TWO

# Microbial Biotechnology: Scope, Techniques, Examples

One can be a good biologist without necessarily knowing much about microorganisms, but one cannot be a good microbiologist without a fair basic knowledge of biology!

– Stanier, R. Y., Doudoroff, M., and Adelberg, E. A. (1957). *The Microbial World*. p. vii, Englewood Cliffs, NJ: Prentice-Hall, Inc.

Microorganisms, whether cultured or represented only in environmental DNA samples, constitute the natural resource base of microbial biotechnology. Numerous prokaryotic and fungal genomes have been completely sequenced and the functions of many genes established. For a newly sequenced prokaryotic genome, functions for over 60% of the open reading frames can be provisionally assigned by sequence homology with genes of known function. Knowledge of the ecology, genetics, physiology, and metabolism of thousands of prokaryotes and fungi provides an indispensable complement to the sequence database.

This is an era of explosive growth of analysis and manipulation of microbial genomes as well as of invention of many new, creative ways in which both microorganisms and their genetic endowment are utilized. Microbial biotechnology is riding the crest of the wave of genomics.

The umbrella of microbial biotechnology covers many scientific activities, ranging from production of recombinant human hormones to that of microbial insecticides, from mineral leaching to bioremediation of toxic wastes. In this chapter, we sketch the complex terrain of microbial biotechnology. The purpose of this chapter is to convey the impact, the extraordinary breadth of applications, and the multidisciplinary nature of this technology. The common denominator to the subjects discussed is that in all instances, prokaryotes or fungi provide the indispensable component. Topics addressed in later chapters of this book are treated briefly. Those not described elsewhere are discussed here in some detail.

## HUMAN THERAPEUTICS

### PRODUCTION OF HETEROLOGOUS PROTEINS

One of the most dramatic and immediate impacts of genetic engineering was the production in bacteria of large amounts of proteins encoded by human genes. In 1982, insulin, expressed from human insulin genes on plasmids inserted into *Escherichia coli*, was the first genetically engineered therapeutic agent to be approved for clinical use in humans. Bacterially produced insulin, used widely in the treatment of diabetes, is indistinguishable in its structure and clinical effects from natural insulin. Human growth hormone (hGH), a protein made naturally by the pituitary gland, was the second such product. Inadequate secretion of hGH in children results in dwarfism. Before the advent of recombinant DNA technology, hGH was prepared from pituitaries removed from human cadavers. The supply of such preparations was limited and the cost prohibitive. Furthermore, there were dangers in their administration that led to withdrawal from the market. Some patients treated with injections of pituitary hGH developed a disease caused by a contaminating slow virus, Jakob–Creutzfeldt syndrome, which leads to dementia and death. hGH can be produced in genetically engineered *E. coli* in large amounts, at relatively little cost, and free from such contaminants.

Human tissue plasminogen activator (tPA), a proteolytic enzyme (a "serine" protease) with an affinity for fibrin clots, is another therapeutic agent made available in large amounts as a consequence of recombinant DNA technology. At the surface of fibrin clots, tPA cleaves a single peptide bond in plasminogen to form another serine protease, plasmin, which then degrades the clots. This clot-degrading property of tPA makes it a life-saving drug in the treatment of patients with acute myocardial infarction (damage to heart muscle due to arterial blockage).

Recombinant human insulin and hGH offered impressive proof of the clinical efficacy and safety of human proteins made by engineered microorganisms. As exemplified by the list in Table 2.1, the list of recombinant human gene products expressed in bacteria or fungi continues to grow rapidly. We devote Chapters 3 and 5 to discussion of the production of heterologous proteins and vaccines in these organisms.

### DNA VACCINES

In the early 1990s, attention focused on the potential wide-ranging opportunities offered by DNA vaccines. DNA vaccines consist of appropriately engineered plasmid DNA prepared on a large scale in *E. coli*. The obvious advantages of DNA plasmid vaccines are that they are not infectious, do not replicate, and encode only the protein(s) of interest. Unlike other types of vaccines, there is no protein component, and hence induction of an immune response against subsequent immunizations is minimized.

