
THE SECOND ANNUAL

ROGER REVELLE

COMMEMORATIVE LECTURE

*The Oceans and Human Health:
The Discovery and Development of
Marine-Derived Drugs*

Featuring

Dr. Shirley A. Pomponi

**National Academy of Sciences Auditorium
Washington, DC
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DR. ROGER REVELLE
(1909 – 1991)

For almost half a century, Roger Revelle was a leader in the field of oceanography. Revelle trained as a geologist at Pomona College and at U.C. Berkeley. Then, in 1936, he received his Ph.D. in oceanography from the Scripps Institution of Oceanography. As a young naval officer, he helped persuade the Navy to create the Office of Naval Research (ONR) to support basic research in oceanography and was the first head of ONR's geophysics branch. Revelle served for twelve years as the director of Scripps (1950-1961, 1963-1964), where he built up a fleet of research ships and initiated a decade of expeditions to the deep Pacific that challenged existing geological theory.

Revelle's early work on the carbon cycle suggested that the sea could not absorb all the carbon dioxide released from burning fossil fuels. He facilitated the first continuous measurements of atmospheric carbon dioxide, leading to a long-term record that makes present-day discussions and research on global warming possible. Revelle kept the issue of increasing carbon dioxide levels before the public and spearheaded efforts to investigate the mechanisms and consequences of climate change.

Revelle was a proponent of daring programs, like Mohole and the International Indian Ocean Expedition, that addressed fundamental scientific questions and pioneered international cooperation. In 1960, Revelle left Scripps for important posts as science advisor to the Department of the Interior (1961-1963) and as the first director of the Center for Population Studies at Harvard (1964-1976). Revelle applied his knowledge of geophysics, ocean resources, and population dynamics to the world's most vexing problems: poverty, malnutrition, security, and education.

In 1957, Revelle became a member of the National Academy of Sciences (NAS) to which he devoted many hours of volunteer service. He served as a member of the Ocean Studies Board, the Board on Atmospheric Sciences and Climate, and many other committees. He also chaired a number of influential Academy studies on subjects ranging from the environmental effects of radiation to the study of sea-level change. Roger Revelle's life, although long, seems far too short to have encompassed all of his accomplishments.



Dr. Shirley A. Pomponi

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Dr. Pomponi received her Ph.D. in Biological Oceanography from the University of Miami, Rosenstiel School of Marine and Atmospheric Science. She has led numerous research expeditions to the tropical western Atlantic Ocean and Caribbean, and to the Galapagos Islands, Australia, New Zealand, American Samoa, Seychelles, Azores, Madeira, Canary Islands, Cape Verde, and Lake Baikal, Russia. Her research focuses on the systematics and cell biology of marine sponges. A major emphasis of her research has been on the development of methods for sustainable use of marine resources for drug discovery and development. Dr. Pomponi is a member of the Society for In Vitro Biology, the Society for Biomolecular Screening, and the American Society for Cell Biology. She recently served on the President's Ocean Exploration Panel.

ABSTRACT

The Oceans and Human Health: The Discovery and Development of Marine-Derived Drugs

The oceans are a rich source of both biological and chemical diversity. During the past two decades, thousands of novel, marine-derived biochemicals have been identified. Many have the potential for development as new pharmaceuticals to treat diseases such as cancer and drug-resistant infections. The challenges facing continued discovery are both technical, such as developing new tools to explore habitats and collect and test organisms never before studied, as well as political, such as complying with regulations related to the rights of a country to its natural resources. Successful discovery and development of marine-derived pharmaceuticals will depend on our ability to address a number of questions. What organism produces the "bioactive" compound, and why? Can we apply this knowledge to our rapidly increasing understanding of the human genome and human disease processes? Are there viable alternatives to harvesting for sustainable use of marine natural resources for drug development? And finally, what constitutes a fair and equitable sharing of revenues resulting from commercialization of marine resources, as mandated by the U.N. Convention on Biological Diversity? Addressing these questions will require the collaboration of marine and biomedical scientists and the cooperation of industry and government.

The Oceans and Human Health: The Discovery and Development of Marine-Derived Drugs

Introduction

I am honored to have been invited to present the Second Annual Roger Revelle Commemorative Lecture. Although I never had the opportunity to meet or work with Dr. Revelle, his depth and breadth of knowledge and scientific investigations have inspired and challenged oceanographers to interpret the results of their research in the context of systems, rather than as isolated phenomena. It is with this challenge in mind that I will discuss the role of the oceans in human health, and specifically, challenges in realizing the potential for the discovery of new, marine-derived drugs to treat human diseases.

