GULF SPECIMEN MARINE LABORATORIES, INC.

In 1968 I published my book "The Sea Brings Forth" (Rudloe, 1968). As part of promoting the book I appeared on the Today television show. I brought specimens along with me for the show. We also discussed the potential of developing natural marine drugs. As fate would have it, Dr. Jonathan L. Hartwell, who headed the US National Cancer Institute (NCI), caught the show over his breakfast. A few telephone calls later and I was on a mission: gather some marine organisms and send them in for testing. I collected a dozen specimens, put them through a meat grinder and sent them off, packed in isopropyl alcohol.

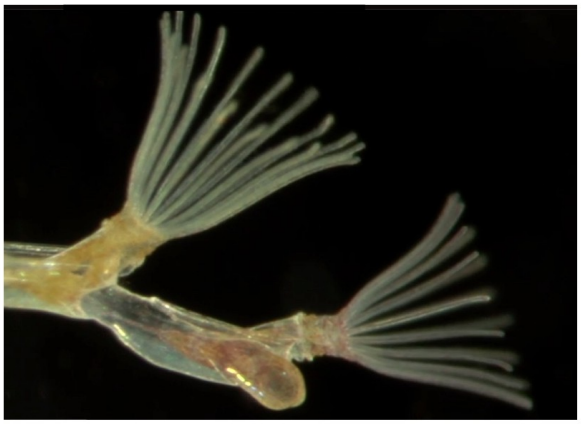
Among the specimens was a tiny filter feeding animal of the bryozoan phylum. This little animal looks like purplish red seaweed and it is called *Bugula neritina*. Before this, the greatest claim to fame the little purple animal had was as an invasive nuisance. It is a fouling organism that likes to take over the underside of docks and boats. After I shipped the ground up parts of this animal to NCI everything about our understanding of *Bugula neritina* changed. NCI discovered compounds from the *Bugula neritina* that stopped cancer in mice. The race was on to use *Bugula neritina* to cure cancer.

Research scientists eventually determined that the cancer fighting compound of *Bugula neritina* contains some twenty complex molecules called macrolide lactones. These are flat molecules with multiple rings and side groups. This family of compounds is named bryostatin. It took 30 years before bryostatin-1 was finally synthesized artificially in 1998. A large number of steps are required to synthesize bryostatin-1. The result is that even though it can be synthesized, the artificial product contains impurities (side products) that make it largely unsuitable for use as a medicine.

Bugula neritina actually contains very small amounts of bryostatin and which of the twenty types of bryostatin depends on where it is harvested. It took 14 tons of *Bugula neritina* collected from deep water off the coast of California to produce 18 grams of pure bryostatin-1 for cancer trials by NCI. The GSML collected *Bugula neritina* from the Gulf of Mexico contains bryostatins 4,5,6,7 and 8. A further complication is that the amount of bryostatin produced by *Bugula neritina* at any given time also varies. Some batches of *Bugula neritina* will give high yields while another batch collected at the same location at another time will give none. *Bugula neritina* is also a seasonally available creature. Sometimes there is so much *Bugula neritina* available it is easy to get pounds of it. A week later it might be impossible to find. GSMLs special knowledge of when and how to harvest *Bugula neritina* is critical for researchers. Over the 2017 Christmas break we collected and shipped 100 pounds of *Bugula neritina* to a pharmaceutical development company. We had two scientists independently come and visit us in order to learn about *Bugula neritina.* Scientists and researchers still need fresh *Bugula*

neritina from the sea. GSML is ready to provide it.

Unfortunately, as is so often the case, the early promise of bryostatin-1 did not get translated into working medicines. Bryostatin-1 did not so much cure cancers as halt their growth as long as the patient kept taking the drug. The cancer growth would resume once the treatment was stopped. Side effects, especially severe muscle pain, meant patients could not tolerate the treatment even though it stopped the cancer. Bryostatin-1 thus fell out of favour as a cancer drug. Nonetheless, research continued. There has been an explosion in our understanding how cells work since 1968. The level of our knowledge has doubled every year and been marked by milestones like the sequencing of the human genome in 2000. Back in 1968, it was not possible it know how bryostatin-1 actually worked. Today, we know exactly what bryostatin-1 does.



