Discorhabdins Revisited: Cytotoxic Alkaloids from Southern Australian Marine Sponges of the Genera *Higginsia* and *Spongosorites*†

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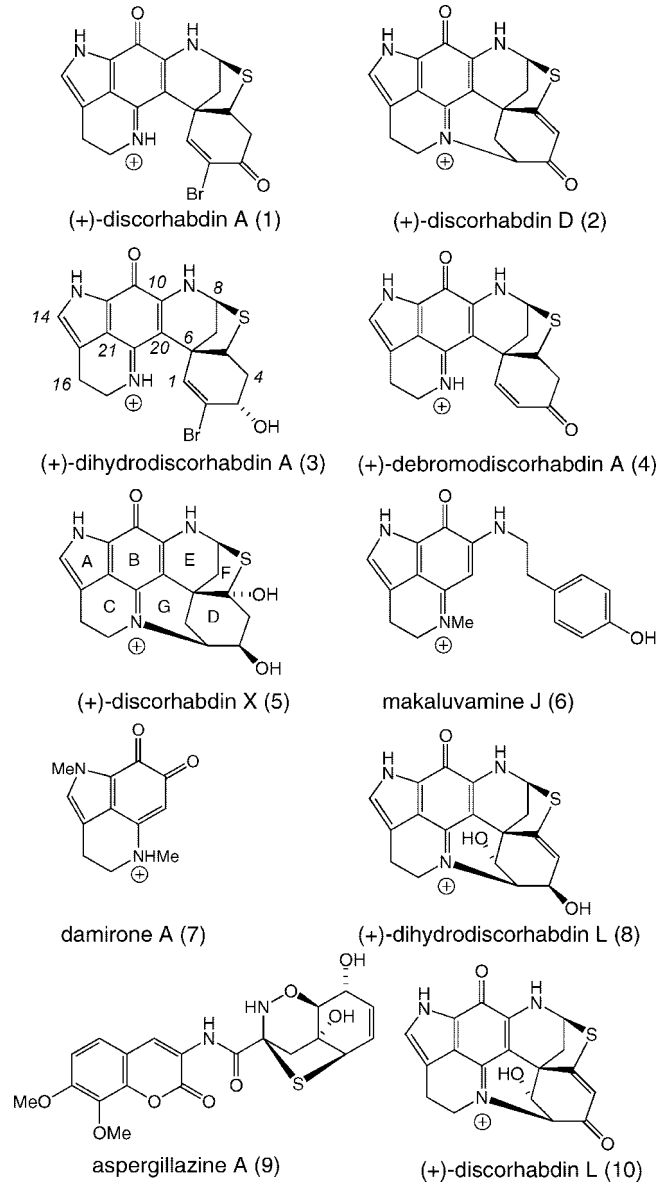
Chemical analysis of southern Australian marine sponges of the genera *Higginsia* and *Spongosorites* has yielded examples of the discorhabdin class of alkaloids. These include the known metabolites (+)-discorhabdin A (**1**), (+)-discorhabdin D (**2**), makaluvamine J (**6**), and damirone A (**7**), together with four new analogues, (+)-dihydrodiscorhabdin A (**3**), (+)-debromodiscorhabdin A (**4**), (+)-dihydrodiscorhabdin L (**8**), and (+)-discorhabdin X (**5**), with the latter compound being the first reported example of a thio heterocycle flanked by oxo-thio-acetal and azo-thio-acetal functionalities. Structures for the new compounds were assigned on the basis of detailed spectroscopic interpretation.

The pyrroloiminoquinone class of marine natural products has attracted considerable attention since discorhabdins A−D were first reported[1−3] from a New Zealand *Latrunculia* sp. more than 20 years ago. In the intervening decades, the array of sponge pyrroloiminoquinones has expanded to include discorhabdins A−W, as well as the related metabolites prianosins, batzellines, isobatzellines, secobazellines, damirones, makaluvamines, epinardins, tsitsikammamines, and veiutamine. Davies-Coleman et al. provided a comprehensive audit of sponge pyrroloiminoquinones in a 2005 review,[4] including commentary on cytotoxic and antibacterial properties. In a more recent 2008 report[5] Copp et al. revealed the first occurrence of *enantio* discorhabdins. Given this latter discovery we have taken the liberty of prefacing known and new discorhabdins with the sign of their rotation, to distinguish enantiomers.

