Part I

PRINCIPLES OF DRUG DISCOVERY

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INTRODUCTION

“Pharmacognosy” is one of the oldest established pharmaceutical sciences, and the term has been used for nearly two centuries. Initially, this term referred to the investigation of medicinal substances of plant, animal, or mineral origin in their crude or unprepared state, used in the form of teas, tinctures, poultices, and other types of formulation (1–4). However, by the middle of the 20th century, the chemical components of such crude drugs began to be studied in more detail. Today, the subject of pharmacognosy is highly interdisciplinary, and incorporates aspects of analytical chemistry, biochemistry, biosynthesis, biotechnology, ecology, ethnobotany, microbiology, molecular biology, organic chemistry, and taxonomy, among others (5). The term “pharmacognosy” is defined on the Web site of the American Society of Pharmacognosy (www.phcog.org) as “the study of the physical, chemical, biochemical, and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin, as well as the search for new drugs from natural sources.”

There seems little doubt that humans have used natural drugs since before the advent of written history. In addition to their use as drugs, the constituents of plants have afforded poisons for darts and arrows used in hunting and euphoriants with psychoactive properties used in rituals. The actual documentation of drugs derived from natural products in the Western world appears to date as far back to the Sumerians and Akkadians in the third century BCE, as well as the Egyptian Ebers Papyrus (about 1600 BCE). Other important contributions on the uses of drugs of natural origin were documented by Dioscorides (De Materia Medica) and Pliny the Elder in the first century CE and by Galen in the second century. Written records also exist from about the same time period on plants...
used in both Chinese traditional medicine and Ayurvedic medicine. Then, beginning about 500 years ago, information on medicinal plants began to be documented in herbals. In turn, the laboratory study of natural product drugs commenced approximately 200 years ago, with the purification of morphine from opium. This corresponds with the beginnings of organic chemistry as a scientific discipline. Additional drugs isolated from plant sources included atropine, caffeine, cocaine, nicotine, quinine, and strychnine in the 19th century, and then digoxin, reserpine, paclitaxel, vincristine, and chemical precursors of the steroid hormones in the 20th century. Even as we enter the second decade of the 21st century, approximately three quarters of the world’s population are reliant on primary health care from systems of traditional medicine, including the use of herbs. A more profound understanding of the chemical and biologic aspects of plants used in the traditional medicine of countries such as the People’s Republic of China, India, Indonesia, and Japan has occurred in recent years, in addition to the medicinal plants used in Latin America and Africa. Many important scientific observations germane to natural product drug discovery have been made as a result (1–4).

By the mid-20th century, therapeutically useful alkaloids had been purified and derivatized from the ergot fungus, as uterotonic and sympatholytic agents. Then, the penicillins were isolated along with further major structural classes of effective and potent antibacterials from terrestrial microbes, and these and later antibiotics revolutionized the treatment of infectious diseases. Of the types of organisms producing natural products, terrestrial microorganisms have been found to afford the largest number of compounds currently used as drugs for a wide range of human diseases, and these include antifungal agents, the “statin” cholesterol-lowering agents, immunosuppressive agents, and several anticancer agents (6,7).

At present, there remains much interest also in the discovery and development of drugs from marine animals and plants. However, to date, marine organisms have had a relatively brief history in serving as sources of drugs, with only a few examples approved for therapeutic use thus far. Although the oceans occupy 70% of the surface of the earth, an intense effort to investigate the chemical structures and biologic activities of the marine fauna and flora has only been ongoing for about 40 years (8).

The term “natural product” is generally taken to mean a compound that has no known primary biochemical role in the producing organism. Such low molecular weight organic molecules may also be referred to as “secondary metabolites” and tend to be biosynthesized by the producing organism in a biologically active chiral form to increase the chances of survival, such as by repelling predators or serving as insect pollination attractants, in the case of plants (9). There have been a number of studies to investigate the physicochemical parameters of natural products in recent years, and it has been concluded that “libraries” or collections of these substances tend to afford a higher degree of “drug-likeness,” when compared with compounds in either synthetic or combinatorial “libraries” (10,11). This characteristic might well be expected, since natural products are produced by living systems, where they are subject to transport and diffusion at the cellular level. Small-molecule natural products are capable of modulating protein–protein interactions and can thus affect cellular processes that may be modified in disease states. When compared to synthetic compounds, natural products tend to have more protonated amine and free hydroxy functionalities and more single bonds, with a greater number of fused rings containing more chiral centers. Natural products also differ from synthetic products in the average number of halogen, nitrogen, oxygen, and sulfur atoms, in addition to their steric complexity (12,13). It is considered that natural products and synthetic compounds occupy different regions of “chemical space,” and hence, they each tend to contribute to overall chemical diversity required in a drug discovery program (13). Fewer than 20% of the ring systems produced among natural products are represented in currently used drugs (10). Naturally occurring substances may serve either as drugs in their native or unmodified form or as “lead” compounds (prototype bioactive molecules) for subsequent semisynthetic or totally synthetic modification, for example, to improve biologic efficacy or to enhance solubility (1–4,6,8,10,11).

