Taxol: A Review

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1. Introduction

Cancer is one of the most feared diseases. It involves the rapid and uncontrolled proliferation of "abnormal" cells in the body. The cancerous cell mass disrupts normal functioning of the organ or tissue in which it is found. Current treatments involve surgery, radiotherapy, and chemotherapy often applied in some combination. Naturally occurring chemotherapeutic agents from plants have been sought during the 20th century. The first success was in the late 1930s when it was discovered that colchicine, an alkaloid obtained from autumn crocus (*Colchicum autumnale*), can disrupt the spindle mechanism during mitosis, thereby blocking cell division (Lewis and Elvin-Lewis, 1977).

Clinical studies with colchicine were disappointing, but compounds with similar inhibitory properties from other plants, especially the forest plant periwinkle (*Catharanthus roseus*) showed more promise (Lewis and Elvin-Lewis, 1977). However, success of commercially available cancer chemotherapeutic drugs of plant origin in 1977 was very limited. There was a compound listed to be of interest in the 1977 text, Medicinal Botany (Lewis and Elvin Lewis 1977), with no further reference. That compound was taxol, which came from *Taxus brevifolia*, the Pacific yew. In less than 20 years when the text, Pharmacognosy, Phytochemistry, Medicinal Plants (Bruneton, 1995), was published, the compound taxol was presented as a highly effective antitumor drug. The following year the cancer-fighting agent taxol made the popular press as one of the most promising treatments for breast and ovarian cancer, with indications of effectiveness against lung cancer and melanoma (Nicolaou *et al.*, 1996). Taxol, in two decades, went from being an obscure compound to becoming a household word, such as aspirin, also a product of tree origin (Nicolaou *et al.*, 1994).

The purpose of this brief review is to outline some of the highlights of the discovery, characterization, clinical trials, and production of this

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non-wood forest product. This type of research begins in the forest where trees, other forest plants, and their microbial associates grow and produce a vast number of complex organic compounds. Screening many thousands of extracts yields activity which may prove useful only in a few cases.

2. Natural History

Possible anticancer activity was discovered in an extract of the bark of the Pacific yew (*Taxus brevifolia* Nutt) tree in the early 1960s. The Pacific yew, also called western yew, is a coniferous tree which is highly shade tolerant, generally growing in the understory of some western forests (Fig. 1). The native range of Pacific yew is the Pacific coastal forests of the northwestern United States, Canada, and the southern tip of Alaska (Fig. 2). Although the wood of the yew is hard, heavy, and decay resistant, it is of little interest to the forest products industry because of the small stature of this understory tree.

Although the commercial interest in Pacific yew was marginal before the discovery of taxol as a highly promising anticancer agent created high demand for its bark, the yew tree had a role to play in the ecosystems in which it was found (Fig. 3). Native American cultures, historically, have also made use of the yew. Once taxol was needed in quantity for clinical trials, the demand for yew bark created great environmental concern (Daly, 1992; USDA Forest Service, 1993). Human health and forest health were brought into conflict; a conflict that needed a quick resolution.

3. Public Health History

A brief chronology of the development of taxol as a cancer-fighting drug begins in 1963 when the National Cancer Institute (NCI) found that yew samples showed activity in a cancer-cell tissue culture assay and sent sub-samples to M. Wall, a medicinal chemist (Table 1). Wall's group found that a crude bark extract had anticancer activity and worked to isolate the primary active principle.

In 1971, Wall working with M.C. Wani, A.T. McPhail, and others published the structure of the antitumor agent from the bark of Pacific yew and called the novel compound, taxol (Wani *et al.*, 1971). They stated that taxol has potent antileukemic and tumor inhibitory properties. Taxol is the first compound possessing the taxane ring that has been demonstrated to have such activity. But, how does taxol work in the cells it inhibits?