Nature as a Source of Drugs

For thousands of years, man has taken advantage of nature's ability to produce remedies to treat infection, inflammation, pain, and many other diseases and ailments. In some parts of the world, natural medicines are still the only treatments available. Scientific research on these ethnomedicinal preparations led to the discovery of purified chemicals, whose beneficial properties provided the foundation of the current pharmaceutical industry. Today, over 50% of the marketed drugs are either extracted from natural sources or produced by synthesis using natural products as templates or starting materials. Drugs such as morphine, Taxol[®], aspirin, penicillin, vincristine, and cyclosporin are but a few examples of drugs derived from terrestrial plants and soil microorganisms¹. The recent emphasis on "alternative" medicines by the National Institutes of Health acknowledges both the history and importance of natural products in the treatment of diseases.

Based on the success of Bergmann and colleagues in the late 1950's², which led to the development of two drugs based on marine sponge nucleosides (Ara-A for herpes infections and Ara-C for leukemia)³, as well as the pioneering research in marine natural products drug discovery by the Roche Research Institute of Marine Pharmacology during the mid-1970's⁴, scientists began to explore the chemical diversity found in marine organisms and the potential for discovery of new drugs from the marine environment. What has become an intense effort in marine natural products drug discovery by academic, government, and industry laboratories, especially in the past 15 years, is based on a number of facts. The oceans are a rich source of both biological and chemical diversity. They cover more than 70% of the earth's surface and contain more than 200,000 described species of invertebrates and algae⁵. It is estimated that this number is only a small percentage of the total number of species that are yet to be discovered and described⁶. Marine microorganisms represent the greatest percentage of undescribed marine species⁷. A relatively small number of marine organisms—primarily algae and invertebrates—has already yielded thousands of novel chemical compounds⁸, yet only a small percentage of these chemicals has been studied for their potential as useful products. Finally, although there is

a major effort by pharmaceutical companies in the design of synthetic chemicals for drug discovery, marine natural products still provide unusual and unique chemical structures upon which molecular modelling and chemical synthesis of new drugs can be based.

Why do marine plants, animals, and microbes produce chemicals that are useful as pharmaceuticals? Many of them are sessile—attached to the bottom, and they live in densely populated habitats where competition for resources, such as space and food, is intense. As a result, they have evolved a unique inventory of metabolites used for their defense, reproduction, and communication^{9,10, 11}. These metabolites also interact with receptors and enzymes involved in human disease processes. If, for example, a sponge is trying to prevent another sponge from invading its space, it may produce a chemical to prevent the other sponge's cells from growing and dividing. It is not unusual, therefore, that these same chemicals may also be effective in inhibiting the uncontrolled growth of cancer cells.

In fact, a major emphasis has been on the discovery of marine-derived anticancer compounds, due in large part to the availability of funding to support marine-derived cancer drug discovery. The National Cancer Institute has led this effort through its programs to support both single-investigator and multi-institutional natural products-based, cancer drug discovery research. As a result, several marine-derived compounds are in preclinical or clinical trials for the treatment of cancer as shown in Table 1. I will highlight just a couple.

Ecteinascidin 743, a complex alkaloid derived from the mangrove tunicate *Ecteinascidia turbinata*^{12,13} shown in Figure 1 and licensed by the University of Illinois to PharmaMar S.A., is in Phase II clinical trials in the U.S. and Europe for the treatment of cancer. Discodermolide, a polyketide isolated from deep-water sponges of the genus *Discodermia* shown in Figure 2, inhibits the proliferation of cancer cells by interfering with the cell's microtubule network¹⁴ as shown in Figure 3. This compound has been licensed by Harbor Branch Oceanographic Institution to Novartis Pharmaceutical Corporation, and is in advanced preclinical trials.

Despite the emphasis on identifying new anticancer compounds, marine natural products have also been found to have other biological activities, including mediation of the inflammatory response. The pseudopterogens are glycosides derived from the Caribbean soft coral *Pseudopterogorgia elisabethae*¹⁵. These are in advanced preclinical trials as anti-inflammatory and analgesic drugs, and purified extracts of this soft coral are available commercially as additives in skin care products. Other marine-derived compounds, such as the prostaglandins, palytoxin, calyculin, okadaic acid, and manoalide, to name just a few, are available commercially as biomedical research probes that give us valuable insights into understanding and treating human diseases.

There are more than 10,000 scientific publications related to marine natural products¹⁶. This fact serves to underscore the vast potential of the oceans as a source of novel chemicals with potential not only as pharmaceuticals, but also as nutritional supplements, cosmetics, agrichemicals, molecular probes, enzymes and fine chemicals shown in Table 2. Each of these classes of marine bioproducts has a potential multi-billion dollar market value¹⁷. Recognizing this potential, President Clinton issued a directive to the Secretary of Commerce on June 12, 2000, requesting recommendations for a national ocean exploration strategy. The President emphasized consideration of the full array of benefits the oceans provide, with "mechanisms to ensure that newly discovered organisms or other resources with medicinal or commercial potential are identified for possible research and development".

