Chapter

13

Secondary Metabolites and Plant Defense

IN NATURAL HABITATS, plants are surrounded by an enormous number of potential enemies. Nearly all ecosystems contain a wide variety of bacteria, viruses, fungi, nematodes, mites, insects, mammals, and other herbivorous animals. By their nature, plants cannot avoid these herbivores and pathogens simply by moving away; they must protect themselves in other ways.

The cuticle (a waxy outer layer) and the periderm (secondary protective tissue), besides retarding water loss, provide barriers to bacterial and fungal entry. In addition, a group of plant compounds known as secondary metabolites defend plants against a variety of herbivores and pathogenic microbes. Secondary compounds may serve other important functions as well, such as structural support, as in the case of lignin, or pigments, as in the case of the anthocyanins.

In this chapter we will discuss some of the mechanisms by which plants protect themselves against both herbivory and pathogenic organisms. We will begin with a discussion of the three classes of compounds that provide surface protection to the plant: cutin, suberin, and waxes. Next we will describe the structures and biosynthetic pathways for the three major classes of secondary metabolites: terpenes, phenolics, and nitrogen-containing compounds. Finally, we will examine specific plant responses to pathogen attack, the genetic control of host–pathogen interactions, and cell signaling processes associated with infection.

CUTIN, WAXES, AND SUBERIN

All plant parts exposed to the atmosphere are coated with layers of lipid material that reduce water loss and help block the entry of pathogenic fungi and bacteria. The principal types of coatings are cutin, suberin, and waxes. Cutin is found on most aboveground parts; suberin is present on underground parts, woody stems, and healed wounds. Waxes are associated with both cutin and suberin.

Cutin, Waxes, and Suberin Are Made Up of Hydrophobic Compounds

Cutin is a macromolecule, a polymer consisting of many long-chain fatty acids that are attached to each other by ester linkages, creating a rigid three-dimensional network. Cutin is formed from 16:0 and 18:1 fatty acids[1] with hydroxyl or epoxide groups situated either in the middle of the chain or at the end opposite the carboxylic acid function (Figure 13.1A).

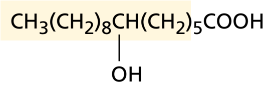
Cutin is a principal constituent of the **cuticle**, a multilayered secreted structure that coats the outer cell walls of the epidermis on the aerial parts of all herbaceous plants (Figure 13.2). The cuticle is composed of a top coating of wax, a thick middle layer containing cutin embedded in wax (the cuticle proper), and a lower layer formed of cutin and wax blended with the cell wall substances pectin, cellulose, and other carbohydrates (the cuticular layer). Recent research suggests that, in addition to cutin, the cuticle may contain a second lipid polymer, made up of long-chain hydrocarbons, that has been named *cutan* (Jeffree 1996).

Waxes are not macromolecules, but complex mixtures of long-chain acyl lipids that are extremely hydrophobic. The most common components of wax are straight-chain alkanes and alcohols of 25 to 35 carbon atoms (see Figure 13.1B). Long-chain aldehydes, ketones, esters, and free fatty acids are also found. The waxes of the cuticle are synthesized by epidermal cells. They leave the epidermal cells as droplets that pass through pores in the cell wall by an unknown mechanism. The top coating of cuticle wax often crystallizes in an intricate pattern of rods, tubes, or plates (Figure 13.3).

Suberin is a polymer whose structure is very poorly understood. Like cutin, suberin is formed from hydroxy or epoxy fatty acids joined by ester linkages. However, suberin differs from cutin in that it has dicarboxylic acids (see Figure 13.1C), more long-chain components, and a significant proportion of phenolic compounds as part of its structure.

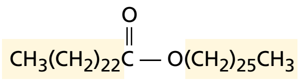
(A) Hydroxy fatty acids that polymerize to make **cutin**:

$HOCH_2(CH_2)_{14}COOH$



$CH_3(CH_2)_8CH(OH)(CH_2)_5COOH$

(B) Common **wax** components:

Straight-chain alkanes	$CH_3(CH_2)_{27}CH_3$ $CH_3(CH_2)_{29}CH_3$
Fatty acid ester	 $CH_3(CH_2)_{22}C(=O)-O(CH_2)_{25}CH_3$
Long-chain fatty acid	$CH_3(CH_2)_{22}COOH$
Long-chain alcohol	$CH_3(CH_2)_{24}CH_2OH$

(C) Hydroxy fatty acids that polymerize along with other constituents to make **suberin**:

$HOCH_2(CH_2)_{14}COOH$

$HOOC(CH_2)_{14}COOH$ (a dicarboxylic acid)

FIGURE 13.1 Constituents of (A) cutin, (B) waxes, and (C) suberin.

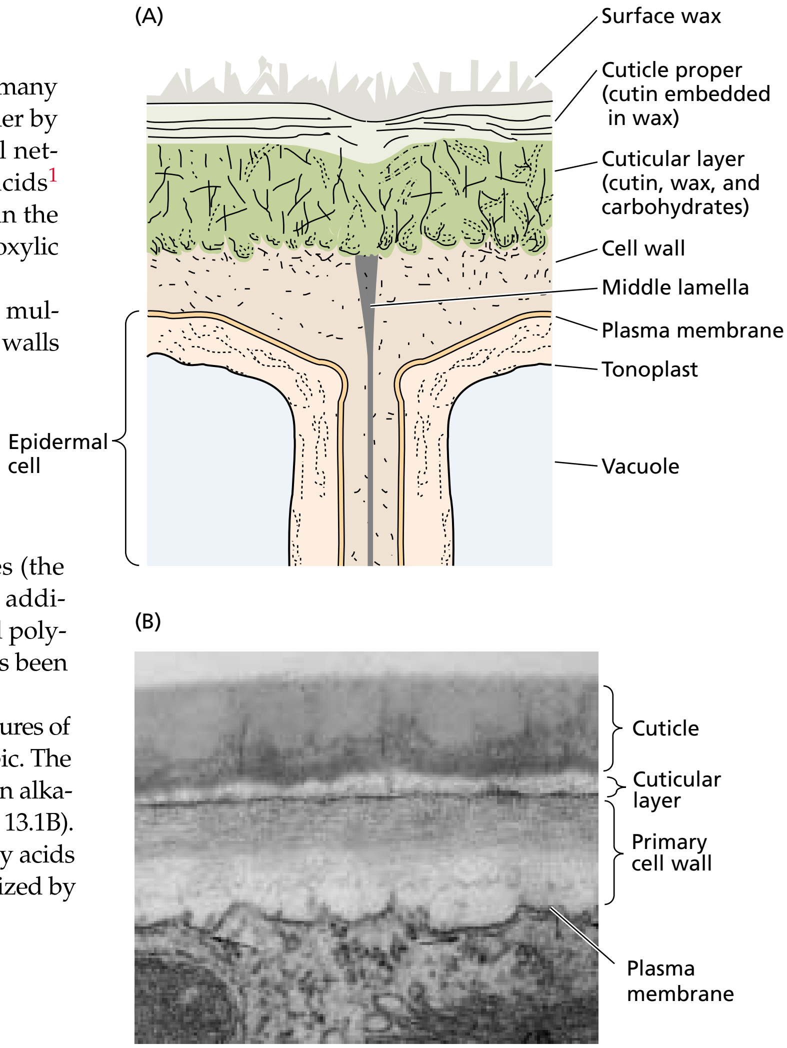


FIGURE 13.2 (A) Schematic drawing of the structure of the plant cuticle, the protective covering on the epidermis of leaves and young stems at the stage of full leaf expansion. (B) Electron micrograph of the cuticle of a glandular cell from a young leaf (*Lamium* sp.), showing the presence of the cuticle layers indicated in A, except for surface waxes, which are not visible. (51,000×) (A, after Jeffree 1996; B, from Gunning and Steer 1996.)

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[1] Recall from Chapter 11 that the nomenclature for fatty acids is X:Y, where X is the number of carbon atoms and Y is the number of *cis* double bonds.

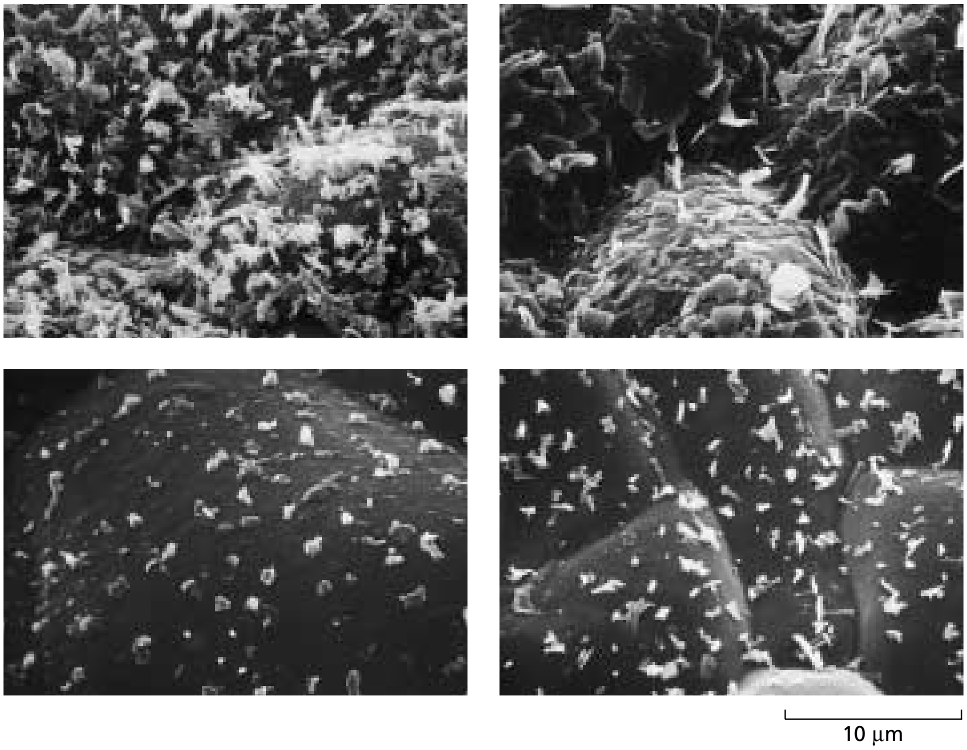


FIGURE 13.3 Surface wax deposits, which form the top layer of the cuticle, adopt different forms. These scanning electron micrographs show the leaf surfaces of two different lines of *Brassica oleracea*, which differ in wax crystal structure. (From Eigenbrode et al. 1991, courtesy of S. D. Eigenbrode, with permission from the Entomological Society of America.)

Suberin is a cell wall constituent found in many locations throughout the plant. We have already noted its presence in the Casparian strip of the root endodermis, which forms a barrier between the apoplast of the cortex and the stele (see Chapter 4). Suberin is a principal component of the outer cell walls of all underground organs and is associated with the cork cells of the **periderm**, the tissue that forms the outer bark of stems and roots during secondary growth of woody plants. Suberin also forms at sites of leaf abscission and in areas damaged by disease or wounding.

Cutin, Waxes, and Suberin Help Reduce Transpiration and Pathogen Invasion

Cutin, suberin, and their associated waxes form barriers between the plant and its environment that function to keep water in and pathogens out. The cuticle is very effective at limiting water loss from aerial parts of the plant but does not block transpiration completely because even with the stomata closed, some water is lost. The thickness of the cuticle varies with environmental conditions. Plant species native to arid areas typically have thicker cuticles than plants from moist habitats have, but plants from moist habitats often develop thick cuticles when grown under dry conditions.

The cuticle and suberized tissue are both important in excluding fungi and bacteria, although they do not appear to be as important in pathogen resistance as some of the other defenses we will discuss in this chapter. Many fungi penetrate directly through the plant surface by mechanical means. Others produce cutinase, an enzyme that hydrolyzes cutin and thus facilitates entry into the plant.

SECONDARY METABOLITES

Plants produce a large, diverse array of organic compounds that appear to have no direct function in growth and development. These substances are known as **secondary metabolites**, *secondary products*, or *natural products*. Secondary metabolites have no generally recognized, direct roles in the processes of photosynthesis, respiration, solute transport, translocation, protein synthesis, nutrient assimilation, differentiation, or the formation of carbohydrates, proteins, and lipids discussed elsewhere in this book.

Secondary metabolites also differ from primary metabolites (amino acids, nucleotides, sugars, acyl lipids) in having a restricted distribution in the plant kingdom. That is, particular secondary metabolites are often found in only one plant species or related group of species, whereas primary metabolites are found throughout the plant kingdom.

Secondary Metabolites Defend Plants against Herbivores and Pathogens

For many years the adaptive significance of most plant secondary metabolites was unknown. These compounds were thought to be simply functionless end products of metabolism, or metabolic wastes. Study of these substances was pioneered by organic chemists of the nineteenth and early twentieth centuries who were interested in these substances because of their importance as medicinal drugs, poisons, flavors, and industrial materials.

More recently, many secondary metabolites have been suggested to have important ecological functions in plants:

- They protect plants against being eaten by herbivores (herbivory) and against being infected by microbial pathogens.
- They serve as attractants for pollinators and seed-dispersing animals and as agents of plant–plant competition.

In the remainder of this chapter we will discuss the major types of plant secondary metabolites, their biosynthesis, and what is known about their functions in the plant, particularly their roles in defense.

Plant Defenses Are a Product of Evolution

We can begin by asking how plants came to have defenses. According to evolutionary biologists, plant defenses must have arisen through heritable mutations, natural selection, and evolutionary change. Random mutations in basic metabolic pathways led to the appearance of new compounds that happened to be toxic or deterrent to herbivores and pathogenic microbes.

As long as these compounds were not unduly toxic to the plants themselves and the metabolic cost of producing them was not excessive, they gave the plants that possessed them greater reproductive fitness than undefended plants had. Thus the defended plants left more descendants than undefended plants, and they passed their defensive traits on to the next generation.

Interestingly, the very defense compounds that increase the reproductive fitness of plants by warding off fungi, bacteria, and herbivores may also make them undesirable as food for humans. Many important crop plants have been artificially selected for producing relatively low levels of these compounds, which of course can make them more susceptible to insects and disease.

