Discorhabdin R: A New Antibacterial Pyrroloiminoquinone from Two Latrunculiid Marine Sponges, *Latrunculia* sp. and *Negombata* sp.

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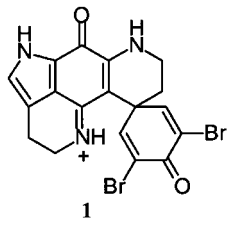
Received May 3, 2000

Two sponges belonging to the family Latrunculiidae (*Negombata* and *Latrunculia* sp.) collected during scientific trawling operations in Prydz Bay, Antarctica, and by scuba off Port Campbell, Victoria, have yielded a new antibacterial pyrroloiminoquinone, discorhabdin R (**2**). The structure was assigned as **2** on the basis of detailed spectroscopic analysis and comparison with the known co-metabolite discorhabdin B (**3**).

More than 60 metabolites featuring a pyrroloiminoquinone moiety have been reported from geographically disperse (tropical, temperate, and Antarctic) sponges of the genera *Latrunculia*,[1−5] *Batzella*,[6,7] *Prianos*,[3] *Zyzzya*,[1,8−11] and *Histodermella*.[12] The structural novelty and biological properties of these pyrroloiminoquinones have prompted continued attention since discorhabdin C (**1**) was first described in 1986 by Perry et al.[2] from an Antarctic sponge of the genus *Latrunculia*. In this report we describe a new example of this structure class, discorhabdin R (**2**), isolated from a southern Australian *Negombata* sp. and an Antarctic *Latrunculia* sp.



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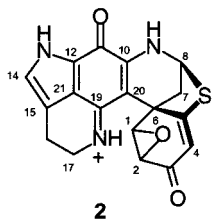

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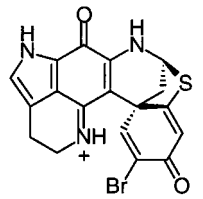

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The EtOH extracts of an Antarctic *Latrunculia* sp. and a southern Australian *Negombata* sp. demonstrated antibacterial activity against Gram positive (*Staphylococcus aureus, Micrococcus luteus*) and Gram negative (*Serratia marcescens, Escherichia coli*) bacteria. The EtOH extract of the *Latrunculia* sp. was decanted, concentrated in vacuo, and subjected to reversed-phase fractionation with H_2O/MeOH modified by TFA to yield the TFA salts of the known metabolite, discorhabdin B (**3**), and a new and yet closely related minor metabolite, discorhabdin R (**2**). Both **2** and **3** proved to be the antibacterial agents in this sponge extract. Similar treatment of the *Negombata* sp. also yielded the TFA salt of discorhabdin R (**2**). Mass spectral analysis of the salt **2** provided a molecular formula for the free base, $C_{18}H_{13}N_3O_3S$, requiring 14 double-bond equivalents. The ^{13}C NMR data for **2** (see Table 1) revealed sp^2 carbon resonances consistent with the pyrroloiminoquinone, along with signals of a trisubstituted double bond and a ketone, confirming discorhabdin R (**2**) to be heptacyclic. Spectroscopic comparisons between discorhabdin R (**2**) and discorhabdin B (**3**), together with 2D NMR analysis (Table 1), suggested both compounds to have a common structural motif from C-3 to C-21, accounting for all but one ring. The remarkable difference in the spectroscopic data of **2** from those of **3** was the absence of both a bromo substituent (as evidenced by the absence of dual isotope peaks in a pseudomolecular ion) and the NMR resonances for the $\Delta^{1,2}$ double bond (most clearly evidenced by absence of a deshielded ^1H resonance observed at δ 7.8 in discorhabdin B (**3**)) and the presence of NMR signals due to an epoxy group composed of two mutually coupled oxymethines. The location of the epoxide was confirmed by HMBC data (Table 1). Thus discorhabdin R (**2**) was proved to be a debromo epoxy analogue of the co-metabolite discorhabdin B (**3**). Careful examination of the extracts of both latrunculiids confirmed that the transformation of **3** to **2** did not occur during isolation and handling, requiring a salt of discorhabdin R (**2**) to be a natural product. The identity of the natural anion was not established. Stereochemistry about all chiral centers in discorhabdin R (**2**), other than those about C-1 and C-2, was assigned on the basis of spectroscopic comparisons to, and the fact that **2** is a co-metabolite with, discorhabdin B (**3**). Clearly, the nature of the fused ring systems requires that H-1 and H-2 be cis disposed; however, the relative orientation of the epoxide oxygen to the rest of the molecule is unresolved. Neither NOE difference or NOESY experiments supported assignment of the epoxide relative stereochemistry.

Discorhabdin R (**2**) is a very highly functionalized heterocyclic metabolite, which adds to the structural diversity encountered in pyrroloiminoquinones of marine origin.

Experimental Section

General Experimental Procedures. For general experimental details, see ref 13.

