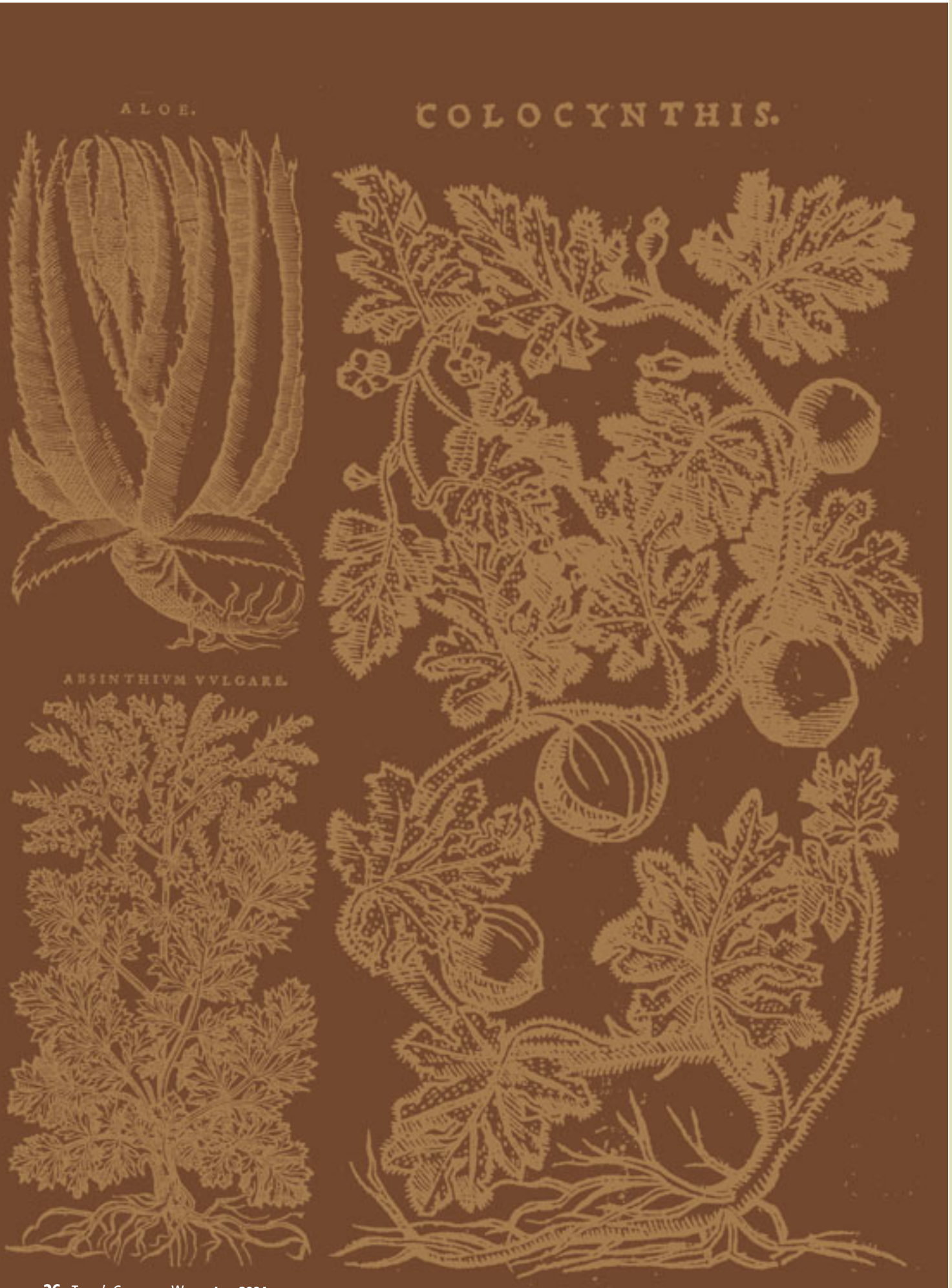
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Nature's Pharmaceuticals

Natural products from plants remain at the core of modern medicinal chemistry.

Mark S. Lesney

For all the biomedical promise of genetic engineering and combinatorial chemistry, the greenhouse, the garden, and the jungle remain key sources of current pharmaceuticals and future drug candidates. Whether directly purified from nature or chemically modified in the lab, these compounds are at the heart of the world pharmacopoeia and the source molecules for directed, knowledge-based medicinal chemistry.

