MARINE RESOURCE DEVELOPMENT:

Pharmacology and Bio-Organic Chemistry

A Proposed National Research Strategy

Sea Grant Working Committee for the Development of Bio-Organic Chemistry and Pharmacology of Marine Resources

PREFACE

The discovery of new pharmaceuticals and other useful chemicals from marine resources is an exciting venture that has the ability to stimulate productivity in the commercial sector. However, the process of isolating, identifying, and commercially developing biologically active substances from marine resources is lengthy and costly. As a result, the close collaboration of university researchers, industry, and government is essential. Only such collaboration can ensure that research results will ultimately find commercial application, thereby stimulating economic growth in the pharmaceutical and other industrial sectors of our economy.

The National Sea Grant College Program has had a long history of supporting research in marine pharmacology and natural product chemistry through several of the state Sea Grant Colleges. The success of these efforts has made it clear that a truly national strategic research initiative is essential if we are to ensure U.S. leadership in this vital field. The intent of this proposal by the Sea Grant Working Committee for the Development of Bio-Organic Chemistry and Pharmacology of Marine Resources is to stimulate development of such an initiative. We urge your support.

Jack R. Davidson, Director
Hawaii Sea Grant College

James J. Sullivan, Director
California Sea Grant College

Manuel Hernandez-Avila, Director
Puerto Rico Sea Grant College

EXECUTIVE SUMMARY

Marine Resources and Drugs for the Future: The Need For This Research

As members of the Sea Grant Working Committee for the Development of Bio-Organic Chemistry and Pharmacology of Marine Resources, we believe that the United States must undertake a national strategic research initiative both to expand research and to develop the human resources necessary for our nation to maintain leadership in the field. In our proposal for a strategic research initiative, we have spelled out the special scientific focus that a U.S. initiative should adopt.

The underwater world is a unique source of new medicinal agents. And, for a number of reasons, the next decade is the time to exploit this resource through a strategic research initiative. While the yield of truly novel chemicals from traditional sources for today's drugs (terrestrial plants and microbial fermentations) has declined, the pioneering studies of marine natural products chemists have shown that almost every class of marine organism contains exciting new molecules. Pharmacologists, physiologists, and biochemists have demonstrated that many of these marine natural products possess properties that modify fundamental life processes in ways that have important biomedical applications. These molecules are the leads that the pharmaceutical and chemical industries have begun to focus on as templates for development of the drugs of tomorrow.

Why is a national strategic research initiative called for? There are four reasons:

•*There is a national need to develop a focused program in marine bio-organic chemistry and pharmacology that provides the economic and intellectual infrastructure required to produce world leadership in this vital research field.* The proposed strategic initiative is not duplicated at any other national funding agency. It is ideally suited to the Sea Grant program, which already has the experience and infrastructure to accomplish this vital task. The strategic research initiative would be designed to take advantage of the individual skills of academic researchers to discover new molecules and to define their basic pharmacological properties so that the pharmaceutical industry can rapidly develop entirely new forms of medical therapy. The emphasis would be on the discovery of unique marine natural products and identification of their role in life processes; such work would provide a bridge leading to their employment as probes and drugs to advance our understanding and control of cellular and molecular processes.

•*There is a need to develop the human resources necessary to achieve national leadership in marine pharmacology and bio-organic chemistry.* A human resource program must be initiated through the development of strategic Sea Grant Post-Doctoral Fellowships. Post-Doctoral Fellows funded directly through Sea Grant would have a unique opportunity to become established at the cutting edge of modern bio-organic chemistry and pharmacology. They would learn to bring the tools of biotechnology, cellular and molecular pharmacology, and chemistry to bear on entirely new questions dealing with the fundamental mechanism of action of marine natural products.

•*If the United States does not take a leadership role, other countries will.* Scientists must also move rapidly to explore the unique resources of the marine environment because of foreign competition. A national effort in the form of a strategic research initiative is needed to provide the U.S. pharmaceutical industry with a competitive edge in the utilization of marine resources. High value-added chemical exports such as pharmaceuticals are one of the few positive trade areas in the U.S. economy. Failure to vigorously explore marine resources could lead to an erosion of this favorable balance.

