated at reduced pressure. The initially received material was purified on the Chromatotron (6:4 hexanes/ethyl acetate) to give 0.94 g (96%) of the furanose **30a** as a low melting yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.63 (br d, 1 H), 5.19 (m, 2 H), 4.64 (t, J = 8.13 Hz, 1 H), 4.02 (t, J = 2.41 Hz, 1 H), 3.40 (s, 3 H), 2.42 (ddd, J = 13.62, 7.47, 4.39, 4.18 Hz, 1 H), 1.96 (d, J = 13.84 Hz, 1 H), 1.50 (d, J = 6.59 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 161.02, 104.95, 90.06, 67.97, 54.63, 50.67, 38.92, 18.55.

(±)-Methyl *N,O*-Bis(trichloroacetyl)-3-amino-α-*ribo*-furanoside (30c). From the furanoside **30a** (0.50 g, 1.64 mmol), trichloroacetyl chloride (2 mL), and pyridine (2 mL) in diethyl ether (15 mL) there was obtained the bis(trichloroacetyl) furanoside **30c** (0.70 g, 95%) as a yellow syrup: ^{1}H NMR (benzene-d_6) δ 7.56 (br d, 1 H), 5.01 (dq, J = 6.59, 2.86 Hz, 1 H), 4.63 (d, J = 4.18 Hz, 1 H), 4.43 (t, J = 8.57 Hz, 1 H), 3.65 (t, J = 2.64 Hz, 1 H), 3.06 (s, 3 H), 1.92 (ddd, J = 18.23, 4.61, 4.17, 7.69 Hz, 1 H), 1.47 (d, J = 13.63 Hz, 1 H), 1.22 (d, J = 6.81 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.95, 161.62, 105.88, 88.48, 77.65, 55.49, 51.32, 39.08, 16.32.

(±)-Methyl *N*-(Trichloroacetyl)-α-ristosaminide (31). The furanoside **30a** (0.91 g, 2.98 mmol) in aqueous acetic acid (50%; 20 mL) was heated on a steam bath for 20 min. Analysis of a TLC indicated that the hydrolysis of the methyl glycoside residue had occurred. The solvent was evaporated, and the residue was taken up in methylene chloride (25 mL) and washed with saturated aqueous sodium bicarbonate (2 × 15 mL). The organic solution was dried ($MgSO_4$), filtered, and evaporated at reduced pressure. The residue was dissolved in anhydrous methanol (15 mL) and chilled in an ice/salt bath, and dry hydrogen chloride gas was bubbled into the reaction for 15 s. The solution was allowed to warm to room temperature and then stand for 1 h. The reaction was transferred to a separatory funnel with methylene chloride (25 mL), and the organic solution was washed with saturated aqueous sodium bicarbonate (2 × 15 mL), then dried ($MgSO_4$), filtered, and evaporated at reduced pressure. The residue, a 3:1 mixture by TLC, was purified by chromatography (silica gel, 50 g). Elution with 6:4 hexanes/ethyl acetate gave 0.26 g (28%) of the methyl pyranoside **31** as a light yellow solid with mp 94–97 °C: ^{1}H NMR ($CDCl_3$) δ 8.52 (br s, 1 H), 4.74 (m, 1 H), 4.18 (m, 1 H), 3.64 (m, 1 H), 3.38 (s, 3 H), 2.57 (br s, 1 H), 2.01 (m, 1 H), 1.65 (m, 1 H), 1.28 (d, J = 5.71 Hz, 3 H). Continued elution gave 0.51 g (56%) of the furanoside **30a** which was identical with the material obtained above.

Acknowledgment. We express appreciation to Henry Kalinoski and Lorne Isabelle for performing mass spectral analyses and to Dr. Bert Fraser-Reid (Duke University) for providing a ^{1}H NMR spectrum of methyl *N*-(trichloroacetyl)ristosaminide. This work was generously supported by the Institute for General Medical Sciences and the National Cancer Institute of the National Institutes of Health (Grant No. GM 26574 and CA 10925).

Supplementary Material Available: A ball-and-stick drawing of the epoxysulfone **27**, a description of the X-ray analysis procedure, tables of fractional coordinates and anisotropic thermal parameters, and figures showing bond distances and bond angles (7 pages). Ordering information is given on any current masthead page.

Metabolites of the Marine Hydroid *Garveia annulata*: Garveatins B and C, 2-Hydroxygarvin A, and Garvin A Quinone

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Four antimicrobial metabolites, garveatins B (**2**) and C (**3**), 2-hydroxygarvin A (**4**), and garvin A quinone (**5**), have been isolated from the marine hydroid *Garveia annulata*. The structures of garveatin B (**2**), garveatin C (**3**), and 2-hydroxygarvin A (**4**) were inferred from their spectral data and chemical derivatization reactions. The structure of garvin A quinone (**5**) was determined by X-ray diffraction analysis of its monoacetate (**12**).