In an ongoing search for cytotoxic metabolites, extracts derived from southern Australian marine sponges of the genera *Higginsia* and *Spongosorites* were examined. Both these extracts displayed significant cytotoxicity against the human colon (HT29), lung (A549), and breast (MDA-MB-231) cancer cell lines. Chemical fractionation of the *Higginsia* extract using a sequence of solvent partitioning and trituration, followed by solid-phase extraction (SPE) and HPLC, yielded the known (+)-discorhabdin A (**1**)[3] and (+)-discorhabdin D (**2**)[2] and the new metabolites (+)-dihydrodiscorhabdin A (**3**), (+)-debromodiscorhabdin A (**4**), and (+)-discorhabdin X (**5**). Comparable fractionation of the *Spongosorites* extract yielded makaluvamine J (**6**)[6] and damirone A (**7**),[7] together with the new metabolite (+)-dihydrodiscorhabdin L (**8**). The alkaloids **1**−**8** were isolated and characterized as their TFA salts. Structures for the known compounds **1** and **2**, and **6** and **7**, were confirmed by spectroscopic analysis and comparison to literature data, with particular attention paid to $[\alpha]_D$ measurements. This latter point warrants further elaboration later in this report. Spectroscopic analysis leading to the structure elucidation of the new metabolites **3**−**5** and **8** is presented below.



Results and Discussion

The positive ion HRESIMS for (+)-dihydrodiscorhabdin A (**3**) exhibited a *m*/*z* ion consistent with the formula $C_{18}H_{17}BrN_3O_2S$ (Δmmu −0.1) and suggestive of a dihydro analogue of the co-metabolite (+)-discorhabdin A (**1**). Supportive of this analysis, the ^{1}H NMR (MeOH-d_4) spectrum for **3** displayed a strong similarity to that for **1**, with the only significant difference being shielding of H-1 (δ_H 6.47), H-4a (δ_H 2.17), and H-4b (δ_H 2.00) and the appearance of a H-3 hydroxy methine (δ_H 4.32), consistent with reduction of the C-3 ketone to a secondary alcohol. The ^{13}C NMR (MeOH-d_4) spectrum for **3** confirmed this interpretation, with

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Table 1. NMR (600 MHz, MeOH-d_4) Data for (+)-Dihydrodiscorhabdin A (**3**)[a]

atom no.	δ_C	δ_H mult. (*J* Hz)	COSY	HMBC (^{1}H−^{13}C)
1	134.3, CH	6.47, s		2, 3, 5, 6, 20
2	128.2, C			
3	71.6, CH	4.32, dd (2.8, 2.8)	4a, 4b	1, 2, 5
4a	39.9, CH_2	2.17, ddd (2.8, 13.6, 13.6)	3, 4b, 5	5, 6
4b		2.00, ddd (2.8, 4.9, 13.6)	3, 4b, 5	5, 6
5	50.6, CH	4.34, dd (13.6, 4.9)	4a, 4b	7, 20
6	50.9, C			
7a	42.3, CH_2	2.60, dd (12.3, 3.6)	7b, 8	5, 6, 20
7b		2.46, brd (12.3)	7a, 8	5, 6
8	60.5, CH	5.20, dd (3.6, 1.1)	7a, 7b	10, 5, 6
10	153.0, C			
11	167.3, C			
12	125.3, C			
14	127.6, CH	7.17, s		12, 15, 21
15	121.1, C			
16	19.6, CH_2	2.96, m	17a, 17b	15, 17
17a	45.2, CH_2	3.95, m	16	15, 16, 19
17b		3.87, m	16	15, 16, 19
19	156.2, C			
20	105.2, C			
21	124.4, C			

[a] Assignments supported by HSQC.

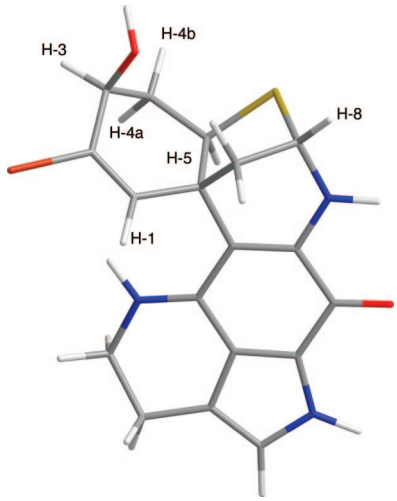

Figure 1. Chem3D minimized model of (+)-dihydrodiscorhabdin A (**3**).

replacement of the C-3 ketone resonance common to **1** (δ_C 168.6)[3] with a C-3 oxymethine carbon in **3** (δ_C 71.6). Full analysis of the 1D and 2D NMR (MeOH-d_4) spectra (Table 1) confirmed **3** as the reduced analogue as shown. Assignment of relative stereochemistry was achieved by consideration of molecular modeling (Figure 1), which indicated a pseudo-equatorial H-3 (expt $J_{3,4a}$ 2.8 Hz and $J_{3,4b}$ 2.8 Hz, calcd $J_{3,4a}$ (53°) 2.8 Hz and $J_{3,4b}$ (60°) 1.8 Hz), and biosynthetic considerations, which suggested a pseudo-axial H-5 in common with the co-metabolite and *putative* biosynthetic precursor **1**. Given its co-occurrence with (+)-discorhabdin A (**1**), on biogenetic grounds we attribute a common absolute stereochemistry to (+)-dihydrodiscorhabdin A (**3**) and propose the complete structure as indicated.

