第 11 章 天然药物的研究与开发

双语教学读物

Introduction to Natural Medicines R & D

What a Natural Product?

Natural products are organic compounds produced by many microbes, plants and marine invertebrates. It is thought by some that toxic natural products are produced by the host as adaptations to deterpredators (chemical defense) or to compete for space in their native environments (alleopathy). Some natural products also exhibit biological activityrelevant to human physiology and disease states. Many terrestrial natural products have found use in medicine (e.g. morphine from the opium poppy, digitalis glycosides from foxglove) and agriculture (e.g. the potent insect anti-feedant azadarichtin from Neem treeoil), however exploration of marine natural products is relatively recent.

- Isolation of pure natural products from various sources
- Structure identification of natural products using a wide variety of NMR and MS techniques.
- Analysis by all types of chromatography *eg*. TLC, PC, HPLC, CC etc.

Chemistry of Pharmaceutical Natural Products

Welcome to the course in natural product chemistry. The course aims at giving a general overview of the chemistry of natural products with the emphasis on secondary metabolites (primary metabolites are commonly regarded as the domain of the biochemists). With the biosynthetic pathways as a basis, knowledge of the major classes of natural products and their chemical and structural characteristics will be provided. The study of pharmaceutical natural

products, classified according to their biosynthetic pathways, will be emphasized on secondary metabolites derived from plants, animals and microbes such as alkaloids, glycosides, tannins, flavonoids, lignin, coumarins, saponins, iridoids, etc. The course will also give some insight into the relation between structure and biological activity of natural products and how this may be used to judge if a synthetic compound is “environmentally friendly” or not.

Plants produce a large number of metabolites that are an abundant source of novel pharmaceutically active agents.

Indeed, many well-defined 20th century drugs were derived from herbal plants. Drugs including salicylic acid (*Salix sp.*), curare (箭毒 *Chondrodendron tomentosum* 1941 年 Bennett 首先在治疗前引用南美箭毒(Curare)作为肌肉松弛剂), digitoxin (*Digitalis purpurea*), taxol (*Taxus brevifolia*), and many others, are well-recognised drugs derived from plants with pharmaceutical and clinical potentials.

The word salicin is derived from the Latin name for the willow, *salix*. Salicin is a white bitter-tasting powder can be obtained by aqueous extraction of mainly willow bark and leaves.

The break through in identifying the pharmacological active compound, salicin (the Latin name for the willow), was made by the Italian chemist Raffaele Piria in 1935 (Piria, 1838). He identified the chemical structure of salicin as 2-(hydroxymethyl)phenyl-1̃ -D-glucopyranoside according to an acid hydrolysis experiment of salicin that claimed to yield pure salicyl alcohol (2-hydroxymethyl phenol) and D-glucose moiety (Fig. 1). Salicin also hydrolyzes in the gastrointestinal tract to give D-glucose and salicyl alcohol (Fig. 1).

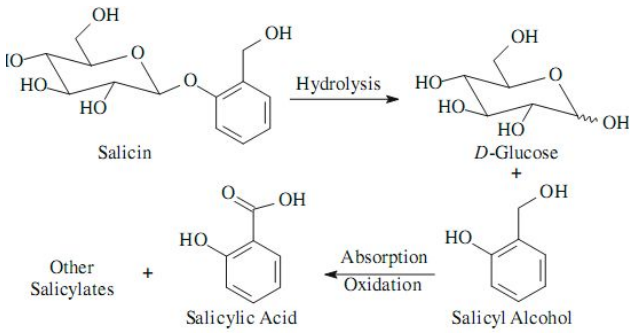


Figure 1 Hydrolysis of salicin.

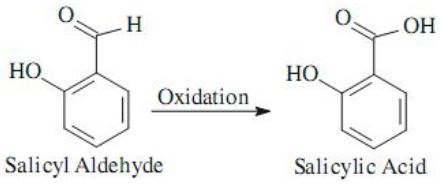


Figure 2 Oxidation of salicyl aldehyde into their acid derivative.

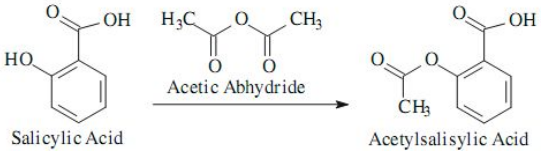
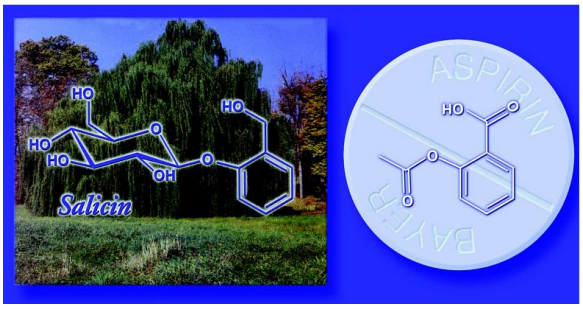


Figure 3 Acetylation of salicylic acid.



Piria, R. Sur des nouveaux produits extraits de la salicin. C.R. *Acad. Sci. Paris* **1838**, *6*, 620–624.

Jassem G. Mahdi. Medicinal potential of willow: A chemical perspective of aspirin discovery. *Journal of Saudi Chemical Society* **2010**, *14*, 317–322.

Literature projects

1. Taxol and its Derivatives, a Plant-Derived Anticancer Agent
2. Natural Products in Different Seasons.
3. Ginseng. Is the Advertisment Empty Words or has it Some Substance?
4. Insects as a Source for Raw Materials.
5. Etoposide (VP-26), a Plant-Derived Anticancer Agent
6. Artemisinin, an Antimalarial Natural Product.
7. The stories of Aspirin, Morphine, Quinine, Penicillin

Part I

The Use of Natural Products as Drugs in History

Natural products appear to have been used to cure illnesses almost since the beginning of time. Possibly the oldest known recipes for such cures are found on a set of 660 clay tablets from the Mesopotamian civilization dating to the third millennium B.C.E. These tablets contain a list of more than a thousand plants used for medicinal purposes.

Natural medicines were being used in China at about the same time. A famous text dating to about 1000 B.C.E., the *Huang Ti Nei Ching Su Wen* (Yellow Emperor's canon of internal medicine), is regarded as the oldest record of traditional Chinese medical techniques and describes treatments that were used as far back as about 2500 B.C.E. The oldest Chinese book containing recipes for herbal treatments is *Shen Nung Pen Ts'ao Ching* (Shen Nung's catalog of herbs), dating to about 1000 B.C.E.

Relatively little progress was made in the West in the treatment of disease from the rise of Christianity to the end of the Middle Ages, to some extent because illness

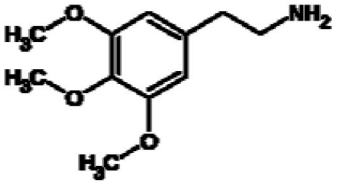
was regarded as punishment for one's sins. As a result, prayer and the hope for miracles were frequently the only methods available for the cure of disease. During the Renaissance, however, a renewed interest in the use of plant materials (usually herbs) sprang up in Europe. The first pharmacopoeia in the modern era, written by the German botanist Valerius Cordus (February 18, 1515 – September 25, 1544), was published posthumously in 1546. (A pharmacopoeia is a list of drugs and medicines, with a description of the illnesses for which they are useful and instructions for their preparation.)

Valerius Cordus （**Valerius Cordus** (February 18, 1515 – September 25, 1544) was a German physician and botanist who authored one of the greatest pharmacopoeias and one of the most celebrated herbals in history. He is also widely credited with developing a method for synthesizing ether (which he called by the poetic Latin name *oleum dulci vitrioli*, or "sweet oil of vitriol").

Cordus wrote prolifically, and identified and described several new plant species and varieties. The plant genus *Cordia* （紫草科破布木属）is named for him.

Cordus's *Dispensatorium* was soon followed by other pharmacopoeia in other parts of Germany and other countries of Europe. The first such book in the United States, the *Lititz Pharmacopoeia,* was published by Dr. William Brown in 1778 for use in military hospitals during the American Revolution.

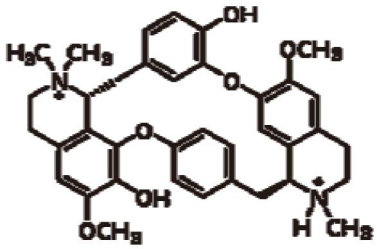
As in the modern world, plant materials were used not only to treat disease but also for other purposes, the most important of which was to produce hallucinogenic, psychedelic, or other "out-of-body" experiences. Many cultures throughout history have made the use of such materials an integral part of their religious ceremonies. For One of the most widely used of all illegal drugs is marijuana, which comes from the *cannabis sativa* plant. (Ted Kinsman/Photo Researchers, Inc.) example, ancient Hindu documents assert that the hallucinogenic effects of marijuana were fi rst discovered by the god Shiva, who ate leaves of the plant and found them very refreshing. Thereafter, the plant was routinely used in many Hindu ceremonies, usually in a form known as *bhang.* In the New World, the peyote cactus has been used in religious ceremonies for at least 10,000 years.



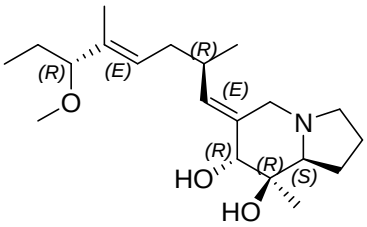
Mescaline, or **3,4,5-trimethoxyphenethylamine**, is a naturally occurring psychedelic alkaloid of the phenethylamine class, known for its hallucinogenic effects comparable to those of LSD and psilocybin.

It occurs naturally in the peyote cactus (*Lophophora williamsii*),[1] the San Pedro cactus (*Echinopsis pachanoi*),[2] the Peruvian torch (*Echinopsis peruviana*),[3] and in a number of other members of the Cactaceae plant family. It is also found in small amounts in certain members of the Fabaceae (bean) family, including *Acacia berlandieri*.[4]

The cactus (仙人掌) contains at least 40 chemicals with mind-altering properties, the most important of which is mescaline (麦司卡林学名三甲氧苯乙胺,是苯乙胺的衍生物。服用2-3小时后出现幻觉). Plant materials have also been used as drugs in some cultures for the killing of prey or in weapons used in battle. Perhaps the best known example of such use is the practice among some South American tribes of using curare, an extract of the plant *Chondrodendron tomentosum,* as a poison for the tips of their arrows used both in hunting and in warfare. The active ingredient in curare is the chemical known as D-tubocurarine.



Other South American tribes use a poison obtained from a group of amphibians known as *poison dart frogs.* These frogs are members of the family *Dendrobatidae* and belong primarily to the genera *Dendrobates, Phyllobates,* and *Epipedobates.* The most toxic member of the group is a frog known as *Phyllobates terribilis,* whose secretions are so toxic that they can cause serious illness to a human simply through contact with the skin. The most important active ingredient in the poison excreted by poison dart frogs is a chemical known as pumiliotoxin (两栖动物毒素).



《有机化学》, 2004, 24(10):1151-1158

JW Daly，F Gusovsky，ET Mcneal，S Secunda，M Bell，... Pumiliotoxin alkaloids: a new class of sodium channel agents. Biochemical Pharmacology, 1990, 40(2):315-326.

Natural Products and the Rise of Modern Chemistry

Prior to the 19th century, practitioners of the healing arts knew essentially nothing about either the chemical composition of natural products or the mechanisms by which they work. They relied entirely on tradition, and trial and error, in the choices they made of the substances they used in their work. That situation began to change in the early 1800s with the rise of organic chemistry. Researchers began to fi nd ways to separate traditional drugs and medicines into their component parts, determine the chemicals of which they were made, elucidate their chemical structures, and, to some extent, synthesize the compounds in the laboratories.

It was a daunting task. In the vast majority of cases, the natural products traditionally used by healers are complex mixtures of dozens of chemical compounds, some of which may have medicinal properties, and some of which may not. At first, the most that chemists could hope to accomplish was to obtain one or more active ingredients of plant materials in a pure form. To determine the chemical structures of these ingredients was, at the time, far beyond their capacity. Indeed, the molecular structures of chemicals obtained in a pure form in the early 1800s were often not determined until more than a hundred years later.

For example, one of the first chemicals to be purified from a natural product for use as a drug was morphine. In 1805, the German chemist Friedrich Wilhelm Sertürner (1783–1841) isolated the compound from opium while trying to find out how that substance induces sleep. He obtained morphine in a pure form, as white

crystals, but had no idea as to its chemical composition. That limitation did not prevent morphine's being put to use as a drug, however. In 1826, Emanuel Merck (1794–1855), founder of the great Merck Chemical company, began producing pure morphine commercially for use as a drug. Still, the compound's chemical structure remained a mystery for more than a century. Finally, in 1925, the English chemist Sir Robert Robinson determined the structural formula for morphine (with the exception of one uncertain atom).

Another historically significant example is the story of quinine. For centuries, quinine was the most effective drug for the treatment of malaria, which has long been one of the world's most serious and widespread infectious diseases. The substance was first used as an antimalarial treatment in the 1600s, although how it was discovered it still not known with certainty. In any case, its importance as a drug inspired the search for methods of extracting it from its natural source and determining its chemical structure so that it could be made synthetically.

The first problem was solved in 1820 when the French chemist Pierre-Joseph Pelletier (1788–1842) and his associate Joseph-Bienaimé Caventou found a way to extract quinine from cinchona bark. That accomplishment gave no clue, however, as to the compound's chemical structure, so chemists were unable to synthesize quinine *analogs* in the laboratory. (In pharmacology, an analog is a drug whose chemical structure is similar to that of another drug, but whose chemical and biological properties may be quite different.)

Indeed, it was not until the 1920s that progress was made in that direction. Then researchers discovered that a number of compounds belonging to the aminoquinoline family were effective in the treatment of malaria. Between the 1920s and the 1950s, these two compounds were the most effective antimalarials available. Still, the search for the chemical structure of quinine itself went on, a pursuit that was not successful until 1944. In that year, the American research team of Robert Burns Woodward (1917–79) and William von Eggers (1917–) completed the monumental task of elucidating the structure of quinine. With this knowledge, it became possible for chemists to begin producing quinine synthetically in the laboratory and, more

important, to develop analogs that were even more effective than the natural product. The most effective of the quinine analogs was mefloquine, developed during the Vietnam War as the result of a program developed by the Walter Reed Army Institute for Research to protect American soldiers against malaria.

The morphine and quinine stories have established a model for the study of natural products that has been repeated many times in recent history, that is, the search for the chemical structure of a biologically active substance so that (1) the compound can then be

made synthetically and (2) analogs of the drug can be produced and tested for biological activity. (The term *biological activity* refers to the benefi cial or adverse effects of a drug on living materials.) One of the most exciting achievements in this type of research involved the study of a natural product that has been used by Chinese herbalists for thousands of years to treat fever. Called qing hoa, it is also known as sweet wormwood, annual wormwood, and sweet annie. Its systematic name is *Artemisia annua.* In addition to its use as an antipyretic (antifever medication), qing hoa has been used effectively as an antimalarial drug.

Beginning in the 1960s, the People's Republic of China initiated an aggressive program to discover the scientifi c basis for many traditional herbal remedies, including qing hoa. As a result of that program, in 1972 Chinese scientists identifi ed the active ingredient in qing hao, a substance they called qinghaosu. Qinghaosu is also known as arteannuin in China and as artemisinine in the West. Artemisinine is a sesquiterpene, a class of naturally occurring compounds with the general formula C15H24. The sesquiterpenes are considered to be chemically derived from the basic compound, isoprene. Chinese researchers have developed a number of derivatives of artemisinine, including the compounds known as artemether, artesunate, arteether and artelinate, all highly effective in the prevention and treatment of malaria.