In the plant itself, these so-called phytochemicals often serve to ward off or poison pathogens or insect predators. In humans, these compounds can be used to ward off, ameliorate, or cure some of our deadlier diseases—often by acting as specific toxins against the causal organisms, aberrant cells, or a physiology out of whack.

A handful of past and current "miracle drugs" from plants can easily illustrate the point—from quinine to Taxol, from aspirin to the birth control pill (see Table 1 for a listing of additional compounds). Many if not most of these have been tremendous challenges to the medicinal chemist to make in the laboratory, much less scale up to factory-level production.

But newer techniques (often using genetically engineered catalysts—whether in living cells or as free enzymes in solutions) are transforming the utility of such drugs, increasing the demand for new candidates, and sending researchers running to the few remaining "wilderness" areas of the planet. From steaming lowland jungles to mist-filled mountainsides, they quest for new candidates by way of either plant discoveries or learning medicinal plant usage from indigenous peoples.

A quick glance at the modern medicine chest tells the story. Natural products have long been the source of the great majority of drugs and drug candidates, and they still are today. Currently, the three most important categories of plant compounds in drug development and sales are the terpenoids (including Taxol and steroids), the glycosides (including digitalis and the various flavonoids), and the alkaloids (including camptothecins and the opiates).

As reported by Frank Petersen, head of Novartis Pharma natural products research, in his report for the 2004 ACS symposium entitled Natural Product Scaffolds as Starting Points for Drug Discovery, "A landmark survey from 2003 published in the *Journal of Natural Products* showed that a whopping 61% of 877 small-molecule new chemical entities (NCEs) introduced worldwide from 1981 to 2002 can be traced to natural products. . . . Seventy-eight percent of the antibacterials and 74% of anticancer compounds are either natural products or inspired by a natural product model" (*1*).

Stealing from Mother (Nature)

At first glance, given the above, it would seem illogical for researchers to look anywhere but nature's own pharmacopoeia for their main sources of drug candidates. But there are problems in dealing with natural products, partly scientific, but also commercial, that have led Big Pharma to significantly abandon the natural products route for technologies such as combinatorial chemistry and high-throughput screening. The reasons are many. One of the most important is the difficulty of synthesizing and characterizing natural compounds from plants, many of which are hard to purify and obtain in quantity from their

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original source material. Equally significant are fears of doing years of research on what may turn out to be a nonproprietary compound due to its natural availability to other researchers in other countries. Artificially created compounds in the laboratory (or virtually in the computer) are far easier to deal with, both chemically and legally (as intellectual property).

Many pundits blame this general shift away from the sources of previous success for the current problems in developing new drug-candidate compounds. And such failures may be starting to lead to a reorientation (although not yet a stampede) in the direction of natural product research (*2*).

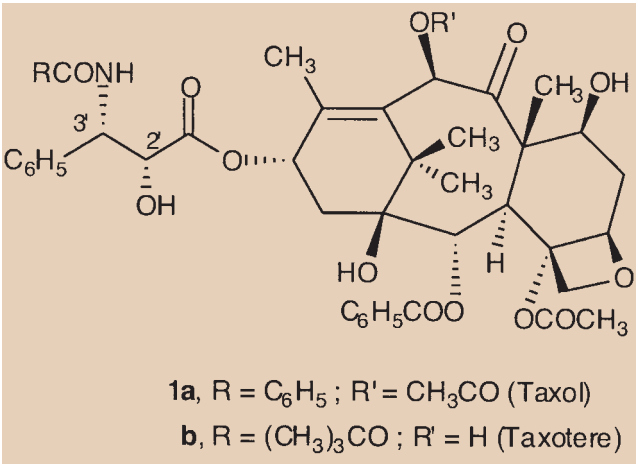

Figure 1. Structure of Taxol (paclitaxel) and Taxotere (docetaxel). (Adapted with permission from Lucatelle, C.; et al. *J. Org. Chem.* **2002**, *67* (26), 9468–9470.)

The Taxol Story

Perhaps one of the most fascinating and socially complex stories of a modern drug derived from nature is that of the cancer-fighting drug paclitaxel (trade name Taxol, Figure 1). It simultaneously illustrates all of the problems inherent in the use of natural product drugs, as well as the tremendous potential they still maintain for saving lives.