The positive ion HRESIMS for (+)-debromodiscorhabdin A (**4**) exhibited a *m/z* ion consistent with the formula $C_{18}H_{16}N_3O_2S$ (Δmmu −0.3) and suggestive of a debromo analogue of discorhabdin A (**1**). Supportive of this analysis, the ^{1}H NMR (MeOH-d_4) spectrum for **4** displayed strong similarity to that for the co-metabolite **1**, with the only significant difference being the appearance of H-2 (δ_H 6.30, d, J = 10.2 Hz) coupled to H-1 (δ_H 7.11, d, J = 10.2 Hz). Again, given its co-occurrence with (+)-discorhabdin A (**1**), on biogenetic grounds we attribute a common absolute stereochemistry to (+)-debromodiscorhabdin A (**4**) and propose the complete structure as indicated.

Table 2. NMR (600 MHz, MeOH-d_4) Data for (+)-Discorhabdin X (**5**)[a]

position	δ_C	δ_H mult (*J* Hz)	COSY	HMBC (^{1}H−^{13}C)
1a	30.9, CH_2	2.56, dd (13.1, 2.5)	1b, 2	6, 20
1b		2.02, dd (13.1, 3.3)	1a, 2	6, 20
2	63.9, CH	4.10, m[b]	1a, 1b, 3	
3	69.4, CH	4.29, m[c]	2, 4a, 4b	
4a	39.7, CH_2	2.37, dd (13.1, 5.1)	3, 4b	2, 3, 5, 6
4b		2.24, dd (13.1, 11.1)	3, 4a	3, 5
5	99.1, C			
6	47.9, C			
7a	38.65, CH_2	2.87, dd (11.9, 4.0)	7b, 8	6, 20
7b		2.05, d (11.9)	7a, 8	6, 8, 20
8	60.8, CH	5.29, d (4.0)	7a, 7b	5, 6, 10
10	147.8, C			
11	168.5, C			
12	125.7, C			
14	126.8, CH	7.11, s		12, 15, 21
15	119.7, C			
16a	20.5, CH_2	3.12, m	16b, 17a, 17b	15, 17, 21
16b		3.05, m	16a, 17a, 17b	15, 17, 21
17a	54.9, CH_2	4.24, m[c]	16a, 16b, 17b	15, 16, 19
17b		4.07, m[b]	16a, 16b, 17a	15, 16, 19
19	154.3, C			
20	103.7, C			
21	123.5, C			

[a] Assignments supported by HSQC experiment. [b] Signals are partially obscured (overlapping). [c] Signals are partially obscured (overlapping).

The positive ion HRESIMS for (+)-discorhabdin X (**5**) exhibited a *m/z* ion consistent with the formula $C_{18}H_{18}N_3O_3S$ (Δmmu 0.3), which as a *putative* ammonium species required 12 double-bond equivalents (DBE). The NMR (MeOH-d_4) data for **5** (Table 2) displayed resonances consistent with the common C-6 to C-20 discorhabdin core skeleton. Particularly diagnostic were resonances for H-14 (δ_H 7.11) and the mutually coupled deshielded diastereotopic methylenes H_2-16 (δ_H 3.05, m; 3.12, m) and H_2-17 (δ_H 4.07, m; 4.24, m), with the latter chemical shifts being consistent with discorhabdins featuring N-18 to C-2 heterocycles. For example, published ^{1}H NMR (MeOH-d_4) data for discorhabdin D (**2**)[2] and discorhabdin L (**10**)[8] (which feature an N-18 to C-2 heterocycle) document chemical shifts for H_2-16 and H_2-17 of δ_H 3.05−3.20 and 3.90−4.00, respectively, whereas comparable resonances for discorhabdin I[8] and discorhabdin B[3] (which have a common C-6 to C-20 core but lack an N-18 to C-2 heterocycle) are less deshielded at δ_H 2.87−2.92 and 3.82−3.94, respectively. Our analysis of the available literature confirms that these empirical observations for H_2-16 and H_2-17 chemical shifts hold true for all relevant sponge pyrroloiminoquinones. Furthermore, NMR (MeOH-d_4) resonances attributed to H-8 (δ_H 5.29) and C-8 (δ_C 60.8) are diagnostic of the C-5 to C-8 thioether functionality common to many discorhabdins [cf. **10**, H-8 (δ_H 5.58) and C-8 (δ_C 63.7)].[8] The observations outlined above account for all sp^2 centers and DBE and require that **5** incorporate a carbon and heterocyclic skeleton common to the co-metabolite discorhabdin D (**2**). Unlike **2**, **5** does not possess a ring D α,β-unsaturated ketone (for ring numbering, see the structure diagram for **5**). Analysis of the 2D COSY NMR (MeOH-d_4) data for **5** (Table 2) revealed a sequence of correlations from a diastereotopic methylene (H_2-1, δ_H 2.02 and 2.56) to a deshielded methine (H-2, δ_H 4.10), to a second deshielded methine (H-3, δ_H 4.29), and finally to a second diastereotopic methylene (H_2-4, δ_H 2.24 and 2.37). The H_2-1 methylene also displayed HMBC NMR (MeOH-d_4) correlations to C-6 (δ_C 47.9) and C-20 (δ_C 103.7), while H_2-4 displayed correlations to C-6 and a significantly deshielded sp^3-hybridized C-5 (δ_C 99.1). As C-5 in **5** is substituted by a thioether (cf. ring F), the observed deshielding is suggestive of further substitution by an OH-5 moiety, to form the oxo-thioacetal, as indicated. The analysis presented above also necessitates