The structural formulas of artemisinine and its derivatives are shown in the following diagram. The formulas show the close structural relationship of the compounds, differing only in the shaded portion of the molecules.

The success in determining the chemical structure of qinghaosu is only one example of the accomplishments of chemists in attaining a better understanding of the relationship between the chemical structure of natural drugs and their pharmacological effects. Those
accomplishments have formed the basis of a whole new phase of the pharmaceutical industry in which natural products and their derivatives provide an extensive source of new drugs.

Microorganisms as the Source of Drugs

Plants remained essentially the sole source of natural product drugs until well into the 20th century. Then in 1928 the discovery of penicillin by the Scottish bacteriologist Sir Alexander Fleming (1881–1955) opened an entirely new area of research in the field of Chemical formulas for artemisinine and its derivatives anti-infective drugs. Quite by accident, Fleming discovered that a sample of staphylococcus bacteria that he had inadvertently left out had begun to die out in certain areas of the culture. He determined that the change had come about in places where mold had fallen into the culture. Fleming isolated the mold and identifi ed it as *Penicillium notatum.* He inferred that the mold produced some chemical with the ability to attack and kill bacteria, a chemical that he later isolated and named *penicillin.*

Penicillin was only the first of a new category of drugs that came to be called *antibiotics* (named by the Russian microbiologist Selman Waksman in 1941). Antibiotics were originally defined as chemical substances produced by microorganisms and able to inhibit the growth of or destroy bacteria and other microorganisms. It took more than a decade for the significance of Fleming's discovery to be appreciated and for penicillin to be adopted by the medical profession as a treatment for infectious diseases. Once scientists turned that corner, however, they discovered a flood of new antibiotics in a relatively short period of time: streptomycin, by Waksman in 1943; bacitracin, by American bacteriologist Frank

Meleney (1889–1963) in 1943; the cephalosporins, by Sardinian medical researcher Giuseppe Brotzu (1895–1976) in 1945; chloramphenicol, the fi rst broad–range antibiotic, by the research team of John Ehrlich, Paul Burkholder and David Gotlieb, in 1947; chlortetracycline, by the American plant physiologist Benjamin Minge Duggar (1872–1956) in 1947; and neomycin by Waksman and his colleague Hubert Lechevalier in 1949.

Today, scientists have a good understanding of the molecular mechanisms antibiotic compounds use to impair or kill these diseasecausing bacteria. In many instances, for example, an antibiotic molecule will bond with one of the enzymes responsible for the

synthesis of a bacterial cell membrane. Openings develop in the cell membrane, water enters, and the cell bursts and dies. More than 150 different antibiotics are now available for treating a host of infectious diseases that had once been considered incurable, diseases such as plague, pneumonia, tuberculosis, typhus, typhoid fever, scarlet fever, staphylococcus infections, gonorrhea, meningitis, pertussis (whooping cough), and urinary tract infections. These antibiotics exist because researchers came to understand how certain microorganisms live and grow.

Marine Organisms as a Source of Drugs

People have long used marine organisms as the source of a limited number of synthetic products used in everyday life. Perhaps the most famous of these organisms has been the mollusk *Murex brandaris,* from which a beautiful purple dye can be extracted. The dye is obtained from a small organ of the mollusk (the hypobranchial gland), and its preparation is so expensive that it was traditionally used as a dye only for clothing worn by the nobility. For that reason, the dye was called *royal purple* or, more commonly, *Tyrian purple,* after the region from which it is obtained.

Traditionally, there has been almost no research into the use of marine organisms as a source of drugs. Beginning in the 1960s, however, that situation changed and

people began to seek out and identify marine organisms that could be used as the source of natural-productbased drugs. One problem that has hindered research in this area is the diffi culty of collecting and identifying marine organisms and of determining both the chemical products that can be extracted from them and the biological effects of those compounds. The 1990s saw a rapid growth of interest in this fi eld of research, however, with almost half as many patents for marine products being granted from 1996 to 1999 as had been granted in the preceding 25 years.

At this point, only a handful of products derived from marine organisms have been approved by the FDA for sale to consumers. The majority of these products have been approved for nondrug use. For example, researchers at the University of California have extracted an anti-infl ammatory agent, which they named *pseudopterosin*, from a Caribbean sea whip called *Pseudopterogorgia elisabethae.* Pseudopterosin is currently used as an additive to a cosmetic skin cream called Resilience® produced by Estée Lauder. Because the compound has undergone study only relatively recently, it is possible pseudopterosin will have important therapeutic applications, and researchers are exploring this possibility. For example, the compound is also being studied for possible use in the treatment of various infl ammatory disorders, such as rheumatoid arthritis, osteoarthritis, rheumatic carditis, bronchial asthma, myasthenia gravis, and psoriasis. It is also being considered for use with insect bites and as additional treatment during organ and tissue transplants.

The diagram above shows how a large number of similar compounds can be produced by making changes in a basic molecule. In this diagram, R1, R2, and R3 represent three positions in the basic pseudopterosin molecule where atoms or groups of atoms can be added. If a hydrogen atom is used as a substituent at all three positions, the compound formed is called pseudopterosin A. If an acetate group is used at position R1 and hydrogen atoms at R2 and R3, the compound is pseudopterosin B, and so on. Each compound in this family has generally similar characteristics but differs from its cousins' efficacy, chemical and physical properties, safety, and other properties.

Another commercially available product containing naturally occurring marine

products is Formulaid®, produced by Martek Biosciences as a nutritional supplement for infant formulas. Formulaid® contains two fatty acids, arachidonic acid (ARA) and docosahexaenoic acid (DHA), extracted from a variety of marine microalgae. ARA and DHA are the most abundant polyunsaturated fatty acids found in breast milk, and they are the most important fatty acids used in the development of brain gray matter. They are especially desirable for use in infant formulas because they come from nonmeat sources and can be advertised as vegetarian additives to the product.

An especially intriguing pair of products obtained from marine organisms in recent years are Vent® and Deep Vent® DNA polymerase. These products are used in DNA research studies. Their special feature is that they are at least 10 times as effi cient as other similar products in polymerase chain reactions because they can tolerate temperatures just below the boiling point of water, a characteristic that comparable research tools lack. Vent® and Deep Vent® DNA polymerases are obtained from the bacterium *Thermococcus litoralis,* which is found around deep-sea hydrothermal vents at the bottom of the ocean.

A number of other products obtained from marine organisms are used in research also. Among the best known of these is green fluorescent protein (GFP), a compound that fl uoresces (gives off light when exposed to radiation) bright green when exposed to blue or ultraviolet light. When GFP is attached to a compound being studied in an experiment, the compound's movement can be followed visually because of the very noticeable green light produced by the GFP. Green fl uorescent protein is obtained from a bioluminescent jellyfish, *Aequora victoria.* Some scientists who study marine organisms believe that they may be at the threshold of an exciting new era in which extracts from such organisms can provide a host of new therapeutic drugs for use against some of the most intransigent diseases known to humans, including cancer and malaria. Two of the most promising of these products were discovered in the early 1950s by W. Bergmann, R. J. Feeney, and D. C. Burke. The products were modifi ed forms of familiar nitrogen bases (aromatic carbon compounds that contain nitrogen) given the names of spongothymidine and spongouridine that demonstrated strong antitumor and antiviral properties. A synthetic analog of these natural products,

arabinosyl cytosine, is now available commercially from the Pharmacia & Upjohn Company under the brand name of Cytosar-U®. As of this writing, it is the only marine-derived anticancer agent available for clinical use.

A number of other marine-derived products are waiting in the wings, however. Among the many compounds that have shown promise and are undergoing further testing for anticancer properties are halichondrin B, isolated from four marine sponge genera, *Halichondria, Axinella, Phakellia,* and *Lissodendoryx;* halomon, from the red alga *Portieria hornemannii;* dolastatin 10, from the sea slug (sea hare) *Dolabella auricularia;* and ecteinascidin 743, from the Caribbean sea squirt *Ecteinashidia turbinata.* One of the compounds furthest along in development is bryostatin-1, derived from the marine bryozoan *Bugula neritina.* In 2001, the FDA granted "*orphan drug*" status to bryostatin-1, reserving marketing rights for the product to the German-based fi rm GPC Biotech AG. The compound has showed great promise for the treatment of esophageal cancer, especially when used in conjunction with another anticancer agent, Taxol®. It also appears to have potential value in the treatment of melanoma, ovarian cancer, and non-Hodgkin's lymphoma.

Drug researchers now hold high hopes for the promise of marine organisms as the source of new drugs. More than 80 percent of all life-forms on Earth exist only in the oceans, so a vast supply of organisms is available for study. Some authorities have stated that the chances of finding new drugs in marine organisms may be 300 to 400 times that of finding drugs in terrestrial organisms.

Plant Products as the Source of New Drugs

Despite all the contributions that microorganisms have made to the development of new drugs and all the promise held by marine organisms for such purposes, many researchers still count primarily on plants as the most likely source for the discovery of new drugs. In some areas, that hope has already been realized. In 2002, authorities estimated that anywhere between one third and one-half of the best-selling prescription drugs used around the world were derived from natural products.

In recent years, however, some of the greatest emphasis has been placed on the

search for anticancer and antiviral agents derived from natural products. Success in that area has not been as great as that achieved in other fi elds. Since 1960, only seven plant-derived drugs have been approved by the FDA for use as anticancer agents. Four of those drugs, vinblastine, vincristine, etoposide, and teniposide, were discovered in the 1950s. The last three—Taxol®, topotecan, and irinotecan—were discovered and approved much more recently. The discovery of vinblastine and vincristine is one of the most intriguing examples of serendipity in scientifi c research in recent years. In 1952, the Canadian medical researcher Robert Laing Noble (1910–90) received a package from his brother, Dr. Clark Noble, containing 25 leaves from the Madagascar periwinkle plant, *Vinca rosea.* Clark had received the leaves from one of his patients in Jamaica, who said that natives on the island often used the plant to control their diabetes when insulin was not available. Clark, who was retired, suggested that his brother study the plant for possible use as a drug for the treatment of diabetes.

When Robert Noble carried out his studies on the periwinkle leaves, he found that they had no effect on blood sugar levels. However, they did appear to significantly reduce a subject's white blood cell count. Perhaps, Dr. Noble reasoned, the product could be used to treat diseases characterized by abnormally high white blood cell counts, such as leukemia. He was successful in isolating two chemicals from the periwinkle leaves, which he named vinblastine and vincristine, that markedly decreased white blood cell counts in patients with certain forms of cancer. The two chemicals were the first anticancer agents derived from natural sources to be approved for use with human patients.

Perhaps the most exciting story about an anticancer agent derived from a natural product is that of Taxol®. That story begins in 1958, when the National Cancer Institute began a program to screen natural products for substances that might have anticancer activity. The plan was to examine more than 35,000 species in the research. Five years later, scientists at the Research Triangle Institute in North Carolina, Monroe Wall and M. C. Wani, found that the bark of the Pacifi c yew tree (*Taxux brevifolia*) demonstrated tumor-suppressing qualities. In 1971, those same scientists

isolated a substance, which

they called *compound 17,* responsible for this antitumor activity. Compound 17 was later renamed *paciltaxel.*

Hopes for using paciltaxel in the treatment of cancer were dampened, however, by the fact that the Pacifi c yew tree is a slow-growing, threatened tree. Its harvest for the collection of paciltaxel from its bark would almost certainly have led to the tree's extinction. Instead, researchers turned to the obvious alternative, characterization of the chemical structure of paciltaxel and its chemical synthesis. That task was a challenge, however, because of the complex structure of the paciltaxel molecule. After more than a decade of research, however, the task was accomplished: Researchers achieved a successful method for the synthesis of the compound in the laboratory. In 1992, the FDA approved paciltaxel for use against cancers that had failed to respond to other treatments. By this time, the compound was being made and marketed by Bristol-Myers Squibb Company under the trade name of Taxol®. Over the next decade, the FDA continued to expand the diseases for which Taxol® could be used, including breast, ovarian, and lung cancer and Kaposi's sarcoma related to HIV infection.

Another success story involving the development of anticancer agents is that of a drug known as *camptothecin.* The same researcher who had begun study of pacitaxel, Dr. M. E. Wall, first studied the natural product from which this drug was originally obtained, a tree native to China called *Camptotheca acuminata,* in the late 1950s. Although initial studies of its effects on tumors were encouraging, later tests were ambiguous, and interest in the anticancer and antiviral properties. In 2007, a number of products are at various stages of testing, including combretastatin A4, isolated from the South African medicinal tree, *Combretum caffrump;* homoharringtonine, from the tree *Cephalotaxus harringtonia* found in mainland China; ingenol 3-O-angelate, originally obtained from a common English and Australian tree *Euphorbia peplus;* and phenoxodiol, a synthetic analog of daidzein, obtained from soybean. This field of research obviously holds great promise for the development of new antiviral and anticancer drugs.

The Search for New Natural Product Drugs Until the 1950s, the world approached the use of drugs to treat disease in either of two ways. Generally speaking, people living in developing nations relied primarily on natural products, especially herbs, to treat disease, while those living in developed nations put their faith in modern scientifi c medicine, usually synthetically produced chemical compounds, for the same purposes. The line between these two practices began to break down when scientific researchers started to search for the chemical compounds in natural products that are biologically active, research characterized by the work of Wall and Wani described in the previous section. In fact, the accomplishments of Wall and Wani prompted the National Cancer Institute (NCI) and the U.S. Department of Agriculture (USDA) to initiate a formal program for the collection and screening of plants with potential anticancer activity.

Between 1960 and 1982, that program was responsible for the collection of more than 35,000 plant samples, from which 114,000 unique extracts were obtained. In addition, field-workers collected 18,000 extracts from marine organisms. NCI and USDA terminated the program in 1982 because of limited success: Only seven plantderived anticancer drugs had been developed as a result of the program, four resulting from Wall and Wani's 1950s research. NCI reactivated the program in 1986 as its Natural Products Branch (NPB), a division that remains in existence today. In 1988, the institute also began to search for drugs that might be effective against AIDS. Since its reactivation, NPB has screened more than 40,000 plant extracts. One of those, Taxol®, has been approved for use, and five others with potential use against AIDS have been isolated. Three of those are now in preclinical trials.

Researchers have adopted three approaches in their search for new drugs among natural products. First, they sometimes use a "broadcast" approach in which they simply collect and study all the plants or marine organisms within a certain geographical area. The advantage of this approach is that large numbers of samples can be collected in a relatively short time. The disadvantage is that there is seldom any particular reason for expecting to find a useful compound in any given area.

A second approach is to focus on plants or marine organisms that are known to

contain biologically active compounds. The hope is that such plants or marine organisms may yield new and different chemicals that may also be effective against certain types of disease.

Finally, researchers may use an ***ethnobotanical*** approach, that is, one that focuses on medicinal plants that have traditionally been used in various cultures. The assumption underlying this approach is that plants on which people have relied for medicines in the past may very well contain biologically active chemicals that can be either isolated, purified, and used as drugs or used as models from which biologically active analogs can be produced.

Once a plant or marine organism sample has been collected, it is labeled, stored, and then treated chemically to remove its primary components. Next, these components (extracts) are tested to determine whether or not they show any biological activity. The tests (*bioassays*) are used to identify any toxicity or other effect an extract may have against target cells (such as cancer cells), organisms (such as parasites), or chemicals (such as allergens). Extracts that seem to be effective against any one of these targets are then analyzed in more detail to fi nd out what chemical(s) they contain that produce the biological activity. When and if such compounds are identified, they are then subjected to the long and detailed series of tests for safety and efficacy that all new drugs undergo.