•*Marine organisms are a rich but threatened source.* The genetic diversity and uniqueness of marine organisms makes them a vital and irreplaceable resource. The majority of plant and invertebrate phyla on Earth are either predominantly or exclusively marine. This is particularly true of the more primitive and ancient groups of organisms that have evolved little and adapted special mechanisms to produce bioactive chemicals for their daily and long-term survival. Many of these organisms are so poorly understood that they have yet to be fully described and named. Many of the larger, more complex marine organisms contain primitive symbiotic microorganisms in their cells that allow them to survive in the ocean environment. The open ocean is also home to a vast array of photosynthetic plants, microorganisms, and bacteria, all capable of synthesizing new molecular structures. Their metabolic activity is as vital to the Earth's well-being as are tropical rain forests. Yet these unique marine resources are rapidly declining because of the changes caused by human activities, which are modifying coastal waters and in some cases destroying the reef habitats that harbor the greatest diversity of marine organisms. Extinction--the death of birth--is removing these chemical factories at a much greater rate than they can be explored. The highest priority must be given to exploration of this vital resource in a focused effort to discover and characterize the most medically relevant organisms and the bioactive chemicals they produce.

For the above reasons, immediate action is essential. We therefore call for a national strategic research initiative in the amount of $1.5 million a year over a six-year period, to support both research and the establishment of Post-Doctoral Fellowships in marine bio-organic chemistry and pharmacology.

RESEARCH PROGRAM IN MARINE NATURAL PRODUCT CHEMISTRY

1. *The Discovery of New Natural Products from Marine Resources*

The strategic research initiative must include exploration of the chemical constituents of smaller and less common marine organisms. It must investigate the potential to culture symbionts from larger invertebrates since it is possible that symbionts may be responsible, in part, for complex chemical biosynthesis. Chemists investigating these phenomena will discover new biochemical pathways and mechanisms of drug biosynthesis in addition to discovering new molecules. The key to a successful drug discovery program, particularly one based on marine natural products, is the availability of highly specific bioassays that require the sacrifice of very small quantities of material. Biotechnology in the form of genetic engineering has begun providing many new bioassay systems that meet these requirements. Some assays might test for activity in genetically altered bacteria or yeast. Other assays can detect inhibition of specific enzymes or binding to receptor sites, showing high specificity and affinity for important biological processes. The new bioassay systems, some of which are becoming available in chemists' and in pharmacologists' laboratories will aid in the discovery of many new molecules. Some of these new methods will also be adapted to field studies.

2. *Defining a Pharmacophore Through Molecular Structure, Synthesis, and Computer Modelling*

The discovery of a potentially useful biological activity leads to the next stage of drug development: structural characterization and analysis. The area of molecular characterization has benefited tremendously from advances in digital electronics, and today's analyst has a suite of instruments of incredible power. Two methods in particular, X-ray diffraction and nuclear magnetic resonance spectroscopy (NMR), are able to completely define the three-dimensional structure of molecules with only micrograms of material. An important aspect of this strategic research initiative will be access to state-of-the-art structural tools.

Once a three-dimensional structure is available, the job of analyzing how the molecule exerts its biological influence can rationally begin. One important question is what part (or parts) of the molecule are responsible for activity? This is what chemists and pharmacologists mean by defining the pharmacophore. A variety of new and powerful aids are being developed to help in such analyses, including powerful calculational and visualization tools. Developments in the linked fields of computer modelling and computer graphics are truly awesome. It is now possible to sketch a molecule on a computer screen and find out its detailed molecular shape through a few minutes of calculation. It is even possible to see how a molecule fits into a particular enzyme. So a scientist can take a lead compound suggested by a marine natural product and determine whether it can be improved to enhance potency and specificity.

3. *New Approaches to Bio-Organic Synthesis: Marine Organisms as Bioreactors*

Many marine natural products possess unique functional groups and structures that are responsible for their biological activity and medical applications. Insufficient quantities of these drugs have often precluded their clinical and industrial development. Even with organisms that can be activated (such as bacteria, fungi, and microalgae), growth rates and drug yields have often been poor. Thus, for largely economic reasons, marine organisms have remained vastly unexploited as a direct resource of new drugs.

Revolutionary advances in molecular biology over the last decade offer potential solutions to production of required quantities of drugs. Isolation of the enzymes in biosynthesis will lead to the isolation of the genes encoding these enzymes. The stage will then be set for the production of large quantities of these enzymes, biosynthetic intermediates, or the drugs themselves. Theoretically it should also be possible to identify and transfer the genes involved in biosynthesis into a more manageable organism for drug production. Thus the aim is to incorporate enzymes, cell-free extracts,

particular cell types or organelles, or whole marine organisms in culture into drug design and synthesis.

It will be necessary to determine the biosynthetic pathways of metabolites in marine organisms, utilizing not only traditional approaches, but also modern molecular techniques. New methodologies will undoubtedly have to be developed and implemented. Ultrasensitive assays for the natural products of interest will need to be developed. Novel enzyme activities and substrate specificities are expected to be discovered. Since secondary metabolites often play important roles in protection and survival of the host organisms, the structures that are frequently found in marine natural products will undoubtedly involve novel biochemistry.