Every cell in the body of virtually all living things (except bacteria) contain a membrane bound protein called Protein Kinase C or PKC for short. PKC acts as a signalling molecule between the outer membrane and the nucleus. When PKC is activated it leaves the membrane and travels down and binds to various receptors triggering entire cascades of signalling molecules in the nucleus. This in turn affects which genes are expressed and by how much. Different cell types have different variants of PKC. Bryostatin-1 is a PKC activator causing a rapid release of PKC from the membrane and that in turns sends it off to be degraded. So by activating the PKC, bryostatin-1 turns off PKC signalling after an initial short boost. Bryostatin-1 works especially well on the type of PKC that exists in nerve and brain cells.

Scientists found that differences in PKC functioning are directly related to disease in humans. PKC malfunctioning is known to be a major cause of Alzheimer's disease. As soon as researchers saw the connection between PKC and disease, they went looking for PKC activators and they rediscovered bryostatin-1. Early human trials have show that bryostatin-1 reverses symptoms of Alzheimers in some patients. Because of the PKC-brain connection, bryostatin-1 is also being tested as a treatment for people with Fragile X syndrome and Parkinson's Disease. PKC activation is furthermore required in awakening the immune system so another exciting area of research is the use of bryostatin-1 to activate T cells in the immune system. This activates and exposes cells carrying latent HIV infection so that the infection can be destroyed in the body with other drugs. This has the potential to effectively cure AIDS by getting rid of all the virus in the body. As of February 2018 there were some 14,700 *Bugula*/bryostatin patents according to Google Patents. Google Scholar shows over 20890 scientific articles on bryostatin and *Bugula.*

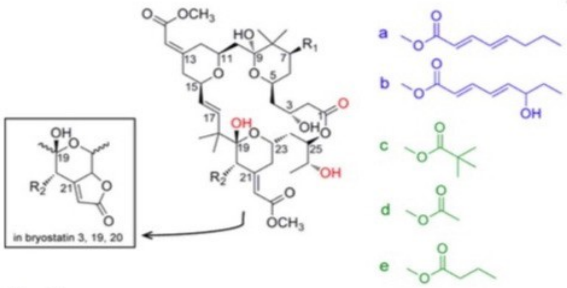
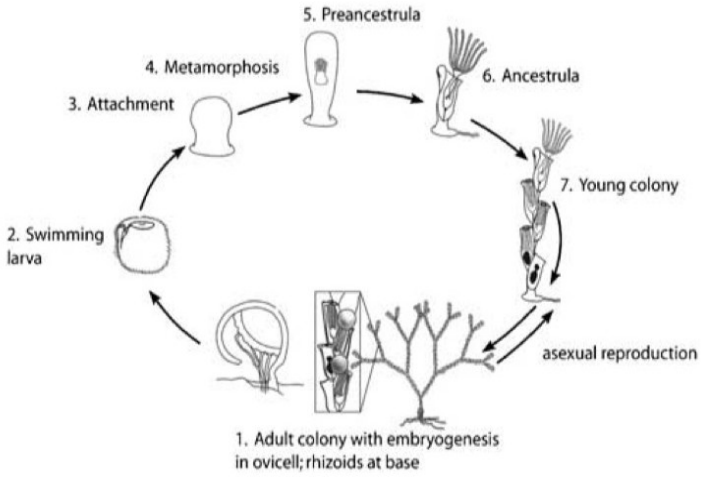


Figure 1.
Structures of bryostatins "0"-20 ("bryostatin 0" is the hypothetical precursor to all the known bryostatins). Bryostatins which contain octa-2,4-dienoate moieties (in blue) are found only in the Deep *B. neritina* sibling species, whereas Shallow *B. neritina* contains bryostatins with other kinds of esterifications (in green). The oxygen groups highlighted in red represent the pharmacophoric elements involved in binding to PKC.

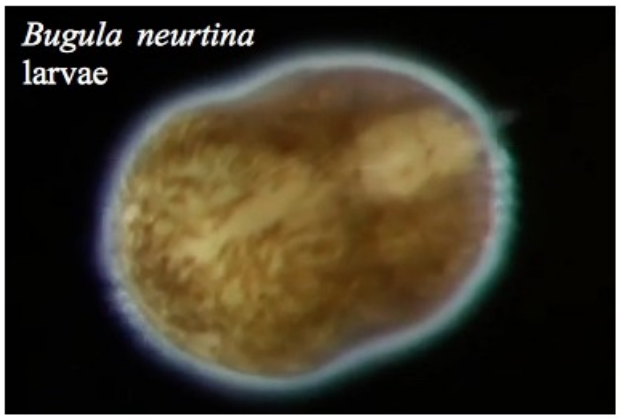
Bryostatin	R^1	R^2
"0"	OH	H
1	d	a
2	OH	a
3	d	a
4	c	d
5	c	d
6	e	d
7	d	d
8	e	e
9	d	e
10	c	H
11	d	H
12	e	a
13	e	H
14	c	OH
15	d	b
16	c	H
17	c	H
18	c	H
19	c	e
20	c	H

