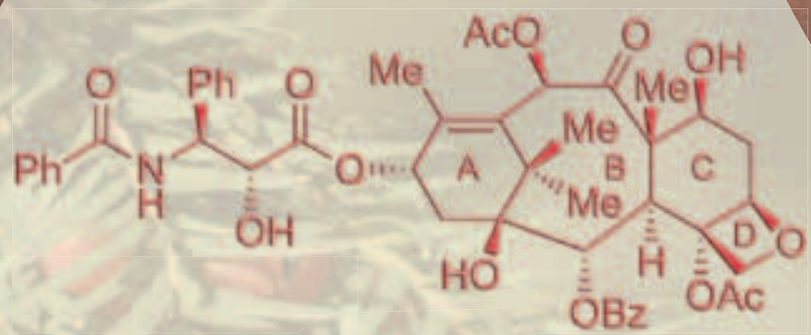
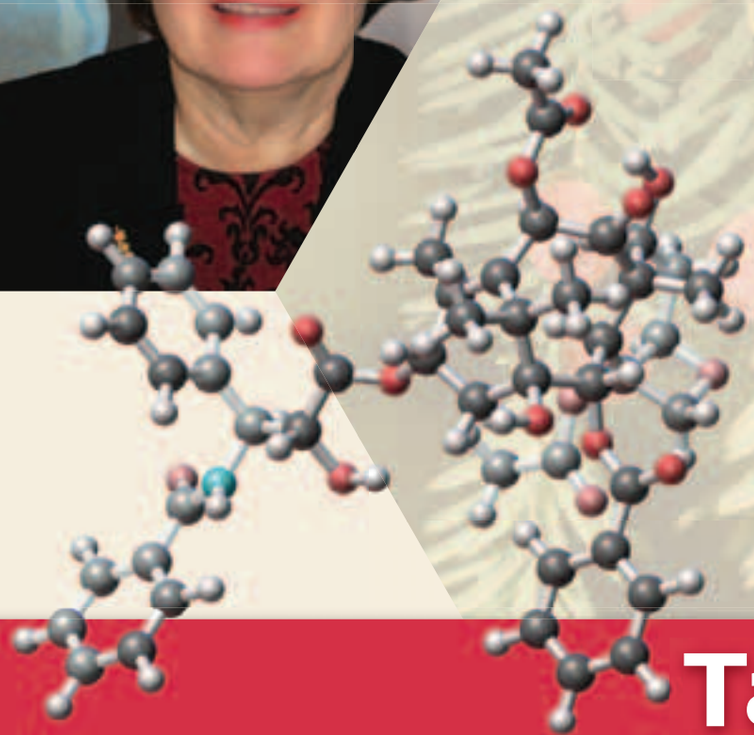


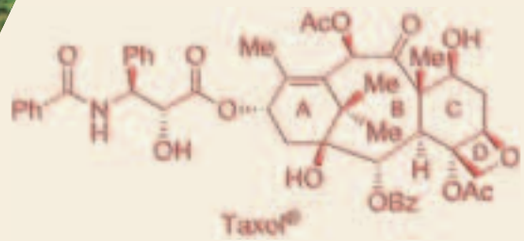


Taxol®

Chapter 25

1994

Taxus brevifolia

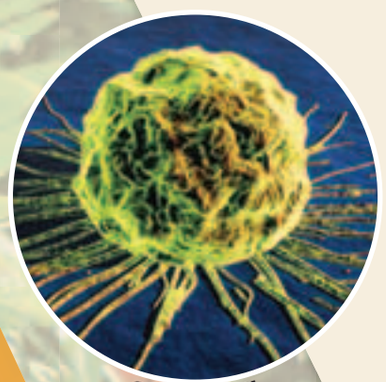


Taxol®

The legend of Taxol® reveals a prime example of how natural products and chemical synthesis influence things at the very heart of our lives. It is a tale of hope for humanity which also brims with scientific drama and intrigue. Taxol® is one of the best-selling anticancer drugs, saving and improving the quality of countless lives around the world. It is also a scarce natural product with a beautiful and complex molecular architecture that chemists were able to isolate and construct in the laboratory, thus learning about its intricate properties and facilitating its advancement to the clinic as an effective cancer-fighting drug.

