Chemical Prospectors Scour The Seas for Promising Drugs

Last month a huge, colorfully patterned lobster from the Central Pacific island of Palau arrived at the National Cancer Institute's (NCI's) lab in Frederick, Maryland. This marine specimen, which came frozen in a plastic bag, waited in line behind a couple of less gaudy creatures for an unattractive fate: to be pulverized in a meat grinder, then soaked in flasks of solvents to leach out soluble compounds. Researchers will take extracts that drip from the lobster and test them against samples of 60 types of human tumors as well as HIV.

The lobster was only one of five or six frozen sea creatures—sponges, mollusks, starfish, and the like—from around the world that arrive daily at the Frederick lab. These marine organisms provide a quarter of about 10,000 different natural products—the rest come from exotic leaves, barks, and other plant matter—the lab tests each year for their therapeutic potential.

With little new ground gained in the war against cancer, no cure in sight for AIDS, and the looming threat of resistance to current antibiotics, modern researchers have become as intrigued by new natural pharmaceuticals as 15th-century explorers were by spices. Collectors search for specimens around the world, in remote jungles, coral reefs, marshes, and the depths of the ocean. And although it costs twice as much to get a specimen from the sea as it does to collect a terrestrial item, the effort is proving worthwhile: Today the majority of natural compounds that show promise as anti-cancer drugs come from the submerged two thirds of the planet. These include six compounds from sea creatures that have reached clinical or late-preclinical trials in the United States and Canada (see chart).

But marine chemists say they've only just begun to tap the chemical diversity of the sea. "Man has been interacting with plants on land for some 3000 years," says chemist William Fenical of the Scripps Institution of Oceanography. "In the ocean there are an incredible number of organisms—and we don't know what their potential is." In the last couple of years, rapid molecular screening methods have allowed Fenical and other researchers in the field to cast a wider net by testing up to a thousand compounds a day.

And it is not just U.S. pharmaceutical researchers who are looking to the oceans. The Japanese government is starting to emphasize marine natural products, and Japan has become the world leader in the use of submersibles for collecting deep-water organisms. So far, however, Japanese researchers haven't found anything that's reached the clinical trial stage, and they don't have a massive screening effort like the one at NCI, say U.S. scientists who have collaborated with them. But Japanese scientists have isolated a number of potential anti-cancer and antibiotic compounds from marine creatures, especially deep-sea bacteria, and used crystallography or other techniques to determine their chemical structures—which are often strange and unique.

This upsurge of interest in chemical prospecting in the oceans is part of a renewed focus on the pharmaceutical potential of all kinds of natural products, says marine chemist Phillip Crews of the University of California, Santa Cruz. "We went through a period of 20 years when people were completely uninterested in natural products as a means of drug discovery—all the energy was in synthetic chemistry and rational drug design." But no cancer cure has emerged from the synthetic chemistry labs, and some people are going back to the natural world, says Crews.

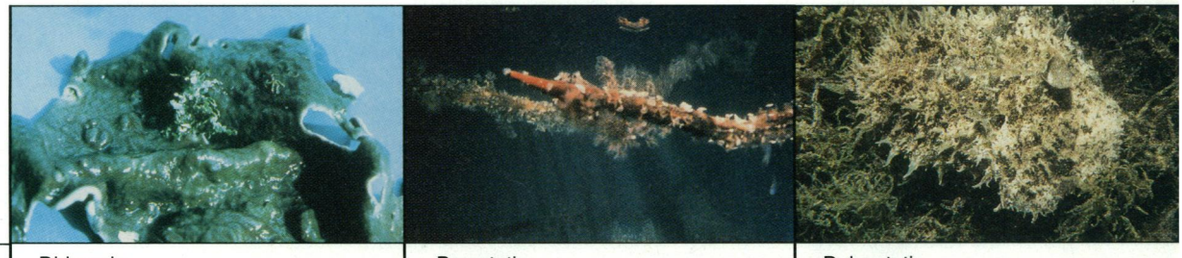
Toxic defense. One reason sea creatures are particularly promising sources of anti-cancer compounds, speculates NCI chemist David Newman, is that natural selection has forced them to develop elaborate chemical arsenals. The sea is full of sitting targets: soft-bodied, stationary, brightly colored creatures that seem to say "eat me," he says. To avoid that fate, those creatures rely on powerful toxins. And toxicity is the reason cancer drugs work—the goal is to poison tumor cells more than healthy cells.