Preclinical work on taxol began in the laboratory of S. Horwitz in 1977. With her graduate student P. Schiff, they discovered how taxol

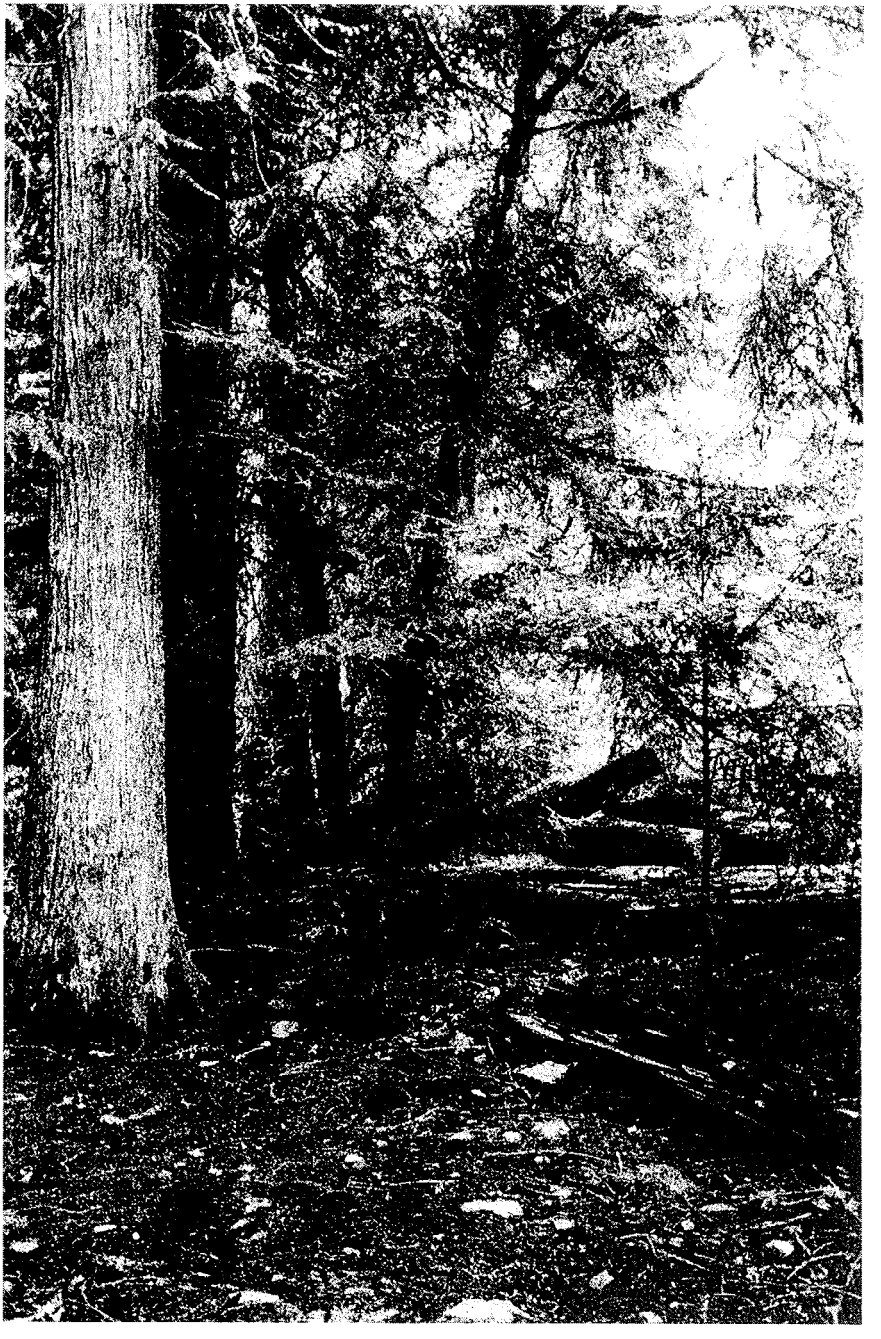

Fig. 1: Prime young yew in the forest (USDA Forest Service, 1993).