Cancer is a word that instills a deep-seated fear because we immediately associate it with grave illness and a high mortality rate. Almost all of us know someone whose life has been blighted by a cancer diagnosis, and who has suffered the prolonged pain of the illness. Cancer patients are forced to tolerate a tough treatment regime with all the accompanying side effects and subsequent problems. Few people are fortunate enough to escape the distress of cancer over their lifetime, since the frightening statistics would suggest that the vast majority of us will either experience it first hand, or have a loved one afflicted. However, as we advance our understanding of the mechanisms involved in causing and propagating cancer, we are gradually uncovering a host of new leads and hopes for cures. Scientists and clinicians diligently and continuously harness such intelligence and powerful resources, laboring to convert them into practical strides forward, giving us hope for a future where cancer is not a death sentence, but a curable disease. The story of the development of Taxol® provides a striking example of how advances in several fields of science and technology can be combined to provide new lifesaving therapies.

Cancer is a collection of a number of rather disparate diseases, all characterized by the uncontrolled proliferation of abnormal cells which invade and disrupt tissues, beginning locally and then spreading through the body to extend the reach of their destructive behavior. Both external causes (e.g. chemicals, radiation, viruses) and internal factors (e.g. hormones, immune conditions, inherited genes), acting either alone or in combination, may be responsible for the initiation and promotion of carcinogenesis. Furthermore, many years can pass between cause and detection, and, with some types of cancer, there exists the obstinate problem of detecting malig-



Cancer cell

nant growths early enough for intervention to stand any chance of success.

The global impact of cancer cannot be overstated. It constitutes a major public health problem with an ever growing worldwide occurrence. In 2003, approximately 1.34 million people were diagnosed with cancer (not including basal and squamous cell skin cancers) and more than half a million patients died from the disease in the United States alone. The only condition attributed with more deaths per annum is cardiovascular disease, which is responsible for one in every four deaths in the industrialized world. The American Cancer Society estimates that men have slightly less than a one in two lifetime risk of developing cancer; for women the risk is slightly more than one in three. Quite apart from the personal suffering caused by this high incidence, there is an immense financial cost to both the individual and society as a whole in the form of direct medical expenses and lost productivity. As a consequence, each small step scientists make in advancing our ability to treat this heinous group of diseases translates into a major unburdening for society, not to mention the lives saved.

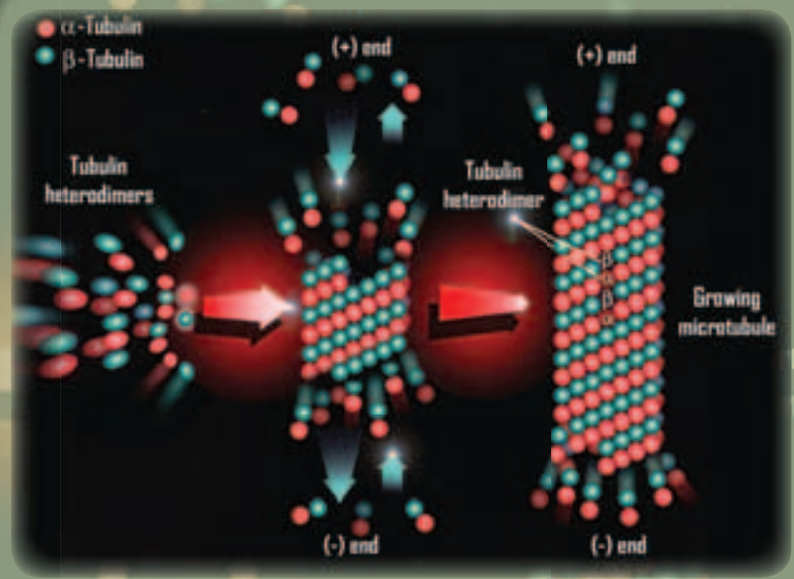
Bark of *Taxus brevifolia*

The seriousness of the problem was recognized in the United States in the 1950s, prompting the rapid passage of new legislation over the ensuing two decades as part of a campaign against the disease. This legislation included the 1971 National Cancer Act which aimed to invigorate cancer research through a directed and substantial increase in funding. As a result of this act proclaiming "the conquest of cancer a national crusade," several new cancer centers were established around the country, and research in the field was accelerated considerably. However, while the long and arduous journey of Taxol® to the clinic would certainly benefit from this infusion of funds, the story begins much earlier. A specific project directed by the National Cancer Institute (NCI), already underway during the 1960s, aimed to conduct a widespread screening of substances and extracts obtained from a variety of natural sources for antineoplastic activity. Thus, United States Department of Agriculture (USDA) botanist Arthur S. Barclay collected samples of bark from the relatively rare Pacific yew, *Taxus brevifolia,* while on a field trip to an Oregon forest in the summer of 1962. Barclay's choice of the yew was an astute one, since these trees had a long and proud history of use by mankind, and buried within this ancient knowledge were the clues that they might contain cytotoxic compounds. For example, Julius Caesar recorded that, after defeat at the hands of the Roman