Animal Material. Two sponge specimens, identified as *Latrunculia* (Museum of Victoria Registry Number F79996) and *Negombata* sp. (F80007), were obtained during scientific expeditions in the central Prydz channel of Prydz Bay, Antarctica, and Port Campbell, Victoria, in February 1997 and March 1998, respectively. The Antarctic specimen was collected by beam trawl at a depth of 544 m, while the second specimen was collected via scuba at a depth of 15−20 m. A

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10.1021/np000220q CCC: $19.00
Published on Web 10/05/2000

Table 1. NMR (MeOH-d_4, 400 MHz) Data for Discorhabdin R (**2**)[a]

				gHMBC	
no.	^{13}C δ	^{1}H δ [m, J(Hz)]	COSY	$^{1}H-^{13}C$	$^{1}H-^{15}N$
1	65.2	4.08 (d, 3.6)	H-2	C-2, C-3, C-4	
2	66.5	4.51 (d, 3.6)	H-1	C-1, C-3, C-5	
3	184.9				
4	111.9	6.06 (s)		C-2, C-5, C-6	
5	172.2				
6	46.7				
7a	35.3	2.69 (dd, 2.5, 12.2)	H-7b, H-8	C-6, C-8, C-20	
7b		2.35 (d, 12.2)	H-7a, H-8	C-5, C-6, C-8, C-20	
8	62.2	5.49 (d, 2.5)	H-7a, H-7b	C-5, C-6, C-10	N-9
10	146.9				
11	166.3				
12	123.2				
14	126.8	6.91 (s)		C-11, C-12, C-15	N-13
15	118.1				
16a	18.9	2.93 (ddd, 17.1, 13.2, 7.7)	H-16b, H-17a, H-17b	C-15, C-17	
16b		2.81 (ddd, 16.9, 7.1, 2.8)	H-16a, H-17a, H-17b	C-15, C-17	
17a	51.2	3.78 (ddd, 14.2, 7.7, 2.8)	H-16a, H-16b, H-17b	C-15, C-16, C-19	N-18
17b		3.68 (ddd, 13.6, 7.0)	H-16a, H-16b, H-17a	C-15, C-16, C-19	N-18
19	148.5				
20	98.9				
21	121.8				

[a] ^{13}C NMR assignments are supported by a DEPT 135 NMR experiment. TOCSY data are supported by COSY analysis and structural assignment of **2**.

taxonomic description of F79996 is as follows: growth form of original specimen not seen; color in life not seen; color in EtOH black-brown; texture firm but compressible, harsh; surface opaque, irregular, minutely hispid; oscules not seen; spicules megascleres styles flexuous (530−580 × 10 μm); microscleres anisodiscorhabds (70−120 μm) with four whirls of spines, the two central whirls of unequal diameter are located closer to one end of the spicule; ectosome a dense, almost acollagenous layer of interwoven megascleres up to 1000 μm thick in parts with an outer single layer of erect microscleres; choanosome a dense, irregular reticulation of single monactinal megascleres occasionally organized into poorly defined plumose tracts; densely collagenous. The taxonomic description of F80007 is as follows: growth form of original specimen not seen; color in life not seen; color in EtOH black-brown; texture firm but compressible, harsh; surface opaque, irregular, minutely hispid; oscules not seen; spicules megascleres oxeas slightly curved occasionally strongylote (250−370 μm × 5 μm); microscleres spinorhabds irregular 2 size classes (10 μm, 25 μm); ectosome a dense, almost acollagenous layer of interwoven megascleres up to 1000 μm thick in parts in which microscleres are sparsely scattered; choanosome is a dense, irregular reticulation of single megascleres occasionally organized into poorly defined plumose tracts; densely collagenous and darkly pigmented.

Extraction and Isolation. The frozen sponges were transported to the laboratory, where they were thawed, documented, diced, and stored in EtOH at −20 °C until required. The decanted EtOH extracts were concentrated in vacuo and then subjected to C_{18} solid-phase extraction (10% stepwise gradient elution from H_2O to MeOH) followed by C_{18} HPLC (2 mL/min isocratic elution with 30% H_2O/MeOH/0.1% TFA through a 5 μm Phenomenex C_{18} Ultracarb 150 × 10 mm column) to yield the known compound discorhabdin B (**3**) (*Latrunculia:* 30.8 mg, 3.4%) and a novel metabolite, discorhabdin R (**2**) (*Latrunculia*: 17.3 mg, 1.37%; *Negombata*: 1.0 mg, 0.01%). Percentage yields are calculated against the mass of crude EtOH extract.

Discorhabdin R (2): green solid; $[\alpha]^{20}_D$ +161° (*c* 0.105, MeOH),); IR (KBr) ν_{max} 3420 (br), 1684 (br) cm^{-1}; UV (MeOH) λ_{max} (ϵ) 202 (7600), 255 (9000), 262 (9200), 287 (5400), 326 (3500), 368 (3800), 567 (600) nm; ^{1}H NMR data (MeOH-d_4, 400 MHz) see Table 1; ^{13}C NMR data (MeOH-d_4, 400 MHz) see Table 1; ESIMS (20 kV) *m*/*z* 352 (salt M^+); HRESIMS 352.0745 (calcd for $C_{18}H_{14}N_3O_3S$, 352.0756).

Acknowledgment. We acknowledge the Australian Antarctic Division for access to the *RSV Aurora Australis* and to C. C. Lu for collection of the *Latrunculia* sp. The *Negombata* sp. was collected with the assistance of L. Goudie, R. Goudie, D. Niclasen, P. Niclasen, and F. Rooney. We also acknowledge L. Goudie for taxonomic analysis, as well as P. Niclasen and T. Mueller for biological screening. This research was funded by the Australian Research Council.

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NP000220Q