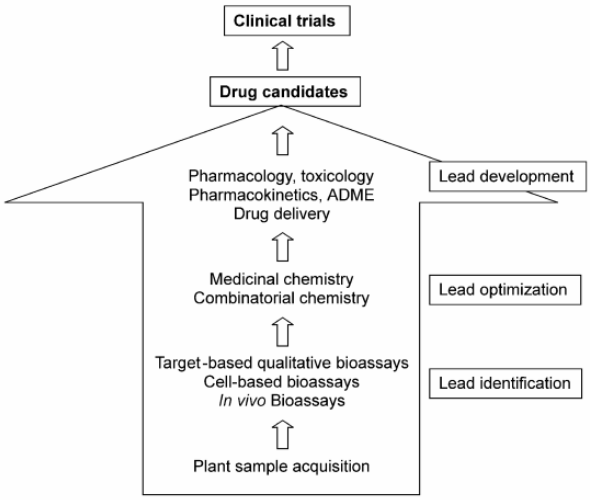
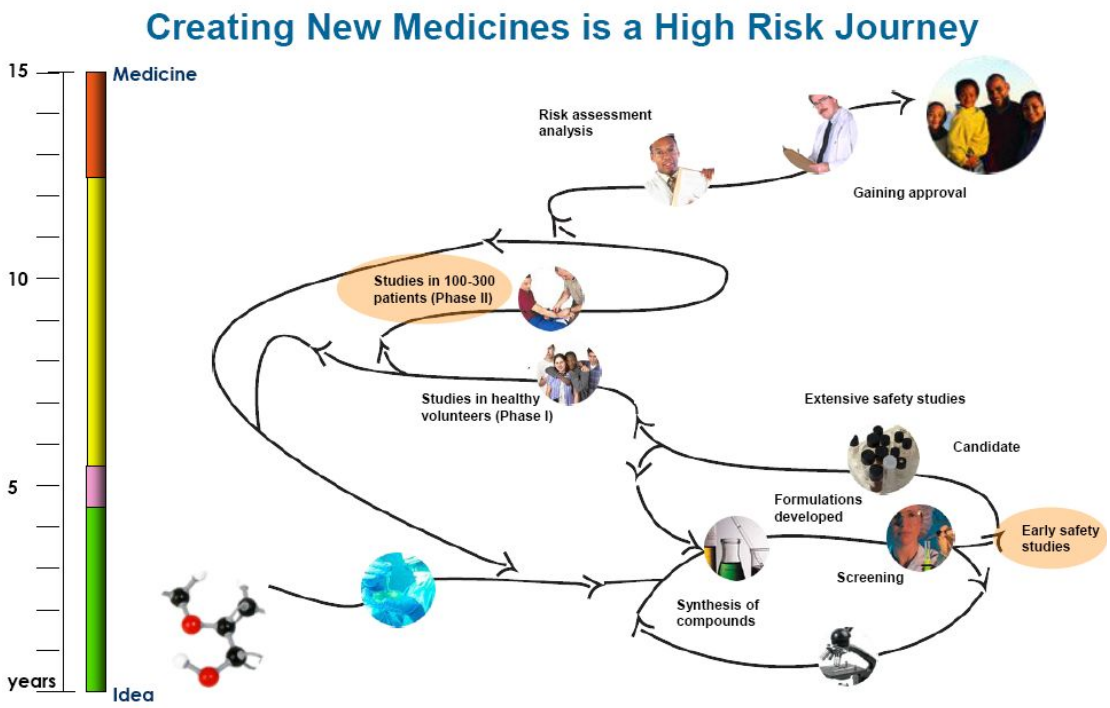


Figure 1. Drug discovery process from plants



Natural Product Research and Biodiversity

Over the past few decades, the use of natural products as drugs and dietary supplements has raised an increasingly important question: What impact does it have on biodiversity? The widespread popularity of some natural products has resulted in their rapid destruction in the environment. One of the best-documented examples of this pattern is the decimation of wild echinacea resources throughout the United States. Sales of the plant in 2002 amounted to more than $32 million, and manufacturers are eager to obtain as much as they can from American sources. As a result, the plant is rapidly being depleted from its natural habitat, which ranges across large parts of the Midwest.

The popularity of ginseng has already led to its extinction in some parts of the world (such as South Korea) and to its classifi cation as an endangered species in other parts (such as China) due to overharvesting.

Today, more than 65 tons of the root are harvested in the United States each year, most of it going to the Far East. At this rate, the plant faces possible extinction in this country also. Goldenseal is yet another threatened herb in the United States and

other parts of the world. It currently sells for about $100 a pound, making it highly popular for individual, independent workers who tear it out of its natural habitat. In 2003, the Convention on International Trade in Endangered Species proposed listing it as an endangered species.

Such losses are potentially serious problems for drug research. Maintaining biodiversity is an essential component of future research efforts to identify possible drugs in the world's plant and marine resources. Scientists have no idea how many species there are in the world, but reasonable estimates place the numbers at about 250,000 plant species and up to 1 million marine species. So far, no more than about 10 percent of all plants and 1 percent of all marine organisms have been studied for possible use as drugs.

Given these circumstances, it is possible that countless numbers of new natural products with potential for use as drugs are still waiting to be discovered. As more and more plants and animals are destroyed each year by deforestation, development, and other forces, those natural products are being to lost for possible future use.

Natural Products as Dietary Supplements

Ask the average person on the street about "natural products," and he or she is likely to mention the kinds of products found on the shelves of grocery stores and stores that specialize in "organic" and "natural" foods. Those items are overwhelmingly plant products, and they range from aconitum napellus (monkshood), alfalfa, allium cepa, aloe vera, angelica, and anise seed to witch hazel, yarrow, yellow dock, yohimbe bark, and yucca. Healers have used many of these products for centuries, and they remain widely popular with people in countries around the world today, both developed and developing.

In many cultures,

Echinacea Stimulates immune system

Ginkgo (*Ginkgo biloba*) Improves concentration and memory; protects against Alzheimer's disease

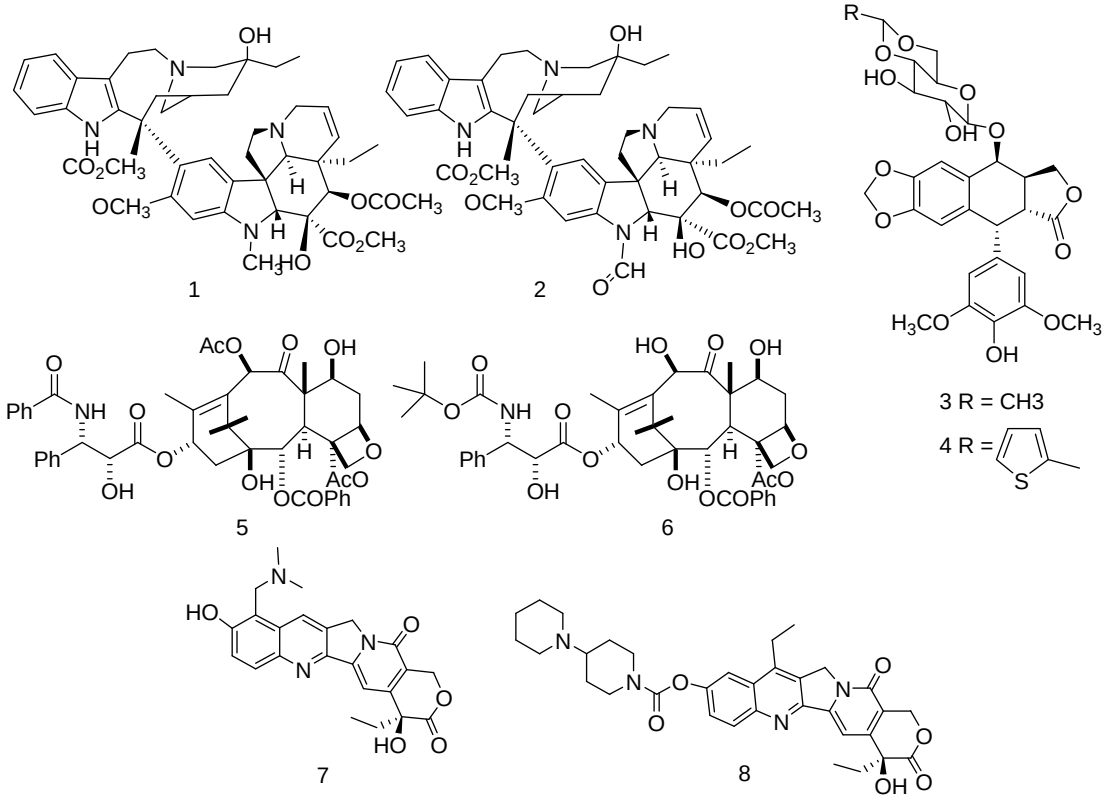
Ginseng (*Panax ginseng*) Increases stamina and concentration

Glucosamine and chondroitin Effective against arthritis and other joint diseases

Part II

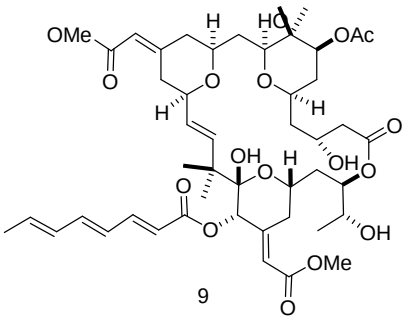
The Significance of Natural Products as Pharmaceuticals

The study of natural products, or "Nature's Combinatorial Library" has a long history as a source of drugs, and especially anticancer drugs. Thus half of all prescriptions dispensed in the U.S.A. contain substances of natural origin, one quarter of all prescriptions contain a plant-derived active principle, and over $8 billion of U.S. prescription drugs in 1980 were estimated to be plant-based ([i]). Using more recent data, a recent authoritative review concluded that 61% of all the new drugs introduced worldwide during 1981-2002 can be traced to or were inspired by natural products ([ii]). Examples of plant-derived clinically used anticancer agents include vinblastine (**1**) and vincristine (**2**), etoposide (**3**) and teniposide (**4**), taxol (**5**) and docetaxel (**6**), and topotecan (**7**) and irinotecan (**8**).

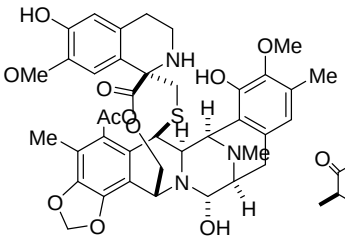


In addition to the plant-derived anticancer agents mentioned above, several marine natural

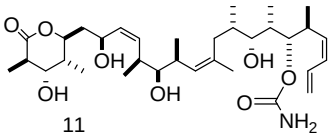
products and related compounds are in clinical and advanced preclinical trials as anticancer agents ([iii]); these include bryostatin 1 (**9**), ecteinascidin 743 (**10**), discodermolide (**11**), and E7389 (**12**).



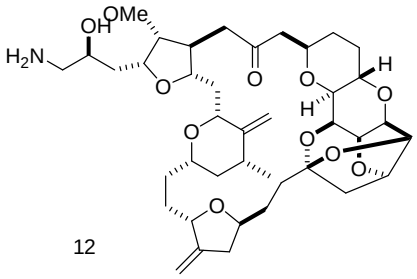
9



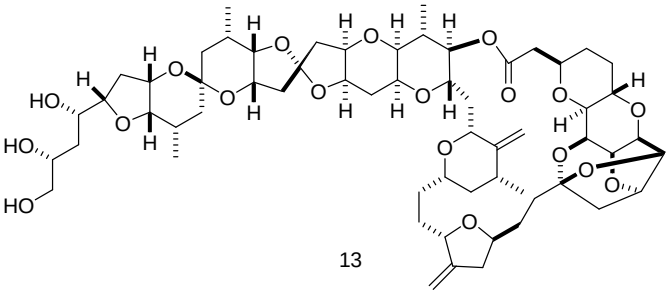
10



11



12



13

It is instructive to ask why it is that natural products, including plant, marine, and microbial products, have proved such a prolific source of bioactive agents. There are several reasons. In the first place, plants and other organisms produce many biologically active substances for defense and other purposes ([iv]). These substances are often so complex that they would never be prepared synthetically as drug candidates, so isolation from natural sources is the only feasible way to access them. Furthermore, most natural products have built-in chirality, whereas most synthetic compounds are achiral. Natural products are thus more “druglike” than most synthetic compounds.

This “druglike” nature of natural products has been demonstrated both by statistical analyses ([v],[vi]) and by various analyses of the importance of natural products as pharmaceuticals. A recent review ([vii]) makes a comparison between natural products and drugs, and shows that there is more similarity between natural products and drugs in several areas (LogP, number of chiral centers, number of nitrogen atoms, number of oxygen atoms, percent of aromatic rings) than between synthetic compounds and drugs.

Natural products were significant from the perspective of a major pharmaceutical company in 1998 ([viii]), and are still significant for some companies. Thus Butler, writing from the perspective

of a small pharmaceutical company that is heavily invested in natural products, writes “Another misconception has been that NP research has failed to deliver many new compounds that have undergone clinical evaluation over the last few years. However, in reality, 15 NP-derived drugs have been launched in the key markets of the United States, Europe, and Japan over the last three years, and an additional 15 NP-derived compounds were in Phase III clinical trials at the end of 2003.” ([ix]).

In addition to the use of natural products as drugs, natural products can also lead to new analogs with greater synthetic accessibility or improved activity. A nice example of this is shown by the synthesis of E7389 (**12**) as a synthetically accessible active analog of halichondrin B (**13**). Compound **12** has been prepared in large quantities in an impressive synthesis, and is currently in clinical trials ([x]). The many examples of taxol analogs in clinical trials ([xi]) also demonstrate the value of synthetic modifications of natural products as anticancer agents, as does the exciting activity of 26-trifluoro-(*E*)-9,10-dehydro-12,13-desoxyepothilone B as an improved epothilone analog ([xii]).

The Decline in Interest in Natural Products as Pharmaceuticals

In spite of the obvious successes of the natural products approach to drug discovery, in recent years it has lost some favor, particularly within the pharmaceutical industry. The reasons for this are complex, but can be summarized as being due to a combination of factors, including the incompatibility of crude extracts with the high throughput assays used in the pharmaceutical industry, the cost of sample collection, problems with the lack of reproducibility and the presence of artifacts in some extracts, the difficulty in isolating active compounds, the long resupply times for active extracts, problems with large scale supply if a drug should emerge, the difficulty of complying with the Rio Treaty on Biodiversity, and last but not least, the diversion of resources to combinatorial chemical approaches to drug discovery ([xiii]). However, there is evidence that some people now realize that the move to discontinue natural products research in favor of combinatorial chemistry may have been a mistake. The authors of the review previously cited (vii) conclude with these trenchant observations: “The early years of combinatorial chemistry suffered from an excess of hype, and a major victim was natural-product screening. Many organizations

went through an irreversible shift in policy, and prematurely discontinued their efforts in this area. We are now seeing the backlash from this knee-jerk reaction. The early combinatorial strategies were flawed and unproven, and have yet to deliver any blockbuster drugs. Meanwhile, we have lost the uniqueness of screening natural-product space as a complement to synthetic compounds. If past indicators are any guide, there are undoubtedly many more unique and potent biologically active natural products waiting to be discovered."

Some Ways to Address the Perceived Problems with Natural Products as Pharmaceuticals

Although as natural products researchers we may decry the apparent decline in interest in our field by the pharmaceutical industry, we must do more than simply lament this decline. The situation is not in fact as bleak as it might appear at first sight, since several small companies have been formed to take up the challenge of drug discovery from natural products. These include Galileo Pharmaceuticals and SelectX Pharmaceuticals in the USA, Ecopia BioSciences in Canada, and MerLion Pharma in Singapore. It is likely that one or more of these and other similar companies will develop an important new drug from a natural product, which will in turn increase the overall level of interest in natural products.

