

Crazy-Looking, But Promising Antibiotic From Deep-Water Sea Sponges



By Josh Bloom — February 8, 2017



Look Out, Evil MRSA!

Just another day at the office.

1. Go to Long Island off the coast of the Bahamas. Coordinates (23°41.12? N, 75°22.18? W) are included, in case you happen to be still using Apple Maps, because you are just as likely to end up here:

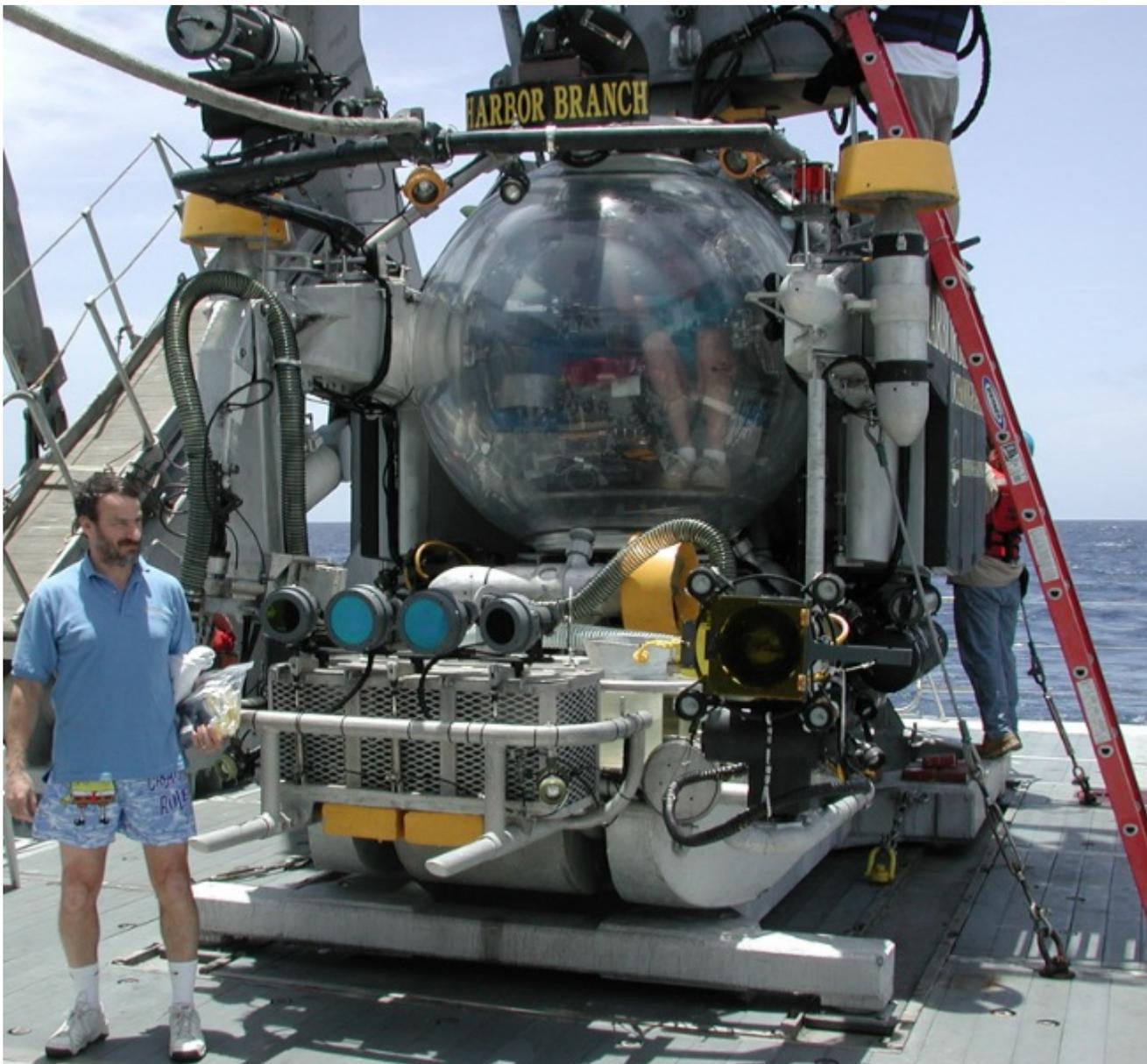


7,483 miles
↔



Apple Maps: Still not all that reliable

2. Get into one of these things.



A Johnson-Sea-Link I manned submersible. Not for claustrophobes.