Secondary Metabolites Are Divided into Three Major Groups

Plant secondary metabolites can be divided into three chemically distinct groups: terpenes, phenolics, and nitrogen-containing compounds. Figure 13.4 shows in simpli-

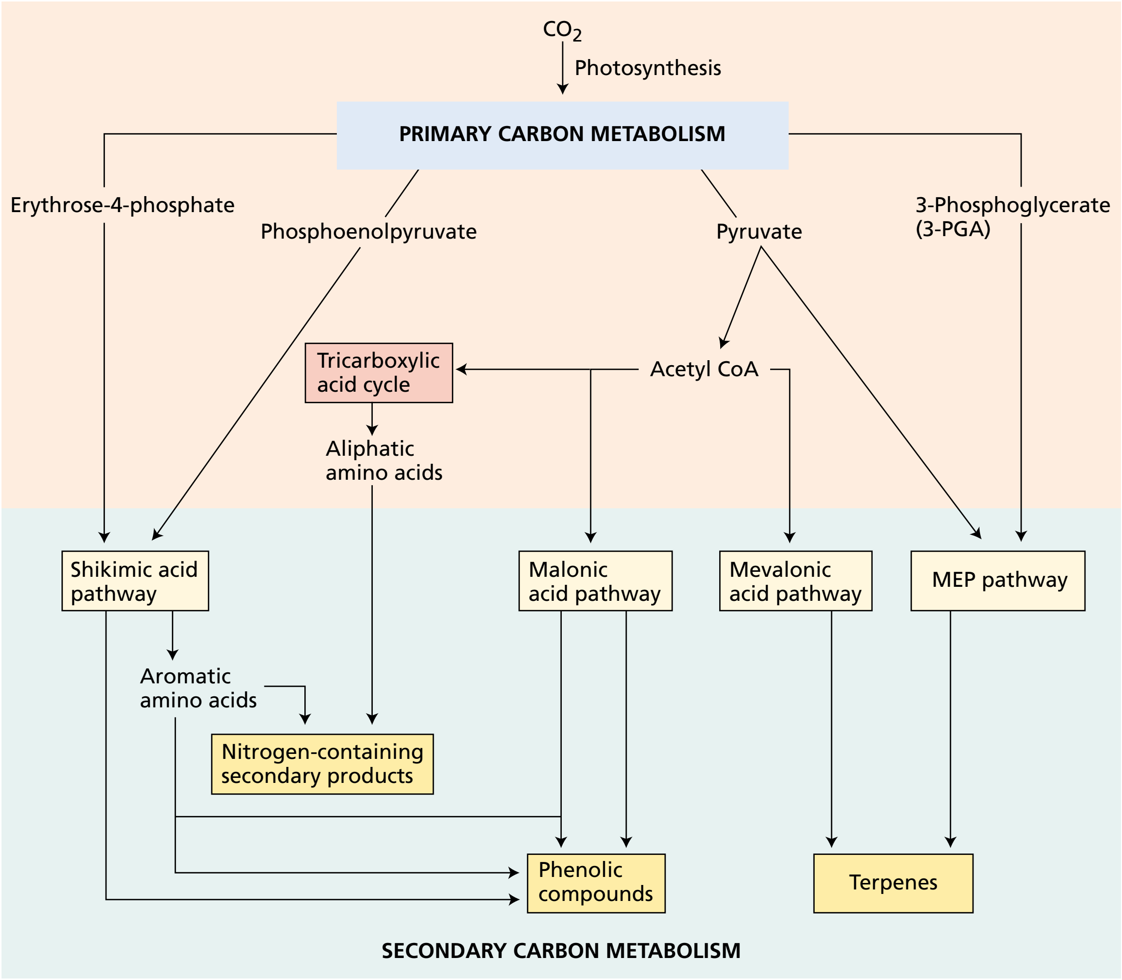


FIGURE 13.4 A simplified view of the major pathways of secondary-metabolite biosynthesis and their interrelationships with primary metabolism.

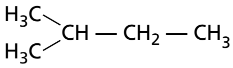
fied form the pathways involved in the biosynthesis of secondary metabolites and their interconnections with primary metabolism.

TERPENES

The **terpenes**, or *terpenoids*, constitute the largest class of secondary products. The diverse substances of this class are generally insoluble in water. They are biosynthesized from acetyl-CoA or glycolytic intermediates. After discussing the biosynthesis of terpenes, we'll examine how they act to repel herbivores and how some herbivores circumvent the toxic effects of terpenes.

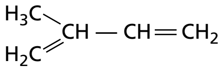
Terpenes Are Formed by the Fusion of Five-Carbon Isoprene Units

All terpenes are derived from the union of five-carbon elements that have the branched carbon skeleton of isopentane:



$$(H_3C)_2CH-CH_2-CH_3$$

The basic structural elements of terpenes are sometimes called **isoprene units** because terpenes can decompose at high temperatures to give isoprene:



$$H_3C(H_2C{=})C-CH{=}CH_2$$

Thus all terpenes are occasionally referred to as *isoprenoids*.

Terpenes are classified by the number of five-carbon units they contain, although extensive metabolic modifications can sometimes make it difficult to pick out the original five-carbon residues. Ten-carbon terpenes, which contain two C_5 units, are called *monoterpenes*; 15-carbon terpenes (three C_5 units) are *sesquiterpenes*; and 20-carbon terpenes (four C_5 units) are *diterpenes*. Larger terpenes include *triterpenes* (30 carbons), *tetraterpenes* (40 carbons), and *polyterpenoids* ($[C_5]_n$ carbons, where $n > 8$).

There Are Two Pathways for Terpene Biosynthesis

Terpenes are biosynthesized from primary metabolites in at least two different ways. In the well-studied **mevalonic acid pathway**, three molecules of acetyl-CoA are joined together stepwise to form mevalonic acid (Figure 13.5). This key six-carbon intermediate is then pyrophosphorylated, decarboxylated, and dehydrated to yield **isopentenyl diphosphate** (**IPP**[2]).

IPP is the activated five-carbon building block of terpenes. Recently, it was discovered that IPP also can be formed from intermediates of glycolysis or the photosynthetic carbon reduction cycle via a separate set of reactions called the **methylerythritol phosphate** (**MEP**) **pathway** that operates in chloroplasts and other plastids (Lichtenthaler 1999). Although all the details have not yet been elucidated, *glyceraldehyde-3-phosphate* and two carbon atoms derived from *pyruvate* appear to combine to generate an intermediate that is eventually converted to IPP.

Isopentenyl Diphosphate and Its Isomer Combine to Form Larger Terpenes

Isopentenyl diphosphate and its isomer, dimethylallyl diphosphate (DPP), are the activated five-carbon building blocks of terpene biosynthesis that join together to form larger molecules. First IPP and DPP react to give geranyl diphosphate (GPP), the 10-carbon precursor of nearly all the monoterpenes (see Figure 13.5). GPP can then link to another molecule of IPP to give the 15-carbon compound farnesyl diphosphate (FPP), the precursor of nearly all the sesquiterpenes. Addition of yet another molecule of IPP gives the 20-carbon compound geranylgeranyl diphosphate (GGPP), the precursor of the diterpenes. Finally, FPP and GGPP can dimerize to give the triterpenes (C_{30}) and the tetraterpenes (C_{40}), respectively.

Some Terpenes Have Roles in Growth and Development

Certain terpenes have a well-characterized function in plant growth or development and so can be considered primary rather than secondary metabolites. For example, the gibberellins, an important group of plant hormones, are diterpenes. Sterols are triterpene derivatives that are essential components of cell membranes, which they stabilize by interacting with phospholipids (see Chapter 11). The red, orange, and yellow carotenoids are tetraterpenes that function as accessory pigments in photosynthesis and protect photosynthetic tissues from photooxidation (see Chapter 7). The hormone abscisic acid (see Chapter 23) is a C_{15} terpene produced by degradation of a carotenoid precursor.

Long-chain polyterpene alcohols known as *dolichols* function as carriers of sugars in cell wall and glycoprotein synthesis (see Chapter 15). Terpene-derived side chains, such as the phytol side chain of chlorophyll (see Chapter 7), help anchor certain molecules in membranes. Thus various terpenes have important primary roles in plants. However, the vast majority of the different terpene structures produced by plants are secondary metabolites that are presumed to be involved in defense.

Terpenes Defend against Herbivores in Many Plants

Terpenes are toxins and feeding deterrents to many plant-feeding insects and mammals; thus they appear to play important defensive roles in the plant kingdom (Gershenzon and Croteau 1992). For example, the monoterpene esters called **pyrethroids** that occur in the leaves and flow-

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[2] IPP is the abbreviation for isopentenyl *pyro*phosphate, an earlier name for this compound. The other pyrophosphorylated intermediates in the pathway are also now referred to as *di*phosphates.

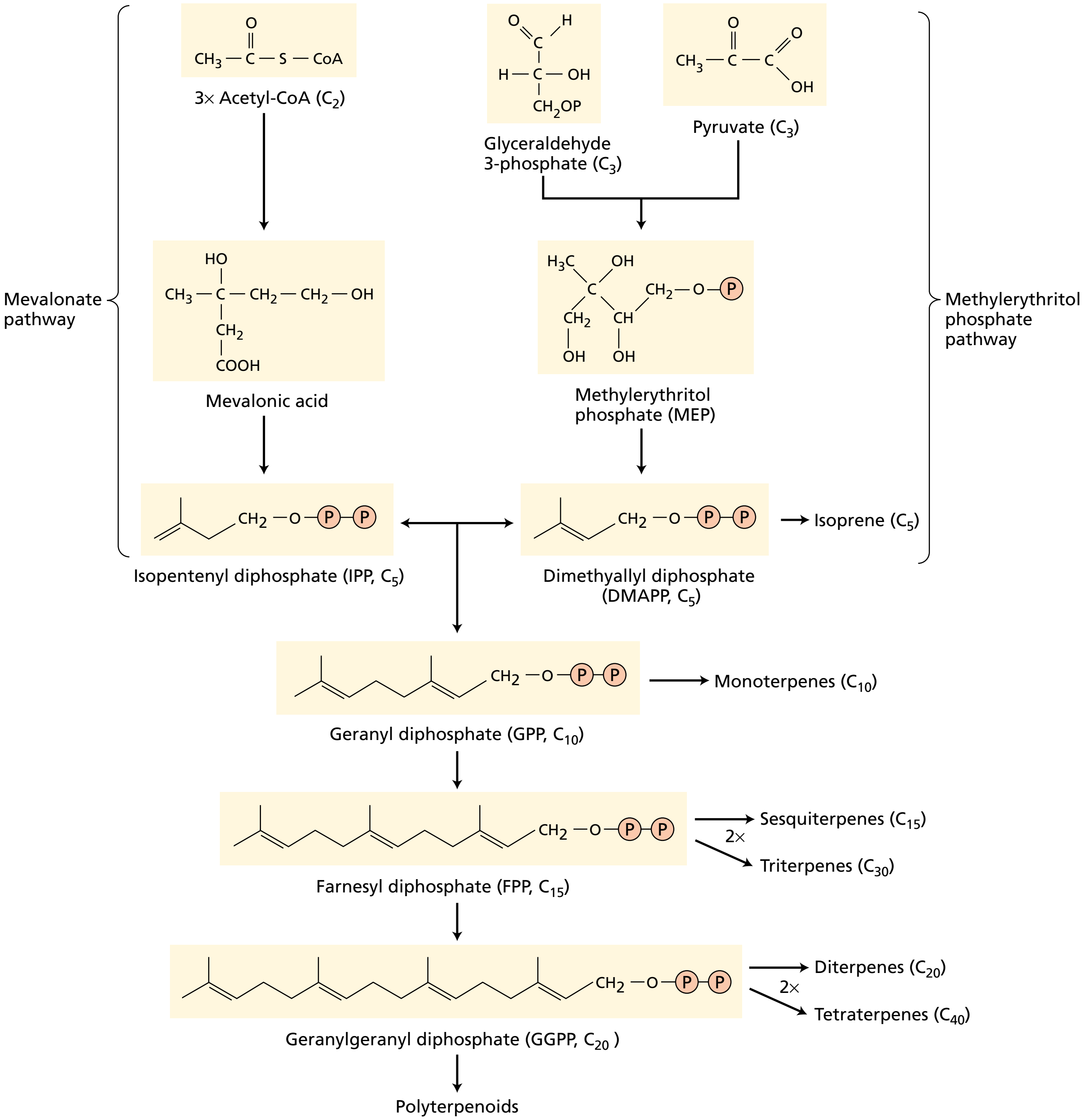


FIGURE 13.5 Outline of terpene biosynthesis. The basic 5-carbon units of terpenes are synthesized by two different pathways. The phosphorylated intermediates, IPP and DMAPP, are combined to make 10-carbon, 15-carbon and larger terpenes.

ers of *Chrysanthemum* species show very striking insecticidal activity. Both natural and synthetic pyrethroids are popular ingredients in commercial insecticides because of their low persistence in the environment and their negligible toxicity to mammals.

In conifers such as pine and fir, monoterpenes accumulate in resin ducts found in the needles, twigs, and trunk. These compounds are toxic to numerous insects, including bark beetles, which are serious pests of conifer species throughout the world. Many conifers respond to bark beetle infestation by producing additional quantities of monoterpenes (Trapp and Croteau 2001).

Many plants contain mixtures of volatile monoterpenes and sesquiterpenes, called **essential oils**, that lend a char-

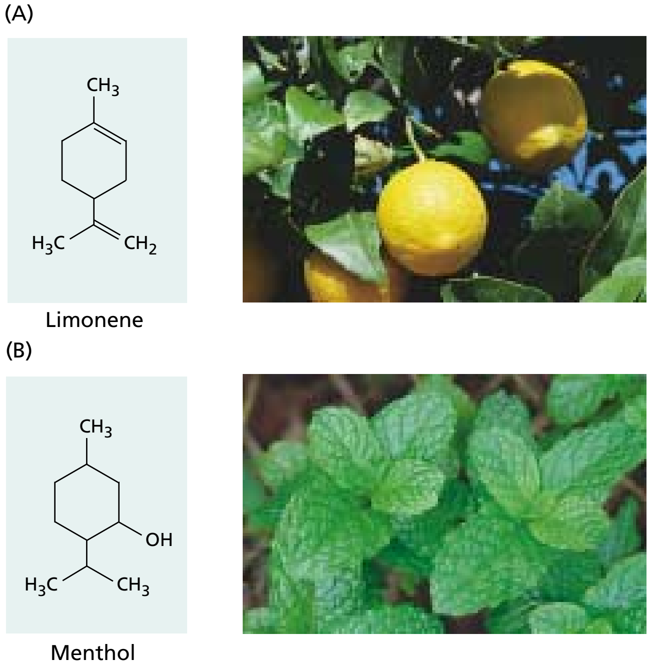


FIGURE 13.6 Structures of limonene (A) and menthol (B). These two well-known monoterpenes serve as defenses against insects and other organisms that feed on these plants. (A, photo © Calvin Larsen/Photo Researchers, Inc.; B, photo © David Sieren/Visuals Unlimited.)

acteristic odor to their foliage. Peppermint, lemon, basil, and sage are examples of plants that contain essential oils. The chief monoterpene constituent of peppermint oil is menthol; that of lemon oil is limonene (Figure 13.6).

Essential oils have well-known insect repellent properties. They are frequently found in glandular hairs that project outward from the epidermis and serve to “advertise” the toxicity of the plant, repelling potential herbivores even before they take a trial bite. In the glandular hairs, the terpenes are stored in a modified extracellular space in the cell wall (Figure 13.7). Essential oils can be extracted from plants by steam distillation and are important commercially in flavoring foods and making perfumes.