Having said this, what can be done now to stimulate drug discovery from natural products? The following suggestions range from those that can be implemented by an individual academic researcher to those that require the resources of a major company or the cooperation of national governments.

The incompatibility of crude extracts with high throughput assays can be addressed by some degree of prefractionation of extracts. This can range from simple polyamide filtration to remove polyphenolics which interfere with enzyme and receptor-based assays to the generation of "peak libraries" of partially purified compounds by automated HPLC of crude extracts. A second approach to this problem is to develop and use "smart" assays that are compatible with natural product extracts. These assays can usefully be cell-based, since the cell wall will limit "hits" to compounds that can pass through it, excluding many nuisance compounds such as tannins. As examples, Roberge and his group have developed cell-based assays that have proven effective for

discovering novel inhibitors of mesenchymal tumor cell invasion and migration ([xiv]) and for antimitotic agents ([xv]). The use of simple yeast-based assays has also proven effective in discovering DNA-damaging agents in our own work ([xvi]). The development of more assays of this type will certainly be beneficial to the academic researcher in the search for bioactive natural products.

The difficulty in isolating and characterizing complex natural products is becoming less of a problem as new methods for isolation and structure elucidation of natural products continue to be developed. Thus it is likely that HPLC-MS and HPLC-NMR will become routine techniques, assisting with the rapid characterization of complex mixtures. In addition, micro-probe NMR will lessen the sample size requirements for structure elucidation; a recent illustration of the power of this technique is the isolation and characterization of thirteen steroids from fifty fireflies ([xvii]).

Problems with resupply and large scale production can be minimized by careful collection work, using GPS to return to the exact location of the original collection. Large scale production can sometimes be carried out by semisynthesis (as in the case of taxol) or by total synthesis (as in the case of E7389). In the case of taxol, the natural product was obtained in only 0.04% yield from yew bark, and there was little initial interest from the pharmaceutical industry, in part because of supply problems. The supply problem was initially solved by semisynthesis from the more abundant 10-deacetylbaccatin III, and taxol is now also available by plant tissue culture (xi).

The geopolitical issues associated with bioprospecting can be approached in various ways. In the first place, it goes without saying that natural product scientists must be careful to collect biomaterial in approved and legal ways, with preservation of the rights of all parties (including those of indigenous peoples). To enable this to be possible with the minimum amount of bureaucracy, it is desirable that the process for obtaining collection permits should be open and transparent. Although this is true in many countries, it is not universally the case, and this is an area where active collaboration with and (in some cases) education of government authorities is necessary.

Another approach to the use of natural products is as herbal medicines. It is estimated that 80% of the world's population use herbal preparations as their main source of medication, and so there is a great need to standardize and validate these preparations. The National Center for Complementary and Alternative Medicine (NCCAM) in the USA actively supports such work; recent awards by NCCAM include grants on phytoestrogens and aging, on the effect of Chinese herbal medicine on food allergy, on estrogen receptor-selective herbs for menopause symptoms, and on natural product therapeutics in Alzheimer's disease.

The Madagascar-Suriname ICBG Project: Development and Biodiversity Conservation

Our own work has been carried out with support from the International Cooperative Biodiversity Group (ICBG) program at the National Institutes of Health, USA. This approach has several important features. Thus, consistent with the Rio Treaty, bioprospecting is done with the full informed consent of all parties, and bioprospecting is combined with economic development activities, since much deforestation is caused directly or indirectly by poverty. The work is done in partnership with a pharmaceutical company (and in our case with an agrochemical company too), so that there is a natural pathway to drug development when a lead compound is discovered. A part of the agreement between the parties involved is a commitment to return any royalty payments in part to the host country in compensation for the use of its biodiversity. Our work was originally based in Suriname, and is now based in Madagascar.

The structure of our group and the activities undertaken by each partner are as follows:

The Missouri Botanical Garden, under Dr. Jim Miller, carries out botanical collections and makes biodiversity surveys. Conservation International-Madagascar is involved with economic development, biodiversity conservation, and benefit sharing activities. The Centre National d'Application des Recherches Pharmaceutiques prepares the plant extracts, and also does screening for antimalarial activity and isolation of compounds with antimalarial activity.

The Centre National de L'Environnement makes collections of marine organisms, and will also carry out some microbial isolations. Eisai Research Institute has an independent program of bioassay drug discovery and development, with an emphasis on the anticancer and immunological

areas. Dow Agrosciences does bioassay and agrochemical discovery and development, while our own program at VPI&SU (Virginia Tech) is focused on bioassay and anticancer drug discovery.

Although our work in Suriname is now over, there has been one significant legacy of our time there, the Central Suriname Nature Reserve. In the early 1990's Suriname had three major National Parks in the center of the country. In the mid 1990's some lumber companies from southeast Asia approached the government of Suriname and offered to pay a significant sum of money for the rights to carry out logging operations in the center of the country. These proposed logging concessions would have logged much of central Suriname, would have completely isolated one National Park, and would have destroyed the forest close to many of the villages, thus denying the indigenous peoples the ability to use this key resource. Our ICBG partner Conservation International argued against this potential catastrophe, and used the ICBG program was an important factor in arguments against logging. Using funds from private donors, Conservation International offered to lease a large section of central Suriname, thus replacing the income that would have been generated by logging, but at no cost to the environment. Conservation International and the government of Suriname then jointly established the Central Suriname Nature Reserve, linking the three major parks into one contiguous unit, and providing a major forest resource for future bioprospecting and biodiversity conservation.

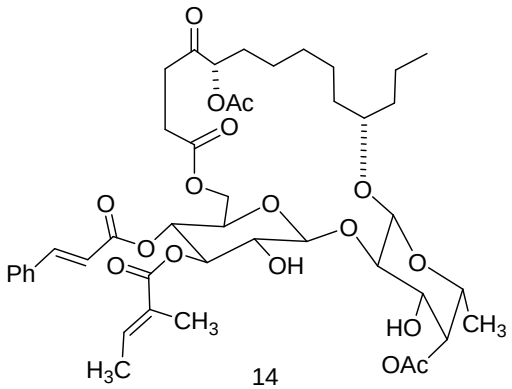
Our conservation and development work in Madagascar has not been on this scale, but it has included several small-scale projects for the villages in the area of our plant collections, namely the Zahamena Park area. These projects were selected by the villagers themselves, and the funds for the work came from upfront compensation funds provided by our industrial partners. Examples of the projects are the construction of an agricultural warehouse in Antanandava, renovation of a primary school in Manakambahiny, and construction of a river bridge to enable villagers to reach a clinic during the rainy season. Other projects have focused on developing ecotourism and farming self-sufficiency.

A further important result of our work in Madagascar has been the assistance our program, and especially the teams from the Missouri Botanical Garden, Conservation International-Madagascar,

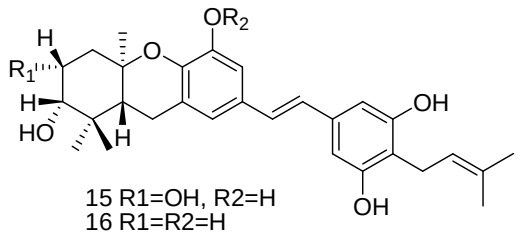
and the Centre National d'Application des Recherches Pharmaceutiques, have been able to give to the government of Madagascar as part of its ambitious program to double the nation's protected areas. The botanical survey work carried out by these partners as part of the overall project has provided a sound scientific basis for the selection of future protected areas.

The Madagascar-Suriname ICBG Project: Drug Discovery

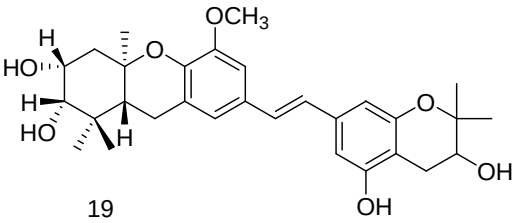
Much of our work in the drug discovery area has been reported previously ([xviii], [xix]), and so it will not be reproduced here. Two results, one each from Madagascar and Suriname, are selected for discussion. Work in Suriname led to the discovery that the leaves of *Ipomoea squamosa* yielded a moderately cytotoxic extract. Fractionation of this extract by flash chromatography on a C18 column followed by HPLC on C18 and then phenyl columns yielded five cytotoxic compounds designated ipomoeassins A-E ([xx]). These compounds turned out to have similar structures, of which **14** is representative. Interestingly, **14** was two orders of magnitude more cytotoxic to the A2780 cell line than some of its close congeners, so there are some interesting SAR effects which we do not as yet understand.



14



15 R1=OH, R2=H
16 R1=R2=H
17 R1=OH, R2=CH3
18 R1=H, R2=CH3



19

Our work in Madagascar has led, among other findings, to the isolation of compounds **15 – 19**. These potent cytotoxins are related to the schweinfurthins ([xxi]), and are of possible interest for drug development. The major stumbling block to further work is the lack of any mechanistic rationale for their activity, but ongoing studies at the National Cancer Institute may resolve this issue and open the way for potential development of these interesting compounds.

Conclusion

The ICBG program represents an ambitious attempt to integrate the areas of biodiversity conservation, economic development, and drug discovery into a coherent whole. The approach that we have taken in Suriname and Madagascar has yielded modest but tangible benefits in the economic development area, and has contributed significantly to biodiversity conservation. This is especially the case in Suriname, where the Central Suriname Nature Reserve stands as a testament to the foresight and diligence of our partners at Conservation International and to the effectiveness of this integrated approach. In the drug discovery area we have isolated over 380 bioactive compounds, over 180 of which are new to science, and several of which have promising bioactivities. At this point none of the isolated compounds are clear drug candidates, but the full story on all of them has not yet been worked out, and so it is still possible that this area of work could eventually be as successful as the other two areas.

Literature Cited

1. Newman, D. J.; Cragg, G. M.; Snader, K. M. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.* **2003,** *66*, 1022-1037.

1. Newman, D. J.; Cragg, G. M. Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.* **2004,** *67*, 1216-1238.

1. Williams, D. H.; Stone, M. J.; Hauck, P. R.; Rahman, S. K. Why are secondary metabolites (natural products) biosynthesized? *J. Nat. Prod.* **1989,** *52*, 1189-1208.

1. Henkel, T.; Brunne, R. M.; Muller, H.; Reichel, F., Statistical investigation into the structural complementarity of natural products and synthetic compounds. *Angew. Chem. Int. Ed.* **1999,** *38*, 643-647.

1. Feher, M.; Schmidt, J. M. Property distributions: Differences between drugs, natural products, and molecules from combinatorial chemistry. *J. Chem. Inf. Comput. Sci.* **2003,** *43*, 218-227.

[1]. Ortholand, J.-Y.; Ganesan, A. Natural products and combinatorial chemistry; back to the future. *Curr. Opin. Chem. Biol.* **2004**, 8, 271-280.

[1]. Shu, Y.-Z. Recent natural products based drug development: A pharmaceutical industry perspective. *J. Nat. Prod.* **1998,** *61*, 1053-1071.

[1]. Butler, M. S. The role of natural products in drug discovery. *J. Nat. Prod.* **2004**, *67*, 2141-2153

[1]. Seletsky, B. M.; Wang, Y.; Hawkins, L. D.; Palme, M. H.; Habgood, G. J.; DiPietro, L. V.; Towle, M. J.; Salvato, K. A.; Wels, B. F.; Aalfs, K. K.; Kishi, Y.; Littlefield, B. A.; Yu, M. J., Structurally simplified macrolactone analogues of halichondrin B. *Biorg. & Med. Chem. Lett.* **2004,** *14*, 5547-5550.

[1]. Kingston, D. G. I. Taxol and its analogs. In "*Anticancer Agents from Natural Products*" Cragg, G. M.; Kingston, D. G. I.; Newman, D. J.; Eds. CRC Press: Boca Raton, FL, 2005, pp. 89-122.

[1]. Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Chou, T.-C.; Dong, H.; Danishefsky, S. J., Discovery of (*E*)-9,10-dehydroepothilones through chemical synthesis: On the emergence of 26-trifluoro-(*E*)-9,10-dehydro-12,13-desoxyepothilone B as a promising anticancer drug candidate. *J. Am. Chem. Soc.* **2004,** *126*, 10913-10922.

[1]. Raskin, I.; Ribnicky, D. M.; Komarnytsky, S.; Ilic, N.; Poulev, A.; Borisjuk, N.; Brinker, A.; Moreno, D. A.; Ripoll, C.; Yakoby, N.; O'Neal, J. M.; Cornwell, T.; Pastor, I.; Fridlender, B., Plants and human health in the twenty-first century. *TRENDS in Biotech.* **2002**, *20*, 2002.

[1]. Roskelley, C. D.; Williams, D. E.; McHardy, L. M.; Leong, K. G.; Troussard, A.; Karsan, A.; Andersen, R. J.; Dedhar, S.; Roberge, M. Inhibition of tumor cell invasion and angiogenesis by motuporamines. *Cancer Res.* **2001**, *61*, 6788-6794.

[1]. Roberge, M.; Cinel, B.; Anderson, H. J.; Lim, L.; Jiang, X.; Xu, L.; Bigg, C. M.; Kelly, M. T.; Andersen, R. J. Cell-based screen for antimitotic agents and

identification of analogues of rhizoxin, eleutherobin, and paclitaxel in natural extracts. *Cancer Res.* **2000**, *60*, 5052-8.

[1]. Gunatilaka, A. A. L.;. Kingston, D. G. I. Johnson, R. K. Mechanism-based isolation and structures of some anticancer natural products, *Pure Appl. Chem.*, **1994**, *66*, 2219-2222.

[1]. Gronquist, M.; Meinwald, J.; Eisner, T.; Schroeder, F. C. Exploring Uncharted Terrain in Nature's Structure Space Using Capillary NMR Spectroscopy: 13 Steroids from 50 Fireflies. *J. Am. Chem. Soc.* **2005**, *127*, 10810 – 10811.

[1]. Kingston, D. G. I.; van der Werff, H.; Evans, R.; Mittermeier, R.; Famolare, L.; Guerin-McManus, M.; Malone, S.; Wisse, J. H.; Vyas D.; Wright, J. J. K. Biodiversity Conservation, Economic Development, and Drug Discovery in Suriname. In *Biologically Active Natural Products: Pharmaceuticals*, Cutler, H. G.; Cutler, S. J.; Eds. CRC Press: Boca Raton, 2000, pp 39-59.

[1]. Recent publications include the following: a) Williams, R. B.; Hoch, J.; Glass, T. E.; Evans, R.; Miller, J. S.; Wisse, J. H.; Kingston, D. G. I. A novel cytotoxic guttiferone analog from *Garcinia macrophylla* from the Suriname rainforest. *Planta Med.* **2003**, *69*, 864-866. b) Cao, S.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.;. Kingston, D. G. I. Cytotoxic compounds from *Mundulea chapelieri* from the Madagascar rainforest *J. Nat. Prod.* 2004, *67*, 454-456. c) Cao, S.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. New cytotoxic triterpenoids from *Acridocarpus adenophorus. J. Nat. Prod.* **2004**, *67*, 986-989. d) Chaturvedula, V. S. P.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.; Kingston D. G. I. New cytotoxic terpenoids from the wood of *Vepris punctata* from the Madagascar rainforest. *J. Nat. Prod.* **2004**, *67*, 895-898. e) Cao, S.; Schilling, J. K.; Miller, J. S.; Randrianasolo, A.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. New cytotoxic alkyl phloroglucinols from *Protorhus thouvenotii*. *Planta Med.* **2004**, *70*, 683-684. f) Chaturvedula, V. S. P.; Farooq, A.; Schilling, J. K.; Malone, S.; Derveld, I.; Werkhoven, M. C. M.; Wisse, J. H.; Ratsimbason, M.; Kingston, D. G. I. New Eudesmane Derivatives from

Melampodium camphoratum from the Suriname Rainforest**.** *J. Nat. Prod.* **2004**, *67*, 2053-2057. g) Murphy, B. T.; Cao, S. G.; Norris A.; Kingston, D. G. I. Cytotoxic flavanones from *Schizolaena hystrix* from the Madagascar rainforest. *J. Nat. Prod.* **2005**, *68*, 417-419.