Needles of *Taxus baccata*

Julius Caesar

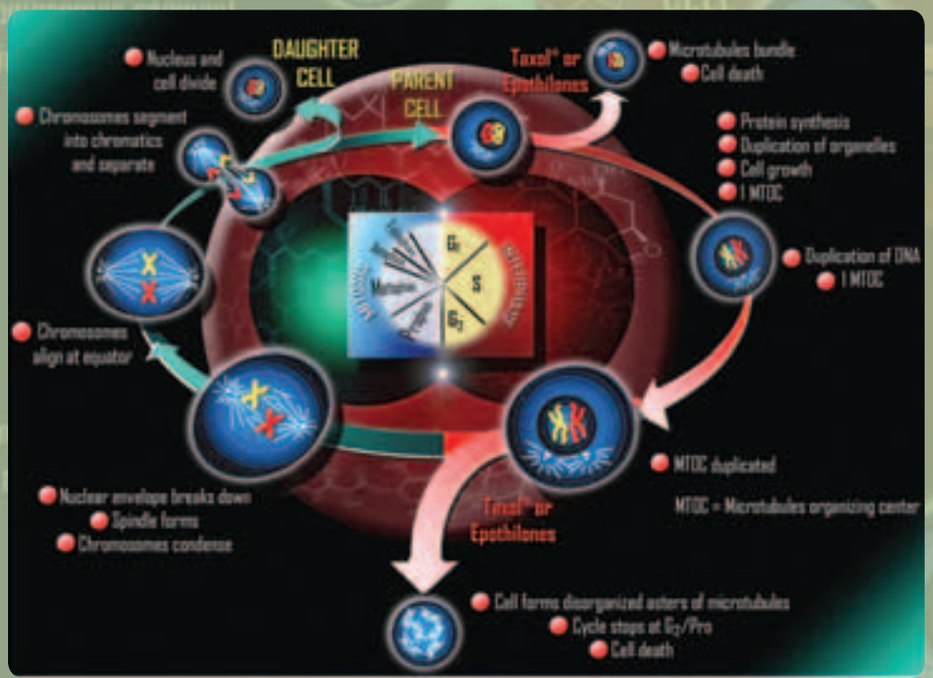
Box 1 Tubulin polymerization and microtubules



Monroe E. Wall and Mansukh C. Wani

Susan B. Horwitz

Box 2 The cell cycle and the mechanism of action of Taxol®



legions, the Gallic chieftain Cativolcus committed suicide by drinking tea made from yew bark. Barclay's samples were eventually packed up and sent to the Research Triangle Institute (North Carolina, USA) in 1964, where two chemists, Monroe E. Wall and Mansukh C. Wani, were charged with their investigation. The crude extracts of the yew bark were shown to exhibit an unusually broad spectrum of cytotoxic activity against leukemia cells, as well as against a variety of other cancer cells. This activity spurred on Wall and his colleagues to isolate the active constituent of the extracts, the complete structure of which was definitively established, in collaboration with researchers at Duke University (North Carolina, USA), by x-ray crystallographic analysis in 1971. Of the more than 110,000 compounds from 35,000 plant species tested by the NCI between 1960 and 1981, this active ingredient, named Taxol®, proved to be the most promising. Despite its promise, however, Taxol® was set to languish on laboratory shelves for almost another full decade before the next advance was made. The reluctance to pursue investigations into the potential therapeutic benefits of Taxol® was largely due to difficulties associated with its isolation and low solubility, as well as the belief that it was simply another microtubule-destabilizing agent akin to the other available natural products, colchicine and the vinca alkaloids, already in use as anticancer agents. However, in 1979, Susan B. Horwitz (Yeshiva University, New York, USA) and coworkers reported in the journal *Nature* that Taxol® actually possessed the unique characteristics of stabilizing and promoting the formation of microtubules. This exciting disclosure led to new research momentum, since this mode of action constituted an entirely new mechanism of intervention against the aberrant replication of cancerous cells (Boxes 1 and 2).