The phylum coelenterata encompasses a diverse collection of marine invertebrates that includes familiar animals such as the hard and soft corals, gorgonians, sea pens, jellyfish, and sea anemones. Hydroids are a large class of coelenterates that are less well-known because they tend to be small and somewhat inconspicuous. To date, marine natural product chemists have successfully isolated a large number of interesting secondary metabolites from soft corals, gorgonians, sea pens, and zooanthids.[1] By contrast, the hydroids have received very little attention.[2] It is not

(1) (a) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 551. (b) Tursch, B.; Braekman, J. C.; Daloze, D.; Kasain, M. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. 2, p 76.

0022-3263/86/1951-0057$01.50/0

Table I. ^{1}H NMR (80-MHz) Data for Garveatins A–C, 2-Hydroxygarvin A, and Garvin A Quinone (Chemical Shifts in ppm from Me_4Si)

proton on carbon no.	1[a]	2[b]	3[b]	4[b,c]	5[b]
5	7.02 br s	7.04 br s	7.35 br s	6.98 s	7.56 s
10	7.15 s	7.12 s	7.15 s	7.21 s	
11	1.98 s	1.99 s	1.49 s	1.66 s	1.98 s
11a			1.49 s		
12	1.63 s	1.62 s	1.58 s	1.81 s	1.61 s
13	1.63 s	1.62 s	1.58 s	1.52 s	1.61 s
14	2.40 br s	2.46 br s	2.38 br s		
15		2.80 q (J = 7 Hz)		3.15 m	2.95 m
16	2.68 s	1.18 t (J = 7 Hz)	2.62 s	1.73 m	1.60 m
17				1.04 t (J = 7 Hz)	1.05 t (J = 7 Hz)
C6 OMe				3.99 s*	4.03 s*
C8 OMe			3.92 s		
C14 OMe				3.98 s*	3.98 s*
OH		17.15	14.45	14.25	
OH		10.25			

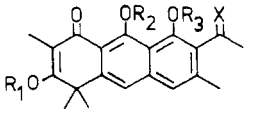
[a] $CDCl_3$ plus one drop of CD_3OD. [b] $CDCl_3$. [c] Asterisk: may be reversed.

clear at present whether the apparent lack of attention is a natural consequence of a general paucity of interesting secondary metabolism in the hydroids or whether it is simply a result of chemists overlooking hydroids in their screening programs.

Garveia annulata is a brilliant orange hydroid that is commonly encountered in rocky subtidal habitats from Alaska to Southern California during the winter and spring months. Our routine antimicrobial screening program revealed that crude methanol extracts of *G. annulata* were potently antibacterial and antifungal. We recently reported the structure of garveatin A (**1**),[3] the major active constituent of the crude extracts, and we now report the structures of four related metabolites, garveatins B (**2**) and C (**3**), 2-hydroxygarvin A (**4**), and garvin A quinone (**5**).

G. annulata was collected by hand using SCUBA (−2 to −10 m) on exposed rocky reefs in Barkley Sound, British Columbia. Freshly collected whole specimens were immediately immersed in methanol and allowed to extract for periods of 1–7 days at room temperature. The methanol extract was decanted, filtered through Celite, evaporated in vacuo, suspended in water, and sequentially extracted with hexane, dichloromethane, and ethyl acetate. Filtration silica gel chromatography of the hexane-soluble material using a hexane/ethyl acetate step gradient gave fractions highly enriched in each of the compounds **2**–**5**. Combinations of silica gel preparative TLC and LH20 chromatography were employed on the enriched fractions to obtain pure samples of each of the metabolites.

Garveatin B (**2**) was obtained as a yellow oil that was shown by HRMS (M^+ 326.1526, calcd 326.1519) to have



1 $R_1=R_2=R_3=H$ X = O

2 $R_1=R_2=R_3=H$ X = <H H

7 $R_1=Me$ $R_2=R_3=H$ X = <H H

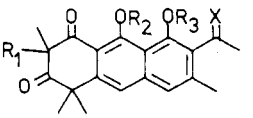
Table II. ^{13}C NMR Data for Garveatins A (1) and C (3) and 2-Hydroxygarvin A (4) (Chemical Shifts in ppm from Me_4Si)

carbon ns.	1[a]	3[b,c]	4[b]
1	200.1	205.8*	199.9
2	103.0	55.3	81.6
3	174.8	211.6	209.9
4	45.7	48.1	46.9
4a	149.3	142.8	142.9&
5	118.2	125.2	116.5
6	138.5	140.0&	157.7
7	120.4	135.0	126.9
8	162.1	155.9	142.1&
8a	106.0	108.8	116.7*
9	170.6	163.7	166.0
9a	114.1	116.3	107.3*
10	109.8	114.7	105.0
10a	136.8	138.0&	142.0
11	7.8	24.6	29.0
11a		24.6	
12	30.2	28.5	28.2‡
13	30.2	28.5	30.6‡
14	21.3	19.5	168.3
15	204.4	204.9*	36.0
16	32.7	32.4	25.3
17			14.7
C6 OMe			55.9
C8 OMe		64.1	
C14 OMe			52.3