4. *Transformations of Molecular Structure in Drug/Receptor Reactions*

A new direction in chemistry is proposed, which involves characterizing the final structure that results when a drug reacts with its receptor. Recent evidence suggests that certain high-affinity interactions of certain drugs with their receptors involve a complex molecular reaction with highly specific amino acid sequences. That is, the reaction of a small molecule with a large macromolecule can entail a high-velocity molecular transformation that can lead to dramatic, unpredicted conformational changes in drug and receptor structures. The challenge to chemists is to devise new approaches to demonstrate the structural features of a drug/receptor conjugate. This can be accomplished through chemical synthesis and development of analogs with unique properties. Direct analysis of drug receptor conjugates is a goal that can be achieved through advances in combined computer simulation, nuclear magnetic resonance, and X-ray crystallography.

In order to accomplish these goals, highly purified and well-defined receptors, fragments of receptors, and enzyme structures will be made available using the tools of biotechnology. These future drug targets and methods to determine their suitability and specificity will be a goal of this research program.

This effort is vital to describe the truly novel characteristics of a natural product and its inherent bioactivity. The basic research is fundamental to generation of designer drugs by the pharmaceutical industry.

5. *Culturing of the Vital Marine Organisms*

Considerable advantage is to be gained by being able to generate or culture organisms which possess a desirable natural product. This is particularly true when the desired organism is from a geographically remote location, from an inaccessible site, or is simply uncommon. Culture offers other advantages beyond reliance on field-collected material as well. Only through culture experimentation can the role played by associated organisms be evaluated. For autotrophic organisms, culture allows evaluation of how nutrients and conditions of light and temperature interact to affect production of a particular metabolite. In this way, controlled conditions may be established which result in optimal laboratory production of a particular metabolite. In cases where a marine natural product has been demonstrated to be a useful drug and where laboratory synthesis of the compound is difficult or impossible, culture of the organism becomes essential to the economic development of that drug. Culturing is also vital to investigating the biosynthesis of a marine natural product and the function it plays in life processes such as respiration, growth, reproduction, and biomineralization. If enzyme modification is fundamental to adaptation and biosynthesis of a marine natural product, culturing represents an ideal way to clone a specially adapted biochemical process.

RESEARCH PROGRAM IN CELLULAR AND MOLECULAR PHARMACOLOGY

1. *Site and Mechanism of Action of Marine Natural Products*

Our understanding of complex disease processes exemplified by aberrant function of components of the immune system that lead to inflammatory disease, drug resistance, and other serious medical problems is advancing rapidly. Such understanding is allowing us to explore how drugs work (i.e., their mechanisms of action) and to provide new leads both to bio-organic chemists and to other biomedical scientists. Based on our collective experience, mechanism-of-action studies of marine natural products define the true importance of a compound. Defining the compound's structure along with its fundamental mode of action is the goal of the collaborative interaction between academic bio-organic chemists and pharmacologists.

The biological analyses of purified marine natural products are to proceed simultaneously to gain pharmacological profiles and to initiate mechanism-of-action studies for specific compounds of interest. This will be approached by exploiting the biological process from which the initial lead was obtained and also by carefully defining the pharmacological properties of the chemical in established experimental models. Collaboration with chemists is to be developed around the need for more structural work; chemists will determine the affinity and specificity of each drug. A large data base is to be incorporated by the use of networked computers to bring together leads from a massive base of available information and to focus on developing information about the effects of a drug on physiological, cellular, molecular, and biochemical processes. Individual scientists who are defining new receptors will be able to utilize these new drugs to dissect the properties of their receptor at the chemical level. This information will lead to further collaborative work to determine the functional units of a natural product necessary for a biological response. Using the powerful new 3-D computer display capabilities, new molecules can be hypothesized and then tested after synthesis. As this fundamental information is developed, the pharmaceutical industry can bring its highly advanced technology to bear, to exploit and develop these new leads.

2. *Drug Targeting and Bioactivation of Marine Natural Products*

Drug targeting and defining the specificity of action of marine natural products requires the latest technology to determine the spectrum of activity of a drug. Certain marine natural products are being shown to act as pro-drugs that are selectively taken up by cells, then bioactivated through cell metabolism into an active drug. Taking advantage of this process in specific ways allows cell or organ specificity to occur. To accomplish these tasks, collaborative synthesis and testing to define the pharmacophore and its special features will be undertaken. A second approach uses the technology of linking an appropriate ligand or antibody to a special cell-surface protein to concentrate a marine natural product at a therapeutic target. This approach is being attempted in cancer treatment, and although it is in a very early stage of development, it promises to provide new avenues for the utilization of marine natural products in the future.

