CHAPTER 8
PHARMACOGNOSY IN ACTION. U. S. RESEARCH INSTITUTIONS

Harbor Branch Oceanographic Institution. Contributions to Pharmacognosy

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The Marine Natural Products Drug Discovery group at Harbor Branch Oceanographic Institution (HBOI), now a Research Institute at Florida Atlantic University, was founded in March of 1984 as the research arm of the start-up biotechnology company, SeaPharm. Using HBOI's fleet of oceangoing research vessels and deep-diving manned submersibles, SeaPharm planned to take discovery of marine natural products with therapeutic properties to ocean depths down to 3000 ft. Under the direction of Vice President of Research Dr. Kenneth Rinehart Jr. and later Drs. Ken Snader, Jake Clement, Gabriel Saucy and Oliver McConnell, a team approach was developed to find natural products with therapeutic potential for the treatment of cancer, infectious diseases (antiviral, antibacterial, antifungal), and immune modulatory agents. In addition to the HBOI team, SeaPharm assembled a team of internationally renowned marine natural products researchers to augment the capabilities of the in-house program, including Drs. Tatsuo Higa, Yoel Kashman, Peter Murphy, Murray Munro and John Blunt.

Pioneering the use of manned submersibles in exploration of deep-water habitats for the collection of organisms producing potential therapeutic agents, HBOI has explored deep-water habitats in the Caribbean, Atlantic, and Eastern Pacific Oceans. Over 975 dives have been conducted using the Johnson-Sea-Link or Clelia submersibles, resulting in a collection of over 12,300 samples of marine invertebrates from deep-water habitats. A culture collection of approximately 17,000 microbes derived from the tissues of these samples has been built. rDNA analysis of a sub-set of the collection has shown representation of many unusual and previously uncultured taxa. In addition to being evaluated for therapeutic potential, the HBOI specimens have been and continue to be used for a variety of research including taxonomic, ecological, evolutionary, and climatology investigations by investigators world-wide. Data collected from these dives (especially for the western Atlantic and Florida) have been used to develop better conservation and management strategies for deep-water habitats and to support the preservation of certain areas as Habitats of Particular Concern and Marine Protected Areas.

Over the course of the past twenty four years, HBOI researchers have published the structures of over 200 compounds with biological activity. These include discodermolide and the ecteinascidins (ET-743), which are discussed in the Milestone section. The structures of just a few of these are shown in the Figure below. The topsentins, first reported by Bartik *et al.* from *Topsentia genetrix*[1], were re-isolated by the Harbor Branch group and initially reported to have moderate cytotoxicity and antiviral properties.[2] Additional members of the bisindole family including the nortopsentins, dragmacidins, hamacanthins and 2,2-bis(indol-3yl)-ethylamine were also isolated from deep-water sponges of the genus *Spongosorites* and *Hamacantha* and their structures defined. Assay of the compounds by the Jacobs group at the UC Santa Barbara indicated that the compounds were potent inhibitors of both immunogenic and neurogenic inflammation with both oral and systemic activity. Lasonolide A, isolated from the sponge *Forcepia* sp., is a potent cytotoxic agent that shows significant affects on cell adhesion.[3]
Although the molecular target of lasonolide A remains elusive it has been shown to induce activation of multiple isoforms of PKC and the MAP kinase signal transduction pathway within

minutes of treatment and apoptosis after 24 hours of treatment. Work is ongoing to define its molecular target.

A deep-water lithistid sponge from the family Neopeltidae was the source of the two potent cytotoxic agents dictyostatin-1[4] and neopeltolide.[5] Dictyostatin-1 was originally reported by the Pettit group as a potent cytotoxic agent.[6] HBOI studies demonstrated that dictyostatin-1 is a potent microtubule stabilizing agent with very significant activity against paclitaxel and multi-drug resistant cancer cell lines. Dictyostatin-1 and its synthetic analogs continue to be the focus of research programs aimed at development of novel microtubule stabilizing agents for the treatment of cancer. Neopeltolide also demonstrates potent cytotoxicity, and cell cycle analysis of tumor cells shows arrest at the G1/S transition; research is ongoing to define its molecular target. Manzamine A, initially discovered by the Higa group during the early days of SeaPharm,[7] recently has been shown to restore an anchorage dependent growth in the AsPC-1 pancreatic cancer cell line; block tumor cell migration through a collagen membrane, and restore sensitivity of the AsPC-1 cell line to TRAIL induced apoptosis; it is being investigated as a possible therapeutic for the treatment of pancreatic cancer.

