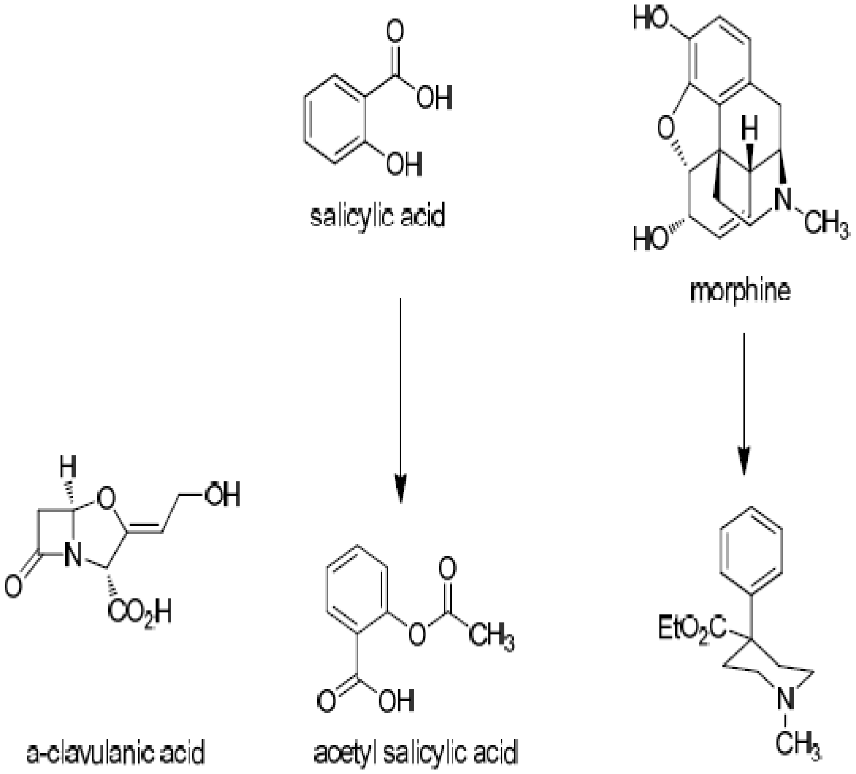
Biosynthetic pathways of Secondary metabolites

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).

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. They serve as attractants (odor, color, taste) for pollinators and seed-dispersing animals. 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.

Historically, natural products form the basis of medicine, and even now, many of the compounds that are medicinally important are derived from natural sources. It is highly likely that these compounds produce by the plant as defense mechanism to protect the plant or animal from attack (**phytoalexins**)

Medicinal agents can be purely natural compound (eg. clavulanic acid form bacteria), or semisynthetic compound like aspirin (acetyl salicylic acid derived from salicylic acid from the tree Salix, or a compound that is totally synthetic based on natural compound like pethidine from morphine (Figure **1**)



salicylic acid

morphine

a-clavulanic acid
fully natural

acetyl salicylic acid

b-aspirin semisynthetic

c-pethidine fully synthetic

Figure 1

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 1** shows in simplified form the pathways involved in the biosynthesis of secondary metabolites and their interconnections with primary metabolism.