3. Submerge 630 meters (2067 feet, seven football fields).

4. Look for an unknown sponge. This is not as easy as you'd think when you're 2067 feet below the water in something that looks like a shop vac with a glandular condition. Which sponge would you pick?



***Spongosorites* sp (a related species)**



***Spongeous Bobeous* (probably unrelated)**

Photo: Biological Education

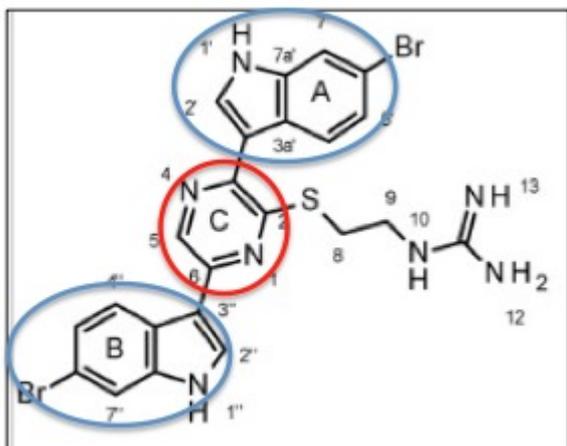
5. Get the stuff out of the sponge:

The sponge specimen was frozen at -20°C immediately after collection and stored frozen until extracted. The frozen material as described above (255 g) was extracted exhaustively with ethanol by macerating in a Waring blender, filtering off tissue and re-extracting the tissue a total of 5 times (2.5 L total). The extract was concentrated to a dark orange oil (15.9 g) by distillation under reduced pressure.

This may not sound so bad, but this does:

A total of 46.5 mg was obtained. Extrapolating back to wet weight of sponge, Dragmacidin G is a major component of the sponge and is present at 0.36% of wet weight of sponge.

If that strikes you like a lot of work to get 1/123rd of the weight of a teaspoon of salt from 16 pounds of an unknown sponge, you are right. But it may be well worth it. Dragmacidin G, the name given to the active chemical in the sponge is both very unusual looking (structurally) and also has some intriguing antimicrobial properties.



Dragmacidin G— a chemist's nightmare.

Dragmacidin G has a unique chemical structure—two 6-bromoindole groups (blue circles), each connected to a pyrimidine ring (red circle). While the image on the left may not have much meaning to you, any organic chemist will tell you that trying to solve (determine) the chemical structure of the abomination of the left will turn you into the guy on the right.

The amount of work this alone required, even with the most modern analytical instruments is your basic nightmare. Just to give you an idea...

Structure Elucidation

Inspection of the ^{13}C NMR spectrum coupled with HRMS data and isotopologue comparison suggested a molecular formula of $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_5$ for 1 ($M^+ \cdot H^+$) with observed 983.9668, calcd. 983.9671 ($\Delta = -0.12$ mmol) requiring 17 degrees of unsaturation. Isotopologue matching algorithms support the presence of sulfur in the molecule (see Figure S2). The ^{13}C NMR spectrum (see Figure S4) has resonances for 21 sp^2 hybridized carbons observed between δ_c 156.8 and 111.8 ppm and 2 sp^3 hybridized carbons observed at δ_c 40.2 and 28.7 ppm. The ^1H NMR spectrum (see Figure S3) coupled with the edited $g\text{-HSQC}$ (see Figures S5 and S6) and ^{15}N -HMBC spectra shows the presence of three protons attached to nitrogen (δ_H 11.85, 11.82, and 7.95), nine olefinic methine protons between δ_H 8.98 and 7.25 ppm, and four methylene protons observed as overlapping resonances between δ_H 3.58 and 3.53 ppm.