[1]. Cao, S. G.; Guza, R.C.; Norris, A.; Evans, R.; Miller, J. S.; Wisse, J. H.; Kingston, D. G. I. Ipomoeassins A-E, five new cytotoxic macrocyclic glycoresins, from the leaves of *Ipomoea squamosa*. *J. Nat. Prod.* **2005**, *68*, 487-492.

[1]. Beutler, J. A.; Shoemaker, R. H.; Johnson, T.; Boyd, M. R. Cytotoxic geranyl stilbenes from *Macaranga schweinfurthii.* *J. Nat. Prod.* **1998**, *61*, 1509-1512

---

[i]. Principe, P. P. The economic significance of plants and their constituents as drugs. In *Economic and Medicinal Plant Research*, Wagner, H.; Hikino, H.; Farnsworth, N. R.; Eds. Academic Press: San Diego, 1985; Vol. 3, pp 1-17.

[ii]. Newman, D. J.; Cragg, G. M.; Snader, K. M. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.* **2003,** *66*, 1022-1037.

[iii]. Newman, D. J.; Cragg, G. M. Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.* **2004,** *67*, 1216-1238.

[iv]. Williams, D. H.; Stone, M. J.; Hauck, P. R.; Rahman, S. K. Why are secondary metabolites (natural products) biosynthesized? *J. Nat. Prod.* **1989,** *52*, 1189-1208.

[v]. Henkel, T.; Brunne, R. M.; Muller, H.; Reichel, F., Statistical investigation into the structural complementarity of natural products and synthetic compounds. *Angew. Chem. Int. Ed.* **1999,** *38*, 643-647.

[vi]. Feher, M.; Schmidt, J. M. Property distributions: Differences between drugs, natural products, and molecules from combinatorial chemistry. *J. Chem. Inf. Comput. Sci.* **2003,** *43*, 218-227.

[vii]. Ortholand, J.-Y.; Ganesan, A. Natural products and combinatorial chemistry; back to the future. *Curr. Opin. Chem. Biol.* **2004**, 8, 271-280.

[viii]. Shu, Y.-Z. Recent natural products based drug development: A pharmaceutical industry perspective. *J. Nat. Prod.* **1998,** *61*, 1053-1071.

[ix]. Butler, M. S. The role of natural products in drug discovery. *J. Nat. Prod.* **2004**, *67*, 2141-2153

[x]. Seletsky, B. M.; Wang, Y.; Hawkins, L. D.; Palme, M. H.; Habgood, G. J.; DiPietro, L. V.; Towle, M. J.; Salvato, K. A.; Wels, B. F.; Aalfs, K. K.; Kishi, Y.; Littlefield, B. A.; Yu, M. J., Structurally simplified macrolactone analogues of halichondrin B. *Biorg. & Med. Chem. Lett.* **2004,** *14*, 5547-5550.

[xi]. Kingston, D. G. I. Taxol and its analogs. In "*Anticancer Agents from Natural Products*" Cragg, G. M.; Kingston, D. G. I.; Newman, D. J.; Eds. CRC Press: Boca Raton, FL, 2005, pp. 89-122.

[xii]. Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Chou, T.-C.; Dong, H.; Danishefsky, S. J., Discovery of (*E*)-9,10-dehydroepothilones through chemical synthesis: On the emergence of 26-trifluoro-(*E*)-9,10-dehydro-12,13-desoxyepothilone B as a promising anticancer drug candidate. *J. Am. Chem. Soc.* **2004,** *126*, 10913-10922.

[xiii]. Raskin, I.; Ribnicky, D. M.; Komarnytsky, S.; Ilic, N.; Poulev, A.; Borisjuk, N.; Brinker, A.; Moreno, D. A.; Ripoll, C.; Yakoby, N.; O'Neal, J. M.; Cornwell, T.; Pastor, I.; Fridlender, B., Plants and human health in the twenty-first century. *TRENDS in Biotech.* **2002**, *20*, 2002.

[xiv]. Roskelley, C. D.; Williams, D. E.; McHardy, L. M.; Leong, K. G.; Troussard, A.; Karsan, A.; Andersen, R. J.; Dedhar, S.; Roberge, M. Inhibition of tumor cell invasion and angiogenesis by motuporamines. *Cancer Res.* **2001**, *61*, 6788-6794.

[xv]. Roberge, M.; Cinel, B.; Anderson, H. J.; Lim, L.; Jiang, X.; Xu, L.; Bigg, C. M.; Kelly, M. T.; Andersen, R. J. Cell-based screen for antimitotic agents and

identification of analogues of rhizoxin, eleutherobin, and paclitaxel in natural extracts. *Cancer Res.* **2000**, *60*, 5052-8.

[xvi]. Gunatilaka, A. A. L.;. Kingston, D. G. I. Johnson, R. K. Mechanism-based isolation and structures of some anticancer natural products, *Pure Appl. Chem.*, **1994**, *66*, 2219-2222.

[xvii]. Gronquist, M.; Meinwald, J.; Eisner, T.; Schroeder, F. C. Exploring Uncharted Terrain in Nature's Structure Space Using Capillary NMR Spectroscopy: 13 Steroids from 50 Fireflies. *J. Am. Chem. Soc.* **2005**, *127*, 10810 – 10811.

[xviii]. Kingston, D. G. I.; van der Werff, H.; Evans, R.; Mittermeier, R.; Famolare, L.; Guerin-McManus, M.; Malone, S.; Wisse, J. H.; Vyas D.; Wright, J. J. K. Biodiversity Conservation, Economic Development, and Drug Discovery in Suriname. In *Biologically Active Natural Products: Pharmaceuticals*, Cutler, H. G.; Cutler, S. J.; Eds. CRC Press: Boca Raton, 2000, pp 39-59.

[xix]. Recent publications include the following: a) Williams, R. B.; Hoch, J.; Glass, T. E.; Evans, R.; Miller, J. S.; Wisse, J. H.; Kingston, D. G. I. A novel cytotoxic guttiferone analog from *Garcinia macrophylla* from the Suriname rainforest. *Planta Med.* **2003**, *69*, 864-866. b) Cao, S.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.;. Kingston, D. G. I. Cytotoxic compounds from *Mundulea chapelieri* from the Madagascar rainforest *J. Nat. Prod.* 2004, *67*, 454-456. c) Cao, S.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. New cytotoxic triterpenoids from *Acridocarpus adenophorus. J. Nat. Prod.* **2004**, *67*, 986-989. d) Chaturvedula, V. S. P.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.; Kingston D. G. I. New cytotoxic terpenoids from the wood of *Vepris punctata* from the Madagascar rainforest. *J. Nat. Prod.* **2004**, *67*, 895-898. e) Cao, S.; Schilling, J. K.; Miller, J. S.; Randrianasolo, A.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. New cytotoxic alkyl phloroglucinols from *Protorhus thouvenotii*. *Planta Med.* **2004**, *70*, 683-684. f) Chaturvedula, V. S. P.; Farooq, A.; Schilling, J. K.; Malone, S.; Derveld, I.; Werkhoven, M. C. M.; Wisse, J. H.;

Ratsimbason, M.; Kingston, D. G. I. New Eudesmane Derivatives from *Melampodium camphoratum* from the Suriname Rainforest**.** *J. Nat. Prod.* **2004**, *67*, 2053-2057. g) Murphy, B. T.; Cao, S. G.; Norris A.; Kingston, D. G. I. Cytotoxic flavanones from *Schizolaena hystrix* from the Madagascar rainforest. *J. Nat. Prod.* **2005**, *68*, 417-419.

[xx]. Cao, S. G.; Guza, R.C.; Norris, A.; Evans, R.; Miller, J. S.; Wisse, J. H.; Kingston, D. G. I. Ipomoeassins A-E, five new cytotoxic macrocyclic glycoresins, from the leaves of *Ipomoea squamosa*. *J. Nat. Prod.* **2005**, *68*, 487-492.

[xxi]. Beutler, J. A.; Shoemaker, R. H.; Johnson, T.; Boyd, M. R. Cytotoxic geranyl stilbenes from *Macaranga schweinfurthii.* *J. Nat. Prod.* **1998**, *61*, 1509-1512

Nature: a vital source of leads for anticancer drug development

G. M. Cragg and D. J. Newman

Abstract Over 60% of the current anticancer drugs have their origin in one way or another from natural sources. Nature continues to be the most prolific source of biologically active and diverse chemotypes, and it is becoming increasingly evident that associated microbes may often be the source of biologically active compounds originally isolated from host macro-organisms. While relatively few of the actual isolated compounds advance to become clinically effective drugs in their own right, these unique molecules may serve as models for the preparation of more efficacious analogs using chemical methodology such as total or combinatorial (parallel) synthesis, or manipulation of biosynthetic pathways. In addition, conjugation of toxic natural molecules to monoclonal antibodies or polymeric carriers specifically targeted to epitopes on tumors of interest can lead to the development of efficacious targeted therapies. The essential role played by natural products in the discovery and development of effective anticancer agents, and the importance of multidisciplinary collaboration in the generation and optimization of novel molecular leads from natural product sources is reviewed.

Keywords Plants - Marine organisms - Microbes - Symbionts - Multidisciplinary collaboration

Introduction

The valuable contributions of nature as a source of potential chemotherapeutic agents has recently been reviewed (Newman and Cragg *2007*). Analysis of the sources of new drugs over the period 01/1981-06/2006 indicated that, while 66% of the 974 small molecule, new chemical entities (NCEs) are formally synthetic, 17% correspond to synthetic molecules containing pharmacophores derived directly from natural products, and 12% are actually modeled on a natural product inhibitor of the molecular target of interest, or mimic (i.e., competitively inhibit) the endogenous substrate of the active site, such as ATP. Thus, only 37% of the 974 NCEs can be classified as truly synthetic (i.e., devoid of natural inspiration) in origin. When considering disease categories, close to 70% of anti-infectives (anti-bacterial, -fungal, -parasitic and -viral) are naturally derived or inspired, while in the cancer treatment area 63% fall into this category.

The United States National Cancer Institute (NCI; http://www.nci.nih.gov) has played a major role in the discovery and/or development of many of the available commercial and investigational anticancer agents. NCI was established in 1937, its mission being “to provide for, foster and aid in coordinating research related to cancer.” In 1955, NCI set up the Cancer Chemotherapy National Service Centre (CCNSC) to coordinate a national voluntary cooperative cancer chemotherapy program, involving the procurement of drugs, screening, pre-clinical studies, and clinical evaluation of new agents. The responsibility for drug discovery and pre-clinical development at NCI now rests with the Developmental Therapeutics Program (DTP; http://dtp.nci.nih.gov), a major component of the Division of Cancer Treatment and Diagnosis (DCTD). Thus, for over 50 years, NCI has provided a resource for the pre-clinical screening of compounds and materials submitted by scientists and institutions, public and private, worldwide, and during this period, more than 500,000 chemicals, both synthetic and natural, have been screened for antitumor activity.

Initially, most of the materials screened were pure compounds of synthetic origin, but the program also recognized that natural products were an excellent source of complex chemical structures possessing a wide variety of biological activities. The original plant collections from 1960 to 1982 were performed by the U. S. Department of Agriculture (USDA) through an interagency agreement with NCI, and involved the random collection of over 35,000 plant samples, mainly from temperate regions. These collections led to the discovery of paclitaxel (Taxol®) and camptothecin which formed the basis for the semisynthesis of several clinically effective drugs. Marine invertebrates were generally collected by academic investigators, mainly funded through grants from the NCI, while microbial samples were obtained from pharmaceutical companies and research institutes in several countries, such as the Institute of Microbial Chemistry in Japan, some of which were funded through contracts with the NCI. From 1960 to 1982, over 180,000 microbial-derived, some 16,000 marine organism-derived, and over 114,000 plant-derived extracts were screened for antitumor activity, mainly by the NCI, and, as mentioned above, a number of clinically effective chemotherapeutic agents have been developed (Newman and Cragg *2005*). In addition, several compounds isolated from plants collected during this period are still in advanced preclinical or clinical development (Fig. 1).

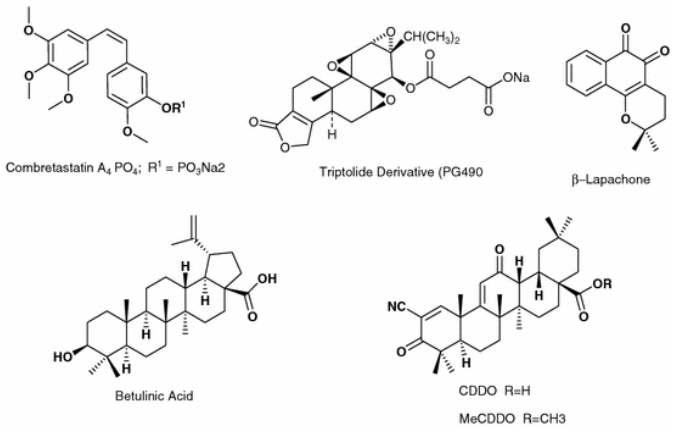


Fig. 1 Early NCI plant-derived agents still in development

Some plant-derived anticancer agents

The structures of some plant-derived anticancer drugs currently in clinical use are shown in Fig. 2. The best known are the so-called vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Gueritte and Fahy *2005*). Of significance is the discovery that these agents can be isolated from endophytic fungi found to be associated

with the source plant (Yang et al. *2004*). More recent semi-synthetic analogs of these agents are vinorelbine and vindesine. These agents act through the inhibition of tubulin polymerization and are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers and Kaposi's sarcoma.