In the present era of efficient drug design by chemical synthesis aided by computational and combinatorial techniques, and with other new drugs obtained increasingly by biotechnologic processes, it might be expected that traditional natural products no longer have a significant role to play in this regard. Indeed, in the past two decades, there has been a decreased emphasis on the screening of natural products for new drugs by pharmaceutical companies, with greater reliance placed on screening large libraries of synthetic compounds (10,11,14,15). However, in a major review article, Newman and Cragg from the U.S. National Cancer Institute pointed out that for the period from 1981 to 2006, about 28% of the new chemical entities (NCEs) in Western medicine were either natural products per se or semisynthetic derivatives of natural products. Thus, of a total of 1,184 NCEs for all disease conditions introduced into therapy in North America, Western Europe, and Japan over the 25.5-year period covered, 5% were unmodified natural products and 23% were semisynthetic agents based on natural product lead compounds. An additional 14% of the synthetic compounds were designed based on knowledge of a natural product “pharmacophore” (the region of the molecule containing the essential organic functional groups that directly interact with the receptor active site and, therefore, confers the biologic activity of interest) (16). The launch of new natural product drugs in Western countries and Japan has continued in the first decade of 21st century, and such compounds introduced to the market recently have been documented (14,16–18).
Thus, it is generally recognized that the secondary metabolites of organisms afford a source of small organic molecules of outstanding chemical diversity that are highly relevant to the contemporary drug discovery process. Potent and selective leads are obtained from more exotic organisms than before, as collection efforts venture into increasingly inhospitable locales throughout the world, such as deep caves in terrestrial areas and thermal vents on the ocean floor. On occasion, a natural lead compound may help elucidate a new mechanism of interaction with a biologic target for a disease state under investigation. Natural products may serve to provide molecular inspiration in certain therapeutic areas for which there are only a limited number of synthetic lead compounds. A valuable approach is the large-scale screening of libraries of partially purified extracts from organisms (11). However, there is a widespread perception that the resupply of the source organism of a secondary metabolite of interest may prove problematic and will consequently hinder the timely, more detailed, biologic evaluation of a compound available perhaps only in milligram quantities initially. In addition, natural product extracts have been regarded as incompatible with the modern rapid screening techniques used in the pharmaceutical industry, and some believe that the successful market development of a natural product–derived drug is too time consuming (10,11,14,15). A further consideration of the factors involved in the discovery of drugs from natural products will be presented in the next section of this chapter. This will be followed by examples of natural products currently used in various therapeutic categories, as well as a few selected representatives with present clinical use or future potential in this regard.

NATURAL PRODUCTS AND DRUG DISCOVERY
Collection of Source Organisms
There are at least five recognized approaches to the choice of plants and other organisms for the laboratory investigation of their biologic components, namely, random screening; selection of specific taxonomic groups, such as families or genera; a chemotaxonomic approach where restricted classes of secondary metabolites such as alkaloids are sought; an information-managed approach, involving the target collection of species selected by database surveillance; and selection by an ethnomedical approach (e.g., by investigating remedies being used in traditional medicine by “shamans” or medicine men or women) (19). In fact, if plant-derived natural products are taken specifically, it has been estimated that of 122 drugs of this type used worldwide from a total of 94 species, 72% can be traced to the original ethnobotanical uses that have been documented for their plant of origin (19). The need for increased natural products discovery research involving ethnobotany should be regarded as urgent, due to the accelerating loss in developing countries of indigenous cultures and languages, inclusive of knowledge of traditional medical practice (20). However, it is common for a given medicinal plant to be used ethnomedically in more than one disease context, which may sometimes obscure its therapeutic utility for a specific disease condition. Another manner in which drugs have been developed from terrestrial plants and fungi is through following up on observations of the causes of livestock poisoning, leading to new drugs and molecular tools for biomedical investigation (21). When the origin of plants with demonstrated inhibitory effects in experimental tumor systems was considered at the U.S. National Cancer Institute, medicinal or poisonous plants with uses as either anthelmintics or arrow and homicidal poisons were three to four times more likely to be active in this regard than species screened at random (22).

Although some shallow water marine specimens may be collected simply by wading or snorkeling down to 20 feet below the water surface, scuba diving permits the collection of organisms to depths of 120 feet. Deepwater collections of marine animals and plants have been made by dredging and trawling and through the use of manned and unmanned submersible vessels. Collection strategies for specimens from the ocean must take into account marine macroorganism–microorganism associations that may be involved in the biosynthesis of a particular secondary metabolite of interest (8). Thus, there seems to be a complex interplay between many marine host invertebrate animals and symbiotic microbes that inhabit them, and it has been realized that several bioactive compounds previously thought to be of animal origin may be produced by their associated microorganisms instead (23).

The process of collecting or surveying a large set of flora (or fauna) for the purpose of the biologic evaluation and isolation of lead compounds is called “biodiversity prospecting” (24). Many natural products collection programs are focused on tropical rain forests, in order to take advantage of the inherent biologic diversity (or “biodiversity”) evident there, with the hope of harnessing as broad a profile of chemical classes as possible among the secondary metabolites produced by the species to be obtained. To exemplify this, there may be more tree species in a relatively small area of a tropical rainforest than in the whole of the temperate regions of North America. A generally accepted explanation for the high biodiversity of secondary metabolites in humid forests in the tropics is that these molecules are biosynthesized (a process of chemical synthesis by the host organism) for ecologic roles, in response to a continuous growing season under elevated temperatures, high humidity, and great competition due to the high species density present. Maximal biodiversity in the marine environment is found on the fringes of the ocean or sea bordering land, where there is intense competition among sessile (nonmoving) organisms, such as algae, corals, sponges, and some other invertebrate animals, for attachment space (25).

Great concern should be expressed about the continuing erosion of tropical rain forest species, which
is accelerating as the 21st century develops (26). Approximately 25 “hot spots” of especially high biodiversity have been proposed that represent 44% of all vascular plant species and 35% of all species of vertebrates in about 1.4% of the earth’s surface (27). At present, many of the endemic (or native) species to these biodiversity “hot spot” areas have been reported to be undergoing massive habitat loss and are threatened with extinction, especially in tropical regions (26,27).

After the United Nations Convention on Biological Diversity, passed in Rio de Janeiro in 1992, biologic or genetic materials are owned by the country of origin (24,28). A major current-day component of being able to gain access to the genetic resources of a given country for the purposes of drug discovery and other scientific study is the formulation of a memorandum of agreement (MOA), which itemizes access, prior informed consent (involving human subjects in cases where ethnomedical knowledge is divulged), intellectual property related to drug discovery, and the equitable sharing of financial benefits that may accrue from the project, such as patent royalties and licensing fees (24,28). When access to marine organisms is desired, the United Nations Convention on the Law of the Sea (UNCLOS) must also be considered (29).