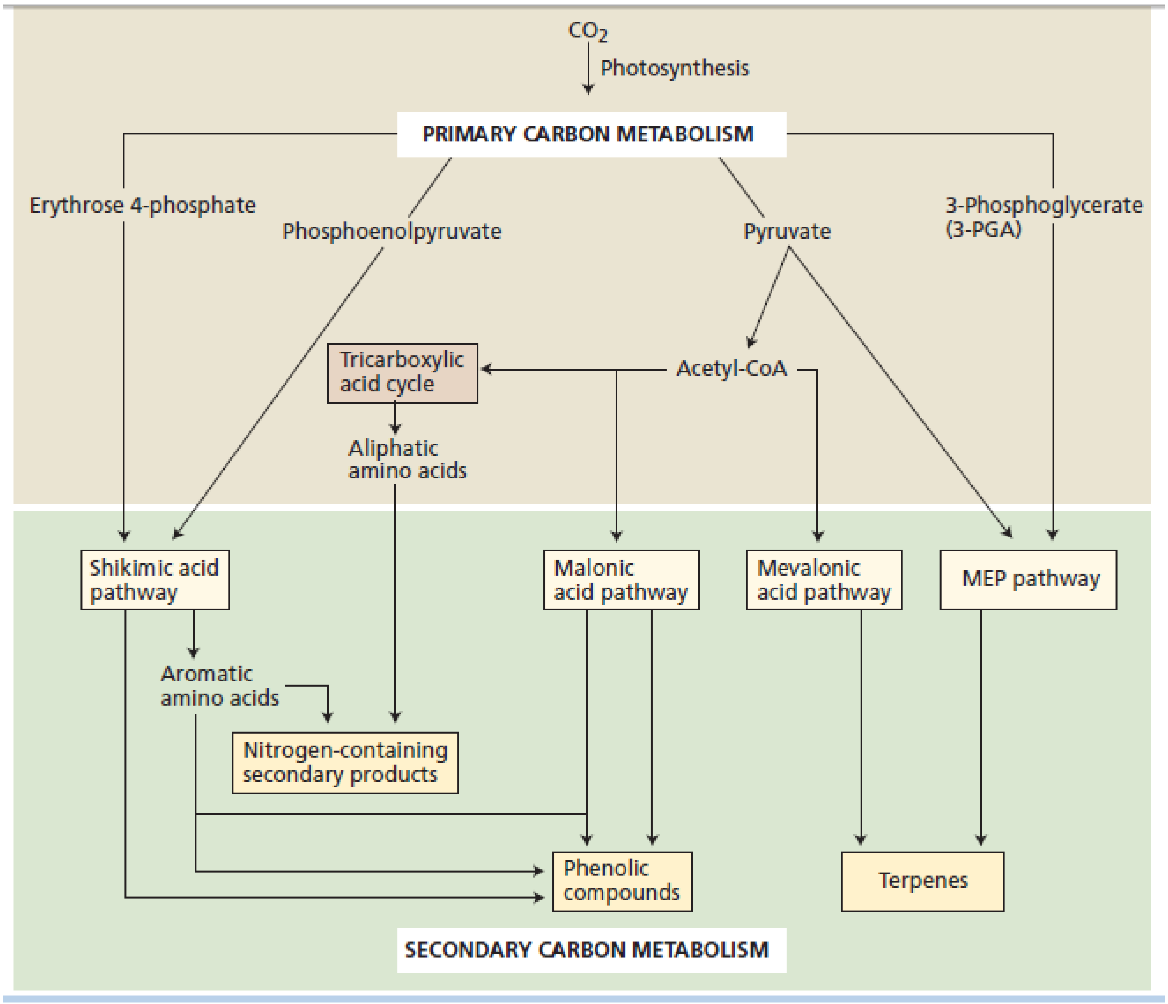


FIGURE.1: A simplified view of the major pathways of secondary-metabolite biosynthesis and their interrelationships with primary metabolism.

Shikimic acid derived natural products

Shikimic acid is the precursor for many natural products and aromatic amino acid (Figure **2**)