A vaccine plasmid includes the following major components: a strong promoter system for expression in eukaryotic cells of an antigenic protein

**TABLE 2.1 Examples of human proteins cloned in *E. coli:* their biological functions and current or envisaged therapeutic use**

| Protein | Function(s) | Therapeutic use(s) |
|---|---|---|
| $\alpha_1$-Antitrypsin | Protease inhibitor | Treatment of emphysema |
| Calcitonin | Influences $Ca^{2+}$ and phosphate metabolism | Treatment of osteomalacia |
| Colony stimulating factors | Stimulate hematopoiesis | Antitumor |
| Epidermal growth factor | Epithelial cell growth, tooth eruption | Wound healing |
| Erythropoietin | Stimulates hematopoiesis | Treatment of anemia |
| Factor VIII | Blood clotting factor | Prevention of bleeding in hemophiliacs |
| Factor IX | Blood clotting factor | Prevention of bleeding in hemophiliacs |
| Growth hormone releasing factor | Stimulates growth hormone secretion | Growth promotion |
| Interferons ($\alpha$, $\beta$,$\gamma$) | A family of 20 to 25 low molecular weight proteins that cause cells to become resistant to the growth of a wide variety of viruses | Antiviral, antitumor, anti-inflammatory |
| Interleukins 1, 2, and 3 | Stimulators of cells in the immune system | Antitumor; treatment of immune disorders |
| Lymphotoxin | A bone-resorbing factor produced by leukocytes | Antitumor |
| Somatomedin C (IGF-I) | Sulfate uptake by cartilage | Growth promotion |
| Serum albumin | Major protein constituent of plasma | Plasma supplement |
| Superoxide dismutase | Decomposes superoxide free radicals in the blood | Prevention of damage when $O_2$-rich blood enters $O_2$-deprived tissues; has applications in cardiac treatment and organ transplantation |
| Tumor necrosis factor | A product of mononuclear phagocytes cytotoxic to certain tumor cell lines | Antitumor |
| Urogastrone | Control of gastrointestinal secretion | Antiulcerative |
| Urokinase | Plasminogen activator | Anticoagulant (dissolution of blood clots) |

(e.g., a viral coat protein), the immediate early promoter of cytomegalovirus is frequently used; a cloning site for the insertion of the gene encoding the antigenic protein; and an appropriately located polyadenylation termination sequence. Most eukaryotic mRNAs contain a polyadenylate (polyA) tail at the 3′ end that appears to be important to the translation efficiency and the stability of the mRNA. The plasmid also includes a prokaryotic origin of replication for its production in *E. coli* and a selectable marker, such as the ampicillin resistance gene, to allow selection of bacterial cells that contain the plasmid.

DNA vaccines are generally introduced by intramuscular injection. It is still not known how cells internalize the DNA after the injection. The encoded antigen is then expressed *in situ* in the cells of the vaccine recipient and elicits an immune response.

Such vaccines have attractive features. The immunizing antigens may be derived from viruses, bacteria, parasites, or tumors. Antigens can be expressed singly or in multiple combinations. In one case, the DNA vaccine contained multiple variants of a highly mutable gene, for example, the gene encoding gp120, a glycoprotein located on the external surface of HIV.

Like all T cells, Th cells arise in the thymus. Th1 cells belong to the $CD4^+$ subset of lymphocytes that participate in cell-mediated immunity. They are essential for combating intracellular pathogens such as viruses and certain bacteria – for example, *Listeria monocytogenes* (causative organism of listeriosis) and *Mycobacterium tuberculosis* (the organism that causes tuberculosis).

BOX 2.1

In other vaccines, the entire genome of the infectious microorganism was introduced into a common plasmid backbone by "shotgun cloning."

DNA vaccines induce both *humoral* responses (the appearance of serum antibodies against the antigen) and *cellular* responses (activation of various T cells). These responses have been documented in animal models of disease in which protection is mediated by such responses.

Important issues remain to be resolved before DNA vaccines can take a regular place alongside other types of vaccines. In clinical trials, vaccines for malaria, hepatitis B, HIV, and influenza elicited only moderate response in human volunteers. An assessment of DNA vaccines encoding certain highly conserved influenza virus proteins concluded that there is a need for considerable enhancement of the immune response to DNA immunization before such vaccines become a promising approach for humans. Moreover, the plasmid DNA itself stimulates T helper 1 (Th1) cells and thereby might contribute to the development or worsening of Th1-mediated organ-specific autoimmunity disorders (see Box 2.1). Other potential concerns have also been identified.