The ^1H NMR spectrum coupled with the edited $g\text{-HSQC}$ spectrum and a series of HMBC experiments support the presence of two 6-substituted-indol-3-yl ring systems in 1. The spectra for 1 have substantial overlap for the indole moieties, but careful analysis of the HSQC and HMBC spectra (including the ^{13}C -HMBC (see Figures S7-S11), a band selective ^{15}N -HMBC (see Figures S12-S14) and the ^{15}N -HMBC (see Figure S15)) allowed for assignment of all atoms in the indol-3-yl rings (see Figure S2).

For the first indole labeled A in Figure 1, residual one bond couplings observed in the ^{15}N - $g\text{-HMBC}$ spectrum assign the proton observed at 11.82 (H-1') as being attached to a nitrogen observed at δ_N 136. Correlations in the 2D- $g\text{-COSY}$ spectrum (see Figures S16-S21) show that this proton is coupled to the proton observed at δ_H 8.08 (H-2'). A 1,2,4-trisubstituted aromatic ring as found in 6-substituted indoles was suggested from the 2D- $g\text{-COSY}$ spectrum in which the resonance observed at 8.10 (δ_H - 8.9 Hz) shows coupling to a proton observed at 7.25 (δ_H - 8.8, 1.4 Hz), which in turn shows coupling to a proton observed at 7.70 (δ_H - 1.4 Hz). This latter proton shows a correlation in the ^{15}N - $g\text{-HMBC}$ to the nitrogen observed at δ_N 136 assigning it as H-2' of the indole ring. The chemical shifts of a number of carbons are very close, but most could be distinguished in a band selective ^{13}C - $g\text{-HMBC}$ experiment selected for carbon resonances between δ_c 130 and 160 ppm. All correlations predicted for a 6-substituted-5-indolyl functionality were observed (Table 1, Figure S25). The chemical shift of C-4' is consistent with bromine substitution (δ_c 115.37) as observed in the toposinins, dragmacidins, and related compounds; therefore, the bromine was assigned to this position.

	δ_c	δ_H	δ_N
1	156.8	11.85	136
2	156.8	11.82	136
3	156.8	7.95	136
4	156.8	8.98	136
5	156.8	8.98	136
6	156.8	8.98	136
7	156.8	8.98	136
8	156.8	8.98	136
9	156.8	8.98	136
10	156.8	8.98	136
11	156.8	8.98	136
12	156.8	8.98	136
13	156.8	8.98	136
14	156.8	8.98	136
15	156.8	8.98	136
16	156.8	8.98	136
17	156.8	8.98	136
18	156.8	8.98	136
19	156.8	8.98	136
20	156.8	8.98	136
21	156.8	8.98	136
22	156.8	8.98	136
23	156.8	8.98	136
24	156.8	8.98	136
25	156.8	8.98	136
26	156.8	8.98	136
27	156.8	8.98	136
28	156.8	8.98	136
29	156.8	8.98	136
30	156.8	8.98	136
31	156.8	8.98	136
32	156.8	8.98	136
33	156.8	8.98	136
34	156.8	8.98	136
35	156.8	8.98	136
36	156.8	8.98	136
37	156.8	8.98	136
38	156.8	8.98	136
39	156.8	8.98	136
40	156.8	8.98	136
41	156.8	8.98	136
42	156.8	8.98	136
43	156.8	8.98	136
44	156.8	8.98	136
45	156.8	8.98	136
46	156.8	8.98	136
47	156.8	8.98	136
48	156.8	8.98	136
49	156.8	8.98	136
50	156.8	8.98	136
51	156.8	8.98	136
52	156.8	8.98	136
53	156.8	8.98	136
54	156.8	8.98	136
55	156.8	8.98	136
56	156.8	8.98	136
57	156.8	8.98	136
58	156.8	8.98	136
59	156.8	8.98	136
60	156.8	8.98	136
61	156.8	8.98	136
62	156.8	8.98	136
63	156.8	8.98	136
64	156.8	8.98	136
65	156.8	8.98	136
66	156.8	8.98	136
67	156.8	8.98	136
68	156.8	8.98	136
69	156.8	8.98	136
70	156.8	8.98	136
71	156.8	8.98	136
72	156.8	8.98	136
73	156.8	8.98	136
74	156.8	8.98	136
75	156.8	8.98	136
76	156.8	8.98	136
77	156.8	8.98	136
78	156.8	8.98	136
79	156.8	8.98	136
80	156.8	8.98	136
81	156.8	8.98	136
82	156.8	8.98	136
83	156.8	8.98	136
84	156.8	8.98	136
85	156.8	8.98	136
86	156.8	8.98	136
87	156.8	8.98	136
88	156.8	8.98	136
89	156.8	8.98	136
90	156.8	8.98	136
91	156.8	8.98	136
92	156.8	8.98	136
93	156.8	8.98	136
94	156.8	8.98	136
95	156.8	8.98	136
96	156.8	8.98	136
97	156.8	8.98	136
98	156.8	8.98	136
99	156.8	8.98	136
100	156.8	8.98	136