Once a formal “benefit sharing” agreement is on hand, the organism collection process can begin. It is usual to initially collect 0.3 to 1 kg of each dried plant sample and about 1 kg wet weight of a marine organism for preliminary screening studies (30). In the case of a large plant (tree or shrub), it is typical to collect up to about four different organs or plant parts, since it is known that the secondary metabolite composition may vary considerably between the leaves, where photosynthesis occurs, and storage or translocation organs such as the roots and bark (31). There is increasing evidence that considerable variation in the profile of secondary metabolites occurs in the same plant organ when collected from different habitats, depending on local environmental conditions, and thus it may be worth investigating even well-studied species in drug discovery projects. Taxa endemic (native) to a particular country or region are generally of higher priority than the collection of pandemic weeds. It is very important never to remove all quantities of a desired species at the site of collection, in order to conserve the native germplasm encountered. Also, rare or endangered species should not be collected; a listing of the latter is maintained by the Red List of Threatened Species of the International Union for Conservation of Nature and Natural Resources (www.redlist.org), covering terrestrial, marine, and freshwater organisms.

A crucial aspect of the organism collection process is to deposit voucher specimens representative of the species collected in a central repository such as a herbarium or a museum, so that this material can be accessed by other scientists, in case of need. It is advisable to deposit specimens in more than one repository, including regional and national institutions in the country in which the organisms were collected. Collaboration with general and specialist taxonomists is very important, because without an accurate identification of a source organism, the value of subsequent isolation, structure elucidation, and biologic evaluation studies will be greatly reduced (31).

Organisms for natural products drug discovery work may be classified into the following kingdoms: Eubacteria (bacteria, cyanobacteria [or “blue-green algae”]), Archaea (halobacteria, methanogens), Prototista (e.g., protozoa, diatoms, “algae” [including red algae, green algae]), Plantae (land plants [including mosses and liverworts, ferns, and seed plants]), Fungi (e.g., molds, yeasts, mushrooms), and Animalia (mesozoa [wormlike invertebrate marine parasites], sponges, jellyfish, corals, flatworms, roundworms, sea urchins, mollusks [snails, squid], segmented worms, arthropods [crabs, spiders, insects], fish, amphibians, birds, mammals) (24). Of these, the largest numbers of organisms are found for arthropods, inclusive of insects (~950,000 species), with only a relatively small proportion (5%) of the estimated 1.5 million fungi in the world having been identified. At present, with 300,000 to 500,000 known species, plants are the second largest group of classified organisms, representing about 15% of our biodiversity. Of the 28 major animal phyla, 26 are found in the sea, with eight of these exclusively so. There have been more than 200,000 species of invertebrate animals and algal species found in the sea (24). A basic premise inherent in natural products drug discovery work is that the greater the degree of phylogenetic (taxonomic) diversity of the organisms sampled, the greater the resultant chemical diversity that is evident.

Interest in investigating plants as sources of new biologically active molecules remains strong, in part because of a need to better understand the efficacy of herbal components of traditional systems of medicine (32). In the last decade, many new natural product molecules have been isolated from fungal sources (6,7). An area of investigation of great potential expansion in the future will be on other microbes, particularly of actinomycetes and cyanobacteria of marine origin, especially if techniques can continue to be developed for their isolation and cultivating in the laboratory (33). Because as many as 99% of known microorganisms are not able to be cultivated under laboratory conditions, the technique of “genome mining” isolates their DNA and enables new secondary metabolite biochemical pathways to be exploited, leading to the possibility of producing new natural products (34). The endophytic fungi that reside in the tissue of living plants have been found to produce an array of biologically interesting new compounds and are worthy of more intensive investigation (35). It is of interest to note that in a survey of the origin of 30,000 structurally assigned lead compounds of natural origin, the compounds were derived from animals (13%), bacteria (33%), fungi (26%), and plants (27%) (12). For the year 2008, it was reported that 24 animal, 25 bacteria, 7 fungal, and
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108 plant-derived natural products were undergoing at least phase 1 clinical trials leading to drug development (36). Therefore, while natural product researchers tend to specialize in the major types of organism on which they work, it is reasonable to expect that the future investigation of all of their major groups mentioned earlier will provide dividends in terms of affording new prototype biologically active compounds of use in drug discovery.

Preparation of Initial Extracts and Preliminary Biologic Screening

Although different laboratories tend to adopt different procedures for initial extraction of the source organisms being investigated, it is typical to extract initially terrestrial plants with a polar solvent like methanol or ethanol, and then subject this to a defatting (lipid-removing) partition with a nonpolar solvent like hexane or petroleum ether, and then partition the residue between a semipolar organic solvent, such as chloroform or dichloromethane, and a polar aqueous solvent (31). Marine and aquatic organisms are commonly extracted fresh into methanol or a mixture of methanol–dichloromethane (30). A peculiarity of working on plant extracts is the need to remove a class of compounds known as “vegetable tannins” or “plant polyphenols” before subsequent biologic evaluation because these compounds act as interfering substances in enzyme inhibition assays, as a result of precipitating proteins in a nonspecific manner. Several methods to remove plant polyphenols have been proposed, such as passage over polyvinylpyrrolidone (PVP) and polyamide, on which they are retained. Alternatively, partial removal of these interfering substances may be effected by washing the final semipolar organic layer with an aqueous sodium chloride solution (31). However, it should be pointed out that there remains an active interest in pursuing purified and structurally characterized vegetable tannins for their potential medicinal value (37). Caution also needs to be expressed in regard to common saturated and unsaturated fatty acids that might be present in natural product extracts, because these may interfere with various enzyme inhibition and receptor binding assays. Fatty acids and other lipids may largely be removed from more polar natural product extracts, using the defatting solvent partition stage mentioned earlier (38).

Drug discovery from organisms is a “biology-driven” process, and as such, biologic activity evaluation is at the heart of the drug discovery process from crude extracts prepared from organisms. So-called high-throughput screening (HTS) assays have become widely used for affording new leads. In this process, large numbers of crude extracts from organisms can be simultaneously evaluated in a cell-based or non-cell-based format, usually using multiwell microtiter plates (39). Cell-based in vitro bioassays allow for a considerable degree of biologic relevance, and manipulation may take place so that a selected cell line may involve a genetically altered organism (40) or incorporate a reporter gene (41). In noncellular (cell-free) assays, natural products extracts and their purified constituents may be investigated for their effects on enzyme activity (42) or on receptor binding (43). Other homogenous and separation-based assays suitable for the screening of natural products have been reviewed (44). For maximum efficiency and speed, HTS may be automated through the use of robotics and may be rendered as a more effective process through miniaturization.