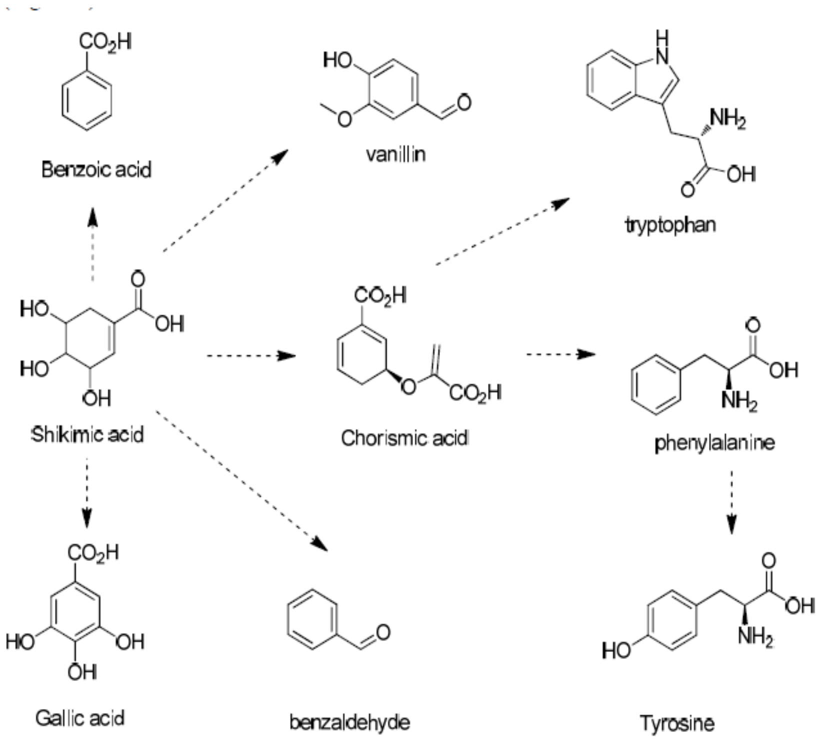


Figure **2**

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.

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

All terpenes are derived from the union of 5-carbon elements (also called *C5 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

Terpenes classified by the number of C5 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 C5 units, are called *monoterpenes*; 15-carbon terpenes (three C5 units) are *sesquiterpenes*; and 20-carbon terpenes (four C5 units) are *diterpenes*. Larger terpenes include *triterpenes* (30 carbons), *tetraterpenes* (40 carbons), and *polyterpenoids* ([C5]*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.3). This key six-carbon intermediate is then pyrophosphorylated, decarboxylated, and dehydrated to yield isopentenyl 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. 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.2).

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.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 (C30) and the tetraterpenes (C40), respectively.