Microtubules are involved in many aspects of cellular biology; they give shape and structure to the cell, assist in reorganizing organelles, and, most importantly for cancer, play an essential role in mitosis, the process of cell division. Their pivotal role in the growth, function, and division of cells led to their description in the 1990s as "the most strategic subcellular targets of anticancer chemotherapeutics." Microtubules are predominantly composed of two similar protein subunits, α- and β-tubulin, which combine to form a heterodimer (Box 1). The tubulin dimer binds two molecules of guanosine 5′-triphosphate (GTP), and in the presence of magnesium these dumbbell-shaped dimers begin to unite in a head-to-tail fashion forming protofilaments. These, in turn, assemble in a staggered manner, leading to the left-handed helices that make up the microtubule. Typically, exchanges are set up at both ends of the microtubule with regular loss and gain of tubulin subunits at relative rates that are often different, thus endowing the tubule with a growing directionality. In general, microtubules are not static structures and, after a certain period of growth, the microtubule and free tubulin will reach a constant equilibrium concentration. At this point, termed the critical concentration, disassembly and growth are finely balanced in a situation regulated by the

bound GTP molecules. What Horwitz's group showed was that Taxol® affects the tubulin-microtubule equilibrium. Taxol® decreases both the concentration of free tubulin (to almost zero) and the induction time for polymerization, with the result that microtubules forming in the presence of Taxol® have a distinctly different morphology from the control variants. They have a shorter average length and are resistant to change under conditions that would depolymerize normal microtubules. In addition, the Taxol® promoted polymerization of tubulin does not require GTP.

