

# APPENDIX 4

## Secondary Metabolites

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. Their first line of defense involves the plant surface. The cuticle (a waxy outer layer) and the periderm (secondary protective tissue), besides retarding water loss, provide passive barriers to bacterial and fungal entry.

A diverse group of plant compounds, commonly referred to as secondary metabolites, also defends plants against a variety of herbivores and pathogenic microbes. Some secondary metabolites serve other important functions as well, such as providing structural support, as in the case of lignin, or acting as pigments, as in the case of the anthocyanins.

Plants produce a large, diverse array of organic compounds that appear to have no direct function in their growth and development. These compounds 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, or differentiation, or the formation of the *primary metabolites*—carbohydrates, proteins, nucleic acids, and lipids—discussed elsewhere in this book.

Secondary metabolites also differ from primary metabolites in having a restricted distribution within the plant kingdom. That is, certain secondary metabolites are only found in 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 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 them because of their importance as medicinal drugs, poisons, flavors, and industrial materials.

Today we know that many secondary metabolites have important ecological functions in plants:

- They protect plants against being eaten by herbivores and against being infected by microbial pathogens.
- They serve as attractants (odor, color, taste) for pollinators and seed-dispersing animals.
- They function as agents of plant–plant competition and plant–microbe symbioses.

The ability of plants to compete and survive is therefore profoundly affected by the ecological functions of their secondary metabolites.

Secondary metabolism is also relevant to agriculture. The very defensive 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 to produce relatively low levels of these compounds (which, of course, can make them more susceptible to insects and disease).

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 defensive roles.

### Secondary metabolites are divided into three major groups

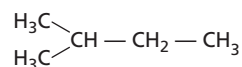
Plant secondary metabolites can be divided into three chemically distinct groups: terpenes, phenolics, and nitrogen-containing compounds. **FIGURE A4.1** shows in simplified form the pathways involved in the biosynthesis of secondary metabolites and their interconnections with primary metabolism. In the next three sections of this chapter, we will discuss each of these groups in turn.

## Terpenes

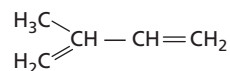
The **terpenes**, or *terpenoids*, constitute the largest class of secondary metabolites. Most of the diverse substances of this class are insoluble in water. They are synthesized from acetyl-CoA or its glycolytic intermediates. After discussing the biosynthesis of terpenes, we will examine how they act to repel herbivores.

### Terpenes are formed by the fusion of five-carbon isoprene units

All terpenes are derived from the union of 5-carbon elements (also called  $C_5$  units) that have the branched carbon skeleton of isopentane:



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

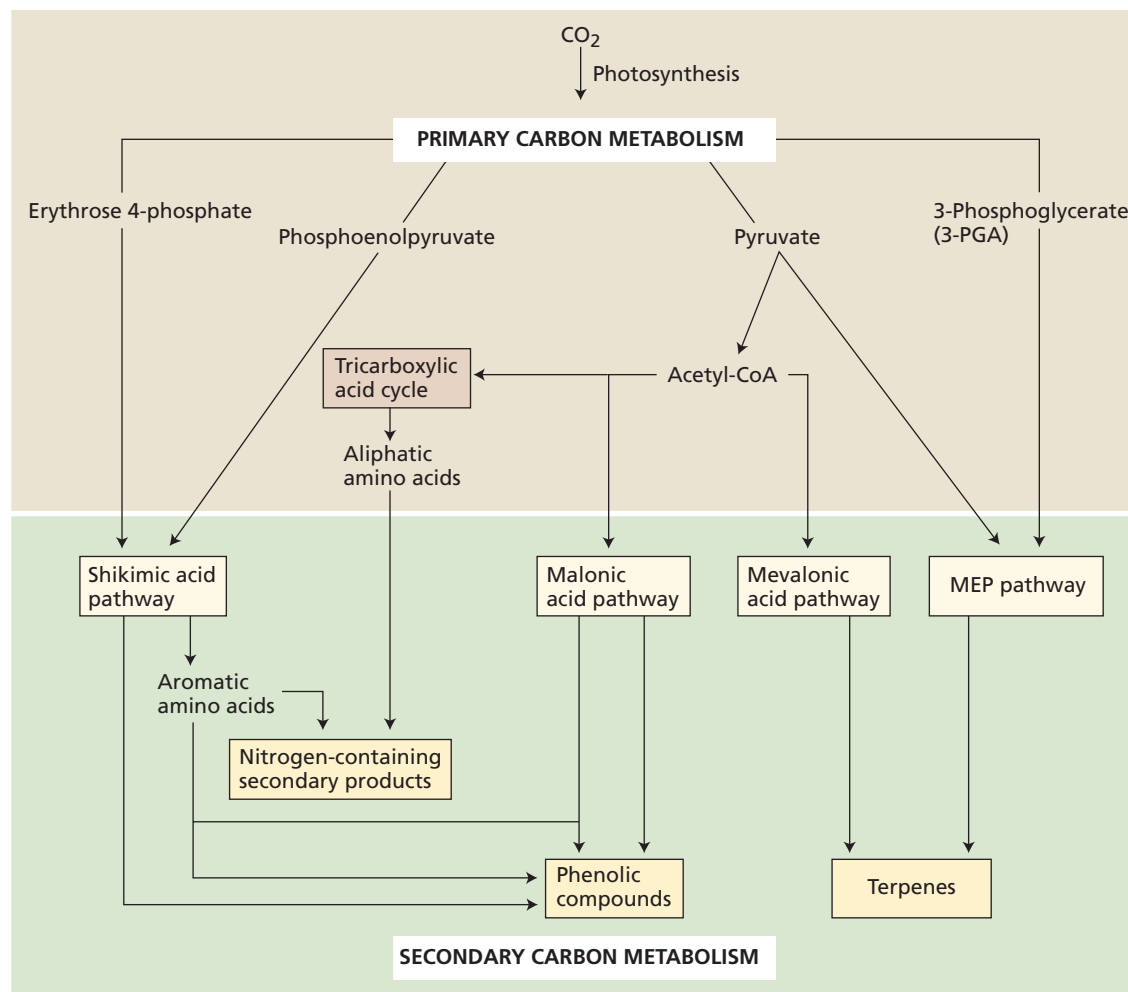


Thus, terpenes are occasionally also referred to as *isoprenoids*.

Terpenes are classified by the number of  $C_5$  units they contain, although extensive metabolic modifications can sometimes make it difficult to pick out the original five-carbon residues. For example, 10-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 synthesized 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 A4.2**). This key six-carbon intermediate is then pyrophosphorylated, decarboxylated, and dehydrated to yield **isopente-**



**FIGURE A4.1** A simplified view of the major pathways of secondary-metabolite biosynthesis and their interrelationships with primary metabolism.

**nyl diphosphate (IPP)\*.** IPP is the activated five-carbon building block of terpenes.

IPP can also 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). Glyceraldehyde 3-phosphate and two carbon atoms derived from pyruvate condense to form the five-carbon intermediate 1-deoxy-D-xylulose 5-phosphate. After this intermediate is rearranged and reduced to 2-C-methyl-D-erythritol 4-phosphate (MEP), it is eventually converted into IPP (see Figure A4.2).