TABLE 1 Anti-tumor agents from marine sources that are currently licensed for development

Organism	Metabolite	Location	Discoverer	Current Status
Bryozoan: <i>Bugula neritina</i>	Bryostatin 1	Gulf of California	Pettit, Arizona State Univ	In Phase I/II clinical trials in US/Europe; NCI sponsored trials.
Sea hare: <i>Dolabella auricularia</i>	Dolastatin 10	Indian Ocean	Pettit, Arizona State Univ	Phase I clinical trials in US; NCI sponsored trials.
Tunicate: <i>Ecteinascidia turbinata</i>	Ecteinascidin 743	Caribbean	Rinehart, Univ Illinois	Licensed to PharmaMar S.A.. In Phase II clinical trials in Europe and in US.
Tunicate: <i>Aplidium albicans</i>	Dehydro-didemnin B [Aplidine]	Mediterranean	Rinehart, Univ Illinois	Licensed to PharmaMar S.A.
Gastropod: <i>Elysia rubefescens</i>	Kahalalide F	Hawaii	Scheuer Univ Hawaii	Licensed to PharmaMar S.A.
Sponge: <i>Discodermia dissoluta</i>	Discodermolide	Caribbean	Gunasekera & Longley, HBOI	Licensed to Novartis.
Sponge: <i>Lissodendoryx sp.</i>	Isohomo-halichondrin B	New Zealand	Munro & Blunt; Univ Canterbury, NZ	Licensed to PharmaMar S.A.
Actinomycete: <i>Micromonospora marina</i>	Thiocoraline	Mozambique Strait	Canedo, Spain	Licensed to PharmaMar S.A.
Tunicate: <i>Didemnum granulatum</i>	Isogranulatimide	Atlantic [Brazil]	Andersen & Bjerinck, Univ British Columbia & Brazil	Licensed to Kinetik, Canada.
Sponge: <i>Jaspis sp.</i>	Bengamide	Fiji	Crews et al Univ California, Santa Cruz	Synthetic derivative licensed to Novartis.
Sponge: <i>Cymbastella sp.</i>	Hemiasterelins A & B	Papua New Guinea	Andersen, Univ British Columbia	Licensed to Wyeth-Ayerst.
Sponge: <i>Pseudaxinyssa cantharella</i>	Girolline	New Caledonia	Potier, France	Licensed to Rhone Poulenc.

Source: Courtesy of Dr. David J. Newman, NIH, National Cancer Institute, Natural Products Branch.

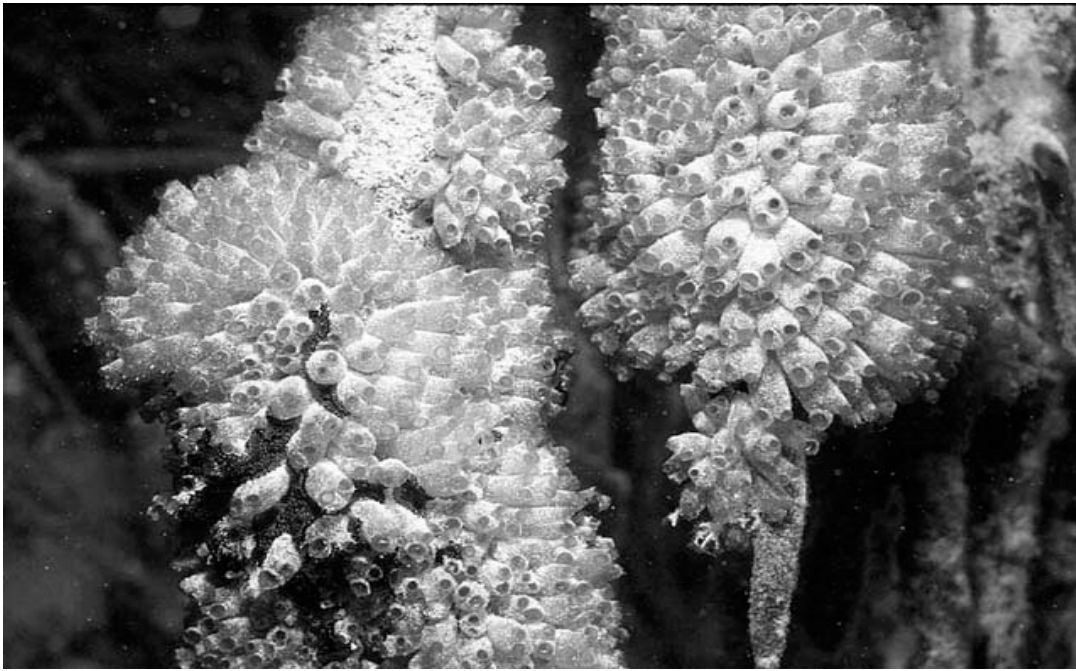


FIGURE 1 The tunicate, *Ecteinascidia turbinata*, source of ecteinascidin 743, is a common inhabitant of mangrove roots throughout the tropical western Atlantic region (photo courtesy of Harbor Branch Oceanographic Institution).