Methods for Compound Purification and Structure Elucidation and Identification

Bioassay-directed fractionation is the process of isolating pure active constituents from some type of biomass (e.g., plants, microbes, marine invertebrates) using a decision tree that is dictated solely by bioactivity. A variety of chromatographic separation techniques are available for these purposes, including those based on adsorption on sorbents, such as silica gel, alumina, Sephadex, and more specialized solid phases, and methods involving partition chromatography inclusive of counter-current chromatography (45). Recent improvements have been made in column technology, automation of high-performance liquid chromatography (HPLC; a technique often used for final compound purification), and compatibility with HTS methodology (46). Routine structure elucidation is performed using combinations of spectroscopic procedures, with particular emphasis on $^1$H- and $^{13}$C-nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Considerable progress has been made in the development of cryogenic and capillary NMR probe technology, for the determination of structures of sub-milligram amounts of natural products (47). In addition, the automated processing of spectroscopic data for the structure elucidation of natural products is a practical proposition (48). Another significant advance is the use of “hyphenated” analytical techniques for the rapid structure determination of natural products without the need for a separate isolation step, such as liquid chromatography (LC)-NMR and LC-NMR-MS (11,46). The inclusion of an online solid-phase extraction (SPE) cartridge is advantageous in the identification of natural product molecules in crude extracts using LC-NMR, coupled with MS and circular dichroism spectroscopy (49).

Dereplication is a process of determining whether an observed biologic effect of an extract or specimen is due to a known substance. This is applied in natural product drug discovery programs in an attempt to avoid the reisolation of compounds of previously determined structure. A step like this is essential to prioritize the resources available to a research program, so that the costly stage of bioassay-directed fractionation on a promising lead crude extract can be devoted to the discovery of biologically active agents representing new chemotypes (46,50). This has been particularly necessary for many years in studies on anti-infective agents from actinomycetes and bacteria and is also routinely applied to extracts from marine invertebrates and higher plants. Methods for
dereplication must be sensitive, rapid, and reproducible, and the analytical methods used generally contain a mass spectrometric component (50). For example, the eluant (effluent) from an HPLC separation of a crude natural product extract may be split into two portions, so that the major part is plated out into a microtiter plate, with the wells then evaluated in an in vitro bioassay of interest. The fractions from the minor portion of the column eluant are introduced to a mass spectrometer, and the molecular weights of compounds present in active fractions can be determined. This information may then be introduced into an appropriate natural products database, and tentative identities of the active compounds present in the active wells can be determined (50).

Metabolomics is a recently developed approach in which the entire or “global” profile of secondary metabolites in a system (cell, tissue, or organism) is catalogued under a given set of conditions. Secondary metabolites may be investigated by a detection step such as MS after a separation step such as gas chromatography, HPLC, or capillary electrophoresis (51). This type of technology has particular utility in systematic biology, genomics research, and biotechnology and should have value in future natural products drug discovery (51,52).

**Compound Development**

A major challenge in the overall natural products drug discovery process is to obtain larger amounts of a biologically active compound of interest for additional laboratory investigation and potential preclinical development. One strategy that can be adopted when a plant-derived active compound is of interest is to obtain a recollection of the species of origin. To maximize the likelihood that the recollected sample will contain the bioactive compound of previous interest, the plant recollection should be carried out in the same location as the initial collection, on the same plant part, at the same time of the year (31). Some success has been met with the production of terrestrial plant metabolites via plant tissue culture (53). For microbes of terrestrial origin, compound scale up usually may be carried out through cultivation and large-scale fermentation (6,7).

Although evaluation of crude extracts of organisms is not routinely performed in animal models because of limitations of either test material or other project resources, it is of great value to test in vitro–active natural products in a pertinent in vivo method to obtain a preliminary indication of the worthiness of a lead compound for preclinical development. There are also a variety of “secondary discriminator” bioassays that provide an assessment of whether or not a given in vitro–active compound is likely to be active in vivo, and these require quite small amounts of test material. For example, the in vivo hollow fiber assay was developed at the U.S. National Cancer Institute for the preliminary evaluation of potential anticancer agents and uses confluent cells of a tumor model of interest deposited in polyvinylidene fluoride fibers that are implanted in nude mice (31,34). It is also important for pure bioactive compounds to be evaluated mechanistically for their effects on a particular biologic target, such as on a given stage of the life cycle of a pathogenic organism or cancer cell. Needless to say, a pure natural product of novel structure with in vitro and in vivo activity against a particular biologic target relevant to human disease acting through a previously unknown mechanism of action is of great value in the drug discovery process.

Once a bioactive natural product lead is obtained in gram quantities, it is treated in the same manner as a synthetic drug lead and is thus subjected to pharmaceutical development, leading to preclinical and clinical trials. This includes lead optimization via medicinal chemistry, combinatorial chemistry, and computational chemistry, as well as formulation, pharmacokinetics, and drug metabolism studies, as described elsewhere in this volume. Often, a lead natural product is obtained from its organism of origin along with several naturally occurring structural analogs, permitting a preliminary structure–activity relationship study to be conducted. This information may be supplemented with data obtained by microbial biotransformation or the production of semisynthetic analogs, to allow researchers to glean some initial information about the pharmacophoric site(s) of the naturally occurring molecule (10,11). Combinatorial biosynthesis is a contemporary approach with the ability to produce new natural product analogs, or so-called “unnatural” natural products, and these may be used to afford new drug candidates. This methodology involves the engineering of biosynthetic gene clusters in microorganisms and has been applied to the generation of polyketides, peptides, terpenoids, and other compounds. New advances in the biochemical and protein engineering aspects of this technique have led to a greater applicability than previously possible (55).

**SELECTED EXAMPLES OF NATURAL PRODUCT–DERIVED DRUGS**

In the following sections, examples are provided of both naturally occurring substances and synthetically modified compounds based on natural products with drug use. It is evident that many of the examples shown reflect considerable structural complexity and that the compounds introduced to the market have been obtained from organisms of very wide diversity. More detailed treatises with many more examples of natural product drugs are available (e.g., see references 1–4). Several recent reviews have summarized natural product drugs introduced to the market in recent years and substances on which clinical trials are being conducted (16–18,36).