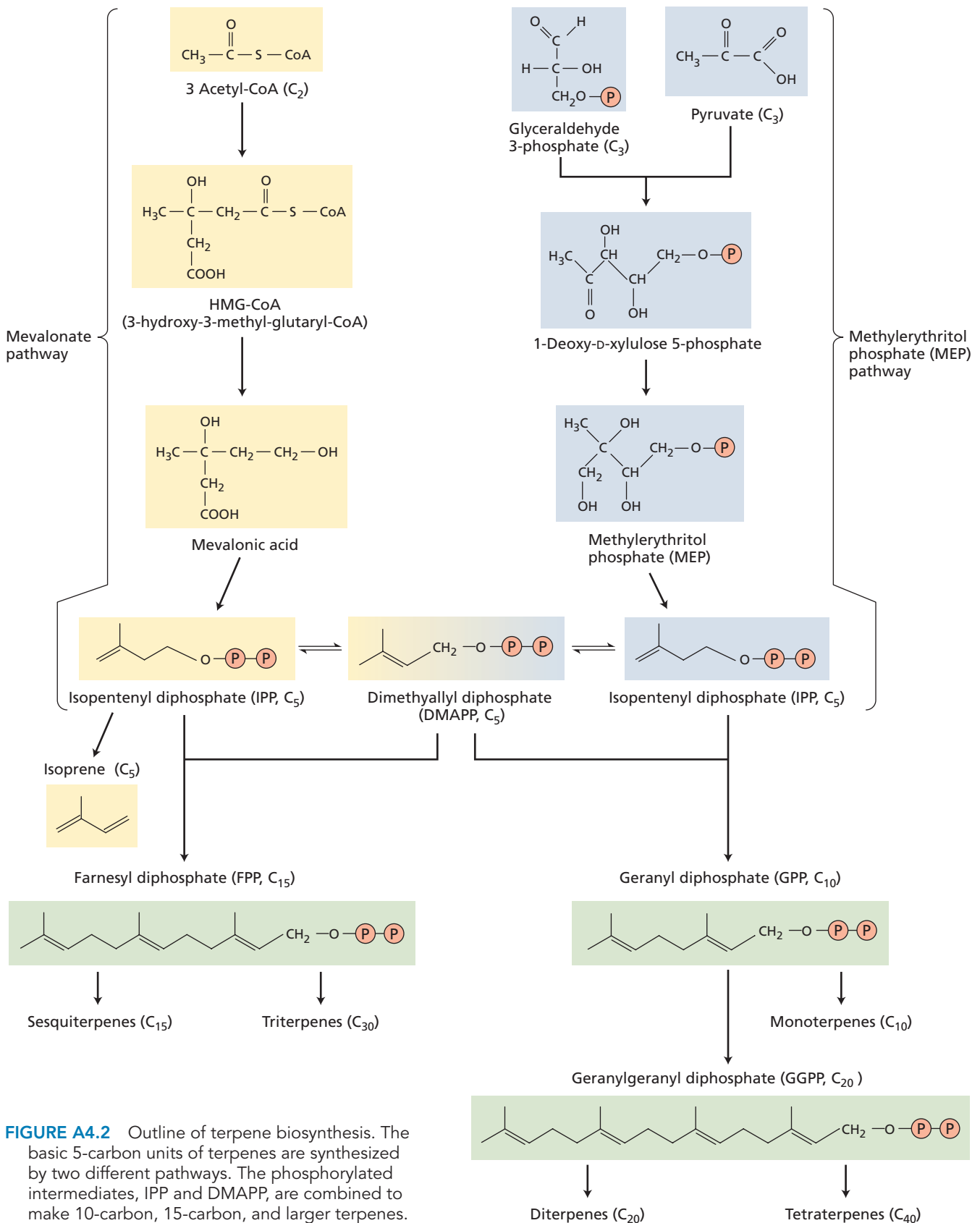
\*IPP is the abbreviation for isopentenyl *pyrophosphate*, an earlier name for this compound. The other pyrophosphorylated intermediates in this pathway are also now referred to as *diphosphates*.

### *IPP and its isomer combine to form larger terpenes*

IPP and its isomer, dimethylallyl diphosphate (DMAPP), are the activated 5-carbon building blocks of terpene biosynthesis that join together to form larger molecules. First IPP and DMAPP react to give geranyl diphosphate (GPP), the 10-carbon precursor of nearly all the monoterpenes (see Figure A4.2). 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.

It is now generally accepted that sesquiterpenes and triterpenes are synthesized through the cytosolic mevalonic acid pathway, whereas mono-, di-, and tetraterpenes are

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**FIGURE A4.2** 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.

derived from the chloroplastic MEP pathway. However, cross talk between these two pathways does occasionally occur, leading to terpenes that are “mixed” with regard to their biosynthetic origin.

### Some terpenes have roles in growth and development

Certain terpenes have well-characterized functions in plant growth or development and so can be considered primary rather than secondary metabolites. For example, the *gibberellins* (see Chapter 25), an important group of plant hormones, are diterpenes. *Brassinosteroids* (see Chapter 15), another class of plant hormones with growth-regulating functions, originate from triterpenes.

Sterols are triterpene derivatives that are essential components of cell membranes, which they stabilize by interacting with phospholipids. 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 15) is a C<sub>15</sub> terpene produced by degradation of a carotenoid precursor.

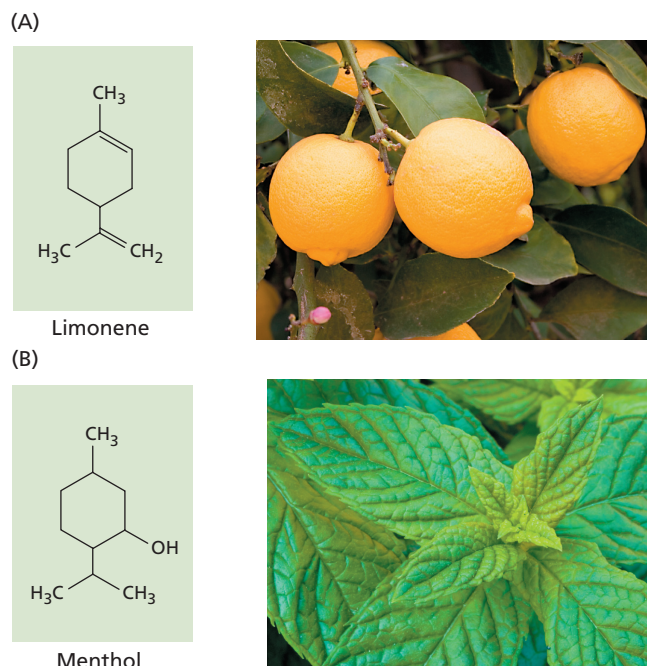
Long-chain polyterpene alcohols known as *dolichols* function as carriers of sugars in cell wall and glycoprotein synthesis (see Chapter 14). Terpene-derived side chains, such as the phytol side chain of chlorophyll (see Chapter 7), help anchor certain molecules in membranes. The vast majority of terpenes, however, are secondary metabolites presumed to be involved in plant defenses.