In addition to the discovery of marine natural products with therapeutic potential, the HBOI group has been actively engaged in research leading toward methods for sustainable production of bioactive marine natural products. Efforts have included the development of new media and methods for the culture of marine invertebrate cells with demonstration of the production of bioactive compounds in cultures; development of conditions for the production of biomass and compounds through aquaculture; development of novel culture methods for marine microbes; research to characterize the microbial community of deep-water sponges that produce bioactive compounds; and characterization of the biosynthetic genes present in invertebrate metagenomes.

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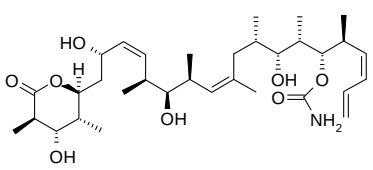
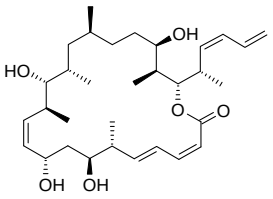
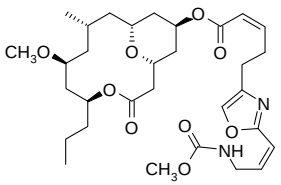
(2) Tsujii, S.; et al. *J. Org. Chem.* **1988**, *53*, 5446-5453.
(3) Horton, P. A. et al. *J. Am. Chem. Soc*. **1994**, *116*, 6015-6016.
(4) Isbrucker, R. A. et al. *Biochem. Pharm*. **2003**, *66*, 75-82.
(5) Wright, A. E.; et al. *J. Nat. Prod.* **2007**, *70*, 412-416.
(6) Pettit G. R. et al. *J Chem Soc Chem Commun* **1994**, 1111-1112.
(7) Sakai, R. et al. *J. Am. Chem. Soc*. **1986**, *108*, 6404-6405.



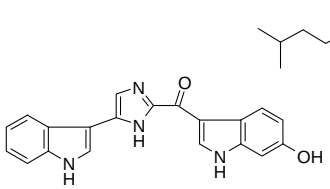
1 Discodermolide



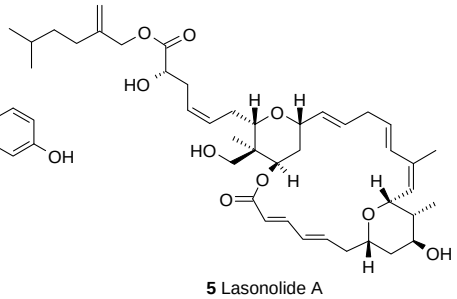
2 Dictyostatin-1



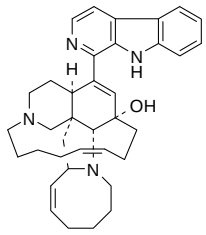
3 Neopeltolide



4 topsentin



5 Lasonolide A



6 Manzamine A

Natural Products Laboratory, Research Triangle Institute

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To describe the Natural Products Laboratory at the Research Triangle Institute (RTI), it is prudent to provide first some context on the founding of the Research Triangle Park (RTP). Histories of both RTI and RTP have been published many places, perhaps most completely by Larrabee[1] and Link.[2] The RTP was conceived and developed by a cadre of forward thinking scientists, business leaders, and politicians in North Carolina in the 1950s, and they probably had two major goals. The first was to create an engine of economic growth, particularly one that could leverage the research expertise of the three area universities (Duke University in Durham, the University of North Carolina at Chapel Hill, and North Carolina State University in Raleigh). The second, related goal was to create a climate that stymied "brain drain," generating viable jobs for the graduates of these institutions, thereby preventing the steady migration of the State's best and brightest individuals to major cities outside of NC. The first entity within the newly established RTP was the RTI, which was founded as a private, non-profit institute with a mission "to improve the human condition" in December, 1958.

In the early years, the first president of RTI, Mr. George Herbert, spent considerable effort in the creation and staffing of RTI, and in this, Monroe E. Wall, Ph.D. was one of his early key hires. Dr. Wall had been working for the Eastern Regional Research Laboratories of the US Department of Agriculture (USDA) near Philadelphia for nearly two decades, and he spent most of the 1950s screening plants for cortisone precursors. Fortuitously, he had saved extracts of many of the samples. The National Cancer Institute (NCI) learned of Dr. Wall's collection and requested aliquots of 1000 plant extracts to test for anticancer activity. Nearly a year later, Dr. Wall received their exciting results, which showed that a sample of *Camptotheca acuminata* had very promising activity.[3] He applied for support from the USDA to begin research on *C. acuminata*, but was denied permission for political reasons. Thus, at the time that RTI was searching for someone to found their chemistry research operations, Dr. Wall was frustrated with the USDA and he was promised support from the NCI, no matter where he went. Mr. Herbert learned of this, and he actively pursued Dr. Wall, luring him to visit RTI in Feb., when the weather in Philadelphia was awful but just turning to spring in NC. In July 1960 Dr. Wall joined RTI as "Head, Natural Products Laboratory." His nascent group began research in the Bacon Street Annex in Durham. Dr. Wani, who joined RTI in 1962 and continues working there today, even as a retiree, jokes that in 1960 RTI was only four bare walls, and it was only when the "fifth wall," Dr. Wall, joined RTI that things began happening.