Taxol had its origins in 1962 as part of the plant procurement and screening program jointly run by the National Cancer Institute (NCI) and U.S. Department of Agriculture. Plant parts were collected from the Pacific yew tree in Gifford Pinchot National Forest in Washington State. Two years later, researchers Mansukh C. Wani and Monroe E. Wall at the Research Triangle Institute's (RTI's) Natural Products Laboratory in Research Triangle Park, NC, discovered the extracts contained cytotoxic activity, and they isolated a crystalline substance that Wall named "taxol," a name since trademarked by Bristol-Myers Squibb. Using MS, X-ray crystallography, and NMR analyses, they detailed the structure of the compound that they published in the *Journal of the American Chemical Society* (**1971**, *93*, 2325). So significant was the discovery of taxol and that of another critically useful antitumor agent—camptothecin—that ACS, in 2003, identified the site of the RTI laboratory as one of its National Historic Chemical Landmarks (*3*).

Because of difficulties in obtaining taxol, and the obvious unlikelihood of developing an easy method of synthesis because of its complexity, research lagged until it was discovered that its activity involved a hitherto unknown mechanism. Taxol was known to be a mitotic poison, but instead of causing disassembly of microtubules, taxol instead prevented microtubule depolymerization, blocking mitosis in a totally different fashion. In 1978, not long after the NCI developed the use of xenografts (in which human tumors are planted in hairless mice bred to lack a functioning immune system), taxol was shown to cause considerable regression in a mammary tumor xenograft.

By the late 1980s, taxol was shown to be effective against ovarian cancer, and the push was on to isolate large quantities of the drug for clinical use. Unfortunately, this had tremendous environmental repercussions. Yields from the Pacific yew from which it could be isolated were incredibly low, and bark-stripping killed the 100-year-old trees—up to six trees were needed per patient per treatment, with multiple treatments usually required. Taxol became a political issue featuring the plight of cancer patients on the one side and old-growth forests on the other, to the point where *The New York Times* headlined one of its articles on the subject: "Save A Life, Kill A Tree?"

This spurred numerous attempts at achieving total organic synthesis. Taxol is an extremely complex molecule to synthesize—a polyoxygenated diterpene with seven asymmetric centers (see Figure 1). Two groups reported the total synthesis of taxol within months of each other. The research group of K. C. Nicolaou published first, and the laboratory of R. H. Holton at Florida State University published a few weeks later. Each used a different mode of synthesis.

Still, at present, semisynthesis is the only commercially viable route for producing this drug in the West. The method relies on the use of a precursor obtained from the less-endangered European yew; and from needles, rather than bark. The compound baccatin III, otherwise known as DAB III, is isolated, and through a still-complex series of synthetic steps, it is transformed into taxol. Today, taxol is one of the most widely used chemotherapy drugs for a variety of cancers (*4*). In 1986, during research on a semisynthetic route to taxol from 10-deacetylbaccatin III—from needles of *Taxus baccata*—another effective chemotherapeutic agent, docetaxel (marketed as Taxotere by Rhône-Poulenc), was developed. This is an important factor to consider in developing synthetic pathways for natural medicinal products—the very process of synthesis may provide alternative testable compounds or scaffolds sufficiently related to the therapeutic molecule to be possible drug candidates themselves.

Because total taxol synthesis is still too inefficient to be used commercially, and semisynthesis is still expensive and (relatively) inefficient, research on extraction continues. For example, in January 2003, U.S. Patent 6,503,396 was issued to Korea's Hanwha Chemical Corp. for isolating taxol using a supercritical fluid and a cosolvent extraction step, a liquid–liquid separation step, and a Sephadex, silica, or RP-18 resin column chromatography step. Significantly, fractions containing baccatin III can be eluted from the RP-18 resin—this is the important starting compound used in producing semisynthetic taxol (*5*).

In this and other research, a wide variety of *Taxus* species are being used worldwide as potential starting points to eliminate the need for using endangered yew species. For example, 9TOP Natural Pharmaceutical Co. works with Chinese researchers to produce paclitaxel from plantation-produced *Taxus chinensis*, which can contain paclitaxel in amounts as high as 0.015–0.02%. The product has passed through Chinese clinical trials and is that

country's main source of the cancer drug for treatments (*6*).