FIGURE 2 The sponge, *Discodermia dissoluta*, source of discodermolide, rarely occurs in shallow water, and is more commonly found at depths of 450-750 ft throughout the tropical western Atlantic region (photo courtesy of Harbor Branch Oceanographic Institution).

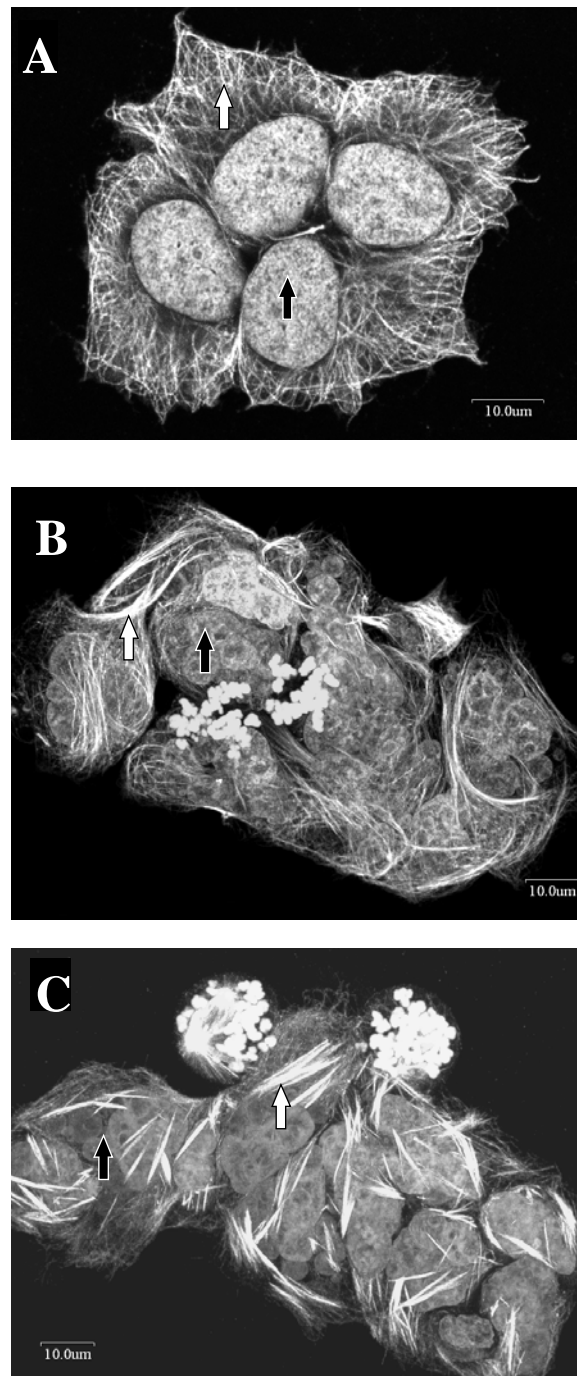


FIGURE 3 MCF-7 human breast adenocarcinoma cells, viewed by confocal microscopy. nuclei (black arrows); “hair-like” structures (microtubules) which comprise the cytoskeleton (white arrows). A. Untreated cancer cells. B. Following treatment with the anti-cancer drug, Paclitaxel, nuclei become fragmented and the microtubules form abnormal “bundled” filaments. These cells will eventually die. C. After treatment with discodermolide, effects are similar to Paclitaxel but are much more pronounced. Nuclei are fragmented and microtubules are formed into severe needle-like “bundled” filaments. These effects induce rapid cell death. (photomicrographs provided by Dr. Ross E. Longley, Harbor Branch Oceanographic Institution).

TABLE 2 Some examples of commercially available marine bioproducts (adapted from Pomponi, 1999¹⁸).

