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Makaluvamine G, a Cytotoxic Pigment from an Indonesian Sponge *Histodermella* sp.

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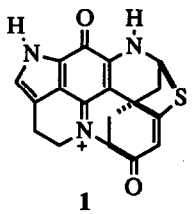
Abstract: A cytotoxic pigment, makaluvamine G (**5**), biogenetically derivable from tryptamine and tyramine and structurally related to the discorhabdins, was isolated from a sponge of the genus *Histodermella* collected in Indonesia. The compound displays significant in vitro cytotoxicity to several tumor cell lines and is a moderate inhibitor of topoisomerase-I, DNA, RNA, and protein synthesis.

The discorhabdins,[1] as e. g. discorhabdin D (**1**), are a series of potent cytotoxic compounds isolated from two sponges of the genera *Latrunculia*[2] and *Prianos*.[3] The carbon skeleton is biogenetically derivable from tryptamine, which forms the pyrroloiminoquinone-containing portion, and tyramine. More recently, related sponge metabolites, the damirones (e. g., damirone A (**2**)),[4] and the highly functionalized batzellines (e. g., batzelline A (**3**)),[5] and isobatzellines (e. g., isobatzelline A (**4**)),[6] all derivable from a single tryptamine unit, have been reported. The discorhabdins and isobatzellines, both of which contain pyrroloiminoquinone moieties, display potent in vitro cytotoxicity to the P388 murine leukemia cell line. No activity was reported for the batzellines or damirones, which lack an iminoquinone moiety, a possible function responsible for cytotoxicity.



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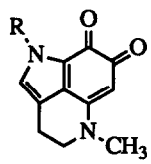
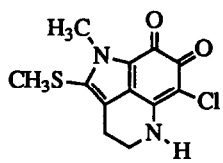


2 R = CH_3
8 R = H



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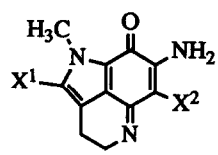
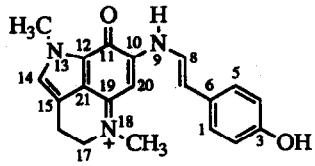


4 $X^1 = CH_3S$, X2 = Cl
9 $X^1 = H$, $X^2 = H$

A recent collection of marine invertebrates from Indonesia included a sponge whose extract was highly cytotoxic to several cell lines. Partitioning of the CH_2Cl_2/2-propanol (1:1) extract and Sephadex LH-20 chromatography with different solvent systems yielded makaluvamine G (**5**) as the major cytotoxic compound. Makaluvamines A (**6**), and C (**7**), whose structures were published while the present work was in progress,[7] and damirones A (**2**) and B (**8**)[4] were also isolated from the sponge. Makaluvamine E,[7] which is 18-demethylmakaluvamine G, was absent.

[+] HBOI Contribution No. 976



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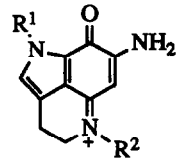

6 $R^1 = CH_3$, $R^2 = H$
7 $R^1 = H$, $R^2 = CH_3$

A molecular formula of $C_{20}H_{20}N_3O_2$ was established for **5** by HRFABMS, indicating 12.5 degrees of unsaturation. DEPT and HMQC NMR experiments revealed 18 protons attached to the 20 carbons observed in the ^{13}C NMR spectrum; two exchangeable protons in the ^1H NMR spectrum at δ 10.70 (bs, N-H) and 9.80 (s, O-H) accounted for the remaining protons. The mass spectral and NMR data indicated that **5** is a discorhabdin-like compound with two additional carbons which could be accommodated by two *N*-methyls (δ 3.92, 3 H, s; 35.9 (q), CH_3N-13, and 3.48, 3 H, s; 39.6 (q), CH_3N-18).