### Terpenes defend many plants against herbivores

Terpenes are toxins and feeding deterrents to many herbivorous insects and mammals; thus they appear to play important defensive roles in the plant kingdom (Gershenzon and Croteau 1992). For example, monoterpene esters called **pyrethroids**, found in the leaves and flowers of *Chrysanthemum* species, show 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 characteristic odor to their foliage. Peppermint, lemon, basil, and sage are examples of plants that contain essential oils.



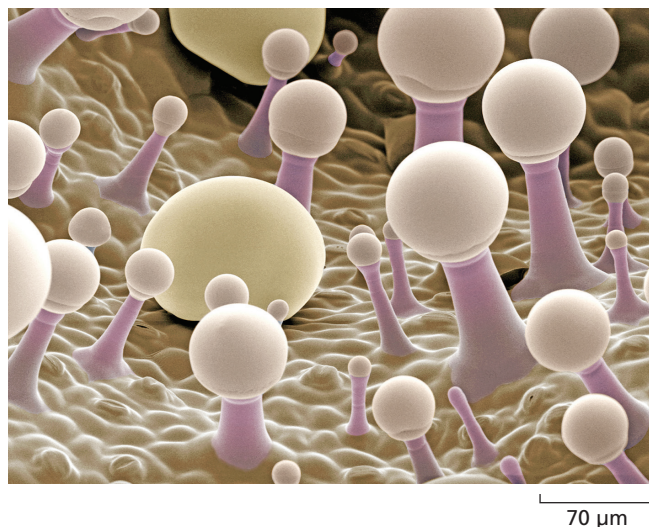
**FIGURE A4.3** Structures of limonene (A) and menthol (B). These two well-known monoterpenes serve as defenses against insects and other organisms that feed on the plants that produce them. (A, lemon tree [*Citrus × limon*], photo © Soren Pilman/istockphoto; B, peppermint [genus *Mentha*], photo © Jose Antonio Santiso Fernández/istockphoto.)

The chief monoterpene constituent of lemon oil is limonene; that of peppermint oil is menthol (**FIGURE A4.3**).

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. Within the glandular hairs, the terpenes are stored in a modified extracellular space (**FIGURE A4.4**). Essential oils can be extracted from plants by steam distillation and are important commercially in flavoring foods and making perfumes.

Among the nonvolatile terpene antiherbivore compounds are the **limonoids**, a group of triterpenes (C<sub>30</sub>) well known as bitter substances in citrus fruits. Perhaps the most powerful deterrent to insect feeding known is *azadirachtin* (**FIGURE A4.5A**), 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; Veitch et al. 2008). 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.





**FIGURE A4.4** Monoterpenes and sesquiterpenes are commonly found in glandular hairs on the surface of a plant. This false-colored scanning electron micrograph shows glandular trichomes (microscopic hairs, purple) on the calyx of a clary sage (*Salvia sclarea*) plant. The trichomes are secreting globules of essential oils (round, white). (© Andrew Syred/Photo Researchers, Inc.)

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 A4.5B**). Ingestion of phytoecdysones by insects disrupts molting and other developmental processes, often with lethal consequences. In addition, phytoecdysones

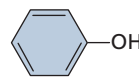
were recently found to have a defensive function against plant-parasitic nematodes (Soriano et al. 2004).

Triterpenes that defend plants 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  $\text{Na}^+/\text{K}^+$ -ATPases. In carefully regulated doses, they slow and strengthen the heartbeat. Cardenolides extracted from foxglove (*Digitalis*) are prescribed to millions of patients for the treatment of some types of heart disease.

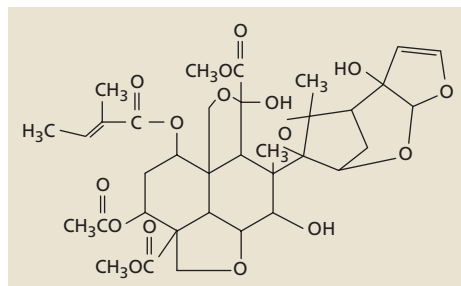
**Saponins** are steroid and triterpene glycosides, so named because of their soaplike properties. The presence of both lipid-soluble (the steroid or triterpene) and water-soluble (the sugar) elements in one molecule gives saponins detergent properties, and they form a soapy lather when shaken with water. 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 compounds that contain a phenol group: a hydroxyl functional group on an aromatic ring:

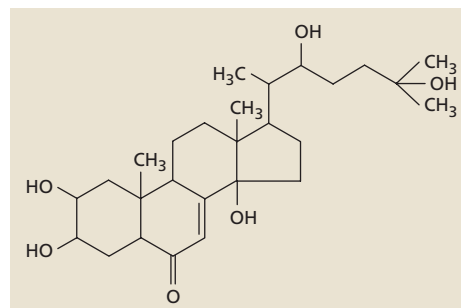


(A) Azadirachtin, a limonoid



**FIGURE A4.5** Structure of two triterpenes, azadirachtin (A) and  $\alpha$ -ecdysone (B), that serve as powerful insecticides. Azadirachtin affects more than 200 species of insects and can be considered a natural insecticide.  $\alpha$ -Ecdysone, a plant-derived steroidal prohormone of the insect molting hormone 20-hydroxyecdysone, can cause irregular molting in insect herbivores. (A, photo of neem leaves © RN Photos/istockphoto; B, photo of *Polypodium vulgare* leaves, © blickwinkel/Alamy.)

(B)  $\alpha$ -Ecdysone, an insect molting hormone



These substances are classified as *phenolic compounds*, or **phenolics**. 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. Many serve as defenses 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. After giving a brief account of phenolic biosynthesis, we will discuss three principal groups of phenolic compounds and what is known about their roles in the plant.

### Phenylalanine is an intermediate in the biosynthesis of most plant phenolics

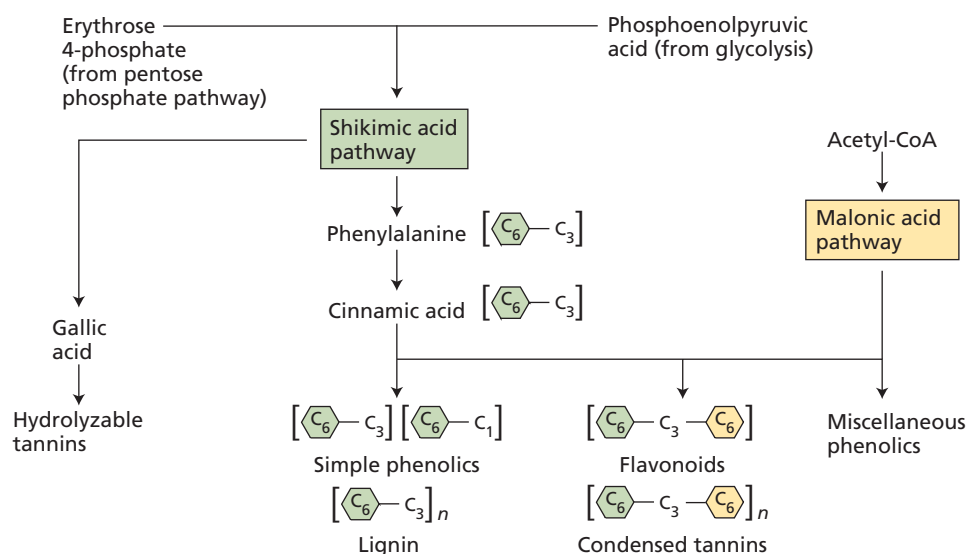
Plant phenolics are synthesized by several different routes and thus constitute a heterogeneous group from a metabolic point of view. Two basic pathways are involved: the shikimic acid pathway and the malonic acid pathway (FIGURE A4.6). The shikimic acid pathway participates in the biosynthesis of most 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 into the three aromatic amino acids: phenylalanine, tyrosine, and tryptophan. 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 Appendix 1). The shikimic acid pathway is present in plants, fungi, and bacteria but is not found in animals. Animals have no way to synthesize aromatic amino acids—phenylalanine, tyrosine, and tryptophan—which are therefore essential nutrients in animal diets.