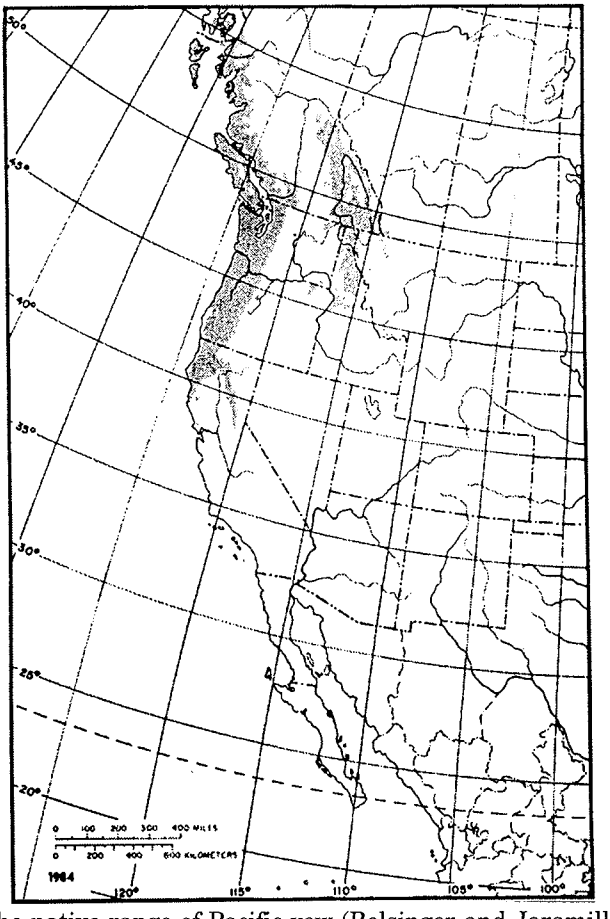

Fig. 2: The native range of Pacific yew (Bolsinger and Jaramillo, 1990).

inhibits the replication of human tumor cells. The findings were published in a series of papers (Schiff, *et al.*, 1979; Schiff and Horwitz, 1980; 1981). Taxol, like some other natural substances, is a mitotic spindle poison. However, its mode of action is very specific. It promotes the formation of microtubules and inhibits their disassembly into tubulin (Bruneton, 1995). This is just the opposite of other plant products such as colchicine and podophyllotoxin, which inhibit assembly. An excellent review of the role of taxol in the cell cycle can be found in Nicolaou *et al.* (1994).

Taxol as a microtubule stabilizing agent, which blocks cell replication, may also have a role in treating human pathogens, such as some protozoan hemoflagellates (Baum *et al.*, 1981), and plant pathogens, especially the Oomycetes (Wagner and Flores, 1994; Elmer, *et al.*, 1994). This is not surprising since taxol in the bark of living yew trees may play a role in