[a] Acetone-d_6. [b] $CDCl_3$. [c] *, &, ‡: may be reversed.

a molecular formula of $C_{20}H_{22}O_4$. The ^{1}H NMR of garveatin B immediately revealed that it was very closely related to garveatin A (**1**) (Table I). Resonances at δ 1.62 (s, 6 H), 1.99 (s, 3 H), 2.46 (br s, 3 H), 7.04 (br s, 1 H), and 7.12 (s, 1 H) in the spectrum of **2** were virtually identical with the corresponding resonances for the protons on C11, C12, C13, C14, C5, and C10 in the spectrum of garveatin A (**1**). The appearance of additional resonances at δ 1.18 (t, J = 7 Hz, 3 H) and 2.80 (q, J = 7 Hz, 2 H) combined with the absence of an acetyl methyl resonance in the region of δ 2.70 in the spectrum of **2** indicated that garveatin B (**2**) differed from garveatin A (**1**) only by replacement of the acetyl substituent at C7 in **1** with an ethyl substituent in **2**. The differences in molecular formulas of **1** ($C_{20}H_{20}O_5$) and **2** ($C_{20}H_{22}O_4$), as well as the similarities in the UV spectra of the two metabolites, were consistent with this structural assignment. Two very weak resonances at δ 1.50 (d, J = 7 Hz) and 3.98 (q, J = 7 Hz) in the ^{1}H NMR spectrum of **2** were assigned to the protons on C11 and C2 in the diketo tautomer **6**, which exists in equilibrium with **2** in $CDCl_3$ but not in acetone-d_6. The phenolic protons appear as doubled peaks at δ 9.90, 10.25 (1:3) and 16.23, 17.15 (1:3) in $CDCl_3$, providing more evidence for the existence of the tautomeric equilibrium. Treatment of garveatin B (**2**) with diazomethane gave monomethylgarveatin B (**7**) in high yield.

Garveatin C (**3**), obtained as orange crystals (mp 125 °C), had a molecular formula of $C_{22}H_{24}O_5$ (M^+ 368.1616, calcd 368.1624). The ^{1}H NMR and UV spectra of **3** showed strong similarities to those of garveatin A (**1**), allowing us



3 $R_1=R_3=Me$ $R_2=H$ X = O

6 $R_1=R_2=R_3=H$ X = <H H

8 $R_1=R_3=Me$ $R_2=Ac$ X = O

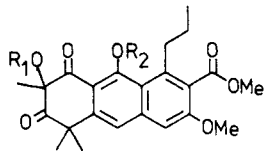
9 $R_1=R_2=R_3=Me$ X = O

(2) Cimino et al. have isolated a highly oxygenated steroid from *Eudendrium sp.* See: Cimino, G.; DeRosa, S.; DeStephano, S.; Sodano, G. *Tetrahedron Lett.* **1980**, *21*, 3303. We are not aware of any other natural products reported from hydroids.

(3) Fahy, E.; Andersen, R. J.; Cun-heng, H.; Clardy, J. *J. Org. Chem.* **1985**, *50*, 1149.

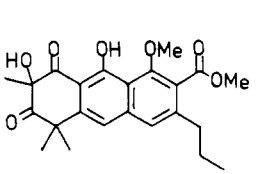
to postulate that garveatin C (**3**) was a dimethyl derivative of **1**. Thus, ^{1}H NMR resonances at δ 2.38 (br s, 3 H), 2.62 (s, 3 H), 1.58 (s, 6 H), 7.19 (s, 1 H), and 7.35 (br s, 1 H) in the spectrum of **3** could be assigned to an aromatic methyl substituent at C6, an acetyl substituent at C7, a *gem*-dimethyl at C4, an aromatic proton at C10, and an aromatic proton at C5 by analogy with the resonances for the corresponding functionalities in **1**. The remaining resonances were assigned to another *gem*-dimethyl functionality [δ 1.49 (s, 6 H)], an aromatic methyl ether [δ 3.92 (s, 3 H)], and a single phenolic proton [δ 14.45 (s, 1 H)]. The downfield shift of the C5 proton in **3**, relative to its position in **1** (δ 7.35 in **3**, 7.10 in **1**), requires that the aromatic methyl ether be attached to C8. Biogenetic reasoning allowed us to place the second *gem*-dimethyl on C2. Hypsochromic shifts in all of the peaks in the UV of **3** relative to those in **1** are consistent with a less extensively conjugated chromophore. The ^{13}C NMR of garveatin C (**3**) shows three ketone carbonyls at δ 211.6, 205.8, and 204.9 and two quarternary sp^3 carbons at δ 48.1 and 55.2 as required by the proposed structure. Acetylation of **3** gave a quantitative yield of monoacetate **8**. Chemical correlation of garveatin A (**1**) and garveatin C (**3**) was achieved by converting both compounds to the dimethoxy derivative **9**.