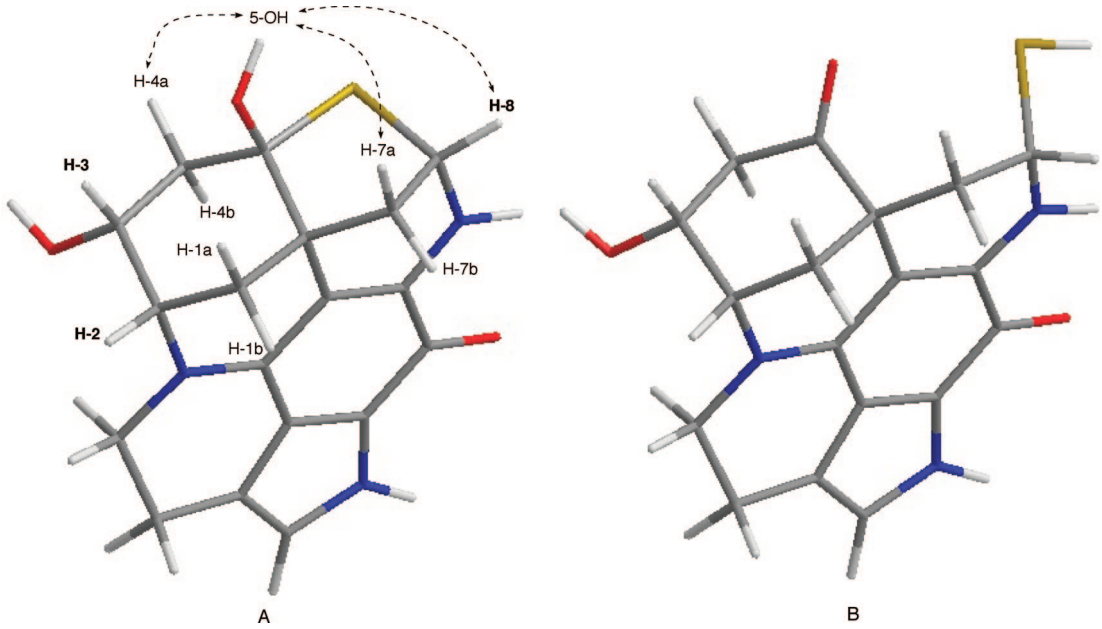

Figure 2. Chem3D minimized model of (A) discorhabdin X (**5**) (thio-acetal form), with key ROESY NMR (DMSO-d_6) correlations (dashed lines), and (B) a hypothetical thio-keto form.

an OH-3 moiety, completing the planar structure of **5** as shown. Supportive of this interpretation, the NMR (DMSO-d_6) data for **5** (Supporting Information) revealed an OH-5 resonance (δ_H 6.61, s) with HMBC correlations to C-4 (δ_C 38.3), C-5 (δ_C 98.3), and C-6 (δ_C 46.1) and ROESY correlations to H-4a (δ_H 2.29), H-7a (δ_H 2.77), and H-8 (δ_H 5.25), placing OH-5, H-4a, H-7a, and H-8 on a common face of the molecule (Figure 2). The relative stereochemistry was further defined by consideration of the rigid heterocyclic framework and diagnostic ^{1}H NMR (MeOH-d_4) couplings, which suggested a pseudo-axial H-3 (expt $J_{3,4a}$ 5.1 Hz and $J_{3,4b}$ 11.1 Hz, calcd $J_{3,4a}$ (46°) 3.7 Hz and $J_{3,4b}$ (163°) 8.4 Hz) and a pseudo-equatorial H-2 (expt $J_{1a,2}$ 2.5 Hz, $J_{1b,2}$ 3.3 Hz, calcd $J_{1a,2}$ (55°) 2.7 Hz, $J_{1b,2}$ (61°) 1.7 Hz) (Table 2 and Figure 2). Given its co-occurrence with (+)-discorhabdin A (**1**), on biogenetic grounds we attribute a common absolute stereochemistry to (+)-discorhabdin X (**5**) and propose the complete structure as indicated.