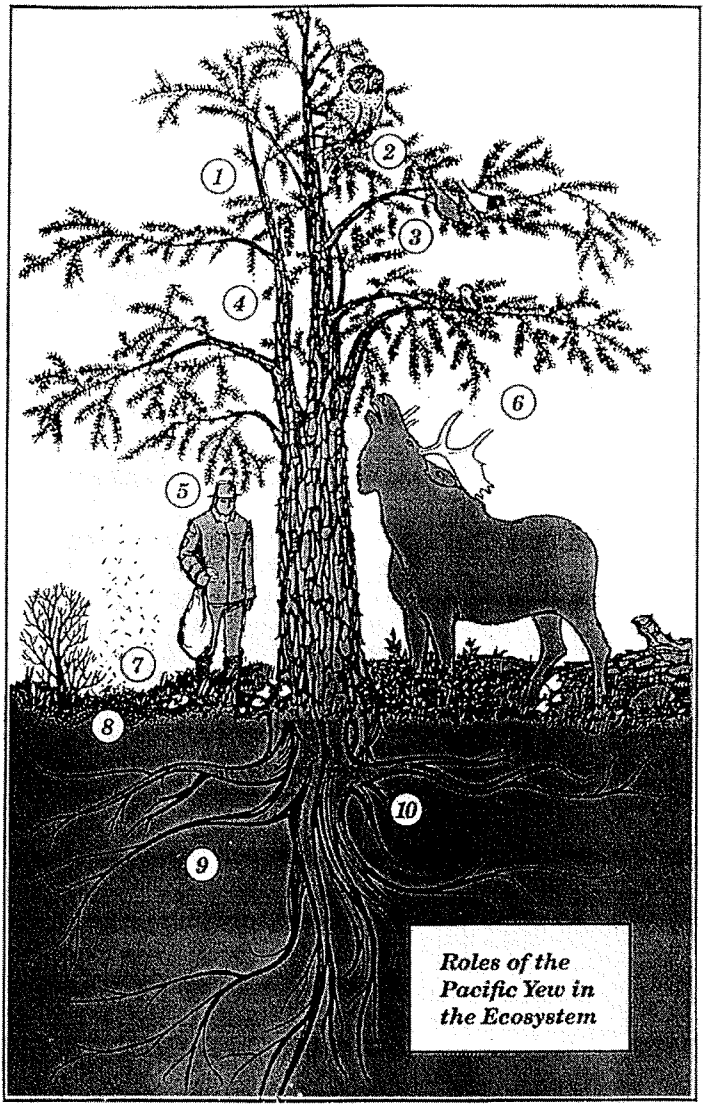

Fig. 3: Roles of the Pacific yew in the ecosystem: (1) habitat for canopy invertebrates (e.g., microspiders, pseudo scorpion, springtail), (2) important roosting habitat for spotted owls and other birds, (3) food for seed-eating birds and squirrels, (4) microclimate influence, (5) traditional and new human uses, (6) an important source of winter food for herbivores such as elk, moose, and deer, especially during heavy snows, (7) litterfall, (8) decomposition, (9) mycorrhizae (beneficial fungi) live in symbiotic association with the root system, and (10) habitat for soil arthropods such as beetles, crickets, and mites.
(USDA Forest Service, 1993)

Table 1: A chronology of the development of taxol as a cancer-fighting drug.

1963:	NCI found that yew samples showed activity against 9KB cancer-cell tissue culture. NCI sent a subsample to Monroe Wall, Ph.D., a medicinal chemist working under contract to NCI at Research Triangle Institute in North Carolina.
1964:	Wall's group found that a crude extract of the yew bark was effective in both the cancer-cell tissue system and against a mouse leukemia. They worked to isolate the primary active principle of taxol.
1966:	Wall asked NCI to give the yew material special priority for research. He isolated the active principle and named it taxol.
1969:	NCI checked the activity of all parts of Pacific yew. They now knew three things—the structure of taxol, its success in cancer screens, and something about how it worked against cancer.
1971:	Wall, with Mansukh Wani (at Research Triangle Institute) and Andrew McPhail (of Duke University), published the structure of the taxol molecule, a complex diterpene with an unusual oxetane ring and an ester side chain.
1974:	Taxol began to show results against a recently developed B16 mouse melanoma system. During the 1970s, cytotoxicity tests continued with tumor lines in new animal screens, including human tumor xenografts (tissues grafted from one species to another).
1977:	Preclinical work on taxol began. NCI contacted Susan Horwitz (professor of molecular pharmacology at Albert Einstein College of Medicine in the Bronx), working under an NCI Cancer Research Emphasis Grant, to ask her to investigate how taxol worked on cancer cells. With graduate student Peter Schiff, she found that taxol inhibited the replication of human tumor cells. (Specifically, taxol induces "tubulin polymerization" and inhibits disassembly of microtubules, an activity necessary to complete cell division.)
1978:	Taxol showed positive results in human cancer xenografts. Taxol showed activity in three systems, including a human breast cancer xenograft developed in the late 1970s.
1979:	Horowitz and Schiff published their findings about taxol's action of freezing microtubules and causing the cell to die.
1980:	Toxicology studies began. Scientists looked for a suitable surfactant formulation for administering the insoluble drug.
1982:	NCI approved taxol for filing an Investigational New Drug Application (INDA) with the Federal Drug Administration.
1983:	Phase I clinical trials began—testing patients who were not responding to other treatments, determining doses and toxicity, and generating data on dose limits of taxol.
1987:	NCI contracted for collection of 60,000 pounds of dry Pacific yew bark.
1988:	Phase II clinical trials showed 30 percent improvement in patients with unresponsive cases of advanced ovarian cancer.