The most abundant classes of phenolic secondary compounds in plants are derived from phenylalanine via the elimination of an ammonia molecule to form cinnamic acid (FIGURE A4.7). 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 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 phytochromes), 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 many plant species is made more complex by the existence of multiple PAL-encoding genes, some of which are



**FIGURE A4.6** Plant phenolics are synthesized 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:  $C_6$  indicates a benzene ring, and  $C_3$  is a three-carbon chain. More detail on the pathway from phenylalanine onward is given in Figure 13.7.

**FIGURE A4.7** Outline of phenolic biosynthesis from phenylalanine onward. The formation of many plant phenolics, including simple phenylpropanoids, coumarins, benzoic acid derivatives, lignin, anthocyanins, isoflavones, condensed tannins, and other flavonoids, begins with phenylalanine.

expressed only in specific tissues or only under certain environmental conditions (Logemann et al. 1995).

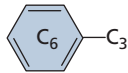
Reactions subsequent to that catalyzed by PAL lead to the addition of more hydroxyl groups and other substituents. The metabolites *trans*-cinnamic acid, *p*-coumaric acid, and their derivatives are simple phenolic compounds called **phenylpropanoids** because they contain a benzene ring:



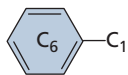
and a three-carbon side chain.

Simple phenolic compounds are widespread in vascular plants and appear to function in various 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 A4.8A**)

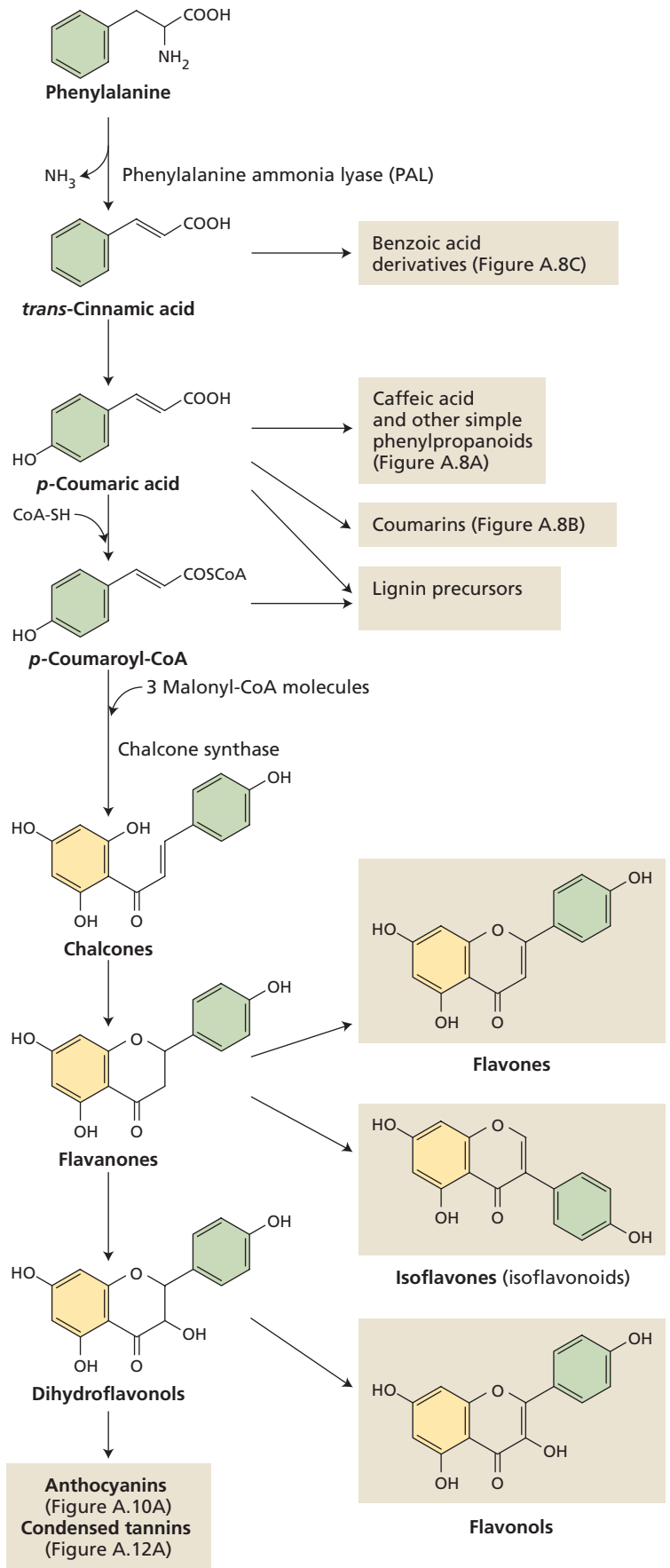


- Phenylpropanoid lactones (cyclic esters) called *coumarins*, which also have a phenylpropanoid carbon skeleton (**FIGURE A4.8B**)
- *Benzoic acid derivatives*, which have a carbon skeleton formed from phenylpropanoids by the cleavage of a two-carbon fragment from the side chain (**FIGURE A4.8C**) (see also Figure A4.7):

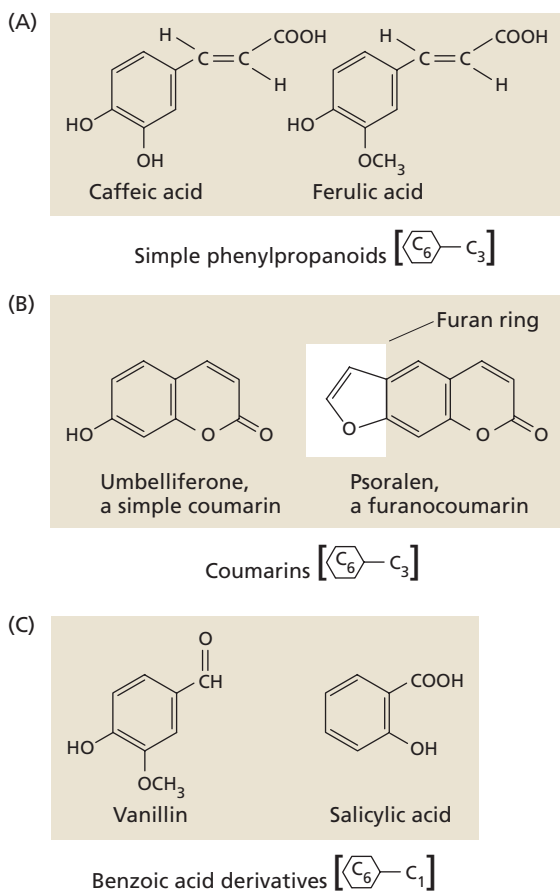


As with many other secondary products, plants can elaborate on the basic carbon skeletons of these simple phenolic compounds to make more complex products.