Discorhabdin X (**5**) represents the first example of a natural pyrroloiminoquinone incorporating a remarkable ring F heterocycle, in which the sulfur atom is simultaneously engaged in both an oxo-thio-acetal (about C-5) and an azo-thio-acetal (about C-8). To the best of our knowledge, such heterocycles are unprecedented, and in proposing such a functionality it is prudent to consider why **5** does not exist in a corresponding ring F opened thio-keto form (see Figure 2). The hypothetical thio-keto form as modeled in Figure 2 suggests a pseudo-1,4-diaxial relationship between the C-5 carbonyl and the SH-8 moiety, well positioned for nucleophilic addition and ring closure to the oxo-thio-acetal, although the spectroscopic data for **5** lack any indication of equilibration to the thio-keto form, being solely consistent with the ring-closed thio-acetal form. In an earlier investigation, Capon et al. encountered a related system in the fungal metabolite, aspergillazine A (**9**),[9] in which a thiol moiety embedded in a rigid heterocyclic skeleton was 1,4-proximal to an oxime functionality. As with **5**, **9** existed exclusively as the azo-thio-acetal heterocycle, as shown. Insofar as the rigid heterocyclic skeletons of both **9** and **5** position a thiol moiety adjacent to a receptive sp^2 center (oxime and ketone, respectively), common chemical principles may drive the thermodynamic bias for ring closure. This observation may provide insights into possible biomimetic strategies for the formation of ring F in **5**, through thio-keto intermediates. In our hands, **5** displayed modest stability, but decomposed on long-term (6 month) storage in DMSO at <−20 °C.

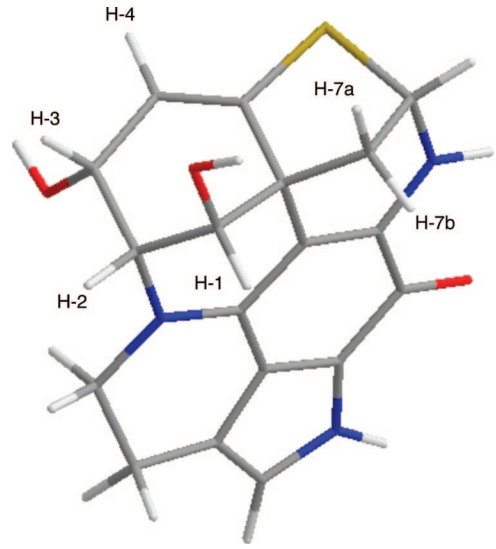

Figure 3. Chem3D minimized model of (+)-dihydrodiscorhabdin L (**8**).

The positive ion HRESIMS for (+)-dihydrodiscorhabdin L (**8**) exhibited a *m/z* ion consistent with the formula $C_{18}H_{16}N_3O_3S$ (Δmmu 0.2) and possessed NMR (MeOH-d_4) resonances suggestive of a dihydro analogue of (+)-discorhabdin L (**10**). More specifically, the NMR data included resonances for the C-6 to C-20 discorhabdin core skeleton with diagnostic resonances for H-14 (δ_H 7.08), as well as H-8 (δ_H 5.36) and C-8 (δ_C 62.0), and the mutually coupled deshielded diastereotopic methylenes, H_2-16 (δ_H 3.08, m) and H_2-17 (δ_H 4.07, m; 4.36, m), all consistent with the presence of rings A−G (see above). Principal differences in the NMR (MeOH-d_4) data for **10** and **8** were attributed to reduction of the C-3 ketone (δ_C 184.9)[8] to a OH-2° (δ_H 4.96; δ_C 67.4), an interpretation further supported by COSY and HMBC correlations that defined the ring D substitution pattern (less stereochemistry) as shown. Steric considerations defined the relative stereochemistry about C-2 and C-8 heteroatom substituents and the heterocyclic carbon skeleton in **8** (Figure 3). The ^{1}H NMR (MeOH-d_4) data for discorhabdin X (**5**), which possesses a common heterocyclic skeleton to **8**, reveals H_2-7 as a diastereotopic methylene with the pseudo-axial H-7a (δ_H