Product	Application	Original Source	Method of Production
Ara-A	antiviral drug	marine sponge, <i>Cryptotethya cryta</i>	microbial fermentation of analog
Ara-C	anticancer drug	marine sponge, <i>Cryptotethya cryta</i>	chemical synthesis of analog
okadaic acid	molecular probe: phosphatase inhibitor	dinoflagellate	cell culture
manoalide	molecular probe: phospholipase A ₂ inhibitor	marine sponge, <i>Luffariella variabilis</i>	wild harvest of sponge
Vent™ DNA polymerase	polymerase chain reaction enzyme	deep sea hydrothermal vent bacterium	recombinant protein
Formulaid® (Martek Biosciences, Columbia, MD)	fatty acids used as additive in infant formula nutritional supplement	marine microalga	cell culture
Aequorin	bioluminescent calcium indicator	bioluminescent jellyfish, <i>Aequora victoria</i>	recombinant protein
Green Fluorescent Protein (GFP)	reporter gene	bioluminescent jellyfish, <i>Aequora victoria</i>	recombinant protein
phycoerythrin	conjugated antibodies used in ELISAs and flow cytometry	red algae	cell culture
Resilience® (Estée Lauder)	“marine extract” additive in cosmetics	Caribbean gorgonian, <i>Pseudopterogorgia elisabethae</i>	wild harvest of gorgonian

I will, therefore, focus the rest of my lecture on the challenges facing discovery and development of new marine organisms with medicinal potential. These challenges are *technical*, such as exploring new environments and developing new platforms, tools, and assays to discover and test organisms never before studied; *financial*, such as obtaining adequate support for both exploration and early-phase, high risk discovery research; and *political*, such as complying with regulations related to the rights of a country to its natural resources, as well as fair and equitable sharing of technologies and revenues resulting from commercialization of products from marine resources. Successful discovery and development of marine-derived pharmaceuticals will depend, of course, on our ability to address a number of scientific questions. What organism produces the bioactive compound, and why? Can we apply this knowledge to our rapidly increasing understanding of the human genome and human disease processes? Are there viable alternatives to prevent over-exploitation and ensure sustainable use of marine resources for drug development? Let’s examine each of these challenges.

Exploring the Unknown

Most of the environments explored for novel organisms containing unique chemicals have been those accessible by scuba. Although bioactive compounds have been identified from higher latitudes, such as the fjords of British Columbia and under the Antarctic ice, the geographic focus of “biodiversity prospecting” has been primarily the tropics. Aside from the fact that it’s just more comfortable to dive in warm water, tropical seas are well-known to be areas of high biological diversity and, therefore, logical targets for high chemical diversity. Marine biodiversity prospectors have focused on shallow water benthic environments—areas that can be accessed with relative ease by snorkelling and scuba diving. Collection of samples from deeper environments has been accomplished using closed-circuit rebreather diving, dredging and trawling, and submersibles. The focus of our drug discovery exploration program at Harbor Branch is deep water organisms that live on the continental shelves and slopes at depths from 400 to 3000 feet, i.e., beyond scuba depths, using our Johnson-Sea-Link manned submersibles. Submersibles provide platforms that enable us to go deeper, stay longer, access unusual sites that cannot be accessed using conventional methods (e.g., steep walls, rocky bottoms, and vent communities), and be more precise and selective in our sampling than conventional deep-water collection methods permit.

There is still most of the deep sea yet to be explored. With rare exceptions (e.g., the analysis of deep sea cores to identify unusual microbes), the deep sea bottom, mid-water habitats, and most of the world’s oceans at depths greater than those accessible by scuba, have not yet been explored. The reason for this deficiency is primarily financial: oceanographic expeditions are expensive, and federal funding to support ship operations (e.g., UNOLS fleet support) is generally not available if the focus of the research is drug discovery. The National Science Foundation does not specifically support drug discovery, and the National Institutes of Health does not support ship operations. The National Oceanic and Atmospheric Administration/National Undersea Research Program has been the single exception, however, budget cuts to that program during the past few years have seriously curtailed funding for ocean-going research. Industry support for oceanographic expeditions and early-phase discovery research has been lacking: some pharmaceutical companies provide matching funds for less expensive scuba collections, but none provides support for oceanographic expeditions.

The President’s Panel on Ocean Exploration, in responding to the President’s directive for a national ocean exploration strategy (cited above), recommended the following: “Relevant federal agencies must ensure support for early phase research to assess the potential and add value to discoveries with potential commercial applications by establishing new programs specifically targeted for research for discoveries from the Ocean Exploration Program. In addition to the programs that currently exist to support short-term, high risk research on the living and non-living “products” of exploration, federal agencies need to emphasize, prioritize, and fast-track research initiatives on these “products” of the Ocean Exploration Program. Private sector involvement is critical. Although mechanisms exist to support and encourage partnerships between industry, academia, and government (e.g., SBIR and STTR programs), these programs are not oriented to support the early phase research that is necessary to identify discoveries with commercial potential. Incentives to industrial sponsors of high risk, early phase, discovery research, including, but not limited to, tax credits, grants and favorable licensing terms, should be provided to industrial sponsors willing to support this research.”

