Taxoids: New Weapons against Cancer

The chemists who developed the cancer-fighting agent taxol are creating a family of similar compounds that may one day help combat the disease

by K. C. Nicolaou, Rodney K. Guy and Pierre Potier

ERIK S. LESSER

Just five years ago the chemical known as taxol made headlines as a breakthrough treatment for ovarian cancer. There was only one problem—the drug was incredibly hard to come by. Researchers had to extract the substance from the bark of the Pacific yew *(Taxus brevifolia)* in a process that inevitably killed the tree. Even more frustrating, yews grow slowly (a full-grown tree is around 25 feet tall), and each plant yields little bark. A 100-year-old tree provides only a gram of the compound, about half the amount needed for a single treatment. In addition, yews that produce taxol exist within the delicate old-growth forest of the Pacific Northwest, and harvesting the endangered trees would cause irreparable harm to this ecosystem. As the number of Pacific yews dwindled, environmentalists argued to protect the few remaining trees, while cancer patients and their families pleaded for more of the drug.

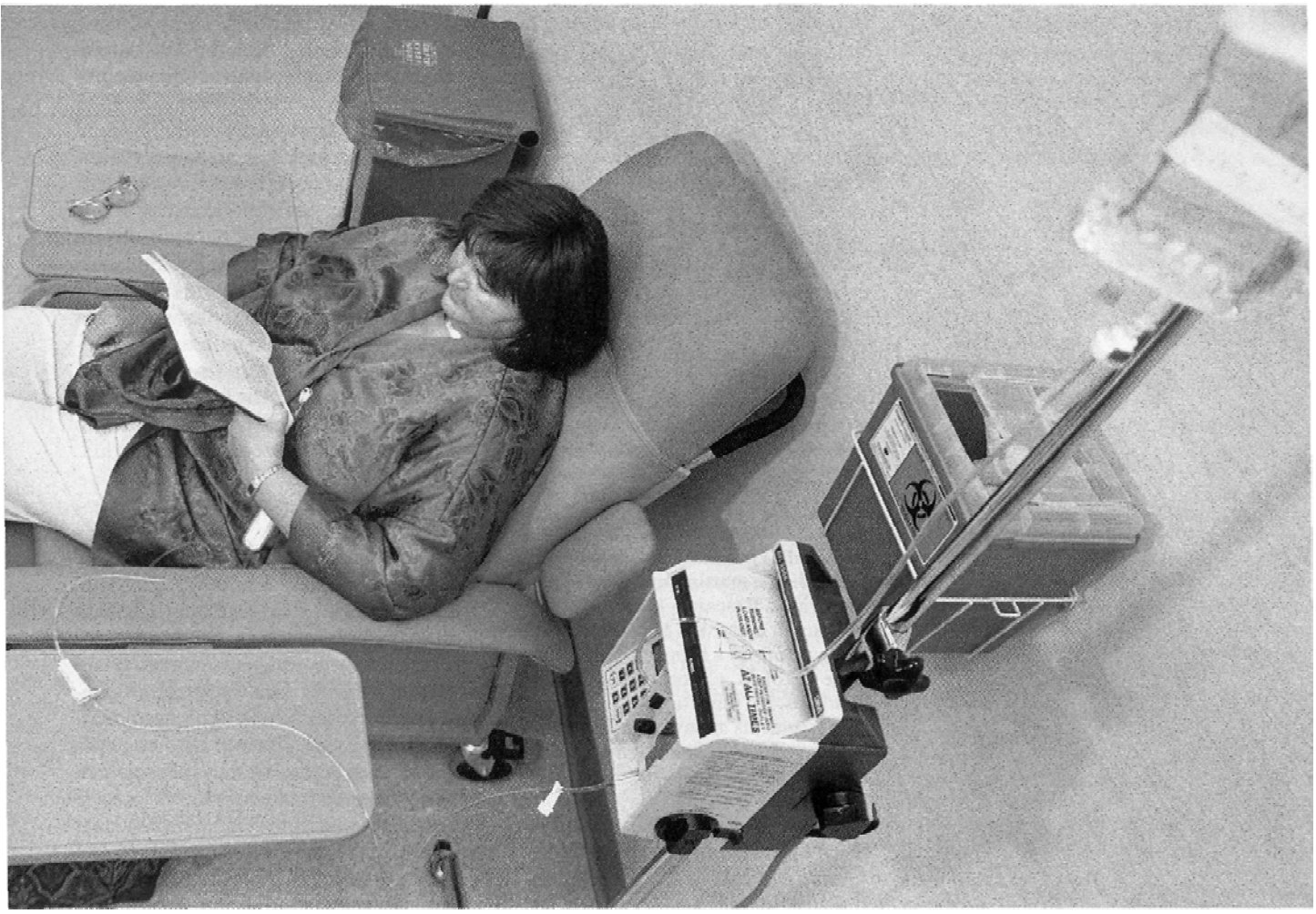
Today the headlines about taxol are quite different. In 1994 the U.S. Food and Drug Administration approved semisynthetic taxol, made in the laboratory and available in unlimited quantities, for use in the treatment of various cancers. Early this year a team of physicians based at Emory University announced results from an extensive study of the drug. Instead of lamenting its scarcity, the researchers emphasized its unexpected potency. According to the findings, women suffering from advanced ovarian cancer who took taxol in combination with another anticancer medication lived an average of 14 months longer than patients who received other therapies. Taxol is now considered one of the most promising treatments for breast and ovarian cancer. Other studies have demonstrated its effectiveness against lung cancer and melanoma. How did taxol, an agent initially known mainly for its absence, become renowned for its powerful presence?