## SECONDARY METABOLITES AS A SOURCE OF DRUGS

Microorganisms produce a huge number of small molecular weight compounds that are broadly described as *secondary metabolites*. A traditional approach to the discovery of new, naturally occurring bioactive molecules utilizes "screens." A screen is an assay procedure that allows testing of numerous compounds for a particular activity. Tens of thousands of secondary metabolites and other compounds have been examined for biological activity in various organisms and many have proved invaluable as *antibacterial* or *antifungal agents*, *anticancer drugs*, *immunosuppressants*, *herbicides*, *tools for research*, and the like (Table 2.2).

Genetically modified microorganisms have been engineered to produce such compounds in large amounts. Among these, antibiotics are the secondary metabolites considered among the most important to human therapeutics, and the most extensive use of screens is in the search for compounds with selective toxicity for bacteria, fungi, or protozoa. It is estimated that natural microbial antibiotics provide the starting point for over 75% of marketed antimicrobial agents. Chapter 10 is devoted to an extensive discussion of antibiotics. The three examples that follow illustrate the exceptional importance of natural products in other important therapeutic applications.

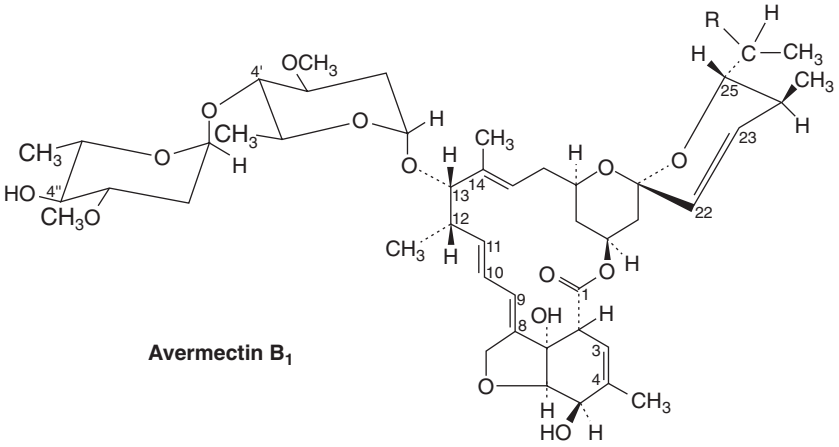
## AVERMECTINS

Many microorganisms indigenous to the soil, especially actinomycete bacteria and many fungi, produce biologically active secondary metabolites. Intensive screening of culture supernatants (usually called "fermentation broths"), rich in secondary metabolites, has led to the discovery of numerous clinically valuable antibiotics, with penicillin as the most famous example, but of many other types of valuable compounds as well. The structures of newly characterized compounds with herbicidal, insecticidal, and

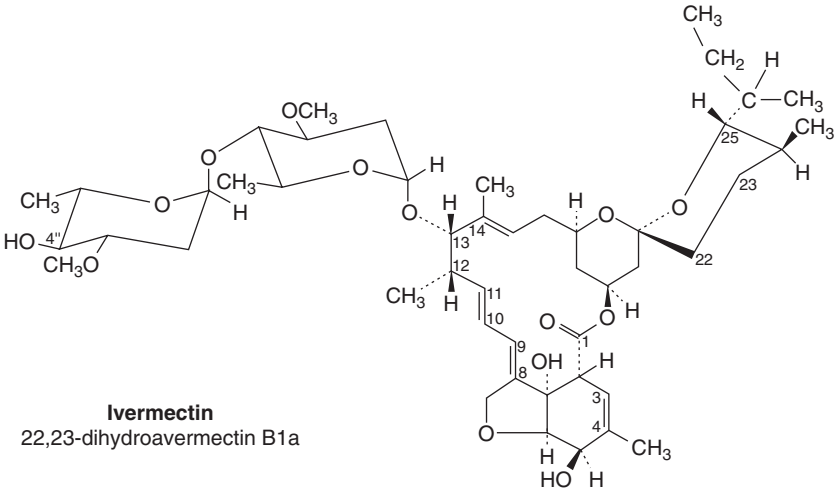
**TABLE 2.2 Bacterial and fungal secondary metabolites**