Table 1. ^1H , ^{13}C and ^{15}N NMR data for dragmacidin G (1) ($\text{DMSO}-d_6$, ^1H 400 MHz; ^{13}C 100 MHz).

Similar arguments led to the assignment of the second 6-bromo-indol-3-yl moiety in 1 (labeled B in Figure 1). For the second indol-3-yl ring, residual one bond couplings observed in the ^{15}N - $g\text{-HMBC}$ spectrum assign the proton observed at 11.98 (H-1'') as being attached to the nitrogen observed at δ_N 136. Correlations in the 2D- $g\text{-COSY}$ spectrum show that this proton is coupled to the proton observed at δ_H 8.33 (H-2''). A 1,2,4-substituted aromatic ring is suggested by the spin system observed in the 2D- $g\text{-COSY}$ spectrum in which the resonance observed at 8.22 (δ_H - 8.9 Hz) shows coupling to a proton observed at 7.31 (δ_H - 8.8, 1.4 Hz), which in turn shows coupling to a proton observed at 7.71 (δ_H - 1.4 Hz). This latter proton shows a correlation in the ^{15}N - $g\text{-HMBC}$ to the nitrogen at δ_N 136 assigning it as H-2'' of the indole ring. Once again, the chemical shift of C-4'' was consistent with bromine substitution (δ_c 114.78) therefore, the second bromine was assigned to this position. As with the first indole ring, all expected correlations were observed in the HMBC spectra supporting the assignment of a second 6-bromo-indol-3-yl ring in 1 (Table 1, Figure S4).