The story of taxol provides an important lesson about how scientists discover and develop new drugs. Chemists first identified the compound almost 30 years ago. Since that time, biologists have determined how taxol works, and physicians have explored its healing properties. Many researchers, including the three of us, are pursuing the challenges of developing an entire family of taxol-like compounds—known as taxoids—that may eventually be easier to manufacture and that may also afford more and better therapeutic options than the parent molecule taxol.

Discovering Taxol, Again

Modern pharmaceutical interest in taxol extends back to the 1960s, but the medicinal properties of the yew tree have been known for centuries. In one of his seven books, collectively entitled *On the Gallic Wars,* published in 51 B.C., Julius Caesar recorded the death of the chieftain Catuvolcus, who committed suicide by drinking tea made from yew bark. In the northwestern U.S., Native American tribes such as the Quinault, Multnomah and Nez Perce utilized the Pacific yew's bark as a disinfectant, an abortifacient and a treatment for skin cancer. Over the past 100 years, however, yew trees attracted little attention—at least until very recently. In the Pacific Northwest, for instance, logging companies simply burned yews after clear-cutting the towering pine and fir trees that surrounded the much smaller yews.

But in 1962 the botanist Arthur Barclay of the U.S. Department of Agriculture started the yew on a long and circuitous journey back to being one of the most valuable trees in the Pacific Northwestern forest. At the time, the National Cancer Institute (NCI) had requested that researchers sample natural sources—such as plants, bacteria and marine life—in hopes of finding substances that might be useful as pharmaceuticals.



CANCER THERAPY WITH TAXOL involves repeated intravenous transfusions, each of which may last up to six hours. Here a woman at the Winship Cancer Center of Emory University receives taxol intravenously for ovarian cancer. Researches hope derivatives of taxol, known as taxoids, will be easier to administer with simple injections or even tablets.

Barclay collected bark from Pacific yew trees in the Gifford Pinchot National Forest, located in Washington State.