2.87) being more deshielded than H-7b (δ_H 2.05) and featuring a coupling to H-8 ($J_{7a,8}$ 4.0 Hz). This pseudo-axial conformation of H-7a in **8** was confirmed by ROESY correlations (Figure 2) and represents a multiplicity and relative chemical shift common to (+)-dihydrodiscorhabdin A (**3**) (Table 1) and (+)-discorhabdin L (**10**).[8] A long-range (coplanar) COSY NMR (MeOH-d_4) correlation to H-4 (δ_H 6.12) and a strong NOE difference response on irradiation of H-1 confirmed a comparable pseudo-equatorial H-7b stereochemistry for **10**.[8] These stereochemical observations with respect to **3**, **5**, and **10** can be used to define the C-1 stereochemistry in **8**. Specifically, a COSY NMR (MeOH-d_4) correlation between H-4 and H-7b and a ROESY correlation between H-1 and H-7b (but not H-7a) in **8** can be interpreted as positioning both H-1 and H-7b in pseudo-equatorial conformations, common with that previously assigned to (+)-discorhabdin L (**10**).[8] Furthermore, comparison of the experimental value for $J_{2,3}$ (4.5 Hz) with that predicted for a pseudo-axial H-3 (~(31°) 5.9 Hz) and a pseudo-equatorial H-3 (~(78°) <1 Hz) conformation permitted assignment of the C-2 to C-3 relative stereostructure for **8** as shown. The absolute configuration of **8** remains unassigned at this time.

The discorhabdins are typically green in color and have UV−vis absorbance that encroaches on the sodium D line (589 nm), a characteristic that is significant when measuring optical rotations. For example, although discorhabdins typically exhibit large $[\alpha]_D$ measurements, some discorhabdins have been reported[2,8] with $[\alpha]_D$ values of zero. While this could indicate a racemic mixture, a more likely explanation is that the concentration of the sample used to acquire the optical rotation was too high. For example, whereas $[\alpha]_D$ measurements have been reported for discorhabdins A,[3] B,[3] G,[10] Q,[11] R,[12] W,[13] and I[8] at concentrations ranging from 0.01 to 0.17 g/100 mL, by contrast discorhabdins D[2] and L[8] returned zero measurements at 0.15 and 0.1 g/100 mL, respectively. Significantly, the $[\alpha]_D$ for discorhabdin D (as prianosin D) measured at 0.01 g/100 mL was reported to be +344.[14] These observations suggest that in acquiring $[\alpha]_D$ measurements discorhabdins featuring a ring G may be particularly susceptible to the choice of concentration. To illustrate this phenomena, we acquired the $[\alpha]_D$ for (+)-discorhabdin D (**2**) in MeOH over a range of concentrations, 0.0008 to 0.04 g/100 mL, recording a zero reading for concentrations > 0.01 g/100 mL and over +400 for concentrations < 0.008 g/100 mL.

The new discorhabdins **3**, **4**, **5**, and **8** described in this report add to our knowledge of the biosynthetic diversity within the discorhabdin family of marine natural products, with discorhabdin X (**5**) being particular noteworthy for its unprecedented heterocyclic system. We attribute the cytotoxic properties of the *Higginsia* and *Spongosorites* extracts to their pyrroloiminoquinone metabolites, with damirone A (**7**) and makaluvamine J (**6**) being the least active (IC_{50} 5−10 μg/mL), followed by (+)-dihydrodiscorhabdin A (**3**) and (+)-dihydrodiscorhabdin L (**8**) (IC_{50} 0.1−0.5 μg/mL), and with (+)-discorhabdin A (**1**) being the most cytotoxic (IC_{50} 0.05−0.1 μg/mL). Lack of material prevented biological testing of **4** and **5**.

Experimental Section

General Experimental Procedures. See ref 15.

Animal Material. The sponge sample (UQ code: CMB-02720) was collected in 1998 at Deal Island (147°21.13′ E, 39°29.3′ S) at a depth of 8−12 m by scuba diving. The sponge sample (UQ code: CMB-02523) was collected in 1998 at Port Campbell (142°49.82′ E, 38°38.02′ S) at a depth of 15−20 m by scuba diving. Freshly collected sponge samples were frozen (−4 °C) for shipping to the laboratory, where they were thawed, catalogued, diced, and steeped in aqueous EtOH at −30 °C for prolonged storage.

Taxonomy. Specimen CMB-02720 was described as follows: Growth form is massive, lobate, liver-like; color in life dark green-black, typical of *Latrunculia*, color in EtOH black-brown; texture firm, compressible, rubbery; oscules not seen; surface opaque, glossy, optically smooth with occasional tubercular processes; spicules megascleres oxeas fusiform, often bent, centrangulate, styloid or deformed (180−320 × 3−5 μm), microscleres discorhabds (25−30 μm), raphides (25 μm); ectosome a continuous palisade of smaller oxeas barely protruding the surface collagen, microrhabds not seen; choanosome a halichondroid plumo-reticulate arrangement of bundles of oxeas, some forming larger tracts in a densely collagenous and pigmented matrix, discorhabds lining the aquiferous system, larvae observed scattered throughout. This specimen was identified as class Demospongiae, order Halichondrida, family Desmoxyidae, genus *Higginsia* (*Higginsia*), and a voucher sample was deposited with Museum Victoria (Reg No. MVF157473).