**Drugs for Cardiovascular and Metabolic Diseases**

There is a close relationship between natural product drugs and the treatment of cardiovascular and
metabolic diseases. The powdered leaves of *Digitalis purpurea* have been used in Western medicine for more than 200 years, with the major active constituent being the cardiac (steroidal) glycoside digitoxin, which is still used now for the treatment of congestive heart failure and atrial fibrillation. A more widely used drug used today is digoxin, a constituent of *Digitalis lanata*, which has a rapid action and is more rapidly eliminated from the body than digitoxin. Deslanoside (deacetyl-lanatoside C) is a hydrolysis product of the *D. lanata* constituent lanatoside C and is used for rapid digitalization (1–4). The “statin” drugs used for lowering blood cholesterol levels are based on the lead compoundLovastatin (formerly known as compactin), produced by cultures of *Penicillium citrinum*, and were discovered using a 5-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase assay. Because hypercholesterolemia is regarded as one of the major risk factors for coronary heart disease, several semisynthetic and synthetic compounds modeled on the mevastatin structure (inclusive of the dihydroxy carboxylic acid side chain) now have extremely wide therapeutic use, including atorvastatin, fluvastatin, pravastatin, and simvastatin. Lovastatin is a natural product drug of this type, isolated from *Penicillium breviceps*compactin and other organisms (3). There is also a past history of the successful production of cardiovascular agents from a terrestrial vertebrate, namely, the angiotensin-converting enzyme inhibitors captopril and enalapril, which were derived from tetrotide, a nonapeptide isolated from the pit viper, *Bothrops jararaca* (56).

Two further new drugs derived from an invertebrate and a vertebrate source, respectively, are bivalirudin and exenatide. Bivalirudin is a specific and reversible direct thrombin inhibitor that is administered by injection and is used to reduce the incidence of blood clotting in patients undergoing coronary angioplasty. This compound is a synthetic, 20-amino acid peptide and was modeled on hirudin, a substance present in the saliva of the leech, *Haementeria officinalis* (57,58). Exenatide is a synthetic version of a 39-amino acid peptide (exenatide-4), produced by a lizard native to the southwest United States and northern Mexico, called the Gila monster, *Heloderma suspectum*, and acts in the same manner as glucagon-like peptide-1 (GLP-1), a naturally occurring hormone. This drug is also administered by injection and enables improved glycemic control in patients with type 2 diabetes (18,59).

**Central and Peripheral Nervous System Drugs**

A comprehensive review has appeared on natural products (mostly of experimental value) that affect the central nervous system (CNS), inclusive of potential analgesics, antipsychotics, anti-Alzheimer disease agents, antitussives, anxiolytics, and muscle relaxants, among other categories. The authors point out that apart from the extensive past literature on plants and their constituents as hallucinogenic agents, this area of research inquiry on natural products is not well developed but is likely to be productive in the future (60). Natural products also have the potential to treat drug abuse (61).

The morphinan isoquinoline alkaloid, (−)-morphine, is the most abundant and important constituent of the dried latex (milky exudate) of *Papaver somniferum* (opium poppy) and the prototype of the synthetic opioid analgesics, being selective for μ-opioid receptors (Fig. 1.1). This compound may be considered the paramount natural product lead compound, with many thousands of analogs synthesized in an attempt to obtain derivatives with strong analgesic potency but without any addictive tendencies (1–4). One derivative now in late clinical trials as a pain treatment is morphine-6-glucuronide (M6G), the major active metabolite of morphine, with fewer side effects than the parent compound (18,62). The pyridine alkaloid epibatidine, isolated from a dendrobatid frog (*Epidendobates tricolor*) found in Ecuador, activates nicotinic receptors and has a 200-fold more potent analgesic activity than morphine. The drug potential of epibatidine is limited by its concomitant toxicity, but it is an important lead compound for the development of future new analgesic agents with less addictive liability than the opiate analgesics (63). A nonopiod analgesic for the amelioration of chronic pain has been introduced to the market recently, namely, ziconotide, which is a synthetic version of the peptide, ω-conotoxin MVIIA. The conotoxin class is produced by the cone snail, *Conus magus*, and these compounds are peptides with 24- to 27-amino acid residues. Ziconotide selectively binds to N-type voltage-sensitive neuronal channels, causing a blockage of neurotransmission and a potent analgesic effect (18,64). This is one of the first examples of a new natural product drug from a marine source.

(−)-Δ²-trans-Tetrohydrocannabinol (tetrohydrocannabinol [THC]) is the major psychoactive (euphoriant) constituent of marijuana (*Cannabis sativa*). The synthetic form of THC (dronabinol) was approved more than 25 years ago to treat nausea and vomiting associated with cancer chemotherapy and has been used for a lesser amount

**Bivalirudin**

D-Phe-L-Pro-L-Arg-L-Pro-Gly-Gly-Gly-L-Asn-Gly-L-Asp-L-Phe-L-Glu-L-Glu-L-Ile-L-Pro-L-Glu-L-Glu-L-Tyr-L-Leu

**Exenatide**

of time to treat appetite loss in HIV/AIDS patients (3). More recently, an approximately 1:1 mixture of THC and the structurally related marijuana constituent cannabidiol (CBD) has been approved in Canada and the United Kingdom for the alleviation of neuropathic pain and spasticity for multiple sclerosis patients and is administered in low doses as a buccal spray (18,65). There is considerable interest in using cannabinoid derivatives based on THC for medicinal purposes, but it is necessary to minimize the CNS effects of these compounds.

Another important natural product lead compound is the tropane alkaloid ester atropine [(±)-hyoscyamine], from the plant Atropa belladonna (deadly nightshade). Atropine has served as a prototype molecule for several anticholinergic and antispasmodic drugs. One recently introduced example of an anticholinergic compound modeled on atropine is tiotropium bromide, which is used for the maintenance treatment of bronchospasm associated with chronic obstructive pulmonary disease (COPD) (66).