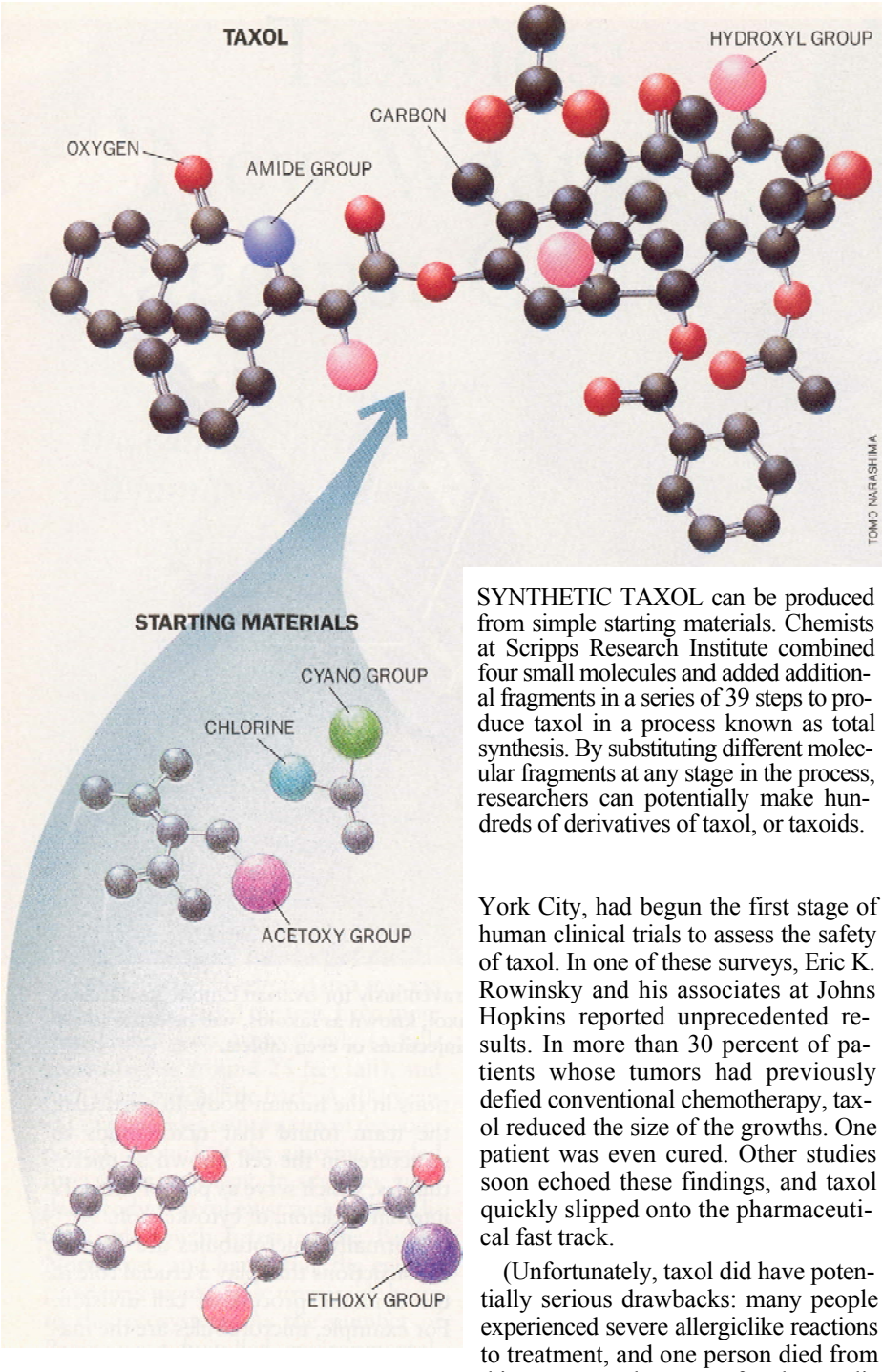
Barclay's yew samples eventually ended up at the Research Triangle Institute in North Carolina. There two chemists, Mansukh C. Wani and Monroe E. Wall, discovered that a mixture containing the yew's bark killed artificially preserved leukemia cells. By 1967 Wani and Wall had isolated the active ingredient from this mixture: a previously unknown chemical that they christened taxol because of its similarities to the family of chemicals known as taxanes and because the substance was found in plants of the genus *Taxus*. (Although the name "taxol" is still widely used generically, the pharmaceutical company Bristol-Myers Squibb has registered "Taxol" as a trademark and wants the scientific community to use "paclitaxel" instead.)