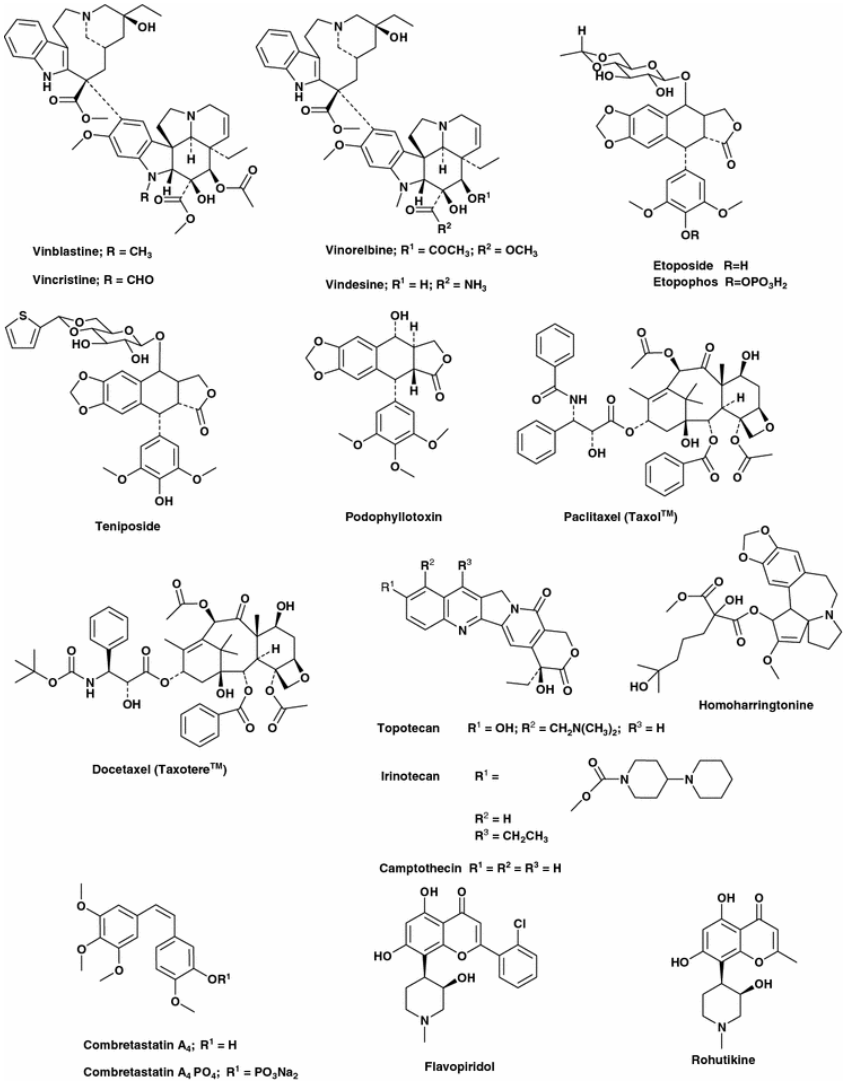


Fig. 2 Some plant-derived anticancer agents

The clinically-active agents, etoposide, etopophos and teniposide are semisynthetic derivatives of the natural product epipodophyllotoxin (Lee and Xiao *2005*), an isomer of podophyllotoxin which was isolated as the active antitumor agent from the roots of various species of the genus *Podophyllum.* Podophyllotoxin has now also been found to be produced by an endophytic fungus isolated from *P. peltatum* (Eyberger et al. *2006*). Extensive research led to the development of

etoposide and teniposide as clinically effective agents which are used in the treatment of lymphomas and bronchial and testicular cancers and which act through inhibition of topoisomerase II, an important enzyme involved in the replication pathway of DNA during cell cycle progression.

More recent additions to the armamentarium of naturally-derived chemotherapeutic agents are the taxanes and camptothecins. Paclitaxel (Taxol®) initially was isolated from the bark of the Pacific yew, *Taxus brevifolia* Nutt. (Kingston *2005*). Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species, and the ready semi-synthetic conversion of the relatively abundant baccatins to paclitaxel, as well as active paclitaxel analogues, such as docetaxel (Taxotere®) (Cortes and Pazdur *1995*) has provided a major, renewable natural source of this important class of drugs. Taxol® has also been isolated from many endophytic fungi (Strobel et al. *2004*), but as yet, these fungi have not been developed as viable sustainable sources. Paclitaxel is used in the treatment of breast, ovarian and non-small cell lung cancer and has also shown efficacy against Kaposi's sarcoma, while docetaxel is primarily used in the treatment of breast cancer and non-small cell lung cancer. Paclitaxel has also attracted attention in the potential treatment of multiple sclerosis, psoriasis and rheumatoid arthritis and as a constituent of stents. In addition, 23 taxanes are in preclinical development as potential anticancer agents (Kingston *2005*).

The clinically-active agents, topotecan (Hycamptin®) and irinotecan (Camptosar®; CPT-11), are semi-synthetically derived from camptothecin, isolated from the Chinese ornamental tree *Camptotheca acuminata* Decne. (Rahier et al. *2005*) Camptothecin has also been reported to be produced by an endophytic fungus of the family Phycomycetes, subsequently identified as *Entrophospora infrequens* (Amna et al. *2006*), isolated from the inner bark of *Nothapodytes foetida* (Puri et al. *2005*). Despite being dropped from clinical trials by NCI in the 1970s due to severe bladder toxicity, extensive research on the structural modification of camptothecin led to the development of the more effective derivatives, topotecan (Hycamptin®) and irinotecan (CPT-11; Camptosar®). Topotecan is used for the treatment of ovarian and small cell lung cancers, while irinotecan is used for the treatment of colorectal cancers. This class of agents acts through inhibition of topoisomerase I, another important enzyme involved in the replication pathway of

DNA during cell cycle progression and, to date, remains by far the most important class of topoisomerase I inhibitors (Cragg and Newman *2004*).

Other plant-derived agents in clinical use or development are homoharringtonine, flavopiridol, and the combretastatins. Homoharringtonine was isolated from the Chinese tree *Cephalotaxus harringtonia* var. *drupacea* (Sieb and Zucc.) (Itokawa et al. *2005*), and a racemic mixture of harringtonine and homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia. Purified homoharringtonine has shown efficacy against various leukemias, including some resistant to standard treatment and has been reported to produce complete hematologic remission in patients with late chronic phase myelogenous leukemia. Flavopiridol is totally synthetic, but the basis for its novel flavonoid structure is a natural product, rohitukine, isolated as the constituent responsible for anti-inflammatory and immunomodulatory activity from *Dysoxylum binectariferum* Hook. f. (Meliaceae) which is phylogenetically related to the Ayurvedic plant *D. malabaricum* Bedd. used for rheumatoid arthritis (Sausville et al. *1999*). It is currently in 18 Phase I and Phase II clinical trials, either alone or in combination with other anticancer agents, against a broad range of tumors, including leukemias, lymphomas and solid tumors (http://www.cancer.gov/Search/SearchClinicalTrialsAdvanced.aspx). Added interest has been stimulated by observation of significant activity against chronic lymphocytic leukemia, a cancer currently lacking efficacious treatment (Byrd et al. *2005*). The combretastatins (e.g., combretastatin A_4) were isolated from the South African "bush willow" *Combretum caffrum* (Eckl. & Zeyh.) Kuntze, collected in Southern Africa in the 1970s as part of a random collection program for the NCI by the USDA, working in collaboration with the Botanical Research Institute of South Africa (Pinney et al. *2005*). A water-soluble analog, combretastatin A_4 phosphate, has shown promise against thyroid cancer in early clinical trials.

Some marine-derived anticancer agents

The marine environment has proven to be a very rich source of potent compounds demonstrating significant antitumor activity. Structures of some marine-derived agents currently in clinical trials are shown in Fig. 3.

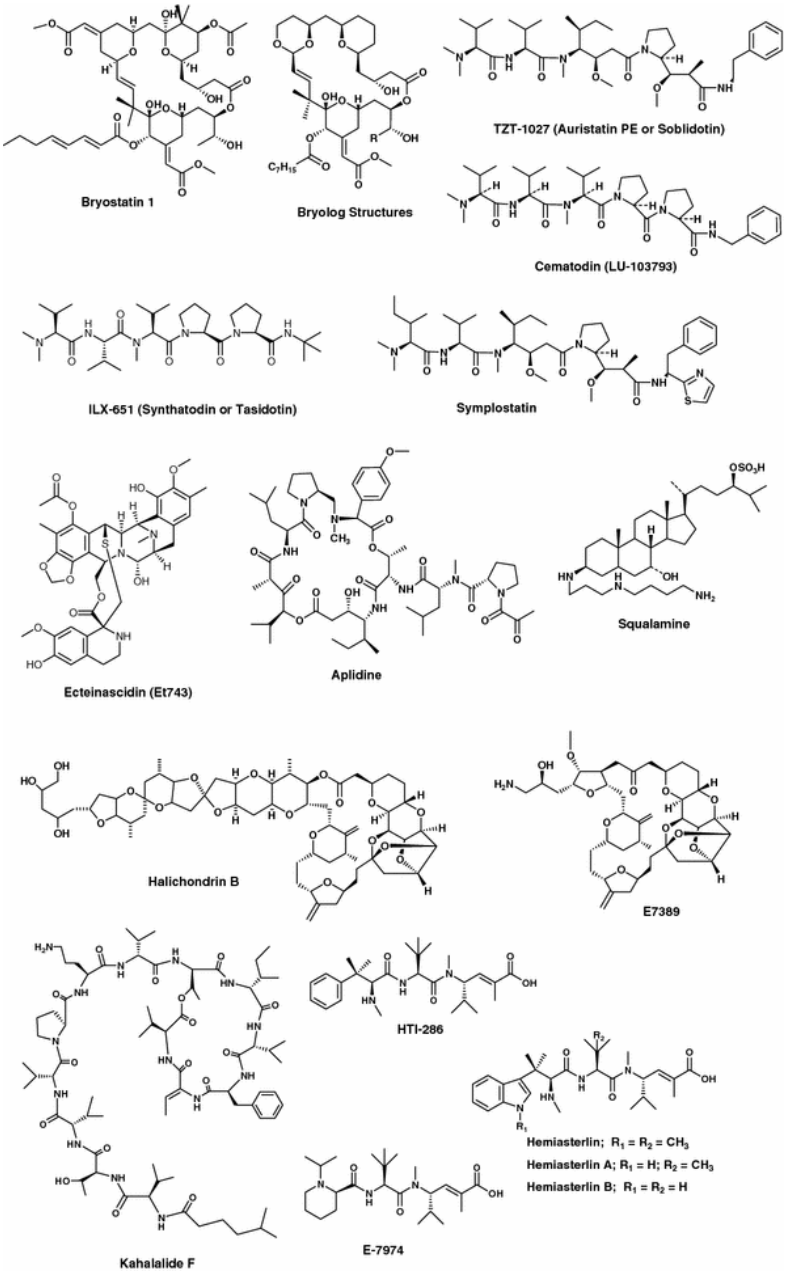


Fig. 3 Some marine-derived anticancer agents

The bryostatins are a series of macrocyclic lactones originally isolated in minute yields from the bryozoan *Bugula neritina* (Hale et al. *2002*; Newman *2005*). Bryostatin 1 of GMP quality has been isolated in sufficient quantities to permit more that 80 clinical trials to date, with 20 being completed at both Phase I and Phase II levels. Despite the observation of some responses to the

compound as a single agent, its use as a single agent is probably not the optimal application for this compound. When administered in combination with other cytotoxins, such as the *Vinca* alkaloids or nucleosides, in the treatment of carcinomas which are leukemic in nature, the response rates, even in Phase I trials, have demonstrated greater efficacy worthy of further investigation (Newman *2005*). The bryostatins have been the target of many synthetic chemistry groups (Mutter and Wills *2000*; Norcross and Patterson *1995*), and extensive studies have also been performed on the synthesis of simpler analogs possessing comparable or better activity, particularly related to binding to some of the molecular targets, protein kinase C isozymes. These have resulted in the preparation of compounds, bryologs (Fig. 3), with greater potency than bryostatin 1 in in vitro cell line assays (Wender et al. *1998a*, *1998b*, *1999*, *2002*, *2003a*, *2003b*; Wender and Lippa *2000*). Use of molecular probes has demonstrated the presence of a putative type I polyketide synthase gene fragment in the microbial flora (*Candidatus Endobugula sertula*) of colonies of the host bryozoan producing bryostatin, but shown to be absent in the corresponding flora of non-producers (Sudek et al. *2007*; Piel *2006*). If successful, the cultivation of the organism, or a surrogate with the bryostatin polyketide synthase system expressed, would potentially solve supply problems that may arise if bryostatin 1 becomes a commercial drug (Newman and Cragg *2004*).

While clinical trials of dolastatin 10 have been terminated, several other dolastatin analogs are currently in clinical development (Flahive and Srirangam *2005*) The synthetic derivative TZT-1027 (auristatin PE or soblidotin) is in Phase II clinical trials and has been shown to exhibit potent anti-vascular effects in addition to anti-tubulin activity, suggesting that a dual mechanism might well be possible with this agent (Shimoyama et al. *2006*). The analog cematodin (LU-103793) has progressed into Phase II clinical trials against malignant melanoma, metastatic breast cancer and non-small-cell lung cancer, and while no objective responses have been reported, there are reports of stable disease being seen in both the melanoma and breast cancer trials and a subjective increase in a quality of life measure in the lung trial (Flahive and Srirangam *2005*). Phase II studies in melanoma, breast and non-small-cell lung cancers have been initiated with ILX651 (synthadotin or tasidotin), which is an orally active third generation dolastatin 15

analogue. There have been two published reports on Phase I studies with this agent, and a profile of the compound showing that it is in phase II trials (Cunningham et al. *2005*; Ebbinghaus et al. *2005*; Rasila and Verschraegen *2005*). It is interesting that the dolastatins have also been shown to be of microbial origin, with the isolation of a dolastatin analogue, symplostatin 1, from the marine cyanobacterium *Simploca hynoides* (Flahive and Srirangam *2005*), and further reports of the isolation of dolastatin-like peptides from different collections of the ubiquitous cyanophyte *Lyngbya majuscula* (Flahive and Srirangam *2005*).

Ecteinascidin 743 (ET743; Yondelis™) was originally isolated in very low yields from the ascidian *Ecteinascidia turbinata* (Henríquez et al. *2005*), and the conduct of basic in vitro and in vivo testing and mechanism of action studies required the collection of large amounts of the ascidian from Caribbean locations. The acquisition of sufficient quantities of ET743 for advanced preclinical and clinical studies was initially achieved by very large-scale aquaculture of *E. turbinata* in open ponds followed by isolation, but later supplies were obtained by means of a 21 step semisynthetic conversion of cyanosafracin B, a metabolite isolated through the large-scale fermentation of the marine microbe *Pseudomonas fluorescens*. Ecteinascidin 743 was approved in the EU for sarcoma in late 2007, and also is currently in a number of Phase II/III clinical trials for ovarian, soft tissue sarcoma, breast, endometrial, prostate, non-small cell lung and pediatric cancers and has been granted orphan drug status by the European Commission for soft tissue sarcoma and ovarian cancer. Ecteinascidin 743 is the first of a novel class of DNA-binding agents, and details of its complex, transcription-targeted mechanism of action are discussed by Henríquez et al. (*2005*).

Aplidine (dehydrodidemnin B) was isolated from the Mediterranean tunicate *Aplidium albicans* and is currently in Phase II clinical trials for a range of cancers, including melanoma, pancreatic, head and neck, small cell and non-small cell lung, bladder and prostate cancers, as well as non-Hodgkin lymphoma and acute lymphoblastic leukemia (Henríquez et al. *2005*). Orphan drug status has been granted by the European Commission for the treatment of acute lymphoblastic leukemia which is a leading cause of death for persons under 35 years of age. The precise

mechanism of action of this agent is not yet known, but details of studies to date are discussed by Henríquez et al. (*2005*).

Halichondrin B has been isolated from several sponges, including *Halichondria okadai* from Japan, an *Axinella* sp. from the Western Pacific, *Phakellia carteri* from the Eastern Indian Ocean, and from a deep water *Lissodendoryx* sp. off the East Coast of South Island, New Zealand (Yu et al. *2005*). Sufficient quantities for early preclinical studies were isolated from large-scale collections of the *Lissodendoryx* sp., and similar yields could also be obtained from this sponge grown by aquaculture in shallow waters off the coast of New Zealand. Halichondrin B and norhalichondrin B were successfully synthesized and the synthetic strategy was adapted to the synthesis of a large number of structurally simpler analogs, some of which maintained the biological activity but were intrinsically more chemically stable, due to the substitution of a ketone for the ester linkage in the macrolide ring. One of these, E7389, was selected for further development and is now in Phase III clinical trials, particularly against refractory breast carcinoma (Yu et al. *2005*; http://www.cancer.gov/Search/SearchClinicalTrialsAdvanced.aspx).