3. *Biosynthesis and Biochemistry: Role of Natural Products in Cellular Processes*

Several examples of cellular processes in marine organisms have been shown to be functionally similar to those in terrestrial organisms; however, marine organisms have adapted to the complex and often hostile environment of the ocean. This adaptation is often accomplished by unique modification of enzymatic pathways regulating fundamental metabolic processes. In a general sense, it appears that this chemical adaptation involves subtle modification of specialized enzymes in critical biochemical pathways. It has been recently demonstrated that during biosynthesis specially adapted marine enzymes modify the substrate in unique ways. Thus, these genetically unique marine organisms can take the same chemical and synthesize dramatically different bioactive natural products. These novel substances are being shown to react with receptors and processes by wholly unpredicted mechanisms. As a result, much deserved attention is being stimulated in the biomedical community by marine natural product research. We wish to develop and expand this field of research by intensifying investigations

of genetically important organisms. We also wish to fully understand the pleomorphic capacity, specificity, and structure of these unique and important enzymes.

4. *Drug Receptors: Specificity and Mechanism of Action*

Marine natural products are among the primary tools a pharmacologist uses to understand the underlying changes drug receptors undergo when responding to or reacting with a drug. These natural products are also used as ligands to isolate, purify, and characterize drug receptors.

One of the areas which requires urgent attention is marine toxin research. Increasing incidents of toxic shellfish and fish resulting from red tides and the occurrence of new types of toxins are endangering fishery industries, public health, and marine ecosystems. In general, marine toxins are highly targeted to specific sites and have unique structural features not found in terrestrial compounds. For example, paralytic shellfish toxins and tetrodotoxin derivatives are specific nerve impulse blockers. The Florida red tide toxins are sodium channel activators with unprecedented polyether structures. Another class of shellfish toxins, known as diarrhetic shellfish poisons, have been shown to be a completely new type of phosphatase inhibitor. Domoic acid, the culprit of the recent Canadian mussel poisoning, is also a unique excitatory amino acid: it acts on the brain glutamate receptors. Most of these compounds have become important tools or lead compounds in the development of therapeutic drugs for such diseases as cancer, Parkinson's disease, and Alzheimer's. Because of their unique properties, many are also being used as ligands to isolate and characterize receptors. There must be a broadening of this research to discover new types of marine toxins and to utilize these substances to discover new receptors.

The Sea Grant effort must focus on the mechanism of action of each toxin, its site of action, and the specific subunits of a receptor affected by the toxin. New receptors must be identified as part of this research program and the gene isolated and expressed in cultured organisms by recombinant DNA techniques. New screening models will be developed for bioassay-guided fractionation of marine natural products, thus providing an important bridge between chemists and biologists both in academia and industrial research laboratories.

5. *Pharmacological Models of Cellular and Molecular Processes*

One of the most exciting areas in marine pharmacology is the investigation of chemical regulators of cellular and molecular processes in marine organisms. Marine models have long been employed to work out details of mammalian processes. Examples of such marine models include the squid axon in neurophysiology and the electric organ of the skate, used as a rich source of acetylcholine receptors and enzymes. Models of nervous system function have been eloquently described in studies of marine invertebrates.

New marine models must be developed to utilize our modern understanding of cellular and molecular processes. Particularly important would be studies of growth (mitosis and cytokinesis), reproduction, respiration and photosynthesis, biomineralization, and other processes under chemical control in marine organisms. Understanding the unique differences between marine organisms and their terrestrial counterparts at the biochemical and molecular level is vital to understanding comparable processes and their relationship to diseases in man. Investigation of marine cellular and molecular processes is also fundamental to understanding biosynthesis of marine natural products.

RECOMMENDATIONS

Academic chemists and pharmacologists recommend the formation of clusters of collaborating scientists for the purpose of discovering new marine natural products through new and novel approaches and identifying important new drugs and toxins. These independent researchers will collectively interact with the pharmaceutical, chemical, and biotechnology industries, thus producing a network of technology transfer under the auspices of Sea Grant. The need for this initiative is very great if we are to expand and maintain U.S. leadership in this important field.

Administrative Recommendations

The proposed research initiative, "Marine Resource Development: Bio-Organic Chemistry and Pharmacology," would be coordinated by the directors of the participating Sea Grant institutions together with a program monitor assigned by the National Office of Sea Grant, Office of Oceanic Research Programs, National Oceanic and Atmospheric Administration. The overall program calls for a six-year cycle. Programmatic interaction and exchange would be facilitated by a workshop for participating scientists in the second, fourth, and sixth years. Meetings in the third and sixth years would focus on presentation of "results to date" and provide for peer and industry feedback and evaluation. Industry, government, and academic scientists would be invited to participate.