Now that the biosynthetic pathways leading to most widespread phenolic compounds have been determined, researchers have turned their attention to studying how those pathways are







**FIGURE A4.8** Simple phenolic compounds play a variety 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 hormone that is involved in systemic resistance to plant pathogens.

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).

### Ultraviolet light activates some simple phenolics

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 A4.8B). These compounds are not toxic until they are activated by light. Sunlight in the ultraviolet A (UV-A) region of the spectrum (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, including celery, parsnip, and parsley. In celery, the concentration of these compounds can increase about one-hundredfold 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 are 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

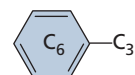
From leaves, roots, and decaying litter, plants release a variety of primary and secondary metabolites into the environment. The release of secondary compounds by one plant that have an effect on neighboring plants is referred to as **allelopathy**. If a plant can reduce the growth of nearby plants by 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 A4.8A) are found 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).

Allelopathy is currently of great interest because of its potential agricultural applications (Kruse et al. 2000). 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 (see **WEB ESSAY 23.7**).

### 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:



Lignin plays both primary and secondary roles in plants. Its precise structure is not known because it is difficult to extract from plants, in which 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, all of 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 phenylpropanoid alcohols 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.

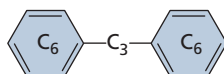
Unlike the monomeric units of polymers such as starch, rubber, or cellulose, those of lignin do not appear to be linked in a simple, repeating way. However, recent research suggests that a guiding protein may bind the individual units during lignin biosynthesis, giving rise to a scaffold that then directs the formation of a large, repeating unit (Davin and Lewis 2000; Hatfield and Vermerris 2001).

Lignin is found in the cell walls of various cell types that make up supporting and conducting tissues, notably the tracheids and vessel elements of the xylem. It is deposited chiefly in the thickened secondary wall, but may also be present 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 synthesize lignin must have been one of the most important adaptations permitting primitive plants to colonize dry land.

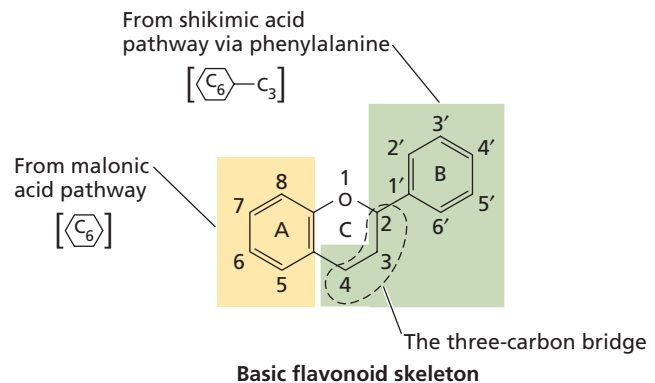
Besides providing mechanical support, lignin has significant protective functions in plants. Its physical toughness deters herbivory, and its chemical durability makes it relatively indigestible. By bonding to cellulose and protein, lignin also reduces the digestibility of those substances. Lignification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

### 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 A4.9).



**FIGURE A4.9** Basic flavonoid carbon skeleton. Flavonoids are synthesized from products of the shikimic acid and malonic acid pathways. Flavonoids contain 15 carbons in the basic molecular skeleton provided by two aromatic rings and one 3-carbon bridge. Positions of carbons on the flavonoid ring system are numbered as shown.

Flavonoids are classified primarily on the basis of the degree of oxidation of the three-carbon bridge. We will discuss four of these groups here: the anthocyanins, the flavones, the flavonols, and the isoflavones (see Figure A4.7).

The basic flavonoid carbon skeleton may have numerous substituents. Hydroxyl groups are usually present at positions 3, 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

The colored pigments of plants provide visual cues that help to attract pollinators and seed dispersers. These pigments 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). The flavonoids also 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 flowers and fruits.

Anthocyanins are glycosides that can have various sugars at position 3 (FIGURE A4.10A) and sometimes elsewhere. Without their sugars, anthocyanins are known as **anthocy-**

**anidins (FIGURE A4.10B).** Anthocyanin color is influenced by many factors, including the number of hydroxyl and methoxyl groups in ring B of the anthocyanidin (see Figure A4.10A), the presence of aromatic acids esterified to the main skeleton, and the pH of the cell vacuole in which the anthocyanins 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 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 A4.10 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 pressure to attract different types of pollinators, many of which have different color preferences.

### Flavones and flavonols may protect against damage by ultraviolet light

Two other groups of flavonoids found in flowers are **flavones** and **flavonols** (see Figure A4.7). These flavonoids generally absorb light at shorter wavelengths than do anthocyanins, 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 visual attractant cues (FIGURE A4.11). 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 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. UV-B radiation is known to induce mutations in the DNA as well as oxidative stress, which has the potential to damage cellular macromolecules.

*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*.

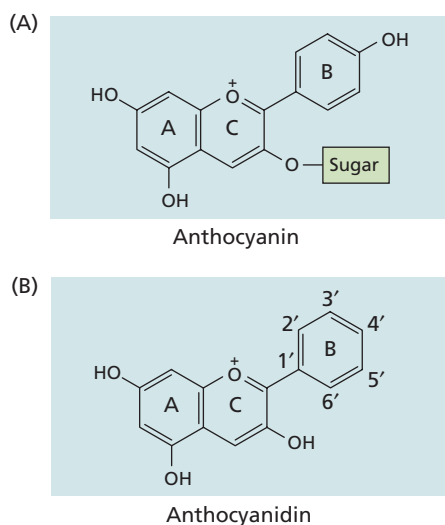
Other functions of flavones and flavonols have also been discovered. For example, these flavonoids, when secreted into the soil by legume roots, mediate the interaction of legumes and rhizobacteria, their nitrogen-fixing symbionts, as described in Chapter 11. As will be discussed in Chapter 17, recent work suggests that these flavonoids also play a regulatory role in plant development as modulators of polar auxin transport.