| Compound | Source organism[a] | Comments |
|---|---|---|
| Actinomycin | *Streptomyces chrysomallus* | Actinomycin, bleomycin, and griseofulvin, inhibit DNA replication |
| Bleomycin | *Streptomyces verticillus* | |
| Griseofulvin | ***Penicillium griseofulvum*** | |
| Rifamycin | *Amycolatopsis mediterranei* | Inhibits transcription by inhibiting DNA-dependent RNA polymerase. Valuable in the treatment of tuberculosis |
| Chloramphenicol | *Streptomyces venezuelae* | Chloramphenicol, tetracycline, lincomycin, and erythromycin inhibit translation by 70S ribosomes |
| Tetracycline | *Streptomyces aureofaciens* | |
| Lincomycin | *Streptomyces lincolnensis* | |
| Erythromycin | *Streptomyces erythreus* | |
| Cycloheximide | *Streptomyces griseus* | Inhibits translation by 80S ribosomes |
| Puromycin | *Streptomyces alboniger* | Puromycin and fusidic acid inhibit translation by 70S and 80S ribosomes |
| Fusidic acid | ***Acremonium fusidioides*** | |
| Cycloserine | *Streptomyces* sp. | Cycloserine, bacitracin, penicillin, cephalosporin, vancomycin, and teicoplanin inhibit peptidoglycan synthesis |
| Bacitracin | *Bacillus licheniformis* | |
| Penicillin | ***Penicillium chrysogenum*** | |
| Cephalosporin | ***Cephalosporium acremonium*** | |
| Vancomycin | *Amycolatopsis orientalis* | |
| Teicoplanin | *Actinoplanes teichomyceticus* | |
| Polymyxin | *Paenibacillus polymyxa* | Polymyxin and amphotericin are polyether surfactants that perturb cell membranes |
| Amphotericin | *Streptomyces nodosus* | |
| Gramicidin | *Bacillus brevis* | Channel-forming ionophore |
| Monensin | *Streptomyces cinnamonensis* | Mobile carrier ionophore; coccidiotic agent |
| Avermectins | *Streptomyces avermitilis* | Avermectins have high activity against helminths and arthropods |
| Clavulanic acid | *Streptomyces clavuligerus* | A penicillinase inhibitor that protects penicillin from inactivation by resistant pathogens; used in conjunction with penicillin |
| Kasugamycin | *Streptomyces kasugaensis* | Kasugamycin and polyoxins are fungicides |
| Polyoxins | *Streptomyces cacaoi* | |
| Nikkomycin | *Streptomyces tendae* | Nikkomycin and spinocins are insecticides |
| Spinosins | *Saccharopolyspora spinosa* | |
| Bialaphos | *Streptomyces hygroscopicus* | Herbicide |
| Cyclosporin A | ***Tolypocladium inflatum*** | Cyclosporin A, FK-506, and rapamycin are immunosuppressants for organ transplant recipients |
| FK-506 (tacrolimus) | *Saccharopolyspora erythrea* | |
| Rapamycin | *Streptomyces hygroscopicus* | |
| Doxorubicin | *Streptomyces peucetius* | An anticancer drug used in treating late-stage tumors |
| Ergot alkaloids | ***Claviceps purpurea*** | Uterocontractants |
| Lovastatin (mevinolin) | ***Aspergillus terreus*** | Cholesterol-lowering agent in humans and animals |
| Acarbose | *Actinoplanes* sp. | Inhibits human intestinal glucosidase |
| Gibberellins | ***Gibberella fujikuroi*** | Plant growth regulators |
| Zearalenone | ***Gibberella zeae*** | Anabolic agent used in farm animals |

[a] Fungi are shown in boldface.



**Avermectin $B_1$**



**Ivermectin**
22,23-dihydroavermectin B1a

**FIGURE 2.1**

Avermectin $B_1$. This compound is the major macrocyclic lactone produced by *Streptomyces avermitilis*. Ivermectin is a synthetic derivative of avermectin $B_1$.

nematocidal activities from soil microorganisms are described in the scientific literature at a rate of several hundred each year.