Therefore, when they're looking for toxins, collectors look for soft bodies and other signs of helplessness. Most of the samples come from warm tropical areas, and although it's likely that those regions hold more biodiversity, a simpler explanation is that divers like the tropics, as Newman says. "I've gone diving off of the coast of England. And it's bloody cold ... and when you go to the waters near Antarctica, there's only one person I know of who enjoys diving there."

Regardless of where the potentially useful compounds come from, researchers working on them have been given a boost in recent years by the development of rapid screening techniques based on the tools of molecular biology. These screens generally look for a compound's effects on a specific chemical "target"—usually an enzyme responsible for the growth of cancer cells. The speed of the molecular screens has revolutionized the search for drugs from natural products, says Crews. "You can screen thousands of things each day now."

Using this new technology, Crews, in collaboration with researchers from Syntex in Palo Alto, California, has identified two possible cancer-fighting compounds from sponges. One is a class of compounds called bastadins that interfere with the growth of leukemic cells and ovarian tumors; the other is a compound called jasplakinolide, which disrupts cell division in cultured renal and prostate cancer cells. Jasplakinolide has a serious drawback as a potential therapeutic agent, however: It is so toxic that it killed all the lab animals exposed to it, says Crews. The hope, however, is that chemists will be able to alter the compound's structure to produce a milder derivative that retains enough toxicity to deal with tumor cells.

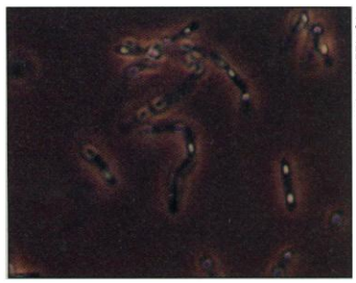
Researchers at SmithKline Beecham are also using fast assays to plow through thou-



Anti-cancer compound	Didemnin	Bryostatin	Dolasstatin
Organism	*Tridemnum solidum*	*Bugula neritina*	*Dolabella auricularia*
Trivial name	Tunicate	Bryozoan	Sea hare
Current status at NCI	Clinical Phase II in Canada	Just finished Phase I in U.S.	Clinical trial application pending

sands of organisms they pluck from coral reefs, estuaries, marine lakes, and even wrecked ships, says Brad Carte, a marine products chemist who works for the company. "We look for anti-cancer drugs, anti-inflammatory, anti-virals, anything you can think of." For one of their many screens, Carte and colleagues test extracts from their creatures to see whether they can inhibit a target enzyme called a topoisomerase, enzymes that, by changing the topology of DNA, are important in the proliferation of cancer cells. They've used this to identify promising small molecules from several sponges from the Philippines and Japan, one of which has shrunk tumors in mice.

The company has also screened for chemicals that bind to an enzyme called protein kinase, which is essential for cell division and signal transduction—the transfer of information from outside chemicals, such as growth factors and hormones, into cells. A drug acting against this enzyme might inhibit either cancer growth or inflammation. Paul Scheuer of the University of Hawaii and his colleagues have identified protein kinase inhibitors from a sponge and an invertebrate called a tunicate. And while NCI has found just one compound from a sea creature that has any effect on HIV, researchers at the University of Chicago have seen sponge extracts that inhibit HIV protease, an enzyme necessary for the replication of the virus.



WILLIAM FENICAL

Combing the depths. Unnamed bacteria yields potential anti-tumor drug.

NCI will continue to use the slower cell-based screens, which Newman says complement the faster, target-based systems. NCI's method of testing the compounds directly against tumor cells gives some information fast screens don't, including which of 60 types of tumor cells are most susceptible to a given compound, he says, and whether the compounds will get into cells. NCI is, however, encouraging the use of new screening technologies through a program called the National Cooperative Natural Products Discovery and Development Program. This program helps support the sponge research Crews does with Syntex, as well as a handful of other industry-academia collaborations.

Microbial prospecting. While most researchers have been testing extracts from large organisms, others, including microbiologist Deborah Steinberg of American Cyanamid, have been thinking small. They are scouring the seas for micro-organisms with potentially useful properties. Researchers have barely begun to tap the diverse chemistry of marine microbiology, says Steinberg. She and her colleagues collect microbes from around the world and use a variety of molecular screens (the company keeps the nature of the screens secret) to test for signs of antibiotic, anti-cancer, or anti-viral properties, as well as potential for cardiovascular and central nervous system drugs. They test thousands per day right on the collection ship and have already found a number of leads, she says. One they've published is bioxalomycin—a compound isolated from shoreline bacteria that shows interesting antibacterial properties in vitro and in vivo.