Recent research has revealed an interesting twist on the role of volatile terpenes in plant protection. In corn, cotton, wild tobacco, and other species, certain monoterpenes and sesquiterpenes are produced and emitted only after insect feeding has already begun. These substances repel ovipositing herbivores and attract natural enemies, including predatory and parasitic insects, that kill plant-feeding insects and so help minimize further damage (Turlings et al. 1995; Kessler and Baldwin 2001). Thus, volatile terpenes are not only defenses in their own right, but also provide a way for plants to call for defensive help from other organisms. The ability of plants to attract natural enemies of plant-feeding insects shows promise as a new, ecologically sound means of pest control (see **Web Essay 13.1**).

Among the nonvolatile terpene antiherbivore compounds are the **limonoids**, a group of triterpenes (C_{30}) well known as bitter substances in citrus fruit. Perhaps the most powerful deterrent to insect feeding known is *azadirachtin* (Figure 13.8A), a complex limonoid from the neem tree (*Azadirachta indica*) of Africa and Asia. Azadirachtin is a feeding deterrent to some insects at doses as low as 50 parts per billion, and it exerts a variety of toxic effects (Aerts and Mordue 1997). It has considerable potential as a commercial insect control agent because of its low toxicity to mammals, and several preparations containing azadirachtin are now being marketed in North America and India.

The **phytoecdysones**, first isolated from the common fern, *Polypodium vulgare*, are a group of plant steroids that have the same basic structure as insect molting hormones (Figure 13.8B). Ingestion of phytoecdysones by insects disrupts molting and other developmental processes, often with lethal consequences.

Triterpenes that are active against vertebrate herbivores include cardenolides and saponins. **Cardenolides** are glycosides (compounds containing an attached sugar or sugars) that taste bitter and are extremely toxic to higher animals. In humans, they have dramatic effects on the heart muscle through their influence on Na^+/K^+-activated ATPases. In carefully regulated doses, they slow and strengthen the heartbeat. Cardenolides extracted from species of foxglove

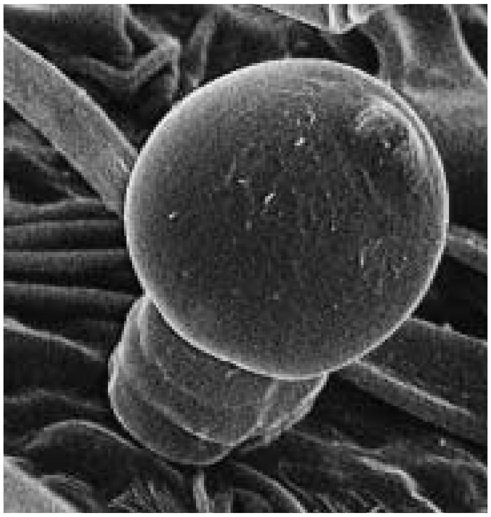
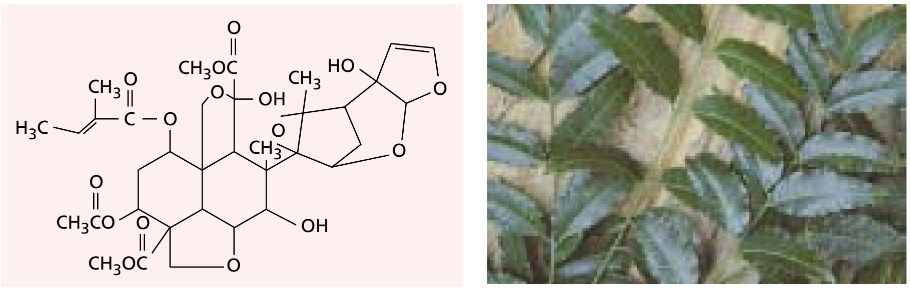


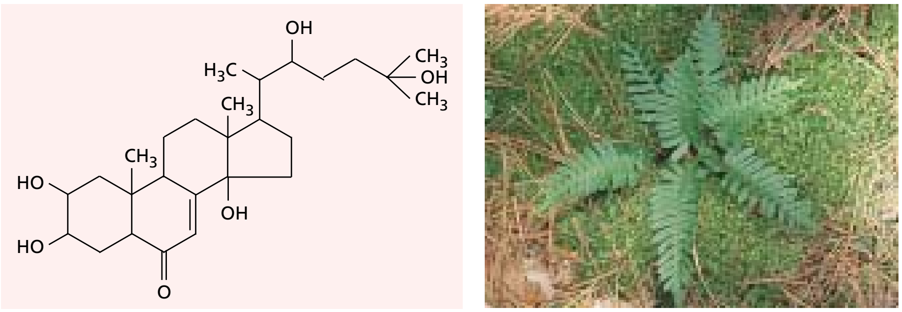
FIGURE 13.7 Monoterpenes and sesquiterpenes are commonly found in glandular hairs on the plant surface. This scanning electron micrograph shows a glandular hair on a young leaf of spring sunflower (*Balsamorhiza sagittata*). Terpenes are thought to be synthesized in the cells of the hair and are stored in the rounded cap at the top. This “cap” is an extracellular space that forms when the cuticle and a portion of the cell wall pull away from the remainder of the cell. (1105×) (© J. N. A. Lott/Biological Photo Service.)

FIGURE 13.8 Structure of two triterpenes, azadirachtin (A), and α-ecdysone (B), which serve as powerful feeding deterrents to insects. (A, photo © Inga Spence/Visuals Unlimited; B, photo ©Wally Eberhart/Visuals Unlimited.)

(A) Azadirachtin, a limonoid



(B) α-Ecdysone, an insect molting hormone

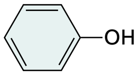


(*Digitalis*) are prescribed to millions of patients for the treatment of heart disease (see Web Topic 13.1).

Saponins are steroid and triterpene glycosides, so named because of their soaplike properties. The presence of both lipid-soluble (the steroid or triterpene) and water-soluble (the sugar) elements in one molecule gives saponins detergent properties, and they form a soapy lather when shaken with water. The toxicity of saponins is thought to be a result of their ability to form complexes with sterols. Saponins may interfere with sterol uptake from the digestive system or disrupt cell membranes after being absorbed into the bloodstream.

PHENOLIC COMPOUNDS

Plants produce a large variety of secondary products that contain a phenol group—a hydroxyl functional group on an aromatic ring:



These substances are classified as phenolic compounds. Plant **phenolics** are a chemically heterogeneous group of nearly 10,000 individual compounds: Some are soluble only in organic solvents, some are water-soluble carboxylic acids and glycosides, and others are large, insoluble polymers.

In keeping with their chemical diversity, phenolics play a variety of roles in the plant. After giving a brief account of phenolic biosynthesis, we will discuss several principal groups of phenolic compounds and what is known about their roles in the plant. Many serve as defense compounds against herbivores and pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or in reducing the growth of nearby competing plants.

Phenylalanine Is an Intermediate in the Biosynthesis of Most Plant Phenolics

Plant phenolics are biosynthesized by several different routes and thus constitute a heterogeneous group from a metabolic point of view. Two basic pathways are involved: the shikimic acid pathway and the malonic acid pathway (Figure 13.9). The shikimic acid pathway participates in the biosynthesis of most plant phenolics. The malonic acid pathway, although an important source of phenolic secondary products in fungi and bacteria, is of less significance in higher plants.

The **shikimic acid pathway** converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids (see Web Topic 13.2) (Herrmann and Weaver 1999). One of the pathway intermediates is shikimic acid, which has given its name to this whole sequence of reactions. The well-known, broad-spectrum herbicide glyphosate (available commercially as Roundup) kills plants by blocking a step in this pathway (see Chapter 2 on the web site). The shikimic acid pathway is present in plants, fungi, and bacteria but is not found in animals. Animals have no way to synthesize the three aromatic amino acids—phenylalanine, tyrosine, and tryptophan—which are therefore essential nutrients in animal diets.

The most abundant classes of secondary phenolic compounds in plants are derived from phenylalanine via the

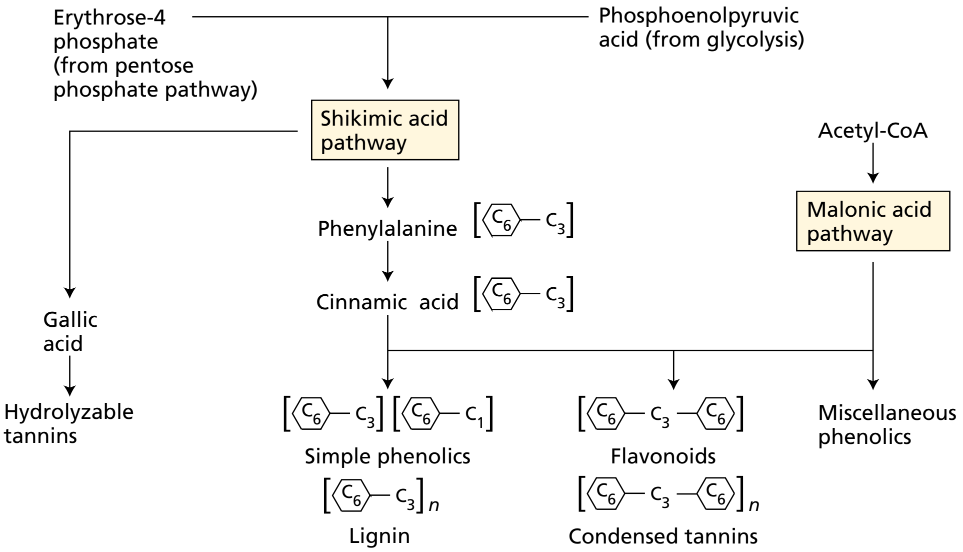


FIGURE 13.9 Plant phenolics are biosynthesized in several different ways. In higher plants, most phenolics are derived at least in part from phenylalanine, a product of the shikimic acid pathway. Formulas in brackets indicate the basic arrangement of carbon skeletons:

indicates a benzene ring, and C3 is a three-carbon chain. More detail on the pathway from phenylalanine onward is given in Figure 13.10.

elimination of an ammonia molecule to form cinnamic acid (Figure 13.10). This reaction is catalyzed by **phenylalanine ammonia lyase** (**PAL**), perhaps the most studied enzyme in plant secondary metabolism. PAL is situated at a branch point between primary and secondary metabolism, so the reaction that it catalyzes is an important regulatory step in the formation of many phenolic compounds.

The activity of PAL is increased by environmental factors, such as low nutrient levels, light (through its effect on phytochrome), and fungal infection. The point of control appears to be the initiation of transcription. Fungal invasion, for example, triggers the transcription of messenger RNA that codes for PAL, thus increasing the amount of PAL in the plant, which then stimulates the synthesis of phenolic compounds.

The regulation of PAL activity in plants is made more complex by the existence in many species of multiple PAL-encoding genes, some of which are expressed only in specific tissues or only under certain environmental conditions (Logemann et al. 1995).

Reactions subsequent to that catalyzed by PAL lead to the addition of more hydroxyl groups and other substituents. *Trans*-cinnamic acid, *p*-coumaric acid, and their derivatives are simple phenolic compounds called **phenylpropanoids** because they contain a benzene ring:

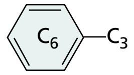
and a three-carbon side chain. Phenylpropanoids are important building blocks of the more complex phenolic compounds discussed later in this chapter.

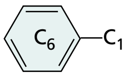
Now that the biosynthetic pathways leading to most widespread phenolic compounds have been determined, researchers have turned their attention to studying how these pathways are regulated. In some cases, specific enzymes, such as PAL, are important in controlling flux through the pathway. Several transcription factors have been shown to regulate phenolic metabolism by binding to the promoter regions of certain biosynthetic genes and activating transcription. Some of these factors activate the transcription of large groups of genes (Jin and Martin 1999).

Some Simple Phenolics Are Activated by Ultraviolet Light

Simple phenolic compounds are widespread in vascular plants and appear to function in different capacities. Their structures include the following:

- Simple phenylpropanoids, such as *trans*-cinnamic acid, *p*-coumaric acid, and their derivatives, such as caffeic acid, which have a basic phenylpropanoid carbon skeleton (Figure 13.11A):

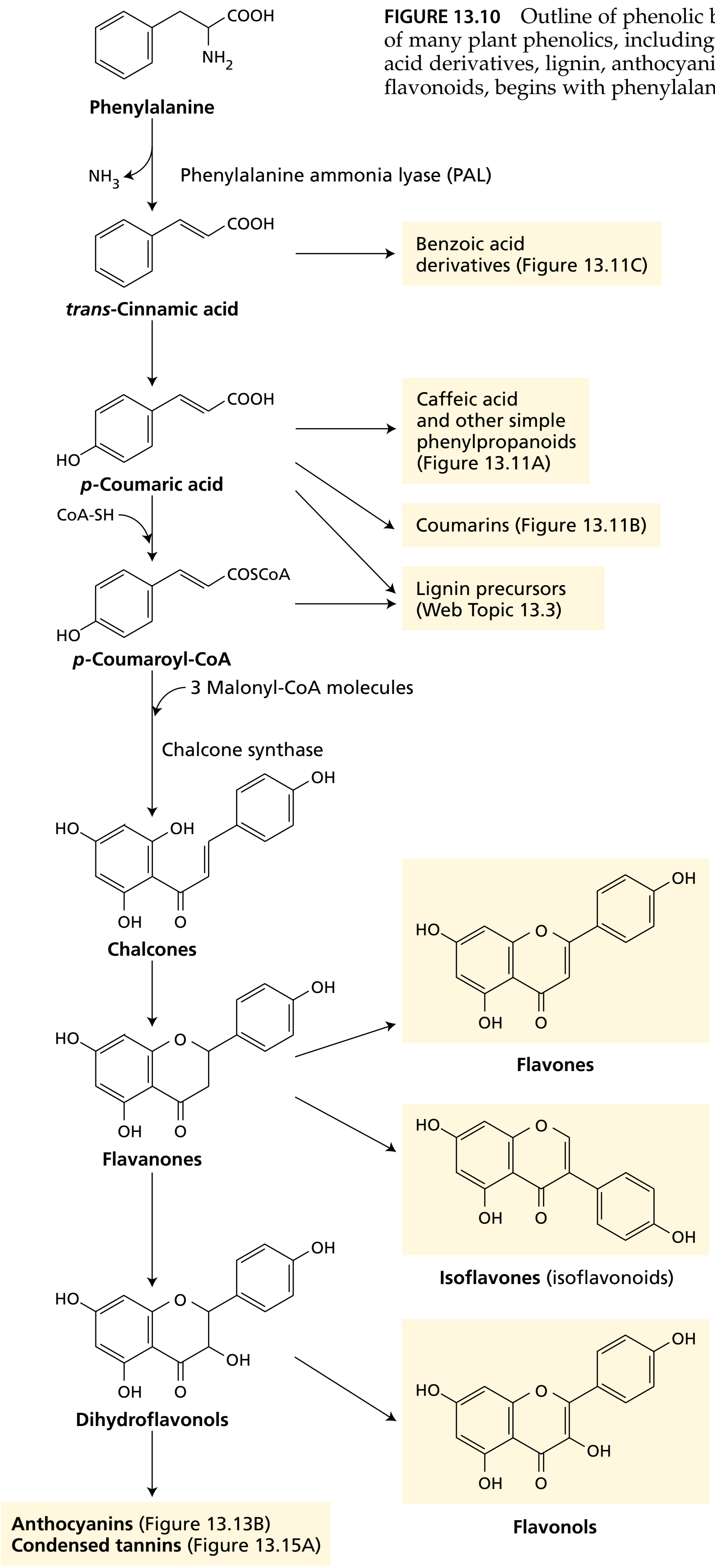


- Phenylpropanoid lactones (cyclic esters) called *coumarins*, also with a phenylpropanoid skeleton (see Figure 13.11B)
- Benzoic acid derivatives, which have a  skeleton: which is formed from phenylpropanoids by cleavage of a two-carbon fragment from the side chain (see Figure 13.11C) (see also Figure 13.10)

As with many other secondary products, plants can elaborate on the basic carbon skeleton of simple phenolic compounds to make more complex products.

