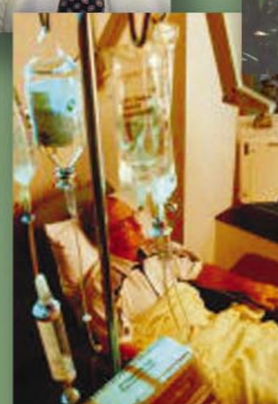
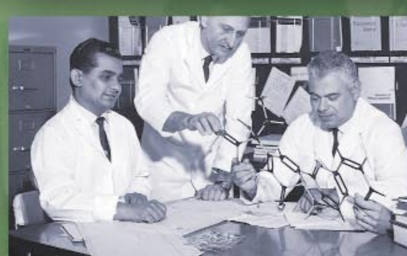
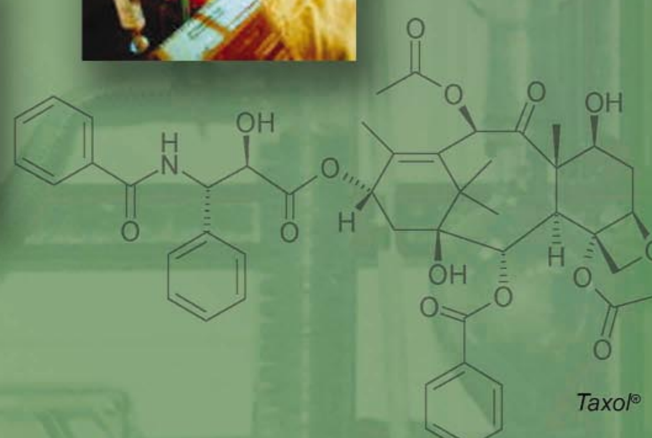
The Discovery of Camptothecin and Taxol®

April 23, 2003

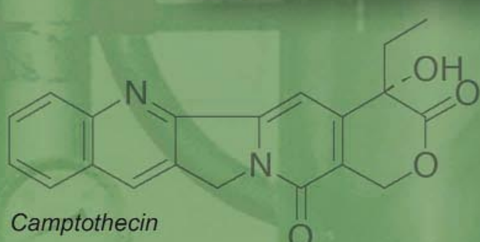
A National Historic Chemical Landmark







Taxol®



Camptothecin

AMERICAN CHEMICAL SOCIETY

SCIENCE THAT MATTERS

The Discovery of Camptothecin and Taxol®

Celebrating Chemistry

The American Chemical Society designated the discovery of camptothecin™ and Taxol® at the Research Triangle Institute a National Historic Chemical Landmark on April 23, 2003. For additional information see our Web site: www.chemistry.org/landmarks.

Monroe E. Wall

Cancer research before 1960

The introduction of antibiotics led to a shift in mortality patterns. By the 1950s deaths from infectious diseases, especially pneumonia, began to decline and heart disease and cancer became major killers. Interest in cancer research, especially chemotherapy, intensified.

To spur the search for anticancer agents, the National Cancer Institute (NCI) created the Cancer Chemotherapy National Service Center (CCNSC) in 1955 to act as a public screening facility. Initially, CCNSC evaluated mostly substances whose chemical structures were well known, such as synthetics and fermentation products. In 1960 the program began to screen extracts of plants and animals as complex mixtures of compounds of unknown structures.

Humans have long used plants for a multitude of medical purposes. But while there was a great deal of folkloric knowledge about the general medicinal properties of plants, virtually nothing was known about the cytotoxic and antitumor potential of plants.

Discovering camptothecin™

In the 1950s Monroe Wall headed a United States Department of Agriculture (USDA) program that examined plants in the search for phyto-steroids that could serve as precursors to cortisone, which was in great demand but of limited supply at that time. Samples of a thousand plant extracts from this project were sent to NCI to test their anticancer potential. One of them, *Camptotheca acuminata,* a tree native to China, demonstrated potent anticancer activity.

The USDA was not interested in anticancer drug research, so Wall's ambition to identify the active component(s) in *C. acuminata* had to be put on hold until July 1960 when the newly founded Research Triangle Institute (RTI) in North Carolina recruited him. Wall lured Mansukh Wani to RTI in 1962, the start of an extremely successful collaboration that spanned four decades. According to Wani, in the beginning RTI was "nothing but four 'walls.' It was not until the fifth 'Wall' arrived that the chemistry programs, in the form of the Natural Products Laboratory, started moving."