2-Hydroxygarvin A (**4**), obtained as optically active yellow needles [mp 195 °C; $[\alpha]_D$ +5.5° (*c* 1.2, $CHCl_3$)] from diethyl ether, had a molecular formula of $C_{23}H_{26}O_7$ (M$^+$



4 $R_1 = R_2 = H$

10 $R_1 = R_2 = Ac$



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414.1677; calcd 414.1679). The UV spectrum (λ_{max} 226, 278, 318, 332, 385 nm) and diagnostic resonances at δ 6.98 (s, 1 H) and 7.21 (s, 1 H) in the ^{1}H NMR spectrum of **4** (Table I) suggested that 2-hydroxygarvin A contained the 1-anthracenone skeleton common to garveatins A–C. The nature of the substituents attached to the ring system could be deduced from the results of a combination of NMR experiments and chemical derivatizations.

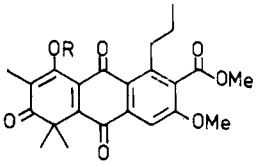
2-Hydroxygarvin A readily formed a diacetate **10** that showed new ^{1}H NMR resonances at δ 2.25 (s, 3 H) and 2.48 (s, 3 H) which could be assigned to one aliphatic and one phenolic acetate. An absence of carbinol methine or methylene protons in the ^{1}H NMR spectrum of underivatized **4** required that the aliphatic alcohol be tertiary. A ^{13}C NMR resonance at δ 81.6 (s) in the spectrum of **4** supports this assignment. The chemical shift of the phenolic proton resonance in **4** (i.e., δ 14.25) implied that it was intramolecularly hydrogen bonded and the downfield shift of the aromatic singlet at δ 7.21 on acetylation required that this aromatic proton be ortho or para to the phenol.

A series of ^{1}H NMR resonances at δ 1.04 (t, J = 7 Hz, 3 H), 1.73 (m, 2 H), and 3.15 (m, 2 H), could be shown by decoupling to belong to an *n*-propyl substituent attached to an aromatic ring. Methyl resonances at δ 3.98 (s, 3 H) and 3.99 (s, 3 H) could be assigned to one aromatic methyl ether [^{13}C NMR, δ 55.9 (q)] and one methyl ester [^{13}C NMR, δ 52.3 (q), 168.3 (s)]. Further support for the methyl ester came from a prominent mass spectral fragment at *m*/*z* 381 (M$^+$ − 31). The remaining ^{1}H NMR resonances [δ 1.52, 1.66, 1.81 (all s, 3 H)] could be attributed to three aliphatic methyls. A gated decoupled ^{13}C NMR spectrum of **4** contained two quartets of quartets at δ 28.2 and 30.6, indicating that two of the aliphatic methyls (δ 1.52 and 1.81) were attached to the same carbon. The third aliphatic methyl (δ 1.66) was assumed to be attached to the carbon bearing the tertiary alcohol. A carbonyl resonance at δ 209.9 in the ^{13}C NMR required that the remaining oxygen atom in **4** be present as a ketone.

A combination of biogenetic arguments and NMR experiments allowed us to place the substituents on the anthracenone nucleus. Irradiation of the aromatic singlet at δ 7.21 produced NOE's in the other aromatic singlet at δ 6.98 and in the two methyl resonances at δ 1.52 and 1.81. This observation is consistent with the placement of the *gem*-dimethyl at C4, the phenolic OH at C9, and aromatic protons at C10 and C5. Irradiation of the methyl resonance at δ 1.52 generated NOE's in the methyl resonance at δ 1.81 and in the aromatic resonance at δ 7.21. Similarly, irradiation of the methyl resonance at δ 1.81 generated NOE's in the methyl resonance at δ 1.52 and in the aromatic resonance at δ 7.21. Neither of the above irradiations induced an observable NOE in the methyl resonance at δ 1.66. We placed the ketone at C3 and aliphatic methyl and tertiary alcohol substituents at C2 based on the NOE results and the requirements of a polyketide biosynthesis.

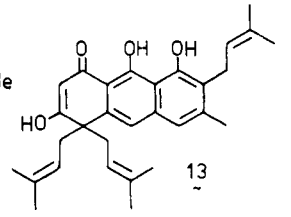
The remaining *n*-propyl, methyl ester, and methyl ether substituents had to be attached to carbons C6–C8. There are only two arrangements of the substituents that are consistent with straightforward polyketide biogenesis. These are represented by structures **4** and **11**. Irradiation of the aromatic proton at δ 6.98 (H5) induced a NOE in the methyl resonance at δ 3.98, allowing us to propose that **4** is the correct structure for 2-hydroxygarvin A. Support for this proposed structure comes from its similarity to the structure of garvin A quinone (**5**), which was solved by X-ray diffraction analysis.