Many simple phenolic compounds have important roles in plants as defenses against insect herbivores and fungi. Of special interest is the phototoxicity of certain coumarins called **furanocoumarins**, which have an attached furan ring (see Figure 13.11B).

FIGURE 13.10 Outline of phenolic biosynthesis from phenylalanine. The formation of many plant phenolics, including simple phenylpropanoids, coumarins, benzoic acid derivatives, lignin, anthocyanins, isoflavones, condensed tannins, and other flavonoids, begins with phenylalanine.



These compounds are not toxic until they are activated by light. Sunlight in the ultraviolet A (UV-A) region (320–400 nm) causes some furanocoumarins to become activated to a high-energy electron state. Activated furanocoumarins can insert themselves into the double helix of DNA and bind to the pyrimidine bases cytosine and thymine, thus blocking transcription and repair and leading eventually to cell death.

Phototoxic furanocoumarins are especially abundant in members of the Umbelliferae family, including celery, parsnip, and parsley. In celery, the level of these compounds can increase about 100-fold if the plant is stressed or diseased. Celery pickers, and even some grocery shoppers, have been known to develop skin rashes from handling stressed or diseased celery. Some insects have adapted to survive on plants that contain furanocoumarins and other phototoxic compounds by living in silken webs or rolled-up leaves, which screen out the activating wavelengths (Sandberg and Berenbaum 1989).

The Release of Phenolics into the Soil May Limit the Growth of Other Plants

From leaves, roots, and decaying litter, plants release a variety of primary and secondary metabolites into the environment. Investigation of the effects of these compounds on neighboring plants is the study of **allelopathy**. If a plant can reduce the growth of nearby plants by releasing chemicals into the soil, it may increase its access to light, water, and nutrients and thus its evolutionary fitness. Generally speaking, the term *allelopathy* has come to be applied to the harmful effects of plants on their neighbors, although a precise definition also includes beneficial effects.

Simple phenylpropanoids and benzoic acid derivatives are frequently cited as having allelopathic activity. Compounds such as caffeic acid and ferulic acid (see Figure 13.11A) occur in soil in appreciable amounts and have been shown in laboratory experiments to inhibit the germination and growth of many plants (Inderjit et al. 1995).

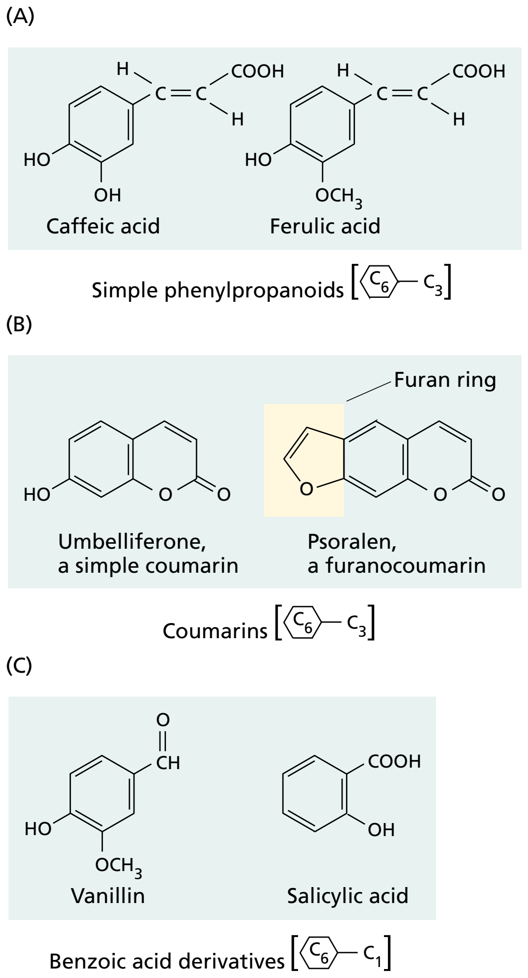


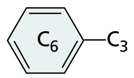
FIGURE 13.11 Simple phenolic compounds play a great diversity of roles in plants. (A) Caffeic acid and ferulic acid may be released into the soil and inhibit the growth of neighboring plants. (B) Psoralen is a furanocoumarin that exhibits phototoxicity to insect herbivores. (C) Salicylic acid is a plant growth regulator that is involved in systemic resistance to plant pathogens.

In spite of results such as these, the importance of allelopathy in natural ecosystems is still controversial. Many scientists doubt that allelopathy is a significant factor in plant–plant interactions because good evidence for this phenomenon has been hard to obtain. It is easy to show that extracts or purified compounds from one plant can inhibit the growth of other plants in laboratory experiments, but it has been very difficult to demonstrate that these compounds are present in the soil in sufficient concentration to inhibit growth. Furthermore, organic substances in the soil are often bound to soil particles and may be rapidly degraded by microbes.

In spite of the lack of supporting evidence, allelopathy is currently of great interest because of its potential agricultural applications. Reductions in crop yields caused by weeds or residues from the previous crop may in some cases be a result of allelopathy. An exciting future prospect is the development of crop plants genetically engineered to be allelopathic to weeds.

Lignin Is a Highly Complex Phenolic Macromolecule

After cellulose, the most abundant organic substance in plants is **lignin**, a highly branched polymer of phenylpropanoid groups



that plays both primary and secondary roles. The precise structure of lignin is not known because it is difficult to extract lignin from plants, where it is covalently bound to cellulose and other polysaccharides of the cell wall.

Lignin is generally formed from three different phenylpropanoid alcohols: coniferyl, coumaryl, and sinapyl, alcohols which are synthesized from phenylalanine via various cinnamic acid derivatives. The phenylpropanoid alcohols are joined into a polymer through the action of enzymes that generate free-radical intermediates. The proportions of the three monomeric units in lignin vary among species, plant organs, and even layers of a single cell wall. In the polymer, there are often multiple C—C and C—O—C bonds in each phenylpropanoid alcohol unit, resulting in a complex structure that branches in three dimensions. Unlike polymers such as starch, rubber, or cellulose, the units of lignin do not appear to be linked in a simple, repeating way. However, recent research suggests that a guiding protein may bind the individual phenylpropanoid units during lignin biosynthesis, giving rise to a scaffold that then directs the formation of a large, repeating unit (Davin and Lewis 2000; Hatfield and Vermerris 2001). (See **Web Topic 13.3** for the partial structure of a hypothetical lignin molecule.)

Lignin is found in the cell walls of various types of supporting and conducting tissue, notably the tracheids and vessel elements of the xylem. It is deposited chiefly in the thickened secondary wall but can also occur in the primary wall and middle lamella in close contact with the celluloses and hemicelluloses already present. The mechanical rigidity of lignin strengthens stems and vascular tissue, allowing upward growth and permitting water and minerals to be conducted through the xylem under negative pressure without collapse of the tissue. Because lignin is such a key component of water transport tissue, the ability to make lignin must have been one of the most important adaptations permitting primitive plants to colonize dry land.

Besides providing mechanical support, lignin has significant protective functions in plants. Its physical toughness deters feeding by animals, and its chemical durability makes it relatively indigestible to herbivores. By bonding to cellulose and protein, lignin also reduces the digestibility of these substances. Lignification blocks the growth of pathogens and is a frequent response to infection or wounding.

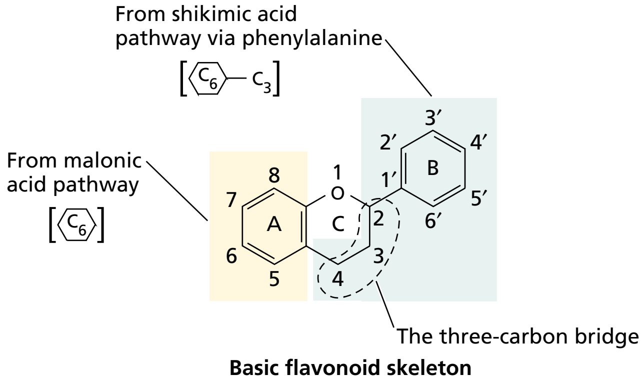


FIGURE 13.12 Basic flavonoid carbon skeleton. Flavonoids are biosynthesized from products of the shikimic acid and malonic acid pathways. Positions on the flavonoid ring system are numbered as shown.

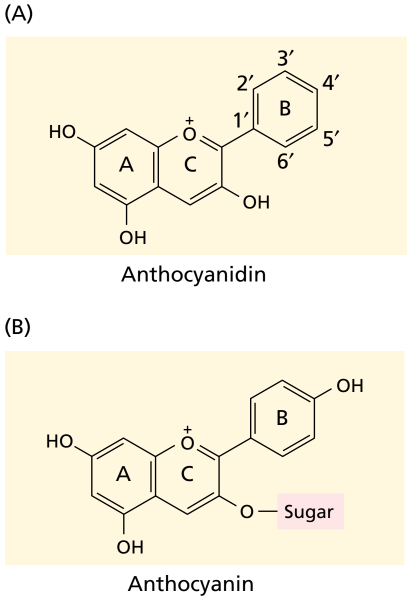
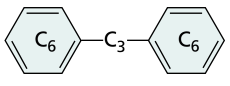


FIGURE 13.13 The structures of anthocyanidins (A) and anthocyanin (B). The colors of anthocyanidins depend in part on the substituents attached to ring B (see Table 13.1). An increase in the number of hydroxyl groups shifts absorption to a longer wavelength and gives a bluer color. Replacement of a hydroxyl group with a methoxyl group (OCH_3) shifts absorption to a slightly shorter wavelength, resulting in a redder color.

There Are Four Major Groups of Flavonoids

The **flavonoids** are one of the largest classes of plant phenolics. The basic carbon skeleton of a flavonoid contains 15 carbons arranged in two aromatic rings connected by a three-carbon bridge:



This structure results from two separate biosynthetic pathways: the shikimic acid pathway and the malonic acid pathway (Figure 13.12).

Flavonoids are classified into different groups, primarily on the basis of the degree of oxidation of the three-carbon bridge. We will discuss four of the groups shown in Figure 13.10: the anthocyanins, the flavones, the flavonols, and the isoflavones.

The basic flavonoid carbon skeleton may have numerous substituents. Hydroxyl groups are usually present at positions 4, 5, and 7, but they may also be found at other positions. Sugars are very common as well; in fact, the majority of flavonoids exist naturally as glycosides.

Whereas both hydroxyl groups and sugars increase the water solubility of flavonoids, other substituents, such as methyl ethers or modified isopentyl units, make flavonoids lipophilic (hydrophobic). Different types of flavonoids perform very different functions in the plant, including pigmentation and defense.

Anthocyanins Are Colored Flavonoids That Attract Animals

In addition to predator–prey interactions, there are mutualistic associations among plants and animals. In return for the reward of ingesting nectar or fruit pulp, animals perform extremely important services for plants as carriers of pollen and seeds. Secondary metabolites are involved in these plant–animal interactions, helping to attract animals to flowers and fruit by providing visual and olfactory signals.

The colored pigments of plants are of two principal types: carotenoids and flavonoids. *Carotenoids*, as we have already seen, are yellow, orange, and red terpenoid compounds that also serve as accessory pigments in photosynthesis (see Chapter 7). *Flavonoids* are phenolic compounds that include a wide range of colored substances.

The most widespread group of pigmented flavonoids is the **anthocyanins**, which are responsible for most of the red, pink, purple, and blue colors observed in plant parts. By coloring flowers and fruits, the anthocyanins are vitally important in attracting animals for pollination and seed dispersal.

Anthocyanins are glycosides that have sugars at position 3 (Figure 13.13B) and sometimes elsewhere. Without their sugars, anthocyanins are known as **anthocyanidins** (Figure 13.13A). Anthocyanin color is influenced by many factors, including the number of hydroxyl and methoxyl groups in ring B of the anthocyanidin (see Figure 13.13A), the presence of aromatic acids esterified to the main skeleton, and the pH of the cell vacuole in which these compounds are stored. Anthocyanins may also exist in supramolecular complexes along with chelated metal ions and flavone copigments. The blue pigment of dayflower (*Commelina communis*) was found

TABLE 13.1
Effects of ring substituents on anthocyanidin color

Anthocyanidin	Substituents	Color
Pelargonidin	4′— OH	Orange red
Cyanidin	3′— OH, 4′— OH	Purplish red
Delphinidin	3′— OH,4′— OH,5′— OH	Bluish purple
Peonidin	3′— OCH_3, 4′— OH	Rosy red
Petunidin	3′— OCH_3, 4′— OH, 5′— OCH_3	Purple

to consist of a large complex of six anthocyanin molecules, six flavones, and two associated magnesium ions (Kondo et al. 1992). The most common anthocyanidins and their colors are shown in Figure 13.13 and Table 13.1.

Considering the variety of factors affecting anthocyanin coloration and the possible presence of carotenoids as well, it is not surprising that so many different shades of flower and fruit color are found in nature. The evolution of flower color may have been governed by selection pressures for different sorts of pollinators, which often have different color preferences.

Color, of course, is just one type of signal used to attract pollinators to flowers. Volatile chemicals, particularly monoterpenes, frequently provide attractive scents.

Flavonoids May Protect against Damage by Ultraviolet Light

Two other major groups of flavonoids found in flowers are **flavones** and **flavonols** (see Figure 13.10). These flavonoids generally absorb light at shorter wavelengths than anthocyanins do, so they are not visible to the human eye. However, insects such as bees, which see farther into the ultraviolet range of the spectrum than humans do, may respond to flavones and flavonols as attractant cues (Figure 13.14). Flavonols in a flower often form symmetric patterns of stripes, spots, or concentric circles called *nectar guides* (Lunau 1992). These patterns may be conspicuous to insects and are thought to help indicate the location of pollen and nectar.