By 1963 RTI began working on a 20 kg sample of the wood and bark of *C. acuminata* using the process of 'bioactivity-directed fractionation.' In this the crude plant extract is purified in an iterative manner. Fractions showing the most potent activity are carried on to the next stage of purification. The process is repeated many times until the compound or compounds responsible for the bioactivity observed in the crude extract are isolated.

All the fractions were analyzed both in an *in vivo* L1210 mouse leukemia life prolongation assay and in an *in vitro* KB (human oral epidermoid carcinoma) cell culture cytotoxicity assay. The pure compound isolated via bioactivity-directed fractionation, termed camptothecin, proved to be very potent against L1210. Its structure was determined by forming crystals that were suitable for X-ray crystallography. These results were reported by Wall, Wani, and colleagues in 1966 in the *Journal of the American Chemical Society,* the first paper they published on a natural product with anticancer potential.

The NCI proceeded with clinical trials. However, problems with formulation, in particular its poor water solubility, and patient side effects sent camptothecin back to the medicinal chemistry laboratory. The use of camptothecin as an anticancer agent languished for almost fifteen years until its unique mode of action for killing tumor cells was determined. Camptothecin traps an important cellular enzyme, topoisomerase I, in complexes with DNA. This prevents cancer cell DNA replication and results in the death of the cancer cell. The discovery of this unique mode of action rekindled interest in developing analogs of camptothecin that were both water soluble and retained anticancer activity. In the mid-1990s, two camptothecin analogs, topotecan and irinotecan, received FDA approval for use against ovarian, lung, breast, and colon cancers.

The Pacific yew

On a hot August day in 1962 Arthur Barclay stood in Washington State's Gifford Pinchot National Forest staring at a small stand of scraggly conifer trees. A Harvard-trained botanist, Barclay worked for the USDA and he was collecting plant samples for the NCI. In the stand, at an elevation of 1,500 feet, Barclay spotted a twenty-five foot tall Pacific yew tree, *Taxus brevifolia,* from which he took samples.

T. brevifolia is an evergreen of medium height with reddish bark and flat, slightly curved needles about an inch long. It has hard wood of limited use. It has few natural pests because most of it is poisonous. And, significantly for the future, the Pacific yew grows very slowly.

Discovering Taxol®

Barclay's samples of *T. brevifolia* were sent to the Wisconsin Alumni Research Foundation for initial extraction. The bark of the tree showed cytotoxicity against KB cells. This gained the attention of the NCI and on September 8, 1964, Barclay returned to the site of the original collection and bagged and shipped to RTI thirty pounds of bark from *T. brevifolia.*

Wall and Wani initiated a bioactivity-directed fractionation study to isolate the pure compound in *T. brevifolia* responsible for its anticancer activity. Early attempts to uncover the secret of the yew's anticancer activity failed. This initial work coincided with the isolation of camptothecin, which was "looking very hot" and on which NCI was ready to begin clinical trials. Moreover, RTI had many other plant extracts to evaluate. At one point, Wall was ready to give up on *T. brevifolia.*

In September 1966 the researchers isolated from one of the fractions a crystalline substance, which Wall named "taxol." This name was fitting for two reasons. First, while the RTI scientists did not yet know the compound's complete structure, they were sure it contained some hydroxyl groups, signifying it was an alcohol. Moreover, it was common to name a molecule after the genus of the plant from which it came. Accordingly, the molecule became known as Taxol® – "*tax*" for *Taxus* and "*ol*" for an alcohol. Besides, the name, as Wall and Wani would later write, "had a nice ring to it."

To definitively elucidate the structure of Taxol®, Wall and Wani used mass spectrometry, X-ray crystallography, and NMR spectroscopy, a technique then in its infancy. These various tools yielded the molecular structure with a formula of $C_{47}H_{51}NO_{14}$. Wall and Wani also discovered that Taxol® was two molecules in one – a large, structurally complex core molecule connected to a small tail or side chain. In 1971 Wall, Wani, and colleagues published the complete structure of Taxol® in their sixth paper on natural products with promising anticancer activity. Interestingly, the structure of Taxol® is so complex that they almost went to press with the side chain of the molecule placed in the wrong position.