Garvin A quinone (**5**), a very minor component of the *G. annulata* extracts, was obtained as an orange-red



5 R = H

12 R = Ac



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amorphous solid. Mass spectrometry indicated that it had a molecular formula of $C_{23}H_{24}O_7$ (M$^+$ 412.1518; calcd 412.1522), and ^{1}H NMR analysis (see Table I) revealed that it contained the *n*-propyl, methyl ester and methyl ether substituents present in 2-hydroxygarvin A (**4**). Acetylation of garvin A quinone (**5**) produced a crystalline monoacetate **12**. The structure of **12** was solved via single-crystal X-ray diffraction analysis.

A computer-generated perspective drawing of garvin A quinone acetate (**12**) is given in Figure 1. The molecule is achiral, and all hydrogens are shown. In general, interatomic distances and angles agree well with accepted values, and there are few surprises in the molecular parameters. There is a pronounced nonplanarity of the quinone ring, which is in a boatlike conformation. If the plane of the ring is defined by atoms C4a, C9a, C8a, and C10a; then C9a is 0.30 Å above this plane. The distances for the other atoms as follows: O8, 0.80 Å; C10, 0.20 Å; O4, 0.40 Å. The O1–O8 distance is 3.20 Å, and the C2–C1–O2–C17 torsional angle is 118°. The propyl sidechain attached to C8 is in the extended conformation.

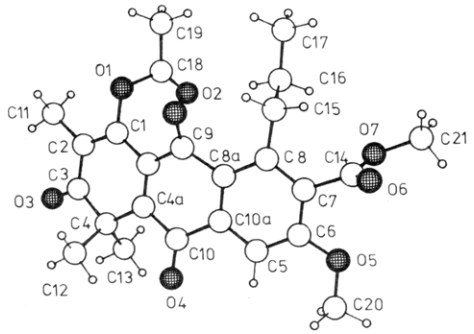
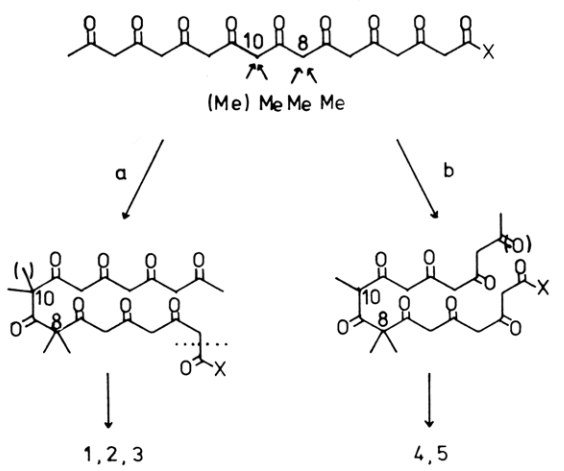


Figure 1. Computer-generated perspective drawing of garvin A quinone acetate. Hydrogens are omitted for clarity.

Scheme I



The garveatins and the garvins are classical examples of polyketide derived metabolites. It is possible that they are all assembled from the same nonaketide precursor that has been dimethylated at C8 and either mono- or dimethylated at C10 (see Scheme I). Folding pattern a can lead to garveatins A (**1**), B (**2**), and C (**3**), while a second folding pattern b can generate 2-hydroxygarvin A (**4**) and garvin A quinone (**5**). The *G. annulata* metabolites are closely related to a series of pigments isolated from the red berries of a group of small tropical trees, belonging to the genus *Vismia*, that grow in Central and South America.[4] Ferruginin A (**13**) is a typical example of the *Vismia* pigments.

Condensed polycyclic aromatic systems of polyketide origin are somethine of a rarity in the chemistry of marine organisms. A few naphthaquinone and anthraquinone pigments have been isolated from echinoderms,[5] and a benz[*a*]anthraquinone antibiotic has been isolated from a marine fungus.[6] The garveatins and garvins represent the first examples of this type of metabolism from coelenterates, which with the exception of the prostaglandins found in gorgonians tend to elaborate terpenoid chemistry.[1]

Experimental Section

^{1}H NMR spectra were recorded on Bruker WP-80, Nicolet-Oxford 270, and Bruker WH-400 spectrometers. ^{13}C NMR spectra were recorded on Bruker WP-80 and WH-400 spectrometers. Me_4Si was used as an internal standard. Low-resolution mass spectra were recorded on an AEI MS902 spectrometer, and high-resolution mass spectra were recorded on an AEI MS50 spectrometer. IR spectra were recorded on a Perkin-Elmer Model 710B spectrometer and a BOMEM Fourier transform spectrometer. UV–visible spectra were recorded on a Bausch & Lomb Spectronic-2000 instrument. Melting points were obtained on a Fisher-Johns apparatus, and values are uncorrected. Merck Silica gel, 230–400 mesh, was used for flash and preparative thin-layer chromatography, and a Whatman Magnum-9 Partisil-10 column was used for preparative HPLC. Sephadex LH-20 was used for molecular exclusion chromatography. R_f values are listed for all compounds in an analytical silica gel TLC system using a 1:49:49 acetic acid/ethyl acetate/hexane eluent.