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

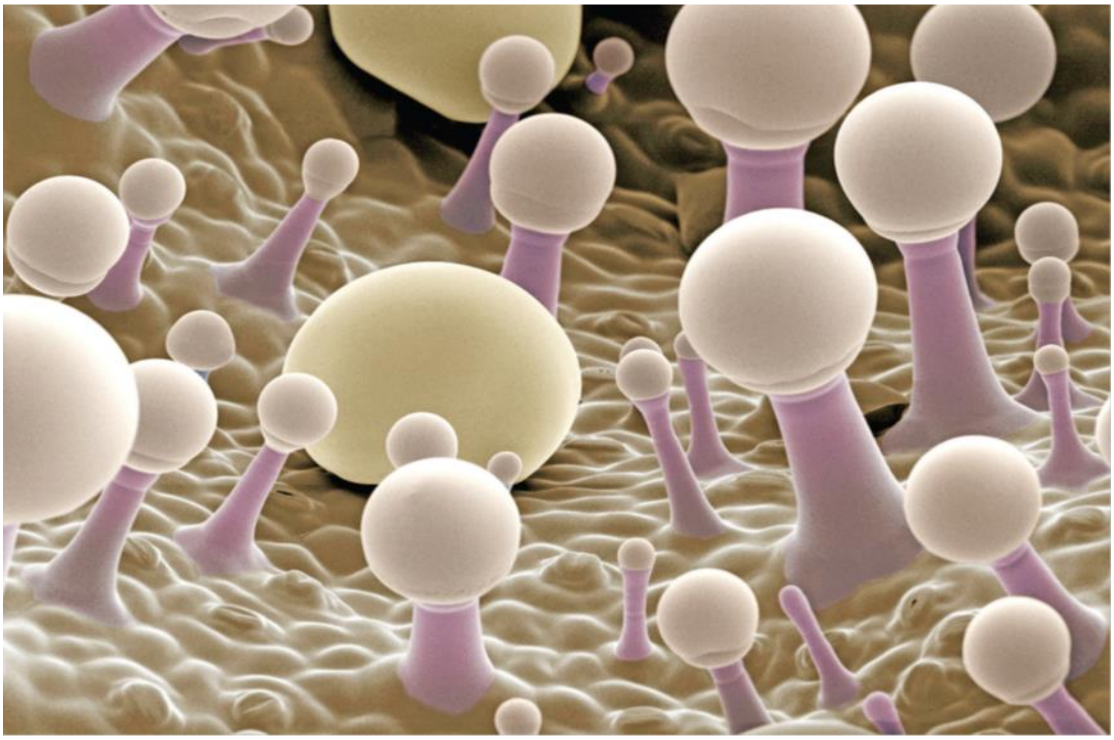


FIGURE.2.1 Monoterpenes and sesquiterpenes are commonly found in glandular hairs on the surface of a plant. This false-colored scanning electron micrograp glandular trichomes (microscopic hairs, purple) on the calyx of a clary sage (*Salvia sclarea*) plant. The trichomes are secreting globules of essential oils (round, white).

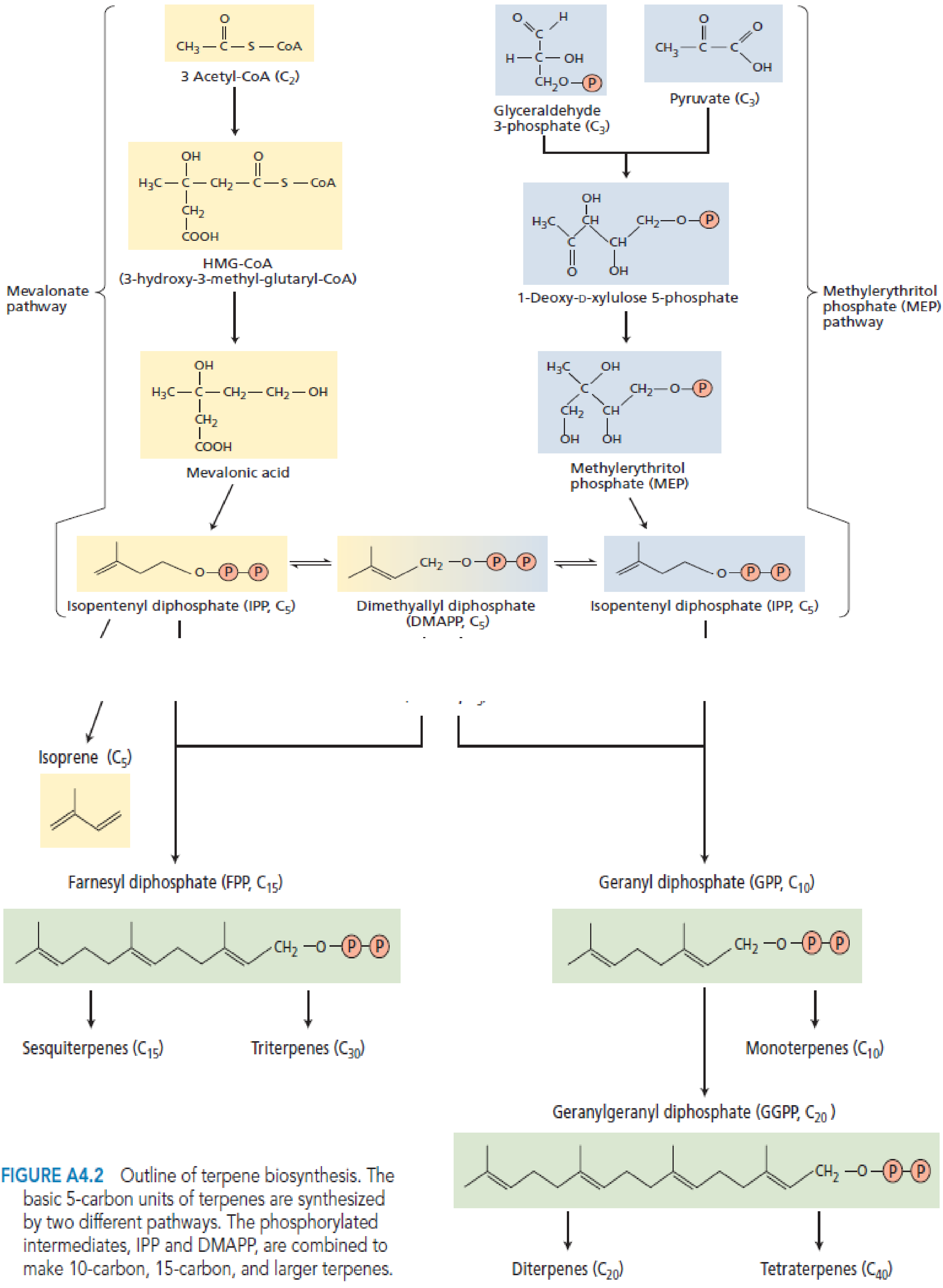
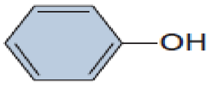