Budgetary Recommendations

The proposed strategic research initiative calls for a total of 10 individual or clustered research proposals. Each proposal should be funded at approximately $110,000 per year. In addition, and vital to the success of the program, would be 10 Post-Doctoral Research Fellowships. These would be about $40,000 per fellow per year. Thus, the estimated annual cost in the first year would be $1.5 million.

BACKGROUND

Marine life is a genetically unique resource--very unlike terrestrial plants and animals--with vast potential for providing new and novel food resources, biological products, pharmaceuticals, agrichemicals, and industrial chemicals. This fact has provided a major rationale and motivation for the National Sea Grant College Program's strong investment, dating back to the early 1970s, in research oriented toward the potential of the oceans to yield new products for the benefit of humanity.

Late in 1977, a project was established within California Sea Grant: the Marine Pharmacology and Natural Product Chemistry Project. It established a collaborative effort between natural products chemists and pharmacologists from various campuses of the University of California. Other Sea Grant programs around the nation also established projects in this field, unique to their scientific interests. These clusters of research projects, each in a unique area of specialization, established leadership roles that soon gained considerable momentum nationally. They focused on several areas of pharmacological development, with an emphasis on inflammatory diseases and cancer.

By the end of the 1980s, the enormous potential of marine natural products had been amply demonstrated. But the work was expensive, especially in a level-funding environment, and it had to compete with other Sea Grant priorities. The California Sea Grant College convened a review of program activities over the previous decade, both to document successes and to determine future research directions. The result was a national meeting, "Workshop in Marine Pharmacology: Prospects for the 1990s," held at the University of California, Santa Barbara, May 7-9, 1990. This Sea Grant-sponsored meeting was attended by academic researchers from around the country as well as representatives of a number of pharmaceutical companies.

The consensus of the group was that the potential of ocean resources to provide new drugs and other biologically important products has been grossly underexplored. Further, that development of these resources is important to the nation's economic competitive future, and that we are in peril of losing our initial lead in this area to major research efforts now under way in Australia, New Zealand, Europe, and Japan. Recent developments in Japan are particularly worthy of note. The Japanese Ministry of International Trade and Industry (MITI) has identified marine biotechnology as a national priority for research and development and established large research centers in Kamaishi (for cold current water systems) and Shimizu (for warm current water systems). The centers are run by monies from the government and industry, mostly conglomerates; the initial investment for them amounted to $42 million. Their primary objectives are the exploitation of marine bioresources, but the emphasis is clearly on organic molecules for drugs and fine chemicals. Though U.S. scientists clearly pioneered the basic research in this field, it may be other nations that position themselves to reap the potentially commercial benefits.

The next step was the formation of a national Sea Grant Working Committee for the Development of Bio-Organic Chemistry and Pharmacology of Marine Resources. Committee members were named by the directors of Sea Grant College Programs in California, Hawaii, Oregon, New York, Puerto Rico, and Rhode Island, with the express purpose of developing recommendations for a national research program. The Committee met in Chicago on October 3, 1990. This proposal is the result of their work.

WORKING COMMITTEE

Professor David Ballantine
Department of Marine Science
University of Puerto Rico
Mayagüez, Puerto Rico

Professor Alison Butler
Department of Chemistry
University of California
Santa Barbara, California

Professor Néstor Carballeira
Departmento de Qurmíca
Universidad de Puerto Rico
Rio Piedras, Puerto Rico

Professor Jon Clardy
Department of Chemistry
Baker Laboratory
Cornell University
Ithaca, New York

Jack R. Davidson
Director
Hawaii Sea Grant College
University of Hawaii
Honolulu, Hawaii

Philip Davies
Executive Director
Inflammation Research
Merck Institute
Rahway, New Jersey

Professor D. John Faulkner
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California

Professor William H. Gerwick
School of Pharmacy
Oregon State University
Corvallis, Oregon

Professor Robert S. Jacobs
Biological Sciences
University of California
Santa Barbara, California
Committee Chair

Manuel Hernandez-Avila
Director
Puerto Rico Sea Grant College
Mayagüez, Puerto Rico

Professor Richard Moore
Department of Chemistry
University of Hawaii
Honolulu, Hawaii

Professor Yuzuru Shimizu
Department of Pharmacognosy and
Environmental Health Sciences
University of Rhode Island
Kingston, Rhode Island

James J. Sullivan
Director
California Sea Grant College
University of California
La Jolla, California

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