Flavones and flavonols are not restricted to flowers; they are also present in the leaves of all green plants. These two classes of flavonoids function to protect cells from excessive UV-B radiation (280–320 nm) because they accumulate in the epidermal layers of leaves and stems and absorb light strongly in the UV-B region while allowing the visible (photosynthetically active) wavelengths to pass through uninterrupted. In addition, exposure of plants to increased UV-B light has been demonstrated to increase the synthesis of flavones and flavonols.

Arabidopsis thaliana mutants that lack the enzyme chalcone synthase produce no flavonoids. Lacking flavonoids, these plants are much more sensitive to UV-B radiation than wild-type individuals are, and they grow very poorly under normal conditions. When shielded from UV light, however, they grow normally (Li et al. 1993). A group of simple phenylpropanoid esters are also important in UV protection in *Arabidopsis*.

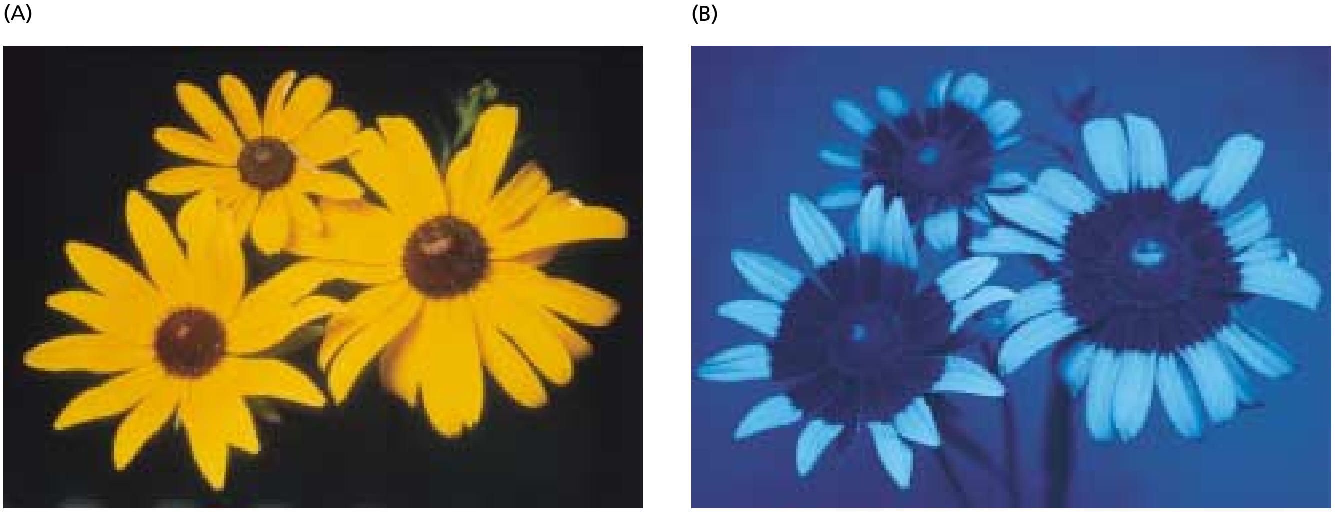


FIGURE 13.14 Black-eyed Susan (*Rudbeckia* sp.) as seen by humans (A) and as it might appear to honeybees (B). (A) To humans, the golden-eye has yellow rays and a brown central disc. (B) To bees, the tips of the rays appear "light yellow," the inner portion of the rays "dark yellow," and the central disc "black." Ultraviolet-absorbing flavonols are found in the inner parts of the rays but not in the tips. The distribution of flavonols in the rays and the sensitivity of insects to part of the UV spectrum contribute to the "bull's-eye" pattern seen by honeybees, which presumably helps them locate pollen and nectar. Special lighting was used to simulate the spectral sensitivity of the honeybee visual system. (Courtesy of Thomas Eisner.)

Other functions of flavonoids have recently been discovered. For example, flavones and flavonols secreted into the soil by legume roots mediate the interaction of legumes and nitrogen-fixing symbionts, a phenomenon described in Chapter 12. As will be discussed in Chapter 19, recent work suggests that flavonoids also play a regulatory role in plant development as modulators of polar auxin transport.

Isoflavonoids Have Antimicrobial Activity

The **isoflavonoids** (isoflavones) are a group of flavonoids in which the position of one aromatic ring (ring B) is shifted (see Figure 13.10). Isoflavonoids are found mostly in legumes and have several different biological activities. Some, such as the rotenoids, have strong insecticidal actions; others have anti-estrogenic effects. For example, sheep grazing on clover rich in isoflavonoids often suffer from infertility. The isoflavonoid ring system has a three-dimensional structure similar to that of steroids (see Figure 13.8B), allowing these substances to bind to estrogen receptors. Isoflavonoids may also be responsible for the anticancer benefits of food prepared from soybeans.

In the past few years, isoflavonoids have become best known for their role as *phytoalexins*, antimicrobial compounds synthesized in response to bacterial or fungal infection that help limit the spread of the invading pathogen. Phytoalexins are discussed in more detail later in this chapter.

Tannins Deter Feeding by Herbivores

A second category of plant phenolic polymers with defensive properties, besides lignins, is the **tannins**. The term *tannin* was first used to describe compounds that could convert raw animal hides into leather in the process known as tanning. Tannins bind the collagen proteins of animal hides, increasing their resistance to heat, water, and microbes.

There are two categories of tannins: condensed and hydrolyzable. **Condensed tannins** are compounds formed by the polymerization of flavonoid units (Figure 13.15A). They are frequent constituents of woody plants. Because condensed tannins can often be hydrolyzed to anthocyanidins by treatment with strong acids, they are sometimes called *pro-anthocyanidins*.

Hydrolyzable tannins are heterogeneous polymers containing phenolic acids, especially gallic acid, and simple sugars (see Figure 13.15B). They are smaller than condensed tannins and may be hydrolyzed more easily; only dilute acid is needed. Most tannins have molecular masses between 600 and 3000.

Tannins are general toxins that significantly reduce the growth and survivorship of many herbivores when added to their diets. In addition, tannins act as feeding repellents to a great diversity of animals. Mammals such as cattle, deer, and apes characteristically avoid plants or parts of plants with high tannin contents. Unripe fruits, for

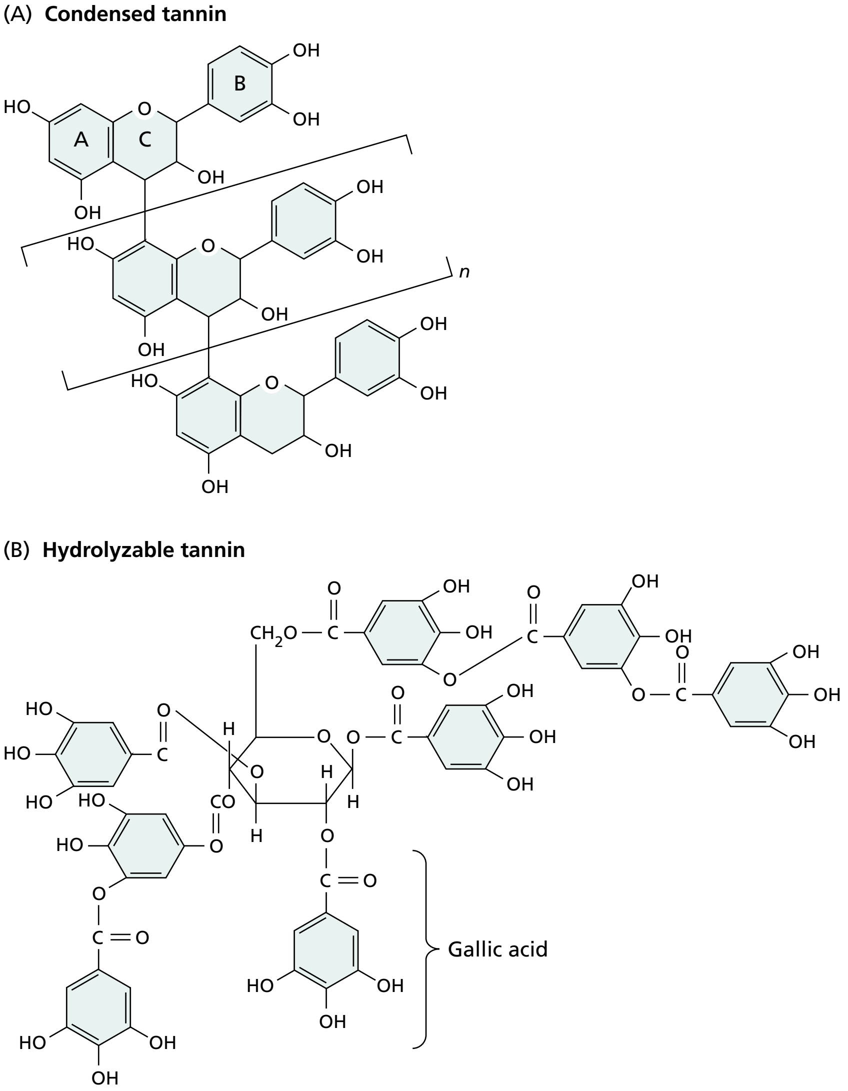


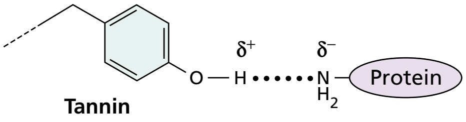
FIGURE 13.15 Structure of some tannins formed from phenolic acids or flavonoid units. (A) The general structure of a condensed tannin, where *n* is usually 1 to 10. There may also be a third —OH group on ring B. (B) The hydrolyzable tannin from sumac (*Rhus semialata*) consists of glucose and eight molecules of gallic acid.

instance, frequently have very high tannin levels, which may be concentrated in the outer cell layers.

Interestingly, humans often prefer a certain level of astringency in tannin-containing foods, such as apples, blackberries, tea, and red wine. Recently, polyphenols (tannins) in red wine were shown to block the formation of endothelin-1, a signaling molecule that makes blood vessels constrict (Corder et al. 2001). This effect of wine tannins may account for the often-touted health benefits of red wine, especially the reduction in the risk of heart disease associated with moderate red wine consumption.

Although moderate amounts of specific polyphenolics may have health benefits for humans, the defensive properties of most tannins are due to their toxicity, which is generally attributed to their ability to bind proteins nonspecifically. It has long been thought that plant tannins complex proteins in the guts of herbivores by forming hydrogen bonds between their hydroxyl groups and electronegative sites on the protein (Figure 13.16A).

(A) Hydrogen bonding between tannins and protein



(B) Covalent bonding to protein after oxidation

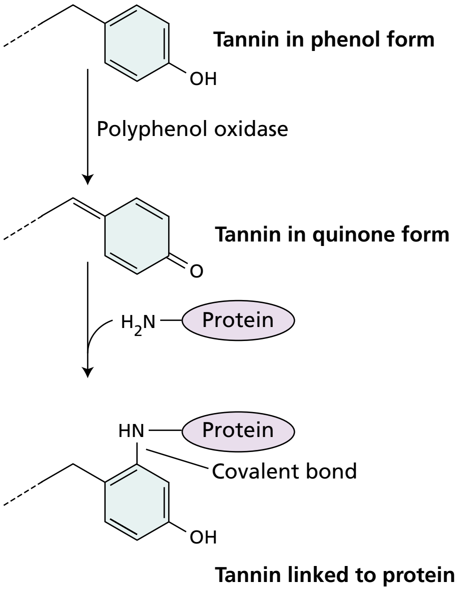


FIGURE 13.16 Proposed mechanisms for the interaction of tannins with proteins. (A) Hydrogen bonds may form between the phenolic hydroxyl groups of tannins and electronegative sites on the protein. (B) Phenolic hydroxyl groups may bind covalently to proteins following activation by oxidative enzymes, such as polyphenol oxidase.

More recent evidence indicates that tannins and other phenolics can also bind to dietary protein in a covalent fashion (see Figure 13.16B). The foliage of many plants contains enzymes that oxidize phenolics to their corresponding quinone forms in the guts of herbivores (Felton et al. 1989). Quinones are highly reactive electrophilic molecules that readily react with the nucleophilic —NH_2 and —SH groups of proteins (see Figure 13.16B). By whatever mechanism protein–tannin binding occurs, this process has a negative impact on herbivore nutrition. Tannins can inactivate herbivore digestive enzymes and create complex aggregates of tannins and plant proteins that are difficult to digest.

Herbivores that habitually feed on tannin-rich plant material appear to possess some interesting adaptations to remove tannins from their digestive systems. For example, some mammals, such as rodents and rabbits, produce salivary proteins with a very high proline content (25–45%) that have a high affinity for tannins. Secretion of these proteins is induced by ingestion of food with a high tannin content and greatly diminishes the toxic effects of tannins (Butler 1989). The large number of proline residues gives these proteins a very flexible, open conformation and a high degree of hydrophobicity that facilitates binding to tannins.

Plant tannins also serve as defenses against microorganisms. For example, the nonliving heartwood of many trees contains high concentrations of tannins that help prevent fungal and bacterial decay.

NITROGEN-CONTAINING COMPOUNDS

A large variety of plant secondary metabolites have nitrogen in their structure. Included in this category are such well-known antiherbivore defenses as alkaloids and cyanogenic glycosides, which are of considerable interest because of their toxicity to humans and their medicinal properties. Most nitrogenous secondary metabolites are biosynthesized from common amino acids.

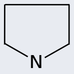
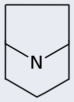
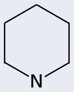
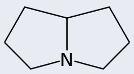
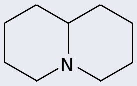
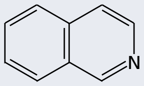
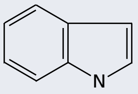
In this section we will examine the structure and biological properties of various nitrogen-containing secondary metabolites, including alkaloids, cyanogenic glycosides, glucosinolates, and nonprotein amino acids. In addition, we will discuss the ability of *systemin*, a protein released from damaged cells, to serve as a wound signal to the rest of the plant.

Alkaloids Have Dramatic Physiological Effects on Animals

The **alkaloids** are a large family of more than 15,000 nitrogen-containing secondary metabolites found in approximately 20% of the species of vascular plants. The nitrogen atom in these substances is usually part of a **heterocyclic ring**, a ring that contains both nitrogen and carbon atoms. As a group, alkaloids are best known for their striking pharmacological effects on vertebrate animals.