FIGURE A4.2 Outline of terpene biosynthesis. The basic 5-carbon units of terpenes are synthesized by two different pathways. The phosphorylated intermediates, IPP and DMAPP, are combined to make 10-carbon, 15-carbon, and larger terpenes.

Figure. 2

Phenolic Compounds

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



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.

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 4**). 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 pentosphosphate 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 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.

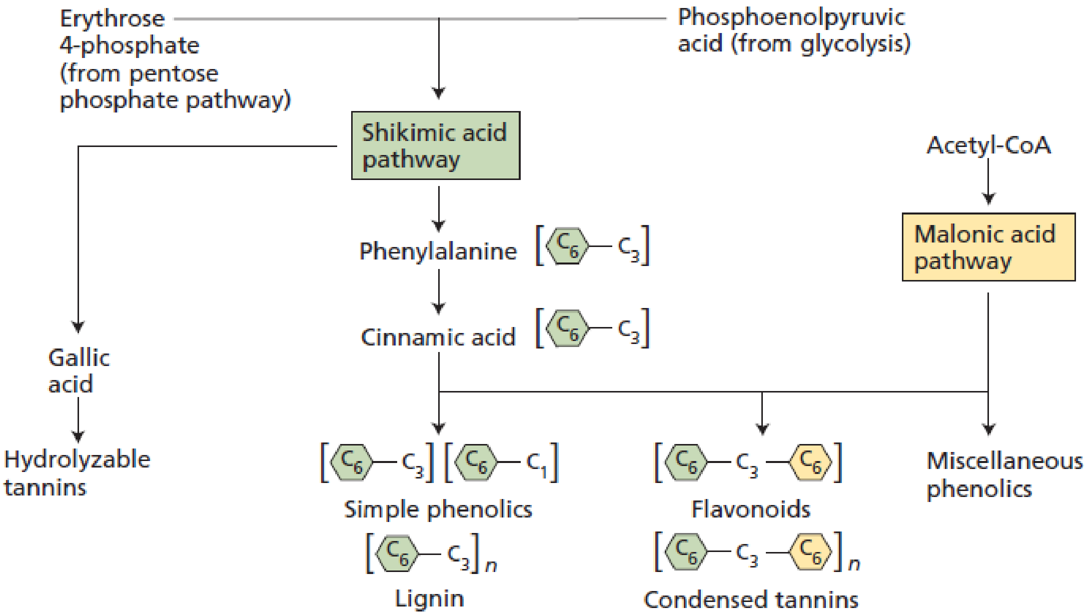


FIGURE 4 Plant phenolics are synthesized in several different ways. In higher plants, most phenolics are derived at least in part from phenylalanine, a product of the shikimic acid pathway. Formulas in brackets indicate the basic arrangement of carbon skeletons: C6 indicates a benzene ring, and C3 is a three-carbon chain.

