Discorhabdin W, the First Dimeric Discorhabdin

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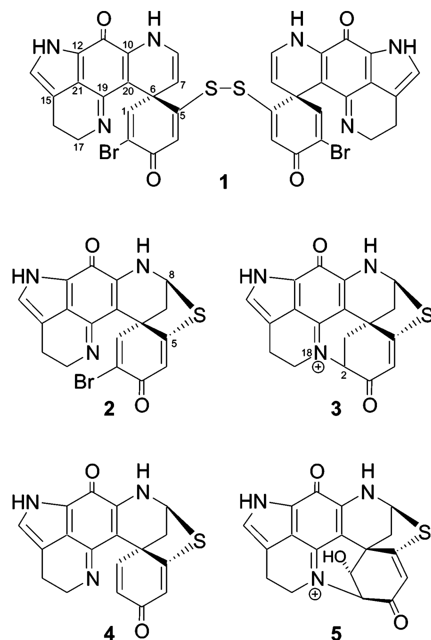
Discorhabdin W (**1**), isolated from a New Zealand *Latrunculia* sp. sponge, is a symmetrical dimer with potent in vitro activity against the P388 murine leukemia cell line. The structure and stereochemistry were assigned by using 1D- and 2D-NMR experiments and mass spectrometry. Discorhabdins B (**2**), D (**3**), G*/I (**4**), and L (**5**) were also isolated from the same sponge.

The discorhabdins A to V constitute, together with the prianosins and epinardins, a group of more than 60 pyrroloiminoquinone alkaloids that are produced by sponges of the families Latrunculiidae and Acarnidae. These metabolites all have a core pentacyclic structure, including a spirocenter, but differ in substitution patterns and often show additional intramolecular ring closures. These additional closures are either via a sulfur bridge between C-5 and C-8 to form a tetrahydrothiophene ring as in discorhabdin B (**2**) or between the 18-imino group and C-2 as in discorhabdin D (**3**).[1] The reported sphere of bioactivity of these alkaloids transverses from antibacterial activity[2] to in vitro[3−5] and in vivo[6] cytotoxicity against a variety of tumor cell lines. Furthermore, the pyrroloiminoquinone metabolites have proved useful as chemotaxonomic markers.[7]

The isolation of the first discorhabdin dimer, discorhabdin W (**1**), along with the known discorhabdins B (**2**), D (**3**), G*/I (**4**), and L (**5**), from a New Zealand *Latrunculia* sp., is described herein.



From the extract of a *Latrunculia* sp. sponge collected in Milford Sound, New Zealand, five compounds were isolated, which on the basis of their dark green to brown color, their UV spectra, and the taxonomy of the sponge could be assigned tentatively to the discorhabdin group of compounds. On the basis of MS and NMR spectroscopic data, four of these compounds were identified as the known discorhabdins B (**2**),[8] D (**3**),[6] G*/I (**4**),[1,5,7] and L (**5**;[5] first published as discorhabdin J[9]).

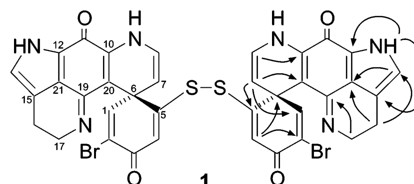


Figure 1. Long-range H,C-couplings for discorhabdin W (**1**).

The ESIMS of the new discorhabdin, trivially named discorhabdin W (**1**), showed two clusters of ions. The highest *m/z* cluster was the pseudomolecular ion $[M + H]^+$ at *m/z* 825. This is approximately double the mass of discorhabdin B (**2**) (M = 413). The second cluster of ions at *m/z* 413 was the doubly charged $[M + 2H]^{2+}$, as indicated by the 0.5 Da spacing between the ions of the isotopic cluster. The isotopic patterns of both clusters showed the presence of two bromine atoms. These data suggested a dimeric structure for **1**.