The cyclic depsipeptide kahalalide F was isolated from the Sacoglossan mollusk *Elysia rufescens* following grazing by the mollusk on a green macroalga, *Bryopsis pennata* (Henríquez et al. *2005*). It was discovered that the depsipeptide also occurs in the alga, though in much lower concentration and thus it appears that the mollusk concentrates the depsipeptide significantly. An efficient synthesis was developed for the compound (Henríquez et al. *2005*) and it entered Phase I clinical trials in Europe in December 2000 for the treatment of androgen-independent prostate cancer, and it is currently in Phase II clinical trials. Activity has also been reported in the treatment of androgen-resistant prostate cancer patients (Rademaker-Lakhai et al. *2005*) and against other advanced solid tumors (Salazar et al. *2005*). Studies have also commenced in the treatment of liver carcinoma. The primary mechanism of action for kahalalide F has not been fully established but progress in this regard is discussed by Henríquez et al. (*2005*).

HTI-286 is a synthetic analog of hemiasterlin and hemiasterlins A and B which were originally isolated from the South African sponge, *Hemiasterella minor*, and shortly thereafter from a Papua

New Guinea sponge *Cymbastela* sp. (Andersen and Roberge *2005*). These compounds interact with tubulin to produce microtubule depolymerization in a manner similar to the Vinca alkaloids, the cryptophycins and the dolastatins (Bai et al. *1990*). HTI-286 was found to be the most effective of a large number of synthetic analogs, and entered Phase I clinical trials; it was scheduled to go into Phase II to investigate its potential in the treatment of non-small cell lung cancer but was then suspended. However, another closely related compound, E7974, synthesized by chemists at Eisai Research Institute, is currently in Phase I clinical trials. It is quoted as not being a good substrate for the MDR complex in tumor cells (Agoulnik et al. *2005*), and hopefully it will progress further along the development pathway than the earlier variations on these structures.

A fairly simple aminosterol, squalamine, isolated from the common spiny dogfish shark, *Squalus acanthias*, collected off the New England coast (Moore et al. *1993*), was shown to possess broad spectrum antibiotic activity, but was also found to exhibit significant anti-angiogenic activity (Sills et al. *1998*). Despite lack of promising activity as a single agent, it has now progressed into Phase II clinical trials in combination with agents such as carboplatin or paclitaxel for the treatment of patients with advanced non-small cell lung cancer; partial responses were observed in 12 (28%) of patients, with 8 (19%) more having stable disease (Herbst et al. *2003*), and later reports of activity in randomized Phase II trials have been presented in abstract form (Rose et al. *2004*). However, significant activity is now being seen in the treatment of wet macular degeneration in Phase III trials using squalamine as a single agent (Melnikova *2005*). Its anti-angiogenic activity stops the unrestrained capillary growth that is the underlying cause of this disease.

Some anticancer agents derived from microbial sources

Antitumor antibiotics (Fig. 4) are amongst the most important of the cancer chemo-therapeutic agents, which include members of the anthracycline and bleomycin classes. Except for the semi-synthetic compounds, all were isolated from various *Streptomyces* species.

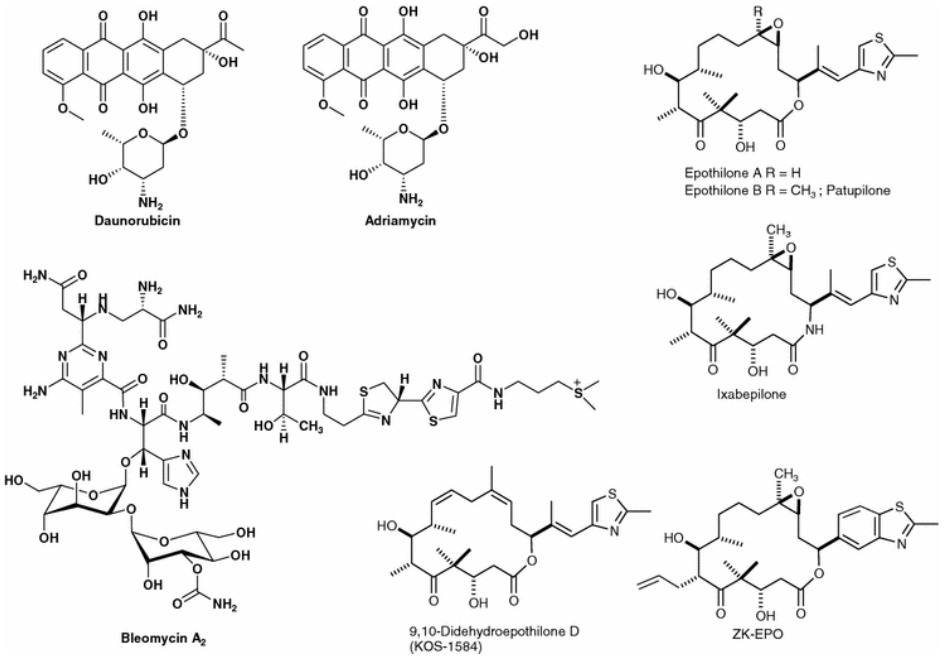


Fig. 4 Some microbial-derived anticancer agents

One of the most important classes of microbial-derived agents is the anthracyclines, with daunorubicin and its derivative doxorubicin (adriamycin) being the best known of these agents currently in clinical use; they are still major components of the treatment regimen for breast cancer (Arcamone *2005*). The mechanism of action of these molecules, aside from their formal identification as intercalators into the DNA helix, is now known to be inhibition of topoisomerase II, one of the important enzymes in the replication pathway of DNA during cell cycling. Derivatives of doxorubicin, such as epirubicin, idarubicin, pirirubicin and valrubicin, have also been approved for clinical use, and the expansion of the efficacy of doxorubicin is being explored through use targeted delivery techniques (Arcamone *2005*).

Another important class is the family of glycopeptolide antibiotics known as bleomycins (e.g., bleomycin A_2, Blenoxane®) (Hecht *2005*). Bleomycin was originally thought to act through DNA cleavage, but recent studies suggest that the major mechanism of action may be inhibition of t-RNA.

Structures based on the epothilones, isolated from the extremely prolific *Myxomycetales* (Höfle and Reichenbach *2005*), are of great interest as potential antitumor agents due to their mechanism of action being the same as that of paclitaxel (vide infra). Though, at first glimpse, appearing to

have quite a different topology, molecular modeling has shown that there are significant common structural features in the two basic molecules. A major impetus behind their development was the realization that the epothilones were active against paclitaxel-resistant cell lines. The aza-analog of epothilone B, ixabepilone, synthesized by Bristol-Myers was approved in late 2007, and epothilone B (patupilone), epothilone D (KOS-862) and ZKEPO are in phase I and/or phase II clinical trials, though the recent comments by de Jonge and Verweji on the epothilones as a class should be borne in mind when assessing their future potential (de Jonge and Verweiji *2005*). These authors noted that: "Activity was not seen in taxane-insensitive tumor types, such as colorectal cancer, melanoma, renal cancer, and others. This activity profile, balanced against their difficult adverse effect profile, creates concern about the potential of further development of the epothilones." However, significant research on both the total synthesis of this class of compounds by chemical means involving modifications around the basic epothilone skeleton (Altmann *2005*; Cachoux et al. *2005*; Van de Weghe and Eustache *2005*; Alhamadsheh et al. *2006*), and biosynthetic modifications using the synthetic gene cluster (Tang et al. *2005*; Wilkinson and Moss *2005*), has been directed at overcoming some of the pharmacological and toxicological problems reported during clinical trials.

Microorganisms: unexplored potential

Until recently, the inability to cultivate most naturally occurring microorganisms has severely limited the study of natural microbial ecosystems, and it has been estimated that much less than 1% of microorganisms seen microscopically have been cultivated. Yet, despite this limitation, the number of highly effective microbially-derived chemo-therapeutic agents discovered and developed thus far has been highly impressive; thus, it is clear that the microbial universe presents a vast untapped resource for drug discovery. In addition, substantial advances in the understanding of the gene clusters encoding multimodular enzymes involved in the biosynthesis of a multitude of microbial secondary metabolites, such as polyketide synthases (PKSs) and/or nonribosomal peptide synthetases (NRPSs), has enabled the sequencing and detailed analysis of the genomes of long-studied microbes such as *Streptomyces avermitilis*. These studies have revealed the presence of additional PKS and NRPS clusters resulting in the discovery of novel secondary metabolites not

detected in standard fermentation and isolation processes (McAlpine et al. *2005*). A recent review discusses the general aspects of genomics in natural product research (Bode and Muller *2005*).

Genomic mining and the metagenome

Despite improvement in culturing techniques, greater than 99% of microscopically observed microbes still defy culture. Extraction of nucleic acids (the metagenome) from environmental samples, however, permits the identification of uncultured microorganisms through the isolation and sequencing of ribosomal RNA or rDNA (genes encoding for rRNA). Samples from soils and seawater are currently being investigated (Rondon et al. *2000*; Venter et al. *2004*), and whole-genome shotgun sequencing of environmental-pooled DNA obtained from water samples collected in the Sargasso Sea off the coast of Bermuda by the Venter group, indicated the presence of at least 1,800 genomic species which included 148 previously unknown bacterial phylotypes (Venter et al. *2004*). Venter et al. are also examining microbial communities in water samples collected by the *Sorcerer II* Global Ocean Sampling (GOS) expedition, and their data predict more than 6 million proteins, nearly twice the number of proteins present in current databases, with some of the predicted proteins bearing no similarity to any currently known proteins, and therefore representing new families (Yooseph et al. *2007*). These methods may be applied to other habitats, such as the microflora of insects (Warnecke et al. *2007*) and marine animals (Fieseler et al. *2007*). The cloning and understanding of the novel genes discovered through these processes, and the heterologous expression of gene clusters encoding the enzymes involved in biosynthetic pathways in viable host organisms, such as *Escherichia coli*, should permit the production of novel metabolites produced from as yet uncultured microbes.

Starting with the pioneering work of Hopwood, it is now evident that the genome of the Streptomycetes and by extension, Actinomycetes in general, contain large numbers of previously unrecognized secondary metabolite clusters. For instance, investigation of the genome of the well known vancomycin producer, *Amycolatopsis orientalis* (ATCC 43491), has resulted in the isolation of the novel antibiotic ECO-0501 (Fig. 5) which was only found by using the genomic sequence to predict the molecular weight, followed by direct detection of the molecule by

HPLC-MS. The compound had a very similar biological profile to vancomycin but was masked by this compound (Banskota et al. *2006*). Many more examples of the value of this type of investigation have been reported in two recent reviews (Lam *2007*; Clardy et al. *2006*) which provide up to date information on the manifold structures that can be found by expression of environmental DNA.

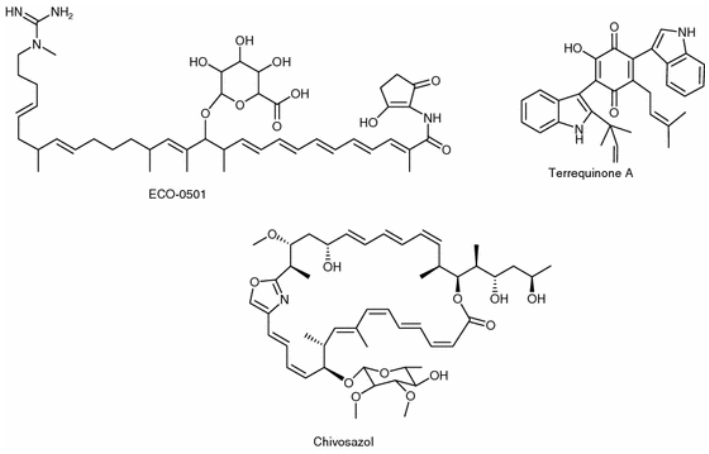


Fig. 5 New compounds from genome mining

The presence of potential gene products controlling metabolite production has been predicted in a recently reported genomic analysis of the fungus *Aspergillus nidulans*. In addition to proposing the presence of clustered secondary metabolite genes having the potential to generate up to 27 polyketides, 14 nonribosomal peptides, one terpene, and two indole alkaloids, Bok and coworkers identified the potential controller of the expression of these clusters (Bok et al. *2006*). This was demonstrated by expressing terrequinone A (Fig. 5), a compound not previously reported from this species. Analysis of the potential number of secondary metabolite clusters in *A. fumigatus* and *A. oryzae* allow similar predictions for these fungi. The control of secondary metabolites in fungi is further discussed in a recent review (Hoffmeister and Keller *2007*).

Even the myxobacteria have now yielded to genomic analyses, and the identification and utilization of *ChiR*, the gene controlling production of chivosazol (Fig. 5), an extremely potent eukaryotic antibiotic, has been reported (Rachid et al. *2007*). This paper also discusses the identification and application of the transcriptional control mechanisms, a major problem in secondary metabolite expression, whether in homologous or heterologous hosts.

Extremophiles

Extremophilic microbes (extremophiles) abound in extreme habitats. These include acidophiles (acidic sulfurous hot springs), alkalophiles (alkaline lakes), halophiles (salt lakes), piezo (baro)- and (hyper) thermophiles (deep-sea vents) (Abe and Horikoshi *2001*; Persidis *1998*; Rossi et al. *2003*; Short *2007*) and psychrophiles (Arctic and Antarctic waters, Alpine lakes) (Cavicchioli et al. *2002*). Thus far, investigations have centered on the isolation of thermophilic and hyperthermophilic enzymes (extremozymes) (Gomes and Steiner *2004*; Hoyoux et al. *2004*; Schiraldi and De Rosa *2002*; van den Burg *2003*; Wiegel and Kevbrin *2004*), but there is little doubt that these extreme environments will also yield novel bioactive chemotypes. Abandoned mine-waste disposal sites have yielded unusual acidophiles which thrive in the acidic, metal-rich waters, polluted environments which are generally toxic to most prokaryotic and eukaryotic organisms (Johnson and Hallberg *2003*). Novel sesquiterpenoid and polyketide-terpenoid metabolites, berkeleydione and berkeleytrione, and berkeleyamides A–D (Fig. 6), showing inhibition against metalloproteinase-3 and caspase-1, activities relevant to cancer, have been isolated from *Penicillium rubrum* Stoll found in the polluted waters of Berkeley Pit Lake in Montana (Stierle et al. *2008*).

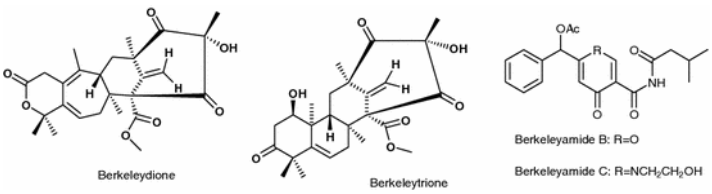


Fig. 6 New compounds from extreme environments

Endophytes

As indicated above, plants have been relatively extensively studied as sources of bioactive metabolites, but the endophytic microbes which reside in the tissues between living plant cells have received little attention. Relationships between endophytes and their host plants may vary from symbiotic to pathogenic, and studies have revealed an interesting realm of novel chemistry (Gunatilaka *2006*; Strobel et al. *2004*; Tan and Zou *2001*). Of particular significance has been the production of various important anticancer agents in small quantities from endophytic fungi isolated from plants, as was mentioned in the section on plant-derived anticancer agents above. It

has been demonstrated that these compounds are not artifacts, and so the identification of the gene/gene product controlling the metabolite production by these microbes could provide an entry into greatly increased production of key bioactive natural products. In addition, a wide range of new bioactive molecules have been discovered, including peptide antibiotics, the coronamycins, isolated from a *Streptomyces* species associated with an epiphytic vine (*Monastera* species) found in the Peruvian Amazon (Ezra et al. *2004*), and the cytotoxic aspochalasins I, J, and K (Fig. 7), isolated from endophytes of plants collected from the southwestern desert regions of the United States (Zhou et al. *2004*).

