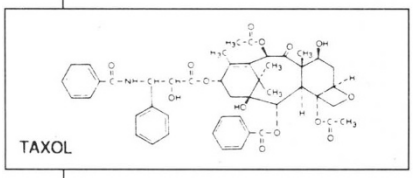
# ISOLATING A SCARCITY

After identifying the Pacific yew, Monroe Wall needed another four years to isolate and purify the active agent in the Pacific yew extract. In 1969 he announced, with his colleagues at the Research Triangle Institute, that a complex di-terpene—dubbed "taxol," a 20-carbon taxane containing a rare oxetane ring and an amide side chain—destroyed tumor cells. Wall and his group published their results without considering patent rights.


TAXOL

Once they could recognize the compound, they began searching for highly concentrated natural sources. The results of a USDA study were disappointing. Thousands of trees had been cut and burned as "trash trees" during logging. Yews seemed to have no preferred habitat and grew under a variety of conditions. Seemingly likely yew habitats were often barren, making per-acre population estimates difficult. Yews were slow-growing and not easily propagated, suggesting that cultivation would be difficult at best.

Minuscule yields compounded these problems. At best, 1 Kg of dried yew bark returned only 50-150 mg of pure taxol. Producing a gram of the substance required three or four trees, each at least 60 years old. The scarcity severely compromised NCI's research. From taxol's discovery until 1989 NCI produced less than three kilograms of the drug.

## How it Works

From its discovery until Susan Horwitz's 1979 *Nature* paper[1], no one had a clue about how taxol might attack cancer. Then Horwitz demonstrated that taxol catalyzes rapid microtubule formation, stabilizing them against depolymerization. This gives the drug two tumor-fighting mechanisms.

First, taxol freezes the mitotic spindle, preventing the depolymerization that pulls chromosomes into the two halves of the rapidly dividing tumor cell. This suspends the cell at G2 or M phase, ultimately killing it.

Second, taxol inhibits cell migration—which may prevent the spread of metastatic cancer cells. Taxol-treated mouse fibroblasts produce mobile lamellipodia and filopodia but can't move[2].

But taxol isn't effective on all tumors or all cell lines. Cells induced to taxol insensitivity display a prominent phosphorylated 135-kD membrane glycoprotein not found in the parental cell line[3]. As researchers gradually remove taxol from the medium over a period of several weeks, the membrane protein disappears and taxol sensitivity returns. Taxol's hydrophobic structure suggests it enters the cell by passive diffusion. Like others in the family of multi-drug resistance proteins, the 135-kD membrane protein apparently acts as a pump, flushing taxol out of the cytoplasm. Obviously, the 135-kD protein may be an important marker.

## The Art of Tissue Culture

Both Bristol-Myers Squibb and Rhone-Poulenc Rorer (RPR) have striven to get more taxol out of natural sources—by increasing the efficiency of bark extraction, by looking to other parts of the plant (usually the needles), and by seeking related molecules in related flora (see sidebar "Other Routes and Effective Analogs"). Despite some progress, supply remains a problem. NCI's Saul Schepartz suggests that in the near future, we may need 250 Kg of purified drug a year—the yield of a whopping 7.5 million pounds of dried bark from 360,000 trees by BMS's methods, or the extract of as much as 750,000 pounds of dried needles by RPR's technique. And that is only for near-term demand.

If ever there was a plain target for plant tissue culture, this is it. On May 28, USDA received a patent for "Production of Taxol or Taxol-Like Compounds" and licensed the technology to Phyton Catalytic (Ithaca, NY). The researchers found that *T. brevifolia* bark or cambial tissue forms callus in tissue culture and secretes taxol (and several other new compounds) into the supernatant. Ammonium nitrate enhanced the growth rate. And off-the-shelf elicitors (*Cytospora abietis*, *Penicillium miniioluteum*, vandyl sulfate, or 3,4-dichlorophenoxy triethyl(amine) ) induced secretion of taxol into the medium. Each liter of supernatant produced 1.0 to 3.0 mg of taxol, the yield of 20 grams of dried bark. While this is still shaker-flask technology, Phyton president Russ Howard estimates that the company is just two to five years away from commercial production.

How cost-effective can such low-yielding processes be—especially when highly purified carbon sources can drive costs up to $90,000 per kilo of finished product?

Maximizing shaker-flask yields is more art than science. Some researchers have documented twenty-five-fold increases in output, however: One must select top-producing progenitor plants, and then subculture the top-producing cells from the initial culture. Conventional mutagenesis (via chemicals, X-rays, or UV) can maximize intrinsic variability within colonies to provide culturable cells with taxol-augmenting mutations.

Downstream processing is a strong point of Phyton's tissue culture system. The single-product-secreting callus makes taxol recovery a simple matter of ether extraction. Continuously filtering taxol from the supernatant, while supplying fresh media, may also increase yields by depleting intracellular taxol stores. Such a continuous-flow system could be scaled up to production levels.

Simplicity is not without its price, however. There are the familiar problems of large-scale cell culture: Slow-growing callus is vulnerable to fungal and bacterial infection, and antibiotic treatment may interfere with growth or production. Complex media are expensive. And callus cells are shear sensitive. Add to that callus's inherent genetic instability, and one has a culture that demands eternal vigilance to keep low-producing (or non-producing) variants from taking over the whole culture.

In short, constant monitoring is paramount.

Until recently, taxol-producing cultures needed too much hand-holding to be worth commercial effort. HPLC or gas-chromatograph monitoring used a lot of time, money, and relatively large sample volumes. Newer ELISA assays, using antibodies sensitive to the 5-ng level, streamline the monitoring and cell selection processes. Hawaii Biotechnology Group (Honolulu, Hawaii) received NCI funding to develop anti-