(Contd.)

Table 1 (*Contd.*)

1989:	Trials of taxol progressed for other forms of cancers: breast, cervical, colon, gastric, non-small-cell lung, prostate, head and neck, small-cell lung, and renal. NCI contracted for an additional 60,000 pounds of dry bark.
1990:	Phase II trials showed 48 percent tumor shrinkage with metastatic breast cancer patients who had at least one prior chemotherapy regime. (Metastatic refers to cancers which tend to spread from one body part to another.)
1992:	Clinical trials were conducted at 20 centers on a number of different cancers, with some experimenting with combinations of chemotherapies.

Source: USDA Forest Service, 1993.

defense against fungal pathogens, insect larvae, or other damaging agents. Although presently taxol production is needed for its anticancer activity, new means of production may make the drug available for other uses in the future.

Phase I clinical trials of taxol began in 1983 (Table 1) with limited supplies of taxol made available by extraction. Phase I trials needed to focus on dose and means of administering this highly toxic, highly insoluble compound to patients (Wiernik *et al.*, 1987). From these Phase I clinical trials would come the recommendations for Phase II clinical trials and a sharp increase in demand for taxol with the primary initial source being dry Pacific yew bark, a poor source with low yield.

The yield of taxol from yew bark is only 0.01%. At best, a 100-year-old tree produces about 3 kg of bark, or 300 mg of taxol. With optimized extraction methods, 15,000 pounds of dried bark is required to yield 1 kg of taxol. A harvest of over 1.6 million pounds of bark was needed to meet the demand in 1992 (139 kg of taxol), and projected needs beyond that would destroy the entire population of Pacific yew in the United States in less than 10 years (Bruneton, 1995)! Although dried Pacific yew bark was needed for Phase II clinical trials in the late 1980s and early 1990s, alternative sources of taxol were also desperately needed.

4. Taxol Supply Crisis

The crisis created by the rapidly rising demand and limited supply of taxol from yew bark has been reviewed (Cragg *et al.*, 1993). The alternative strategies to extraction from the bark included total synthesis from simple starting materials, partial synthesis from readily available taxol precursors, identification of simple drug analogs, extraction of *Taxus* needles, cultivation of *Taxus* plants, and cell culture production (Borman, 1991).

5. Plant Tissue Extraction

A relatively simple approach to meet short-term needs was to extract taxol and its precursors, from bark, wood, root, leaf, twig, and seedlings of a variety of *Taxus* species (Vidensek *et al.*, 1990). It was found that needles of *Taxus* spp. contain amounts of taxol comparable to bark of *Taxus brevifolia* (Witherup *et al.*, 1990). Genetic studies of *T. brevifolia* were undertaken, but the authors concluded that there was no advantage in manipulating Pacific yew, when several common ornamental cultivars of *Taxus* were readily available to yield needles for the production of taxol and its precursor from which taxol can be produced by semi-synthesis (Wheeler *et al.*, 1995). For a more extensive review of these tissue extraction and cultivation techniques consult Heinstein and Chang (1994).

6. Chemical Synthesis

Taxol is a highly complex molecule with 11 asymmetric centers, which allows for 2,048 potential steric isomers (Fig. 4). Total synthesis of taxol was finally achieved in 1994 (Holton *et al.*, 1994a; 1994b; Nicolaou *et al.*, 1994), 23 years after its structure was initially proposed (Wani *et al.*, 1971). A semi-synthesis from 10-deacetyl baccatin III (Fig. 4), which can be readily extracted in high yield from leaves of *Taxus baccata* L., was achieved in 1988 (Denis *et al.*, 1988). This procedure became an attractive solution to the serious supply problem. Many taxol analogs were synthesized along the way to total synthesis, (for example, see Guéritte-Voegelein *et al.*, 1991; Swindell *et al.*, 1991) with taxotére (Fig. 4) being the most useful to date. For a more complete review of the chemistry of taxol and taxotére consult Guénard *et al.* (1993).

