A New Discorhabdin from Two Sponge Genera

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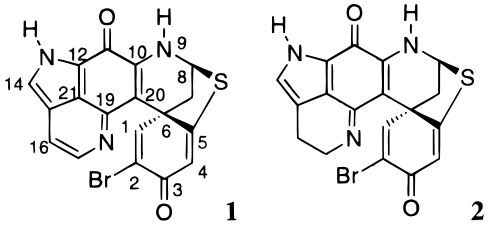
A new discorhabdin, discorhabdin Q (16,17-dehydrodiscorhabdin B, **1**), has been isolated from cytotoxic extracts of the sponge *Latrunculia purpurea* and numerous collections of *Zyzzya massalis, Z. fuliginosa,* and *Z.* spp. The structure was solved by spectroanalytical methods and comparison to known compounds in the series. It is not the principal cytotoxin in any of the extracts examined, yet it is apparently widely distributed.

As part of our effort to identify new leads to antitumor agents from marine organisms,[1,2] we observed that extracts from several collections of the sponge genera *Zyzzya* and *Latrunculia* exhibited potent and differential cytotoxicity[3] in the NCI 60 cell line human tumor screen.[4] Bioassay-guided fractionation of selected extracts from this group led to varying ensembles of pyrroloiminoquinones[5] of the makaluvamine, batzelline, and isobatzelline classes (Dijoux et al., manuscript in preparation). A common denominator in these extracts, however, was the previously unreported discorhabdin Q, 16,17-dehydrodiscorhabdin B, **1**. Dereplication of additional extracts of the genus *Zyzzya* revealed that **1** was frequently present and typically the most prevalent or only discernible discorhabdin.



1

2

Compound **1** was usually found in the organic extracts of the sponges, and could be isolated by solvent−solvent partitioning,[6] gel permeation on Sephadex LH-20, and VLC or HPLC on C_{18}-bonded phase. Compound **1** gave a pseudomolecular ion [MH^+] at *m/z* 411.9760 by HRFABMS analysis, consistent with the molecular formula $C_{18}H_{10}BrN_3O_2S$. Well-resolved resonances for all eighteen carbons were observed in the ^{13}C NMR spectrum. The construction of the pyrroloiminoquinone substructure via HMQC, HMBC, and NOE experiments revealed that the pyrrole nitrogen was unsubstituted and that the usual pair of vicinally coupled methylene signals at C-16 and C-17 were replaced by a pair of mutually coupled olefinic methines at δ 7.51 (H-16) and 8.27 (H-17). According to the DEPT data, the remaining structural elements, C_8H_5BrOS (six unsaturations), were composed of four quaternary carbons at δ 127.5, 173.3, 177.4, and 53.9, three methines at δ 115.9, 149.1, and 64.9, and one methylene at δ 42.7. HMQC experiments established that a methine proton at δ 5.72 ppm (H-8) was attached to the carbon at δ 64.9, the pair of diastereotopic methylene protons at δ 2.54 (dd, J = 3.5, 11 Hz, H-7α) and δ 3.03 (d, J = 11 Hz, H-7β) resided on the carbon at δ 42.7, and two olefinic protons at δ 7.75 (H-1) and δ 5.95 (H-4) were attached to the carbons at δ 149.1 (C-1) and 115.9 (C-4), respectively. These last two correlations showed that **1** contained two additional CH=C fragments. A $^1H-^1H$ COSY experiment established that the H-8 methine proton was vicinally coupled to the two H-7 methylene protons, consistent with the observed NOE between H-7 and H-1 (δ 7.75). HMBC data showed that H-7 correlated to the carbons at δ 53.9 (C-6), 64.9 (C-8), 111.4 (C-20), 149.4 (C-1), and 173.8 (C-5) and that H-1 correlated with C-5, C-6, C-7, and C-20. On the other hand, C-6 was correlated with H-1, H-4, H-7, and H-8. The correlations of H-1 and H-7 to C-5 and C-20 confirmed the connectivities of C-6 to C-5 and to C-20; therefore, C-6 was a juncture point for a CH-CH_2 and a pair of CH=C fragments. ^{13}C NMR and $^1H-^1H$ COSY spectra showed that H-1 and H-4 were situated in two similar CBCH=C fragments. The carbonyl resonance at δ 177.4 was placed at C-3 on the basis of an HMBC correlation to H-1. Additional long-range correlations observed between H-4 and C-2, C-5, and C-6 supported the assembly of the fragment from C-3 to C-6. Thus, H-1 and H-4 resided on opposite sides of a cross-conjugated ketone fragment. HMBC correlations to H-8 from C-5, C-6, C-7, and C-10 provided evidence for the spiral ring junction at C-6. As in discorhabdin B (**2**),[5] the chemical shifts of C-5, C-8, and C-10 allowed the introduction of a sulfur atom between C-5 and C-8, and a nitrogen atom between C-8 and C-10. The bromine was, therefore, placed on the quaternary C-2. HMBC correlations between H-8 and C-10, and between C-20 and H-1 and H-7, completed the attachment of the C_8H_5BrOS substructure to the pyrroloiminoquinone subunit. Dreiding models of **1** revealed a very rigid structure; as in **2**, the C-8/S and C-6/C-5 bonds could only be cis. Thus, the structure of **1** was established as 16,17-dehydrodiscorhabdin B and assigned the trivial name discorhabdin Q; the absolute configuration, while not rigorously established, is proposed to be the same as that of **2**.[7,8]