In the early years, the NCI supported much of the chemistry research at RTI, including a contract to examine plants for anticancer activity, which ran from March 1961 until early 1979. This contract led to the discovery of camptothecin in 1966[4] and taxol in 1971.[5] These seminal discoveries are described in more detail in their own separate chapters of this book, and a National Historic Chemical Landmark was bestowed upon the Natural Products Laboratory for these discoveries by the American Chemical Society in 2003.[6] These compounds have had a huge impact on cancer chemotherapy, and as just one indicator, in 2002, the global marketplace for antineoplastic agents was estimated at $9 billion annually with the combined sales of taxol and the camptothecin analogs totaling at least a third of that market.[6] RTI never profited from these agents. This was not because Dr. Wall did not recognize their value. Rather, at the time those discoveries were made the US laws were structured such that inventions originating from

government sponsored research could not be patented, a rule that was only supplanted with the passage of the Baye-Dole Act in 1980.

The Natural Products Laboratory grew steadily over the years, with the name for the entire enterprise being changed to Chemistry and Life Sciences (CLS) in 1966, and Dr. Wall as its first vice president. He catalyzed many areas of research outside of natural products within CLS that continue today, such as the analysis and metabolism of cannabinoids, the synthesis of steroids, and radiochemistry. In nearly all cases, he would get the project started and then turn it over to one of the many promising scientists he hired, often from one of the local universities, fulfilling one of the early goals of RTP.

With respect to natural products research, Dr. Wall always led the Natural Products Laboratory. He never retired, serving as Chief Scientist until his death in 2002 at the age of 85. Working with his long-time colleague, Dr. Wani, the Natural Products Laboratory (NPL) focused on two main areas of research. One of these, which was initiated soon after the discovery of camptothecin and has continued in many different guises until the present day, was the synthesis of camptothecin analogs that had improved solubility and minimized side effects;[7] the Wall and Wani team have been awarded more than 20 patents on second- and third-generation camptothecin analogs. The other was the discovery of new, bioactive compounds from plants. After the NCI contract expired in 1979, they worked on chemopreventive agents in collaboration with Dr. Pezzuto from the University of Illinois at Chicago (UIC) for a few years, and from 1990 to 2005, they were part of a National Collaborative Drug Discovery Group (NCDDG) to uncover anticancer agents from plants, largely in collaboration with a team of researchers from UIC, including Drs. Cordell, Farnsworth, Kinghorn, Pezzuto, and Soejarto.[8,9] Today, the NPL continues to focus on drug discovery, typically in the anticancer realm, but from varied source materials, such as filamentous fungi and predator bacteria, and also on the analysis of herbal drugs, particularly towards the development of reference standards.

(1) Larrabee, C. X. *Many Missions: Research Triangle Institute's First 31 Years*; Research Triangle Institute: Research Triangle Park, NC, 1991; pp 208.
(2) Link, A. N. *A Generosity of Spirit: The Early History of the Research Triangle Park*; Research Triangle Foundation of North Carolina: Durham, NC, 1995; pp 149.
(3) Wall, M. E. *Camptothecin and Taxol*; In *Chronicles of Drug Discovery*; Lednicer, D., Ed.; American Chemical Society: Washington, DC, 1993; Vol. 3, pp 327-347.
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(7) Wall, M. E.; Wani, M. C. *Camptothecin*; In *The Monoterpenoid Indole Alkaloids*; Saxton, J. E., Ed.; John Wiley & Sons, Ltd.: Sussex, England, 1994, pp 689-712.
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Natural Products Chemistry at the Scripps Institution of Oceanography

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The study of marine natural products chemistry is not a discipline embraced by the oceanography research community. But over the years, this discipline has become one of the strongest programs at the Scripps Institution of Oceanography (SIO). As the school of oceanography for UC San Diego, SIO evolved from a small marine biology group in the early 1900s to a major research institution with more than 1400 faculty, professional researchers, staff and students. At the time of writing, SIO had attracted some of the major figures in marine natural products research and, arguably, established itself as the major center for this discipline.

In 1968, the classical marine chemists at SIO decided they wanted to attract a first-class organic chemist to study the organic components of seawater. They decided upon D. John Faulkner, a young British synthetic organic chemist with superb credentials. Faulkner had received his Ph.D. from Imperial College under Derek Barton and had just completed postdocs with R. B. Woodward and William S. Johnson. Academically, Faulkner was a superb recruitment for SIO, but the marine chemists soon found that he was not going to be directed to examine the mundane topic of seawater organics. Instead, he struck out to continue his work in synthesis by establishing a small synthetic program with targets such as tetrodotoxin. Needless to say, the senior faculty at SIO did not see this turn of events in a positive light…synthesis was simply not appropriate for SIO!