Over the next several years, taxol almost faded back into the forest. The NCI did not consider the compound particularly promising. In early tests, other drugs worked just as well or better than taxol for the treatment of cancer. Taxol was also rare and difficult to obtain. But Wall, acting on a strong belief about its potential, continued to champion the substance to the NCI. In 1977 the agency agreed to investigate the matter further. But even after additional study, taxol still did not stand out from drugs already in the anticancer pipeline.

Rigid Microtubules

Soon after the second round of tests at the NCI, however, a pair of biologists at the Albert Einstein College of Medicine in Bronx, N.Y., uncovered a new facet of taxol. In 1978 Susan B. Horwitz and one of her graduate students, Peter B. Schiff, demonstrated that taxol killed cancer cells in a manner unlike any other drug known at the time. Over the next 10 years, Horwitz's group probed the details of how taxol functions in the human body. In particular, the team found that taxol binds to structures in the cell known as microtubules, which serve as part of the cell's internal skeleton, or cytoskeleton.

Normally, microtubules are flexible constructions that play a crucial role in the dynamic process of cell division. For example, microtubules are the major constituents of the cellular apparatus known as the mitotic spindle, which helps to separate the chromosomes during cell mitosis. When taxol attaches to microtubules, they become extremely stable and static, making cell division impossible, thus killing the cells just as they begin to divide. Cancer cells divide more frequently than healthy cells, so the drug primarily attacks tumors, in which runaway cell division occurs. Bui other rapidly dividing cells, such as white blood cells or hair cells, can also be affected; consequently, taxol is not without side effects when used to treat cancer. For example, taxol can suppress



SYNTHETIC TAXOL can be produced from simple starting materials. Chemists at Scripps Research Institute combined four small molecules and added additional fragments in a series of 39 steps to produce taxol in a process known as total synthesis. By substituting different molecular fragments at any stage in the process, researchers can potentially make hundreds of derivatives of taxol, or taxoids.

patients' immune systems, deaden sensory nerves or cause nausea and hair loss.

The news of taxol's unusual method of attacking cancerous cells excited the research community. Cancer tends to become resistant to treatment over time; because taxol killed tumor cells in a novel fashion, it might offer hope to patients whose disease was not responding to current therapy. By 1984 physicians at a number of hospitals, including the Dana-Farber Cancer Institute in Boston, the Johns Hopkins Oncology Center in Baltimore, and Memorial Sloan-Kettering Cancer Center in New York City, had begun the first stage of human clinical trials to assess the safety of taxol. In one of these surveys, Eric K. Rowinsky and his associates at Johns Hopkins reported unprecedented results. In more than 30 percent of patients whose tumors had previously defied conventional chemotherapy, taxol reduced the size of the growths. One patient was even cured. Other studies soon echoed these findings, and taxol quickly slipped onto the pharmaceutical fast track.

(Unfortunately, taxol did have potentially serious drawbacks: many people experienced severe allergiclike reactions to treatment, and one person died from this response. The cause of such complications remains unclear, but doctors have adjusted the dosage of the drug and how it is administered to minimize the risk of adverse reactions. Nevertheless, as with all chemotherapies, the side effects of taxol continue to trouble physicians and patients.)

When the promising stories of taxol's benefits surfaced, the NCI found itself faced with two challenges. First, although taxol appeared to be excitingly effective, it was far from perfect. But this problem was typical for new drugs. Second, and more unusual, the supply of taxol was running short. Consequently, between 1984 and 1989 physicians could conduct only a limited number of extensive clinical trials. In 1989 the NCI and Bristol-Myers Squibb established a contract that arranged for the company to produce the compound for the NCI in exchange for gaining access to the results of the NCI's clinical trials. Soon after, Bristol-Myers Squibb began large-scale harvesting of the Pacific yew but predicted that supplies would last only five years. Faced with this impending shortage, scientists in many fields, including horticulture, forestry, cellular biology and chemistry, scrambled to find new ways to produce taxol.