The avermectins were discovered in the early 1980s as a result of a deliberate search for antihelminthic compounds produced by soil microorganisms. Helminths are parasitic worms that infect the intestines of any animal unfortunate enough to ingest their eggs. There were two particularly notable features of the screening program. First, the microbial fermentation broths were tested by being administered in the diet to mice infested with the nematode *Nematospiroides dubius*. Nematodes are a subclass of helminths that includes roundworms or threadworms. Although such an *in vivo* assay was expensive, it simultaneously tested for efficacy of the preparation against the nematode and toxicity to the host. Second, to increase the chance of discovering new types of compounds, the selection of microorganisms for testing was biased toward those with unusual morphological traits and nutritional requirements. The morphological characteristics of *Streptomyces avermitilis*, the producer of avermectins, were unlike those of other known *Streptomyces* species. *S. avermitilis* produces a family of closely related macrocyclic lactones (Figure 2.1), compounds that are

active against certain nematodes and arthropods at extremely low doses, but have relatively low toxicity to mammals. These avermectins and their derivatives, as the compounds came to be called, are highly effective in veterinary use and in treating infestations in humans.

Avermectins act on invertebrates by activating glutamate-gated chloride channels in their nerves and muscles, disrupting pharyngeal function and locomotion. The paralyzed parasite most likely starves to death. Their selective toxicity – they do not harm vertebrates – has led to the conclusion that avermectins affect a specific cellular target either absent or inaccessible in the resistant organisms. The avermectins do not migrate in soils from the site of application and are subject to both rapid photodegradation and microbial decomposition. Consequently, avermectins are not expected to persist for a long time in the feces of treated animals. The biological activity and selective toxicity of the avermectins could not have been anticipated even if the structures of these compounds had been known.

**River Blindness (Onchocerciasis) and Lymphatic Filariasis**

Onchocerciasis, first described in 1875, is caused by a filarial nematode (*Onchocerca volvulus*), a parasite transmitted by the bite of infected blackflies of the genus *Simulium*. Onchocerciasis is a leading cause of eye disease in Africa, the Eastern Mediterranean area, and Latin America. In 2002, it was estimated that 17.7 million people were infected; of these, about 250,000 went blind and another 250,000 suffered significant visual impairment. Ivermectin kills the infectious larvae of *O. volvulus* but not the adult worms. The disease is controlled by an annual dose of IVM of 150 $\mu$g/kg.

Lymphatic filariasis is caused by the nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. The disease is endemic in most of the warm, humid regions of the world, including South America, Africa, Asia, and the Pacific Islands. The principal vectors are mosquitoes. Infections may lead to a wide variety of symptoms, including acute recurrent fever, lymphadenitis, and blood disorders. IVM controls lymphatic filariasis in a manner similar to that described for onchocerciasis.

Unexpectedly, endosymbiotic bacteria make the decisive contribution to the onset of river blindness. Bacteria of the genus *Wolbachia* are essential endosymbionts in all the pathogenic nematodes mentioned above. In humans infected with *O. volvulus*, adult worms survive for up to 14 years in subcutaneous nodules and release millions of microfilariae over this time. The microfilariae migrate through the skin and enter the eye. When some of these filariae die, the host response may result in eye inflammation that causes progressive loss of vision and ultimately leads to blindness. The host immune response plays a critical role in the inflammatory response associated with the pathogenesis of river blindness. This response is initiated by the release from the dead and degenerating worms of endotoxin-like molecules originating in the *Wolbachia* endosymbionts. Consequently, elimination of *Wolbachia* by antibiotic treatment may prevent onchocerciasis.

*Sources:* Benenson, A. S. (ed.) (1990). *Control of Communicable Diseases in Man*, 15th Edition, Washington, D.C.: American Public Health Association; Cooper, P. J., and Nutman, T. B. (2002). Onchocerciasis. *Current Treatment Options in Infectious Diseases*, 4, 327–335; Brown, R. K., Ricci, F. M., and Ottesen, E. A. (2000). Ivermectin: effectiveness in lymphatic filariasis. *Parasitology*, 121, S133–S146; Saint André, A., et al. (2002). The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. *Science*, 295, 1892–1895.

BOX 2.2

The structure of a naturally occurring small molecule with desirable biological activity is generally used as the starting point for the design and preparation of semisynthetic derivatives with improved activity, selectivity, and stability characteristics. This has proved to be the case for avermectins. Ivermectin (IVM; 22,23-dihydroavermectin $B_{1a}$, Figure 2.1), a semisynthetic derivative of avermectin $B_{1a}$, is an indispensable drug in mass treatment programs to eradicate two widespread serious diseases that affect millions of people and that are caused by nematodes: river blindness (onchocerciasis) and lymphatic filariasis (Box 2.2).