In the category of anti-Alzheimer disease agents, galantamine hydrobromide is a selective acetylcholinesterase inhibitor that slows down neurologic degeneration by inhibiting this enzyme and by interacting with the nicotinic receptor (67). Galantamine (also known as “galanthamine”) is classified as an Amaryllidaceae alkaloid and has been obtained from several species in this family. Because commercial synthesis is not economical, it is obtained from the bulbs of Leucojum aestivum (snowflake) and Galanthus species (snowdrop) (1–4). There is some evidence that there is an ethnomedical basis for the current use of galantamine (68).

**Anti-infective Agents**

Since the introduction of penicillin G (benzylpenicillin) to chemotherapy as an antibacterial agent in the 1940s, natural products have contributed greatly to the field of anti-infective agents. In addition to the penicillins, other classes of antibacterials that have been developed from natural product sources are the aminoglycosides, cephalosporins, glycopeptides, macrolides, rifamycins, and tetracyclines. Antifungals, such as griseofulvin and the polyenes, and avermectins, such as the antiparasitic drug ivermectin, are also of microbial origin (1–4). Of the approximately 90 drugs in this category that were introduced in Western countries, including Japan, in the period from 1981 to 2002, almost 80% can be related to a microbial origin (16). Despite this, relatively few major
pharmaceutical companies are currently working on the discovery of new anti-infective agents from natural sources, due to possible bacterial resistance against new agents and concerns regarding regulation (17). Higher plants have also afforded important anti-infective agents, perhaps most significantly the quinoline alkaloid quinine, obtained from the bark of several Cinchona species found in South America, including Cinchona ledgeriana and Cinchona succirubra. Quinine continues to be used for the treatment of multidrug-resistant malaria and was the template molecule for the synthetic antimalarials chloroquine, primaquine, and mefloquine (1–4).

The following examples have been chosen to represent an array of different structural types of antibacterial agents recently introduced into therapy (Fig. 1.2) (6,14,17,18). Meropenem is a carbapenem (a group of β-lactam antibiotics in which the sulfur atom in the thiazolidine ring is replaced by a carbon) and is based on thienamycin (Fig. 1.2), isolated from Streptomyces cattleya. It is a broad-spectrum antibacterial that was introduced into therapy in the last decade as a stable analog of the initially discovered thienamycin (69). Tigecycline (Fig. 1.2) is a member of the glycylcycline class of tetracycline antibacterials and is the 9-tert-butylglycylamido derivative of minocycline, a semisynthetic derivative of chlorotetracycline from Streptomyces aureofaciens. This is a broad-spectrum antibiotic with activity against methicillin-resistant Staphylococcus aureus (70). Daptomycin (Fig. 1.2) is the prototype member of the cyclic lipopeptide class of antibiotics and, although isolated initially from Streptomyces roseosporus, is produced by semisynthesis. This compound binds to bacterial cell membranes, disrupting the membrane potential, and blocks the synthesis of DNA, RNA, and proteins. Daptomycin is bactericidal against gram-positive organisms including vancomycin-resistant Enterococcus faecalis and Enterococcus faecium and is approved for the treatment of complicated skin and dermal infections (71). Telithromycin (Fig. 1.2) is a semisynthetic derivative of the 14-membered macrolide erythromycin A from Saccharopolyspora erythraea and is a macrolide of the ketolide class that lacks a cladinose sugar but has an extended alkyl-aryl unit attached to a cyclic carbamate unit. It binds to domains II and V of the 23S rRNA unit of the bacterial 50S ribosomal unit, leading to inhibition of the ribosome assembly and protein synthesis. This macrolide antibiotic is used to treat bacteria that infect the lungs and sinuses, including community-acquired pneumonia due to Streptococcus pneumoniae (72).

Natural products have been a fruitful source of antifungal agents in the past, with the echinocandins being a new group of lipopeptides introduced recently (73). Of these, three compounds are now approved drugs, including the acetate of caspofungin, which is a semisynthetic derivative of pneumocandin B0, a fermentation product of Glarea lozoyensis. Caspofungin inhibits the synthesis of the fungal cell wall β(1,3)-d-glucan, by noncompetitive inhibition of the enzyme β(1,3)-d-glucan synthase, producing both a fungistatic and a fungicidal effect (73). The compound is administered by slow intravenous infusion and is useful in treating infections by Candida species (74).

![FIGURE 1.2 Examples of Natural and Semisynthetic Anti-infective Agents.](image-url)
Malaria remains a parasitic scourge that is still extending in incidence. In 1972, the active principle from *Artemisia annua*, a plant used for centuries in Chinese traditional medicine to treat fevers and malaria, was established as a novel antimalarial chemotype. This compound, artemisinin (*qinghaosu* in Chinese), is a sesquiterpene lactone with an endoperoxide group that is essential for activity, and it reacts with the iron in haem in the malarial parasite, *Plasmodium falciparum* (Fig. 1.3). Because this compound is poorly soluble in water, a number of derivatives have been produced with improved formulation, including arteether and artemether. Although animal experiments have suggested that artemisinin derivatives are neurotoxic, this may not be the case in malaria patients (1–4). Artemisinin-based combination treatments such as coartemether (artemether and lumefantrine) are now widely used for treating drug-resistant *P. falciparum* malaria (75). Coartemether is also known as arteether (*Artemisia annua*), a plant used for centuries in Chinese traditional Chinese medicine. This compound is now registered in the Netherlands (76).

There are now about 30 approved drugs or drug combinations used to treat HIV/AIDS infections, with most of these being targeted toward the viral enzymes reverse transcriptase or protease. Bevirimat is a semisynthetic 3′,3′-dimethylsuccinyl derivative of the oleanane-type triterpenoid betulonic acid, which is found widely in the plant kingdom, including several species used in traditional Chinese medicine. This compound is now undergoing clinical trials as a potential HIV maturation inhibitor (77,78).