In 1970, I was a postdoc with Jim Sims at UC Riverside. Jim is a classic plant natural products chemist, who I had convinced to investigate the chemistry of marine plants and animals. Although, in 1970, there was little evidence that this field would be rewarding, he and I visited SIO and asked for the assistance of Ralph Lewin to locate some of the local red algae. One study in Japan (Toshie Irie) had shown a terpene produced by red alga *Laurencia*, hence we thought that our local seaweeds warranted study. At that time, the Southern California organic chemistry community was small and most were acquainted. Consequently, Sims and I visited Faulkner and made the connection that would, in 1973, facilitate my joining the staff at SIO.

I was hired at SIO by a visionary director, John D. Issacs, who loved to read articles on economic botany. Immediately, he recognized the future of marine natural products chemistry and provided me with the elements needed to start my career. Faulkner and I realized that we had a significant challenge ahead of us. Not only did the SIO community lack interest in this field, but it was a serious uphill battle to also convince the granting agencies (NSF in particular) that this would be a field of growing and undeniable importance. Couple this with the fact that instrumentation for organic chemistry (NMR, UV, IR, etc.) was non-existent, and that we were a quorum of two, the prognosis for creating a sizable effort was bleak. Nonetheless, we made inroads, albeit slowly. In the early 1970s, the SeaGrant Program was established and a UC office was established at SIO. SeaGrant, which had in its charter "drugs from the sea" was the first agency to see the benefits of our discipline and we began a strong program in SeaGrant-funded collaboration with Bob Jacobs at UC Santa Barbara.

We both had a strong focus on training students and worked closely to establish a joint program, which included a full complement of courses in chemistry and marine chemical ecology. We were proud of our program and, still today, teaching students continues to be a strong focal point of the marine natural products program (now within the Center for Marine Biotechnology and

Biomedicine). Our research programs grew, and the faculty began to realize the importance of this field, especially in the chemical ecology of defense, chemical communication and symbiosis. Chemicals were slowly being recognized as the foundation of trophic level interactions even in the open oceans, and the field of marine natural products chemistry became one of respect and importance at SIO and throughout the world. Eventually, we convinced the SIO administration and the granting agencies that instrumentation for our work was important. This was never easy, however, as with just two PIs, it continued to be very difficult to fund NMR instrumentation and to support the instruments we had. Even today, with five faculty in this field, instrumentation remains a significant challenge. By the 1990s, we had sizable teaching (about 20 graduate students, 10 postdocs) and research programs that had depth and impact. We had begun to attract NIH funding for our cancer drug discovery efforts and had made significant discoveries in anti-inflammatory drug discovery. SIO was on the map as a major center of marine natural products research.

One Friday evening in 2002, all of us at SIO were shocked to learn that John Faulkner had passed away during a surgical procedure. The community, and especially I, was stunned by this. What would we do without our colleague? What would I do with his 8 graduate students, 3 postdocs and 2 career technicians? I had promised John to be sure that his students were well taken care of "should any problems arise" (in his surgery). Thus, at the beginning of 2003, most of his students had joined my group, which now consisted of 18 graduate students, 10 postdocs, and 6 career technicians. How would all of these people be funded and be given the proper attention? It was the biggest challenge of my career. Hopefully, it was done well. I was reeling with the loss of John, but I knew that progress at SIO had to continue. The director was fairly positive about replacing John, but he was being pressured from many programs to allocate the position to them. Finally, after over 6 months of lobbying, I was told that he would offer half of a position, and then only if I could acquire the other half elsewhere. Fortunately, the founding dean of the newly founded UCSD school of pharmacy, Palmer Taylor, was receptive to providing half of a position to create a joint appointment between SIO and the new Skaggs School of Pharmacy and Pharmaceutical Science (SSPPS). The more he thought about this linkage, the more enthusiastic he became, and shortly afterward he proposed that two new positions be created and that he and SIO contribute equally.

With two positions in hand, SIO recruited Bill Gerwick and Brad Moore, both having pharmacy appointments but focused on marine topics. New research faculty were also hired. Paul Jensen and Lena Gerwick joined our program, as did Ted Molinski and Pieter Dorrestein (both later recruited to UCSD in pharmacy and chemistry). Research at SIO has never been more vibrant; the new additions dramatically changed what we do and how we do it. The fields of biosynthesis, genomics, and semi-synthesis have now become exciting new elements of our discipline, and SIO has maintained its leadership in marine natural products chemistry by making significant new strides to take this discipline into the next generation of multidisciplinary science.