The presence of an N-(2-mercaptoethyl) guanidine moiety in 1 was suggested by the data that follows. A broad four-proton multiplet was observed between δ_H 3.58 and 3.53 ppm in the ^1H NMR spectrum. Careful inspection of the edited $g\text{-HSQC}$, ^{13}C - $g\text{-HMBC}$, and ^{15}N - $g\text{-HMBC}$ allow for the assignment of one methylene group at δ_H 3.56 attached to a carbon observed at δ_c 28.7 and a second methylene group observed at δ_H 3.54 ppm attached to a carbon observed at δ_c 40.2. These ^1H resonances are too close to each other to detect scalar coupling between them, but each proton shows a correlation to the other carbon in the ^{13}C - $g\text{-HMBC}$ spectrum, suggesting the presence of ethyl functionality. Correlations observed in the 2D- $g\text{-COSY}$ spectrum between the protons observed at δ_H 3.54 ppm and δ_H 7.93 (δ_H - 4.8 Hz) extend this chain. The proton observed at δ_H 7.93 is attached to a nitrogen observed at δ_N 80 based upon residual one bond coupling observed in the ^{15}N - $g\text{-HMBC}$ experiment. The ^{13}C - $g\text{-HMBC}$ shows a correlation from the methylene group observed at δ_H 3.54 to the quaternary carbon observed at 156.8 ppm, suggesting that this carbon is attached to the nitrogen (N-10). The proton resonance observed at 7.93 has long range coupling to an additional nitrogen resonance observed at 72 ppm in the ^{15}N - $g\text{-HMBC}$ spectrum. The presence of an additional nitrogen resonance as well as the chemical shift of 156.8 is consistent with the presence of an N-ethyl guanidine functionality in 1. The molecular formula of 1 calls for the presence of a sulfur atom. A search of the literature reports the presence of an N-(2-mercaptoethyl)guanidine unit in phalloidin B isolated from the sponge *Phalloichthys* sp. [13]. Comparison of the NMR data to that of 1 shows a fairly close correlation (1: δ_H 3.56, 3.56, 7.93; δ_c 28.7, 40.2, 156.8; phalloidin B: δ_H 3.46, 3.46, 8.25; δ_c 31.7, 40.8, 158.6). The presence of guanidine functionality was confirmed by reaction of 1 with 2,4-pentanedione to form the 3,5-dimethyl pyrimidine derivative 2 (Figure 1).

The remaining atoms in the molecule are an olefinic methine group (δ_H 8.98 s, δ_c 156.8), three quaternary olefinic carbons (δ_c 130.2, 145.8, 143.3), and two nitrogen atoms (δ_N 324, 299) whose chemical shifts are consistent with being part of a heteroaromatic ring [14]. Correlations observed in the ^{13}C - $g\text{-HMBC}$ spectrum from H-2' to the non-protonated olefinic carbon observed at δ_c 143.3 suggests the attachment of the "A" indole to this carbon. Similarly, a correlation observed in the ^{13}C - $g\text{-HMBC}$ spectrum between H-2'' of the "B" indole and the carbon observed at δ_c 143.9 suggests the attachment of the "B" indole to this carbon.

The heteroaromatic methine proton observed at δ_H 8.98 has a number of correlations in both the ^{13}C - $g\text{-HMBC}$ and ^{15}N - $g\text{-HMBC}$ spectra that allow for construction of the "C" ring (Figure 2). Correlations observed in the ^{13}C - $g\text{-HMBC}$ spectrum between the methine proton observed at δ_H 8.98 and both C-3' of the "B" indole (δ_c 112.6), and the carbon observed at 143.9 suggested the attachment of the methine group (C-5) to the carbon observed at 143.9 (C-6), which is in turn attached to C-3'. The $^1\text{J}_{\text{CH}}$ coupling constant for this methine group was observed through residual one bond resonances in the ^{13}C - $g\text{-HMBC}$ experiment (see Figure S22) and was found to be 183 Hz, which is consistent with substitution of a single nitrogen atom [15]; therefore, a nitrogen substituent was placed as the final substituent on this carbon (C-4). The methine proton observed at δ_H 8.98 (H-5) showed a strong correlation in the ^{15}N - $g\text{-HMBC}$ spectrum to two nitrogens observed at δ_N 324 and 299. One of the nitrogens must be N-4, while the other must be attached to the carbon observed at δ_c 143.9. These nitrogen chemical shifts are consistent with nitrogens in a pyrazine ring [16]. The final atom to be placed in the molecule is the olefinic carbon observed at δ_c 130.1. By default, it has been placed between N-1 and C-3 to form the pyrazine ring. A correlation observed in the ^{13}C - $g\text{-HMBC}$

NMR analysis used to establish the structure of Dragmacidin G. Don't try this at home.

(Note: Since organic chemists use these same basic techniques to solve structures of run-of-the-mill compounds, we are at least somewhat proficient in doing so. But not with this bad boy. This one is like kicking an 80-yard field goal into the wind, wearing ballet slippers.)