## ZARAGOZIC ACIDS (SQUALESTATINS)

Over 93% of the cholesterol in the human body is located in cells, where it performs indispensable structural and metabolic roles. The remaining 7% circulates in the plasma, where it contributes to atherosclerosis (formation of

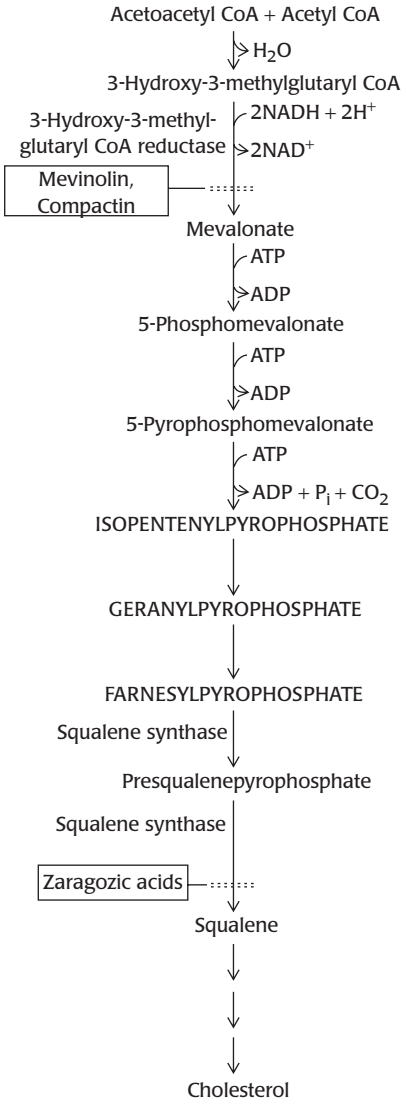



**FIGURE 2.2**

Biosynthetic pathway leading to cholesterol in humans. Isopentenyl, geranyl, and farnesyl pyrophosphate are precursors not only of sterols but also of several important isoprenoid derivatives. The fungal fermentation products mevinolin (from *Aspergillus terreus*) and compactin (from *Penicillium* spp.) are highly effective drugs used to reduce serum cholesterol in humans. These compounds are potent inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase and block formation of all products of the mammalian polyisoprenoid pathway. In contrast, the zaragozic acids inhibit squalene synthase, which catalyzes the first committed step in sterol synthesis, and do not affect the formation of other isoprenoids.

plaques on the walls of the arteries supplying the heart, the brain, and other vital organs). For delivery to tissues, plasma cholesterol is packaged in lipoprotein particles; two thirds is associated with low-density lipoprotein (LDL) and the balance with high-density lipoprotein.

The disorder *familial hypercholesterolemia* occurs in one in 500 of the population and results in elevated plasma levels of cholesterol-bearing LDL. Male heterozygotes with dominant familial hypercholesterolemia have an 85% chance of occurrence of heart attacks (myocardial infarction) before the age of 60. (Homozygotes of either sex die of heart disease at an early age). A much larger number of people, who do not have familial hypercholesterolemia, have plasma levels of LDL at the upper limit of the normal range and are also at high risk for atherosclerosis. The goal of therapy in these subjects is to reduce the level of LDL without impairing cholesterol delivery to cells. This is achieved by partial inhibition of cholesterol biosynthesis.

Cholesterol is a product of the isoprenoid pathway in mammals. In addition to cholesterol and other steroids, this pathway produces several key metabolic intermediates essential to cells – dolichol, ubiquinone, the farnesyl and geranylgeranyl moieties of prenylated proteins, and the isopentenyl side chain of isopentenyl adenine. The pathways for the synthesis of these compounds diverge from the synthesis of cholesterol either at or before the farnesyl diphosphate branch point (Figure 2.2). The first committed step in cholesterol biosynthesis is the squalene synthase–catalyzed conversion of two moles of farnesyl pyrophosphate to one mole of squalene. Therefore, squalene synthase is an attractive target for selective inhibition of cholesterol biosynthesis.

Screening of fungal cultures led to the discovery of three structurally related and very potent inhibitors of squalene synthase. Zaragozic acid A (squalestatin S1; Figure 2.3) was obtained from an unidentified fungus found in a water sample taken from the Jalon River in Zaragoza, Spain, hence the name. Soon after, zaragozic acids B and C were obtained from fungi isolated elsewhere: *Sporomiella intermedia*, a coprophilous fungus isolated from cottontail rabbit dung in Tucson, Arizona, and *Leptodontium elatius*, isolated from wood in a forest in North Carolina, respectively.