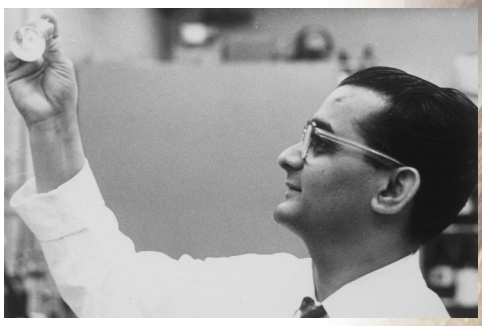
Taxol® becomes a drug

It would be another twenty years before Taxol® reached market as a chemotherapy drug. There were many reasons for the delay, but the primary one was supply: extracting Taxol® from *T. brevifolia* was arduous and the slow growing tree yielded only small amounts of the compound. Moreover, bark is a finite resource; a tree stripped of its bark dies.

Despite the supply problem, research continued. Studies showed Taxol® active against B16 melanoma. Further tests demonstrated its ability to cause considerable regression when used against mammary tumors transplanted in mice. A significant breakthrough came in the late 1970s when Susan Horwitz, a molecular pharmacologist in the Albert Einstein College of Medicine at New York's Yeshiva University, discovered Taxol's® unique mode of action. Previous antitumor agents killed cancer cells by inhibiting their division; they did this by preventing the production of ultrafine filaments called microtubules that enable cells to divide. Horwitz discovered that Taxol® worked differently. Instead of preventing the formation of microtubules, it stimulated their development. Cells treated with Taxol® churn out so many microtubules that they are unable to coordinate cell division. As a result, cells die of continued attempts to replicate their DNA in the absence of the ability to divide.

In clinical trials against the most virulent forms of ovarian cancer,

Mansukh C. Wani



Taxol® showed a previously unheard of response rate of thirty percent. But to make it available to all ovarian cancer patients, the NCI would have had to produce 240 pounds of the drug. That would mean killing 360,000 trees. It did not take a mathematical prodigy to understand that this was an equation without a future.

The solution came in the form of a semi-synthesis in which scientists succeeded in extracting 10-deacetylbaccatine III, commonly known as 10-DAB, from the ubiquitous *Taxus baccata*, or English yew. This compound constitutes the complex core of Taxol® minus the relatively simple side chain. Most importantly, 10-DAB comes from the needles, a renewable resource. Researchers developed a way to marry 10-DAB to a synthetic version of the side chain to achieve a semi-synthesis of Taxol®.

With the clinical trials going well, the NCI began to look for a pharmaceutical company to take a chance on turning Taxol® into a marketable drug. In 1989 the Institute reached an agreement with Bristol-Myers, soon to merge with Squibb, another giant in the field. In December 1992, the Food and Drug Administration granted approval for Taxol's® use in refractory ovarian cancer. It has since been approved for use in the treatment of breast cancer and AIDS-related Kaposi's sarcoma.

Note: Camptothecin is a trademark of the Research Triangle Institute. Taxol® is a registered trademark of Bristol-Myers Squibb.

National Historic Chemical Landmark

The American Chemical Society designated the discovery of camptothecin and Taxol® a National Historic Chemical Landmark on April 23, 2003. The plaque commemorating the event reads:

The research team of Monroe E. Wall, Mansukh C. Wani, and colleagues discovered two life-saving anticancer agents from natural products. In 1966, they reported the first of these, Camptothecin, from the Chinese tree* Camptotheca acuminata. *In 1971, they reported the structure of Taxol from the Pacific yew,* Taxus brevifolia. *These natural products were isolated by a novel bioactivity-directed fractionation of the crude plant extracts. Both of these compounds kill cancer cells by unique and previously unknown mechanisms of action, and together they have been approved for treatment of ovarian, breast, lung, and colon cancers and Kaposi's sarcoma.

About the National Historic Chemical Landmarks Program

The American Chemical Society, the world's largest scientific society with more than 161,000 members, has designated landmarks in the history of chemistry for more than a decade. The process begins at the local level. Members identify milestones in their cities or regions, document their importance, and nominate them for landmark designation. An international committee of chemists, chemical engineers, museum curators, and historians evaluates each nomination. For more information, please call the Office of Communications at 202-872-6274 or 800-227-5558, e-mail us at nhclp@acs.org, or visit our web site: www.chemistry.org/landmarks.

A nonprofit organization, the American Chemical Society publishes scientific journals and databases, convenes major research conferences, and provides educational, science policy, and career programs in chemistry. Its main offices are in Washington, DC, and Columbus, Ohio.

Acknowledgments:
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