As research continued, scientists also learned that it wasn't the actual *Bugula neritina* animal that was producing the bryostatin. Inside the *Bugula neritina* are numerous symbiotic bacteria called Candidatus *Endobugula sertula.* Unfortunately this bacteria cannot survive outside of the *Bugula neritina* animal in a laboratory culture. The bacteria produce bryostatin which then collects in the tips of the adult. Bryostatin coats the swimming larvae when they are released. The bacteria is thought to produce bryostatin as a noxious substance that discourages fish and other predators from eating the

larvae. When *Bugula neritina* are mechanically disturbed they appear to almost explode, releasing clouds of purple red liquid into the water in a manner reminiscent of a disturbed octopus or squid. This liquid contains mixtures of bryostatin along with red and blue pigments and lots of little larvae. The blue pigments have been found to have their own antimicrobial properties which may function to protect the larvae from fungus and bacteria and the dark cloud of pigments may hide the larvae from predators like fish.



The mixture of bryostatin and pigments released from grinding *Bugula neritina* may have curative and restorative properties. When we were collecting for that huge order of *Bugula neritina* we dumped what we collected into sinks for cleaning and sorting. The sinks also contained two sick remora. We had isolated the fish for treatment but they were badly off enough that we had assumed they would die and we needed the tank. Imagine our astonishment when the last of the *Bugula neritina* was packed and shipped and the water cleared and we found the two sick fish were completely cured! We think *Bugula neritina* "blood" in sea water may work as a curative on fish with fungus and bacteria infecting wounds. We are working on some of our own experiments to test this. We know that most green sea turtles actively seek out and eat patches of pure *Bugula neritina.* Sea urchins and certain shrimp also dine on *Bugula neritina.* Maybe these animal know something about *Bugula neritina* that we don't. We are also planning on experiments to learn how to reliably grow large quantities of *Bugula neritina* so regular harvests can take place instead of just by the whim of the sea. Just as researchers have found that whole plant marijuana has healing properties not found just in TCH or other single constituents, we suspect that whole *Bugula* extract has properties of interest. We encourage the research community to look at the whole product. We are proud of the role we played in bringing *Bugula* to the medical research community. Gulf Specimen Marine Lab is ready to continue that partnership.



Jack Rudloe with Natalie K Gordon

Selected References

Clamp, A., Jayson, G.C., 2002. The clinical development of the bryostatins. Anti-Cancer Drugs 13(7), 673-683.

Coyne, M.S., 1994. Feeding Ecology of Subadult Green Sea Turtles in South Texas Waters [Master of Science Thesis]. Texas A&M University.

Halford, B., 2011. The bryostatins' tale. With the promise of treating cancer, Alzheimer's, and HIV this family of marine natural products continues t intrigue scientists more than four decades after its discovery. Chemical & Engineering News 89(43), 10-17.

Nelson, T.J., Alkon, D.L., 2009. Neuroprotective versus tumorigenic protein kinase C activators. Trends in Biochemical Sciences 34(3), 136-145.

Nelson, T.J., Sun, M.K., Lim, C., Sen, A., Khan, T., Chirila, F.V., Alkon, D.L., 2017. Bryostatin effects on cognitive function and PKCε in Alzheimer's disease phase IIa and expanded access trials. Journal of Alzheimer's Disease 58(2), 521-534.

Ruan, B.F., Zhu, H.L., 2012. The chemistry and biology of the bryostatins: Potential PKC inhibitors in clinical development. Current Medicinal Chemistry 19(16), 2652-2664.

Rudloe, J., 1968. The Sea Brings Forth. Alfred A. Knopf, New York.

Sharp, K.H., Davidson, S.K., Haygood, M.G., 2007. Localization of '*Candidatus Endobugula sertula*' and the bryostatins throughout the life cycle of the bryozoan *Bugula neritina*. ISME Journal 1(8), 693-702. (Lifecycle figure from this article)

Trindade-Silva, A.E., Lim-Fong, G.E., Sharp, K.E., Haygood, M.G., 2010. Bryostatins: biological context and biotechnological prospects.Current Opinion in Biotechnology 21834-842. (Bryostatin article from this article.)