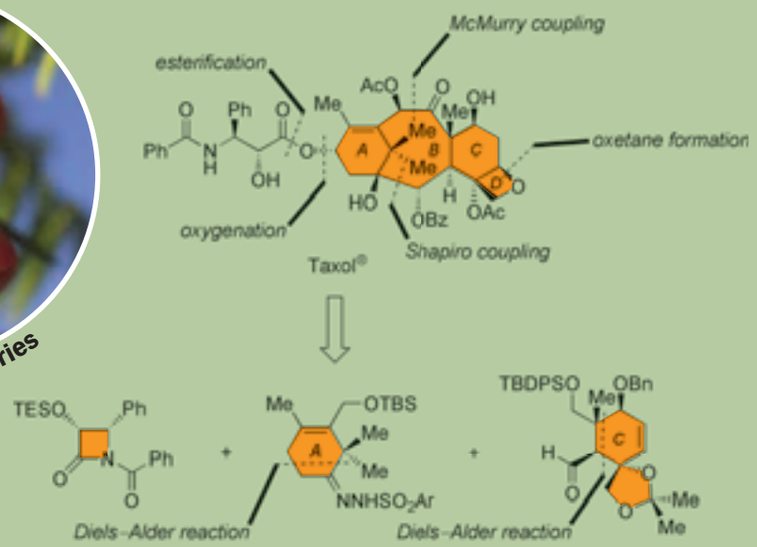
K. C. Nicolaou

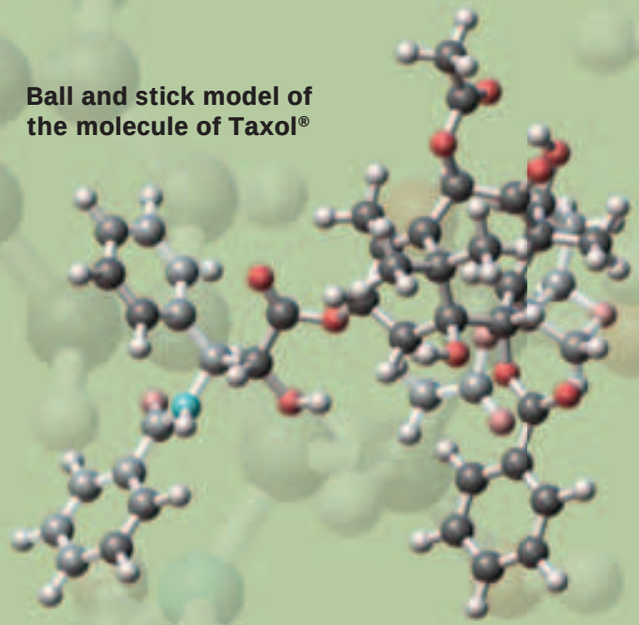
From the brief discussion above, we can now understand the interactions of Taxol® with tubulin and the subsequent effects on the gross architecture of microtubules, but how does this translate into the powerful cytotoxicity of the drug? Most cells, excluding a few that cannot replicate, spend the majority of their time in a nonproliferative state called quiescence, yet they are able to switch rapidly into a reproductive cycle (mitosis) when their populations are low and in need of a boost. The mitotic cycle itself has a number of defined stages (Box 2). Cancer cells, unlike their normal counterparts, do not enter the quiescent state when the population density is high, and furthermore, during the mitotic cycle, cancer cells suffer from a deficiency in the checkpoints which regulate the rate of division so that the problem of excessive expression of these cells is grossly exacerbated. Horwitz showed that tumor cells treated *in vitro* with Taxol® were arrested at the transition from metaphase to anaphase. Morphologically these cells exhibited unnatural bundles of microtubules and no mitotic spindle. Other research groups quickly extended these findings, proving that Taxol® causes the general and irreversible formation of bundles of microtubules at several cell lifecycle stages, visually reminiscent of a log jam. The net effect of this phenomenon is the sequestering of tubulin in the form of stable structures, ultimately preventing the formation of a mitotic spindle, halting cell division, and leading to rapid cell death by apoptosis (suicidal death). Furthermore, the disturbance to microtubule mobility was also shown to have dramatic effects on other processes within the cell, such as inhibiting the secretion of certain proteins, leading to the general conclusion that the full nature of the attack of Taxol® on cancerous cells is likely to be a complex conglomeration of cell function shutdowns.

The Nicolaou Taxol® team

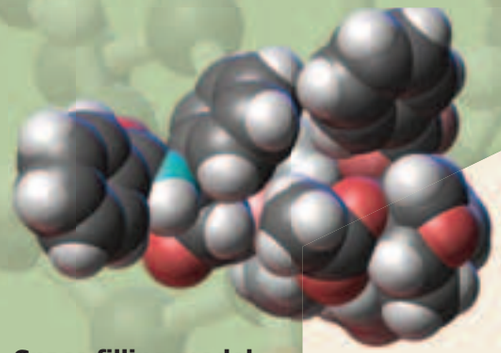
Box 3 The Nicolaou retrosynthetic analysis of Taxol®