G. annulata was collected by hand using SCUBA (−2 to −15 m) on exposed rocky reefs in Barkley Sound, British Columbia. Freshly collected whole specimens were immediately placed in methanol and stored at room temperature. The methanol extract was decanted and filtered through Celite. The filtrate was evaporated in vacuo to give an aqueous suspension that was diluted with distilled water to 400 mL and extracted successively with hexane (3 × 400 mL), methylene chloride (3 × 400 mL), and ethyl acetate (2 × 400 mL).

The hexane (600 mg) and methylene chloride (4 g) extracts were fractionated separately by step gradient flash chromatography using a 3.5-cm silica pad in a sintered-glass funnel (10-cm diameter). Fractions eluting with the same solvent from each separation were combined. Elution with 20% ethyl acetate/hexane, 50% ethyl acetate/hexane, and 100% ethyl acetate gave fractions A (140 mg), B (500 mg), and C (1.5 g), respectively. These fractions were further purified as described below to give pure garveatins and garvins.

Purification of Garveatin B (2) and Garvin A Quinone (5). Fraction C was subjected to LH-20 chromatography in 9:1 MeOH/CH_2Cl_2 and preparatory TLC (50% ethyl acetate/hexane) to give partially purified garveatin B and garvin A quinone. Final purification via HPLC (silica gel, ethyl acetate/hexane gradient) afforded pure garveatin B (**2**) (15 mg, R_f 0.48) and garvin A quinone (**5**) (10 mg, R_f 0.50).

Purification of Garveatin C (3). Fraction A was subjected to LH-20 chromatography in 9:1 methanol/CH_2Cl_2 followed by preparative TLC (40% ethyl acetate/hexane) to give garveatin C (**3**) (20 mg, R_f 0.54).

Purification of 2-Hydroxygarvin A (4). Fraction B was subjected to LH-20 chromatography in 9:1 methanol/CH_2Cl_2 followed by preparative TLC (10% ethyl acetate/$CHCl_3$) to give 2-hydroxygarvin A (**4**) (100 mg, R_f 0.31).

An ethanol extract of *G. annulata* yielded 2-hydroxygarvin A (**4**) containing the methyl ester and methyl ether functionalities that were observed in the methanol-extracted material.

Garveatin B (2): yellow oil; UV (MeOH) 240 nm, 260, 317, 417; IR ($CHCl_3$) 2960, 1710, 1625, 1600, 1430 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 3 H), 1.62 (s, 3 H), 1.99 (s, 3 H), 2.46 (brs, 3 H), 2.80 (q, J = 7 Hz, 2 H), 7.04 (brs, 1 H), 7.12 (s, 1 H), 10.25 (s, 1 H), 17.15 (s, 1 H), [other tautomer: δ 1.50 (d, J = 7 Hz, 3 H), 3.98 (q, J = 7 Hz, 1 H), 9.90 (s, 1 H), 16.23 (s, 1 H)]; ^{1}H NMR (acetone-d_6) δ 1.15 (t, J = 7 Hz, 3 H), 1.65 (s, 6 H), 1.98 (s, 3 H), 2.43 (s, 3 H), 2.78 (q, J = 7 Hz, 2 H), 7.10 (s, 1 H), 7.31 (s, 1 H); HRMS, obsd m/z 326.1526, $C_{20}H_{22}O_4$ calcd 326.1514.

Methyl Garveatin B (7). Garveatin B (**2**) (5 mg) in diethyl ether was treated with excess diazomethane for 2 h. Preparative TLC chromatography yielded methyl garveatin B (**7**) (3 mg, R_f 0.54): orange oil; UV (MeOH) 210 nm (12 400), 238 (16 100), 265 (9300), 287 (8500), 320 sh (3900), 426 (3800); ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 3 H), 1.55 (s, 6 H), 2.05 (s, 3 H), 2.44 (br s, 3 H), 2.80 (q, J = 7 Hz, 2 H), 4.00 (s, 3 H), 7.03 (br s, 1 H), 7.10 (s, 1 H), 10.19 (s, 1 H), 17.08 (s, 1 H); LRMS, M$^+$ m/z 340.

Garveatin C (3): orange crystals (hexane); mp 125 °C; UV (MeOH) 228 nm (13 700), 274 (19 700), 305 sh (3300), 389 (4400); IR ($CHCl_3$) 2930, 1700, 1620, 1455, 1390 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 6 H), 1.58 (s, 6 H), 2.38 (br s, 3 H), 2.62 (s, 3 H), 3.92 (s, 3 H), 7.19 (s, 1 H), 7.35 (br s, 1 H), 14.45 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 211.6 (s), 205.8 (s), 204.9 (s), 163.7 (s), 155.6 (s), 142.8 (s), 140.0 (s), 138.0 (s), 135.0 (s), 125.2 (d), 116.3 (s), 114.7 (d), 108.8 (s), 64.1 (q), 55.3 (s), 48.1 (s), 32.4 (q), 28.5 (q, 2 C), 24.6 (q, 2 C), 19.5 (q); HRMS, observed M$^+$ m/z 368.1616, $C_{22}H_{24}O_5$ calcd 368.1624.