Political Considerations

The exploration of new geographic areas will undoubtedly focus on areas outside of US jurisdiction. Indeed, since most of the tropical seas—the areas of focus of most biodiversity prospecting—are under the jurisdiction of other countries, compliance with national and international regulations regarding access, extraction of natural resources, and exporting materials has been an essential element of marine natural products drug discovery to date. Working in a country's territorial water, to collect samples and to export those samples involves obtaining permits from and coordinating the interaction of several national and state agencies, e.g., defense, fisheries, and national parks, to name a few. It is often a lengthy process, and plans for foreign collections must be made several months or even years in advance of the expedition.

Balancing the responsibilities of the stewards of marine resources with the requests of marine bioprospectors is indeed a challenge. Environmental resource managers are charged with protecting their resources from over-exploitation. Legislators and policy-makers must decide whether bioprospecting is consistent with their plans for resource management. If they decide it is, they must draft and implement policies for access, use, and development of the resources.

Still unresolved, for the most part, are issues dealing with fair and equitable sharing of technologies and revenues related to the discovery and commercialization of products from marine resources. As a result of the recommendations of the U. N. Convention on Biological Diversity, legislators, bioprospectors, and environmental resource managers have begun to discuss what actually does constitute a “fair and equitable sharing”. How does one place a value on a resource? If, for example, a drug was developed based on a chemical from an organism that is endemic to the country in which it was collected, should the revenue sharing be greater than if the organism occurs (and was collected) throughout a geographic region and across jurisdictional boundaries? If the organism is harvested to provide the materials for clinical development and commercialization, should the revenue sharing be greater than if only a few samples were collected to identify the active compound and subsequent supply was based on alternative methods? Since the answers to these questions are usually not known at the time an agreement must be negotiated to access the resources, the approach taken to date has been simply to agree upon a royalty to be paid by the bioprospector to the country. Expectations for the amount of such a royalty may sometimes be unrealistic, especially in view of the fact that pharmaceutical companies will generally agree to pay royalties of less than 5% of net sales to the licensor of the chemical. This is (perhaps unfairly) based on the industry average of \$50 to \$200 million to develop a drug, compared with the licensor's costs of perhaps less than \$1 million to collect the organism, test it for medicinal potential, add value to the compound by characterizing its physical, chemical, and biological properties, and protect its proprietary value by patenting the discovery. Undoubtedly, these considerations arise during negotiations to determine fair and equitable sharing between the bioprospector and the country of origin of the source organism. To be fair, neither party should be unreasonable in its expectations. Each party must recognize both the value added to the resource (and the revenue stream as a result of the value added), as well as the original, continuing, and perhaps intangible value of the resource as part of an ecosystem.

Of course, the chances of a drug being commercialized are very low, and the pay-off could be several years from the original discovery. In the spirit of respect for, and acknowledgment of, the scientific capabilities and contributions of the source country, alternative scenarios to revenue sharing may include more near-term benefits, such as support for development of the scientific infrastructure (particularly for developing nations), training of

students, scientist exchange programs, and technology transfer. These are often key elements in a revenue- and technology-sharing agreement.

New Tools for Discovery

To optimize our chances for identification of marine resources with medicinal potential, we must use the best tools for discovery at all stages: exploration of new locations, collection of organisms never before sampled, and identification of chemicals with pharmaceutical potential. Increased sophistication in the tools available to explore the deep sea has expanded the habitats that can be sampled and has greatly improved the opportunities for discovery of new species and the chemical compounds they produce. New and improved platforms (such as autonomous, remote, and human-occupied underwater vehicles) to take us farther and deeper are in development. These platforms need to be equipped with even more sophisticated and sensitive tools (e.g., cameras, sensors, and manipulators) to enable us to identify an organism as new, to assess its potential for novel chemical constituents, and if possible, to non-destructively remove a sample of the organism. Tools and sensors that have been developed both for space exploration and for diagnostic medicine need to be applied to the study of the inhabitants of our last frontier on Earth.

Although there exist some specialized tools and chambers that allow deep water organisms to be maintained under ambient conditions, i.e., high pressure and low temperature, there is still a need for the development of versatile bioreactors that can be deployed and operated in extreme environments (e.g., hypersaline, vent, anoxic, and deep sea habitats). Such bioreactors could be used for collection, at-sea maintenance, and evaluation of both macroorganisms and microorganisms so that their metabolites could be studied under physiological conditions that are as similar as possible to ambient conditions.