The tryptamine-derived portion of **5** was suggested by comparison of NMR data with those for **9**, a derivative prepared for the structure elucidation of the isobatzellines; it was supported by extensive HMBC correlations. One methyl group showed HMBC correlations to carbons at δ 122.7 (C-12) and 130.8 (C-14). A proton resonating at δ 7.31, attached to C-14, showed correlations to CH_3N-13 and to signals at δ 116.9 (C-15) and 123.0 (C-21). The other methyl group was correlated in the HMBC experiment to a methylene carbon at δ 52.6 (C-17) and a quaternary carbon at 154.0 (C-19). The ^{13}C chemical shift of C-17 was consistent with a tertiary iminium nitrogen at position 18.[2b] The protons attached to C-17 (3.90, 2 H, t, J =7.6 Hz) were coupled to H_2-16 (2.93, 2 H, t, J =7.6 Hz) and exhibited HMBC correlations to C-16, C-15, and C-19. HMBC correlations between a proton at δ 6.30 (H-20), attached to a carbon at δ 85.7, and signals at 146.7 (C-10) and 167.8 (C-11), and also to C-19 and C-21 established the structure of the tryptamine-derived portion of **5**.

That **5** contained a *para* substituted phenol was suggested by two mutually coupled two-proton doublets at δ 7.43 (2 H, d, J = 8.2 Hz, H-1 and H-5) and 6.78 (2 H, d, 8.2 Hz, H-2 and H-4), each attached to carbons resonating at δ 128.4 and 115.6, respectively. Both aromatic proton signals displayed correlations to C-3 (δ 158.0); H-2 and H-4 correlated to a signal at δ 126.2 (C-6).

Two olefinic methine carbons (δ 124.3, C-7; 121.8, C-8) remained to be placed. The coupling constants of the attached protons (δ 7.05, 1 H, d, J =13.8 Hz, H-7; 7.44, 1 H, d, 13.8 Hz, H-8) indicated a *trans* double bond.[8] The C-8 proton signal changed to a broad triplet upon addition of a drop of TFA to the NMR sample solution, demonstrating that N-H was adjacent to this proton. HMBC correlations of H-1, H-5 to C-7, and H-8 to C-6 linked the olefin to the aromatic ring. An HMBC correlation of H-8 to C-10 connected the tryptophan- and tyramine-derived portions, thus securing the structure of makaluvamine G (**5**).

In common with other pyrroloiminoquinone-containing compounds, **5** was moderately cytotoxic to several tumor cell lines, with an IC_{50} value of 0.50 μg/mL against P388 (murine leukemia), A549 (human non-small cell lung cancer), HT-29 (human colon cancer), and MCF-7 (human breast cancer), and 0.35 μg/mL against KB (human oral epidermoid carcinoma). Compound **5** was not antifungal to *Aspergillus oryzae, Candida albicans, Penicillium notatum, Saccharomyces cerevisiae* and *Trichophyton mentagrophytes*, or antiviral to Herpes simplex 1 and 2 and polio viruses, but it displayed moderate immunomodulatory activity in

the mixed lymphocyte reaction (IC_{50} = 0.28 μg/mL) with only mild cytotoxicity to resting lymphocytes (IC_{50} = 24.4 μg/mL). The compound was not active, however, in the graft-vs.-host in vivo immunomodulatory assay and was surprisingly not toxic to mice at the highest dose tested (210 mg/kg total dose). In vivo antitumor assays are in progress.

In contrast to the topoisomerase-II inhibition reported for makaluvamines A, C, E, and F,[7] **5** was found to be a moderate inhibitor of topoisomerase-I (IC_{50} = 3.0 μM); it did not significantly inhibit topoisomerase-II. Compound **5** also moderately inhibited RNA (IC_{50} = 15 μM), DNA (15 μM), and protein (21 μM) synthesis.

Table 1. NMR Data for **5** (DMSO-d_6)