Garveatin C Acetate (8). Garveatin C (3) (5 mg) was added to 250 μL of acetic anhydride and 250 μL of pyridine, and the

(4) (a) Monache, F. D.; Mac-Quhae, M. M.; Bettolo, G. B. M. *Tetrahedron* **1979**, *35*, 2143. (b) Pinheiro, R. M.; Mac-Quhae, M. M.; Bettolo, G. B. M.; Monache, F. D. *Phytochemistry* **1984**, *23*, 1737.
(5) Singh, H.; Moore, R. E.; Scheuer, P. J. *Experientia* **1967**, *23*, 624.
(6) Kitahara, T.; Naganawa, H.; Okazaki, T.; Okami, Y.; Umezawa, J. *J. Antibiotics* **1975**, *28*, 280.

reaction mixture was stirred overnight at room temperature. The reagents were removed in vacuo, and the residue was purified via preparatory silica TLC (50% ethyl acetate/hexane) to give garveatin C acetate (**8**) (4 mg, R_f 0.48): pale yellow oil; UV (MeOH) 224 nm, 257, 295, 350; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 6 H), 1.55 (s, 6 H), 2.37 (br s, 3 H), 2.43 (s, 3 H), 2.57 (s, 3 H), 3.84 (s, 3 H), 7.48 (br s, 1 H), 7.66 (s, 1 H); HRMS, obsd M^+ m/z 410.1735, $C_{24}H_{26}O_6$ calcd 410.1730.

Methyl Garveatin C (9). Garveatin A (**1**) (8 mg) was dissolved in acetone (10 mL) to which K_2CO_3 and methyl iodide (500 μL) had been added. The reaction mixture was refluxed overnight. The residue obtained after filtration and concentration in vacuo was purified by preparative silica gel TLC (50% ethyl acetate/hexane) to give methyl garveatin C (**9**) as the major product (2 mg): pale yellow oil; UV (MeOH) 225 nm, 255, 295 sh, 351; ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 6 H), 1.54 (s, 6 H), 2.38 (br s, 3 H), 2.61 (s, 3 H), 3.84 (s, 3 H), 4.01 (s, 3 H), 7.45 (br s, 1 H), 7.52 (s, 1 H); LRMS, M^+ m/z 382.

Garveatin C (8.8 mg), K_2CO_3 (125 mg), and methyl iodide (500 μL) were added to 10 mL of HPLC-grade acetone. The mixture was refluxed overnight after which it was filtered, and the filtrate was evaporated to dryness. Chromatography as above gave pure **9**, which was identical by TLC, ^{1}H NMR, UV, and mass spectral comparison with the material prepared from garveatin A.

2-Hydroxygarvin A (4): pale yellow needles (diethyl ether); mp 195 °C; UV (MeOH) 226 nm, 278, 318, 332, 385; IR ($CHCl_3$) 3490, 2990, 1720, 1700 sh, 1610, 1380, 1210 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.04 (t, J = 7 Hz, 3 H), 1.52 (s, 3 H), 1.66 (s, 3 H), 1.73 (s, m, 2 H), 1.81 (s, 3 H), 3.15 (m, 2 H), 3.98 (s, 3 H), 3.99 (s, 3 H), 6.98 (s, 1 H), 7.21 (s, 3 H), 14.25 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 209.9 (s), 199.9 (s), 168.3 (s), 166.0 (s), 157.7 (s), 142.9 (s), 142.1 (s), 141.9 (s), 126.9 (s), 116.7 (s), 116.5 (d), 107.3 (s), 105.0 (d), 81.6 (s), 55.9 (q), 52.3 (q), 46.9 (s), 36.0 (t), 30.6 (q), 28.9 (q), 28.2 (q), 25.3 (t), 14.7 (q); HRMS, obsd M^+ m/z 414.1677, $C_{23}H_{26}O_7$ calcd 414.1679.

Diacetate 10. 2-Hydroxygarvin A (**4**) (5 mg) was dissolved in 500 μL of acetic anhydride and 500 μL of pyridine and stirred overnight at room temperature. The reagents were removed in vacuo, and the residue was purified via silica gel preparative TLC (10% ethyl acetate/chloroform) to yield diacetate **10** (4 mg, R_f 0.35): pale yellow oil; UV (MeOH) 226 nm, 268, 327, 356 sh; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, J = 7 Hz, 3 H, 1.50 (s, 3 H), 1.60 (s, 3 H), 1.70 (m, 2 H), 1.83 (s, 3 H), 2.25 (s, 3 H), 2.48 (s, 3 H), 2.94 (m, 2 H), 3.99 (s, 6 H), 7.06 (s, 1 H), 7.73 (s, 1 H); LRMS, M^+ m/z 498.