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.

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 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 5**). 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 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 plant defensive compounds and even use them in their own defense .

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 of their general toxicity and deterrence capability .

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 nonmedical 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.

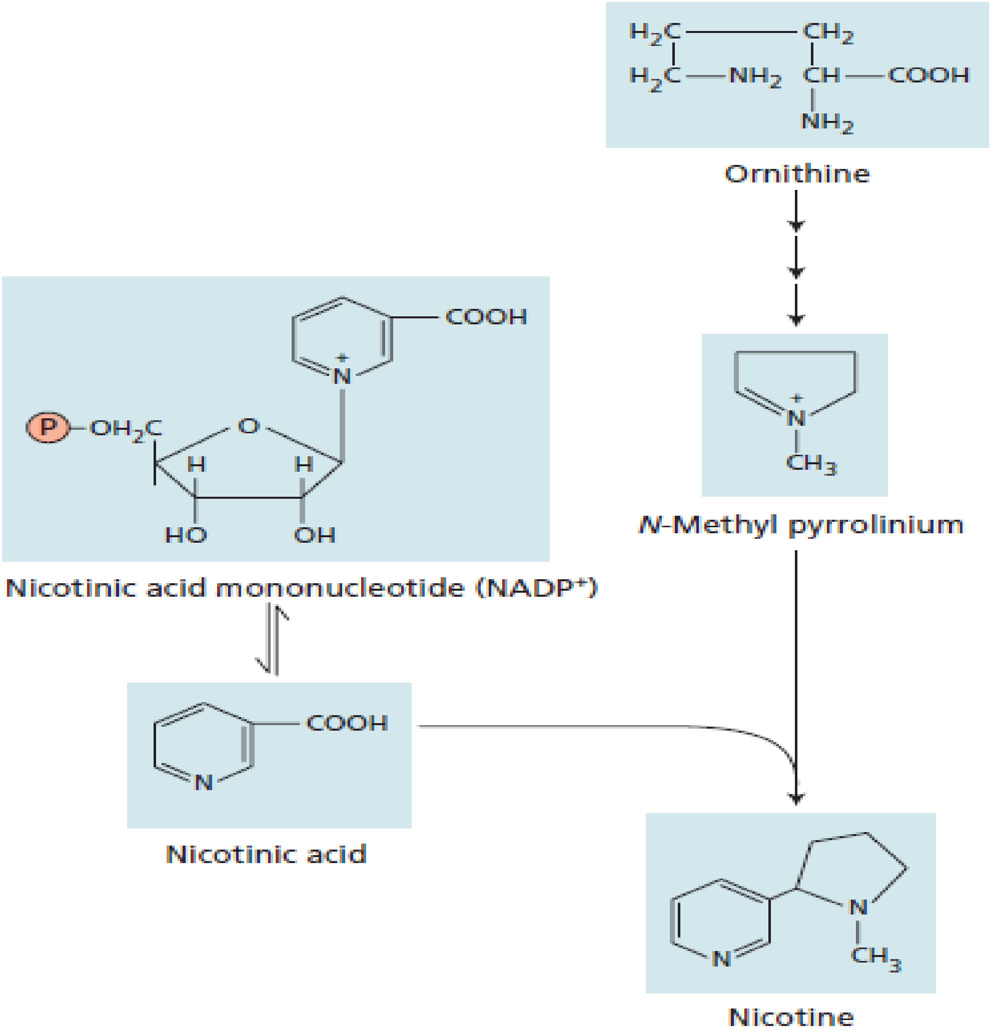


FIGURE 5. Nicotine biosynthesis begins with the synthesis of nicotinic acid (niacin) from aspartate and glyceraldehyde 3-phosphate. Nicotinic acid is also a component of NAD+ and NADP+, important participants in biological oxidation–reduction reactions. The five-membered ring of nicotine is derived from ornithine, an intermediate in arginine biosynthesis.