Taxus baccata berries



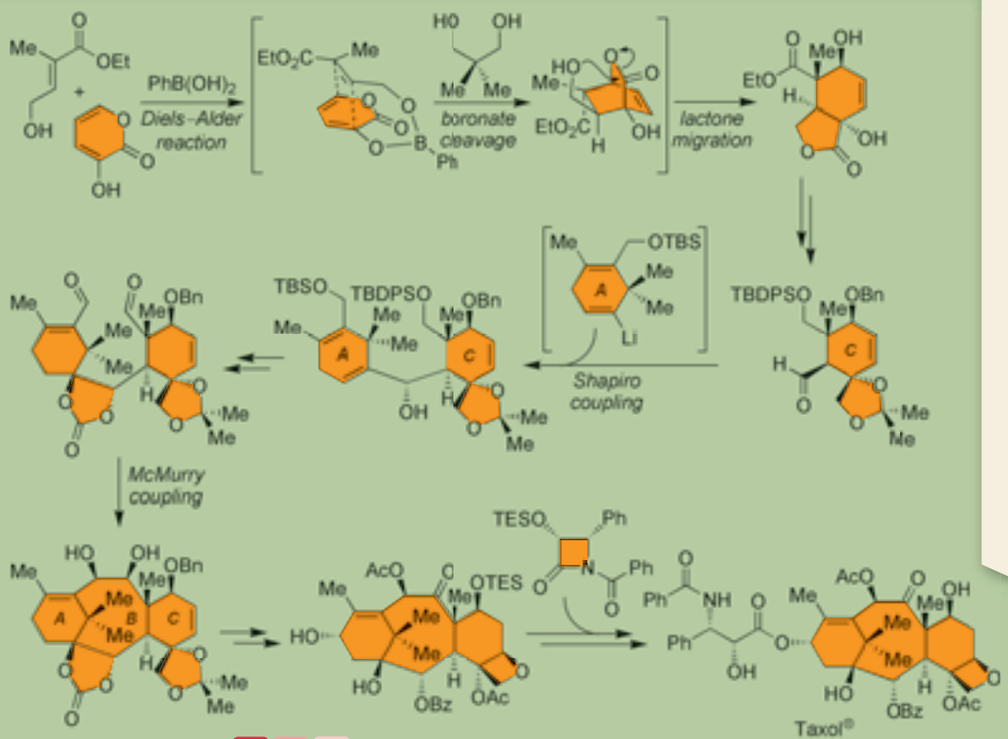


Ball and stick model of the molecule of Taxol®



Space filling model of the molecule of Taxol®

Box 4 Highlights of the Nicolaou group's total synthesis of Taxol®



As these groundbreaking investigations were gradually revealed, scientists became increasingly excited about Taxol® because of its emerging portrayal as a long sought-after ally in the fight against cancer. In the 1980s and 1990s, the media was buzzing with stories of a miraculous cure and a drug that could change the face of cancer treatment forever. However, before these dreams could be realized, passage around a number of difficult logistical obstacles still had to be navigated. The most pressing of these issues was that of supply. The Pacific yew is a slow growing tree and has a tendency to grow in dispersed microsites hidden in ecosystems containing mostly older growth and larger tree species. Prior to the discovery of Taxol®, these trees were regarded as scrub and were, for the most part, ignored in primary logging operations, instead being destroyed in the slash and burn disposal fires that followed. Large numbers of mature specimens concentrated in sizeable and harvestable regions were therefore not available. The extraction of Taxol® itself was also a tedious process requiring large quantities of tree bark to yield relatively small amounts of the compound. For example, 38,000 *Taxus brevifolia* trees were sacrificed to obtain just 25 kg of Taxol®, enough to treat 12,000 patients (that is more than three trees per person) in one of the initial clinical studies of this new medication. Such short supplies were hampering the progress of these early clinical trials, and yet the problem was complicated even further by environmental concerns. Indeed, there was a major controversy and a lively debate surrounding the harvest of so many trees from the delicate forest environments. The concern was voiced that the success of Taxol® would drive the Pacific yew to the verge of extinction and cause irreparable damage to other members of these fragile ecosystems, including the endangered Northern Spotted owl.

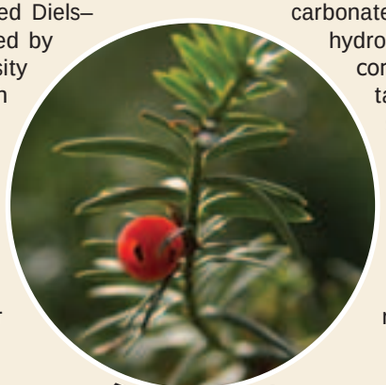
The frustrated medicinal promise that Taxol® held in these heady days created unprecedented interest among synthetic organic chemists, who were motivated by the recognition that a chemical synthesis could potentially provide a solution to the supply problem. This urgency spurred additional teams of researchers to follow the many groups, from a host of countries, who had already embarked on the quest for a total synthesis of Taxol®. The lure was not only the issue of the limited stocks, but also the intrigue and challenge presented by the highly complex and unique architecture of this molecule. The structure of Taxol® consists of a diterpene core that is distinguished by its ring arrangement, a periphery densely populated with oxygen functionalities, and nine asymmetric centers. Appended to this core, at a relatively hindered site, is an ester side chain bearing two additional asymmetric carbon atoms. Taxol® became the Holy Grail of total synthesis, and the race to reach it took on many twists and turns until it finally ended in success in 1994.