**FIGURE 1.3** Artemisinin and two derivatives used for the treatment of malaria.

**Anticancer Agents**

For several decades, natural products have served as a useful group of structurally diverse cancer chemotherapeutic agents, and many of our most important anticancer agents are of microbial or plant origin. Thus, the antitumor antibiotics include the anthracyclines (daunorubicin, doxorubicin, epirubicin, idarubicin, and valrubicin), bleomycin, dactinomycin (actinomycin D), mitomycin C, and mitoxantrone. Four main classes of plant-derived antitumor agents are used: vinca (*Catharanthus*) bisnucleo alkaloids (vinblastine, vincristine, and vinorelbine); the semisynthetic epipodophyllotoxin derivatives (etoposide, teniposide, and etoposide phosphate); the taxanes (paclitaxel and docetaxel); and the camptothecin analogs (irinotecan and topotecan) (Fig. 1.4) (1–4,79).

The parent compounds paclitaxel (originally called “taxol”) and camptothecin were both discovered in the laboratory of the late Monroe E. Wall and of Mansukh Wani at Research Triangle Institute in North Carolina (Fig. 1.4). Like some other natural product drugs, several years elapsed from the initial discovery of these substances until their ultimate clinical approval in either a chemically unmodified or modified form. One of the factors that served to delay the introduction of paclitaxel to the market was the need for the large-scale acquisition of this compound from a source other than from the bark of its original plant of origin, the Pacific yew (*Taxus brevifolia*), because this would involve destroying this slow-growing tree. Paclitaxel and its semisynthetic analog docetaxel may be produced by partial synthesis. To enable this, the diterpenoid “building block,” 10-deacetylbaccatin III, is used as a starting material, which can be isolated from the needles of the ornamental yew, *Taxus baccata*, a renewable botanical resource that can be cultivated in greenhouses (80). A major pharmaceutical company now manufactures paclitaxel by plant tissue culture. The initial source plant of camptothecin, *Camptotheca acuminata*, is a rare species found in regions south of the Yangtze region of the People’s Republic of China. Today, camptothecin is not only produced commercially from cultivated *C. acuminata* trees in mainland China, but also from the roots of *Nothapodytes nimmoniana* (formerly known as both *Nothapodytes fordi* and *Mathia fordi*), which is found in the southern regions of the Indian subcontinent (81). It is of interest to note that these two antineoplastic agents are particularly important not only because of the clinical effectiveness of their derivatives as cancer chemotherapeutic agents, having a significant proportion of the market share (80), but...
also because they are prominent lead compounds for synthetic optimization. There are several taxanes and camptothecin derivatives in clinical trial (17,18). Interestingly, endophytic fungi have been reported to produce paclitaxel (82) and camptothecin (83), so it may be possible in the future to produce these important compounds by fermentation rather than by cultivation or other existing methods. Paclitaxel and camptothecin were each found to exhibit a unique mechanism of action for the inhibition of cancer cell growth, with paclitaxel shown to promote the polymerization of tubulin and the stabilization of microtubules and with camptothecin demonstrated as the first inhibitor of the enzyme DNA topoisomerase I (84).

Several other natural product molecules or their derivatives have been introduced to therapy recently (Fig. 1.5) (17,18,85). Ixabepilone, a semisynthetic derivative of epothilone B, is now marketed in the United States for the treatment of locally advanced and metastatic breast cancer (86). The epothilones are derived from the terrestrial myxobacterium Sorangium cellulosum.
and have a similar type of action on tubulin as paclitaxel (87). Trabectedin (ecteinascidin-743 or ET-743) is an isoquinoline alkaloid obtained originally from the marine tunicate, Ecteinascidia turbinata, but now produced by partial synthesis from a microbial metabolite, cyanosafacin B, of Pseudomonas fluorescens. Trabectedin is an alkylating agent that binds to the minor groove of DNA and blocks cells in the G2-M phase; it is used in Europe as second-line therapy for patients with soft tissue sarcoma (88,89). Romidepsin is a depsipeptide isolated from the soil bacterium, Chromobacterium violaceum, in 1994 (90). This compound is an inhibitor of histone deacetylase and was approved in the United States for the treatment of T-cell lymphoma (91). Temsirolimus is a semisynthetic ester derivative of sirolimus (rapamycin), with the latter compound isolated some time ago from Streptomyces hygroscopicus (92,93). Recently, temsirolimus was approved in the United States for treating advanced renal cell carcinoma, and mechanistically, this compound is an inhibitor of the mammalian target of rapamycin kinase (92,93). A promising new anticancer agent still in advanced clinical trials is combretastatin A4 phosphate, a water-soluble prodrug of combretastatin A4 from the South African plant, Combretum caffrum (94). Combretastatin A4 phosphate binds to tubulin and also affects tumor blood flow as a vascular disrupting agent (94,95).

Cancer chemoprevention is regarded as the use of synthetic or natural agents to inhibit, delay, or reverse the process of carcinogenesis through intervention before the appearance of invasive disease. This relatively new approach toward the management of cancer has involved gaining a better understanding of the mechanism of action of cancer chemopreventive agents (96). Among the natural products that have been studied for this purpose, there has been a renewed interest in the effects of the phytochemical components of the diet, and some of these compounds have been found to block cancer initiation (blocking agents) or reverse tumor promotion and/or progression (suppressing agents) (97). Members of many different structural types of plant secondary metabolites have been linked with potential cancer chemopreventive activity (97,98). Approximately 35 foods of plant origin have been found recently to produce cancer chemopreventive agents, such as curcumin from turmeric, epigallocatechin-3-O-gallate from green tea, trans-resveratrol from grapes and certain red wines, and d-sulfaphane from broccoli (Fig. 1.6) (97,98).