no.	^{13}C	^{1}H	HMBC
1	128.4 (d)[a]	7.43 (1 H, d, J = 8.2 Hz)	H-5, H-7
2	115.6 (d)	6.78 (1 H, d, 8.2 Hz)	H-4, O-H
3	158.0 (s)		H-1, H-2, H-4, H-5
4	115.6 (d)	6.78 (1 H, d, 8.2 Hz)	H-2, O-H
5	128.4 (d)	7.43 (1 H, d, 8.2 Hz)	H-1, H-7
6	126.2 (s)		H-2, H-4, H-8
7	124.3 (d)	7.05 (1 H, d, 13.8 Hz)	H-1, H-5
8	121.8 (d)	7.44 (1 H, d, 13.8 Hz)	H-7
9		10.70 (1 H, bs)	
10	146.7 (s)		H-8, H-20
11	167.8 (s)		H-20
12	122.7 (s)		CH_3N-13
13			
14	130.8 (d)	7.31 (1 H, s)	CH_3N-13
15	116.9 (s)		H-14, H_2-16, H_2-17
16	18.8 (t)	2.93 (2 H, t, 7.6 Hz)	H_2-17
17	52.6 (t)	3.90 (2 H, t, 7.6 Hz)	H_2-16
18			
19	154.0 (s)		H_2-17, H-20, CH_3N-18
20	85.7 (d)	6.30 (1 H, s)	
21	123.0 (s)		H-14, H_2-16, H-20
CH_3N-13	35.9 (q)	3.92 (3 H, s)	H-14
CH_3N-18	39.6 (q)	3.48 (3 H, s)	
O-H		9.80 (1 H, s)	

[a]Multiplicities were determined by DEPT and HMQC NMR spectra.

EXPERIMENTAL SECTION

Sponge Collection and Taxonomy. The sponge, collected in Manado Bay, Sulawesi, Indonesia, on October 2, 1992, from depths between 20 and 120 ft., is an undescribed species of *Histodermella* (Porifera, Demospongiae, Poecilosclerida, Coelosphaeridae). A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalog No. 003:00879).

Isolation. The freeze-dried sponge (340.8 g) was extracted overnight with 3 L of 2-propanol/CH_2Cl_2 (1:1). The black residue (6.072 g) was partitioned between 600 mL of hexanes/MeOH (2:1), and the resulting lower layer residue (3.532 g) was partitioned between 180 mL of EtOAc/heptane/MeOH/H_2O (7:4:4:3). The lower phase was concentrated in vacuo, yielding 1.412 g, of which a 516 mg portion was chromatographed on Sephadex LH-20 (120 mL void volume, 18 mL fractions), eluting with CH_2Cl_2/MeOH (1:1). Fraction 6 was rechromatographed on LH-20, eluting with MeOH, to give 31.4 mg of **5**.

Makaluvamine G (5): green-black powder, mp > 250 °C; FABMS *m/z* 334 (M^+); HRFABMS 334.1563, $C_{20}H_{20}N_3O_2$ (Δ -0.7 mmu); IR (neat) 3600-2640, 1600, 1575, 1505, 1430, 1410, 1235, 1160 cm^{-1};

UV-vis (MeOH) λ_{max} 280 (log ε 4.2), 346 (4.1), 450 (3.9), 624 (4.0) nm; UV-vis (MeOH, NaOH) λ_{max} 286 (4.1), 332 (sh), 612 (4.1) nm, with decomposition; ^1H and ^{13}C NMR (DMSO-d_6), see Table 1; ^1H NMR (CD_3OD) δ 3.00 (2 H, t, J =7.7 Hz, H_2-16), 3.50 (3 H, s, CH_3N-18), 3.95 (2 H, t, J = 7.7 Hz, H_2-17), 3.98 (3 H, s, CH_3N-13), 6.18 (1 H, s, H-20), 6.78 (2 H, d, J = 8.6 Hz, H-2, H-4), 6.88 (1 H, d, J = 13.6 Hz, H-7), 7.11 (1 H, s), 7.43 (2 H, d, J = 8.6 Hz, H-1, H-5), 7.49 (1 H, d, J = 13.6 Hz, H-8); ^{13}C NMR (CD_3OD) δ 20.4 (C-16), 36.5 (CH_3N-13), 39.9 (CH_3N-18), 54.3 (C-17), 86.2 (C-20), 116.5 (C-2, C-4), 118.9 (C-15), 122.0 (C-8), 124.7 (C-12), 124.9 (C-21), 126.1 (C-7), 128.2 (C-6), 129.5 (C-1, C-5), 131.6 (C-14), 148.6 (C-10), 156.6 (C-19), 159.6 (C-3), 169.0 (C-11).

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