Conquering a Molecular Mount Everest

Chemists in particular exhibited a serious interest in taxol. To them, molecules as large and complex as taxol, which contains 112 atoms, are aesthetically as well as scientifically appealing. Its intricate architecture presented a unique challenge to researchers—such as the three of us—who specialize in synthesizing natural products. We knew that the task of making artificial taxol would be a lengthy one, requiring years of work. In the course of the project, we would most likely come to understand the compound's idiosyncrasies—what parts of the structure were particularly stable or fragile and how the molecule interacted with other chemicals. Such information would help us address broader questions about the precise molecular function of taxol in the body of a cancer patient. Eventually, we hope scientists will understand the architecture of taxol and how the compound attaches to microtubules so thoroughly that they will be able to custom-design new drugs with the benefits of taxol but with fewer harmful side effects.

Between 1983 and 1993, more than 30 research groups struggled to synthesize taxol or simpler, related compounds. But taxol proved to be an exceedingly difficult molecule to construct; at times, it seemed unconquerable. Initially, many groups explored the technique known as semisynthesis in their attempts. In this process, chemists essentially start at about the halfway point in the synthesis; rather than joining many small fragments together to produce the final product, they begin with a substance that is very similar to the desired structure (and that is, ideally, cheap and available

in large quantities). Then, by slightly altering this molecule, they obtain the compound of interest in only a few steps.

In the early 1980s one of us (Potier) at the National Center of Scientific Research in France, along with Andrew E. Greene and his colleagues at the Joseph Fourier University in Grenoble, carried out the first successful semisynthesis of taxol. The investigators observed that taxol could be dissected into two parts: the complex center of the molecule, known as the taxane core, and a simpler structure known as the side chain, which is connected to the core. While Potier and his group were screening the European yew *(T. baccata)* for taxollike substances, they realized that the taxane core could be isolated from the needles of this plant. They then figured out a straightforward way to attach the side chain. Because the team obtained the taxane core from the needles, which grow back after harvesting, the procedure offered hope that supplies of taxol might not always be limited.

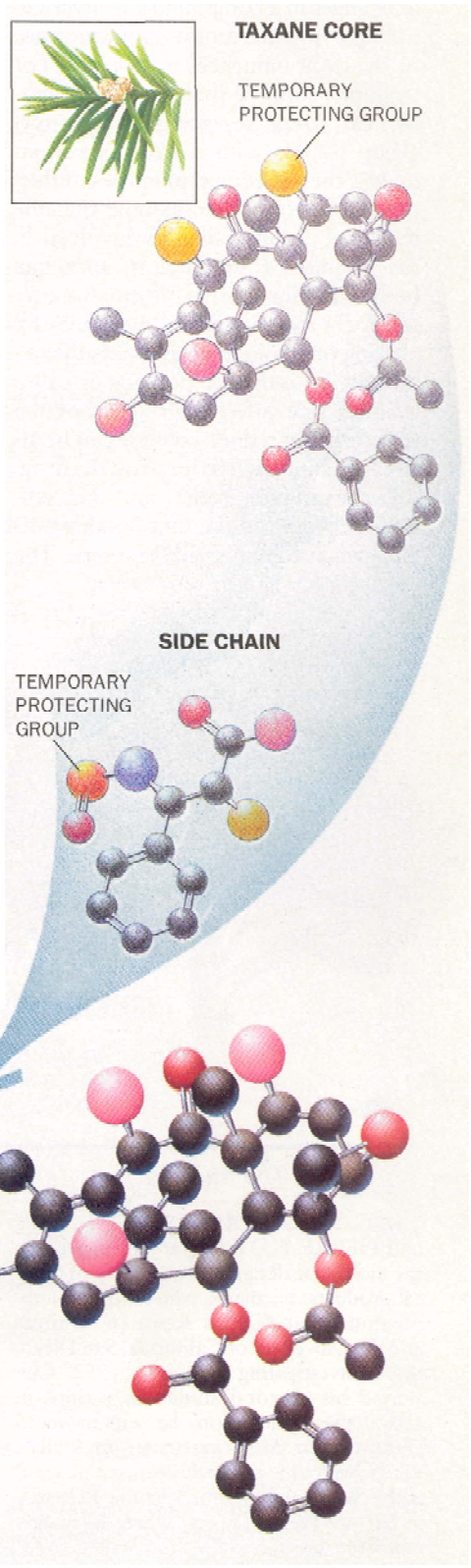
Such hope proved justified when, in 1993, Bristol-Myers Squibb announced that it would no longer harvest Pacific yews. The company had adopted a process for the commercial production of taxol that was initially developed independently by Iwao Ojima of the State University of New York at Stony Brook and by Robert A. Holton of Florida State University. These two researchers also employed a semisynthesis, but their side chain and the method they used to attach it to the core differed from the French version.