Garvin A Quinone (5): red oil; UV (MeOH) 216 nm, 276, 384; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7 Hz, 3 H), 1.60 (m, 2 H), 1.61 (s, 6 H), 1.98 (s, 3 H), 2.95 (m, 2 H), 3.98 (s, 3 H), 4.03 (s, 3 H), 7.56 (s, 1 H), 12.15 (s, 1 H); HRMS, obsd M^+ m/z 412.1518, $C_{23}H_{24}O_7$ calcd 412.1522.

Garvin A Quinone Acetate (12). Garvin A quinone (**5**) was dissolved in acetic anhydride (250 μL) and pyridine (250 μL) and stirred overnight at room temperature. The reagents were removed in vacuo and the residue was purified by preparative silica gel TLC (50% ethyl acetate/hexane) to yield acetate **12** (3 mg, R_f 0.55): light yellow needles (acetone); UV (MeOH) 224 nm, 276, 299 sh, 344 sh; ^{1}H NMR ($CDCl_3$) δ 1.02 (t, J = 7 Hz, 3 H), 1.59 (m, 2 H), 1.61 (s, 6 H), 1.94 (s, 3 H), 2.37 (s, 3 H), 2.83 (br s, 2 H), 3.94 (s, 3 H), 3.96 (s, 3 H), 7.48 (s, 1 H); LRMS, M^+ m/z 454.

Single-Crystal X-ray Diffraction Analysis of Garvin A Quinone Acetate (12). Preliminary X-ray photographs displayed only triclinic symmetry. Accurate lattice constants of a = 9.397 (1) Å, b = 11.754 (1) Å, c = 12.301 (1) Å, α = 75.625 (8)°, β = 77.162 (8)°, and γ = 104.745 (8)° were determined from a least-squares fit of moderate 2θ values. An approximate density measurement indicated that 2 molecules of composition $C_{25}H_{26}O_8$ formed the unit cell. The space group could be either $P1$ or $P\bar{1}$. We assumed that the centrosymmetric choice was the correct one based on our earlier work with the garveatins, and the successful solution and refinement fully confirmed this. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using graphite-monochromated Cu K$\bar{\alpha}$ radiation (1.54178 Å) and variable-speed 1° ω scans. Of the 3159 reflections collected in this fashion, 2837 (90%) were judged observed after correction for Lorentz, polarization, and background effects ($F_o \geq 3\sigma(F_o)$). A phasing model was found uneventfully using the MULTAN series of programs.[7] The model was partially refined, and hydrogens were located on a difference synthesis. Block-diagonal least-squares refinements with anisotropic non-hydrogen atoms and fixed isotropic hydrogens have converted to a standard crystallographic residual of 0.0652 for the observed reflections. Additional crystallographic details are available and are described in the paragraph at the end regarding supplementary material.

Antimicrobial Activity of the Garveatins and Garvins. A standard in vitro disk (0.25 in.) bioassay was used to assess the antibacterial and antifungal activity of the *G. annulata* metabolites. Activities are reported as minimum inhibitory concentrations (MIC) in μg/disk. *Staphylococcus aureus*: **2**, 10; **3**, 30; **4**, ≫500; **5**, 3. *Bacillus subtilus*: **2**, 10; **3**, 30; **4**, 190; **5**, 12. *Rhizoctonia solani*: **2**, 80; **3**, 30; **4**, ≫500; **5**, 3. *Pythium ultimum*: **2**, 80; **3**, ≫500; **4**, 190; **5**, ≫500.

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Supplementary Material Available: HETCOR experiment for garveatin A (**1**), partial gated decoupled spectra for 2-hydroxygarvin A (**4**) and tables of fractional coordinates, thermal parameters, interatomic distances, and interatomic angles for garvin A quinone acetate (**12**) (9 pages). Ordering information is given on any current masthead page.

(7) All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were REDUCE and UNIQUE [data reduction programs by M. E. Leonowicz, Cornell University, 1978], MULTAN 78, MULTAN 80, and RANTAN 80 [systems of computer programs for the automatic solution of crystal structures of X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson synthesis) written by P. Main, S. E., Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1978 and 1980], DIRDIF [written by P. T. Beruskens et al., University of Nijmegen, Netherlands, 1981], MITHRIL [an automatic solution package written by C. J. Gilmore, University of Glasgow, Scotland, 1983], BLS78A [an anisotropic block-diagonal least-squares refinement written by K. Hiratsu and E. Arnold, Cornell University, 1980], PLUTO78 [a crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978], and BOND [a program to calculate molecular parameters and prepare tables written by K. Hiratsu, Cornell University, 1978].