7. Cell Culture Production

Perhaps the most promising long-term solution to taxol production is its production in cell suspension culture using modern biotechnology approaches. Yields of taxol continued to improve through the mid-1990s (Fett-Neto, *et al.*, 1994a, 1994b). Methyl jasmonate-induced overproduction of taxol, and its precursor baccatin III in *Taxus* cells in suspension culture was achieved by Yukimune *et al.* (1996). Further advances are expected in the near future as biotechnology continues to develop.

Taxol production by microorganisms in culture is another approach which has been tried. An endophytic fungus isolated from Pacific yew bark was found to produce taxol in low yield (Stierle, *et al.*, 1993). Other fungi that might produce taxol, or related compounds, are being sought along with ways to enhance production (Stierle *et al.*, 1995).

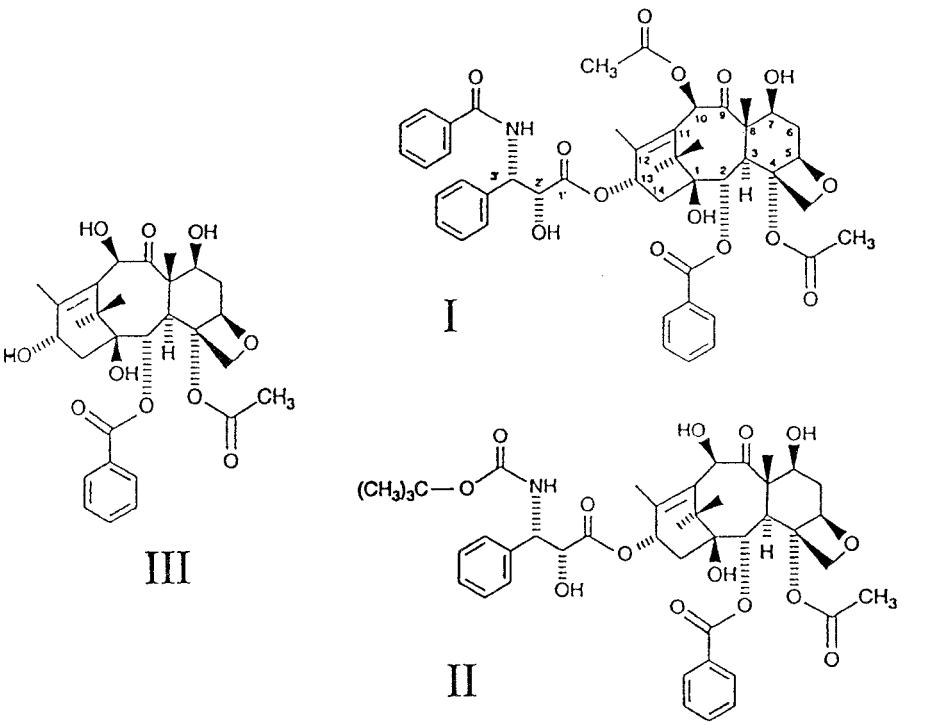

Fig. 4: Taxol (I) and its analog Taxotére (II) can be synthesized from the precursor 10-Deacetylbaccatin III (III).

8. Commercialization

In the late 1990s, more advanced clinical trials on taxol were in progress (Gan *et al.*, 1996; Hennequin *et al.*, 1996), attempts to understand the highly complex biochemical pathways of taxol and other taxanes are being made (Eisenreich *et al.*, 1996; Lin *et al.*, 1996), and taxol is being studied as a case history in pharmaceutical prospecting and potential for pharmaceutical crops (Cragg *et al.*, 1995).

Taxol is a naturally occurring compound first named in 1966. Since 1992, Taxol is also a registered trade name for a formulated drug based on the chemical name for taxol, paclitaxel, marketed by Bristol-Myers Squibb (Wheeler and Hehnen, 1993). Forestry companies, such as Weyerhaeuser, which have traditionally focused on wood products, began new business ventures to produce materials yielding the non-wood forest product, taxol. Perhaps the discovery of other valuable non-wood forest products will stimulate further opportunities for commercialization and expand the forest based industry.

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