### Isoflavonoids have widespread pharmacological activity

The **isoflavones** (*isoflavonoids*) are a group of flavonoids in which the position of one aromatic ring (ring B) is shifted (see Figure A4.7). Isoflavonoids, which are found mostly in legumes, have several different biological activities. Some, such as rotenone, can be used effectively as insecticides,

**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 <sub>3</sub> , 4'— OH	Rosy red
Petunidin	3'— OCH <sub>3</sub> , 4'— OH, 5'— OCH <sub>3</sub>	Purple



**FIGURE A4.10** The structures of anthocyanins (A) and anthocyanidins (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<sub>3</sub>) shifts absorption to a slightly shorter wavelength, resulting in a redder color.

(A)



(B)



**FIGURE A4.11** Black-eyed Susan (*Rudbeckia* sp.) as seen by humans (A) and as it might appear to honeybees (B). (A) To humans, the flowers have 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.” UV-absorbing flavonols are

found in the inner parts of the rays, but not in the tips. The distribution of flavonols in the rays creates a “bull’s-eye” pattern visible to 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.)

pesticides (e.g., as rat poison), and piscicides (fish poisons). Other isoflavones have anti-estrogenic effects; for example, sheep grazing on clover rich in isoflavonoids often suffer from infertility. The ring system of isoflavones has a three-dimensional structure similar to that of steroids (see Figure A4.5B), allowing these substances to bind to estrogen receptors. Isoflavones may also be responsible for the anticancer benefits of foods prepared from soybeans.

In the past few years, isoflavones 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 lignin, 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, thereby 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 A4.12A). They are common constituents of woody plants. Because condensed tannins can often be hydrolyzed into 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 (FIGURE A4.12B). 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 Da.

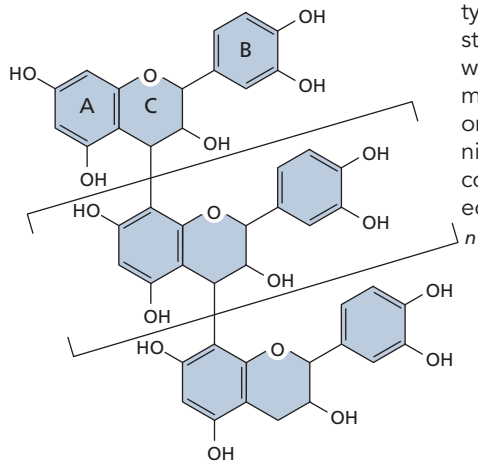
Tannins are general toxins that can reduce the growth and survival of many herbivores when added to their diets. In addition, tannins act as feeding repellents to a great variety of animals. Mammals such as cattle, deer, and apes characteristically avoid plants or parts of plants with high tannin contents. Unripe fruits, for instance, frequently have very high tannin levels, which deter feeding on the fruits until their seeds are mature enough for dispersal.

Although crop plants generally produce fewer secondary metabolites, there are exceptions. Humans often prefer a certain level of astringency in tannin-containing foods, such as apples, blackberries, tea, and grapes. The tannins in red wine have been 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. In recent years, however, another phenolic compound, the stilbene phenylpropanoid resveratrol, has also been identified as a health benefit factor in red wine.

Moderate amounts of specific tannins may have health benefits for humans, but the defensive properties of most tannins are due to their toxicity, which is generally attrib-

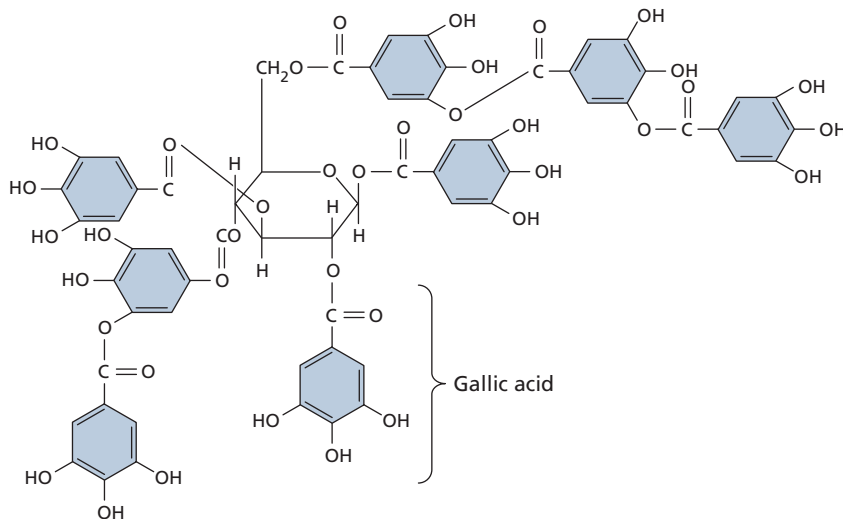


(A) Condensed tannin



**FIGURE A4.12** Structure of two types of tannins. (A) The general structure of a condensed tannin, where  $n$  is usually 1 to 10. There may also be a third hydroxyl group on ring B. (B) The hydrolyzable tannin from sumac (*Rhus semialata*) consists of glucose and eight molecules of gallic acid.

(B) Hydrolyzable tannin



uted to their ability to bind proteins nonspecifically. It has long been thought that plant tannins bind proteins in the guts of herbivores by forming hydrogen bonds between their hydroxyl groups and electronegative sites on the proteins (FIGURE A4.13A). More recent evidence indicates that tannins and other phenolics can also bind to dietary protein in a covalent fashion (FIGURE A4.13B). 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  $\text{—NH}_2$  and  $\text{—SH}$  groups of proteins (Appel 1993). By whatever mechanism protein–tannin binding occurs, this process has a negative effect 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, which is induced by ingestion of food with a high tannin content, 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, which facilitate binding to tannins.

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

## Nitrogen-Containing Compounds

A large variety of plant secondary metabolites have nitrogen as part of 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 as well as their medicinal properties. Most nitrogenous secondary metabolites are synthesized from common amino acids.

In this section we will examine the structures and biological properties of various nitrogen-containing secondary metabolites, including alkaloids, cyanogenic glycosides, glucosinolates, and nonprotein amino acids.

### Alkaloids have dramatic physiological effects on animals

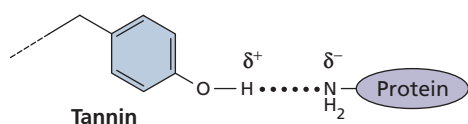
The **alkaloids** are a large family of more than 15,000 nitrogen-containing secondary metabolites. They are found in approximately 20% of vascular plant species. The nitrogen atom in these compounds is usually part of a **heterocyclic ring**, a ring that contains both nitrogen and carbon atoms. As a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

As their name would suggest, most alkaloids are alkaline. At the pH values commonly found in the cytosol (pH 7.2) or the vacuole (pH 5–6), the nitrogen atom is protonated; hence alkaloids are positively charged and are generally water soluble.