Another approach to the identification of new products is the incorporation of miniaturized biosensors into both collecting tools and bioreactors for rapid, *in situ* analysis of marine organisms for bioactive molecules. A number of miniaturized biosensors and probes to study human disease processes are in development. Adaptation or development of new biosensors and probes for rapid, *in situ* screening of marine organisms would involve specialized tools, ideally for non-destructive sampling and analyses of the target organisms¹⁸. Development of *in situ* biosensors would enhance our ability to probe the expression of metabolites in response to various stimuli, lead to a better understanding of the role of the secondary metabolites in nature, and perhaps provide clues to the potential biomedical utility of these compounds.

New Models, New Paradigms

The biological evaluation of marine-derived extracts and pure compounds for pharmaceutical development has been based on assays developed by the pharmaceutical industry for high-throughput screening of large libraries of synthetic compounds. They measure a number of end-points, such as activation or inhibition of enzymes or receptors involved in human disease processes, inhibition of growth of human pathogenic microorganisms, and toxicity against human cancer cells^{4, 8, 16}. As our understanding of the biochemistry behind a variety of diseases has improved, better methods have been developed for rapidly determining the biomedical potential of the metabolites produced by marine organisms. None of the assays used in major pharmaceutical drug discovery programs, however, takes into account the role of marine-derived compounds in nature, i.e., the *in situ* biochemical functions of the metabolites, and how those functions may be applied to the discovery of new drugs and probes to study human disease processes.

Marine organisms as model systems offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites. For example, the mechanisms of action of the toxins of *Conus*, the poisonous snail, are well-known¹⁹, and are currently being applied to the development of new classes of drugs to treat diseases such as epilepsy.

Marine organisms have already demonstrated their utility as biomedical models, the results of which have been applied to understanding normal and disease processes in humans²⁰. Can we continue to apply this knowledge to our rapidly increasing understanding of the human genome and human disease processes? Or perhaps a more relevant question is this: can we use our rapidly increasing understanding of the human genome and human disease processes to guide our discovery of novel marine-derived chemicals that can be used against newly-discovered disease targets? Considering the evolutionary conservation of molecular signalling pathways, one can hypothesize that our understanding of molecular pathways and disease targets in mammalian systems can help us to not only elucidate the ecological roles of marine-derived metabolites, but can also provide a more rational approach to the discovery of drugs to treat a variety of diseases. How does a simple organism, like a sponge, through which seawater and associated bacteria, fungi, and viruses constantly flow, defend itself against microbial infections? How does it signal its cells to divide rapidly enough to heal a damaged surface in a few hours, or spread out over a substrate in a matter of days or weeks, yet control this proliferation so that the sponge itself does not get cancer? How, with only a primitive immune system, does it recognize an invading organism as “non-self” and send out chemicals to kill the invading cells, without killing its own cells? We know so little about these processes in the organisms from which we have identified literally thousands of chemicals with biomedical potential. Molecular tools and approaches that have been developed to understand similar processes in mammalian systems must be applied to understanding these processes in marine organisms. A multidisciplinary approach—involving molecular biologists, pharmacologists, and chemical ecologists—to discovery of marine-derived drugs will likely result in the discovery of unique bioactive chemicals and new ways to address the treatment of human disease.

Sustainable Use of Marine Resources with Commercial Potential

With the enormous potential for discovery, development, and marketing of novel marine bioproducts comes the obligation to develop methods by which these products can be supplied in a way that will not disrupt the ecosystem or deplete the resource. Supply of most marine-derived compounds is a major limiting factor for further pharmaceutical development. Often, the metabolite occurs in trace amounts in the organism, and a steady source of supply from wild harvest cannot provide enough of the target compound for preclinical studies. In general, the natural abundance of the source organisms will not support production based on wild harvest. Unless there is a feasible alternative to harvesting, promising chemicals will remain undeveloped.

Some options for sustainable use of marine resources are chemical synthesis, controlled harvesting, aquaculture of the source organism, *in vitro* production through cell culture of the macroorganism or microorganism source, and transgenic production. Each of these options has its advantages and limitations, not all methods will be applicable to supply of every marine bioproduct, and most of the biological supply methods are still in development¹⁸. The approach to be used will be based on a number of factors:

1. ***Complexity of the molecule: can it be synthesized using an industrially-feasible process?*** Chemical synthesis of a compound gives the pharmaceutical company ultimate control in the supply of materials for clinical development and eventual marketing of the drug. Synthetic processes have been published for many marine bioproducts in development as pharmaceuticals^{21,22}. Unfortunately, most of these are multi-step processes that are not amenable to economic, industrial-scale synthesis. To overcome this problem, major research programs are undertaken by the pharmaceutical industry to identify structurally similar compounds that are easier to synthesize, and perhaps even more potent, less toxic, and more bioavailable than the parent molecule.
2. ***Abundance of the organism in nature: what do we know about the impact of collections on the environment?*** Prior to large-scale wild harvest of an organism for recovery of a bioproduct, harvesting feasibility studies should be conducted. These should define factors such as the standing stock of the organism, its growth rate and the factors that affect growth, and the effects of harvesting and post-harvesting recovery of the target organism. These impact data should then be used to assess the potential of supply from wild harvest, and may also be useful in developing models for aquaculture of the source organism. Unfortunately, this is rarely done. One notable exception is the survey of the New Zealand sponge, *Lissodendoryx* sp., which produces the halichondrins, potent antitumor compounds in preclinical trials. The sponge is restricted in occurrence to one locality off the Kaikoura Peninsula. Research conducted by Battershill²³ demonstrated that harvesting by dredging significantly reduced the standing stock of the sponge, but that the population may recover rapidly as a result of asexual propagation of sponge fragments dispersed as a result of the dredging. This study suggested that harvesting is only feasible for smaller quantities of the sponge.
3. ***Source of the compound: is it microbially produced?*** A significant number of marine bioproducts with pharmaceutical potential have been identified from heterotrophic marine microorganisms isolated from coastal sediments^{24, 25, 26}. In addition, some marine bioproducts originally isolated from macroorganisms, such as sponges, have been subsequently discovered to be localized in microbial associates^{27,28}. If these microorganisms can be isolated and cultivated, optimization of production in marine microbial bioreactors could lead to an industrially-feasible supply option.

If the source of the compound is the macroorganism itself, development of *in vitro* production methods could provide bulk supply of the compound. Research in progress in our laboratory focuses on establishing cell lines of bioactive marine invertebrates that can be used as models to study *in vitro* production of bioactive metabolites and the factors which control expression of production²⁹. This could ultimately lead to *in vitro* production of marine bioproducts. More importantly, an understanding of the cellular and molecular processes that control production of these metabolites could be used to enhance culture optimization or to stimulate production of “unnatural” natural products—i.e., chemicals that the organism would not produce under normal conditions, but which may be more potent than the “natural” product.

4. ***In situ growth conditions: is aquaculture an option for deep-water organisms?*** Both in-the-sea and land-based aquaculture methods have been developed for production of bioproducts from shallow-water organisms. CalBioMarine Technologies (Carlsbad, CA) has successfully aquacultured the bryozoan, *Bugula neritina*, the source of the antitumor compound bryostatin, and *Ecteinascidia turbinata*, the ascidian from which the antitumor compound, ecteinascidin 743 has been isolated (Figure 1). These are both common, shallow-water

organisms for which reproduction and growth have been studied, but the factors controlling production of the compounds are not yet completely known. The New Zealand deepwater sponge, *Lissodendoryx* sp., is the source of the antitumor compounds, called halichondrins. The sponge occurs at 85-105 meters depth, but has been cultured successfully from cuttings on lantern arrays in shallower water, maintaining production of the bioactive halichondrins²³. This indicates that aquaculture of some deep water sponges is feasible, however, species from deeper water may have more critical growth requirements, such as high pressure and low temperature. In-the-sea aquaculture is a cost-effective method of production with opportunities for technology transfer and economic development in the source country, however, it may not afford the opportunity for complete control of environmental parameters or the over-expression of production of the compounds. Development of closed-system bioreactors for aquaculture of both shallow water and deep water organisms is a particularly challenging opportunity.

5. ***Biosynthetic pathway: is genetic engineering realistic for the compound?*** If the biosynthesis of the target compound is understood, it may be possible to identify, isolate, clone, and express the genes responsible for production of the metabolite in another host. In many cases, of course, biosynthesis of the product is not known, or it is a multi-step process involving several enzymatic reactions. For these cases, transgenic production is not a trivial process. Alternatively, chemoenzymatic synthesis, by which marine bioproducts are synthesized in cell-free, enzyme-based systems, offers a complementary technique to *in vitro* and transgenic production methods for marine bioproducts³⁰

Summary

The Ocean Studies Board, in its review of the ocean's role in human health²⁰, recommended a greater focus on marine organisms as sources of new pharmaceuticals, new funding initiatives to support this effort in all therapeutic areas—not just cancer—and new interdisciplinary research and education programs to provide innovative approaches to marine-based drug discovery.

Recent U.S. legislation and executive directives support a renewed emphasis on exploring the vast unknown areas of our oceans, discovering species new to science, identifying marine resources with potential medicinal value, and ensuring the protection and sustained use of these resources. We now have the unique opportunity to capitalize on new technological advances developed to explore the oceans of Earth and other planets, recent discoveries in medicine, molecular biology, and marine biotechnology, and a greater appreciation for benefit sharing and the value of marine resources to realize the full potential of our oceans and human health.

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