The first total synthesis of Taxol® to be published was that emanating from the Nicolaou laboratories at The Scripps Research Institute in La Jolla, California (USA), reported in *Nature* early in 1994. This achievement, together with another

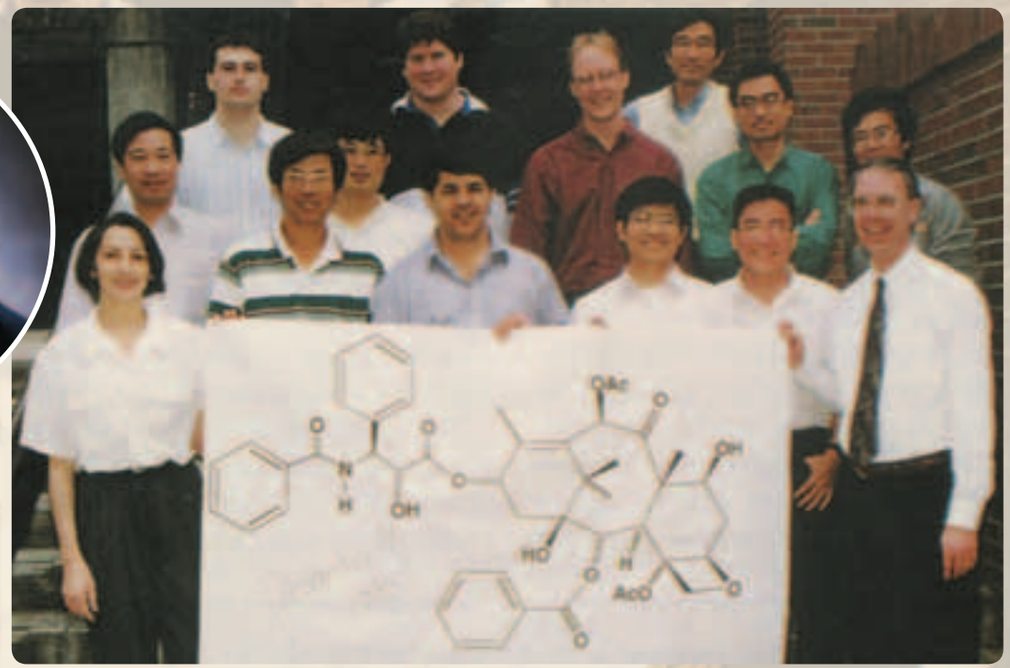
total synthesis which appeared at almost the same time and was accomplished in the laboratories of Robert A. Holton at Florida State University in Tallahassee (USA), ended nearly two decades of recalcitrance exhibited by Taxol®. The Nicolaou group's success relied on the implementation of a highly convergent strategy devised by retrosynthetic analysis (Box 3). Thus, five strategic bonds had been identified and retrosynthetically disconnected, disassembling the molecule of Taxol® into three key building blocks: a β-lactam corresponding to the side chain, a hydrazone encoding the A-ring, and an aldehyde containing the necessary carbon framework and functionality to complete rings C and D. It was anticipated that ring B, which perhaps posed the thorniest problem of the synthesis, might be cast through an intermolecular Shapiro reaction followed by an intramolecular McMurry coupling process. Finally, the venerable Diels–Alder reaction was to be called upon twice, to assemble each of the two requisite cyclohexene rings.

This strategy was successfully implemented, more or less the way it was designed, after a relentless campaign that included many twists and turns and dramatic moments, as summarized in Box 4. Thus, the six-membered C-ring was assembled through the application of an innovative boron-tethered Diels–Alder reaction, developed by Koichi Narasaka (University of Tokyo, Japan), which caused a switch of regioselectivity from the unwanted natural bias of the reactants towards the desired isomer. The A-ring was prepared through a different Diels–Alder reaction, and was then coupled to the C-ring in a subsequent Shapiro reaction. Between each of these key stages adjustment of oxidation levels and functional group manipulations steered the growing molecule towards its final destination. Pleasingly, the challenge of cyclization to furnish the highly strained and crowded 8-membered B-ring was met, as projected, by the McMurry reaction. This remarkable process proved capable of uniting the two aldehyde groups to close the highly strained ring, yielding the tricyclic framework of Taxol® molecule, despite the significant opposing forces. A more advanced intermediate exhibiting all of the structural motifs of the Taxol® was then reached after a number of sensitive and frequently treacherous chemical modifications. Included among these stringent tests were the generation of the highly strained oxetane ring and the regioselective cleavage of the cyclic carbonate to form the desired hydroxy benzoate moiety. To complete the Herculean task of Taxol®'s total synthesis, the hindered ester side chain was finally installed by the reaction of the completed tetracyclic core with an appropriate electrophilic β-lactam ring, according to the

Robert A. Holton



Taxus baccata



The Holton Taxol® team

Box 5 Highlights of the Holton group's total synthesis of Taxol®