The presence of only 18 signals in the ^{13}C NMR spectrum, the “normal” number for a discorhabdin, showed that the structure had to be symmetrical. In the ^{1}H NMR spectrum, two signals of exchangeable protons at 9.05 and 12.42 ppm could be attributed to the amine protons H-9 and H-13. An AB spin system of two *cis*-olefinic protons (H-7 and H-8) appeared at 4.81 and 6.56 ppm, and the signals of two neighboring methylene groups (H-16 and H-17) could be seen between 2.8 and 4.4 ppm. The remaining three olefinic singlet signals were assigned to the positions H-1, H-4, and H-14. The ^{13}C NMR chemical shifts of the three annelated rings comprising C-10 to C-20 were similar to those of discorhabdin B (**2**); the greater differences observed for C-10 and C-17 may be due to differences in protonation of the imino nitrogen. This structural similarity was corroborated by long-range H,C-couplings (see Figure 1) from the pyrrole proton H-13 to C-12 and C-15, from H-14 to C-21, from H-16 to C-14 and C-21, and from H-17 to C-19. The C-7/C-8 olefinic system could next be connected to the tricyclic system on the basis of the long-range couplings observed from H-8 to C-10 and from H-7 to C-20. The molecular mass and isotopic pattern of **1** required the remaining 2,5-cyclohexadienone ring bound to the spirocenter C-6 to be substituted with one bromine and one sulfur group. As there was no vicinal coupling observed between the two protons of the 2,5-cyclohexadienone, these protons could not be situated on the “same

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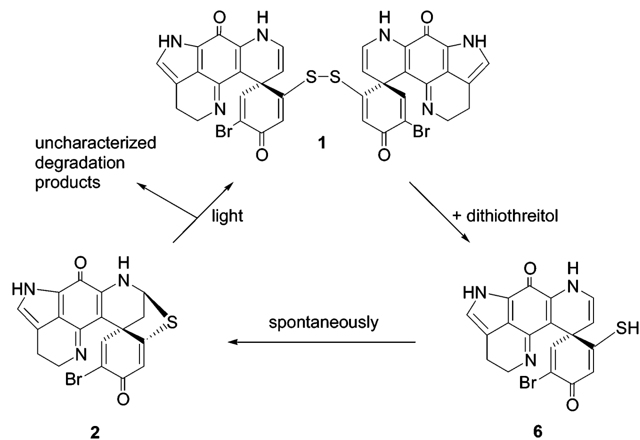

Figure 2. Formation and cleavage of discorhabdin W (**1**).

Table 1. Cytotoxicity of Compounds **1**−**5** for P388 Lymphocytic Leukemia Cells

compound[a]	IC_{50}, μM/μg/mL
discorhabdin W (**1**)	0.084/0.089
discorhabdin B (**2**)	0.087/0.046
discorhabdin D (**3**)	1.6/0.70
discorhabdin G*/I (**4**)	0.51/0.23
discorhabdin L (**5**)	1.1/0.49

[a] All compounds tested as TFA salts.

side" of this unit. Long-range couplings from H-7 to the methine carbon C-1 and the sulfur-bearing carbon C-5 showed that these carbons were both close to the spirocenter and, therefore, established the substitution pattern to be as shown. This structure left only the sulfur as a possible connection site for the second half of the molecule, with discorhabdin W (**1**) consequently being a disulfide-linked discorhabdin dimer. The optical rotation of **1** ($[\alpha]^{20}_D$ +220) precluded the possibility of an achiral dimer with different configurations of "left" and "right" halves.

To confirm the presence of the disulfide bond, **1** was reduced with dithiothreitol (on an analytical scale) and the reaction mixture analyzed by HPLC-UV and -MS. The intermediate product of the reduction had an UV spectrum similar to that of **1** and a molecular mass of 413 Da, which is in agreement with the expected thiol **6** (Figure 2). Under the reaction conditions **6** was not stable and cyclized spontaneously to form discorhabdin B (**2**).

Preliminary studies have established that discorhabdin B (**2**) also appears to be involved in the formation of **1**. Upon irradation of discorhabdin B (**2**) solutions with sunlight, and depending on factors such as pH, concentration, and solvent, **2** is decomposed in what seems to be a radical reaction and the dimer **1** is among the products (Figure 2). Further studies are in hand on the photochemistry of discorhabdin B to better establish the steps involved.