As their name would suggest, most alkaloids are alkaline. At pH values commonly found in the cytosol (pH 7.2)

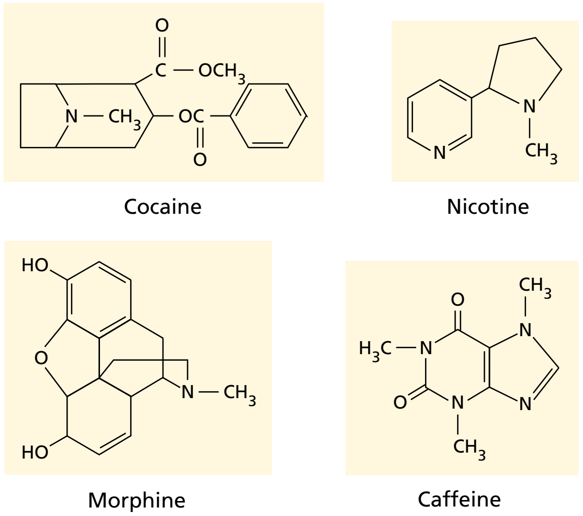
TABLE 13.2
Major types of alkaloids, their amino acid precursors, and well-known examples of each type

Alkaloid class	Structure	Biosynthetic precursor	Examples	Human uses
Pyrrolidine		Ornithine (aspartate)	Nicotine	Stimulant, depressant, tranquilizer
Tropane		Ornithine	Atropine	Prevention of intestinal spasms, antidote to other poisons, dilation of pupils for examination
			Cocaine	Stimulant of the central nervous system, local anesthetic
Piperidine		Lysine (or acetate)	Coniine	Poison (paralyzes motor neurons)
Pyrrolizidine		Ornithine	Retrorsine	None
Quinolizidine		Lysine	Lupinine	Restoration of heart rhythm
Isoquinoline		Tyrosine	Codeine	Analgesic (pain relief), treatment of coughs
			Morphine	Analgesic
Indole		Tryptophan	Psilocybin	Halucinogen
			Reserpine	Treatment of hypertension, treatment of psychoses
			Strychnine	Rat poison, treatment of eye disorders

or the vacuole (pH 5 to 6), the nitrogen atom is protonated; hence, alkaloids are positively charged and are generally water soluble.

Alkaloids are usually synthesized from one of a few common amino acids—in particular, lysine, tyrosine, and tryptophan. However, the carbon skeleton of some alkaloids contains a component derived from the terpene pathway. Table 13.2 lists the major alkaloid types and their amino acid precursors. Several different types, including nicotine and its relatives (Figure 13.17), are derived from ornithine, an intermediate in arginine biosynthesis. The B vitamin nicotinic acid (niacin) is a precursor of the pyridine (six-membered) ring of this alkaloid; the pyrrolidine (five-membered) ring of nicotine arises from ornithine (Figure 13.18). Nicotinic acid is also a constituent of NAD$^+$ and NADP$^+$, which serve as electron carriers in metabolism.

The role of alkaloids in plants has been a subject of speculation for at least 100 years. Alkaloids were once thought to be nitrogenous wastes (analogous to urea and uric acid in animals), nitrogen storage compounds, or growth regulators, but there is little evidence to support any of these functions. Most alkaloids are now believed to function as defenses against predators, especially mammals, because


Cocaine Nicotine
Morphine Caffeine

Representative alkaloids

FIGURE 13.17 Examples of alkaloids, a diverse group of secondary metabolites that contain nitrogen, usually as part of a heterocyclic ring. Caffeine is a purine-type alkaloid similar to the nucleic acid bases adenine and guanine. The pyrrolidine (five-membered) ring of nicotine arises from ornithine; the pyridine (six-membered) ring is derived from nicotinic acid.

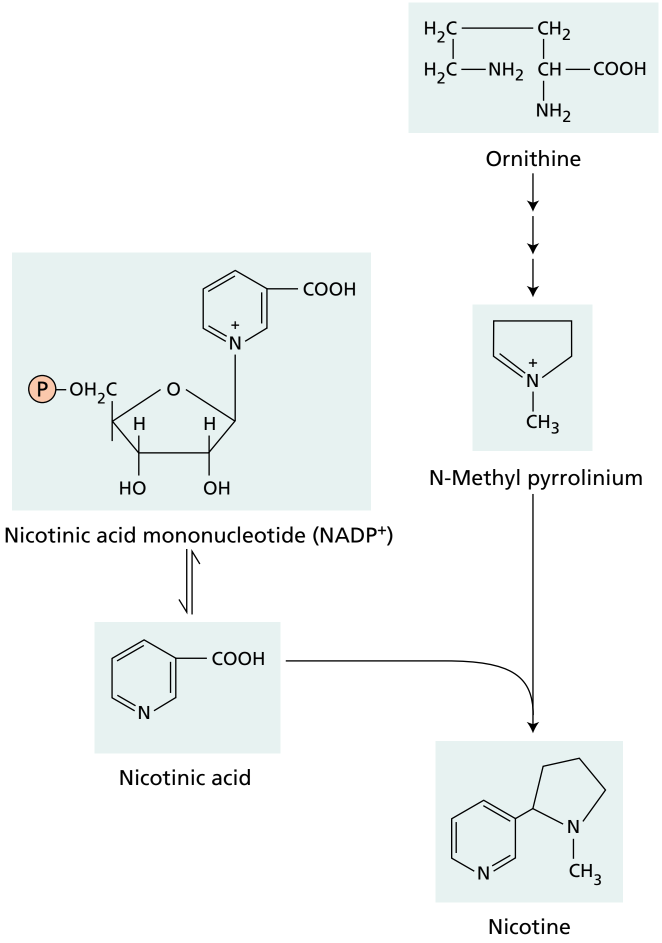


FIGURE 13.18 Nicotine biosynthesis begins with the biosynthesis of the nicotinic acid (niacin) from aspartate and glyceraldehyde-3-phosphate. Nicotinic acid is also a component of NAD$^+$ and NADP$^+$, important participants in biological oxidation–reduction reactions. The five-membered ring of nicotine is derived from ornithine, an intermediate in arginine biosynthesis.

Indeed, some livestock actually seem to prefer alkaloid-containing plants to less harmful forage.

Nearly all alkaloids are also toxic to humans when taken in sufficient quantity. For example, strychnine, atropine, and coniine (from poison hemlock) are classic alkaloid poisoning agents. At lower doses, however, many are useful pharmacologically. Morphine, codeine, and scopolamine are just a few of the plant alkaloids currently used in medicine. Other alkaloids, including cocaine, nicotine, and caffeine (see Figure 13.17), enjoy widespread nonmedical use as stimulants or sedatives.

On a cellular level, the mode of action of alkaloids in animals is quite variable. Many alkaloids interfere with components of the nervous system, especially the chemical transmitters; others affect membrane transport, protein synthesis, or miscellaneous enzyme activities.

One group of alkaloids, the pyrrolizidine alkaloids, illustrates how herbivores can become adapted to tolerate plant defensive substances and even use them in their own defense (Hartmann 1999). Within plants, pyrrolizidine alkaloids occur naturally as nontoxic N-oxides. In herbivore digestive tracts, however, they are quickly reduced to uncharged, hydrophobic tertiary alkaloids (Figure 13.19), which easily pass through membranes and are toxic. Nevertheless, some herbivores, such as cinnabar moth (*Tyria jacobeae*), have developed the ability to reconvert tertiary pyrrolizidine alkaloids to the nontoxic N-oxide form immediately after its absorption from the digestive tract. These herbivores may then store the N-oxides in their bodies as defenses against their own predators.

Not all of the alkaloids that appear in plants are produced by the plant itself. Many grasses harbor endogenous fungal symbionts that grow in the apoplast and synthesize a variety of different types of alkaloids. Grasses with fungal symbionts often grow faster and are better defended of their general toxicity and deterrence capability (Hartmann 1992).

Large numbers of livestock deaths are caused by the ingestion of alkaloid-containing plants. In the United States, a significant percentage of all grazing livestock animals are poisoned each year by consumption of large quantities of alkaloid-containing plants such as lupines (*Lupinus*), larkspur (*Delphinium*), and groundsel (*Senecio*). This phenomenon may be due to the fact that domestic animals, unlike wild animals, have not been subjected to natural selection for the avoidance of toxic plants.

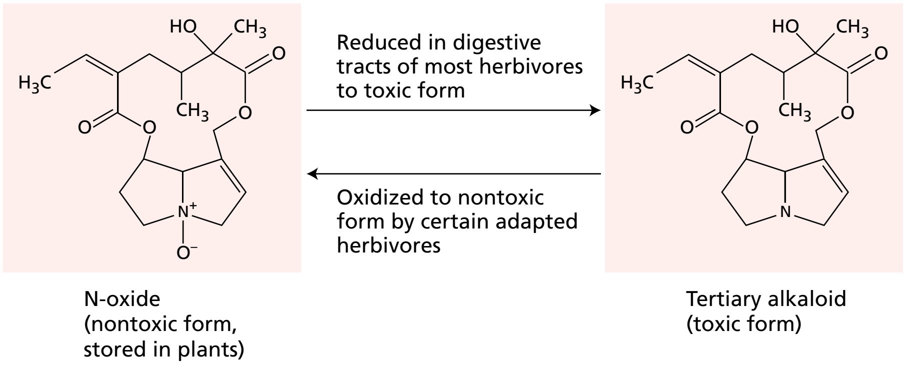


FIGURE 13.19 Two forms of pyrrolizidine alkaloids occur in nature: the N-oxide form and the tertiary alkaloid. The nontoxic N-oxide found in plants is reduced to the toxic tertiary form in the digestive tracts of most herbivores. However, some adapted herbivores can convert the toxic tertiary alkaloid back to the nontoxic N-oxide. These forms are illustrated here for the alkaloid senecionine, found in species of ragwort (*Senecio*).

against insect and mammalian herbivores than those without symbionts. Unfortunately, certain grasses with symbionts, such as tall fescue, are important pasture grasses that may become toxic to livestock when their alkaloid content is too high. Efforts are under way to breed tall fescue with alkaloid levels that are not poisonous to livestock but still provide protection against insects (see **Web Essay 13.2**).

Like monoterpenes in conifer resin and many other antiherbivore defense compounds, alkaloids increase in response to initial herbivore damage, fortifying the plant against subsequent attack (Karban and Baldwin 1997). For example, *Nicotiana attenuata,* a wild tobacco that grows in the deserts of the Great Basin, produces higher levels of nicotine following herbivory. When it is attacked by nicotine-tolerant caterpillars, however, there is no increase in nicotine. Instead, volatile terpenes are released that attract enemies of the caterpillars. Clearly, wild tobacco and other plants must have ways of determining what type of herbivore is damaging their foliage. Herbivores might signal their presence by the type of damage they inflict or the distinctive chemical compounds they release. Recently, the oral secretions of caterpillars feeding on corn leaves were shown to contain a fatty acid–amino acid conjugate that induced the plant to produce defensive terpenes when applied to cut leaves.

Cyanogenic Glycosides Release the Poison Hydrogen Cyanide

Various nitrogenous protective compounds other than alkaloids are found in plants. Two groups of these substances—cyanogenic glycosides and glucosinolates—are not in themselves toxic but are readily broken down to give off volatile poisons when the plant is crushed. Cyanogenic glycosides release the well-known poisonous gas hydrogen cyanide (HCN).

The breakdown of cyanogenic glycosides in plants is a two-step enzymatic process. Species that make cyanogenic glycosides also make the enzymes necessary to hydrolyze the sugar and liberate HCN:

1. In the first step the sugar is cleaved by a glycosidase, an enzyme that separates sugars from other molecules to which they are linked (Figure 13.20).
2. In the second step the resulting hydrolysis product, called an α-hydroxynitrile or cyanohydrin, can decompose spontaneously at a low rate to liberate HCN. This second step can be accelerated by the enzyme hydroxynitrile lyase.

Cyanogenic glycosides are not normally broken down in the intact plant because the glycoside and the degradative enzymes are spatially separated, in different cellular compartments or in different tissues. In sorghum, for example, the cyanogenic glycoside dhurrin is present in the vacuoles of epidermal cells, while the hydrolytic and lytic enzymes are found in the mesophyll (Poulton 1990).

Under ordinary conditions this compartmentation prevents decomposition of the glycoside. When the leaf is damaged, however, as during herbivore feeding, the cell contents of different tissues mix and HCN forms. Cyanogenic glycosides are widely distributed in the plant kingdom and are frequently encountered in legumes, grasses, and species of the rose family.

Considerable evidence indicates that cyanogenic glycosides have a protective function in certain plants. HCN is a fast-acting toxin that inhibits metalloproteins, such as the iron-containing cytochrome oxidase, a key enzyme of mitochondrial respiration. The presence of cyanogenic glycosides deters feeding by insects and other herbivores, such as snails and slugs. As with other classes of secondary metabolites, however, some herbivores have adapted to feed on cyanogenic plants and can tolerate large doses of HCN.

The tubers of cassava (*Manihot esculenta*), a high-carbohydrate, staple food in many tropical countries, contain high levels of cyanogenic glycosides. Traditional processing methods, such as grating, grinding, soaking, and drying, lead to the removal or degradation of a large fraction of the cyanogenic glycosides present in cassava tubers. However, chronic cyanide poisoning leading to partial paralysis of the limbs is still widespread in regions where cassava is a major food source because the traditional detoxification methods employed to remove cyanogenic glycosides from cassava are not completely effective. In addition, many populations that consume cassava have poor nutrition, which aggravates the effects of the cyanogenic glycosides.

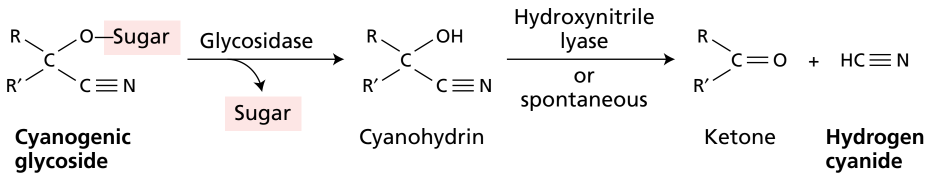


FIGURE 13.20 Enzyme-catalyzed hydrolysis of cyanogenic glycosides to release hydrogen cyanide. R and R′ represent various alkyl or aryl substituents. For example, if R is phenyl, R′ is hydrogen, and the sugar is the disaccharide β-gentiobiose, the compound is amygdalin (the common cyanogenic glycoside found in the seeds of almonds, apricots, cherries, and peaches).

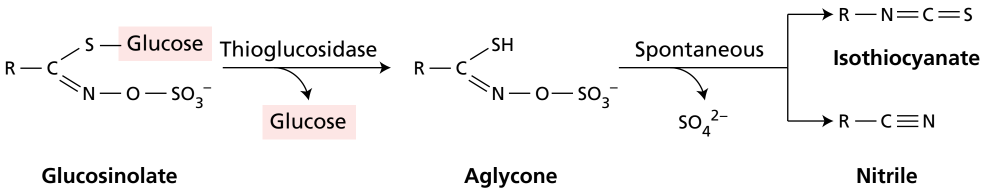


FIGURE 13.21 Hydrolysis of glucosinolates to mustard-smelling volatiles. R represents various alkyl or aryl substituents. For example, if R is $CH_2{=}CH{-}CH_2^-$, the compound is sinigrin, a major glucosinolate of black mustard seeds and horseradish roots.

Efforts are currently under way to reduce the cyanogenic glycoside content of cassava through both conventional breeding and genetic engineering approaches. However, the complete elimination of cyanogenic glycosides may not be desirable because these substances are probably responsible for the fact that cassava can be stored for very long periods of time without being attacked by pests.