Squalene synthase catalyzes a two-step reaction. Farnesyl pyrophosphate is converted to presqualene diphosphate and then to squalene. The zaragozic acids are potent inhibitors of squalene synthase competitive with farnesyl pyrophosphate. Their inhibition constants ($K_i$s) are extraordinarily low, about $10^{-11}$ M, and they are at least $10^3$ times more potent inhibitors of the catalytic activity of squalene synthase than any previously described compound. Structural comparisons suggest that the zaragozic acids bind to squalene synthase in a manner similar to that of presqualene pyrophosphate (Figure 2.2). Experiments in laboratory animals indicate that zaragozic acids are promising therapeutic agents for hypercholesterolemia. They have also proved valuable as specific inhibitors of squalene synthase in studies of the regulation of hydroxymethylglutaryl–coenzyme A (CoA) reductase and of other aspects of lipoprotein metabolism.

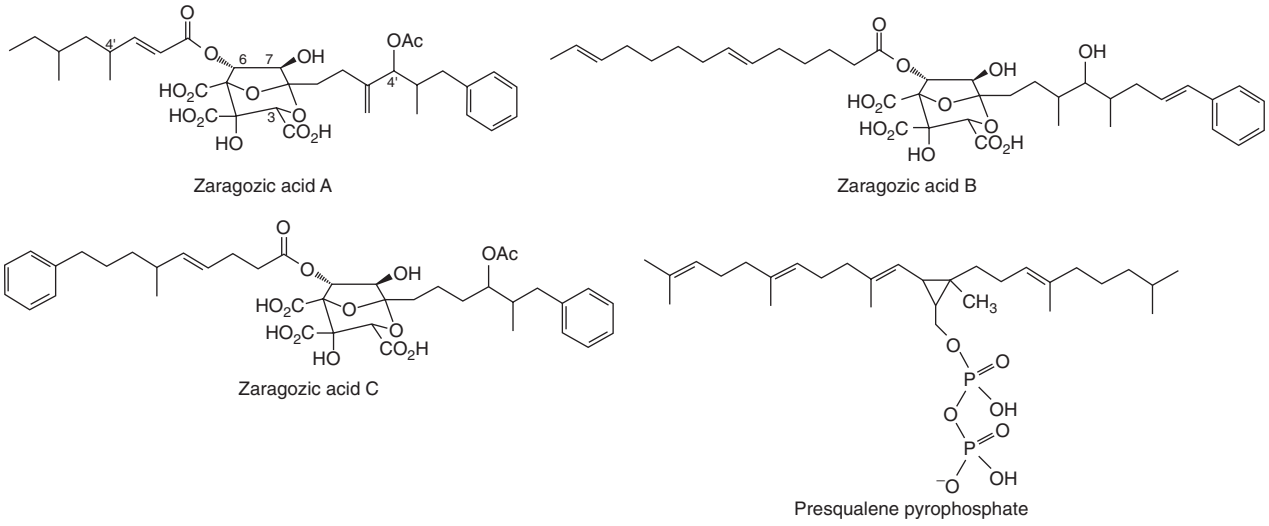



**FIGURE 2.3**
Structure of zaragozic acids and of presqualene pyrophosphate. [From Wilson, K. E., Burk, B. M., Biftu, T., Ball, R. G., and Hoogsteen, K. (1992). Zaragozic acid A, a potent inhibitor of squalene synthase: initial chemistry and absolute stereochemistry. *Journal of Organic Chemistry*, 57, 7151–7158.]

A recent study reveals that zaragozic acids have unexpected promise in other therapeutic applications. Squalestatin was shown to cure prion-infected neurons and to protect against prion neurotoxicity. Prion diseases (or transmissible spongiform encephalopathies) are fatal neurodegenerative disorders that include kuru and Creutzfeldt–Jakob disease in humans. In prion diseases, the normal cellular prion, $PrP^{c}$, is converted into a $\beta$-sheet–rich conformer, $PrP^{Sc}$, whose aggregation is believed to lead to neurodegeneration. Low concentrations of squalestatin reduced the cholesterol content of the neurons and prevented the formation of $PrP^{Sc}$. These observations suggest that squalestatin is a potential drug for the treatment of prion diseases.