**Immunomodulators**

The fungal-derived cyclic peptide cyclosporin (cyclosporine A) was found some years ago to be an immunosuppressive agent in organ and tissue transplant surgery. Another compound with this same type of use and that also acts by the inhibition of T-cell activation is the macrolide tacrolimus (FK506) from Streptomyces tsukubaensis (3).
care system. Over the last 15 years, there has been a large influx of botanical products into community pharmacy practice and health food stores in the United States as a result of the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994. Such products are regulated by the U.S. Food and Drug Administration as foods rather than drugs and must adhere to requirements regarding product labeling and acceptable health claims (101). Currently, among the most popular botanical products used in the United States are those containing black cohosh, cranberry, echinacea, evening primrose, garlic, ginkgo, ginger, ginseng, green tea, milk thistle, saw palmetto, soy, St. John’s wort, and valerian. These are purchased as either the crude powdered form in compressed tablets or capsules or as galenical preparations, such as extracts or tinctures, and are frequently ingested in the form of a tea (101). In addition to the United States, a parallel increased interest in herbal remedies has occurred in all countries in Western Europe, Canada, and Australia, in part because of a greater awareness of complementary and alternative medicine (CAM). Many clinical trials on these products have been conducted in Europe, and some are occurring in the United States under the sponsorship of the National Institutes of Health.

The recent widespread introduction of a large number of botanical dietary supplements has opened a new door in terms of research inquiry for natural product scientists in the United States. Not all of these products have a well-documented efficacy, however. Three important needs in the scientific investigation of herbal remedies are the characterization of active principles (where these are not known), the development of rigorous and validated analytical methods for quality control procedures, and the determination of their potential toxicity and interactions with prescription medications (102). Unlike compounds approved as single chemical entity (SCE) drugs, it is accepted that combinations of plant secondary metabolites may be responsible for the physiologic effects of herbal medicines. For example, both the terpene lactone (e.g., ginkgolide B; Fig. 1.8) and the flavonoid glucoside constituents of ginkgo (Ginkgo biloba) leaves are regarded as being necessary for alleviation of the symptoms of peripheral vascular disease for which this phytomedicine is used in Europe (101). Moreover, an acetone-soluble extract of G. biloba containing standardized amounts of flavone glycosides (24%) and terpene lactones (6%) has been used in many clinical trials on this herb (101). If the “active principles” of an herbal remedy are known or can be discovered, these substances can act as reference standards, and their specified concentration levels can be quantified in chemical quality control procedures, which are predominantly performed by HPLC. A number of official monographs for the standardization of botanical dietary supplements have been developed over the last decade in the United States (103). Other scientific challenges on herbal remedies are to establish more completely their dissolution, bioavailability, and shelf life. For example, it has been found that co-effectors such as certain procyandins present in St. John’s wort (Hypericum perforatum) can increase the bioavailability of hypericin (Fig. 1.8), one of the constituents of this plant known to exert antidepressant activity (104). These herbal products should be free of adulteration (the deliberate addition of nonauthentic plant material or of biologically active or inactive compounds), as well as free of other additives such as herbicides, pesticides, heavy metals, solvent residues, and microbial and biologic contaminants (101,102).

Unfortunately, many herbal remedies may pose toxicity risks or may be involved in harmful drug interactions. A drastic example of toxicity was caused by the herbal Chinese medicinal plant, Aristolochia fangchi, which was substituted in error for another Chinese plant in a weight-reducing regimen taken by a number of women in Belgium several decades ago. Years later, this was linked to the generation of severe renal disease characterized as interstitial fibrosis with atrophy of the tubules, as well as the development of tumors. These toxic symptoms, also known as “Chinese herb nephropathy,” were attributed to the presence of the phenanthrene derivatives aristolochic acids I and II (Fig. 1.8) produced by A. fangchi, which have been found experimentally to intercalate with DNA (105). The presence of high levels of the phloroglucinol derivative hyperforin (Fig. 1.8) in St. John’s wort (Hypericum perforatum) products has been found to induce cytochrome P450 enzymes (in particular CYP34A), leading to decreased plasma concentrations of prescription drugs that may be coadministered, such as alprazolam, cyclosporin, digoxin, indinavir, irinotecan, simvastatin, and warfarin, as well as oral contraceptives (106). In 2006, the first example of a new class of natural product prescription drugs was approved by the U.S. Food and Drug Administration, namely, a mixture of

![FIGURE 1.8 Chemicals found in various botanical dietary supplements.](image-url)
sinecatechins present in a standardized extract of the leaves of green tea (Camellia sinensis). This product is approved for the topical treatment of external genital and perianal warts. Stringent criteria must be followed in the manufacture and quality control of this product, and it was subjected to rigorous clinical trials (107).

**FUTURE PROSPECTS**

The beginning of the second decade of the 21st century seems to be an opportune time for renewed efforts to be made regarding the discovery of new secondary metabolite prototype, biologically active compounds from animals, fungi, microorganisms, and plants of terrestrial and marine origin. Although many pharmaceutical companies have reduced their investment in natural products research, in favor of screening libraries of synthetic compounds and combinatorial chemistry, this has coincided with disappointing numbers of new drug being introduced in recent years (11,14–16). Fortunately, many smaller “biotech” companies have actively taken up the challenge of contemporary natural products drug discovery from organisms. There continues to be a steady stream of new natural product–derived drugs introduced into therapy for the treatment of many common human diseases (e.g., cancer, cardiovascular diseases, neurologic conditions) (17,18). However, there is ample potential for much greater utilization of natural product–derived compounds in the treatment or prophylaxis of such major worldwide scourges as HIV/AIDS, tuberculosis, hepatitis C, and tropical diseases (e.g., lymphatic filariasis, leishmaniasis, schistosomiasis). The search for such agents should be enhanced by the availability of extensive libraries of taxonomically authenticated crude extracts of terrestrial and marine origin, as well as of pure secondary metabolites from microorganisms, plants, and animals. In addition, this will be facilitated by recently developed techniques such as biocatalysis, combinatorial biosynthesis, combinatorial and computational chemistry, metabolic engineering, and tissue culture. The high “drug-like” quality of natural product molecules stands as a constant, and it only remains for natural product chemists and biologists to investigate these substances in the most technically ingenious and expedient ways possible.

It should not be thought that after approximately 200 years of investigation, the prospects of finding new drugs of natural origin are nearing exhaustion; there is still a large scope for success in this type of endeavor. For example, if plants are taken specifically, less than 20% have been evaluated chemically or biologically. The urgency to perform this type of work cannot be understated in view of the increasing erosion of natural resources that will accelerate as the 21st century progresses.

**References**


CHAPTER 1 / DRUG DISCOVERY FROM NATURAL PRODUCTS