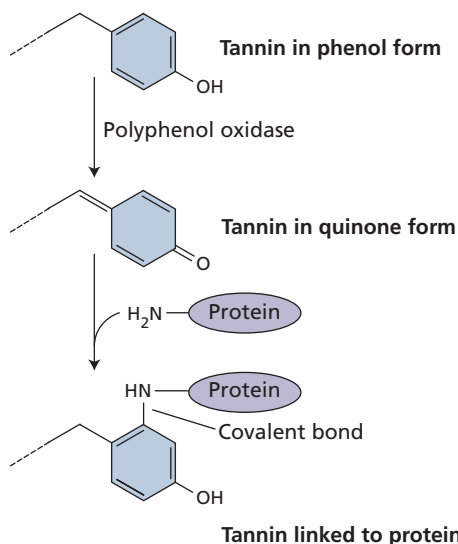
Alkaloids are usually synthesized from one of a few common amino acids—in particular, lysine, tyrosine, or



(A) Hydrogen bonding between tannin and protein



(B) Covalent bonding to protein after oxidation



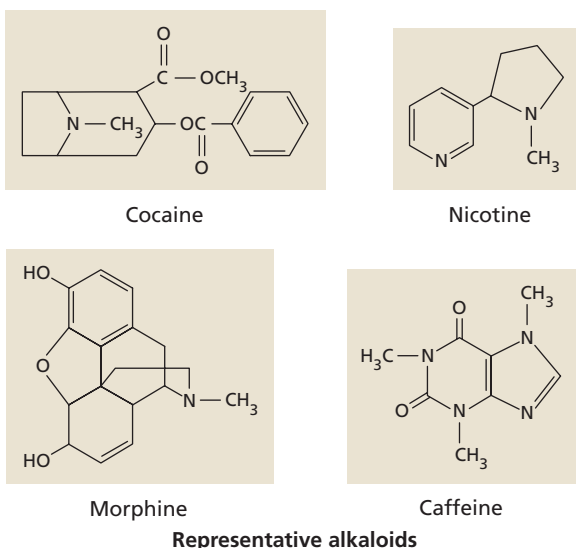
**FIGURE A4.13** Proposed mechanisms for the interaction of tannins with proteins. (A) Hydrogen bonds may form between the phenolic hydroxyl groups of the tannin and electronegative sites on the protein. (B) Phenolic hydroxyl groups may bind covalently to proteins following activation by oxidative enzymes, such as polyphenol oxidase.

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 A4.14**), 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 A4.15**). Nicotinic acid is also a constituent of NAD<sup>+</sup> and NADP<sup>+</sup>, which serve as electron carriers in metabolism.

The role of alkaloids in plants has been a subject of speculation for at least a century. 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 herbivores, especially mammals, because

**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 Cocaine	Prevention of intestinal spasms, antidote to other poisons, dilation of pupils for examination 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 Morphine	Analgesic (pain relief), treatment of coughs Analgesic
Indole		Tryptophan	Psilocybin Reserpine Strychnine	Hallucinogen Treatment of hypertension, treatment of psychoses Rat poison, treatment of eye disorders



**FIGURE A4.14** 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 nucleotide bases adenine and guanine. The pyrrolidine (five-membered) ring of nicotine arises from ornithine; the pyridine (six-membered) ring is derived from nicotinic acid.

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

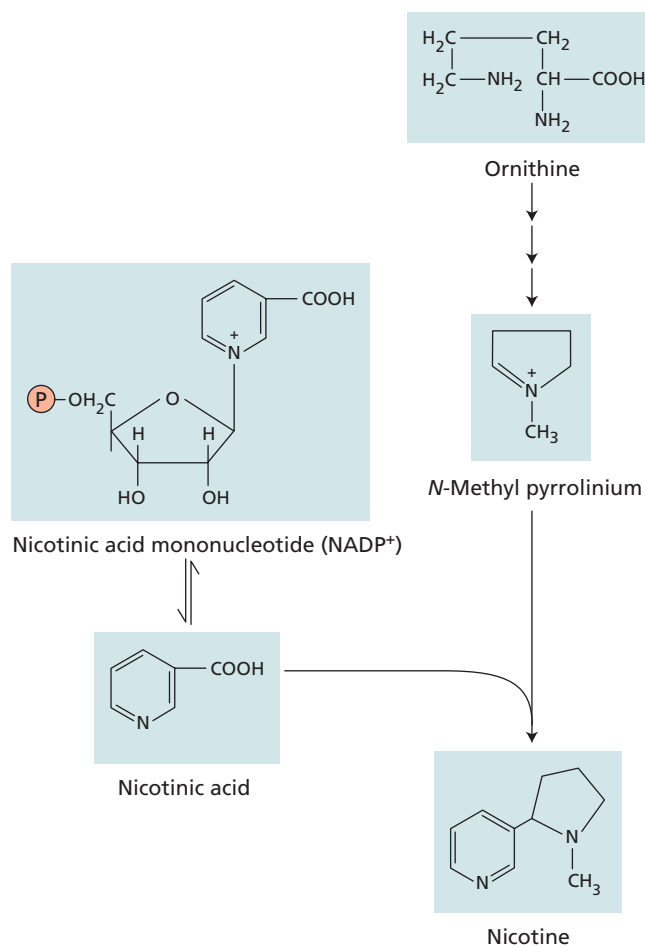
Nearly all alkaloids are also toxic to humans when taken in sufficient quantities. For example, strychnine, atropine, and coniine (from poison hemlock, *Conium maculatum*) are classic alkaloid poisons. At lower doses, however, many are useful pharmacologically. Morphine, codeine, and scopolamine are just a few of the plant alkaloids currently used in medicine. Other alkaloids, including cocaine, nicotine, and caffeine (see Figure A4.14), have widespread non-medical uses 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 neurotransmitters; 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

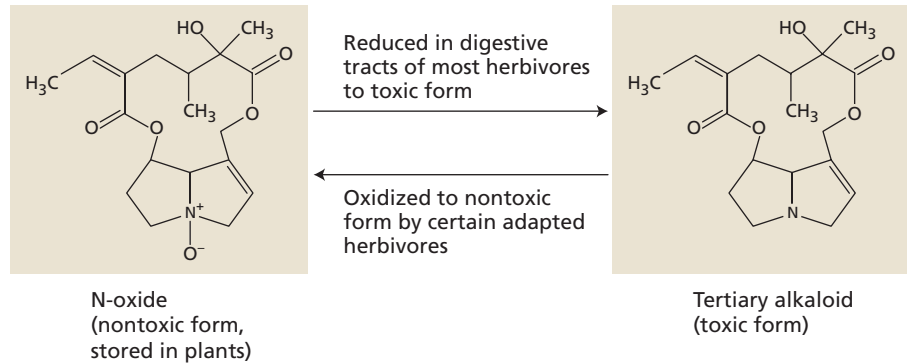
plant defensive compounds and even use them in their own defense (Hartmann 1999). Within plants, pyrrolizidine alkaloids occur naturally as nontoxic N-oxides. In the alkaline digestive tracts of many insect herbivores, however, they are quickly reduced to uncharged, hydrophobic tertiary alkaloids (FIGURE A4.16), which easily pass through membranes and are toxic. Nevertheless, some insect herbivores, like the cinnabar moth (*Tyria jacobaeae*), have developed the ability to reconvert tertiary pyrrolizidine alkaloids into the nontoxic N-oxide form immediately after their 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 (endophytes) that grow in the apoplast and synthesize a variety of different alkaloids. Grasses



**FIGURE A4.15** Nicotine biosynthesis begins with the synthesis of nicotinic acid (niacin) from aspartate and glyceraldehyde 3-phosphate. Nicotinic acid is also a component of NAD<sup>+</sup> and NADP<sup>+</sup>, important participants in biological oxidation-reduction reactions. The five-membered ring of nicotine is derived from ornithine, an intermediate in arginine biosynthesis.