Scripps's Fenical is now working with Bristol-Myers Squibb to screen marine microbes for both anti-cancer and anti-inflammatory agents. Though his team searches water and sand, he's actually found the most interesting samples on the bodies of other organisms. "We look on the surfaces of plants and animals," he says, "in the tissues of fish and the digestive tracts of shrimp."

Indeed, some of the toxins associated with macroscopic animals come from microbes that live in or on their bodies. Take tetrodotoxin, a deadly substance that concentrates in the puffer fish—and occasionally kills daredevil gourmets who indulge in this high-risk "delicacy." Japanese biochemists Takeshi Yasumoto of Tohoko University and Michlo Murata of the University of Tokyo recently discovered that tetrodotoxin in fact comes from a type of unicellular marine bacteria living in the fish's internal organs.

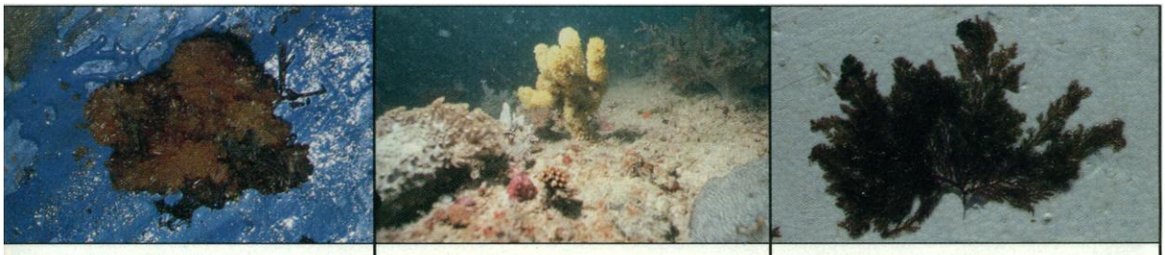
Fenical has found that many marine creatures and their eggs are covered with bacteria that protect their hosts from disease: "If you put organisms in polluted seawater and destroy the surface bacteria, the animals die because they get infected with pathogens." That finding, he says, argues for seeking new antibiotics in such protective microbes. Already, he has interesting leads, including a powerful anti-fungal compound, called istatin, isolated from bacteria that protect shrimp eggs, and two possible antibiotics, salinamide A and B, in bacteria living on the body of an undescribed jellyfish found in the Florida Keys. "This is just an open area," he says. "I think you are going to see a lot of activity in the near future."

Supply and demand. If a useful drug does turn up, researchers could quickly face a problem: Demand may outstrip supply. Crews and other researchers are trying to prevent that from happening by developing techniques for farming marine organisms. It can be a challenging task, however, for some creatures—sponges, for example—are notoriously difficult to cultivate, says Crews. One group, headed by marine chemists Murray Munroe and John Blunt at the University of Canterbury in New Zealand, has, however, managed to start the first sponge farm in Wellington Harbor, growing the sponge that produces halochondrin—one of the six marine-based compounds that are moving toward clinical trials.

Microbes should be easier to grow, but even here, the strangeness of the marine world presents a challenge. "You have to reinvent microbiology," says Fenical. Terrestrial bacteria are often grown in protein compounds or glucose. But "there's no glucose in the sea." So to grow marine microbes, he says, "we've developed about 25 media based on all kinds of concoctions—fish meals and powders, special oils, and crab shells. ... You have to think about what's out there."

Chemical prospecting among marine organisms is still in its early stages. But with several compounds showing promise in the lab and a handful moving toward clinical trials, researchers like Fenical are confident that it is just a matter of time before they hit pay dirt. And, when that happens, the big drug companies will follow. "Within the next 5 years companies will look into the ocean as they've never done before," he says.

–Faye Flam



Halichondrin B	Ecteinascidin	Halomon
Lissodendoryx (and others)	*Ecteinascidia turbinata*	*Portieria hornemannii*
Sponge	Tunicate	Red alga
Preclinical	Clinical trial application pending	Preclinical

SOURCE AND PHOTOS: NATURAL PRODUCTS COLLECTION/NCI