Was it worth the effort? It would seem so. Finding new classes of antibiotics is as important an endeavor as any in pharmaceutical research.

(See: "A New Class Of Antibiotics From Lichens? Maybe [1].")

The article above is a few weeks old, but is useful for contrasting and comparing the two discoveries. The following table shows the antibacterial activity of the lichen antibiotics:

	<i>P. gingivalis</i>		<i>S. mutans</i>	
	MIC ^b (μm)	MBC ^c (μm)	MIC (μm)	MBC (μm)
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	—	—	80	80
7	80	80	80	80
8	40	40	80	—
9	80	80	80	80
10	80	80	80	—
11	20	20	10	20
13	80	80	20	80
penicillin G ^a	0.29	2.3	0.15	4.7

The antibacterial activity of compounds isolated from lichens. MIC values are expressed in micrograms/mL

I noted in the earlier article that while lichens did contain two chemical compounds that had antibacterial properties, they were not potent enough to become drugs. The orange circles indicate the MIC (minimum inhibitory contraction—the concentration of drug required to stop bacteria from growing—of compounds 11 and 13 against two different oral bacteria. By comparison, the reference sample penicillin G had MIC values ranging from 5-10 times lower, meaning that penicillin G is 5-10 times more potent than the lichen-derived compounds. For antibiotics with MICs in the 20-80 microgram/mL range to be effective, the required dose would have to be huge. Probably enough so to be toxic.

But, the table below tells a radically different story.

The MIC values of six well-known antibiotics from five different classes are given. **Here you can see resistance with your own eyes** by comparing the concentration of the antibiotic required to kill both susceptible and resistant versions of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*—the infamous and deadly MRSA*.

Antibiotic	<i>S. aureus</i> (susceptible)	MRSA* (resistant)
Penicillin	16.0	64
Methicillin	1.0	64
Ceftriaxone	4.0	>64
Meropenem	0.1	8
Azithromycin	1.0	>128
Cipro	0.5	32
Dragmacidin G	1.0	0.62

Antibacterial activity of common antibiotics (in micrograms/mL)

Source: <http://www.antimicrobe.org/b237tabrev.htm> [2]

Penicillin, which is rarely used, and methicillin belong to the penicillin class. Ceftriaxone is cephalosporin **(1)**. Meropenem is a carbapenem **(2)**. Azithromycin (Zithromax) is a macrolide, and Cipro is a fluoroquinolone. These are critically important antibiotics that were previously used to wipe out staph infections, but cannot do so anymore, as indicated by the 50-100 fold loss of activity against MRSA. Now look at Dramacidin G. It kills not only staph but MRSA too—something that other the six drugs can no longer do.

Will Dramacidin G make it to the pharmacy at your hospital? There is a long way to go. Toxicity is one of many potential pitfalls. But the discovery of a new class of antibiotics that can kill MRSA is quite an achievement by any measure.

Notes:

(1) Ceftriaxone is the last line of defense against gonorrhea. (See: [The coming gonorrhea epidemic](#) [3], New York Post, September 5, 2012)

(2) The even scarier infection, carbapenem-resistant enterococcus (CRE), which has a 50 percent mortality rate is resistant to Meropenem and other carbapenem antibiotics.

Original Source: Wright, *A Mar. Drugs* **2017**, 15(1),

COPYRIGHT © 1978-2016 BY THE AMERICAN COUNCIL ON SCIENCE AND HEALTH

Source URL: <https://www.acsh.org/news/2017/02/08/crazy-looking-promising-antibiotic-deep-water-sea-sponges-10840>

Links

[1] <http://www.acsh.org/news/2017/01/23/new-class-antibiotics-lichens-maybe-10734>

[2] <http://www.antimicrobe.org/b237tabrev.htm>

[3] <http://nypost.com/2012/09/05/the-coming-gonorrhea-epidemic/>