Starting from Scratch

As Potier, Greene and others focused their efforts on producing taxol by semisynthesis, researchers elsewhere, including the two of us—Nicolaou and Guy—at Scripps Research Institute, continued to work on a total synthesis. By constructing taxol with simple building blocks, we would be able to modify the compound's structure at any position, thereby creating a variety of taxol derivatives, or taxoids, some of which might prove less expensive and more potent than taxol itself. In early 1994 two groups almost simultaneously reported a total synthesis of taxol. Nicolaou, Guy and their colleagues first published the results of their work in the journal *Nature;* Holton's group recounted its success in the *journal of the American Chemical Society.*

Any synthesis of taxol must take into account the inherent symmetry, or "handedness," of natural products. Structures with this property—like our two hands—exist as mirror images of each other; we refer to each mirror image as an enantiomer. But often only one enantiomer can produce an effect in the human body. Not surprisingly then, scientists believe only one form of taxol can combat cancer. The proper enantiomer can be selected early on, by starting with chemicals of the appropriate configuration and then maintaining this orientation at every step of the synthesis. This approach restricts the choice of starting material, however, and thereby limits the versatility of the synthesis. To avoid such constraints and to reserve the option of constructing both enantiomers, our group at Scripps employed the technique known as resolution, which allowed us to distinguish between enantiomers. We were then free to work with a mixture of enantiomers and to select the relevant configuration near the end of the synthesis.

To further streamline the efficiency of our method, we assembled taxol using what is called convergent synthesis. Using this approach, one begins with several small pieces and joins them together to obtain the desired product; in contrast, linear synthesis involves modifying a single starting compound sequentially. The final structure can be altered in



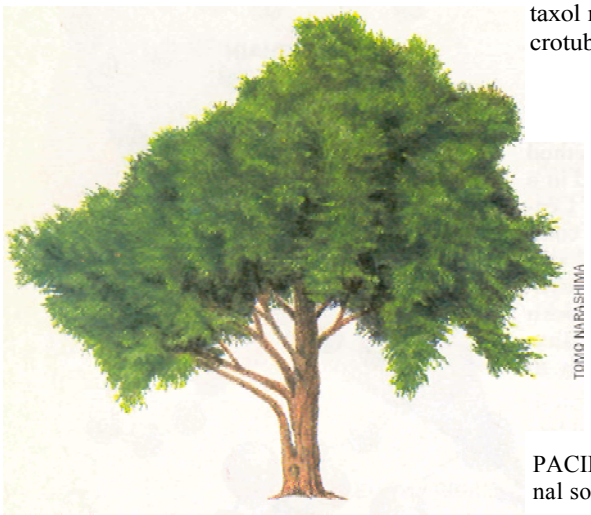
MAKING TAXOTERE from a complex substance similar to both taxol and Taxotere, which is found in the needles of the European yew *(inset),* offered a method of producing a taxollike compound in a few steps. Scientists at the National Center of Scientific Research in France combined the taxane core with a small side chain in a technique known as semisynthesis. Although semisynthesis provides a quick way to produce taxol and Taxotere, it does not lend itself as easily to making a variety of taxoids.

a convergent synthesis fairly easily by introducing different building blocks at any stage of the process; in a linear synthesis, the choice of building blocks is much more restricted. In this way, we could make small, systematic changes in taxol's central core or side chain.