Discorhabdin W (**1**) was found to be strongly cytotoxic against P388 cells,[10] with an IC_{50} in the same range as that of the very active discorhabdin B (**2**; see Table 1). Cytotoxicity data are also shown for compounds **3**−**5**.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV data were obtained with a Varian Cary 50 UV−visible spectrometer. NMR spectra were recorded on a Varian INOVA 500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 2.6 and δ_C 39.6 ppm for DMSO-d_6). HRESIMS were acquired using a Micromass LCT TOF mass spectrometer. Solvents used for extraction and isolation were distilled prior to use. Cytotoxicity against the P388 cell line was measured using a standard protocol.[10]

Sponge Material. *Latrunculia* sp. (code 03MI02-12) was collected by scuba on November 25, 2003, at Anita Bay, in Milford Sound, Fiordland, New Zealand, at a depth of ~22 m. The sample was stored at −20 °C until extraction. A spicule preparation of the dark green sponge showed anisodiscorhabd microscleres and anisostyle megascleres indicative of a *Latrunculia* species.[11] The microscleres differed in shape and conformation of the whorls from those found in a previously investigated New Zealand *Latrunculia* sp.[8]

Extraction and Isolation. The sponge (wet wt 375 g) was homogenized and exhaustively extracted with MeOH−CH_2Cl_2 (1:1). The crude extract was dissolved in EtOAc and partitioned against H_2O. The brown EtOAc phase was extracted with *n*-BuOH, concentrated (680 mg), and partitioned again between petroleum ether and MeOH−H_2O (10:1). The precipitate that formed in the MeOH phase was dissolved in CH_2Cl_2, dried (160 mg), and subjected to gel chromatography on a Sephadex LH-20 column eluting with CH_2Cl_2−MeOH (1:1). The fractions containing discorhabdins W (**1**) and B (**2**) were further purified using gradient elution HPLC (Phenomenex Luna C_{18}, 250 × 10 mm, 5 μm; solvents: A water + 0.05% TFA, B MeCN; linear gradient: 0 min 20% B, 15 min 80% B; 5 mL min^{-1}). Compounds **1** (11.8 mg) and **2** (1.5 mg) were eluted at 6.3 and 9.1 min, respectively.

An aliquot of the *n*-BuOH phase (40 mg) was subjected to semipreparative HPLC, under the same conditions as mentioned above. Compounds **3** (1.2 mg), **4** (1.3 mg), and **5** (1.6 mg) were eluted at 7.7, 8.1, and 6.6 min, respectively.

Discorhabdin W (1): $[\alpha]^{20}_D$ +220 (*c* 0.05, MeOH); UV [H_2O + 0.05% TFA−MeCN (1:1)] λ_{max} (rel int %) 203 (100), 241 (91), 301(51), 444 (27) nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.4 (1H, s, H-13), 9.05 (1H, d, J = 4.5 Hz, H-9), 8.01 (1H, s, H-1), 7.25 (1H, s, H-14), 6.56 (1H, dd, J = 7.3, 4.5 Hz, H-8), 5.95 (1H, s, H-4), 4.43 (1H, m, H-17b), 4.08 (1H, d, J = 7.3 Hz, H-7), 3.91 (1H, m, H-17a), 2.83 (2H, m, H-16); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 175.9 (C, C-3), 168.9 (C, C-5), 168.3 (C, C-11), 156.0 (CH, C-1), 155.2 (C, C-19), 138.6 (C, C-10), 128.0 (CH, C-8), 125.0 (CH, C-14), 122.3 (C, C-21), 121.6 (C, C-12), 117.9 (C, C-2), 117.3 (C, C-15), 114.6 (CH, C-4), 105.4 (C, C-20), 102.9 (CH, C-7), 50.2 (CH_2, C-17), 49.7 (C, C-6), 17.9 (CH_2, C-16); HRESIMS *m/z* 824.9417 [M + H]$^+$ (calcd for $C_{36}H_{23}N_6O_4S_2{}^{79}Br_2$, 824.9589).

Discorhabdins B[8] (2), D[6] (3), G*/I[1,5,7] (4), and L[5] (5): ^{1}H, ^{13}C NMR and UV data and results from ESIMS were identical with, or consistent with, reported data.

Reduction of Discorhabdin W (1). To an aqueous solution of **1** (0.2 mg in 1 mL) was added 1 mg dithiothreitol. The reaction mixture was kept at room temperature and analyzed by analytical HPLC (Phenomenex Luna C_{18}, 250 × 4.6 mm, 5 μm; solvents: A water + 0.05% TFA, B MeCN; linear gradient: 0 min 10% B, 2 min 10% B, 14 min 75% B, 24 min 75% B; 1 mL min^{-1}) 0, 1, 2, 5, and 24 h after adding the reagent. After 24 h an additional HPLC-MS analysis (ESI pos.) was carried out.

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