Glucosinolates Release Volatile Toxins

A second class of plant glycosides, called the **glucosinolates**, or mustard oil glycosides, break down to release volatile defensive substances. Found principally in the Brassicaceae and related plant families, glucosinolates give off the compounds responsible for the smell and taste of vegetables such as cabbage, broccoli, and radishes.

The release of these mustard-smelling volatiles from glucosinolates is catalyzed by a hydrolytic enzyme, called a thioglucosidase or myrosinase, that cleaves glucose from its bond with the sulfur atom (Figure 13.21). The resulting aglycone, the nonsugar portion of the molecule, rearranges with loss of the sulfate to give pungent and chemically reactive products, including isothiocyanates and nitriles, depending on the conditions of hydrolysis. These products function in defense as herbivore toxins and feeding repellents. Like cyanogenic glycosides, glucosinolates are stored in the intact plant separately from the enzymes that hydrolyze them, and they are brought into contact with these enzymes only when the plant is crushed.

As with other secondary metabolites, certain animals are adapted to feed on glucosinolate-containing plants without ill effects. For adapted herbivores, such as the cabbage butterfly, glucosinolates often serve as stimulants for feeding and egg laying, and the isothiocyanates produced after glucosinolate hydrolysis act as volatile attractants (Renwick et al. 1992).

Most of the recent research on glucosinolates in plant defense has concentrated on rape, or canola (*Brassica napus*), a major oil crop in both North America and Europe. Plant breeders have tried to lower the glucosinolate levels of rapeseed so that the high-protein seed meal remaining after oil extraction can be used as animal food. The first low-glucosinolate varieties tested in the field were unable to survive because of severe pest problems. However, more recently developed varieties with low glucosinolate levels in seeds but high glucosinolate levels in leaves are able to hold their own against pests and still provide a protein-rich seed residue for animal feeding.

Nonprotein Amino Acids Defend against Herbivores

Plants and animals incorporate the same 20 amino acids into their proteins. However, many plants also contain unusual amino acids, called **nonprotein amino acids**, that are not incorporated into proteins but are present instead in the free form and act as protective substances. Nonprotein amino acids are often very similar to common protein amino acids. Canavanine, for example, is a close analog of arginine, and azetidine-2-carboxylic acid has a structure very much like that of proline (Figure 13.22).

Nonprotein amino acids exert their toxicity in various ways. Some block the synthesis or uptake of protein amino

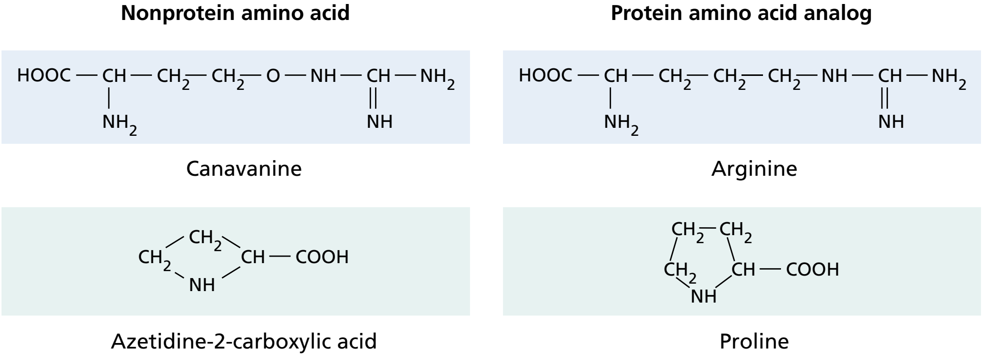


FIGURE 13.22 Nonprotein amino acids and their protein amino acid analogs. The nonprotein amino acids are not incorporated into proteins but are defensive compounds found in free form in plant cells.

acids; others, such as canavanine, can be mistakenly incorporated into proteins. After ingestion, canavanine is recognized by the herbivore enzyme that normally binds arginine to the arginine transfer RNA molecule, so it becomes incorporated into proteins in place of arginine. The usual result is a nonfunctional protein because either its tertiary structure or its catalytic site is disrupted. Canavanine is less basic than arginine and may alter the ability of an enzyme to bind substrates or catalyze chemical reactions (Rosenthal 1991).

Plants that synthesize nonprotein amino acids are not susceptible to the toxicity of these compounds. The jack bean (*Canavalia ensiformis*), which synthesizes large amounts of canavanine in its seeds, has protein-synthesizing machinery that can discriminate between canavanine and arginine, and it does not incorporate canavanine into its own proteins. Some insects that specialize on plants containing nonprotein amino acids have similar biochemical adaptations.

Some Plant Proteins Inhibit Herbivore Digestion

Among the diverse components of plant defense arsenals are proteins that interfere with herbivore digestion. For example, some legumes synthesize α-amylase inhibitors that block the action of the starch-digesting enzyme α-amylase. Other plant species produce **lectins**, defensive proteins that bind to carbohydrates or carbohydrate-containing proteins. After being ingested by an herbivore, lectins bind to the epithelial cells lining the digestive tract and interfere with nutrient absorption (Peumans and Van Damme 1995).

The best-known antidigestive proteins in plants are the proteinase inhibitors. Found in legumes, tomatoes, and other plants, these substances block the action of herbivore proteolytic enzymes. After entering the herbivore's digestive tract, they hinder protein digestion by binding tightly and specifically to the active site of protein-hydrolyzing enzymes such as trypsin and chymotrypsin. Insects that feed on plants containing proteinase inhibitors suffer reduced rates of growth and development that can be offset by supplemental amino acids in their diet.

The defensive role of proteinase inhibitors has been confirmed by experiments with transgenic tobacco. Plants that had been transformed to accumulate increased levels of proteinase inhibitors suffered less damage from insect herbivores than did untransformed control plants (Johnson et al. 1989).

Herbivore Damage Triggers a Complex Signaling Pathway

Proteinase inhibitors and certain other defenses are not continuously present in plants, but are synthesized only after initial herbivore or pathogen attack. In tomatoes, insect feeding leads to the rapid accumulation of proteinase inhibitors throughout the plant, even in undamaged areas far from the initial feeding site. The systemic production of proteinase inhibitors in young tomato plants is triggered by a complex sequence of events:

1. Wounded tomato leaves synthesize **prosystemin**, a large (200 amino acid) precursor protein.
2. Prosystemin is proteolytically processed to produce the short (18 amino acid) polypeptide called **systemin**, the first (and so far only) polypeptide hormone discovered in plants (Pearce et al. 1991) (Figure 13.23).
3. Systemin is released from damaged cells into the apoplast.
4. Systemin is then transported out of the wounded leaf via the phloem.
5. In target cells, systemin is believed to bind to a site on the plasma membrane and initiate the biosynthesis of **jasmonic acid**, a plant growth regulator that has wide-ranging effects (Creelman and Mullet 1997).
6. Jasmonic acid eventually activates the expression of genes that encode proteinase inhibitors (see Figure 13.23). Other signals, such as ABA (abscisic acid), salicylic acid, and pectin fragments from damaged plant cell walls also appear to participate in this wound-signaling cascade, but their specific roles are still unclear.

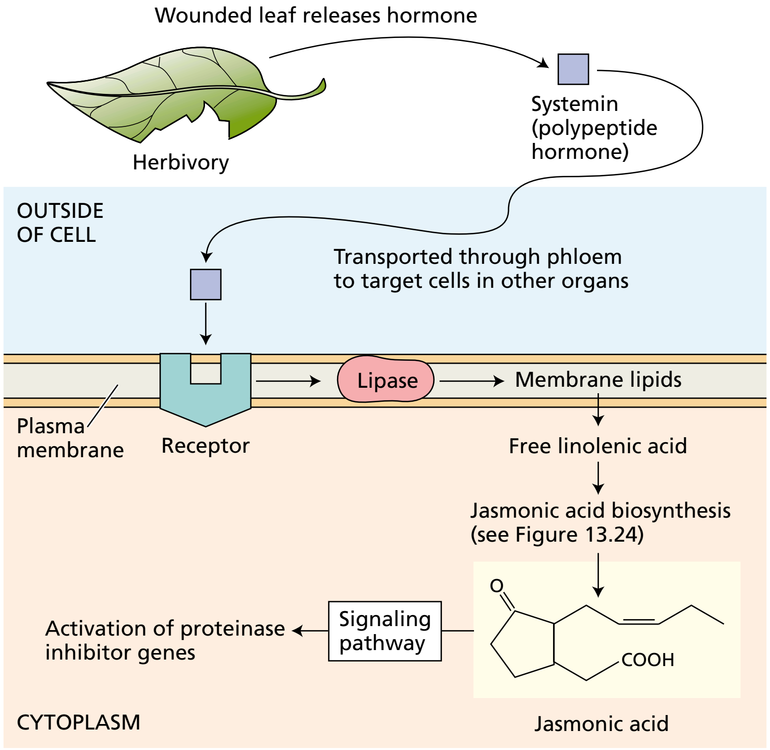


FIGURE 13.23 Proposed signaling pathway for the rapid induction of proteinase inhibitor biosynthesis in wounded tomato plants.

Jasmonic Acid Is a Plant Stress Hormone That Activates Many Defense Responses

Jasmonic acid levels rise steeply in response to damage caused by a variety of different herbivores and trigger the formation of many different kinds of plant defenses besides proteinase inhibitors, including terpenes and alkaloids. The structure and biosynthesis of jasmonic acid have intrigued plant biologists because of the parallels to some eicosanoids that are central to inflammatory responses and other physiological processes in mammals (see Chapter 14 on the web site). In plants, jasmonic acid is synthesized from linolenic acid (18:3), which is released from membrane lipids and then converted to jasmonic acid as outlined in Figure 13.24.

Jasmonic acid is known to induce the transcription of a host of genes involved in plant defense metabolism. The mechanisms for this gene activation are slowly becoming clear. For example, recent research on the Madagascar periwinkle (*Catharanthus roseus*), which makes some valuable anticancer alkaloids, identified a transcription factor that responds to jasmonic acid by activating the expression of several genes encoding alkaloid biosynthetic genes (van der Fits and Memelink 2000). Interestingly, this transcription factor also activates the genes of certain primary metabolic pathways that provide precursors for alkaloid formation, so it appears to be a master regulator of metabolism in Madagascar periwinkle.

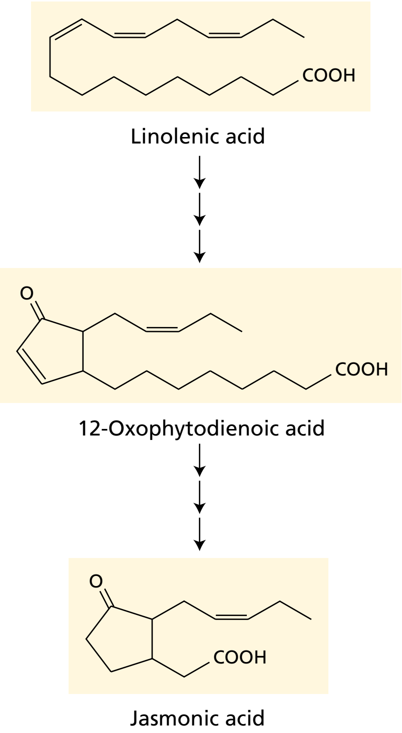


FIGURE 13.24 Steps in the pathway for conversion of linolenic acid (18:3) to jasmonic acid.

Direct demonstration of the role of jasmonic acid in insect resistance has come from research with mutant lines of *Arabidopsis* that produce only low levels of jasmonic acid (McConn et al. 1997). Such mutants are easily killed by insect pests, such as fungus gnats, that normally do not damage *Arabidopsis*. However, application of exogenous jasmonic acid can restore resistance nearly to the levels of the wild-type plant.

PLANT DEFENSE AGAINST PATHOGENS

Even though they lack an immune system, plants are surprisingly resistant to diseases caused by the fungi, bacteria, viruses, and nematodes that are ever present in the environment. In this section we will examine the diverse array of mechanisms that plants have evolved to resist infection, including the production of antimicrobial agents and a type of programmed cell death (see Chapter 16) called the *hypersensitive response*. Finally, we will discuss a special type of plant immunity called *systemic acquired resistance*.

Some Antimicrobial Compounds Are Synthesized before Pathogen Attack

Several classes of secondary metabolites that we have already discussed have strong antimicrobial activity when tested in vitro; thus they have been proposed to function as defenses against pathogens in the intact plant. Among these are saponins, a group of triterpenes thought to disrupt fungal membranes by binding to sterols.

Experiments performed in the laboratory of Anne Osbourn at the John Innes Centre (Norwich, England) utilized genetic approaches to demonstrate the role of saponins in defense against pathogens of oat (Papadopoulou et al. 1999). Mutant oat lines with reduced saponin levels had much less resistance to fungal pathogens than wild-type oats. Interestingly, one fungal strain that normally grows on oats was able to detoxify one of the principal saponins in the plant. However, mutants of this strain that could no longer detoxify saponins failed to infect oats, but could grow successfully on wheat that did not contain any saponins.

Infection Induces Additional Antipathogen Defenses

Some defenses are induced by herbivore attack or microbial infection. Defenses that are produced only after initial herbivore damage theoretically require a smaller investment of plant resources than defenses that are always present, but they must be activated quickly to be effective. Like proteinase inhibitors, other induced defenses appear to be triggered by complex signal transduction networks, which often involve jasmonic acid.

After being infected by a pathogen, plants deploy a broad spectrum of defenses against invading microbes. A common defense is the **hypersensitive response**, in which cells immediately surrounding the infection site die rapidly, depriving the pathogen of nutrients and preventing its spread. After a successful hypersensitive response, a small region of dead tissue is left at the site of the attempted invasion, but the rest of the plant is unaffected.

The hypersensitive response is often preceded by the production of **reactive oxygen species**. Cells in the vicinity of the infection synthesize a burst of toxic compounds formed by the reduction of molecular oxygen, including the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH). An NADPH-dependent oxidase located on the plasma membrane (Figure 13.25) is thought to produce $O_2^{\bullet -}$, which in turn is converted to •OH and H_2O_2.

The hydroxyl radical is the strongest oxidant of these active oxygen species and can initiate radical chain reactions with a range of organic molecules, leading to lipid peroxidation, enzyme inactivation, and nucleic acid degradation (Lamb and Dixon 1997). Active oxygen species may contribute to cell death as part of the hypersensitive response or act to kill the pathogen directly.

Many species react to fungal or bacterial invasion by synthesizing lignin or callose (see Chapter 10). These polymers are thought to serve as barriers, walling off the pathogen from the rest of the plant and physically blocking its spread. A related response is the modification of cell wall proteins. Certain proline-rich proteins of the wall become oxidatively cross-linked after pathogen attack in an H_2O_2-mediated reaction (see Figure 13.25) (Bradley et al. 1992). This process strengthens the walls of the cells in the vicinity of the infection site, increasing their resistance to microbial digestion.