Specimen CMB-02523 was described as follows: Growth form massive, irregular; color in life dark brown with an aerophobic color change after collection, color in EtOH dark purple; texture firm, compressible, harsh, difficult to tear; oscules not seen; surface opaque, porous, irregular, lumpy; spicules megascleres strongyles (270−400 × 3−5 μm) occasionally deformed ends, microscleres none; ectosome a dense crust-like layer 250−1000 μm thick of packed strongyles; choanosome a mass of strongyles not as densely packed as in the ectosome with occasional thick (150 μm) tracts of strongyles ascending to the surface, collagen dense and darkly pigmented. This specimen was identified as class Demospongiae, order Halichondrida, family Halichondriidae, genus *Spongosorites*, and a voucher sample was deposited with Museum Victoria (Reg No. MVF157472).

Bioassay. The in vitro cytotoxicity screening methodology carried out on lung (A549), colorectal (HT29), and breast (MDA-MB-231) cancer cell lines has been described.[16]

Extraction and Isolation. The aqueous EtOH extract of specimen CMB-02720 (genus *Higginsia*) was decanted and concentrated in vacuo, and the residue (2.60 g) partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble fraction was concentrated in vacuo (1.08 g) and the residue further triturated into light petroleum (1.1 mg), CH_2Cl_2 (1.2 mg), MeOH (1077 mg), and water (1520 mg) solubles. The MeOH solubles were subjected to HPLC fractionation (a 5 μm Zorbax SB Phenyl 250 × 9.4 mm column, with a 4 mL/min gradient elution, 95−70% H_2O/MeCN with a constant 0.01% TFA modifier), over 20 min, to yield four fractions. HPLC resolution of fraction 3 (11.4 mg) (a 5 μm Zorbax SB-C_{18} 250 × 9.4 mm column, with a 4 mL/min isocratic elution, 65% H_2O/MeOH + 0.01% TFA over 17 min) yielded the known (+)-discorhabdin A (**1**) (7.1 mg, 0.65%) together with the new analogue (+)-dihydrodiscorhabdin A (**3**) (3.7 mg, 0.34%). HPLC resolution of fraction 2 (12.9 mg) by HPLC (a 5 μm Zorbax SB-C_{18} 250 × 9.4 mm column, with a 4 mL/min isocratic elution, 80% H_2O/MeOH + 0.01% TFA, as well as a 5 μm Zorbax SB Phenyl 250 × 9.4 mm column, with a 4 mL/min isocratic elution, 85% H_2O/MeOH + 0.01% TFA) afforded (+)-discorhabdin D (**2**) (1.5 mg, 0.13%), (+)-debromodiscorhabdin A (**4**) (1.8 mg, 0.16%), and (+)-discorhabdin X (**5**) (3.3 mg, 0.30%). Fractions 1 and 4 did not resolve into pure compounds. (Note: % yields are expressed as a mass to mass % compared to the weight of the *n*-BuOH solubles).

The aqueous EtOH extract of specimen CMB-02523 (genus *Spongosorites*) was decanted and concentrated in vacuo, and the residue (2.13 g) partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble fraction was concentrated in vacuo (0.19 g) and the residue further triturated into light petroleum (82 mg), CH_2Cl_2 (22.8 mg), MeOH (77 mg), and water (3.2 mg) solubles. The MeOH solubles were subjected to SPE fractionation (a 900 mg Alltech C_8 SPE Cartridge with a 10% stepwise gradient elution, 100% H_2O to 60% H_2O/MeCN) to afford five fraction, of which fraction 4 was pure makaluvamine J (**6**) (8.4 mg, 4.4%). HPLC purification of fraction 2 (a 5 μm Zorbax RX-C_8 250 × 9.4 mm column, with a 4 mL/min isocratic elution, 80% H_2O/MeCN + 0.01% TFA) yielded dihydrodiscorhabdin L (**8**) (1 mg, 0.5%), while HPLC purification of fraction 5 (a 5 μm Zorbax SB Phenyl 250 × 9.4 mm column, with a 4 mL/min isocratic elution, 57% H_2O/MeOH + 0.01% TFA) yielded damirone A (**7**) (0.7 mg, 0.3%). (Note: % yields are expressed as a mass to mass % compared to the weight of the *n*-BuOH solubles).

(+)-Discorhabdin A (1): green solid; $[\alpha]_D$, UV−vis, ^{1}H NMR (600 MHz, MeOH-d_4), and (+) ESIMS data consistent with published data.[3]

(+)-Discorhabdin D (2): green solid; $[\alpha]_D$, UV−vis, ^{1}H NMR (600 MHz, MeOH-d_4), and (+) ESIMS data consistent with published data.[2]

(+)-Dihydrodiscorhabdin A (3): green solid; $[\alpha]_D$ +115 (*c* 0.006, MeOH); UV−vis (MeOH) λ_{max} (log ε) 249 (3.84), 351 (3.55), 425 (3.35), 567 (2.71); 1D and 2D NMR (600 MHz, MeOH-d_4) see Table 1; (+) HRESIMS *m*/*z* 418.0220 (calcd $C_{18}H_{17}S^{79}BrO_2N_3S$ 418.0219).