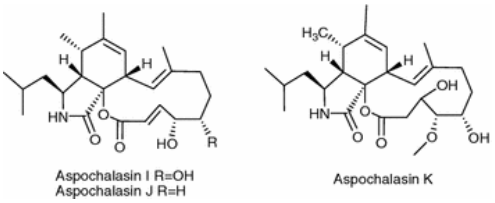


Fig. 7 Some compounds from endophytes

Marine microbes

Deep ocean sediments are proving to be a valuable source of new actinomycete bacteria that are unique to the marine environment **(**Udwary et al. *2007*). Combination of culture and phylogenetic approaches has led to the description of the first truly marine actinomycete genus named *Salinispora* (Gontang et al. *2007*); its members are proving to be ubiquitous, being found in concentrations of up to 10^4/ml in sediments on tropical ocean bottoms and in more shallow waters, as well as appearing on the surfaces of numerous marine plants and animals. Culturing, using the appropriate selective isolation techniques, has led to the observation of significant antibiotic and cytotoxic activity, and has resulted in the isolation of a potent cytotoxin, salinosporamide A (Fig. 8), a very potent proteasome inhibitor (IC_{50} = 1.3 nM) (Feling et al. *2003*), currently in Phase I clinical trials. More recently, the isolation and cultivation of another new actinomycete genus, named *Marinispora*, has been reported, and novel macrolides called marinomycins have been isolated. Marinomycins A–D (Fig. 8) show potent activity against drug-resistant bacterial pathogens and some melanomas (Kwon et al. *2006*). Recent publications by the Fenical group on the novel and diverse chemistry of these new microbial genera include the isolation of potential chemopreventive agents, saliniketals A and B from *Salinispora arenicola* (Williams et al. *2007*),

and two new cyclic peptides, thalassospiramides A and B, possessing immunosuppressive activity from a new member of the marine alpha-proteobacterium *Thalassospira* (Oh et al. *2007*).

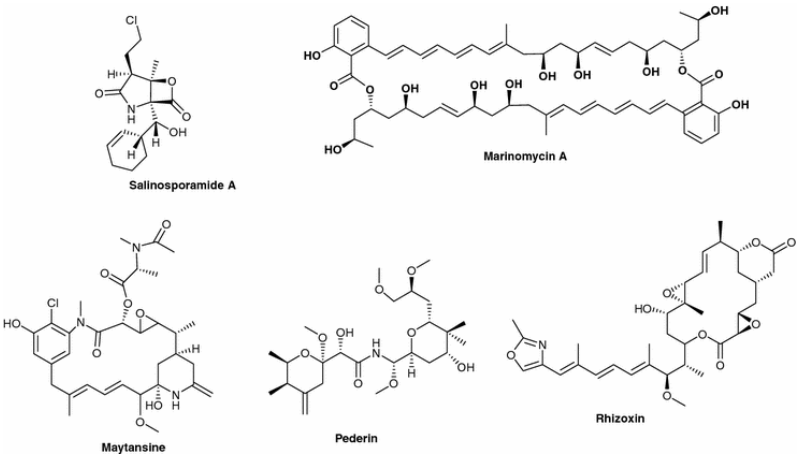


Fig. 8 Some metabolites from marine and symbiotic microbes

Microbial symbionts

There is mounting evidence indicating that many bioactive compounds isolated from various macro-organisms are synthesized by symbiotic bacteria (Piel *2004*). These include the anticancer compounds, the maytansanoids (Fig. 8), originally isolated from several plant genera of the Celastraceae family (Yu and Floss *2005*), and the pederins (Fig. 8), isolated from beetles of genera *Paederus* and *Paederidus*, as well as from several marine sponges (Piel et al. *2004a*, *2004b*, *2005*). In addition, a range of antitumor agents isolated from marine organisms closely resemble bacterial metabolites (Newman and Cragg *2004*).

An interesting example of a complex symbiotic–pathogenic relationship involving a bacterium–fungus-plant interaction has been discovered in the case of rice seedling blight. The toxic metabolite, rhizoxin (Fig. 8), originally isolated from the contaminating *Rhizopus* fungus, has actually been found to be produced by an endo-symbiotic *Burkholderia* bacterial species (Partida-Martinez and Hertweck *2005*). Rhizoxin exhibits potent antitumor activity, but its further development as an anticancer drug has been precluded by toxicity problems. Thus, in addition to offering potentially new avenues for pest control, this unexpected finding has enabled the isolation of rhizoxin as well as rhizoxin analogs through the cultivation of the bacterium independently of

the fungal host. This may have significant implications in development of agents of this class with improved pharmacological properties.

Multidisciplinary collaboration: an essential factor

The probability that a directly isolated natural product (e.g. adriamycin or taxanes in the antitumor area) will be the drug used for the treatment of a given disease in the future is relatively low. In many instances, however, these natural molecules can serve as lead compounds which can be optimized through the application of methodologies such as combinatorial biosynthesis and/or combinatorial chemistry to give products suitable for drug development. In addition, novel methods of total chemical syntheses of the natural molecules can yield intermediates possessing equal or superior preclinical activity to that observed for the natural product; these leads can be optimized for drug development using medicinal or combinatorial chemistry approaches. Of course, all these approaches require suitable biological assays to follow the optimization process, and thus a truly multidisciplinary, collaborative approach is required for effective drug development That these ideas are not just pipe dreams can be seen in the following examples.

Combinatorial biosynthesis

The substantial advances made in the understanding of the role of multifunctional polyketide synthase enzymes (PKSs) in bacterial aromatic polyketide biosynthesis have led to the identification of many of them, together with their encoding genes (Khosla *2000*; Staunton and Weissman *2001*; Walsh *2004*, *2007*). The same applies to nonribosomal peptide synthases (NRPS) responsible for the biosynthesis of nonribosomal peptides (NRPs) (Walsh *2004*; Everts *2008*). The rapid developments in the analysis of microbial genomes has enabled the identification of a multitude of gene clusters encoding for polyketides, NRPs and hybrid polyketide-NRP metabolites, and have provided the tools for engineering the biosynthesis of novel “non-natural” natural products through gene shuffling, domain deletions and mutations (Walsh *2004*; Clardy and Walsh *2004*). Results of the application of these combinatorial biosynthetic techniques to the production of novel analogs of anticancer agents, such as the anthracyclines, ansamitocins, epothilones,

enediynes, and aminocoumarins, have been reviewed by Shen and coworkers (Thomas et al. *2005*).

Total synthesis

The total synthesis of complex natural products has long posed challenges to the top synthetic chemistry groups worldwide, and has led to dramatic advances in the field of organic chemistry. Nicolaou and his coauthors put it well: “Today, natural product total synthesis is associated with prudent and tasteful selection of challenging and preferably biologically important target molecules; the discovery and invention of new synthetic strategies and technologies; and explorations in chemical biology through molecular design and mechanistic studies. Future strides in the field are likely to be aided by advances in the isolation and characterization of novel molecular targets from nature, the availability of new reagents and synthetic methods, and information and automation technologies” (Nicolaou et al. *2000*).

The process of total synthesis can often lead to the identification of a sub-structural portion of the molecule bearing the essential features necessary for activity (the pharmacophore), and, in some instances, this has resulted in the synthesis of simpler analogs having similar or better activity than the natural product itself. As mentioned in the section on marine-derived anticancer agents, one of the most notable examples is that of the marine-derived antitumor agent, halichondrin B (Fig. 3), where total synthetic studies revealed that the right hand half of the molecule retained all or most of the potency of the parent compound, and the analog, E7389 (Eribulin) (Fig. 3), is currently in Phase III clinical trials (Yu et al. *2005*). In some instances, clinical trials of the original natural product may fail, but totally synthetic analogs continue to be developed. The example of dolastatin 10 has been discussed in the section on marine-derived anticancer agents. The epothilones, discussed in the section on anticancer agents derived from microbial sources, provide further examples of the power of total synthesis in generating improved candidates for clinical trials.

Combinatorial and parallel synthesis

While there are claims that combinatorial chemistry is generating new leads (Borman *2003*), the declining numbers of new New Chemical Entities (NCEs) (Class *2002*) indicate that the use of *de novo* combinatorial chemistry approaches to drug discovery over the past decade have been disappointing, with some of the earlier libraries being described as "poorly designed, impractically large, and structurally simplistic" (Borman *2003*). As stated in this article, "an initial emphasis on creating mixtures of very large numbers of compounds has largely given way in industry to a more measured approach based on arrays of fewer, well-characterized compounds" with "a particularly strong move toward the synthesis of complex natural-product-like compounds—molecules that bear a close structural resemblance to approved natural-product-based drugs". The importance of the use of natural product-like scaffolds for generating meaningful combinatorial libraries has been further emphasized in a recent article entitled "Rescuing Combichem. Diversity Oriented Synthesis (DOS) aims to pick up where traditional combinatorial chemistry left off" (Borman *2004*). In this article it is stated that "the natural product-like compounds produced in DOS have a much better shot at interacting with the desired molecular targets and exhibiting interesting biological activity".

For instance, the combretastatin chemical class has served as a model for the synthesis of a host of analogs containing the essential trimethoxy aryl moiety linked to substituted aromatic moieties through a variety of two or three atom bridges including heterocyclic rings and sulfonamides (Fig. 9). It also provides an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry (Li and Sham *2002*). A number of combretastatin mimics are being developed; three analogs are in clinical trials while 11 are in preclinical development.

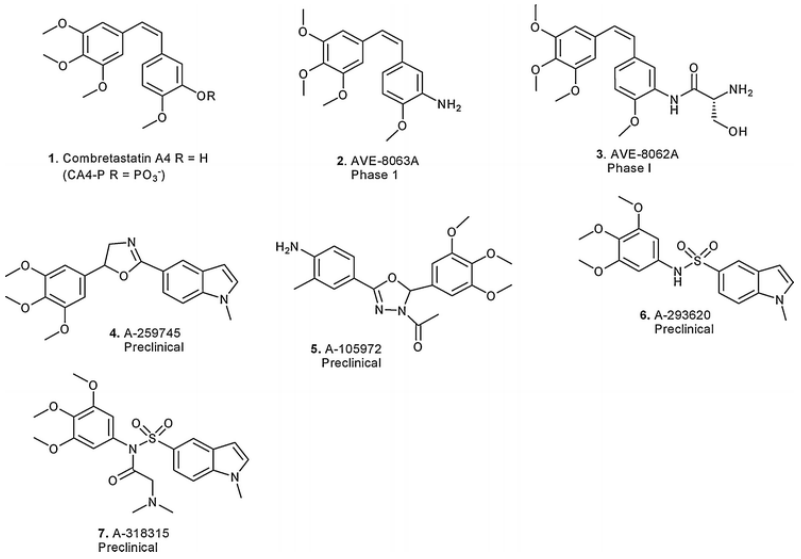


Fig. 9 Combretastatin analogs

Another synthetic agent based on a natural product model is roscovitine which is derived from olomoucine, originally isolated from the cotyledons of the radish, *Raphanus sativus* L. (Meijer and Raymond *2003*) (Fig. 10). Olomoucine was shown to inhibit cyclin-dependent kinases, proteins which play a major role in cell cycle progression. Roscovitine is currently in Phase II clinical trials in Europe as CYC202. The basic structural motif led to the purvalanols which are even more potent (Chang et al. *1999*) and which have now led to even more selective agents such as NU6140 which targets survivin, thus acting synergistically with paclitaxel (Pennati et al. *2005*).

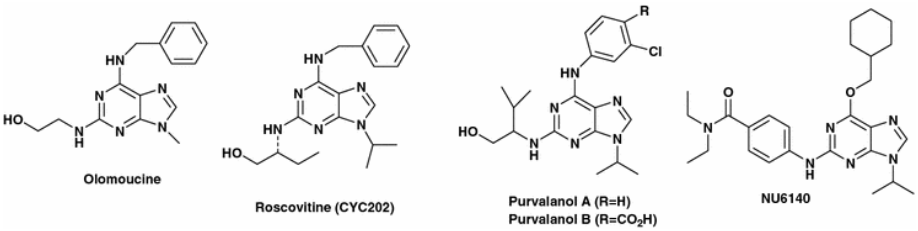


Fig. 10 Olomoucine analogs

Targeted delivery of natural products

In the area of cancer chemotherapy, natural products may often exhibit potent cytotoxicity, but may suffer from pharmacological liabilities due their limited solubility in aqueous solvents and narrow therapeutic indices. These factors have led to the initial demise of a number of pure natural products, such as the plant-derived agents bruceantin and maytansine (Fig. 8). An alternative

approach to successful development, however, is to investigate the potential of such potent agents as “warheads” which may be attached to monoclonal antibodies or polymeric carriers specifically targeted to epitopes on tumors of interest (Duncan *1997*; Engert et al. *1998*).

A promising case is that of maytansine (Fig. 8), originally isolated in the early 1970s from the Ethiopian plant, *Maytenus serrata* (Hochst. Ex A.Rich.) Wilczek (Cassady et al. *2004*). It exhibited extreme cytotoxicity against cancer cell lines and very promising activity in preclinical animal testing, but unfortunately this preclinical promise did not translate into significant efficacy in clinical trials and it was dropped from further study in the early 1980s. The subsequent isolation of related compounds, the ansamitocins, from a microbial source, *Actinosynnema pretiosum*, posed the question as to whether the maytansines are actually plant products or are produced through an association between a microbial symbiont and the plant, a question which is a topic of continuing study (Yu and Floss *2005*). This microbial source of closely related compounds, however, has permitted the production of larger quantities of this class of compounds which, combined with their extreme potency, has stimulated continued interest in pursuing their development. A derivative of maytansine, DM1, conjugated with a monoclonal antibody (Mab) targeting small cell lung cancer cells, is being developed as huN901-DM1 for the treatment of small cell lung cancer, while another conjugate of DM1 to J591, a Mab targeting the prostate-specific membrane antigen, is in clinical trials against prostate cancer. A conjugate known as SB408075 or huC242-DM1 (also known as Cantuzumab Mertansine) has been prepared by the coupling of DM1 to huC242, a Mab directed against the *muc*1 epitope expressed in a range of cancers, including pancreatric, biliary, colorectal and gastric cancers, and is currently in Phase I clinical trials in the USA (Yu and Floss *2005*).

Another interesting case is that of thapsigargin (Fig. 11) isolated from the umbelliferous plant *Thapsia garganica* L., collected on the Mediterranean island of Ibiza (Denmeade et al. *2003*). Thapsigargin induces apoptosis in quiescent and proliferating prostate cancer cells but does not show selectivity for prostate cancer cells. Conjugation to a small peptide carrier, however, produces a water-soluble prodrug which is specifically activated by prostate specific antigen protease at metastatic prostate cancer sites. Treatment of animals bearing prostate cancer

xenograft tumors demonstrated complete tumor growth inhibition without significant toxicity. Given that the prodrug is stable in human plasma, it holds promise as a treatment for human prostate cancer (Sohoel et al. *2006*; Janssen et al. *2006*).

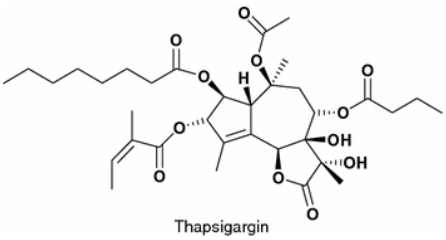


Fig. 11 Targeted delivery