**FIGURE A4.16** Two forms of pyrrolizidine alkaloids occur in nature: the N-oxide form and the tertiary alkaloid. The nontoxic N-oxide form found in plants is reduced to the toxic tertiary form in the digestive tracts of most insect herbivores. However, some adapted herbivores can convert the toxic tertiary form back into the nontoxic N-oxide. The two forms are illustrated here for senecionine, a pyrrolizidine alkaloid found in ragwort (*Senecio*).



with fungal symbionts often grow faster and are better defended 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 .

### 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 themselves toxic but are readily broken down to give off poisons, some of which are volatile, 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, a hydrolytic enzyme that separates sugars from other molecules to which they are linked (FIGURE A4.17).

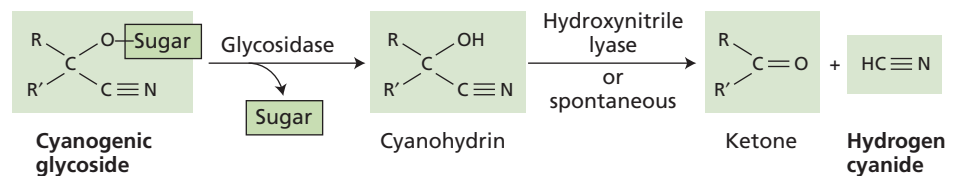
2. In the second step the resulting hydrolysis product, called an  $\alpha$ -hydroxynitrile or cyanohydrin, can decompose spontaneously at a low rate to liberate HCN. This second step can be accelerated by the lytic enzyme hydroxynitrile lyase.

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 compartmentalization 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 found in many 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.

**FIGURE A4.17** 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  $\beta$ -gentiobiose, then the compound is amygdalin (the common cyanogenic glycoside found in the seeds of almonds, apricots, cherries, and peaches).



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.

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 pest resistance of stored cassava.

### Glucosinolates release volatile toxins

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

Glucosinolate breakdown is catalyzed by a hydrolytic enzyme, called a *thioglucosidase* or *myrosinase*, that cleaves glucose from its bond with the sulfur atom (FIGURE A4.18). The resulting aglycone—the nonsugar portion of the molecule—loses the sulfate and rearranges itself to give pungent and chemically reactive products, including isothiocyanates and nitriles, depending on the conditions of hydrolysis. These defensive products function as toxins and herbivore 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

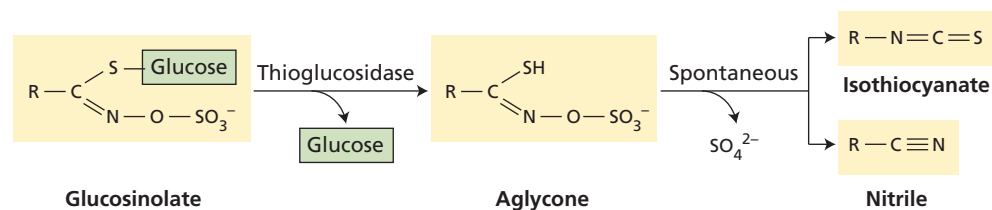
without ill effects. For adapted herbivores such as the cabbage butterfly, glucosinolates serve as stimulants for adult feeding and egg laying, and the isothiocyanates produced after glucosinolate hydrolysis act as volatile attractants (Renwick et al. 1992). In addition, the caterpillars can redirect the glucosinolate hydrolysis reaction to the production of the less toxic nitriles (Wittstock et al. 2004).

Most of the recent research on glucosinolates in plant defense has concentrated on rape, or canola (*Brassica napus*), a major oilseed 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 more resistant to pests and still provide a protein-rich seed residue for animal feeding.

### Nonprotein amino acids are toxic to herbivores

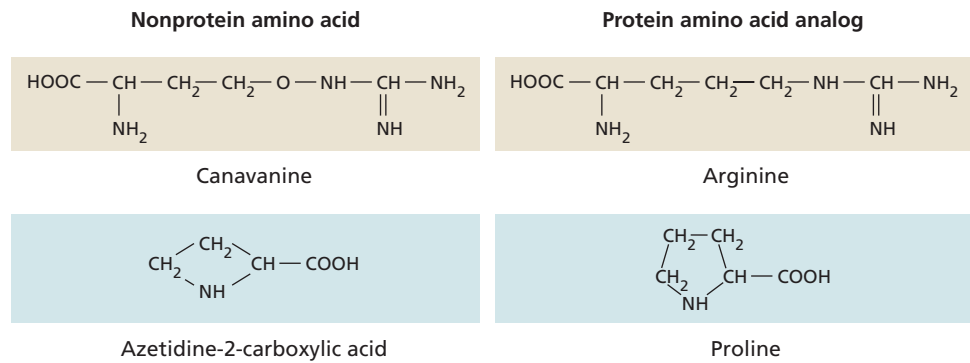
Plants and animals incorporate the same 20 amino acids into their proteins. However, many plants also contain unusual amino acids, called **nonprotein amino acids**, that are not incorporated into proteins. Instead, these amino acids are present in the free form and act as defensive substances. Many nonprotein amino acids are 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 A4.19).

Nonprotein amino acids exert their toxicity in various ways. Some block the synthesis or uptake of protein amino acids. Others, such as canavanine, can be mistakenly incorporated into proteins. After ingestion by an herbivore, canavanine is recognized by the enzyme that normally binds arginine to the arginine transfer RNA molecule, so it becomes incorporated into herbivore proteins in place of arginine. Canavanine is less basic than arginine and its incorporation usually results in a nonfunctional protein



**FIGURE A4.18** Hydrolysis of glucosinolates into mustard-smelling volatiles. R represents various alkyl or aryl substituents. For example, if R is  $\text{CH}_2=\text{CH}-\text{CH}_2^-$ , the compound is sinigrin, a major glucosinolate of black mustard seeds and horseradish roots.

**FIGURE A4.19** 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. Their activity ranges from interference with the uptake of amino acids to the disruption of translation.



because either its tertiary structure or its catalytic site is disrupted (Rosenthal 1991).

It has also been shown that several nonprotein amino acids, including 4-*N*-oxalyl-2,4-diaminobutyric acid, diaminobutyric acid (DABA), 2,3-diaminopropionic acid (DAPA), 3-*N*-oxalyl-2,3-diaminopropionic acid, and 2-amino-6-*N*-oxalylureidopropionic acid, as well as acetylated forms of some of these amino acids, occur in certain fodder legumes and have been linked to toxicity in ruminants (McSweeney et al. 2008).

Plants that synthesize nonprotein amino acids are not susceptible to the toxicity of these compounds. The jack bean (*Canavalia ensiformis*), which has high concentrations 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 are adapted to eat plants containing nonprotein amino acids have similar biochemical adaptations.