## TAXOL

Microbial endophytes (bacteria and fungi) are an enormous, highly diverse component of the microbial world. Plant endophytes live in plant tissues between living plant cells but generally can be isolated and cultured independent of the host. For some endophytes, there is evidence that genetic exchange takes place in both directions between the plant and the endophyte. Such exchange raises the possibility that higher plant pathways for the synthesis of complex organic molecules that have desirable biological activities might be transferred to their endophytes.

The story of the highly effective anticancer drug taxol provides proof of the validity of this notion. Taxol, a highly substituted diterpenoid with multiple asymmetric centers (Figure 2.4) was isolated in 1965 from the Pacific yew (*Taxus brevifolia*). In human cells, taxol prevents the depolymerization of microtubules during cell division. It has the same effect in fungi. Consequently, in nature, taxol is a fungicide.

Taxol proved to be an exceptionally effective anticancer drug, and demand far exceeded the amount that could be produced from the Pacific yew

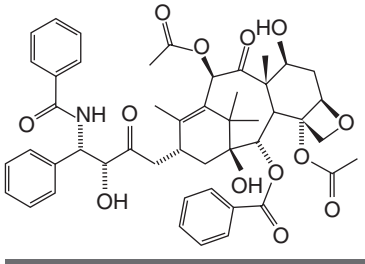


**FIGURE 2.4**
Taxol.

"When the NCI-USDA (National Cancer Institute – U.S. Department of Agriculture) screening program finally was shut down in 1981, taxol was about all the government had to show for more than 20 arduous years of sifting through natural products. From 1960 to 1981, the program had screened 114,045 plant extracts and more than 16,000 extracts from animals. Yet of all these exquisite molecules made by nature, in the rarefied air of advanced testing, taxol stood alone."

*Source:* Stephenson, F. (2002). *A Tale of Taxol*. Florida State University Office of Research http://www.research.fsu.edu/researchr/fall2002/taxol.html.

BOX 2.3

(Box 2.3). Moreover, the level at which these slow-growing trees were being utilized for taxol production threatened them with extinction. The development in 1989 of a commercially viable organic synthesis of taxol resolved the problem. In the early 2000s, a plant cell fermentation process for taxol production displaced the chemical synthesis. Here, calluses of a specific *Taxus* cell line are propagated on a simple defined medium to produce taxol.

Even so, it would be advantageous if taxol could be produced by an inexpensive microbial fermentation. The Pacific yew is not the only tree that produces taxol. This compound is in fact found in each of the world's *Taxus* species. The possibility was then explored that a taxol-producing endophyte might be discovered in a *Taxus* species. In 1993, a taxol-producing endophytic fungus, *Taxomyces andreanae*, was discovered in *T. brevifolia*. Subsequently, fungal endophytes in a wide variety of higher plants were found to make taxol. In culture, these endophytes make taxol in submicrogram per liter amounts. A great deal of work remains to be done to achieve high levels of microbial taxol production.

## AGRICULTURE

Methods dependent on microbial biotechnology greatly increase the diversity of genes that can be incorporated into crop plants and dramatically shorten the time required for the production of new varieties of plants. It is now possible to transfer foreign genes into plant cells. Transgenic plants that are viable and fertile can be regenerated from these transformed cells, and the genes that have been introduced into these transgenic plants are as stable as other genes in the plant nuclei and show a normal pattern of inheritance. Transgenic plants are most commonly generated by exploiting a plasmid vector carried by *Agrobacterium tumefaciens*, a bacterium that we discuss in detail in Chapter 6. Foreign DNA carrying from one to 50 genes can be introduced into plants in this manner, with the donor DNA originating from different plant species, animal cells, or microorganisms.

Higher plants have genes whose expression shows precise temporal and spatial regulation in various parts of plants – for example, leaves, floral organs, and seeds that appear at specific times during plant development and/or at specific locations, or whose expression is regulated by light. Other plant genes respond to different stimuli, such as plant hormones, nutrients, lack of oxygen (anaerobiosis), heat shock, and wounding. It is therefore possible to insert the control sequence(s) from such genes into transgenic plants to confine the expression of foreign genes to specific organelles or tissues and to determine the initiation and duration of such expression. Microorganisms that live on or within plants can be manipulated to control insect pests and fungal disease or to establish new symbioses, such as those between nitrogen-fixing bacteria and plants.