Other researchers are trying to produce taxol and taxol precursors in yew cell cultures (*7*). Research on improving medicinal drug production in cell cultures includes attempts to select or engineer improved pathways and controls into these cells to elicit the production of commercially viable quantities of the compounds. These include attempts to induce overexpression of genes involved in rate-limiting synthetic steps, down-regulation of undesirable taxoids that contaminate preparations and siphon off needed precursors, and attempts to produce new taxanes that may even be better than taxol.

As supplies of taxol become more routinely available, research on taxol conjugates and analogs becomes possible—opening up new avenues in the search for cancer-controlling drugs. This is especially important because cancers often develop multidrug resistance to taxol and other effective cancer drugs.

Table 1. Some Representative Plant-Derived Medicinal Compounds

Compound	Type	Source	Disease treated/use
Artemisinin	Terpenoid	*Artemisia annua*	Antimalarial
Camptothecin	Alkaloid	*Camptotheca acuminata*	Breast, colon cancer, etc.
Colchine	Alkaloid	*Colchicum autumnale*	Antitumor agent, gout
Digitalin	Glycoside	*Digitalis purpurea*	Cardiotonic
Docetaxel (Taxotere)	Terpenoid	*Taxus* sp.	Antitumor agent
Etoposide	Glycoside	*Podophyllum peltatum*	Antitumor agent
Irinotecan	Alkaloid	*Camptotheca acuminata*	Anticancer, antitumor
Paclitaxel (Taxol)	Terpenoid	*Taxus* sp.	Breast, colon cancer, etc.
Quinine	Alkaloid	*Cinchona ledgeriana*	Antimalarial, antipyretic
Reserpine	Alkaloid	*Rauvolfia serpentina*	Antihypertensive, tranquilizer
Theobromine	Alkaloid	*Theobroma cacao*	Diuretic

For a more complete listing, see www.hort.purdue.edu/newcrop/proceedings1990/v1-491.html#Table 1.

In one example of an attempt to derive improved cancer drugs from a taxol-like chemical core, U.S. and Italian researchers Arturo Battaglia and colleagues have recently reported on a series of methylated taxoid compounds (created through modifying the diterpene moiety and the isoserine appendants of taxol) during semisynthesis from the bacattin III precursor. Several of these compounds appear to have significantly improved toxicological and therapeutic characteristics in human ovarian cancer cell lines resistant to cisplatin, paclitaxel, and doxorubicin (*8*).

Problems and Promise

There remain tremendous difficulties in the use of plants in drug discovery. These include the technical issues of the haphazard nature of the initial selection of promising species in the wild and the often-significant problems that occur when trying to extract

complex and still-active metabolites from crude mixtures of interfering compounds in plant sap. The full gamut of separation science techniques is frequently required, including HPLC, SFC, filtration, planar and countercurrent chromatography, size exclusion chromatography, and ion exchange. This is not to mention the need to characterize and identify the isolated compounds with every form of spectroscopy and X-ray crystallography, as well as MS and NMR, making the job of the medicinal chemist one of the most complex in modern chemistry.

On the social side, there are ever more complex issues of intellectual property rights of native peoples and developing countries, as well as the fear of unpatentable, nonproprietary products on the one hand, and an industrial commitment to high-tech approaches such as combinatorial chemistry and "virtual discovery" rather than the "old-fashioned" medicinal chemistry approach on the other. A commitment to melding new technologies with the more traditional plant-based medicinal chemistry approach is evident in one of the key programs of the NCI.

In response to perceived demand from pharmaceutical researchers, the Natural Products Branch of the NCI (which maintains a repository of nearly 60,000 plant and marine organism samples collected from around the world) developed its Natural Products Set, consisting of 235 key compounds from its repository of 140,000 compounds (*www.nci.nih.gov*). These compounds were chosen not only because they had a natural origin but also because they were structurally diverse and relatively available. Given the history of success that natural products have had as drug compounds, the logic of mining the natural world for candidates is obvious. This meshes with the tremendous current interest in finding plausible scaffold compounds with appropriate and preferably multiple functional groups to serve as a starting point for combinatorial libraries (real and virtual) for the purpose of drug discovery.

With the commitment of entities such as the NCI to natural product compounds, and given their history and continuing promise—not to mention the failure of many of the so-called modern approaches to give the promised results—the use of plants as a source of medicinal compounds is likely to remain key to the discovery of new candidates for the human pharmacopoeia.

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KEY TERMS: biotech, LC, MS, pharmaceutical, separation science