Another defensive response to infection is the formation of hydrolytic enzymes that attack the cell wall of the pathogen. An assortment of glucanases, chitinases, and other hydrolases are induced by fungal invasion. Chitin, a polymer of *N*-acetylglucosamine residues, is a principal component of fungal cell walls. These hydrolytic enzymes belong to a group of proteins that are closely associated with pathogen infection and so are known as **pathogenesis-related** (**PR**) **proteins**.

Phytoalexins. Perhaps the best-studied response of plants to bacterial or fungal invasion is the synthesis of **phytoalexins**. Phytoalexins are a chemically diverse group of secondary metabolites with strong antimicrobial activity that accumulate around the site of infection.

Phytoalexin production appears to be a common mechanism of resistance to pathogenic microbes in a wide range of plants. However, different plant families employ different types of secondary products as phytoalexins. For example, isoflavonoids are common phytoalexins in the legume family, whereas in plants of the potato family (Solanaceae), such as potato, tobacco, and tomato, various sesquiterpenes are produced as phytoalexins (Figure 13.26).

Phytoalexins are generally undetectable in the plant before infection, but they are synthesized very rapidly after microbial attack because of the activation of new biosynthetic pathways. The point of control is usually the initiation of gene transcription. Thus, plants do not appear to store any of the enzymatic machinery required for phytoalexin synthesis. Instead, soon after microbial invasion

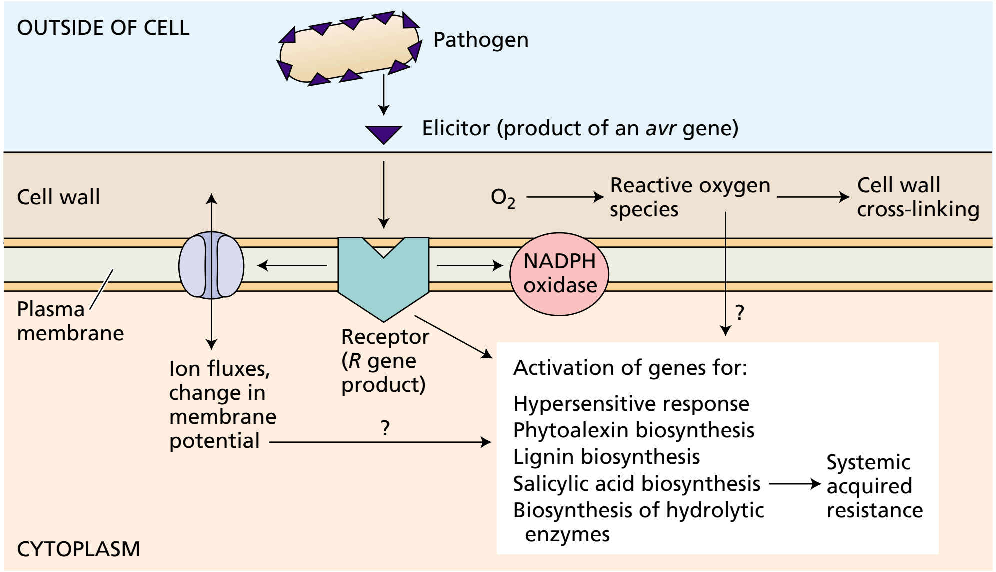
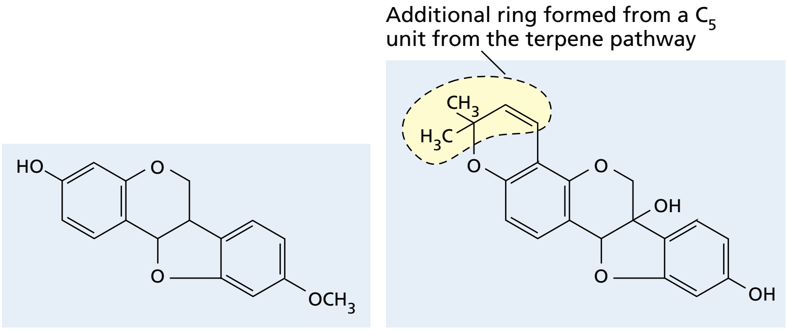


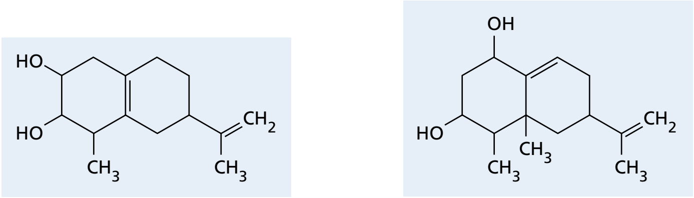
FIGURE 13.25 Many modes of antipathogen defense are induced by infection. Fragments of pathogen molecules called elicitors initiate a complex signaling pathway leading to the activation of defensive responses. Some bacterial protein elicitors are injected directly into the cell, where they interact with *R* gene products.



Medicarpin (from alfalfa)

Glyceollin I (from soybean)

Isoflavonoids from the Leguminosae (the pea family)



Rishitin (from potato and tomato)

Capsidiol (from pepper and tobacco)

Sesquiterpenes from the Solanaceae (the potato family)

FIGURE 13.26 Structure of some phytoalexins—secondary metabolites with antimicrobial properties that are rapidly synthesized after microbial infection.

they begin transcribing and translating the appropriate mRNAs and synthesizing the enzymes de novo.

Although phytoalexins accumulate in concentrations that have been shown to be toxic to pathogens in bioassays, the defensive significance of these compounds in the intact plant is not fully known. Recent experiments on genetically modified plants and pathogens have provided the first direct proof of phytoalexin function in vivo. For example, when tobacco was transformed with a gene catalyzing the biosynthesis of the phenylpropanoid phytoalexin resveratrol, it became much more resistant to a fungal pathogen than nontransformed control plants were (Hain et al. 1993). In contrast, *Arabidopsis* mutants deficient in the tryptophan-derived phytoalexin camalexin were more susceptible than the wild-type to a fungal pathogen. In other experiments, pathogens that had been transformed with genes encoding phytoalexin-degrading enzymes were then able to infect plants that were normally resistant to them (Kombrink and Somssich 1995).

Some Plants Recognize Specific Substances Released from Pathogens

Within a species, individual plants often differ greatly in their resistance to microbial pathogens. These differences often lie in the speed and intensity of a plant's reactions. Resistant plants respond more rapidly and more vigorously to pathogens than susceptible plants. Hence it is important to learn how plants sense the presence of pathogens and initiate defense.

In the last few years, researchers have isolated over 20 different plant resistance genes, known as ***R* genes**, that function in defense against fungi, bacteria, and nematodes. Most of the *R* genes are thought to encode protein receptors that recognize and bind specific molecules originating from pathogens. This binding alerts the plant to the pathogen's presence (see Figure 13.25). The specific pathogen molecules recognized are referred to as **elicitors**, and they include proteins, peptides, sterols, and polysaccharide fragments arising from the pathogen cell wall, outer membrane, or a secretion process (Boller 1995).

The *R* gene products themselves are nearly all proteins with a leucine-rich domain that is repeated inexactly several times in the amino acid sequence (see Chapter 14 on the website). Such domains may be involved in elicitor binding and pathogen recognition. In addition, the *R* gene product is equipped to initiate signaling pathways that activate the various modes of antipathogen defense. Some *R* genes encode a nucleotide-binding site that binds ATP or GTP; others encode a protein kinase domain (Young 2000).

R gene products are distributed in more than one place in the cell. Some appear to be situated on the outside of the plasma membrane, where they could rapidly detect elicitors; others are cytoplasmic to detect either pathogen molecules that are injected into the cell or other metabolic changes indicating pathogen infection. *R* genes constitute one of the largest gene families in plants and are often clustered together in the genome. The structures of *R* gene clusters may help generate *R* gene diversity by promoting exchange between chromosomes.

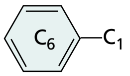
Studies of plant disease have revealed complex patterns of host relationships between plants and pathogen strains. Plant species are generally susceptible to the attack of certain pathogen strains, but resistant to others. This specificity is thought to be determined by interaction between the products of host *R* genes and pathogen ***avr*** **(*avir*ulence) genes** believed to encode specific elicitors. According to current thinking, successful resistance requires the elicitor, a product of the pathogen *avr* gene, to be rapidly recognized by a host plant receptor, the product of an *R* gene. Despite their name, *avr* genes appear to encode factors that promote infection.

Exposure to Elicitors Induces a Signal Transduction Cascade

Within a few minutes after pathogen elicitors have been recognized by an R gene, complex signaling pathways are set in motion that lead eventually to defense responses (see Figure 13.25). A common early element of these cascades is a transient change in the ion permeability of the plasma membrane. *R* gene activation stimulates an influx of Ca^{2+} and H^+ ions into the cell and an efflux of K^+ and Cl^- ions (Nürnberger and Scheel 2001). The influx of Ca^{2+} activates the oxidative burst that may act directly in defense (as already described), as well as signaling other defense reactions. Other components of pathogen-stimulated signal transduction pathways include nitric oxide, mitogen-activated protein (MAP) kinases, calcium-dependent protein kinases, jasmonic acid, and salicylic acid (see the next section).

A Single Encounter with a Pathogen May Increase Resistance to Future Attacks

When a plant survives the infection of a pathogen at one site, it often develops increased resistance to subsequent attacks at sites throughout the plant and enjoys protection against a wide range of pathogen species. This phenomenon, called **systemic acquired resistance** (**SAR**), develops over a period of several days following initial infection (Ryals et al. 1996). Systemic acquired resistance appears to result from increased levels of certain defense compounds that we have already mentioned, including chitinases and other hydrolytic enzymes.

Although the mechanism of SAR induction is still unknown, one of the endogenous signals is likely to be **salicylic acid**. The level of this benzoic acid derivative, a  compound rises dramatically in the zone of infection after initial attack, and it is thought to establish SAR in other parts of the plant, although salicylic acid itself is not the mobile signal (Figure 13.27).

In addition to salicylic acid, recent studies suggest that its methyl ester, methyl salicylate, acts as a volatile SAR-inducing signal transmitted to distant parts of the plant and even to neighboring plants (Shulaev et al. 1997). Thus, even though plants lack immune systems like those present in many animals, they have developed elaborate mechanisms to protect themselves from disease-causing microbes.

SUMMARY

Plants produce an enormous diversity of substances that have no apparent roles in growth and development processes and so are classified under the heading of secondary metabolites. Scientists have long speculated that these compounds protect plants from predators and pathogens on the basis of their toxicity and repellency to herbivores and microbes when tested in vitro. Recent experiments on plants whose secondary-metabolite expression has been altered by modern molecular methods have begun to confirm these defensive roles.

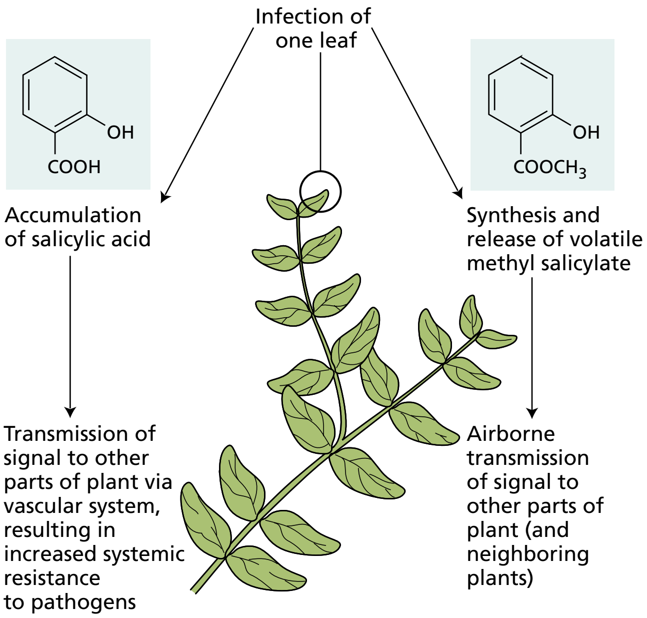


FIGURE 13.27 Initial pathogen infection may increase resistance to future pathogen attack through development of systemic acquired resistance.

There are three major groups of secondary metabolites: terpenes, phenolics, and nitrogen-containing compounds. Terpenes, composed of five-carbon isoprene units, are toxins and feeding deterrents to many herbivores.

Phenolics, which are synthesized primarily from products of the shikimic acid pathway, have several important roles in plants. Lignin mechanically strengthens cell walls. Flavonoid pigments function as shields against harmful ultraviolet radiation and as attractants for pollinators and fruit dispersers. Finally, lignin, flavonoids, and other phenolic compounds serve as defenses against herbivores and pathogens.

Members of the third major group, nitrogen-containing secondary metabolites, are synthesized principally from common amino acids. Compounds such as alkaloids, cyanogenic glycosides, glucosinolates, nonprotein amino acids, and proteinase inhibitors protect plants from a variety of herbivorous animals.

Plants have evolved multiple defense mechanisms against microbial pathogens. Besides antimicrobial secondary metabolites, some of which are preformed and some of which are induced by infection, other modes of defense include the construction of polymeric barriers to pathogen penetration and the synthesis of enzymes that degrade pathogen cell walls. In addition, plants employ specific recognition and signaling systems enabling the rapid detection of pathogen invasion and initiation of a vigorous defensive response. Once infected, some plants also develop an immunity to subsequent microbial attacks.

For millions of years, plants have produced defenses against herbivory and microbial attack. Well-defended plants have tended to leave more survivors than poorly defended plants, so the capacity to produce effective defensive products has become widely established in the plant kingdom. In response, many species of herbivores and microbes have evolved the ability to feed on or infect plants containing secondary products without being adversely affected, and this herbivore and pathogen pressure has in turn selected for new defensive products in plants.

The study of plant secondary metabolites has many practical applications. By virtue of their biological activities against herbivorous animals and microbes, many of these substances are employed commercially as insecticides, fungicides, and pharmaceuticals, while others find uses as fragrances, flavorings, medicinal drugs, and industrial materials. The breeding of increased levels of secondary metabolites into crop plants has made it possible to reduce the need for certain costly and potentially harmful pesticides. In some cases, however, it has been necessary to reduce the levels of naturally occurring secondary metabolites to minimize toxicity to humans and domestic animals.

Web Material

Web Topics

13.1 Structure of Various Triterpenes

The structures of several triterpenes are given.

13.2 The Shikimic Acid Pathway

The biochemical pathway for the synthesis of aromatic amino acids, the precursors of phenolic compounds, is presented.

13.3 Detailed Chemical Structure of a Portion of a Lignin Molecule

The partial structure of a hypothetical lignin molecule from European beech (*Fagus sylvatica)* is described.

Web Essays

13.1 Unraveling the Function of Secondary Metabolites

Wild tobacco plants use alkaloids and terpenes to defend themselves against herbivores.

13.2 Alkaloid-Making Fungal Symbionts

Fungal endophytes can enhance plant growth, increase resistance to various stresses, and act as "defensive mutualists" against herbivores.

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