Chemists routinely make these kinds of changes in a compound's structure to evaluate how the molecular framework of the drug influences its potency. For example, suppose that for some hypothetical drug, replacing a hydroxyl group (-OH) with a hydrogen atom makes the substance much less effective. One would then assume that the hydroxyl group is directly involved in the chemical's interaction with the body. Drawing on this information, researchers can make new molecules by altering or eliminating segments that either do not influence potency or cause harmful side effects. Or parts of the structure that reduce potency can be altered or eliminated to improve the drug.

For example, Potier and his colleagues produced the first notable taxoid, which they named Taxotere. The structure of Taxotere differs from taxol at two sites, but fortunately the taxoid also combats the growth of tumors. Physicians in Japan and Europe commonly use Taxotere as a therapy for breast and ovarian cancers; in late 1995 the FDA approved Taxotere for women with drug-resistant or metastatic breast cancer. Taxotere and taxol appear to have subtle differences in their ability to treat certain cancers. Extensive use of both drugs in clinical trials should allow scientists to define any advantages that one may have over the other.

Nicolaou, Guy and their colleagues at Scripps have produced two important classes of taxol derivatives that might one day yield functional pharmaceuticals. First, they simplified taxol's structure and produced a taxoid that is somewhat easier to make than taxol but, in preliminary tests, can still kill certain types of cancer cells. Second, the group has developed a class of taxoids that differs slightly at what seems to be the region of taxol that attaches to microtubules. Scientists are continuing work to improve taxol's potency by tinkering with this binding site and thus making taxol more efficient at connecting to microtubules and preventing cell division.



PACIFIC YEW TREE provided the original source of the anticancer agent taxol.

Improving Taxol

All three of us have also been attempting to solve one of taxol's major pharmacological drawbacks: its inability to dissolve in water. This property makes administering taxol to patients complex and difficult. Currently doctors administer taxol intravenously over several hours; the liquid medium used in this process, Cremophor El, has caused complications in some patients. A water-soluble compound would be much easier to handle. One new taxoid developed at Scripps dissolves in water and could possibly be administered with fewer side effects.

Other water-soluble taxoids produced in the laboratory allow us to examine in greater detail how taxol actually attaches to microtubules. Because taxol itself is so resistant to solubility, investigators have typically analyzed its crystalline, or solid, structure. Unfortunately, the solid form of a molecule does not always accurately reflect the way the compound exists in the aqueous environment of the cell. By observing how dissolved taxoids attach to microtubules, we can get a sense of which segments of the taxoid molecules are most likely to interact with cells. Obviously, if we want to manipulate taxol's structure to better its effectiveness, we need to know where and how this binding occurs. We may be able to enhance taxol's ability to latch onto microtubules and thus kill cells. At the very least, we would not want to alter the binding site in such a way as to diminish taxol's potency.

Clearly, the story of taxol is not complete. But in the discovery of new drugs, one rarely cries "Eureka!" Rather the process takes years of detailed research to determine how a drug works and how to improve its potency. In the case of taxol, scientists have made significant progress, not only figuring out how to produce large quantities of the originally scarce drug but also finding new applications for its use in cancer therapy. Now we have turned to yet another challenge—tinkering with taxol's structure until we find a less expensive, more effective medication.

The Authors

K. C. NICOLAOU, RODNEY K. GUY and PIERRE POTIER share an interest in the molecular design and chemistry of natural products. Nicolaou, who holds joint appointments at Scripps Research Institute and the University of California, San Diego, began investigating taxoids in 1992. Guy started his doctoral studies at Scripps in 1991; after graduation, he will move to Southwestern Medical Center in Dallas. Potier began his research on taxol in 1980 at the National Center of Scientific Research in Gif-sur-Yvette, France, where he is currently the director.

Further Reading

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