(+)-Debromodiscorhabdin A (4): green solid; $[\alpha]_D$ +207 (*c* 0.009, MeOH); UV−vis (MeOH) λ_{max} (log ε) 250 (3.95), 350 (3.51), 400

(3.38) nm; ^{1}H NMR (600 MHz, MeOH-d_4) δ 7.11 (1H, d, J = 10.2 Hz, H-1), 6.30 (1H, d, J = 10.2 Hz, H-2), 2.90 (1H, m, H-4a), 2.68 (1H, dd, J = 2.68, 17.3 Hz, H-4b), 4.49 (1H, dd, J = 6.5, 12.3 Hz, H-5), 2.91 (1H, m, H-7a), 2.58 (1H, d, J = 12.5 Hz, H-7b), 5.36 (1H, d, J = 3.5 Hz, H-8), 7.18 (1H, s, H-14), 2.94 (1H, m, H-16), 3.92 (1H, m, H-17a), 3.79 (1H, m, H-17b); ^{13}C NMR (150 MHz, MeOH-d_4) δ 150.3 (CH, C-1), 132.1 (CH, C-2), 196.8 (C, C-3), 46.1 (CH_2, C-4), 55.8 (CH, C-5), 48.3 (C, C-6), 42.5 (CH_2, C-7), 60.4 (CH, C-8), 153.0 (C, C-10), 167.2 (C, C-11), 125.5 (C, C-12), 127.6 (CH, C-14), 121.4 (C, C-15), 19.5 (CH_2, C-16), 45.4 (CH_2, C-17), 156.4 (C, C-19), 105.6 (C, C-20), 124.7 (C, C-21); (+) HRESIMS m/z 338.0961 (calcd for $C_{18}H_{16}N_3O_2S$ 338.0958).

(+)-Discorhabdin X (5): green solid; $[\alpha]_D$ +86 (c 0.011, MeOH); UV−vis (MeOH) λ_{max} (log ε) 252 (3.48), 370 (3.30), 560 (2.12) nm; 1D and 2D NMR (600 MHz, MeOH-d_4) see Table 2; 1D and 2D NMR (600 MHz, DMSO-d_6) see Supporting Information; (+) HRESIMS m/z 356.1060 (calcd for $C_{18}H_{18}N_3O_3S$ 356.1063).

Makaluvamine J (6): deep red solid; UV−vis, ^{1}H NMR (600 MHz, DMSO-d_6), and (+) ESIMS data consistent with published data.[6]

Damirone (7): red-brown solid; UV−vis, ^{1}H NMR (600 MHz, MeOH-d_4), and (+) ESIMS data consistent with published data.[7]

(+)-Dihydrodiscorhabdin L (8): green solid; $[\alpha]_D$ +256 (c 0.002, MeOH); UV−vis (MeOH) λ_{max} (log ε) 232 (4.10), 375 (3.67) nm; ^{1}H NMR (600 MHz, MeOH-d_4) δ 4.61 (d, J = 4.0 Hz, H-1), 4.14 (dd, J = 4.0, 4.5 Hz, H-2), 4.96 (dd, J = 2.4, 4.5 Hz, H-3), 5.32 (d, J = 2.4 Hz, H-4), 2.87 (dd, J = 3.6, 11.5 Hz, H-7a), 2.52 (d, J = 11.5 Hz, H-7b), 5.36 (dd, J = 1.2, 3.6 Hz, H-8), 7.08 (s, H-14), 3.08 (m, H-16), 4.36 (m, H-17a), 4.07 (m, H-17b); ^{13}C NMR (150 MHz, MeOH-d_4) δ 70.3 (C-1), 65.6 (C-2), 67.4 (C-3), 111.8 (C-4), 146.0 (C-5), 47.2 (C-6), 38.9 (C-7), 62.0 (C-8), 148.2 (C-10), 168.8 (C-11), 125.3 (C-12), 126.6 (C-14), 119.3 (C-15), 21.0 (C-16), 55.6 (C-17), 153.1 (C-19), 106.1 (C-20), 122.7 (C-21); (+) HRESIMS m/z 354.0905 (calcd for $C_{18}H_{16}N_3O_3S$ 354.0907).

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Supporting Information Available: Images of ^{1}H NMR (600 MHz, MeOH-d_4) spectra for **1**−**8** and additional tabulated 1D and 2D NMR data for **4**, **5**, and **8** are available free of charge via the Internet at http://pubs.acs.org.

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