In the NCI 60 cell line antitumor screen,[4] **1** exhibited moderate, generalized cytotoxicity (mean panel GI_{50} = 0.5 μg/mL) but essentially no differential cytotoxicity profile.[3] It is our hypothesis that full aromatization of the pyrroloiminoquinone system confers reduced cytotoxicity relative to the 16,17-saturated members of the family, but this remains to be confirmed. Perhaps this reduced potency has

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permitted this seemingly widely distributed compound to escape detection until now.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter in $CHCl_3$ and CH_3OH. UV spectra were recorded on a Beckman DU-64 spectrophotometer. FT-IR spectra were obtained on a Perkin-Elmer 267 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-500 spectrometer using acetone-d_6 as solvent and internal standard. The number of attached protons for each carbon was determined from DEPT experiments. Mass spectra were determined on a JEOL SX102 spectrometer.

Sponge Material. *Zyzzya* sp. (voucher Q66C4282) was collected at Assail Bank, between North Island and the Wallab Group, Australia, in September 1990. It was originally described in the field as an *Inflatella* sp. but was reclassified as *Zyzzya* sp. by R. van Soest. *Zyzzya massalis* (= *Zyzzya fuliginosa*,[9,10] voucher Q66C6218) was collected on the northeast side of a small island in the bay south of Sphinx Head, Wessell Island, Australia, in November 1990. *Z. fuliginosa* (voucher 0CDN4242) was collected from Bega Lagoon, Fiji, in October 1996. *Latrunculia purpurea* (voucher Q66C2463) was collected on Horseshoe Reef west northwest of Margaret Brock Lighthouse, Australia, in February 1989. All voucher specimens are maintained at the Smithsonian Sorting Center, Suitland, MD.

All sponge samples were extracted with the standard National Cancer Institute (NCI) protocol. Frozen sponge was ground to small pellets in a meat grinder with dry ice and soaked in distilled H_2O at 4 °C for 4 h. The aqueous extract was removed in a basket centrifuge, lyophilized, and weighed. The marc was freeze-dried and then extracted successively with CH_2Cl_2−MeOH (1:1) and MeOH. The combined organic extracts were evaporated in *vacuo* and weighed.

Isolation Example of *Zyzzya* sp. The organic extract (1.99 g, 2.3% dry weight) was partitioned to give cytotoxic $CHCl_3$ (522 mg) and MeOH−H_2O (638 mg) fractions. A sequence of VLC (C_{18}, MeOH−H_2O/HOAc step gradient), Sephadex LH-20 gel permeation (CH_2Cl_2−MeOH, 1:1), and VLC on silica gave 17 mg (0.9% crude extract) of 16,17-dehydrodiscorhabdin B (**1**): orange solid, UV (MeOH) λ_{max} 222 (ϵ = 28 200), 428 (9900) nm; $[\alpha]_D = -452.4°$ (*c* 0.0042, $CHCl_3$), $[\alpha]_D = -904°$ (*c* 0.0125, MeOH); ^1H NMR (acetone-d_6) δ 2.54 (dd, J = 4, 11 Hz, H-7b), 3.03 (br d, J = 11 Hz, H-7a), 5.92 (dd, J = 1, 4 Hz, H-8a), 5.95 (s, H-4), 7.51 (d, J = 5.5 Hz, H-16), 7.75 (s, H-1), 8.23 (s, H-14), 8.27 (d, J = 5.5 Hz, H-17), H-9 and H-13 were not observed; ^{13}C NMR (acetone-d_6) δ 177.4 (C-3), 173.3 (C-5), 166.6 (C-11), 149.1 (C-1), 147.9 (C-19), 145.0 (C-10), 143.1 (C-17), 128.1 (C-14), 127.5 (C-2), 125.5 (C-15), 120.1 (C-12), 119.4 (C-21), 115.9 (C-4), 113.6 (C-16), 111.4 (C-20), 64.9 (C-8), 53.9 (C-6), 42.7 (C-7); HRFABMS *m/z* 411.9764 (MH^+, calcd for $C_{18}H_{11}{}^{79}BrN_3O_2S$, 411.9759).

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